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## Mosses as biomonitors of POPs pollution: spatial trends of PAH concentrations in mosses from France, Switzerland and Spain

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**Centre for  
Ecology & Hydrology**

NATURAL ENVIRONMENT RESEARCH COUNCIL



## **24<sup>th</sup> Task Force Meeting**

**31 January – 2 February, 2011  
Rapperswil-Jona, Switzerland**

*HSR - Hochschule Rapperswil, Oberseestrasse 10.*

# **Programme & Abstracts**



Working Group on Effects  
of the  
Convention on Long-range Transboundary Air Pollution



## Organizers:



**Centre for  
Ecology & Hydrology**

NATURAL ENVIRONMENT RESEARCH COUNCIL

ICP Vegetation Programme Coordination Centre  
Centre for Ecology and Hydrology  
Bangor, UK.

*Harry Harmens  
Gina Mills  
Felicity Hayes*

## Local organizers



Forschungsstelle für Umweltbeobachtung (FUB) –  
Research Group for Environmental Monitoring

*Lotti Thöni*

***With financial support of:***

***Swiss Federal Office for the Environment (FOEN)***

***Community of Rapperswil-Jona***



# Programme

## Monday 31<sup>st</sup> January, 2011

- 18:30**      **Registration (throughout evening) in Circus Museum in the harbour of Rapperswil** (*first floor of building of Tourist Information, Fischmarktplatz 1*).
- 19:00**      **Welcome reception: Circus Museum**
- ca. 20:00      Welcome address: *Rahel Würmli*, town councillor Rapperswil-Jona.

## Tuesday 1<sup>st</sup> February, 2011

- 08:30**      **Registration for late arrivals**
- Session 1: 9:00 – 10:30 Plenary session      Chair: Beat Achermann**
- 09:00      Welcome address:  
- *Hermann Mettler*, Head Master of HSR.  
- *Beat Achermann*, Swiss Federal Office for the Environment FOEN – Recent developments under the Convention.
- 09:20      *Harry Harmens* et al. – Overview of the achievements of the ICP Vegetation in 2010.
- 09:50      *Patrick Büker* et al. – Plans for a future pan-Asian ozone and food security ‘Research for Policy Programme’: potential linkages to UNECE LRTAP Convention and ICP Vegetation.
- 10:05      *Marcus Schaub* et al. – The ICP Forests ozone programme: what’s new?
- 10:20      General discussion.
- 10:30 – 11:00 Coffee/tea (in the canteen of HSR) and poster viewing**
- Session 2: 11:00 – 12:45 Two parallel sessions: Ozone and Heavy Metals/N/POPs**
- Session 2a: Ozone      Chair: Gina Mills**
- 11:00      *Gina Mills* et al. – Update on ozone activities, including:  
- Chapter 3 Modelling and Mapping Manual;  
- Discussion of ‘Ex-post’ maps of current and future ozone effects on vegetation;  
- Food security report, including results of visible injury and bean biomonitoring;  
- Ozone effects on carbon sequestration report (to be published in the autumn of 2011).
- 12:00      **Poster introductions.** Lead author of each poster to stand by poster and introduce its content for two minutes (only!).

**Session 2b: Heavy metals/N/POPs**                      **Chair: Harry Harmens**

11:00      **Poster introductions.** Lead author of each poster to stand by poster and introduce its content for two minutes (only!).

11:45      *Harry Harmens et al.* – Update and discussion on heavy metal/N/POPs activities, including:

- Participation 2010/11 survey and outline of the report;
- POPs and nitrogen developments;
- Data use, MossMet, publications.

**12:45 – 14:00 Lunch** (*in the canteen of HSR*)

**Session 3: 14:00 – 15:30 Two parallel sessions: Ozone and Heavy Metals/N/POPs**

**Session 3a: Ozone**    **Chair: Håkan Pleijel**

14:00      *Sally Wilkinson et al.* – Interactions between ozone and drought stress in plants: mechanisms and implications.

14:20      *Serena Wagg et al.* – Ozone and drought stress interactions in bean and grassland species.

14:40      *Sabine Braun et al.* – Stomatal ozone uptake of Norway spruce: validation of the functions using sap flow.

15:00      *Patrick Büker et al.* – Updates on further developments and application of the DO<sub>3</sub>SE model, including a demonstration of the revised online version of DO<sub>3</sub>SE.

**Session 3b: Heavy metals/N/POPs**                      **Chair: Jesús Santamaría**

14:00      *Iliia Ilyin* – Local-scale modeling of heavy metal atmospheric pollution levels for selected European countries: pilot results.

14:20      *Eiliv Steinnes* – Is it possible to estimate atmospheric deposition of heavy metals by analysis of terrestrial mosses?

14:40      *Louise Foan et al.* – Mosses as biomonitors of POPs pollution: Spatial trends of PAH concentrations in mosses from France, Switzerland and Spain.

15:00      *Jesús Santamaría et al.* – Source apportionment of nitrogen by stable isotope analysis.

15:20      General discussion.

**15:30 - 16:00 Coffee/tea** (*in the canteen of HSR*) **and poster viewing**

**Session 4: 16:00-18:00 Two parallel sessions: Ozone and Heavy Metals/N/POPs**

**Session 4a: Ozone Chair: Gina Mills**

16:00 Ozone Discussion Groups: 3 groups to discuss future ozone work programme of the ICP Vegetation

**Group 1: Impacts of ozone on C sequestration and feedbacks to climate (2011 report)**

**Chair: Håkan Pleijel Rapporteur: Karine Vandermeiren**

Report content  
Contributions from ICP Vegetation participants  
Horizon scanning

**Group 2: Impacts of ozone on biodiversity and ecosystem services**

**Chair: Jürg Fuhrer Rapporteur: Felicity Hayes**

Report content  
Contributions from ICP Vegetation participants  
Horizon scanning

**Group 3: Role of biomonitoring, outreach and “horizon-scanning”**

**Chair: Gina Mills Rapporteur: Patrick Büker**

Future role and requirements of the Convention for biomonitoring  
Outreach – ongoing activities in other regions and links with the ICP Vegetation  
Horizon scanning, including impacts of black C on vegetation

17:15 Discussion groups report back

**Session 4b: Heavy metals/N/POPs Chair: Harry Harmens**

16:00 Moss Survey Discussion Groups: 2 groups to discuss future moss survey work of the ICP Vegetation

**Group 1: Relationship element concentration in mosses and impacts on ecosystems**

**Chair: Ludwig De Temmerman Rapporteur: Sebastien Leblond**

Report content  
Contributions from ICP Vegetation participants  
Horizon scanning: heavy metals, N and POPs work

**Group 2: Role of the European moss survey, outreach and “horizon scanning”**

**Chair: Harry Harmens Rapporteur: Gunilla Phil-Karlsson**

Future role within the Convention  
Outreach – ongoing activities in other regions and links with the ICP Vegetation  
Horizon scanning: heavy metals, N and POPs work

17:15 Discussion groups report back

## Wednesday 2<sup>nd</sup> February, 2011

**Session 5: 8:30 – 10:15 Two parallel sessions: Ozone and Heavy Metals/N/POPs**

**Session 5a: Ozone Chair: Karine Vandermeiren**

- 08:30 *Verena Blanke, Matthias Volk, Serena Bassin et al.* - Ozone and nitrogen effects on a subalpine pasture: novel insights into belowground processes.
- 09:00 *Sally Power et al.* – Effects of ozone on mesotrophic grassland communities under climate change.
- 09:20 *Samina Madkour et al.* – A new Egyptian common bean O<sub>3</sub> bioindicator pair comparable in performance to the (S156/R123) system.
- 09:40 General discussion, including conclusion of discussion of future work programme (*Gina Mills*).

**Session 5b: Heavy metals/N/POPs Chair: Marina Frontasyeva**

- 08:30 *Eero Kubin et al.* – The Finnish environmental specimen bank Paljakka - an archive for heavy metal survey samples.
- 08:50 *Ivan Suchara et al.* – A comparison of element concentrations in moss, grass, spruce needles and forest floor humus.
- 09:10 *Willy Werner et al.* – Patterns of wet deposition types and element concentration in mosses in Germany.
- 09:30 *Dinesh Saxena et al.* – Assessment of metal accumulation by moss in relation to atmospheric emission sources in Uttarakhand, India.
- 09:50 General discussion, including conclusion of discussion of future work programme (*Harry Harmens*).

**10:15 - 10:45 Coffee/tea (in the canteen of HSR) and poster viewing**

**Session 6: 10:45-12:30 Two parallel sessions: Ozone and Heavy Metals/N/POPs**

**Session 6 a: Ozone Chair: Patrick Bükér**

- 10:45 *Sheikh Ahmad* – Measurements of tropospheric ozone in rural/semi rural sites of Rawalpindi and Islamabad, Pakistan.
- 11:05 *Håkan Pleijel* – Reduced ozone by air filtration consistently improved grain yield in wheat.
- 11:25 *Ludger Grünhage et al.* – CRO<sub>3</sub>PS - an ozone risk evaluation model for winter wheat at local scale.
- 11:45 *Ignazio González-Fernández et al.* – Modelling ozone stomatal flux for wheat under Mediterranean conditions.
- 12:05 *Towhid Islam et al.* – Impacts of tropospheric ozone on crop losses using white clover in farming areas of Mymensingh, Bangladesh.
- 12:20 General discussion.

**Session 6b: Heavy metals/N/POPs**

**Chair: Eiliv Steinnes**

- 10:45 *Marina Frontasyeva* et al. – JINR (Dubna, Russia) contribution to the moss surveys in Europe.
- 11:05 *Ivan Suchara* et al. – Concentration of elements in moss along forest roads: the effect of distance and traffic volume.
- 11:25 *Dinesh Saxena* et al. – Large scale atmosphere monitoring in mosses in India: a conceptual approach.
- 11:45 *Stephanie Boltersdorf* et al. – Regional variation in the nitrogen content and  $\delta^{15}\text{N}$  signatures of lichens in relation to atmospheric nitrogen deposition.
- 12:05 *Eero Kubin* et al. – *Hylocomium splendens* and *Pleurozium schreberi* as biomonitors of nitrogen deposition in boreal forests.
- 12:15 General discussion.

**12:30 – 13:45 Lunch** (*in the canteen of HSR*)

**Session 7: 13:45 – 15:30 Plenary session**

**Chair: Harry Harmens**

- Reporting back from ozone and heavy metals sessions.
- ICP Vegetation work programme 2012 – 2014.
- Collaboration with other relevant bodies/organizations.
- Conclusions and review of the 24<sup>th</sup> Task Force Meeting.
- Next Task Force Meeting.
- Any other business.

**16:00 – 17:30 Sight Seeing Tour through the Old Town of Rapperswil-Jona**

(*Meeting point in front of building of Tourist Information, Fischmarktplatz 1*)

**18:15 10 min walk to conference dinner place: Raiffeisen Pavillon in the "Lido"**

(*Meeting point in front of building of Tourist Information, Fischmarktplatz 1*)

Menu: Salad, Käsefondue (melted cheese with potatoes and bread).  
Alternative for those who can't eat cheese: Ghackets mit Hörnli und  
Öpfelmues (pasta with minced meat and apple sauce).

**Thursday 3<sup>rd</sup> February, 2011**

Departure: participants are to make their own travel arrangements to the airport.



**Abstracts**

**Oral**

**Presentations**

## MEASUREMENTS OF TROPOSPHERIC OZONE IN RURAL/SEMI RURAL SITES OF RAWALPINDI AND ISLAMABAD, PAKISTAN

Ahmad S.S.

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Tropospheric ozone formation ( $O_3$ ) is chemically coupled to the emissions of major precursor gases, nitrogen oxides and volatile organic compounds. Highly multifarious and chemically interdependent reaction provides non-linear and coupled pollutant formation processes. In the present study, an attempt has been made to measure the ozone concentration in rural areas of Rawalpindi/Islamabad. Furthermore certain meteorological parameters were also studied to find out correlation between ozone concentration and environmental variables. The study presents five months data from January to May, 2010, along five zones. The general trend in accumulation of monthly  $O_3$  concentration in all zones is same. It can be seen that  $O_3$  levels vary throughout the different seasons. There was continuous increase in the level of  $O_3$  from January to May, 2010. The low concentration values of  $O_3$  were recorded in January and February, while the high concentration values were recorded in May. The average variation of  $O_3$  shows that maximum  $O_3$  concentration is 24.34 ppb. The average monthly concentration from January to May was 16.93, 18.20, 19.39, 21.08 and 22.96 ppb respectively. The variation in concentration of  $O_3$  is either due to possible increase in precursor gases by anthropogenic activities or by the influence of metrological activities. Results indicated that  $O_3$  concentration was maximum in May (Pre-monsoon) and lowest in January (winters). Bivariable correlation analyses indicated a significant association between  $O_3$  concentration and climatic variables. Spatial interpolation also showed variations graphically in  $O_3$  concentration in rural areas of twin cities in different months. Moreover,  $O_3$  concentration level of all the sampling points was less than WHO limits of  $O_3$ . But due to continuous urbanization and pollution, it is important to take appropriate steps before it exceeds the limits.

## IS IT POSSIBLE TO ESTIMATE ATMOSPHERIC DEPOSITION OF HEAVY METALS BY ANALYSIS OF TERRESTRIAL MOSSES?

Berg, T. and Steinnes, E.

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Since the pioneer work of Rühling and Tyler (1971) the moss technique has been used regularly in the Nordic countries to assess the geographical distribution of heavy metal deposition from the atmosphere. In Norway it has been shown that the concentrations of key metals in moss correlates well with the bulk deposition during the corresponding time period (Berg et al., 1995; Berg and Steinnes, 1997). This has also been shown to hold for other Scandinavian countries (Berg et al., 2003). Extension to larger parts of Europe however appeared to be difficult because most EMEP precipitation stations are situated close to the coast where the uptake of metals in moss suffers from competition with marine cations (Gjengedal and Steinnes, 1990).

In a recent paper Aboal et al. (2010) raise the question shown in the present title. Based on a critical review of the moss literature they conclude: “Except for certain elements and specific cases atmospheric deposition of elements (*i.e.* Pb and Cd) cannot be accurately assessed from the concentration of metals and metalloids in moss tissues”.

More than 30 years of experience with the moss technique in Norway has revealed that there are several limitations and sources of error that Rühling and Tyler originally did not realize (Steinnes, 1995; Økland et al., 1999). According to our experience however there is strong evidence that the geographical distribution in moss samples of several elements associated with long-range atmospheric transport is closely related to the spatial variation in atmospheric deposition of these elements. Although Aboal et al. (2010) have correctly pointed to some limitations of the moss biomonitoring technique, their main conclusion is not generally valid.

### References

- Aboal, J.R., Fernández, J.A., Boquete, T. and Caballeira, A.: *Sci. Total Environ.* 408 6291-6297 (2010).
- Berg, T. and Steinnes, E.: *Sci. Total Environ.* 208, 197-206 (1997).
- Berg, T., Røyset, O. and Steinnes, E.: *Atmos. Environ.* 29, 353-360 (1995).
- Berg, T., Hjellbrekke, A., Rühling, Å. and Steinnes, E.: *J. Phys. IV France* 107, 155-158 (2003).
- Steinnes, E.: *Sci. Total Environ.* 160/161, 243-249 (1995).
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- Økland, T., Økland, R.H. and Steinnes, E.: *Plant and Soil* 209, 71-83 (1999).

## OZONE AND NITROGEN EFFECTS ON A SUBALPINE PASTURE: NOVEL INSIGHTS INTO BELOWGROUND PROCESSES

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To investigate the effects of ozone (O<sub>3</sub>) and nitrogen (N) deposition on a subalpine pasture, 180 turf monoliths were exposed during 7 years at Alp Flix, Switzerland (2000 m a.s.l.) in a free air fumigation system to a combination of three O<sub>3</sub> levels (ambient, 1.2 x ambient, 1.6 x ambient) and 5 N loads (ambient, +5, +10, +25, +50 kg ha<sup>-1</sup> a<sup>-1</sup>). Elevated N deposition stimulated aboveground and belowground biomass production, shoot:root ratio, and altered the plant species composition in favour of the functional group of sedges, which accounted almost alone for the observed biomass increase. In contrast, elevated O<sub>3</sub> concentration had no effect on any of the above mentioned parameters. However, first results show slightly increased soil microbial biomass in response to both elevated O<sub>3</sub> and N deposition.

