Italian Society of Soil Science
School of Soil Biodiversity and Bioindication
XI cycle

BIODIVERSITY AND BIOINDICATORS IN MONITORING AND MANAGEMENT OF CONTAMINATED SOILS

IN-VITRO AND IN-VIVO TESTING TO ASSESS HUMAN BIOACCESSIBILITY OF TRACE METALS IN SOIL

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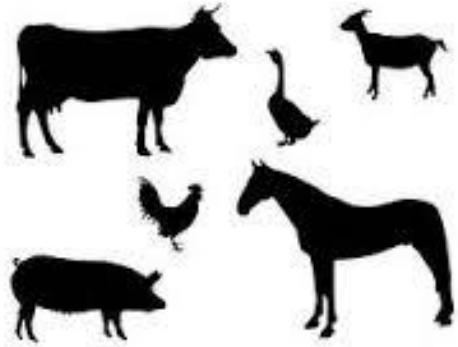
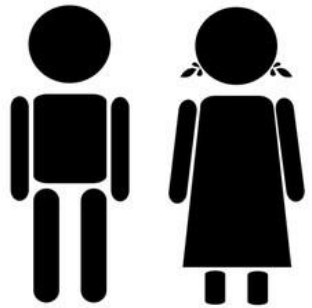
Soil contamination

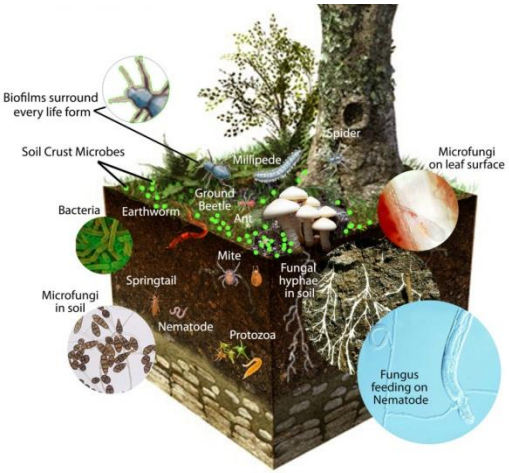
Soil contamination by potentially toxic elements (PTEs) poses serious risks to surrounding environment, water bodies, soil biota, plants and human health

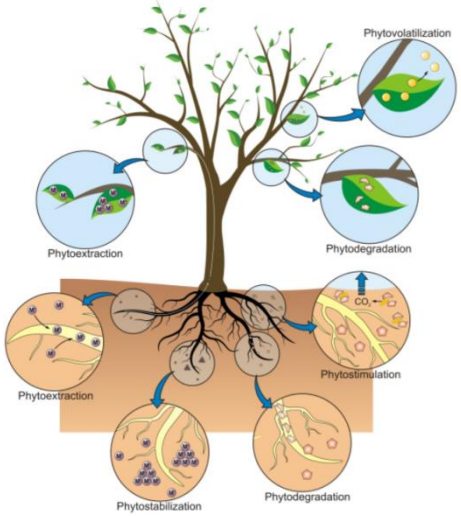


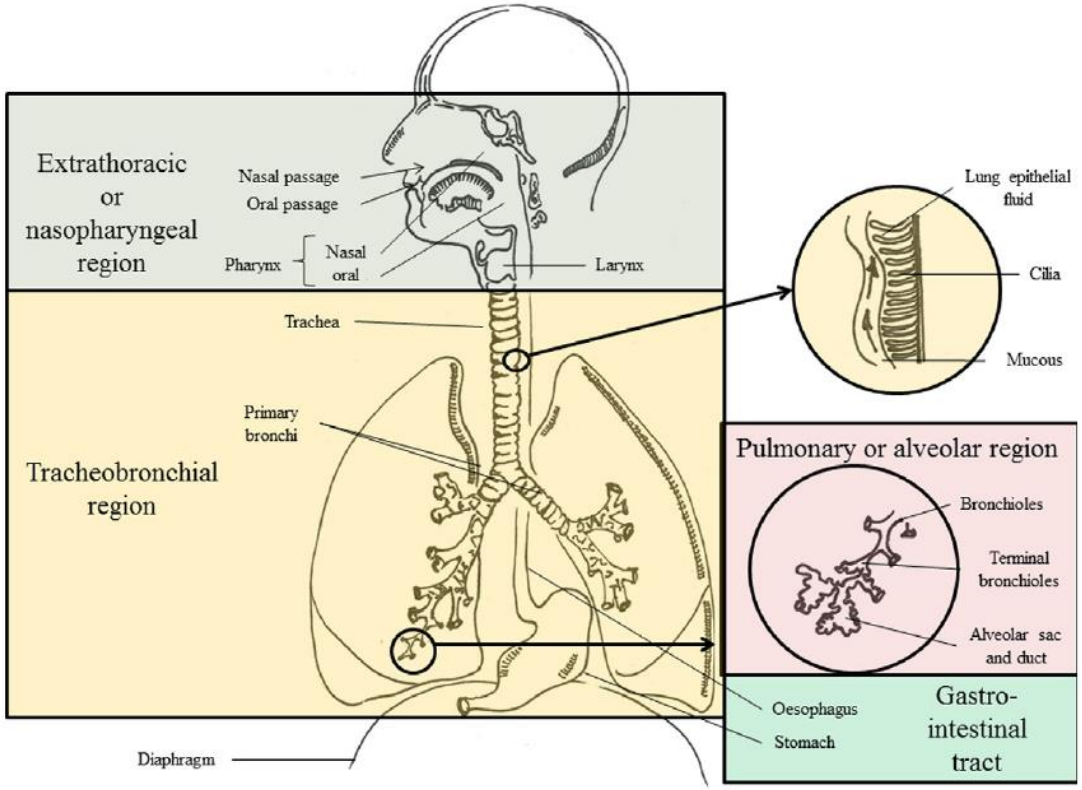


Risk-management of potentially contaminated sites

A proper management of risks for environment and human beings of potentially contaminated sites implies the comprehension of contaminant bioaccessibility to soil biota, plants and humans living or working nearby the sites

