

# 7<sup>th</sup> International Workshop on Biomonitoring of Atmospheric Pollution

14<sup>th</sup>-19<sup>th</sup> June 2015 - Lisbon, Portugal



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## BOOK OF ABSTRACTS

Instituto Superior Técnico  
Universidade de Lisboa



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## FOREWORD

The BIOMAP 7 National Organizing Committee from the C<sup>2</sup>TN - Centro de Ciências e Tecnologias Nucleares (Instituto Superior Técnico, Universidade de Lisboa) will hold, with the high patronage of his Excellency, the President of the Republic of Portugal, the 7<sup>th</sup> International Workshop on Biomonitoring of Atmospheric Pollution (BIOMAP 7). The event will take place in the Pavilion of Knowledge - Ciência Viva (Lisbon) between 14<sup>th</sup> and 19<sup>th</sup> of June 2015.

This is the third time that BIOMAP is organized in Portugal and it is a pleasure and an honor for C<sup>2</sup>TN - Centro de Ciências e Tecnologias Nucleares (Instituto Superior Técnico, Universidade de Lisboa) to organize such an important meeting of researchers, policy makers and practitioners in environmental sciences from all over the world.

There is a growing need for air and biological monitoring within the preventive context of identifying health hazards at the environment and of keeping them under control. Biomonitoring is a sensitive, selective and user-friendly method for air quality management. The objective of BIOMAP 7 is to promote knowledge on methods and strategies for workplace, indoor and ambient air monitoring. In addition to the specific issues related to biomonitoring as a technique, this workshop specifically addresses the potential use of biomonitoring data in assessing the human exposure to toxic substances.

BIOMAP 7 aims to provide an opportunity for an interchange of ideas among researchers, policy markers and practitioners in environmental sciences.

Therefore, the Organizing Committee is pleased to announce an exciting innovative congress, with scientific presentations covering a wide range of topics.

The Organizing Committee looks forward to your presence and participation to continue the Excellency of the previous BIOMAP series.

Lisbon, 14<sup>th</sup> June 2015.

The National Organizing Committee

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# PROGRAM

7<sup>th</sup> International Workshop on Biomonitoring of Atmospheric Pollution  
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## GENERAL

	15 <sup>th</sup> June	16 <sup>th</sup> June	17 <sup>th</sup> June	18 <sup>th</sup> June	19 <sup>th</sup> June
10:00 - 11:00	Opening ceremony (10:00-10:30)	<b>Session 3: Organic pollutants</b> Plenary with Sofia Augusto	<b>Session 5: Monitoring long- term and large- scale deposition of pollutants</b> Plenary with Marina Frontasyeva	<b>Session 6: Air quality management</b> Plenary with Roeland Samson	<b>Session 8: Biomonitoring of atmospheric pollution using magnetic properties</b> Plenary with Aldo Winkler
11:00-11:20	<b>Session 1: Biomonitoring - Principles &amp; perspectives</b> Plenary with Bert Wolterbeek (10:30-11:30)	Coffee break			
11:20-11:50		S3-LO	S5-LO	S6-LO	S8-LO
11:50-12:05	Coffee break (11:30-11:50)	S3-01	S5-01	S6-01	S8-01
12:05-12:20	S1-LO (11:50-12:20)	S3-02	S5-02	S6-02	S8-02
12:20-12:35	S1-01	S3-03	S5-03	S6-03	<b>Round Table</b> (12:20-12:50)
12:35-12:50	S1-02	S3-04	S5-04	S6-04	Closing ceremony (12:50-13:05)
13:00-14:15	Lunch				
14:15-15:00		Poster Session		Poster Session	
15:00-16:00	<b>Session 2: Exposure, human health &amp; epidemiology</b> Plenary with Cristina Branquinho	<b>Session 4: Geostatistical on biomonitoring science</b> Plenary with Amílcar Soares	Social Programme (Tour and dinner)	<b>Session 7: Human biomonitoring</b> Plenary with Fátima Reis	
16:00-16:20	Coffee break			Coffee break	
16:20-16:50	S2-LO	S4-LO		S7-LO1	
16:50-17:05	S2-01	S4-01		S7-LO2 (16:50-17:20)	
17:05-17:20	S2-02	S4-02		S7-01 (17:20-17:35)	
17:20-17:35	S2-03	S4-03		S7-02 (17:35-17:50)	
17:35-17:50	S2-04	S4-04		S7-03 (17:50-18:05)	
18:00-19:00	Walking meeting			S7-04 (18:05-18:20)	



# ABSTRACTS



# **SESSION 1**

## **Biomonitoring: Principles and Perspectives**





# Biomonitoring: Purpose, Pitfalls and Prospects

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Biomonitoring may be defined as the use of bio-organisms or –materials to obtain information on (selected) properties of the biosphere. The information is deduced from either changes in the behaviour of the biomonitor, or from the concentrations of specific substances in the monitor tissues. General advantage of the use of biomonitors is argued as related to their common and permanent occurrence, the ease of sampling, and the absence of the need of expensive technical equipment.

Mosses and lichens are assumed to be among the most suited biomonitors of atmospheric metal pollution, for which mostly an approach is chosen that implies the assessment of metals in the biomonitor's tissues.

It should be realized, however, that mosses and lichens (or any other living organisms) are dynamic systems that accumulate and release substances such as metals, at rates depending on the organism's vitality, its physiology and morphology, the metal's metabolic pathways inside the tissues, and, probably, the metal's properties, levels, and speciation in aerosols or deposition.

The above prompts thoughts on the status quo and future (purpose, pitfalls and prospects) of biomonitoring. After four decades of development, biomonitoring is still far from its trustworthy and routine use as a (quantitative) tool in metal air pollution studies, and alternatives are emerging such as small solar-powered air filter set-ups or ion-exchange collector materials. Furthermore, the specific goals in biomonitor studies are shifting from a "mere" assessment of time/geography-related variability in metal concentrations towards a more extended use of informative data in mostly health-related research.

Set in this context, the present paper addresses the possible future purpose of biomonitors, the purpose-related pitfalls, and tries to arrive at considerations towards the prospects in biomonitoring.

**Keywords:** biomonitoring, metal air pollution, bio-systems, dynamics

# The MOSSclone Project: Creating and Testing a Novel Biotechnological Tool to Monitor Air Quality Based on a Devitalized Moss Clone

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Session 1

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The technique of “moss bags” for monitoring atmospheric pollution largely employed so far shows some limitations: among others, a certain environmental impact of the collection sites and the lack of standardization of the exposure protocol, making data sets derived not directly comparable. To overcome these limits, the “MOSSclone” project (EU-FP7; [www.mossclone.eu](http://www.mossclone.eu)) has developed a new biotechnological tool based on the use of a devitalised moss clone to detect inorganic (metals and metalloids) and organic (PAHs) pollutants in the air. The research consortium, led by the University of Santiago de Compostela (USC, Spain), involves academic institutions and SMEs from Italy, Spain, Germany, France, Ireland. Aims of the project are: 1) selection and culture of a particularly performing peat moss clone (*Sphagnum palustre*); 2) morphological, molecular, physical, chemical and multi-elemental characterization of the selected clone; 3) large-scale production of the clone; 4) design and production of new concept moss-bags for transplants; 5) methodological standardization to develop a protocol for using moss-bags; 6) tool validation by comparison between data collected using moss-bags and traditional techniques (i.e. bulk deposition collectors, airborne particles and gaseous pollutants samplers); 7) test of the tool for identification of pollution sources. The exposure standardization assay has taken into account aspects concerning bag preparation (net mesh, shape, size and moss weight) and exposure (height above ground and duration time). In order to evaluate the influence of land use and climate on moss uptake, the test exposures were performed in natural background, urban, industrial, and agricultural sites selected among three European countries characterised by continental (Austria), mediterranean (Italy), and oceanic (Spain) climate. The main outcome of the MOSSclone project was that, having standardised the moss material and the exposure protocol, the uptake measured by the *S. palustre* clone in different environmental conditions can be directly compared giving indications on the levels of airborne pollutants. Future perspectives raised by the conclusions of the present project concern the test of the moss matrix with other pollutants (i.e. dioxins, PCB, radionuclides), the possible ascertainment of a correlation function between concentration in the air and moss uptake, at least for some pollutants, and the test of the moss matrix in a water environment.

**Keywords:** moss bags, standardization, devitalised moss clone, *Sphagnum palustre*

# Are Feathers of Birds Useful as Indicators of Metal Pollution?

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Feathers of birds have often been used as indicators of environmental pollution. They are easy to collect and sampling does not harm living birds. Atmospheric pollution can be traced in both terrestrial and aquatic food chains. In most cases feathers seem to reflect the environmental metal burden in different food chains well. Mercury is especially bioaccumulated in aquatic food chains which is seen in e.g. fish-eaters like the osprey and sea eagle. For other heavy metals the bioaccumulation is less pronounced. It may be difficult to separate between internal (food) and external pollution (air, dust). Chick feathers reflect more reliably local conditions compared to feathers of adult birds. Interpretation of the results requires good knowledge on food habit, molting and migration patterns. In some cases museum samples can be used for monitoring temporal changes. Special attention must be paid to clean sampling and preparation of samples. We conclude that feathers of birds can be successfully used as biological indicators when the before mentioned requirements are met.

**Keywords:** bird feather, indicator, metal pollution, monitoring

# Radiocaesium and Fungi: Evolution of Contamination, Soil-to-Fungus Transfer and Expression of Results

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## Session 1

Nearly one half-life of caesium 137 has now passed since the major accident at Chernobyl power plant. Although analyses of radioactive caesium in fungi have been quite numerous it appears now appropriate to draw a wider picture of the question. Different mycological societies have agreed to collect samples in different areas in France; to be complete some species from different origins were also bought on markets. Priority was given to mycorrhizal species, especially *Boletus edulis* and *Hydnum repandum*. Analyses were performed using a hyper-pure Germanium detector and results were compared first according to the origin; then authors compared the evolution of radiocaesium concentration in species having been monitored in the past on the same spot, some of them quite regularly since 1986. Results from recent years showed that activity in saprotrophic species is now very low compared with what was measured in the eighties; as regards mycorrhizal species, decrease in activity results mostly of the physical decay of the radionuclides: the effective half-life appears to be very similar to the physical one. This puts the question of the role of fungal mycelia to keep some elements in the same soil layer. The evolution between the early sixties and 1986 (when research on that subject started on a wider scale) could be investigated if compared with the evolution in the last 29 years so as to have an idea of the contamination of fungi some 60 years ago. Another question is the soil-to-fungus transfer: it is related to the expression of results. This has often been done on the basis of dry soil mass (sometimes under a certain grain size) vs dry fungal mass. It may however be more appropriate to estimate the concentration factor by comparing the activity of pore water and the activity of water in the fungus. Differences in temperature and humidity could interact with soil texture and chemistry to explain differences in contamination.

**Keywords:** fungi, mushrooms, pore water, radiocaesium, evolution of contamination.

**SESSION 2**  
Exposure, Human Health and  
Epidemiology



# The Contribution of Environmental Biomonitoring to Human Health Studies

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During the last decades, awareness regarding the effect of atmospheric pollutants on human health has become a cutting-edge topic due to their toxicity effects (e.g. carcinogenicity, mutagenicity and endocrine disruption character). Once emitted, atmospheric pollutants disperse, deposit on environmental compartments (air, soil, water), bio-accumulate and contaminate the food-chain, causing severe human health problems. Human exposure to atmospheric pollutants deposition, which occurs through inhalation of air and soil particles, ingestion of food, water and soil, and dermal contact, is assessed using estimated values for each environmental compartment. Usually, these data are based on low spatial resolution information, as: a) the number of air monitoring stations is insufficient; b) only few industries measure these compounds in their emissions; c) models do not account with diffuse and unknown sources (such as traffic). Thus, data of human exposure to pollutants has a large proportion of uncertainty which does not allow taking adequate measures to protect the population. To assess which populations are under a high and low exposure level, it's crucial to know, with a high spatial resolution, what is actually being deposited on each environmental compartment? Moreover, as pollutants may exist at very low concentrations, most of times they're not detectable using conventional reference methods; but still, humans are exposed to low concentrations of these compounds. One of the main challenges of modern environmental sciences is to relate long-term environmental exposure to low levels of pollutants to human health effects. Most epidemiological studies have focused on occupational and accidental exposures to pollutants, among workers and residents of affected areas. However, individuals potentially more susceptible, such as infants and children, are not normally occupationally exposed. In line with the European Environment and Health Strategy it is important to provide new insights into human exposure to environmental pollutants in non-occupationally environments, making use of pioneering research methods at the forefront of environmental monitoring and health science. In this talk, a review of the contribution that environmental biomonitoring such as lichens, mosses, tree leaves, etc can make to human health studies will be performed. An historical perspective will be addressed reporting the first studies in which biomonitoring were used in an indirect way as signals for human health problems. The advantageous and limitations of the use of biomonitoring in relation to traditional measures of atmospheric pollutants. A presentation of the most important case studies addressing biomonitoring and human health studies will be described. Finally a critical analysis concerning the difficulties in using routinely environmental biomonitoring to estimate human health impacts due to atmospheric pollution will be performed future lines of research will be suggested.

**Keywords:** lichens, biomonitoring, bioindicators, human exposure, air pollutants

# Biomonitoring and Risk Assessment: the Case of Cement Production

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## Session 2

Risk assessments of combustion emission sources typically focus on heavy metals such as arsenic, cadmium, lead, and mercury (Hg) and products of incomplete combustion such as polychlorinated dibenzo(p)dioxins/furans (PCDD/Fs) and polycyclic aromatic hydrocarbons (PAHs). As these compounds persist and bioaccumulate, multi-pathway risk assessments typically use a series of sequential, interconnected models to predict pollutant dispersion in air, deposition to soil/water, and foodweb transfer. The distribution of pollutant concentrations among environmental media is then used as the basis of estimating exposure among all potentially relevant pathways. For contaminants such as Hg and PCDD/Fs, top-of-food chain pathways are predicted to be most important. Environmental monitoring studies (EMSs) are sometimes used in an attempt to confirm the predictions of fate-and transport models and seek evidence of the impacts of specific emission sources. Our talk will focus on the challenges associated with environmental monitoring studies, with specific application to the cement kiln industry in Portugal. As there are many sources of combustion-related pollutants, a facility based EMS must account for both natural and anthropogenic background (i.e., signal to noise issues).

Emissions from modern, well-controlled cement kilns have been greatly reduced such that fate-and transport models predict lower-than-background impacts, leading to the expectation that facility specific impacts cannot easily (if at all) be distinguished. Statistical sampling considerations thus become important, as well the design of sampling studies based on patterns of projected impacts. Use of biomonitoring (e.g., lichens) has been investigated in an attempt to focus on bioaccumulation endpoints. Our talk will explore potential relationships between bioaccumulation modeling and biomonitoring using recent data collected around operating cement kilns. It will be discussed the integration between the lichens data and the output of the QRA in the same site.

**Keywords:** biomonitoring, risk assessment, QRA, integration



# Determination of Estrogenic Activity in PM<sub>10</sub> Air Samples from Flanders (Belgium) with the BG1 Ovarian Cancer Cell Line

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Exogenous substances that act like hormones and disrupt the physiologic function of endogenous hormones in the endocrine system are called endocrine disrupting compounds (EDCs). Due to their possible health effects, there is growing attention for EDC pollution in our environment. In this study, a novel methodology to measure the overall response of estrogenic disruptors in PM<sub>10</sub> air samples was developed and the effect of different extraction solvents was investigated. To obtain more information about the EDCs present in our environment and their effects on the human hormone system, a bioassay technique using a BG1Luc4E2 ovarian carcinoma cell line was employed to quantify the potency of the mixture of estrogenic compounds in PM<sub>10</sub> air samples. Between April 2013 and January 2014, samples were taken in a rural, industrial and urban area in Flanders (Belgium). Estrogen disruptive potencies were reported as biologic equivalents (BEQ), expressed in picograms 17 $\beta$ -estradiol per cubic meter of air. Non-parametric tests (Kruskal-Wallis and Wilcoxon rank tests) were used to determine possible differences between sampling areas and time periods. Spearman's rank correlations were calculated to examine relationships with meteorological data and environmental pollutants. No statistical significant differences in estrogenic activity were found between the locations, but large day-by-day variations were seen in all locations. The estrogenic activity was positively related with PM<sub>10</sub> levels ( $p=0.035$ ), black carbon ( $p=0.024$ ) and the sum of pyrene and fluoranthene ( $p=0.044$ ), while a significant negative correlation with air temperature was found ( $p=0.04$ ). Furthermore, the estrogenic potency was generally higher when a polar extraction solvent was used (ethanol or acetonitrile) instead of a mixture of a polar and non-polar solvent (hexane/acetone, 50/50). This study shows that this in vitro bioassay is a suitable tool to monitor estrogenic disruption in air samples. Since relatively large day-by-day fluctuations were observed, it seems that on all locations a certain background estrogenic activity is present with on certain occasions high peak levels originating from a mixture of estrogen-active compounds. More long term studies on PM<sub>10</sub>, or even PM<sub>2.5</sub> and PM<sub>1</sub>, are thus needed to investigate these fluctuations in estrogenic potency in relation to meteorological and environmental data and to define possible human health effects. However, it seems that changes in sample pre-treatment methods can significantly influence the observed potencies and that a harmonized approach for sampling, extraction and in vitro measurement is thus necessary.

**Keywords:** PM<sub>10</sub>, CALUX, EDC, estrogen, bioassay

# Mutagenicity assessment of aerosols in emissions from domestic combustion processes

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Domestic biofuel combustion is one of the major sources of regional and local air pollution, mainly regarding particulate matter and organic compounds, during winter periods. Studies have already shown that PAHs and their derivatives generated from domestic cooking and domestic heating are mutagenic agents, whose mutagenic potential varies regarding the combustion appliances, the type of used fuels and extraction methods. This study proposes to evaluate the toxic effect of the fraction of PAHs of emitted coarse particles (PM<sub>10</sub>) from domestic combustion. The mutagenicity assessment was done by the Ames assay. Two bacterial strains of *Salmonella typhimurium*, TA98 and TA100, were used to determine frameshift mutations and base-pair substitution mutations, respectively. To evaluate the mutagenicity of metabolic products of the studied chemicals, an in vitro metabolic activation is studied by adding the S9 mix (rat liver microsomal fraction) to the strains. Seven fuels, including four types of pellets (one commercially certificated and the other 3 with different percentages of residues from furniture industry – 25, 35 and 50%) and three agro-fuels (olive pit, almond shell and shell of pine nuts), were tested in an automatic pellet stove and two types of wood (maritime pine and eucalypt) were tested in a woodstove (studying two phases of the combustion: devolatilisation and flaming/smouldering). PAHs extracts of PM<sub>10</sub> collected from combustion of the different types of solid biofuels were then tested by the Ames assay described above.

The eucalypt samples of both phases of combustion along with the devolatilisation phase of the pine combustion imposed direct mutagenicity towards TA98. Although almond shell did not comply with both requirements to confirm its direct mutagenicity towards TA98, it presented a ratio of 1.7±0.3, which may suggest a mutagenicity effect. The Pellet Type IV (with 50% of residues from furniture industry) presented cytotoxic effects towards TA100, with a decrease of 30% of the revertant colonies comparatively to the negative control, along with the sample of pine devolatilisation, which presented a decrease of 25%. Regarding the indirect-acting mutagenic effect (strains with S9 mix), the combustion phase of devolatilisation for both samples of pine and eucalypt presented similar high ratios in both strains (between 1.3 and 1.4), however the existence of mutagenic activity of these samples towards both strains could not be confirmed. This study allows to identify which fuel poses the least mutagenic risk and, therefore, to be recommended for domestic use.

