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Biomonitoring of atmospheric pollution (with emphasis on trace elements) — BioMAP

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FOREWORD

Some organisms accumulate atmospheric contaminants over certain periods of time and concentrate them, thus allowing reliable analytical measurements if the organisms are suitably chosen. Measurements of contaminants accumulated by such organisms (biomonitors) provide information on the integrated exposure over an extended period of time. They may also be present in remote areas, and no expensive technical equipment is involved in collecting them. Therefore, biomonitoring can be an effective tool for pollutant mapping and trend monitoring by real time and retrospective analysis.

The IAEA is making concerted efforts to promote the practical use of nuclear and related analytical techniques in studies of non-radioactive environmental pollutants that may impact human health, and one of the main emphases is on studying air contaminants.

The idea of organizing a workshop on biomonitoring atmospheric pollution arose during an IAEA Technical Co-operation Project on Monitoring of Trace Element Air Pollution, carried out at the Instituto Tecnológico e Nuclear (ITN), Portugal, with substantial technical support by the Interfaculty Reactor Institute (IRI) of the Delft University of Technology (TUDelft), Netherlands.

The International Workshop on Biomonitoring of Atmospheric Pollution (With Emphasis on Trace Elements) — BioMAP, was held in Lisbon, Portugal, from 21 to 24 September 1997. The meeting was organized in co-operation with the Instituto Tecnológico e Nuclear. The workshop was also sponsored by the Grupo EDP/Companhia Portuguesa de Produção de Electricidade, Caixa Geral de Depósitos, Dias de Sousa Lda, Fundação Luso-Americana para o Desenvolvimento, Fundação Oriente, Sociedade Portuguesa de Física-Divisão de Física Nuclear e Partículas, and the Junta Nacional de Investigação Científica e Tecnológica. This TECDOC contains the papers presented at the workshop.

It is hoped that this publication will contribute to general knowledge on the use of bio-organisms in monitoring of (trace element) atmospheric pollution.

The members of the BioMAP International Committee were H.T. Wolterbeek and P. Bode from IRI, TUDelft, B. Smodiš, IAEA, and M.C. Freitas from ITN, Sacavém.

The IAEA staff member responsible for this publication was B. Smodiš of the Division of Human Health.

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SUMMARY

BACKGROUND

Ongoing concern about atmospheric pollutants underlies the efforts to establish control programmes in many countries. In general, the policy is both source-oriented (e.g. technology-based emission management), and effect-oriented (e.g. risk assessment). In most countries, various regulatory instruments are combined into a co-ordinated control programme. In practice, controlling (anthropogenic) air pollutants is a very complex problem: sources and emissions have to be identified, analytical methods have to be evaluated, risks have to be assessed, critical emissions have to be controlled, and economical aspects have to be integrated.

The necessary information on air pollutants can be obtained by (1) modelling of their atmospheric dispersion and deposition, based on *a-priori* known emission sources (source orientation), or (2) by measurements of actual atmospheric occurrences and/or deposition of the pollutants (receptor-orientation). In many countries, the dispersion modelling approach is attracting more and more interest, also for economic reasons: the receptor-oriented measurements require expensive equipment and manpower, and are generally associated with high costs. The receptor measurements, however, should be regarded as necessary and indispensable: they may be used to validate dispersion models, and the data obtained may indicate the presence of sources and/or emissions which were not known or registered. In order to ensure that temporal and spatial in-field measurements are representative, sampling is required on a long term basis, and at a large number of sites.

Biomonitoring, in a general sense, may be defined as the use of bio-organisms/materials to obtain information on certain characteristics of the biosphere. The relevant information in biomonitoring (e.g. using plants or animals) is commonly deduced from either changes in the behaviour of the monitor organism (species composition and/or richness, ecological performance, morphology) or from the concentrations of specific substances in the monitor tissues. For monitoring purposes, appropriate organisms should be selected, and also commonly and permanently available in the field (i.e. at the sampling sites chosen). Organisms may be selected on the basis of their accumulative and time-integrative behaviour towards the atmospheric compounds of interest. Providing these prerequisites, the general advantage of the biomonitoring approach is the ease of sampling and no requirement for the use of complicated and expensive technical equipment.

Long term sampling and measurements of air particulate matter and deposition carried out at a large number of sites using technical equipment have been few, mainly due to the high costs and the lack of sufficiently sensitive and inexpensive techniques permitting simultaneous measurements of many air contaminants at a large number of stations. Providing that certain criteria are fulfilled, biomonitoring can be an efficient supplement or even replacement for such investigations.

In carrying out biomonitoring surveys, apart from these obvious advantages, a number of difficulties are encountered. Many of them are related to general survey questions, which are not specifically associated with the use of organisms. They include the definition of monitoring goals (and associated experimental design of the survey), appropriate selection and the use of analytical techniques, as well as interpretation of the vast amount of data obtained. Questions are also to be answered on how to select an appropriate biomonitor, and on how the chosen organism reflects the atmospheric and polluting compounds of interest. As much as possible should be known about the "dose-response" relationships and about the possible interferences.

In order to address many of the above scientific problems, the International Workshop on Biomonitoring of Atmospheric Pollution (BioMAP), was held in Lisbon, Portugal, 21–24 September, 1997.

THE WORKSHOP

The workshop was about both general survey questions and problems and advantages specifically related to biomonitors. The workshop was focused on biomonitoring of *trace element* air pollution, thereby emphasising not only the difficulties related to surveys, organisms and tools for data interpretation, but also underlining the necessary attention for adequate and reliable (multi)elemental analytical techniques.

Biomonitoring practices in many countries were presented, providing guidance to newcomers in this area on how to properly design a biomonitoring network, which kind of organisms to use, and what information may be obtained from the surveys. Several approaches for data interpretation have been presented, from relatively simple graphic techniques to more sophisticated mathematical models. The papers presented re-emphasised the importance of using biomonitors for estimating atmospheric trace element pollution. Although there is still much information to be gathered, especially on the quantitative relationships between pollutant occurrences in atmosphere and biomonitor and the physiological responses of organisms to elevated pollution, it is clear that biomonitoring of trace elements has come to stay for the foreseeable future. It is hardly possible by any other approach to obtain such a detailed picture of variations in time and space within reasonable limits of costs.

The majority of the presented surveys was multi-elemental, the materials to be analysed were mostly solids, where non-destructive techniques such as neutron activation analysis (NAA) and X ray fluorescence spectrometry (XRF) are advantageous over other analytical techniques where sample destruction need to precede the measurement. These survey aspects and the presented analytical approaches (data for more than half of the contributions were obtained by nuclear and related analytical techniques) strongly underline the importance of nuclear and related analytical techniques in present-day and future biomonitor studies, in regular surveys as well as in the calibration of reference materials.

The workshop gave an overview of current applications of biomonitoring techniques. Biomonitoring is used throughout the world, both in urban and rural areas, and both in base-line approaches and in strongly source-oriented applications. Bio-materials used most are mosses, lichens and tree bark. The selected materials were discussed as advantageous in terms of occurrence, (trace) element levels, ease of handling, and dependence of atmospheric inputs. Biomonitoring was presented as being used in a variety of studies: effect-studies on (coal fired) power plants, base-line studies over larger geographical areas, up to an Europe-wide focusing (NORDIC), effect-studies on specific plants (e.g. metal smelters), studies into the salinity variability in sea-side countries, studies on specific (urban) areas (e.g. cities), chronology-studies (tree wood annual growth rings), and general time-trend analyses.

The workshop also comprised a large number of contributions focusing on biomonitoring as a technique: in these contributions the approach as such was discussed, and underlying assumptions were critically investigated. On the one hand, studies are focused on the physiology of the metal sorption processes, on retention, release and on tolerance and toxicity phenomena. Here, the basic underlying reasoning is that detailed knowledge is necessary before any extensive use of the organisms in metal deposition monitoring. On the other hand, quantification studies are carried out, in which, on a merely phenomenological basis, quantitative relationships are demonstrated between atmospheric availability parameters (e.g. aerosols, deposition) and the metal concentrations in (parts of) the monitoring organism. In addition, studies are focused on response modeling of availability, thereby making use of simplified modeling of monitor behavior, which still fully covers atmospheric parameters.

The general consensus may be that both approaches should be pursued: the latter one to further demonstrate, substantiate and determine the practical applicability of the method, and the first one to generally increase knowledge, thus allowing for future more sophisticated use of the monitor organisms.

In a number of contributions, specific biomonitoring approaches or specific aspects were presented. One of them concerned the vertical profiling of radionuclides in mosses; the results were interpreted in terms of moss growth and chronology. Another one was focused on dendroanalysis, which is the use of annual growth rings in tree wood to perform chronological recording of trace element pollution. Main (element specific) problems in these approaches were the basically assumed stability of element distribution patterns. Another point of attention was the (local) effects of soil dusts on elemental concentrations in the monitor sample mass: implicit assumption is that organisms reflect atmospheric input. Both problems could not be put to an end: they should receive further attention in future work.

Attention was given to the interpretation of biomonitoring data. Contributions ranged from the quantification of data on the basis of simultaneously recorded atmospheric availability parameters, to the statistical handling of the numerous data coming from larger-scaled surveys. Computer handling comprised both mapping and source-profiling, the latter carried out by graphic methods and by dedicated factor-analytical (e.g. MCTTFA) techniques. The robustness of techniques should be the topic of future research, to fill in the need for adequate and sophisticated, but fully reliable interpretation of data.

A large number of the studies presented were based on multi-elemental approaches. The reasoning is that multi-elemental monitoring set-ups were generally recognized as being most effective. This conclusion may be drawn because: (1) the analysis of a large number of elements may generally increase the modes for interpretation, mat permit a more reliable recognition of source fingerprints, and may show effects which are not a-priori anticipated, and (2) the resources needed for the field-work will not or hardly depend on the number of elements of interest. This indicates that the selection of a large number of elements principally emerges from many survey's goals: clear and unequivocal interpretation of data on specific elements may largely depend on the simultaneous presence of data on various other elements.

- The role of nuclear analytical techniques

As illustrated by the nature of the majority of the presentations in the workshop, the materials analyzed in biomonitoring surveys are mostly solids. Furthermore, data were predominantly obtained by nuclear and related analytical techniques. As such, the Workshop underlined that NAA, XRF and other *non-destructive* multi-elemental techniques are principally well-suited for the routine analysis of the solid samples encountered in the biomonitoring research: it is not necessary to bring the sample into solution, with all the associated problems ranging from incomplete digestion (elemental losses) to impurities in the applied chemicals (elemental contamination) and hardly any mass-related problems (homogeneity) are encountered (viz. particle induced X ray emission, PIXE). Apart from NAA/XRF, a large number of elemental analyses are carried out by inductively coupled plasma - mass spectrometry (ICP-MS), based on aspects of sensitivity, and on the fact that certain relevant elements (e.g. Pb) can not be assessed easily/routinely by NAA/XRF.

- The need for appropriate reference materials

A multitude of (1) analytical techniques (*e.g.* NAA/XRF/PIXE/atomic absorption spectrometry (AAS)/ICP-MS), (2) sample materials (various moss/lichen/tree species), and (3) laboratories engaged in larger-scaled surveys (up to 30 in the European-scaled moss NORDIC survey) presented, signaled the need for continuous efforts to produce new types of reference materials (RMs). Although results for some RMs were presented, efforts to establish new reference materials should be continued to bridge nondestructive and destructive techniques, and to permit the further substantiation of quantitative PIXE in the framework of biomonitoring research.

- The main conclusions
    - Biomonitoring of atmospheric trace elements will be continued for the foreseeable future;
    - Biomonitoring identifies atmospheric variations in trace elements occurrence in time and in space, with adequate level of quality and within reasonable limits of costs;
    - Much progress has been made in the quantification aspects of the approach, thereby also including behavior modeling and source-profiling;
    - Efforts on quantification should be pursued (including research into physiological processes); and attention should also remain focused on the production of reference materials, combined with further aspects of compatibility and comparability of the diverse analytical approaches.

IMPROVING THE USE OF LICHENS AS BIOMONITORS OF ATMOSPHERIC METAL POLLUTION

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Abstract

Improving the use of lichens as biomonitors of atmospheric metal pollution is the main objective of this work. For that a better insight into the availability and location of heavy metals in lichens subjected to polluted conditions both under laboratory and field conditions was attained. The data in this work supported the hypothesis that the absence of the lichen *Ramalina fastigiata* near a copper mine site, located in the south of Portugal, was related with toxic levels of Cu-dust near the centre of the mine. Lichen biodiversity, measured by the parameter *number of lichen species*, reflected the impact of the copper-mine dust emissions. The extent of the impact was more widespread towards east, which appears to be correlated with the frequency of the winds. The chemical analysis of *in-situ* samples of *R. fastigiata* collected at different distances and in different directions from the mine showed that Cu, K and Mg were emitted from the centre of the mine site, confirming that major sources of atmospheric dust were located there. Total inhibition of PSII (photosystem II) photochemical reactions occurred in *R. fastigiata* both under field and controlled conditions, when intracellular Cu concentrations exceeded a threshold of *ca.* 2.0 μmol g^{-1}. No samples of this species were found under field conditions beyond the Cu threshold, suggesting that the absence of the lichen *R. fastigiata* might be used as an indicator of intracellular Cu concentrations above 2.0 μmol g^{-1} in the surroundings of the copper-mine. The fluorescence parameter F_v/F_m proved to be a good estimator of the survival capacity of *R. fastigiata* under field conditions and thus a useful parameter in determining the sensitivity of the lichens (photobiont) to Cu pollution. The intracellular location of Cu, K and Mg allowed to explain the physiological changes and the survival of the species in the surroundings of the copper-mine.

1. INTRODUCTION

Research on the effects of dust pollution on plant communities has never received the same degree of attention as that of phytotoxic pollutants such as SO_2, NO_2 and O_3. However, most of the plant communities are affected by dust deposition, so that community structure is altered [1]. Dust pollution may affect photosynthesis, respiration, transpiration and allows the penetration of phytotoxic gaseous pollutants. It is, therefore, important to assess the impact of dust deposition upon vegetation. Epiphytic lichens and *Sphagnum* communities are the most sensitive of the studied vegetation groups [1]. The main processes that regularly cause dust pollution are those concerned with mineral extraction.

Epiphytic lichens have been traditionally used as bioindicators of atmospheric pollution because they show differential sensibilities to air pollution, *i.e.*, the most sensitive lichens tend to disappear from polluted areas whereas the most tolerant species can be seen in areas with moderate pollution emissions [2, 3].

Despite the large number of publications dealing with the interaction of lichens with heavy metals [4], a detailed knowledge on their physiology related to the form and cellular location of metals is needed before lichens can be extensively used for monitoring metal deposition patterns [5]. However, most studies concerning this matter relate to total metal content [6, 7], irrespective of its availability to the lichen. It is important to know the physico-chemical form of the metal in lichens because that influences its mobility and toxicity. It is well established that lichens acquire elements by interception of either soluble or particulate material although soluble ions are expected to have the major impact on lichens on a short-term basis [5, 8, 9, 10]. The relative contribution of these accumulation mechanisms to the total lichen element burden has not been sufficiently evaluated in the field situation [8]. Only [11] reported that lichen total metal content shows no seasonal pattern, whereas data on leachable elements showed seasonal variations with pollution.

Soluble metals in lichens may be located extracellularly or intracellularly [9]. The extracellular metals are more related to environmental sources whereas those located within the cell are more related to the effects on the physiological processes [12]. In biomonitoring studies, it is important to quantify the amount of extracellular soluble metals in lichens since it is physico-chemically related to environmental sources [12]. To locate the metals in lichens, a sequential elution technique has been used in routine studies [13, 14, 15, 16]. Most of these studies deal with uptake of artificial solutions in the laboratory and only a few studies have been performed under field conditions [17].

Quantification of the amount of metals associated with extracellular exchange sites on the cell wall of plants, fungi, bryophytes and lichens has been established through cation exchange techniques [18, 19, 20, 21]. In lichens, the absence of a cuticle allows the location and quantification of extracellular and intracellular metals, by using a sequential elution technique [14, 16, 22]. The cations bound to the wall can be displaced efficiently by other cations with a strong affinity for those binding sites [13, 23] or by chelating agents [14]. What is subsequently released corresponds to the amount of intracellular elements retained by the cell membrane [13].

Work on the physiological effects of metals has often emphasized the inhibition of photosynthesis and membrane damage both in lichens [24, 26, 29] and higher plants [18, 27]. Ion loss from the cell interior of lichens, particularly K, has been widely used as an indicator of the state of membrane integrity [28]. Chlorophyll fluorescence is becoming a valuable, non-destructive procedure with which to measure changes associated with photosystem II (PSII) of lichens due to gaseous pollutants [29, 30] and heavy metals [15, 16]. The chlorophyll fluorescence parameter ratio of variable fluorescence to maximal fluorescence (F_v/F_m), has been widely used to assess the state of the photosynthetic apparatus and to reflect the efficiency of the primary photochemical reactions in PSII in higher plants [31, 32] and in lichens [33, 34, 35].

Improving the use of lichens as biomonitors of atmospheric metal pollution is the main objective of this work. For that a better insight into the availability and location of heavy metals in lichens subjected to polluted conditions both under laboratory and field conditions is attained.

2. MATERIALS AND METHODS

2.1. Site characterization

This study was conducted in the mine site of the Neves-Corvo underground copper-mine. This is the largest operating mine in Portugal, probably the most significant base metal mine in Western Europe, and is one of the world's top 15 Cu producers. This mine is located in the Alentejo region. The mine produces Cu by processing mostly massive sulphide ore in two on-site concentrators.

The underground primary crushers reduce the Cu-ore to particle sizes of less than 250 mm. After being crushed, the ore is transported by a conveyor belt to a silo storage system prior to hoisting. Following secondary crushes (through cone crushers) the Cu-ore attains particle sizes less than 19 mm and is discharged to an open air raw ore stockpile. The raw ore is then transported to ore concentrators where it goes through several processes of size reduction (milling), concentration (flotation, hydroclassification, etc.) and drying before the final concentrate is discharged in a concentrate stockpile. The Cu-concentrate is characterised by a size distribution with 80% of the particles under 20 μm of size. In this mine, the tailings from the concentrators are pumped through a sealed pipeline to a tailings pound, 3 km away from the mine site. Tailings are discharged under water to avoid oxidation of the pyrite.

The Cu-concentrate stockpiles and the waste heaps, which are affected by climatic long dry periods, create potential conditions for the dispersion of windblown dust from the operation site.

Thus, the main source of dust at the mine are fugitive dust emissions from the stockpiles and waste heaps. The Cu-concentrate occurs mainly as chalcopyrite, $CuFeS_2$, with high Cu (24%), Fe (31%) and S (35%) levels. The waste heaps are composed of barren rock, which have Fe but not significant amounts of Cu. Since the mine is located in a rural area far away from any other industry or major city, there are no other local significant sources of air pollution. The average of the maximum SO_2 emissions measured at the mine site on a daily basis is 15 $\mu g\ m^{-3}$ for 1993 and 19 $\mu g\ m^{-3}$ for 1994, whereas the maximum limit both for Portugal and EU is 100-150 $\mu g\ m^{-3}$. The values reported for the mine region are lower than SO_2 values reported for other rural areas in south of Portugal [36].

The climate is semi-arid warm (annual total rainfall of 400-500 mm and air temperatures range between 4 and 32ºC). The topography of the region is mainly smooth (altitude range 190-250 m) and consequently altitude will not be considered in this study as an important factor. The landscape is dominated by low density *Quercus ilex* woodlands. From July 93 to July 95 the wind speed and wind direction was obtained at the nearest national meteorological station Beja, 45 km NE from the mine.

2.2. Biodiversity sampling

The impact of the mine on the lichen biodiversity was evaluated by studying the variation of the number of epiphytic lichen species with distance from the centre of the mine. The 20 sampling points were distributed along two transects on the north-south and east-west directions, *ca.* 2 km away from the centre of the mine. At each sampling point the number of epiphytic lichen species was recorded from 6-10 *Quercus ilex* mature trees. The sampling campaign occurred in 1993. The sampling within each tree occurred for all directions of trunks and branches. The sampling campaign occurred in 1993. The frequency of *R. fastigiata* was calculated by divinding the number of sampling sites where we had observed this species by the total number of sampling sites for each different class of distance.

2.3. Analysis of *in-situ* lichens around a copper-mine

2.3.1. Lichen sampling

Samples of the *in-situ* lichen *Ramalina fastigiata* (Pers.) Ach., of similar size (5-7 cm), were collected in October 1994 from tree trunks and branches of *Quercus ilex* facing a copper mine site at a height greater than 1.5 m. The spatial sampling occurred along two transects on the north-south and east-west directions, comprising 20 sampling points distributed at varying distances up to *ca.* 5 km from the centre of the mine.

2.3.2. Sequential elution analysis

Before the sequential elution, samples were stored in a high relative humidity atmosphere for 24 h (over water in a closed box), to reactivate the physiological activity and reduce membrane permeability [28].

Soluble extracellular Cu, either bound or unbound to the extracellular surfaces of the lichen, were obtained by washing lichen samples with Na_2-EDTA at pH 4.5 as displacing agent [14, 15, 16]. Lichen samples (30-80 mg) were shaken for 40 min in 10 ml of 20 mM Na_2-EDTA followed by a second washing with 20 mM Na_2-EDTA solution (5 ml) for 30 min. All lichen samples, after this extraction, were dried overnight at 80ºC and weighed. The oven drying process was shown to rupture cell membrane without alterations in the distribution of the elements [21]. Then, the *soluble intracellular fraction* was obtained by shaking the samples in 10 ml of 20 mM Na_2-EDTA, for 2 h [21]. *The particulate fraction* was then obtained by digestion of the samples in 3 ml of 65% HNO_3 on a hot plate at *ca.* 120ºC. The digestion was completed after all organic material had disappeared.

Extracellular K and Mg concentrations were extracted based on the sequential elution developed by [13].

Each fraction was analysed by atomic absorption spectrophotometry (Varian Techtron AA6, UK) using an air/acetylene flame and with added CsCl and $LaCl_3$ (1g l^{-1}) to both samples and standards, as suppressants of ionization and refractory compound formation, respectively. Plant metal concentrations were expressed on a total dry weight basis. The blanks were all below the detection limit. All the material in contact with the lichens during laboratory processing were acid washed to avoid metal contamination.

2.3.3. Fluorescence measurements

A PAM 101 Chlorophyll Fluorometer (Walz, Effeltrich, Germany) was used to measure chlorophyll fluorescence in different thalli of the lichen *R. fastigiata*. During storage, the samples were kept at room temperature (*ca.* 15ºC) in a closed box at high relative humidity. Lichen samples were dark-adapted for 5 min before the measurements of fluorescence to maximize oxidation of the primary quinone electron acceptor of PSII. After this period, the minimum fluorescence level with open PSII reaction centres (F_o) was measured by a weak red measuring beam, followed by a saturation light pulse to determine the maximum fluorescence (F_m) level with closed PSII reaction centres. Variable fluorescence, F_v, is the difference between F_m and F_0, and it was calculated in order to obtain the parameter F_v/F_m.

2.3.4. Data treatment and statistical analysis

The variation in concentration of elements with distance from an emission source was represented by the function: $Y=b+(a/d)$, where Y is the concentration of the element, b is the control value or background value, a is the slope and d the distance from the source. This equation is preferred to the exponential function because non-zero backgrounds exist [37]. Significant fittings were considered whenever significant correlation coefficients (Pearson *r*), for $P<0.05$, were found between the data fitted to the respective function and the observed values. Correlation coefficients between variables were calculated through Pearson *r* ($P<0.05$), and one-way ANOVAs (for $P<0.05$) were performed to test differences between means.

2.4. Effect of Cu concentration on *Ramalina fastigiata* under controlled conditions

Samples of the fruticose epiphytic lichen *Ramalina fastigiata* (Pers.) Ach. were collected in February 1994, from branches of *Olea europaea* L. located in an unpolluted site at Serra de Grândola, SW Portugal. The presence of the *Lobarion* community in this area showed that this site was pollution-free, since this community is very sensitive to general air pollution [38]. All lichen samples were air dried in the laboratory for no more than 7 days.

Samples of *R. fastigiata* (200-350 mg each) were incubated and agitated for 2 h in 1 l of different Cu concentrations (deionized water, 0.0157, 0.079, 0.157, 0.393, 0.786, 1.573, 3.934, 7.868 and 15.737 mM), to reach concentrations of *ca.* 400 μmol g^{-1} [39]. Samples were then separately rinsed with 2 l deionized water and divided into five replicates before subsequent sequential elution extraction procedures.

The cation location and analysis followed the procedure described in section 2.2.2. However intracellular cations (Cu, K and Mg) were then extracted by shaking the samples in 3-5 ml of 1 M HNO_3 for 2 h at *ca.* 15ºC [13].

Chlorophyll fluorescence in each lichen species was measured 24 h after incubation in different Cu concentrations, following the procedures described in section 2.2.3. Effects on photosynthesis are observed more clearly 24 h after incubation with potentially toxic elements [15, 16].

To test whether the relationship between the intra- or extracellular Cu concentration in lichens with the Cu concentrations supplied or incubation time is described by saturation kinetics, the data were fitted to a rectangular hyperbola [40]. Correlation coefficients between variables were calculated by Pearsons *r*.

3. RESULTS AND DISCUSSION

3.1. Lichen biodiversity around a copper-mine

The total number of epiphytic lichens species, increased with increasing distances from the centre of the mine (Fig. 1). At the different sampling points the total number of epiphytic lichen species varied between 5 and 35. The impact on the lichen biodiversity was clearer around the centre of the mine and it was more extended towards east (Fig. 1).

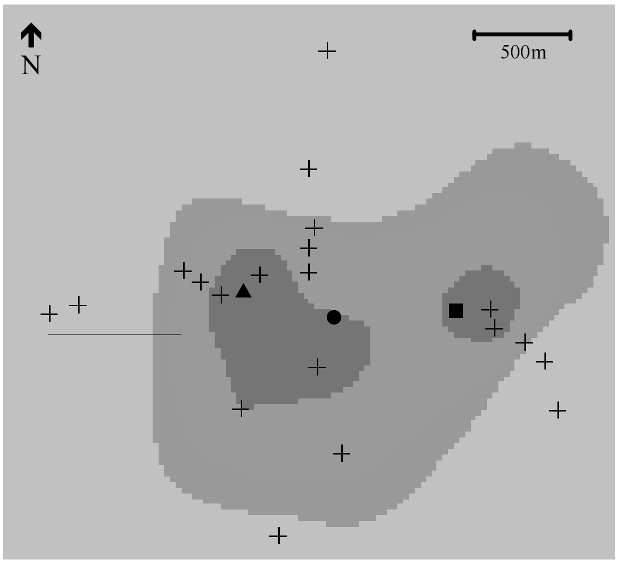

Fig. 1. Estimated spatial distribution of the total number of epiphytic lichens in the surroundings of a copper-mine obtained by kriging of data based on 20 sampling points (+). The centre of the mine is represented by ●, the Cu-concentrate stockpile by ▲, and the waste heaps by ■. The studied area was divided into classes with different numbers of lichen species: ■<10, ▨ 10-18 and □ >18 species.

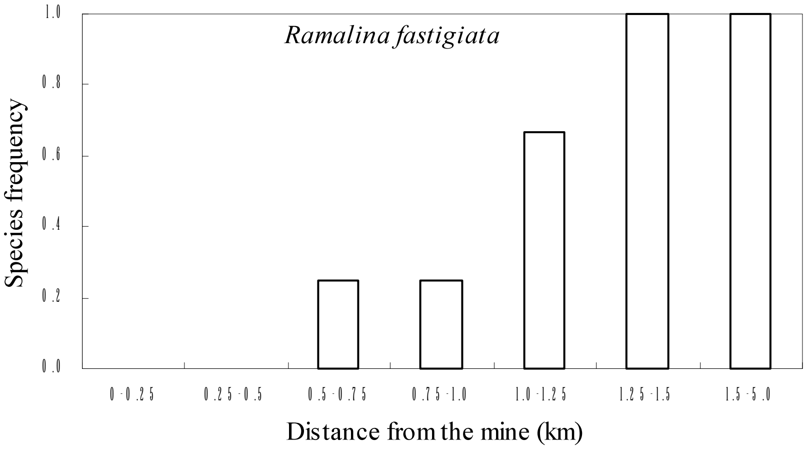

Fig. 2. Frequency of Ramalina fastigiata for different classes of distances from the centre of the mine.

The frequency of the fruticose lichen *Ramalina fastigiata* was calculated for classes of different distances from the mine (Fig. 2). The lichen *R. fastigiata* was observed at distances greater than 500 m (Fig. 2).

One of the most usual approaches in biomonitoring with lichens is by means of indices of atmospheric purity (IAP). Most of these indices require, for their calculation, a toxitolerance factor for each species, which is determined on a rather subjective basis [3]. Furthermore, there are evidences that the tolerance of a given species to air pollution may differ according to the general climatic conditions [3]. Thus, even in the rare cases where a toxitolerance factor for calculating IAP index is based on experimental data, its extrapolation to another area, with different climatic conditions, may not be accurate and it is not recommended [3]. In this work, the number of lichen species was shown to be a reliable and objective measure of impact on lichen flora, since it varied widely (5-35) within short distances from the pollution source (Fig. 1). Lichen biodiversity, measured by the parameter *number of lichen species*, reflects the impact of the copper-mine dust emissions (Fig. 1). The distribution of epiphytic lichen species has been studied in the area of a copper-smelter in Canada and around a Zn-smelter in the USA [41,42]. Rather than affected by metal pollution, it is suggested from these studies that the lichen distribution was greatly influenced by SO_2 emissions. Few studies reported the effects of metal pollution on lichen flora without gaseous emissions [43].

The biodiversity pattern can be influenced by distance from the source and wind direction [3]. The main source of pollution was focused on the centre of the mine (Fig. 1). At the sampling points observed, the maximum number of epiphytic lichen species found (35) was within the range of those found by other authors in the south of Portugal for unpolluted sites [44].

The estimated spatial distribution of the number of epiphytic lichen species around the mine site allows areas with different levels of impact on lichen flora to be distinguished (Fig. 1). The extent of the impact was more widespread towards east, which appears to be correlated with the frequency of the winds (Fig. 3) and thus with a greater dispersion of particles in that direction. No relationship with the wind speed was found due to the lack of variation in this factor between the different directions (Fig. 3). Lichens are slow-growing organisms, hence, they may be used as long-term integrators of environmental conditions. This lichen biodiversity study allowed an easy identification of the zones of the mine which reflected the long-term emissions (Fig. 1).

