

TXRF WEBINAR

Can TXRF be a valuable tool in biomonitoring?

Armin Gross¹, Hagen Stosnach¹ and Ignazio Allegretta²

- 1) Bruker Nano GmbH, Berlin
- 2) "Micro X-ray Lab", Department of Soil, Plant and Food Sciences, University of Bari, Italy

October 2021

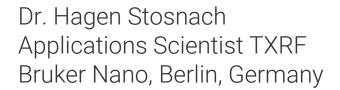


Welcome

Speakers

Dr. Armin Gross Global Product Manager TXRF Bruker Nano, Berlin, Germany

Dr. Ignazio Allegretta Researcher University of Bari











Agenda

Principles of TXRF

Overview
Environmental analysis

Biomonitoring and TXRF

04 Q&A

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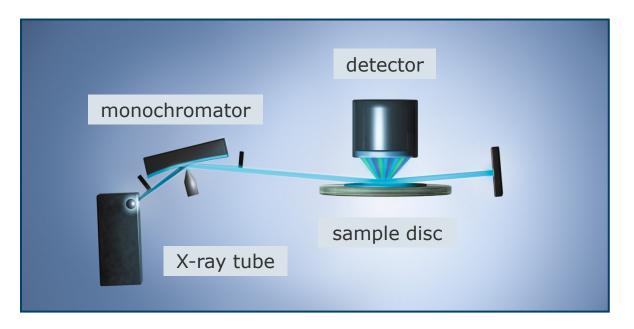


1 Principles of TXRF

Principles of TXRF Technical background

Total reflection X-ray fluorescence spectroscopy

- Samples must be prepared on a reflective media
- Polished quartz glass or polyacrylic glass disc
- Dried to a thin layer, or as a thin film or microparticle
- Matrix effects are negligible



Beam angle: 0°/90°

- confidential -

Principles of TXRF Quantification



$$C_{i} = \frac{C_{IS} \cdot N_{i} \cdot S_{IS}}{N_{IS} \cdot S_{i}}$$

C_i: Element concentration

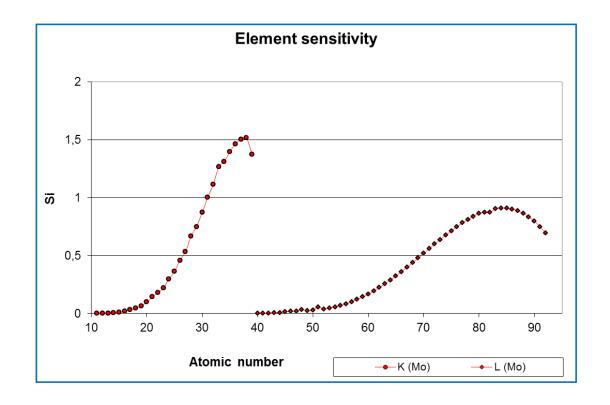
C_{IS}: Internal standard concentration

N_i: Element net countrate

N_{IS}: Internal standard net countrate

S_i: Element sensitivity factor

S_{IS}: Internal standard sensitivity factor



Principles of TXRF Bruker Product Portfolio



S2 PICOFOX

 Most compact design transportable, for on-site analysis

 Fixed excitation mode easy to use, most suitable for teaching

about 300 installations well established technology worldwide

S4 T-STAR

Multiple excitation to detect most elements modes of the PSE

Large area detectors improved sensitivity for lowest limits of detection

Motorized beam path automatic beam adjustment and QC procedures

 Large sample capacity up to 90 sample discs, multi-user operation







02 Overview – Environmental analysis

Overview **Environmental analysis**



Air

Airborne particulates

Soil

Contaminated and uncontaminated

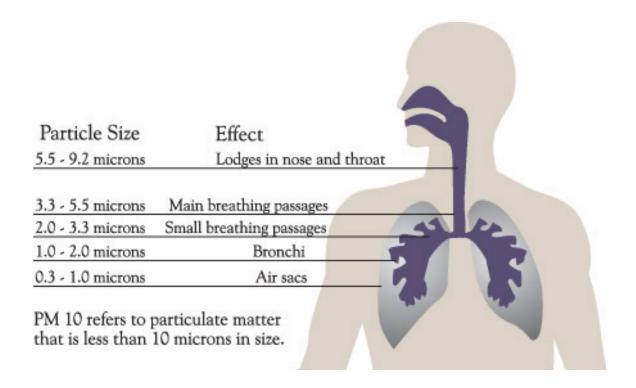
Water

- Surface, fresh and drinking water
- Wastewater, sewage

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Overview Environmental analysis

Air, airborne particulates

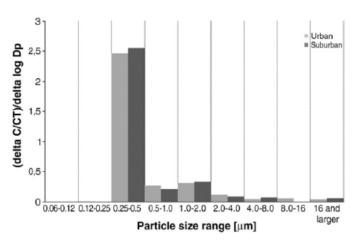


- Suspended: > 10 μm
- Respirable suspended: < 10 μm (PM10)
- Fine particles: < 2,5 μm (PM2.5)
- Ultrafine particles: < 1 μm
- Soot: < 0,3 μm

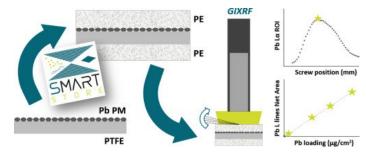
Overview **Environmental analysis**

Air, airborne particulates

- Samples collected of filters can be analyzed after extraction or complete digestion
- Direct analysis is only possible qualitatively with small filter segments applying external calibrations or qualitatively
- Results can be applied for environmental monitoring or research on origin analysis of airborne particulates



Wagner & Mages (2010)



Borgese et al. (2020)

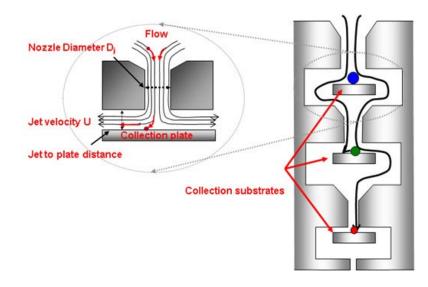
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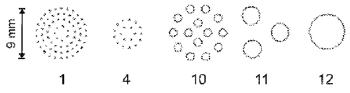
Overview Environmental analysis

Air, airborne particulates

 Direct analysis after particle-size fractionated sampling directly in sample carriers in multiple stage impactors







Dekati stage

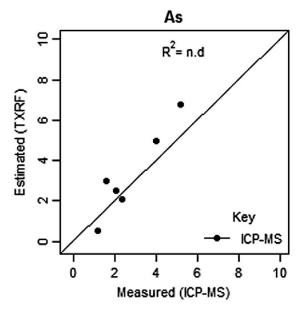
Seeger et al.(2021)

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Overview **Environmental analysis**

Soil

- Soil samples can be analysed directly after slurry preparation, after complete digestion or extraction
- Due to line interferences the heavy metals Cd,
 Sn and Sb can only be analysed with W-Brems excitation with detection limits in the higher mg/kg range



Towett et al.(2013)

Overview **Environmental analysis**

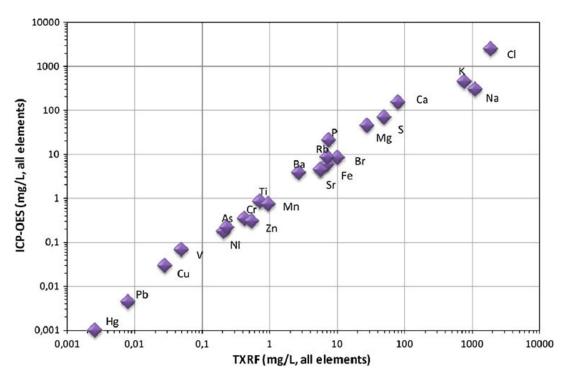
Water

- Surface-, rain-, ground-, drinking waters can be analyzed nearly directly (acidification, internal standardization, preparation on sample carrier)
- Typical detection limits are in the µg/l, sometimes pg/l range
- Mercury analysis is difficult because of its high vapor pressure
 - Complexation of Hg with thiourea at pH = 10 (Margui et al., 2002)
- Cadmium analysis is difficult because of line interferences
 - \triangleright Analysis with W-Brems excitation (LLDs in the high μ g/l range)
 - ➤ Liquid-liquid microextraction (Margui et al., 2012)

Overview Environmental analysis

Water

- Water samples with high matrices (wastewater, sewages etc.) demand some additional sample preparation (adddition of detergents like polyvinyl alcohol)
- TXRF results are typically similar to those, derived by methods like ICP-OES or ICP-MS



Cataldo (2012)

Overview **Environmental analysis**

- The routine analysis of air particulates, water and soil only gives a limited actual evaluation
- For a more detailed assessment more complex investigations e.g. on biomonitors must be made

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Biomonitoring and TXRF

Can TXRF be a Valuable Tool in Biomonitoring?

