6th INTERNATIONAL WORKSHOP on BIOMONITORING of ATMOSPHERIC POLLUTION

15-19 October 2012

Çeşme-İzmir, TURKEY

ABSTRACTS

Organized by;

Middle East Technical University and Abant Izzet Baysal University

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(BIOMAP)
October 15-19, 2012
Çeşme, Turkey

PREFACE

Biomonitoring involves measurement of chemicals in biological matrices including human tissues. It has been shown to be an excellent approach to quantify the exposure to a wide range of chemicals. Biomonitoring studies routinely revealed novel, and sometimes surprising, information about the accumulation of chemicals in living receptors.

The 6th International Workshop on Biomonitoring of Atmospheric Pollution will take place in Çeşme (50 km to the city of İzmir)/Turkey between 15-19 October, 2012.

Attention will be focused on qualitative and quantitative aspects of biomonitoring in all segments of the environment. Topics that will be discussed in the workshop are not limited to biomonitoring for air pollution but include biomonitoring for water pollution, human exposure to environmental pollutants, biodiversity, metabolic responses, and use of biomonitoring for emission source identification. Furthermore, new methodologies and analytical techniques, and the use of geographical information in biomonitoring studies are in the content of this workshop.

The workshop will be of interest not only to specialists in the field but also to people working in regulatory agencies, engineers working for consulting firms and general public interested in environmental issues, particularly in spatial distribution of pollutants.

Topics of the Workhop are:

- -Human biomonitoring and health related studies
- -Biomonitoring in water systems
- -Monitoring air pollution using different monitors, air quality and biodiversity

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- -Methodological aspects
- -Metabolic responses
- -Analytical techniques
- -Combination of source profiles and geographical information

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ORAL PRESENTATIONS

Arsenic in Garcinia gardneriana Trees from Atlantic Forest

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Brazil has a mega biodiversity concentrated primarily in forest ecosystems like the Atlantic Forest which is one of the most endangered biomes of the world. The Serra do Mar State Park (PESM) in the São Paulo state is the largest conservation unit of this biome comprehending eight administrative nuclei with examples of impacted and preserved areas. The Picinguaba nucleus is one of the most preserved area of this conservation unit. In another hand, the Itutinga-Pilões nucleus is exposed to different pollution sources namely the biggest Latin American industrial complex, located in Cubatão city, and the Anchieta-Imigrantes highway system, the main route for the Brazilian production to the Santos harbor. In fact, severe impacts caused by human activities led to consider the Itutinga-Pilões nucleus as a great natural laboratory for investigating the effects of chemical changes on a wet tropical forest. In this study, instrumental neutron activation analysis was used to evaluate the bioaccumulation of As in this ecosystem. The tree species Garcinia gardneriana was selected as a biomonitor since it is one of the most abundant tree species in the nucleus. Leaves were sampled from Garcinia gardneriana trees with breast height perimeter higher than 15 cm. Under the Garcinia gardneriana crown projection, soil samples were collected at 0-10 cm depth in a permanent parcel of Picinguaba nucleus and at 100 m from the highway in Itutinga-Pilões nucleus. The maximum value for As $(125 \pm 16 \text{ mg kg}^{-1})$ was observed in Itutinga-Pilões, about 36 times higher than the maximum value measured in Picinguaba $(3 \pm 1 \text{ mg kg}^{-1})$. The maximum As value obtained in Itutinga-Pilões nucleus was higher than the national reference value (3.5 mg kg⁻¹) established by the National Environment Council (CONAMA) for soil in natural areas. In Itutinga-Pilões nucleus, the higher As mass fraction observed in Garcinia gardneriana leaves was 0.92 ± 0.52 mg kg⁻¹, while in Picinguaba all the values were below the detection limit. Therefore, there are evidences that the presence of heavy traffic highway system as the Anchieta-Imigrantes has a negative impact on the Atlantic Forest.

Keywords: conservation unit, biomonitoring, trace elements, pollution

Optimization of Some Methodological Aspects in Moss Bag Techniques

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Moss bag technique has been employed since 1971. The use of this technique is mainly to biomonitor the air quality showing several advantages comparing with chemical and physical techniques. The use of moss bags is special suitable to biomonitor urban and industrial areas where native moss is scarce and even absent. However, despite the advantages of the technique there is still an important lack of the standardization in the methodology. In the present study some of the most important variables, not still fully investigated, were evaluated: i) selection of a moss species (Sphagnum denticulatum vs. Pseudoscleropodium purum); ii) quantity of moss employed and size of bags (2,5, 5 and 20 mg/cm2); iii) height of exposure (0,5, 1, 1,5, 2, 2,5, 3, 4 and 5 m) and iv) duration of exposure (1, 2 and 3 months). The concentration of Hg and Zn were determined in moss bags exposed (two periods of exposure) in areas with different degrees of pollution (i.e. unpolluted, semi-urban and industrial areas. The coefficients of variation of the concentrations (CV) for different options of these variables were calculated. To identify which of them introduce more variability in the technique, the results were represented with a Pareto chart. For both measured elements, Sphagnum denticulatum showed higher variability than Pseudoscleropodium purum. Moreover, as much quantity of moss was employed lower variability was found in both cases. For the height and duration of exposure no clear pattern was detected. The Pareto chart shows that the variable which introduced more variability in the technique is the period of exposure for Hg, while for Zn is the height of exposure. The results of the present study should be taking into account to prioritize the optimization process of the methodological variables in the moss bag technique. This process should mostly lead to obtain a greater replicability of the results allowing the routine use of the technique as a low cost and flexible environmental tool.

Keywords: air quality, moss bag technique, methodological standardization, Pareto chart.

Global and Transdisciplinary Networking in Bioindication and Biomonitoring Studies—Past, Present and Future Approaches

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Living or formerly living organisms are being used to obtain information on quality of the general (health) status of our environment by bioindication and biomonitoring methods for many decades. Thus different roads towards this common scientific goal were developed by a lot of different international research groups. Especially the global cooperation in between various scientific teams throughout the world has produced common ideas, scientific definitions, and highly innovative results of this extremely attractive working field. The transdisciplinary approach of different and multifaceted scientific areas - starting from biology, (analytical) chemistry, via health physics, up to social and economic issues - have surpassed (in parts existing) mental barriers of individual scientists, so that now "production" of straightforward (common) results related to the influence of material and immaterial (environmental) factors to the wellbeing of organisms and human life is in forefront of international thinking. For further sustainable development of our common scientific "hobby" of bioindication and biomonitoring, highest personal energy has to be given by us, being teachers (in universities and other institutions) to our students and to convince (strategically) decision makers as politicians to invest (financially) into the development of education and research of this innovative technique. Young people have to being intensively convinced on the "meaning" of our scientific doing, f.e. by extended forms of education. One example of multilingual education of students on a global scale and perspective is given here, which we started about 3 years ago. International colleagues have translated a general paper on bioindication and biomonitoring, which had been published in the English (and German) languages before. The languages include for example Arabic, Chinese, French, Latvian, Lithuanian, Persian, Polish, Russia, Spanish (for sure, much more languages are in preparation). In addition to translation the colleagues of participating countries have to include a special case study of bioindication investigated in their own country into the translated paper. The translated papers then were published in national journals. So, the students can find innovative scientific ideas in their own mother language with a strong international relevance to get mentally convinced by this fantastic and profitable "biological green" technique, because by our teaching methods we meet the emotional and rational intelligence of our young people and motivate them to move into our international working field. In addition to this form of international scientific exchange we find an excellent side-effect pertinent to intercultural exchange.

Keywords: bioindication and biomonitoring, globalization, transdisziplinarity, (multilingual) international education, (intercultural) side effects

Levels and Size Fractions of Fine Particulate Matter in Indoor and Outdoor Air Environments and Human Health Effects

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Particulate matter (PM) in ambient air is responsible for adverse health effects in adults and children. Relatively little is known about the concentrations, sources and health effects of PM in indoor air. Epidemiological studies of the effects of ambient PM on human health explore associations between changes in ambient levels of PM and changes in the occurrence of cardiovascular and respiratory health problems in the population. A monitoring study was conducted in Ankara in order to measure PM levels in indoor and outdoor environments and to identify and quantify associations between sources and concentrations. Approximately 120 homes, two rooms (living room and baby room) and outdoor ambient at each home were sampled with Grimm Environmental Dust Monitoring (EDM) 107, during different seasonal periods of 2011 and 2012. The relationship between indoor and outdoor size fraction and levels for particulate matter less than 10 µm (PM₁₀), particulate matter less than 2.5 µm (PM_{2.5}) and particulate matter less than 1.0 µm were investigated. The mean concentration of PM₁₀ at living room is higher than baby room (or bedroom) whereas there is no statistically significant variation of PM2.5 and PM1.0 levels at living and baby room. It has been found that the household activities and environmental conditions are very important for PM concentrations in the indoor environments during the sampling periods. Smoking, use of stove, room spray or vacuum-cleaner, interior rebuilding or renovation, local traffic, building construction in the vicinity, type of residential heating, inner city residence and cold seasonal increased the fine PM concentration. Our study will be assessed that the relationship between indoor and outdoor PM levels and effects of their levels on infants health.

