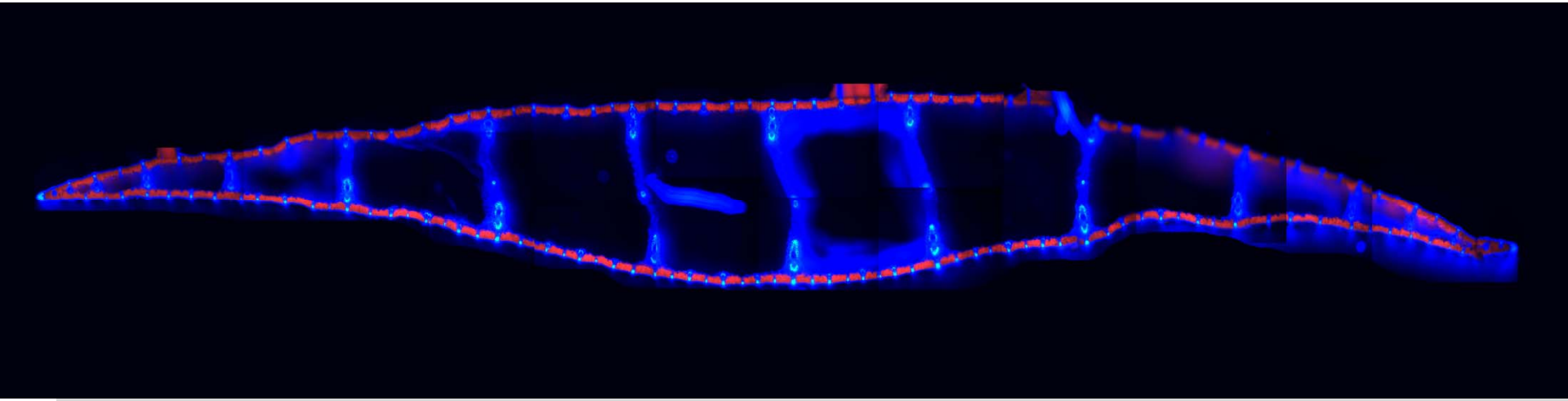
HelmholtzZentrum münchen
German Research Center for Environmental Health

Localization and quantification of heavy metals and nutrients in cattail (*Typha latifolia)*



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Department Microbe Plant Interactions

Bordeaux, 05/03/10

HELMHOLTZ ASSOCIATION

Project details

SPIRIT TNA - Proposal 008:

Localization and quantification of cadmium, lead and arsenic in *Typha latifolia* plants. Effects of individual and mixed pollution

