INCORPORATING IN VITRO BIOACCESSIBILITY FOR ARSENIC AND LEAD IN SOIL IN HUMAN HEALTH RISK ASSESSMENT

14th Annual SABCS Workshop

Sept 25, 2024

Royal Roads UNIVERSITY

Questions to be Addressed

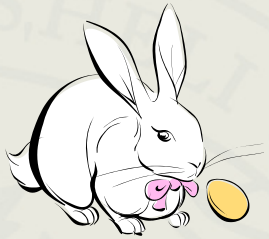
- Which specific bioaccessibility tests are best for As and Pb contaminated soils?
- What are the pros and cons of the various tests?
- What is the relative cost of the tests?
- Which tests should we include in the lab manual?

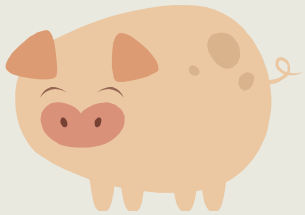
What is Bioavailability

- The amount of a contaminant that is absorbed into the body following skin contact, ingestion, or inhalation (Ng et al., 2005)
- The fraction of an ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs (USEPA, 2007)

How is Bioavailability Determined?

- In vivo assays
  - Rabbits, primates, rodents and swine*





- In vitro assays
  - Biological fluids
  - Extraction using simulated gastric and intestinal fluids - Physiologically Based Extraction Test (PBET) or In Vitro Bioavailability Assay (IVBA)

What is Bioaccessibility?

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- In vitro tests measure bioaccessibility
- Fraction of the contaminant that is released from soil into solution during digestion making it available for absorption

$$\text{Bioaccessibility} = \frac{\text{Amount released into solution}}{\text{Total concentration in the soil}} \times 100\%$$

- Less expensive and less time consuming compared to in vivo tests
- Surrogate for bioavailability

What is the Relationship between Bioavailability and Bioaccessibility?

- Bioaccessibility can be related to bioavailability by comparing in vivo model (RBA) to in vitro (IVBA)
- Regression equation developed e.g., for USEPA Method 1340

As RBA = 0.84 (IVBA %) + 3.56

Pb RBA = 0.878 (IVBA %) – 2.8

Common Bioaccessibility Methods

- In Vitro Bioaccessibility Assay (USEPA 1340)
- PBET Gastrointestinal Model (Ruby et al., 1999)
- BARGE Unified Bioaccessibility Method (UBM) (ISO Technical Specification 17924)
- Ohio State University In Vitro Gastrointestinal Method (OSU-IVG)
- Deutsches Institut für Normung e.V. (DIN)

In Vitro Bioaccessibility Assay (IVBA) - SBRC, SBET, RBALP, USEPA METHOD 1340

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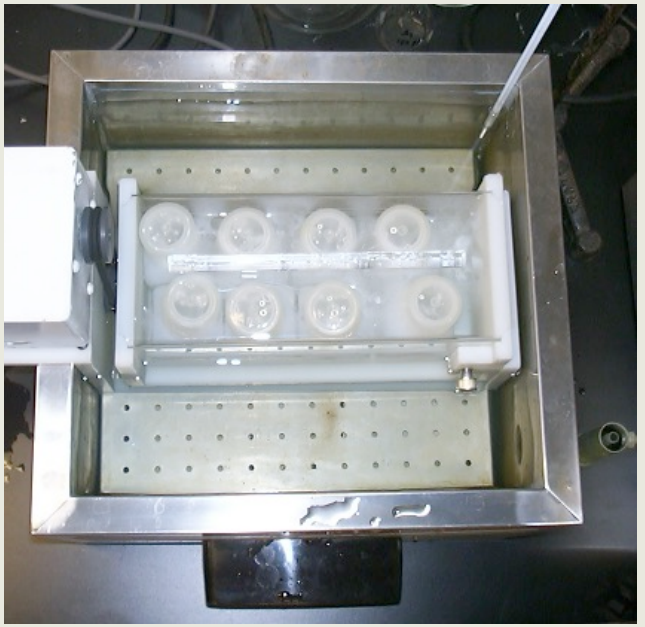
- 1.0 g soil: 100 mL glycine/HCl buffer
- pH 1.5
- 1-h extraction at 37 ^{O}C
- End-to-end rotation
- Filter through 0.45 µm
- Analyze extracts
  - One per sample



PBET - Gastrointestinal (GI) Model

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Gastric Phase

- Pepsin, citrate, malate, lactic acid, acetic acid and HCl at pH 2
- Rotate at 37^{O}C end-over-end for 1 h
- Collect 10 mL and filter through 0.45µm

Intestinal Phase

- Adjust pH to 7 with saturated sodium bicarbonate solution
- Add bile salts and pancreatin
- Rotate end-over-end for 3 h
- Collect 20 mL and filter through 0.45µm

- Analyze extracts - 2 for each sample

Ohio State University In Vitro Gastrointestinal Method (OSU-IVG)

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Gastric Phase

- 1 g soil
- NaCl, porcine pepsin, pH 1.8, 37^{O}C
- Stir (100 rpm) for 1 h, pH monitored and kept at 1.8 ± 0.1
- Remove 10 mL, centrifuge and then filter (0.45 µm).

Intestinal Phase

- Adjust pH of remaining solution to 6.1 ± 0.1 by dropwise additions of a saturated Na_2CO_3 solution
- Add porcine bile extract porcine pancreatin.
- Mix and adjust pH (6.1 ± 0.1)
- Remove 10 ml solution after 2 h and treat as per gastric extracts

- Analyze extracts – 2 for each sample

Deutsches Institut Für Normung E.V. (DIN)

- Gastric Phase
  - 1 g soil
  - NaCl, pepsin, mucin, KCl, KH_2PO_4, pH 2, 37^{O}C
  - Stir (100 rpm) for 1 h, pH monitored and kept at 1.8 ± 0.1
  - Remove 10 mL, centrifuge and then filter (0.45 µm).
- Intestinal Phase
  - Adjust pH of remaining solution to 7.5
  - Add bile, pancreatin, trypsin, urea, KCl, $CaCl_2$, $MgCl_2$.
  - Extract for 6 h
- Analyze extracts – 2 for each sample

Unified BARGE Method (UBM)