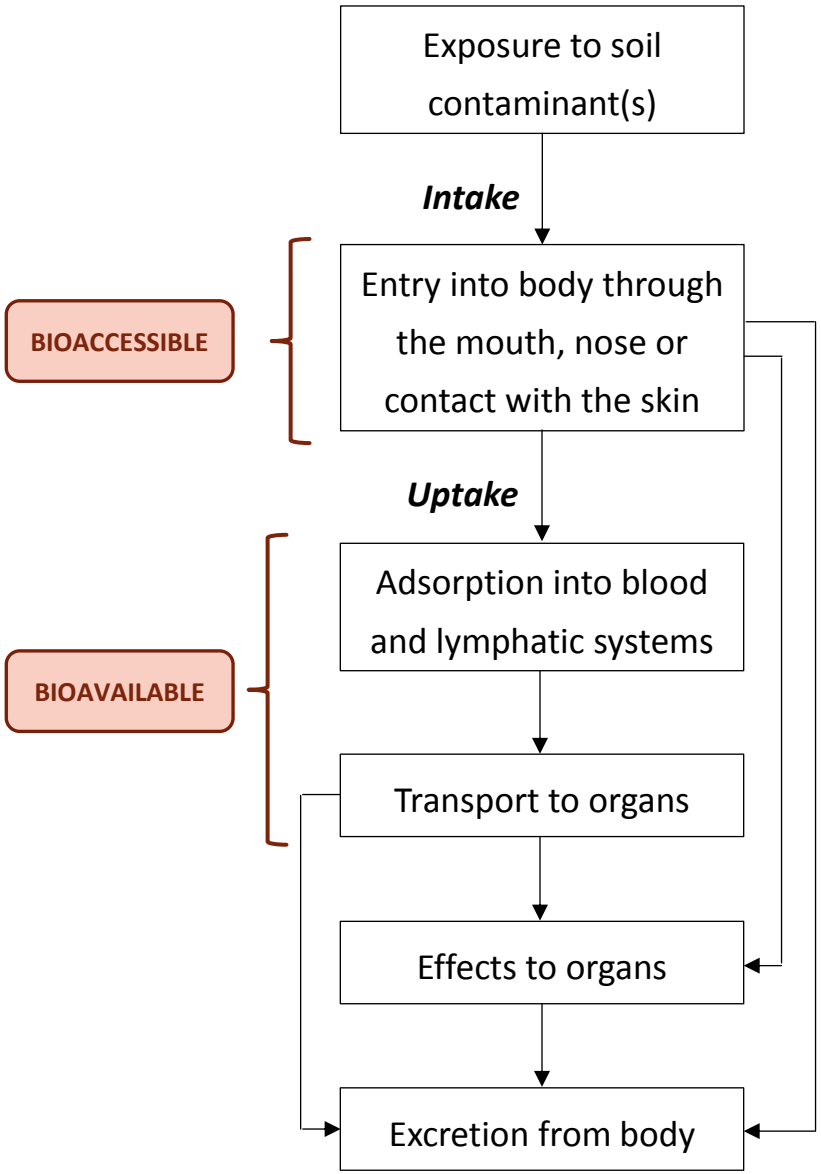




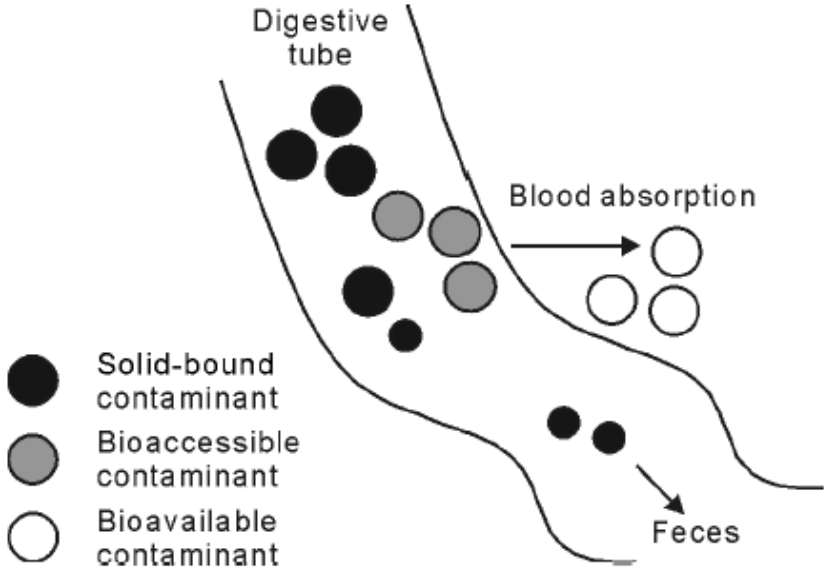


The study of contaminant bioaccessibility is only partially recognised by environmental regulations and stakeholders

Bioaccessibility *vs* bioavailability of soil contaminants



The International Union of Pure and Applied Chemistry (IUPAC) defines as bioaccessible a substance *'able to come in contact with a living organism and interact with it'* and bioavailable a substance '*able to be absorbed by living organisms*'

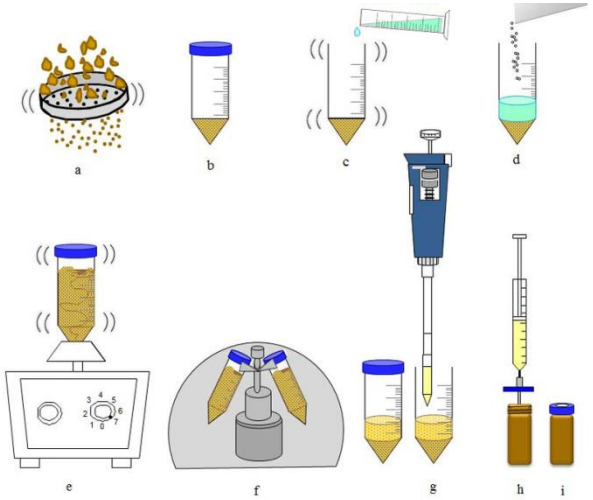


Source: Kumpiene et al., 2017. Pedosphere 27(3): 389-406

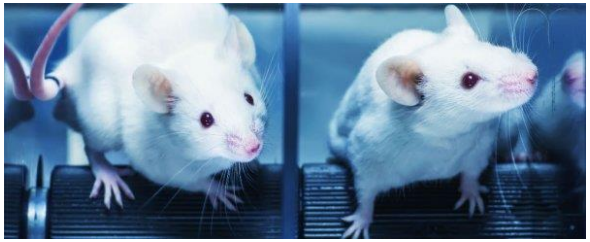
Bioaccessibility *vs* bioavailability of soil contaminants

Bioaccessibility and bioavalability of soil contaminants depends on the contaminant chemistry, soil properties and chemical conditions in the human digestive and respiratory systems or body skin

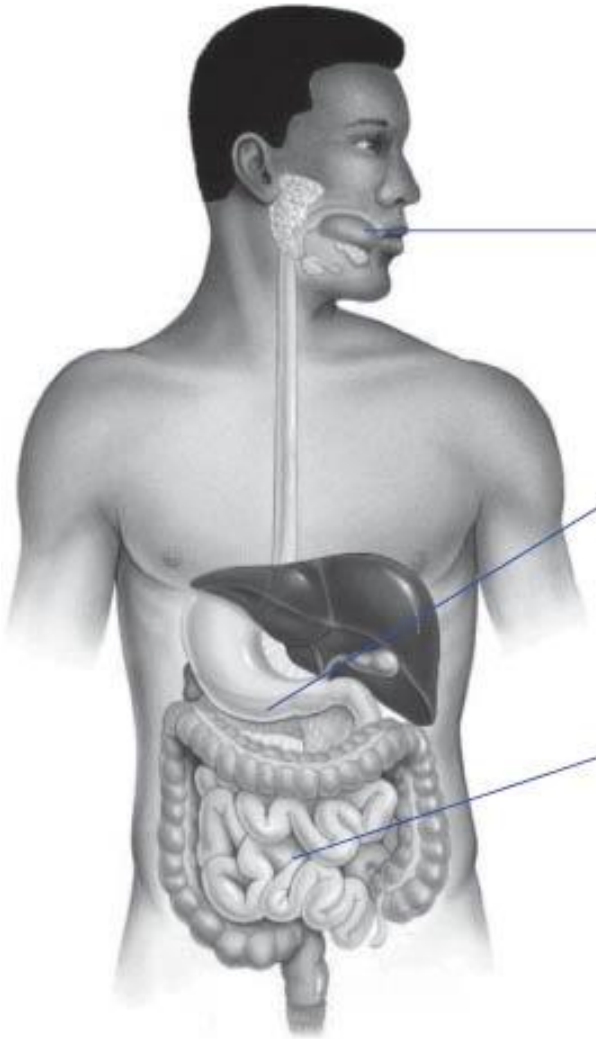
In-vitro tests for bioaccessibility of soil contaminants in soil must enable quantification of the dissolution under 'realistic worst case conditions', meaning that the test would simulate the highest possible bioaccessibility in extractions with one or more simulants of body fluids, at body temperature (37 °C), for realistic reaction times



In-vivo tests are generally considered the best bioavailability tests available, as the animal uptake measured in these tests is believed to resemble the conditions applied during toxicity testing. However, these assays are time- and resource-consuming and comprise lots of ethical issues



Oral uptake processes of soil contaminants



Accidental ingestion of heavy metal

Mouth
pH: 6.5
Process involve: Grinding
Temperature: 37°C

Stomach
pH: 1–5
Process involve: Dissolution
Temperature: 37°C

Intestine
pH: 4–7.5
Process involve: Absorption, precipitation, re-absorption
Temperature: 37°C

Digestion time

Few minutes

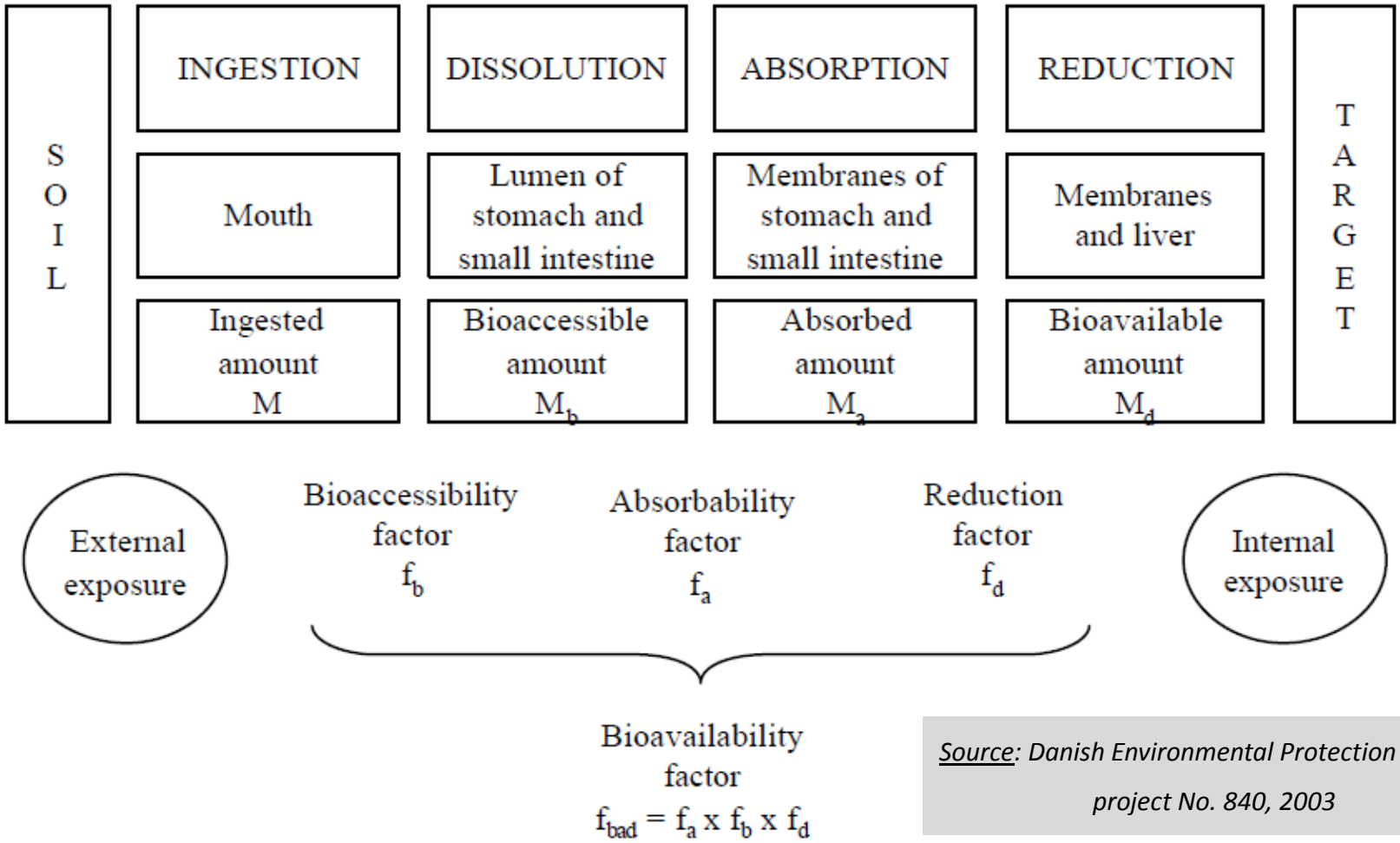
Carbohydrates → 1-2 h
Proteins → 3-4 h
Fats → 5-6 h