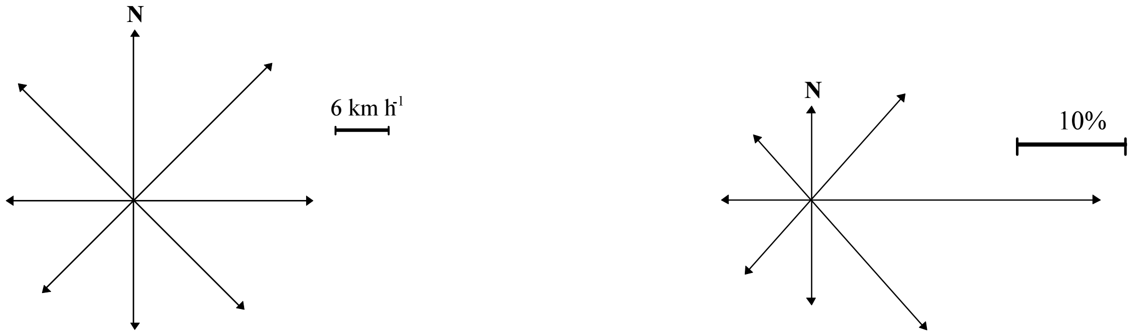

Fig. 3. Wind speed (left) and wind frequency (right) referred to 1951-1980 at the nearest meteorological station Beja (ca. 45 km NE from the mine).

This biodiversity study suggested that pollutants emission from the centre of the mine might be important in terms of the lichen survival at short distances around the mine site. It was then hypothesised that the absence of the lichen *R. fastigiata* near the mine site was related with toxic levels of Cu-dust near the centre of the mine.

3.2. Effects of Cu pollution on lichen physiology

3.2.1. Source of elements in lichens around a copper-mine

The Cu concentration in different fractions, soluble extracellular, soluble intracellular and particulate with increasing distances from the mine site also significantly fitted the function *Y=b+(a/d))* (Fig. 4). At the sampling points nearest to the centre of the mine, Cu was in greater proportion in the particulate fraction, followed by the extracellular and the intracellular fraction (Fig. 4). However the predicted background intracellular soluble Cu concentrations in *R. fastigiata* (0.20 µmol g^{-1}) were greater, when compared to the either extracellular or particulate, that showed a trend to zero values (Fig. 4). Approximately 2.0 µmol g^{-1} was the highest intracellular Cu concentration found in samples of *in-situ R. fastigiata* (Fig. 4B). The highest Cu concentrations in the three fractions could be found within the firsts 2 km radius from the centre of the mine site (Fig. 4).

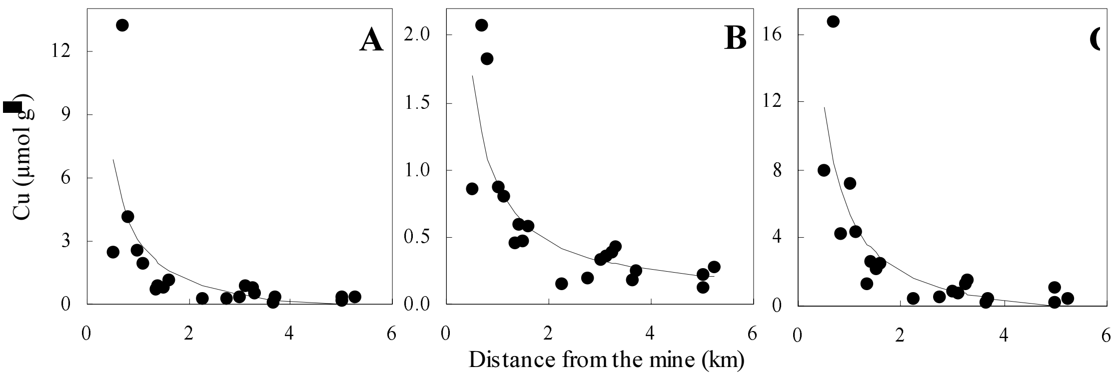

Fig. 4. Mean concentration of Cu in extracellular (A), intracellular (B) and particulate (C) fractions in the lichen Ramalina fastigiata collected at different distances from the centre of the mine. Lines represent the function Y=b+(a/d), fitted to the observed values. Significant (P<0.05; n=20) correlation coefficients (r) were found for Cu concentration in extracellular (r=0.65), intracellular (r=0.78) and particulate fractions (r=0.81). Symbols are the means of five replicates.

Far away from the centre of the mine (> 2 km), the low total Cu background concentrations appear to be related mostly with the physiological Cu requirement, since the intracellular soluble Cu fraction (Fig. 4B) had the greatest background concentration at remote sites as compared to extracellular or particulate fractions (Figs. 4A and 4C).

In order to establish whether K and Mg were being emitted from the mine site, their concentrations in extracellular, intracellular and particulate fractions were plotted against distance from the mine and were fitted to the function *Y=b+(a/d)* (Fig. 5). Extracellular K and Mg concentrations and Mg concentration in the particulate fraction showed a significant fitting with the previous function (Figs. 5A, 5D and 5F). No significant fittings were found between the concentrations of K and Mg in intracellular fraction and K in the particulate fraction (Figs. 5B, 5C and 5E).

Despite observing that the extracellular K and Mg concentrations decrease with increasing distances from the mine, intracellular K and Mg were not influenced by the distance to the mine site (Figs. 5B and 5E), suggesting that their concentrations were controlled under certain limits due to their physiological role.

The chemical analysis of *in-situ* samples of *R. fastigiata* collected at different distances and in different directions from the mine showed that Cu, K and Mg were emitted from the centre of the mine site, confirming that major sources of atmospheric dust were located there (Figs. 4 and 5);

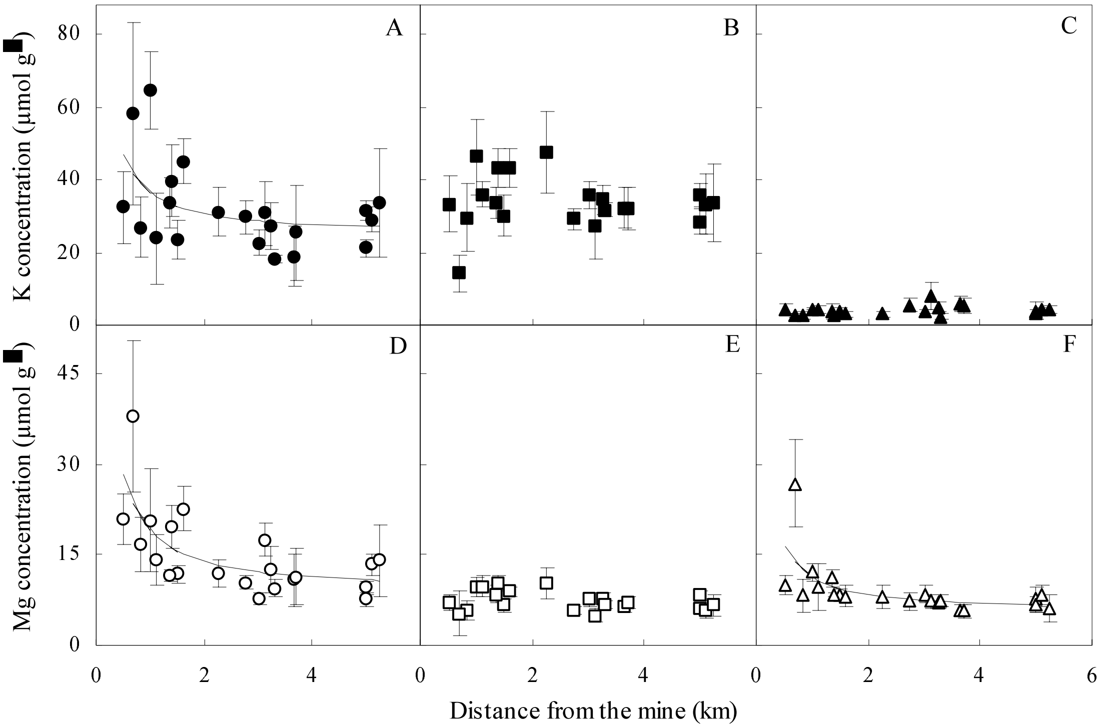

Fig. 5. Mean concentrations of K (A, B, C; closed symbols) and Mg (D, E, F; open symbols) in extracellular (A, D; circles), intracellular (B, E; squares) and particulate (C, F; triangles) fractions in the lichen Ramalina fastigiata collected at different distances from the centre of the mine. Lines represent the function Y=b+(a/d), fitted to the observed values. Significant (P<0.05; n=21) correlation coefficients were found for extracellular K (r=0.46), extracellular Mg (r=0.69) and particulate Mg (r=0.61). Symbols are the means and bars the standard deviations of five replicates.

lichens intercepted Cu and Mg in soluble and particulate forms, whereas K was only intercepted in the soluble form (Figs. 4 and 5).

3.2.2. Impact on lichen physiology under field and controlled conditions

To investigate possible alterations in cellular membrane permeability due to Cu dust, intracellular K and Mg concentrations were correlated with intracellular Cu concentrations in lichens (Table I). Increasing concentrations of intracellular Cu in samples of *in-situ R. fastigiata* were significantly correlated with the decrease in intracellular K (Table I). The increase in intracellular Cu concentration was correlated with K-loss from the cell but not with Mg-loss (Table I). This result was in total agreement with a controlled experiment performed for the same species (Table II). The effects of Cu on membrane integrity were tested by measuring the loss of cations from the cell with increasing supplied Cu concentrations [15,16]. The intracellular K concentrations in *R. fastigiata* were significantly (*P*<0.05) correlated with increasing supplied Cu concentrations (Table II). No significant (*P*>0.05) linear correlation between intracellular Mg concentration and intracellular Cu concentration in *R. fastigiata* was observed (Table II). Thus, the high intracellular Cu concentration found in lichens near the mine site might be responsible for the K-loss.

Although many investigators have used only K loss as a measure of membrane integrity [24, 25], it is important to note that we found no losses of intracellular Mg. Some proportion of this element is readily available within the cell, and is lost when generalised membrane damage [28]. Therefore, we suggest that Cu induces specific K loss in lichens rather than a non-selective membrane damage [22].

TABLE I. CORRELATION COEFFICIENT (PEARSON *r*) OF INTRACELLULAR COPPER CONCENTRATION WITH INTRACELLULAR POTASSIUM AND MAGNESIUM CONCENTRATIONS IN THE LICHEN *Ramalina fastigiata* COLLECTED AT DIFFERENT DISTANCES FROM THE MINE SITE

	Correlation coefficient (Pearson *r*)	
Fraction	Cu-K	Cu-Mg
Intracellular	-0.40*	-0.24

n=21, *$P<0.01$

TABLE II. CORRELATION COEFFICIENT (PEARSON *r*) OF INTRACELLULAR COPPER CONCENTRATIONS WITH CHANGES IN THE DISTRIBUTION OF INTRACELLULAR POTASSIUM AND MAGNESIUM CONCENTRATIONS IN *Ramalina fastigiata* AFTER INCUBATION IN DIFFERENT COPPER CONCENTRATIONS FOR 2 HOURS

	Correlation coefficient (Pearson *r*)	
Fraction	Cu-K	Cu-Mg
Intracellular	-0.82*	-0.47

[#] Cation measurements were performed after incubation for 2 h in solutions of different Cu concentrations. Significant linear correlations are marked * $P < 0.05$, n=9.

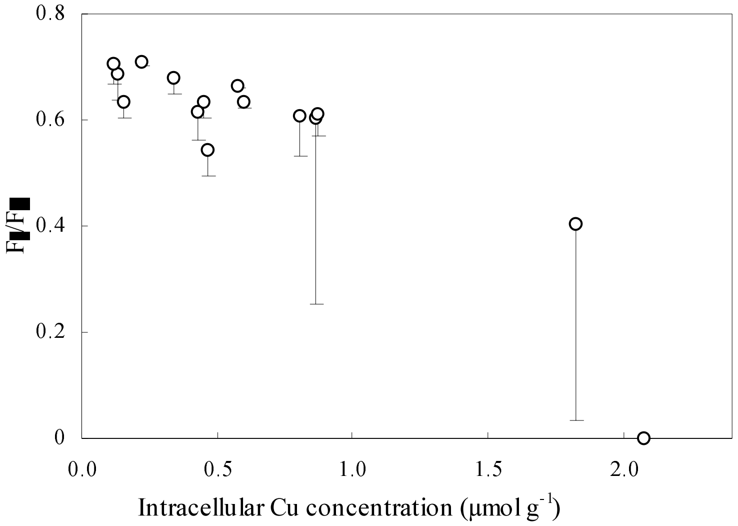

Fig. 6 - Variation of chlorophyll fluorescence parameter (F_v/F_m) with intracellular Cu concentration in the lichen Ramalina fastigiata collected at different distances from the mine ($r=-0.88$; $P<0.05$; $n=14$). Symbols represent the mean and bars the standard deviations of five replicates.

The effect of the Cu dust on the lichen PSII photochemical reactions was investigated (Fig. 6). There was a significant correlation between the decrease in (F_v/F_m) and the increase in intracellular Cu concentrations under field conditions (Fig. 6). The zero value of F_v/F_m corresponded to intracellular Cu concentrations beyond 2.0 μmol g^{-1} (Fig. 6). The observed decrease in F_v/F_m might be a consequence of excessive intracellular Cu concentrations in the lichens near the mine site (Fig. 6). For intracellular Cu concentrations above *ca.* 2.0 μmol g^{-1} the lichen *R. fastigiata* showed a complete inhibition of the photochemical reactions at PSII level (Fig. 6). These results were in total agreement with controlled Cu uptake experiments performed in *R. fastigiata* (Fig. 7). *Ramalina fastigiata*

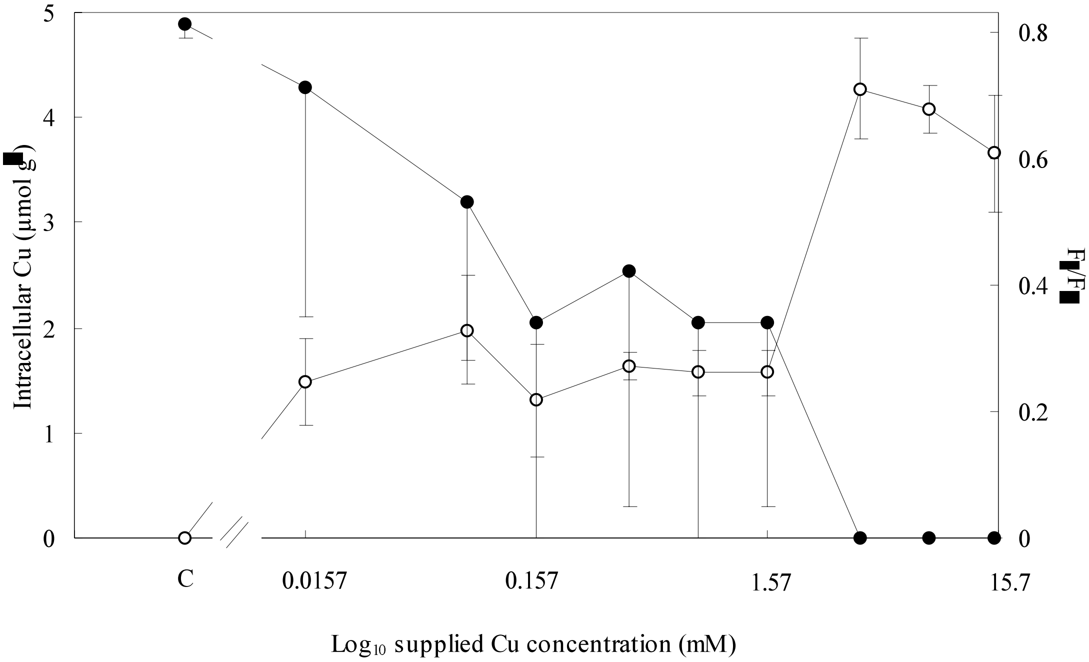

Fig. 7 - (●) - Response of chlorophyll fluorescence (ratio F_v/F_m) in the lichens Ramalina fastigiata after incubation for 2 h in different Cu concentrations. Symbols are the means and bars the standard deviations of three replicates. (O) - Response on incubation for 2 h in different Cu concentrations of intracellular mean concentrations of Cu in Ramalina fastigiata. Symbols are means and bars are the standard deviations of five replicates. The control sample (C) was incubated in deionized water for 2 h.

samples submitted to Cu addition under controlled conditions, showed F_v/F_m values equal to zero for supplied Cu concentrations above 1.6 mM (with intracellular Cu concentrations between 3.7-4.3 µmol g^{-1}) (Fig. 7). A highly significant ($P<0.01$) linear correlation (correlation coefficient, $r = -0.90$, $n = 10$) was found between the F_v/F_m values and the intracellular Cu concentration in *R. fastigiata*.

Note that F_v/F_m values showed an increase in variability with increasing intracellular Cu concentrations (Fig. 6). A similar result was also observed with the Cu uptake by *R. fastigiata* in the laboratory (Fig. 7). These results might be related with an heterogeneous response of the lichen thallus to Cu uptake and deserve future research.

The data in this work supported the hypothesis that the absence of the lichen *R. fastigiata* near the mine site was related with toxic levels of Cu-dust near the centre of the mine. Total inhibition of PSII photochemical reactions occurred in *R. fastigiata* both under field and controlled conditions, when intracellular Cu concentrations exceeded a threshold of *ca.* 2.0 µmol g^{-1} (Figs 6 and 7). No samples of this species were found under field conditions beyond the Cu threshold (*ca.* 2.0 µmol g^{-1}), suggesting that the absence of the lichen *R. fastigiata* might be used as an indicator of intracellular Cu concentrations above 2.0 µmol g^{-1} in the surroundings of the copper-mine. The fluorescence parameter F_v/F_m proved to be a good estimator of the survival capacity of *R. fastigiata* under field conditions and thus a useful parameter in determining the sensitivity of the lichens (photobiont) to Cu pollution. The results also showed that variation in independent physiological responses to Cu (losses of intracellular K and chlorophyll fluorescence) were concomitant with changes in intracellular Cu concentration for both species. Although the proportion of intracellular Cu uptake was much smaller (6%) than extracellular [16], the intracellular Cu concentration appeared to be a relevant factor in explaining F_v/F_m changes due to Cu uptake (Fig. 7). The intracellular location of Cu allowed to explain the physiological changes and the survival of the species in the surroundings of the copper-mine.

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LICHENS AS ENVIRONMENTAL BIOMONITORS IN ISRAEL: TWO DECADES OF RESEARCH

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Abstract

The present paper describes a series of studies dealing with lichens as environmental biomonitors in Israel in the years 1974-1997. In the first years of research we focused on a tolerant epilithic crustose species, *Caloplaca aurantia* in an attempt to classify locations according to their level of contamination by airborne heavy metals accumulated in the lichen. Later on our studies centered around the epiphytic fruticose lichen *Ramalina duriaei* which is rather sensitive to air pollution. This lichen has the capability to absorb and accumulate remarkable amounts of airborne mineral elements but undergoes physiological deterioration by their impact. The presence of airborne pollutants leads to a degradation of cell membranes and chlorophyll, a decrease of ATP content and an increase of stress-ethylene formation. Suggested by the compilation of data available upon two decades of research, we proposed the application of an element enrichment factor (EEF) to distinguish between polluted and unpolluted sites.

Lichens are known to be sensitive to various kinds of pollution and excel as biological monitors of air-quality. Lichens absorb and accumulate high concentrations of airborne mineral elements including heavy metals and sulphur [1, 2]. Innumerable investigations and surveys led to a classifi-cation of lichens into three categories with regard to their reaction to air pollution: (1) sensitive species, damaged and annihilated by air pollution; (2) species succeeding annihilated lichen communities; (3) tolerant species, *i.e.* belonging to the native community and resistant to air pollution.

We have found that the epilithic crustose lichen *Caloplaca aurantia* (Pers.) Hellb. var. *aurantia,* which is rather common in Israel, is a good representative of the third group. A comparison of the concentrations of Mn, Zn, Fe, Pb, Ni, Cu, Cr and Cd in lichens growing on roof-tiles indicated high levels in urban and suburban sites over and above rural sites [3]. Lichen species like *C. aurantia*, growing both in clean and in metal-contaminated areas, were established as comparative monitors especially suitable for an assessment of changes in metal concentrations. In addition, we demonstrated the extracellular deposition of particulate metallic fall-out accumulated in *C. aurantia* with the aid of scanning and transmission electron microscopy and an application of an energy dispersive X-ray analysis. We were able to prove that the bulk of the accumulated elements is concentrated in the medulla [4].

The epiphytic fruticose (shrub-like) lichen *Ramalina duriaei* (De Not.) Bagl. is abundant in Israel. Since 1974 it is utilized as a tool for an estimation of air-quality in various parts of the country. We focused our attention on analyses of the mineral element content of this lichen growing on twigs of carob trees (*Ceratonia siliqua* L.) in the HaZorea Forest (Ramot Menashe, 1 km south of the HaZorea Kibbutz, Esdraelon Valley, NE Israel). In addition to a determination of the elemental content of *in situ* thalli collected in HaZorea, we relocated lichen thalli with their substrate (detached carob twigs) in different sites to monitor the contamination produced by power plants, oil refineries, car traffic, agricultural activity and a steel smelter. We examined different physiological parameters: degradation of chlorophyll, concentration of ATP, integrity of cell membranes, production of ethylene and leakage of essential ions (*i.e.* K). A comparative analysis of physiological parameters and the corresponding concentrations of heavy metals and S after a 8-12 month period of transplantation yielded significant correlations.

The first series of studies applying *R. duriaei* as a biomonitor was carried out around a coal-fired electricity generating station under construction [5, 6]. A second series was performed prior to its operation [7] and a third at the beginning of its operation [8-11].

In the years 1978-1985 we performed assessments of air-quality in the environment of Tel Aviv utilizing *R. duriaei* transplants. The transplants contained high concentrations of heavy metals like Pb, Ni, Cr, Zn and Cu, especially following exposure at busy roadsides and intersections [5, 7-12]. In the absence of instrumental measurements, our findings, providing evidence on the presence of contaminating particles in the air, were an exclusive source of information at that time.

As lead is still in use as an additive to gasoline, we studied the localization of this toxic heavy metal in *R. duriaei* [13]. Specimens of the lichen were exposed to $Pb(NO_3)_2$ solutions and a buffered solution (tartaric acid/sodium bitartrate) of sodium rhodizonate was used to locate Pb in their thalli. Cross sections of vegetative parts of the thallus and the apothecia revealed that Pb penetrated into the cortical cells of the thallus but not into the algal cells of the photobiont nor into the ascopores or medullary cells. The observed massive penetration of Pb into cortical cells supports the notion that *R. duriaei* is sensitive to atmospheric Pb pollution.

Measurements of the chlorophyll integrity in algal cells (*Trebouxia* sp.) of *R. duriaei* allowed us to observe an inverse correlation between the OD435 nm/OD415 nm ratio in lichen transplants and the concentration of Br, Pb, Fe and Ti [7] and between this ratio and the concentration of Pb, Zn and Cu [12].

Combined methods were applied to measure the impact of air pollution on the integrity of cell membranes and chlorophyll in transplanted *R. duriaei* in industrial sites in the Haifa Bay. Temporal and spatial alterations of physiological parameters were analyzed in comparison with a determination of the presence of Mg on/in cortical cells, by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX) [14]. This study revealed symptoms of injury to cell membranes in *R. duriaei* long before any indication of damage was apparent in the photobiont chlorophyll. It appears that the presence of Mg is indicative of a significant leakage from inter-cellular sites of the thallus whereas the accumulation of S on/in cortical cells of *R. duriaei* indicates contamination by SO_2.

In a pilot study transplanted *R. duriaei* from HaZorea were found to produce large amounts of ethylene in a polluted site at a busy road intersection near Tel Aviv, relative to control thalli left in the HaZorea Forest where no industry is founded [15]. In polluted industrial sites in the Haifa Bay, *R. duriaei* produced greater amounts of ethylene than control specimens in HaZorea [16].

Recently, we focused our attention on the region of Ashdod, an industrial town in the south-western part of the country. We wished to determine whether transplanted thalli of *R. duriaei* from HaZorea would provide information on the air-quality in this area [17]. For this purpose the concentrations of Pb, Cu, Cd, Ni, Mn, Fe, S, Ca, Mg, Na and K were determined in *in situ* thalli collected in the HaZorea forest in March 1993 and in *in situ* and transplanted thalli retrieved in June 1993. The concentration of these elements was analyzed in comparison with physiological parameters: integrity of cell membranes and chlorophyll content. Transplanted thalli in industrial sites in the town of Ashdod for a period of 100 d accumulated high concentrations of Pb, Cd, Ni, Fe, S, Mg, Na, Ca and K. The concentration of S correlated with damage caused to cell membranes and was in inverse correlation with the chlorophyll content. The electric-conductivity values, corresponding to membrane integrity in the lichen thallus, showed an inverse correlation with the ratio of chlorophyll *a* to phaeophytin *a*, thus indicating the degradation of the photobiontic chlorophyll. Magnesium accumulated in lichen thalli in dusty sites was found to correlate positively with damage caused to membranes.

In February 1994 we transplanted thalli of *Ramalina duriaei* from HaZorea to the Ashdod region (Table I) to be retrieved in November 1994. The concentrations of 11 elements in these thalli were determined. Thus we were able to assess the capability of *R. duriaei* to accumulate airborne elements upon a long-term exposure. We divided the mean concentration value for each of the elements detected in the transplanted thalli in nine biomonitoring sites, by the corresponding value for resuspended thalli in the control site in HaZorea.

Table II presents the element enrichment factors (EEF). The following is suggested by our findings:

(1) The high EEF for *R. duriaei* which represent its noticeable capacity to accumulate V, Ni, Pb and Cu whereas the absolute concentrations of these metals were not very high.

(2) The low EEF for K and P which should be attributed to their tendency to leach out from transplanted thalli in polluted sites as a result of damaged cell membranes.

(3) The EEF for sites in or near industrial areas in the Ashdod region (especially sites 1-5 and 9) suggest the existence of a high rate of pollution. Two of these sites (5 and 9), rural by definition, are actually contaminated by main road traffic, by a power station and by various industries at some distance. The EEF obtained for transplants from three other rural sites (6 ,7 and 8) indicate an air quality which is rather good.

TABLE I. DESCRIPTION OF THE BIOMONITORING SITES (FEBRUARY-NOVEMBER 1994)

Site No.	Site name	Description	Distance from Eshkol Power Plant, Ashdod (km)	Distance from the oil refineries, Ashdod (km)
1	Ashdod, Nature Reserve (center)	Near an industrial area, northern Ashdod (urban-industrial)	3.8	2.0
2	Ashdod, Nature Reserve (east)	About 200 m west of the Tel Aviv-Ashdod highway, northern Ashdod (suburban)	4.2	2.2
3	Between a chemical plant and the main motorway entering Ashdod	About 50 m north of the main motorway entering the town and 50 m south of the chemical plant (urban-industrial)	2.5	2.2
4	Ashdod, 1.4 km east of the Eshkol Power Plant	Near a dusty road among sand dunes (urban-industrial)	1.4	1.2
5	Kevutzat Yavneh Kibbutz	Close to the local cemetery (rural)	7.1	5.2
6	Sand dunes, south of Ashdod	Out of the town (suburban)	7.0	6.9
7	Nitzanim Sand Dunes	(rural)	12.2	13.5
8	Hafetz Hayyim Kibbutz	Two km west of the kibbutz (rural)	14.3	12.6
9	Nir Galim Village	West part of the village, near the interior road (rural)	3.2	2.5
10	HaZorea Forest	Carob tree plantation, 1 km south of the kibbutz (rural)	100 km from the city of Ashdod	

TABLE II. ELEMENT ENRICHMENT FACTORS (EEF): RATIO OF MEAN CONCENTRATIONS OF ELEMENTS DETECTED IN TRANSPLANTED *RAMALINA DURIAEI* IN THE ASHDOD REGION AND THE MEAN CONCENTRATIONS OF THE SAME ELEMENTS IN RESUSPENDED THALLI IN HAZOREA (SITE 10). Mean = mean value of the ratio for element (horizontal) and for site (vertical).

Element	1/10	2/10	3/10	4/10	5/10	6/10	7/10	8/10	9/10	Mean (horizontal) EEF for element
S	1.32	1.18	1.63	1.20	1.15	1.17	1.11	1.09	1.31	1.23
V	2.71	2.00	2.85	2.42	1.57	1.28	1.00	1.28	3.71	2.09
Ni	1.30	1.87	3.72	2.87	2.33	1.36	0.63	1.21	3.54	2.09
K	1.17	0.96	0.46	0.63	0.87	0.93	0.93	0.90	1.09	0.88
Pb	4.10	2.40	14.90	2.80	1.80	1.40	1.50	2.00	2.50	3.71
P	1.08	0.87	0.70	0.95	0.92	0.97	0.84	0.78	1.25	0.92
Cu	2.36	1.51	2.26	1.23	1.25	0.98	1.01	1.00	1.78	1.48
Cr	1.44	0.93	1.45	0.86	0.95	0.91	0.90	1.03	1.21	1.07
Fe	1.12	0.81	1.28	0.83	0.94	1.05	0.91	1.19	1.02	1.01
Mn	1.10	0.86	1.33	1.03	1.13	1.00	1.00	1.13	1.13	1.07
Mg	1.07	0.83	1.20	0.90	1.10	1.31	1.08	1.02	1.22	1.08
Mean (vertical) EEF for sites. K and P are excluded	1.83	1.37	3.40	1.57	1.35	1.16	1.01	1.21	1.93	

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EPIPHYTIC LICHENS AS QUANTITATIVE BIOMONITORS FOR ATMOSPHERIC ELEMENT DEPOSITION

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Abstract

Epiphytic lichens are being used as passive and active biomonitors of trace elements in Slovenia. The lichen *Hypogymnia physodes* (L.) Nyl. was exposed at three locations (two in the vicinity of a coal fired-power plant, and one at a reference location) for 8 months. At the same locations air particulate matter and total deposition were collected on a monthly basis. The k_0-method of neutron activation analysis, using the TRIGA Mark II reactor at the "Jozef Stefan" Institute, was employed for multielemental nondestructive analysis of all samples. The influence of the power plant on the concentration levels of some elements in the transplanted lichens, air particulates and total deposition is discussed and their correlation presented.

1. INTRODUCTION

Growing awareness of the consequences of air pollution on the environment has led to an increased interest in epiphytic lichens, which are regarded as suitable tools for monitoring levels of atmospheric pollution [1-3]. Unlike higher plants, they have neither roots, a waxy cuticle nor stomata, hence for mineral nutrition they are largely dependent on wet or dry deposition from the atmosphere. The concentration levels in lichens are usually higher than levels in precipitation or in air filters, sampling is easier and no expensive equipment is needed. Although some literature exists about quantitative relationships between elemental levels in biomonitors and those measured in atmospheric deposition [4-7], these relationships are not yet known in detail. The aim of the present study was to obtain insight into the response of lichens exposed in the vicinity of a thermal power plant to trace element air pollution by comparing the levels in lichens with atmospheric levels as measured by air particulates (fine and coarse) and total deposition.

