Ecology at the interface
Science-based solutions for human well being

Rome, September 21-25, 2015

A NOVEL BIOTECHNOLOGICAL TOOL TO MONITOR TRACE ELEMENTS AND PAHs BASED ON A DEVITALIZED MOSS CLONE: THE clone MOSS OUTPUT

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The use of ***transplants*** of mosses and lichens is relatively common in urban and industrial environments due to the absence or scarcity of autochthonous biomonitors.

This approach, also called ***active biomonitoring***, offers several advantages because:

i) the best exposure schemes can be adopted;

ii) true enrichment rates can be calculated;

iii) the monitoring can be repeated over time.

The use of transplants often needs the adoption of the so-called '**bags**', small envelops made in a number of forms and materials containing the biological sample.

Previous results…

1) biological matrices are more efficient than various synthetic materials

2) dead biological samples accumulate as much as alive samples

3) mosses are more efficient than lichens for their greater exposed surfaces

4) the particulate trapped by the material exposed in green areas is essentially PM_{10}

5) the careful water-washing of moss and lichen materials selected for bag preparation reduces the variability of the results

…problems related to moss-bags preparation:

i) the sampling of native mosses is problematic for untrained people;

ii) the bag preparation is often a home-made affair;

iii) among materials of different origin there is an intrinsic variability in elemental composition;

iv) standardized protocols for bag preparation and use are still missing.

http://www.mossclone.eu/

Monitoring Air Quality using Moss

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COOPERATION

Welcome to the MOSSclone Research Consortium

The consortium aims to invent and test a novel, precise and inexpensive method to monitor air contamination, especially by heavy metals.

The MOSSclone partners from universities as well as small and medium enterprises situated across Europe collaborate to create this new biotechnological tool which will function as a passive contaminant sensor consisting of a devitalized moss clone.

The MOSSclone project is funded by the European Union in the Seventh Framework Programme (FP7) for Research and Technological Development.

more: About

News

23rd September 2013 Category: Press Release

News & Events

all news

Mid Term Meeting: Freiburg, Germany, September 26th-28th 2013

Social Media

Facebook Group MOSSclone

MOSSclone News on Twitter

The main MOSSCLONE objectives were:

1) design and standardize the moss-bags exposure

2) select a highly performant moss on the basis of literature data;

3) establish a clone of this species under highly controlled conditions;

4) characterize the moss clone molecularly and physical-chemically;

5) compare the accumulation performance of the moss clone with traditional materials (e.g. other wild mosses) and physical-chemical techniques (e.g. passive and active samplers).

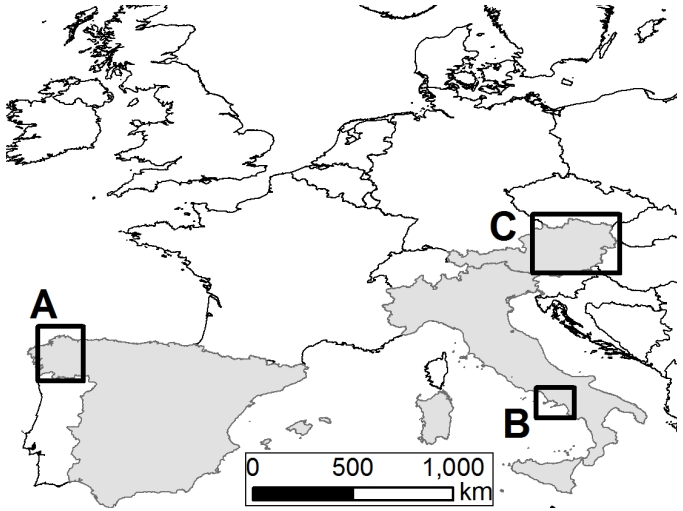
1) Standardization assay

Pseudoscleropodium purum
(Hedw.) M.Fleisch.

Standardization assay Task:

8 elements: Al, Ba, Cr, Cu, Fe, Ni, Sr, Zn

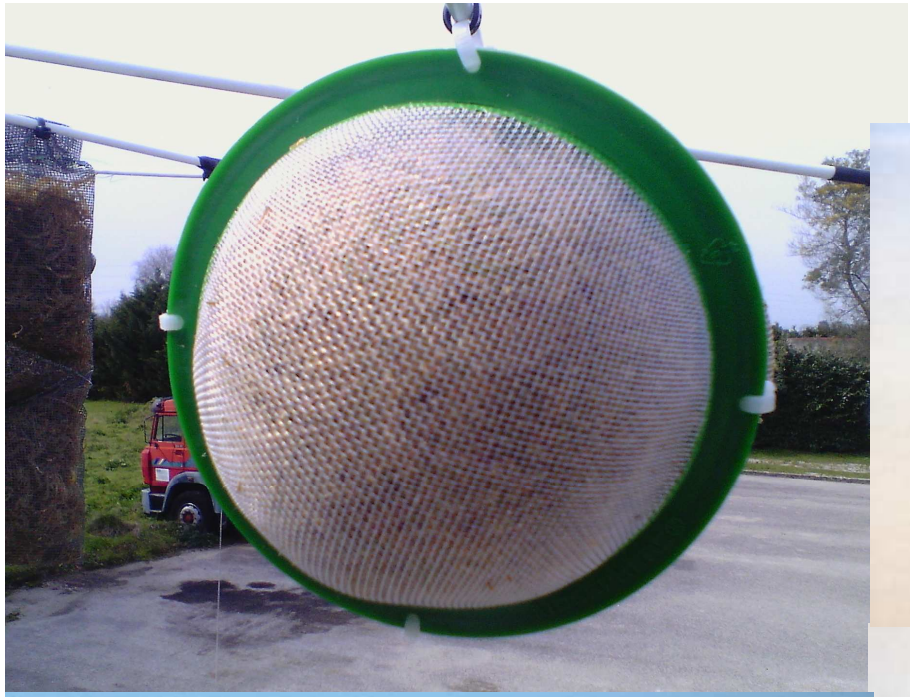
- **5 items**
  - Shape of the bags (3 types)
  - Mesh size (1, 2, 4 mm)
  - Weight (15, 30, 45 mg/cm^2)
  - Height above ground (4, 7, 10 m)
  - Exposure time (3, 6, 12 weeks)
- **3 countries**
  - Spain (A)
  - Italy (B)
  - Austria (C)
- **7 exposure sites x country**
  - 2 urban
  - 2 industrial
  - 2 rural
  - 1 background
- **3 replicates for each item**