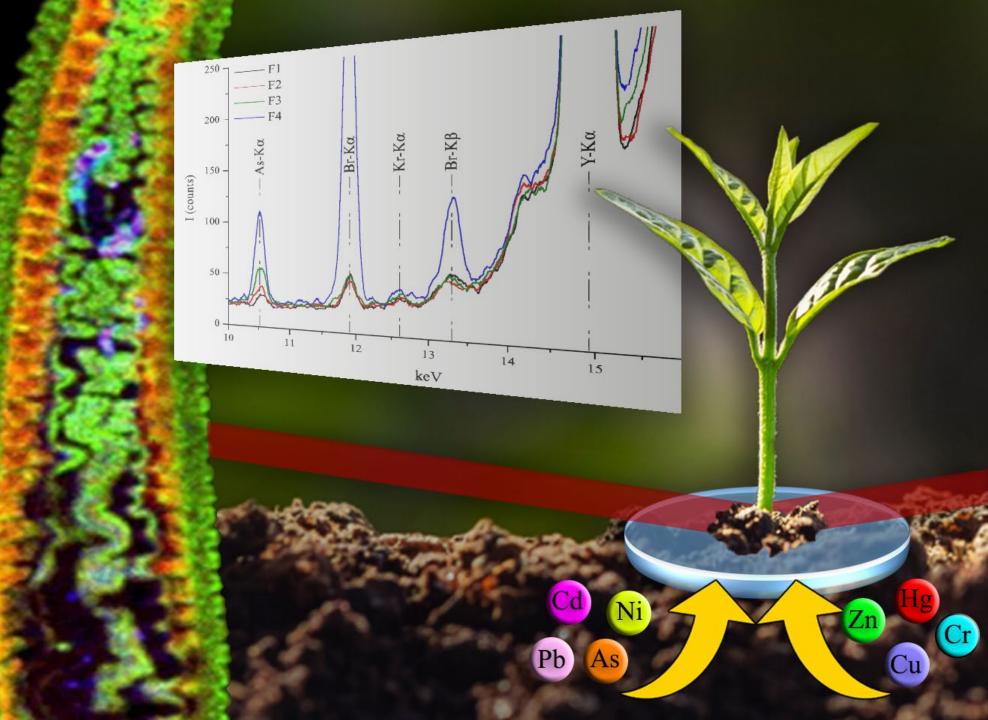
Ignazio Allegretta

Micro X-ray Lab, DiSSPA, University of Bari, Italy









Biosphere Hydrosphere Atmosphere Lithosphere



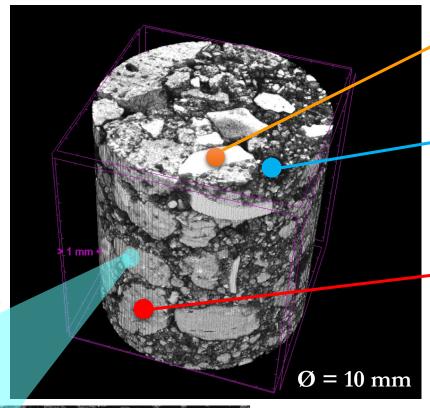


Soil system

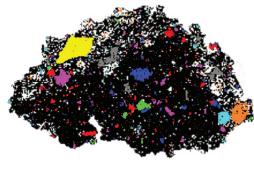
Organisms



Microorganisms



100 µm EHT = 15.00 kV Signal A = HDESD Date :31 van 2019 WD = 7.5 mm Mai = 1/11 X Tran :11-34.91



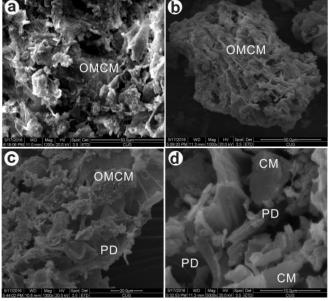
Minerals

Pores

(water, solutions, air, gases)

Aggregates

(OM, clays and other minerals)



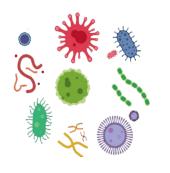
Hong et al. (2019) Appl. Clay Sci. 105125

Soil and Organisms

Photosynthesis vs respiration

Multiple interactions

Different kind of cells, tissues and organs

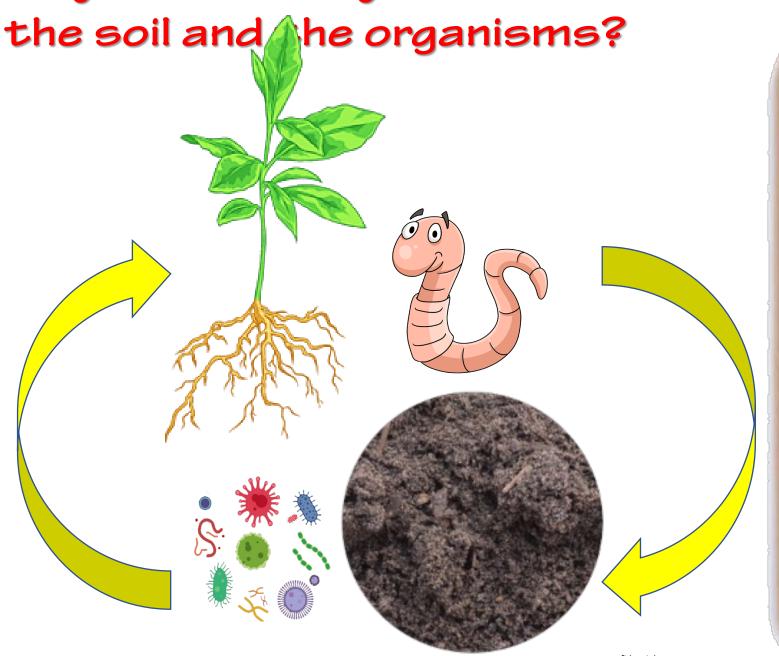




Different sample matrix

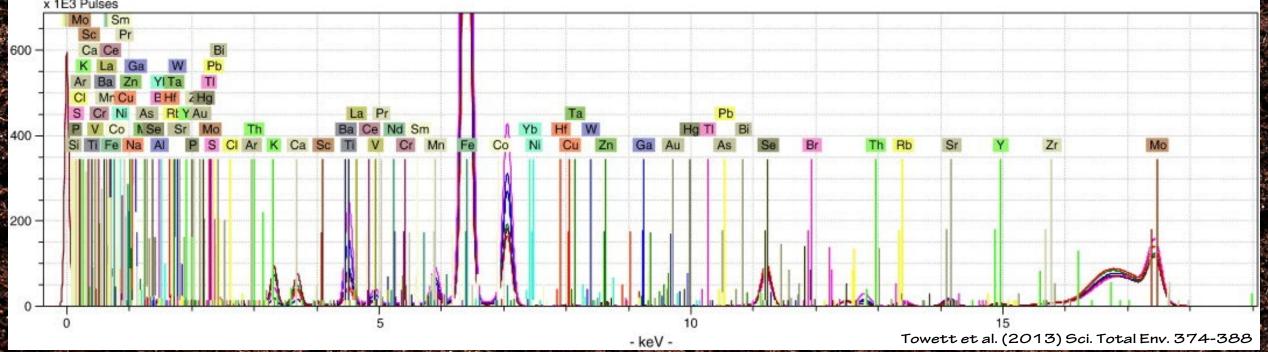
(saps/fluids, parts rich in fibers, Si, +/-water)

Why do we study the interaction between



Physiology Nutrition/fortification Synergy/Competition Response to pathogens attack Food security Environmental studies Fate of PTE Strategies for soil remediation



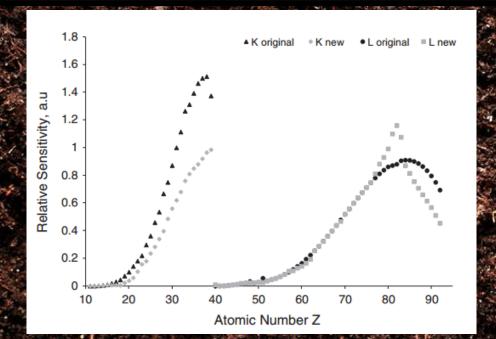


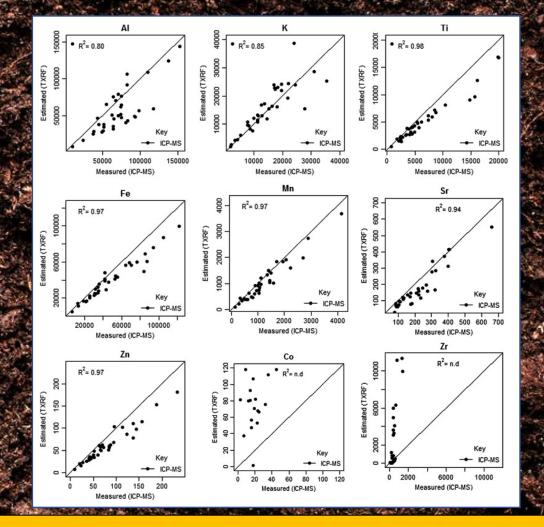


Matrix effect can't be neglected

J

Sensitivity recalibration using ICP-MS data



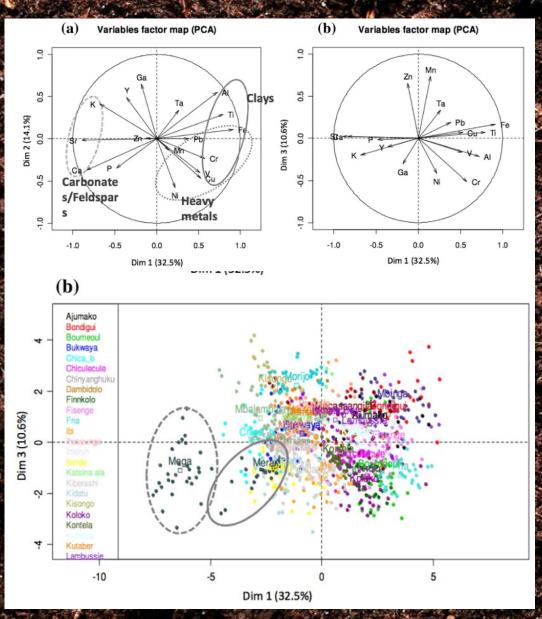


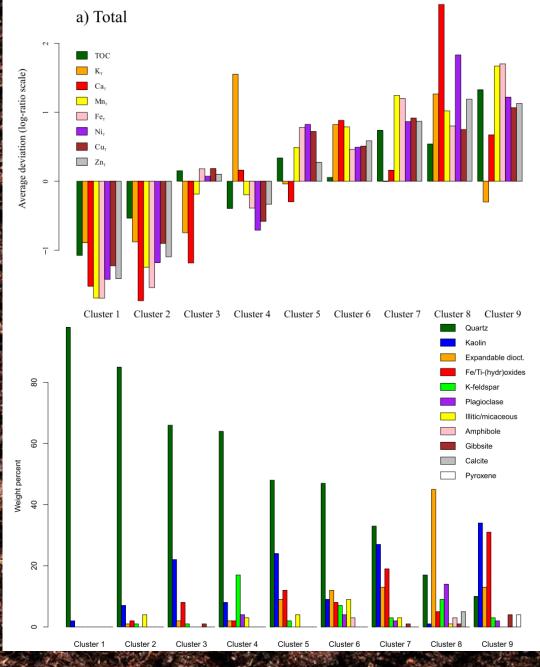
Light elements (but Na and Mg)