- Saliva
- Gastric
- Intestinal
- 4 extracts for each sample

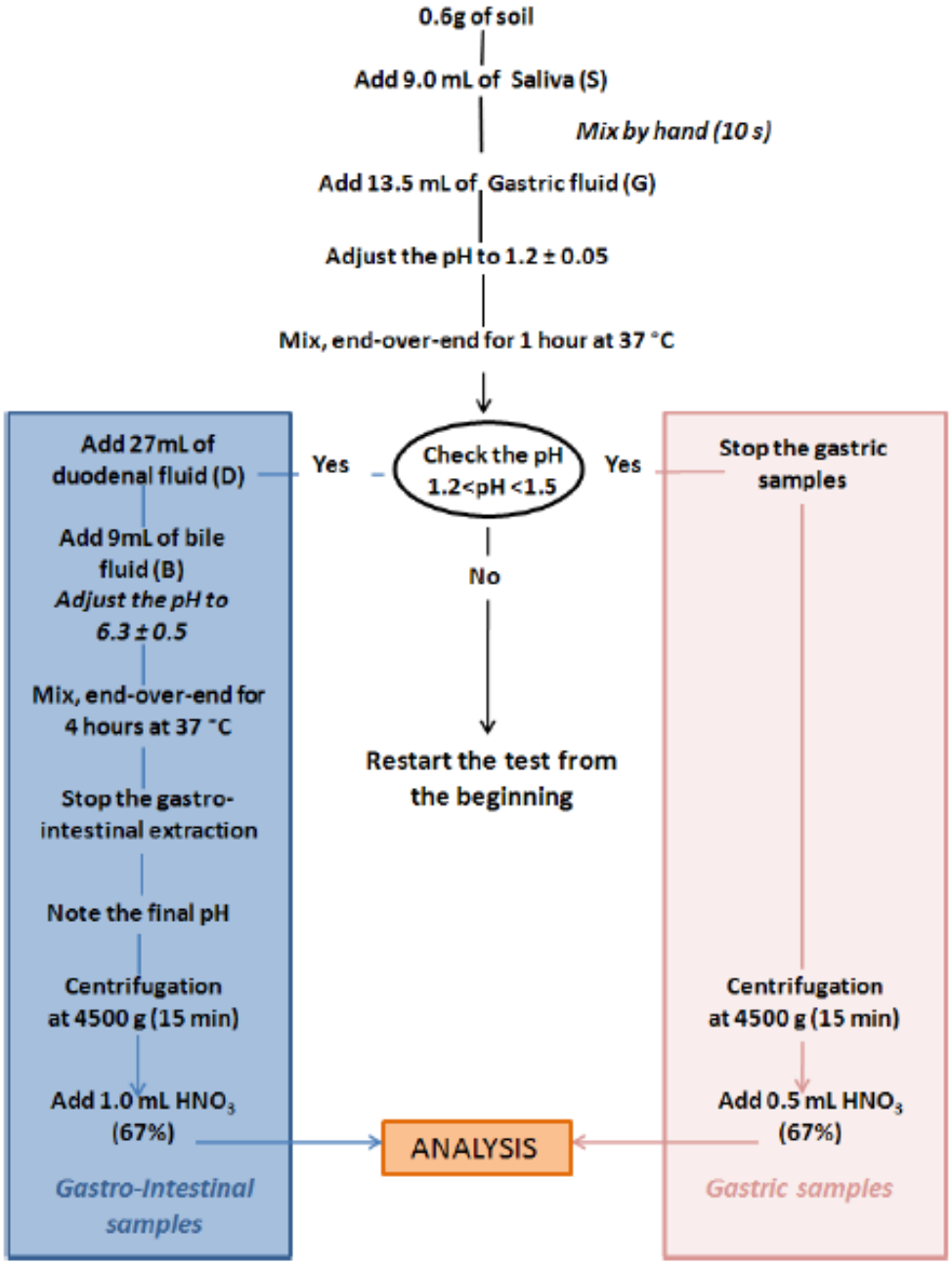

Figure 1 Schematic diagram of the UBM methodology

UBM Reagents

INERIS controlling risks for sustainable development

BARGE

4.3. Reagents

Table 1 shows the various reagents used in the synthesis of the digestive fluids. All pH adjustments are performed with HCl 37% or NaOH 1-5 M purchased from Analytika Ltd or other suitable supplier.

Table 1 Reagents involved in the synthesis of the digestive fluids.

Reagents	Supplier	Product Code	CAS N°
NaH_2PO_4	Merck	1.06342.0250	13472-35-0
NaCl	Prolabo	27810.262	7647-14-5
KSCN	Sigma	P2713	333-20-0
Na_2SO_4	VWR	28114.230	7757-82-6
KCl	Merck	1.04936.1000	7447-40-7
$CaCl_2.2H_20$	VWR	1.02382.0250	10035-04-8
NH_4Cl	Prolabo	21236.291	12125-02-9
$NaHCO_3$	Prolabo	27778.293	144-55-8
KH_2PO4	Prolabo	26936.236	7778-77-0
$MgCl_2.6H_20$	Sigma	M8266	7786-30-3
NaOH	Prolabo	28244.295	1310-73-2
HCl	Analytika Ltd.		
Urea	Merck	108487	57-13-6
D + Glucose	VWR	101174Y	50-99-7
D – Glucuronic acid	Sigma	49335	6556-12-3
D-glucosaminehydrochloride	Sigma	G4875	66-84-2
Pepsine (porcine)	Merck	107185	9001-75-6
Bovine Serum Albumen	Merck	112018	90604-29-8
Mucin (porcine)	Sigma	M2378	84082-64-4
Uric Acid	Sigma	U2625	69-93-2
Pancreatin (porcine)	Merck	107130	8049-47-6
α-amylase (bacillus)	Sigma	A-6814	9000-90-2
Lipase (porcine)	Sigma	L-3126	9001-62-1
Bile (bovine)	Sigma	B-3883	8008-63-7

Table 1. Compositions and parameters in the gastric (GP) and intestinal phases (IP) of in vitro assays SBRC, IVG, DIN, PBET, and UBM for metal bioaccessibility assessment.

Method	Phase	Composition (L^{-1})	pH	Soil/solution ratio	Extraction time (h)	Reference
SBRC	GP	glycine 30.0 g	1.5	1:100	1	Kelley et al. (2002)
	IP	bile 1.75 g, pancreatin 0.50 g	7.0	1:100	4	
PBET	GP	pepsin 1.25 g, sodium malate 0.50 g, sodium citrate 0.50 g, lactic acid 420 µL, and acetic acid 500 µL	2.5	1:100	1	Ruby et al. (1996)
	IP	bile 1.75 g, pancreatin 0.5 g	7.0	1:100	4	
IVG	GP	pepsin10 g, NaCl 8.77 g	1.8	1:150	1	Rodriguez et al. (1999)
	IP	Bile 3.5g, pancreatin 0.35g	5.5	1:150	1	
DIN	GP	1 g pepsin, 3 g mucin, 2.9 g NaCl, 0.7 g KCl, 0.27 g KH_2PO_4	2.0	1:50	2	DIN (2000)
	IP	9.0 bile, 9.0 g pancreatin, 0.3 g trypsin, 0.3 g urea, 0.3 g KCl, 0.5 g $CaCl_2$, 0.2 g $MgCl_2$	7.5	1:100	6	
UBM	Saliva	KCl 0.90 g, NaH_2PO_4 0.89 g, KSCN 0.20 g, Na_2SO_4 0.57 g, NaCl 0.30 g, urea 0.2 g, amylase 0.145 g, mucin 0.05 g, uric acid 0.015 g	6.5	1:15	10 s	Wragg et al. (2009)
	GP	KCl 0.824 g, NaH_2PO_4 0.266 g, NaCl 2.752 g, $CaCl_2$ 0.4 g, NH_4Cl 0.306 g, urea 0.085 g, glucose 0.65 g, glucuronic acid 0.02 g, glucosaminehydrochloride 0.33 g, bovine serum albumin 1.0 g, mucin 3.0 g, and pepsin 1.0 g	1.2	1:37.5	1	
	IP	KCl 0.94 g, NaCl 12.3 g, $NaHCO_3$ 11.4 g, KH_2PO_4 0.08 g, $MgCl_2$ 0.05 g, urea 0.35 g, $CaCl_2$ 0.42 g, bovine serum albumin 2.8 g, pancreatin 3.0 g, lipase 0.5 g, and bile 6.0 g	6.3	1:97.5	4	