Small intestine → 3-10 h

Large intestine → 15-70 h

Oral uptake processes of soil contaminants

Thus, oral bioavailability of soil contaminants depends primarily on the ability of stomach and small intestine to dissolve the contaminant (bioaccessibility) and then on the ability of intestinal membranes to absorb the contaminants



Source: Danish Environmental Protection Agency, project No. 840, 2003

In-vitro methods to assess oral bioaccessibility of metals

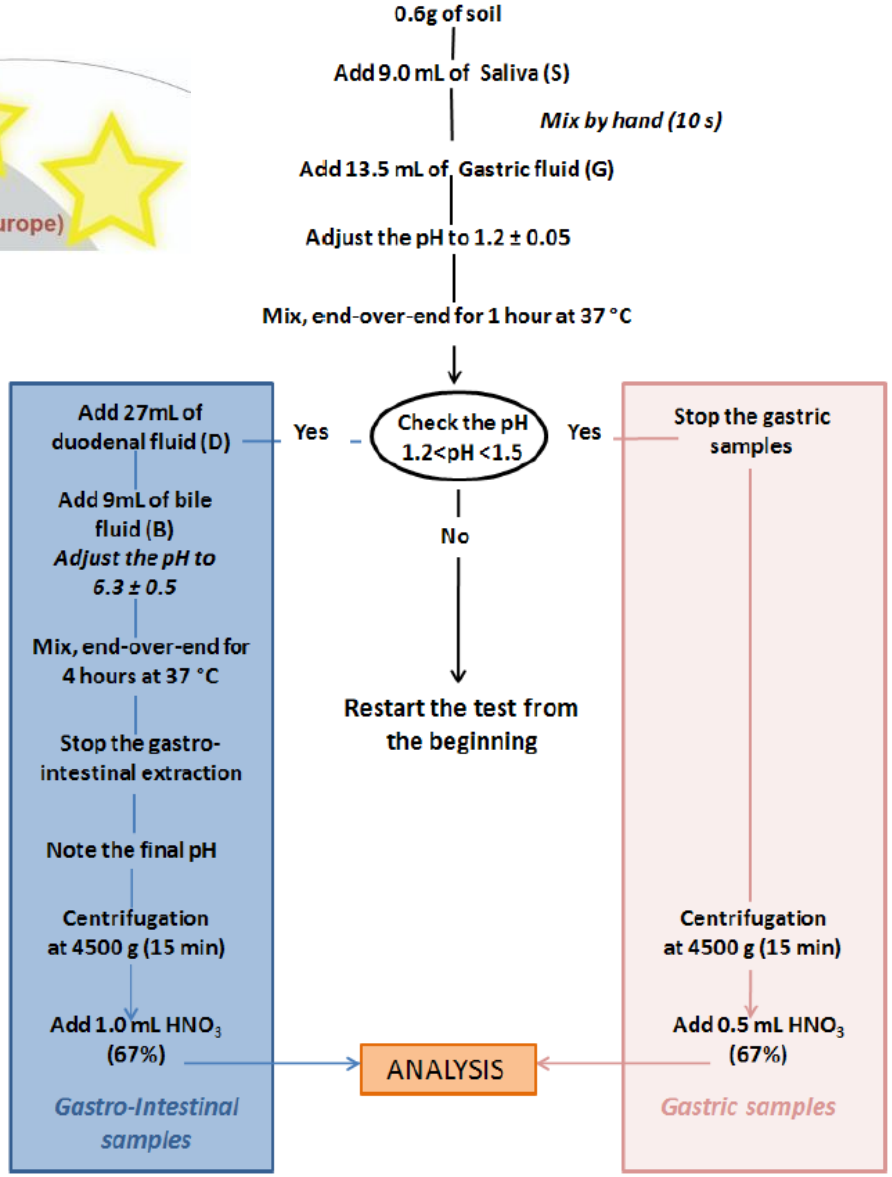
Method[a)]	TE(s)	Extraction procedure	Simulated digestive compartment	pH	Temperature	L/S ratio[b)]	Residence time	Reference
					°C			
PBET	As, Pb	Batch	Stomach	2.5	37	100:1	1 h	Ruby *et al.*, 1992,
			Small intestine	7.0	37	100:1	4 h	1996
SBET	As, Cd, Pb	Batch	Stomach	1.5	37	100:1	1 h	Drexler, 1999
IVG	As	Batch	Stomach	1.8	37	150:1	1 h	Rodriguez and
			Small intestine	5.5	37	150:1	1 h	Basta, 1999
USP	Pb, Cr, As, Cd, Ni	Batch	Stomach	1.0	37	1 000:1	2 h	Hamel *et al.*, 1998
MB&SR	Pb, Cr, As, Cd	Batch	Mouth	6.4	37	160:1	5 s	Hamel *et al.*, 1999
			Stomach	2.0	37	2 160:1	2 h	
			Small intestine	7.5	37	4 770:1	4 h	
DIN	As, Cd, Pb, Cr, Hg	Batch	Mouth	6.4	37	15:1	0.5 h	Hack and
			Stomach	2.0	37	50:1	2 h	Selenka, 1996
			Small intestine	7.5	37	100:1	6 h	
SHIME	As, Cd, Pb	Batch	Stomach	5.2	37	2.5:1	3 h	Molly *et al.*, 1993
			Small intestine	6.5	37	4:1	5 h	
RIVM	As, Cd, Pb	Batch	Mouth	6.5	37	15:1	5 min	Sips *et al.*, 1998
			Stomach	1.5	37	37.5:1	2 h	
			Small intestine	5.5	37	97.5:1	2 h	
TGM	As, Cd, Pb	Dynamic	Mouth	5.0	37	5:1	5 min	Minekus *et al.*, 1995
			Stomach	2.0	37	30:1	1.5 h	
			Small intestine	7.0	37	51:1	6 h	
AOACPD	Cu, Zn, Mn, Fe, Al	Batch	Stomach	1.1, 2.0	37	150:1	16 h	AOAC, 2000
UBM	As, Cd, Sb, Pb,	Batch	Mouth	6.5	37	15:1	20 s	BARGE-INERIS,
	Montana		Stomach	1.2	37	37.5:1	1 h	2010
	NIST[c)] 2711 soil		Small intestine	6.3	37	97.5:1	4 h	

Source: Kumpiene et al., 2017. Pedosphere 27(3): 389-406

Methodological complexity of in-vitro assays

BARGE (the Bioaccessibility Research Group of Europe)

	REAGENTS	Saliva (S)	Gastric (G)	Duodenal (D)	Bile (B)	Volume (mL)
Inorganic (I)	KCl	448	412	282	188	250
	NaH_2PO_4	444	133	-	-	
	KSCN	100	-	-	-	
	Na_2SO_4	285	-	-	-	
	NaCl	149	1376	3506	2630	
	$CaCl_2$	-	200	-	-	
	NH4Cl	-	153	-	-	
	$NaHCO_3$	-	-	2803.5	2893	
	KH_2PO_4	-	-	40	-	
	$MgCl_2$	-	-	25	-	
	NaOH (1M)	0.9 mL	-	-	-	
	HCl (37%)	-	4.15 mL	90 uL	90 uL	
Organic (O)	Urea	100 mg	42.5	50	125	250
	Glucose		325	-	-	
	Glucuronic acid		10	-	-	
	Glucosamine hydrochloride		165	-	-	
Enzymes	Alpha amylase	72.5 mg	-	-	-	250+250= 500
	Mucin	25 mg	1500	-	-	
	Uric acid	7.5 mg	-	-	-	
	Bovine Serum Albumin	-	500	500	900	
	Pepsin	-	500	-	-	
	$CaCl_2$	-	-	100	111	
	Pancreatin	-	-	1500	-	
	Lipase	-	-	250	-	
	Bile	-	-	-	3000	
pH	I+O	6,5 +/- 0,5	1,1 +/- 0,1	7,4 +/-0,2	8 +/- 0 .2	