2. EXPERIMENTAL

2.1.Sampling and sample preparation

The experiment was performed from September 1992 to May 1993 at three sampling locations; 2 locations were selected within a 10 km radius around the coal fired thermal plant (TPP) at Šoštanj and one in a rural area at Podvolovljek (35 km SW of the TPP). At each sampling location three types of samples were collected, namely air particulate matter, total deposition and epiphytic lichens.

Branches of apple trees covered with *Hypogymnia physodes* were collected at a remote location, about 20 km NE of the TPP and the same day exposed to the atmosphere at each location for 3, 6 and 8 months. They were tied with nylon thread onto a wooden holder at a height of 1.5-2 m above the ground. Some lichen material was transported to the laboratory to determine the initial concentration of elements. It was planned that the exposure period would be one year; however due to visible damage to the transplanted lichens, especially at Veliki vrh which is only 2.5 km distant from the power plant, the experiment was terminated after 8 months. In the laboratory the lichen samples were moistened with distilled water and carefully separated from the bark substratum using nylon tweezers and lyophilized.

TABLE I. TRACE ELEMENT CONCENTRATIONS (µg g^{-1} DRY WEIGHT) IN TRANSPLANTED *H. PHYSODES* EXPOSED FOR 3. 6 AND 8 MONTHS (3M. 6M. 8M) AND 3. 6 AND 8 MONTH AVERAGES IN TOTAL AIR PARTICULATES (COARSE + FINE FRACTION).

LICHENS (µg/g dry weight)

El.	**Initial value**		**Podvolovljek**			**Veliki vrh**			**Zavodnje**		
	0 M	**std**	**3M**	**6M**	**8M**	**3M**	**6M**	**8M**	**3M**	**6M**	**8M**
As	0.72	0.07	0.88	1.00	1.08	1.21	2.01	1.70	1.01	1.32	1.15
Fe	835	115	1038	1207	1453	2223	2429	3531	1169	1443	1477
K	4187	1145	4452	3482	3334	3619	1840	1229	4146	3630	2081
Mo	0.51	0.27	0.56	0.58	0.66	0.99	1.42	1.55	0.71	0.50	0.90
Hg	0.05	0.01	0.06	0.07	0.09	0.08	0.16	0.14	0.06	0.08	0.09
Sb	0.20	0.02	0.29	0.23	0.31	0.29	0.46	0.58	0.22	0.27	0.30
Sc	0.25	0.04	0.30	0.35	0.42	0.39	0.54	0.67	0.35	0.42	0.42
Th	0.20	0.04	0.24	0.30	0.30	0.34	0.43	0.51	0.28	0.36	0.33
U	0.09	0.01	0.10	0.12	0.13	0.24	0.40	0.35	0.14	0.17	0.19
W	0.12	0.01	0.75	0.77	0.76	0.18	0.30	0.38	0.18	0.23	0.28
Zn	59.3	6.0	78.0	71.3	70.0	68.1	75.9	52.0	67.6	74.4	66.9
AIR PARTICULATES (**ng m^{-3}**)											
As			0.57	0.93	0.97	0.88	1.38	1.41	0.72	0.89	0.89
Fe			56.2	95.4	84.2	174.3	163.0	158.6	206.2	139.7	126.8
K			104	137	131	217	200	191	158	131	127
Mo			0.26	0.27	0.28	0.44	0.79	0.70	0.32	0.29	0.28
Hg			0.02	0.02	0.02	0.02	0.03	0.03	0.04	0.03	0.03
Sb			0.30	0.37	0.39	0.76	1.04	1.00	0.60	0.57	0.54
Sc			0.01	0.02	0.02	0.04	0.03	0.03	0.05	0.04	0.03
Th			0.01	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02
U			0.01	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.02
W			0.16	0.13	0.14	0.25	0.20	0.19	0.39	0.25	0.21
Zn			27.7	26.6	26.5	117.5	75.5	65.3	65.5	42.9	38.8

The samples were not washed, as our aim was to measure the elements that were physically trapped on the surface of the thallus as well as chemically bound to the cell wall. The samples were made brittle by immersion in liquid nitrogen and were then crushed and ground in a zirconium mortar with Zr ball in a Fritch vibration micro-pulverizer. About 100-200 mg of dry lichen powder was then used to make tablets for neutron activation analysis.

Air particulate matter (APM) was collected at each station using an in-house constructed single jet facility, with a cut-off point of ~2.5 µm [8]. The fraction ~2.5 µm to 15 µm was collected on a Nuclepore polycarbonate membrane filter, 37 mm diameter, pore size 0.45 µm. The particulate fraction finer than ~2.5 µm was collected on a Nuclepore polycarbonate membrane filter, 47 mm diameter and the same pore size. Both filters were held in the original Nuclepore holders. The arithmetic median value of the flow rate was about 400 L h^{-1}. The flow rate was measured by a rotameter at the beginning of sampling, checked every second day and finally at the end of the sampling period. Sampling times varied, depending on the minimal flow rate necessary for successful operation of the separator and the content of air particulates, being from 2 to 7 days. Filters were weighed before and after exposure and kept in petri dishes. The APM loaded filters collected during each month were pressed in a pellet die of 5 mm using a manual press, for neutron activation analysis.

Total deposition was collected monthly in a 5 L high density polyethylene sampler with a funnel of 20 cm diameter and vertical collar of 10 cm. The funnel-bottle system was mounted inside a stainless steel cylinder placed on a concrete base plate, keeping the upper edge of the funnel 1.5 m above ground level. The opening of the funnel was protected by a coarse filter mesh made of polyethylene and a nylon net (pore size of 250 µm) in order to prevent inlet of coarse particles and insects. Before each sampling

TABLE II. TOTAL EXPLAINED VARIANCE (R^2) AND ITS SIGNIFICANCE (P VALUE) USING A MULTIPLE LINEAR REGRESSION MODEL TO DESCRIBE THE RELATIONSHIP BETWEEN ELEMENT CONCENTRATIONS IN LICHENS AND 3 INDEPENDENT VARIABLES: AVERAGE TOTAL AIR PARTICULATE MATTER (NG M^{-3}), TOTAL DEPOSITION AND TOTAL AMOUNT OF PRECIPITATION DURING THE EXPOSURE PERIOD. ($P \le 0.001$ HIGHLY STATISTICALLY SIGNIFICANT ; $P \le 0.01$, $P \le 0.05$ STAT.SIGNIFICANT; $P > 0.05$ NOT STAT. SIGNIFICANT)

Element	R^2	P value
As	88.1	0.0095
- APM (ng m^{-3})		0.002
- deposition (μg m^{-2})		0.91
- precipitation (mm m^{-2})		0.21
Mo	88.7	0.0084
- APM (ng m^{-3})		0.003
- deposition (μg m^{-2})		0.529
- precipitation (mm m^{-2})		0.599
Hg	84.3	0.0187
- APM (ng m^{-3})		0.04
- deposition (μg m^{-2})		0.0036
- precipitation (mm m^{-2})		0.017
Sb	91.7	0.0039
- APM (ng m^{-3})		0.0009
- deposition (μg m^{-2})		0.465
- precipitation (mm m^{-2})		0.013
Se	74.2	0.06
- APM (ng m^{-3})		0.073
- deposition (μg m^{-2})		0.648
- precipitation (mm m^{-2})		0.881
W	94.8	0.0012
- APM (ng m^{-3})		0.809
- deposition (μg m^{-2})		0.03
- precipitation (mm m^{-2})		0.0038

period, 10 ml conc. suprapur HNO_3 was added to the bottle to preserve the precipitation collected. In the laboratory the samples were filtered through Nuclepore polycarbonate 0.45 μm membrane filters, and the water and filtrate analysed separately.

2.2. Sample analysis

The k_0-standardisation method was used for analysis of all samples. All irradiations were performed in the carousel facility of the TRIGA Mark II reactor of the Jozef Stefan Institute (IJS) at a thermal fluence rate of 1.1×10^{16} m^{-2}s^{-1}. The irradiation time for each sample was 18 hours. All samples were irradiated together with an Al-0.1 % Au alloy disc and Zr foil, serving as comparator and fluence rate monitor, respectively. After irradiation samples were transferred to clean 5 ml polypropylene mini scintillation vials for measurement. The radionuclides used in the determination of 50 elements in each sample , their half lives and gamma energies measured are given elsewhere [9]. The samples were measured on an absolutely calibrated Ortec HP Ge detectors, connected to a Canberra Series 90 multichannel analyser. Each sample was measured twice; for 1 hour after 2 days of cooling time and for 20 hours 8 days after the end of irradiation. The details of the measurement procedures and quality assessment of the method applied are given elsewhere [9-10].

3. RESULTS AND DISCUSSION

More than 40 elements were determined in each sample but only the most indicative results for those elements in transplanted lichens which showed a significant increase or decrease (twice the standard deviation of the initial value) after 3 months of exposure are presented in Table I. The results are not corrected for the initial concentration, which is presented as the mean value of three subsamples collected from the reference location, and its standard deviation, in the first column of Table I. In the second part of Table I the results for mean total air particulate matter for the exposure period of the lichens are presented. The mean values were calculated from the monthly results for coarse and fine fractions. The results for the levels of trace elements in precipitation were presented elsewhere [11].

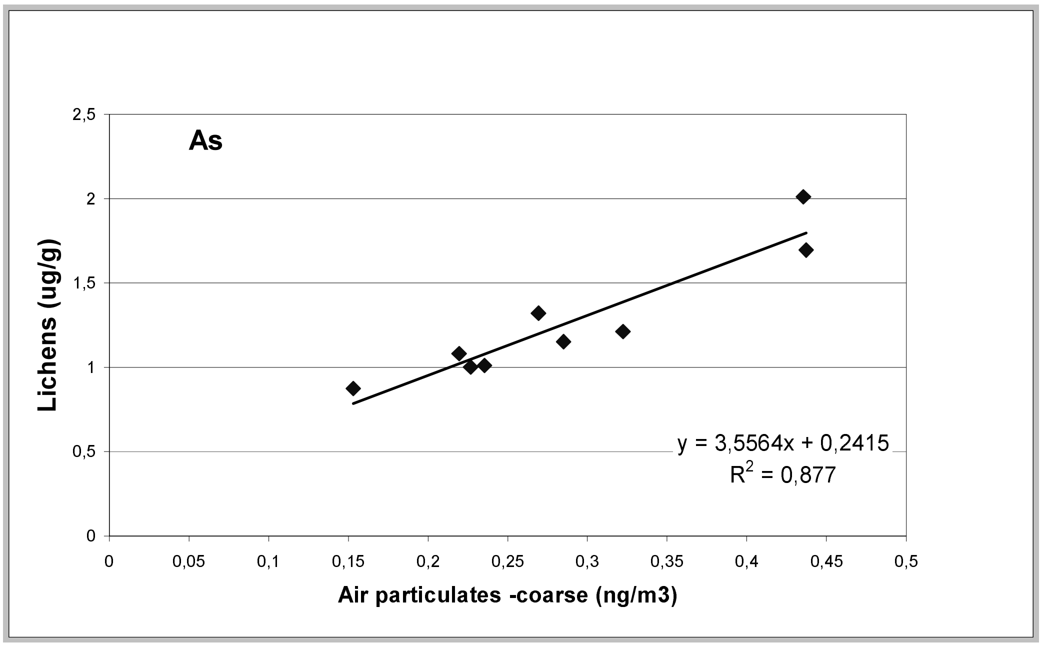
As shown in Table I, except for K, the elemental levels in lichens at all locations slightly increase with increasing exposure time. As expected the highest elemental levels were found at Veliki vrh, the sampling location only 2.5 km distant from the TPP. The elements that increase most significantly are

TABLE III. CONCENTRATIONS OF ELEMENTS (μg/g) IN BAUXITE, RED MUD AND SOIL SAMPLES

	Bauxite	**Red mud**	**soil (site 7)**
Al	134000	9010	5250
As	74.7	127	9.5
Au	<0.06	<0.04	<0.016
Br	2.4	7.2	11.1
Ca	21700	<2000	-
Ce	403	662	59.1
Cl	<50	<50	<100
Co	45.4	67.8	13.5
Cr	409	715	740
Cs	3.5	6.3	2.6
Dy	23.2	0.15	3.35
Eu	2.3	0.057	0.68
Fe	149000	258000	27700
Ga	59.0	87	8.52
Hf	17.2	88.1	5.45
K	1230	304	9560
La	134	227	25
Mg	<2000	<1400	1100
Mn	1170	59	33
Na	430	4240	5380
Nd	115.5	49.9	12.1
Rb	27.3	41.5	47
Sb	4.9	9.1	1.23
Sc	70.1	121	8.7
Se	0.31	<1.3	15.8
Sm	2.04	-	4.14
Tb	2.8	4.62	2.38
Th	57.1	104	12.3
Ti	6570	348	256
U	<4	-	<4
V	123	<18	9.2
W	7.3	12.7	1.4
Zn	370	556	161

TABLE IV. CONCENTRATIONS OF ELEMENTS (μg/g) IN THREE DIFFERENT BIOINDICATORS FROM THE SAME SITE

	Lichen	**Grass**	**Pine needles**
Al	11000	253	506
As	2.25	<1.0	<0.8
Au	<0.005	0.013	<0.003
Br	34	2.8	1.55
Ca	22600	14300	26000
Ce	11.2	<1.5	0.45
Cl	415	4720	200
Co	2.43	0.13	0.16
Cr	32	2.2	2.6
Cs	0.85	<0.4	<0.5
Dy	0.62	<0.18	<0.01
Eu	0.165	<0.09	<0.10
Fe	6200	229	410
Ga	3.9	<0.4	0.27
Hf	0.62	<0.05	0.04
K	5000	23000	3100
La	5.6	<0.7	0.25
Mg	2000	1800	920
Mn	77	28	15.6
Na	585	2100	91
Nd	4.3	<1.1	<0.75
Rb	<15	18.9	<12
Sb	0.55	<0.035	0.06
Sc	2.05	0.06	0.09
Se	<2.0	<0.5	<1
Sm	0.68	<0.08	0.04
Tb	<0.15	<0.15	<0.1
Th	1.33	0.07	0.07
Ti	530	<120	<45
U	<0.7	<0.8	<1
V	20.5	<1	0.95
W	<0.4	<0.2	<0.2
Zn	76	43	15.6



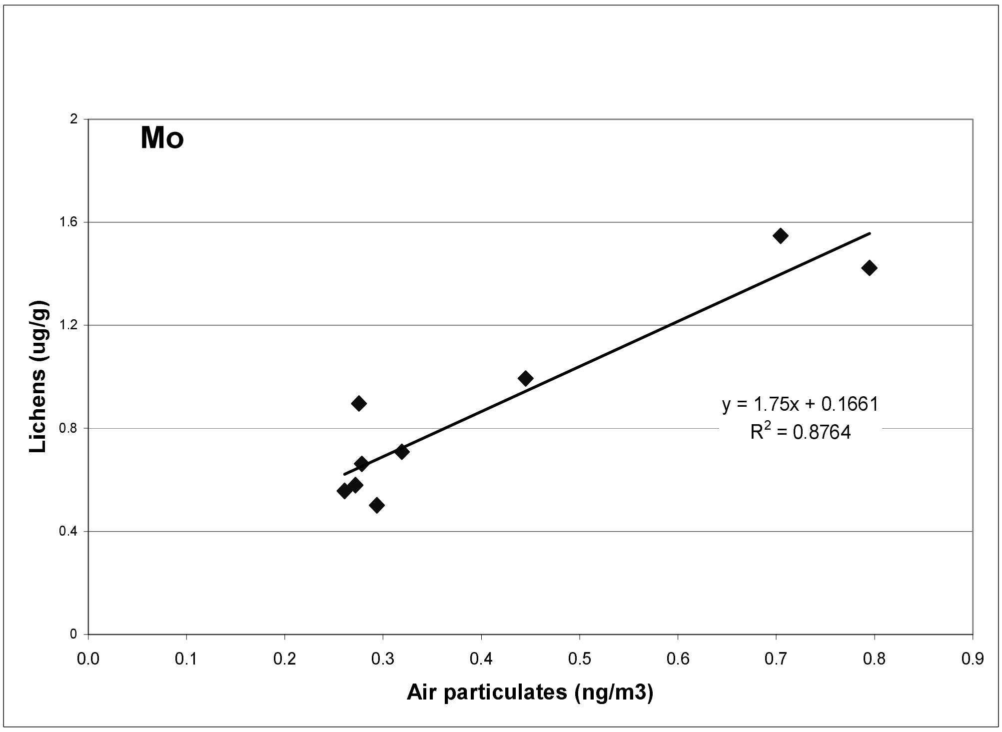


Fig.1. Plots of As and Mo concentrations in lichens exposed for 3, 6 and 8 months and mean air particulate matter (ng m^{-3} per exposure period, coarse fraction for As and total (fine + coarse) for Mo).

U, Fe, Sb, Mo, Hg and As. These elements are typical constituents of coal and are released into the atmosphere by coal combustion. However, at the reference location of Podvolovljek an extremely high value of W was obtained in all 3 exposure periods. As can be seen from the results, the concentrations of K decreased from 4187 μg g^{-1} at the reference location to 1229 μg g^{-1} after 8 months of exposure at Veliki vrh, most probably due to damage to the cell membranes and thus leaching of intracellular

K ions. In April 1993 there were elevated emissions of SO_2 from the TPP caused by full operation of all 5 units due to demand for electricity. Since the exposed lichens became completely white at the Veliki vrh sampling location but were slightly less injured at Zavodnje, it can be concluded that even for one of the most tolerant epiphytic lichens (*Hypogymnia physodes*) the levels of SO_2 in the gaseous emissions were too high.

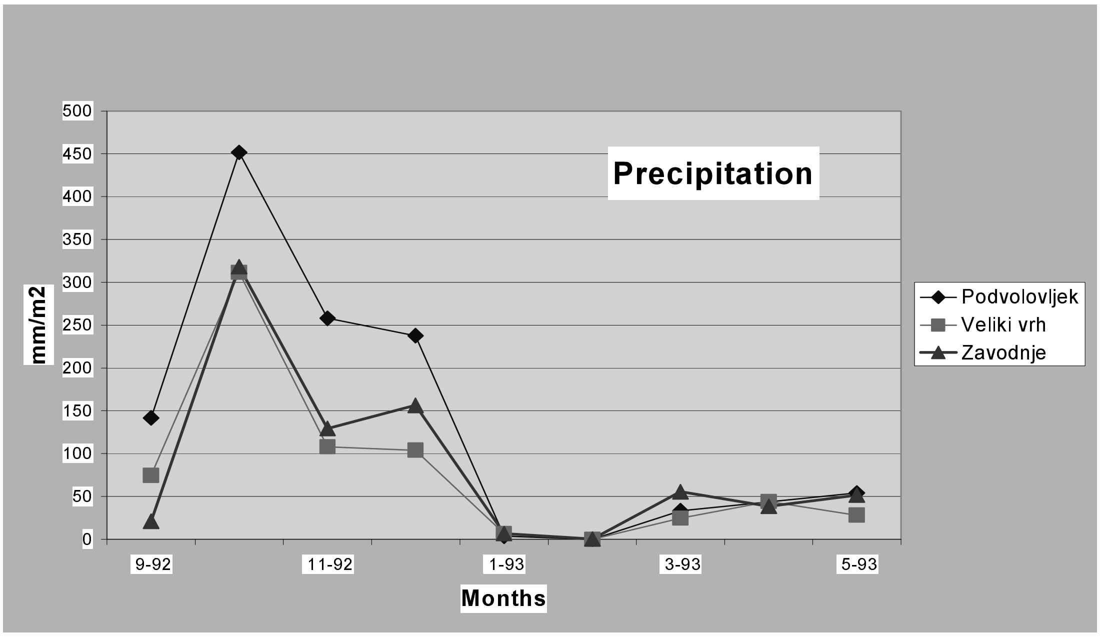


Fig.2. The amount of precipitation (mm/m^2) at the sampling stations.

Knowing that trace elements are deposited on the lichen surface either as dry particulates or as material dissolved or suspended in wet precipitation, some initial multiple linear regression analysis was performed to find out which process was the dominant one at this particular environment. Since the experiment was performed only at three locations (two of them very close to the emission source) and because of the limited data set no other statistical analysis was meaningful.

The approach in the present field study was to compare the elemental concentrations in lichens exposed for 3, 6 and 8 months with air particulate matter (mean value per exposure period), total deposition (wet and dry for the exposure period) and the total amount of precipitation. Statgraphics routines [12] were used and a summary of the results are presented inTable II. For As, Mo, Hg, Sb, Se, and W significant relationships between the variables used in the model were found (expressed as P value), and the model explained from 74 (Se) to 95 % (W) of the total variance of elemental concentration in lichens. For Ca, Ce, Cs, Fe, K, La, Na, Rb, Sc, Sm, Th, U, and Zn the total explained variance was less than 65 %. Based on the P value which was the highest for total deposition (except for Hg and W), the initial statistics showed good correlation between the concentrations in air particulates, the amount of precipitation and the concentrations in lichens; for example for Sb, Fe, Sc, and Th these relationships were highly significant ($0.01 > P > 0.0001$). However, for As and Mo it was found that a simple linear model can be applied (Fig. 1) which explained more than 82 % (depending on particle size) of the total variance in the lichen data. For example for As better correlation was obtained between lichens and the coarse fraction of APM, but in the case of Mo with total APM (fine + coarse). Our results are in a way contrary to some literature data where a good correlation between lichen values and bulk precipitation was obtained [4-7]. However, one reason for this discrepancy could be the vicinity of the emission source and entrapment of fine or coarse particles directly onto the lichen surface, and another the meteorological conditions during the experiment (Fig.2). There was heavy rain in October, but the winter period was very dry with low levels of precipitation. The first statement appears to be in agreement with Ross [6] who found no correlation between wet deposition and the levels for Mn, Cr and Ni in mosses and suggested than some other factors are more important than adsorption of wet deposition. He also concluded that atmospheric wet deposition is of primary importance only at baseline stations, not near local sources.

Our field experiment, which as explained, was performed under the extreme environmental conditions (high SO_2 emission, heavy rain in one month) showed that even in such conditions, good correlation between physical measurements and lichen values exist.

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LICHEN *PARMELIA SULCATA* TIME RESPONSE MODEL TO ENVIRONMENTAL ELEMENTAL AVAILABILITY

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Abstract

Transplants of lichen *Parmelia sulcata* collected in an area previously identified as non polluted, were placed at six stations, five of which were near Power Plants and the other in an area expected to be a remote station. Together with the lichen transplants, two total deposition collection buckets and an aerosol sampler were installed. Lichens were recollected two every month from each station. At the same time the water collection buckets were replaced by new ones. The aerosol sampler filter was replaced every week, collection being effective only for 10 minutes out of every two hours; in the remote station aerosol filters were replaced only once a month, the collection rate being kept. Each station was run for a period of one year. Both lichens and aerosol filters were analysed by PIXE and INAA at ITN. Total deposition samples were dried under an infrared lamp, and afterwards acid digested and analysed by ICP-MS at the National Geological Survey of The Netherlands. Data for the three types of samples were then produced for a total of 16 elements. In this work we used the data set thus obtained to test a model for the time response of lichen *Parmelia sulcata* to a new environment.

1. INTRODUCTION

Biomonitoring is an environmental study approach which is being applied already for more than 20 years. Nevertheless there is not yet a physical or mathematical model about the processes involved. In this work we try to present an "as simple as possible" model for the lichen elemental uptake. The model developed is tested against with data provided by an experiment where six lichen transplants stations were explored during a 12 months period. Total deposition data was elected as the type of availability data for this test although other types of data is also available.

In this work we restrict the model testing to Al, Pb and Th. A more exaustive test and application of the model to other elements and other types of availability data is planed to be pulished in a near future.

2. PROCEDURE

For this experiment 26x6 samples of lichen *Parmelia sulcata* were prepared. Lichens were collected in one site previously identified as scarcely polluted [1, 2]. The selected lichen substrate was Olive tree and all lichens were picked-up at a distance between 1 and 2 meters above the ground. Lichens still on bark substrate were then mounted in nylon net bags and kept in the laboratory while waiting to be exposed in the polluted area. For logistic reasons, experimental sites were selected nearby power plants except for one which was *a priori* assumed as a remote clean air environment site. At each site 24 lichen samples were suspended. The additional 2 lichen samples from the set were at the same time separated and prepared for analysis. Afterwards, at intervals of approximately one month, two additional lichen samples were brought to laboratory and prepared for analysis. This was done by carefully removing the lichens from any remaining Olive tree substrate used during transplantation, rinsing the lichens in 18MΩ water for about 30 seconds, for cleaning of dust, freeze drying and grinding in a Teflon balls mill cooled to LN temperature. In each site two buckets with 25 cm diameter funnels were also installed for total deposition collection which would be replaced every time a new set of lichens was recollected. Besides lichens and total deposition, also airborne particles were collected by a Gent Aerosol Sampling Unit [3].

In this work no reference will be made to the aerosol data. We will present a very simple physical model of the lichen response to environmental elemental availability in section 2, and in section 3 the total deposition data will be used for comparison to the measured results.

3. THEORETICAL MODEL

In this work our objective is to develop a physical or mathematical model that aims at reproducing the time response of lichen elemental concentrations to a new environment. We are looking for a general model so we want not to use complex atmospheric processes nor biological complex functions.

The most simple approach would be to say that the uptake (or the increase in the amount) of element *i* in the lichen, N_L^i, is proportional to its environmental availability, N_E^i:

$$\frac{dN_L^i}{dt} = \alpha N_E^i \qquad (1)$$

The solution to this equation is $N_L^i(t) = N_L^i(0) + \alpha\ N_E^i\ t$ which is not acceptable because N_L^i goes to infinity as time does.

The second most simple approach is to state that the uptake is proportional to the difference between the availability and the concentration inside the lichen:

$$\frac{dN_L^i}{dt} = \alpha\left(N_E^i - N_L^i\right) \qquad (2)$$

Better still is to consider that there is a release proportional to the lichen concentration. This provides an equation similar to eq.(2), namely:

$$\frac{dN_L^i}{dt} = \alpha\ N_E^i - \gamma\ N_L^i \qquad (3)$$

Solving this equation we obtain

$$N_l(t) = e^{-\gamma\ t}\ N_l(0) + \frac{\alpha}{\gamma}\ N_E\left(1 - e^{-\gamma\ t}\right) \qquad (4)$$

which is not much different from the solution of eq.(2). Unfortunately none of these approaches behaves properly when compared to our data. By “proper behaviour” we here mean that:

(i) an acceptable fit to the data can be obtained considering the start of the experiments as the start of the times,

(ii) once obtained the values for the parameters from the fitting procedure, it should be possible to produce, from the initial concentration of the lichen and the sequence of availability values, a time sequence of concentration values which correlates well with the lichen concentration time series measured

After several attempts we manage to define a simple structure, independent of the lichen biological processes and based on very simple physical assumptions. We will now describe the model and in the next section we present the results obtained by applying it to Al, Pb from the six stations and to several elements from one station.

Consider the scheme presented in Figure 1. We assume that the lichen has an interior, I, which does not contact the outside (exterior, E) and an interface where all exchanges take place. Under this point of view, the elements outside the lichen must be deposited at the surface, S, of the lichen, where they accumulate before being incorporated in the lichen interior. Due to the method we use for sampling preparation we assume that we only analyse the lichen interior. Although this statement might be questionable, its drawbacks reside mainly on how we define interior. In order to avoid this discussion which is not important for the validity or not of this model at the present stage, we will define interior as the total portion of lichen we are analysing after following the sample preparation method described above. The surface (which acts like a buffer for the lichen) is assumed to become empty during the 30 seconds rinsing.

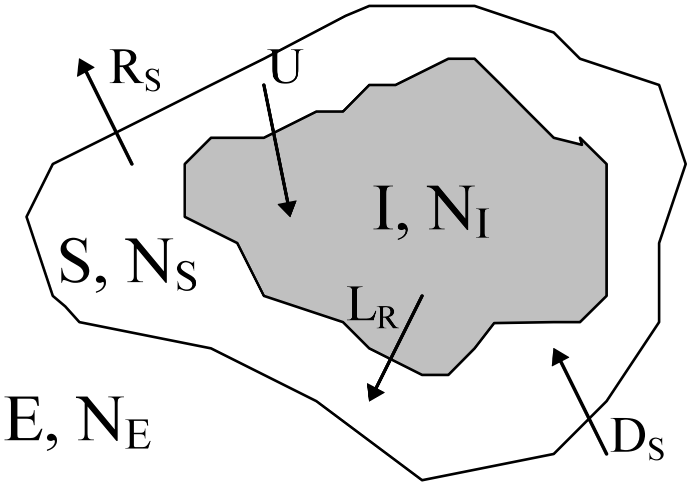


Figure 1. Scheme of the physical structure of the lichen assumed in the model. Four time rates define the model: D_s , the deposition rate, U, the uptake rate, R_s and L_r the rates at which the material is lost

In this model all the absorption processes of the element *i* are then supposed to be regulated by four time rates: D_s is the rate at which a given amount is deposited on the lichen surface, U is the rate at which the element once deposited on the lichen surface is absorbed, and R_s and L_r the rates at which the element is lost from the surface to the exterior and from the lichen interior to its surface, respectively. Because we cannot state *a priori* if these coefficients are element dependent or element independent, for simplicity we will not use the upper index *i* in the coefficients. We can then equate this model by a system of two linear differential equations:

$$\begin{cases} \dfrac{dN_S^i}{dt} = D_s N_E^i - U N_S^i - R_s N_S^i + L_r N_I^i \\ \dfrac{dN_I^i}{dt} = U N_S^i - L_r N_I^i \end{cases} \tag{5}$$

In order that we can speak of proportionality in a straight forward way, the element amount terms above must represent absolute values and not concentrations. N_E, N_S and N_I must then be total amounts of element (number of atoms or total mass) in the exterior, in or at the surface and in the interior of (or inside) the lichen. But this raises another problem because we have no direct access to the total amounts. We can rewrite the system of equations considering now a surface concentration S_S and a bulk concentration C_L defined as:

$$N_S^i = A_S\,\Sigma_S^i \qquad ; \qquad N_I^i = M_L\,C_L^i \tag{6}$$

where A_S and M_L are respectively the lichen surface and the lichen mass. We then obtain a different system:

$$\begin{cases} \dfrac{d\Sigma_S^i}{dt} = \dfrac{D_S}{A_S} N_E^i - U\Sigma_S^i - R_S\Sigma_S^i + L_r \dfrac{M_L}{A_S} C_L^i \\ \dfrac{dC_L^i}{dt} = U\Sigma_S^i - \left(L_r + \dfrac{d\ln\left(M_L\right)}{dt} \right) C_L^i \end{cases} \tag{7}$$

The term in $d\ln(M_L)/dt$ appears because the lichen mass also varies in time and unlike the lichen surface, it does change whenever the lichen composition changes. However the mass change is very small, thus we can at first assume M_L as constant and let this term vanish.