**Keywords:** mutagenicity, polycyclic aromatic hydrocarbon, PM<sub>10</sub>, biomass burning, ames assay

# Emerging Concern on Siloxanes – Using Air, Soil and Vegetation to Assess their Environmental Behaviour

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Persistent organic pollutants (POPs) are, by definition, compounds highly resistant to biological, photolytic or chemical degradation. In 2001, the Stockholm Convention has defined the first list of 12 POPs, and since then several compounds have been included or under consideration for inclusion. One of these groups of so-called "emerging pollutants" are siloxanes, profusely used in personal care products and in numerous industrial applications. Although little is known about the effects of these compounds, they are recognized great potential risk to the environment, including carcinogenic and endocrine disruption, as well as direct or indirect toxic effects in several biological processes. Their volatility makes the atmosphere especially prone to host (and transport) the bulk of emissions, whether localized or dispersed. Therefore, this study aimed to contribute to enhance the knowledge of levels, trends, and behaviour of eight siloxanes (four linear and four cyclic) in the environment. Adding to the priority scrutiny of the incidence in the atmosphere through passive samplers (sorbent-impregnated polyurethane foam disks –SIPs– in this case), the sampling of pine needles and soil was also performed, thus closing the circle of atmospheric exposure in the areas of study and enhancing the knowledge of cycles and levels of pollution in different matrices. Two sampling campaigns (one in summer and one in winter) were done in a total of 8 sampling points in the Portuguese territory, which covered a wide range of human presence and an extensive characterization as to the uses of the soil (urban, industrial, rural, remote and beach areas).

In terms of analytical methods, a "green" approach was adopted, namely reducing the clean-up steps for the passive air samples and using the QuEChERS technology for soils and pine needles. Mean recoveries of 74%, 70% and 72% were obtained for the three matrices, respectively. Regarding the total concentration of siloxanes found in the samples, for soils values ranged between 5 and 80 ng/g (dry weight) whereas for the pine needles varied from 10 to 160 ng/g (dw). In none of these matrices was possible to draw a clear seasonal trend. For the passive air samples, it could be seen that in the two beach areas the values in summer were higher than in winter, probably due to the considerable increase in the population of these areas during the summer campaign. Overall, the levels varied from 10 to 130 ng/sample. In all matrices, the cyclic siloxanes were found in much higher concentrations, with D5 and D6 being the most predominant in a great majority of cases. Also, the urban and industrial areas had the highest incidence, as could be expected from their main uses. An initial modelling approach for the atmospheric distribution of D5 on the west of the Iberian Peninsula showed difficulties due to the lack of emission data for the domain of study and portrayed differences using the parameters available from other parts of the world. In consequence, the knowledge on the behaviour of siloxanes still has considerable gaps and more integrated studies have to be conducted both at local and at wider domains.

**Keywords:** siloxanes, air, soil, vegetation, emerging pollutants

# Associations Between Birth Outcomes and Outdoor Air Quality Measured with High Spatial Resolution Lichen Data

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## Session 2

Many environmental epidemiology studies suggest that birth outcomes like low birth weight (weight<2500 gr) or preterm births (gestation<37 weeks) might contribute to poor infant's health outcomes, like increased infant morbidity and mortality. At same time there is extended scientific literature suggesting significant associations between outdoor air pollution levels and these birth outcomes. Majority of studies trying to measure these associations are hampered by the lack of spatial resolution in air quality data, because they commonly use traditional air quality monitoring stations, which are expensive to operate and exist only in few locations selected for regulation purposes. However, cities are very heterogeneous regarding air quality, thus increasing the spatial resolution of air quality data is a critical issue to improve efficiency of spatial methods in environmental epidemiology. In the scope of the GISA project (Gestão Integrada Saúde Ambiente) we conducted an environmental epidemiology study in small Mediterranean cities located in Coastal Alentejo Region (Portugal), to measure associations between air quality during pregnancy and birth outcomes. To overcome the lack of spatial resolution in air quality data, we measured air quality levels using lichen data available in those cities, as they acted as a surrogate of maternal exposure to air pollution during pregnancy. Historical individual data on birth weight, duration of gestation, night and day-time addresses of mothers during pregnancy, demographic, social and clinical covariates were collected by questionnaire (n=860) from mothers enrolled on GISA project (860 out of 1393 enrolled mothers). We gathered health and lichen data in a Geographic Information System and applied geostatistical methods (estimation and sequential simulation methods) to lichen diversity data to derive a continuous metric of integrated outdoor air quality exposure. We applied multivariate regression methods (generalized linear models framework) to estimate the odds of birth outcomes at different air quality levels controlling for the effect of other covariates, such as maternal lifestyle factors (smoking habits, weight gain during gestation), maternal age or maternal body mass index before pregnancy. Results suggest differences in both air quality and birth outcomes between cities. Rationale and results obtained emphasize the ability of high spatial resolution lichen data to be included in design of an epidemiological study on health and air quality.

**Keywords:** lichens, pollution, health exposure, birth weight

# **SESSION 3**

## **Organic Pollutants**



# Biomonitoring Persistent Organic Pollutants: One Decade at a Glance

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Persistent organic pollutants (POPs) are organic chemical substances, carbon based, whose awareness has become a cutting-edge topic during last decades. Features such as toxicity, bioaccumulation and persistence of these compounds in the environment contributed for international agreements aiming to eliminate and/or restrict the production and use of selected POPs, in order to protect human health and the environment. Most of POPs have been intentionally produced for commercial purposes, such as pesticides (DDT, lindane, etc.) but some have never been intentionally produced. Examples are PCDD/Fs (dioxins and furans) and PAHs (polycyclic aromatic hydrocarbons), which are considered unintentionally produced substances whose emissions should be reduced and whose monitoring in the environment is essential. Though it is simple to measure POPs in industrial emissions, emissions from diffusive sources, such as traffic and other urban activities, are difficult to quantify. Moreover, once emitted, POPs will disperse in atmosphere, travel for long distances (depending on their size and weight) and suffer chemical modifications (influenced by temperature, sunlight, and other factors) before their deposition. In this sense, monitoring what is actually being deposited is important to assess risk to ecosystems and human health. Monitoring of POPs in air has proven to be an unsatisfactory method to capture the real picture of the dispersion and deposition of these compounds. Because air is sampled for short periods, concentrations of POPs are many times not detectable. However, since POPs are lipophilic compounds with tendency to bioaccumulate, even if not detectable in air, they can be found in high concentrations in biota and soil as a result of a long term atmospheric deposition. Soil, on the other hand, is considered a natural sink for persistent organic pollutants; POPs absorb to soil organic carbon, and once absorbed, remain relatively immobile. For this reason, concentrations of POPs in soil are a reflex of a long-term deposition during decades and consequently are not helpful to assess the impact of the measures to reduce emissions that have been taking place. The use of biomonitors (living organisms) arises as a useful tool to fulfil the conventional air and soil monitoring methods. Over time, different biomonitors have been used to monitor POPs. In terrestrial environments, the most frequent has been the use of pine needles and other vegetation, and more recently moss and lichens (symbioses of fungi and algae). The main aim of this presentation is to provide an overview of the use of biomonitors to assess POP atmospheric deposition onto the environment, focusing on the last decade, and stretching aspects such as their advantages and limitations, factors that influence interception and accumulation of POPs, and how have they been used to track different pollution sources.

**Keywords:** POPs, PCDD/F profile, PAH profile, air pollution, bioindicator

# Modelling the Air-Vegetation Levels of Benzo[a]pyrene for Different Land Uses

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Benzo(a)pyrene (BaP), with a 5-aromatic ring molecular structure (and thus present in the atmosphere mainly as particulate matter) was defined by the European Commission (Directive 2004/107/EC, amended by Regulation 219/2009) as a reference for air quality standards regarding polycyclic aromatic hydrocarbons (PAHs), setting an average limit of 1 ng m<sup>-3</sup> of BaP over one year. Although legal limits for atmospheric PAHs are scarce, some guidelines have been presented by studies in literature that can help in the assessment of their harmful potential. Biomonitoring using plant species has been used to evaluate the levels of PAHs in the environment since the late 80s. Pine trees have a worldwide presence, and the Iberian Peninsula is no exception. Consequently, there is a great potential to set up small to large-scale spatial studies, enhanced by the life span of the needles (up to several years, depending on the species), which continuously accumulate organic pollutants [25]. However, studies reporting a wide geographic distribution of accumulation patterns of PAHs using coniferous needles are scarce and use different approaches. Even fewer are those dealing with the estimation of air-vegetation partitioning phenomena. Thus, combining field data from biomonitoring campaigns (or atmospheric sampling networks such as the European Monitoring and Evaluation Programme, EMEP) and chemistry transport models (CTMs) can be a way to obtain reliable estimates of the air-vegetation loads of PAHs, as well as a more comprehensive insight on their geographical and temporal distribution. An innovative concept using the WRF+CHIMERE modelling system, coupled to emission data from EMEP and compared to data from 70 pine needles sampling sites is then reported in this study. Apart from evaluating the presence and hazardous potential of atmospheric BaP in the Iberian Peninsula, the main objective is also to evaluate if different land uses (urban, industrial, rural and remote) can be accurately portrayed by field and modelling approaches. The climatology of the canopy levels of BaP was evaluated against the concentrations in pine needles, showing a good accuracy (biases lower than 30%). The ability of pine needles to act as biomonitoring markers for the atmospheric concentrations of BaP was estimated by converting the levels obtained in pine needles to air concentrations, taking into account the different land uses of the sampling sites and also those included in the modelling approach.

**Keywords:** polycyclic aromatic hydrocarbons, land use, biomonitoring, air sampling.



# Biomonitoring Research of Heavy Metals and PAHs in the Air of Agricultural Landscape of Italy's Campania Region

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In recent years the Campania region, one of the most fertile Italian agricultural landscapes has been under the attention of media because for decades its soils were illegally used to dump potentially lethal toxic wastes, as result of waste crisis. Moreover, with time people began setting fire to the dumps, making matters worse. Public opinion claims that this situation is the cause of increase of some cancers rates and shorter lifespan of people living in this area. Authoritative researchers have recently suggested that Campania region could be a perfect field study for a biomonitoring research programme, as their poisoned fields could serve as a giant experiment in the new science of 'exposomics'. In pilot sites of "Litorale Domizio-Agro Aversano" NIPS, recognized by the Italian State as a National Interest Priority Site in Campania, the Life ENV-IT ECOREMED project is working to implement eco-compatible protocols for agricultural soil remediation. In this framework we present the data from a first biomonitoring survey to detect inorganic (metals and metalloids) and organic (PAHs) pollutants in the air using moss transplants. The methodology applied follows a harmonised protocol of exposure in which devitalised moss *Hypnum cupressiforme* was used to prepare sub-spherical moss bags that were exposed in the sites of interest for six weeks starting on March 2014. The exposure design was set taking into account the inventory of known emissions to the atmosphere, and the diffuse urbanization of the area in which agricultural and urban/residential sites are strictly intermingled and was applied to five municipalities, Acerra, Casal Di Principe, Giugliano, Maddaloni, Teverola. Results for metals and metalloids indicate that all the investigated area is highly homogeneous, and for most of analysed elements the uptake rates were higher in agricultural sites compared to urban/residential ones. The highest uptakes were measured for Pb, Zn, Cu, major pollutants in the soils of the area, the last one with large application in agricultural practices. Limited PAH uptakes were evidenced with the most represented components, pyrene and benzo(b)fluoranthene following a similar trend in soils. Comparison with previous monitoring surveys indicates moss uptake rates largely lower than those measured in the urban area of Naples city.

**Keywords:** moss bags, *Hypnum cupressiforme*, trace metals, PAHs, Campania region

# Uptake and Toxicity of Glyphosate in the Lichen *Xanthoria parietina* (L.) Th.Fr.

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Glyphosate (N-(phosphonomethyl)glycine) is the most important herbicide worldwide. It is a post-emergent, systemic, and non-selective herbicide applied directly to plant foliage. Glyphosate is a glycine derivate that target a key enzyme (5-enolpyruvyl-shikimate-3-phosphate synthase, EPSPS) for the production of the aromatic aminoacids essential for the growth of most plants. This pathway is also present in non-target organism, like some microorganism, but is absent in mammals. Lichens are also non-target organism but glyphosate treatments have showed to affect the abundance of some species. For this reason, physiological responses of lichens treated with this herbicide should be investigated. The objective of this study was to test the uptake and toxicity of a common commercial glyphosate-based herbicide (Glifene-SL) in the lichen *Xanthoria parietina* (L.) Th. Fr. To this purpose, three concentrations were used for the treatments (0, 10 and 100 mL/L of the commercial herbicide, corresponding to 0, 20 and 200 mM/L of glyphosate) and the effects were checked under three different times of incubation (24, 48 and 96h). Glyphosate accumulation was constant over the 2 treatment concentrations, with accumulation differences of an order of magnitude (4.07 and 60.5 µg/mg dw respectively), reflecting the concentration of the treatment; control samples were below the detection limit. Also physiological parameters (chlorophyll integrity, photosynthetic efficiency, viability, concentration of ergosterol and chlorophyll a and b) showed both a dose- and time-depend effect, with decreasing values of physiological parameters with increasing glyphosate concentrations and increasing exposure time.

**Keywords:** glyphosate, lichens, uptake, chlorophyll, ergosterol

# Monitoring PAHs in the Petrochemical area of Tarragona County, Spain, using Air Passive Samplers and Lichens

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The most important chemical/petrochemical industrial complex in Southern Europe is located in Tarragona County, Spain. Since 2002, the concentrations of polycyclic aromatic hydrocarbons (PAHs) are periodically assessed in a number of environmental monitors. The main aim of this study was to determine the levels of 16 PAHs in air at different areas of Tarragona County, by means of 2 different passive sampling techniques: polyurethane foam (PUF)-based passive air samplers (PAS) and lichens. PAS were deployed at 8 sampling points for a period of 2 months, from November 2014 to January 2015). Two samples were collected in each one of the areas under study (petrochemical, chemical, urban/residential, and unpolluted). In parallel, fruticose lichens were collected from a background, non-polluted area, and transplanted to the same sampling points during the same time span. After two months, the samples (PUFs and lichen transplants) were collected and analysed for the 16 priority PAHs. Based on data of air and lichens, a PAH profile was achieved for each area under study reflecting different pollution sources.

**Keywords:** passive air samplers, *Ramalina* sp., biomonitoring, pollution, petrochemical area

# Elemental Concentrations under Different Heights and Weather Conditions on Marine Island – Lichen Response to Different Aerosols Deposition Levels

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## Session 3

To investigate the altitude influence in the detection of local and remote sources of pollution, lichen transplants of *Parmotrema bangii* were exposed and collected at Pico Mountain, Azores, approximately every 250 m starting from 50m to 2250 m during one year period. This was done also horizontally around the mountain in order to detect the possible pollutant trajectory passing the Pico Mountain. Conductivity of the lichens samples were measured and compared with weather factors. The chemical elemental concentrations were determined by INAA. This transplant study demonstrates that comparing elemental concentration in time-or spatial series in lichens surveys along altitude transects requires a comparison with lichen vitality as the different stages could affect variability in metal uptake. Significant differences in lichen elemental concentrations between different layers and different directions confirms different deposition levels, correspondent to the aerosol data collected which suggests the ability of using lichens in atmosphere deposition studies along different altitudes.

**Keywords:** biomonitors, atmosphere deposition, trace elements, vitality, Azores

# **SESSION 4**

## Geostatistical on Biomonitoring Science



# New Geostatistical Methodologies to Deal with Different Space/time Support and Uncertainty of the Data

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One of the main challenges to create spatial numerical models of a given air pollutant, is the integration of different information in one unique and coherent model. Available data of air quality—biomonitoring, diffusive tubes, monitoring stations— have different spatial and temporal support and different uncertainty about the measurements. In this presentation we introduce some new geostatistical methods to integrate such diversity of data properties: block sequential simulation and simulation with point distributions. Some examples of air quality characterization will illustrate the methods.

**Keywords:** geostatistics, space-time modelling, uncertainty

# Integrity of Forest Ecosystems in Germany Exposed to Climate Change and Atmospheric Nitrogen Deposition

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In Germany, an integrative approach to cope with the integrity of ecosystems exposed to climate change and atmospheric nitrogen deposition using monitoring data is still lacking. Therefore, based on a definition of ecosystem integrity, a comprehensive and spatial explicit methodology for evaluating the integrity of natural and near-natural forest ecosystems was developed. The approach integrates data on vegetation, chemical and physical soil conditions as well as on climate change and atmospheric deposition of nitrogen collected from respective monitoring programmes in Germany. The key component for evaluation ecosystem integrity is a classification system for potential natural and current near-natural ecosystem types. The classification system contains information on ecological functions (habitat, net primary production, carbon sequestration, nutrient and water flow, resilience) covering the years 1961-1990, which are regarded as reference. The assessment of ecological integrity relies on comparing a respective current or future ecosystem state with the reference status of the given ecosystem type. Whilst the current ecosystem status was quantified by use of data collected within monitoring programmes, potential future developments were projected using a geo-chemical soil modelling technique and data from a regional climate change model. The ecosystem types were referred to the potential natural vegetation of Germany and mapped additionally using geo-data on current tree species coverage and land use. The current ecosystem types were related to geo-data on elevation above sea level (a), soil texture (b), climate in terms of air temperature and humidity, evapotranspiration, and precipitation across the years 1961-1990 (c) and were determined / identified by using Classification and Regression Trees. The relations determined by this and represented by if then else-rules were used to map the spatial pattern of ecosystem type clusters for 1961-1990 and, in terms of if then else-rules, applied to the above mentioned geo-data (a), (b) and (c) and then used to map the spatial pattern of ecosystem type clusters for 1961-1990 and. Thereby, the climate data were replaced in the following three analyses by results from a regional climate model for two climate change scenarios covering initially 1991-2010, then 2011-2040, and finally 2041-2070. Accordingly, for each of the four time periods each one map of ecosystem type clusters were produced and evaluated with regard to the development of areal coverage of ecosystem clusters across time due to climate change. This evaluation of structural aspects of ecological integrity in terms of bio-geographical coverage and distribution of ecosystem types on the national level was added by projecting potential future values of indicators for ecological functions at site-level. This was achieved by using the Very Simple Dynamics soil modelling technique using the above mentioned climate data and two scenarios of atmospheric nitrogen deposition as input. The results were compared to the reference (1961-1990) and enabled to evaluate site-specifically ecosystem integrity across time.

**Keywords:** biological and chemical aspects, biological monitoring, exposure, spatial and temporal patterns



# Assessment of a Steelwork Impact Based on Three Different Approaches: Biomonitoring Technique, PM<sub>10</sub> Monitoring and Soil Contamination

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Steel production is a key sector for Europe's economy and competitiveness and its use is critical in enabling man to move towards a sustainable future. However, production of steel is associated with a number of significant environmental challenges, one of which is the emission of airborne particles (PM) to the atmosphere. The present work was developed within the project ASEMIS "Assessment of emissions and impact of steel processes" that aimed to provide improved understanding concerning the steelworks' emissions and their impacts on ambient air quality. The specific objectives of this work were: 1) to identify the steelworks emission profiles, associating emissions to specific production units in the steelwork complex and 2) to assess heavy metal deposition in the vicinity of a steelwork. Biomonitoring with lichens and soil monitoring were performed according to a specific grid, of 2.5 km × 2.5 km within a rectangle of 18 km × 20 km, composed by 68 sampling points. PM<sub>10</sub> sampling was carried out in one single sampling point located adjacent to an integrated steelwork. Element contents in lichens, soils and filters were determined by Nuclear Analytical Techniques. Results showed that the emissions from integrated steelmaking facilities are complex. Firstly, there is a mixture of stationary and diffuse emissions that produce important amounts of particles with very particular characteristics. Secondly, the major steelworks processes are located in very close proximity to each other making it difficult to distinguish between the processes. The situation is further complicated by the fact that some processes operate continuously while others are batch processes, and the influence of other external sources like traffic and other industries.