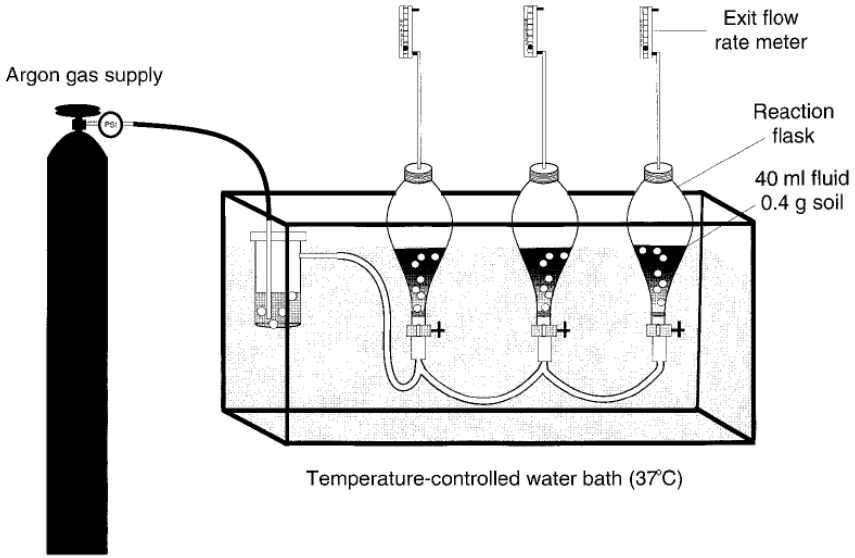
Simplified in-vitro bioaccessibility tests

Physiologically based extraction test (PBET)

(Ruby et al., 1992; 1996)

Stomach phase → 6 chemicals (pH 2.5 - 1 h)

Small intestine phase → + 3 chemicals (pH 7 - 4 h)



Simplified bioaccessibility extraction test (SBET)

(Drexler, 1999)

Stomach phase → Glycine and HCl

(only 2 chemicals, pH 1.5 - 1 h)

SBET was validated for Pb by an in-vivo swine study

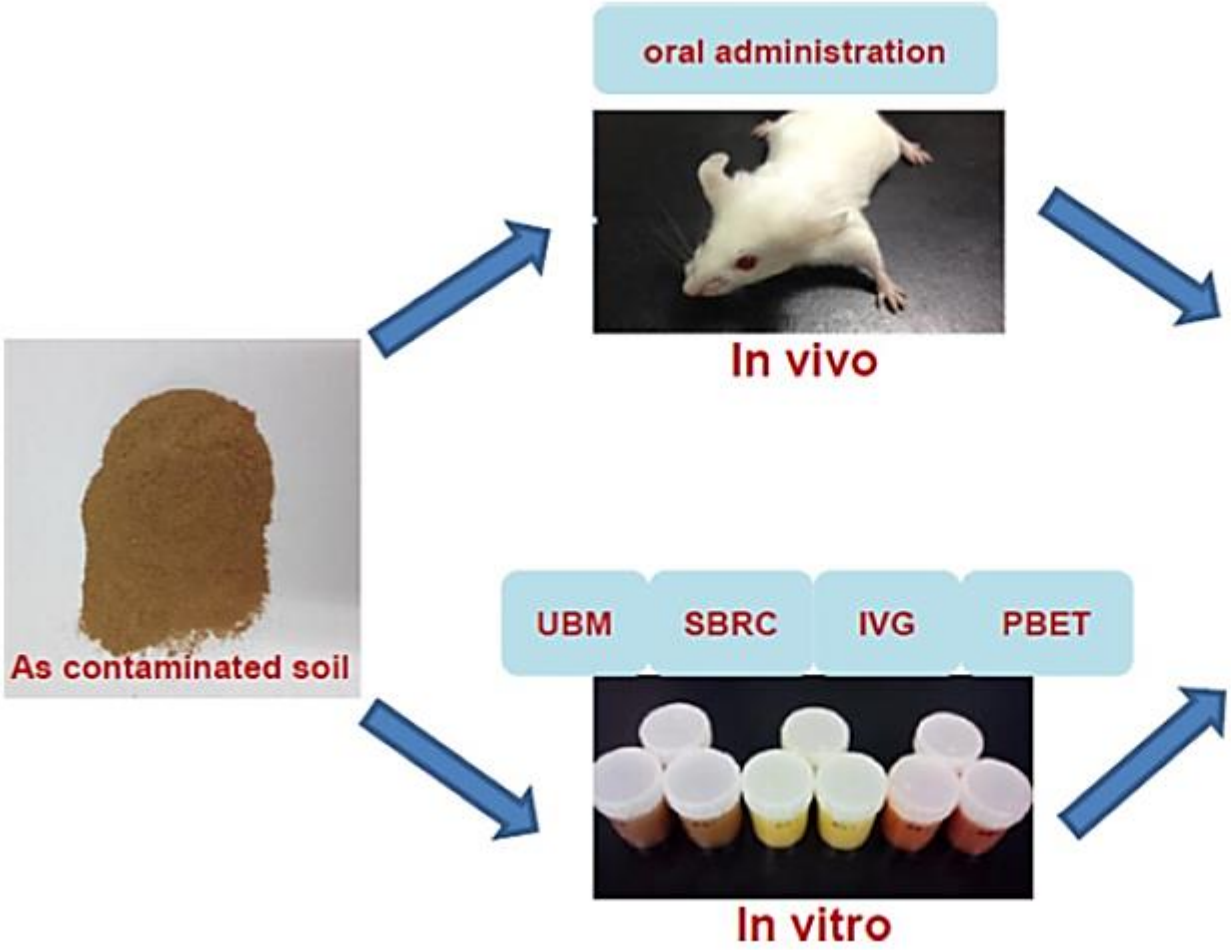
(Ruby et al., 1999)

In-vivo ingestion studies using metal-containing feed

Soil type	Pb concentration range (mg/kg)	Specimen and biomarker	Dose, period, state	RBA (%)	Reference
Mining	4482–40,214	Swine (5 weeks age, BW = 9.5 ± 1.2 kg), kidney/liver/bone/urine	50–4000 μg Pb/kg BW day, 14 days, fasting	8.25–58.67[b]	(Denys et al., 2012)
	1270–14,200	Swine (5–6 weeks age, BW = 8–11 kg), blood/liver/kidney/femur	15 days, fasting	6–105	(Casteel et al., 2006)
	1270–14,200	Swine (5–6 weeks age, BW = 10 ± 12 kg), blood/liver/kidney/bone	15 days, fasting	0.75–97.75	(Schroder et al., 2004)
	3900	Rabbits (BW = 2.1 kg), blood/liver/kidney/bone	2.0 ± 0.02 g Pb/kg BW, 36 h, fasting	9	(Ruby et al., 1993)
	3908–10,230	Rats	fed	8.7–36	(Ruby et al., 1996)
	200–6330	Minipigs (10 weeks age, BW = 4.8 kg), kidney/liver/bone/urine	500 μg Pb/kg BW day, 28 days, fasting	17–63	(Marschner et al., 2006)
	810, 3908	Rats (7–8 weeks age), blood/liver/bone	30 days, fed	8.95, 13.57	(Freeman et al., 1992)
	2924	Human	Fast and fed	26.2(fast), 2.52 (fed)[a]	(Maddaloni et al., 1998)
	3870, 14,200	Swine (BW = 8–9 kg), kidney/liver/bone	75, 225 and 675 μg Pb/kg BW day, 15 days, fasting	63, 64	(Casteel et al., 1997)
	516–4163	Mice (BW = 20–25 g), blood	2150 -10700 μg Pb/kg BW, 48 h, fasting	7–26	(Li et al., 2015)
Smelter	1388, 2090	Rats		35, 41	(Ruby et al., 1996)
	1460–30,155	Swine (5 weeks age, BW = 9.5 ± 1.2 kg), kidney/liver/bone/urine	50–4000 μg Pb/kg BW day, 14 days, fasting	32.25–94.5[b]	(Denys et al., 2012)
	536–3200	Mice (BW = 20–25 g), blood	48 h, fasting	10–63	(Smith et al., 2011a)
	2154	Rats blood/liver/kidney/bone	15 days, fed	35.5[c]	(Hettiarachchi et al., 2003)
	250–25,329	Mice (BW = 20–25 g), blood	2150 -10700 μg Pb/kg BW, 48 h, fasting	30.8–84.3	(Li et al., 2015)
	237–6330	Swine (6–8 weeks age, BW = 20–25 kg), blood	5 days, single dose, fasting	17–63	(Juhasz et al., 2009)
Small arm range	4503–23,409	Swine, blood/liver/kidney/femur	15 days	77–140[c]	(Bannon et al., 2009)
Gaswork	1343	Mice (BW = 20–25 g), blood	48 h, fasting	43	(Smith et al., 2011a)
Shooting range	576, 1801	Mice (BW = 20–25 g), blood	48 h, fasting	85, 89	(Smith et al., 2011a)
Dust	29–738	Mice (BW = 18–20 g), blood	340-6220 μg Pb/kg BW, 48 h, fasting	29.1–60.1	(Li et al., 2014)
	1693–6799	Children		11.25–21.48[d]	(Oliver et al., 1999)
Incinerator and residential	646–3905	Swine (6–8 weeks age, BW = 20–25 kg), blood	5 days, single dose, fasting	10.1–19.1	(Juhasz et al., 2009)
Urban soil	12.6–1198	Female mice (BW = 20–22 g), kidney	10 days, repeat dose, fasting	17.3–86.6	(Li et al., 2016)
Farming	215–1543	Mice (BW = 20–25 g), blood	2150–10,700 μg Pb/kg BW, 48 h, fasting	51.4–60.5	(Li et al., 2015)
Spiking and aging soils	1500	Swine (BW = 20–25 kg), blood	5 days, single dose, fasting	34–59	(Wijayawardena et al., 2014)