Affecting assimilation and respiration processes of plants and heterotrophes, both N and O<sub>3</sub> deposition may alter C pool sizes and turnover rates throughout the ecosystem. As a result of photosynthetic discrimination against <sup>13</sup>C, SOM has a characteristic δ<sup>13</sup>C signature that depends on environmental conditions during assimilation of the C and on the stability/age of the respective pools.

The δ<sup>13</sup>C of soil air CO<sub>2</sub> reflects the δ<sup>13</sup>C signature of the substrate primarily respired in microbial metabolism. Our δ<sup>13</sup>C analysis revealed a strong seasonality of δ<sup>13</sup>C and large absolute differences between N treatments. Seasonality is characterised by resupply of labile substrates very early in the growing season and a growing CO<sub>2</sub> limitation of photosynthesis while plants approach maturity. The N effect in δ<sup>13</sup>C within seasonality is a consequence of increased productivity under N fertilisation. The database also allows to speculate that mitigating the N limitation of microbial growth may have accelerated the consumption of the favoured C substrates. Thus, increased N supply may have lead to a depletion of labile SOM pools.

Arbuscular mycorrhizal fungi (AMF) play an important role in ecosystems, as they colonize roots of the majority of vascular plants, and provide them with nutrients in exchange for photosynthetically fixed carbon (C), on which the fungi are completely dependent.

To examine N and O<sub>3</sub> effects on the percentage of root length colonized (% RLC) by AMF, in-growth cores (Johnson *et al.* 2001) containing abundant resident plant species were introduced into monoliths with highest and lowest N and O<sub>3</sub> treatments. N addition prevalently increased % RLC, presumably because P limitation of the respective plant species was amplified and therefore, they increased C allocation to the symbionts. O<sub>3</sub> fumigation decreased % RLC in some plant species, as it may lead to a reduced C transfer below-ground, even when no damages are visible above-ground. In some instances, NxO<sub>3</sub> interactions indicated weaker N effects at elevated O<sub>3</sub>, maybe indicating an impeded ability of an O<sub>3</sub>-stressed plant to regulate AMF colonization.

Fungal effects on plants and their responses to N and O<sub>3</sub> deposition were investigated in a climate chamber experiment, in which O<sub>3</sub> fumigation, N fertilization and inoculation with

AMF abundant at Alp Flix were combined in a full-factorial design. Several plant species appeared to be very responsive to AMF while others grew better in the absence of the fungi. In some cases, fungal effects interacted with other treatments, demonstrating how N or O<sub>3</sub> effects on plants may differ depending on the presence or absence of AMF.

Given all these plant species-specific differences in regulating and responding to the AM symbiosis under different N and O<sub>3</sub> levels, it can be concluded that at Alp Flix, AMF are an important factor in mediating abiotic effects on plants and that changes in their abundance, which likely will happen following increased N and/or O<sub>3</sub> depositions, will strongly affect vegetation composition.

***Reference:***

Johnson D., Leake J.R., Read D.J., 2001: Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. *New Phytologist* 152: 555-562.

# REGIONAL VARIATION IN THE NITROGEN CONTENT AND $\delta^{15}\text{N}$ SIGNATURES OF LICHENS IN RELATION TO ATMOSPHERIC NITROGEN DEPOSITION

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Anthropogenic activities have led to major increases in global emissions of nitrogen (N). This change has led to large increases in deposition of atmospheric N to the terrestrial biosphere. Such increases in N deposition pose a outstanding ecological threat to plant community composition in many natural and semi-natural ecosystems. N deposition is known to be an important driver of biodiversity loss. For this reason N deposition should be monitored extensively. Because "conventional" deposition measurement methods are afflicted with methodical and analytical sources of error, the present study researched the effects of atmospheric N deposition on epiphytic lichens. These have been proven to be important bioindicators of air pollution. In contrast to physical-chemical methods, they integratively indicate long-term effects of deposition on biological patterns and processes. In the present study, we tested whether the N concentrations and  $\delta^{15}\text{N}$  ratios of lichens can be used to estimate deposition rates and to locate various sources of N compounds.

In winter 2008/09 and 2009/10 epiphytic lichens (*Physcia sp.* and *Xanthoria parietina*) were sampled from different sites located in the northwestern parts of Germany. Samples were collected within a radius of 2 km around field stations for deposition measurement, from trees that met the requirements for bioindication with lichens (VDI DIRECTIVE 3799/1). Besides, we sampled lichens in 174 recording units (25 km x 25 km) for geostatistical analyses.

We were able to show that the N content of epiphytic lichens reflects the N deposition circumstances at various sites in the northwest of Germany. We found ranges between high N concentrations in *X. parietina* and *Physcia sp.* under high N deposition. Lower N concentrations we found in unpolluted areas.  $\delta^{15}\text{N}$  signatures of both researched epiphytic lichen species are more negative under high ammonium deposition. The verification of highly negative  $\delta^{15}\text{N}$  ratios at sites with local (agriculture) and regional emitters (Benelux through Western winds) shows that source attribution by comparing different  $\delta^{15}\text{N}$  signatures is possible. With geostatistical methods, we were able to underline our results.

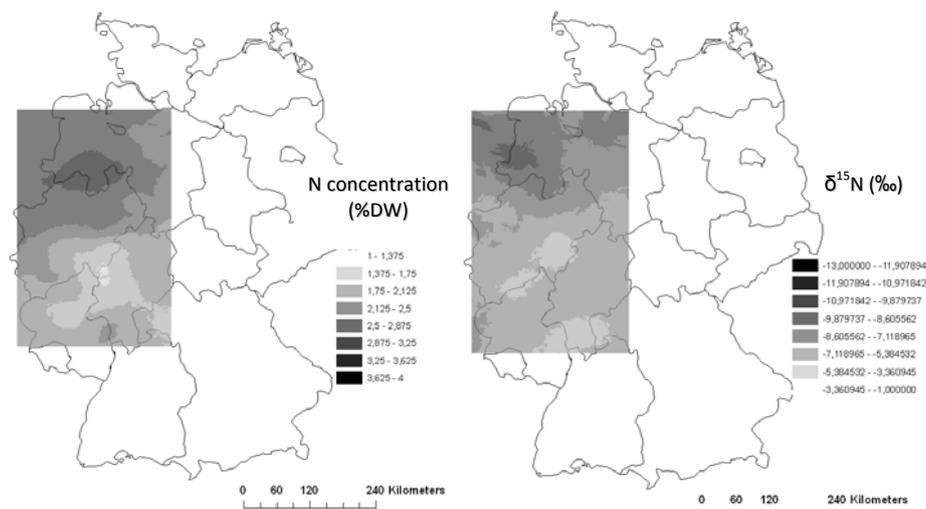


Fig. 1: Geostatistical analyses of N concentration and  $\delta^{15}\text{N}$  signatures of *Xanthoria parietina*.

# STOMATAL OZONE UPTAKE OF NORWAY SPRUCE: VALIDATION OF THE FUNCTIONS USING SAP FLOW

Sabine Braun<sup>1)</sup>, Christian Schindler<sup>2)</sup>, Raphael Mainiero<sup>1)</sup>

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<sup>2)</sup> Swiss Tropical and Public Health Institute, University of Basel, Switzerland

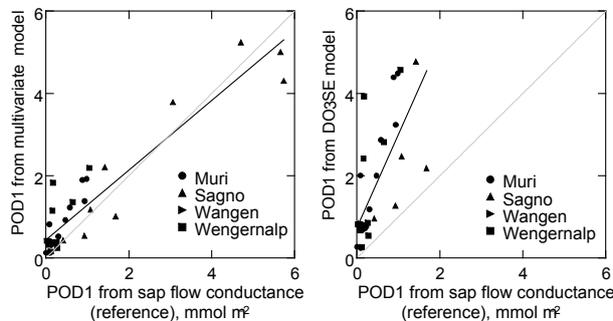
For a quantitative estimate of the ozone effect on vegetation reliable models for ozone uptake through the stomata are needed. Because of the analogy of ozone uptake and transpiration it is possible to utilize measurements of water loss such as sap flow for quantification of ozone uptake. Canopy conductance from sap flow allow either direct uptake calculation or a derivation of stomatal functions using multivariate statistics. This technique has been applied for *Fagus sylvatica* in Switzerland (1) where it corroborated the functions used by the DO3SE model (2). The same procedure has now been used also for Norway spruce (*Picea abies*) with data from four sites at different altitudes and in different regions of Switzerland. The following multiplicative nonlinear equation was applied to the data to derive a stomatal factor:

$$f_{sto} = \left[ 1 - e^{(PAR \times light_a)} \right] \times \left[ 1.5 - \frac{1}{1 + e^{\beta \cdot VPD}} \right] \times \left[ e^{(b_1 \times (T - T_{opt})^2)} \right] \times f_{SWP} \quad (1)$$

where PAR is photosynthetic active radiation ( $\mu\text{mol m}^{-2}$ ), VPD vapour pressure deficit (kPa) and T air temperature ( $^{\circ}\text{C}$ ). The factor for soil water potential ( $f_{SWP}$ ) was not estimated by the model but calculated separately from modelled soil water. An additional term for the minimum daylight conductance ( $f_{min}$ ) was not significant and therefore removed from the model. The resulting estimates of the coefficients are listed in Table 1. For validation, ozone uptake was then calculated using either the standard DO3SE model or the model presented here and compared to ozone uptake calculated directly from sap flow data. The comparison suggest an overestimate of ozone uptake using the standard DO3SE model whereas the multivariate model presented here performs well. This indicates that further validation of the DO3SE model is needed.

**Table 1:** Coefficients of the functions in formula 1 as estimated by the nonlinear model.

Parameter	Estimate	95% confidence interval		p-value
light <sub>a</sub>	-0.012	-0.019	-0.005	0.0007
beta	-5.08	-7.04	-3.12	<.0001
b <sub>1</sub>	-0.00721	-0.00872	-0.00570	<.0001
T <sub>opt</sub>	14.90	13.57	16.22	<.0001



**Figure 1:** Comparison of ozone uptake (Phytotoxic Ozone Dose with threshold 1) estimated using the multivariate model (left) or the DO<sub>3</sub>SE model (right) to uptake calculated directly from sap flow. Seasonal sums per tree ( $\text{mmol m}^{-2}$ )

## References

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- UNECE (2010). Mapping Critical Levels for Vegetation. International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops, Bangor, UK,

**PLANS FOR A FUTURE PAN-ASIAN OZONE AND FOOD SECURITY  
'RESEARCH FOR POLICY PROGRAMME':  
POTENTIAL LINKAGES TO UNECE/LRTAP AND ICP VEGETATION**

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In November 2010 we organised and facilitated a symposium in Delhi on “Ground-level ozone and food security in Asia”. This symposium was funded by Sida (the Swedish International Development Cooperation Agency) and hosted by the Global Atmospheric Pollution Forum (GAP Forum) in collaboration with the Stockholm Environment Institute (SEI) and the United Nations Environment Programme (UNEP).

It was attended by 35 participants from 9 Asia countries as well as Sweden and the UK. The participants included representatives from ministerial departments, science institutes and universities, research funding agencies and UNEP. The variety of participants emphasised the level of international cooperation that currently exists to disseminate evidence and raise awareness of the issue of ozone (O<sub>3</sub>) and food security across South Asia. Based on the scientific evidence presented at the seminar, it was concluded that there is i) sufficient scientific evidence that ambient levels of ground-level O<sub>3</sub> are high enough to cause substantial yield losses in South Asia, but the magnitude of the problem is still unknown; ii) a lack of rural O<sub>3</sub> monitoring stations and ample emission inventories; iii) a lack of robust dose-response relationships that have been derived under South Asian conditions; iv) a need to continue awareness raising in the region; v) a need for risk assessments which would enable cost-benefit analyses to indicate whether mitigation or adaption efforts would be more cost effective; where mitigation efforts are favoured, such studies could provide information necessary to establish an effects based emission reduction policy programme

A potential research programme should, include:

- an O<sub>3</sub> monitoring programme in rural areas
- the establishment of standardised protocols for experimental studies, such as visible injury observations, chemical protectant monitoring, open top chamber (OTC) filtration and fumigation and Free Air Concentration Enrichment (FACE)
- model approaches to extrapolate site-specific experimental studies to regional risk assessments
- the investigation of interactions between O<sub>3</sub> pollution impacts and other stresses to agriculture (e.g. drought, temperature stress, pests and pathogens, other pollutants)
- a continuous dialogue with key stakeholders, incl. farmers, crop breeders and policy makers

Such a novel “research for policy programme” could benefit from the intensification of the existing collaboration between the LRTAP Convention (and hence the ICP Vegetation) and the South Asian Malé Declaration, as well as from direct cooperation of European and Asian research teams. Such cooperation would mainly consist of a knowledge transfer in both directions as well as potentially an equipment transfer from Europe to Asia (e.g. OTCs that are no longer used in Europe).

## UPDATES ON FURTHER DEVELOPMENTS AND APPLICATION OF THE DO<sub>3</sub>SE MODEL

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The main focus of the DO<sub>3</sub>SE model development in 2010 was on further developing its internal soil moisture deficit (SMD) module. The SMD module is based on i) the energy balance method of Penman-Monteith to drive water cycling through the soil-plant-atmosphere system and ii) empirical data describing stomatal conductance relationships with pre-dawn leaf water status to estimate the biological control of transpiration. An internally consistent SMD method is especially important for the realistic prediction of gaseous ozone flux from the atmosphere to trees and forests. As such, the SMD module was developed for, and tested against, eleven forest stands of different tree species (deciduous, coniferous and Mediterranean broadleaf evergreen) and geographical provenances (representative of Scandinavia, Continental and Mediterranean Europe).

This evaluation work specifically assesses different peer-reviewed methods that relate soil water content to stomatal conductance ( $g_{sto}$ ) to assess the limiting influence on water transfer from soil through the tree to the atmosphere. These methods either directly relate soil water status, characterised either as i) soil water potential (the fSWP method) or ii) plant available volumetric soil water content (the fPAW method) to  $g_{sto}$ ; or introduce a iii) steady state or iv) non-steady state plant hydraulic resistance method which controls the relationship between soil and leaf water content and subsequent  $g_{sto}$ . Model results using site-specific parameterisations show that the DO<sub>3</sub>SE model using either the fSWP or fPAW method tend to show more consistency in capturing seasonal trends of soil water content, soil water potential and transpiration as compared to the steady state and non-steady state method.

The implications of SMD, not only on ozone deposition but also on remaining atmospheric ozone concentrations, will be assessed for the UK using CMAQ, a photochemical model, to assess the importance of vegetation acting as a sink for ozone in relation to risks to both ecosystems and human health.

**MOSSES AS BIOMONITORS OF POPS POLLUTION:  
SPATIAL TRENDS OF PAH CONCENTRATIONS IN MOSSES FROM FRANCE,  
SWITZERLAND AND SPAIN**

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Regulation of persistent organic pollutants (POPs) emissions and reliable monitoring of POP concentrations in ambient air is of paramount importance because of their slow rates of degradation, toxicity and potential for both long-range transport and bioaccumulation in living organisms (Aarhus protocol, 1998). Polycyclic aromatic hydrocarbons (PAHs) in particular have shown carcinogenic, mutagenic and immunotoxic effects detrimental to health and appear to be ubiquitous in the environment. Indeed, these compounds are released through combustion of fossil fuels in power plants, domestic heating, waste incineration, industrial processes and, most importantly, motor vehicle exhaust.

Studies have shown that spatial patterns and temporal trends in the concentration of POPs (of which PAHs) in mosses resembled those of concentrations in ambient air (Holoubek et al., 2007). However, in contrast to heavy metals, the use of mosses for monitoring atmospheric deposition of organic compounds at an international scale has so far received little attention. This study was carried out to investigate whether mosses can be used as biomonitors of atmospheric deposition of POPs at the European scale, by determining PAH concentrations in mosses from several countries.

Mosses *Hypnum cupressiforme* Hedw. were collected during October 2010 in 3 regions of Europe: Île-de-France (France), Navarra (Spain) and the Swiss Plateau (Switzerland). 20 sites were chosen in each region in aim to collect at least 1.5 moss samples/1000 km<sup>2</sup>. Three composite samples (of 5 to 10 sub-samples) were collected from each site to evaluate the variability due to the sampling procedure. All samples were sent in insulated boxes containing ice to the LCA in Toulouse. The green and green-brown shoots from the last three years growth were taken using stainless steel tweezers and scissors. The unwashed samples were freeze-dried and then ground to a fine powder in a stainless steel mill. After treatment, the samples were stored at -20°C until analysis. PAHs were extracted from the mosses by pressurized liquid extraction. Extracts were purified by a solid phase extraction clean-up before analysis by high-performance liquid chromatography associated with fluorescence detection. The preliminary results obtained will be discussed.