Source: https://www.tandfonline.com/doi/full/10.1080/10643389.2019.1656512

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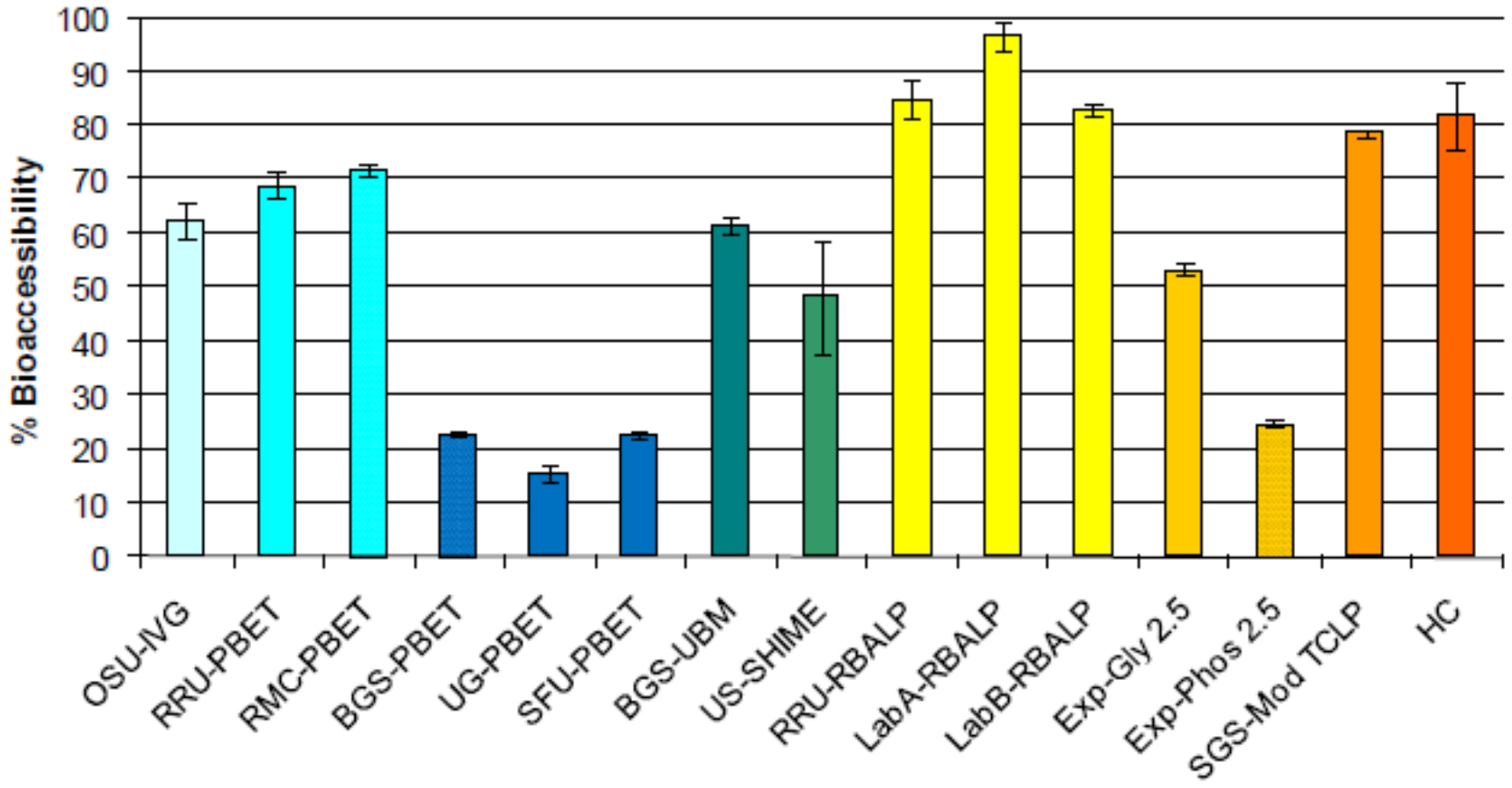
Variability of bioaccessibility results using seventeen different methods on a standard reference material, NIST 2710

Bioaccessibility Lead – Gastric Phase



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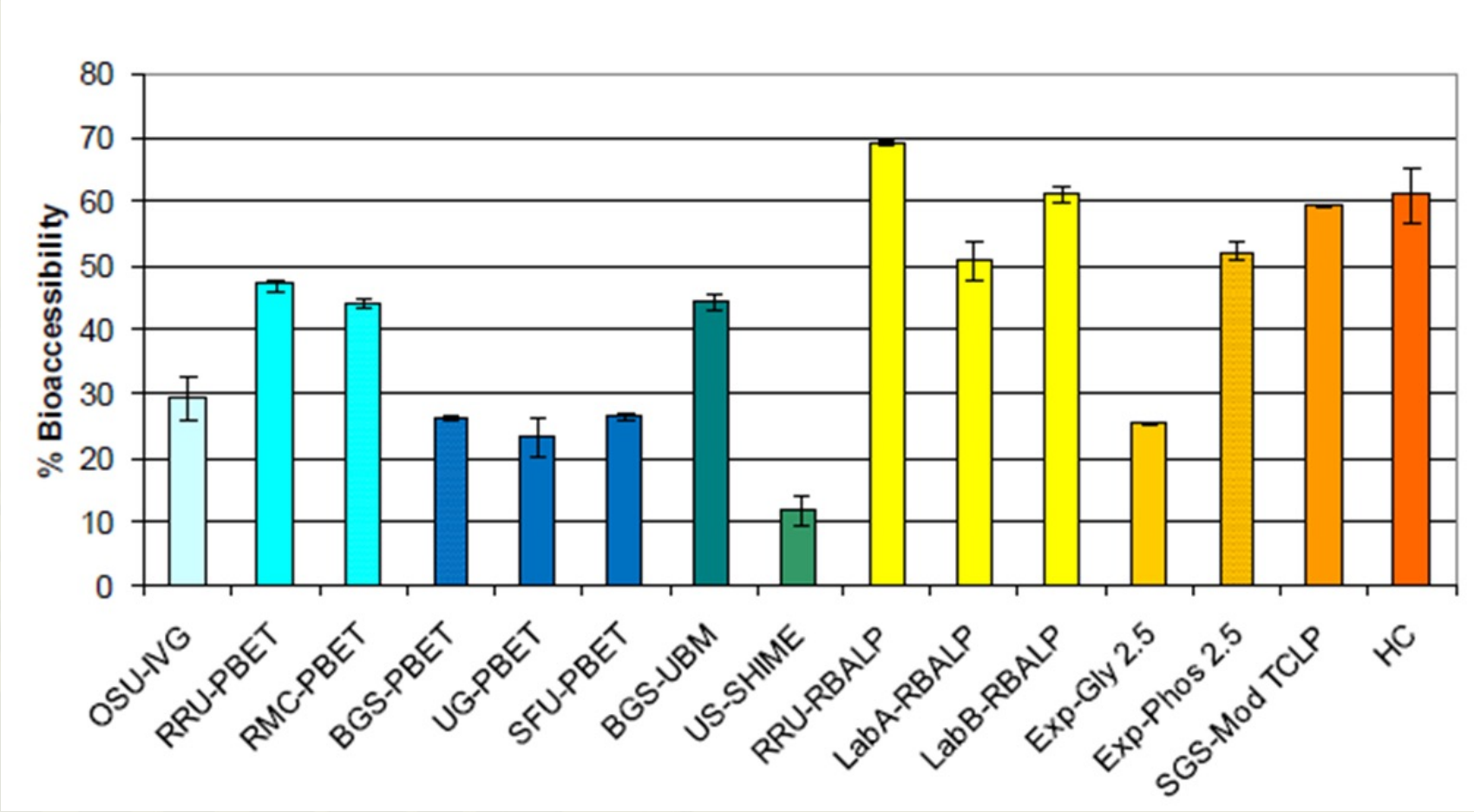
Variability of bioaccessibility results using seventeen different methods on a standard reference material, NIST 2710