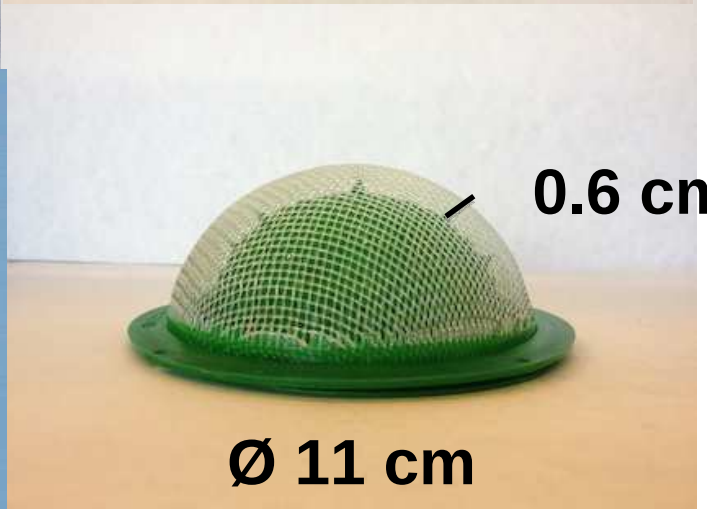


We used c. 1 thousand bags…

…a new device:

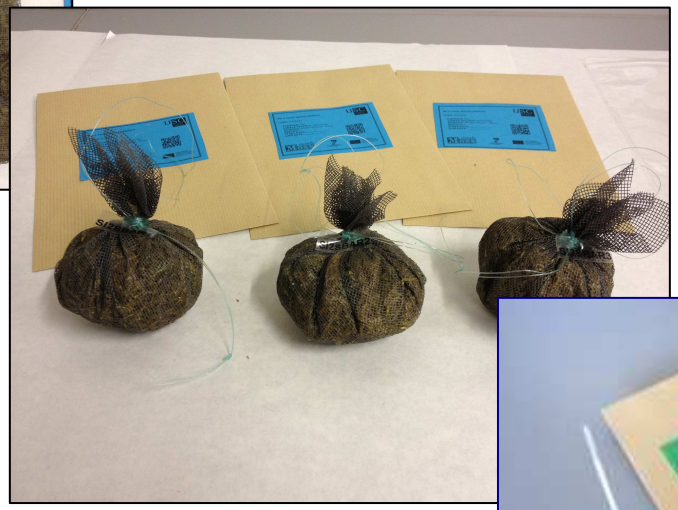
the MOSSPHERE





“Shape Effect”

Envelope



Bag

Mossphere

clone Moss

- **Shape** of the moss-bags not always affects the accumulation performance of airborne elements in a significant way.
The **mossphere** should be preferred because it secures an improved standardization of bag preparation.

- **Mesh size**: the mesh size does not affect the accumulation of elements.

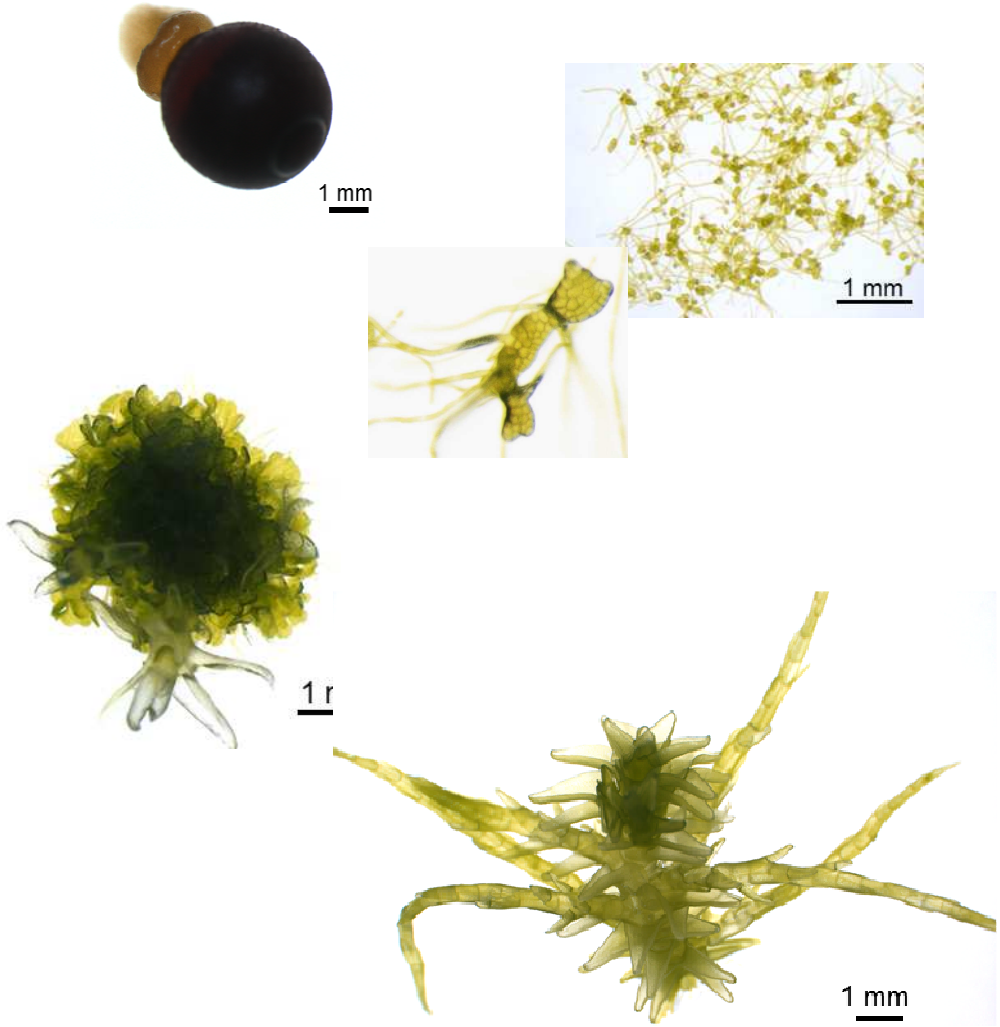
- **Weight effect**: a small amount of moss seems to ensure an improved accumulation performance, with a slight increase in data spread.