**Keywords:** steelworks, industrial pollution, biomonitoring, soil contamination, PM<sub>10</sub>

## Effects of Canopy Drip on Nitrogen Concentration in Mosses: Comparative Statistical Analyses of Biomonitoring Sampling Data from Seven European Countries

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Reactive nitrogen can have multiple negative effects on the environment. High inputs of nitrogen from the air (deposition) are a significant risk for biodiversity and functionality of (semi-) natural ecosystems, especially for nitrogen limited habitats and habitats with low soil pH buffering capacity. Forests are particularly affected due to filter effects of their canopy. Our study uses sampling data compiled in seven European countries according to the guidelines of the European moss survey, adds new data and presents a synoptic evaluation. It is assessed what is the impact of canopy drip on the accumulation of nitrogen (N) in moss specimen used as biomonitors for atmospheric deposition. Therefore, the moss samples were collected and analysed using a standard protocol. Possible canopy drip effects were additionally analysed for ammonium, nitrate and total N deposition measured with technical deposition samplers used as comparators. For the statistical evaluation, descriptive statistical analyses were conducted taking possible canopy drip effects into account. Furthermore, the ratio between the total N concentrations in moss specimen sampled at sites located in open land (e.g. grassland, heathland, and wetland) and at sites influenced by canopy drip was calculated. The results were used to execute spatial extrapolation to sites that were not sampled. In addition, multivariate analyses were carried out to uncover relationships between the total N concentration in moss as well as total N deposition, ammonium and nitrate measured in deposition samplers and potentially influencing environmental predictors such as population density, elevation above sea level, distance to sea, precipitation, modelled atmospheric N deposition and land use indices derived by Corine Land Cover maps. The findings for the different European countries are quantitatively described. Therefore, the sampling locations were grouped according to the influence of canopy drip. An increasing amount of N deposition could be substantiated for areas influenced by higher vegetation in the course of canopy drip that leads to higher amounts of nitrogen accumulated in bioindicators and collected in deposition samplers. The multivariate analyses confirmed that the sampling site category (site with/without canopy drip) was the factor most associated with the N concentration in moss. To date, there are only few peer reviewed studies analysing canopy drip effects on the accumulation of N in moss. This study aimed at investigating such effects because it allows a better data interpretation concerning the regional variation. Functional implications for the regional nutrient budgets may take into account the role of vegetation cover and its local heterogeneity. The results support the assumption that forest ecosystems show higher N concentrations in moss and N deposition in technical deposition samplers due to filtering of dry deposition within the canopy leading to an increased absorption and accumulation of N in the biomass by contrast with open-land deposition that mainly covers wet and humid deposition. These spatial variances also uncovered by the multivariate analyses and should be considered in future monitoring networks.

**Keywords:** biomonitoring, moss survey, nitrogen, canopy drip

# Airborne Heavy Metal Deposition and Accumulation in Mosses in Estonia – Spatial and Temporal Trends

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In Estonia, the main sources of airborne heavy metal pollution are oil shale fired power stations, the chemical and the building materials industry and road traffic. The two principal power stations and most of the heavy industry are concentrated in the north-eastern part of the country. The relative deposition of Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in Estonia has been monitored since 1989 by the method of bioindication, using the mosses *Pleurozium schreberi* or *Hylocomium splendens* as indicator species. Both of the species are well suited for monitoring atmospheric heavy metal pollution as heavy metals accumulate in them in proportion with their concentration in air. The relative deposition of N has been monitored since 2005 and the relative deposition of Al, As, Hg and Ti has been monitored since 2011 using the same method. The general biomonitoring network of heavy metal accumulation in mosses in Estonia consists of 99 permanent sampling sites. The denser network of sampling sites for monitoring the deposition of heavy metals and accumulation in mosses in North-East Estonia consists of 33 permanent sampling sites, among with the transects in the vicinity of Estonian Power Plant and Kunda Cement Factory analysed separately. There is also denser network of sampling sites on an area of up to 35 km from the city centre of Tallinn, the capital of Estonia (37 sampling sites) and also in surroundings of 4 larger cities: Kohtla-Järve, Pärnu, Viljandi and Tartu (30 sampling sites). In 1989, the concentration of heavy metals in mosses was the highest in the moss samples collected from North-East Estonia. By 1995, the surroundings of Tallinn had become the second most polluted area in Estonia, probably due to increased traffic density. During 1989-2006, the concentration of heavy metals in mosses in Estonia had decreased significantly in correlation with the decrease of power production as well as the decrease of emission of pollutants due to the implementation of environmentally friendlier technologies. According to the latest monitoring conducted in 2013 the mean concentrations of Cd, Cr, Fe, Ni, Pb, Zn, V and Hg in the mosses of the sampling sites in the Tallinn region were at the same level as the mean values for the whole Estonia in 2010/2011. The mean concentrations of Cu, N, Al and Ti in the mosses of the Tallinn region in 2013 were higher than the mean contents of those elements in the moss samples collected from entire Estonia in 2010/2011. The minimum and median levels of Cd, Cr, Fe, Ni, Pb, Zn and V in the mosses of the Tallinn region had not changed over the period 2003-2013. The median level of the Cu concentration increased 1.3 times from 2003 to 2013, but was unchanged compared to 2008. The maximum levels of Cd, Fe, Ni, Pb and V had decreased from 2003 to 2013. The steepest decrease – 5.3 times – was observed in the moss concentration of V.

**Keywords:** atmospheric pollution, heavy metals, emission sources, biomonitoring, mosses as biomonitors

# Validating and Mapping Atmospheric Deposition of Heavy Metals Using LOTOS-EUROP Model Calculations and Data from Biomonitoring Programmes in Germany

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Impacts of heavy metal (HM) emissions on air quality and ecosystems can be assessed by numeric modelling, by measuring HM deposition sampled by technical devices or by measuring HM concentrations in biomonitors. As part of the research and development project “Impacts of Heavy Metal Emission on Air Quality and Ecosystems across Germany - Sources, Transport, Deposition and potential Hazards”, funded by the Federal Environmental Agency of Germany, numeric model LOTOS-EUROS were used to calculate data on HM deposition at a spatial resolution of 25 km by 25 km throughout Europe. The European Monitoring and Evaluation Programme (EMEP) provides model calculations with grid size of 50 km by 50 km. With respect to monitoring data, the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation Moss Survey) and the German Environmental Specimen Bank (ESB) provide corresponding data on HM concentration in mosses, leaves and needles. LOTOS-EUROS and EMEP modelling were evaluated by comparing them with data from ESB / ICP Vegetation Moss Survey. The modelled atmospheric HM deposition and respective concentrations in moss, leaves and needles were investigated for their statistical relationships. Correlations were investigated, whether they are statistically different. The strongest relationships between modelled and observed data were quantified by regression analysis. Regression equations were applied on geostatistical surface estimations of HM concentration in mosses and then the residuals were interpolated by use of Kriging interpolation. Finally, both maps were summed up to a map of atmospheric Pb and Cd deposition across Germany. Data from biomonitoring programmes are higher correlated to LOTOS-EUROS than to EMEP. For HM concentrations in mosses, the highest correlations were found for the association between geostatistical surface estimations of HM concentration in moss and LOTOS-EUROS model calculations. This could be utilized for data integration by use of Regression-Kriging-Technique, which leads to Cd and Pb deposition maps at a high spatial resolution (3 km x 3 km).

**Keywords:** biomonitoring, deposition modelling, EMEP, environmental specimen bank, European moss survey, LOTOS-EUROS

# **SESSION 5**

## Monitoring long-Term and Large-Scale Deposition of Pollutants



# Monitoring Long-term and Large-scale Deposition of Pollutants Based on Moss Analyses

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A brief historical review is given on the development and milestones of the moss biomonitoring technique used to study atmospheric deposition of trace elements, nitrogen, organic persistent pollutants (PAHs, PCBs, PBDEs, dioxins, PFOS, *etc.*) and radionuclides of technogenic origin in Europe. The relevance of these studies to the UN Convention on long-range transboundary air pollution (LRTAP) is enlightened. Examples of the long-term activity of the UNECE International cooperative programme Vegetation established in 1987 are given to illustrate the tendencies in behavior on a large scale of such air pollutants as Pb, Cd, and other significant ones. In agreement with the long-term strategy of the LRTAP Convention to enhance participation and improve air quality in Eastern Europe, the Caucasus, Central Asia and South Eastern Europe, efforts to extend the moss survey for former republics of the USSR such as Armenia, Azerbaijan, Georgia, Moldova, Kazakhstan, and Uzbekistan were successfully undertaken. Around 15 teams are formed in Russia to cover with moss sampling Northern and Central Russia, Western Siberia, and Far East of Russia (Kamchatka and Sakhalin). JINR will continue support for the moss survey program in its member states: Bulgaria, Slovakia, Poland, Romania, Mongolia, Vietnam, as well as in non-member states: Albania, Croatia, Hungary, Thailand, South Korea, and China. Up-to-date 36 countries expressed their desire to participate in the coming moss survey. In spite of the growing interest in assessment of the deposition of persistent organic pollutants (PAHs, PCBs, PBDEs, dioxins, PFOS, *etc.*) using moss, only a limited number of the Western European countries intend to determine POPs. Radioecological laboratories in JINR (Dubna, Russia), Institute of Nuclear Physics (Alma Ata, Kazakhstan), University of Novi Sad (Serbia), Bratislava University (Slovakia) and Opole University (Poland) will be used to measure natural and man-made radionuclides (<sup>137</sup>Cs, <sup>210</sup>Pb, *etc.*) under individual agreements with the interested countries. Some details are given on the newly established database for storage of information about the European and Asian moss survey, conducting and storing analytical results on heavy metals, nitrogen, persistent organic compounds and radionuclides based on moss analysis.

**Keywords:** moss biomonitoring, trace elements, nitrogen, POPs, radionuclides

# Spatial Patterns and Temporal Trends of Trace Element Deposition in Norway Studied by Analysis of Moss, Humic Surface Soils, and Peat Cores

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Atmospheric deposition of trace elements on the national scale has been monitored in Norway at regular intervals using the moss *Hylocomium splendens* and a network of around 470 sites distributed over the country. Simultaneous sampling of the 0-3 cm layer of natural surface soils was carried out in 1977, 1985, 1995, and 2005. In the southern part of the country, where trans-boundary pollution from other parts of Europe is the main source of elements such as V, Zn, As, Cd, Sb, and Pb, the humic surface soil exhibits a similar geographic distribution as that observed in the moss, confirming that these ecosystems have been, and still are, significantly affected by trans-boundary pollution. The humic surface soil – as well as the moss - is also useful monitor of the geographic distribution of elements derived from the marine environment and supplied to land areas by atmospheric transport, such as I and Se. Vertical distribution of anthropogenic elements in peat cores from ombrotrophic bogs serves for monitoring of temporal trends after dating of peat layers. Results from 40 years of studies combining these techniques will be presented.

**Keywords:** atmospheric deposition, trace elements, moss, humic surface soil, peat



## A Quarter Century of Biomonitoring the Atmospheric Pollution Loads at the Scale of the Czech Republic. What Have Analyses of Moss, Bark and Humus Bioindicators Shown?

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The registered major emission amounts in the Czech Republic peaked in the mid 1980s. In the first half of the 1990s, industry in the country was restructured, leading to a reduction in the amounts of pollutants that were emitted. Since 1990, changes in the chemical and physical properties of moss, spruce bark and forest floor humus related to air pollution levels, have been repeatedly monitored at more than 200 permanent forest plots situated across the Czech Republic. The content of 13–40 elements in moss and humus, and the pH, electric conductivity and sulphate content in bark have been determined, usually at 5-year intervals. The moss analyses have shown a significant reduction in the content of all investigated elements in the 1990–2000–2010 time series (e.g., median of As: 1.70–0.29–0.26, median of V: 5.40–1.52–1.18 mg.kg<sup>-1</sup>), caused mainly by reduced amounts of combusted brown coal and by introducing desulphurization of coal power plants in the mid 1990s. Moss showed a significant decrease in the Pb content in the 1990–2000–2010 time series (16.60–5.66–2.85 mg.kg<sup>-1</sup>), reflecting the distribution of petrol with a limited Pb content, and later a total ban on leaded petrol distribution in 2000. Fine-scale mapping of the element content in mosses near selected pollution sources documented, for example, reduced levels in the zone with very high deposition of Pb around a secondary lead smelter after the introduction of environmental engineering measures, and revealed the position and the shape of a previously unknown hot spot of U, Th and other lithogenic elements caused by the use of a mill to grind stones from former uranium pits. Spruce bark analysis indicated a significant decrease in acidity, electric conductivity and sulphate content between 1990 and 2010. For example, the territorial pH medians increased in time as follows: 2.50 in 1987, 2.66 in 1995, 3.12 in 2005 and 3.33 in 2010. The figures clearly indicate diminishing effects of acid rains in Central Europe in recent decades. The median of electric conductivity and sulphate content in bark decreased threefold between 1995 and 2010 (760 and 213 μS.cm<sup>-1</sup> and 760 and 213 mgSO<sub>4</sub><sup>2-</sup>.g<sup>-1</sup>), while territorial annual SO<sub>2</sub> emissions decreased sixfold (2,250.10<sup>3</sup> tons in 1995 and 300.10<sup>3</sup> tons in 2010). The hot spots of these bark parameters, located mainly near coal power plants, have been fading. The spruce bark investigation revealed increased activity of Chernobyl-derived radionuclides, mainly <sup>137</sup>Cs, persisting in areas of raised fallout caused by local rains in spring 1986. In these areas, increased <sup>137</sup>Cs activity has persisted for a long time, and the <sup>137</sup>Cs activity distributions showed the same patterns in bark and in humus even 9 and 24 years after the fallout. Forest floor humus has accumulated atmospheric element deposition rates for decades, and can reveal hidden contamination in areas where former air pollution sources have been removed or limited. Mapping the element content in forest humus indicated, for example, Pb accumulation in a 35-km radius around a former lead smelter, areas of increased Hg accumulation around a chlor-alkali plant, and the position of hot spots of Chernobyl-derived <sup>137</sup>Cs throughout the Czech Republic.

**Keywords:** bioindicators, heavy metals, radionuclides, acid rain, Czech Republic

# Twelve Years of Ecotoxicologic Monitoring in the Disposal Area of an Oil Terminal in Northern Coast of São Paulo, Brazil

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Petrobras is one of the largest oil companies in the world. The largest marine terminal of Petrobras is in São Sebastião Channel, North coast of São Paulo State. This terminal moves 130.000 m<sup>3</sup> of oil per day and since 1998 disposes its effluent in the channel through marine outfall. Since that time a monitoring program, requested by the environmental agency is being carried out. The present study aims to report results of ecotoxicological assays conducted in the period between 1998 and 2010 as part of the evaluated parameters in São Sebastião Channel monitoring program after the installation of the submarine outfall of Petrobras Waterway Terminal. This monitoring program began in 1998 and is still in progress. The toxicity tests were applied to the evaluation of the water toxicity (surface and bottom) and of the sediment (whole sediment and pore water). The chronic toxicity assay based on the embryolarval development of the sea urchin *Lytechinus variegatus* was applied to the liquid samples. Whole sediment samples were tested by the acute assay using the amphipod *Tiburonella viscana*. In the period between 1998 and 2006, the sampling points were distributed along the entire channel length. In the period between 2007 and 2010 the sampling schedule was redefined, by the environmental agency solicitation. The sampling stations were distributed in a radius of 1 km around the outfall opening. Results point out the increase in the sediment and water toxicity both in surface and bottom water, but mainly in the pore water. The conclusion obtained through the data is that the effluent has been affecting the environment, but due to the large hydrodynamism the pollutant probably has been carried out to other places. We suggest a monitoring program in a more comprehensive sampling area.

**Keywords:** oil terminal, effluent, monitoring program, toxicity tests

# Environmental pollution and pine stand condition in Mežaparks, Rīga in the beginning of the 21st century

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Pine stands in Mežaparks have been developing under long-term pollution. Yet, it is unknown whether the regeneration process is long term. The aim of the study is to determine the progress of regeneration following reduction of anthropogenic stress. Atmospheric pollution in the entire territory is indicated by limited distribution of lichens and acidification of tree bark and soil. Heavy metal distribution in mosses and bark confirms that the highest pollution level is near a road. Historical pollution has remained in the northern part in soil O horizon and in the southern part in B horizon and does not show the influence of Riga superphosphate factory. Spatial distribution of macrountrients in the soil is similar to the heavy metal distribution that might be both industrial pollution and fertilization effects. A trend of positive mean relative additional annual increment is mainly determined by shutdown of phosphate factory.

**Keywords:** urban woodlands, air pollution, recovery, bioindication

# Quality Means Decency – On the Quality of Teaching on B&B Technologies through Accreditation of the Course?

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The term “accreditation” applies to attempts in various fields of scientific work and administration. It refers to acknowledgement of some particular positive (wanted) property being present in or brought about by either a procedure or technical system, an acknowledgement which then is formally issued by some authority generally accepted to be able to prove and file such statements. To give an example, analytical measurement results from IMAP 20 International Measurement Program and the construction of the university course “Integrative Sustainable Management: Quality, Environmental, Health, Safety and Risk Management (QUAR)” are reviewed in this respect. It was a serious finding by the multi-participant IMAP study that neither quality control measures nor accreditation actually ensure improved quality of measurement results, i.e. data produced by analytical laboratories. Accordingly, even though accreditation of study curricula is often done with utmost diligence and precision, and while it is fairly important for making study curricula throughout Europe (and the world at all) fitting and comparable to each other to get study results produced and tested at university X also be accepted when changing to university Y, we cannot assume or take for granted that the mere protocol of accreditation will do anything positive to improve the chance to get better or “more correct” results. A single member of lab staff or professor changing to some other position might seriously compromise the turnout of some analytical lab or university course at least in the short term regardless whether accreditation was done or not.

**Keywords:** B&B technologies, bioindication/biomonitoring, accreditation, quality control, lecturing, integrative sustainable management

# **SESSION 6**

## Air Quality Management



# The Potential of Biomonitoring for Urban Air Quality Management

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Urban air pollution, and especially particulate matter (PM) pollution causes serious health problems, especially in densely populated areas, like cities. The composition and level of this air pollution largely depends on the (spatial distribution of the) various emissions sources, while its spatial distribution depends on e.g. the location of the major traffic axes, the urban planning and characteristics of e.g. urban street canyons. Although the well-known adverse effects of air pollution the number of air quality monitoring stations is mostly very limited in urban environments, do not give a good idea about the spatial PM distribution at the urban and neighbourhood level, and do certainly not provide any insight into the distribution of PM inside street canyons. However, knowledge of the spatial distribution of air pollution in general, and PM pollution in particular, at these different spatial scales is important as it gives information about the PM exposure for those who live, work and pass at these places. Moreover, also in an international context knowledge on these factors is important to support international legislation issues. In this presentation we will discuss PM distribution at street level, urban scale and at the European level, thereby considering major urban PM sources and factors influencing PM distribution, the role of urban vegetation to mitigate air pollution and how biomonitoring can contribute to air quality management in urban areas. Moreover, the potential of a citizen science driven biomonitoring approach will be discussed.

**Keywords:** Particulate matter, urban air pollution, European biomonitoring network, citizen science, biomagnetic monitoring

# European Standard for Assessing Epiphytic Lichen Diversity: a Common Useful Tool

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Lichen biomonitoring can help assessing the impact of anthropogenic activity, particularly the effect of atmospheric pollution. A new CEN (European Committee for Standardization) European Standard has been recently developed aiming at providing a reliable, repeatable and objective method for assessing epiphytic lichen diversity (CEN/TC 264, EN 16413:2014).