Bioaccessibility Arsenic – Gastric Phase



Which Method(s) should we Include in the BC Lab Manual?

- IVBA? PBET? IVG? UBM? DIN?
- Parameters for method selection
  - In vivo/in vitro validation for the test method and the metals of interest
  - Cost
  - Conservatism
  - Reproducibility
  - Availability of certified standard reference materials (SRMs)
- BCELTAC Bioaccessibility Subcommittee selected IVBA (USEPA Method 1340)

Cost Estimates

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	IVBA	PBET	IVG	DIN	UBM
Drying, sieving, Extraction	100	150	150	150	250
Total metals	75	75	75	75	75
Extracts	75	150	150	150	300
Total	250	375	375	375	625

BCELTAC Round Robin

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- Evaluate the capabilities of BC-based laboratories to conduct USEPA Method 1340
- Investigate the suitability and acceptance of using other SRMs for the BC Environmental lab manual
- Draft bioaccessibility method for BC lab manual incorporating results from first round robin
- Use draft method to analyze field collected samples
- Update draft method and post for review
- Finalize and publish method

Methodology

Round Robin I

- Five labs participated
- USEPA Method 1340 used to analyze As and Pb IVBA for NIST 2710a, NIST2711a, BGS119 & Enviromat SS-2
- Draft IVBA method for BC Environmental Laboratory Manual that incorporates findings

Round Robin II

- Four labs participated
- Draft BC IVBA method used to analyze 10 field collected samples

Results for Round Robin I

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BGS119

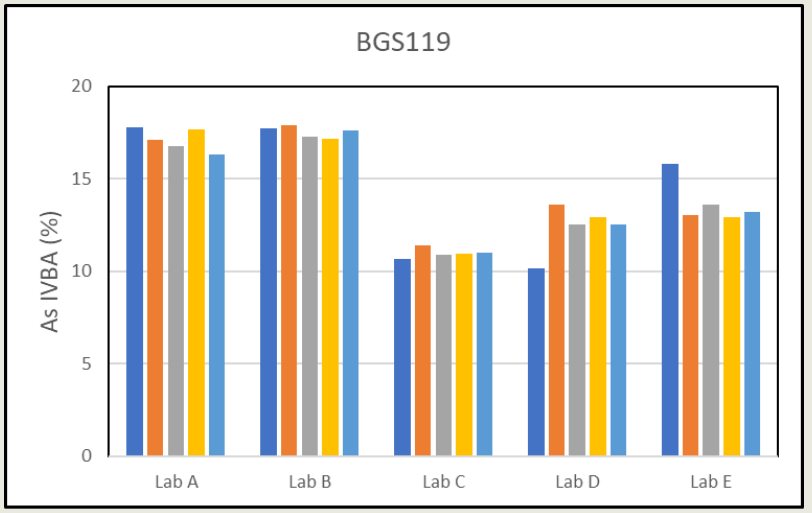
- As IVBA: 10.2% to 17.8%.
- Pb IVBA: 31.9% to 86.7%.
- Pb IVBA significantly lower for Lab D.

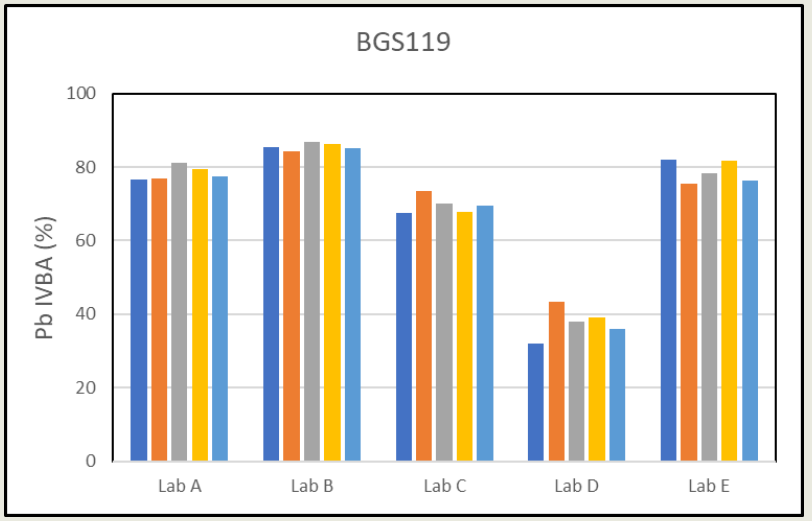
Enviromat SS-2

- As (3.2 mg/kg) too low for use in draft method.

Reproducibility

- Good inter-lab and intra-lab reproducibility (RSD <20%).