Location: Jožef Stefan Institute, Microanalytical center, Ljubljana, Slovenia

Sample preparation: 10-14.08.2009

Sample measurements: 10-11.2009

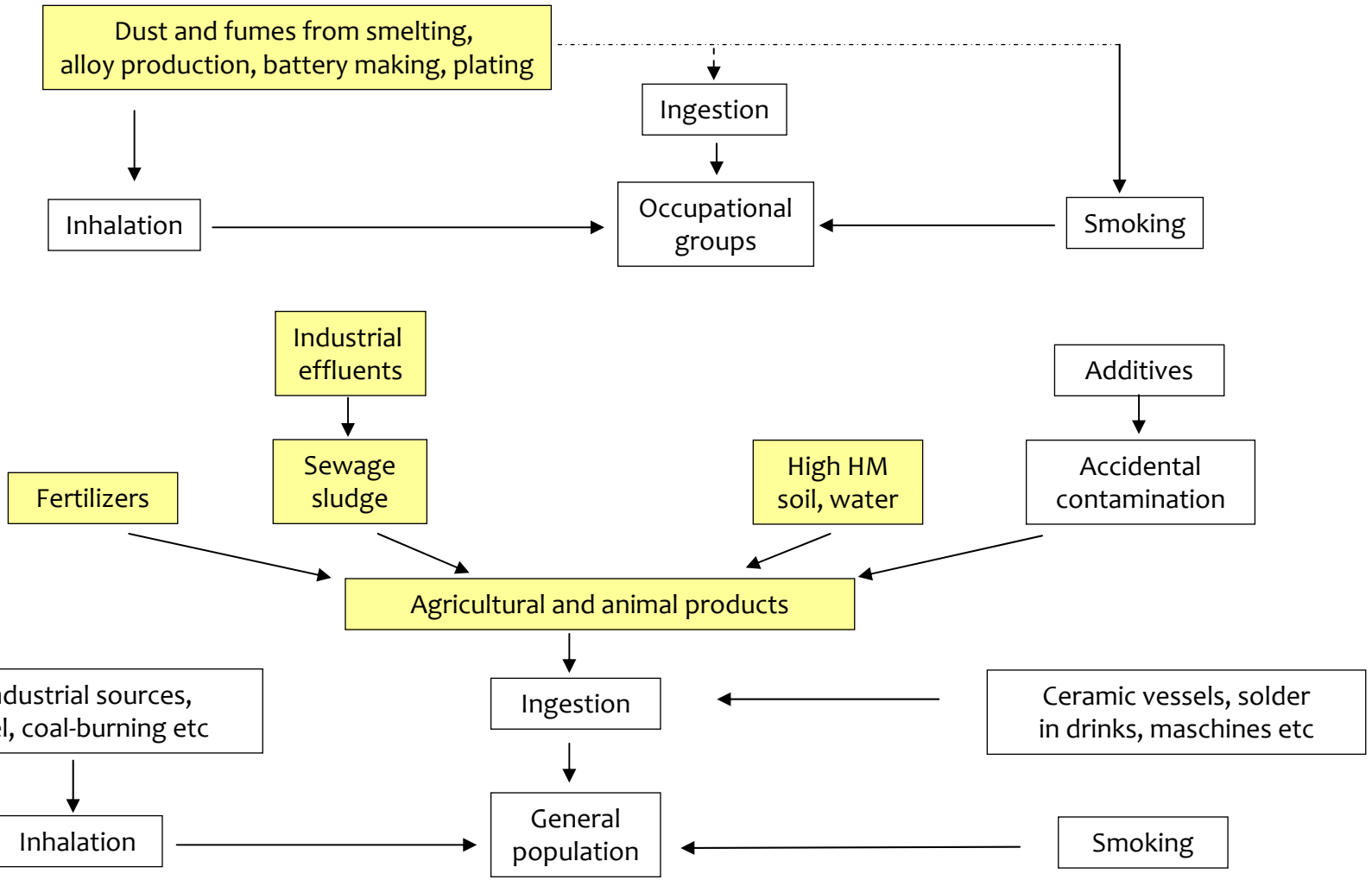
Quantification of data: 01-02.2010

Why cattail?

- the most common european aquatic plant
- relatively high biomass
- its co-cultivation is encouraged to maintain species diversity
- good for wetland ecology
- phytoremediates agricultural pollutants (nitrogen fertiliser and pesticides)
- usefull in wastewater cleaning by rhizofiltration (method of the phytoremediation)

Field of study: Can cattail cope with heavy metals?

Heavy metals: sources and pathways to the environment and to humans



Symptomatology

I) Visible symptoms of metal toxicity in plants are an expression of preceding metal-induced alterations.

II) The vizualisation of the heavy metal effects at the structural and ultrastructural level contributes to identification of the sites of the primary metal toxicity effects, the tolerance mechanisms in plants and their consequences for plants.

Previous experiments in cattail

I) uptake and accumulation mechanisms of different heavy metals such as cadmium, lead and the metaloid arsenic

II) reactions of antioxidative enzymes after heavy metal stress

By now we know details about the detoxification mechanisms of heavy metals in cattail but detailed information about the spatial distribution of Cd, Pb and As in the plants is missing.

New aspects

Information about the translocation and localization of heavy metals in cattail gives

I) the opportunity for better understanding the detoxification

II) idea about the plant adaptations to heavy metals in contaminated sites

The method of **micro-PIXE**

I) gives the opportunity to localize and indicate the heavy metal accumulation by providing important information that can not be obtained using other analytical procedures

II) permits quantitative studies of element distribution, with lateral resolution of the order of 1µm for elements from **Na** to **U**

Plant material and treatments

- 30 days old plants
- plants were grown on perlite
- metals were add as: $PbCl_2$, $CdSO_4$ and $NaAsO_2$

Green house treatments during one week

I) 50µM, 100µM and 250µM $PbCl_2$

II) 100µM and 250µM As+Cd+Pb

III) control

Sample preparation

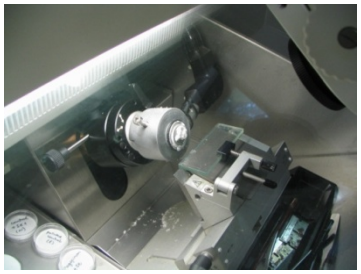
Preparation of thin samples for micro-PIXE consists of rapid freezing,

Cryo-sectioning and freeze-drying

embedding the tissue in a drop of bidistilled water

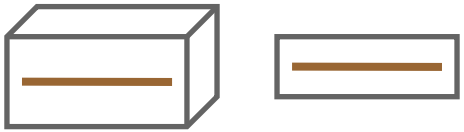
sample freezing

cryo-sectioning



freeze-drying

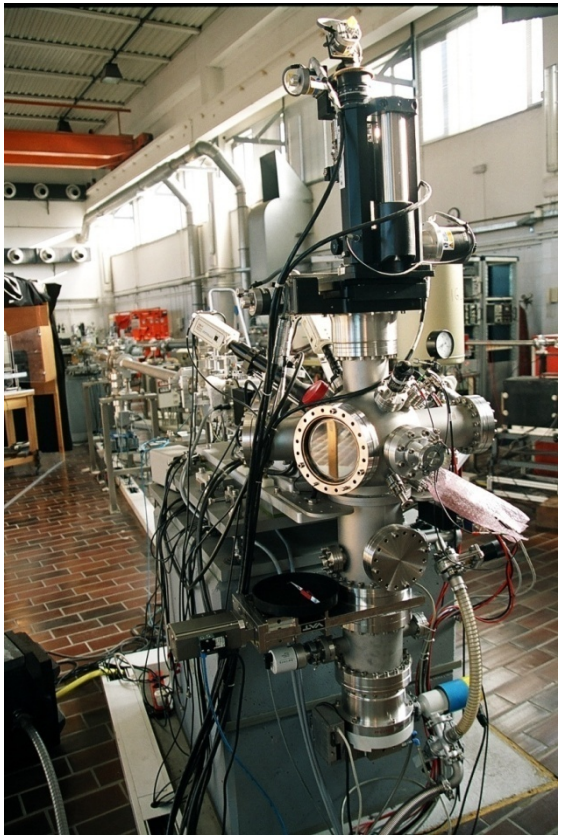
embedding the tissue in tissue freezing medium