Here we show the results of ring tests which were carried out applying the proposed CEN standard operating procedures to evaluate data consistency among teams from different European countries. Several exercises were performed for estimating the effects of different sources of errors (location of plot, selection of trees, position of the sampling grid, identification of taxa) on the data quality. We also review and discuss open points, which should be addressed for further improving the reliability of the method. Moreover, we explore the applicability of the new CEN standard in the framework of national and European legislations, and we propose new common tools for periodic standard quality assurance of biomonitoring surveys throughout Europe.

**Keywords:** Biomonitoring, lichen diversity, standard procedures



# Particle Exposure and Dose While Cycling: Biomonitoring and Active Sampling Approaches

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Benefits that come from the physical activity to health and human well-being are recognized all over the world (prevention of chronic disease, premature death cardiovascular primary and secondary diseases, etc.). For adults aged between 18–64, the WHO recommend at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week. The use of cycling paths promotes not only the active transportation but also the practice of physical activity outdoors. However, in urban areas people are more exposed to air pollutants, especially to traffic related air pollutants once cycling paths are usually placed along roads. This study aims to describe the personal exposure in different cycling paths in Lisbon combining instrumental sampling and biomonitors. Three cycling paths from Lisbon cycling network, representing different backgrounds, were selected to assess PM<sub>10</sub> and PM<sub>2.5</sub>. PM<sub>10</sub> and PM<sub>2.5</sub> were sampled using an AM510 SidePak from TSI. For each path sampling was performed in five different days during weekdays and in five different days on weekends at 8am and 11am, from May to August, resulting in a total of 30 days of sampling. The epiphytic lichen *Flavoparmelia caperata* was transplanted from Ponte de Sôr (Portugal) to the same cycling paths. Lichens were placed along the paths and exposed from April 7<sup>th</sup> to July 21<sup>th</sup>. Electric conductivity was measured before and after exposure. Elemental concentrations (As, Br, Ca, Co, Fe, K, La, Na, Sb, Sc, Se and Zn) were obtained using the k<sub>0</sub>-Instrumental Neutron Activation Analysis (INAA) technique. Results showed that cyclists are exposed to very high levels of PM, which vary considering the location of the cycle lane. The conductivity levels indicated city hotspots with intense traffic or in under construction sites. This study supplied information to improve urban planning policies, not only providing the best places to build new cycling lanes but also identifying alternatives for the requalification of the existent infrastructures.

**Keywords:** Physical activity, exposure, cycling, biomonitoring, particles

# Assessing Air Quality in Small Mediterranean Cities by Using Lichen Diversity

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Small cities, considered as those with less than 15000 inhabitants, present different patterns of atmospheric pollution compared to big cities, due to the absence of intensive pollution sources. Monitoring the air quality in those cities is often neglected, because it would be necessary implementing a network of measuring devices, which can result too costly. We propose to use lichen biodiversity in order to reduce costs and provide a survey of air quality with high spatial resolution. Among current applied air quality indicators, lichens have shown up as a useful tool to evaluate atmospheric conditions in agricultural, forest and urban areas. The application of a standard protocol based on a European guideline for mapping epiphytic lichen diversity as an indicator of environmental stress provides an easy and quite fast way to assess air quality. The protocol calculates an index called Lichen Diversity Value (LDV), that takes into account species richness but also abundance. In addition to measures of total diversity, we analysed LDV of functional traits related to different ecological factors. This results in a more precise indication of which ecological factors are affecting lichen communities; therefore, we can produce a better evaluation of the atmospheric quality. The protocol has been implemented in 6 small cities located in Coastal Alentejo Region (Portugal) as part of the of the GISA project (Gestão Integrada Saúde Ambiente). The location of the trees was classified into three different classes: residential, green and traffic, depending on the main occupation of the nearby area. Two more factors were taken into consideration, the species of tree and the aspect of the bark (smooth vs rugose). Despite the high heterogeneity of trees, this factor did not affect the lichen diversity; neither did the aspect of tree bark. The main factor driving significant differences on LDV was location. Those trees located in areas with higher traffic presented lower values of LDV, as well as on species richness, indicating the effect of the stress generated by the vehicles emission. Moreover, the stress caused by traffic resulted in a marked increase on the abundance of species adapted to high levels of eutrophication and lower requirements of humidity. At the same time those species adapted to low levels of eutrophication decrease significantly, almost disappearing from traffic areas.

**Keywords:** Bioindicators, functional traits, pollution

# Lichen Biomonitoring and Waste Management

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There is a high public concern for the environmental and health impact related to waste management. Source reduction, reuse, recycling, composting, energy recovery and disposal in landfill are part of the integrated system for waste management. Waste incineration, landfilling and industrial composting are practices associated to a certain degree of air pollution. Biomonitoring with lichens is a valuable tool for the implementation of environmental policy on air quality and atmospheric pollution control. Evidences from case studies carried out in Italy using lichens as bioindicators around incinerators, landfills and compost plants are reported. Three different lichen based methods were used to assess the biological effects of air pollution around a municipal solid waste incinerator: lichen diversity, bioaccumulation of trace elements and physiological status, overall reflecting the environmental quality of the area and detecting ongoing processes and the origin of element depositions. Lichen samples (*Evernia prunastri*) exposed around the incinerator showed increased cell membrane damage and reduced vitality and accumulated several trace elements (As, Cu, Fe, Hg, Mn and V), but the use of enrichment factors (lichen/soil) allowed detecting the atmospheric origin chiefly for Hg, although at low environmental concentrations. The abundance of non-nitrophilous lichen species corresponded to sites with higher environmental quality, while high frequencies of nitrophilous species corresponded to sites with higher eutrophication. In a study carried out around a composting plant we found out that N-tolerant (*Xanthoria parietina*) and N-sensitive (*E. prunastri*) lichens reacted differently to short-term exposures (up to 3 months) to atmospheric ammonia. The N-tolerant lichen was not affected by the proximity to the facility, while the N-sensitive species showed higher performances with increasing distance from the source. These data suggested a local influence of air pollutants from the facility, which is leading to a shift of lichen communities from meso-acidophilous to nitrophilous at the sites facing the facility. In a long-term study carried out around a solid waste landfill, the diversity of epiphytic lichens and the accumulation of trace elements in *Flavoparmelia caperata* were used as indicators along 18 years of waste management. Lichens revealed a local increase of depositions for some heavy metals and a decrease in the diversity at sites directly facing the landfill. In a recent experiment, physiological and morphological alterations in native samples of *F. caperata* were recorded. We simulated the closure of the landfill and hence the removal of the pollution source. To this purpose, we selected and removed *F. caperata* thalli from the surroundings of the landfill and exposed them in a clean site for one year. We investigated the physiological performance of the lichen as indicator of the effectiveness of the simulated environmental recovery. The study focused on heavy metal contents, photosynthetic performance, chlorophyll integrity, cell membrane integrity, dehydrogenase activity, production of secondary metabolites. The results showed that lichens could be profitably used as indicator of the effectiveness of environmental recovery policies. An integrated use of lichen based methods can provide useful biological outputs for decision-makers to establish correct science-based environmentally sustainable waste management policies.

**Keywords:** Bioaccumulation, biodiversity, composting, landfill, waste incinerator

## Is Moss Bag Technique a Suitable Approach for Monitoring of Air Pollution in Urban Environment?

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Nowadays air pollution is substantial environmental concern in most cities across the world. Instrumental monitoring of air quality with high spatial-temporal resolution is difficult to implement because it requires expensive equipment and ongoing maintenance of the monitoring stations. On the other hand, biomonitoring has been investigated as a complementary tool to physico-chemical measuring of air quality. Bryophytes have proved to be suitable for trace element and PAH air pollution biomonitoring. In urban areas, where mosses are often scarce or even absent, active biomonitoring, i.e. “moss bag technique”, has been developed. Since 2005, in the Belgrade urban area, the investigation of moss bag application for air quality assessment has been taking place with special reference to development and adjustment of the methodology. Series of experiments were carried out with the aim to test the possibility of trace elements capture by *Sphagnum girgensohnii* moss bags depending on different exposure periods and water supply, as well as its relation to atmospheric bulk deposition. According to the results, for about 30 elements determined by instrumental neutron activation analysis (INAA), the **significant** increase of concentration was obtained in exposed moss bags, especially those irrigated. Significant correlation were found for certain elements (Cu, Zn, V) in moss sample and bulk deposits. In regard to different exposure time, both dry and irrigated moss bags showed a linear trend of accumulation during 1–5 months of exposure for a majority of the 50 elements measured by high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). Further, several experiments were performed in specific urban microenvironments - street canyons, city tunnel and parking garages in order to explore the answer of moss bag to the element ambient concentration under disturbed conditions of natural ventilation. About 15 elements, determined by inductively coupled plasma optical emission spectrometry (ICP-OES), showed statistically significant decrease with height in the moss exposed in street canyons while the highest element content were found in moss exposed inside the city tunnel. These studies were followed by an extensive survey conducted over the whole urban area of Belgrade in 2013, where two moss species *Sphagnum girgensohnii* and *Hypnum cupressiforme* were exposed to atmospheric deposition. The results showed distinctive spatial resolution of trace element concentrations throughout different densely populated, traffic burden and green zones of the city. Additionally, new hotspots of pollution, omitted by current instrumental monitoring network, were discovered by this moss bag survey. The results of all performed studies contribute to validation of active moss biomonitoring of trace elements and PAH air quality. This discipline responds to growing societal demand and should be constantly adapted to scientific and regulatory development in the domain of air quality: emergence of new pollutants, indoor air quality, changes in regulations, standardization, etc.

**Keywords:** Trace elements, PAHs, moss biomonitoring, *Sphagnum girgensohnii*, *Hypnum cupressiforme*

# **SESSION 7**

## Human Biomonitoring



## S7 PLENARY SESSION

# Human Biomonitoring in Europe and Portugal: What is going on?

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Human biomonitoring (HBM) has been defined as a methodology in human beings, using biological indicators (biomarkers) – that focus on environmental exposures, diseases and/or disorders and genetic susceptibility – and analysing the existing links between biomarkers and exposures or pathologies. It allows a more accurate and direct assessment of exposure than environmental monitoring and enhances the knowledge of health risks related to environmental exposures. As a result, HBM can be used to inform the public wishing to know their exposure, estimate the exposure to pollutants and evaluate the impact of policy measures for better public health decision-making, and assess total exposure to emerging substances that might need regulation. The relevance of HBM has been recognised (2004) by the European Commission in its EU Environment and Health Action Plan (2004-2010). In 2005, 2009 and 2010, respectively, three EU funded projects on HBM were started – ESBIO (Expert team to Support BIOmonitoring in Europe), COPHES (Consortium to Perform Human Biomonitoring on a European Scale) and the EU pilot study DEMOCOPHES (DEMOstration of a study to COordinate and Perform Human biomonitoring on a European Scale) – with the aim of developing a framework for gathering HBM data throughout Europe, according to a harmonized approach to obtain comparable results as far as possible. Building on this experience and taking advantage of the Horizon 2020 funding possibilities, the Commission’s DG Research and Innovation is currently exploring opportunities for a European Human Biomonitoring Initiative (EHBMI) with the mission to “*establish a sustainable HBM system for Europe which is of direct use for the EU policy to protect man and the environment against chemicals impact on health, control EU legislations success and further develop knowledge, state of science and a health oriented chemicals policy*”. Specifically the EHBMI will bring together relevant national and EU-level research and policy stakeholders, in order to create a European Joint Programme to strengthen the coordination and collaboration between national initiatives, stimulate research on novel approaches and innovative biomarkers for new/emerging chemicals and mixtures, and generate and use high quality HBM data and information on exposure to chemicals in the European population, including the analysis of spatial and temporal differences within Europe. At Member State level, the EHBMI will rely on the National Hubs established to build the national HBM capacity, to optimize the link between national research and collaborative research carried out across Europe, to represent the different national stakeholders in a coordinated way, and to facilitate the interaction between the national and European stakeholders, providing a single contact point for discussions at EU level. Since the very beginning (2003), Portugal has contributed for the establishment of a European joint initiative on HBM through the active participation of the Faculty of Medicine, University of Lisbon (FMUL) in all concurrent efforts. FMUL integrated the ESBIO project, leading one of its Work Packages (WP) and participating actively in other four; the “Implementation Group on HBM” that was given technical support by ESBIO; and the COPHES and the DEMOCOPHES Projects, as Member State representative for the scientific side and National Management Unit, respectively. A national representative for the EHBMI has already been officially nominated among the FMUL researchers, thereby ensuring that research will be involved in the National Hub to be structured. How to structure the National Hub, ensuring the involvement of all relevant actors – from research to policy stakeholders – is currently under discussion and will be presented in this communication.

**Keywords:** Human Biomonitoring (HBM), European HBM Initiative (EHBMI), European harmonization and comparable data, Evidence-based policy making

Session 7

# Exhaled Breath Condensate: A Tool for Non-invasive Evaluation of Air Pollution Exposure

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Extensive epidemiological evidence supports the association of air pollution with adverse health effects. Aerosol particles or their chemical compounds may interact with the cells of exposed organs and tissues causing a range of various deteriorations. In industrial scenarios, particles are a major concern. Human exposure in the workplace is insidious as dust concentrations are higher compared to outdoors, and workers are closer and stay longer near the emission sources. Assessing the retention of aerosol particles in the human lung, one of the most important pathways of absorption, is a demanding issue. Standard methods are often indirect or invasive, which limits their applicability to monitoring exposed populations. At present, there is no direct biomarker of exposure for the respiratory system. The collection of exhaled breath condensate (EBC) constitutes a new non-invasive method for sampling from group. The relationship between the elemental concentrations measured in the EBC matrix and those measured in blood and in the fine and coarse fractions of airborne particulate matter in the workplace were established. The changes of Pb concentrations in EBC, and other elements that are relevant for the specific work environment, during the working day and throughout the working week period evidenced the sensitivity of EBC to assess the temporal evolution of the exposure levels. The persistence of Pb in EBC supports the use of EBC in assessing target organ levels of inhaled particulate matter. The results of this study highlight the potential of EBC as a medium for assessing lung dose after exposure to inhaled deleterious substances, such as Pb. The integrated use of EBC and classic biological matrices such as urine and blood, which reflect systemic exposure, may therefore allow the completion of the biological monitoring of pneumotoxic compounds.

**Keywords:** Exhaled Breath Condensate, occupational exposure, lead processing industries, inhalation biomarker, particulate matter



# Biomonitoring of Occupational Exposure to Styrene - Induced Genetic Lesions and Individual Genetic Polymorphisms

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Styrene is a commercially important chemical widely used in the manufacture of synthetic rubber, resins, polyesters, and plastics. Occupational exposure to styrene occurs in the styrene-butadiene rubber, styrene monomer and polymerisation, and reinforced plastics industries. Although the genotoxic potential of styrene is known, very limited and inconclusive information is available regarding its dose-dependent genotoxic effects in humans. The objective of this work was to study occupational exposure to styrene in a multistage approach, in order to integrate the following end-points studied: styrene in workplace air, mandelic and phenylglyoxylic acids (MA+PGA) in urine, haemoglobin (Hb) adducts, sister-chromatid exchanges (SCE), micronuclei (MN), DNA damage (comet assay) and genotypes of polymorphic genes of some metabolising enzymes. Seventy-five workers from a fibreglass-reinforced plastics factory and seventy-seven unexposed controls took part in the study. The mean air concentration of styrene in the breathing zone of workers (30.4ppm) was higher than the threshold limit value of 20ppm recommended by the ACGIH, and the biological exposure index adopted by the ACGIH for exposure to styrene prior to the next shift (MA+PGA=400mg/g cr) was exceeded, indicating that styrene exposure for this group of workers was higher than recommended. The level of Hb adducts and SCE in exposed workers were significantly increased as compared with controls. The DNA damage was higher among styrene-exposed workers than in controls. No significant differences were observed in the MN. Concerning the effect of the genetic polymorphisms on the different exposure and effect biomarkers studied, we observed the effect of microsomal epoxide hydrolase activity on Hb adducts of highly exposed individuals and on the levels of SCE of exposed workers. The present results suggest the importance of individual susceptibility factors in modulating genotoxicity, although cautious interpretations are required since the size of the study population limits the power of many of the analyses. Because the effects of these polymorphisms are relatively subtle, and some important alleles are relatively rare, a much larger study population will be necessary to evaluate their effects on biomarkers, especially when gene-gene interactions are considered.

**Keywords:** Biomonitoring, styrene, DNA Damage, genetic polymorphisms

## Airborne Particulate Pollution and Urinary Biomarkers of Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) among Kindergarten Children in Chiang Mai Province, Thailand

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We investigated the exposure to ambient airborne particulate matter with aerodynamic diameter less than 10  $\mu\text{m}$  ( $\text{PM}_{10}$ ) and  $\text{PM}_{10}$ -bound polycyclic aromatic hydrocarbons (PAHs) among 555 kindergarten children. The study sites were 4 rural areas of Chiang Mai province including Mae Chaem, Om koi, Chiang Dao and Phrao districts where frequent open burning in agricultural and forest areas in dry season occurred. An urban kindergarten in Chiang Mai city was chosen as the reference site.  $\text{PM}_{10}$  and urine samples were collected in 2 periods; dry season during January to March 2012 and wet season during August to September 2012.  $\text{PM}_{10}$  samples were collected 24 hour on quartz filter with 5 mL/min flow rate using a portable sampler (MiniVol TAS Air Sampler, USA). Sixteen PAHs bound to  $\text{PM}_{10}$  were analyzed using gas chromatograph- mass spectrometer (GC-MS, Agilent 6890, USA). Parents and children were signed informed consent and assent forms, respectively, for study participation. Children mostly aged 6-7 years old and boys were 52.6%. Urines, total volume, from the children were collected at school from 8 to 16 hrs of 2 school days (the first school day of the week and mid-week day). The total collected urines were pooled and 10 mL aliquots were kept in  $-20\text{ }^{\circ}\text{C}$  prior analysis. Urinary biomarkers, 1-hydroxypyrene (1-OHP) and 3-hydroxybenzo (a) pyrene (3-OHBP), was analyzed using high performance liquid chromatograph-fluorescence detector (HPLC-FLD, HP1100, USA). Concentrations of 1-OHP and 3-OHBP were normalized with urinary creatinine. Structured questionnaires were employed to collect demographic, environmental and behavioral factors from their parents. In dry season, 24 hour mean  $\text{PM}_{10}$  levels were elevated in all rural sites and the highest levels in February and March were 416.8 and 458.2  $\mu\text{g}/\text{m}^3$ , respectively. While the reference site in Chiang Mai city also had the highest  $\text{PM}_{10}$  levels in February and March but at 249.3 and 288.5  $\mu\text{g}/\text{m}^3$ , respectively. Please note that Thailand 24 hour mean  $\text{PM}_{10}$  standard level is 120  $\mu\text{g}/\text{m}^3$ . In wet season, the  $\text{PM}_{10}$  samples were collected only in Mae Chaem site and the reference site. The  $\text{PM}_{10}$  levels at Mae Chaem and the reference site were not significant difference: the highest levels in August and September were 42.7 and 78.7  $\mu\text{g}/\text{m}^3$  and 42.7 and 43.4  $\mu\text{g}/\text{m}^3$ , respectively. Levels of total  $\text{PM}_{10}$ -bound PAHs in February and March were highest in rural site at 64.1 and 54.6  $\text{ng}/\text{m}^3$  and significantly higher than the reference site ( $p < 0.05$ ). Only in dry season that levels of total PAHs very significantly correlated with 24- hour mean  $\text{PM}_{10}$  levels. The increment lifetime cancer risk (ILCR) from exposure to peak air pollution (February and March) was  $1.87 \times 10^{-6}$  in rural site and  $0.95 \times 10^{-6}$  in reference site which was about 2 fold greater. Levels of urinary 1-OHP were significantly correlated with ILCR ( $r = 0.793$ ) but not the levels of 3-OHBP. Therefore, the present study results support urinary 1-OHP as the biomarker of exposure to PAHs from airborne particulate pollution.