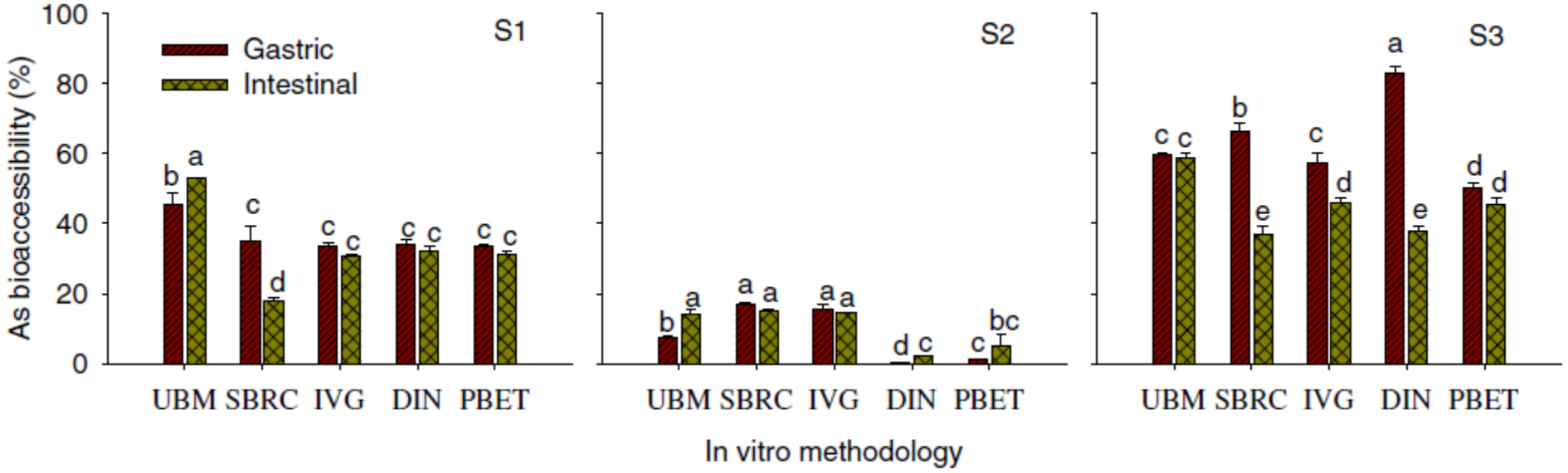
Source: Yan et al., 2017. Chemosphere 184: 27-42

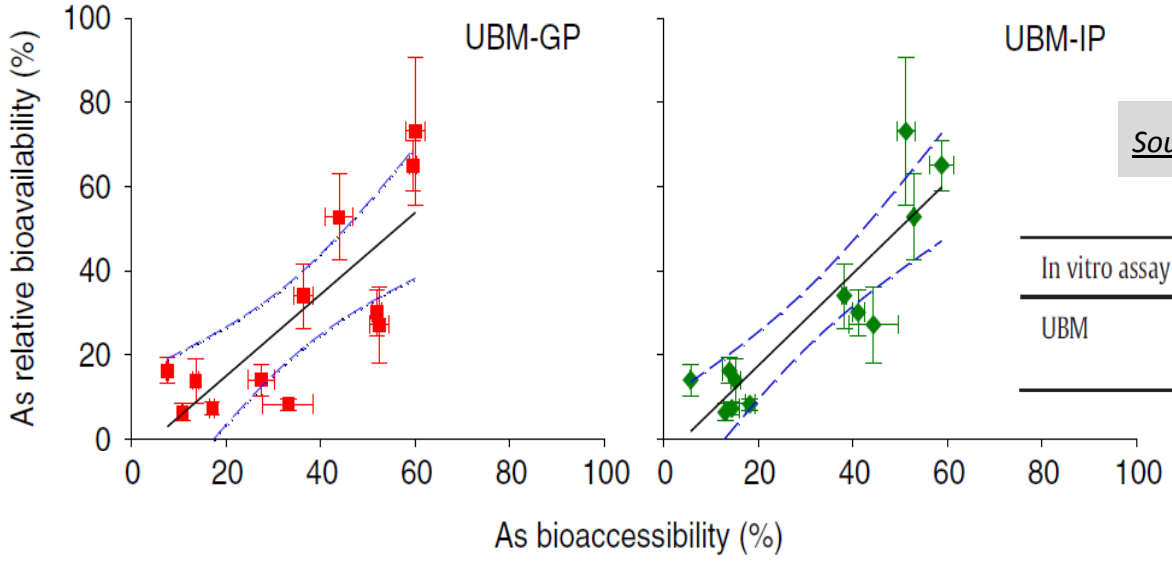
In-vitro oral bioaccessibility *vs* in-vivo bioavailability



Source: Li et al., 2015. Sci Total Environ 532: 812-820

In-vitro oral bioaccessibility *vs* in-vivo bioavailability

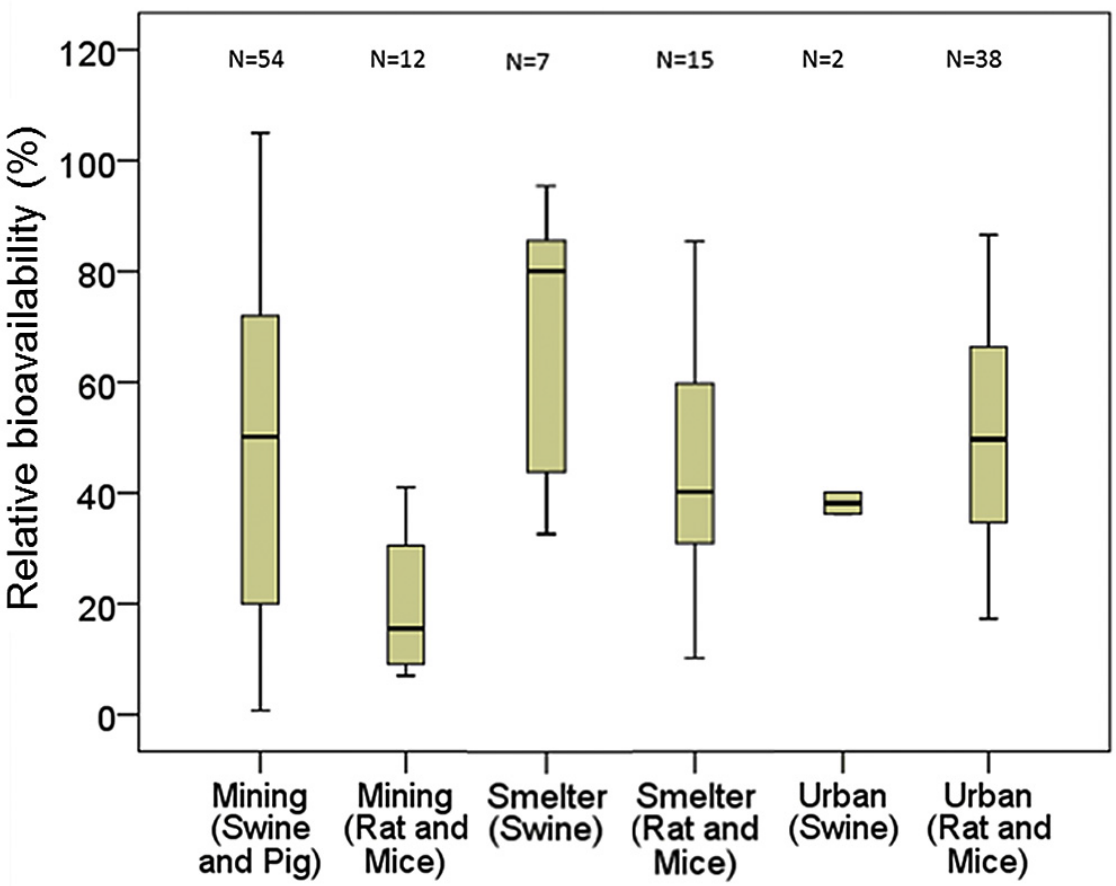




Source: Li et al., 2015. Sci Total Environ 532: 812-820

In vitro assay	Phase	In vivo–in vitro predictive model	r^{2a}
UBM	Gastric	RBA = −4.40 + 0.97 (UBM-GP)	0.66
	Intestinal	RBA = −4.43 + 1.09 (UBM-IP)	0.80