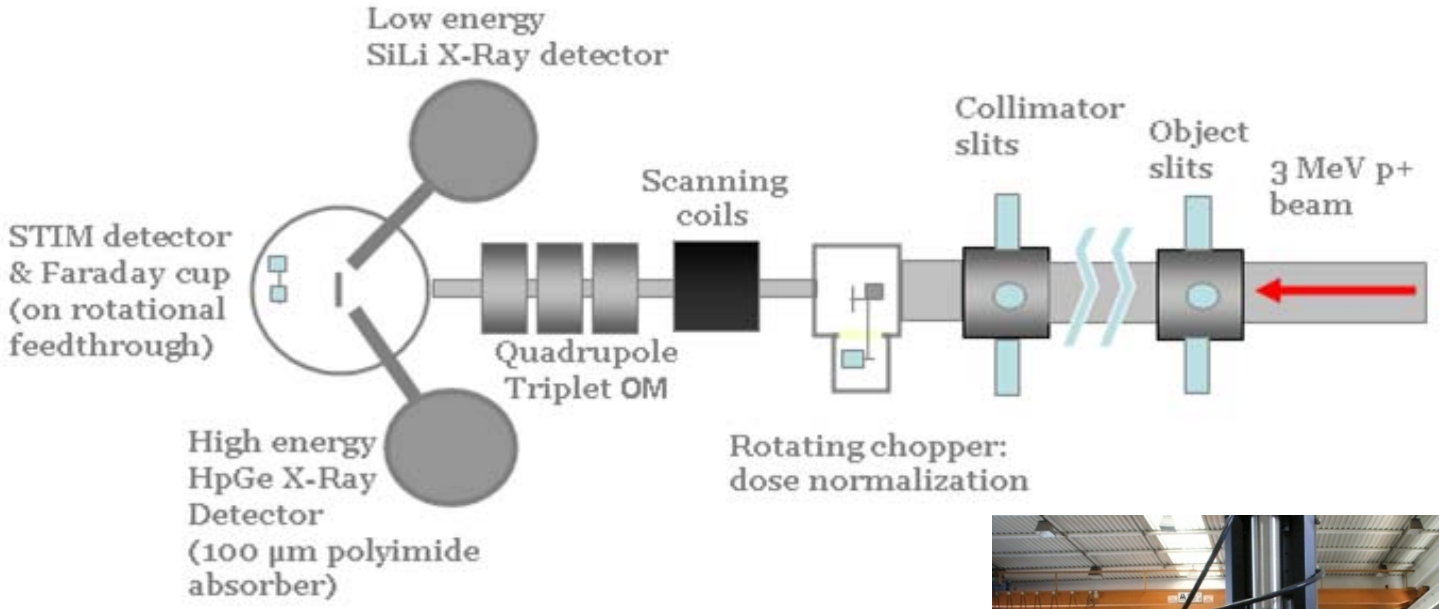
mounting the samples on Pioloform foil on Al holders

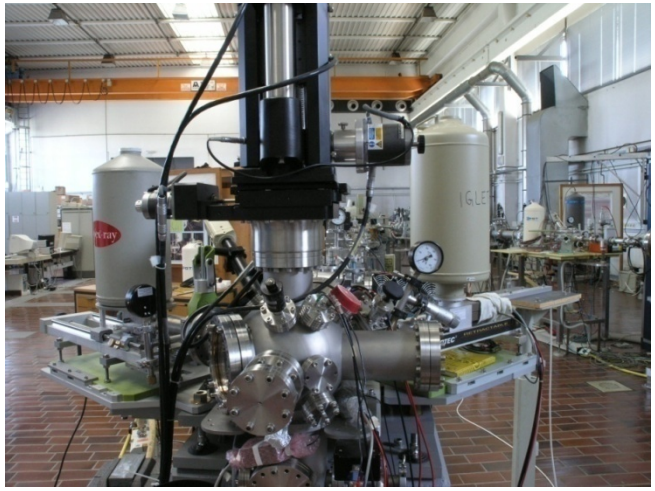
Vogel-Mikuš et al. 2009, A. Varma and A.C. Kharkwal (eds.), Symbiotic Fungi, Soil Biology 18, Symbiotic Fungi - Principles and Practice, pp: 227-242

Instruments and settings at Microanalytical center at Jožef Stefan Institute



Simplified description of the nuclear microprobe at Jožef Stefan Institute





STIM - scanning transmission ion microscopy

Faraday cup - collector for electrons in a vacuum; gives the direct relation between the measured current and number of ions.