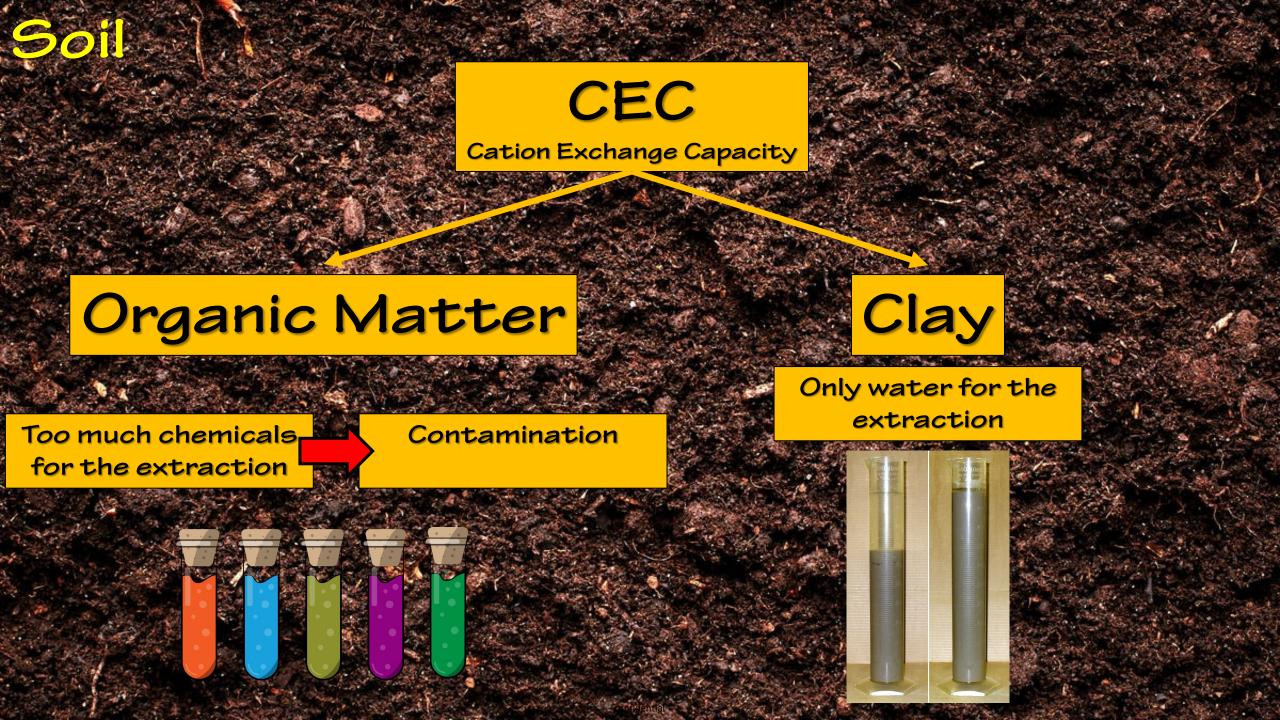
High-Z elements

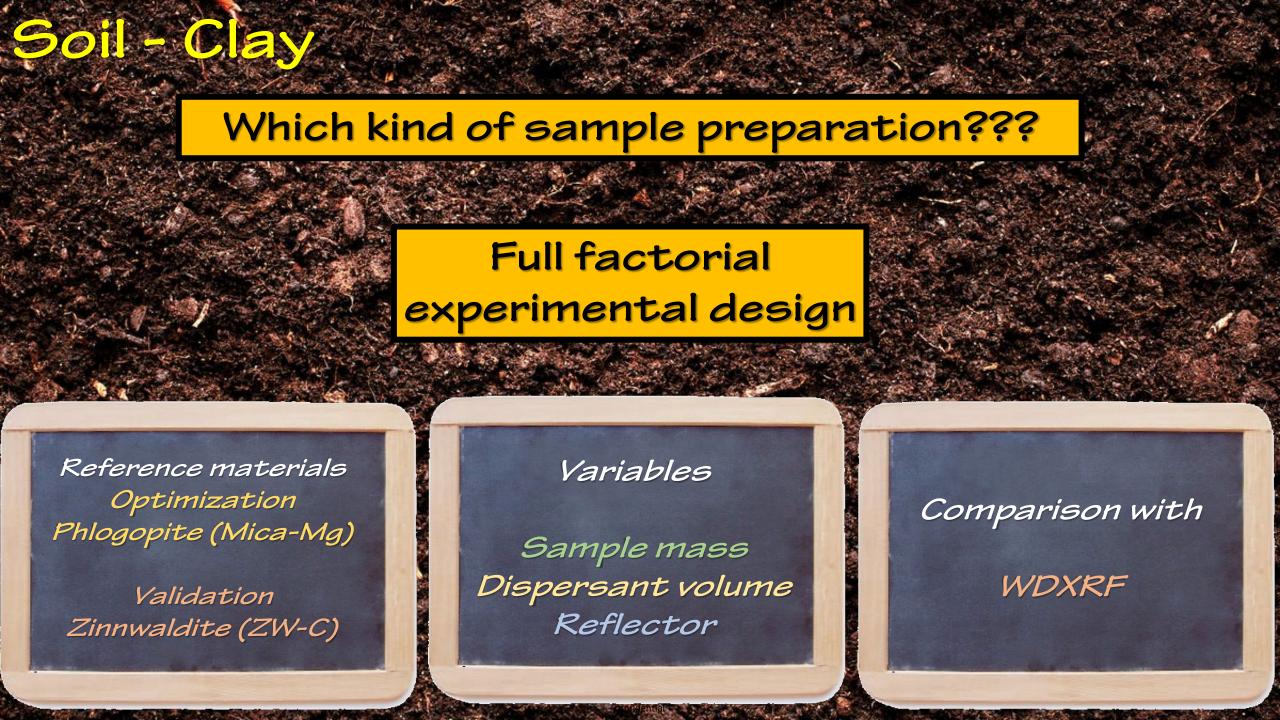
Problems in case of overlapping

Soil









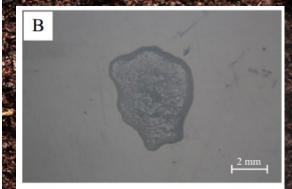
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Variables	Levels							
	Low	Centre point	High					
Sample weight (mg)	50	75	100					
Dispersant volume (ml)	2.5	3.75	5					
Reflector	Quartz		Plexiglas					

Table 3

Recovery (%) obtained for each element after the preparation of slurries using different parameters and TXRF analysis. A recovery of 80–120% was considered acceptable for the correct element detection. The certified concentration of each element is also reported.

Sample weight (mg)	50	50	50	50	75	75	100	100	100	100	Reference Value
Triton solution volume (ml)	2.5	2.5	5	5	3.75	3.75	2.5	2.5	5	5	_
Reflector	Quartz	Plexiglas	mg/kg								
Mg	111	222	132	214	131	246	51	172	110	236	12.30 (%)
Al	102	185	123	192	115	204	49	137	98	194	8.04 (%)
Si	85	126	123	127	100	139	42	92	86	132	17.89 (%)
CI	100	179	132	169	110	200	59	118	97	184	800
K	107	149	117	132	112	154	74	118	94	142	8.25 (%)
Ca	36	42	36	27	42	42	37	34	30	40	0.06 (%)
Ti	84	112	87	95	87	114	64	90	71	104	0.98 (%)
V	133	185	145	106	156	167	163	137	105	170	90
Cr	65	110	64	99	56	82	46	90	64	76	100
Mn	82	105	82	87	84	105	67	87	68	95	0.20 (%)
Fe	92	116	90	97	93	116	76	97	76	105	6.62 (%)
Ni	96	122	94	104	96	119	79	100	80	108	110
Cu	86	46	21	39	17	28	32	23	39	24	4
Zn	104	126	97	104	101	126	86	108	83	112	290
Ga	86	111	84	94	86	110	72	92	71	101	21
Rb	105	128	98	104	103	127	91	110	85	113	1300
Sr	113	126	103	106	108	119	96	112	89	107	27
Ba	75	92	71	81	72	91	54	79	63	81	4000
Pb	109	159	86	94	117	181	112	123	83	129	9
Total number of elements with a recovery in the range 80–120%	15	6	10	12	13	7	4	12	11	9	





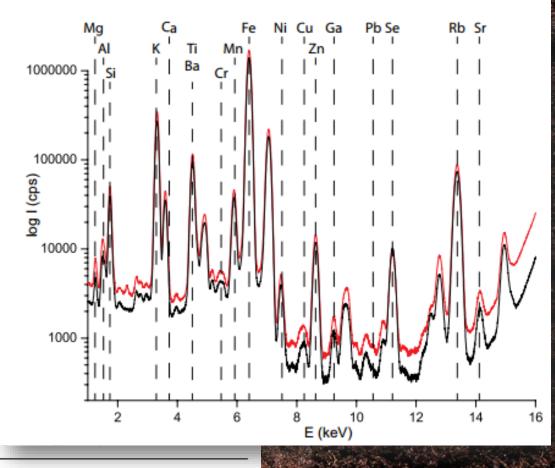


Variables	Levels	Levels							
	Low	Centre point	High						
Sample weight (mg)	50	75	100						
Dispersant volume (ml)	2.5	3.75	5						
Reflector	Quartz		Plexiglas						

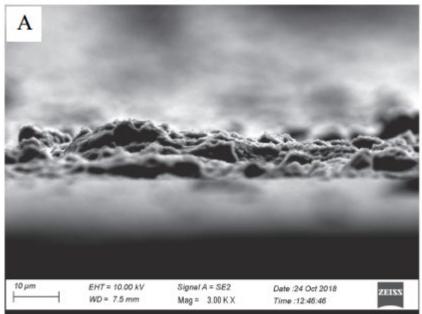
Table 3

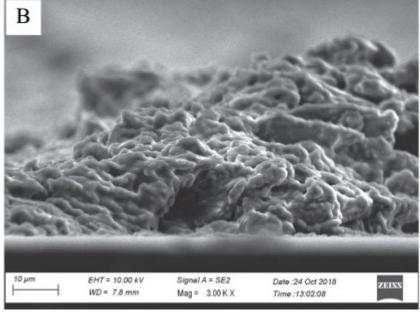
Recovery (%) obtained for each element after the preparation of slurries using different parameters and TXRF analysi acceptable for the correct element detection. The certified concentration of each element is also reported.