- **Height effect**: the selection of the exposure height may depend on practical questions (e.g. to avoid vandalism) or specific aims of the study.

- **Time effect**: a 3-week exposure period is not adequate. As the differences between 6- and 12-week exposures were scarce and taking into account operational criteria (i.e. increased temporal resolution, seasonality), it is recommended to use a **6-week exposure**.

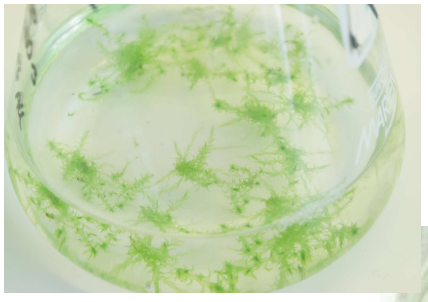
2-3) Establishing mosses in axenic culture

- sterilization from spores in capsules
- germinating spores within a few days
- isolation of one single protonema
- cultivation of independent clones



Mosses in axenic *in vitro* culture

- *Sphagnum palustre* (more than 20 clones)
  collected by Schulze/Krebs/Joosten (University of Greifswald)
- *Rhynchostegium murale*
  collected by Frahm (University of Bonn)
- *Brachythecium rutabulum*
  collected by Frahm (University of Bonn)
- *Hylocomium splendens*
  collected by Ares-Pita, Zechmeister (UDC, University of Vienna)
- *Pseudoscleropodium purum*
  collected by Ares-Pita (UDC)
- *Hypnum cupressiforme*
  collected by Frahm (University of Bonn)