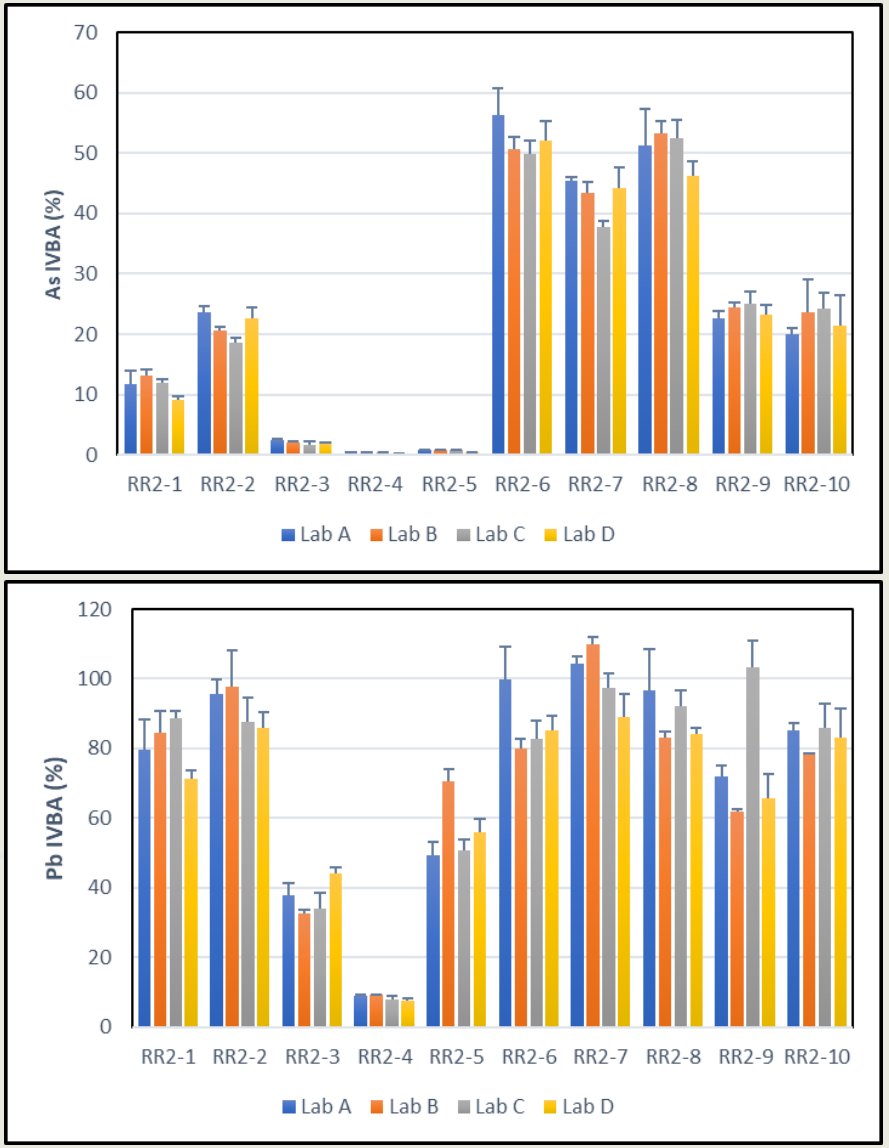


Recommendations: Round Robin I

- Include US EPA Method 1340 for the assessment of As and Pb IVBA - Prescriptive Method format
- Use BGS 119 as SRM for As and Pb IVBA along with NIST 2710a and NIST 2711a
- Include NIST 2711a as SRM for As IVBA in the BC Env Lab Manual
- Develop Lab Manual method and use for Round Robin II

Results Round Robin II

- Good recoveries for SRMs (NIST 2710a, NIST 2711a and BGS 1190).
- As IVBA: 0.1 to 60.4%.
- Pb IVBA: 7.0 to 121.6%.
- Good intra-lab and inter-lab reproducibility (RSD ≤30%).



Synopsis for Round Robin Studies

- Labs in BC can use the BC Lab Manual method to provide reproducible and comparable As and Pb IVBA
- Labs should use one digestion method (SALM) and end-to-end rotation for the IVBA extraction
- Labs should ensure appropriate soil size fraction (<150 µm) is used for both the total metal analysis and the IVBA extraction
- Lab manual method published

Publications

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Metals
Revision Date: Nov 30, 2021

In Vitro Bioaccessibility (IVBA) for Arsenic and Lead in Soil - Prescriptive

Parameter	Arsenic soil IVBA, Lead soil IVBA
Analytical Method	IVBA extraction, Nitric – Hydrochloric acid digestion, Instrumental analysis
Introduction	Bioavailability is the fraction of an ingested contaminant that is absorbed by the body and

https://www2.gov.bc.ca/assets/gov/environment/research-monitoring-and-reporting/monitoring/emre/methods/in_vitro_bioaccessibility_ivba_for_as_and_pb_in_soil_prescriptive.pdf

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Environmental Policy & Regulation

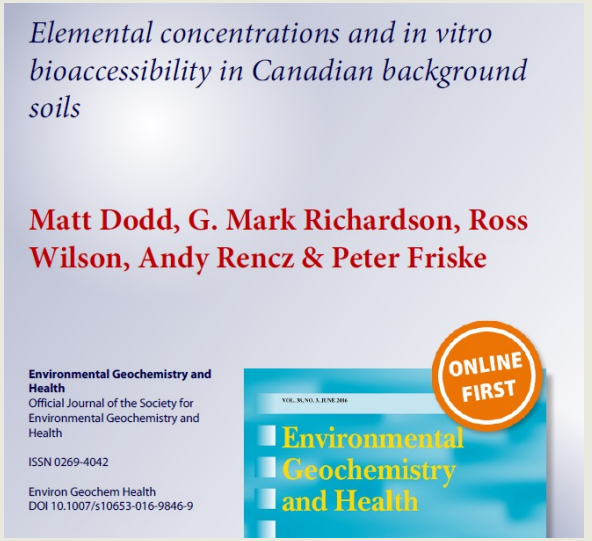
In vitro bioaccessibility round robin testing for arsenic and lead in standard reference materials and soil samples

Matt Dodd,[1] Deanna Lee,[2] Jasen Nelson,[3] Sergei Verenitch,[4] and Ross Wilson[5]

[1]*Royal Roads University, Victoria, British Columbia, Canada*
[2]*Health Canada, Vancouver, British Columbia, Canada*
[3]*BC Ministry of Environment and Climate Change Strategy, Victoria, British Columbia, Canada*
[4]*Aquatech EnviroScience Laboratories Inc., Victoria, British Columbia, Canada*
[5]*Wilson Scientific Consulting Inc., Vancouver, British Columbia, Canada*

RRU Examples of Bioaccessibility Applications

- HHRA for metal contaminants at various sites
  - Canada, Ghana, Mexico, Sweden
- Tri-National Survey (background samples)
- Urban soils – playgrounds, parks, garden soils