Sample weight (mg)	50	50	50	50	75	75	100	1
Triton solution volume (ml)	2.5	2.5	5	5	3.75	3.75	2.5	2
Reflector	Quartz	Plexiglas	Quartz	Plexiglas	Quartz	Plexiglas	Quartz	P
Mg	111	222	132	214	131	246	51	1
Al	102	185	123	192	115	204	49	1
Si	85	126	123	127	100	139	42	9
CI	100	179	132	169	110	200	59	1
K	107	149	117	132	112	154	74	1
Ca	36	42	36	27	42	42	37	3
Ti	84	112	87	95	87	114	64	9
V	133	185	145	106	156	167	163	1
Cr	65	110	64	99	56	82	46	9
Mn	82	105	82	87	84	105	67	8
Fe	92	116	90	97	93	116	76	9
Ni	96	122	94	104	96	119	79	1
Cu	86	46	21	39	17	28	32	2
Zn	104	126	97	104	101	126	86	1
Ga	86	111	84	94	86	110	72	9
Rb	105	128	98	104	103	127	91	1
Sr	113	126	103	106	108	119	96	1
Ba	75	92	71	81	72	91	54	7
Pb	109	159	86	94	117	181	112	1
Total number of elements with a recovery in the range 80–120%	15	6	10	12	13	7	4	1
								_



Soil - Clay Mg Si C C. B AB AB AC AC AB AC ABC BC ABC BC ABC BC 15 10 12 14 10 Fe Sr Ba 2,20 A В AB AB AC AC ABC BC AB AC-ABC B B BC ABC BC 2.0 A = sample weight B = dispersant volume C = reflector

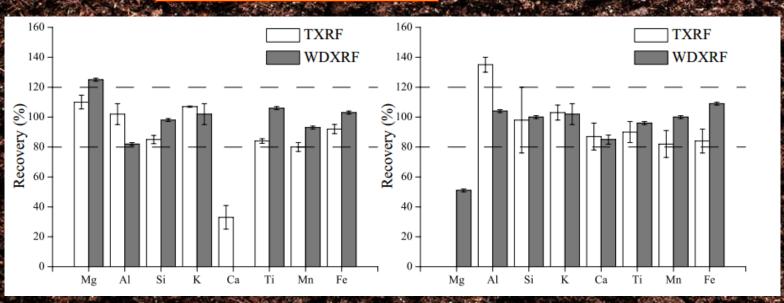


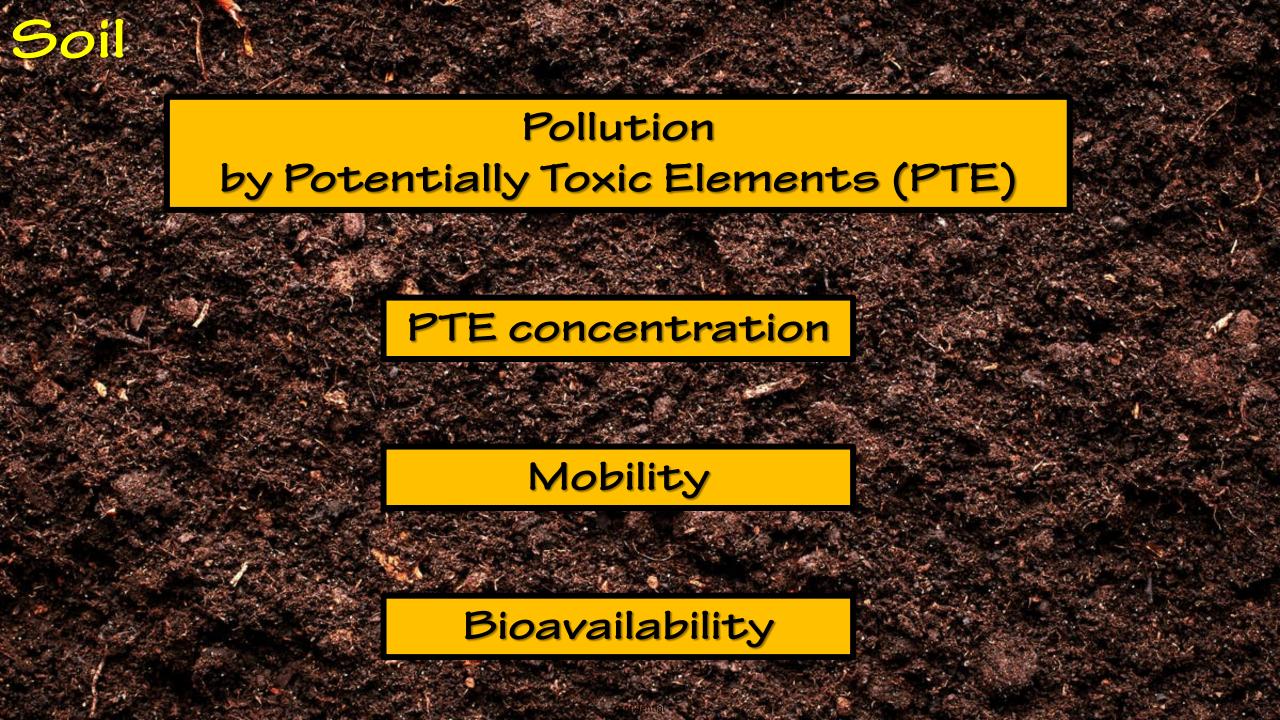


Soil - Clay

	Elements							_							
	Al	Si	К	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Ga	Rb	Sr	Pb
RV (mg/kg)	97,600	252,200	63,600	2600	300	56	7500	66,200	11	39	1050	99	8500	17	80
Mean (mg/kg)	131,938	247,412	65,803	2264	270	63	6175	55,359	14	26	1052	81	7734	18	126
Recovery (%)	135	98	103	87	90	112	82	84	127	68	100	82	91	108	158
RSD _{WLR} (%)	5	24	7	11	9	95	12	10	37	12	11	10	11	15	18
RSDr (%)	5	21	4	5	6	95	6	6	37	12	7	5	6	10	18
Day	0	21	65	79	52	0	77	66			63	68	69	58	
Sample	19	8	9	9	28	95	13	21	1	26	21	21	20	28	0
Instrument	81	71	26	12	20	5	10	13	99	71	16	11	11	14	100
LOD (mg/kg)	1153	279	24	8	5	4	4	4	1	1	1	1	1	1	1
LOQ (mg/kg)	3845	930	81	25	17	12	14	12	3	3	3	2	3	2	2

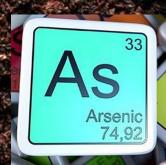
Overlapping with Rb L-lines and K escape peak





Soil - Pollution

As pollution in mining









As concentration

S1 = 145 ppm

S2 = 4640 ppm

S3 = 13300 ppm

S4 = 40200 ppm

Is it mobile?

Soil - Pollution

Sequential extraction

- 1. Non-specifically adsorbed, extracted with (NH₄)₂SO₄ 0.5 M for 4 h at 20 °C;
- 2. Specifically sorbed on minerals, extracted with NH₄H₂PO₄ 0.5 M for 16 h at 20 °C;
- 3. Associated to amorphous and scarcely ordered Fe and Al oxides and hydroxides, extracted with NH₄-oxalate 0.2 M for 4 h at 20 °C;
- 4. Associated to well-crystallized Fe and Al oxides and hydroxides, extracted with NH₄-oxalate 0.2 M and ascorbic acid 0.1 M for 30 min at 96 °C;
- 5. Residual, extracted using acid microwave-assisted digestion with HNO₃ and H_2O_2 (7:1, v/v).

Extraction step	Description	S1 % of total As	S2	S3	S4
1	Non-specifically sorbed	2.2 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
2	Specifically sorbed	10.7 ± 2.1	25.2 ± 2.7	11.9 ± 2.3	7.6 ± 3.9
3	Associated to amorphous Fe oxides/hydroxides	49.8 ± 0.8	67.2 ± 2.9	85.5 ± 1.5	87.1 ± 4.8
4	Associated to well crystalline Fe oxides/hydroxides	27.9 ± 1.1	1.6 ± 0.3	1.4 ± 0.8	4.7 ± 1.2
5	Residual	9.4 ± 1.2	5.4 ± 1.1	1.0 ± 0.6	0.3 ± 0.2