In-vivo oral bioavailability in different animal studies

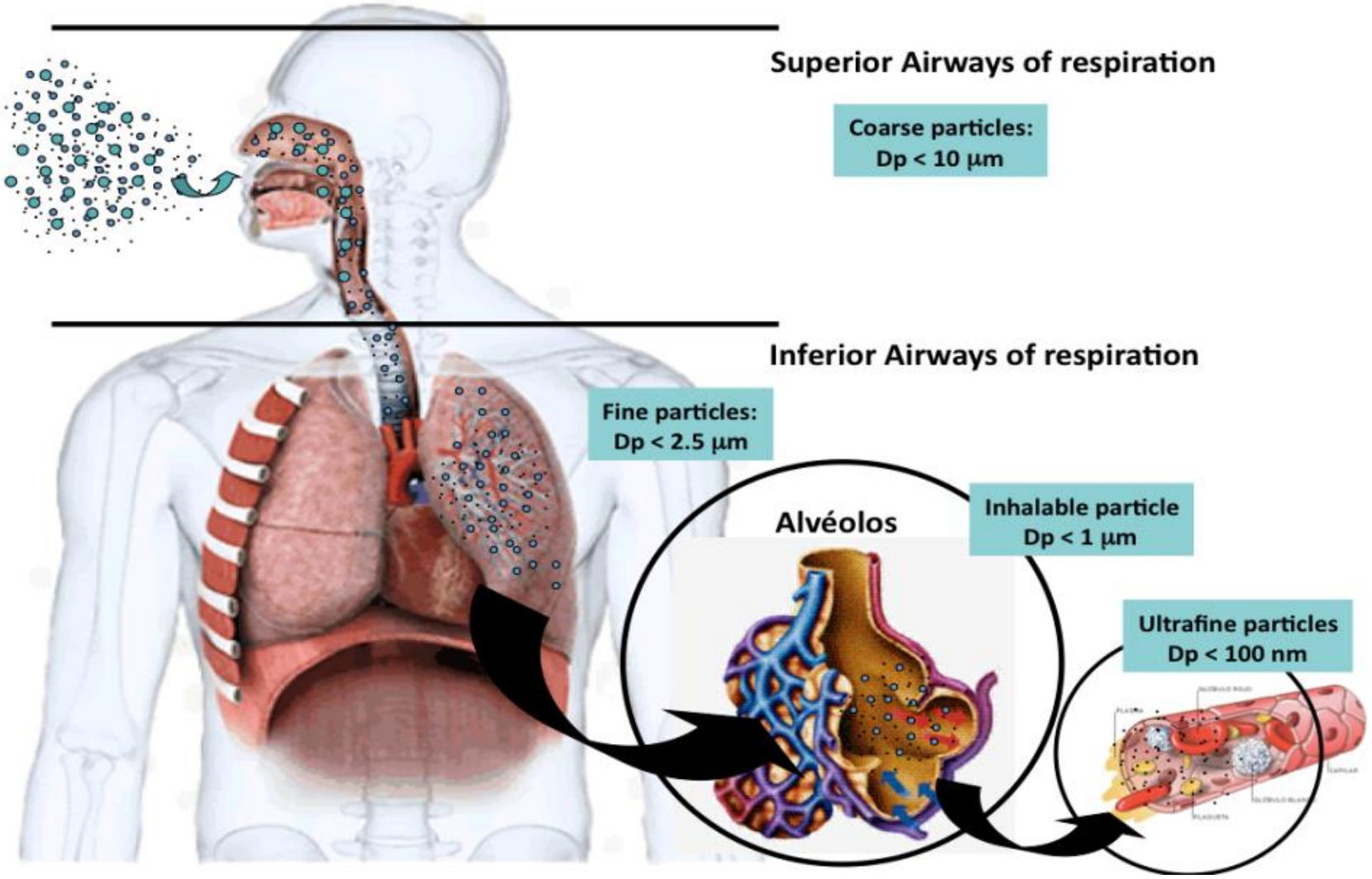


Source: Yan et al., 2017. Chemosphere 184: 27-42

In both mining and smelter soils, the swines showed a higher oral bioaccessibility of Pb than rats and mice

Compared to swine, small animals (rats and mice) are economic and have been widely used in tests for assessing oral bioaccessibility of metals in farmlands, mining soils, smelters, gasworks, shooting ranges and house dust

Lung bioaccessibility of metals depends on soil particle-size



In-vitro methods to assess lung bioaccessibility of metals

Reference	Study type	Sample type	Elements of interest	Leaching agents used (pH)	Sample/solution amounts	Extraction time/ temperature	Extraction procedure	Analytical method[a]
Boisa et al. [20]	ENV	Fractionated Pb-contaminated soil, tailing and smelter samples	Pb	Simulated epithelial lung fluid (SELF) (7.4 ± 0.2)	0.3 g/20 mL	Variable: up to 170 h/37 °C	Samples rotated @ 30 RPM; then centrifuged @ 3000 RMP for 10 min	ICP-MS
Wiseman and Zereini [32]	ENV	Airborne PM_{10}, $PM_{2.5}$ and PM_1	As, Ce, Co, Cr, Cu, Mn, Ni, Pb, Sb, Ti and V	Artificial lysosomal fluid (4.3), Gamble's solution (7.4)	50 mL solution (mean sample amount of 43 mg)[b]	24 h and 30 days/ 37 °C	Sample filters shaken daily; then filtered	ICP-MS
Witt et al. [26]	ENV	Road dust samples; fractionated via air-suspension into >1 and <1 μm	Pb	Artificial lysosomal fluid (4.5)	Not specified	Not specified	Not specified	ICP-MS
Lima et al. [25]	ENV	Slag from a metallurgical facility	Ni, Cd, Zn and Mn	Gamble's solution (7.4)	50 mg/50 mL	Rapid test: 10, 30, 60, 120, 240, 360 and 720 min Slow test: 1, 3, 5, 7, 14, 21, 30, 60, 90, 120, 160, 210, 280 and 360 days/22–25 °C	Samples placed in flow through Teflon cylinders; then filtered	PIXE
Potgieter-Vermaak et al. [44]	ENV	Fractionated road dust (63–125 μm)	Cr and Pb	Artificial lysosomal fluid (4.5), Gamble's solution (7.4) and water	Not specified	Ranging from 1 h to 4 weeks/37 °C	Samples shaken @ 150 RPM; aliquots taken directly	GF-AAS
Zereini et al. [34]	ENV	Urban airborne PM (PM_{10}, $PM_{2.5}$ and PM_1)	Pt, Pd and Rh	Artificial lysosomal fluid (4.4) and Gamble's solution (7.8)	50 mL solution (sample amount not specified)[b]	24 h and 30 days/ 37 °C	Sample filters shaken daily; then filtered	ICP-MS
Caboche et al. [18]	ENV	SRMs: NIST 1648a (urban PM), NIES 8 (vehicle exhaust particulates), BCR 038 (fly ash powder), NIST 2584 (indoor dust)	Ba, Cd, Ce, Co, Cu, La, Mn, Mo, Ni, Pb, Rb, Sb and Zn	Gamble's solution (7.4) and water	1–2 mg/20 mL (to achieve S/L values of 1/30, 1/500, 1/2000, 1/4000, 1/10,000, 1/ 20,000 and 1/ 50,000)	Variable: 15 min, 30 min, 60 min, 120 min, 240 min, 300 min, 24 h, 48 h and 72 h/37 °C	Samples shaken on an orbital shaker @ 40 cycles/min; then centrifuged for 20 min @ 14,600 RMP	ICP-MS
Colombo et al. [27]	ENV	SRMs: BCR 723 (road dust), SRM 2557 (used milled auto catalyst); synthetic PGE-loaded hydroxides	Pt and Rh	Artificial lysosomal fluid (4.5), Gamble's solution (7.4)	1 g/100 mL	Variable (up to 700 h)/37 °C	Samples shaken (rate not specified); then filtered	ICP-MS