Holoubek I., Klánová J., Jarkovský J., Kohoutek J., 2007. Trends in background levels of persistent organic pollutants at Kosetice observatory, Czech Republic - Part II. Aquatic air and terrestrial environments 1996–2005. *Journal of Environmental Monitoring* 9, 564-571.

## JINR (DUBNA, RUSSIA) CONTRIBUTION TO THE MOSS-SURVEYS IN EUROPE

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Since 1995 Department of NAA of FLNP JINR participates in the European moss surveys contributing to the European Atlas «Atmospheric Heavy Metal Deposition in Europe – Estimations Based on Moss Analysis» on collaborative studies with the research groups from several Eastern European and Balkan countries: Central Russia, Romania, Bulgaria, Poland, Slovakia, Northern Serbia and Bosnia, Macedonia, Croatia, and the European part of Turkey (Thrace Region). Moss-survey 2010/2011 is extended for Albania, Montenegro and some new areas in Russia. A combination of instrumental ENAA at the IBR-2 reactor in JINR, Dubna, and AAS in relevant counterpart laboratories provides data for concentrations of about 40 chemical elements (**Al**, **As**, Au, Ba, Br, Ca, **Cd**, Ce, Cl, Co, **Cr**, Cs, **Cu**, Dy, Eu, **Fe**, Hf, **Hg**, I, In, La, Lu, Mg, Mn, Na, Nd, **Ni**, **Pb**, Rb, **Sb**, Sc, Se, Sm, Ta, Tb, Ti, Th, **V**, W, Yb, **Zn**) that substantially exceeds the requested number of elements (marked as bold) by the European Atlas. The above trace elements are not all strictly relevant as air pollutants, but most of them can be used as air-mass tracers of long-range atmospheric transport. Examples of multivariate statistical analysis applied to the data sets are given. Distribution of determined elements over the sampled areas is illustrated by contour maps created by the Russian software package GIS-INTEGRO with raster and vector graphics.

## MODELLING OZONE STOMATAL FLUX FOR WHEAT UNDER MEDITERRANEAN CONDITIONS

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The latest approaches to model the ozone (O<sub>3</sub>) risk for vegetation in Europe are based on the cumulative flux of ozone absorbed through the stomata of an upper-canopy leaf (POD). Following the DO<sub>3</sub>SE methodology (UNECE 2010), calculation of POD involves the parameterization of the stomatal conductance (g<sub>sto</sub>) model. This parameterization presents significant challenges at wide geographic scales (i.e. European scale) because of the range of climatic conditions and intra-specific variability of ecophysiological parameters that must be considered.

Much attention has focused on assessing the risk posed to wheat by O<sub>3</sub> pollution. Current g<sub>sto</sub> models have been developed using data from Central and Northern Europe (UNECE, 2010). However, climatic conditions of Southern Europe differ from other areas mainly due to the higher temperature and vapour pressure deficit conditions, less rain and severe summer droughts. Furthermore, the spread of agriculture has triggered regional and local adaptations of wheat to a wide array of environmental conditions (Araus et al., 2007). Several studies indicate that wheat productivity in dry environments of the Mediterranean basin was associated with relatively high values of g<sub>sto</sub> and lower canopy temperature (Giunta et al., 2008).

A new parameterization for wheat is proposed in order to account for climatic and ecophysiological characteristics of wheat growing under Mediterranean conditions. This parameterization is based on field measurements of g<sub>sto</sub> collected for 5 years in central Spain and local phenological information. Differences between two wheat species widely cultivated, *Triticum aestivum* and *T. durum*, will be analyzed.

It is concluded that climatic conditions and ecophysiological characteristics of wheat cultivars growing under Mediterranean conditions need to be represented by g<sub>sto</sub> models in order to provide good estimates of POD for ozone risk assessment in Southern Europe.

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UNECE, 2010. Manual on Methodologies and Criteria for Modelling and Mapping Critical Loads and Levels and Air Pollution Effects, Risks and Trends, Convention on Long-Range Transboundary Air Pollution. Available on line at: <http://www.icpmapping.org>.

# CRO<sub>3</sub>PS - AN OZONE RISK EVALUATION MODEL FOR WINTER WHEAT AT LOCAL SCALE

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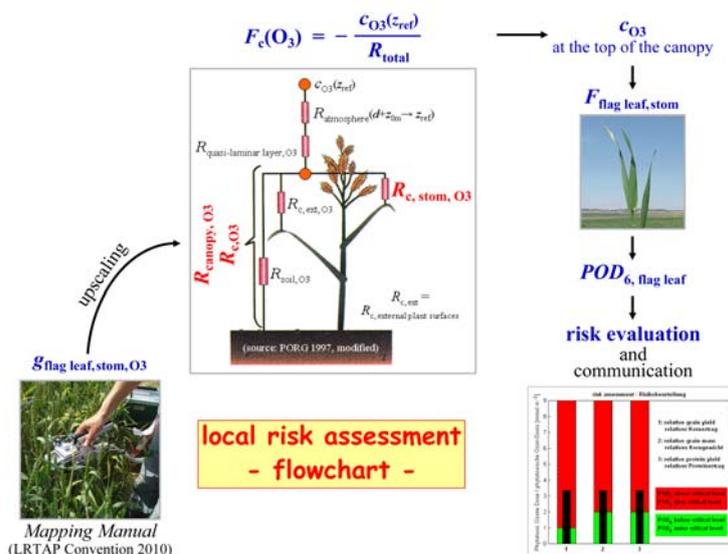
The recent data synthesis, published by the ICP Vegetation Programme Coordination Centre, suggested widespread occurrence of O<sub>3</sub> effects on vegetation at ambient concentrations in Europe over the period 1990 - 2006. In principle, an O<sub>3</sub> risk evaluation can be performed at the European/EMEP, at national as well as at local level. According to the Council Directives of the European Union, the air quality has to be assessed and managed by means of sampling points for fixed measurement of O<sub>3</sub> concentrations in our case. In this context, local risk assessments for O<sub>3</sub> have to be based on the parameters routinely measured by the European air quality monitoring networks.

The presentation gives an overview of an O<sub>3</sub> risk assessment approach for winter wheat at local scale. The model CRO<sub>3</sub>PS is based on the *big-leaf* model PLATIN (PLant-ATmosphere INteraction) and on the flux-based critical level concept described in the LRTAP Convention's Mapping Manual.

The Mapping Manual's stomatal flux algorithm for wheat is based on the assumption that the O<sub>3</sub> concentration at the top of the canopy provides a reasonable estimate of the O<sub>3</sub> concentration at the upper surface boundary of the laminar boundary layer near the flag leaf, if the roughness sublayer near the canopy is not taken into account. Because O<sub>3</sub> concentrations are not measured at the canopy top by the European air quality monitoring networks, the O<sub>3</sub> concentrations measured in the monitoring networks at a standard reference height above the canopy must be transformed to that at the top of the wheat canopy.

Fig. 1 summarizes the four steps of the risk assessment approach at local scale:

- (i) Upscaling the stomatal conductance of the flag leaf to canopy level
- (ii) Modelling total O<sub>3</sub> flux and calculation of O<sub>3</sub> concentration at canopy top
- (iii) Calculation of flag leaf stomatal uptake and Phytotoxic Ozone Dose (POD<sub>6</sub>)
- (iv) Risk evaluation



**Figure 1.** Flowchart for a local O<sub>3</sub> risk assessment for winter wheat.

## OVERVIEW OF THE ACHIEVEMENTS OF THE ICP VEGETATION IN 2010

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The ICP Vegetation is an international programme that reports on the effects of air pollutants on natural vegetation and crops [1]. It reports to the Working Group on Effects (WGE) of the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). In particular, the ICP Vegetation focuses on the following air pollution problems: quantifying the risks to vegetation posed by ozone pollution and the atmospheric deposition of heavy metals and nitrogen to vegetation. In addition, the ICP Vegetation is taking into consideration impacts of nitrogen on vegetation (including interactions with ozone), consequences for biodiversity and interactions between air pollutants and climate change.

At the 24<sup>th</sup> Task Force Meeting we will report on the achievements of the ICP Vegetation in 2010, in particular regarding progress made with items to be reported to the WGE in 2011 [2]:

- 2010 biomonitoring exercise for ozone;
- Ozone impacts on food security;
- Progress with European heavy metals and nitrogen in mosses survey 2010/11;
- Mosses as biomonitors of POPs.

We will also report on the revised chapter 3 of the Modelling and Mapping Manual of the Convention ([http://www.rivm.nl/thema/images/Ch\\_3\\_revised\\_summer\\_2010\\_final\\_221010\\_tcm61-48386.pdf](http://www.rivm.nl/thema/images/Ch_3_revised_summer_2010_final_221010_tcm61-48386.pdf)), which now includes 10 new/revised stomatal flux-based critical levels of ozone for vegetation. In addition, we will discuss the contribution of ICP Vegetation to the common workplan items of the WGE for 2011 [2].

Apart from looking back to our achievements in 2010, throughout the Task Force Meeting we will be discussing our future plans, in particular the medium-term workplan of the ICP Vegetation (2012 – 2014).

### **Acknowledgement**

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- [2] Working Group on Effects. Draft 2010 workplan. ECE/EB.AIR/2010/5. <http://www.unece.org/env/documents/2010/eb/eb/ece.eb.air.2010.5.e.pdf>

## **LOCAL-SCALE MODELING OF HEAVY METAL ATMOSPHERIC POLLUTION LEVELS FOR SELECTED EUROPEAN COUNTRIES: PILOT RESULTS**

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There are a number of factors complicating the assessment of heavy metal pollution. They include uncertainties of anthropogenic emissions, insufficient coverage of monitoring network in some regions of the EMEP domain, uncertainties associated with wind re-suspension parameterization. In order to improve quality of the heavy metal pollution information submitted to countries by EMEP a Case Study was initiated under CLRTAP. The Case Study is focused on complex assessment of heavy metal pollution in one or a few EMEP countries involving all available information on European, national and local scales. Six countries (the Czech Republic, Croatia, then Netherlands, Spain, Italy and Slovakia) expressed their wish to participate in this activity.

In order to prepare more detailed information on spatial distribution of concentrations and deposition in countries-participants the resolution of the model was decreased. Input data (emissions, meteorology, land-cover, etc.) with fine (5x5 km and 10x10 km) spatial resolution were prepared in the framework of Case Study project for the Czech Republic and Croatia, respectively. Modelling of heavy metal pollution levels was carried out and comparisons with measured concentrations and deposition as well as concentrations of heavy metals in mosses were performed. In particular, the modelled deposition with fine spatial resolution was compared with heavy metal concentrations in mosses measured in the Czech Republic and Croatia.

The purpose of this presentation is to inform about the current status and future plans of the EMEP Case Study, and to demonstrate its pilot results.

## IMPACTS OF TROPOSPHERIC OZONE ON CROP LOSSES USING WHITE CLOVER IN FARMING AREAS OF MYMENSINGH, BANGLADESH

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A study was conducted to examine the ozone status and its effects on crops in Bangladesh. In the study ozone concentrations were measured at 4 weeks interval using passive samplers in Mymensingh during April 2007 -2008. The 4-weekly mean O<sub>3</sub> concentration varied between 17 ppb (December) and 35 ppb (May/June). Low and high O<sub>3</sub> concentrations corresponded well with low and high temperatures, with the average monthly air temperature ranging from 18°C (January) to 29°C (August) and the average monthly relative humidity ranging from 76 % (February) to 91 % (July). Not surprisingly, the first growing season, which covered the summer monsoon months, was much wetter (2011 mm) than the second growing season, which occurred during wintertime (126 mm). This difference is also mirrored in the shorter average sunshine hours during the cloudy summer as compared to the sunny winter growing period. The biomonitoring study used O<sub>3</sub> sensitive (NC-S) and O<sub>3</sub> resistant (NC-R) white clover genotypes and worked on the principle that the difference in plant foliar injury as well as the biomass ratio between the O<sub>3</sub> sensitive and O<sub>3</sub> resistant clover genotypes can be directly related to the prevalent O<sub>3</sub> concentrations during the exposure period. The experimental results revealed that the ozone-resistant clover genotype NC-R had on average a higher biomass as compared to the biomass of the ozone-sensitive clover genotype NC-S at all harvests. Accordingly, the biomass ratio NC-S/NC-R genotype was between 0.50 and 0.69, and 0.75 and 0.87 in the first and second experiment respectively. However, the difference in the average biomass per pot between the two genotypes was only significant at harvest 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> of the 2007 and harvest 2<sup>nd</sup> and 3<sup>rd</sup> of the 2007/08 growing period. The medium foliar injury class of the NC-S plants varied slightly during the growing periods with the peak damage being reported for the 2<sup>nd</sup> harvest during the 2007 and the 2<sup>nd</sup> and 4<sup>th</sup> harvest during the 2007/08 experiment. The NC-R plants never expressed ozone-induced foliar injury.

**Key words:** Tropospheric ozone, white clover, crop losses, biomass.

# THE FINNISH ENVIRONMENTAL SPECIMEN BANK PALJAKKA - AN ARCHIVE FOR HEAVY METAL SURVEY SAMPLES

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The Finnish Forest Research Institute (Metla) established a network of 3009 permanent monitoring sites all over the country in 1985-1986 for monitoring environmental changes in the forests. During the first two years 6 877 moss, lichen and pine bark samples were collected. A half of the samples were used for chemical analysis and the rest was stored for future purpose. The monitoring continued every five years and the Paljakka ESB was established in 1994. Soon a large number of forest litter collected since 1958 was also placed to the Paljakka ESB and it became necessary to build an extension part in 1999.

Paljakka ESB provides a high quality facility for environmental samples. Total floor area of 770 m<sup>2</sup> includes room for pre-treatment and nine fireproof storage rooms. At the beginning of 2010 there were 306 153 litter samples and when the litter will be sorted components the total amount of stored samples is about 700 000 (Table 1). There are also offices, auditorium, exhibition hall, accommodation rooms, small kitchen, and sauna. Only dried plant material is stored in the Paljakka ESB currently.

**Table 1.** Number of samples in Paljakka Environmental Specimen Bank.

Samples	Number	Collection year	Responsible researcher
<b>INTERNATIONAL CO-OPERATION</b>			
<b>ICP-Vegetation</b>			
Mosses	10 500	1985-2010	E. Kubin & J. Piispanen
Epiphytic lichen	5 200	1985-1990	E. Kubin
Bark	2 000	1985	J. Poikolainen
Humus	1 000	1995	E. Kubin
<b>ICP-Forests (Forest Focus, FutMon)</b>			
Needles	75 000	1990	H. Raitio
BioSoil (coming 2010)	60 000		H. Ilvesniemi
<b>REGIONAL PROJECTS</b>			
Biomass	10 000	1990-2000	H.-S. Helmisaari
Forest litter	306 153	1946-	T. Hokkanen
Forest litter (in pre-treatment)	300 000		
			M. Kauppi, M. Kozlov, E. Ohenoja, A. Mäkinen
<b>UNIVERSITY SAMPLES</b>	2 000		
<b>Total</b>	<b>771 853</b>		

Stored samples originate from several national and international projects like UNECE ICP Vegetation and ICP Forests. On the European scale samples represent mostly background areas sensitive for environmental changes. At present the value of environmental specimen banking is becoming more broadly understood with growing number of ESBs. Future prospects for Paljakka ESB and environmental specimen banking in general will be discussed in the Task Force Meeting.