Keywords: Particulate matter (PM), particle diameter, indoor air

Physiological Effects of Carbon Nanotubes Exposure in a Lichen Photobiont

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The potential of nanotechnology to bring benefits to many areas of research, industry and health has been largely recognized, which explains the increase in investments by governments and businesses all around the world. New materials and nanoproducts are introduced, increasing the source of engineered nanoparticles to the environment, when very few data on the environmental impact and/or ecotoxicology are available. In this study we analyzed the effects of a functionalized multiwalled carbon nanotube (f-MWNT) on a lichen photobiont, as a first contribution in a project aimed at verifying the potential use of lichens as biomonitors of carbon nanotubes (CNT) aerosols. The coccoid green alga Trebouxia sp., isolated from the lichen Parmotrema perlatum (Huds.) M. Choisy and cultured in axenic conditions, was exposed to f-MWNTs under two interaction conditions - re-suspended in a nanotube dispersion before inoculation and inoculated on acetate or glass fiber discs containing nanotubes on the surface - at 0.01, 1.0 and 100 µg/mL. After the inoculation, the cultures were maintained in a growing chamber for 21 days at optimal conditions in closed petri dishes, on solid 3 Nitrogen Trebouxia medium, together with controls, i.e. other cultures obtained from the original liquid suspension, not exposed to f-MWNT. The following parameters were monitored: (i) growth (at six-day intervals, by taking color photographs with a Nikon Coolpix 8400 digital camera), (ii) chlorophyll content (spectrophotometrical readings of DMSO crude extracts), (iii) chlorophyll a fluorescence emission of dark- and light-exposed samples (by means of a pulse-

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amplitude-modulated fluorimeter Mini-PAM; Walz, Effeltrich, Germany), (iv) membrane damage (Sytox Green S-7020 stained cells observed with a confocal laser scanning microscope C1-si, Nikon, Tokyo, Japan). The presence of carbon nanotubes on the surface of both types of discs did not affect the cultures of *Trebouxia sp.* On the contrary, those derived from algae re-suspended in the CNT dispersions had lower chlorophyll content than the control, and most of their cells showed consistent membrane damage, although the photosynthetic efficiency, expressed by the F_{ν}/F_{m} ratio, was not significantly affected. Surprisingly, no dosedependent response was observed comparing the 3 different concentrations. The environmental implications suggested by these results are shortly addressed also on the basis of the most recent literature.

Cytogenetic Biomonitoring of Primary School Children in an Urban City in Turkey

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Genotoxic effects of air pollution through buccal epithelial cell (BEC) micronucleus (MN) assay primary school children were investigated. Children from two schools at high polluted and less polluted sites were investigated in winter and summer seasons for BEC-MN frequencies. It was found that BEC-MN frequencies of children in high polluted and less polluted regions did not show any significant difference. In summer period, BEC-MN frequencies (‰) of the urban (high polluted) children were found to be significantly higher (mean±SD, 2.68±1.99) than in winter period (1.64±1.59;p=0.004). On the other hand, no seasonality was observed for the suburban (less polluted) children. The study population was chosen among MATRA Project subjects', personal BTEX (benzene, toluene, ethylbenzene, m+p-xylene, o-xylene), NO₂, ozone (O₃) and pulmonary function data were also available. The personal BTEX and NO₂ exposures of children were significantly increased whereas personal O₃ concentrations were significantly decreased in the high polluted area (p<0.05) in winter period. In summer, BEC-MN frequencies were significantly increased with

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the decrease in pulmonary function levels based on forced expiratory flow between 25% and 75% of vital capacity (FEF 25-75%) (p<0.05). It was concluded that children living in high polluted and less polluted areas in the city of Eskişehir exhibited similar genotoxicity. Seasonal change might be a factor increasing genotoxicity since children are exposed to ambient air pollutants more in summer time due to increasing time spent at the outdoors.

Keywords: Air pollution, children, personal biomonitoring, buccal epithelial micronucleus assay

Atmospheric Deposition of Pollutants by Moss Sampling: Experience from 35 Years of Monitoring in Norway

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Monitoring atmospheric deposition of trace metals has been carried out on a regular basis in Norway since 1977 as a multi-element survey covering 470 sites all over the country. Initially neutron activation analysis was employed, but since 1990 the survey has been based on ICPMS. Deposition of a majority of elements has shown a substantial decrease over this period, particularly in the south where the main source of most metals is long-range transport from areas elsewhere in Europe. Calibration of concentrations in moss versus bulk deposition from precipitation samples has been successful for most key heavy metals. An exception is Hg where the moss samples appear to concentrate some Hg⁰ from the atmosphere whereas precipitation samples show deposition of oxidised Hg forms. Monitoring of metal deposition has also proved useful in local studies around metal factories such as aluminium industries, iron and iron alloy smelters, and copper and zinc refineries, and about 15 factories have been monitored since 2000. In the most recent survey in 2010 moss samples were also collected for determination of POPs such as PCB and other chlorinated compounds, PBDE, and PAH. Comparison of these data with geographically matching data from SPMDs (average air concentrations) and surface soils (integrated deposition) is in progress, and preliminary conclusions will be presented at the conference.

Keywords: terrestrial moss, monitoring, deposition, metals, persistent organic pollutants

Ecophysiology of Tilia americana Under Ozone Stress

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Ozone (O₃) is an environmental emergency, for its impact on biological systems and non-living materials. In recent decades, the current annual average of O₃ levels ranges from 20 to 45 ppb across the globe, which is roughly double the concentration that preceded the Industrial Revolution. Future mixing ratios are expected to raise to even more dangerous levels. Under specific conditions, such as in urban environment, plant systems are affected by this pollutant gas and it is important to understand (i) how these organisms respond to this stress factor, and (ii) which species should be chosen for planting in urban areas, especially in the Mediterranean basin. Generally speaking, the sensitivity/resistance to O₃ of trees has only received little attention, so our knowledge of the mechanisms underlying the capacity of these plants to tolerate or avoid oxidative stress is very fragmentary. This study reports on the effects at the physiological and biochemical level of a chronic realistic exposure to O₃ (120 ppb of O₃ for 5 h days⁻¹ for 45 consecutive days) in a controlled environment facility, in order to assess the variations in the magnitude of responses of the photosynthetic apparatus of Tilia americana saplings. At the end of the exposure, no foliar symptoms were evident, but the pollutant induced relevant biological effects on treated plants in comparison with control individuals: (i) a significant (-41%) reduction in the photosynthetic capacity, starting from 28 days after the beginning of exposure, due to a partial stomatal closure (-28%); (ii) an alteration of non-stomatal photosynthetic parameters derived from CO₂ response curve of photosynthetic activity after prolonged exposure (the maximum carboxylation rate of Rubisco and the lightsaturated rate of electron transport values became lower than that observed in control plants: -35 and -21%, respectively), (iii) an impairment of the efficiency of photosystem II (PSII), attributable to an increase in minimal fluorescence associated to maximal fluorescence similar to controls that induces a decrease of the F_{ν}/F_{m} ratio that reached a mean value of 0.75; (iv) a damage to the chlorophyll pigments system, as confirmed by the reduction of the chl a/b ratio, that increased

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by a 58%, to indicate an evident reduction of light harvest complex of PSII and by the concomitant decrease of the total chl/carotenoids ratio (-12%), showing that there was a need for plants to invest in an enhancement of photoprotective deexcitation pathways mediated by carotenoids. On the basis of the data given here it is confirmed that: (i) biochemical and physiological changes occur during or at the end of O_3 treatment, even in the absence of foliar visible effects; (ii) in asymptomatic species, like *T. americana*, photosynthesis is down-regulated as a response to oxidative stress; (iii) the absence of foliar injury is a confirmation that this species shows a relatively low sensitivity to this contaminant and, for this reason, it can be recommended as a shade tree for streets, parks and lawn planting in urban areas, even under future pollution scenarios.

Keywords: air pollution, PSII photochemistry, oxidative stress, carboxylation efficiency, xanthophylls cycle

Source Apportionment of Allergenic Ragweed (Ambrosia) Pollen Observed in Istanbul, Turkey

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This research recently published in the journal STOTEN deals with the detection of sources of ragweed (Ambrosia) pollen found in the atmosphere of Istanbul, Turkey. We aim to summarise our findings and results in BIOMAP2012.Ragweed is an invasive taxon and detrimental to human health with its highly allergenic pollen. So far, Turkish Thrace and Istanbul lackknown populations of ragweed. Aerobiological records in our pollen monitoring, however, indicate a close source. Moreover, ragweed pollens from distant sources reach Istanbul through air mass movements. Pollens were sampled with a Burkard trap and identified under the light microscope. Bi-hourly concentrations were obtained by counting samples on 12 transverse traverses. The highest count of the season was made on August 28 with 20 grains m⁻³. Ragweed was observed on 22 days during August and September. Circadian ragweed concentrations peaked around midday and during the night. We used the results on peak day as case study to identify origins by means of a HYSPLIT backward trajectory model. To solidify results, we used a frequency analysis of back trajectories covering the entire ragweed season. The Istanbul Province and Turkish Thrace resulted in being the local source, the overregional source was Bulgaria and remote sources of ragweed pollen are in the Ukraine, the Russian coastal region of the Black Sea and Moldova. We provide evidence that ragweed grains detected on our receptor site have both local and remote origins.