Source: Wiseman, 2015. Anal Chim Acta 877: 9-18

In-vivo inhalation studies using metal-containing aerosols

Reference	Matrix	Particle size	Metal(loid)s	Dose (exposure duration)	Exposure mode	T est. animal (strain)	Exposure endpoint
Damon et al. (1984)	Yellowcake	1.4–1.6 μm	U	0.014–0.12 mg/kg of U/rat body weight (once)	Nose only	Rats (Fischer-344)	Absorption into blood, tissues, excreta, cytotoxicity, metabolic activity
Ansoborlo et al. (1990)	UF_4	6.6 μm	U	35 mg/m^3 (20 min)	Nose only	Rats (OFA strain)	Absorption into blood, tissues, excreta, initial lung and body burden
Kodavanti et al. (1998, 2000, 2002)	ROFA	<3 μm	As, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn	15 mg/m^3 (6 h/d, 3 d/wk. for 1–4 wk)	Nose only	Rats (Spontaneously hypertensive & normotensive Wistar-Kyoto)	Airway hyperreactivity, injury, inflammation, oxidative stress, pulmonary and cardiac histological lesions, cytokine gene expression
Artelt et al. (1999)	Pt coated onto Al_2O_3 powder	M2O3: < 5 μm, Pt:4 nm	Pt	4 & 12 mg/m^3 (5 h/d, 5 d/wk., 90 days)	Nose only	Rats (Lewis)	Absorption into blood, tissues, excreta, stomach content and speciation in BALF
Campen et al. (2001)	ROFA associated metal solution	nd	Ni, V, or Ni + V	0.3, 0.6, 1.2, 2.1 mg/m^3 (6 h/d, 4 days)	Whole body	Rats (Sprague-Dawley)	Cardiovascular and Thermoregulatory Effects
Hamada et al. (2002)	ROFA leachate suspended in PBS ROFA associated $NiSO_4$, VSO_4, $ZnSO_4$, $CuSO_4$, $MnSO_4$, $CoCl_2$	nd	Ni, V, Zn, Cu, Mn, Co, Cd, Fe	10, 50, or 100 mg/ml Similar concentrations to those found in ROFA suspended in PBS	Whole body	Mice (BALB/c)	Airway responsiveness in presence and absence of an antioxidant 'Dimethylthiourea'
Muggenburg et al. (2003)	APM associated transition metal solutions	<3 μm	Cu, Fe, Mn, Ni, V	0.05 mg/m^3 (3 h/d, 3 days)	Oronasal	Dogs (Beagle)	Electrocardiograms, heart rate, heart rate variability, and abnormalities of waveforms
Kodavanti et al. (2005)	Fine concentrated ambient particle (CAP)s (40–60 × ambient level)	<2.5 μm	Al, As, Ba, Be, Cd, Co, Cu, Pb, Mn, Ni, Ag, Ti, Zn	144–2758 μg/m^3 (2–4 h/day, 1–2 d, 6–7 repeats)	Whole body	Rats (Wistar Kyoto & Spontaneously hypertensive)	Pulmonary ventilation, injury and inflammation, cytotoxicity, enzyme activity, protein quantification
Lippmann et al. (2006)	Fine concentrated ambient particle (CAP)s (10 × ambient levels)	<2.5 μm	Ni, V	85 μg/m^3 (6 h/d, 5 d/wk., 6 months)	Whole body	Mice (ApoE −/−)	Cardiovascular and pulmonary mortality
Wallenborn et al. (2008)	APM associated Zn solution	nd	Zn	10, 30 or 100 μg/m^3 (5 h/d, 3 d/wk. for 72 days)	Nose only	Rats (Wistar Kyoto)	Cytotoxicity, lung & heart , enzyme activity and cardiac gene expression
Zhong et al. (2010)	Soot and Fe oxide	72 nm	Fe	Total particle conc. 250 mg/m^3; Fe conc.30 & 100 μg/m^3 (6 h/d for 3 days)	Whole body	Rats (Sprague-Dawley, neonatal)	Cytotoxicity, intracellular ferritin levels, oxidative stress, level of proinflammatory cytokines

Source: Kastury et al., 2017. Sci Total Environ 574: 1054-1074

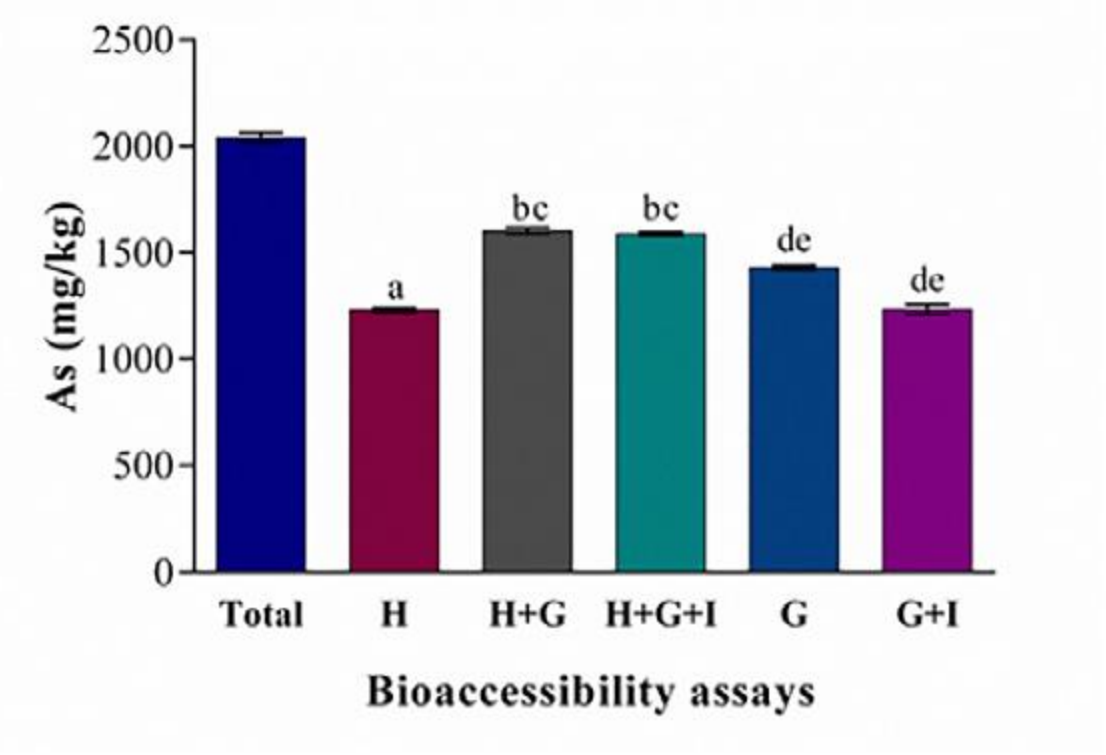
In-vitro lung bioaccessibility *vs* in-vivo bioavailability

In literature, there is a lack of comparison between in-vitro and in-vivo data on concerning metals in soil. This is a knowledge gap for the application of inhalation bioaccessibility data for refining exposure assessment

Main limitations of in-vivo lung bioavailability studies:

- ✓ Intra-nasal or intra-tracheal instillation are often necessary to overcome issues related to inhalation assays
- ✓ Doses in many animal studies are several times higher than ambient concentrations, diminishing relevance or validity to human exposure scenarios
- ✓ Significant intra and interspecies differences exist in lung physiology, retention and respiratory uptake between model animals and humans, so the translation of animal data to humans is often inaccurate
- ✓ Synergistic or antagonistic behaviour of metals may affect bioavailability and subsequent toxicity in-vivo, which may not be reflected in in-vitro assays

Combined inhalation-ingestion bioaccessibility assay



PM_{10}

+

Simulated lung fluid: Hatch's solution (H) pH 7.4

24 hours

→

Simulated gastric solution (G) pH 1.5

1 hour

+

Simulated intestinal tract solution (I) pH 7

4-5 hours

→

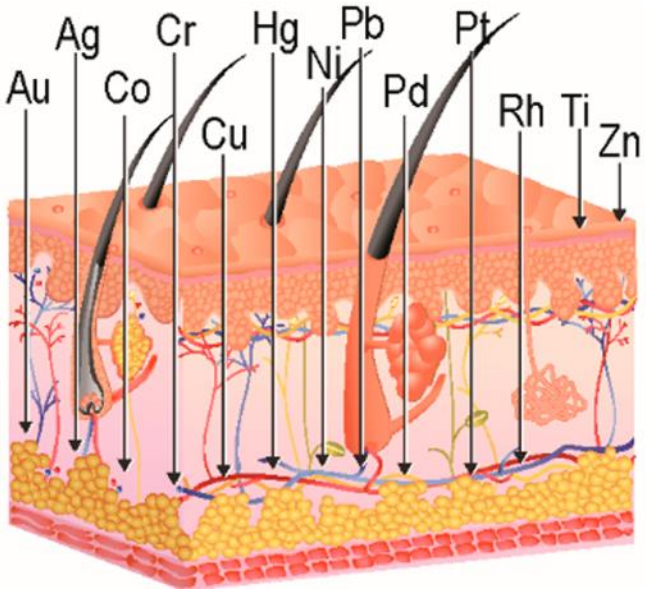
Separation of solid and liquid

→

Elemental analysis

Source: Kastury et al., 2018. Sci Total Environ 631-632: 92-104

In-vitro dermal bioaccessibility of metals



Dermal exposure to contaminated sites has generally received less attention than oral/inhalation exposure due to limited exposure scenarios and less perceived potential for toxicity, however, the risk can be significant for specific contaminants and scenarios

In-vitro dermal bioaccessibility of metals can be assessed through soil extraction by synthetic sweat formulations, such as EN 1811 and NIHS 96-10

Sweat formulation NIHS 96-10

Sample	Cr		Ni		Pb		Zn	
	2 h	8 h	2 h	8 h	2 h	8 h	2 h	8 h
S1	0.7	1.6	0.4	0.7	3.6	2.6	0.24	0.1
S3	3.3	5.6	3.6	7.0	10	6.6	26	34
S4	3.0	5.6	3.8	7.8	13	8.7	6.6	8.2
S5	1.7	2.7	1.3	2.0	8.2	7.6	0.12	<0.05
S7	0.9	1.4	10	6.8	4.3	3.8	45	39
S8	<0.05	<0.05	7.5	6.1	13	10	0.4	<0.05
S9	<0.05	0.1	3.4	1.7	1.5	2.0	9.5	8.5
BGS 102	<0.05	<0.05	2.5	0.9	4.5	4.6	2.6	0.3