The above system was solved by applying Laplace transforms. During the inverse transform process we use the following approximation, (let $g(\tau)$ be any function with existing convolution) :

$$\int_0^t g(\tau)\cdot N_E(t-\tau)\,d\tau = N_E(\xi)\int_0^t g(\tau)\,d\tau \cong \left\langle N_E \right\rangle \int_0^t g(\tau)\,d\tau \tag{8}$$

we then obtain the solutions:

$$\begin{cases} \Sigma_S(t) = D_s \cdot \dfrac{1-e^{-R_s t}}{R_s\ A_s} \left\langle N_E \right\rangle + e^{-\frac{A_1}{2}t}\left(Ch_S + Sh_S\right) + \left(1 - R_s\right) e^{-R_s t} \left[\dfrac{M_L C_L(0)}{A_s} + \Sigma_s(0) \right] \\ C_L(t) = \dfrac{UD_s}{L_r R_s} \cdot \dfrac{\left\langle N_E \right\rangle}{M_L} + \dfrac{2\,Sh_C\ e^{-\frac{A_1}{2}t}}{B_1} + e^{-\frac{A_1}{2}t} \left(C_L(0) - \dfrac{UD_s}{L_r R_s} \cdot \dfrac{\left\langle N_E \right\rangle}{M_L} \right) \left[Cosh\left(\dfrac{B1}{2} t \right) + \dfrac{A_1}{B_1} Sinh\left(\dfrac{B1}{2} t \right) \right] \end{cases}$$

(9)

where :

$$A_1 = U + R_s + L_r$$

$$B_1 = \sqrt{A_1{}^2 - 4 L_r R_s}$$

$$Sh_C = Sinh\left(\frac{B_1\,t}{2} \right) \left[\frac{U\,A_s}{M_L} \Sigma_S^i(0) - L_r C_L^i(0) \right]$$

$$Ch_S = Cosh\left(\frac{B_1\, t}{2}\right)\left[R_s \Sigma_S^i(0) - \frac{D_s \langle N_E \rangle}{A_s} - (1 - R_s) \cdot \frac{M_L\, C_L^i(0)}{A_s}\right]$$

$$Sh_S = Sinh\left(\frac{B_1\, t}{2}\right)\left\{\left[\frac{(1 - R_s) \cdot A_1}{B_1} + 2\frac{L_r R_s}{B_1}\right] \cdot \frac{M_L\, C_L^i(0)}{A_s} - \left(R_s \Sigma_S^i(0) + \frac{D_s \langle N_E \rangle}{A_s}\right) \cdot \frac{A_1 - 2L_r}{B_1}\right\}$$

$C_L^i(0)$ and $\Sigma_S^i(0)$ are the initial bulk and surface concentrations of the lichen.

When time goes to infinity, the above expressions converge to:

$$\begin{cases} \Sigma_S^i(\infty) = \dfrac{D_s}{R_s\, A_s} \cdot \langle N_E \rangle \\ C_S^i(\infty) = \dfrac{U\, D_s}{L_r\, R_s\, M_L} \cdot \langle N_E \rangle \end{cases} \tag{10}$$

These expressions are independent of the initial values. They also show that the lichen concentration is proportional to the mean environmental availability, as long as the coefficients involved are constant. This is a very important result. It implies that if the model is proved to reproduce the lichen response, it becomes automatically true that the lichens, when transplanted from one place to another, evolve from one equilibrium condition to another, each of them being independent of the past history of the lichen.

A simple relation between $C_L^i(0)$ and $\Sigma_S^i(0)$ is also established because the initial values of lichen concentrations can be assumed to correspond to lichens in equilibrium after an infinite time in the same environment. Under this hypothesis we can look for the values of the parameters by fitting a simpler expression to the data, namely:

$$\boxed{C_L(t) = \frac{UD_s}{L_r R_s} \cdot \frac{\langle N_E \rangle}{M_L} + e^{-\frac{A_1}{2}t}\left(C_L(0) - \frac{UD_s}{L_r R_s} \cdot \frac{\langle N_E \rangle}{M_L}\right) \cdot \left[Cosh\left(\frac{B1}{2}t\right) + \frac{A_1}{B_1} Sinh\left(\frac{B1}{2}t\right)\right]}$$

(11)

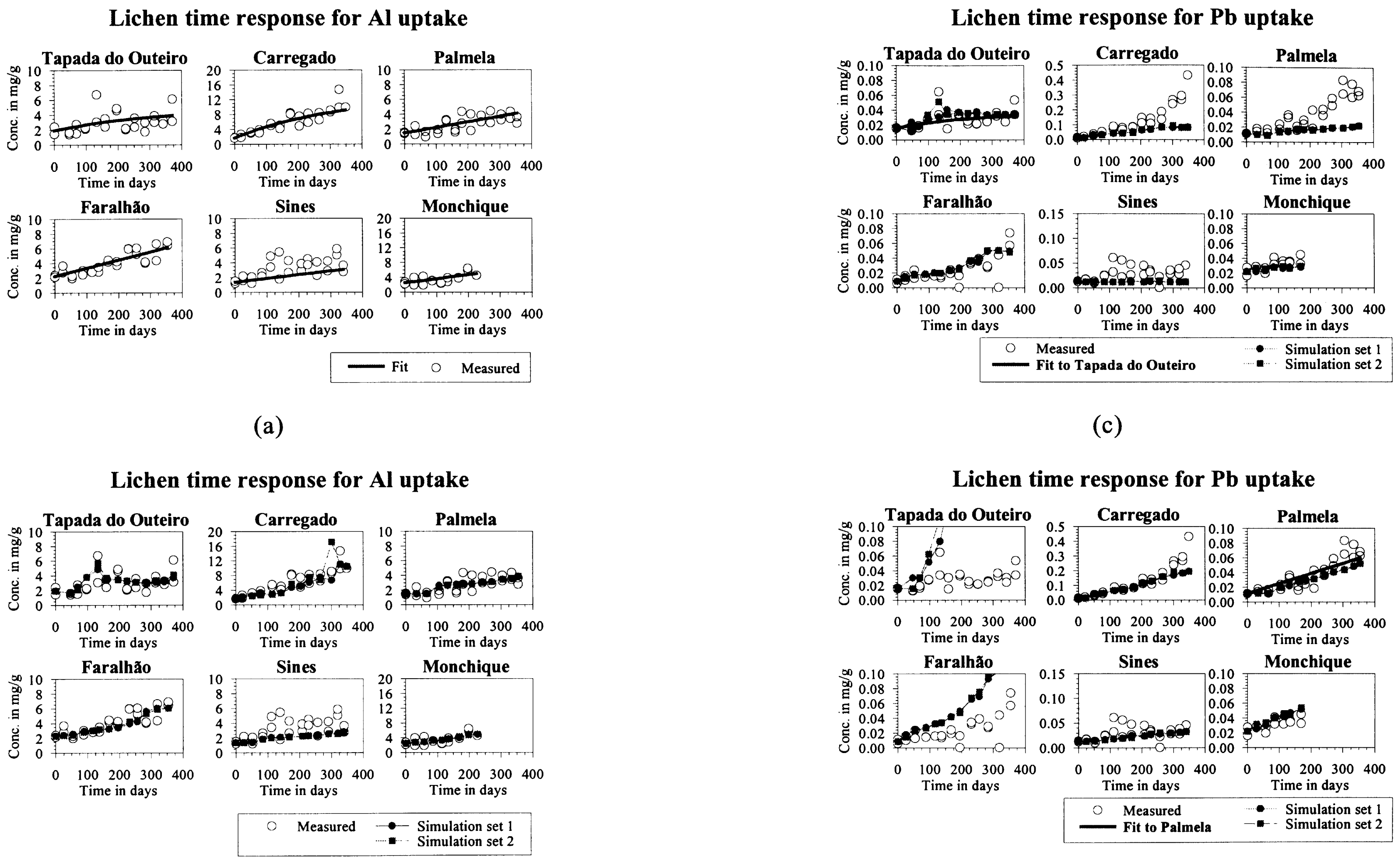
In fitting this expression a numerical problem has still to be solved because the right hand side becomes equal to $C_L(0)$ when $L_r R_s \to 0$. To solve this numerical problem, another expression, namely:

$$C_L(t) = \frac{UD_s}{L_r R_s} \cdot \frac{\langle N_E \rangle}{M_L} + \left(C_L(0) - \frac{UD_s}{L_r R_s} \cdot \frac{\langle N_E \rangle}{M_L}\right) \cdot \left[\left(1 - \frac{L_r R_s}{A_1^2}\right) e^{-\frac{A_1}{2}t} - \frac{L_r R_s}{A_1^3}\, e^{-2A_1\left(1 - \frac{L_r R_s}{A_1^2}\right)t}\right] \cdot \left(1 - 2\frac{L_r R_s}{A_1^2}\right)^{-1}$$

must be used when $L_r R_s / A_1^2 < x$ for small values of x. We used x=0.05

4. MODEL TESTING

In order to be able to fit data using expression (11), the parameters must be grouped to increase the robustness of the fitting procedure. We used as fitting parameters: D_s/M_L , U, $R_s + L_r$ and $R_s \cdot L_r$. Due to the symmetry of the problem relative to R_s and L_r, it is not possible



(a)

(c)

(b)

(d)

Figure 2. Lichen transplants concentrations time series, fit and simulations. (a) stations were separated in two groups for this fit; (b) Al concentration simulations using the parameters obtained in (a) ; (c) simulations of Pb concentrations using the parameters obtained by fitting data from Tapada do Outeiro; (d) simulations of Pb concentrations using the parameters obtained by fitting data from Palmela.

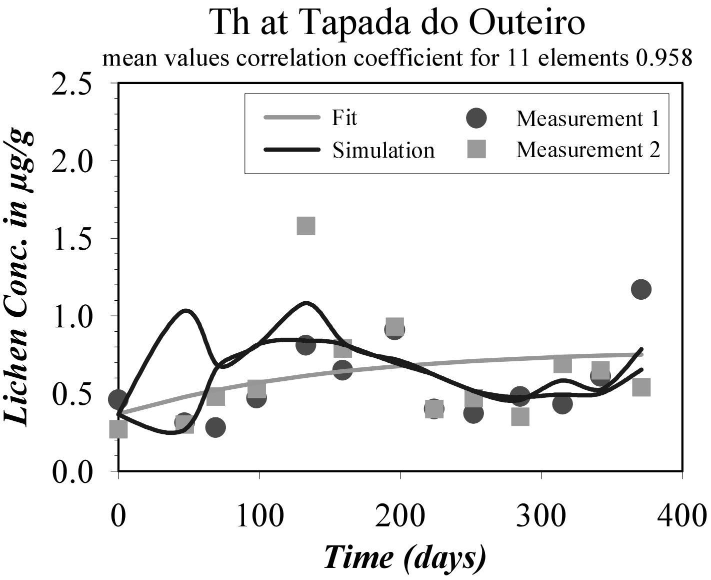


Figure 3. Results of fitting and simulation of Th concentration time series obtained by fitting 11 elements data from Tapada do Outeiro station.

to extract any of them separately from this model. Even so the model still presents itself very critical on the initial values used for the non-linear least squares process used used (the SigmaPlot package non-linear least squares Marquardt method). This might be intrinsic to the mathematical problem, will it be the case that there is no well defined minimum for the least squares process. This kind of conditions have been reported by Williams and Kalogiratou [4], to appear in several biological mathematical problems. Nevertheless it was possible to obtain values for the parameters by fitting the experimental data using the whole year average of availability and the start of exposure as the time reference.

The parameter values so obtained were used to reconstruct the lichen concentration time series that could be expected from the model and the availability time series. The approach $t \gg 1$ (or $t \to \infty$) was assumed for the relation between the surface concentration and the bulk concentration (independently of the time step considered). In each iteration the time lag between measurements was considered as evolution time under a given condition of mean availability. The new (predicted) lichen concentration value was obtained from the previous by applying the model. In the cases we are now reporting, the availability variable considered was the amount of total deposition collected in the buckets. Two values were thus available and were used to provide some insight to the variability that can be expected by applying this model. The lichen concentration initial value for each iteration was taken as the mean of the predicted values obtained in the previous iteration.

In Figure 2 the results obtained for Al and Pb are presented. In Figure 2(a) only the fitted curves are shown, in this case the six stations were separated in two groups: (i) Tapada do Outeiro, Carregado and Palmela and (ii) Faralhão, Sines and Monchique. No other reason then better fit was behind this grouping. In Figure 2(b) the simulations based on each group parameters are shown. Although no good result can be claimed for the Sines station, it becomes clear from the Tapada data set that this simple model does reproduce some fast variations which were both not expected *a priori* nor specially accounted for during the model development. In order to access the quality of the simulation (or prediction), the overall correlation coefficient between the time series of the mean values of predicted and observed concentration was calculated. In the case of Al data, a value of 0.78 was obtained. This is a good value once 136 (148 lichen concentration values used minus the 12 corresponding to the instant zero) measured values were predicted from only 8 fitted parameters.

In Figures 2(c) and 2(d) results for lead are presented. In this case only one station data was used to obtain the model parameters, Tapada do Outeiro in the case of Figure 2(c) and Palmela in the case of Figure 2(d). The simulation of the whole six stations data was then made using these values of the parameters. The parameters obtained from Tapada do Outeiro data provide a simulation for Faralhão which reproduces very well the measured data. The overall correlation coefficient for the six stations is 0.82. For the parameters obtained by fitting the data from Palmela, good results can be obtained for itself and three other stations: Carregado, Sines and Monchique . But the Tapada do Outeiro parameters do not reproduce well the data for Palmela, Carregado, Sines and Monchique, and neither do the Palmela parameters reproduce well the concentration series from Tapada do Outeiro or Faralhão. The parameters obtained from fitting the Palmela data provide an overall correlation coefficient of 0.65 that in spite of being quite meaningful (taking into account the number of degrees of freedom, 136), is not as nice as the 0.82 obtained from the Tapada do Outeiro data.

These results of comparison of predicted and observed values do show the validity of the model. Nevertheless the values of the parameters obtained are affected by large errors which are due to the fact that the fitting routine is either not robust enough or not the appropriate tool to access this specific problem.

To better access the potentialities of the model still another type of test was made. The data from Tapada do Outeiro, relative to 11 elements was fitted using the same set of 4 parameters. The elements selected were: Al, Ce, Cs, Ba, Hf, La, Lu, Mn, Th, Ti, Yb. In Figure 3 the results obtained for Th are presented. The correlation coefficient between the mean values of simulation and the mean values of measured concentrations found for the joint data of eleven elements was 0.96.

5. CONCLUSION

In this work a simple two box working model was developed for the lichen time response to a new environment. The application of the model to measured data permitted to verify that fast variability patterns identified in the concentration time series can be reproduced from the deposition data by using such a simple model. Although no good values could be obtained for each individual parameter, due to problems in the fitting procedures, the *a posteriori* tests of the model using the parameters that could be extracted from the fitting routines revealed that: (a) the time series data obtained for one element can be reproduced from the availability time series by using the values for the parameters obtained from fitting the whole concentration series with a function of the annual mean availability (b) the values obtained for one set of data can reproduce time series from an *a priori* different experimental conditions, but not all different experimental environments; (c) lichen concentrations time series for several elements within the same environmental conditions can be well reproduced using a unique set of 4 fitted parameters.

We thus conclude that the model is good although improvements must be made both on the fitting procedure used and towards introducing the effects of different environments into the model. In fact, the above (c) item can be interpreted by assuming that the parameters are essentially element independent, while the (b) item shows that the parameters are not environment independent.

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AN EXPERIENCE IN MONTENEGRO WITH ATMOSPHERIC POLLUTION BY MEANS OF LICHENS[*]

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Abstract

The very first study of the atmospheric pollution by means of bioindicators in the region of Montenegro was performed in 1992-93. The study, supported by the Commission of the European Union, was focused to the impact of an aluminium smelter plant to the air pollution in its surroundings. The plant (KAP) is situated 10 km to the south of Podgorica (capital of Montenegro) and produces some 100 000 tons of cast aluminium per year, using French (Pechine) technology. The technological process contributes in several manners to the atmospheric pollution. We investigated an area up to 50 km distances from the plant. Epiphytic lichens (*Hypogymnia caperata*) were collected from the tree bark 2 m above the ground. In the near vicinity of the plant no epiphytic lichens could have been found (died out due to high pollution levels). Instead, the more resistant lithophytic varieties (*Lecanora expallens*) were collected therein. Samples of wild grasses and pine needles were taken at the same spots, so as to compare the three indicators. We further found 5-10 times higher pollutant accumulation factors in lichens than in grasses or pine needles, emphasising thus the monitoring sensitivity of the lichens. Soil samples were also taken at the spots and analysed, but no impact factors for the soil were calculated. Samples were analysed by means of the k_0-method of neutron activation analysis at "Pierre Sue" Laboratory, Nuclear Research Centre, Saclay/Paris, France. Three types of analyses were made: (i) with short (matter of a few minutes) irradiations at the reactor ORPHEE , followed by analysing the short lived nuclides; (ii) with long (matter of hours) irradiations at the reactor OSIRIS, followed by analysing the long lived nuclides and occasionally - when necessary - (iii) epithermal analyses, in order to suppress the induced activities of certain matrix elements (e.g.Na) and obtain better sensitivity for the other elements. The following elements were determined down to μg/g levels or lower: Al, As, Au, Br, Ca, Ce, Cl, Co, Cr, Cs, Dy, Eu, Fe, Ga, Hf, K, La, Mg, Mn, Na, Nd, Rb, Sb, Sc, Se, Sm, Tb, Th, Ti, U, V, W, Zn. Characteristic samples from the technological process of the aluminium production (bauxite ore, pulp, alumina, additives and catalysts, electrodes) are analysed as well in the same way. A clear correlation is found between the elemental composition of the bioindicators (lichens, grasses, pine needles) at one side, and the pollution originating from the smelter, from the other. Samples from the unpolluted areas, taken as the blanks, differ from the affected ones just for the elements which characterise the technological process: Al, As, Ca, Ce, Cr, Eu, Fe, Ga, La, Nd, Sm, Th, Zn... (rare earths being constituents of the bauxite ore). The differences are higher for the sites closer to the plant and also reflect the dominant directions of the wind. So we conclude that lichens could be useful biological indicators for the air pollution control.

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SALINE VARIABILITY AT THE SOUTH-WEST OF PORTUGAL AFTER KRIGING DATA FROM *RAMALINA* SPP. BIOMONITORS

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Abstract

Sea-salt input over the land masses may have deleterious effects on man-made structures, vegetable organisms (crops and vegetation) and soil/water resources. Recent work has shown the ability of some lichen species to monitor the deposition of airborne salts of marine origin. The atmospheric transport and deposition of sea salts can be assessed by measuring saline elements in lichens growing over coastal areas. The concentration of Cl^- and Na^+ was determined in lichen thalli collected in three different dates on the south-west region of Portugal. The extracellular fraction of Na^+ was obtained by a sequential elution procedure, and Cl^- was determined after leaching samples with water. The dispersion of element grades in the area was studied through geostatistical analysis. Numerical values were estimated by two interpolation methods: ordinary kriging and kriging with an external drift. The latter method used the logarithm of the distance to the coast as an auxiliary variable and yielded more reliable results. Every set of data produced a similar spatial pattern, showing a steep gradient in the first three kilometres from the coast. However, considerable variation can be observed between surveys. The variability of results is interpreted using the precipitation data obtained for the region under consideration.

1. INTRODUCTION

Airborne salinity is one factor that most contributes to accelerating the degradation of materials and the desertification of land, in the latter case namely through salt-assisted deforestation, aquifer contamination and direct soil impoverishment. However, setting up a classical network to monitor deposition rates of saline elements is all but an easy task, due to its installation and operation costs.

The use of lichens as biomonitors has gone through major developments in the past few decades, as a result of advances in the knowledge of uptake and retention of elements by lichens, especially cations. Also, there has been arising an acute environmental conscience, leading not only to an enhanced control of pollutant emissions but also to the assessment of environmental risk factors. There are many examples referring to the application of lichens in the monitoring of air pollution [1-3].

The total concentration of Cl^- and Na^+ has already been measured in lichens growing in coastal areas [4-7]. For both elements, higher values were obtained in sites near to the coast and their concentrations showed a negative gradient with the distance inland. Recent studies with lichen transplants in the south-west region of Portugal achieved a calibration between lichen and physical monitors, for Cl^- and Na^+ acting as sea-salt tracers [8]. These studies also indicate that an essential parameter to be included in any calibration model is some measure of the (wet) precipitation amount. Another calibration study on mine-derived pollution came to the same conclusion [9].

After sampling over a regional area, the use of geostatistical methods for interpolating the grades of saline elements in lichens shows some advantages. These data-driven methods, born as a supporting tool of mineral-deposit evaluation, turned into an important discipline for environment risk assessment in the nineties. The application of geostatistics is based on the existence of some spatial correlation between sampling points: the smaller the distance, the higher the correlation between a variable measured in both locations. This behaviour can be described by the variogram function. The spatial information from the variogram can then be used for estimating the variate in non-sampled points by kriging. Several kriging procedures were developed to meet different needs in estimation, depending on the properties of the variable in hand. Generally speaking, they rule out bias and minimise the residual variance: in this sense, they give optimal results and perform better than other interpolation methods.

Both the ordinary kriging [10] and the kriging with an external drift [11] estimate the unknown variates as a linear combination of the available samples. However, the latter method also conditions the estimation to another (auxiliary) variable, which must have a well-known relationship with the (primary) variable being processed. In the present study, the lichen concentrations of Cl^- and Na^+ in south-western Portugal were estimated by either method for three sampling campaigns (same locations, different dates). The logarithm of the distance from the coast was taken as an auxiliary variable for the external drift procedure. A comparison between the resulting maps as well as an interpretation of the spatial and temporal variation in the lichen concentrations are given herein.

2. MATERIAL AND METHODS

2.1. Field procedures

The study area is located in the south-western part of Portugal, ranging 16 km along the coast and 10 km inland. This is an almost straight, near-flat region wide open to the westerly winds from the Atlantic ocean. The collection of lichens took place in September 1994, March 1995 and February 1996 at an average of 69 sampling sites, allowing for minor variations between campaigns. Samples of *Ramalina canariensis* Steiner were collected; whenever unavailable, other lichens of the same genera were selected.

2.2. Laboratory procedures

Surface chloride was obtained by shaking *ca.* 50 mg of lichens twice in plastic flasks with 10 ml deionised water. The weight of each sample (dry weight) was determined after drying at 80ºC for 16 h. Chloride was measured by mercurimetric titration [12].

The method for assessing the extracellular sodium followed the sequential elution technique by Brown and Wells [13], with some modifications. Prior to elution, complete thalli (about 50 mg) were stored for 24 hours in a high-humidity chamber. The extracellular fraction was obtained by shaking samples in two plastic flasks with 10 and 5 ml of $NiCl_2$ (20 mM) for 40 and 30 minutes, respectively. Through this procedure, all extracellular ions (on the surface, in intercellular spaces and bound to the cell wall) are removed into the nickel solution. Samples were dried at 80ºC for 16 h before taking their dry weight. Sodium concentration was determined by atomic absorption spectrophotometry.

The results are given in micromole per gram dry weight (µmol/g d.w.) of lichen, and they are based on five independent samples for each sampling site.

2.3. Statistical analysis

Continuous maps of chloride and sodium concentrations were drawn for the whole study area by geostatistically processing the discrete field data. Before interpolation, experimental variograms were calculated for the variables $[Cl^-]$ and $[Na^+]$. They are the semi-variance for all pairs of samples as a function of the distance (or lag) between sampling points and thus provide a measure of the spatial dependence between samples. The variogram function of a variable $Z(x)$ is defined as

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[Z(x_i) - Z(x_i + h) \right]^2 \qquad (2.1)$$

where $\gamma(h)$ is the estimated semi-variance for lag h and $N(h)$ is the number of pairs of points separated by h. The variograms for the study variables were determined in the four main directions, which are the parallel and perpendicular to the coast, plus the two half-quarters in between.

Theoretical models of spherical form were fitted to the experimental variograms. The parameters of these models (nugget effect, sill and range) were found by an interactive process and then used in the estimation by ordinary kriging and kriging with an external drift. The former estimator should be used only with stationary variables, *i.e.*, variables that show the same mean and variance for the whole field. However, it can be applied to non-stationary variables in sub-areas of the field where they show stationary behaviour. Should the non-stationarity be described by some well-known process, typified by an auxiliary variable all over the estimation domain, then the inclusion of such an external drift into the kriging algorithm can be a suitable device to overcome the problems caused by a less stringent application of ordinary kriging.

The kriging estimator is given by a weighted linear combination of the available samples:

$$Z^*(x_0) = \sum_{i=1}^{n} \lambda_i Z(x_i) \tag{2.2}$$

where $Z^*(x_0)$ represents the estimated value, $Z(x_i)$ the value of the variable at location i and λ_i the weights assigned to that location. The kriging estimator must be unbiased, which means null differences between actual and estimated values:

$$E\left[Z^*(x_0) - Z(x_0)\right] = 0 \tag{2.3}$$

Should the variable be stationary, *i.e.*, $E\{Z(x_i)\} = m$ (constant), then the sum of weights must equal one:

$$\sum_{i=1}^{n} \lambda_i = 1 \tag{2.4}$$

This is called the universality condition. The optimal solution is obtained by minimising the estimation variance constrained by the universality condition, as follows:

$$E\left[\left(Z^*(x_0) - Z(x_0)\right)^2\right] \min \tag{2.5}$$

The kriging system appears as a set of equations

$$\begin{cases} \displaystyle\sum_{j=1}^{n} \lambda_j \gamma_{ij} + \mu = \gamma_{i0} \\ \displaystyle\sum_{j=1}^{n} \lambda_j = 1 \end{cases} \tag{2.6}$$

where μ stands for the Lagrange parameter that enables the transformation of the constrained minimisation problem into an unconstrained one, with the estimation variance given by

$$\sigma^2 = \sum_{i=1}^{n} \lambda_i \gamma_{i0} + \mu \tag{2.7}$$

Kriging with an external drift follows the same procedure, but the weights for each location are conditioned to an auxiliary variable as well. Now, let the relationship between $Z(x)$ and the auxiliary variable $Y(x)$ be linear and known for the whole estimation field, that is

$$E\left[Z(x_i)\right] = aY(x_i) + b \qquad \forall i = 1,\ldots,n \tag{2.8}$$

where a and b are constants. Using Eq. 2.3, Eq. 2.8 can be rewritten as follows:

$$a\left[\sum_{i=1}^{n} \lambda_i Y(x_i) - Y(x_0)\right] + b\left[\sum_{i=1}^{n} \lambda_i - 1\right] = 0 \tag{2.9}$$

This brings about a new universality condition (one for each auxiliary variable)

$$\sum_{i=1}^{n} \lambda_i Y(x_i) = Y(x_0) \tag{2.10}$$

to be accounted for by the kriging system

$$\begin{cases} \sum_{j=1}^{n} \lambda_j \gamma_{ij} + \mu_1 + \mu_2 Y(x_i) = \gamma_{i0} \\ \sum_{j=1}^{n} \lambda_j = 1 \\ \sum_{j=1}^{n} \lambda_j Y(x_j) = Y(x_0) \end{cases} \tag{2.11}$$

and by the kriging variance as well

$$\sigma^2 = \sum_{i=1}^{n} \lambda_i \gamma_{i0} + \mu_1 + \mu_2 Y(x_0) \tag{2.12}$$

3. RESULTS AND DISCUSSION

Several factors may influence the dispersion and deposition of marine salts on land: the distance from the coast, the altitude and the number/type of natural barriers between the sampling location and the coast, among others. The effect of these factors in the accumulation of saline elements by lichens was ascertained through the correlation between such factors and the concentrations of Cl^- and Na^+ in the organisms. Every factor was determined for each sampling point using a digital elevation model of the whole study area as support information. The best correlation

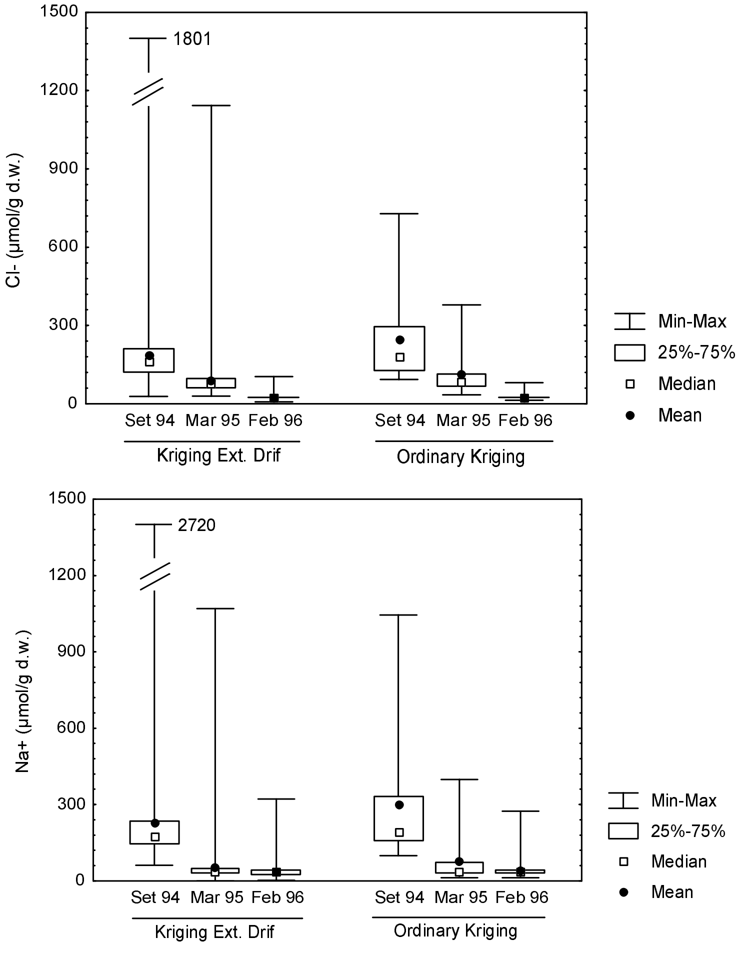

Fig. 1. Statistical parameters for the interpolation results of [Cl^-] (above) and [Na^+] (below), after kriging with an external drift and ordinary kriging.

was obtained for the natural logarithm of the distance from the coast with coefficients of -0.80 for [Na^+] and -0.75 for [Cl^-], which are significant at $p<0.05$. This logarithmic behaviour was already observed in other studies relating not only to saline elements [4, 5] but to heavy metals as well [3, 14].