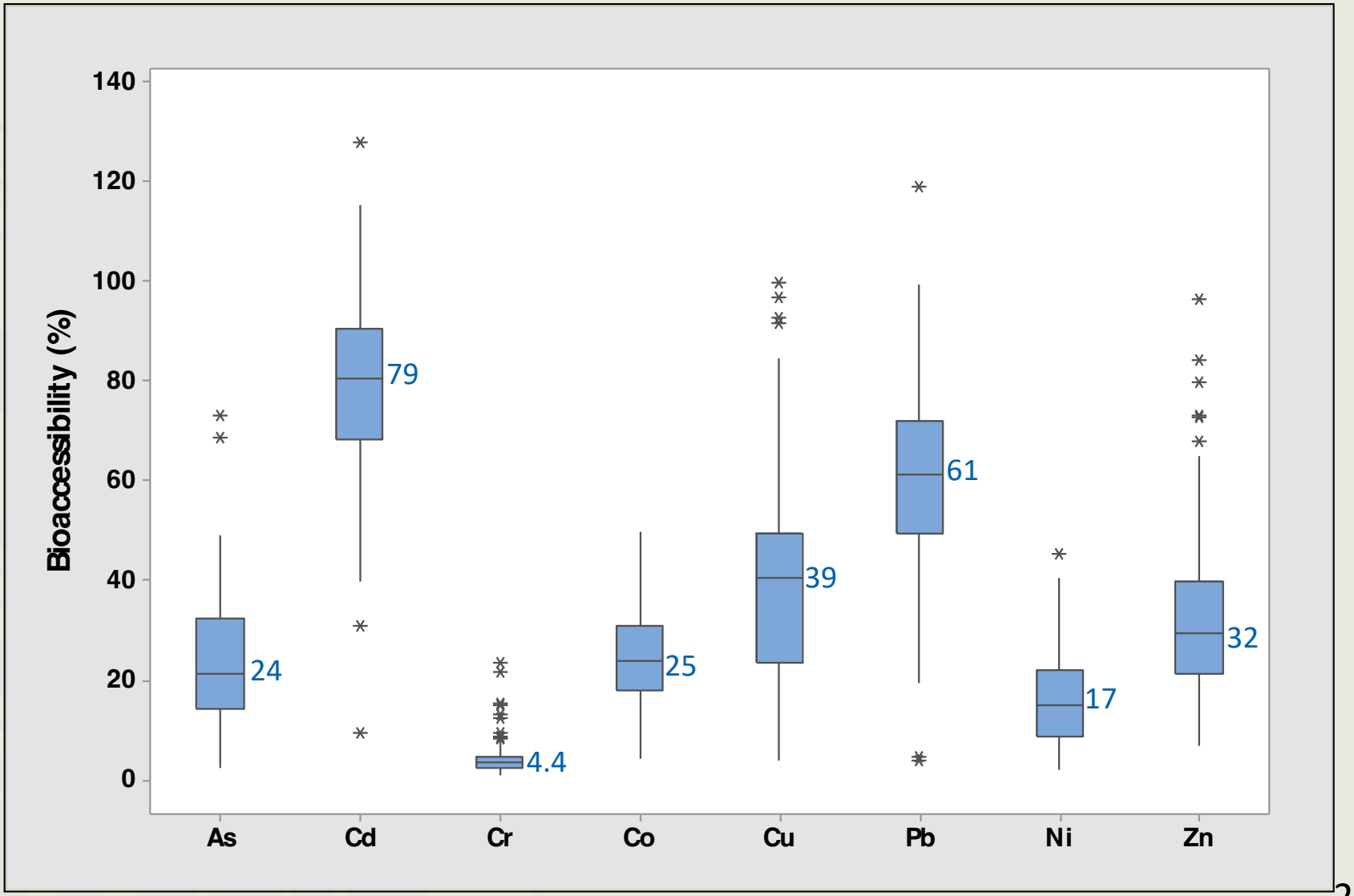


Potential Risk Associated with Exposure to Metal Contaminants at Urban Parks and Playgrounds in Canada

Sampling and Analysis

- Surface soils (0-5 cm)
- Accessible areas in parks
  - Playgrounds
  - Picnic areas
- Different surrounding land use
- Site history
- Total metals
- IVBA
- HHRA

Boxplots of Metal Bioaccessibility and Mean Values



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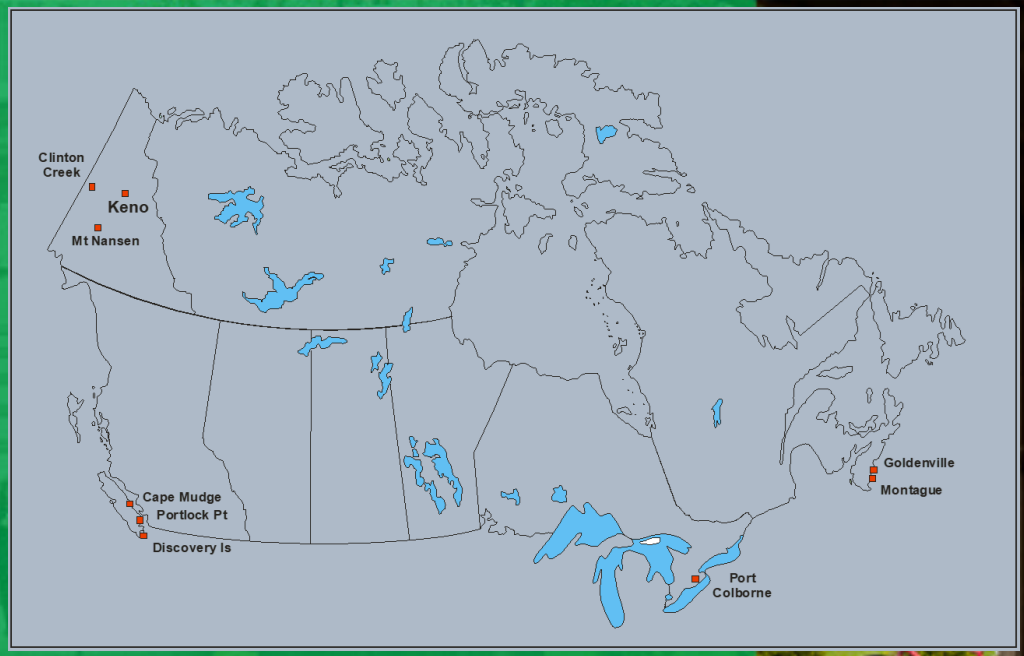
MINE SITES AND LIGHTSTATIONS IN CANADA




Mt Nansen Tailings Pond


Cape Mudge Lighthouse


Clinton Creek Waste Rock

Summary of Metals Bioaccessibility (%) for Mine Sites and Lightstations (n = 158)

	As	Cd	Cr	C0	Cu	Pb	Ni	Zn
Mean	**14**	**38**	**8.1**	**18**	**37**	**48**	**14**	**24**
Std Dev	14	30	9	14	21	22	11	20
Median	**37**	**5**	**18**	**35**	**4**	**21**	**20**	**20**
Min	0.1	0.2	0.1	0.1	1.1	0.4	0.4	0.3
Max	55	100	80	74	105	106	74	86
95%	43	81	23	44	71	79	34	59