Centrifugation 15min 1700xg Filtering (0.45 µm) IS (Se 1000g/L) 40µL

1 ml o sample + 10µL of Ga (100 mg/l) 1000 s

Soil - Pollution

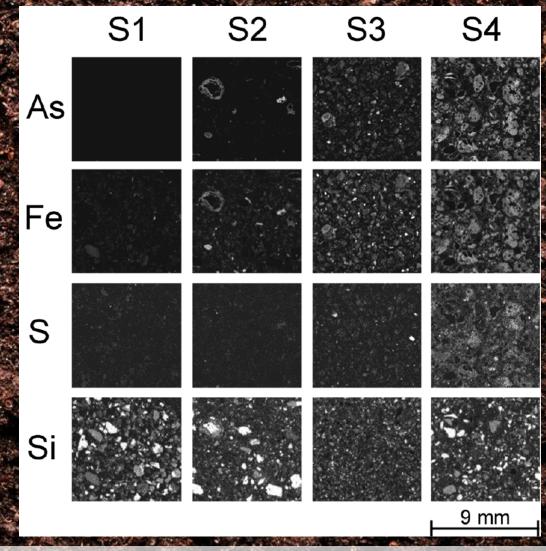
No mobile

Mobile ≠

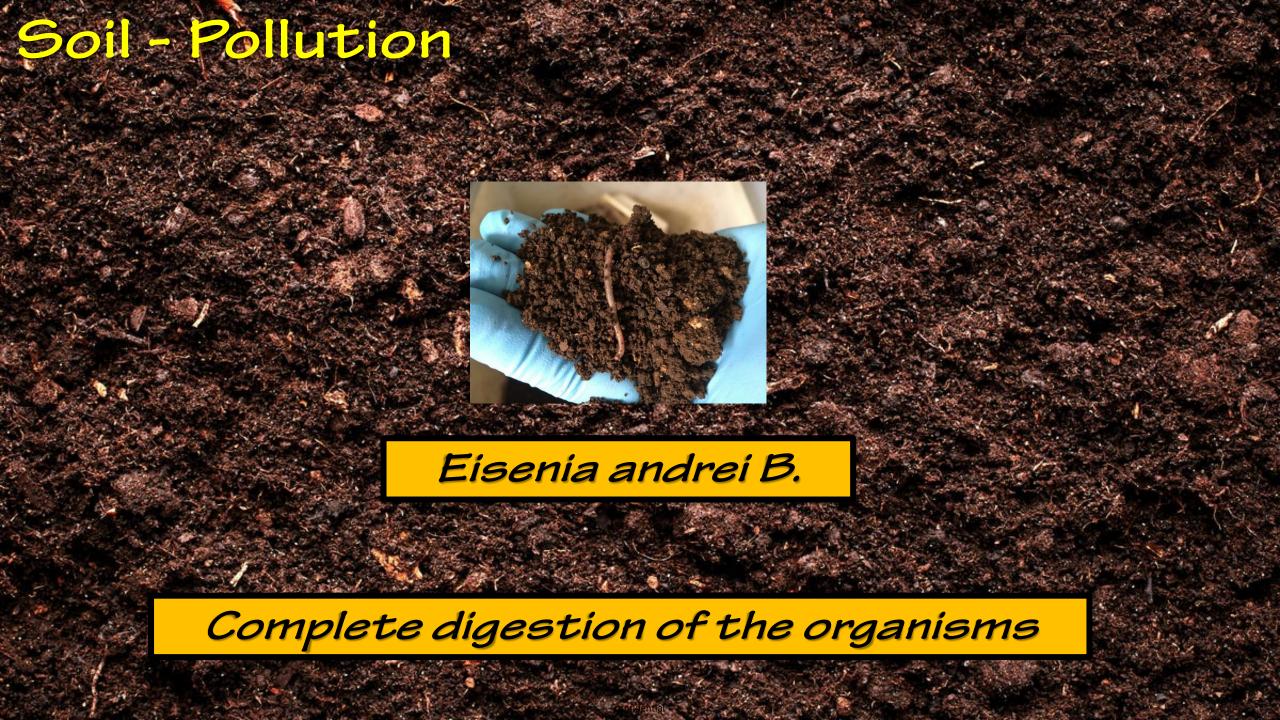
Sentinel Organism

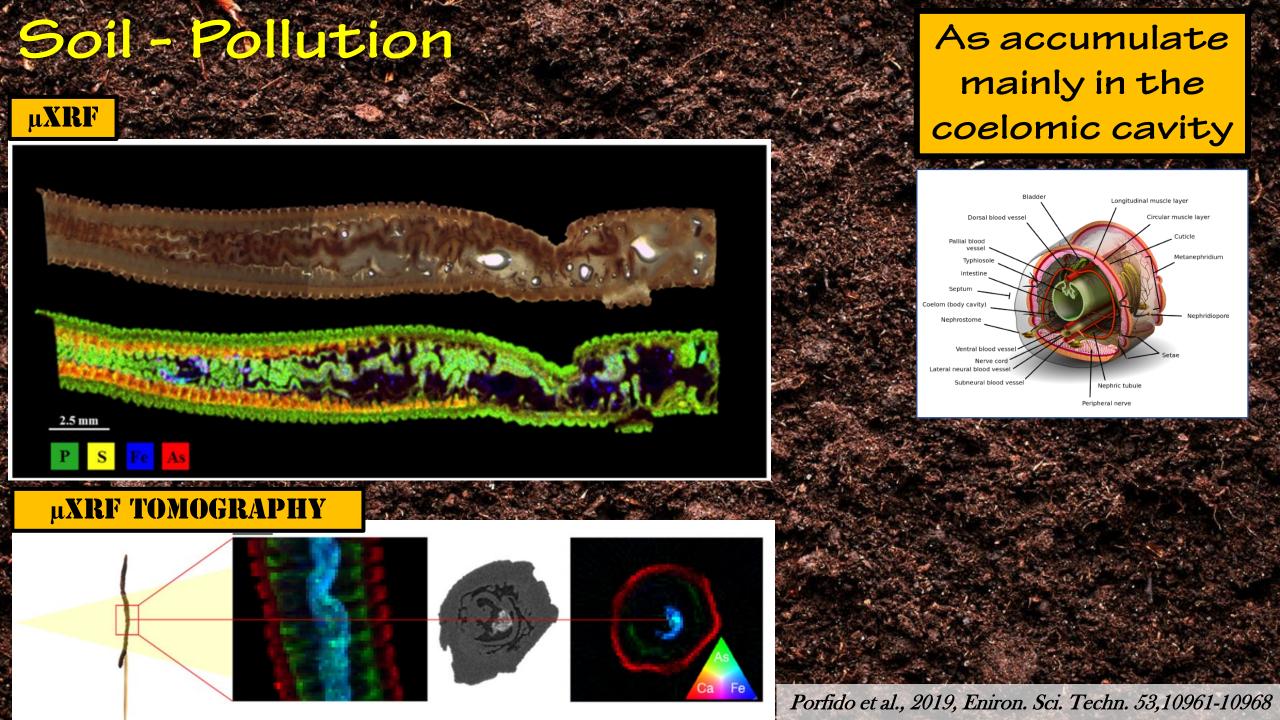
Extraction step	Description	S1 % of total As	S2	S3	S4
1	Non-specifically sorbed	2.2 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
2	Specifically sorbed	10.7 ± 2.1	25.2 ± 2.7	11.9 ± 2.3	7.6 ± 3.9
3	Associated to amorphous Fe oxides/hydroxides	49.8 ± 0.8	67.2 ± 2.9	85.5 ± 1.5	87.1 ± 4.8
4	Associated to well crystalline Fe oxides/hydroxides	27.9 ± 1.1	1.6 ± 0.3	1.4 ± 0.8	4.7 ± 1.2
5	Residual	9.4 ± 1.2	5.4 ± 1.1	1.0 ± 0.6	0.3 ± 0.2

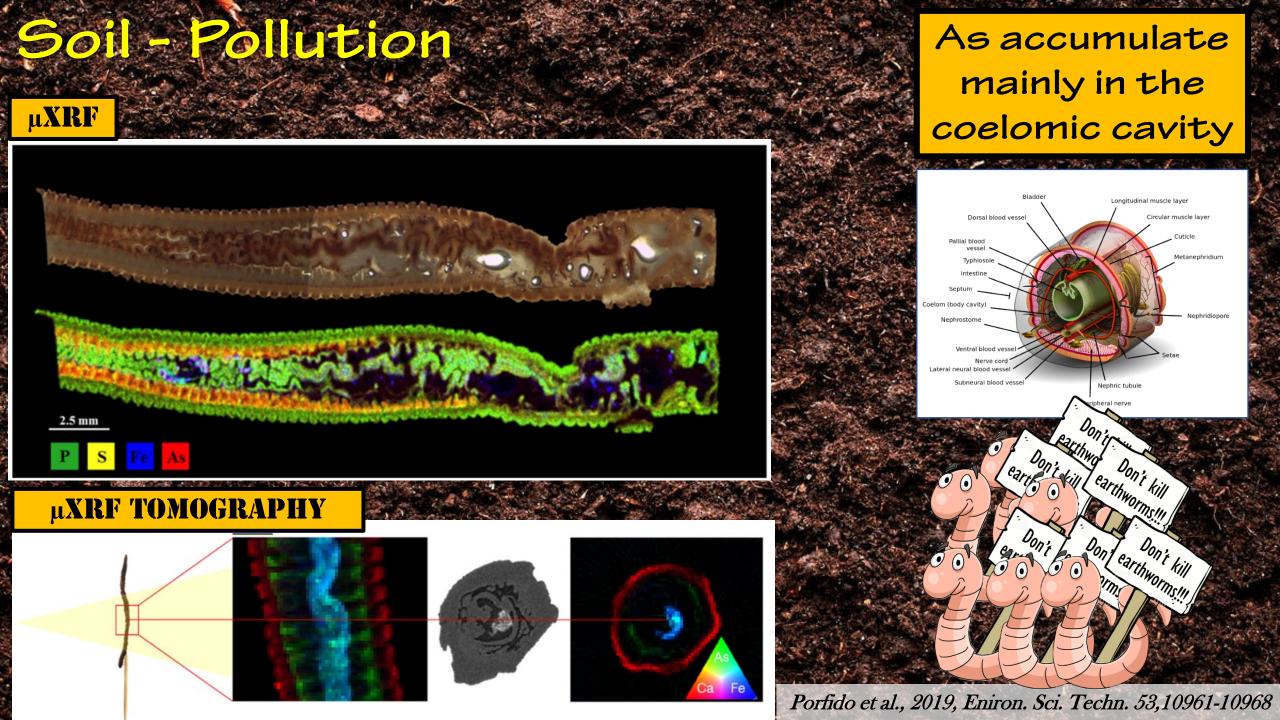
μXRF



Allegretta et al., 2018, Environ. Sci. Poll. Res., 25:25080-25090







Soil - Pollution

Dilution: 1(CF):8(PVA):1(IS=Y)

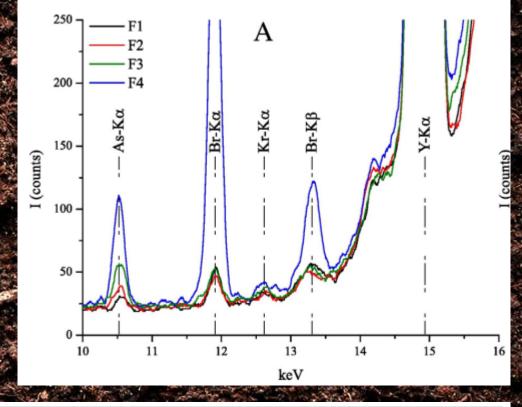


Extraction (5V, 10sec)

Don't worry!!!

For microsamples
you can use TXRF!!!