## A NEW EGYPTIAN COMMON BEAN O<sub>3</sub> BIOINDICATOR PAIR COMPARABLE IN PERFORMANCE TO THE (S156/R123) SYSTEM

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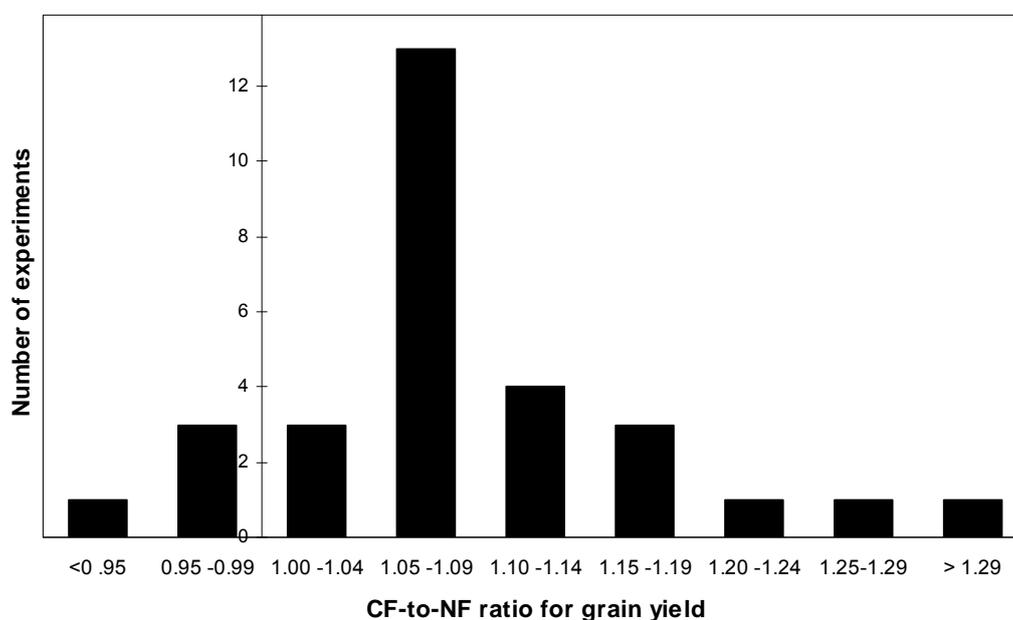
Currently cultivated Egyptian common bean genotypes were screened for sensitivity to ozone. Our chief goal was to identify at least one Egyptian pair of cultivars (sensitive / tolerant) to be further tested for use as a bioindicator system and to compare the response and sensitivity of this system with those of the worldwide adopted bean bioindicator system (S156 / R123). Four Egyptian genotypes were selected for this purpose (Nebrasca, Paulista, Bronco and Contender) along with the O<sub>3</sub>-bioindicator pair (S156 / R123) and a European genotype (Pinto) known for its sensitivity to O<sub>3</sub>. Tests for O<sub>3</sub>-sensitivity were conducted using short-term acute exposure to O<sub>3</sub> at 150 ppb for 3 days (8h/d). Screening tests yielded three sensitive genotypes: Pinto, S156 and Nebrasca, two intermediate candidates: Paulista and Bronco and two tolerant R123 and Contender, when ranked on the basis of % foliar injury to the 1<sup>st</sup> trifoliolate leaves. The pair (Nebrasca / Contender) responded to O<sub>3</sub> fumigation in a manner that was equal in type and magnitude to the pair (S156 / R123). Data collected using the 1<sup>st</sup> trifoliolates reflected the true genotype sensitivity ranking in all the parameters under study. A close relationship was established between the intensity of visible symptoms and degradation of plant pigments, especially leaf chlorophylls. The magnitude of total chlorophylls, C<sub>a</sub> and C<sub>b</sub> reduction correlated positively with the plant sensitivity. A decrease in quantum yield (QY) was recorded and could be positively correlated with the degree of foliar injury and O<sub>3</sub> sensitivity ranking only in the 1<sup>st</sup> trifoliolate leaves. The depression in QY could be partly attributed to the degradation of C<sub>a</sub> molecule which is the center of photosystems I and II or to structural damage of the thylakoids by ozone. More stomatal closure was observed in the tolerant genotypes (Bronco, R123 and Contender) indicating that a control of stomatal aperture could be part of the defense strategies of those plants aimed at restricting O<sub>3</sub> entry into the leaf mesophyll and thus avoiding injury.

# REDUCED OZONE BY AIR FILTRATION CONSISTENTLY IMPROVED GRAIN YIELD IN WHEAT

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Most studies of ozone effects on crops used elevated concentrations. This study only considers effects of reduced  $[O_3]$  based on data in the scientific literature. Open-top chamber (OTC) charcoal-filtered air treatments were compared with non-filtered air treatments for experiments with field-grown wheat. In the literature 30 experiments meeting the requirements were found, published 1985-2010. They represent nine countries in North America, Europe and Asia, 18 cultivars and one set of four cultivars. 26 experiments reported improved yield and 4 experiments reduced yield by air filtration, a strongly significant positive effect of air filtration (Figure 1). The average yield improvement was 9%. There was a significant association between the filtration effects on grain yield and grain mass. Average daytime  $[O_3]$  was reduced by filtration from  $35 \text{ nmol mol}^{-1}$  to  $13 \text{ nmol mol}^{-1}$ . The average filtration efficiency of OTC air was 63% for  $O_3$  and 56% for  $SO_2$ . For  $NO_x$  ( $NO + NO_2$ ) it was consistently observed that  $NO_2$  was reduced and  $NO$  increased by charcoal filtration. Detailed study of  $NO$  and  $NO_2$  in the air entering OTCs revealed that the net effect of charcoal filtration on  $[NO_x]$  is small and that the filters rather convert  $NO_2$  to  $NO$ . Most experiments reported low  $[SO_2]$  and  $[NO_x]$ . Thus,  $O_3$  can be concluded to be the main phytotoxic component in the experiments. Substantially elevated  $[NO_2]$  was observed in one experiment. The main conclusion of this study is that current levels of  $O_3$  over large parts of the world adversely affect wheat yield.



**Figure 1.** Frequency distribution of the CF-to-NF ratio for grain yield over 30 experiments with field-grown wheat.

## EFFECTS OF OZONE ON MESOTROPHIC GRASSLAND COMMUNITIES UNDER CLIMATE CHANGE

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Future climate scenarios include an increase in factors that contribute to tropospheric ozone formation and an increased likelihood of prolonged summer drought. Individually, these stresses reduce plant productivity and growth, while inter-specific differences in sensitivities to ozone and low soil water availability can lead to changes in plant community composition and biodiversity loss. However, little is known about the combined effects of these two important global change drivers at either the species or the community level.

An experiment was therefore carried out exposing model mesotrophic grassland communities to differing levels of ozone, under two levels of simulated rainfall. Mesocosm communities were established in spring 2009 by planting equal biomass ratios of three grasses (*Holcus lanatus*, *Agrostis capillaries*, *Trifolium repens*) and four forb species (*Lotus corniculatus*, *Plantago lanceolata*, *Crepis biennis* and *Hypochaeris radicata*) in replicate 42 L pots filled with local soil. Ozone fumigation was carried out in 16 open top chambers at Silwood Park (Ascot, UK), from May to September 2009, and May-August 2010. Daytime target ozone concentrations were 0 ppb, 30 ppb, 60 ppb, and 90 ppb in each of four replicate chambers, with nighttime concentrations falling to 30ppb in all except filtered air treatments. The DO<sub>3</sub>SE model was parameterized with site-, and species- specific environmental and physiological data to derive ozone flux estimates for all pots.

Watering regimes represented 1) average summer rainfall at Silwood Park (UK) over the last 20 years or 2) levels experienced during the summer of 2003, a particularly dry year in southern England; droughted pots received 40% less water than their paired, non-droughted counterparts over the course of the fumigation.

Results indicate species-level differences in responses to ozone and watering regime.

Physiological data confirm lower stomatal conductances in water stressed plants, with an associated reduction in ozone flux under droughted conditions. This is supported by the slower and less pronounced development of visible ozone injury symptoms of plants growing under water stress. Generally increasing levels of ozone exposure resulted in a progressive reduction in aboveground biomass in both well watered and droughted mesocosms. However, there was consistent evidence of interactions between ozone and drought, with a greater negative effect of ozone in well watered (compared to droughted) pots.

Overall, results provide empirical evidence that predicted changes in climate are likely to modify the effects of ozone in some species, with implications for the composition, diversity and functioning of semi-natural grasslands.



## SOURCE APPORTIONMENT OF NITROGEN BY STABLE ISOTOPE ANALYSIS

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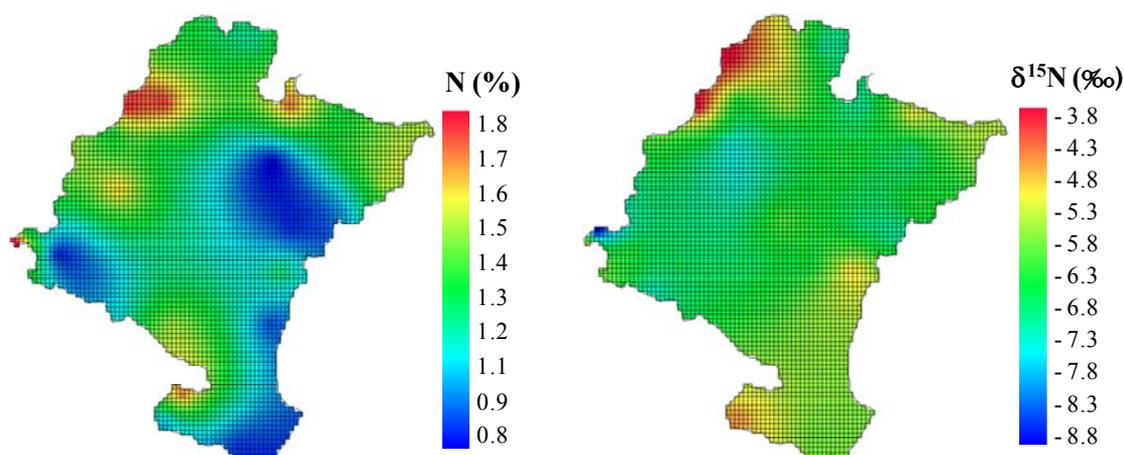
Over the 20<sup>th</sup> century, anthropogenic reactive N emissions have led to a drastic increase in atmospheric N deposition, which has potentially altered natural N cycle. Compared with vascular plants, mosses are characterized by higher sensitivities to atmospheric N supply due to the lack of a true root system to acquire N from substratum.

$\delta^{15}\text{N}$  signatures are being used to investigate N supply in ecosystems. In this context, recent interest in moss %N and  $\delta^{15}\text{N}$  is enhancing our knowledge of the spatial variation, main sources and N species of N deposition.

The main objective of this study was to investigate the existence of different sources of N deposition based on moss analysis. Samples from *Hypnum cupressiforme* were collected in September 2010 at 39 sites distributed in a regular grid (20x20 km) across the province of Navarra. Samples were analyzed for N concentration and  $\delta^{15}\text{N}$  signatures by isotope ratio mass spectrometry (Vario Micro EA coupled to an Isoprime IRMS).

The mean N content in moss tissue was  $1.3 \pm 0.3\%$ , while the average of  $\delta^{15}\text{N}$  reached  $-6.13 \pm 1.0\text{‰}$ . These values are in agreement with other obtained in similar studies.

No significant correlations were detected between N content and  $\delta^{15}\text{N}$  signatures. However, %N was positively correlated with altitude ( $R^2 = 0.34$ ,  $P < 0.05$ ) and precipitation ( $R^2 = 0.43$ ,  $P < 0.01$ ).



Kriging maps of %N and  $\delta^{15}\text{N}$  signatures in Navarra (Spain)

The highest N content in moss tissue was detected in the NW of Navarra, an area influenced by the emissions coming from the Basque country (a very industrialized region). Moreover, high N levels were found in the SW of the province, where two power plants are located.

This source attribution was deduced from  $\delta^{15}\text{N}$  signatures, as both areas showed the highest values of  $\delta^{15}\text{N}$ , thus suggesting the influence of oxidized N forms (combustion sources). On the contrary, in other areas of Navarra N content was linked to farm activities, as can be deduced from the low  $\delta^{15}\text{N}$  values (marked in blue) related to the presence of reduced N forms ( $\text{NH}_3$ ) emitted in such activities.

## LARGE SCALE ATMOSPHERE MONITORING BY MOSSES IN INDIA: A CONCEPTUAL APPROACH

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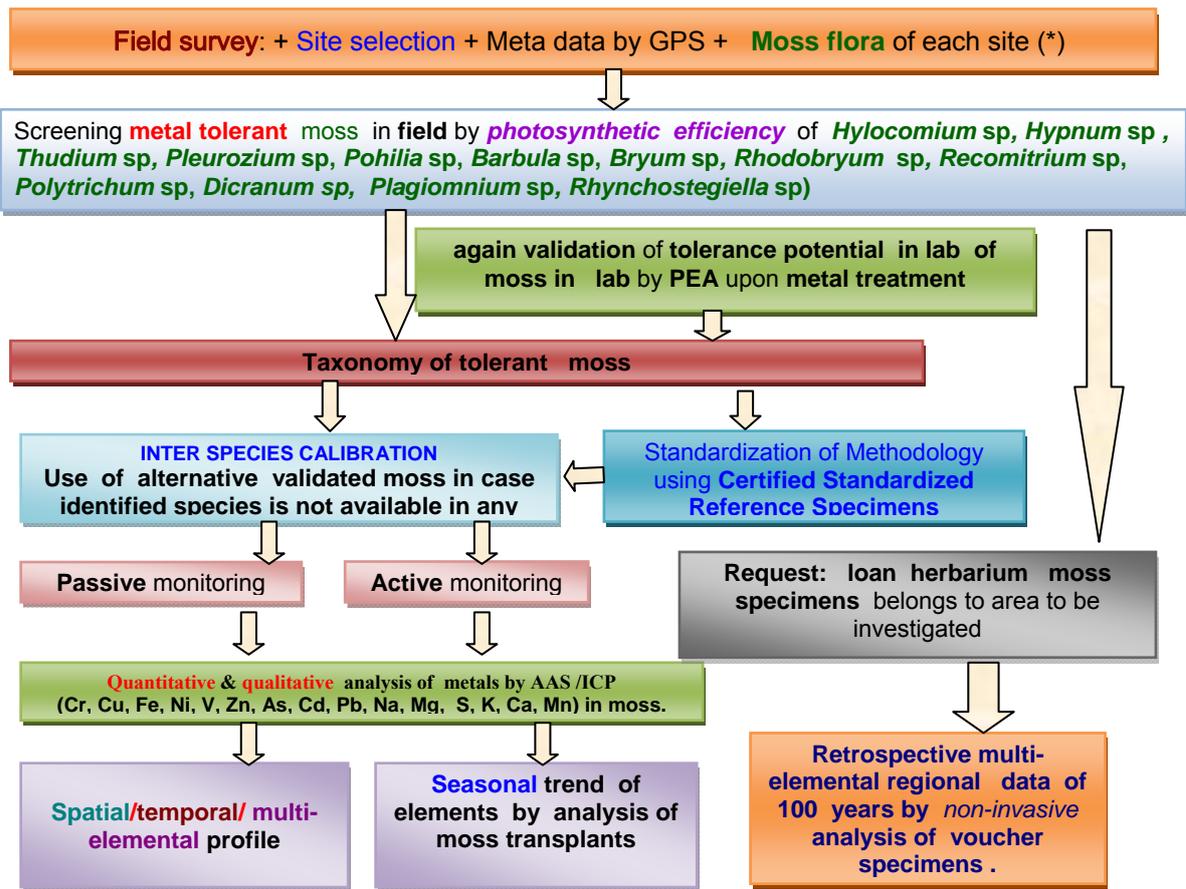
**Key Words:** metals, bio-monitoring, moss, validation of tolerance species, chlorophyll fluorescence

A conceptual approach was developed to be used as a protocol for large scale atmospheric monitoring of metals to validate the tolerant moss species based on their photosynthetic activity. This approach would be suggested for annual, seasonal and for large scale multi-elemental monitoring. Measurements were carried out at Uttarakhand (Kumaon and Garhwal), Himachal, Punjab and Upper Gangetic plains by using moss *Thuidium cyambifolium* C. Muell.

To achieve the same, widely distributed moss species (*Rhodobryum roseum* (Hedw.) Limpr, *Distichium capillaceum* (Hedw.) B.S.G., *Bryum argenteum* L., *Hypnum cupressiforme* Hedw., *Dicranum undulatum* Schrad. Ex Brid., and *Thuidium cyambifolium* C. Muell.) were shortlisted first. Later on, validation for tolerance potential of moss against metals (Pb, Cd Ni and Cu) were carried out by measuring the chlorophyll *a* fluorescence signals in the field and under laboratory conditions. Based on above mentioned measurements, sensitive moss species were omitted out while tolerant ones were calibrated for metal uptake before inducting for active seasonal monitoring. Metal intake calibration was made amongst short listed tolerant moss species and same is desired to optimize the choice of the alternative species in case selected species is not available. Moss species having nearly same accumulation potential were finally short listed for large scale bio-monitoring program. Moss transplants were carried out for three different weather seasons representing summer, winter and monsoon. Each season represents the data of four months. Certified reference materials (CRM) were used for standardization of methodology and instrument as well.