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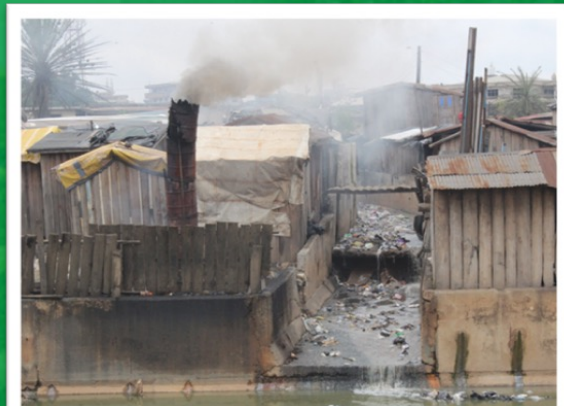
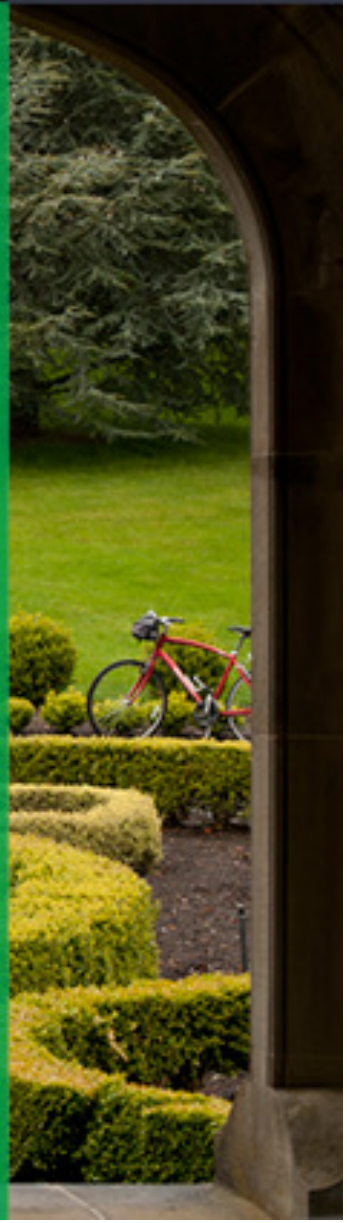
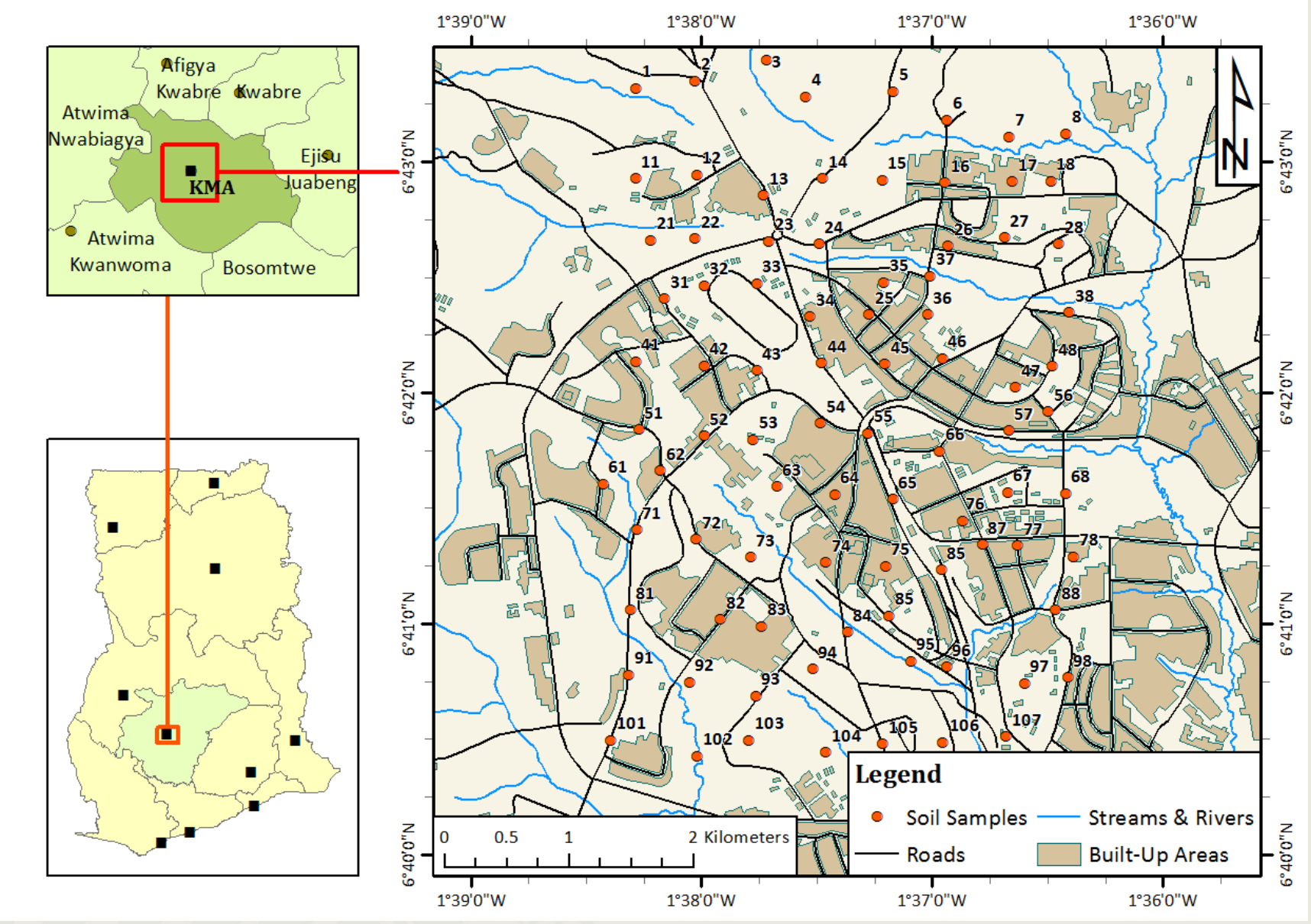
EXAMPLE:
KUMASI, GHANA