**Keywords:**  $\text{PM}_{10}$ , PAHs, urinary biomarkers, kindergarten, Thailand

# Human Biomonitoring Initiatives Under the Government of Canada's Chemicals Management Plan

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The Chemicals Management Plan (CMP) is part of the Government of Canada's comprehensive environmental agenda to ensure the safe management of chemicals. Under the CMP, Health Canada performs health-based monitoring activities (including biomonitoring) to inform policy to help Canadians maintain and improve their health. The objective is to establish nationally representative concentrations of chemicals in Canadians, including vulnerable populations, to inform exposure assessments and chemicals management. This presentation will present an overview of the Government of Canada's human biomonitoring initiatives and highlight some key results. The cornerstone of Health Canada's biomonitoring program is the Canadian Health Measures Survey. This nationally-representative ongoing health survey, carried out in collaboration with Statistics Canada and the Public Health Agency of Canada, includes a biomonitoring component measuring a number of chemicals in the blood and urine of Canadians. Results from the third cycle of the CHMS will be available in Spring 2015. Biomonitoring is also performed in certain targeted vulnerable populations, including pregnant women, children, and northern populations. One of the largest, the Maternal-Infant Research on Environmental Chemicals Study, measures a range of factors, including environmental chemicals, in pregnant woman and their babies. Health Canada also contributes to the Northern Contaminants Program which conducts health research and biomonitoring in Canada's northern populations. Smaller targeted studies include monitoring and surveillance in populations living in geographic areas of concern, research and targeted environmental monitoring to support biomonitoring. Data from selected chemical groups from some of the major national biomonitoring initiatives will be presented. These data will serve as a baseline for comparison with future surveys, will help to determine trends over time, and help assess the effectiveness of risk management actions. Data will also contribute to the evaluation of chemical exposure and the development of policies to help Canadians maintain and improve their health.

**Keywords:** Biomonitoring, exposure

# Blood Lead Levels and Birth Weight: Results from Thirteen Years of Human Biomonitoring

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Human exposure to lead can result in a wide range of biological adverse effects, mainly in children. Sub toxic lead levels during pregnancy can also be responsible for delays in foetal development, reflected in lower birth weight, a factor that predicts infant growth, development, and even survival. In the ambit of two Environmental Health Survey Programs related to solid waste incineration developed in the Lisbon Region and Madeira Island, a study has been developed with the main objective of investigating the relationship between maternal blood lead levels and children's birth weight. Apparently healthy pregnant women living or working in the study areas were recruited during pregnancy or at the delivery time, along the thirteen years of the Programs development. Besides the blood collection, a questionnaire was applied by interview at the recruitment time or during a proximal posterior contact, for gathering relevant information on study participants (age, residence, parity, smoking habits, use of medicines, dietary information, and occupational exposure). Complementary information on the newborns was obtained from the mothers and the maternity records. The global sample along the thirteen years of the study included 1414 women (29% from Madeira Island) with a mean age of 29 years, the majority (86,4%) being Caucasian. In absolute terms, the blood lead levels are significantly lower than the established action level and show a tendency to reduction over the monitoring period, either in Madeira Island or Lisbon Region. For the global study groups no statistical associations have been found between maternal lead levels and children's birth weight. However, stratifying the sample for other factors that can also influence the birth weight (mainly smoking and duration of pregnancy), differences were found between groups, which are discussed in the present work.

**Keywords:** Lead, human biomonitoring, maternal blood lead, birth weight, Portugal

# Lead in up to 6 years Children living in Lisbon Region or Madeira Island

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Human exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Children are most at risk than adults because their body is still developing, their nervous system is more vulnerable and sensitive, and they have a higher intestinal absorption and lower renal excretion. Moreover, children are exposed to higher dose of toxicant relative to their body weight, having more years of future life where the adverse effects can manifest. Previous studies showed that children with high lead levels may have problems like difficulty in learning and delayed growth, permanent brain damage and even death. Based on these demonstrated risks, blood levels  $\geq 10$   $\mu\text{g}/\text{dl}$  were defined as action levels. However, accumulated evidence shows adverse health effects (for example, hyperactive or being irritable) even when exposed to small amounts of lead, leading to the establishment of a lower limit (5  $\mu\text{g}/\text{dl}$ ). Lead poisoning is one of the most preventable childhood illness, making it imperative monitoring children's exposure to lead, mainly in the neighbourhood of polluting sources. In the present work, two projects on exposure to lead of children under six, developed in the ambit of Environmental Health Survey Programs in Lisbon and Madeira Island are described. Children's exposure to lead has been assessed by determining blood lead levels. Based on these values, either stratified by age or living area, temporal trends of lead exposure were evaluated. Altogether, 836 apparently healthy children, aged 1 to 6 years, 459 from Lisbon and 377 from Madeira Island, participated in the study, after written informed consent from their legal responsables. Results show that only a small percentage of children from the whole group have blood lead levels above the limits, higher mean levels in Lisbon Region than in Madeira Island, and a tendency to reduction of lead levels over the monitoring period in both regions. From a public health perspective and mainly in relation to children, periodic and systematic biomonitoring of childrens's exposure to lead should be carried out in the future, to support policy measures to reduce and/or control human exposure to lead.

**Keywords:** Human Biomonitoring, children, blood lead levels, infant exposure to lead, Portugal



**SESSION 8**

**Biomonitoring of  
Atmospheric Pollution Using  
Magnetic Properties**





# Biomonitoring of Airborne Particulate Matter Pollution with Magnetic Measurements on Leaves and Lichens: Principles, Methods and Experimental Results

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In recent years, the magnetic properties of soils, snowpacks, street dusts, air filters and tree leaves have been widely analyzed for characterizing the pollution arising from different vehicular and industrial sources. Magnetic particles may originate from both combustion processes, related to industrial, domestic and vehicle emissions, and from abrasion, as for vehicle brakes. They are usually spherules or irregularly shaped grains, containing variable amount and grain-size of iron oxides. In anthropic/polluted aerosols, magnetite is usually the main magnetic carrier and is often associated to heavy metals like zinc, cadmium and chrome and even to dangerous mutagenic organic compounds. Usually, the air quality is monitored by means of specific stations; however, biomonitoring on tree leaves, needles, tree ring cores, mosses is recently establishing as a complementary and effective way for investigating the airborne pollution and its biological effects. Tree leaves are efficient particulate matter (PM) receptors and they act as widespread natural biomonitors of airborne PM; their magnetic properties can be studied to provide high-resolution pollution data, since environmental magnetism techniques are sensitive, reproducible, rapid, and relatively cheap. Moreover, magnetic analyses are particularly effective for discriminating different anthropic and natural sources of PM. The aim of this talk is to introduce, after a brief foreword on the basic principles of magnetism of matter, the main laboratory methods involved in environmental magnetism. It will be presented a brief overview of the main case studies concerning magnetic biomonitoring, and a review of the results achieved at INGV on the study and the comparison of the magnetic properties of leaves, air quality filters and vehicle related powders (fuel, brakes). Up-to-date magnetic analyses on lichens from cement plants and industrial contexts will be introduced to encourage the discussion on this promising direction of biomonitoring research.

**Keywords:** Biomonitoring, particulate matter, magnetic properties, leaves, lichens

# Magnetic Properties, Bioaccumulation, Physiological status and Ultrastructure of Lichens Exposed around a Cement Plant

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A cement industry is a source of dust pollution, from quarrying and grinding of the raw material, kiln operations, transportation, and packing and dispatch of the cement. Airborne pollutants related to combustion processes are also emitted, especially during kiln operations and power generation. The use of biomonitors can provide valuable information about the impact of airborne pollutants released during cement production. In this framework, lichens are suitable bioindicators of the effects of air pollution, providing reliable information on the quality and characteristics of the environment. We investigated the biological effects of airborne pollutants released during cement production by means of epiphytic lichens at selected sites (a cement mill, two quarries, agricultural areas, villages) in SW Slovakia. Using *in situ* samples (*Xanthoria parietina*) we found out that Ca, Ti, Fe, V, Al and Ni were significantly higher around the cement mill and the quarries than in the surrounding environment. Calcium was the best tracer for dust contamination around the quarries and the cement plant and a clear decrease in its content with increasing distance from the source emerged, with background values reached at ca. 1,700 m from the cement mill. In a second step, the content of selected elements was measured in samples of the lichen *Evernia prunastri* exposed up to 180 days at the same sites. Dust-associated elements, i.e. Ca, Fe, Ti, rapidly (30 days) accumulated in the thalli, while airborne pollutants (S) progressively increased. Concerning the physiological response of the samples, dust pollution influenced the efficiency of the primary photochemistry of PSII and the vitality of the mycobiont. TEM sections were made to observe ultrastructural effects. In parallel, we investigated the magnetic properties of both transplanted and *in situ* lichens, bark, soil and rock samples from the same sites, as well as pre-transplant samples. *Evernia prunastri* transplanted in the study area showed excellent correlations between the values of magnetic saturation and Fe concentrations. *Evernia prunastri* samples are magnetically homogeneous, with marked differences only for the sample from the basalt quarry. *Xanthoria parietina* samples have a similar magnetic mineralogy, but magnetization saturations are two orders of magnitude higher, implying increased concentration of magnetic particles according to the different lichen species and to the prolonged exposure. The analyses of magnetic mineralogy pointed out the influence of soil and bedrock, when they are highly magnetic, as in the case of the basalt. In biomonitoring studies, magnetic methods can be valuable for discriminating different natural and anthropogenic sources; for this reason, it is essential to verify the nature of the substrate and to select suitable pre/post-transplant sites.

**Keywords:** Calcium, dust, *Evernia prunastri*, magnetization, *Xanthoria parietina*

# Biomagnetic Monitoring of Urban Air Pollution Using Moss Bags (*Sphagnum girgensohnii*)

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Plant magnetic properties such as susceptibility as well as remanence has been estimated as a good proxy for particulate matter (PM) air pollution. Although mosses are widely used in biomonitoring studies, magnetic analysis on these bioindicators were only reported in few cases and for industrially polluted areas predominantly. This study explored the suitability of the moss *Sphagnum girgensohnii* for biomagnetic monitoring in cities. To this end *Sphagnum girgensohnii* moss bags were exposed at three different urban microenvironments characterized by heavy traffic in the city of Belgrade. The moss bags were mounted in the street canyons at heights of 4, 8 and 16 m with the aim to study vertical pollutant distribution. On the other side, horizontal pollutant distribution was tested in semi enclosed spaces such as a city tunnel and parking garages. In the city tunnel, moss bags were hung at 4 m inside - 100 m from the entrance; in front of the entrance; and outside of the tunnel. In the parking garages, at 2.5 m above ground, moss bags were suspended at two places: near the entrance/tollbooth and in the garage interior. During the experiment – summer and autumn of 2011, traffic flows in the studied microenvironments were counted. After 10 weeks of exposure, the concentrations of Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, V and Zn were determined in the moss samples by inductively coupled plasma optical emission spectrometry. The ferro(i)magnetic PM fraction in the moss samples was quantified by Saturated Isothermal Remanent Magnetization (SIRM) using a Molspin Minispin magnetometer. The measured SIRM were compared with the trace element concentration in the moss samples. According to the results, moss SIRM values were significantly different across the considered urban microenvironments. SIRM values varied between 5.1 – 9.9 A m<sup>2</sup> kg<sup>-1</sup>, 10.5 – 19.1 A m<sup>2</sup> kg<sup>-1</sup> and 30.9 – 93.6 A m<sup>2</sup> kg<sup>-1</sup> in the parking garages, street canyons and city tunnel, respectively. The vertical and horizontal trends of pollutant concentrations were observed. Very high correlation coefficients ( $R \geq 0.95$ ) between moss SIRM and concentrations of Cr, Cu, Fe and Ni were demonstrated. Moss SIRM values exhibited a relatively high linear correlation ( $R = 0.72$ ) with the average traffic intensity counted in the studied urban microenvironments, as well. The results emphasized that for biomagnetic monitoring of air pollution using moss bags it is crucial that the exposition of the moss bag is representative for the particulate load at the sites of interest which depends on the structure of the site and its specific air ventilation. Finally, in urban microenvironments, *S. girgensohnii* is capable to reflect moss SIRM enrichment depending on traffic intensity, as well as its relation to the ambient element concentrations.

**Keywords:** biomagnetism, SIRM, air pollution, trace elements, moss *Sphagnum girgensohnii*

# A Comparative Study between Biomagnetic Analysis and Scanning Electron Microscopy of Particulate Matter Deposition on Urban Green

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In an urban environment, particulate matter (PM) is mainly due to anthropogenic activities such as transportation, construction and industrial emissions. The particulates are formed by a panoply of small particles (including magnetic iron-based particles) that can be immobilized on the existing vegetation through leaf deposition or in-wax encapsulation. Biomagnetic monitoring of tree leaves, through the measurement of saturation isothermal remanent magnetization (SIRM), already proved to be a good bio-indicator for ambient PM, providing a rapid and cost-effective method to investigate both temporal and spatial PM loadings. On the other hand, PM source attribution requires a deeper understanding on the composition and particle size distribution. This study demonstrates the analytical potential of combining biomagnetic analysis with scanning electron microscopy (SEM) on the assessment of PM<sub>2.5-10</sub> deposition for different pollution sources on leaves collected in the city of Antwerp, Belgium. While SIRM is an estimator of the ferro(i)magnetic content, SEM allows the chemical characterization of particles deposited at the surface level. In order to analyze different urban activities, common ivy leaves were collected from a forested area, a rural area, next to a high traffic crossroad, an industrial complex and a train line. Their bulk SIRM values for normalized leaf area were obtained, with mean values ranging from 24.0  $\mu$ A (forested area) to 399.5  $\mu$ A (train line). For each sampling site, SEM combined with Energy Dispersive X-ray Spectroscopy was applied to approximately 40,000 leaf deposited particles, so that chemical composition was estimated according to the different sites. Within each sampling site, both adaxial and abaxial leaf sides were analyzed with SEM, revealing similarities in terms of particle distribution and composition. In a subsequent experiment, ivy leaves were hand washed so that the PM deposited on the leaf surface was retained in a washing solution and then submitted to filtration using two different pore size filters (10 and 3  $\mu$ m). The resultant filters and washed leaves were SIRM analyzed to study both the leaf surface deposited particles and leaf encapsulated particles from the total SIRM signal. Their relative contribution appeared to vary with the source of pollution. Size distribution analysis on the deposited PM suggested the importance of fine PM (PM<sub>2.5</sub>). The relation between the SIRM signal and the iron content registered by SEM was not completely clear, suggesting that the iron-based particles (which should be source-dependent) result in different SIRM signals according to their chemical structure. Further research must be pursued on this topic to disclose the remaining unanswered questions.

**Keywords:** Urban air pollution, PM deposition, pollution sources, saturation isothermal remanent magnetization (SIRM), scanning electron microscopy (SEM)

# POSTER SESSION



# Active Moss Biomonitoring: An Approach for Extensive Screening of Air Pollution over Urban Area - Belgrade Case Study

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Nowadays air pollution is a substantial environmental concern in most cities across the world. Instrumental techniques conventionally applied for air quality measurements gives instantaneous value of pollutants at a sampling time and with limited spatial resolution due to practical constraints. On the other hand, biomonitors act as long-term integrators of air pollution and could be used as transplants enabling high density of sampling sites over the wide area of interest. In this study, over the whole metropolitan area of Belgrade, active magnetic biomonitoring of particle air pollution, as well as trace elements and PAHs content assessment, were performed. Two moss species were used, one from the most recommended biomonitor moss genera - *Sphagnum* and another common for the studied area – *Hypnum*. The moss bags were exposed at 153 sampling sites during a summer season (June – August 2013). The magnetic particles captured by the exposed mosses were quantified by saturation isothermal remanent magnetization (SIRM). In the moss samples, concentration of Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, V and Zn were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) while 16 US EPA priority PAHs were determined by gas chromatography mass spectrometry (GC-MS).

Significantly higher levels of all measured pollutants were recorded by *S. girgensohnii* in comparison with *H. cupressiforme*. There was a visible distinction in moss SIRM as indication of magnetic particle distribution over the Belgrade urban area depending of a prevailing public transportation type - buses, trams and trolleys. The highest moss SIRM for both species were observed in streets occupied with tram traffic. In general, the highest moss element concentrations (Cu, Cr, Fe) were observed at the sites influenced by local industry and very busy roads. Significantly high correlation coefficients ( $R \geq 0.7$ ) were obtained between the measured SIRM and Cu and Cr in the moss samples. The most abundant PAHs in the moss samples were those of low and medium molecular weights. Nevertheless, according to the relative contamination factors obtained by both moss species, similar city zones related to high, intermediate and low level of the measured pollutants as well as green zones were distinguished. Moreover, new hotspots of pollution, omitted by current instrumental monitoring network, were discovered by the performed active moss biomonitoring.

The results of this study confirmed that the use of moss bag technique could be a simple, sensitive and inexpensive way of obtaining extensive information on deposition levels of certain airborne pollutants in urban area and could be recommended for screening of air quality within cities.

**Keywords:** *Sphagnum girgensohnii*, *Hypnum cupressiforme*, SIRM, trace elements, PAHs

## Chemical Element Accumulation in Tree Bark grown in Volcanic Soils of Fogo Island (Cape Verde)

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Fogo island belongs to the Cape Verde archipelago (Atlantic Ocean), circa 800 km westwards of the Senegal coast on the Cape Verde Rise. This island corresponds to an active volcano with the maximum altitude of 2829 m. The climate is semi-arid, with a rain period mainly between July and October. Soils are developed in three major geological units: 1) a carbonatite; 2) a pre-caldera formation; and 3) a post caldera sequence including several historic eruptions.

Bark from several types of trees including *Acacia albida* Del. (*acacia*), *Jatropha curcas* Linnaeus (*purgueira*), *Eucalyptus Tereticornis* (*eucalyptus*), *Acacia Dealbata* Mimosa (*mimosa*) and *Malus Pumila* P. Mill (*apple tree*), were collected in eighteen sites corresponding to different geological formations. Elemental contents in biological samples and soils were assessed through k(0)-standardised instrumental neutron activation analysis (INAA) and comparative method. The main goals are: (i) the bark response of the same tree to differences in soil composition and climate conditions; and (ii) the evaluation of eventual differences on the chemical elements uptake of different biomarkers within the same site. This work is a first contribution to the biomonitoring of the Fogo island.

Among the chemical elements studied, iron was selected to normalize chemical contents and calculate the enrichment factors (EF), due to its precise and accurate determination by INAA and conservative behaviour in this type of soils/climate.

The results obtained showed significant accumulations (EF>10) of most of the chemical elements studied: maximum EF value - K (EF= 6469); and minimum EF value – Sm (EF=2.65).

In general in *Acacia* bark: (a) Lu, K and Br uptake is similar in all geological formations; (b) sodium is more enriched in soils developed in carbonatites and nephelinites; (c) the lower EF values were found in pyroclasts soils; and (d) Zn is more enriched in historic lavas. Concerning *Purgueira* bark, a similar high accumulation of Lu, K and Br occurs in all soils. EF>10 are found for Na and Ba (particularly in nephelinite soils), and Cr and Zn in nephelinites and historic lavas. The higher EFs were also found for Lu, K, and Br in *eucalyptus* bark. Zn, Yb and Cr are also enriched in pyroclasts.

In *apple trees* grown in pyroclasts soils, K, Lu, Yb and also Cr are enriched. *Lichens* collected in the less arid area of the island showed significant enrichments only for K and Br.

Among the rare earth elements (REE), EFs of the heavy REE (EF<sub>Lu</sub> up to 4439) are much higher than the light ones in bark, which agree with their preferential release after the breakdown of primary minerals like pyroxenes.

The results obtained indicate that the chemical elements may be significantly accumulated by plants grown in volcanic soils of Fogo island, which can be explained by the semi-arid climate and a consequent bioavailability of chemical elements when rain drops fall in this non-pollutant environment.