Keywords: ragweed, pollen, long-distance transport, HYSPLIT model, source apportionment.

Bioaerosols in the Environment: Composition, Health Effects and Analysis

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Bio-aerosols are airborne particles that are living (bacteria, viruses and fungi) or originate from living organisms. Bio-aerosols are natural or artificial particles of biological (microbial, plant, or animal) origin suspended in the air. The sampling and analysis of airborne microorganisms has received attention in recent years due to concerns with mould contamination in indoor environments, the threat of bioterrorism and the occurrence of associated health effects, including infectious diseases, acute toxic effects, allergies and cancer. Bio-aerosols contribute to about 5-34% of indoor air pollution. Their presence in air is the result of dispersal from a site of colonization or growth. The evaluation of bio-aerosols includes use of variety of methods for sampling depending on the concentration of microorganisms expected. There have been problems in developing standard sampling methods, in proving a causal relationship and in establishing threshold limit values for exposures due to the complexity of composition of bio-aerosols, variations in human response to their exposure and difficulties in recovering microorganisms. Currently bio-aerosol monitoring in various indoor/outdoor environments is carried out to generate baseline data and explore the link to infectious diseases and other health problems. In Turkey, there is little awareness regarding the quality of indoor air, mould contamination in indoor environments, potential source for transmission of nosocomial infections in daily care and health care facilities. This presentation will be a review on composition, sources, modes of transmission, health effects and sampling methods used for evaluation of bio-aerosols, and also suggests control measures to reduce the loads of bio-aerosols.

Effects of Air Pollution on Children's Lung Function Living in Eskisehir, Turkey

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Health effects of ambient air pollution were studied in three groups of schoolchildren living in areas (sub-urban, urban, urban-traffic) with different air pollution levels in Eskisehir, Turkey. This study involved children aged between 9 and 13 years from 16 public primary schools. This two-season study was conducted from January 2008 through March, 2009. Two lung function tests were performed by each child for summer (n=1841) and winter (n=1497) seasons with simultaneous ambient air measurements of ozone (O₃), nitrogen dioxide (NO₂) and sulfur dioxide (SO₂) by passive sampling. Effect of air pollution on children lung function was investigated by multivariate logistic regression analyses. Girls with impaired lung function (only for the summer season evaluation) were more observed in higher concentration ozone areas (sub-urban and urban) when compared to urban-traffic area with the lowest concentration of ozone, albeit not significant. The prevalence of impaired lung function in boys varied significantly among three regions neither in summer nor in winter. A significant positive association between impaired lung function and two week average ozone concentrations was found for the summertime evaluation only for the girls. The results of this study showed that increasing ozone concentrations may cause shortterm impairment in lung function of school aged children.

Keywords: air pollution, ambient ozone, children, lung function

Combining Biomonitoring Techniques and Geographic Information Systems to Assess Air Pollution

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The aim of this study was to combine biomonitoring techniques with geographic information systems (GIS) in order to assess the impact of an industrial area from the North of Spain on the air quality. Samples of the epiphytic lichen Parmelia sulcata were collected from olive trees in an unpolluted Portuguese rural area (Montargil, Portugal) and transplanted to the north of Spain, in a grid of 18 km per 20 km, having an industrial area at its centre. In addition, a higher density of lichens was exposed near the three main sources of air pollution in the studied area - a cement mill, a power plant and a steelwork. Lichens were exposed during 5 months, starting in April and ending in September 2010. After exposure the technique Instrumental Neutron Activation Analysis, using the k₀ methodology, was used to determine the concentrations of the elements Al, As, Br, Ca, Cl, Cu, Fe, I, La, Na, Sb, Sc, Sm, U, V and Zn. The spatial distribution pattern of the elements concentrations was modelled using the programme ArcGIS 10. Based on samples results, continuous surfaces of elements distributions were estimated using an inverse distance weighted (IDW) interpolation technique. The GIS provided the framework for geospatial integration of elements concentrations, land topography, land use and existing roads. This approach contributed for the identification of emission sources and for the assessment of the topography effect in the dispersion of pollutants.

Keywords: Lichens, Biomonitoring, Trace elements, Geospatial distribution, INAA

Source Apportionment of Trace Elemental Air Pollution by Employing Biomonitoring Techniques

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Monitoring of elemental atmospheric pollution is essential due to its impact on both ecosystem performance and human health. Source apportionment is regarded as an important parallel issue in order to establish air pollution control programmes. Biomonitoring techniques can be advantageously used to indicate geographical related variances in trace element air pollution and to identify emission sources, with a reduced infra-structure and manpower costs associated with the survey of large areas. The objective of this study was to assess the contribution of natural and anthropogenic emission sources to the levels of trace elements in the atmosphere of an industrial region (that includes a power plant, a steelwork, a cement mill and a harbor) placed in the North of Spain. The epiphytic lichens *Parmelia sulcata* were used as passive biomonitors to evaluate the relation between elemental accumulation and emission sources. Lichens were collected in olive trees from a considered unpolluted zone in Portugal and transplanted to the studied area in a grid of 18 km per 20 km (n=68). After an exposure of five months (since April to September of 2010), the concentrations of the elements Al, As, Br, Ca, Cl, Cu, Fe, I, K, La, Na, Sb, Sc, Sm, U, V and Zn were determined in transplanted lichens by the technique Instrumental Neutron Activation Analysis using the k₀ methodology. Principal Component Analysis combined with Multilinear Regression Analysis were subsequently applied in order to assess the contribution of local emission sources for these elements. Results showed that multiple sources influenced the total variance of trace elements concentrations: the soil (Al, Fe, La, Sc, Sm), the sea (Cl, I, Na), the traffic (Br, K, Sb and Zn) and the industry (Al, As, Cu, V and Zn).

Keywords: Biomonitoring, Lichens, Trace elements, INAA, Source apportionment

Biomonitoring the Effects of Air Pollutants Released During Cement Production with Lichens

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This study investigated the effects of airborne pollutants released during all steps of cement production on lichen physiology. The aim was selecting physiological parameters and functional groups of lichens suitable for bioindication purposes around similar sources of pollution. Thalli of lichens with different anatomical and ecological features, namely Xanthoria parietina, Evernia prunastri and Peltigera praetextata, have been exposed for 1 – 6 months at several locations in the area of Rohožník, near Bratislava (Slovakia), where an important cement mill is operating. These locations include guarries, cement mill, rural and inhabited areas and remote sites. We considered trace elements deposition in native and transplanted samples, physiological responses of the transplants by means of the analysis of secondary metabolites and chlorophyll fluorescence a emissions. We also measured microclimatic and chemical-physical parameters and lichen diversity as a function of distance from dust sources and at remote sites. We outlined the increase of xeronitrophilous species and the bioaccumulation of trace elements in the environment influenced by dust depositions respect to remote sites and detected changes of some secondary metabolites in lichens influenced by dust, which could be used as early stress markers. We developed a model to estimate the influence of dust from cement production which could be helpful in biomonitoring around similar sources.

Keywords: air pollution, cement industry, dust deposition, early indicators, lichens

Chemical Element Accumulation in Tree Bark Grown in Volcanic Soils of Cape Verde – a First Biomonitoring of Fogo Island

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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 (476 km²) corresponds to an active volcano with the maximum altitude of 2829 m, and is under a semi-arid climate with a rain period mainly between July and October (annual precipitation < 600 mm). Soils are developed in: 1) a carbonatite unit exposed in fluvial valleys near S. Filipe; 2) a major volcanic sequence related to the sub-aerial shield-building of the volcano (nephelinites and associated lavas with layers of scoria or tuffs, previous to the caldera formation); and 3) a post caldera sequence including several historic eruptions. Bark from several types of trees including Acacia, Jatropha curcas (Purgueira), eucalyptus, mimosa and apple tree, were collected in twenty sites corresponding to different geological formations. Bushes (oloendro) and lichens (Parmelia Sulcata Taylor) were also studied. Elemental contents in biological samples and soils were assessed through k(0)-standardised instrumental neutron activation analysis (INAA) 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 values - K (EF= 6470) > Lu > Br > Na > Yb > Cr > Zn >Ba > Eu > Hf > Tb > Cs > Ce (EF=19.7). 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

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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 (EF_K=109, EF_{Br}=20). The chemical contents in bark indicate that a significant portion of the elements may derive from the soils of the Fogo island. This may be due to the presence of bioavailable elements in soils (not leached due to the lack of precipitation) that are accumulated by plants when water intervenes. Among the rare earth elements (REE), EFs of the heavy REE are much higher than the light ones in bark, which agree with their preferential release after the breakdown of primary minerals like pyroxenes. According to these conclusions, lichens are not enriched in HREE, neither on most of the other elements studied.