Sphagnum palustre

Rhynchostegium murale

Brachythecium rutabulum

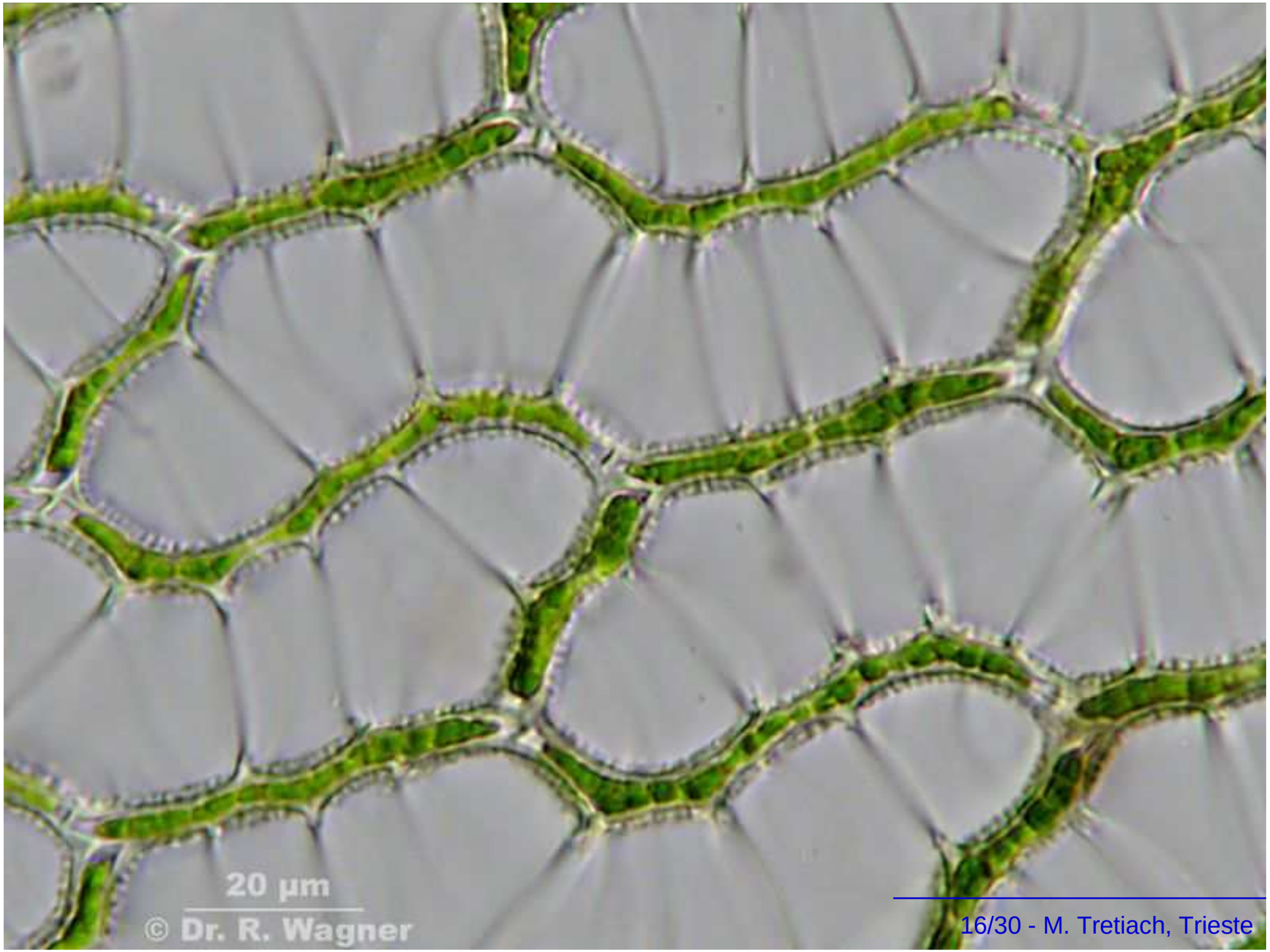
Pseudoscleropodium purum

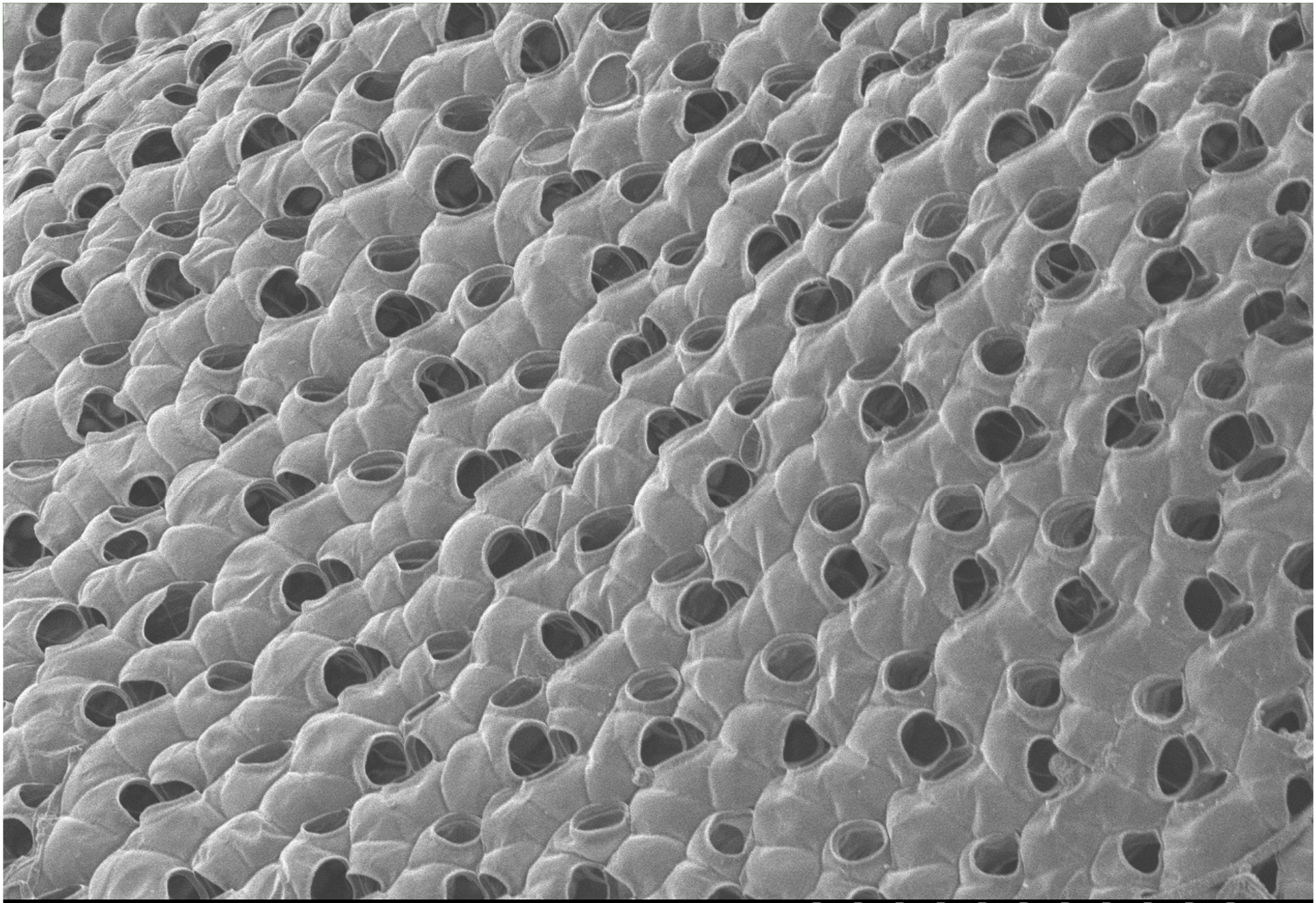
Hypnum cupressiforme

plant-biotech.net

The WINNER is…..

Sphagnum palustre L.


20 µm
© Dr. R. Wagner


SE 26-Mar-13 007227 WD22.9mm 5.00kV x400 100um

Cultivation in the bioreactor (pH not adjusted)

initial density (pH 4)

1 week (pH 3)

2 weeks (pH 3)

4 weeks (pH 4)

- volumes of 5 L
- pH adjustable
- temperature: 25 ° C
- 120 µmol photons m^{-2} s^{-1}

Scaling up to large-scale clone production

Net biomass production

Optimize

Initial inoculation
Moss cut before inoculation
Moss cut inside the bioreactor
Sucrose control

4 bioreactors, 10 L each

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4) Molecular characterization of the mossclone