Source: Chaparro Leal et al., 2018. Chemosphere 197: 42-49

Scientific study on metal bioaccessibility

LUNG AND ORAL BIOACCESSIBILITY OF POTENTIALLY TOXIC ELEMENTS IN TWO ITALIAN CASE STUDIES OF SOUTH ITALY

- ✓ Study carried out by soil chemists of DiA-UniNA, involving two Master students writing and discussing theses on this research topic
- ✓ Orally-presented at European Geosciences Union General Assembly 2019 in Vienna, Austria (16,273 participants from 113 countries)

The case studies

'land of fires'

SITE A

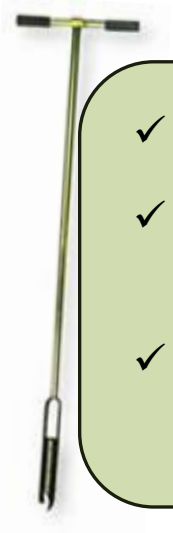
6 ha of farmland currently confiscated by the Italian Judiciary due to past illegal burial of tannery wastes

SITE B

3.5 ha of industrial soil inside an automobile-battery recycling plant in operation since 1970

Soil sampling strategy

Site A



- ✓ Sampling grid: **20 x 20 m**
- ✓ Depths at site A:
  0-20, 30-60, 70-90 cm
- ✓ Depths at site B:
  0-10, 10-40 cm

Site B

AT THE BEGINNING

↓

- diffuse contamination

- soil compaction (site B)

- fertility decline

Assisted phytoremediation plants

Phytoremediation plants consisting of poplar trees (*Populus nigra* L.) and permanent grass cover, assisted by compost amendment and irrigation system, were then implemented on both sites few years ago

IMPLEMENTATION OF ECO-COMPATIBLE PROTOCOLS FOR AGRICULTURAL SOIL REMEDIATION IN LITORALE DOMIZIO-AGRO AVERSANO NIPS (LIFE11/ENV/IT/275 – ECOREMED)

Site A

3-year phytoremediation plants (August 2018)

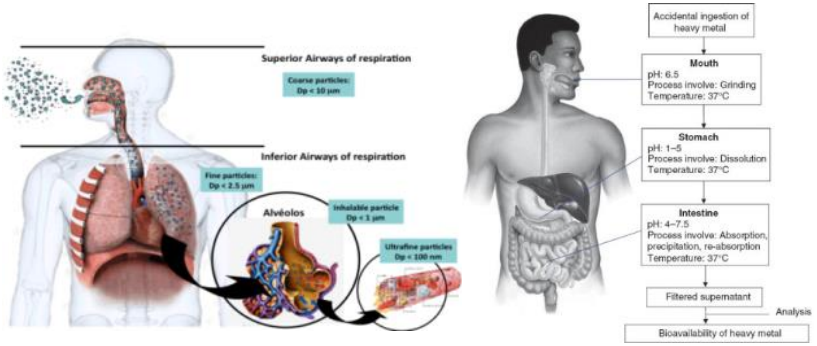
Site B

Aims of the work

- To separate selected topsoil samples (previously sieved at 2 mm) into particle-size fractions (250-2000, 50-250, 20-50, 10-20, 2-10 and <2 µm)

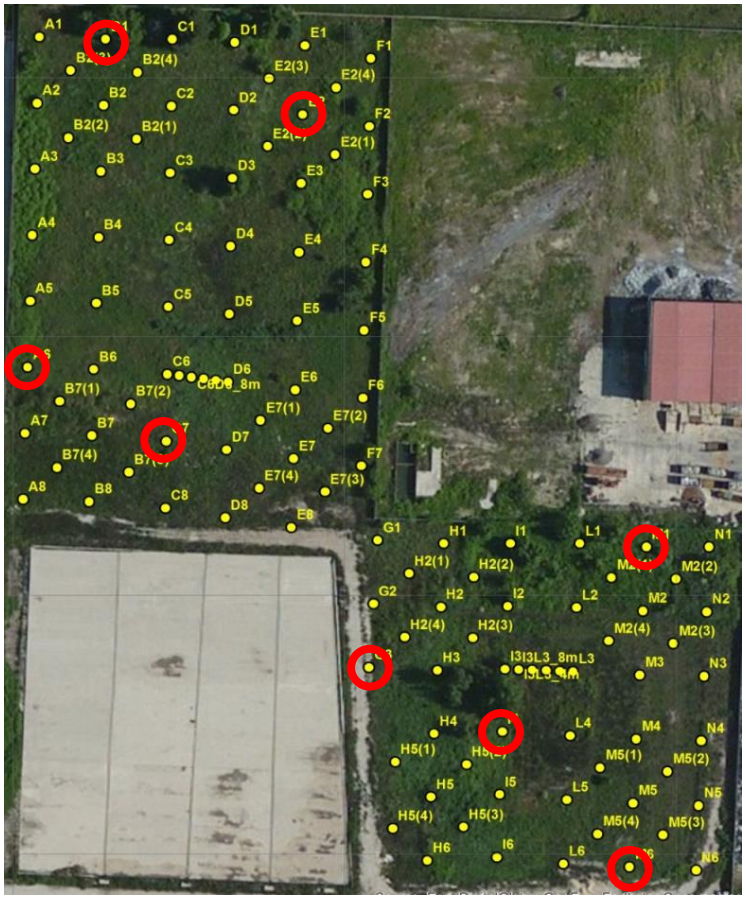
- To assess the concentration and the distribution of the potentially toxic elements (PTEs) in the different soil particle-size fractions

- To assess the human lung and oral bioaccessibility of the PTEs in the different soil particle-size fractions, in order to study the risks for health of residents and workers living or working nearby the potentially contaminated sites



Selection of topsoil samples

Only topsoil samples were taken into account, since they could be interested by wind erosion and soil-particle dispersion in the atmosphere



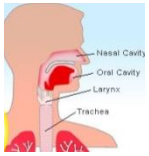
8 topsoil samples from each site were selected on the basis of spatial distribution in the site, physicochemical properties and extent of contamination

Analytical methods

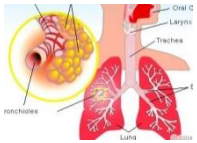
- ✓ **Soil separation in particle-size fractions:** sonication, centrifugation (<2 µm) and sedimentation cycles (courser particles)

- ✓ **Pseudo-total contents:** microwave-assisted *aqua regia* digestion (ISO 11466, 1995)

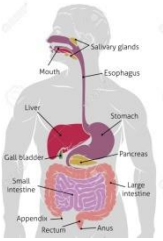
- ✓ **Bioaccessibility in upper respiratory tract:** Simulated Epithelium Lung Fluid (**SELF**, Boisa *et al.*, 2014)



- ✓ **Bioaccessibility in lower respiratory tract:** Artificial Lysosomal Fluid (**ALF**, Stopford *et al.*, 2003)



- ✓ **Oral bioaccessibility in gastric (G) and gastro-intestinal (GI) tracts:** Unified BARGE Method (UBM, Barge-Ineris, 2010)



- ✓ **Analysis of all the extracts:** Atomic Absorption Spectroscopy (FAAS, HG-AAS or GF-AAS)

Main remarks

- Our findings highlight the potential risk of contaminated topsoil particles of both sites for human health, if air-dispersed by wind

- The PTEs distributed in the finer soil fractions (PM10 and PM2.5) are those of major concern, since these particles are most easily erodible by wind and can reach the upper respiratory tract (PM10 and PM2.5) or even enter the pulmonary alveoli (PM2.5). These soil particles are effective sinks of PTEs due to their higher surface area and reactivity

- Bioaccessibility methods employed in this study guarantee a high degree of similarity with the digestive and respiratory systems of the human body, in terms of biochemical composition and fluid pH, residence times and temperature

- It is thus clear how is important to properly manage the phytoremediation plants implemented in both sites to efficiently cover the contaminated topsoils over time

Acknowledgements

- ✓ Italian Society of Soil Science (SISS)
- ✓ Invited Speakers, Lecturers, Local Organising and Scientific Commitees of School Bio-Bio 2019
- ✓ *All the Pedologists, Soil Chemists, Agronomists and Microbiologists involved in the characterisation and phytoremediation of sites A and B → human resources which have recently built a transdisciplinary bridge in soil science for scientific interests and even for environmental and cultural purposes*
- ✓ *LIFE11/ENV/IT/275 (ECOREMED) and After-LIFE projects financing field and lab activities*
- ✓ ***YOU ALL FOR YOUR KIND ATTENTION!***

Greetings

Italian Society of Soil Science
School of Soil Biodiversity and Bioindication
XI cycle

BIODIVERSITY AND BIOINDICATORS IN MONITORING AND MANAGEMENT OF CONTAMINATED SOILS

4 - 7 JUNE 2019

Department of Agricultural Sciences
University of Naples Federico II, Portici, Italy

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