Keywords: Fogo island, biomonitoring, volcanic soils; semi-arid climate; INAA

Baseline Levels and Potential Sources of Lesser Elements in Portuguese Cereals

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Cereals are by far the most significant agricultural crops, not only on a gross-tonnage basis, but also by what they really represent in terms of energy supply and dietary intake for human nutrition worldwide. One of the drawbacks of human diets based mostly on cereals is that the intake of essential elements may be inadequate regarding their average daily requirements – when cultivation soils become exhausted or are already poor in such elements – while, in turn, others can be present in levels that may be questionable for human ingestion on a daily basis. Within the framework of a research contract by the Portuguese Foundation for the Science and the Technology (PTDC/QUI/65618/2006; FCT, Lisboa), an extensive investigation of elemental concentrations in home-grown cereals and their cultivation soils has been carried out across the main production areas of mainland Portugal, for baseline-data purposes and with a view to an eventual improvement of major cultivars as well. The present paper deals with maize (or corn; Zea mays L.), rice (Oryza sativa L.) and barley (Hordeum vulgare L.) from the 2009 campaign. Cereal and soil samples from distinct areas that feature significant productions for each cereal were collected through the summer of 2009. Elemental levels in all samples were determined by k_0 -standardized, instrumental neutron activation analysis (k₀-INAA), in the Portuguese Research Reactor of the Technological and Nuclear Institute (RPI-ITN, Sacavém; pool-type reactor; maximum nominal power: 1 MW). Quality control of the instrumental procedure has been asserted with concurrent analyses of NIST-SRM® 1567a (Wheat Flour), NIST-SRM® 1568a (Rice Flour) and GBW 07404 (Limy-yellow Soil). Concentrations of As, Br, Cr, Co, Fe, K, Na, Rb and Zn – all within their safe intervals for human consumption – are reported and discussed with respect to former biomonitoring (epiphyte- and barkbased) surveys of the corresponding production areas.

Keywords: biomonitoring surveys; cereal crops; lesser elements; mainland Portugal; k_0 -INAA

Passive Biomonitoring Study and Effect Biomarker in Oysters Crassostrea brasiliana (Lamark, 1819: Mollusca, Bivalvia) in Santos and Cananéia Estuaries in São Paulo State, Brazil

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Estuaries are widely used for the disposal of urban and industrial effluents, causing water pollution and threat to marine life by several pollutants. This study assessed the exposure and effects of trace elements in sentinel organisms, using the bivalve Crassostrea brasiliana (Lamark, 1819: Mollusca, Bivalvia) in two estuarine areas in São Paulo State, Brazil: Santos Estuary (23° 58'S - 46° 19'W) (Bertioga and Canal de Santos), one of the most polluted in the world, as Canal de Santos is strongly impacted by industrial and domestic effluents and it has high harbour activities. Cananéia Estuary (25° 01'S - 47° 55'W), site of oyster farms, was used as reference site since it is one of the most preserved in the coast of the State of São Paulo. Seasonally, oysters were collected between September/2008 and July/2009 in each study site. Oysters were evaluated for bioaccumulation of As, Co, Cr, Fe, Se and Zn by Instrumental Neutron Activation Analysis (INAA) and Cd, Pb and Hg by Atomic Absorption Spectroscopy (AAS). Effect biomarker was assessed by evaluation of lysosomal membrane stability, using the Neutral Red assay (NR). In the study of bioaccumulation of trace elements among the sites of oyster collection, it was verified that Canal de Santos presented the largest concentrations of elements studied in most cases. Regarding the seasonal variation. it was observed that, in winter there was a greater accumulation of the elements. From the study of the cellular biomarker, it was verified that Canal de Santos and winter showed higher stress to organisms in the present study. Correlations between trace elements and the integrity of lysosomal membrane were evaluated.

Keywords: Crassostrea brasiliana, oyster, estuaries, biomonitoring, effect biomarker

Assessment of Polycyclic Aromatic Hydrocarbon Exposure by Hair Analysis of Monohydroxylated Metabolites Using Gas Chromatography Coupled with Tandem Mass Spectrometry (GC-MS/MS)

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Polycyclic Aromatic Hydrocarbon (PAHs) emissions are considered an important environmental and occupational health problem. As the exposure to PAHs is associated with diseases ensuing from long-term exposure (e.g.cancer), relevant biomonitoring has to provide quantitative information on chronic exposure. The use of hair as a biomarker of long-term exposure to PAHs allows the assessment of an average exposure integrating all exposure routes over several months. Moreover, the hair sampling can be easily collected which is compatible with epidemiological investigations. The aim of this work was the development of a sensitive method for the determination of monohydroxylated PAHs (OH-PAHs) in hair by Gas Chromatography coupled with tandem Mass Spectrometry.OH-PAH levels were assessed in hair samples of Lister Hooded rats sub-acutely exposed to a mixture of 16 PAHs (0.01 to 1 mg/kg, intraperitoneal, for 28 days). The suitability of the method for the determination of OH-PAHs in rat hairwas demonstrated by the detection of 20 OH-PAHs out of the 54 analytes investigated. Limits of quantification ranged from 0.2 to 10 pg/mg of hair. Mean concentrations of OH-PAHs in animal hair samples exposed to 1 mg/kg of PAH mixture ranged from 0.6 ± 0.2 pg/mg for 8-OH-benzo[b]fluoranthene to 6.7 ± 1.0 pg/mg for 1-OH-pyrene. This animal experiment demonstrates how OH-PAH metabolites can be internally incorporated into hair. With the exception of 4-OHphenanthrene and 1-OH-pyrene, hair pigmentation does not appear to affect the concentration values of most of the OH-PAHs investigated. These findings confirm the possibility to assess chronic PAH exposure by the detection of OH-PAHs in hair. The results of OH-PAHs analysis in urine and plasma which are currently investigated on the exposed rats will be compared to those obtained in hair in order to confirmhair analysis as a trustworthy biomarker for the human biomonitoring of exposure to PAHs.

Keywords: Hair, OH-PAHs, PAHs, Biomonitoring, GC-EI-MS/MS

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Are biomarkers really reflects true?

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Biomonitoring is possible with the acceptable and sensitive biomarkers in the right selected tissues. For example to monitor the mercury pollution in the sea, our experiences, as being stuck in there to choose bivalvia is better then fishes. Because they are living there, but fishes constantly migrate. To determine δ -aminolevulinic acid (ALA) level in the hepatopancreas of mussels are beter then determine directly mercury level. After mercury exposure, although some of mercury binds to proteins but most of them are relased to water again. To look if mercury toxication exist in the resident alive materials, are giving better information then to determine mercury itself. So there are many biomarkers for mercury pollution in the sea. An important point is to decide which one is better. Unless to clarify sensitivity, specificity, positive predictive value and negative predictive values of those tests, it is very difficult to decide and find true.

Interpretation of Data of Biogeochemical Indication: Models and Causes of Atmospheric Pollution

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Development and application of new methods of quantitative interpretation of data, in particular modeling of the spatial structure and causes of air pollution, play a large role in the further development of biogeochemical indication. In our studies mosses and epiphytic lichens were used as a biogeochemical indicator plants. The content of 23 chemical elements in them was determined using the ICP-OES method. During our study of heavy metal deposition (As, Cd, Cr, Cu, Hg, Fe, Ni, Pb, V, Zn) in the Ukrainian Carpathians in order to identify the causes of air pollution, we used such modern statistical and mathematical methods of data processing and modeling of content of the mentioned chemical elements as compositional biplot and geostatistical interpolation. Geostatistical interpolation has been performed by mapping of the logarithms of ratios between the content in the samples of various chemical elements. The model was developed, which expressed the ballance (v) between the content of lead and mercury in mosses: $v = (1/20, 5)\ln (Pb/[Hg])$. Geostatistical interpolation and mapping of the results of this ratio for the mosses *Pleurozium schreberi* and Hylocomium splendens, sampled at 115 points in the Ukrainian Carpathians, proved the existence of the western air transport of pollutants from Western Europe into the territory of Ukraine. The statistical analysis of elements content in indicator lichens (Parmelia sulcata, Xanthoria parietina, Hypogymnia physodes, Evernia prunastri) by biplot method was also used in the study of atmospheric pollution in Ukrainian arboretums "Alexandria" (Bila Tserkva), "Trostianets" (Chernihiv region). and natural boundary "Feofaniya" (Kyiv). The typomorphic concept of chemical elements regarding a chemical element as a marker of the causes and process of environmental pollution, which is conventional in modern geochemistry of technogenesis, was used as a basis. This allowed to demonstrate the different structure of the atmogeochemical fields of contamination, as well as to identify the causes leading to air pollution in the studied areas.