The directional variograms show large differences for both elements between the range of the parallel to the coast and the perpendicular one. The former is over three times higher than the latter, indicating the existence of a strong anisotropy, *i.e.*, there is much more continuity along the coastal direction than in its perpendicular.

Statistics for the results of kriging with an external drift and ordinary kriging are given in Fig. 1.

Concerning either element, the amplitude between maximum and minimum is always higher for the external-drift results than for those from ordinary kriging. Kriging with an external drift yielded higher values near the coast and lower values deep inland, due to the inclusion of the logarithm of the distance from the coast as an auxiliary variable. This seems to improve the quality of the estimation, since it was possible to identify a good and comprehensive relationship between the variables being estimated and the auxiliary variable. However, as aforesaid, care must be taken when dealing with an external drift. The relationship between variables should be well understood and physically sound [15], otherwise the results are most likely to turn meaningless.

Considerable variations can be observed on the values of [Cl^-] and [Na^+] referring to different sampling dates. The main reason for this can be the amount of (wet) precipitation observed before each sampling campaign. Figure 2 shows the total rainfall over the three-month period prior to lichen collection. The highest values for both elements were obtained after the first campaign, which took place in the end of a particularly dry summer. Lower values were found for the second and third campaigns, especially for the latter. Both surveys were done after a rainy winter, yet they differ as to the mean rainfall in the three months before lichen collection: the average for the 1996 survey is five times higher than for the 1995 one. The chloride and sodium levels in these lichens thus seem to reflect the variations in the amount of wet precipitation, which is likely to act as a leaching agent rather than an input medium for saline elements. The importance of this factor in the lichen uptake and release of atmospheric constituents has also been addressed by its inclusion in calibration models of lichen contents *versus* atmospheric fluxes [9, 10].

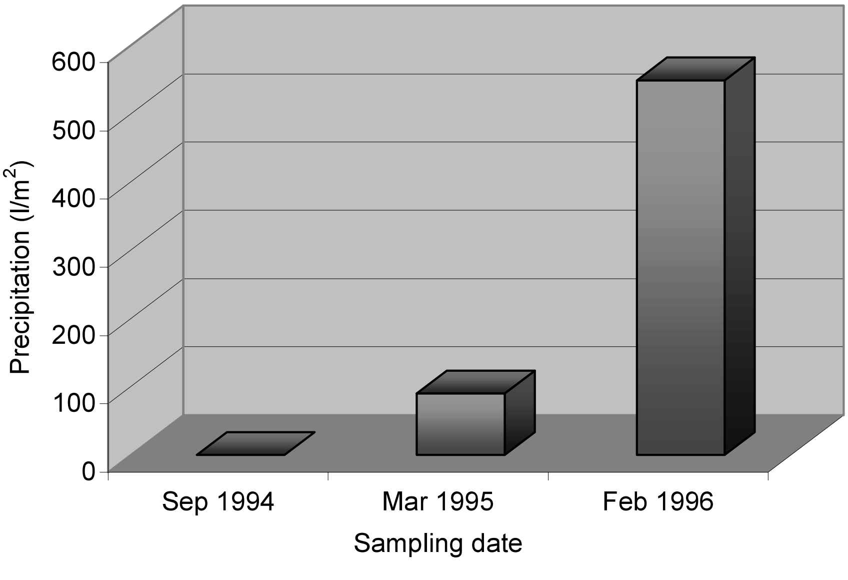

Fig. 2. Rainfall measured in the three-month period prior to lichen-sampling dates.

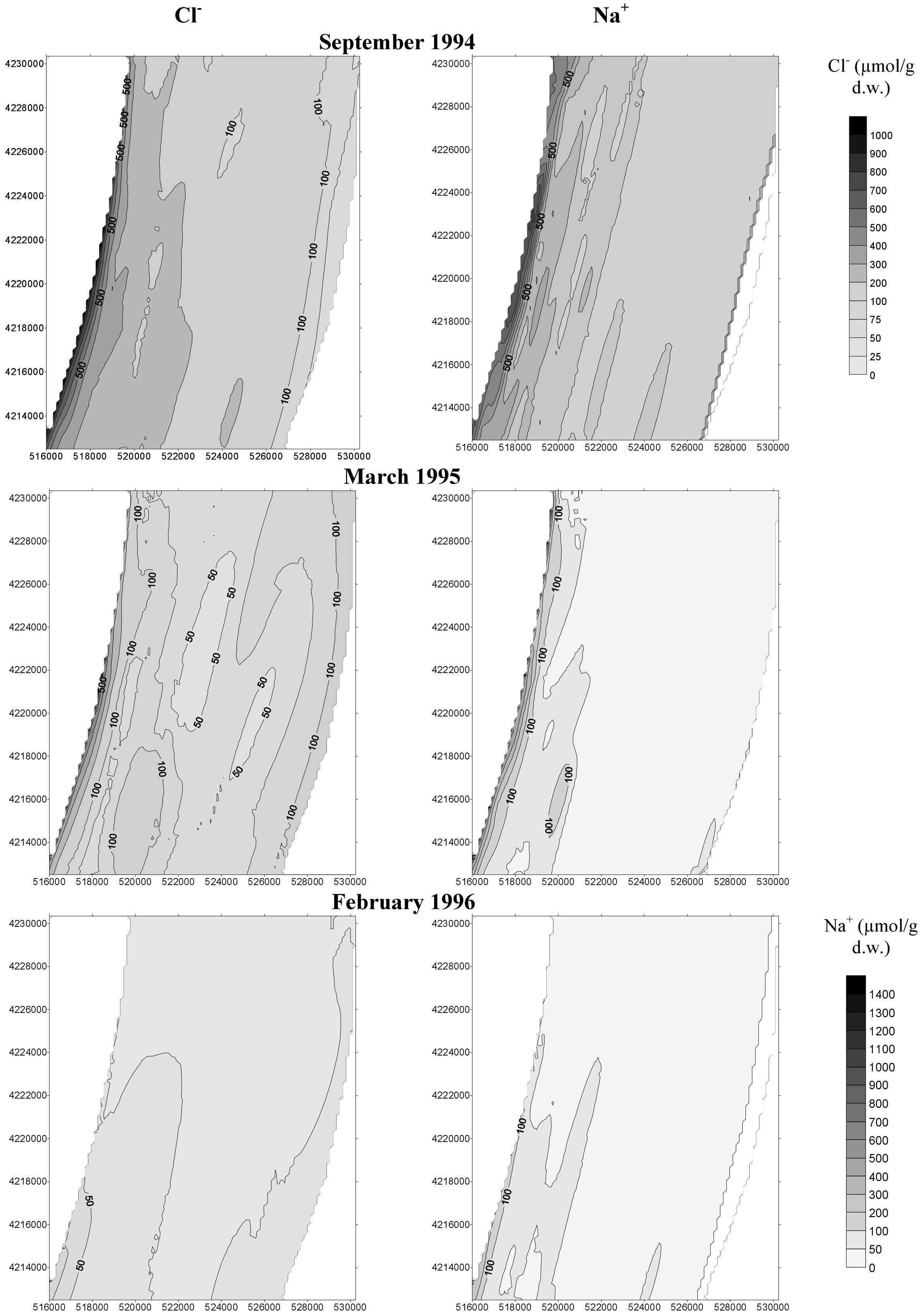

Fig. 3. Maps of [Cl$^-$] and [Na$^+$] obtained by kriging with an external driftafter three lichen surveys in south-western Portugal.

The estimation of [Cl^-] and [Na^+] in lichens by external-drift kriging produced the maps shown in Fig. 3. The spatial variation of the estimated values follows a similar pattern for all field surveys: there is always a steep gradient for either element across the first 3 km from the coast. The larger salt nuclei, which are likely to represent the major fraction of the marine aerosol, should be deposited in this area through gravitational settling. Between 3 and 10 km from the coast, the negative gradient is much less pronounced, and little variation can be found for extensive patches within this section. There is also little variation in the north-south direction, though concentration values appear to increase southwards. This is probably due to the existence of a swamp system (Santo André lagoon plus some marshy land), which may act as an entry channel for the maritime air masses over this low-altitude zone.

4. CONCLUSIONS

The concentration of Cl^- and Na^+ in lichens declines with increasing distance from the coast in a logarithmic mode. This behaviour naturally translates into the variograms, which show high anisotropy. Interpolation by kriging with an external drift, using the logarithm of the distance from the coast as a conditioning variable, gives better results than ordinary kriging, especially in areas close to the sea.

In the first three kilometres from the coast, a steep gradient is obtained for either element. Little variation is noticed further inland. This pattern is recurrent in every survey; however, great alteration in the concentration levels is observed from survey to survey. The values show an increasing depletion with the amount of rainfall that occurred before lichen collection, probably due to wash-out effects.

ACKNOWLEDGEMENTS

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A GRAPHICAL TECHNIQUE FOR DISTINGUISHING PLANT MATERIAL AND SOIL FROM ATMOSPHERIC DEPOSITION IN BIOMONITORS

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Abstract

The paper explores the limits to which a new graphical technique can distinguish the various hierarchical levels of sources of trace elements within biomonitors. When applied to data from Portuguese lichens, it appears to resolve four levels of sources, from plant material down to individual types of pollution. Careful factor analysis appears to offer very similar results, being weaker than the graphical method in some aspects and stronger in others. As a result, it now seems possible to determine sources for elements in lichens with better precision and confidence than was available previously.

1. BACKGROUND

1.1. Genesis of this study

This study is an attempt to apply to biomonitors a graphical technique for distinguishing components of elements in environmental media that I have been exploring for the past few years [1]. After developing the technique as a way to deduce the composition of the crustal component of the aerosol from the aerosol itself, I found that it also worked for precipitation, streamwater, soils, stream sediments, marine sediments, and even coal. Having in the meantime become familiar with elemental data for Russian mosses,[1] I wondered whether the technique could be used there, too, perhaps to help address the longstanding problem of distinguishing plant material from deposition. Although the first results were encouraging, they were limited by the small numbers of samples available.

After I had been invited to describe the technique further at this workshop, correspondence with Dr. M.C. Freitas revealed that she had a large, high-quality elemental data set for Portuguese lichens, supplemented by data on deposition and soils throughout Portugal, that she would be willing to share with me. These data have proven so satisfactory that I will focus my entire talk on them.

1.2. The graphical technique

The graphical technique is binary, that is, it separates two components of a mixture at a time. A log-log scatter diagram of a large data set is constructed, with the vertical axis being the crustal ratio of some element X (i.e., X/Al, where Al is the crustal reference element) and the horizontal axis being the ratio to Al of the reference element in the other source (say, Na for the sea or Se for pollution). This form of the horizontal axis creates a crustal left end and, depending on the other element being used, a marine or pollution right end. Thus the more crustal a sample is, the farther to the left of center it will be located, and the more marine or pollution the sample is, the farther to the right it will be located. Given enough samples and a great enough range of compositions, clear crustal and marine or pollution ends can be created. At the crustal end, a horizontal crustal “tail” appears and frequently points asymptotically to the X/Al ratio of the pure crustal component of the samples. At the marine or pollution end, a second asymptotic tail appears, having unit slope. It represents the pure marine or pollution component of the samples, i.e., the other component represented on the horizontal axis. Thus a single plot extracts the compositions of two pure components in mixed samples.

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[1] Data generated by Dr. M.V. Frontasyeva of the Joint Institute for Nuclear Research, Dubna.

1.3. Applying the graphical technique to biomonitors

The simplest way to apply the graphical technique to biomonitors is to distinguish the plant component from atmospheric deposition. Plant material can be represented by K or some other element enriched in plants relative to aerosol (Rb, Cs, Sr. and Ca are obvious possibilities), deposition by Al (which has crustal and pollution components in deposition). Using Al allows us to keep X/Al on the vertical axis and use K/Al for the horizontal axis. The resulting plot would have an atmospheric left end and a plant right end.

2. BASIC GEOCHEMISTRY OF THE PORTUGUESE LICHENS

2.1. The data sets

The basic data set consisted of 235 lichen samples taken over all Portugal during July and August 1993. Sampling grids of 10 km x 10 km were used near the coast and 50 km x 50 km in the interior. Samples were analyzed by INAA and TTPIXE; 44 elements were reported. Details are given in Freitas, Reis, Alves, et al. [2]. The data set for soils was generated from 89 samples taken throughout Portugal along with the lichens and analyzed by INAA at ITN (Dr. Isabel Prudencio; see paper by Prudencio et al. in these proceedings). Data for total deposition consisted of annual-average concentrations taken in duplicate at six sites in Portugal. Samples were taken monthly with funnels and plastic bottles and analyzed by ICP-MS at the Geological Survey of the Netherlands (Dr. Bertil van Os).

2.2. Choosing the reference element for plant material in the lichens

The easiest way to choose the best elemental indicator for plant material in the Portuguese lichens is to compare their average composition with that of atmospheric aerosol or deposition. Figure 1 does this by showing elemental enrichment factors of the average lichens relative to average urban aerosol [3], where the enrichment factor of element X is defined as $[X/Al]_{lichens}/[X/Al]_{urban\ aerosol}$. Phosphorous is most strongly enriched in lichens relative to aerosol, and has enrichment factors of 6–7. Below P comes a series of elements with enrichments near unity (K, Ca, Rb, Sr, Cd (artifact of data), REE, Hf, Th, U) and a series of elements depleted by factors of up to 10–100 (the "transition elements" V–Br plus the pollution elements Sb, Hg, and Pb). These results imply that relative to aerosol or deposition, P will be the clearest indicator of plant material, and perhaps the only clear indicator, that a small number of additional elements may be mixtures of plant and aerosol, and that most other elements will come mostly from the aerosol (or deposition). Note that the conventional indicator for plants, K, is predicted to be a distinctly poorer reference element for plant material in these lichens than is P. Therefore, we tried P rather than K as our reference element for plants. The best indicators for deposition are predicted to be Pb and Sb, with Cu, Zn, Se, Br not as good. Overall, the plant contributions to elements in the lichens are predicted to be small.

2.3. Finer levels of discrimination

Distinguishing plant material from atmospheric material within lichens is not the only possible application for the graphical technique. The atmospheric material is itself composed of two components: windblown local soil and true atmospheric deposition, both wet and dry. Atmospheric deposition typically contains three major components: crustal, marine, and pollution. The pollution component itself typically contains contributions from coal combustion, oil combustion, traffic, incineration, industry, etc. Each component of this entire hierarchy, comprising at least three levels below the basic plant-atmosphere pair, is potentially separable graphically.

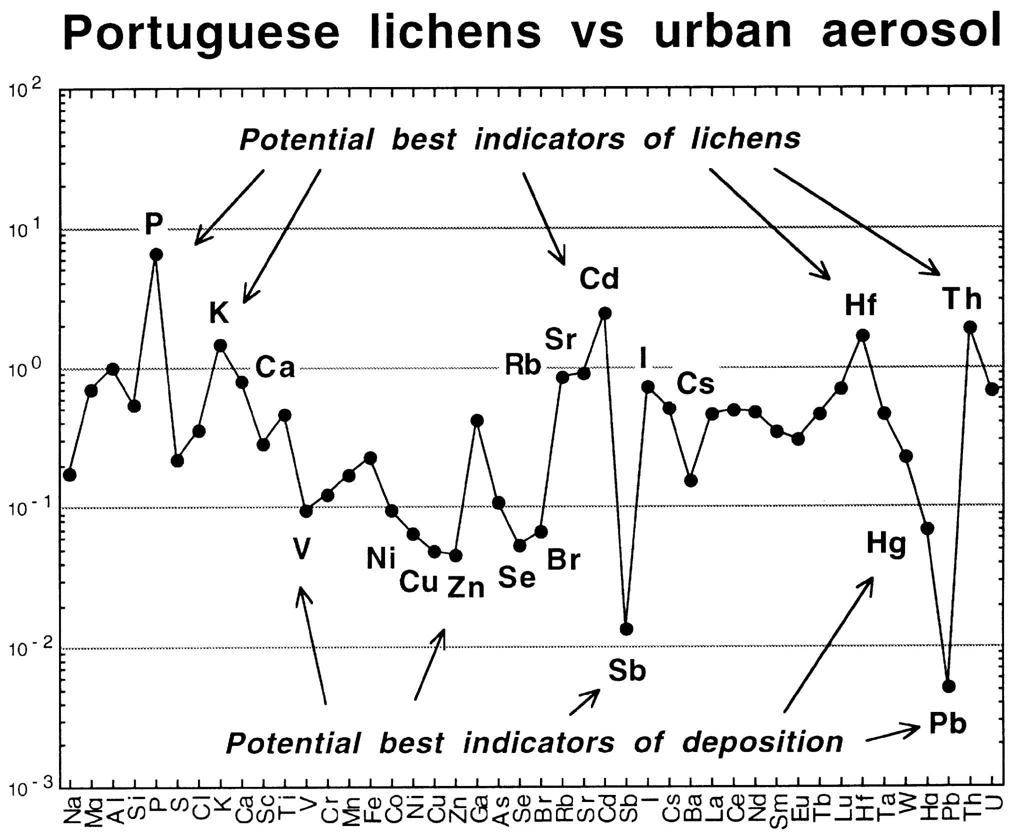

FIG. 1. Enrichment factors of elements in Portuguese lichens with respect to urban aerosol.

2.4. Goals and assumptions of this work

There were three main goals for this exploratory work. The main goal was to see whether the plant and atmospheric components of Portuguese lichens could be distinguished graphically, qualitatively and perhaps even semiquantitatively. (True quantitative resolution will be the next step.) The second goal was to see whether we could distinguish the finer levels of components: the soil and depositional components of the atmospheric component; the crustal, marine, and pollution components of the depositional component, and the coal, oil, and other components within the pollution component. The third goal was to see how the graphical results compared to results from factor analysis.

The major assumption of this exploratory work is that elemental ratios of the atmospheric components of lichens are the same as in the soil and the deposition, i.e., that the elements are deposited to the lichens with the same efficiency and are retained by the lichens to the same degree. Although this assumption is almost certainly violated at times, the extent of the violations and their practical effects on the discriminations will have to wait for later, more quantitative treatment of the data.

3. CAN WE GRAPHICALLY DISTINGUISH PLANT AND ATMOSPHERIC COMPONENTS?

To distinguish the plant and atmospheric components of the lichens requires two steps. First we use scatter diagrams of one element versus another to confirm that the suspected plant elements do indeed come from plants. Then we use scatter diagrams of elemental ratios to search for plant and atmospheric tails, from which we can estimate the compositions of those components.

If the predicted plant elements truly represent plant material, they must meet three criteria: They must have narrow ranges of concentrations relative to atmospheric elements, they must be enriched relative to atmospheric elements, and they must be in plant proportions with each other.

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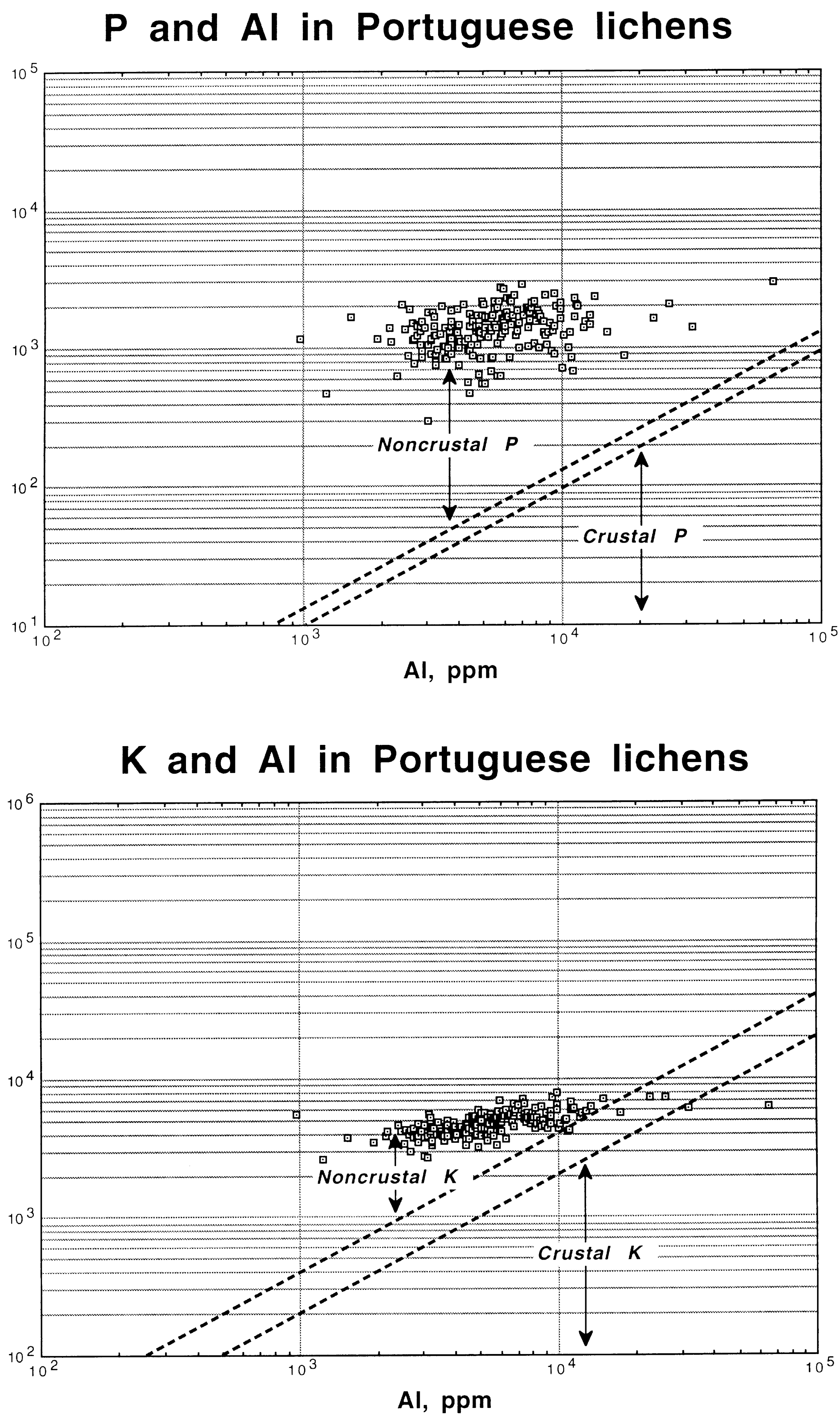


FIG. 2. Scatter diagrams of P vs. Al and K vs. Al in Portuguese lichens.

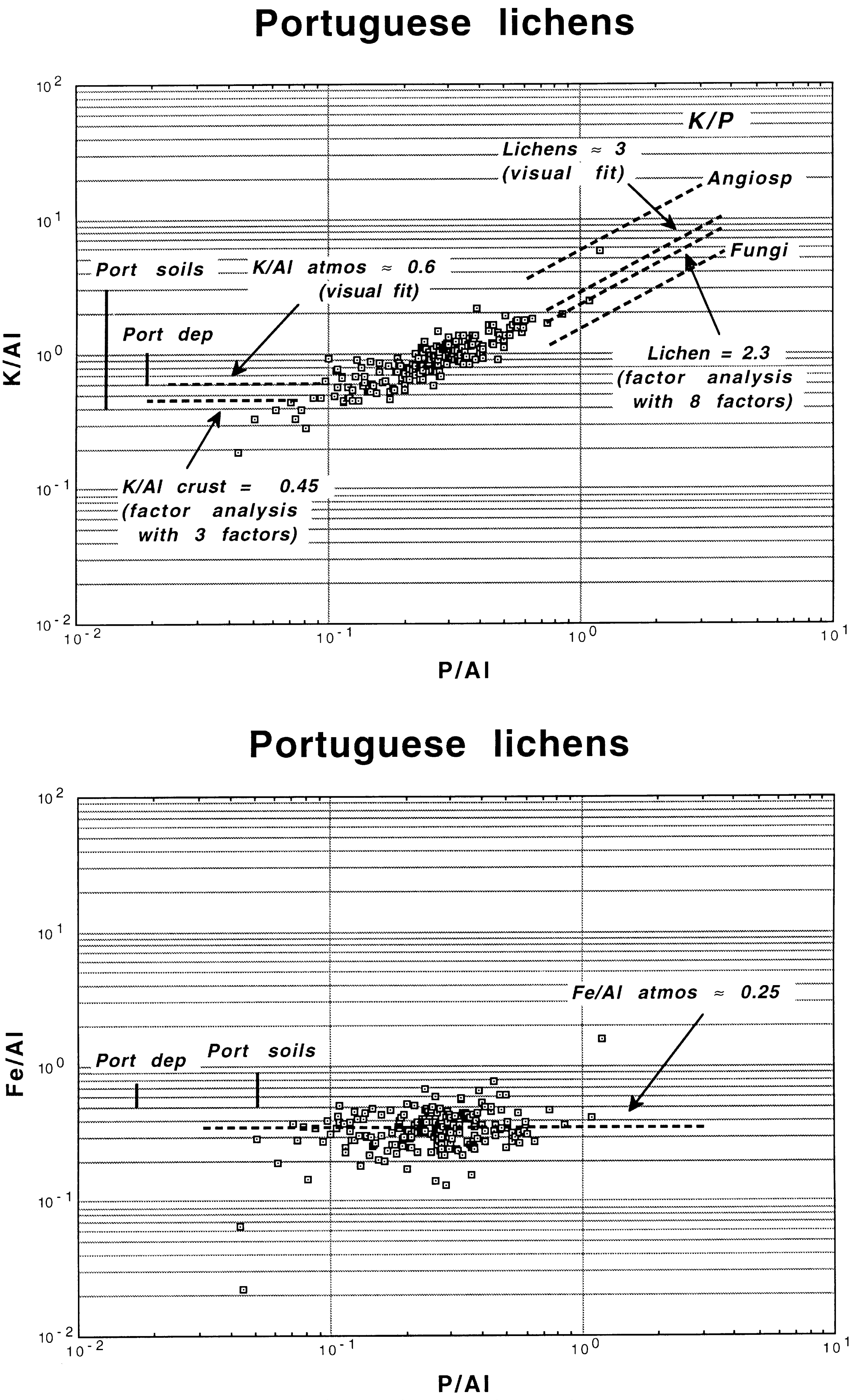


FIG. 3. Scatter diagrams of K/Al vs. P/Al and Fe/Al vs. P/Al in Portuguese lichens.

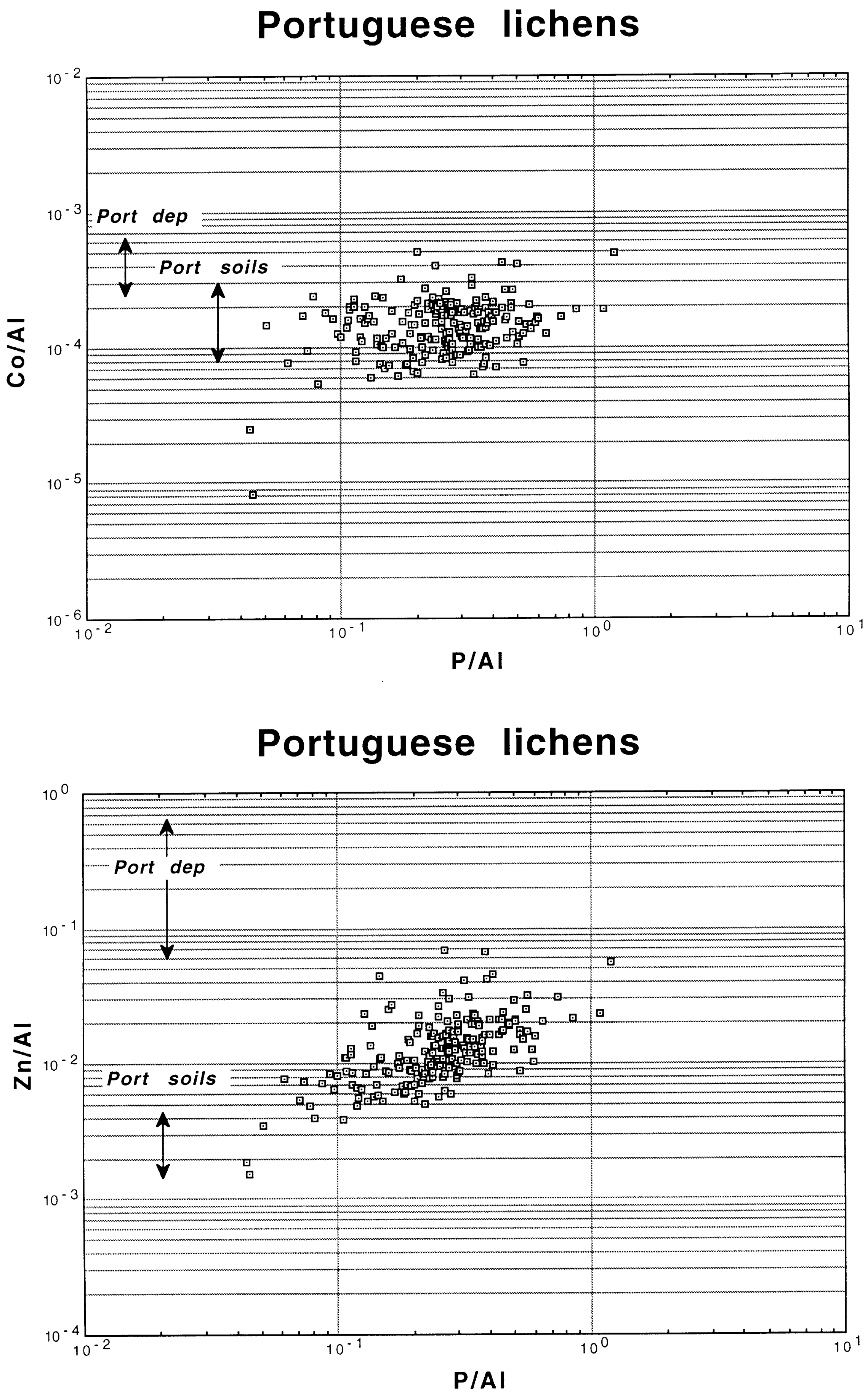
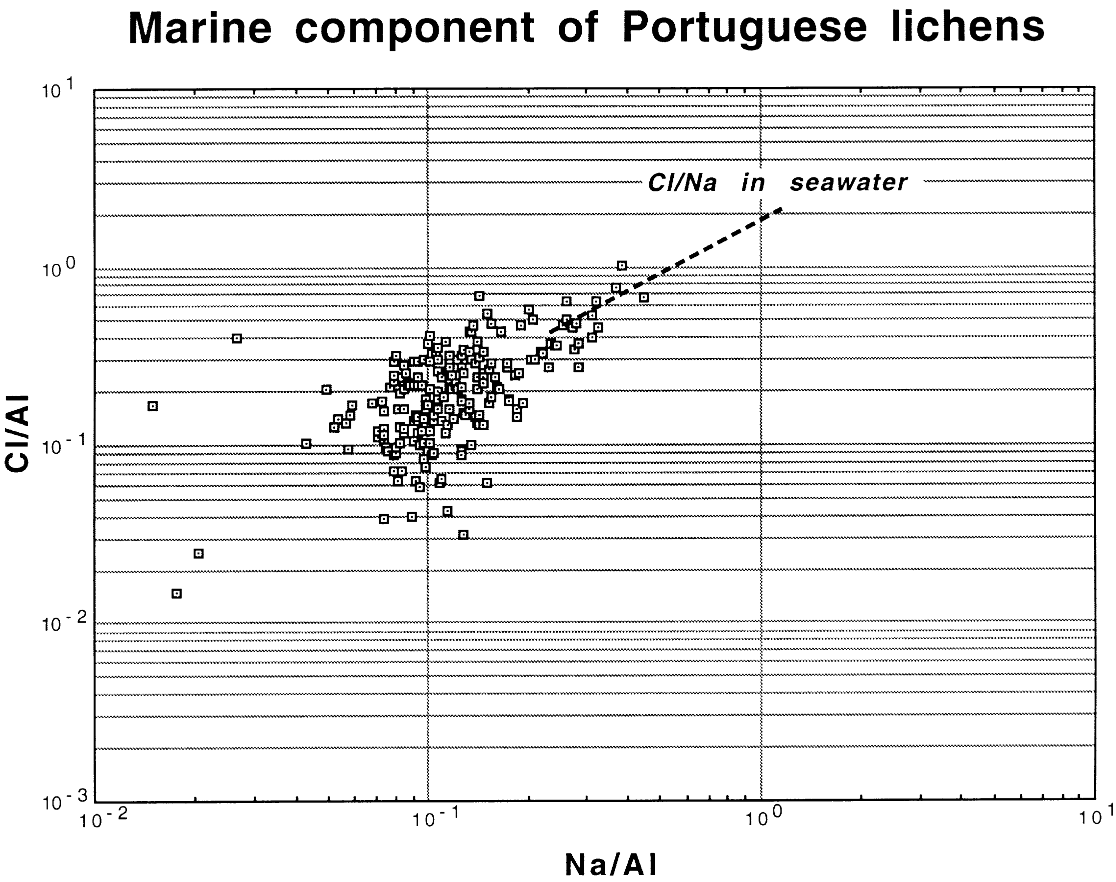


FIG. 4. Scatter diagrams of Co/Al vs. P/Al and Zn/Al vs. P/Al in Portuguese lichens.