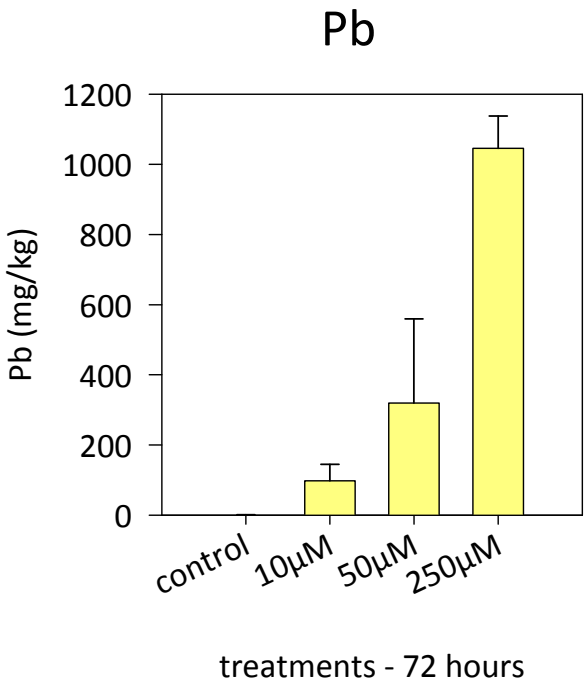
Some impacts of elements in plants

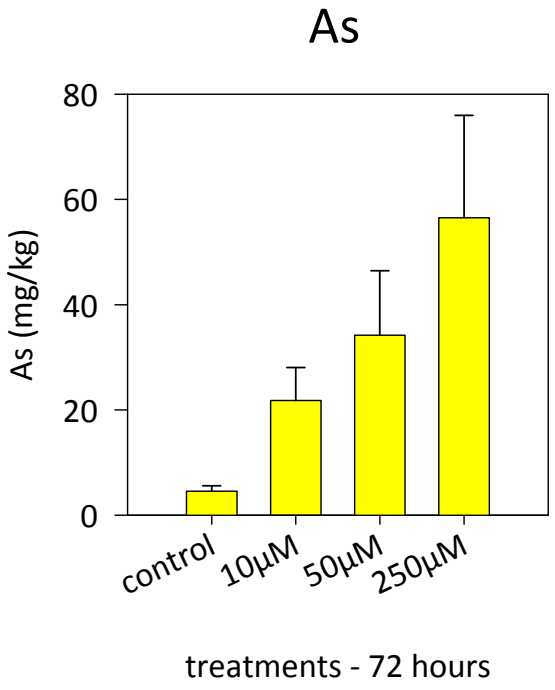
Ca	enzyme activation
K	maintenance of the membran potencial
Na + Cl	uptake and transport of nutrients, regulation of the water household and the balance between acids and bases
Mg	component of enzymes and phosphate energy sources
P	ATP, phospholipids
S	component of amino acids and vitamines

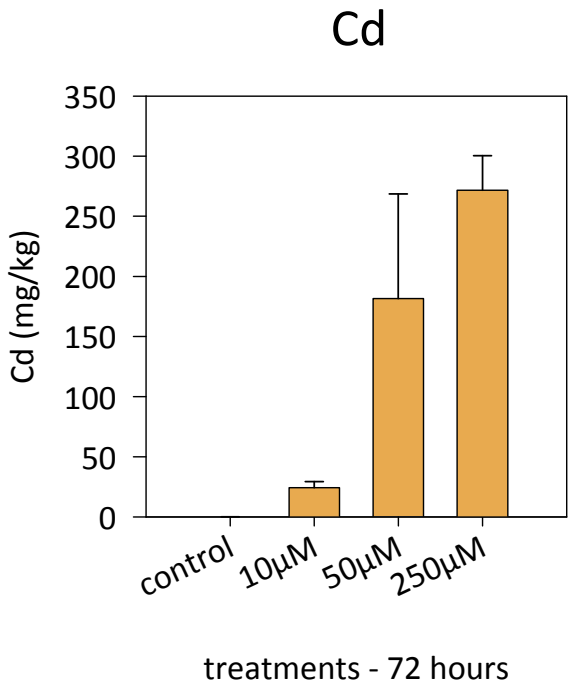
4 organic bacis elements | nutrients | trace elements | probably essential trace elements

H																		He
Li	Be												B	C	N	O	F	Ne
Na	Mg												Al	Si	P	S	Cl	Ar
K	Ca	Sc		Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y		Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	**	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg							
			*	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	
			**	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	

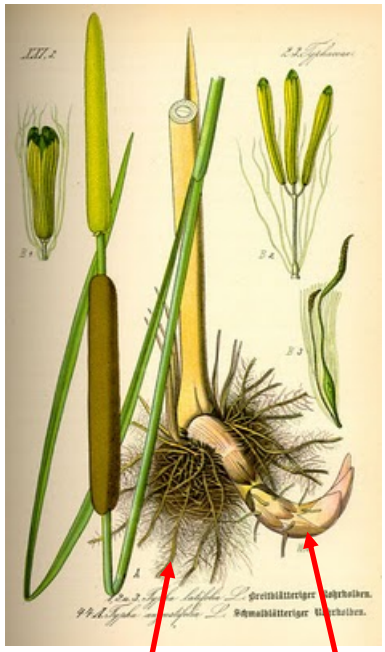
Pb, As and Cd concentrations in cattal - rhizomes measured via Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)