Keywords: Air pollution, Lichens, Biogeochemical indication, Chemical elements, Geostatistical methods

The Regression Models of Species Specific Accumulation of Chemical Elements by Epiphytic Lichens Used in Bioindication

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It is known that various species of lichens have different ability to uptake and accumulate chemical elements. Therefore, one of the most important requirements of biogeochemical lichenoindication (BGCHLI) is the presence of an indicator species of lichen in the maximum possible number of sampling points across the study area. However, in practice, during lichenoindication field work, especially large-scale one, this requirement often cannot be met due to the lack of the necessary indicator species in a number of sampling points. At the same time, other lichens grow here, which can be used in BGCHLI. Experimental grounding of the simultaneous use of several species of lichens in the BGCHLI is an important methodological problem. It is necessary to perform the statistically significant normalization of the content of pollutants in various species of lichen on a certain species of normalization. Unfortunately, this classical problem of searching biogeochemistry with respect to the lichens remains unsolved up to now, although some attempts have been made. We carried out intercalibration of contents of 23 chemical elements (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sb, Se, Sr, Ti, V, Zn) in 4 species of epiphytic lichens, which are most often used in BGCHLI in Ukraine: Parmelia sulcata, Xanthoria parietina, Hypogymnia physodes and Evernia prunastri. Parmelia sulcata has been chosen as normalization standard. The rest of the lichen species were compared with it. The content of chemical elements in lichens was determined by ICP-OES method. Pairwise comparison of the contents of elements in these lichens (8-22 pairs), sampled in the same locations on the same phorophyte, was carried out using a simple regression analysis by means of Statgraphics 5.0 software. Seven various regression models were tested. The calculated correlation coefficients allowed to estimate the significanse of the correlation dependenses of chemical elements between P. sulcata and species under comparison.

Keywords: Air pollution, Biogeochemical indication, Chemical elements, Lichens, Interspecis calibration

Comparison Study of Several Decontaminations and Extractions Methods for Multi-Class Pesticides Analysis in Human Hair

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Even if a growing interest is currently observed in hair analysis for the biomonitoring of human exposure to pesticides, researches on specific aspects such as hair decontamination and reduction of interferences are still needed. The objectives of the present study were 1) developing a decontamination procedure to eliminate atmospheric pesticides deposited on hair surface, which might interfere with the analysis of hair incorporated molecules; and 2) developing a purification method in order to limit the analytical background noise generally associated with this complex matrix. Sixty-seven pesticides and metabolites: organochlorines, organophosphates, carbamates, pyrethroids, ureas, azoles, phenylpyrazoles and neonicotinoids; have been determined using gas- and liquid- chromatography tandem mass spectrometry. Several solvents have been tested with regard to their efficiency to clean the surface of hair samples artificially contaminated by either dusts or solutions containing pesticides. The most efficient procedure was found to be successive washings with sodium dodecylsulfate, water and methanol. For sample purification, 7 solid phase extraction (SPE) columns were tested and compared to liquid-liquid extractions using ethyl acetate, hexane and dichloromethane. The choice of the most suitable purification method was depending on the targeted molecules and the separation method. ENVI-Carb-II/PSA column was appropriate for organochlorines, organophosphates, pyrethroids and phenylpyrazoles extraction (recovery: 45.9% to 117.1%). Taking into account the recovery (10.3% to 93.1%) and the chromatographic separation, Bond Elut Plexa cartridge was found to be suitable for the extraction of organophosphates and pyrethroids metabolites. The highest recovery percentages for carbamates, neonicotinoids, ureas and azoles were obtained using SAX/PSA columns, 52.1% to 100.9%. The use of SPE cartridges have reduced the interferences due to matrix and derivatization products, resulting in better chromatographic separation.. This work provides new insights on pre-analytical treatment of hair samples in order to improve the use of this matrix for the biomonitoring of human exposure to pesticides

Keywords: Pesticide, Biomonitoring, Sample preparation, Hair decontamination, Solid phase extraction

Volatile Organic Compounds Dependent Indoor Air Pollution and Infant Health

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Volatile Organic Compounds (VOCs) are chemicals that easily turn into gases and contaminate indoor air. Some of these chemicals cause developmental damage as well as damage to the liver and central nervous, respiratory, and reproductive systems. Many products we have in our homes release VOCs. Some examples of sources of VOCs are building materials, home and personal care products and behaviors. Indoor air VOCs were collected simultaneously from variety of indoors (e.g. living rooms, baby's rooms) and outdoor environments which were voluntarily selected throughout Ankara. Studies have shown that the level of indoors VOCs is generally higher than the level of outdoors. Indoor air quality can be 2 to 5 times worse than the air outside. Babies spend about 95% of their time in indoor environment, the time has been spent in indoor might be more during winter times. Their organs, immune system and neurological system are still developing, and because of their lower body weight, they breathe in a relatively greater volume of air than adults. This is the first comprehensive study done in Turkey including prenatal and postnatal periods. VOCs levels were measured over 94 homes. Average of total VOCs in the living rooms and baby's rooms varied between 84.41 to 133.83 µg/m³. According to the sampled indoor environments of the study, it was found that the levels of indoor air volatile organic compounds in the baby's rooms were higher than the living rooms. Our findings suggest that there is a relationship between indoor VOCs levels and infants health. As there are no standards for indoor residential air quality, additional researches are needed to assess the impacts of indoor pollution exposure for infants.

Keywords: Indoor air, Volatile Organic Compounds, Infant health, Living Rooms, Baby's Rooms

Evaluating the Impact of Urbanization in Urban and Peri-urban Forests Using Lichen Functional Groups

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Environmental changes caused by urbanization and disturbances caused by human daily activities are among the major threats to biodiversity in urban ecosystems. Urban and peri-urban forests besides playing a main role in the sustainable development of cities and in guaranteeing a high quality life to the citizens represent fragments of habitat in which plants and animals can survive and be conserved. There is however a need to find reliable ecological indicators that can be used in urban and peri-urban areas to evaluate the impact of urbanization in urban forests. To that, we tested lichens and several animal taxa as potential indicators in a case study carried out in forest remnants of a Mediterranean urban area (Almada, Portugal). We present the results concerning lichen diversity. The city of Almada is located near Lisbon, with a density of near 2500 habitants/km². The area is bordered by the river Tagus to east and north and by the Atlantic Ocean to west. The urbanization that occurred mainly over the last decades led to a fragmentation of the original forest. Nowadays, the area includes both dense and scattered urban areas, mixed with remnant patches of forested areas and a large natural area committed to the conservation of a natural pine woodland forest. A GIS was used to design a stratified random sampling taking into account location and size of the forest fragments. A total of 48 patches were selected. At each sampling site, lichens were sampled on *Pinus pinaster* and *P. pinea*-trees, the most common tree species in the area, and lichen functional diversity was assessed. Lichen functional diversity resulted mostly correlated with fragment dimension and surrounding land use confirming to be a suitable indicator of the impact of urbanization on urban and peri-urban forests. This can help the identification of critical areas and forest fragments with high potentiality for conservation and provide fundamental information for decision-makers and urban management to develop strategies for a sustainable urbanization.

Assessment of Adaptations to High Heavy Metal Deposition Rates by Means of Cross-Transplants of the Terrestrial Moss *Pseudoscleropodium purum*

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Development of heavy metal tolerances in terrestrial mosses, similar to those found in vascular plants, would constitute an important limitation for the use of these organisms as biomonitors of heavy metal deposition of these elements. However, nowadays it has not been made any experience to confirm or refute the possible existence of these adaptations. In order to investigate the existence of differences in concentration capacity of heavy metals between different populations of the terrestrial moss Pseudoscleropodium purum, we determined tissue concentrations of Cu, Hg and Zn in cross transplants of this organism exposed during 30, 60, 120, 240, 480 y 840 days, between various polluted and unpolluted areas. Our results showed a clear increase of heavy metal concentrations in mosses from the unpolluted area but transferred to the polluted area and, in case of Hg, achieved those of the native moss autotransplanted in the same area (although differences were not significant). Furthermore, concentrations of heavy metals in transplants from the polluted area located in the unpolluted area, decreased close or even lower than those for the autotransplants from the unpolluted area. According to these results, we could not find any difference in concentration capacities of these elements between mosses grown in environments with different levels of atmospheric heavy metal deposition. For this reason, by analysing heavy metal concentration in moss tissues we cannot assume the existence in mosses of any mechanism of adaptation to different levels of pollution. Therefore further investigations (e.g. genetic variability, proteomic, etc.) should be conducted to confirm these results.

Keywords: Heavy metals, Native moss, Transplants, Autotrasplants.

Landscape-Specific Correlation Analysis between the Atmospheric Deposition of Nitrogen and Metals and their Concentrations in Mosses

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The presentation at hand deals with the landscape-specific analysis of the statistical association between the concentration of Cadmium (Cd), Mercury (Hg), Lead (Pb) and Nitrogen (N) in mosses and corresponding modelling results for the atmospheric deposition. Until now, such analyses had either been done for all of Europe or for single administrative units such as federal states or countries but not for spatial units taking into account the spatial variability of ecological criteria such as soil and climate. To reach this goal, measurement data from the 2005 European moss monitoring survey and modelled data on atmospheric depositions from the European Monitoring and Evaluation Programme (EMEP) were intersected with a European wide landscape map structuring Europe into 40 ecologically defined landscape classes. The classification map was calculated with help of data on vegetation, elevation, climate and soil texture using GIS techniques and decision tree models. Spearman correlation analysis was applied to quantify the correlation between the concentrations of Cd, Hg, Pb and N in mosses and corresponding deposition loads within each of the 40 landscape units. The results thereby revealed different coefficients of correlation for Cd (between -0.23 and 0.77), Hg (between -0.46 and 0.53), Pb (between -0.13 and 0.67) and N (between -0.49 and 0.8). Decision tree models furthermore allowed investigating the multivariate interactions between the moss concentrations and chosen predicting variables on moss-specific criteria, land use, climate, elevation and deposition. This study 6th International Workshop on Biomonitoring of Atmospheric Pollution
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proves that further work should be done to investigate landscape specific patterns of the correlation between the atmospheric bioaccumulation and deposition. Corresponding knowledge may improve the understanding of the spatial variability of the concentrations of metals and N in mosses.