Analyzed mosses exhibit high concentration of Zn in rural areas, on the contrary, Pb and Cd was higher in moss harvested from urban areas, however, values were not critical. Indeed, Pb values were higher in proximity to the highways. That could be due to emissions spewed out from automobile despite lead free fuel. Nearly same trend was observed by all the sites examined. At certain sites Ni and Pb values were high and could not be ruled its source from domestic waste dumping grounds and also Pb and Ni batteries.

Our results revealed that photosynthetic activity of moss could help to validate their tolerance in monitoring program through developed conceptual approach. Study was useful to sense the seasonal variations in atmospheric metal concentrations.



## ASSESSMENT OF METAL ACCUMULATION IN MOSS IN RELATION TO ATMOSPHERIC EMISSION SOURCES IN UTTARAKHAND, INDIA

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**Key words:** Biomonitoring, metal precipitation, *Isopterygium elegans*, active & seasonal monitoring.

Monitoring of atmospheric seasonal and annual metal precipitation program has been in progress since 2002. The present work is the outcome of the above program on Garhwal hills (Uttarakhand). The climate of Garhwal hills (Uttarakhand) is influenced by metal precipitation due to many fold increase in vehicles and tourist activity. The data presented belongs to atmospheric deposition of metals by moss *Isopterygium elegans* exposed 2004-2005 for every four months (representing one season) in all the four directions to evaluate the atmospheric deposition of metals. All the analytical results are above the detection limits for metals studied. ANOVA revealed significant difference in the metals concentration during different seasons. Zn and Pb was measured maximum in moss samples transplanted in Garhwal hills (Uttarakhand) during summer where as Cu and Cd was found moderately high. The over all trend of metals in different seasons was in order of Zn>Pb>Cu>Cd. The maximum concentration of all metals was measured in moss during summer followed by winter and minimum during the monsoon. Indeed moss harvested from the Petrol Pump had high value for the metals, however, Zn and Cd did not show significant difference in metal for winter and summer seasons. The result obtained after analysis suggests the potential of an epiphytic moss *Isopterygium elegans* as reliable biomonitor of atmospheric deposition of elements and its suitability for biomapping. This also implies that the active transplant technique is a cost effective and reliable tool, for mapping of metal precipitation. The novel aspect of this study is that it delivers information on atmospheric deposition in India and Asia at all, where very limited information is available in this field, therefore, present finding is a gap filling one.

## CONCENTRATION OF ELEMENTS IN MOSS ALONG FOREST ROADS: THE EFFECT OF DISTANCE AND TRAFFIC VOLUME

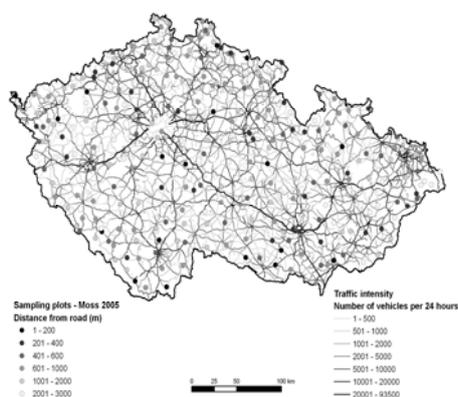
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Cars emit coarse and fine particles carrying compounds of metals and persistent organic pollutants, gaseous pollutants, etc. In order to eliminate the effect of traffic pollution, the moss monitoring manual states that moss samples are not to be collected closer than 100 m from a local road and 300 m from a busy road. We may ask whether the element content of moss is indeed not affected beyond these distance limits.

The concentrations of 37 elements (Ag, Al, As, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Hg, In, La, Li, Mn, Mo, N, Nd, Ni, Pb, Pr, Rb, S, Sb, Se, Sn, Sr, Th, Tl, U, V, Y and Zn) were determined in moss specimens (largely *Pleurozium schreberi*) collected at 282 sites in the Czech moss monitoring campaign in 2005. Figures for traffic volume (Czech Road Traffic Census 2005) for major and local roads were available. Post hoc, all moss samples collected (100 m) 300 m - 2 000 m from the nearest roads with a known traffic volume were found using GIS methods. The concentrations of elements in moss were correlated with the distance of the sampling site from the nearest road, and with the traffic volume counts for cars, trucks and motorcycles.

The concentrations of 17 elements (Al, As, Bi, Hg, Ga, In, La, Li, Mn, Mo, Ni, Se, Sn, Sr, U, V and Zn) correlated significantly ( $p \leq 0.05$ ) and surprisingly positively with distance from the road in the zone (100 m) 300 m - 2 000 m. Sampling plots far away from roads are frequently situated nearer to forest edges with less filtering of the background air pollution. In the same zone, the concentration of Cr, Cs, Cu, Fe, Mo and Zn correlated significantly and positively with the volume of cars, and the concentration of Fe in moss correlated with truck volumes. No significant effect of motorcycle volumes was found. We also investigated the effects of traffic volumes on element concentrations in moss in a zone closer to the roads (100 m) 300 m - 500 m. A significant effect of motorcycle volumes on Cu, Hg, Mn, N and S concentrations was revealed, while car and truck volumes significantly affected only the total N concentrations in moss in this zone. The increased accumulation of these elements in moss at a greater distance from roads may be due to transport of the elements by fine particles, which in contrast to coarse particles may be segregated and deposited in wider zones rather than on a narrow exponential gradient.



Data of the Czech Road Traffic Census 2005 and the positions of the moss sampling plots.

## A COMPARISON OF ELEMENT CONCENTRATIONS IN MOSS, GRASS, SPRUCE NEEDLES AND FOREST FLOOR HUMUS

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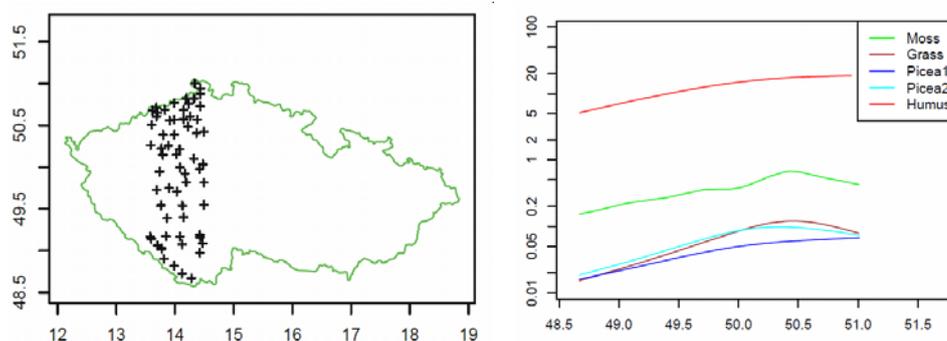
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The distribution of 37 - 41 elements (Ag, Al, As, Ba, Be, Bi, (Ca), Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Hg, In, (K), La, Li, (Mg), Mn, Mo, N, (Na), Nd, Ni, Pb, Pr, Rb, S, Sb, Se, Sn, Sr, Th, Tl, U, V, Y and Zn) was determined in four indicators: forest floor humus (H, Oh), moss *Pleurozium schreberi*, grass *Avenella flexuosa*, and annual and biennial spruce needles (*Picea abies*). Representative composite samples of each material were collected at the same approx. 250 permanent moss monitoring plots in developed coniferous forests across the Czech Republic. Isopleth maps of the element distributions and the concentration trends of the elements in the indicators in areas with different local land use, geology, geomorphology, climatic districts, etc. were compared.

The highest concentrations of most of the elements were determined in forest floor humus, followed by moss, 2-year-old needles and grass. Concentrations of Ca, K, Mg and Na were not determined in moss, but data available in the literature indicates a high accumulation of these elements in moss. Some elements were accumulated in higher amounts by bioindicators other than moss. For example, the Ba, Mn and Ni concentrations were higher in spruce needles and the Mo, Rb and S concentrations were higher in grass leaves than in moss.

The element concentrations in moss and humus were affected most by local industrial (traffic) pollution sources. Two-year-old spruce needles seem to be a good indicator of an urban type of contamination in the Czech Republic. One-year-old needles contain higher concentrations of nutrient elements (e.g., K) and their chemical analogs (e.g., Cs, Rb) than older needles, due to active annual retranslocation of elements in trees.

Surprisingly, the element composition of all investigated indicators was considerably affected by the operation of nearby industrial regions and cities, and by major individual emission sources. In spite of the different efficiency of each indicator for accumulating the elements, the distribution of specific combinations of elements or groups of elements in any indicator pointed more or less markedly to the position of nearby significant anthropogenic pollution sources.



Arsenic concentration in the indicators along a south-north air pollution gradient in CZ.

## OZONE AND DROUGHT STRESS INTERACTIONS IN BEAN AND GRASSLAND SPECIES

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Two characteristic temperate grassland species *Dactylis glomerata* and *Ranunculus acris*, grown in competition, and the ozone-sensitive (strain S156) and ozone-resistant (strain R123) lines of *Phaseolus vulgaris* used in the ICP Vegetation experiments, were exposed to well- and reduced-watered conditions together with increasing background ozone concentrations for between 5 to 20 weeks. Experiments were carried out in the CEH-Bangor ozone exposure facilities and the mean 24 hr ozone concentration in the 8 solardomes ranged from pre-industrial to predicted 2100 values (ca. 15 to 95 ppb).

There was a significant ozone effect on below ground biomass in *D. glomerata*, *R. acris* and *P. vulgaris*, with the watering treatment - ozone interaction being a strong modifier of response. However, for all species above ground biomass was not significantly altered despite there being a significant increase in rates of foliar injury and senescence under increasing ozone concentrations.

Stomatal conductance measurements were obtained throughout the exposures, under similar climatic conditions, using a Delta-T (AP4) porometer. The combined effects of increasing ozone and drought appear to have a direct effect on stomatal conductance with increasing ozone reducing the ability of plants to close their stomata under stress conditions. This trend was more apparent in *D. glomerata* and *P. vulgaris* than *R. acris*.

Impairment of stomatal functioning, due to disruption of abscisic acid (ABA, the so-called “drought hormone”) signalling, is a reported response of some plant species to ozone toxicity. ABA bioassay studies for *P. vulgaris* showed that after 7 days ozone exposure detached leaves were able to close their stomata in response to exogenously supplied 3µm ABA solution, whereas in the control treatment rates of transpiration significantly increased with increasing ozone concentrations. This trend was still evident after 14 days ozone exposure in the ozone resistant cultivar but there was a significant increase in transpiration rates with increasing ozone in ABA treated ozone-sensitive plants. This finding suggesting that ABA signalling is being disrupted in the ozone sensitive cultivar and that disruption of stomatal functioning may potentially reduce the ability of plants to respond to drought.

Exogenous application of ABA would be anticipated to close the stomata, thereby reducing the influx of ozone into the plant and consequently reducing rates of foliar damage associated with enhanced ozone. Response of *P. vulgaris* cultivars to 10µm ABA foliar spray resulted in a significant decrease in rates of ozone injury in the ozone resistant cultivar across all the ozone concentrations in comparison to the control, however there was no change in rates of injury with ABA application in the ozone sensitive strain.

**Acknowledgement:** We are grateful to DEFRA and NERC for supporting this study.

## **PATTERNS OF WET DEPOSITION TYPES AND ELEMENT CONCENTRATION IN MOSSES IN GERMANY**

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Atmospheric deposition rates are measured or modeled on many stations across Germany and Europe with the focus of acidification and eutrophication processes especially nitrogen and acid deposition. Quotations of heavy metal and POP deposition are not so frequently. These trace substances are often difficult to analyze and on account of their greater proportion on dry deposition their total deposition rates are dependent on physico-chemical interactions between acceptor surface and deposited particles. To distinguish spatial patterns of heavy metals and or POPs biomonitoring methods e.g. moss sampling or the investigation of dry deposition (Bergerhoff) are more suitable. So the question arises, if there exist a coherence between frequently determined acid and nitrogen deposition rates and element concentration in mosses.

In October 2008 on 18 deposition measurement stations in the net of German environmental agency (UBA) across Germany moss samples of *Hypnum cupressiforme*, *Pleurozium schreberi* and *Hyloconium splendens* were taken according to the ICP moss manual in a 2 km vicinity around the measurement stations. After analysis of Al, Ca, Cd, Cu, Fe, K, Mg, Mn, N, Na, Ni, P, Pb, V and Zn in the moss samples principal component analyses were done to extract main deposition factors from moss sampling (factors: traffic and combustion, soil, agriculture, sea spray, metalliferous industry) and from deposition rates (sea spray, agriculture, combustion/acidity).

With cluster analysis separately calculated for mosses and deposition rates the 18 stations were classified into 4 depositions types which correspond with each other. These types are characterized by: (1) sea spray (2) agriculture in low lands (3) midrange mountains characterized by high Pb concentration in mosses and small Ca, and K deposition (3) midrange mountains with high K, N and Ca deposition (4) like group 2 but in greater altitudes with higher K Deposition.

## INTERACTIONS BETWEEN OZONE AND DROUGHT STRESS IN PLANTS: MECHANISMS AND IMPLICATIONS

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Ozone concentrations equivalent to those frequently found in summer in the northern hemisphere either open the stomata of some sensitive species, and/or prevent stomata from closing as fully as usual in response to stresses such as drought (Mills et al 2009; Wilkinson & Davies 2009, 2010). We predict that this reinforces and exacerbates the “dose” of ozone that a plant receives, and may alter the delicate balance of plant tissue water status required for healthy plant development, eventually causing secondary reductions in growth and yield, and increasing injury and senescence (Wilkinson & Davies 2009, 2010), particularly during additional/subsequent stress.

It has long been known that ozone increases synthesis of the plant stress hormone ethylene from the leaves of sensitive plants, and sensitivity to ozone is linked to the extent of this, usually with respect to foliar injury or senescence (e.g. Overmyer et al 2008). We proposed (Wilkinson & Davies 2009) that ozone-induced ethylene could be responsible for the ozone-induced reductions in stomatal sensitivity to the “drought hormone” abscisic acid (ABA), that we observed. We confirmed ethylene’s role in uncoupling ABA and drought from stomatal closure in ozone-exposed plants (Wilkinson & Davies 2009, 2010), by demonstrating that agents which prevented ethylene perception/generation restored the stomatal closure response to soil drying or applied ABA. We predict that this occurs in response to ozone in combination with any stress that produces ABA, such as high VPD/temperature, high salinity, high light stress or nutrient deficiency.

Some genetic variability in ozone tolerance has already been attributed to inherent rates of stomatal conductance (eg. Biswas et al. 2008). We have shown that the extent of the ozone-induced loss of stomatal sensitivity to stress is also genetically determined: an ozone sensitive white clover clone (NC-S) consistently had more open stomata in the presence of ozone plus a steady soil water deficit, than its ozone resistant counterpart NC-R. We have predicted that many crop plants will be susceptible to ozone via disruption of the stomatal signalling mechanism in this way, by searching the literature for those in which ozone is known to up-regulate ethylene.

We propose that ethylene/ABA ratios also have a more direct impact on grain yield in response to ozone stress, particularly in combination with other stresses. Outside of ozone biology, a clear link between ethylene/ABA ratios and yield parameters has been demonstrated in stressed wheat (e.g. Yang et al. 2006). The possibility that ozone-induced ethylene will also have detrimental effects on grain yield and quality via this mechanism needs to be tested.

**Literature** Biswas D. K. et al. 2008. *J. Exp. Bot.*, 59, 951-963; Mills G. et al. 2009. *Global Change Biol.* 15, 1522-1533; Overmyer K. et al. 2008. *Plant, Cell & Environ.* 31, 1237-1249; Wilkinson S. & Davies W.J. 2009. *Plant, Cell & Environ.* 32, 949-959; Wilkinson S. & Davies W.J. 2010. *Plant, Cell & Environ.* 33, 510-525; Yang J. 2006. *New Phytol.* 171, 293-303.