Morphological structures of cattail roots and rhizomes
Optical microscopy



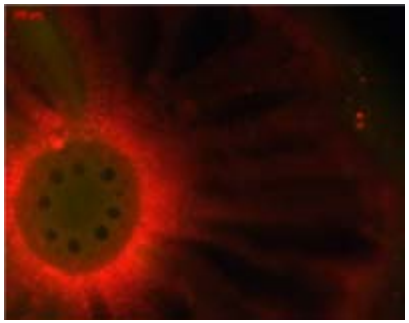
root

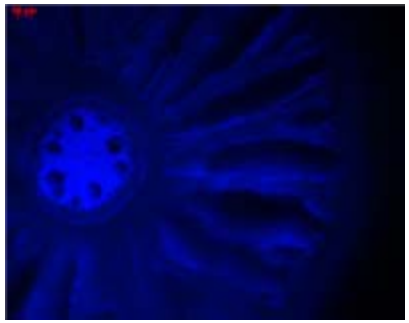
rhizome

visible

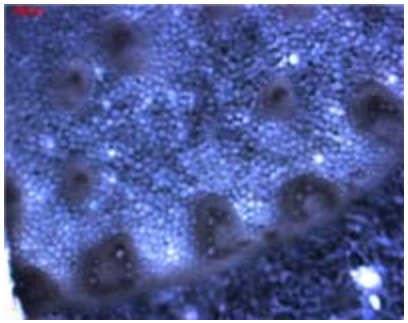
blue

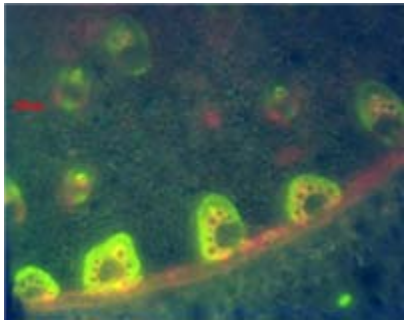
UV

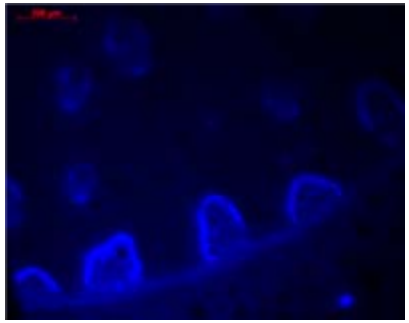




ROOTS



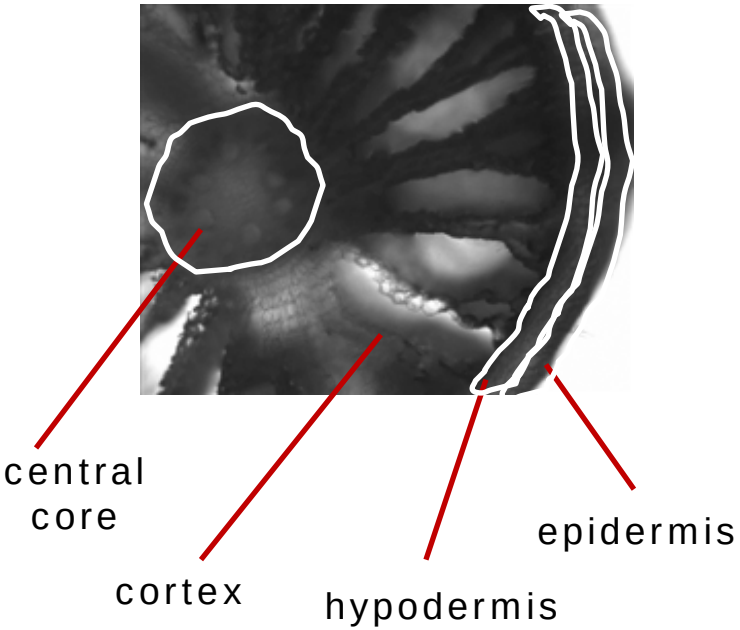


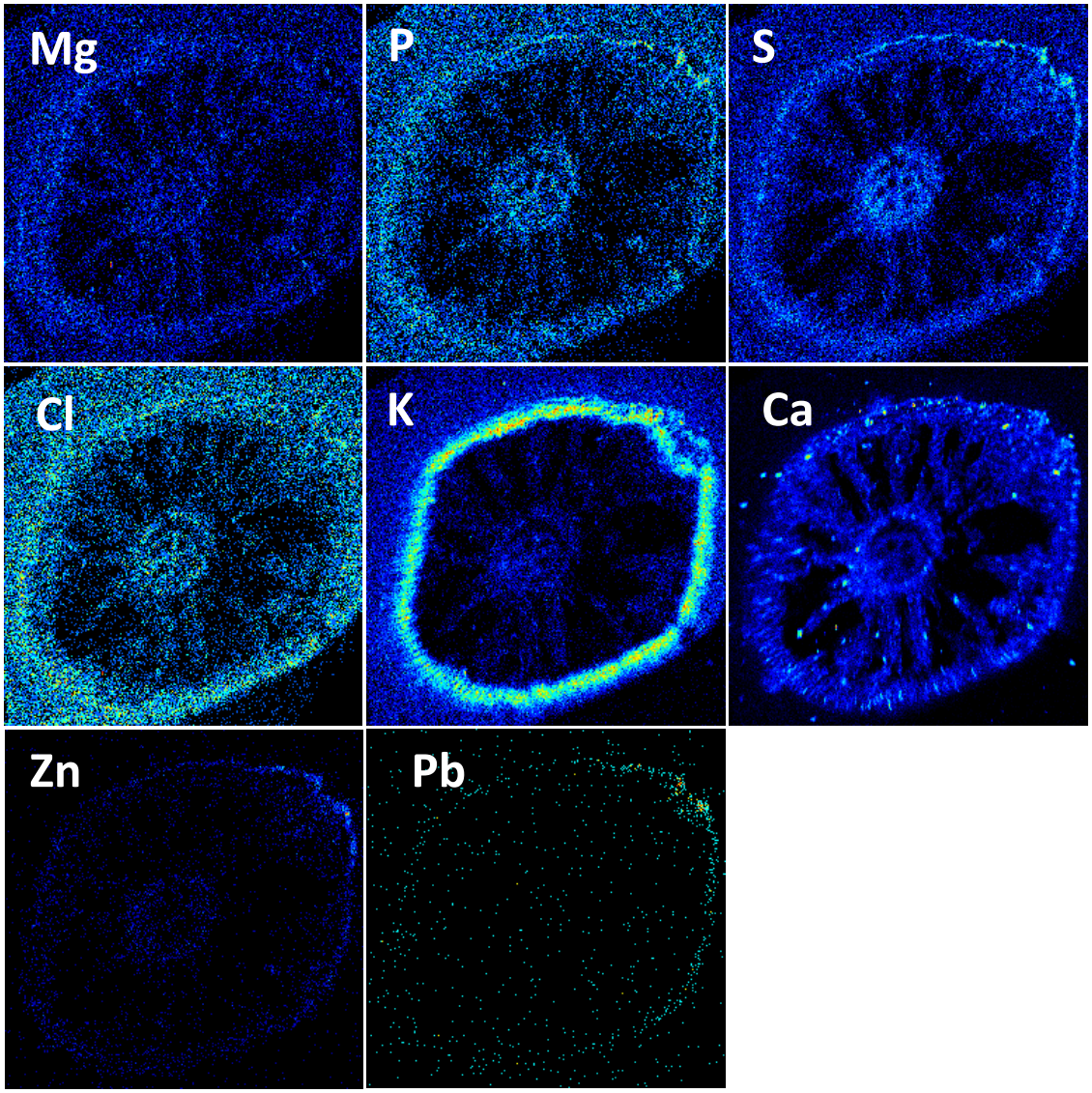