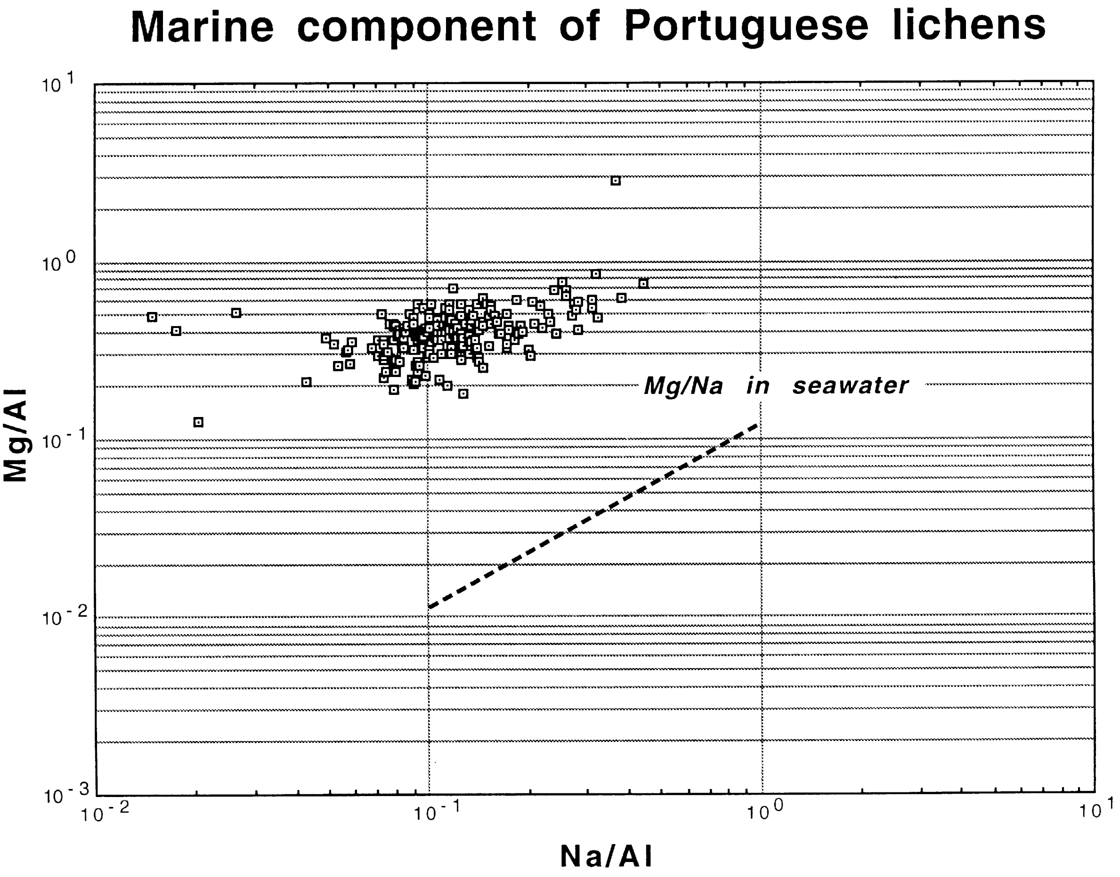
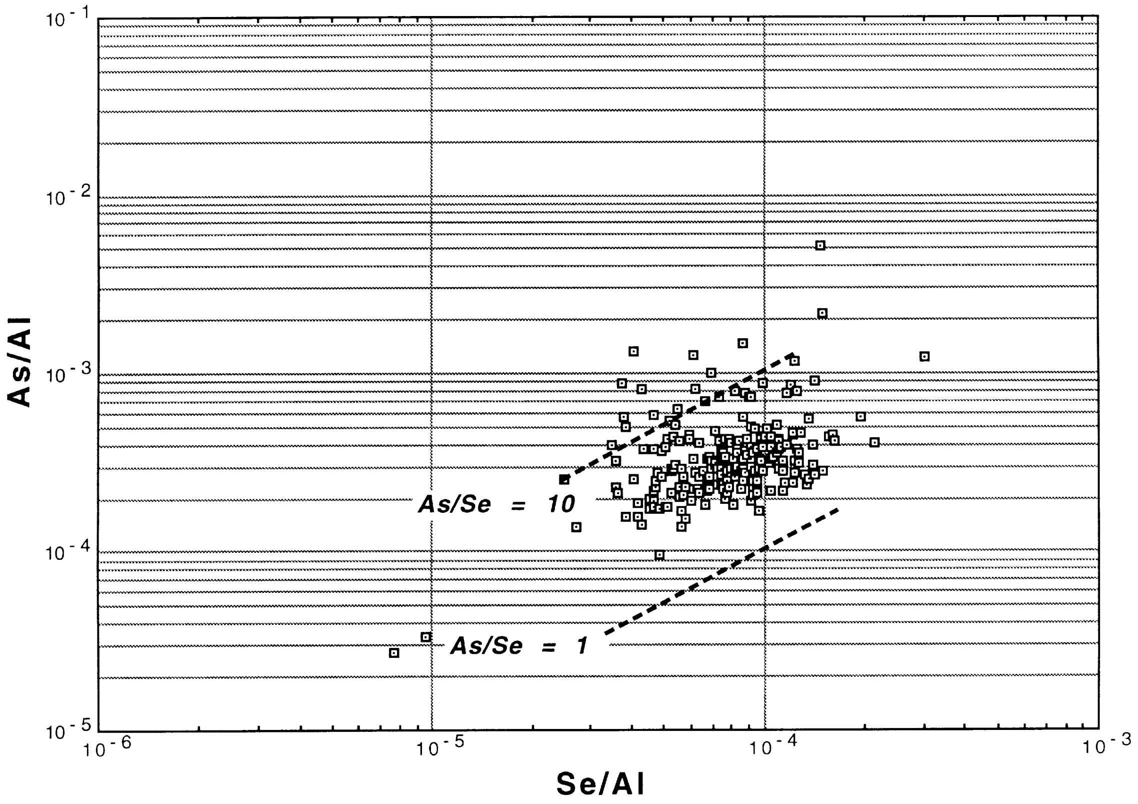


FIG. 5. Scatter diagrams of Cl/Al vs. Na/Al and Mg/Al vs. Na/Al in Portuguese lichens.

General pollution of Portuguese lichens



Oil component of Portuguese lichens

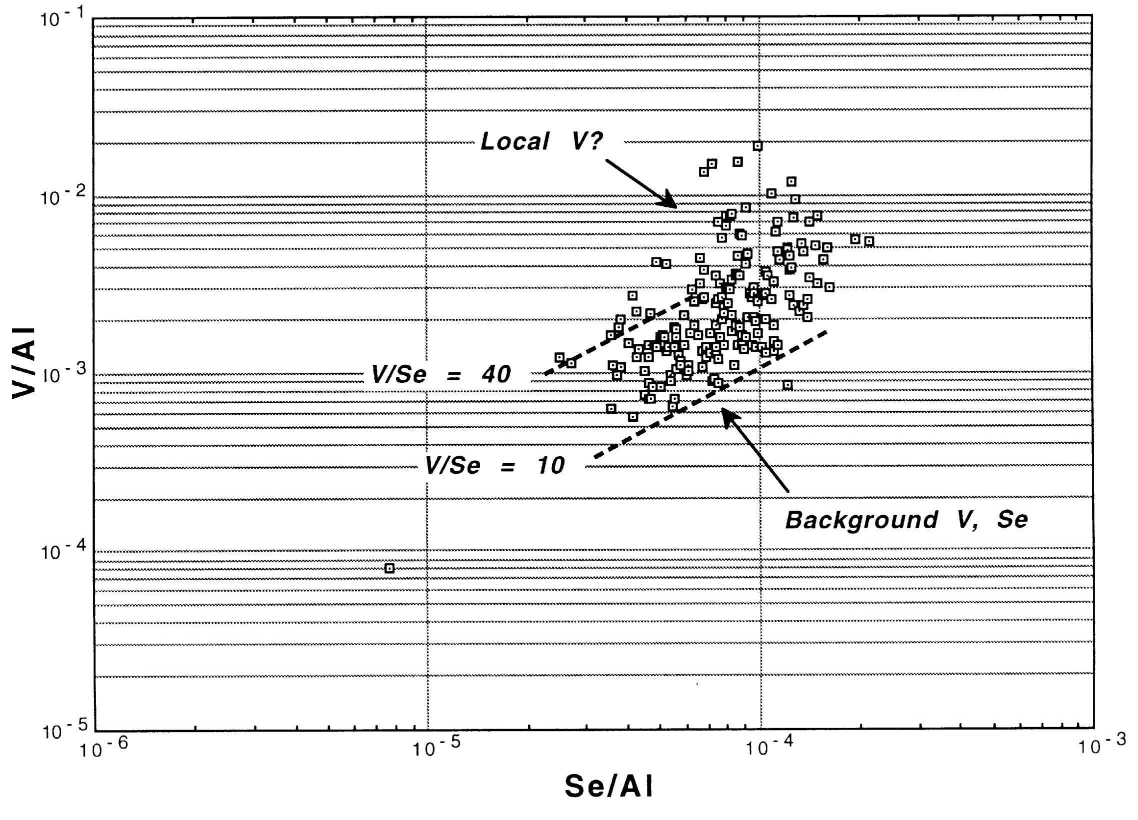
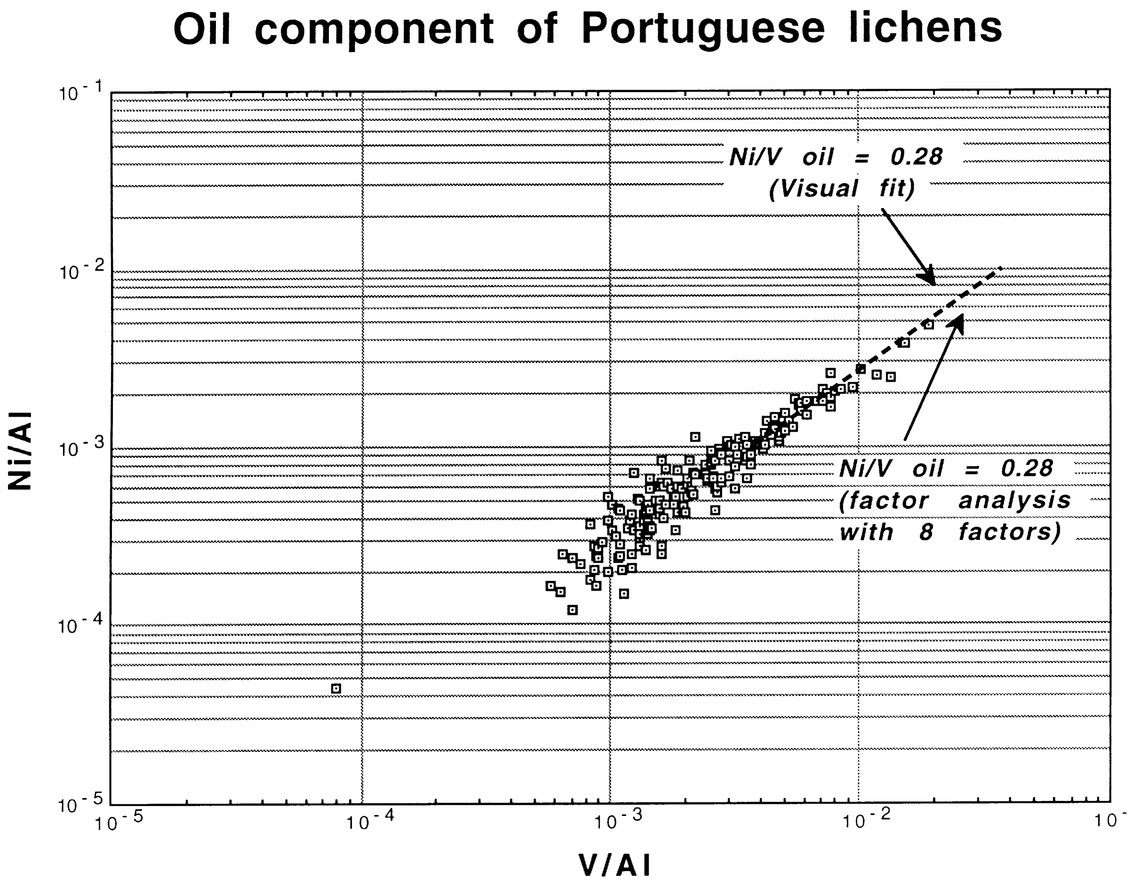


FIG. 6. Scatter diagrams of As/Al vs. Se/Al and V/Al vs. Se/Al in Portuguese lichens.



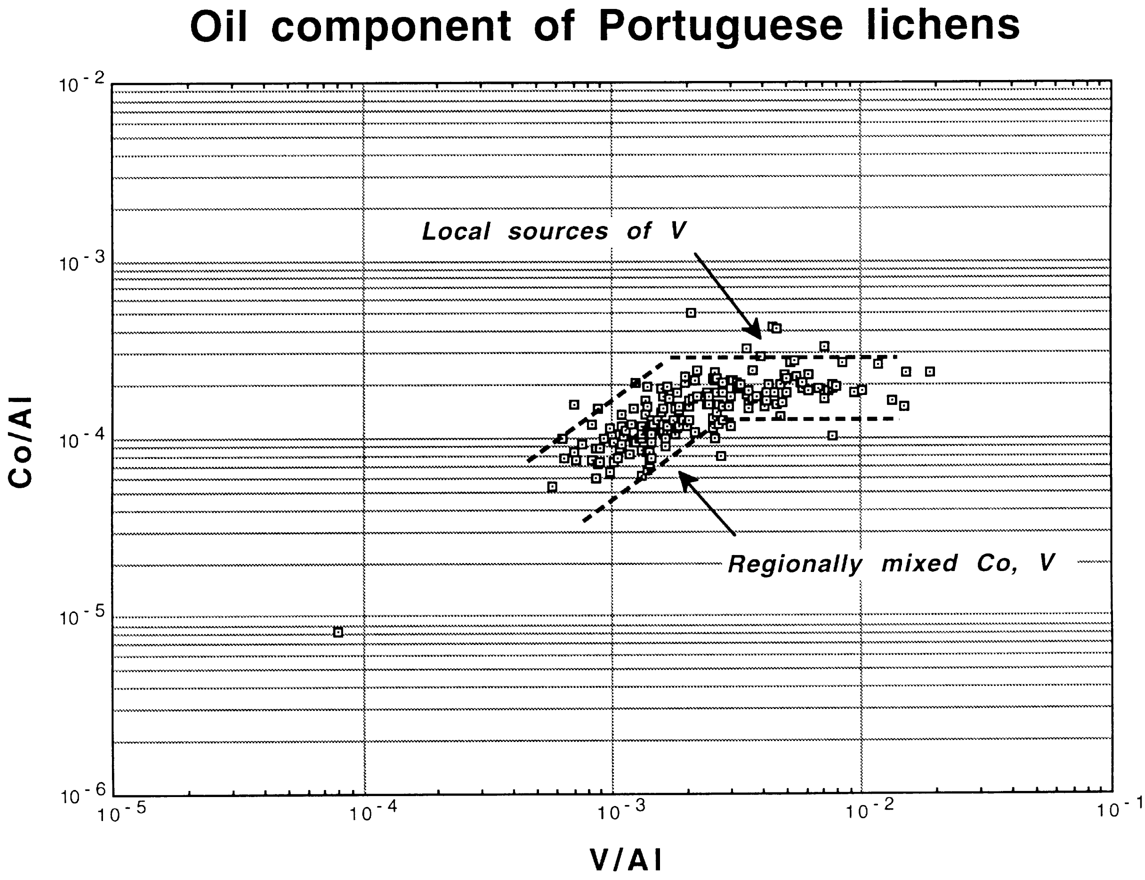


FIG. 7. Scatter diagrams of Ni/Al vs. V/Al and Co/Al vs. V/Al in Portuguese lichens.

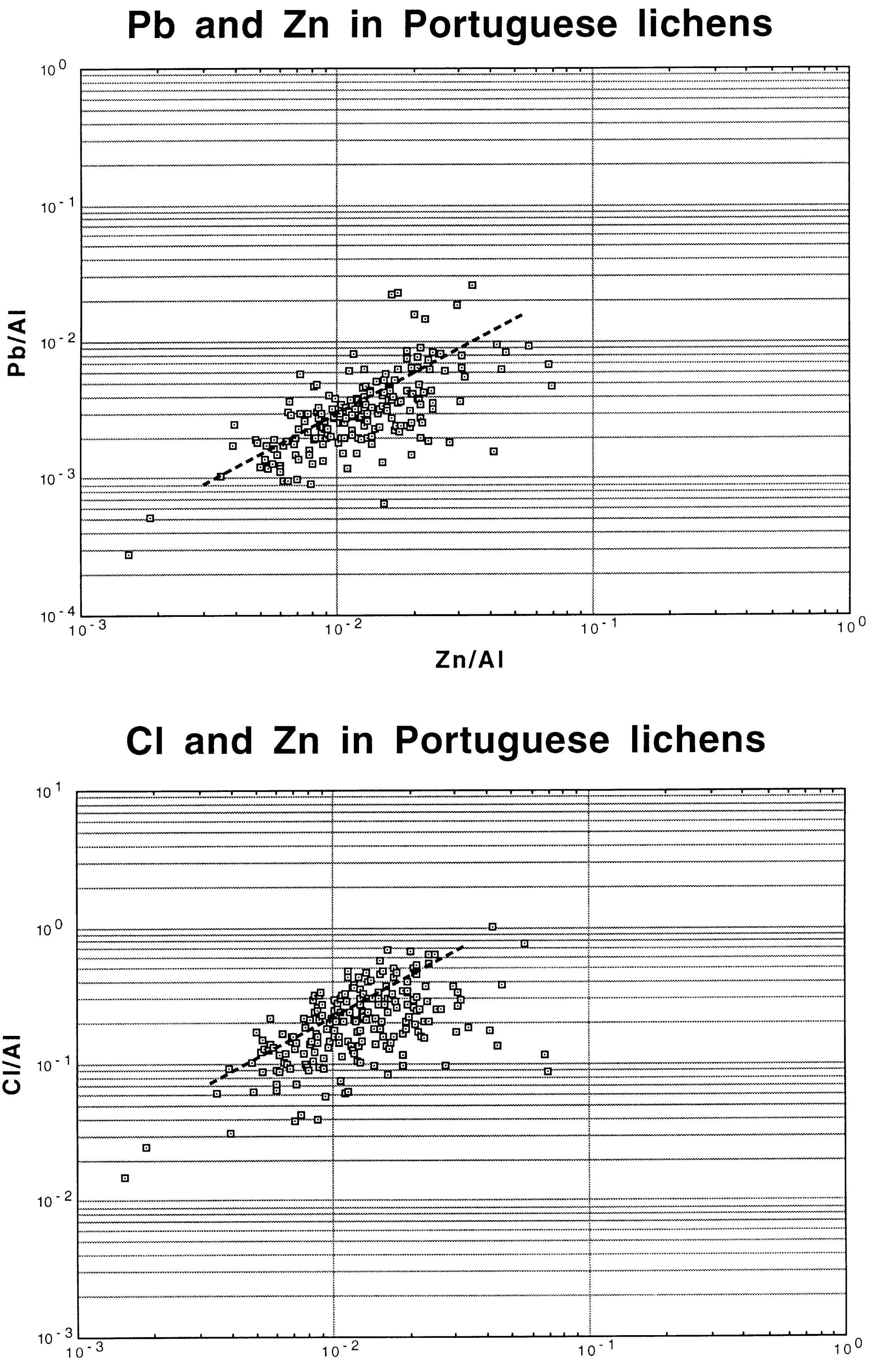


FIG. 8. Scatter diagrams of Pb/Al vs. Zn/Al and Cl/Al vs. Zn/Al in Portuguese lichens.

The first two of these conditions can be checked from plots of the elements versus Al, the third from plots of plant elements versus one another. Figure 2 shows plots for P and K, which from Figure 1 appeared to be the best indicators of plant material, versus Al. Dashed lines indicate the zones of crustal ratios for each pair, as derived from bulk crustal rocks and soils compiled in [3]. Each element has a much smaller range of concentrations than does Al—factors of 2–3 for the elements vs 5 for Al. Each is clearly enriched relative to the crust—typically by a decade for P and by factors of two or so for K. As a bonus, each element shows a clear horizontal tail at low Al, which indicates a substantial nonatmospheric component for each. Thus P and K meet their first two criteria for being plant elements. Although P's graph is noisier than K's, P's greater enrichments relative to the crust would make it a superior indicator of lichens. The third criterion can be addressed by a scatter diagram of K vs P that also shows their concentrations in bryophytes and angiosperms (not shown here). The elements are tightly associated, with nearly all points for each element falling within the range of the two types of plants. While this test is imperfect, it does reassure us that P and K are likely lichen elements.

Plots for two other potential plant elements, Ca and Rb, were less persuasive. Ca, while essentially horizontal, was very noisy and closer to crustal proportions than even K was. Rb's pattern was parallel to the crustal zone and extending into it, which presumably meant that it was strongly crustal and without a detectable plant component. S was a puzzle, being horizontal but well below the zone shown by precipitation in Narragansett, RI. The plot for Sb fell in the zones for Portuguese deposition and Portuguese soils and was parallel to them. After considering these elements and others, I concluded that P and K were the best indicators for plant material in Portuguese lichens, with Ca and possibly S running a distant second.

The second step in distinguishing plant and atmospheric components uses scatter diagrams of X/Al versus P/Al. Plant components of X will appear as tails of unit slope on the right, atmospheric components as horizontal tails on the left. For the Portuguese lichens, K shows the best plant tail, followed by less-distinct tails for S and possibly Ca. Of these elements, only K shows a clear atmospheric tail. Rb, Sr, and Cs show no clear tails, and so appear to be of mixed origins. Pure atmospheric patterns (single long, narrow horizontal tails) are shown by the classical crustal elements (in aerosol) Sc, Fe, Co, the rare earths, etc. Patterns for pollution elements (in aerosol) such as Mn, Zn, As, and Se were generally nearly horizontal, with greater vertical scatter for those elements most pollution-derived in aerosol. Figure 3 shows the plots for K and Fe. The plot for K also depicts rough values for the atmospheric tail (K/Al = 0.6) and the plant tail (K/P = 3). Note how the atmospheric tail falls within the ranges of the Portuguese soils and deposition and how the lichen tail falls between the values for angiosperms and fungi [4]. For Fe, I have estimated the atmospheric Fe/Al ratio to be about 0.35. The upper end of the zone of points overlaps the ranges of Fe/Al in Portuguese soils and deposition. The Fe plot shows no indication of a plant tail.

4. CAN WE DISTINGUISH SOIL FROM DEPOSITION WITHIN THE ATMOSPHERIC COMPONENT?

Within the atmospheric component of lichens, deposition can potentially be distinguished from soil by the large suite of pollution elements enriched in deposition relative to soil by factors of 10–1000 or more. One need only examine the atmospheric ends of plots of X/Al versus P/Al (or the entire plot if there is no plant component) to see whether the X/Al ratios correspond to the lower values in soil, to the higher values in deposition, or to some combination of the two. A practical limitation to this step was that only eight good pollution elements were available for Portuguese deposition (Mn, As, Sb, Zn, Cu, Ni, Cr, Co), three of which were not available in Portuguese soils (Mn, Cu, Ni). Thus, conclusions distinguishing soil from deposition had to be drawn largely from the five pollution elements As, Sb, Zn, Cr, and Co. (Because Al was not available for the soils, it was estimated as 5000xSc.)

Plots for these elements indicated that their sources were mostly soil plus some deposition (Cr and Co), similar parts soil and deposition (Zn, Sb), and soil or deposition (As). Thus it appears that both sources can be detected in lichens, with the relative contributions of deposition depending on the degree of enrichment over soil in the deposition. For example, Zn and Sb would have greater contributions from deposition than Cr and Co would have because the former are much more enriched butions from deposition than Cr and Co would have because the former are much more enriched in aerosol and deposition. The plots for Co and Zn are given in Figure 4. The points for Co extend throughout the range of Portuguese soils and into the lower end of the range for Portuguese deposition. By contrast, nearly all the points for Zn fall above the range for soil but below the range for deposition.

To quantify the relative contributions of soil and deposition to the various elements, standard source-apportionment techniques such as chemical element balance (CEB) could be used.

5. CAN WE DISTINGUISH CRUST, MARINE, AND POLLUTION SOURCES WITHIN THE DEPOSITIONAL COMPONENT?

The three main types of sources for aerosol and deposition are crust, marine, and pollution. They can be found at nearly all locations. In the previous section, we found crustal sources within the depositional component of the lichens. To search for evidence of marine and pollution sources, we need only change the numerator of the horizontal axis to a reference element of that source and search for the appropriate tail at the right end of the plots.

To search for marine components, we used Na/Al on the horizontal axis. Of the four potential marine elements Cl, Br, I, and Mg, only Cl appeared to show a marine component, and it was very weak. Figure 5 shows the marine plot for Cl along with that of Mg, the element likely to have the next-strongest marine signal. Whereas the Cl/Na ratio of the marine tail corresponded well to that of seawater, the analogous tail for Mg fell an order of magnitude above its seawater ratio. Thus Mg in the Portuguese lichens had a main source other than the sea.

To search for a general pollution component (or a coal component) within the deposition, we used Se/Al as the horizontal axis. Plots of all the pollution elements in the lichen data (S, V, Zn, Sb, As, Pb, and even Mn) displayed a pollution tail of unit slope on their right sides. In most plots, the tails were smooth and well-behaved; for V, however, the right end of the tail rose nearly vertically, as though in response to strong specific sources of V. Figure 6 shows the plots for As and V.

The pollution proportions of at least S, Se, V, Zn, Sb, As and Br shown by these plots are essentially indistinguishable from those in European aerosol. Typical proportions were: S/Se = 3000 to 10,000, V/Se = 10 to 40, Zn/Se = 75 to 250, Sb/Se = 0.3 to 1.5, As/Se = 2 to 5, and Br/Se = 30 to 100. Ratios to Se for Pb, for which we have fewer data in aerosol, were 20 to 100. Thus it appears that the pollution elements as a class generally preserve their proportions when deposited to lichens. By contrast, ratios to Se for less-polluted elements such as Mn, Co, and Cr were higher by up to an order of magnitude in lichens than in aerosol, perhaps because of influences from their large crustal components.

6. CAN WE DISTINGUISH SPECIFIC SOURCES WITHIN THE POLLUTION COMPONENT?

6.1. Oil component

The previous section offered preliminary evidence that the lichens showed evidence of a specific oil component. This component can be sought more definitively by placing V/Al on the horizontal axis. These kinds of plots for Ni, the other major element from oil, and Co, an element sometimes found associated with V in aerosol, shown in Figure 7. The plot for Ni is essentially one long oil tail . Its Ni/V ratio of 0.28, as read visually, agrees well with ratios for European aerosol (0.4 to 0.7) and for North American aerosol (about 0.3). The plot for Co is very different. At low Co/Al and V/Al, it shows a broad area of unit slope that is presumably from regionally mixed Co and V in the deposition rather than specific oil sources for Co. At the higher V/Al associated with strong oil signals, Co/Al flattens out and remains constant, further demonstrating that Co in the deposition is not being strongly affected by oil sources. Thus in Portuguese lichens V and Ni but not Co appear to come primarily from oil.

6.2. Incineration component

The prime indicator element for refuse incineration is usually considered to be Zn. Thus we can search for a refuse signal by placing Zn/Al on the horizontal axis. When this is done, Pb and Cl show reasonable tails on the right (Figure 8). These elements are also strongly associated with refuse incineration, Pb from batteries and Cl from PVC. Sb, another element associated with refuse incineration, shows a less-distinct plot than Cl does. On balance, there seemed to be reasonable evidence for a detectable incineration component in the lichens.

6.3. Smelter component

An alternative source for the Pb and Zn might be smelters. With Pb/Al on the horizontal axis, however, Sb, also frequently from nonferrous smelters, failed to show a clear tail. As, even a better smelter element, showed no inclination at all toward a tail. Thus the lichens showed no evidence for a distinct smelter component.