**Abstracts**

**Posters**

## **TRACE ELEMENT ATMOSPHERIC DEPOSITION STUDY IN BELARUS BASED ON MOSS BIOMONITORING TECHNIQUE**

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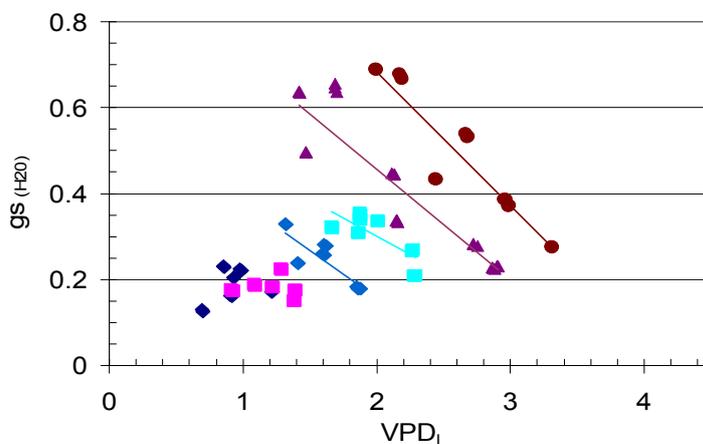
For the first time atmospheric deposition of trace elements in Belarus was monitored by the moss biomonitoring technique widely used in Europe for air pollution studies. Samples of moss species *Pleurozium shreberi* and *Hylocomium splendens* were collected at 200 sites over the Minsk, Gomel, Vitebsk and Grodno regions. A total of 40 elements was determined by Instrumental Neutron Activation Analysis carried out at the reactor IBR-2 in Dubna, Russia and at the BR1 reactor of SCK•CEN, Belgium. Geographical Information System technology was applied to construct maps of elemental distributions over the sampled areas. Principal Component Analysis allowed distinguishing heavy and light crust elements, and those of vegetation and anthropogenic origin. Comparison of the results obtained with the analogous data for the neighbouring countries showed relatively low contamination levels for most of heavy and toxic elements.

## ESTIMATION OF THE REDUCTION IN PHOTOSYNTHETIC PARAMETERS ( $V_{P_{MAX}}$ , $V_{C_{MAX}}$ , $J_{MAX}$ ) IN THE LEAVES OF MAIZE PLANTS EXPOSED TO INCREASING OZONE CONCENTRATIONS

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The toxicity of tropospheric ozone ( $O_3$ ) on maize physiology is not well known. To estimate the negative impact of  $O_3$  on maize photosynthetic parameters, a new model for the determination of stomatal conductance ( $g_s$ ) based on a multiplicative Jarvis-Stewart approach and including an original temperature/ $VPD_1$  function was designed (Fig. 1). Phytotoxic Ozone Dose values were derived from this model.



**Fig. 1:** Maize stomatal conductance as a function of leaf vapour pressure deficit at various leaf temperatures during a growth chamber experiment (●: 38.5°C, ▲: 35°C, ■: 30°C, ◆: 25°C, ■: 20°C, ◆: 15°C). Measurements were carried out on the upper leaves, using a LiCor 6400.

Leaf photosynthetic parameters were deduced from  $A/CO_2$  curves established in three experimental setups (Open Top Chamber, Growth Chamber, field Face system), using a LiCor 6400. A mean  $O_3$  concentration of approximately  $80 \text{ nmol mol}^{-1}$  induced a 50% reduction and a 30% reduction in the maximal PEP carboxylation rate ( $V_{pmax}$ ) and the maximal Rubisco carboxylation rate ( $V_{cmax}$ ), respectively (Table 1).

**Table 1:**  $V_{cmax}$  and  $O_3$  exposure indices (accumulated exposure over a threshold concentration for ozone of  $40 \text{ nmol mol}^{-1}$  (AOT40) and  $POD_0$ ) for maize leaves located near the ear. In the OTC and field experiments, stomatal conductance ( $g_s$ ) and  $POD$  values were determined according to a new model. In the Growth Chamber experiment,  $g_s$  values were measured.

	Open Top Chamber			Growth Chamber			Field Crop 2008		
AOT40 (ppm h)	0.5	10.0	12.4	0	1.4	11.5	1.2	11.7	17.8
$POD_0$ ( $\text{mmol m}^{-2}$ )	1.5	2.9	3.6	0	1.7	3.5	2.49	4.26	5.04
$POD_6$ ( $\text{mmol m}^{-2}$ )							0.18	1.37	2.13
Decrease in $V_{cmax}$ (%)	0	-5	-26	0	-7	-23.9	0	-36.8	-36.2

$POD$  values were lower than those generally determined for wheat, as a result of a more limited stomatal conductance in maize. As a consequence, maize yield parameters were not affected. In all three experimental setup,  $O_3$ -induced reductions in  $V_{cmax}$  were equivalent. To include leaf  $O_3$  detoxification capacity in  $POD$  values, a  $6 \text{ nmol m}^{-2} \text{ s}^{-1}$  threshold was arbitrarily set. However, further field experiments are required to accurately determine the extent of maize  $O_3$  scavenging capacities.

## FIRST EXPERIENCE WITH BEAN BIOMONITORING OF AMBIENT OZONE IN UKRAINE

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For the first time ambient ozone phytotoxicity has been monitored using bean (*Phaseolus vulgaris* L., ozone-sensitive (S156) and ozone-resistant (R123) genotypes) as a bioindicator plant in Ukraine, at a semi-urban monitoring station (Kyiv city) during summer period of 2009–2010. Simultaneously ambient ozone concentrations were measured using the Thermo Environmental UV Photometric Ozone Analyzer Model 49i at the experimental site. Temperature and relative humidity of the atmospheric air were also measured and recorded. During the period of biomonitoring experiments the dose of ozone was calculated using index of AOT 40. The duration of the experiments was from 22 June till 21 August in 2009 and from 27 July till 23 September in 2010. The AOT 40 index for these periods was 4124 ppb·h and 4175 ppb·h respectively.

The ambient ozone concentrations observed during the summer of 2009-2010 caused visible foliar injuries on the both ozone-sensitive and ozone-resistant genotypes of bean (Fig. 1). However, the degree of injury was significantly higher in 2010 as compared to 2009. Though, ozone-sensitive plants were injured more severely than ozone-tolerant ones, the total number of leaves per plant was higher on ozone-sensitive genotype. But these leaves were of lower size as compared to ozone-tolerant plants. There was not observed any significant differences in mean number of pods per plant between the two genotypes during the both experimental periods.



**Fig. 1.** Ozone-induced visible foliar injuries on ozone-sensitive (S156) and ozone-tolerant (R123) bean genotypes (A), ozone injuries on a leaf of ozone-sensitive (S156) bean plant (B)

Though during the biomonitoring experiments in 2010 the mean weight of pods > 4 cm was noticeably higher in ozone-sensitive plants as compared to the tolerant ones and the number of failed pods in plants of ozone-tolerant genotype was almost twice as much as in ozone-sensitive plants. Another interesting thing observed in 2010 was the earlier ripening of seeds in ozone-sensitive plants as compared to ozone-tolerant ones, which resulted in higher values of number and mean weight of seeds in the former at the end of biomonitoring experiment. Such acceleration in the seeds development observed in ozone-sensitive bean genotype is a typical adaptive reaction to environmental stress for plant species with R-strategy, such as bean. Therefore it could be considered as another evidence that ozone-sensitive plants experienced more severe stress conditions than ozone-tolerant ones.

## ESTIMATING PHOTOSYNTHESIS AND YIELD REDUCTION OF WHEAT EXPOSED TO CHANGING OZONE CONCENTRATIONS

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Two field-grown winter wheat cultivars (Premio and Soissons) were fumigated with ozone by means of a linear free air fumigation device, from anthesis to the end of grain filling stage. The mean ozone concentration at the flag leaf level was enhanced by 40% close to the linear source of ozone, and decreased downwind with the distance from the fumigation line.

Both flag leaf stomatal conductance and photosynthesis were significantly reduced by ozone exposure. No significant difference in ozone impact could be detected between the two varieties. The stomatal conductance measurement values taken on flag leaves of each variety were not significantly different from the conductance values calculated using the Jarvis-type “Mapping manual” model. Therefore, this model was used to calculate ozone stomatal uptake over the fumigation period.

The grain yields of the two varieties were not strongly affected by ozone exposure (table I).

**Table I.** Ozone exposure indicators (AOT40 and POD<sub>6</sub>) and mean grain yield of two French winter wheat cultivars exposed to free-air ozone fumigation from anthesis to grain filling. The values are means (and standard deviation) of 6 measurements.

Distance to the fumigation line (m)	2	3.5	5	Control
AOT40 (ppb.h)	8779	3731	2502	1596
POD <sub>6</sub> (mmol.m <sup>-2</sup> )	5.42	4.49	4.03	3.56
Yield (t/ha) – cv Premio	9.873 (1.069)	11.751 (0.376)	10.896 (1.448)	11.284 (0.978)
Yield (t/ha) – cv Soissons	8.341 (0.424)	9.187 (0.608)	9.928 (0.608)	10.215 (1.201)

Even though the detoxification capacity of plants may vary with time and ozone concentration levels, these results support the hypothesis of a simple linear relationship between yield loss and POD<sub>6</sub> values.

## **DOES OZONE EXPOSURE LEAD TO BIOCHEMICAL CHANGES IN BRASSICA SPECIES?**

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Tropospheric ozone is an important air pollutant with known detrimental effects on crops. This study focuses on spring oilseed rape (*Brassica napus* cv Ability) and broccoli (*Brassica oleracea* L. cv. Italica cv. Monaco); the first being important for oil production and the second crop is an important vitamin source in the human diet. Ozone can influence both yield and physiological performance of crops; this is true for oilseed rape, but no effects are reported in broccoli (De Bock et al, in press). These changes can be the result of ozone induced biochemical changes. Both crops were grown in open-top chambers, ozone was added for 8 hours per day (ambient air +20 ppb (oilseed rape) and +40 ppb (both crops)). A micro-array experiment was conducted on leaf samples taken at several point in time, to analyze which pathways are most affected by chronic ozone exposure. In addition, determination of glucosinolates and different antioxidants like ascorbate, glutathione and tocopherols were performed to correlate with the transcriptome changes.

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## TRANSFER OF ATMOSPHERIC ARSENIC, CADMIUM AND LEAD TO VARIOUS VEGETABLES

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Atmospheric deposition of trace elements has an impact on their concentration in vegetables. Leafy vegetables accumulate most efficiently deposited trace elements on their exposed leaf blades and only part of it can be removed by thoroughly washing. Exposure of vegetables for biomonitoring proved to be a good tool to study the impact of atmospheric deposition. Transfer functions have been calculated for the atmospheric deposition of Cd and Pb on leafy vegetables used for biomonitoring purposes (De Temmerman and Hoenig, 2004). To meet the conditions referring to the maximum tolerable values in the European legislation (EU, 2001; EU, 2008) it is better to establish transfer functions on washed instead of unwashed leafy vegetables. The vegetables studied were spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), endive (*Cichorium endivia*), lamb's lettuce (*Valerianella locusta*), celery (*Apium graveolens* var. *secalinum*) and curly kale (*Brassica oleracea* convar. *acephala* var. *laciniata*) as leafy vegetables, carrots (*Daucus carota*), and celeriac (*Apium graveolens* var. *rapaceum*) as root crops (peeled storage organs), and also some preliminary results about onion (*Allium cepa*) and bean (*Phaseolus vulgaris*) were added.

The test plants were grown in a reference area with low atmospheric deposition and then exposed in a polluted area around a lead smelter for 14 days (spinach and lettuce), 28 days (endive, green beans and lamb's lettuce), 2 months (onion celery, curly kale and carrots) or 4 months (celeriac). In most cases the containers were placed near gardens where vegetables were cultivated.

At each experimental plot, the containers were exposed in triplicate allowing statistical treatment of the results. After exposure, the edible parts, leaves, fruits, bulbs were cleaned, washed and analyzed. Also the unwashed leaves were analyzed for comparison reasons. The storage organs of carrot and celeriac were cleaned, washed thoroughly and peeled. The peels and the inner part (pulp) were analyzed separately.

At each site, bulk deposition was measured in order to be able to link the results to atmospheric deposition.

Leafy vegetables accumulate most efficiently air borne trace elements and primarily the late season vegetables are showing the highest accumulation rate due to their reduced growth and biomass increase. The storage organs of celeriac and carrot accumulate airborne Cd, Pb and As but in the inner part only a significant accumulation of As and Cd was observed. For lead there is no significant increase. Preliminary results for green beans revealed a clear impact of airborne As and Pb but not for Cd. For onion bulbs, however, there is a clear impact of As and Cd deposition but no effect of Pb deposition was noticed.

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## A REVIEW OF MOSS BIOMONITORING IN ROMANIA

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The first systematic study in Romania of atmospheric pollution from heavy metals and other toxic elements based on moss analysis was undertaken as a Romanian–Russian–Norwegian collaboration, in order to assess the general state of heavy metal pollution in Romania in the period 1995–2001 (Lucaciu et al., 2004 and the citations herein). The participating institutions were: National Institute of Physics and Nuclear Engineering (NIPNE) “Horia Hulubei” Bucharest and “Al.I. Cuza” University of Iasi (UAIC), Romania; Joint Institute for Nuclear Research (JINR) Dubna, Russia, and Norwegian University of Science and Technology, Trondheim, Norway. The results on moss samples collected in different regions of Romania in 1990, 1995 and 2000 were unified and reported by Harmens et al., 2008; Romania did not submit data for the 2005/6 European moss survey.

In present in Romania are carried out activities related to implementation of nuclear and related techniques for the analysis of environmental bio-monitors, including mosses (Ene A., 2009; Popescu et al., 2009), by a consortium consisting of “Valahia” University of Targoviste (UVT), “Dunarea de Jos” University of Galati (UDJG) and NIPNE (National Project, 2008) and moss sampling from the sites covered in previous surveys (Bilateral Project JINR-Romania, 2010). The Romanian teams from UVT, UDJG, UAIC and University of Baia Mare (UBM) collected approx. 300 samples (specified by Lucaciu et al., 2004) in summer-autumn time during 2010, in the Carpathian Mountains, Transylvanian plateau, and Prut river catchment, following internationally accepted guidelines. The goal is to contribute to the European moss survey 2010/11, conducted under the auspices UNECE ICP Vegetation, covering some “white areas” on the map of atmospheric deposition of heavy metals in Europe (Harmens et al., 2008). The concentrations of elements will be determined using NAA (at JINR) and AAS (at UVT) and compared to those observed in previous surveys.

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## SNAP BEAN PERFORMANCE IN CRETE/GREECE

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The performance of the S156/R123 snap bean (*Phaseolus vulgaris* L.) as a potential bioindicator system was evaluated in Crete (Greece) in the summers of two consecutive years (August – September in 2009 and 2010). Seeds were supplied by the ICP-Vegetation Co-ordination Centre at Bangor, UK. Plants were grown at a field site (latitude 35°20'N, longitude 25°8'E, 10 m.a.s.l.) situated in the suburbs of Heraklion, using a standardised protocol developed by the UNECE ICP-Vegetation appropriate to the proposed investigations. Pods were separated into (a) mature or sterile and (b) two size classes, larger than 4cm and smaller than 4cm, counted, dried to constant weight at 70°C, and weighed. Stomatal conductance and physical variables were monitored and ozone injury was assessed. Meteorological data, ozone exposure information, pod biomass and visible injury assessment are summarized in Table 1.

**Table 1.** Meteorological data, ozone exposure information, biomass data and visible injury assessment over the course of the field experiment conducted on snap bean (*Phaseolus vulgaris* L.) S156 and R123 biotypes in the suburbs of Heraklion, Crete.

Growth period	1/8 – 30/9/2009	23/7 – 29/9/2010
24 h mean temperature (°C)	24	27.1
24 h mean VPD (kPa)	1.1	1.3
Mean daytime PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	485	398
AOT40 (ppb h)	418	4,223
S156 mature pod (number per plant)	25.9	100.8
S156 sterile pod (number per plant)	10.9	39.1
S156 mature pod weight (g dry wt. plant <sup>-1</sup> )	20.1	61.8
S156 average mature pod weight (g dry wt. pod <sup>-1</sup> )	0.8	0.6
S156 damaged leaves (scored as >25% injury on 29/9)	57.1	52.0
R123 mature pod (number per plant)	25.7	74.2
R123 sterile pod (number per plant)	5.0	207.4
R123 mature pod weight (g dry wt. plant <sup>-1</sup> )	30.1	59.4
R123 average mature pod weight (g dry wt. pod <sup>-1</sup> )	1.1	0.8
R123 damaged leaves (scored as >25% injury on 29/9)	14.7	1.0
Yield ratio (S156:R123)	0.67	1.04

Genotypes R123 and S156 developed the same number mature pods but the biomass per mature pod was greater for R123 in both years. The sensitive genotype showed more extensive visible injury and that led to yield ratio 0.67 (S156:R123) in the first experimental year. In contrast, in 2010, despite the higher O<sub>3</sub> concentration (Table 1), no differences were found in the yield. The discrepancy may be a result of the early harvest of resistant plants' pods and might be related to the protocol changes. Abaxial surface stomatal conductance measurements were recorded on the 25<sup>th</sup>, 33<sup>rd</sup>, 40<sup>th</sup> and 48<sup>th</sup> experimental day on the most recently fully-expanded leaf. No differences were found between the two genotypes apart from the last measurements in the second year when stomatal conductance was greater for R123 genotype and this was consistent with the younger plant phenological stage.