•ISSR amplification

•RAPD amplification

→ Sequencing (anonymous regions) → SNPs, CAPs

→ Banding profile by capillary electrophoresis

SSR → Analysis of polymorphisms in DNA regions containing microsatellite motives

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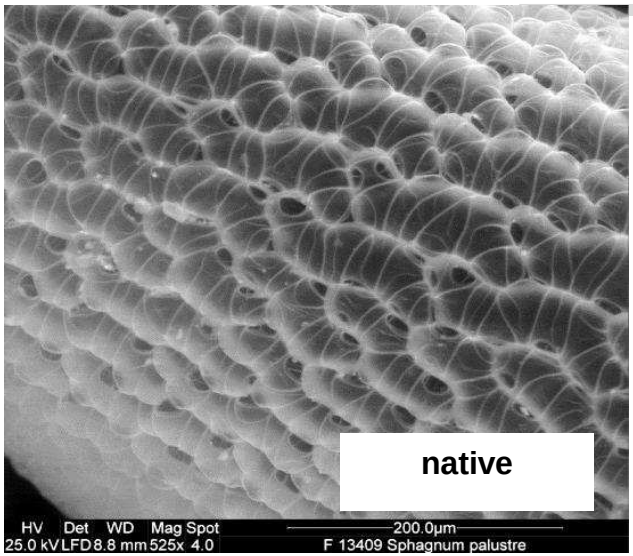
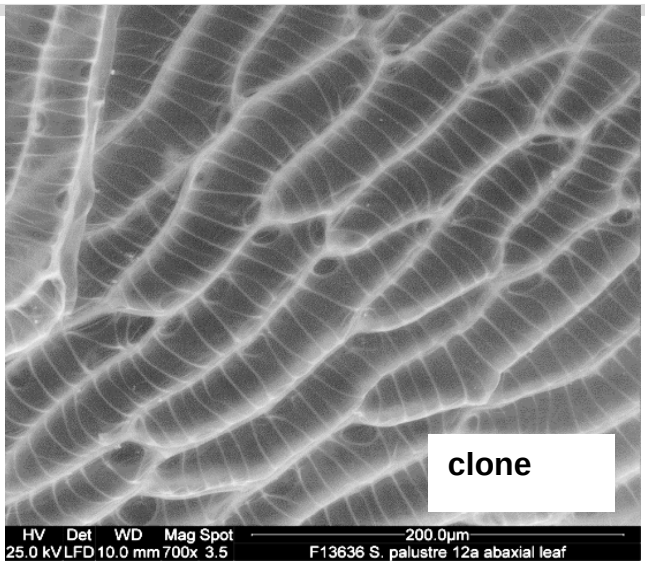
4) Morphological and ultrastructural characterization of the moss clone





Spanish Native

Clone-12A

10.8 ± 0.3 m^2/g

B.E.T. Specific Surface Area

28 ± 1 m^2/g

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Danish peat (13.3 m^2 g^{-1}) and Heilongjiang peat (9.67 m^2 g^{-1}) *Qin et al., 2006*

Physical-chemical characterization of our *Sphagnum palustre* clones

Materials

ALU-FR *S. palustre* clones (2a and 12a)

Biovia *S. palustre* clones (12a)

Methods

EDTA-washing and devitalization at 100 ° C for 8 h

Microwave HNO_3 digestion

ICP-MS

- ✓ Element composition
- ✓ abundance of proton-binding sites at the cell surfaces
- ✓ metal adsorption capacity

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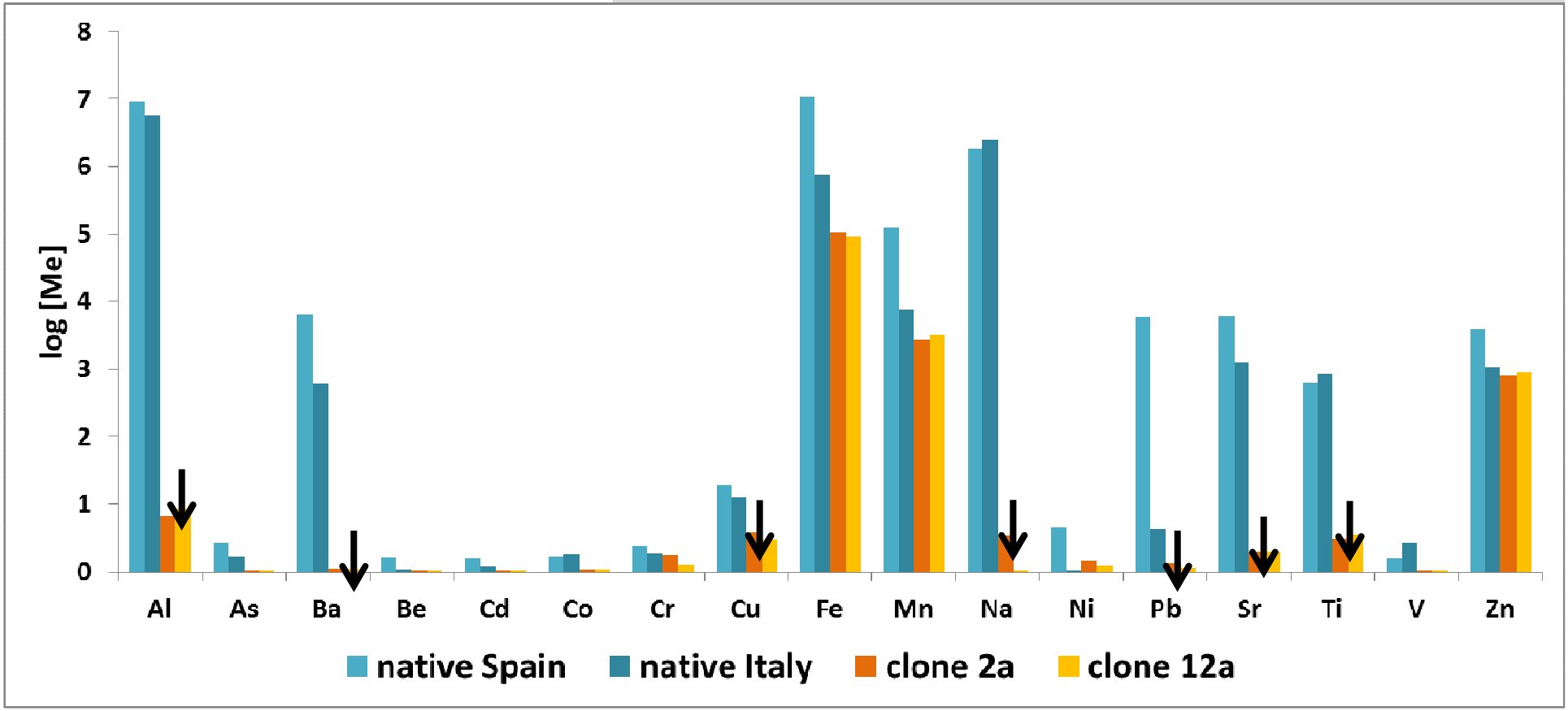
Chemical Composition of our clones *vs* native *Sphagnum palustre*