7. HOW DO THESE GRAPHICAL RESULTS COMPARE WITH THOSE FROM FACTOR ANALYSIS?

The other major way to distinguish sources within lichens is factor analysis. Factor analysis has the advantages of being much more established than graphical analysis and of treating all the elements simultaneously. It has the disadvantage of being a black-box technique whose procedure cannot be visualized or easily validated. By contrast, the graphical approach is simple, direct, highly visual, and does not have the problems caused by missing data the way that factor analysis does. Graphical analysis is still being developed, however, is slower and more trial-and-error than factor analysis is, and can deal with only three elements at a time.

7.1. Our general procedure for factor analysis

Our procedure for factor analysis is highly iterative. In general, we use a conventional package such as Statistica to get a quick, basic idea of the solution, and then refine the calculations with the newer PMF2 [5]. We have found that the most important aspects of factor analysis are the choice of elements and the number of factors used. The elements must be of the right type and the right number. Concerning type, elements with poor analytical data or too many points missing (not measured or below detection limit) must be avoided—when too many "bad" elements are included, they create "junk factors" that mean nothing and degrade the other factors as well. It appears to us that the effects of missing data cannot be properly compensated for when more than a very small percentage of the points, possibly as low as 20–25%, are missing. The number of good elements must not be too small, either, or else the results become unstable. The number of factors to retain is very difficult to determine. We seem to be finding that published criteria are often insufficient—we need to examine various numbers of factors for each data set and evaluate their reasonableness based on their stabilities and our experience. We prefer to use the smallest number of factors before nonsensical elements or groups of elements begin to split off.

By contrast, we appear to be finding that while the choice of factor-analysis program is important, it is not so critical as often thought. Once the number of elements has been properly restricted, standard commercial programs perform nearly as well as the larger, research-level programs. For example, we find little difference between Statistica and Positive Matrix Factorization [6]: Whereas PMF2 produces factors with less crossover, Statistica's remain more stable as their numbers change.

Concerning missing data points, by far the best approach is not to let elements with a significant fraction of missing points into the analysis in the first place. We find that the few missing points for the other elements are best handled by substituting mean values. Lastly, we nearly always use logs of concentrations when dealing with atmospheric data, having found that log-transformed data correspond better to what we see graphically. Although logs of concentrations are less important for bio

TABLE 1. FACTOR SOLUTIONS FOR LOG CONCENTRATIONS, 29 ELEMENTS, AND MEAN SUBSTITUTION

Number of factors	Names	Elements
4	Crust heavy	Na, **Al, Si**, K, Sc, **Ti**, Mn, **Fe**, Co, As, Se, **Rb, Cs, La, Ce, Hf, Th, U**
	Crust light	Sc, Ti, **V**, Fe, Co, **Ni**, Sb, I, Pb
	Lichen	**P**, S, K, **Zn**
	Secondary/gas	-Ca, **Br**, I
5	Crust heavy	Na, **Al, Si**, K, Sc, Ti, Mn, Fe, Co, Se, **Rb, Cs, La, Ce, Hf, Th, U**
	Crust light	Sc, Ti, **V, Fe, Co, Ni**, Sb, Pb
	Plant	**P**, S, K, **Zn**
	Secondary/gas	S, **Br, I**
	Sea/land	**Cl, -Ca**
6	Crust light	Al, Si, **Sc, Ti**, V, Mn, **Fe, Co**, Ni, La, Ce, Hf
	Industry/oil	V, Ni, Zn, As, **Sb, Pb**
	Lichen	**P, K**
	Secondary/gas	S, **Br, I**
	Land/sea	**Cl, -Ca**
	Crust heavy	Na, Al, Si, K, **Rb, Cs, La, Ce**, Hf, **Th, U**
7	Crust heavy	Na, **Al, Si**, K, Sc, **Ti**, Mn, **Fe**, Co, Se, **Rb, Cs, La, Ce, Hf, Th, U**
	Oil/crust light	Sc, Ti, **V**, Fe, Co, **Ni**
	Lichen	**P, K**
	Secondary/gas	S, **Br, I**
	Cl/marine	**Cl**
	Industry	Zn, As, **Sb, Pb**
	Ca	**-Ca**
8	Crust light	Al, **Si, Sc, Ti**, Mn, **Fe, Co**, Se, **La, Ce, Hf**, Th
	Oil	**V, Ni**, Pb
	Lichen	**P, K**
	Secondary/gas	S, **Br, I**
	Cl/marine	**Cl**
	Crust heavy	**Rb, Cs**, La, Ce, Hf, **Th, U**
	Ca	**-Ca**
	Industry	**As, Sb**, Pb
9	Crust light	Al, **Si, Sc, Ti**, Mn, **Fe, Co**, Se, **La, Ce, Hf**
	Oil	**V, Ni**
	Lichen	**P, K**
	Secondary/gas	**Br, I**
	Cl/marine	**Cl**
	Crust heavy	**Rb, Cs**, La, Ce, Hf, **Th, U**
	Ca	**-Ca**
	Industrial	As, **Sb**, Pb
	S	**S**
10	Crust light	Al, **Si, Sc, Ti**, **Fe, Co**, Se, **La, Ce, Hf**, Th
	Oil	**V, Ni**
	Lichen	**P, K**
	Secondary/gas	**Br, I**
	Cl/marine	**Cl**
	Crust heavy	**Rb, Cs**, La, Ce, **Th, U**
	Ca	**Ca**
	Industry	As, **Sb**, Pb
	S	**S**
	Mn	Mn

monitoring data because of the latter's narrow ranges, we chose to use them here for consistency and ease of comparing with our graphical results.

7.2. Iterations

We use iteration to determine whether we should be using logarithmic concentrations, to find the optimal suite of elements, and to find the optimal number of factors. In general, to check the effects of logarithmic versus linear concentrations, we try various combinations of elements and factors in both modes. To find the optimal suite of elements, we make tables of analytical uncertainties and percent missing data for the elements, start with too many elements, identify the junk factors, and progressively remove the offending elements until all the factors are justifiable. To find the optimal number of factors, we start with too few factors, usually three or four, and progressively increase the number until meaningless single-element factors begin to appear. (Single-element factors can be meaningful, however, if confirmed graphically and environmentally.) Our actual iterative procedure is a compromise that balances all the above the needs. The steps listed below are generally followed separately for logarithmic and linear concentrations.

First, we determine a preliminary list of elements to watch out for by examining the number of data points available for each. For 34 of the 44 elements, 96–100% of the data points were available (Na, Br, Sc, Cr, Co, As, Se, Cs, La, Ce, Sm, Tb, Lu, Hf, Ta, Th, Rb, Eu, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Sr, Hg, Pb, Sb). For another 8 of the elements, between 85% and 91% were available (Nd, W, U, Al, Mg, I, V, Ga). For the two remaining elements, only 58% and 7% were available (Ba, Cd). Taking the first cut at 96% thus tells us to watch out for Nd, W, U, Al, Mg, I, V, Ga, Ba, and Cd. General knowledge of analytical uncertainties of NAA tells us also to be careful for Lu, Hf, Ta, Hg, Eu, Ca, Ni, Cu, and Sb. Beginning with 44 elements, linear concentrations, and four factors eventually yielded six justifiable factors, four junk factors, and 15 problem elements for the ten-factor solution: Mg, Cr, Cu, Ga, Sr, Cd, Ba, Nd, Sm, Eu, Tb, Lu, Ta, W, Hg. Removing these elements and repeating the analysis with 29 elements gave for the preferred eight-factor solution seven defensible factors (crust heavy, oil, lichens, secondary/gas, crust light, sea/land, industry) and only one junk factor (Al and I). Repeating the process with logarithmic concentrations and 44 elements gave seven defensible factors and three single-element junk factors for the ten-factor solution. Removing the same 15 problem elements as before gave eight defensible factors (crust light, oil, lichens, secondary/gas, Cl/marine, crust heavy, Ca, and industry) and no junk factors for the preferred eight-factor solution. Table 1 summarizes the solutions for 4 through 10 factors for the logarithmic case with 29 elements and shows the elements most characteristics of each factor. The eight-factor solution comprised crust light (Al through Hf), oil (V, Ni), lichens (P, K), secondary combustion (Br, I, some S, Se), Cl/marine (Cl, some Na), crust heavy (Rb through U), Ca, and industry (As, Sb, some Pb). All these factors are confirmed graphically, except for possibly the industry or smelter source.

How do the compositions of the factors compare with compositions of the corresponding graphical components? Quite well, it seems, at least for elements in their major sources. To convert factor loadings based on logs of concentrations to masses, one need only multiply together the loading for an element, the exponentiated standard deviation of that element's (logarithmic) concentrations in the lichen samples, and the exponentiated average of the element's (logarithmic) concentrations in the samples. One must be careful, though, to always choose the appropriate factor solution to use for this task. For pollution elements and lichen elements, the eight-factor solution is appropriate, whereas for crustal elements, the three-factor solution is probably best (so as to avoid splitting the crustal factor into heavy and light components). Figures 3 and 7 show two of the better cases, K and V-Ni. For K, I had originally visually estimated the K/Al of its atmospheric component to be about 0.6; factor analysis with three factors subsequently gave 0.45. I had visually estimated the K/P ratio in lichens (the right-hand tail) to be about 3; the eight-factor analysis gave 2.3. On the V-Ni plot, I had estimated the Ni/V ratio in the oil tail to be 0.28; the eight-factor analysis gave 0.28 as well. Thus at least for elements in their major factors, compositions determined graphically and with factor analysis can agree to within 30–40%, and sometimes much better than that. For elements not in their major factors, the situation is less clear-cut and will require careful study.

8. CONCLUSIONS

In brief, this work seems to show that careful graphical analysis of elements in large suites of lichens can distinguish the plant component from the atmospheric component, the soil component from the depositional component within the atmospheric component, the crustal, marine, and pollution components within the depositional component, and at least some types of pollution within the pollution component. Careful factor analysis confirms the plant component, the crustal component but not soil), the marine component, the oil component, the industrial component, and adds a secondary combustion component and a calcium component. These preliminary results are extremely encouraging.

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GEOSTATISTICAL MODELS FOR AIR POLLUTION

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Abstract

The objective of this paper is to present geostatistical models applied to the spatial characterisation of air pollution phenomena. A concise presentation of the geostatistical methodologies is illustrated with practical examples. The case study was conducted in an underground copper-mine located on the southern of Portugal, where a biomonitoring program using lichens has been implemented. Given the characteristics of lichens as indicators of air pollution it was possible to gather a great amount of data in space, which enabled the development and application of geostatistical methodologies. The advantages of using geostatistical models compared with deterministic models, as environmental control tools, are highlighted.

1. INTRODUCTION

Air quality modelling is an essential tool for most air pollution studies. Monitoring data are indispensable for inferring theories or parameters and calibrating or validating computer simulation packages. However, only a well-tested simulation model can be a good representation of the real world, its dynamics and its responses to perturbations. The monitoring design and activities should be integrated with numerical models in order to avoid investments and efforts to collect data that remain unused or even turn out useless.

Hence, air quality modelling is an indispensable tool for prevention and control of air pollution [1], covering the following tasks:

- planning the control of air pollution episodes;
- selecting locations of future sources of pollutants, in order to minimise their environmental impacts;
- assessing responsibility for existing air pollution levels;
- establishing emission control strategies;
- evaluating proposed emission control techniques and strategies.

Air quality models can be divided into physical and mathematical models. The physical models consist on small-scale laboratory prototypes that try to reproduce the real phenomena. The mathematical models are a set of analytical or numerical algorithms that describe chemical and physical processes. Often, the data obtained through physical models may be very useful for understanding the processes and helping the developers of mathematical models. Mathematical models can be subdivided into deterministic and statistical models. A general distinction between deterministic and statistical approaches in air pollution is that deterministic models concept are based on physical principles, while statistical models are characterised by their direct use of air quality measurements to infer statistical relationships. Nevertheless, some deterministic models are based on statistical diffusion theories.

In the category of statistical models are included a large set of approaches namely time series analysis, Kalman filters, receptor modelling techniques and interpolation algorithms. In the last years, the number of applications using geostatistical models applied environmental sciences have been increasing with successful results on modelling soil [2, 3], water [4, 5] and air pollution [6–9].

Geostatistics offers a way of describing the spatial continuity that is an essential feature of many natural phenomena [10]. In the former years, geostatistics was used mainly as an interpolation technique, which took advantage of the ability to incorporate spatial continuity. More recently geostatistical models for the uncertainty assessment have been developed and applied to environment sciences in particular for the polluted sites characterisation. Such models based on the indicator formalism allow the risk evaluation related with the impact of the pollutant [11, 12].

Two practical examples illustrate these geostatistical methodologies. This case study was conducted in an underground copper mine where a biomonitoring programme with lichens has been implemented.

2. BIOMONITORING PROGRAMME

The study was conducted in the area of influence of a large industrial facility that produces copper concentrates from ore, located in southern Portugal. The main air pollution source at mine site is fugitive dust emissions from the concentrate stockpiles with high copper grade and a particle size of about 20 μm.

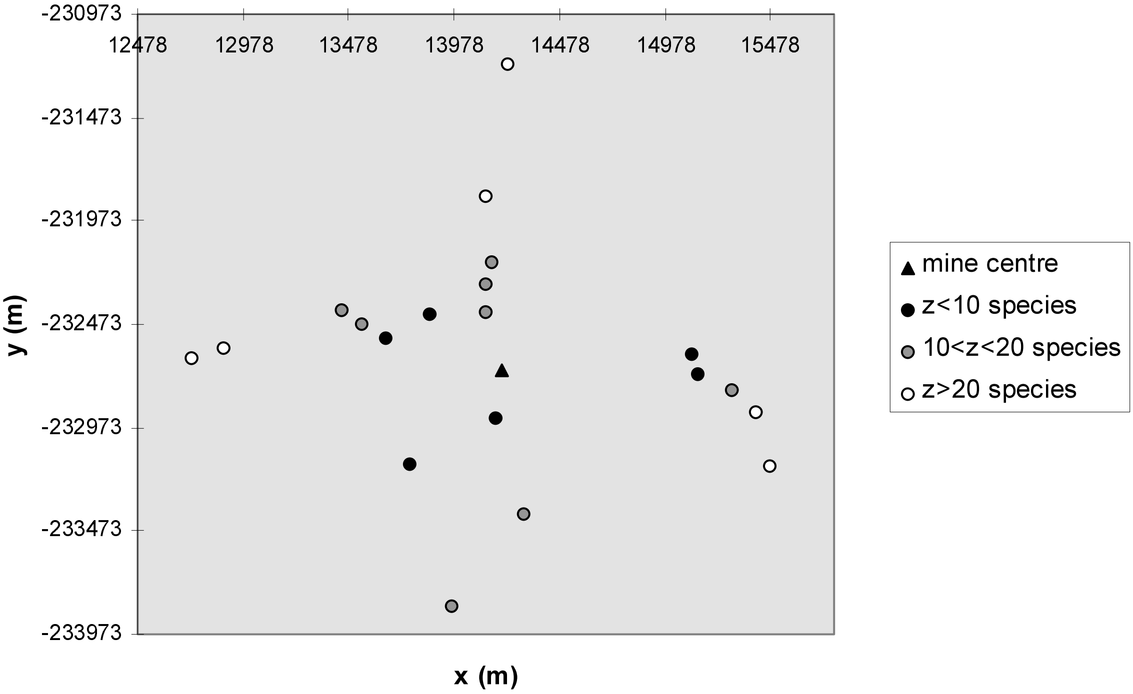
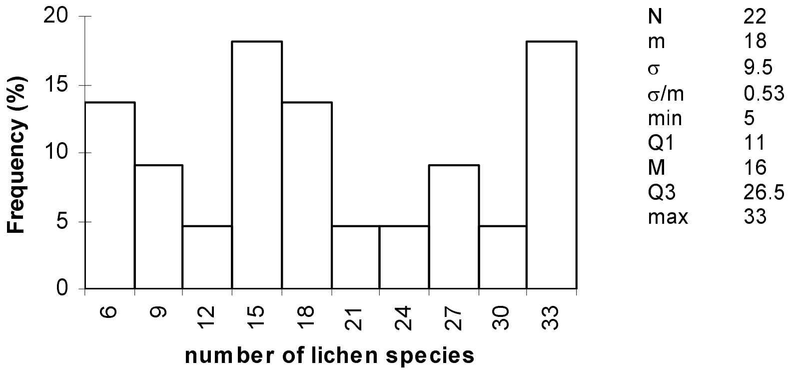


Figure 1. Studied area around the mine site and sampling locations.



N	22
m	18
σ	9.5
σ/m	0.53
min	5
Q1	11
M	16
Q3	26.5
max	33

Figure 2. Histogram and basic statistics of the studied variable (number of lichen species).

Epiphytic lichens show differential sensitivities to air pollution, i.e., the most sensitive lichens tend to disappear from polluted areas whereas the more tolerant species can be seen in areas with moderate pollution emissions [13, 14, 15]. Moreover, since lichens are slow-growing organisms they may be used as long-term integrators of environmental conditions. Because species loss occurs in areas with heavy air pollution, in this work the number of epiphytic lichen species was used, as a direct measure of biodiversity, to evaluate the impact of the copper mine on lichen flora [16]. The lichen biodiversity study consisted in recording at each sampling point the number of epiphytic lichen species from 6-10 Quercus ilex trees and exceptionally from Olea europeae. The sampling was conducted according the pattern shown in Figure 1, in 1993.

The number of lichen species varies from 5 at the centre of the mine (near the stockpile location) to 20-30 species at 2 km from the mine (Figure 2). A detailed study description about the biodiversity can be found in [17, 18].

The relationship between the number of lichen species and their thallus growth forms with distance from the centre of mine confirms that the main source pollution was focused on the centre of mine site [18].

3. GEOSTATISTICS

3.1 Spatial dispersion of lichens

The main geostatistical tool for the spatial continuity characterization of a given attribute is the spatial covariance or the variogram [10]. In fact, spatial features of the attribute like preferential patterns of continuity (anisotropic behaviour), spatial dispersion at diferent scales, etc., can be visualised and quantified by the variogram. In Figure 3, one can see the variogram of the number of lichens species. Two discontinuities reveal two structures at different scales. Up to 400 m from the mine site (1^{st} structure) only crustose lichens occur given their robustness to adverse conditions. Foliose lichens and some fruticose lichens only appear at distances beyond 400 m. More fruticose lichens, such as usnea species, occur beyond the 1200 m (2^{nd} structure).

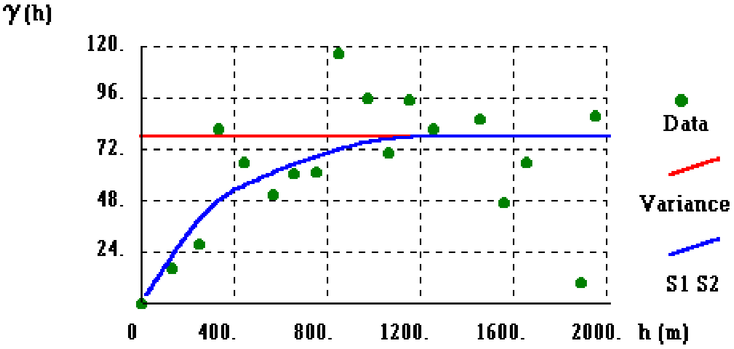


Figure 3. Semi-variogram of the number of lichen species and theoretical model fitted.

The variogram was modelled by a sum of two spherical structures:

$$\gamma(h) = Sph_1(a_1 = 400, c_1 = 50) + Sph_2(a_2 = 1200, c_2 = 40.25)$$

3.2 Estimation of main attribute

The number of lichens was estimated by Kriging in a regular grid of points covering the entire area around the mine, shown in Figure 4.

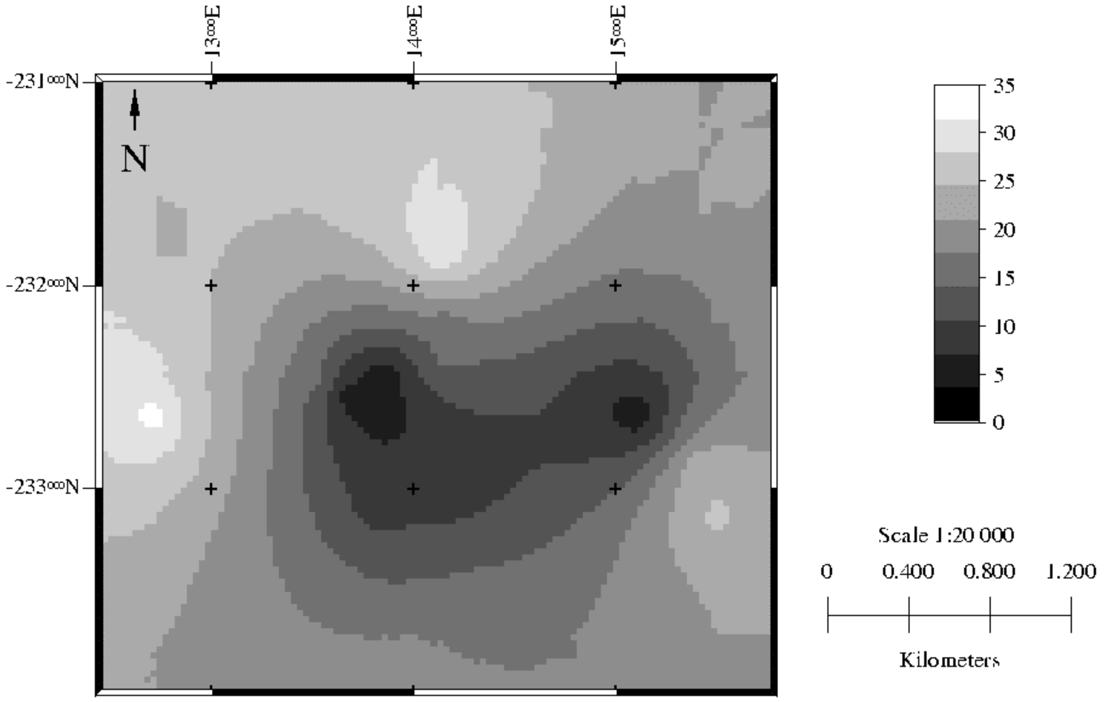


Figure 4. Estimated map representing the number of lichen species around the main mine site.

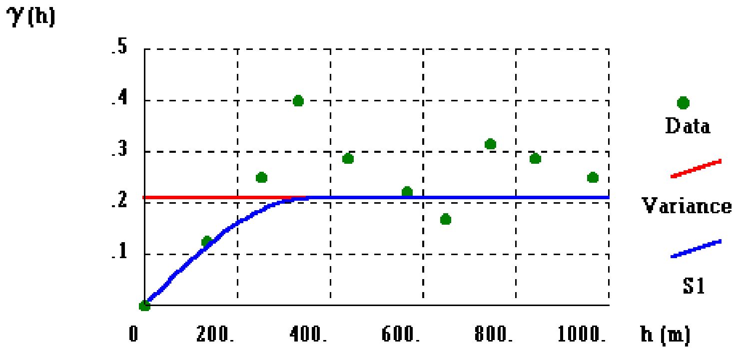


Figure 5. Experimental indicator variogram and theoretical model fitted:
$C_0 = 0$, $C_1 = 0.21$, $a_1=350$ m.

3.3 Uncertainty assessment with indicator formalism

With these models one intend to assess the uncertainty about the unknown, rather than calculating the best estimator of it. In fact, the objective is to estimate the probability of the main attribute $Z(x_0)$ to be lower than a threshold z at a given location x_0, given the sample values $Z(x_\alpha)$, $\alpha=1$, N:

$$\mathrm{Pr}\,ob\ \{Z(x_0) < z \mid Z(x_\alpha),\ \alpha = 1, N\}$$

Different geostatistical models can be used for the estimation of local conditional distribution functions [11, 19]. To estimate these local conditional probabilities one has used the indicator formalism. An indicator variable is obtained through a binary transform of the original variable Z(x) with a threshold z.

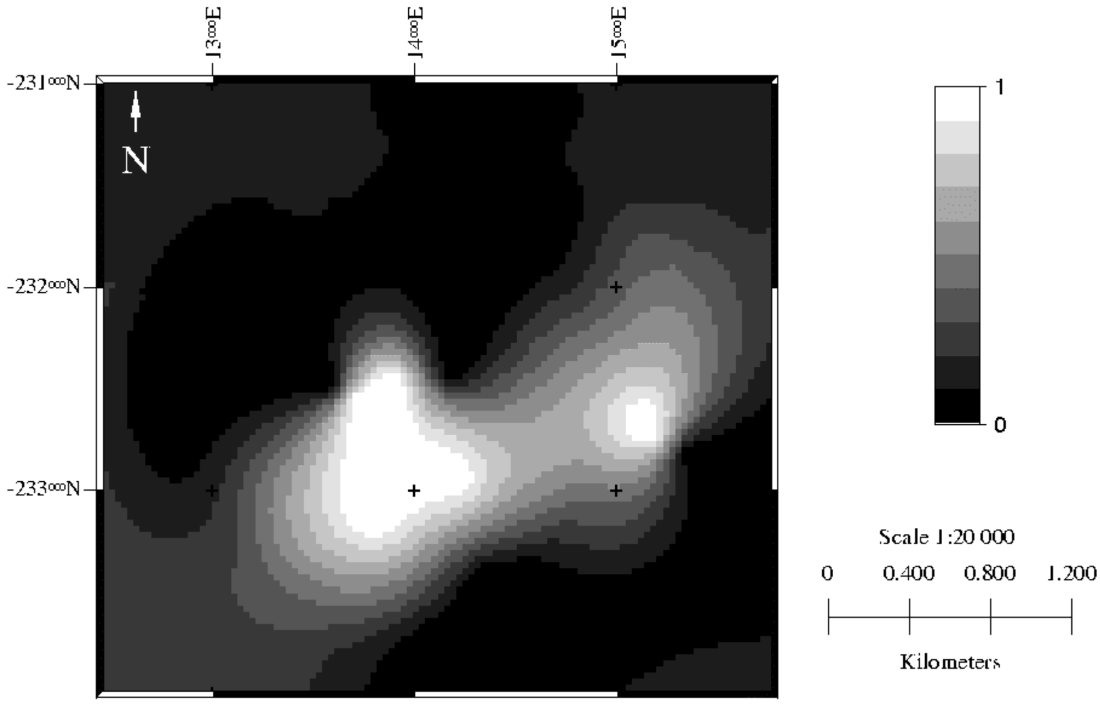


Figure 6. Probability map of occurring less than 10 lichen species.

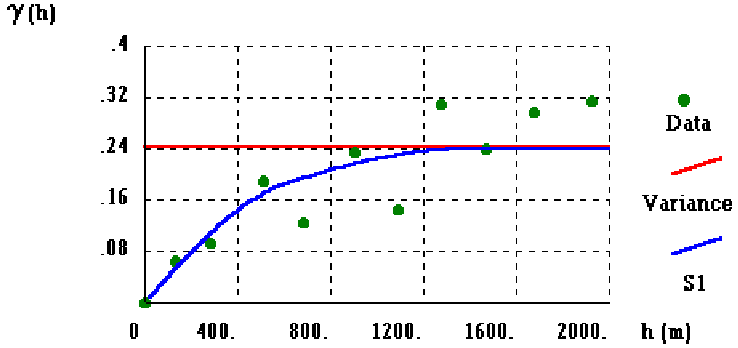


Figure 7. Experimental indicator variogram and theoretical model fitted:
$C_0 = 0$, $C_1 = 0.15$, a_1=600 m.; $C_2 = 0.09$, a_2=1400 m.

$$I_z(x) = \begin{cases} 1 & \text{if} \quad z(x) < z \\ 0 & \text{otherwise} \end{cases}$$

In the sample locations, $I(x_\alpha)$, α=1,…N has the meaning of the probability of sample x_α to not exceed the threshold z:

$$I(x_\alpha) = prob\{z(x_\alpha) < z\}$$

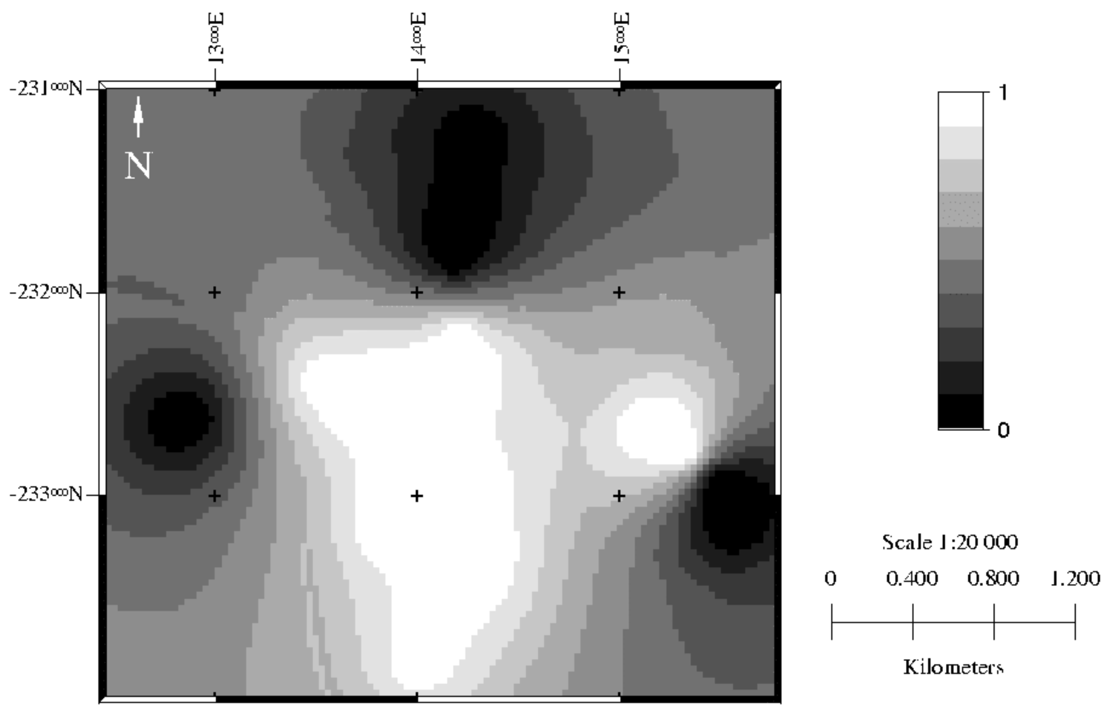


Figure 8. Probability map of occurring less than 20 lichen species.

The indicator Kriging of any point x_0 is an estimator of the local conditional probability of x_0 to exceed z.

$$[I(x_\alpha)]^* = \sum_\alpha \lambda_\alpha I(x_\alpha) = [prob\{z(x_0) < z \mid (I(x_\alpha), \alpha = 1, N\}]$$

In the present case study one intend to calculate, just for illustrative purposes, the areas below and above the threshold 10 and 20 (number of identified lichen species).