Keywords: Moss monitoring, biomonitoring, nitrogen, heavy metals, atmospheric deposition

Country-Specific Correlations across Europe between Modelled Atmospheric Cadmium and Lead Deposition and Concentrations in Mosses

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Mosses have been used as biomonitors of atmospheric heavy metal deposition since the late 1960s. The application is based on the fact that especially carpetforming species obtain metals directly from precipitation and dry deposition and there is little uptake of metals from the substrate. Since 1990, the European moss survey has been conducted every five years to assess spatial patterns and temporal trends in heavy metal deposition at a high spatial resolution. Up to 29 countries have taken part and mosses were sampled from up to 7,300 sites across Europe per survey year. The results of the 2010/11 survey will be reported in 2013. Since 2000, the European moss survey is coordinated by the International Cooperative Programme on Effect of Air Pollution on Natural Vegetation and, a subsidiary body of the Working Group on Effects of the Convention on Long-range Transboundary Air Pollution. Statistical analyses have shown that total deposition is a main determinant of cadmium and lead concentrations in mosses. Further country-specific analyses show that Spearman rank correlations between the concentration in mosses and the deposition modelled by the European Monitoring and Evaluation Programme (EMEP) are country and metal-specific. Significant positive correlations were found for about two thirds or more of the participating countries in 1990, 1995, 2000 and 2005 (except for Cd in 1990). Correlations were often not significant and sometimes negative in countries where mosses were only sampled in a relatively small number of EMEP grids (50 km x 50 km).

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Correlations frequently improved when only data for EMEP grids with at least three moss sampling sites per grid were included. It was concluded that spatial patterns and temporal trends agree reasonably well between lead and cadmium concentrations in mosses and modelled atmospheric deposition. Hence, mosses provide a complementary method to indicate spatial patterns and temporal trends of the atmospheric deposition of cadmium and lead.

Keywords: biomonitoring; EMEP; heavy metals; metal deposition; bryophytes

Compositional Analysis of Pollution Sources by Means of Terrestrial Moss, *Pseudoscleropodium purum*

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Compositional analysis arises appropriate for studies that attempt to describe the processes of atmospheric pollutants dispersion and observe patterns of emission of pollution sources. As the original data are transformed into proportions, the results emphasize in their differences in composition and not in the total amount of it. So this analysis is suitable before the PCA (Principal Component Analysis) for this type of research. The main aim of this study is to check if the terrestrial moss Pseudoscleropodium purum can find differences between different industrial sectors according to levels of 17 heavy metals accumulated by the moss (Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, V, Zn). For this purpose, 237 samples were collected in the surroundings of industries in different sectors (Canning factory, Cement works, Ceramic factory, Chlor-alakali plant, Chemical industry, Ferrous smelter, Non-ferrous smelter, Paper and wood production processing, Urban waste incinerator and Waste oil treatment) and also in backgrounds areas, in Galicia (NW Spain). The results obtained show that there certainly is a cluster based on industry sector. The set of samples located around the Ceramic factories seems to be linked to concentrations of V, while the Non-Ferrous and Ferrous smelter to concentrations of Pb. Moreover, samples of background areas are grouped in a wide cluster from samples of industries. These results lead to the conclusion that the moss do not respond in the same way to different elements in the atmosphere even with the same concentration. The moss has a preference to retain some heavy metals from other ones so it does not reflect the actual pattern of emissions of the industries.

Keywords: Atmospheric contamination, bryophytes, Compositional data analysis, heavy metals. PCA.

Mosses as Biomonitors of Trace Element Deposition in Slovenian Forests

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Since 1995 Slovenia has been regularly involved in the European international moss survey. As some discrepancies were found in the results from previous surveys, a systematic sampling approach was performed in 2010 aiming to test how the environmental characteristics (type of forest, canopy closure, , inclination, local climate, precipitation) of different sites influence metal levels in mosses. Moss samples of Hypnum cupressiforme were collected at more than 100 locations distributed on a regular 8 x 16 km grid, which is also part of the grid for national forest inventory, including 11 locations near plots of the Intensive Monitoring Programme of Forest Ecosystems in Slovenia (UN-ECE ICP-Forest Level II plots). At each sampling plot two types of samples, namely below the canopy and in the open were taken and analysed for trace elements using ICP-MS. Results of the statistical evaluation for some selected elements (As, Cd, Cr, Pb, Sb, Zn) in mosses sampled below the canopy and in the open are presented and discussed. Consequently, models which could most significantly explain cause-and-effect relationships between the metal levels in mosses sampled below the canopy (inside forest) and in the open (outside the forest) are presented.

Keywords: Hypnum cupressiforme, Trace elements, Forest type, Canopy effect, Modelling, biomonitoring

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POSTER PRESENTATIONS

Epiphytic Bromeliads from Atlantic Rainforest as Accumulators of Chemical Elements

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Epiphytes directly respond to changes in the atmosphere, thus becoming an important tool for environmental studies as biomonitors. Epiphytic bromeliads have different strategies for the uptake of nutrients from the litterfall or from the atmosphere, presenting accumulation of chemical elements at various levels. This work aimed at selecting a bromeliad species with high accumulation of chemical elements, therefore showing a good potential for application in biomonitoring studies. Eleven bromeliad species were collected in four conservation units of São Paulo State, Brazil, i.e. Parque Estadual Ilha do Cardoso (PEIC), Parque Estadual Carlos Botelho (PECB), Estação Ecológica dos Caetetus (EEC) and Estação Ecológica de Assis (EEA), respectively representing restinga forest, dense ombrophilous forest, seasonal semideciduous forest and savanna woody. Bromeliad leaves were analyzed by instrumental neutron activation analysis. Results showed significant differences for Ca, Co, Na, Sc, Sr and Zn amongst bromeliad species at a 95% confidence level. There was no common distribution pattern of chemical elements in the leaves, even for species of the same gender. Accumulation indexes were calculated for each bromeliad, with a higher average value (0.6) for Canistropsis billbergioides from PECB, which was therefore identified as accumulator of chemical elements in the Atlantic Forest.

Effects of the Urban Environmental Conditions on the Physiological Parameters of the Transplanted Lichen *Evernia* prunastri

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Urban environments are subjected to intensive anthropogenic pollution. The aim of the study was to evaluate the changes in physiological parameters under the urban environment pollution. Thalli of epiphytic lichen *Evernia prunastri* were transplanted from unpolluted environment to urban environment (Kaunas, Lithuania). Chlorophyll *a* and *b*, ergosterol and MDA in lichen thalli were determined after 1 month exposure period. The biomonitoring sites were determined according to pollution levels and divided into three groups: inner and outer city sites and control. The concentration of chlorophyll a was higher in samples exposed in the city center and was significantly different from samples exposed in the outer city sites. Chlorophyll b degradation was detected in the inner and outer city sites (16.6% and 27.8%, respectively). Cell membrane damage was also observed in the urban environment. It was concluded that pollution induced oxidative stress in the transplanted lichen due to the increase in MDA concentrations.

Keywords: Evernia prunastri, oxidative stress, chlorophyll, ergosterol

Determination of PAH Residues in Striped Red Mullet (Mullus Surmuletus) from the West Black Sea Coast of Turkey

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Polycyclic aromatic hydrocarbons (PAHs) are lipid soluble chemicals. They tend to accumulate in living organisms. They are metabolized into highly reactive metabolites. These metabolites may cause effects at the molecular and cellular levels. Industrial, anthropogenic, and agricultural activities threat the Black Sea. The presence of PAHs has been shown previously in flathead mullet (Mugil cephalus) by total PAH analysis [1]. The striped red mullet (Mullus surmuletus) are economically important and highly consumed fish species in Turkey. The aim of this study was to determine the levels of specific polycyclic aromatic hydrocarbon residues in striped red mullet samples caught from the West Black Sea Coast of Turkey. The striped red mullet samples were caught from Zonguldak and Eregli Harbours, Kefken and Amasra in 2009-2011. PAHs were extracted from the liver tissues and then the levels of PAHs were measured by HPLC. Acenaphthene, fluoranthene, pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, benzo(k)fluoranthene. indeno(1,2,3,-cd)pyrene, dibenzo(a,h)anthracene and benzo(g,hi)perylene were below detection limits in striped red mullet caught from Amasra in 2009. Benzo(b)fluoranthene was the highest PAH measured in all sampling stations except for striped mullet caught from Amasra in 2009. Its concentration ranged between 2.99 and 3.48 µg g⁻¹ dry weight. Phenanthrene was present in all sampling stations and all samples caught in 2009-2011. The concentration of phenanthrene changed between 0.44 and 2.68 ug g⁻¹ dry weight. Anthracene was also present in all sampling stations. Acenaphthylene was present in all sampling stations except for striped red mullet caught from Amasra in 2010. It ranged between 0.87 and 3.38 µg g⁻¹ dry weight. This result indicated the presence of PAHs in the West Black Sea coast of Turkey and exposure of living organisms to these chemicals. This research was supported by TUBITAK (The Scientific and Technological Research Council of Turkey) (Project No:104Y083).