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Comparison our clone with native *Sphagnum spp.*

Sphagnum species	Geographical area	Metal concentrations – mean values (mg/kg)																				*min 1*	*min 2*
		Al	As	B	Ba	Be	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Pd	Pt	Rh	Sn	Sr	V	Zn
BIOVIA *clone 12a*		23	<0.1	6.7	1.1	<0.2	<0.05	1.1	0.8	6.1	461		218.7	11.4	2.9	<0.2	<0.3	<0.2	<0.2	<0.5	3.2	<0.2	99
BIOVIA *clone (EDTA)*		20	<0.1	3.7	1.5	<0.2	<0.05	<0.1	0.7	5.4	266		14.7	9.4	2.7	<0.2	<0.3	<0.2	<0.2	<0.5	3,0	<0.2	20.5
BIOVIA *clone (EDTA-oven)*		14	<0.1	4.0	1.2	<0.2	<0.05	<0.1	0.5	5.1	243		15.3	9.6	2	<0.2	<0.3	<0.2	<0.2	<0.5	2.8	<0.2	19
ALU-FR *clone 2a*		21	<0.1	5.5	1.3	<0.2	<0.05	0.18	0.8	2.6	658	<0.5	138.5	11.9	0.6	<0.2	<0.3	<0.2	<0.2	<0.5	1.4	<0.2	78
ALU-FR *clone 12a*		17	<0.1	7	1.0	<0.2	<0.05	0.17	0.6	2.5	674	<0.5	133.5	13.6	0.7	<0.2	<0.3	<0.2	<0.2	<0.5	1.3	<0.2	71
S. palustre [2]	Poland		0.19		6.4		0.09				150			0.16									28
S. olafii [3*]	Greenland	322	0.11				0.04		0.71	1.0				0.04		0.16		0.0001		0.01			5,0
S. cristatum [4] *(a.w.)*	New Zeland						0.001			0.004	2		0.003			0.1							0.02
S. girgensohnii [5]	Russia	254	0.11		17.0			0.37	0.26		297		114		2.5						7.6	0.5	21
S. girgensohnii [6]	Russia	167	0.1		19.5	0.01	0.18	0.17	0.26		150	0.03	341	0.13		1.69					6.9	0.52	23.7
S. girgensohnii [7]	Russia	254	0.11				0.18		0.25	2.1	297		113		2.4	2.2						0.54	20
S. girgensohnii [8]	Finland										132												
S. girgensohnii [9]	Bulgaria	1043	0.67		56		0.3	0.33	1.7	7.6	864		173		1.4						26	2.1	36
S. girgensohnii [10]	Russia	347	0.13					0.28	1.4		219		372		2.5							1.0	27
S. girgensohnii [11]	Bulgaria	1187	0.52					0.3	1.6		665		302		2.1							3.0	60
S. capillifolium [12]	Bulgaria	600					0.54	0.4		6.3	1200		198		2.5	24							35
S. capillifolium [13]	NE Italy	1108	0.11				0.38	0.43	1.6	5.54	675		552	0.41	2.4	18.9						1.6	83
S. capillifolium [14]	NE Italy	1108	0.11				0.38	0.43	1.6	5.54	675		552	0.41	2.4	18.9						1.6	83
S. angustifolium [15]	Canada	537		4.8	233			0.2	1.1	8.2	383		10.5	0.5	0.8	0.1						1.3	46
S. auriculatum [16]	Portugal								0.2	7.5	156		0.22		3.1	30							71
S. teres [17]	Bulgaria	500					0.48	0.2		5.1	600		246		2.4	21							62
S. fuscum [18]	Canada															5.7							20
S. fallax [19]	Poland						0.25									4							27
S. fallax [20]	Poland						0.16									2.5							