RHIZOMES

Obtained results with micro-PIXE

Roots treated with 100µM Cd+Pb+As





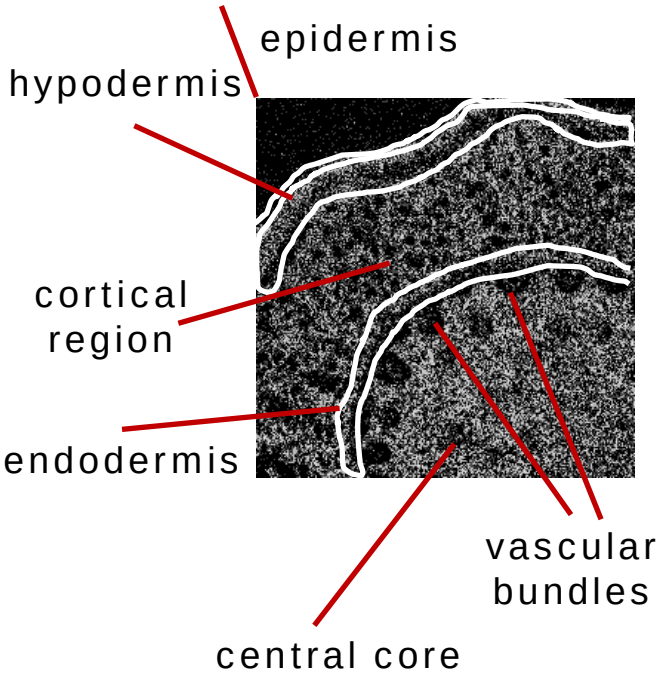
scan size : 1200×1200 µm²

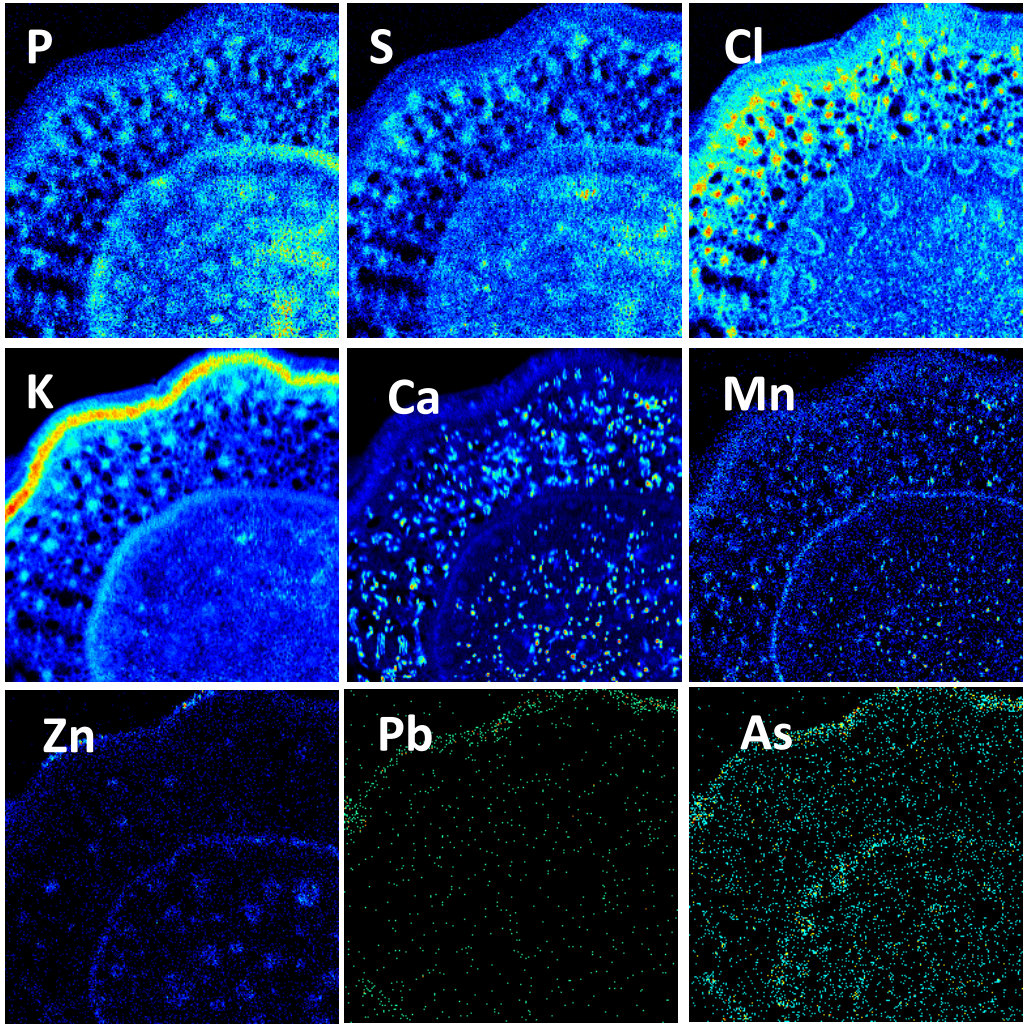
Roots treated with 100µM Cd+Pb+As: concentrations (ppm)

	Epi-dermis	Stat error %	LOD	Hypo-dermis	Stat error %	LOD	Cortex	Stat error %	LOD	Central core	Stat error %	LOD
As	253.7	19.24	131.4	<LOD	-	52.9	<LOD	-	39.3	32	34.07	20.1