Keywords: Black Sea, Polycyclic aromatic hydrocarbons (PAHs), Striped red mullet (*Mullus surmuletus*)

Leucaena leucocephala as Passive Biomonitor in Urban Area Around Steel Industry

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The species Leucaena leucocephala is a native leguminous tree from Mexico and Central America, which was introduced in Brazil in the last century to feed livestock due to the high protein content of its leaves. Nowadays the species is spread over almost the entire country. It is considered one of the most aggressive invasive species based on its efficient accumulation of nutrients in different soil conditions and production of allelopathic substances that inhibit the growth of other species. These characteristics coupled with its wide occurrence in the city of Piracicaba supports its use as a passive biomonitor of trace elements. The Piracicaba city, located in São Paulo state, Brazil, is a highly industrialized area comprising several metal industries. The concern with the environment protection has pointed out priority actions to establishing an environmental monitoring programme in the region. In this context, Leucaena leucocephala was used as a passive biomonitor of an iron-steel industrial complex located in a very populous urban area of the city. Therefore, this work focused at evaluating the influence of anthropogenic contributions on the soil and atmospheric uptake by Leucaena leucocephala. Tree leaves were collected in several points around the steel industry. Soil samples were collected at the 3 depths under the tree crown projection. Soil-to-leaf transfer factor (TF) was measured to observe the statistical dependence of chemical elements bioaccumulation in function of the plant substract. Sc and Th were used as tracers of leaf surface contamination by atmospheric deposition. Several elements could be determined in the samples by instrumental neutron activation analysis (INAA). Among them, Br, Fe, Cr, Sb and Zn showed concentrations higher than usually found in plants and soils thereby indicating a potential contamination of the area.

Keywords: biomonitoring, soil, NAA, transfer factor

Antimony Toxicity in the Lichen Xanthoria parietina (L.) Th.Fr.

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Antimony (Sb) is a chemical element which is known to cause adverse effects to humans and the environment. Sb³⁺ is considered as possibly carcinogenic to humans by the IARC. We have investigated the physiological, cytological and anatomical effects of short term exposures to excess Sb³⁺ on the lichen *Xanthoria* parietina. Samples were incubated for 1 h in 50 mL of solutions 0.1 mM, 1 mM, 10 mM and 100 mM and treatments were repeated three times. Concentrations of total and intracellular Sb were measured, as well as sample viability (by means of the triphenyltetrazolium chloride reduction to triphenylformazan, which is a good indicator of dehydrogenase activity), membrane lipid peroxidation (using the thiobarbituric acid reactive substances assay), photosynthetic efficiency (evaluated by the chlorophyll fluorescence F_V/F_M and performance index), and the content of water-soluble proteins. Cytological and anatomical features were investigated by means of TEM and ESEM analysis. The results showed that Sb content in the thalli increased with increasing concentration in the treatment solutions. Treatments with increasing Sb concentrations induced membrane lipid peroxidation since the concentration 0.1 mM. Lichen viability decreased and the content of water-soluble proteins was affected in thalli incubated with 10 mM Sb-solutions. Photosynthetic parameters (F_V/F_M and performance index) were affected only in thalli incubated with 100 mM Sb³⁺. Sb treatments affected cell ultrastructural features in a dosedependent way: at the lowest Sb concentration (0.1 mM) ultrastructural changes were already visible and treatments with 100 mM Sb determined the complete loss of the ultrastructure in the algal cells.

Keywords: antimony, chlorophyll fluorescence, lichens, membrane lipid peroxidation, water soluble proteins

Biomonitoring the Effects of Ammonia from Industrial Composting Using Lichens

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Aerobic treatment of organic wastes (composting) transforms crop residues, organic municipal wastes, industrial organic wastes and animal feces to composts. Composting is an environmental friendly process, however, during composting of organic wastes, a certain amount of nitrogen can be locally released as NH₄⁺/NH₃ to the environment. We are investigating whether ammonia emissions from industrial composting influence lichens in the surrounding environment. The aims are: detecting early signs of environmental changes around point sources of ammonia by means of lichens; establishing a link between selected physiological parameters and ammonia in the air and foresee future variation in lichen communities due to ammonia; extending the results of the present study to other environments concerned by point sources of ammonia. We transplanted thalli of Ntolerant and N-sensitive lichens, namely *Xanthoria parietina* and *Evernia prunastri* for 1 – 3 months along transects at increasing distance (from 0 to 350 m) from a compost facility in Tuscany (central Italy). We investigated the physiological response of the transplants by means of the damage endured by cell membranes (measured as electrolyte leakage), the chlorophyll a fluorescence emission and the overall viability assessed by the enzimatic activity of dehydrogenase. We measured ammonia emissions with passive samplers around the facility and assessed the condition of lichen communities, actually dominated by meso-acidophilous lichens, using diversity indexes. N-tolerant and N-sensitive lichens reacted differently: the N-tolerant X. parietina was not affected by the proximity to the facility and some parameters even suggested higher performances, while the

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mesophilous N-sensitive *E. prunastri* showed higher performances with increasing distance from the source. This would suggest a shift from meso-acidophilous to nitrophilous lichens in the near future only in the sites facing the facility.

Keywords: ammonia, chlorophyll fluorescence, early indicators, industrial composting, lichens

Lichen Biomonitoring around a Solid Waste Incinerator

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There is a high public concern for the environmental and health impact of municipal solid waste incinerators (MSWIs). During the incineration process, heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzop-dioxins (PCDDs) and dibenzofurans (PCDFs) are of particular concern. A monitoring programme around any MSWI should mandatory include also monitoring of the biological effects. Lichen biomonitoring can be used as a complementary system that integrates instrumental monitoring of air pollution around MSWIs. A monitoring programme was implemented around a MSWI located in an industrial area in south Italy that included also the use of bioindicators, i.e., analysis of heavy metal depositions in lichens, mosses and soil, a standardized assessment of lichen communities according to the lichen diversity as an indicator of global environmental quality, an evaluation of the above pollutants by means of passive samplers and lichens. This study reports on the physiological and chemical response of the lichen Evernia prunastri (L.) Ach. (a commonly used monitoring tool) transplanted for six months in and around the industrial area and its surroundings. We investigated the physiological response of the transplants by means of the damage endured by cell membranes (measured as electrolyte leakage), the chlorophyll a fluorescence emission, the overall viability assessed by the enzimatic activity of dehydrogenase. Lichen transplants were used also as bioaccumulators of heavy metals. The experiment allowed detecting early 6th International Workshop on Biomonitoring of Atmospheric Pollution
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signs of environmental stress in some parts of the industrial area, at stations also featuring high frequencies of nitrophilous lichen species. A shift from meso-acidophilous to xero-nitrophilous lichen communities with proximity to the industrial area was observed. Functional response groups, i.e. species that have a similar response to an environmental factor seemed even more reliable than the total diversity, depicting the ecological status of the area.

Keywords: air pollution, early indicators, lichen transplants, waste incineration

Contributions from Vehicles and Oil Refinery to Emissions of PAHs in the Industrial City of Cubatão (Brazil)

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PAHs can be formed during the incomplete combustion of organic materials, such as oil, gas and wood, and are emitted primarily by anthropogenic sources in all around the world, inclusively in Brazil. Due to the potential of plants to accumulate PAHs, it has been suggested their use as passive samplers in biomonitoring studies. Although Lolium multiflorum spp. italicum 'Lema' is one standardized grass for the biomonitoring of inorganic substances adsorbed on the atmospheric particulate matter, it has also been recommended as a biomonitor of PAHs. The leaf contents of individual PAHs in such biomonitor plant may be markers of their different emission sources. The most appropriate PAHs for this purpose are then source markers. Therefore, the objectives of this work were: to verify the contamination of PAHs in two sites in the industrial city Cubatão (southeastern Brazil), by measuring the PAH accumulation on the leaves of L. multiflorum, and to determine possible source markers. Site 1 is away from the emissions of the industrial complex, but is next to a highway with heavy traffic toward the Atlantic coast. Site 2 is in the vicinity of an oil refinery. Cultures of L. multiflorum were exposed during twelve consecutive periods of 4 weeks each (April 2009 to April 2010). The following PAHs were analyzed: naphthalene (NAP), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CRY), benzo[b]-fluoranthene

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(BbF), benzo[k]fluoranthene (BkF) and benzo[a]pyrene (BaP). The sum of concentrations of PAH accumulated on the leaves of L. multiflorum ranged from 2426 ng.g-1 to 9185 ng.g-1 and from 2525 ng.g-1 to 9185 ng.g-1 in sites 1 and 2 respectively. The plants accumulated more ACE, NAP and ANT than the other compounds analyzed. CRY, PYR, BbF and BkF were the most abundant among the heaviest compounds. During the summer, increasing proportions of PAH, among them NAP, ACE, FLU, FEN, PYR, BaA, CRY, BbF and BkF, were observed on the leaves of plants from the site 1 than in plants exposed in the site 2, indicating the predominance of vehicular emissions in the period, probably due to the large number of vehicles that moved along the highway toward the coast. During the autumn and winter, increased proportions of all PAHs were measured on the leaves of the plants exposed in the site 2, with respect to site 1, revealing the high contribution of the cracking and refining of oil to the atmospheric emissions of PAHs. NAP, ACE, PIR and BkF, which predominated in the leaf samples from one site or from the other depending on the experimental period, probably indicated both vehicular and petrochemical emissions. Therefore, they should be used with caution as source markers. FLT and BaP, which appeared in higher levels in the plants from the site 2, marked appropriately the petrochemical source. However, these emission markers can be altered due to the reactivity of some PAH with other atmospheric species, restricting the identification of emission sources.