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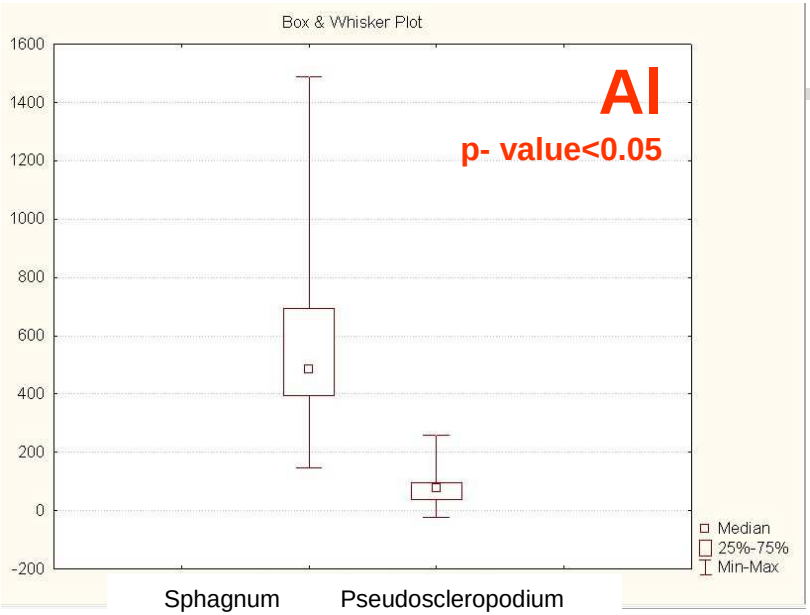
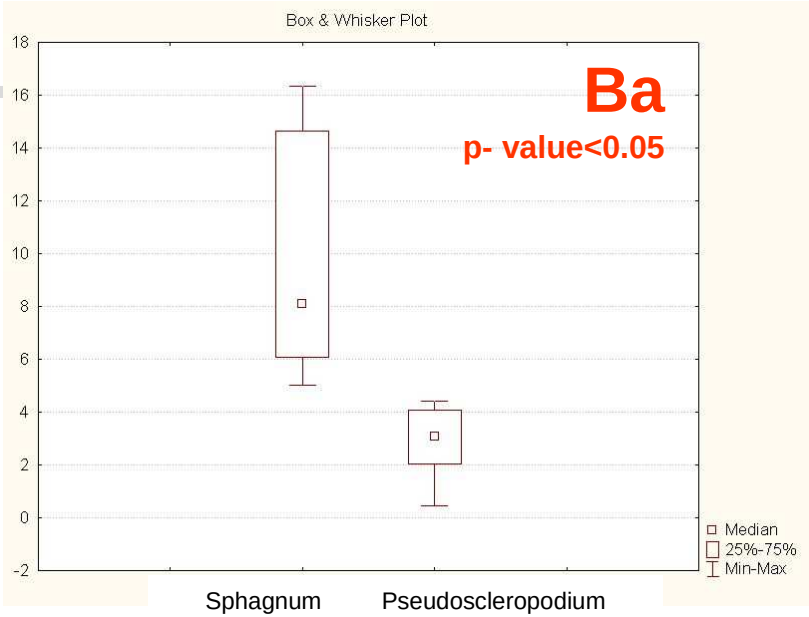
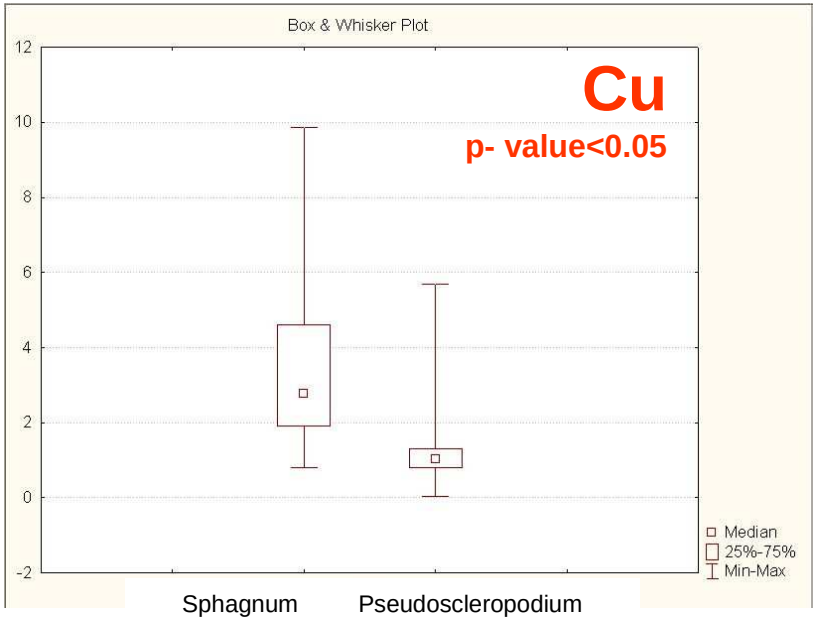
Mosspheres filled with the *Sphagnum palustre* clone vs. mosspheres filled with *Pseudoscleropodium purum*







POST-EXPOSURE CONTENT VALUES
Wilcoxon matched pairs test
n=10

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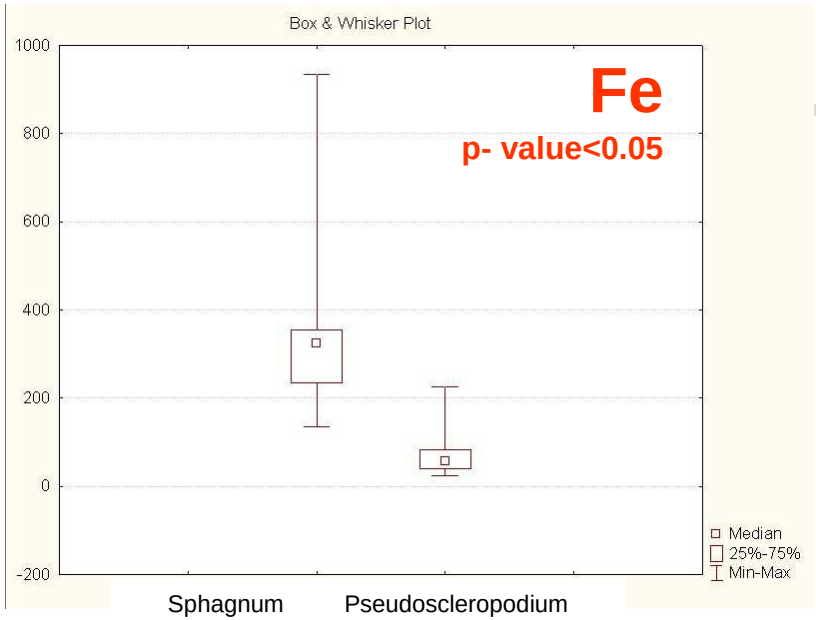