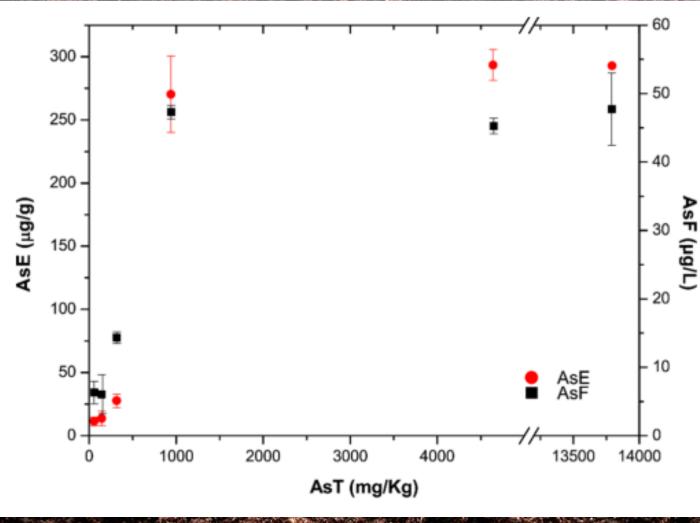


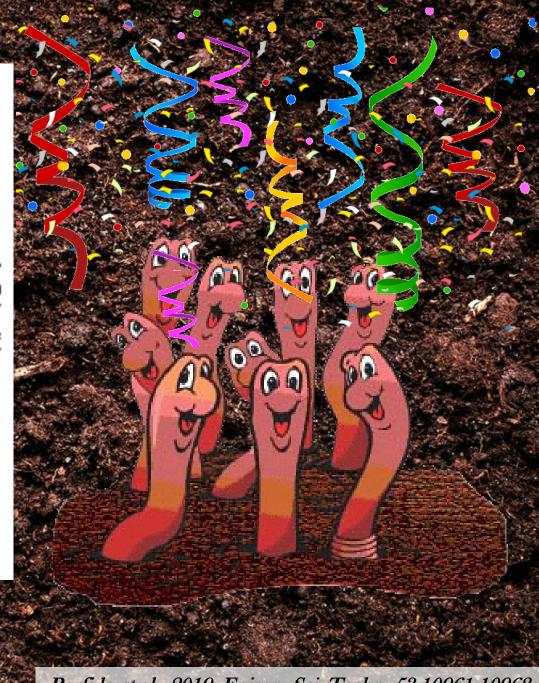


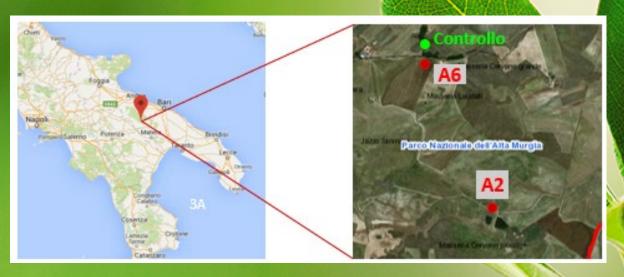
50		0	T
50	_	00	μ L

Sample	As concentration in the soil (mg/kg)	Analytical technique	Final		Diluted	Diluted		
			Conc (µg/l)	SD (µg/l)	Conc (µg/l)	SD (µg/l)	DL (μg/l)	
F1ª	18	TXRF	5.3	1.0	0.5	0.1	0.2	
		ICP-MS	3.3	0.4	0.02	0.002	0.01	
F2 ^a	21	TXRF	7.0	1.0	0.7	0.1	0.2	
		ICP-MS	4.7	0.4	0.02	0.002	0.01	
F3 ^a	400	TXRF	25.3	1.6	2.5	0.2	0.2	
		ICP-MS	24.8	0.2	0.1	0.001	0.01	
F4 ^b	600	TXRF	47.0	0.9	4.7	0.1	0.3	
		ICP-MS	42.0	1.2	0.21	0.006	0.01	

Soil - Pollution







 Cr concentration

 C
 65 mg/kg

 A2
 3807 mg/kg

 A6
 5160 mg/kg

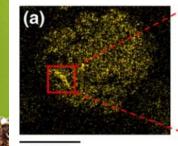
Agricultural soils

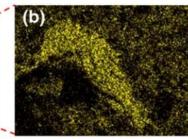


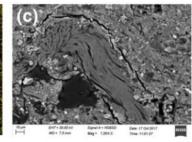
(agricultural practice)

What's app to Cr??? Is it bioavailable?

Tannery Sludge





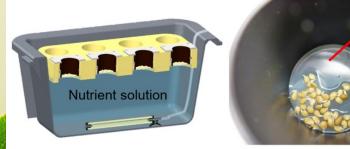


50 µ

Gattullo et al., 2020, Environ Sci Pollut Res 27, 22967-22979

Plant - Pollution RHIZOTESTS





SOIL(9g)



Phase 2







Membrana di nylon

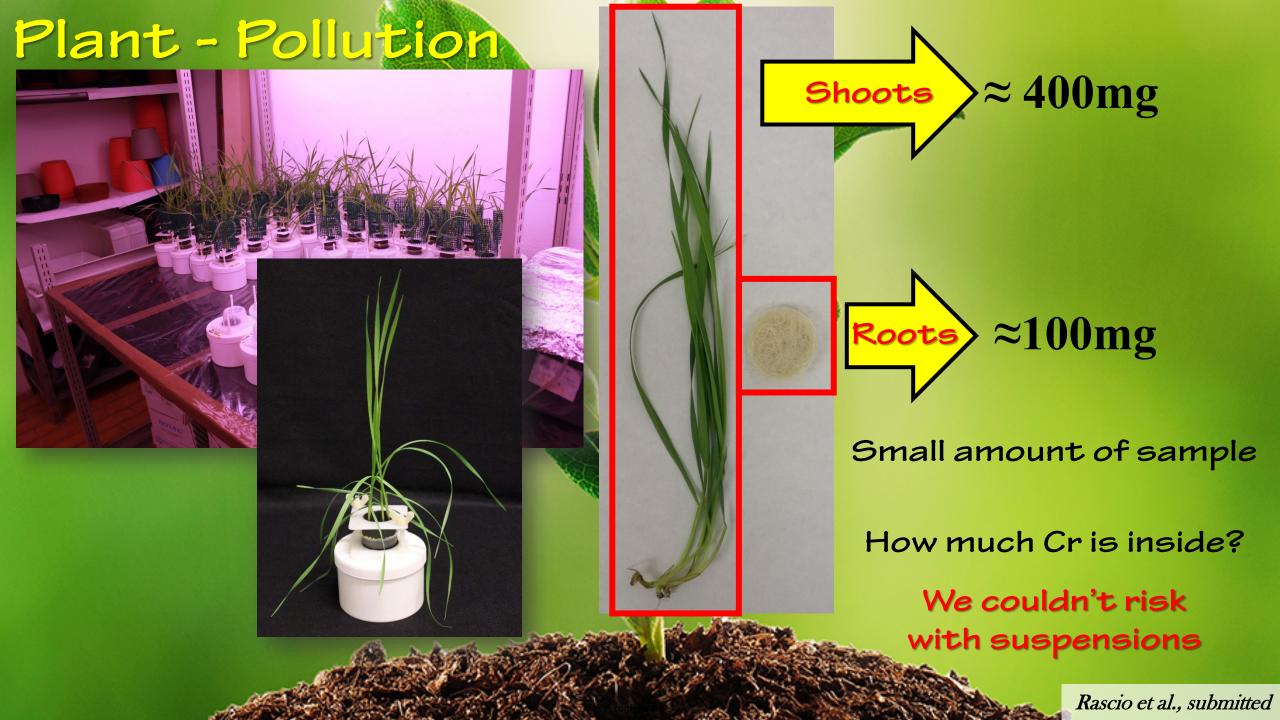
con pori di 30µm



https://rhizotest.cirad.fr/en/the-rhizotest http://www.metrhizlab.com/

27.677 10.00 (Sept. 1981) (Sept. 10.00 Mills 10.00 Mil

rascio et al., submued



Digestion

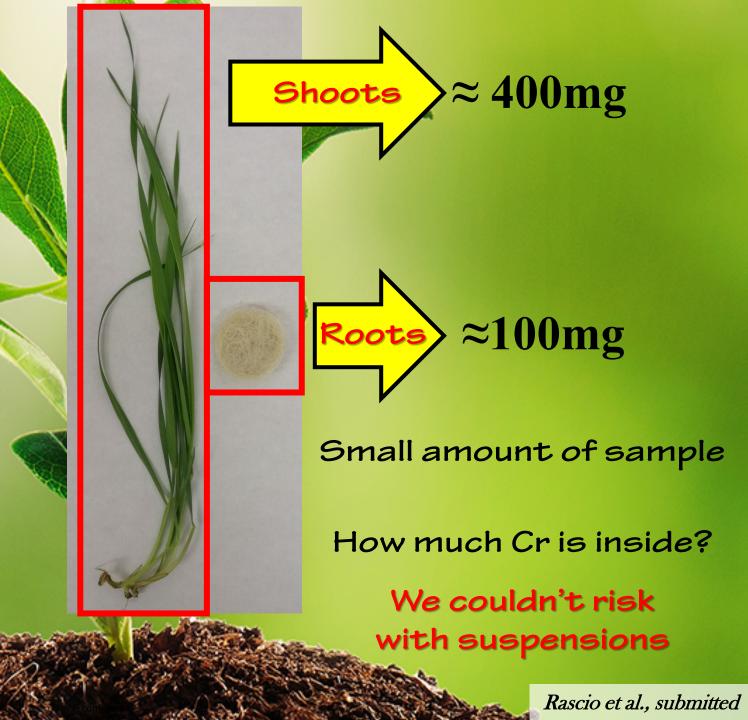
100-150 mg of sample

1 ml of H_2O_2 7ml of HNO_3

TXRF analysis

Solution

Ga (15) 10 µl 1g/l

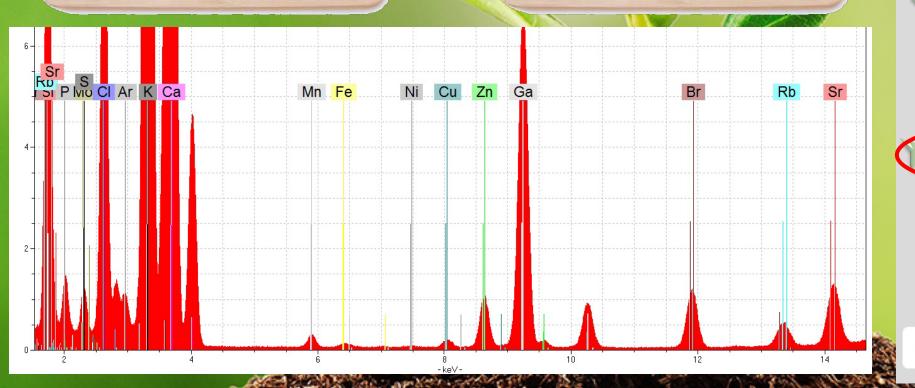


Plant - Pollution Xylem Saps



TXRF analysis

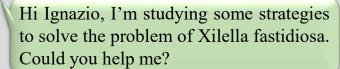
1 ml of sap Ga (IS) 10 µl 100 mg/ Mn= 20-200 mg/l Fe = 10-100 mg/l Ni = 0.7-10 mg/L $Cu = 10-70 \, \text{mg/l}$ $Zn = 70-500 \, mg/l$











Hi Dr Plant, it depends...what's the problem?

should the elemental analyze composition of the olive trees' xylem saps. But I can give you just 1-2 ml.

OK no problem!!! We can use TXRF!!!



