**Keywords:** Fogo island, elemental accumulation, volcanic soils, semi-arid climate, INAA

P3

# Evaluation of Contamination by Selected Elements in Moss and Soil in Moscow and Tver Oblast's

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The results of trace element atmospheric deposition study in two regions of Central Russia based on moss and soil analyses are presented. The sampled territory included Moscow and Tver Oblast's. Epithermal neutron activation has enabled determination of up to 40 elements including heavy metals and rare earth elements in 170 moss and soil samples collected in the summer of 2014.

The goal of this study was to evaluate the local pollution sources and to reveal possible pollution hot-spots in the study area, as well as to conduct a comparative analysis of the present results and those from the previous moss survey in 2009. Multivariate statistical analysis has been applied to the obtained datasets. *Contamination factors* for selected elements and *ecological risk indexes* were calculated. The comparison of elemental concentrations in atmospheric deposition in different parts of Russia was carried out.

**Keywords:** moss biomonitoring, soil, heavy metals, environmental pollution, contamination factor

Poster Session

# The Moss Technique and Neutron Activation Analysis for Trace Element Atmospheric Deposition Study in Tikhvin District, Leningradskaya Oblast'

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For the first time the moss biomonitoring technique was used to study trace element atmospheric deposition in the area affected by a ferroalloy (Fe-Cr) plant in the town of Tikhvin, Leningradskaya Oblast', to appportion its deposition patterns and to reveal previously unknown pollution sources located in and within the sampled territory. Moss samples were collected in the summer of 2011 from 36 sites evenly distributed over the Tikhvin District in accordance with the guidelines of the UNECE ICP Vegetation. A total of 35 elements were determined by means of epithermal neutron activation analysis at the reactor IBR-2 FLNP, JINR, Dubna. Multivariate statistical analysis was applied to characterize the sources of determined elements over the examined territory. *Contamination factors* (CF) for selected elements and *ecological risk index* were calculated using their concentrations for the Tverskaya Oblast' considered as a relatively unpolluted territory. The results obtained are compared to the data of the atmospheric deposition of trace elements in Central Russia and in some countries of Eastern Europe. Distribution maps of most hazardous element-pollutants over the surveyed territory created using GIS technologies are demonstrated. These maps show that the main source of contamination in the investigated area is Tikhvin ferroalloy plant. In the close vicinity of Tikhvin the highest content of Al, As, Co, Fe, Cr, Ni, and V in moss samples was observed. Factor analysis revealed that high content of these elements had one and the same source. In the 10 km zone around the town of Tikhvin the CF is 2 times higher than for the rest of the investigated territory. Maximum value of CF for Cr was determined in the close vicinity of the Tikhvin ferroalloy plant. The main source of contamination in the sampled area is the metallurgic plant in the town of Tikhvin. Another source is located close to the military aerodrome and metallurgic waste dump in the north of the investigated territory. In general, the investigated area is quite pristine in comparison with the other Russian territories.

**Keywords:** moss biomonitoring, heavy metals, atmospheric deposition, risk index

# X-ray Fluorescence Analysis in Leaves of *Nerium oleander L.* used as Environmental Biomonitor

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Since the early 20<sup>th</sup> century, the air pollution in the major centers worsened in consequence of the industrialization and urbanization process, along with the fast population growth and the motorized transport. Some plants are used in the detection of the air pollutants due to leaves ability to absorb the pollutants through their stomata and the cuticle, thus, the plants leaves work as a natural sampler of the air emissions that are deposited in their self. Therefore, the foliar analysis of these vegetal species can be used to environmental monitoring. The *Nerium oleander L.* is a specie of plant commonly used as an environmental biomonitor.

In this study *Nerium oleander L.* leaves were used to measure the environmental pollution levels in a sub-region in the Rio de Janeiro Metropolitan Region - Brazil using X-ray Fluorescence (EDXRF). The EDXRF system was developed in the own laboratory and consist of a portable XRF system formed by a mini X-ray tube of low power (anode of Ag and operated in 15 kV/50  $\mu$ A and 35 kV/50  $\mu$ A) and a SiPIN detector. The samples of *Nerium oleander L.* were collected from adult plants drawn randomly so as not favouring one side of the plant. The samples were collected during the year seasons (summer, fall, winter and spring). It was possible to determinate the elemental concentration of 13 elements: S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Rb, Sr, Ba and Pb.

From the concentrations of each element, the elemental maps of the studied areas in each season were obtained. The Pearson's correlation analysis shows a significant correlation between the elements Fe, Zn, Ba and Pb; Ca and Sr and between the elements Cl, K and Rb. The Principal Components Analysis shows that there are two mainly factors of the environmental pollution emissions: emission through the resuspension of the soil (Cl, K, Ca, Mn, Rb and Sr) and vehicular/industrial emission (Fe, Zn, Ba and Pb). So, the *Nerium oleander L.* leaves analysis by XRF shows to be efficient in the environmental evaluation of several elements.

Therefore, *Nerium oleander L.* can be indicated as a low cost option to the ornamentation of inside and outside areas in the industry with a polluter potential with the intension of preservation and alert in case of an incident with the release of potentially polluting materials such as heavy metals.

**Keywords:** X-ray Fluorescence, *Nerium oleander L.*, heavy metals, environmental pollution, environmental biomonitoring

# Zn at Ecosystem Level as Heavy Metal and Bio-element

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Zn is a bio-element, highly necessary for plants functioning, but on the other hand, a heavy metal prone for accumulation in ecosystems at higher air pollution levels. In order to characterise Zn and its connectedness to different parts of coniferous ecosystem, data from different UN ECE International Cooperative Programmes were compared: moss carpet (ICP Vegetation moss survey data), litter layer, and organic layer (mor humus) of coniferous forest (ICP Forest soil survey data). In addition *Pinus sylvestris* and *Picea abies* fine roots and litterfall data from 3 ICP Integrated Monitoring programme plots and deposition data of 18 local precipitation stations was used. Objectives of this study are 1) to assess differences in Zn accumulation in various aged media 2) to estimate the variation of concentration and storages of Zn in different media and parts of ecosystems.

The comparison of studied media revealed that Zn has a different retention pattern compared to highly accumulative Pb or Ni. The retention characteristic to Zn could be described in the increasing order of mor layer < moss layer < soil litter layer, where the concentrations in moss layer are a half and in mor layer three times lower than Zn concentrations determined in soil litter layer. The mor/moss layer ratio was calculated for detecting the level of retention in the organic layer of soil. Unexpectedly, the ratio for Zn is extremely low (0,6) in comparison to other heavy metals (for Pb 10; for Cr 34). The highest concentrations of Zn were measured in the second year's fine roots (16 mg/kg). In comparison to litterfall, the average concentrations in fine roots were 30% higher for Zn (5 times higher for Cd and Pb). The largest stocks of heavy metals were found embedded in the organic layer of soil.

Compared to the annual input of HMs in litterfall and uptake by fine roots, the stock of the metals in fine roots was about 50% higher than in litterfall. The age of fine roots is estimated to be only 2 years and after the decomposition process starts. Considerable uptake of heavy metals by roots promotes retention of metals in soil, as the decomposition rate of root litter is low in older coniferous forests. The retention of Zn in mosses is relatively low in comparison to other metals - according to Steinnes (1985) approximately 41% of Zn, 84% of Cr and 100% of Pb is retained in mosses. Low retention of Zn in mosses explains the outstandingly low mor/moss ratio, which may lead to underestimation of air pollution related Zn. Our data shows that considerable yearly uptake of heavy metals by roots promotes retention of metals and delays purification processes of forest soils from previously accumulated heavy metals. Zn is a heavy metal very prone to long-range trans-boundary transport and simultaneously Zn is one of the most reused heavy metal at ecosystem level. That makes it hard to estimate the actual amount of air pollution originated from Zn cycling in ecosystems.

**Keywords:** Zn, moss carpet, soil organics, fine roots, litterfall

# Soil Pollution Analysis in the Rio de Janeiro State

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Increased pollution from vehicle emissions, industrial waste, atmospheric deposition of dust and aerosols and other activities has caused severe disturbances in the natural geochemical cycle of ecosystems and present risks to environment and the human health. The metal contamination is one of the most severe forms of pollution of the land ecosystems. Due to the severity of the problem, the assessment of pollution requires efficient and sensitive analytical techniques to obtain results quickly and accurately. Recently, X-ray fluorescence technique has proven to be very versatile and can be applied in different complex samples, such as leaves, wood and animal waste. This technique offers the great advantage of evaluation samples, qualitatively and quantitatively, allowing simultaneous and quick multi-elemental analysis. The aim of this study is to evaluate, through the X-ray fluorescence technique, the degree of soil contamination of a site under intense urban pollution, order to provide data for the development of biomonitoring in one of the most important cities in Brazil.

Soil samples were obtained from: (1) *campus* of the Oswaldo Cruz Foundation (FIOCRUZ), located on Brasil Avenue, one of the main urban thoroughfares of the city of Rio de Janeiro, and (2) Tinguá Biological Reserve, a preserved remnant of Atlantic Forest of Rio de Janeiro State (control site). The samples were collected in five areas of each studied site. In each area, five samples were obtained, totaling 25 samples from each site. Each sample was homogenized and dried in oven at 60 °C for 24h. After drying, the samples were triturated, sieved through a nylon mesh in order to obtain a granulometry of 325 mesh (44µm) and pressed by 8 tons in compactors of 2.5 cm diameter, building tablets of 500 mg. Each tablet was analyzed in a portable EDXRF system, formed by a mini X-ray tube of low power (anode of Ag and operated in 15 kV/100 µA and 35 kV/100 µA) and a SiPIN detector. It was possible to determinate the concentration of the 15 elements: K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Ga, Rb, Sr, Zr, Nb e Pb. The mean comparison tests, Test t and Mann-Whitney, showed significant differences between the studied sites for all elements, except for Ca, Ti, Fe and Ga. The soil sampled in the polluted area showed lower concentration of essential elements for plant nutrition, as K and Mn, and higher levels of heavy metals: Cr, Ni, Cu, Zn, Zr and Pb, when compared to the preserved area. The concentrations of the elements chromium, zinc and lead, observed in both studied sites, are above the reference normal values for Brazilian soils. However, in the polluted site, we observed that zinc and lead concentrations are above the tolerance limits established for Brazilian soils. This study produced inexistent data for the studied sites and confirmed the potential of the technique for evaluation of soil samples.

**Keywords:** urban pollution, heavy metals, environmental health, lead contamination

# Determination of Metals in Air Samples using the APDC Technique Preconcentration and Analysis with X-ray Fluorescence

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Air pollution has become one of the leading quality degradation factors of life for people in large urban centers. Studies indicate that the suspended particulate matter in the atmosphere is directly associated with risks to public health, in addition, it can cause damage to fauna, flora and public / cultural patrimonies. The inhalable particulate materials, especially the ultrafine fractions that are below 2.5 microns in diameter, can cause the emergence and / or worsening of chronic diseases related to respiratory system and other diseases, such as reduced physical strength. Thus, knowledge of the levels of atmospheric pollutants is critical to the health and welfare of the population.

The present study proposes the development of a low cost method to measure the concentration of metals in the air by Total Reflection X-ray Fluorescence (TXRF) technique using a bubbler as sample collection instrument. The metallic elements are normally present in air in trace levels thereby it is necessary to use the preconcentration technique to increase the detection limit of these elements. The complexing agent APDC (ammonium pyrrolidine dithiocarbamate) was used to preconcentration of samples. The collection of air was performed for 24 h with a volumetric flow rate of 1.8 L/min. Samples were collected from five random points in the State of Rio de Janeiro/Brazil. Analyses of TXRF were performed at the Brazilian Synchrotron Light Laboratory (LNLS).

It was possible to determine the volume concentration of the metallic elements Cr, Fe, Ni, Cu and Zn. The concentrations of these elements ranged 2-200 ng/m<sup>3</sup>. These elements are closely linked to urban traffic and emissions from industries. Although the collection points are in residential areas, its proximity to roads with heavy flow of cars and also the urban restructuring works of the city, with the construction of some important roads, may have influenced the presence of these elements in the sampling points.

It can be concluded that the metals analysis technique in air samples using a bubbler as sample collection instrument associated with a complexing agent (APDC) was viable and it was possible the detection of five important metal elements in environmental studies associated with industrial emissions and urban traffic. Moreover, this technique has the advantage of being low-cost.

**Keywords:** TXRF, particulate matter, impinger, APDC preconcentration, environmental pollution

# Holm Oak Leaves Effectively Point Out Spatial and Temporal Trends in Pollutant Depositions

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In the last two decades, holm oak (*Quercus ilex* L.) leaves for their morphological characteristics, such as hairs and waxes, have been proved to be feasible biomonitors to assess air contamination by heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs). The wide distribution of this species in the Mediterranean, area where it represents the potential climax community in natural areas and it is used as ornamental plant in urban areas, makes this evergreen oak widely employing in the monitoring of both inorganic and organic pollutants. Despite the many advantages, the International and National legislations do not include the biomonitoring among the methods to assess air pollution.

In this framework, this work aims to present a historical data set of selected HM and PAH concentrations in *Q. ilex* leaves collected at different sites (remote, periurban, urban, industrial) of Campania region (southern Italy) during several monitoring campaigns from 1986 to 2009.

During all the monitoring campaigns, the same sampling protocol was applied making possible a comparison: fully developed and healthy one-year old leaves were collected around the canopies of 4-5 trees for each site at about 3 m of height, and analysed without washing. In particular, oven-dried leaves were mineralised with a mix of nitric:hydrofluoric acids to determine Cd and Pb concentrations by Atomic Absorption Spectrometry or Inductively Coupled Plasma Atomic Emission Spectroscopy. Contextually, fresh leaves were sonicated in a mix of dichloromethane:acetone and the extracts analysed for acenaphthylene, phenanthrene, fluoranthene, pyrene, chrysene, benzo(a)pyrene, indeno(1,2,3 cd)pyrene concentrations by gas chromatography coupled with a mass detector.

The results showed a clear trend among differently affected sites, as well as a spatial gradient among urban sites regarding both HM and PAH leaf concentrations. The findings also showed a continuous decrease of Pb concentrations over the time, overall attributable to the use of unleaded fuel from 1986 until now.

These findings, highlighting both spatial and temporal trends in pollutant depositions, can contribute to promote the use of biomonitoring in the assessment of air pollution, suggesting its introduction in the legislation guidelines to monitor air quality.

**Keywords:** pollution maps, heavy metals, PAHs, biomonitoring, evergreen oak

## Air Pollution Biomonitoring with the Lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale in Industrial Areas from Southern Brazil

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Lichens are widely used as biomonitors and bioindicators, since they are among the most sensitive group of organisms in the world. One of their important usages is to monitor the effects of environmental changes, especially the ones that are caused by atmospheric pollution. Among the possible ways to monitor air pollution with lichens is the transplant method, which has the advantage to be easily standardized along sampling sites. Considering this, the present work has as main objectives: a) to evaluate the air quality in different industrial areas, and b) to relate the changes caused in the lichen thallus with the pollutants absorbed by the lichen. The biomonitoring was carried out with the transplant of some thalli of *Parmotrema tinctorum* from areas with no industrial atmospheric pollution to other areas with two conditions of atmospheric pollution: one with a greater number of industries and expected as more polluted (cities of Esteio, Triunfo and Charqueadas); and other with less industries and expected as less polluted (cities of Montenegro, Santo Antônio da Patrulha and Caraá) all located in the state of Rio Grande do Sul, southern Brazil. The lichens were exposed in proper and standardized structures from March to October 2013 and from March to May 2014. The following pollutants were analyzed in the lichen thalli: Sulfur, Copper, Zinc, Iron, Manganese, Chromium, Nickel, Lead, Vanadium and Aluminium. We also analyzed the percentage of live, dead and plasmolysed algae cells, as well the content of organic carbon and the content of Chlorophyll a and b, in order to test the physiological response of lichens to the atmospheric pollution of each studied site. A Canonical-Correlation Analysis (CCA) was made to test the relation between the pollutants (environmental matrix) and the physiological effects (biological matrix) to observe how each pollutant measured were related to the sampled sites. This analysis was made including all variables and the significance of all canonical axes were tested through a Monte Carlo test, all made in the software CANOCO. The correlation found between the two matrices was of 0.90 for the first axis and 0.65 for the second, being the cumulative percentage variance equal to 80% considering the two of them. The test of significance showed that both axes were also significantly different ( $F = 1.96$ ;  $P = 0.03$ ). Considering the pattern observed in the canonical ordination diagram it is possible to note that there is a real difference between most of the more polluted sites and the less polluted ones, according to the pollutants and the physiological damages on the lichens. It also shows that even the sites where we expected to have very low concentrations of pollutants are somehow contaminated with important components, such as Chromium. As a previous conclusion, we can affirm that the lichen *Parmotrema tinctorum* showed a good response to atmospheric pollution and that the pollutants tested in the studied region are spread all over it, affecting even less industrial cities. The particular sources of pollution of each site are under investigation yet.

**Keywords:** industrial pollution, lichenized fungi, monitoring



# Accumulation of Organic and Inorganic Pollutants in Moss and Lichen bags: Preliminary results of an exposure in London and Naples

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Mosses and lichens have been largely used so far to detect environmental alterations and to monitor deposition of airborne pollutants as heavy metals and more recently, organic pollutants as polycyclic aromatic hydrocarbons (PAHs) and dioxins. The dependence of moss and lichens on atmospheric inputs makes them able to accumulate not only nutrients but also xenobiotics via air depositions. There are a lot of studies that demonstrate the possibility to use transplanted mosses and /or lichens to monitor deposition of pollutants at different scales.

Here the results of a biomonitoring study carried out in two cities, London (UK) and Naples (Italy), by four biomonitors (two lichens and two mosses) are presented.

Lichens, *Pseudevernia furfuracea* (L.) Zopf var. *furfuracea* and *Parmelia sulcata* Taylor, and mosses, *Sphagnum capillifolium* (Ehrh.) Hedw. and *Hypnum cupressiforme* Hedw., were exposed in bags for 10 weeks at urban streets of Naples and London cities, semi-rural sites and, rural areas chosen as controls. Pre and post exposure mosses and lichens were powdered and analysed for heavy metals (Cd, Cu, Cr, Fe, Ni, Pb, V and Zn) by Atomic Absorption Spectrometry after acid digestion, and for PAHs (16 EPA PAHs) by gas chromatography coupled to mass spectrometry after matrix solid-phase dispersion.

Regarding the heavy metals, the comparison between the selected mosses highlights that, in all the exposure sites of the two cities, *S. capillifolium* had a better accumulation performance than *H. cupressiforme*, whereas for the lichens it is *P. furfuracea* to accumulate a greater number of metals respect to *P. sulcata*. Also for total PAHs, *Sphagnum* showed a good accumulation capability compared to the other investigated biomonitors, overall if compared to the other moss. The analyses of metal and PAH concentrations in moss and lichen bags indicate an overall higher pollutant accumulation in biomonitors exposed in Naples compared to London sites. Moreover, the findings highlight a spatial trend of heavy metal and PAH concentrations in the exposed biomonitors from rural to urban sites, both in London and Naples cities.