## VISIBLE LEAF INJURIES ON "BABY LEAF" SPINACH LEAVES IN SWEDEN

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Visible leaf injuries were surveyed in collaboration with a commercial grower of “baby-leaf” spinach leaves in southwest Sweden from May until September 2010. Local ozone concentrations as well as meteorology were measured nearby with an hourly time resolution.

Visible leaf injuries were detected on spinach leaves at two occasions, 2010-06-01 and 2010-06-17. The injuries were typical for ozone damage (Figure 1) and the experienced commercial growers confirmed that they could not explain these injuries with other factors. The main batch of spinach leaves had been harvested before the ozone-induced leaf damage started to develop, so there was no economic damage. Preliminary assessments of the ozone concentrations during the weeks before these occasions indicated that the ozone concentrations had probably not exceeded 55 ppb. On 2010-07-10 there was an ozone episode in southern Sweden with ozone concentrations up to 80 ppb for two days. No leaf visible injuries could be detected on the spinach leaves after this ozone episode. These differences in ozone induced leaf injuries between June and July was probably explained by differences in the rotation period between sowing and harvests. In May/June the growth rates of the spinach were slower and the leaves were harvested about 8 weeks after sowing. In July, however, the growth rates were very high and the spinach leaves were harvested already about 3 weeks after sowing. Most likely time did not permit ozone injury to develop before the leaves were harvested in July.

Hence, the conclusion so far is that the ozone induced leaf visible injuries did not cause major economic damage to the commercial growers, since the leaves were harvested before the damage started to develop.

**Figure 1.** Ozone-induced leaf visible injuries on spinach leaves detected in southwest Sweden 2010-06-01.



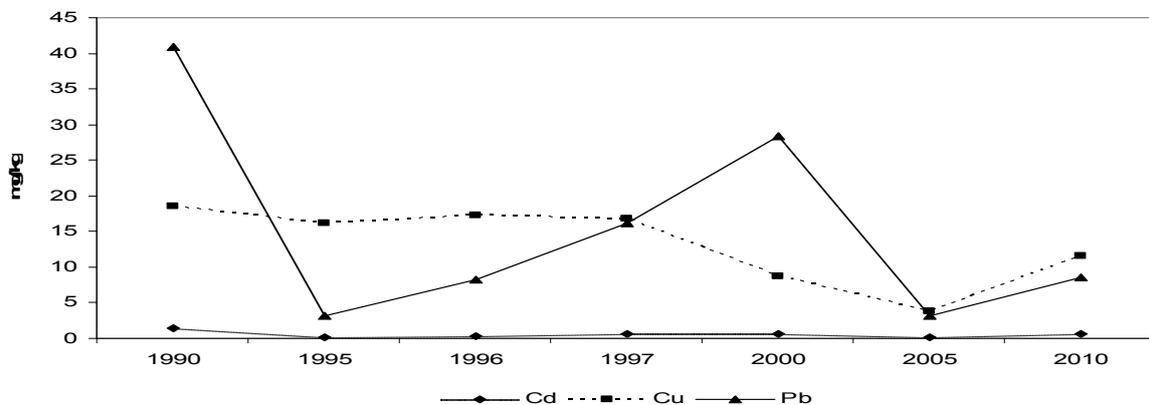
# TEMPORAL AND SPATIAL TRENDS (1990- 2010) IN CD, CU, N, PB AND S ACCUMULATION IN MOSSES IN SLOVAKIA

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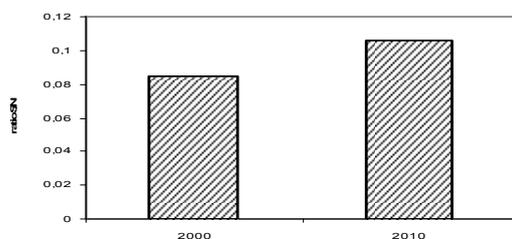
Biomonitoring of multielement atmospheric deposition using terrestrial moss is a well-established technique in Europe. The moss samples of *Hylocomium splendens*, *Pleurozium schreberi* and *Dicranum* sp. were collected in the Slovakia. In comparison to the median northern Norway values of heavy metal contents in moss the Slovak atmospheric deposition loads of the elements were found to be the survey has been repeated and in this paper we report on the temporal trends in the concentration of Cd, Cu, Pb, between 1990 and 2010. Metal- and sites -specific temporal trends were observed. In general, the concentration of Cd, Cu, and Pb in mosses decreased between 1990 and 2010; the decline was higher for Pb than Cd. The observed temporal trends for the concentrations in mosses were similar to the trends reported for the modelled total deposition of Cd, Pb in Europe. The level of elements determined in bryophytes reflects the relative atmospheric deposition loads of the elements at the investigated sites. Factor analysis was applied to determine possible sources of trace element deposition in the Slovakian moss. Ratio S/N transform also between 2000 and 2010.

Concentration of Cd, Cu and Pb (median) in mosses for Slovakia in all survey years



Note: Year (number of PMP): 1990(58);1995(79); 1996(69); 1997 (74); 2000 (86); 2005(82);2010 (69)  
 PMP- permanent monitoring plots

Ratio S/N in mosses for Slovakia in 2000 and 2010



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**Keywords:** Air pollution, Bryomonitoring, Heavy metals; Moss survey

## TESTING THE RESPONSE OF SOME EGYPTIAN PLANT SPECIES TO OZONE

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Currently cultivated varieties of two Egyptian ozone bioindicator plant species (Jute and Garden rocket) previously identified and tested only in environmentally controlled conditions (Madkour and Laurence, 2002), were further screened to identify their relative sensitivity/tolerance both under controlled and field conditions.

Screening tests under controlled conditions (80 ppb O<sub>3</sub> for 3days, 8h/day) ranked the sensitivity of the four Jute ecotypes in the descending order: Balady, Fallahy, Saigy and Siwi; and that of the four Garden rocket genotypes: Wady, Wahat, Menufiya and Nahya. These experiments revealed that none of the varieties of the two plant species currently cultivated in Egypt were significantly tolerant. In both plant species, a decrease in total chlorophyll content, leaf greenness, chlorophyll *a* and *b*, carotenoids and quantum yield of photosynthesis was recorded as a result of ozone exposure and found to be proportional to the degree of foliar injury on the different entries. Decrease in stomatal conductance was recorded in both Jute and Garden rocket plants, pointing to the fact that stomatal closure may be involved with the inhibition of photosynthesis in those plant species. The suitability of those selected bioindicator plant species for use as ozone biomonitors under European (Greek) open field conditions was tested. AOT40's values within the critical range for crops (3 - 4 ppm h) caused both Jute and Garden rocket plants to exhibit symptoms of O<sub>3</sub> injury in the open field. Both Jute ecotypes and Garden rocket genotypes retained their sensitivity in open field experiments. They showed distinctive symptoms. The percentage of leaf injury reflected the same ranking obtained in environmentally controlled experiments. Foliar injury was even more severe in the open field. Jute and Garden rocket varieties have proved to be stable as active bioindicators of O<sub>3</sub> pollution close to critical background levels (40 ppb).

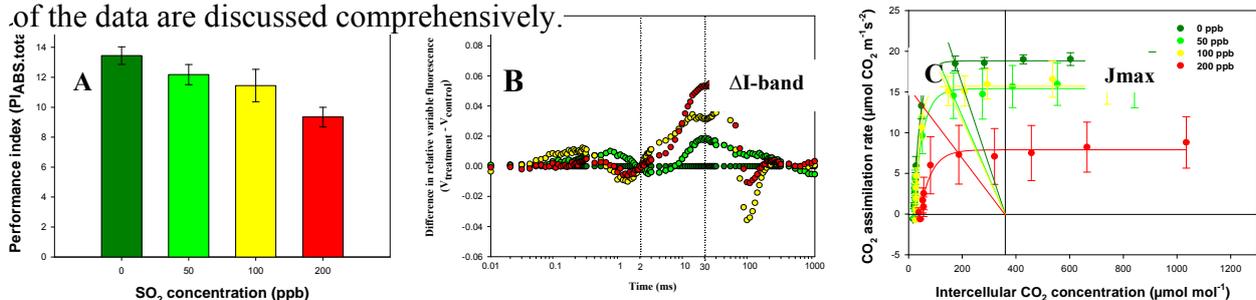
# CONSTRAINTS ON PS II FUNCTION AND CO<sub>2</sub> ASSIMILATION OF THE C<sub>4</sub> CROP, *Zea mays*, IMPOSED BY SO<sub>2</sub> AND DROUGHT

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Considerable increases in fossil fuel burning have led to increases in tropospheric air pollutants such as SO<sub>2</sub>, O<sub>3</sub> and NO<sub>2</sub>. These increases, combined with climatic variations are the subject of much concern in agricultural sectors of southern Africa [1]. The aim of this study was to quantify the effect of increasing SO<sub>2</sub> concentrations and the interaction with drought on the photosynthetic capacity of maize plants. Maize plants were cultivated in open top chambers (OTCs) for an entire growth season and subjected to SO<sub>2</sub> enriched air (50, 100 and 200 parts per billion) for eight hours/day. Control plants received carbon filtered (CF) air. Drought stress was induced in half of the plants of each treatment. Chlorophyll *a* fluorescence induction and photosynthetic gas exchange measurements were conducted in parallel on weekly basis. Gas exchange parameters were deduced from CO<sub>2</sub>-response curves, whereas chlorophyll *a* fluorescence transients were analysed according to the JIP-test [2] and translated into parameters of PS II function. The measured SO<sub>2</sub>-induced decrease in photosynthetic capacity was mainly due to inhibition of phosphoenol pyruvate (PEP) regeneration rate (54% decrease in J<sub>max</sub>, Fig. 1C) and secondly to inhibition of PEPcarboxylase, namely the SO<sub>2</sub>-concentration dependant decrease of the initial slope of the demand function (Fig. 1C). The fluorescence data complemented gas exchange data, showing that the SO<sub>2</sub> concentration dependent decrease in PSII function (decrease in the photosynthetic performance index, PI<sub>ABS.total</sub> (Fig. 1A)) could be attributed to the decrease in the reduction of end electron acceptors beyond PS I such as NADP<sup>+</sup> (Fig. 1B). A significant decrease in yield could be seen in both well watered and drought stressed plants. Implications of the data are discussed comprehensively.



**Figure 1A:** Effect of 7 weeks' fumigation with different SO<sub>2</sub> concentrations on the photosynthetic performance index (PI<sub>ABS.total</sub>) of drought treated maize plants. The performance index (PI<sub>ABS.total</sub>) is based on the fast phase chlorophyll fluorescence rise. The PI<sub>ABS.total</sub> is a multi-parametric function taking into account all partial processes of primary photochemistry.

**Figure 1B:** Average fluorescence transients normalised between F<sub>0</sub> (0.001 ms) and F<sub>J</sub> (2 ms). ΔV curves were obtained by deducting the variable fluorescence of the treatment from that of the control plants (ΔV = V<sub>treatments</sub> - V<sub>control</sub>). Each plot represents a mean (± SE) of 4 plants measured.

**Figure 1C:** CO<sub>2</sub>-response curves of drought stressed maize plants exposed to different SO<sub>2</sub> fumigation levels at the 7<sup>th</sup> week of fumigation.

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# MOSES (*HYLOCOMIUM SPLENDENS* AND *PLEUROZIUM SCHREBERI*) AS BIOMONITORS OF ATMOSPHERIC NITROGEN DEPOSITION IN BOREAL FORESTS

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The suitability of the boreal forest mosses, *Hylocomium splendens* and *Pleurozium schreberi*, as biomonitors of nitrogen deposition will be investigated in the years 2009-2013 in Finland on the plots of National Forest Inventory (NFI) and on the intensive monitoring plots of forest ecosystems (ICP Forests, Level II). The studies contribute to use of mosses as biomonitors, and will provide new information about the biological response of forest plants to nitrogen deposition. In this paper, we present preliminary results of nitrogen concentrations in mosses in relation to nitrogen deposition on the intensive monitoring plots in 2009.

The studies were carried out on six Norway spruce plots and on five Scots pine plots in the different parts of the country. Moss samples were collected monthly (V–X) as 10 x 10 cm quadrates (three repetitions) from the pure moss carpets between the trees. The last three full year's growth (= practically green parts) was separated monthly and the most recent growth in October for nitrogen analysis. Furthermore, the number of mosses and dry weight of the last three full year's growth per dm<sup>2</sup> were estimated. Nitrogen deposition (N<sub>tot</sub>, NH<sub>4</sub>-N, NO<sub>3</sub>-N) was measured at 4-week periods (bulk deposition in open area and in stand throughfall).

There was a remarkable difference in the density of *H. splendens* and *P. schreberi* in pure moss carpets per area. The mean number of *H. splendens* was on the plots 58 per dm<sup>2</sup> and the mean number of *P. schreberi* 163 per dm<sup>2</sup>. The difference in the densities between these moss species was similar in spruce and pine stands. The average weight of the last three full year's growth was for *H. splendens* 3.4 g and for *P. schreberi* 4.5 g per dm<sup>2</sup>. Nitrogen concentration in *H. splendens* (on an average 0.89 %) was on the same plots usually slightly greater than in *P. schreberi* (0.86 %). Furthermore, nitrogen concentrations were higher in spruce stands as in pine stands on the same locality. Nitrogen concentration in the senescing segments decreased to some extent during the growing season, and the concentration of the new segment was in autumn clearly higher as those of the senescing ones.

Nitrogen deposition varied greatly on the plots monthly. However, nitrogen concentration of both moss species correlated very well with the total nitrogen deposition of the growing season in stand throughfall ( $r^2$  for *H.s* = 0.94;  $r^2$  for *P.s* = 0.94), and with NH<sub>4</sub>-N deposition in stand throughfall ( $r^2$  = 0.83; 0.79) and in the open area ( $r^2$  = 0.85; 0.88). It did not correlate so well with NO<sub>3</sub>-N deposition in stand throughfall ( $r^2$  = 0.67; 0.62) and with total nitrogen deposition ( $r^2$  = 0.69; 0.50) and especially with NO<sub>3</sub>-N deposition ( $r^2$  = 0.18; 0.17) in the open area.

The regional differences in nitrogen concentrations of mosses in the similar habitats were caused mainly by nitrogen deposition. The concentrations in mosses varied also in relation to the characteristics of the tree stand. The density of mosses had probably partly an influence on the differences between moss species in accumulation of nitrogen.

**SPATIAL DIFFERENCES IN DEPOSITION PATTERNS OF SOME ELEMENTS  
MEASURED IN TWO SUBSEQUENT MOSS SURVEYS IN THE NORTHERN PART  
OF SERBIA (2000-2005)**

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The pilot study of trace element deposition using the moss technique was carried out in the northern part of Serbia in 2000. The study was repeated in 2005 covering almost all area of the Republic of Serbia. Both sets of samples were subject to instrumental neutron activation analysis (INAA) for determination of more than 40 elements. Significantly larger amounts of some specific pollutants were emitted into the atmosphere in the spring of 1999 (when two largest oil refineries and several chemical plants were bombarded) than in the period till 2005. Comparison of the 2005 and 2000 results for the same sites and the same moss species allowed exploring difference in spatial deposition patterns of several elements of interest. Ratios of values measured in the first and the second survey were calculated for all elements. Calculated ratios show significant changes in the depositional pattern of several oil burning elements. For example concentrations of V and Cl in samples collected in the vicinity Oil refinery near to Novi Sad town were three times higher in the 2000 than in 2005 moss survey. The both set of moss data (2000 and 2005) from Serbia were subjected to principal component factor analysis as well as the set of the 2000/2005 ratio data. Factor analysis of the 2000/2005 ratio indicated several groups of the elements having similar trend of changes in two subsequent moss surveys.