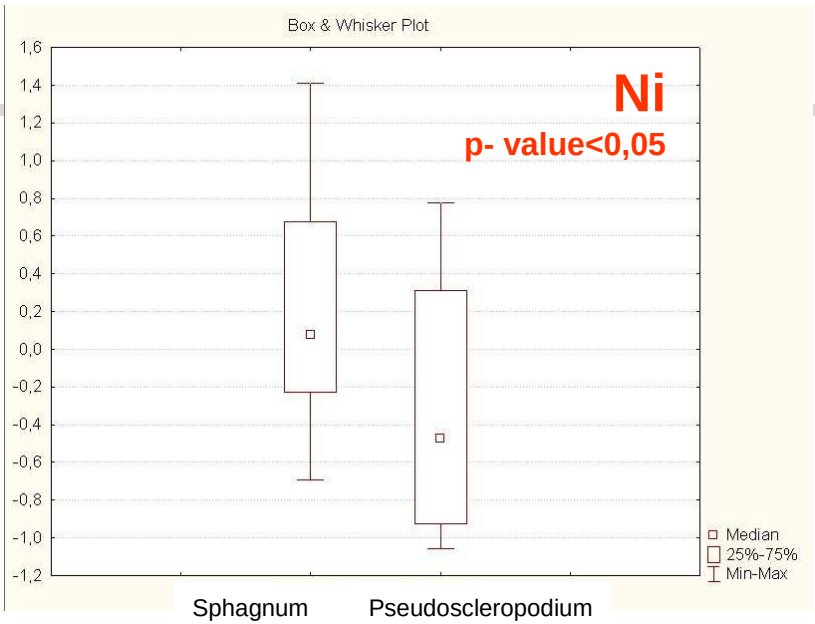

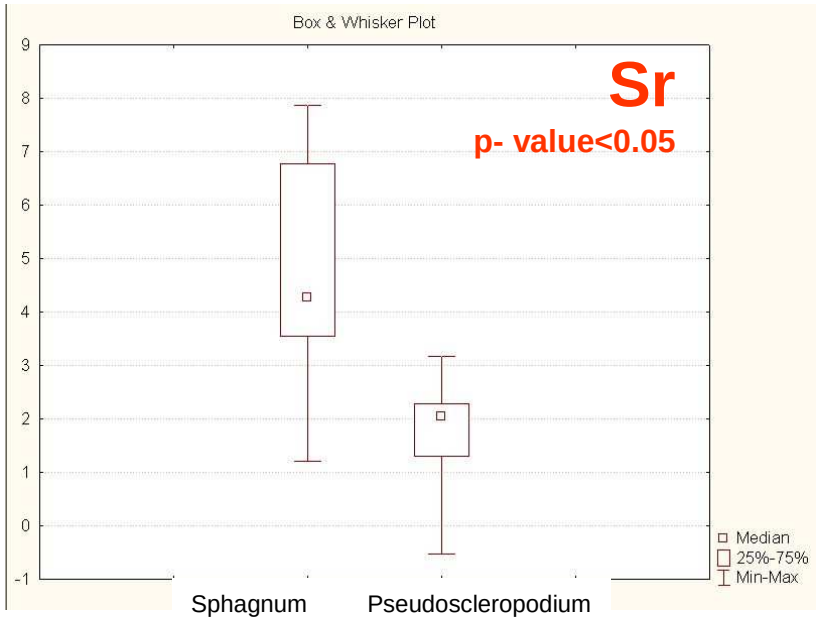

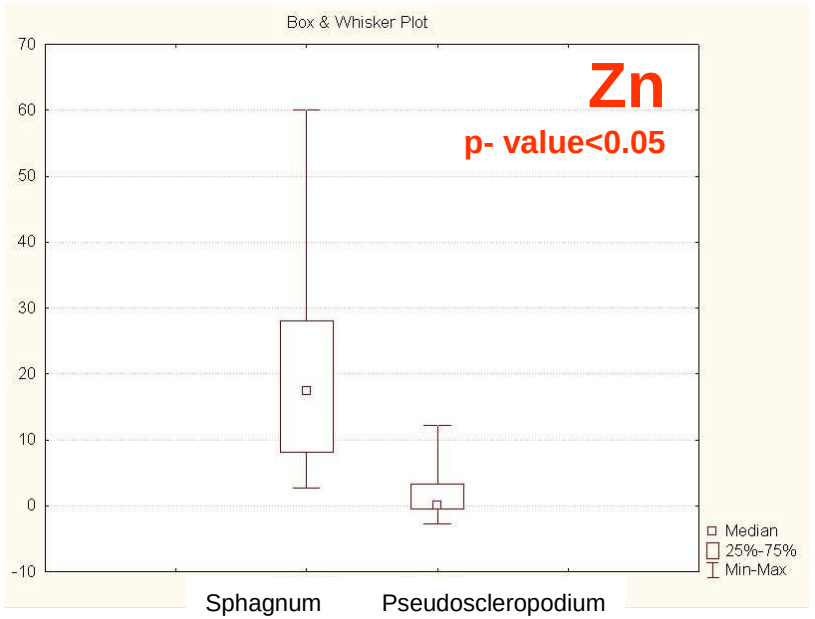

Conclusions (1/2)

The clone provides excellent devitalized material for the production of a highly standardized mossphere:

- ✓ higher SSA
- ✓ comparable metal adsorption capacity
- ✓ smaller element content variability
- ✓ 3 to 100 times lower metal content

Conclusions (2/2)

Mossphere is under patenting, and is already available on the market, at a reasonable price, commercialized by Biovia, the spin-off of Universidade de Santiago de Compostela (Spain).

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…problems related to moss-bags preparation:

i) the sampling of native mosses may have high environmental impact and is problematic for untrained people SOLVED

ii) the bag preparation is often a home-made affair SOLVED

iii) among materials of different origin there is an intrinsic variability in elemental composition SOLVED

iv) standardized protocols for bag preparation and use are still missing SOLVED

Thanks for your attention by

the MOSSclone Research Consortium

…and by all the people who joined us in these years