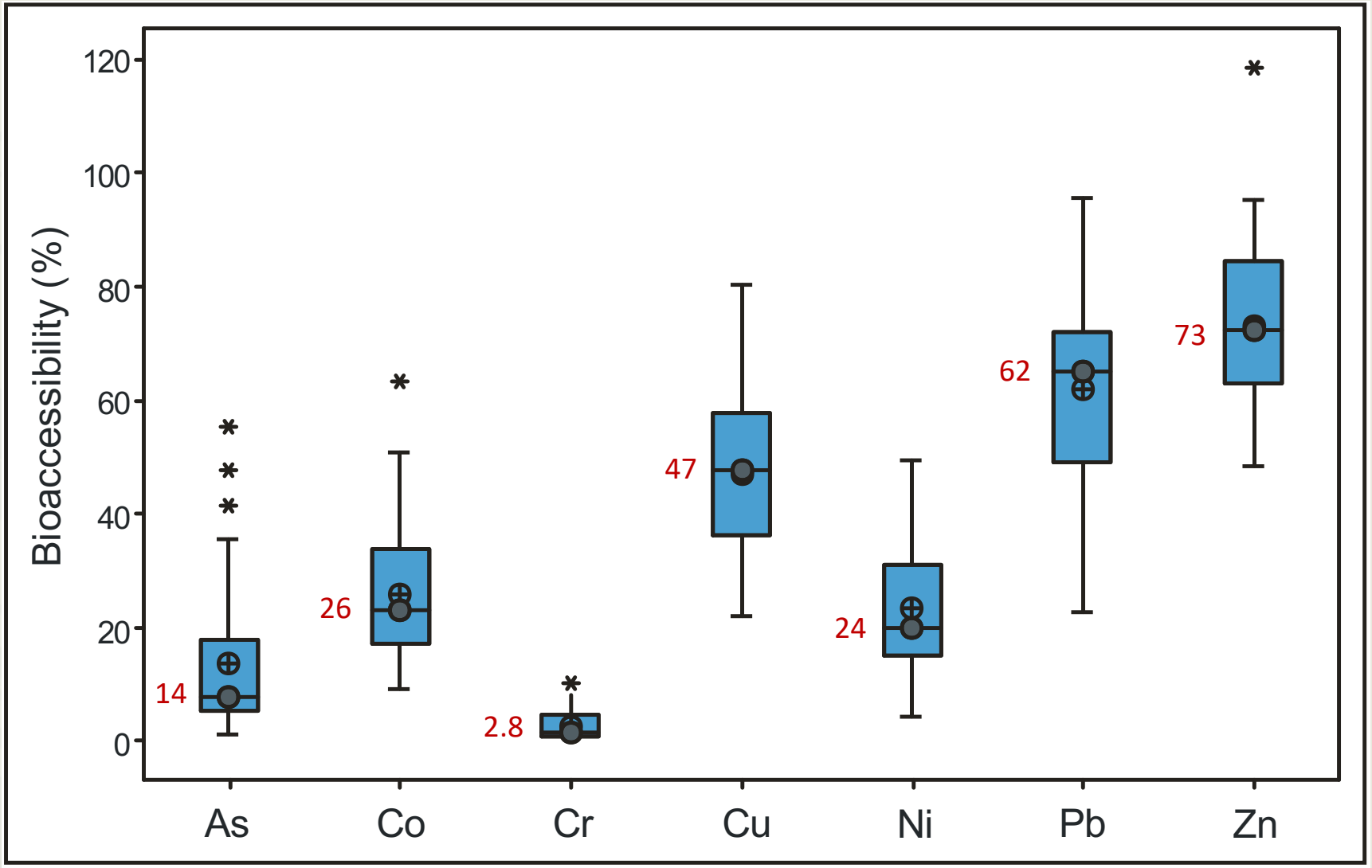




Kumasi Urban Soils Sampling Locations



Boxplots of Kumasi Urban Soils Metal Bioaccessibility



E-Waste Recycling Sites, Dagomba Line, Kumasi, Ghana

- Kumasi is the second largest city in Ghana
- Over 1000 people work in e-waste and metal recycling at Dagomba Line
- Electronic equipment dismantled – ICS and metals recovered
- Materials burnt in locally constructed stacks or open fires to recover metals

Sampling


1°37'0"W
1°36'30"W
1°36'0"W
1°35'30"W
6°42'30"N
6°42'0"N
6°41'30"N
N
E-waste recycling workshops
E-waste recycling workshops
E-waste recycling workshops.
E-waste recycling workshops.
x=0.
0
0.175
0.35
0.7
1.05
1.4
Kilometers
Legend
Background
Soil
Water / Sediment
Road
River
Railroad
Boundary

Descriptive Stats for Select Metals (mg/kg) in Dagomba Line Soils

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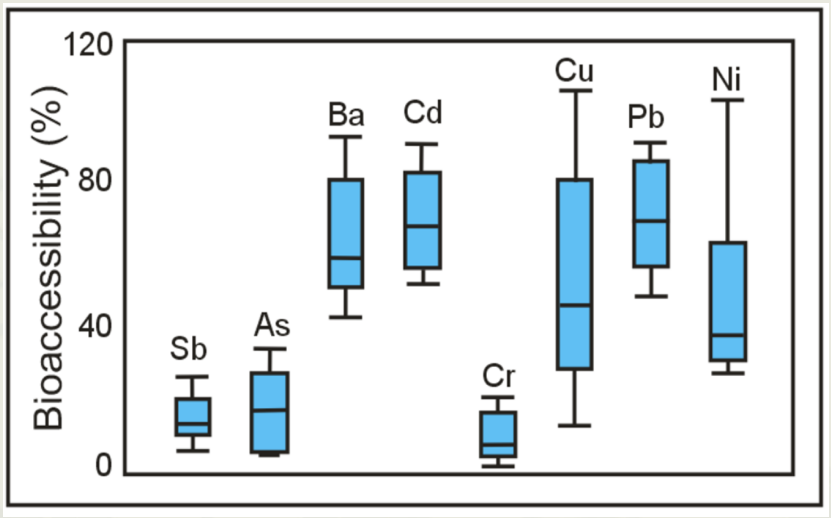
	Ag	As	Ba	Cd	Cr	Cu	Ni	Pb	Sb	Zn
Mean	9.3	80	518	7.2	116	2608	83	1273	151	1714
Std Dev	12	144	459	7.9	41	3834	63	1609	286	1573
Median	2.3	33	357	3.2	119	643	62	364	29	758
Max	65	634	2305	30	205	18618	276	6141	1514	5232
95%tile	24	387	1245	26	186	9010	200	5073	559	4832
CCME R/P	*20*	*12*	*500*	*10*	*64*	*63*	*50*	*140*	*20*	*200*

Risk Characterization

- Variable IVBA



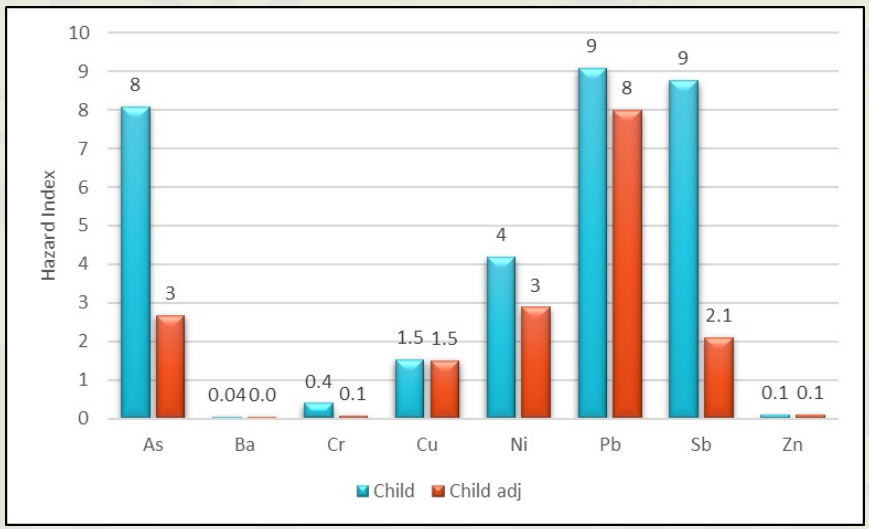
Home > Environmental Geochemistry and Health > Article

Gastric bioaccessibility and human health risks associated with soil metal exposure via ingestion at an E-waste recycling site in Kumasi, Ghana

Original Paper | Published: 03 November 2020

Volume 44, pages 497–509, (2022) Cite this article

- Bioaccessibility adjustments resulted in reduced HI



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Human health risk associated with metal exposure at Agbogbloshie e-waste site and the surrounding neighbourhood in Accra, Ghana

Original Paper | Open access | Published: 28 February 2023

Volume 45, pages 4515–4531, (2023) Cite this article

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Matt Dodd, Lydia Otoo Amponsah, Stephen Grundy & Godfred Darko

WHERE DO WE GO FROM HERE?

- Variable metal bioaccessibility among samples
- Risk associated with soil ingestion is reduced when IVBA data is incorporated in the HHRA
- Currently IVBA use in HHRA acceptable for As & Pb in Canada, US
- In vivo/in vitro models required for other metals to meet regulatory requirements

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- BCELTAG Bioaccessibility subcommittee
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  - RRU/KNUST

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Li H-B, Li M-Y, Zhao D, Li J, Li S-W, Xiang P, Juhasz AL. & Ma LQ. (2020) Arsenic, lead, and cadmium bioaccessibility in contaminated soils: Measurements and validations, Critical Reviews in Environmental Science and Technology, 50:13, 1303-1338, DOI: 10.1080/10643389.2019.1656512

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Risk Characterization

- Assumption: ingestion is the predominant operating pathway at most contaminated sites
- Estimated daily intake (EDI) for incidental ingestion of contaminated soil:

$$EDI = \frac{C_S \times IR_S \times EF \times ED \times CF \times RBA}{BW \times LE}$$

- Non-carcinogenic hazard index = EDI/RfD
- Carcinogenic risk = EDI x CSF

C_S = conc in soil
IR_S = soil ingestion rate
EF = exposure frequency
ED = exposure duration
CF = conversion factor

BW = body weight
LE = life expectancy
RBA = relative bioavailability
CSF = cancer slope factor
RfD = reference dose