S, P, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn

TOP TOP TOP TOP TOP TOP TOP







Xylem Saps: SE-HPLC + TXRF

Table 5
Concentrations of the metals present in the selected freeze-dried and concentrated SEC fractions of xylem sap of cucumber artificially contaminated with nickel

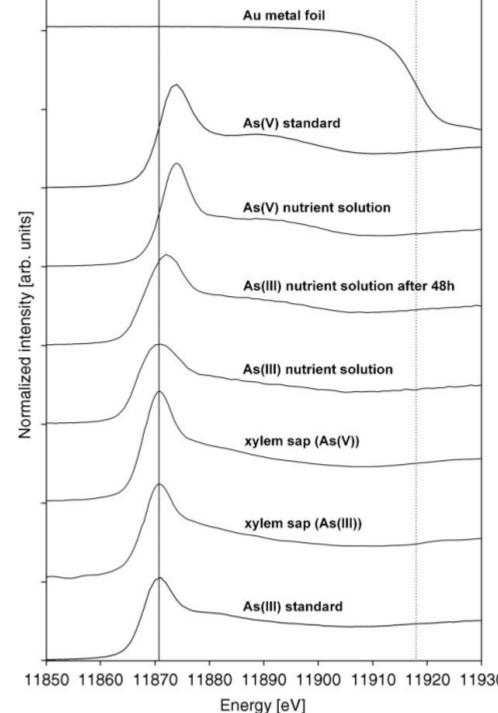
ΔV elution	n (cm ³)					
	2.0-2.5	2.5-3.0	3.0-3.5	3.5-4.0	4.0-4.5	4.5-5
c [μg cm	$^{3} \pm \text{R.S.D.}(\%)$					
Ca	0.22 ± 8.7	0.13 ± 4.0	14.5 ± 10.3	18.2 ± 4.0	0.33 ± 5.4	0.52 ± 1.5
K	0.34 ± 3.2	0.69 ± 7.0	46.7 ± 11.3	80.3 ± 3.7	1.38 ± 1.2	2.83 ± 3.3
c [ng cm ⁻¹	$^{3} \pm \text{R.S.D.}(\%)$					
Cu	20.0 ± 6.6	n.d.	81.1 ± 10.5	41.7 ± 2.2	12.1 ± 9.4	8.10 ± 2.6
Fe	n.d.	60.0 ± 12.2	42.4 ± 10.5	41.5 ± 22.4	10.6 ± 58.3	12.8 ± 20.6
Mn	n.d.	n.d.	45.5 ± 5.8	239.9 ± 14.0	n.d.	n.d.
Ni	15.6 ± 2.1	138.5 ± 1.2	433.9 ± 12.1	57.6 ± 9.3	20.1 ± 8.1	32.4 ± 6.5
Zn	50.2 ± 0.03	33.5 ± 8.4	38.5 ± 2.8	62.7 ± 6.3	66.5 ± 2.3	100.2 ± 2.8

TXRF Xylem Saps
Sample 100 µl C_{Ga} 3 µg/cm³
25µl on Qtz disk
300 s

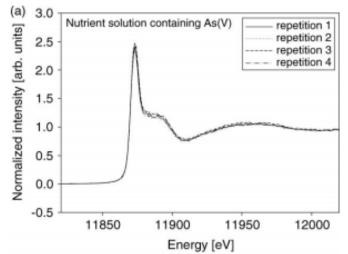
EXTRA IIA ATOMIKA
(Mo)

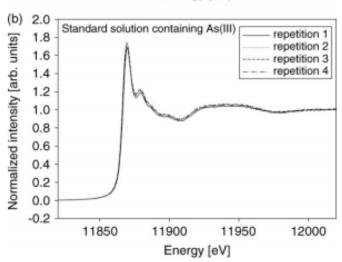
SE-HPLC
+ TXRF analysis of the
fractions
500 µl
Freeze-drying C_{Ga} 3 µg/cm³
25µl on Qtz disk
500 s

Plant - Pollution Xylem Saps: SR-TXRF-XANES Nutrient solution containing As(V) Normalized intensity [arb. units] units] Normalized intensity [arb. 0.5 0.0 -0.511850 11900 11950 12000 Energy [eV] Standard solution containing As(III) units] repetition 1 repetition 2 repetition 3 Normalized intensity [arb. repetition 4 0.8 0.6 0.4 0.2 0.0 -0.211900 12000 11850 11950 Energy [eV] Meirer et al. (2007) X-ray Spectrometry 36, 408-412



Xylem Saps: SR-TXRF-XANES

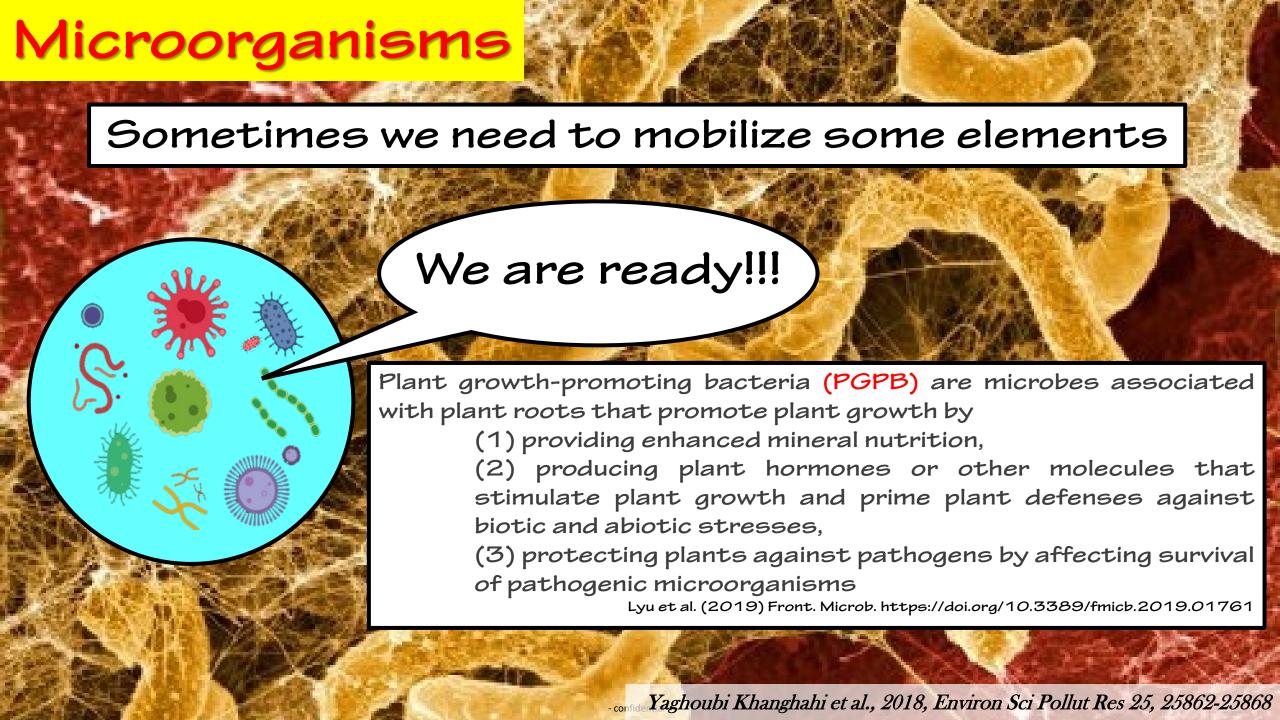


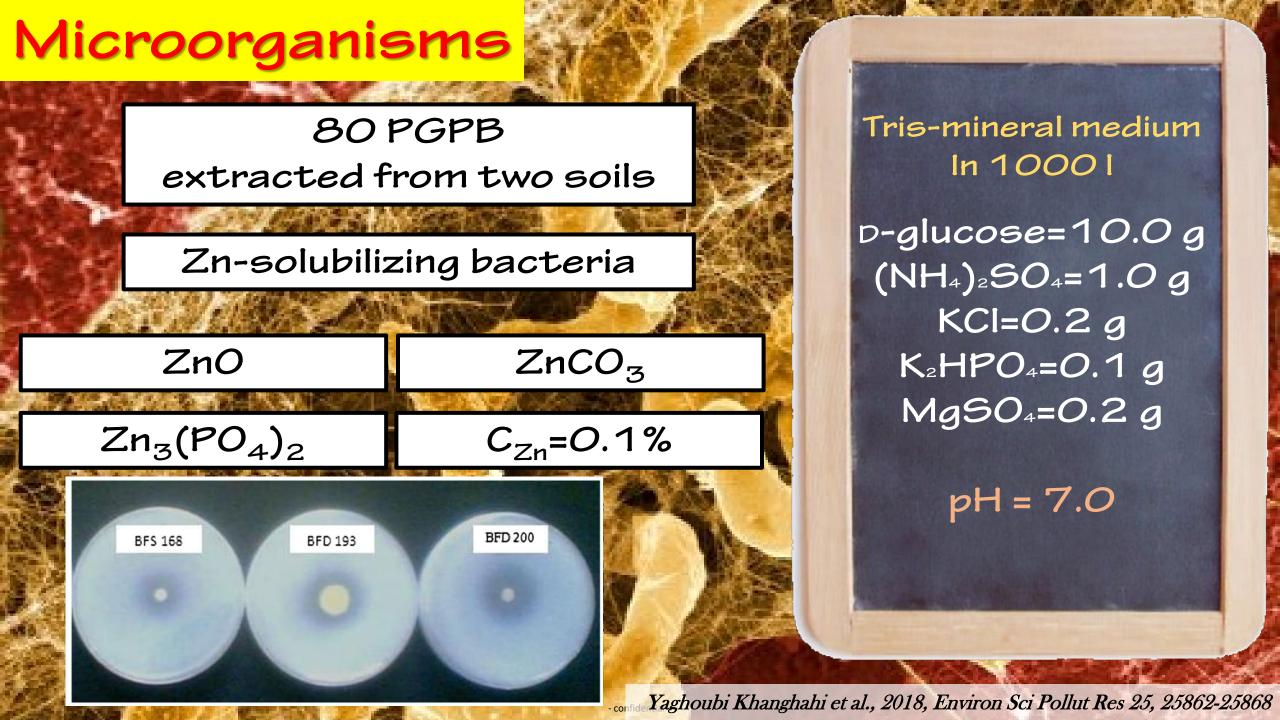


C_{As} 20-50ng
Deposition 1-20µl
Vacuum drying
Kept in Ar atm
Measurement under
vacuum at HASYLAB DESY

