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

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


Dipartimento di Scienze della Vita, Università degli Studi di Trieste
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:

1. the best exposure schemes can be adopted;
2. true enrichment rates can be calculated;
3. 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.

4/30 - M. Tretiach, Trieste
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 derivatized moss clone.

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

News

23rd September 2013  Category: Press Release
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:

- 5 items
  - Shape of the bags (3 types)
  - Mesh size (1, 2, 4 mm)
  - Weight (15, 30, 45 mg/cm²)
  - 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

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

8/30 - M. Tretiach, Trieste
We used c. 1 thousand bags...
...a new device:
the MOSSPHERE

Ø 11 cm

0.6 cm

spike

10/30 - M. Tretiach, Trieste
Envelopes
Bag
Mossphere

“Shape Effect”
- **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*. 

12/30 - M. Tretiach, Trieste
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)
The WINNER is.....

Sphagnum palustre L.
Cultivation in the bioreactor (pH not adjusted)

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
4) Molecular characterization of the mossclone

- ISSR amplification
- RAPD amplification
- Sequencing (anonymous regions)
- Analysis of polymorphisms in DNA regions containing microsatellite motives
- Banding profile by capillary electrophoresis
- SNPs, CAPs

amra
analysis and monitoring of environmental risk
cloneMoss
4) Morphological and ultrastructural characterization of the moss clone

Danish peat (13.3 m² g⁻¹) and Heilongjiang peat (9.67 m² 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
Chemical Composition of our clones vs native *Sphagnum palustre*
Comparison our clone with native *Sphagnum* spp.

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<th><em>Sphagnum</em> species</th>
<th>Geographical area</th>
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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

- *Al*: p-value < 0.05
- *Ba*: p-value < 0.05
- *Cu*: p-value < 0.05
Mosspheres filled with the *Sphagnum palustre* clone vs. mosspheres filled with *Pseudoscleropodium purum*.

- **Fe**: p-value < 0.05
- **Ni**: p-value < 0.05
- **Sr**: p-value < 0.05
- **Zn**: p-value < 0.05
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
**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).
...problems related to moss-bags preparation:

i) the sampling of native mosses may have high environmental impact and 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

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Thanks for your attention by

the MOSSclone Research Consortium

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