## A THREE YEARS PILOT STUDY TO EVALUATE FLUORESCENCE ANALYSIS IN OZONE BIOMONITORING WITH BEAN CLONES NEAR ROME, ITALY

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An ozone (O<sub>3</sub>) biomonitoring experiment was conducted during the years 2008, 2009 and 2010 in a natural area inside the Castelporziano Presidential Estate (Rome, Italy), characterized by a typical Mediterranean climate (Manes et al., 1997). Following the UNECE ICP Vegetation protocols (UNECE, 2008-2010), the O<sub>3</sub>-sensitive (S156) and ozone-resistant (R123) genotypes of *Phaseolus vulgaris* L. were used. 10 to 12 replicates plants per clones, fully irrigated, were exposed to ambient air for approximately 60 days from mid July 2008 mid June 2009 and end of June, 2010. O<sub>3</sub> concentrations and climatic parameters were continuously monitored in the experimental site, leaf visible injury was evaluated weekly every year and, at the end of each experiments, pods were harvested and yield evaluated. Moreover, during the years 2009 and 2010, gas exchanges and direct chlorophyll “a” fluorescence were measured on fully developed leaves, during selected phenological phases, and in particular close to the onset of flowering. AOT40 during the experimental periods (from day 0 to harvest) was 3588, 9273, and 3324 ppb in 2008, 2009 and 2010, respectively. A clear distinction in the extent of visible leaf injury symptoms between the S and R biotypes was apparent (UNECE- ICP Vegetation, 2009), although during 2010 also the R variety showed injury symptoms. However, differently from what reported in previous studies (Flowers et al., 2007) no clear relationship between the extent of leaf injury, pod yield and ozone levels were evident across years: production of developed pods was in fact higher in the R clone only in 2008 (S156/R123 = 0.86), and higher in the S clone during the years 2009 and 2010 (S156/R123 = 3.46 and 1.12 in 2009 and 2010, respectively). Stomatal conductance and net photosynthesis in the 2009 assessment were slightly lower in the S than in the R clone, while in the 2010 assessment the S clone had slightly higher gas exchange values, both before and after flowering. The chlorophyll fluorescence measurements and the application of the JIP-test (Strasser et al., 2010) give important insight for early evaluation of differences in photosynthetic efficiency between genotypes. The fluorescence transients analysis highlights a similar behaviour of both clones, and the OJIP fluorescence parameters did not show the typical ozone-induced stress response. Both clones appear instead affected by the summer climatic conditions, and particularly by high temperatures and irradiance values. Moreover, this response seems to vary with plant developmental stages (Elagöz and Manning, 2005). Further studies are therefore needed to better investigate the applicability of this biomonitoring system under environmental conditions typical of the Mediterranean area.

### References

- Elagöz V., Manning W.J., Environmental Pollution 136 (2005) 371-383.  
Flowers M.D. et al., Environmental and Experimental Botany 61 (2007) 190–198

# EFFECT OF OZONE ON PHOTOSYNTHESIS AND SEED YIELD OF SENSITIVE (S156) AND RESISTANT (R123) *Phaseolus vulgaris* L. GENOTYPES IN OPEN-TOP CHAMBERS

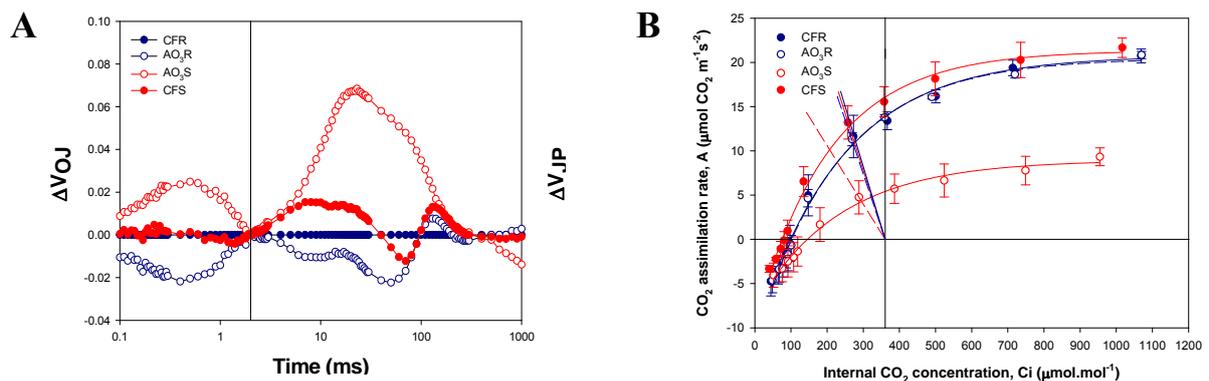
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Rising tropospheric ozone (O<sub>3</sub>) concentrations have been identified as a significant threat to crop production. In the present study two snap bean (*Phaseolus vulgaris* L.) genotypes with known difference in sensitivity to O<sub>3</sub>, namely S156 (sensitive) and R123 (resistant) were compared with respect to their response to O<sub>3</sub> using open-top chambers (OTCs) to elucidate the physiological and biochemical basis of O<sub>3</sub> effects. Seedlings were exposed to two different controlled levels of O<sub>3</sub>, namely 0 and 80 nmol.mol<sup>-1</sup>. Chlorophyll *a* fluorescence and photosynthetic gas exchange were measured in parallel throughout the growing season. Yield data were collected at physiological maturity. The physiological O<sub>3</sub> induced effects were evident long before visible damage appeared in the S156 genotype. While limited data on photosynthesis is available for S156, no data could be found for R123 [1]. Photosynthesis was largely inhibited in the S156 genotype, mainly due to inhibition of the photosynthetic electron transport chain as indicated by the prominent ΔK and ΔI fluorescence bands (fig. 1A) and large decrease of the demand function resulting in decreased reduction of end electron acceptors with consequential reduced carboxylation efficiency and regeneration capacity of RuBP (fig. 1B). Our study corroborated the results of previous OTC studies showing that under moderate O<sub>3</sub> stress, seed yield in S156 is suppressed, while the effects on R123 are minimal [2]. The seed yield data corresponded well to the photosynthetic response of the test plants. Though the JIP-test is conducted in the dark adapted state it provides a measure of the potential of the whole photosynthetic electron transport chain.



**Figure 1A:** Fluorescence transients, normalised between steps O and J (2ms) and between J and P. Both these partial transients were plotted as difference kinetics,  $\Delta V = V(\text{treatment}) - V(\text{control R})$ .

**Figure 1B:** Average CO<sub>2</sub> response curves (A: Ci) for intact leaves of R123 and S156 *P. vulgaris* genotypes after 25 days exposure to filtered air (CF) and 80 nmol.mol<sup>-1</sup> ozone (AO<sub>3</sub>) respectively.

[1] Heagle AS, Miller JE, Burkley KO, Eason G. and Pursley WA. (2002). Growth and yield responses of snap bean to mixtures of carbon dioxide and ozone. *J. Environ. Qual.* 31, 2008-2014.

[2] Burkley KO. and Eason G. (2002). Ozone tolerance in snap bean is associated with elevated ascorbic acid in the leaf apoplast. *Physiol. Plant.* 114, 387-394.

## MOSS SURVEY 2010 IN CROATIA

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For the second consecutive time Croatia participate in moss 2010 survey in the framework of the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops heavy metals in Europe. Moss samples were collected during the summer/autumn of 2010 from 124 sites evenly distributed over the country (Fig. 1).

Moss sampling was carried out according to the guidelines of the ICP Vegetation using a 23x23 km network. Areas with expected higher air pollution were covered with a denser sampling network. Samples were prepared for the analytical measurements (Fig. 2).



**Figure 1** Moss samples locations in Croatia



**Figure 2** Moss samples prepared for analytical measurements

This study is undertaken in order to provide a reliable assessment of air quality throughout Croatia and to better characterize the pollution sources identified in the first moss survey in Croatia in 2006 [1]. Information on atmospheric deposition of trace elements obtained by two complementary analytical techniques – AAS and NAA - will serve for assessing the environmental and health risks in Croatia associated with toxic elements.

[1] Spiric Z., Frontasyeva M.V., Steinnes E., Stafilov T.: Multi-element atmospheric deposition study in Croatia, International Journal of Environmental Analytical Chemistry (2010) - In press.

## MULTI-ELEMENT DISTRIBUTIONS IN PLANT, HUMUS AND TOP SOIL COMPARTMENTS OF MATURE CONIFEROUS FOREST STANDS IN THE CZECH REPUBLIC

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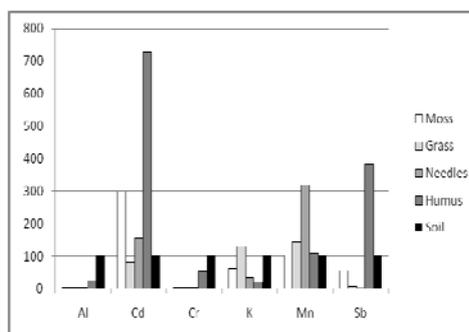
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The element distribution in compartments of a coniferous forest, at the interface between the atmosphere and the geosphere, results from element uptake by plants from soil, humus and atmospheric deposition, and redistribution of the elements in plants and in the forest ecosystem. Only a small amount of data on multielement distribution in compartments of coniferous forests has been published.

Samples of Schreber's big red stem moss (*Pleurozium schreberi*), leaves of Wavy Hairgrass (*Avenella flexuosa*), two-year-old Norway spruce needles (*Picea abies*), forest floor humus (H, Oh) and top soil (B1 horizon) were collected at 254 - 280 plots in mature coniferous forests across the territory of the Czech Republic, which is highly variable in geological, geomorphological and climatic conditions. The total concentrations of 37 - 41 elements (Ag, Al, As, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Hg, In, K, La, Li, Mg, Mn, Mo, N, Na, Nd, Ni, Pb, Pr, Rb, S, Sb, Se, Sn, Sr, Th, Tl, U, V, Y and Zn) were determined in the samples, using ICP-MS methods.

In forests, the mineral topsoil is the main potential pool of most of the investigated elements (Ag, Al, Ba, Be, Ce, Co, Cr, Cs, Fe, Ga, K, La, Li, Mg, Na, Nd, Pr, Rb, Sr, Th, Tl, U, V and Y). No significant difference in median concentrations of As, Mn, Ni and Zn was found for forest floor humus and mineral top soil. However, humus was revealed to be the most effective element accumulator, containing Bi, Co, Cd, Cu, Hg, Mo, Pb, S, Sb, Se and Sn in amounts 2-7 times higher than mineral top soil. The plant species accumulate mainly biogenic elements (Ca, K, Mn, S) and some toxic elements, e.g. Cd.

In industrial areas with higher atmospheric deposition levels, the concentrations of the elements in the indicators increased substantially in the humus and plant compartments, in the order humus > moss > biennial needles > grass, while the element concentrations in top soils remained unaffected.



Relative median concentrations (soil = 100 %) of selected elements in the analysed materials.

## OVERVIEW OF THE SLOVENIAN BEAN EXPERIMENT IN THE LAST YEARS

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The ICP-Vegetation programme study “Yield response and ozone injury on *Phaseolus vulgaris*” has been running for three subsequent years in Ljubljana, Slovenia. In the study we are using ozone resistant (R123) and ozone sensitive (S156) genotypes of bush bean selected in North Carolina, USA. Each year 10 plants of R type and 10 plants of S type were potted alternately in two rows of self watered pots. The setup of experiment, assessment of ozone injuries and final harvest procedure were performed according to ICP-Vegetation protocol.

Some comparison between measurements and observations of ozone concentration and impact on bean plants in the years 2008, 2009 and 2010 are presented. The plants were exposed on 3<sup>rd</sup> of July 2008, 15<sup>th</sup> of June 2009 and on 16<sup>th</sup> of June 2010. AOT40 values were relatively low in all three years, exceeding 250 ppbh only on few occasions each year. The AOT40 values were higher in the first half of the exposure period, especially so in year 2010.

Higher weekly cumulative AOT40 values were gathered in the first 5 weeks in 2008 and 2010 and between the 4<sup>th</sup> and 6<sup>th</sup> week in 2009, with highest values around 1200 ppbh. The “Injury index”, calculated as a function of the number of leaves in each injury class, showed some inconsistency between years. In 2008 the indices of both genotypes were highest in the 8<sup>th</sup> week. The differences between genotypes were small. In 2009 the injuries started to show later in the experiment (in accordance with maximal AOT40 values) and were almost twice as high in the sensitive genotype, compared to the resistant one. In 2010 the senescence of sensitive genotype was obvious, and in the 8<sup>th</sup> week plants were completely leafless. The results suggest difficult direct comparison of the injuries between years and complex relations between visible condition of plants and ozone concentrations.

Very variable results were found counting the seedless pods and pods with seeds. In 2008 the average number of all pods on resistant plants was 38 (32 with seeds, 6 without) and on sensitive plants 26 (20 with seeds, 6 without). In 2009 the average total number of pods was much higher in sensitive plants with 56 (29 with seeds, 27 without) than in resistant with 35 (29 with seeds, 6 without). In 2010 average total number of pods in resistant type was 26 (12 with seeds, 14 without) and in sensitive clone 28 (13 with seeds, 15 without). As one can see from the mentioned numbers, the ratio between fertile and sterile pods can vary greatly.

More consistent were the results on the number and weight of seeds. In all three years the average number and average mass were approximately twice as high in resistant plants as in sensitive ones. Due to some unresolved reason, the number of seeds in 6 resistant plants in year 2010 was extremely low (0, 6, 9, 9, 9 and 19 seeds).

The quick conclusions of this three year overview would be that the visual condition of plants, number and weight of seeds, number of fertile and sterile pods and their ratio, and the senescence of plants can vary greatly, between the years and between the individuals, so the ozone effects can remain hidden in the bias.

## USE OF MOSSES IN AN URBAN AREA, BULGARIA

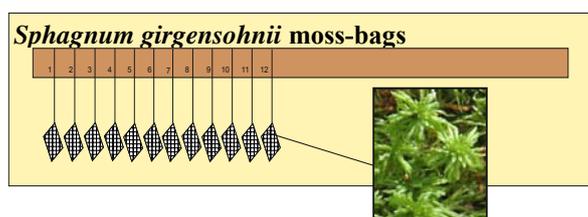
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This study is a small part of a large program for passive and active biomonitoring with tree leaves, tree-rings, herbaceous species, mosses and lichenized fungi applied for a wide evaluation of the urban conditions in various towns within the Balkan Peninsula.

*Sphagnum girgensohnii* Russ., grown in a background montane area (42°37' N, 23°19' E, altitude 1710 m), was used as a suitable active biomonitor of air pollution. Moss-bags were exposed at four important sites in the town of Plovdiv (42°08' N and 24°45' E; north, east, west and central part) from May to October 2010. *Hypnum cupressiforme* Hedw. on ground was collected at three main hills in the town area.



*Hypnum cupressiforme*



The concentrations of 26 micro- and macroelements were analyzed by FAAS (Zn, Fe, K, Mg, Mn, Na, Cu, Ca) using Atomic Absorption Spectrometer PERKIN-ELMER 4000 and ICP-MS (Be, B, Al, S, P, Cr, V, Co, Ni, As, Se, Sr, Mo, Cd, Hg, Pb, Bi, U) with instrument Agilent 7700 (2009).

For the 30, 90 and 150 days periods the elements Na, Al, Ca, V, Cr, Fe, Co, Ni, Cu, Zn, Sr, Cd, Pb, and Hg increased their concentrations in moss-bags. The accumulation found was with highest significance for Zn, Cd, Pb, Cr, Cu and Na, due to heavy motor vehicle traffic, power plants and non-ferrous metallurgy located at 5 km (SE) from the town. The relative accumulation factors (RAF), calculated as mean content of each element after 150 days of exposure in all locations divided by the content of each element before exposure, was highest for Cr (6), followed by Al, Co, Fe, Na, Ni, V and Zn (3-4). The elements K (up to 7 times), B, P and Mo decreased their values in comparison with the unexposed *Sphagnum girgensohnii*. No significant changes were proved for the rest of analyzed elements.

Between different sites was found a tendency to enlarge the number of maximal values of chemical elements by approaching to the central part, along one of the major traffic arteries.

*Hypnum cupressiforme* in Plovdiv accumulated more Cd (9-fold), Hg (7-fold), Pb (4-fold), and Zn, V, Cu (3-fold) as compared to the medium values in EU Moss Survey, 2005.

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