Cd	n.d.	-	1258	1962.3	32.33	792.2	n.d.	-	-	2686.9	23.15	878.5
Pb	2764.2	5.07	231.3	162.4	23.79	53.3	n.d.	-	-	<LOD	-	86.2

LOD – level of detection (ppm)
n.d. – not detected

Rhizomes treated with 100µM Cd+Pb+As





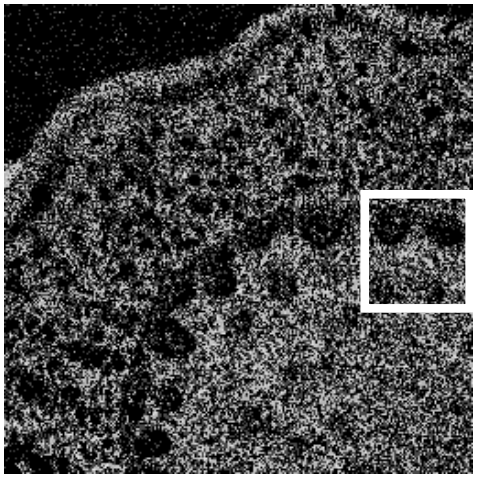
scan size : 2000×2000 µm²

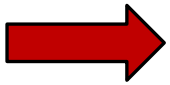
Rhizomes treated with 100µM Cd+Pb+As concentrations (ppm)