**Keywords:** mosses, lichens, heavy metals, PAHs, pollutant accumulation capability

# Genetic Diversity of *Pinus sylvestris* (L.) and *Pinus nigra* (Arn.) Growing in the Area Polluted by Heavy Metals

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Damage caused to pine forests by industrial pollution is observed even several decades after emissions have stopped down. *Pinus nigra* Arn. and *P. sylvestris* L., important European coniferous trees, differ in their adaptability to environmental pollution. The sources of this difference are still not sufficiently known. The aim of the study was the comparison of the above-mentioned species, which needles are used as biomonitors of heavy metals pollution, in terms of their genetic diversity reflected in the ability to survive in the same area under extremely harmful conditions. Studies were conducted within the protective zone at a zinc smelter and a copper smelter (S Poland). Both selected areas manifested a high extent of anthropogenic pollution-induced environmental degradation until the end of the 90s. Control group were natural populations of Scots and Black pine from the region of National Park of Wielkopolska. A simple morphological feature - the leaf fluctuating asymmetry - was used as an assessment of the bioindication of pine stands growing in a heavily degraded area condition. A study was performed on the developmental instability of needles. Among both examined species sensitive and tolerant trees were observed, each one being different in terms of health status. To assess a genetic structure of populations a common set of chloroplast microsatellite (cpSSR) markers was used. Polymorphism of cpSSR markers was tested and basic genetic parameters i.e. mean number of alleles, number of haplotypes, Nei's gene diversity and Shannon index were determined. The highest genetic diversity was observed in *Pinus sylvestris* (sensitive group) and the lowest in *Pinus nigra* (sensitive group). The AMOVA recognize a strong and significant separation among observed species less pronounced into sensitive and tolerant groups. These results in combination with the overall health condition of examined trees may indicate the action of selection pressure directed against genotypes specific for Scots and Black pine and connected with different mechanism of reaction on stress. It demonstrates that cpSSR analyses are a useful tool explaining the pattern of genetic differentiation in heavy metal resistance of pine species. The results confirm also the usefulness of fluctuating asymmetry as a highly sensitive indicator of non-specific stress.

**Keywords:** *Pinus nigra*, *Pinus sylvestris*, fluctuating asymmetry, genetic differentiation, heavy metals

## ***Tillandsia usneoides*: a Successful Alternative for Biomonitoring Changes in Air Quality due to a New Highway in São Paulo-Brazil**

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*Tillandsia usneoides* L. (Bromeliaceae) is an aerial epiphytic bromeliad that absorbs water and nutrients directly from the atmosphere by scales that cover its surface. Although this species has been widely used for monitoring metal atmospheric pollution, its broader use as biomonitor based on chemical, structural and physiological markers to detect changes in air quality is scarce. The usefulness of such comprehensive approach was tested around a new peripheral highway in São Paulo state, Brazil. Previous results showed that increasing concentrations of Cr, Mo, Ni, Pb, Sb and Zn were observed in *T. usneoides* after the highway inauguration, indicating that these elements originated from vehicular emissions. The objective of this study was to verify if this metal accumulation was followed by structural and physiological changes in the plant. *T. usneoides* samples were collected in a forest fragment near the highway southern side, where the species occur on trees inside and at the forest fragments edge. The remaining plants in this forest were taken as control. *T. usneoides* samples were transferred to tree branches located about 5m from the highway, and a sample was taken for further comparisons, representing the time zero (T0). The biomonitoring study was performed from 2009 to 2012, thus comprising the period during the construction and after the highway inauguration. In both control and highway sites, subsamples were collected after 5 (T1), 13 (T2), 25(T3) and 37 (T4) months after T0. T0 and T1 represented the periods before the highway inauguration and T2 to T4 those after the inauguration. The material was fixed in FAA70 for structural purposes or grinded for chemical analysis. To verify the percentage of anomalous scales, leaves fragments were submitted to maceration techniques and quantified under light microscopy. Emission patterns of secondary metabolites' fluorescence (mainly phenolic compounds) were obtained by deconvolution analysis of leaf cells images by confocal microscopy. In situ localization of heavy metals was performed by analysis of leaves' fragments under scanning electron microscopy with energy-dispersive X-ray spectroscopy detector. The mean percentage of anomalous scales did not change significantly in the control plants during the exposure time (T0=13%, T1 =2%, T2 =3%, T3 = 2% and T4 = 5%). The significant increases in the percentage of anomalous scales (T2 = 45%, T3 = 14%, T4= 33%) were observed in samples exposed near the new highway after its inauguration. An induction of the constitutive defences was observed by emission peak variations, referring to phenolic compounds that exhibit antioxidant properties, when comparing the control and the highway exposure sites, showing that this species reacted to the impact of air pollutants. In addition, heavy metals were observed in regions of the tissue that are coherent with the absorption through scales. These results point to increasing oxidative stress to the plants, which may be attributed not only to the metal accumulation previously observed, but also to gaseous pollutants. In conclusion, the results showed that *T. usneoides* presented morphological and biochemical alterations that indicated the highway building impacts.

**Keywords:** biomonitors, traffic-related atmospheric pollution

# Development of a methodology to characterize emissions sources in an urban area surrounded by industrial activities – Setúbal, Portugal

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The fast urbanization of the cities and the concentration of industrial and principally road traffic activities resulted in increased levels of air pollution and consequently have relevant impacts in the environment, climate and human health. The health effects of Particulate Matter (PM) have been subject of intense study in recent years. In particular, epidemiological and toxicological research have sought to establish the chemical components primarily responsible for particle toxicity and have frequently implicated the metal content as a possible harmful component of PM. The objective of this work was to develop a methodology to characterize PM emission sources in the city of Setúbal- Portugal. Setúbal is surrounded by several industries, traffic and ports that have an important environmental impact, since they are major hubs of economic activity. The methodology was based: 1) in the national emission inventory reported by the Portuguese Environmental Agency (APA) for the period of 2003-2009; 2) in the characterization of the emissions from all industrial activities located in the area; 3) in the accounting of the number of vehicles per category and in the determination of their emissions by using the model TREM; 4) in the identification of the principal fugitive sources, that largely contribute for the uncontrolled PM emissions in port activities, and in the determination of their emissions by using AP-42 emission factors; 5) in the PM sampling performed both in an urban traffic and in an industrial station during the winter and summer of 2011; and 6) in source apportionment techniques. Results showed that the integration of different approaches in the developed methodology is essential due to the complexity of the emissions in this region. Firstly, there is a mixture of stationary and fugitive emissions that produce important amounts of particles with very particular characteristics. Secondly, the major industrial processes are located in very close proximity to each other making it difficult to distinguish between the processes. The situation is further complicated by the fact that some processes operate continuously (principally in industries) while others are batch processes (principally in ports). The traffic was identified as the principal source of PM<sub>2.5</sub> whereas in coarse fraction the soil and the sea were the main identified emission sources.

**Keywords:** PM, emissions, urban, industry, monitoring, models

# Effects of Pb on Essential Oil Contents in Sweet Basil (*Ocimum basilicum* L.)

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Sweet basil (*Ocimum basilicum* L., Lamiaceae) is a common herb, used for culinary and medicinal purposes. The essential oils of different sweet basil chemotypes contain various proportions of the allyl phenol derivatives estragole, eugenol, and methyl eugenol, as well as the monoterpene alcohol linalool. The hypothesis tested in this study was that some essential oil crops could be grown as alternatives to edible crops in heavy metal enriched soils. Experiment was conducted to evaluate the effect of Pb on yield and essential oils of basil. Metal treatments of basil consisted of (in mg.L<sup>-1</sup>): pb at 50, 100, and 150, 200 and an unamended control. In this study, secretory activity on the adaxial surface of the leaves of the *Ocimum basilicum* was investigated, using light, scanning and transmission electron microscopy. The results indicate basil yields were not affected by the treatments. The tested treatments slightly altered chemical composition of the essential oils of basil. The chemical composition of hexane and methanol extracts of the leaves, analyzed by gas chromatography coupled with mass spectrometry (GC-MS), revealed the presence of diterpenes, phenolic compounds and fatty acids. Compounds in the hexane fraction included camphor,  $\beta$ -cadinene, eugenol, methyl eugenol,  $\beta$ -pachoulene, 1,8-cineol, L- $\alpha$ -terpeniol, linolenic acid, stearic acid, tricyclo[5.2.1.0(1,5)]decane and an unknown substances. Compounds in the methanol fraction included furfural, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, trans geraniol,  $\beta$ -Linalool, 9,12,15-octadecatrienoic acid, methyl ester,  $\beta$ -elemene, bicycle[3.1.1]hept-2-ene, 2,6-dimethyl-6-[4-methyl-3-pentenyl]- and an unknown substances. Our results support the use of aromatic plants as alternative crops for Pb enriched soils.

**Keywords:** essential oil, sweet basil, methyl eugenol, lead, aromatic plants

# Urinary Benzophenone Concentrations and Their Association with Demographic Factors in South Korean Population

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Benzophenone and its derivatives (BPs) have been widely used in sunscreen, a variety of cosmetics and personal care products. The aim of this study was to evaluate urinary BPs in a representative South Korean population aged  $\geq 6$  years. The total of 1,576 urine samples was collected in national wide scale during 2010 to 2011. Six kinds of urinary BPs: BP-1, BP-2, BP-3, BP-4, BP-8 and 4-OH-BP were analyzed using liquid chromatography-mass spectrometry. The detection rates for BP-1, BP-3 and 4-OH-BP were 56%, 25% and 88%, respectively, whereas the detection rate for BP-2, BP-4 and BP-8 were below 14%. The geometric means of the urinary BP-1, BP-3 and 4-OH-BP concentrations were 1.24 ng/ml (95% confidence interval [CI]: 1.14-1.35), 6.23 ng/ml (95% CI: 5.78-6.71), 0.31 ng/ml (95% CI: 0.29-0.34), respectively. The multiple linear regression analysis revealed a significant relationship between the concentration of BP-1, BP-3 and 4-OH-BP and some demographic factors (gender and age group). The regression coefficients in BP-1 and BP-3 levels, female were significantly higher than male BP-1, BP-3 and 4-OH-BP levels significantly correlated in children, adolescents and adults group. Differences by gender and age reflect differences in use of cosmetics and personal care products containing BPs. This is the first study to evaluate occurrence of BPs in urine for South Korean population. The above results may be used to study and assess human health risk from benzophenone exposure.

**Keywords:** benzophenone, urine, biomonitoring, demographic characteristics, Korean population

# Biomonitoring of technogenic pollution in industrialized urban ecosystems using biogeochemical activity of woody plants

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Biomonitoring by means of different kinds of woody plants growing under industrialized urban ecosystems in the Russian non-black soil zone and nuclear and related analytical techniques were used to assess the level of technogenic pollution in case of a city of Tula located 180 km south of Moscow. Ecosystem, which includes the city area of 154 sq. km and a population of 500 thousand, represents an area with developed metallurgical, chemical, engineering and defense industries with the city's infrastructure and network of roads with heavy traffic. The risk of impact on the environment determines the relevance of research carried out with the use as biomonitors of more than 50 species of woody plants growing both in areas with high technogenic impact and in the conditionally pristine area (Central Park of Culture and Rest located in the city center). Neutron activation analysis (NAA) and atomic absorption spectrometry (AAS) were used as analytical methods for studying plant samples. A total of 35 elements were determined. The results of correlation and factor analyses of the data showed that woody plants are good biomonitors of technogenic pollution of the urban environment. The investigations have shown that the analysis of the biogeochemical activity of woody plants leaves and shoots and the subsequent statistical processing of data allow to reveal the basic regularities of the air transport of toxic elements and soil contaminants caused by aerosol emissions and to assess the state of urban ecosystems of the Russian non-black soil zone. The developed method can be used to characterize the environmental situation in other similar urban ecosystems with high levels of technogenic pollution.

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**Keywords:** woody plants, biomonitoring, toxic elements, urban ecosystem

# Trace Element Determinations in Tree Bark Samples for Biomonitoring Atmospheric Pollution in the City of São Paulo

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Concern about air pollution and its effects on public health has become important programs to reduce the levels of pollutants in many countries and, Brazil is no exception. Within this context, the objective of this study was to investigate on use of tree barks as biomonitor to evaluate the levels of element pollutants. Tree bark are considered to be a promising indicators of air pollution monitoring, because of its accumulation of aerosol particles, simplicity of species identification and wide geographical distribution. However, there are no established protocols for its sampling as well as about its capabilities to be used as an alternative or complementary indicator of aerial pollution. Besides, there are contradictory opinions about the accumulation capability of bark as and its adequacy as a bioindicator of atmospheric pollution. In this study barks from very common and dominant arboreal species in urban areas of São Paulo city were analyzed in order to define adequate experimental conditions for their use as biomonitor of air pollution. Tree bark samples collected from Sibipiruna (*Caesalpinia peltophoroides*) and Tipuana (*Tipuana tipu*) species at a height of 1.5 m from the soil were cleaned using a nylon brush. A surface layer of 2 mm was removed using a Ti grater and ground in an agate mortar for the analysis. Instrumental neutron activation analysis (INAA) was applied for the determination of As, Br, Ca, Co, Cr, Fe, K, Na, Sb, Sc, Zn and graphite furnace atomic absorption spectrometry (GF AAS) for Cd and Pb determinations. For analytical control of results certified reference materials were analyzed. Findings of this study demonstrated that the techniques of NAA and AAS provide reliable data for element concentrations with standardized differences,  $|Zscore| < 2$ . Results obtained in bark samples indicated that sample characteristics such as tree species or bark porosity, bark surface layer taken for analysis as well as tree trunk diameter or age of tree should be considered in its sampling and treatment for use in air pollution monitoring.

**Keywords:** tree bark, trace element, pollution biomonitoring, biomonitors



# Odour Monitoring: How to Measure Odour and Gas Emissions at Source with an Electronic Nose and See Its Dispersion in the Environment?

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Industrial odours can significantly impair the quality of life of residents. Odour appears as the second ground for complaint after noise. To reduce odour pollution and meet regulatory requirements, industrial operators seek to implement effective corrective and preventive actions. In this context, odour measurement is essential to identify, target and characterise the emission sources, to understand the phenomena of emission and dispersion involved, to obtain real-time indicators of odour pollution and to take action in case of threshold exceedance. One of the solutions to monitor the olfactive pollution from the source to the environment where odours are detected by residents is the electronic nose with a dispersion modelling. Questions are often raised by the industrial about the return in routine use of this kind of equipment. What reliability for the data measured? What correlation with the perception of local residents? What can we do with the results? How can we use the electronic nose diagnostics to optimize our odour treatment solution? How electronic nose solutions can reduce complaints from local residents? We will see the technical possibilities of an electronic nose in terms of results, and especially what can be the benefits for an industrial during the daily use.

**Keywords:** air, odour, electronic nose, olfactometry, dispersion

# Assessment of Metal Atmospheric Pollution in Forest Remnants of the Campinas Metropolitan Region, São Paulo State, SE Brazil, Using Tree Species as Biomonitors

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Forest remnants in urban areas are highly impacted by emissions of particulate matter originated from many human activities. The presence of potentially toxic elements in these atmospheric pollutants may affect the vegetation of these ecosystems. The scenario is not different in Brazil, especially in the Campinas Metropolitan Region, São Paulo State (SE Brazil). This region comprises 19 cities with a total population of 2.9 million inhabitants in an area of 3,645.16 km<sup>2</sup>, with a modern and diversified industrial complex, which includes one of the biggest petroleum refinery of the country, besides a significant agricultural and agro-industrial structure surrounding the remaining forests. The objectives of this study were: a) to evaluate foliar concentrations of Ba, Ca, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb and Zn in the three most abundant species (*Piptadenia gonoacantha*, *Croton floribundus* and *Astronium graveolens*) in two remnants of the Semideciduous Atlantic Forest located in the cities of Campinas and Paulínia, in the Campinas Metropolitan Region; b) to verify which species was more adequate to biomonitoring metal atmospheric pollution; c) to evaluate seasonal and spatial variations using this species. Leaves were collected in both forest remnants during the dry (Aug / Sep 2011) and wet (Jan /Feb 2012) seasons. Leaf concentrations of Cu, Mn, Ni and Pb were determined by Inductively Coupled Plasma Mass Spectrometry after aqua regia digestion and the other elements by Instrumental Neutron Activation Analysis. Analysis of variance followed by Holm-Sidak method identified differences in the leaf concentrations among locations and/or seasons. Statistical tests (Dunn's or Turkey tests) showed that *C. floribundus* presented, for most elements, higher leaf concentrations than the other species. These results indicated that *C. floribundus* was the most efficient accumulator species, probably due to the high contents of epicuticular wax and a dense layer of stellate trichomes on leaf surfaces, as already observed in previous studies. Therefore, the leaf concentrations measured in this tree species were used for biomonitoring temporal and spatial variations of the studied elements in the remnants. Higher levels of Co, Fe, Pb and Sb were observed during the dry season and Cu was the only element that occurred in higher proportion during the wet season. Significantly higher concentrations of Ca, Co, Cu, Fe, Sb and Pb were observed in the forest remnant located in Campinas. In the case of Pb, this result was only verified during the dry season. Enrichment factor, calculated for both seasons and remnants, using local background values of the three species, demonstrated that leaves of *C. floribundus* were enriched with Mn and the same was observed with Co, except in the wet season in Paulínia. Both elements are usually associated to coal burning. In Campinas, during the dry season, leaves of *C. floribundus* were enriched also with the traffic-related elements Cr, Fe, Pb and Sb. These results indicated that most elements showed higher levels in the Campinas remnant, mainly in the dry season, indicating that this forest is more affected by the industrial and vehicular sources than Paulínia.

**Keywords:** metal atmospheric pollution, biomonitors, forest remnants

# Biomonitoring of Selected Regions of the São Paulo State Seashore Using *Perna perna* Mussels: a Comparison Between the Passive and Active Experiments

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Bivalve mussels such as *Perna perna* have been many times used as biomonitors of water contamination, for many trace elements, due to their filtering capabilities. In the present work, *Perna perna* was used for passive and active biomonitoring experiments, in two regions of the São Paulo state seashore: Caraguatatuba (Cocanha Beach mussel farm), which was the reference region and Santos bay (Ilha das Palmas and Ponta de Itaipu), where contamination from domestic and industrial effluents occur. In the period from the fall of 2005 to the summer of 2006, the active biomonitoring experiments were carried out and from the spring of 2008 to the winter of 2009 the passive biomonitoring was performed. In the active biomonitoring experiments, mussels were transplanted from Cocanha beach to the contaminated sites, in ropes, and left in each site for three months, corresponding to the four seasons of the year. In the case of the passive biomonitoring, mussels were collected from the rocks with titanium knives. After the sample treatment, the following trace elements were determined: As, Co, Cr, Fe, Se and Zn by instrumental neutron activation analysis (INAA) and Cd, Hg and Pb by atomic absorption spectroscopy (AAS). It was observed, in the passive biomonitoring, that most of the higher values were found in mussels collected in the Cocanha mussel farm. In the active biomonitoring with *Perna perna*, in the same areas and seasons of the year a similar situation was observed for Cd (spring), As (spring, summer and winter), Se (summer and winter) and Fe (summer). The high values obtained in the Caraguatatuba region could be related to the fact that the region presents an economy based on services related to tourism, and occupation of illegal housing occurs, including in a permanent protection area. According to reports of the São Paulo environmental agency, Cocanha beach has been impacted over time since its classification ranged from “good” (1999- 2006) to “regular” (2007- 2009).

It can be concluded that both experimental approaches of biomonitoring, can yield important results concerning the environmental quality. The active biomonitoring is viable, although more complex from the experimental point of view. Since the passive biomonitoring was carried out in a period after the active biomonitoring and the values obtained for Cocanha beach were higher for most of the elements, in the first case, these results seem to accompany the worsening of the beach quality.