Keywords: Polycyclic aromatic hydrocarbons, Cubatão, *L. multiflorum*, traffic emission, oil refinery emission

Biomonitoring of Piracicaba River Basin, SP, Brazil: Assessment of Chemical Elements in a Riparian Area

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Rivers are the main carriers of chemical elements in a terrestrial ecosystem and the maintenance of riparian vegetation is considered vital for ensuring the resilience of water resources due to its bioaccumulation and depuration abilities. Furthermore, several chemical elements from different pollution sources can be bioaccumulated in riparian vegetation and cycled into the environment. Unfortunately this is the reality of most rivers of the Piracicaba River basin, São Paulo state, Brazil. This river basin is located in one of the most urbanized regions of the country receiving large discharge of industrial and domestic effluents. Due to the potential risk of contamination of this important water resource, efforts have been expended by government agencies to implement monitoring programs in the Piracicaba River basin. In this context, this work aimed at evaluating the chemical composition of sediments from different points of the Piracicaba River as well as their bioaccumulation by the riparian vegetation. Trees species of Leucaena leucocephala were randomly selected, identified and labeled for sampling of leaves and the sediments under the tree crown projection. Chemical elements were determined in analytical portions of dried and powered materials by instrumental neutron activation analysis (INAA). Biological and geological certified reference materials were also analyzed for analytical quality control. Several elements, i.e. As, Ba, Br, Ce, Co, Cr, Cs, Eu, Fe, Hf, La, Lu, Na, Nd, Rb, Sb, Sc, Sm, Ta, Tb, Th, Yb and Zn, could be determined in the samples of leaves and sediments. Among such elements, Br, Fe, Sb, and Zn should be emphasized for presenting high concentrations in both compartments indicating changes of anthropogenic nature in the Piracicaba River basin.

Keywords: metals, sediments, bioaccumulation, INAA

Monitoring in Forested Natura 2000 Sites: is the Lichen Diversity Value (LDV) Effective to Assess Local Air Pollution?

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Several lichen methods are usually used to indicate pollution from phytotoxic gaseous substances in urban, periurban and industrial areas while they are not recommended in natural and semi-natural habitats because of the complex interactions among environmental factors affecting the lichen biota. However, in these areas lichen biomonitoring can potentially respond to a variety of requirements and objectives of European environmental policy primarily in the fields of pollution control and conservation. Main aims of this investigation were identify possible critical issues and improve consistency of lichen biomonitoring in forested areas using European standard operating procedures, which are still in preparation. The case study is represented by the environmental impact assessment of a waste incinerator plant in Molise (Central Italy). The study area includes two Natura 2000 Sites where the fallout of pollutants from the incinerator is presumed by mathematical modeling results. A stratified random sampling design based on the two major land cover types (Forests and Agricultural areas) and on the distribution of standard trees, has been adopted. The number of plots has been calculated considering preliminary information on data variability in the study area. The sampling objective was to obtain an estimate of LDV over the study area with a confidence interval $\pm 10\%$ of the mean value, at a probability level of 95%. Analysis of the data shows that the diversity of epiphytic lichens is strictly correlated with the distance from the incinerator. Applying Wilcoxon and Sign tests to the results, the study shows that there are no significant differences between the two land cover types. Despite a high variability of values, results suggest the possibility to use the approach to assess local air pollution in forested areas. The work was funded by the Joint Consultative Body of the Consortium for Industrial Development of Isernia-Venafro and the society Energonut SpA.

Keywords: Forested habitats, Lichen biomonitoring, Pollution

Effects of Freshwater Pollution in *Leptodictyum riparium* Moss Bags Exposed in a Polluted River of S Italy

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The pollution of air, water and soil is a major environmental and human health issue. Contamination of freshwaters is of special concern since rivers may transport pollutants away from source, even to areas far removed from any pollution source. Plants reacts to pollutants in a very complex way, activating a number of parallel and/or consecutive reactions at molecular, physiological and morphological levels. This study aimed at evaluating biological effects of freshwater pollution in the highly contaminated river Solofrana (Campania, S Italy), using bags containing the aquatic moss Leptodictyum riparium Hedw. Homogeneous samples of 750 mg of L riparium, collected at the Botanical Gardens of the University of Naples, were accurately washed and disposed in 10x10 cm wide nylon 1 mm² - mesh bags. The moss bags were exposed for one week at 25 cm of water depth in the river, to determine morpho-physiological and genetic modifications as a response to pollutants. A number of parameters were measured and observed on these samples: heavy metal accumulation, ultrastructural organization, HSPs induction and repetitive DNA organization. The results showed that L. riparium is very effective in accumulating heavy metals. Morphological alterations concern overall chloroplasts (shape and thilacoidal organization) and vacuoles. HSPs and repetitive DNA were influenced by exposure. The results suggest the possibility of using this moss species and this method both in biomonitoring and phytoremediation projects.

Keywords: Bioaccumulation, Freshwaters, Italy, Moss, Pollution

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Source Apportionment of Polycyclic Aromatic Hydrocarbons (PAHs) in Surface Sediments of İkizcetepeler Dam Lake, Balıkesir, Turkey

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Hierarchical cluster analysis (HCA) and factor analysis (FA) were applied to apportion sources of polycyclic aromatic hydrocarbons (PAHs) in surface sediments of the İkizcetepeler Dam Lake, Balıkesir, Turkey, based on the measured PAH concentrations of 44 samples collected at 15-16 September, 2009. The sum PAH concentrations was 0.19 ± 0.07 mg/kg. The FA and diagnostic analyses results indicated that pyrogenic and petrogenic sources are the important sources of PAHs. Further analysis showed that contributions of Factor 1 (mixed) and Factor 2 (pyrogenic) are 37,9 % and 26,9 % respectively. Factor 3 (explains 15,4 of the total variance), represents petrogenic (spills of oil products) source.

Keywords: PAH; Sediment; FA; Diagnostic Ratios

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Determination of Some Organochlorine Pesticides in Balıkesir (İkizcetepeler) Dam Lake Sediments

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Seventeen Organochlorine Pesticides (OCPs) were evaluated in 14 surface sediment samples collected from Balıkesir (İkizcetepeler) Dam Lake. After ultrasonic bath extraction of the sediment samples GC-MS was used as analytical tool. The quality control (QC) and quality assurance tests were applied by the analysis of standard reference materials (SRMs), surrogate standards and analysis replicates. Average OCP concentration was found in the range of 0.06-22.94 mg/kg. Total OCP contamination in lake is 58.00 ± 45.44 mg/kg. Although the use of organochlorine pesticides has been banned in Turkey, the results of this study show the fresh usage of these pollutants continued in the environment around İkizcetepeler Lake after the regulation. The p,p'- DDT isomer covers the 87.72% of the total DDTs which is the pesticide that the usage of it was banned 40 years ago. Comparison of organochlorine pesticides concentrations in sediment samples with other lakes in Turkey implies the higher concentration therefore higher usage of these chemicals.

Keywords: Organochlorine Pesticides (OCPs), Gas Chromatography-Mass Spectrometry (GC-MS), sediment

Speciation of Organochlorine Pesticides in Ankara, Turkey by Using GC-MS

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Pesticides are used to protect the crops from insects and other organisms in order to increase the yield of agricultural products. But, pesticides are damaging environment and human health through food chain. The Stockholm Convention on Persistent Organic Polluters was signed by the Turkish Government in 2001 in order to provide the withdrawal and reduction of the releases of some organochlorines including DDT, aldrin, endrin, dieldrin, heptachlor and hexachlorobenzene. Therefore, contamination of irrigation water, soil, and residue on the vegetables are observed in in some agricultural areas of Turkey. The aim of this study is the determination of the extent and distribution of chlorinated pesticides in water, soil and tomato samples. Therefore, the concentrations of alpha-HCH, beta HCH, gamma-HCH, Aldrin, p.p'-DDD, Endrin Aldehyde, p.p'-DDT, Methoxychlor were determined by using Solid Phase Extraction (SPE) technique for water samples, Ultrasonic Bath for soil and tomato samples. The analyses of the samples were performed with Gas Chromatography - Mass Spectrometry. To check the efficiency of the extraction methods and the stability of the instrument surrogate and internal standards were used, respectively. Risk calculations will be applied to the produced data to evaluate health risk through food chain.

Keywords: Organochlorine pesticides, GC-MS, soil, water, tomato