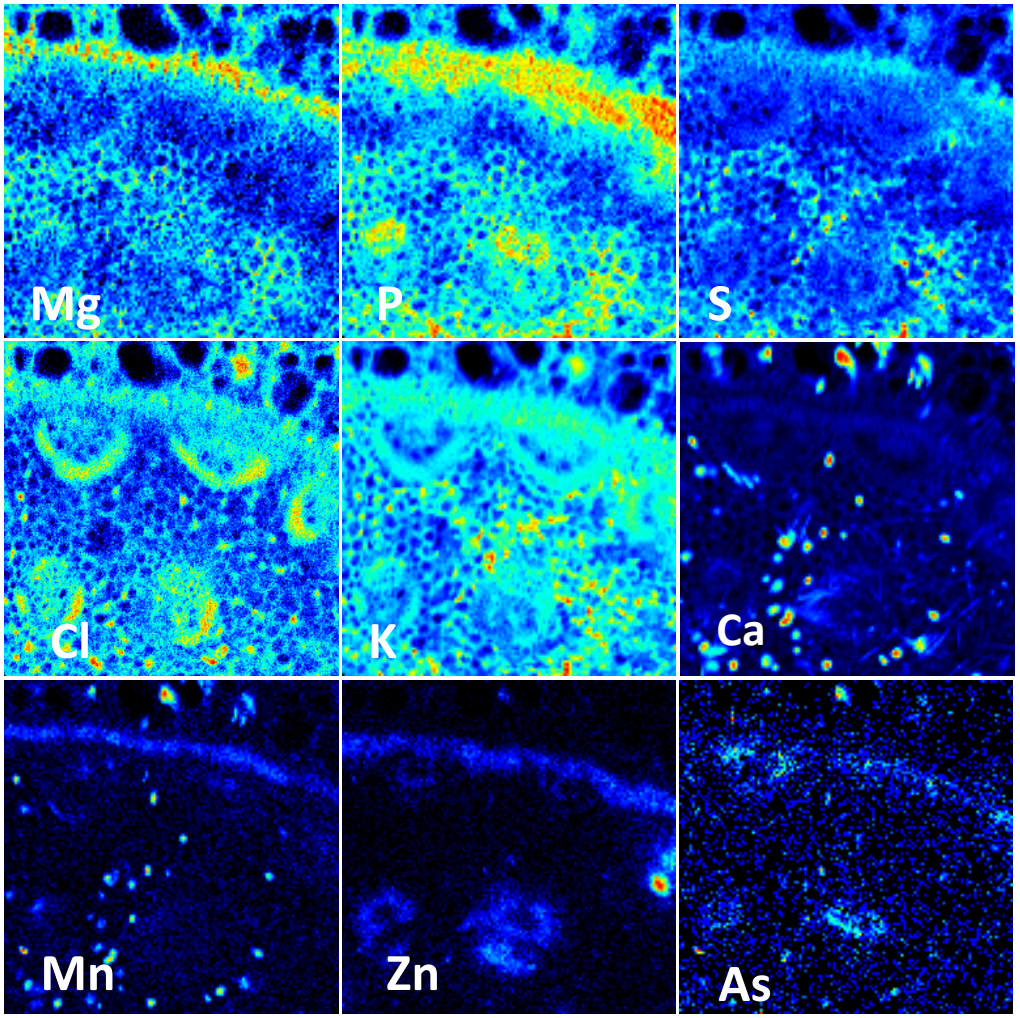
	Epi-dermis	Stat error %	LOD	Hypo-dermis	Stat error %	LOD	Endo-dermis	Stat error %	LOD	Central core	Stat error %	LOD
As	<LOD	-	87.4	29.3	13.9	7.1	72.7	9.11	6.2	63.3	3.33	2.2
Cd	1912.9	16.29	284.8	<LOD	-	210.4	<LOD	-	179.2	<LOD	-	91.7
Pb	1154.4	4.34	21.4	<LOD	-	38.1	<LOD	-	66	<LOD	-	24.9

LOD – level of detection (ppm)

Rhizomes treated with 100µM Cd+Pb+As zoomed maps







scan size : 550×550 µm²

Conclusions

according to the method

1) highly sophisticated method suitable for the spatial analysis of elemental distribution in plants
2) the sample preparation is a critical point

   - the distribution of the elements has to be preserved

   - the sample sections have to be flat

according to the physiology of the investigated samples

1) detection and localization of macronutrients in the epidermis of roots and rhizomes
2) identification of specific tissues that attract or inhibit element accumulation
3) verification of the hypothesis that heavy metals distribute proportional from roots to leaves
4) clarification of the antioxidative defence in cattail after heavy metal stress

General conclusion and outlook

1. The micro-PIXE analysis paints a complex picture of the heavy metal accumulation, transport and distribution, which helps us to understand the processes of metal recycling and export in cattail.

2. The obtained results give information about the homeostasis of heavy metals and micro and macro nutrients in cattail.

3. The studies of metal homeostasis in plants used for phytoremediation will lead to better application of the knowledge about the transport mechanisms and distribution of nutrients for the objects of these green technology.

4. This type of investigations should benefit further evaluation of hyperaccumulators used as green filters of numerous contamination types.

Outlook

Analysis of the metallothioneins and the phytochelatins which have the capacity to bind both, physiological and xenobiotic heavy metals and help in their detoxification