**Keywords:** biomonitoring, *Perna perna*, São Paulo, active biomonitoring, passive Biomonitoring

# The Exposure of Bosk's Fringe-Toed Lizard (*Acanthodactylus boskianus*) to Heavy Metals in Relation to Habitat and Diet in a Polluted Coastal Area in Southern Tunisia

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Since the early 1970s, huge quantities of phosphogypsum, charged with heavy metals, have been released in the sea by Gabès-Ghannouche factory complex of phosphate treatment, in southern Tunisia. The possible transfer of these pollutants from the marine ecosystem to the terrestrial one, through littoral organisms, has never been investigated. In this work we investigated the possible role of the Bosk's Fringe-toed lizard *Acanthodactylus boskianus*, a common lacertid in the coastal area of the gulf of Gabès, in exporting heavy metals from the marine to the terrestrial food webs. We sampled lizards living close to the factory complex but in two different habitats (coastal dunes vs backshore), and we investigated the concentrations of Cd, Pb and Zn in their stomach contents (preys) and various tissues (liver, kidney and tail). We found that the diet of lizards living on the coastal dunes included amphipods (85%), while lizards living on the backshore feed exclusively on terrestrial invertebrates. The concentrations of Cd, Pb and Zn in the stomach contents and tissues were higher in lizards living on coastal dunes than in those living in the backshore. Overall, our results suggest that by preying on contaminated amphipods, lizards living on coastal dunes close to the Gabès-Ghannouche factory complex are more exposed to heavy metals than those living in the backshore and preying on terrestrial preys. The former lizards may thus accumulate heavy metals in their tissues and transfer these pollutants to their terrestrial predators, such as snakes and birds.

**Keywords:** *Acanthodactylus boskianus*, environmental pollution, heavy metals, Tunisia

# Scenario Based Analysis of Traffic Related PM<sub>2.5</sub> Concentration: Lisbon Case Study

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Road transportation has become a major contributor to air pollution and, since more than 50% of world population lives in cities, this is the cause of approximately two million deaths per year. Therefore, several strategies are being introduced to reduce the impact of vehicle emissions, such as low emission zone (LEZ), parking fees and congestion pricing (tolls). Consequently, modelling methodologies to quantify traffic related Particulate Matter (PM) concentration are essential, as they offer a prompt tool to assess human exposure over different temporal scales or hypothetical conditions. The objective of this research work is to evaluate the impacts of changing traffic related parameters on PM concentration for several hierarchical street levels (from arterial to local roads) using real-world data for the city of Lisbon, Portugal. For that purpose, 2014 data regarding meteorological conditions (temperature, wind speed and humidity in a 30 minutes basis), traffic volumes (in a 15 minute basis), typical vehicle speed (from large-scale vehicle monitoring) and vehicle fleet (based on the average Lisbon fleet composition) was used. The available data allows building a baseline case for several streets that are representative of the traffic circulation conditions in Lisbon. The baseline case is compared with different scenarios that consist on: introduction of a cordon toll (drivers are charged when crossing the boundary of a predefined tolling area, with consequences in terms of traffic volumes); and the implementation of a low emission zone by restricting the access of older (and more polluting) vehicles to the city centre. Copert4 (a European reference vehicle emission model) is used to estimate the PM emission (in grams per kilometre) for the existing Lisbon average vehicle fleet and for the LEZ scenarios. Copert4 outputs are used as input in air quality dispersion models (e.g. AERMOD, CALINE4 and CAL3QHCR, etc.) as well as meteorological data, traffic volumes and road characteristics. This methodology allows performing a seasonal comparison of PM exposure for different hierarchical street levels, as well as a monthly comparison basis to test the impacts of two scenarios: Scenario 1 - traffic reductions between 5 to 20% (justified by the possible implementation of cordon tolls); and Scenario 2 - implementation of a low emission zone. LEZ take into consideration both the exclusion of vehicles within older EURO standards and the correspondent adjustment in traffic volumes and vehicle fleet arising from the exclusion of these vehicles. LEZ have been introduced in Lisbon in 2011 and since then the geographical coverage of the measure has been increasing. The third stage of LEZ will take effect from January 2015 and vehicles registered before 1 January 2000 will not be allowed to circulate in the city centre, affecting ~30-40% of the Lisbon fleet. Preliminary results indicate that a 5% traffic volume reduction can result in a 7% reduction in PM<sub>2.5</sub> concentration, while a 20% traffic reduction can result in 17-21% reductions in PM exposure. Overall, this study demonstrates that modelling tools based on real-world data can provide a reliable approach to study urban policies regarding traffic related PM exposure.

**Keywords:** PM<sub>2.5</sub> concentration, policy scenarios, modelling, LEZ

# Impact of Urban Pollution on the Vascular Cambium of *Ceiba Speciosa* (A. St.-Hil.) Ravenna

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Air pollution is considered one of the main causes of forest decline and the vascular cambium of trees has been highlighted for being the tissue responsible for the increase in thickness of plant species and being sensitive to environmental variations. However, no significant reduction in the number of cells in cambial zone (radial layers of fusiform and ray initials, including phloem and xylem mother cells) was observed in literature. In general, the studies have been carried out only in the dormancy period and only in polluted areas, thus without simultaneous observations in unpolluted sites. This study compares the cambium histology of *Ceiba speciosa* (A. St.-Hil.) Ravenna (Malvaceae) in polluted (Rio de Janeiro City – one of the most developed city of Brazil) and unpolluted (natural biological reserve) sites of the Atlantic Rain Forest domain. Samples were obtained in two periods (cambial activity and dormancy) in each area and they were processed and examined according to usual techniques of light microscopy. The seasonality of the cambial activity and the structure of the cambial zone were similar in two sites, but despite of the differences regarding the formation of cells in the period most favourable to growth or less favourable, the fusiform initials were shorter and thicker-walled in trees of the polluted site. The groups of ray initials were the structural components of the cambium that had the most significant difference between the studied sites. This parameter was also the one with the highest phenotypic plasticity index in both sites, reinforcing its importance in cambial activity studies. These results show that this species is flexible in survive and adapt to different environmental conditions and therefore is an important biomarker in environmental monitoring studies. This is the first report about the pollution effects on cambial activity for South American species and evidenced the pollutants and the climate dynamics related to the high urbanization of Rio de Janeiro city caused alterations on the cambium of *C. speciosa*. The studies of these impacts widen and gather knowledge about plant biodiversity and contribute to defining global strategies of conservation and preparation of modern man to changes that will be necessary, regarding the limitation of natural resources and of plant growth.

**Keywords:** environmental change, phenotypic plasticity, xylogenesis, tropical forest, wood production

# Using Lichen Diversity to Evaluate the Alleviation of Urban Heat Island Effect Provided by Green Spaces

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Urban areas are associated with higher temperatures than their surroundings, which is known as Urban Heat Island (UHI) effect. The great increase in buildings, streets and other structures, as well as the heat and pollution released by human activities, leads to this change on local climate in urban areas. The UHI effect enhances the impact of heat waves, which is particularly relevant considering that most of world population lives in urban areas. It is known that increasing the amount of green spaces in a city, decreases the magnitude of the UHI effect. However, the importance of the size and/or of the type of vegetation in green spaces is still poorly known. Using ecological indicators that evaluate the impact of microclimate changes is a way of unavailing the real importance of green spaces to microclimate changes in urban areas. Lichens communities are sensitive to environmental changes that occur in the ecosystems since they are poikilohydric. In particular lichen response functional groups, i.e. groups of species with a common response to an environmental factor, have been shown to give an integrated response to the microclimatic variations occurring in urban areas. Our aim was to use epiphytic lichens diversity to understand the effect that different types of green spaces (e.g. with different sizes, tree densities, density of the surrounding urban areas) have on alleviating the UHI effect. We expect that small green spaces are dominated by xerophytic and nitrophytic lichen functional groups whereas larger green spaces are dominated by hydrophytic and oligotrophytic lichen functional groups. All forested areas within the city of Lisbon, Portugal, were mapped and a number of areas (N=40) was selected in a random way. The areas were stratified to: 1) the location within the city of the green space; 2) the size of the green space; and 3) the density of artificial areas surrounding the green space. In the 40 selected areas epiphytic lichens were sampled using a European standard protocol in the four trees closest to the centroid. Total species richness, lichen diversity value (LDV) and lichen functional groups regarding humidity requirements and eutrophication tolerance were calculated. We found that lichen functional groups based on water and nutrient availability significantly responded to the quality of green spaces. Using these results we were able to support the decision makers of Lisbon urban area about what type of urban green spaces are able to alleviate the UHI effect, thus optimizing the urban green infrastructure.

**Keywords:** ecological indicators, urban heat island, cities, urban parks

# The Pollutant Spreading Model AUSTAL 2000 is Not Validated

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AUSTAL 2000 is recommended in Germany, the European Union and other countries for purposes of calculation of spreading of air pollutants. Authors and distributors (and therefore applicants) of AUSTAL 2000 thereby claim to have provided a reference solution for all other spreading models. It must, however, be noticed that this ambitious claim is not met; both actual results and theoretical foundations are incapable of meeting such an end. Analytical solutions and numerical calculations of deposition and sedimentation both are flawed. The floor-level concentrations are calculated with an average deviation of 50% to 100% and even more. Homogeneity tests prove to be meaningless trivial cases with exactly the same solutions. Even these are described providing absurd compensation times (since) they were obtained by faulted calculation assumptions and methods. E.g., the so-called turbulence approaches used prove to be inconsequential. AUSTAL uses to be validated assuming some three-dimensional wind field and on the other hand it uses the flat speed distribution of a rotating solid. Given iterations do not converge, this suggests unstable behaviour of equations included in the code. STABILITY, CONVERGENCE and CONSISTENCY are not proved for Austal. Wind speeds being supposed to be less than 1 m/s are excluded although one really should know that these are substantially responsible for limiting value transgressions. Such strange concepts are prone to produce erroneous results or predictions. Homogenizing rather describes a work process of the food or process engineering than a technical term in the field of the air-pollution control, for example. Stationary conditions only shall adapt when no outer forces act or forces/flows obtained from the responsible differential equations are assumed/set to balance mutually. BERLJAND solutions describe three-dimensional concentration distributions. Although people claim to have validated AUSTAL with BERLJAND solutions, the model rather uses an inferior two-dimensional solution instead. Applicants charge to obtain approx. 70% the concentration distribution at a height of 5 m with a fault in the local position of pollutant accumulation of less than 500 m. Using maximum concentration close to surface the point of source emission is determined with an error of approx. 32%. One charges to calculate density distributions and mean concentration regimes even though neither mass balance criteria nor the second law of thermodynamics are respected in the model framework. Turbulence approaches are deliberately used for resting liquids although turbulent movements neither exist then nor can appear. In addition, one denies that the approaches used for it are anyway insignificant. In short, AUSTAL 2000 is not validated from neither theoretical nor empirical points of view.

**Keywords:** air-pollution control, spreading of air pollutants, particle model, 3D wind country, AUSTAL 2000



# Sources Influencing Elderly Exposure to PM<sub>10</sub> Components

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Several researches have been studying the particles composition in different micro-environments, such as offices, schools, fitness centers, outdoor, etc. Some of those studies had also tried to identify the main emission sources associated to each micro-environment. However, such kind of study is still scarce regarding elderly population. This evidence is quite relevant since the elderly population is consider susceptible and, consequently, more vulnerable to possible exposure to air pollutants. The present study aimed to investigate the elderly exposure to PM<sub>10</sub> components and emission sources that may influenced those exposure. To achieve the presented goal it was selected one Elderly Care Center (ECC), hosting 69 elders, due to its singular localization: near two important highways and International Airport of Lisbon. Here, physical and chemical characterization of air pollutants were done in bedroom, living-room and outdoor, and the daily exposure and inhaled dose of elders to PM<sub>10</sub> components were also calculated. Thus, two 2-weeks sampling campaigns were conducted – one during early fall (warm phase) and another throughout the winter (cold phase) in order to perform the apportionment of the PM<sub>10</sub> components. Chemical analysis of the particles was performed by Thermal Optical technique for Organic Carbon (OC) and Elemental Carbon (EC) analysis; Ion Chromatography for the determination of inorganic water soluble ions and Instrumental Neutron Activation Analysis (INAA) for the elemental characterisation of particles. Finally, the contribution of indoor and outdoor sources was assessed by Principal Component Analysis (PCA). Results showed that the PM<sub>10</sub> indoor concentrations in ECC did not exceed the national and the international limit values and that PM<sub>10</sub> concentration in living-room were significantly higher than in bedrooms. Zn and Cr presented higher concentrations in the indoor environments indicating the existence of indoor sources for these elements. PCA showed the importance of the highways and the International Airport of Lisbon located less than 500 m of the Elderly Care Center for both indoor and outdoor air quality. This approach allows the identification of the micro-environments with highest impacts on elderly exposure and proved to be an essential tool to identify health risks, set and review air quality standards and evaluate effective policy interventions.

**Keywords:** sources, air pollution, elderly, PM<sub>10</sub>.

Poster Session

# Air pollution by PM<sub>10</sub> in Portugal between 2001 and 2011

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The good quality of the air we breathe is considered a basic need for human health and well-being. During last three decades special efforts have been made in Europe aiming to reduce air pollution and more importantly, to reduce the adverse impacts of atmospheric pollutants. Although these efforts led to a reduction of risks and effects, air pollution in Europe is still a matter of concern. The aim of this study was to study the air pollution by PM<sub>10</sub> in Portugal between 2001 and 2011. Data were collected from 60 stations of air quality monitoring throughout mainland Portugal, divided by 5 regions – 1) North, 2) Centre, 3) Lisbon and Tejo Valley, 4) Alentejo and 5) Algarve. Each station was divided into 5 different types, namely: a) Traffic, b) Industrial, c) Background - Urban, d) Background - Sub-Urban and e) Background - Rural. These data were provided by the Portuguese Environment Agency, with contribution from the Committee for Coordination and Regional Development (CCDR). The results showed that the average PM<sub>10</sub> concentrations in Portugal declined from 57 µg/m<sup>3</sup> in 2001 to 27 µg/m<sup>3</sup> in 2011 and that the annual averages since 2004 were below the current limit value of 40µg/m<sup>3</sup>. This data are in agreement with the European Environment Agency who showed, in 2012, that Portugal was the country with main reported reduction between 2000 and 2010 (29%). This decrease may be related with the implementation of the Best Available Technologies in the industry, with the development of low emission vehicles and with the crisis experienced in recent years that is associated with the decrease of consumption and production. It is possible to observe that monitoring stations located along the high traffic areas had higher concentrations of PM<sub>10</sub>. The stations near the industries and sub-urban areas were the second type of station that presented higher particles concentration. The largest and most important cities of Portugal were the ones with the largest pollution outbreaks and where levels were still below the European targets. Between 2001 and 2011 the annual levels of PM<sub>10</sub> decreased in Portugal. However, both the annual and the daily limit values, defined by the Portuguese Legislation, were still exceeded principally in urban and industrial areas. Moreover, the levels were also far from the narrower limit value of 20µg/m<sup>3</sup> defined by the WHO. It is fundamental to continue with efforts reducing emissions of these pollutants, in order to protect public health, since it is well known that particles may promote serious diseases upon human health.

**Keywords:** PM<sub>10</sub>, air pollution, Portugal.

# Biomonitoring of Air Pollution Using Bark and Lichen in an Urban Area – Lisbon, Portugal

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Biomonitoring of air pollution has been used extensively in the past in order to assess air pollution levels. This study focused on two types of biomonitors: lichens and tree bark. Lichens are known to have the ability to accumulate elements in excess of physiological requirements in close correlation with atmospheric element levels and tree bark accumulates atmospheric particulate matter (PM) in its outermost structure, which are accumulated over time. The biomonitoring methodology used in this study was transplanting of both biomonitors from a clean area to the study area, which was the urban area of Lisbon. Therefore, the main goal of this work was to assess the main pollution sources of Lisbon area using transplanted biomonitors. The biomonitors used were the epiphytic lichen *Parmotrema bangii* (Vain.) Hale and bark from its phorophyte, the Japanese cedar *Cryptomeria japonica* (L. fil.) D. Don, which were collected in São Miguel Island (Azores, Portugal), which is known to have a clean atmosphere. Few days after collection, the biomonitors were prepared in the laboratory and exposed in the courtyard trees of 22 primary schools of the urban of Lisbon. Two exposure campaigns of the biomonitors were conducted, winter and summer, with a total period of exposure of 5 months each, in 2008. After exposure, the biomonitors were both analysed regarding the chemical elements using Instrumental Neutron Activation Analysis – INAA, which allowed to assess a total of 15 chemical elements (Br, Ce, Co, Cr, Eu, Fe, Hf, La, Na, Rb, Sb, Sc, Sm, Sr and Zn).

Since electrical conductivity of lichen's leachate is a key parameter to evaluate the general lichen vitality, which is influenced by stress that lichens are subjected to at the transplanting site due to atmospheric conditions and due to local environmental pollution impact, this parameter was also assessed for the lichens. Higher values of electrical conductivity were found in the summer season at specific locations, such as international Lisbon airport and downtown. Both biomonitors showed similar behaviour regarding the accumulation of, for example, Sb and Zn, which are chemical elements related to traffic emissions. Significant correlations between chemical elements in both biomonitors were found for elements associated with soil sources only in the winter season (namely, Ce, Eu, Fe, La, Na and Sm). This fact may be explained by a higher damage of lichens in summer, due the lower atmospheric humidity and higher temperatures, as confirmed by the higher values of electrical conductivity in summer. Factor analysis (MCTFA) was applied to the database of our study, which compromised chemical elements of both biomonitors and both seasons. This source apportionment technique allowed to identify several sources, such as, arsenic emission sources, soil with anthropogenic contamination, a Se source, traffic, industry and a sea contribution.

This study showed the importance of biomonitors to assess atmospheric pollution in order to identify their main sources. Moreover, the use of transplanted bark as a biomonitor, with a total of 5 months of exposure period, was possible and supplied results similar to the obtained by the lichens.

**Keywords:** biomonitors, bark, lichen, INAA, chemical elements, emission sources

# Indoor and Outdoor Biomonitoring Using Lichens at Urban and Rural Primary Schools

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Monitoring particulate matter (PM) and its chemical constituents in classrooms is a subject of special concern within the scientific community in order to control and minimize child exposure. Regulatory sampling methods have presented several limitations in their application to larger number of classrooms due to operational and financial constraints. Consequently, passive sampling methodologies using filters were developed for indoor sampling. However, such methodologies could not provide parallel information for outdoors, which is important to identify pollution sources and assess outdoor contribution to the indoors. Therefore, biomonitoring with transplanted lichens, a technique usually applied for outdoor studies, was used both indoor and outdoor of classrooms. Three main objectives were proposed, to (i) characterize simultaneously indoor and outdoor of classrooms regarding inorganic air pollutants, (ii) investigate spatial patterns of lichen conductivity, and (iii) assess pollution sources that contribute to a poor indoor air quality in schools.

Lichens *Flavoparmelia caperata* were transplanted to indoor and outdoor of classrooms for 59 days, in both urban and rural environments of Portugal (Lisbon and Ponte de Sor, respectively). After exposure, electric conductivity of lichens leachate was measured to evaluate lichen vitality and cell damage. Outdoors lichen conductivity was higher near the main highways, and indoors there was great variability in levels, which indicates different emissions sources and different ventilation patterns. Chemical content of lichens was assessed by instrumental neutron activation analysis (INAA), and As, Br, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Na, Rb, Sb, Sc, Sm, Sr, Ta, Th, Yb, and Zn were determined. Elemental gain and losses were found in exposed lichens in both outdoor and indoor environments. This finding illustrates that the use of lichens is possible in indoor environments, despite the higher physiological stress that lichens are under in these types of environments, as evidenced by higher values of electric conductivity. Element accumulation, crustal enrichment factors, and spatial variability of elements were analyzed and contaminants from anthropogenic sources, such as traffic (As, Sb, and Zn) and indoor chalk (Ca) found. Classrooms with potential indoor air quality problems were identified by presenting higher accumulations of inorganic pollutants in exposed biomonitors.

**Keywords:** biomonitors, lichen, classrooms, indoor, outdoor

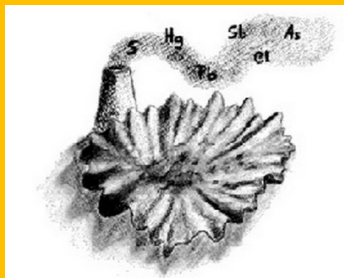
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