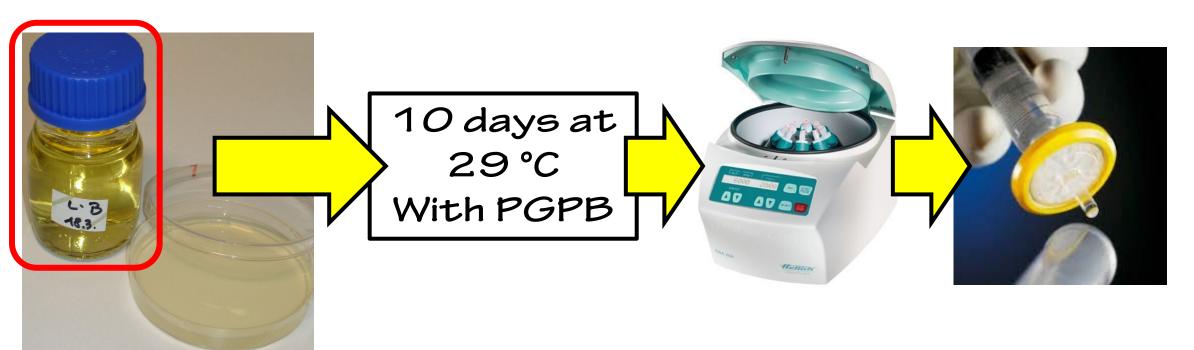
			Reduced	
	%	%	chi	chi
Sample	As(III) ^a	As(V) ^a	square	square
xylem sap (As(III))	88	12	0.0115	1.09
xylem sap (As(V))	83	17	0.0112	1.06
As(III) nutrient solution	100	0	0.0072	0.68
As(III) nutrient solution after 48h	71	29	0.0063	0.60
As(V) nutrient solution	2	98	0.0066	0.63

Meirer et al. (2007) X-ray Spectrometry 36, 408-412



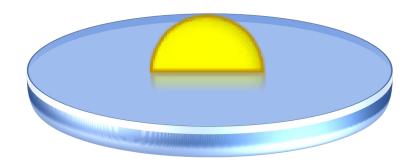


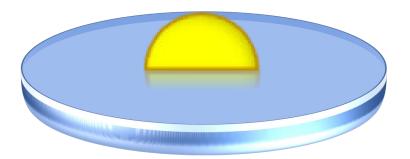
Microorganisms



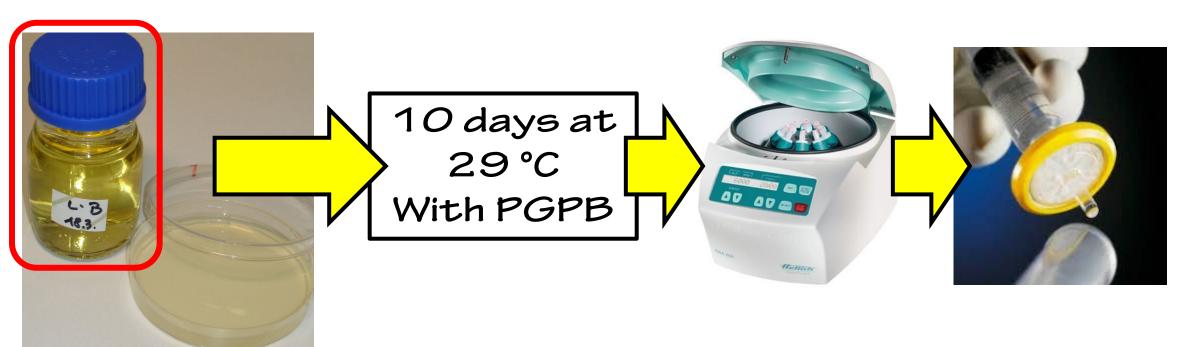
Direct

200 µl CM + 800 µl H20



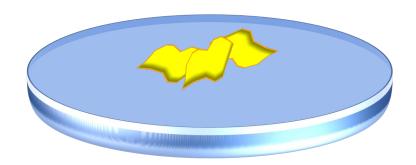


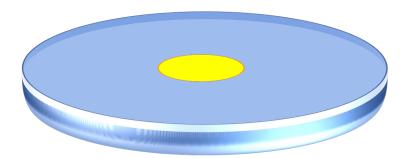
Microorganisms



Direct

200 µl CM + 800 µl H20









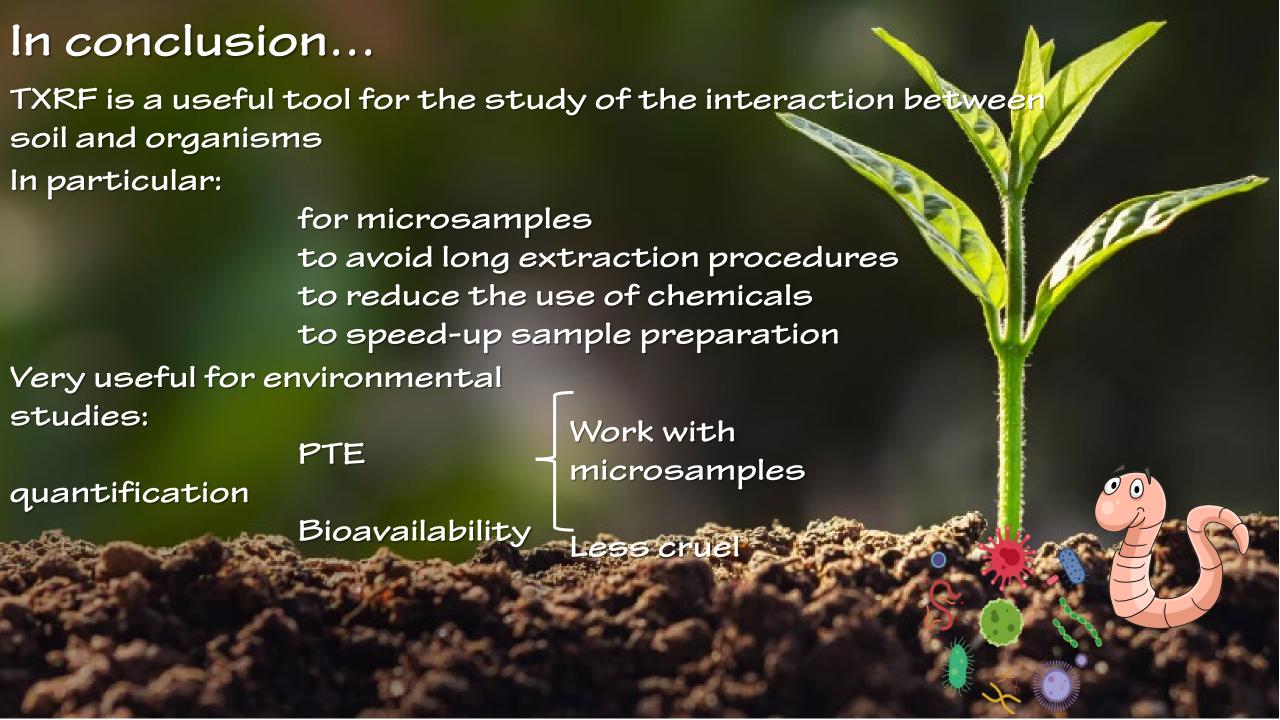
30- MO		
Ga S Mo A I Ca	Zr Ga	Mo
20-		
		<u> </u>
10		
	10 -keV-	15

Strain	pH value	Zinc solubilization (mg/L) ± SE
Agrobacterium tumefaciens	8	1.95 ± 0.02
Agrobacterium tumefaciens	9	51.39 ± 1.73
Agrobacterium tumefaciens	10	13.03 ± 1.54
Rhizobium sp.	8	2.06 ± 0.11
Rhizobium sp.	9	72.07 ± 2.88
Rhizobium sp.	10	52.69 ± 2.31
LSD value		5.33



They can mobilize Zn in a pH range in which Zn is not mobile

Yaghoubi Khanghahi et al., 2018, Environ Sci Pollut Res 25, 25862-25868



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www.microxraylab.com





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enforcetxrf.eu



All the people with whom I collaborate













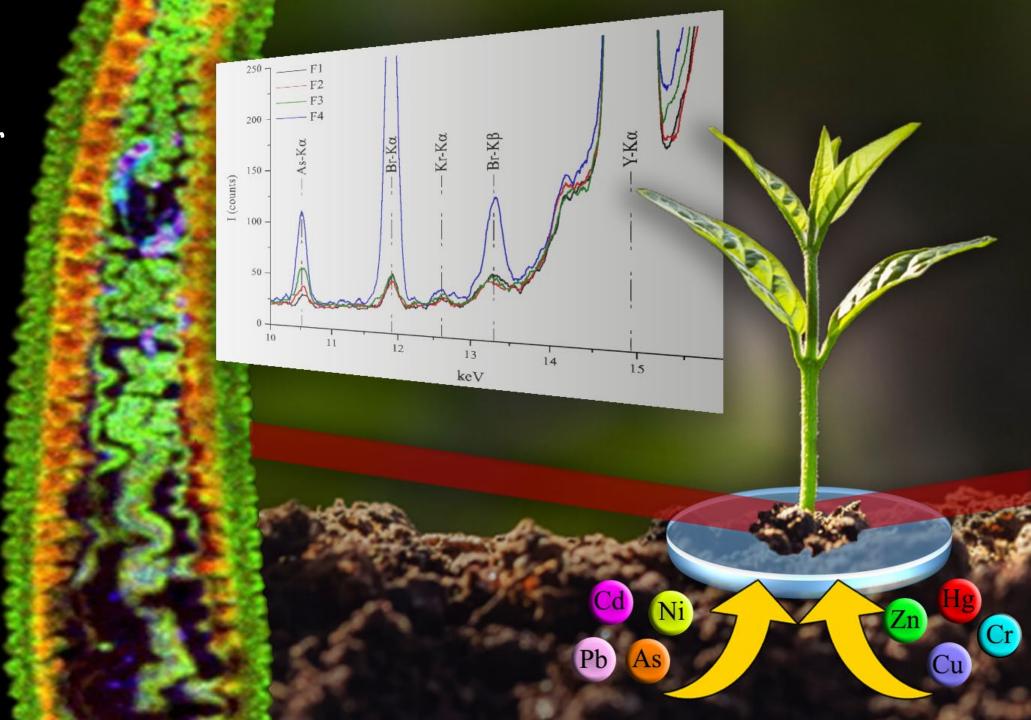
Thank you for your kind attention!!!

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Q & A

Any Questions?

Please type in the questions you may have for our speakers in the **Questions Box** and click Submit









Thank you for your attention!

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