3.3.1 Threshold of 10 lichen species

For the threshold 10 (lichen species) the following indicator variable was defined at experimental sampling points (see Figure 1):

$$I_z(x) = \begin{cases} 1 & if \ \mathrm{Z(x)} \leq 10 \\ 0 & if \ \mathrm{Z(x)} > 10 \end{cases}$$

The indicator values have the following statistics:

$$m_{I10} = 0.30 \qquad \sigma_{I10}^2 = 0.21$$

The calculated omnidirectional variogram (see Figure 5) reflects basically the shape of the affected vegetation coverage around the mine site. A spherical model with 350 m of range and null nugget effect was fitted to the experimental variogram.

Figure 6 represents the map of probabilities of occurring less than 10 lichen species. The uncertainty related with the impact of dust emissions around the mine can be visualised with this map or other identical maps using different thresholds.

3.3.2 Threshold of 20 lichen species

For the threshold 20 (lichen species) the following indicator variable was defined at experimental sampling points (see Figure 1):

$$I_z(x) = \begin{cases} 1 & if \quad Z(x) \leq 20 \\ 0 & if \quad Z(x) > 20 \end{cases}$$

The indicator values have the following statistics:

$$m_{I20} = 0.64 \qquad \sigma_{I20}^2 = 0.24$$

A sum of two spherical models with respectively 600 m and 1400 m of range and null nugget effect was used to fit the experimental omnidirectional variogram (see Figure 7).

Figure 8 represents the map of probabilities of occurring less than 20 lichen species.

4. CONCLUSIONS

The lichen biodiversity sampling enabled the development and application of geostatistical methodologies to spatially characterise the air quality. It is important to remark, however, that lichens are slow growing organisms and they must be used only as long term integrators of the environmental conditions.

Output maps of estimated number of lichen species represent the average air quality during a long period of time. Maps of estimated probabilities of occurring less than a certain number of species (i.e., a given threshold) represent the uncertainty related with the impact of dust emissions around the mine.

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LARGE SCALE AIR MONITORING: BIOLOGICAL INDICATORS VERSUS AIR PARTICULATE MATTER

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Abstract

Biological indicator organisms are widely used for monitoring and banking purposes since many years. Although the complexity of the interactions between bioorganisms and their environment is generally not easily comprehensible, environmental quality assessment using the bioindicator approach offers some convincing advantages compared to direct analysis of soil, water, or air. Direct measurement of air particulates is restricted to experienced laboratories with access to expensive sampling equipment. Additionally, the amount of material collected generally is just enough for one determination per sampling and no multidimensional characterization might be possible. Further, fluctuations in air masses have a pronounced effect on the results from air filter sampling. Combining the integrating property of bioindicators with the world wide availability and uniform matrix characteristics of air particulates as a prerequisite for global monitoring of air pollution will be discussed. A new approach for sampling urban dust using large volume filtering devices installed in air conditioners of large hotel buildings is assessed. A first experiment was initiated to collect air particulates (300 to 500 g each) from a number of hotels during a period of three to four months by successive vacuum cleaning of used inlet filters from high volume air conditioning installations reflecting average concentrations per three months in different large cities. This approach is expected to be upgraded and applied for global monitoring. Highly positive correlated elements were found in lichen such as K/S, Zn/P, the rare earth elements (REE) and a significant negative correlation between Hg and Cu was observed in these samples. The ratio of concentrations of elements in dust and *Usnea spp.* is highest for Cr, Zn, and Fe (400 - 200) and lowest for elements such as Ca, Rb, and Sr (20-10).

1. INTRODUCTION

Bioindicators are organisms or organs of such organisms that respond to a certain level of pollution by a change in their life cycle or accumulation of the particular pollutant. They reflect, contrasting to direct analysis, *complex effects* of harmful substances, as such organisms not only show synergistic effects of a sum of parameters, but also reflect a time-integrated picture of the history of their life span [1]. Another advantage is the selective uptake of such substances, as an organism exposed to an environmental pollutant, either through air or direct uptake, absorb the bioavailable fraction only, and hence reflect readily the portion of the substance which might be hazardous to man as well.

Bioavailability is generally accepted as the extent and the rate of absorption of matter and its availability at the site of reaction [2]. Chemicals can be accumulated in organisms via the direct uptake from the surrounding medium (e.g. air, water) by gills, skin, stomata etc. or by ingestion of particle-bound chemicals (bioconcentration, or bioaccumulation) as well as via food chain following various pathways along different trophic levels (biomagnification). These processes will not always manifest themselves as direct adverse effects, e.g. mortality, but complex phenomena may occur, e.g.

reduced fertility, malformation of offsprings, decoloration of leaves and dwarf growth, which constitute a risk potential for humans and the environment [3].

Many examples are known where indicator organisms or communities have been used to assess environmental quality by phenomenological description of their appearance, shape or behavior [4-8]. More interesting with respect to monitoring, however, is the analysis of the concentration of a particular substance accumulated by the organism and the assessment of trends following environmental input or exclusion of this material. Early recognition of compositional disturbances of our environment sometimes can only be achieved using the accumulation and magnification properties of food chains by sensitive and accurate analysis of parts of these bioindicators. Long term specimen banking programs as the one going on since 1985 at the Environmental Specimen Bank of Germany at the Research Center in-Juelich help to maintain continuity in sampling, processing of samples and the analysis of hazardous substances in order to elucidate such trends [9]. Carefully selected biological indicators for such programs assure a meaningful data interpretation and could even serve as pieces of evidence in future trials as for the responsibility in a certain case of pollution or the effect of a legislative action.

The main criteria for the selection of bioindicators include the wide-spread availability, some information about the dose/response relationship and the accumulation properties of the species.

A more serious aspect in the selection of any indicator organism is its selectivity for the absorption of environmentally interesting substances. It should have a certain tolerance and respond more or less linearly to the increase or decrease of that substance's concentration. If a lethal threshold concentration in the environment is exceeded no further material can be sampled for investigation. Therefore, well adapted and dominant biological specimens should be preferred to more occasional species even if they accumulate to a lower extent. Additionally, nature conservation aspects should not be violated.

Finally analytical aspects as to the possibility to produce a homogeneous sample by milling, grinding or sieving or the digestion properties of the material should be mentioned. If a particular organ of an organism should be targeted (e.g. liver or kidney of mammals or fish) proper dissection and separation from adhering material is mandatory.

The analysis of air particulate matter in principal does not raise any serious problems to the analyst, particularly when nuclear analytical methods are available. Here the sampling of adequate amounts of material is the critical factor. Filters for air dust sampling can introduce contamination especially when small volume samplers are used. Multidimensional characterization of air filter materials is hardly possible as the few milligrams collected are consumed in one analytical run. The interest in organic, inorganic and radioisotope analysis from the same sample urged us to look out for high volume sampling facilities which might be available in many places around the world to be used for the collection of large amounts (up to 500 g) of air particulate matter. The air conditioning in large hotels is one possible source for the collection of adequate quantities of material suitable to be used for air quality assessment. By simply vacuum cleaning of the filter supporting material from such large volume air filtering devices within a period of four to twelve weeks an amount of 300 to 500 g of material can be collected. By appropriate selection of the hotels a more or less world-wide net of sampling stations, - both urban sites as well as holiday resorts at remote places - can be used for sampling.

2. MATERIALS AND METHODS

Usnea spp., a fruticose lichen hanging from trees in bundles of thalli is abundant in almost all continents and remote areas of the world having moderately humid climate. Particularly in elevated mountainous regions it can be found and collected without risk of contamination from the host tree. As lichens possess no roots they totally rely on nutrient uptake from air constituents (wet or dry). Samples of *Usnea* were collected in Siberia, near Lake Baikal and in Calgary, Canada, in boreal forests and in Sri Lanka in a mountain rain forest at about 1200 m above sea level. In the Bavarian

Forest, Germany, a sample was taken by the local forestry personnel, air dried and sent to the laboratory in Juelich, where the milling and analysis of all the samples were performed.

All samples, including the reference material IAEA 336 were acid digested and analyzed using a PE ELAN 5000 ICP-MS and a PE Optima-500 ICP-AES system. The analytical parameters and settings used are described elsewhere [10].

Air dust samples were collected from the air purification device at the Atominstitut in Vienna and from the air conditioning system at the Hilton Hotel in Antananarivo, Madagascar. Vacuum cleaning of the filtering mat yielded enough material to determine inorganic, organic and radio nuclide contaminants. The material was sieved (through 70 mesh) and encapsulated for neutron irradiation with 10^{13} n cm^{-2} s^{-1} at the research reactor DIDO in Juelich. INAA was performed according to the procedure described in [11].

3. RESULTS AND DISCUSSION

3.1. Lichen

From the many elements determined in the lichen *Usnea spp.* only a few can be presented and discussed here for lack of space. In Figures 1 and 2 some environmentally relevant elements are displayed. It can be seen that for Cu, Zn, Cd, Sn, Pb, and Th concentrations in the sample from Bavarian Forest far exceed the results from the other samples. Only Ba is higher in the Canadian lichen and Hg tends to be highest in the Siberian sample. Co as well as some rare earth elements (not shown here) are highest in the sample from Sri Lanka. This might cause the distinct difference in grouping of northern and southern hemisphere samples in the discriminant analysis as shown by the dendrogram in Figure 3.

Many highly correlated elements were found and some examples are displayed in Figures 4-6. Potassium correlates to sulfur in our samples at a very high significant level as does Nd to Ce and Zn to P also. The only negative correlation we found is between Hg and Cu as shown in Figure 7. Reports of a negative correlation between the two elements could not be found in the literature so far.

3.3. Urban dust

The comparison of results from Austria, Madagascar and the certified reference material NIST 1648 is shown in Figure 8 in logarithmic scale. The NIST material, originating from St. Louis, MO, seems to be highest for As, Se, Br and Ag whereas the material from Madagascar tends to show elevated levels of Na, Rb, Sr, REE, and Th respectively. The origin of the different pattern of trace constituents can be elucidated only after more relevant data are collected and statistically evaluated. The comparison of results from PAH analysis clearly shows a much lower burden of the Madagascar sample compared to Austrian material with such residues from fossil fuel burning (see Figure 9).

3.3. Comparison of results from lichen and dust samples

If the range of concentrations found for various elements in dust and lichen samples is displayed (Figure 10) it becomes obvious that the analytical results differ by nearly two orders of magnitude in the two distinct materials. The ratio of mean concentrations in both materials are shown in Figure 11. Cr, Zn, and Fe are enriched in urban dust by a factor of 200 to 400 and Ca, Rb, and Sr by a factor of 10 to 20 only.

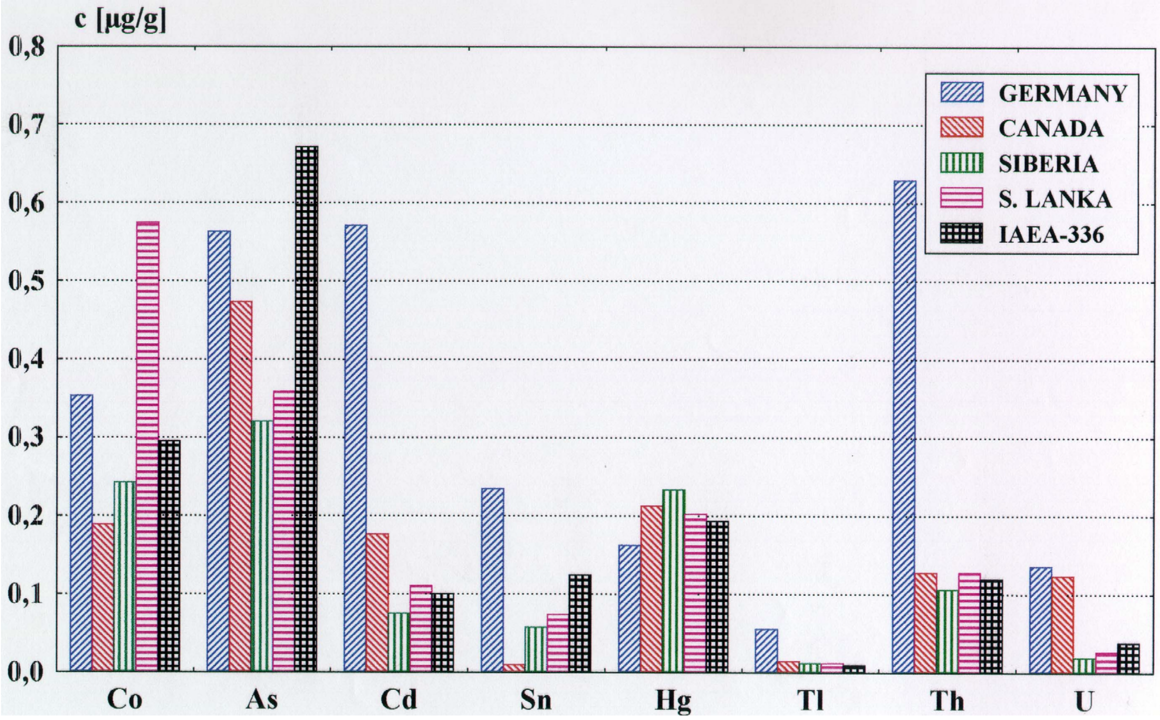

Figure 1: Selected trace elements in lichen (Usnea spec.) from different places of the world.

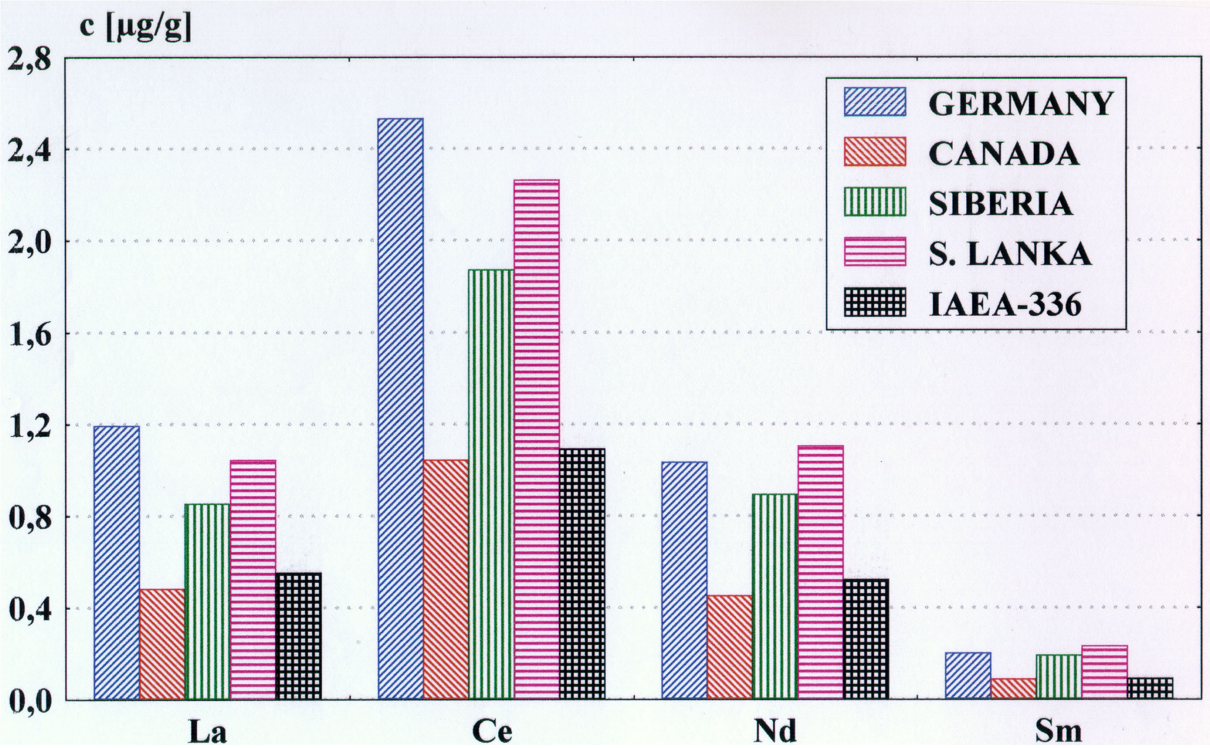

Figure 2: Selected trace elements in lichen (Usnea spec.) from different places of the world.

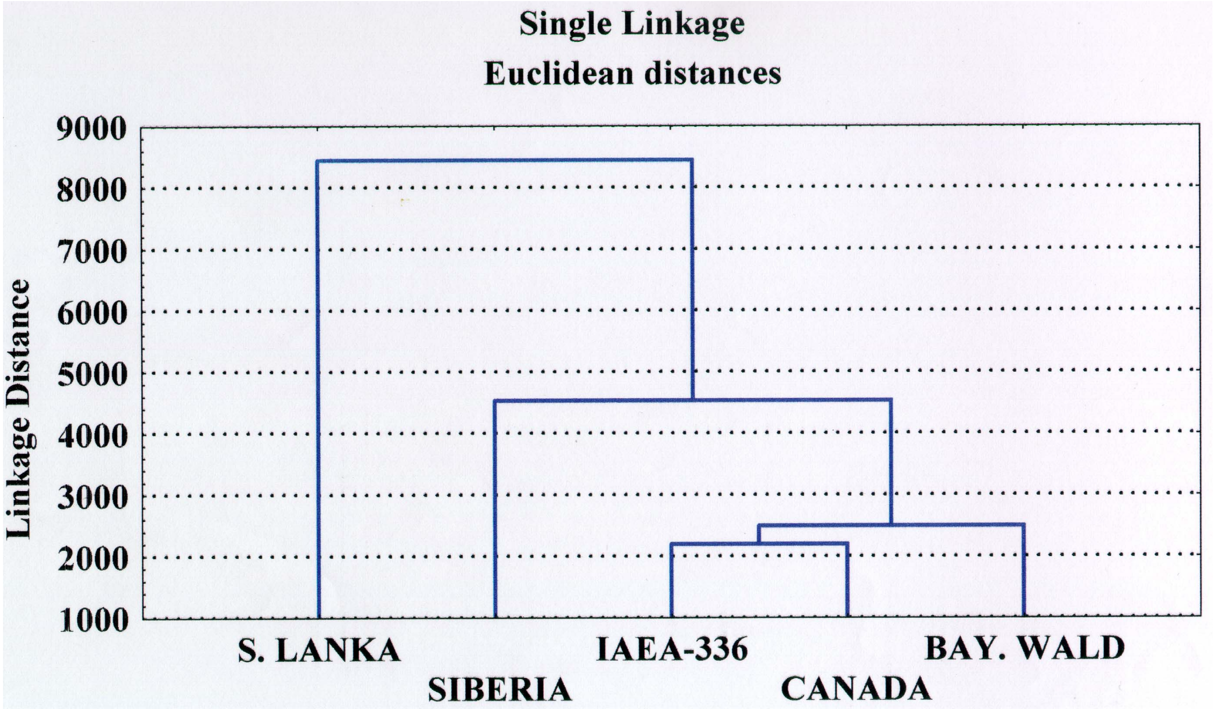

Figure 3: Dendrogram of the discriminant analysis using all elemental results in lichen from various places.

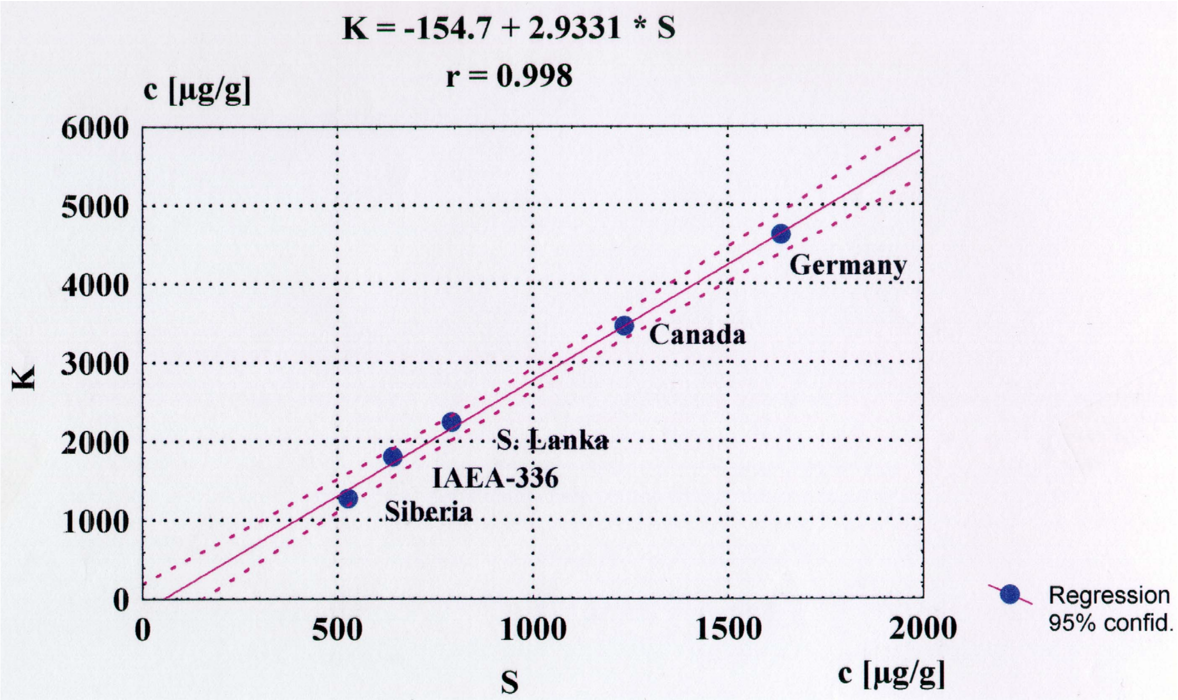

Figure 4: Correlation of K and S in lichen from various places of the world.

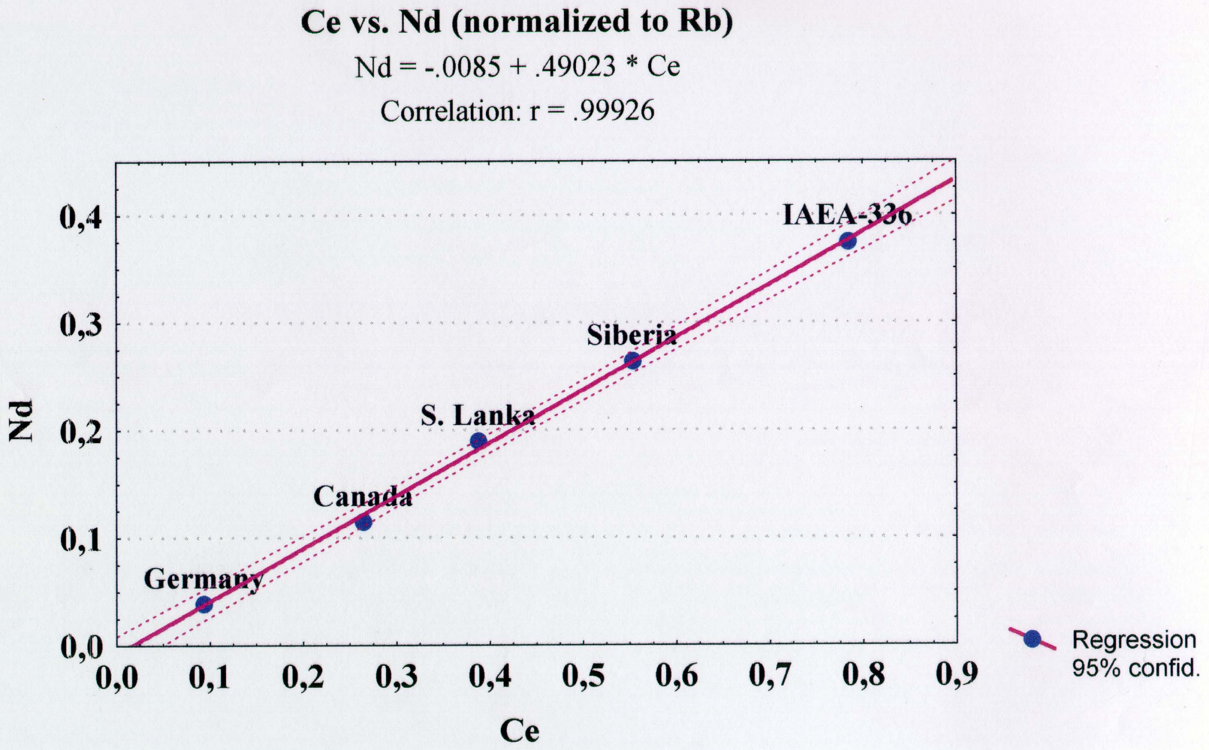

Figure 5: Correlation of normalized (Rb) lichen data for Ce and Nd.

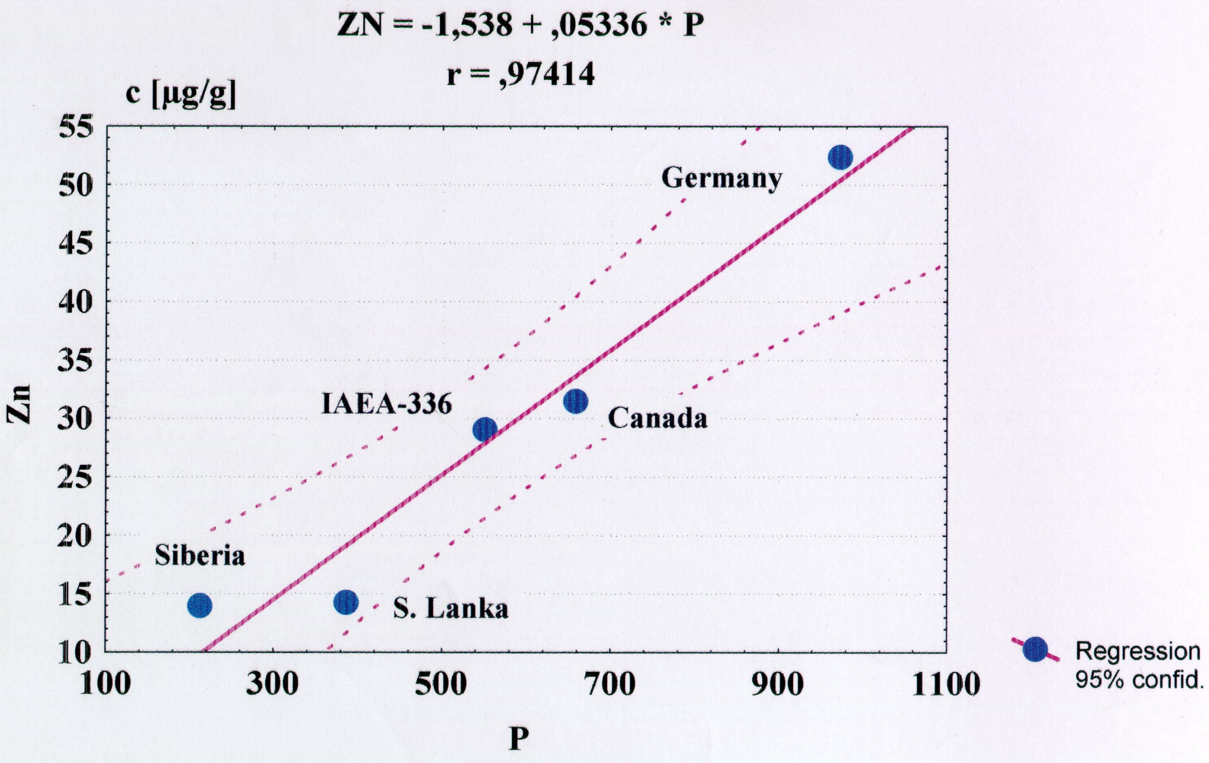

Figure 6: Correlation of Zn and P in lichen from various places of the world.

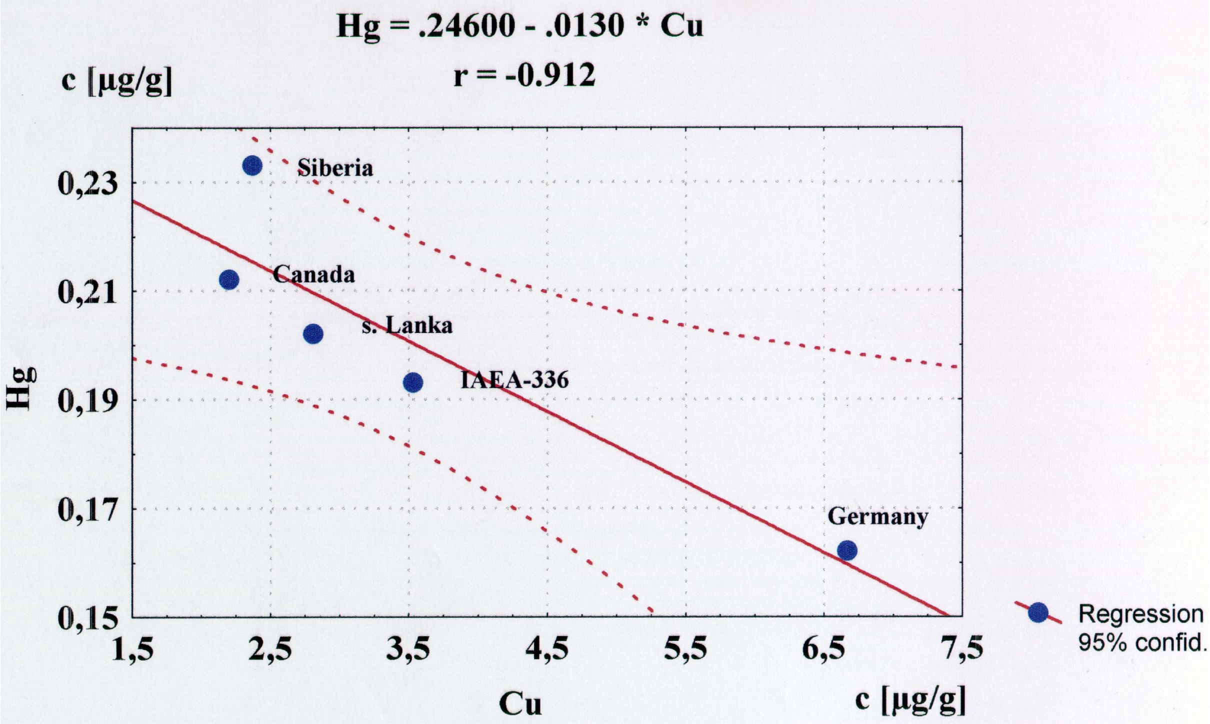

Figure 7: Negative correlation of Hg and Cu in lichen from various places of the world.

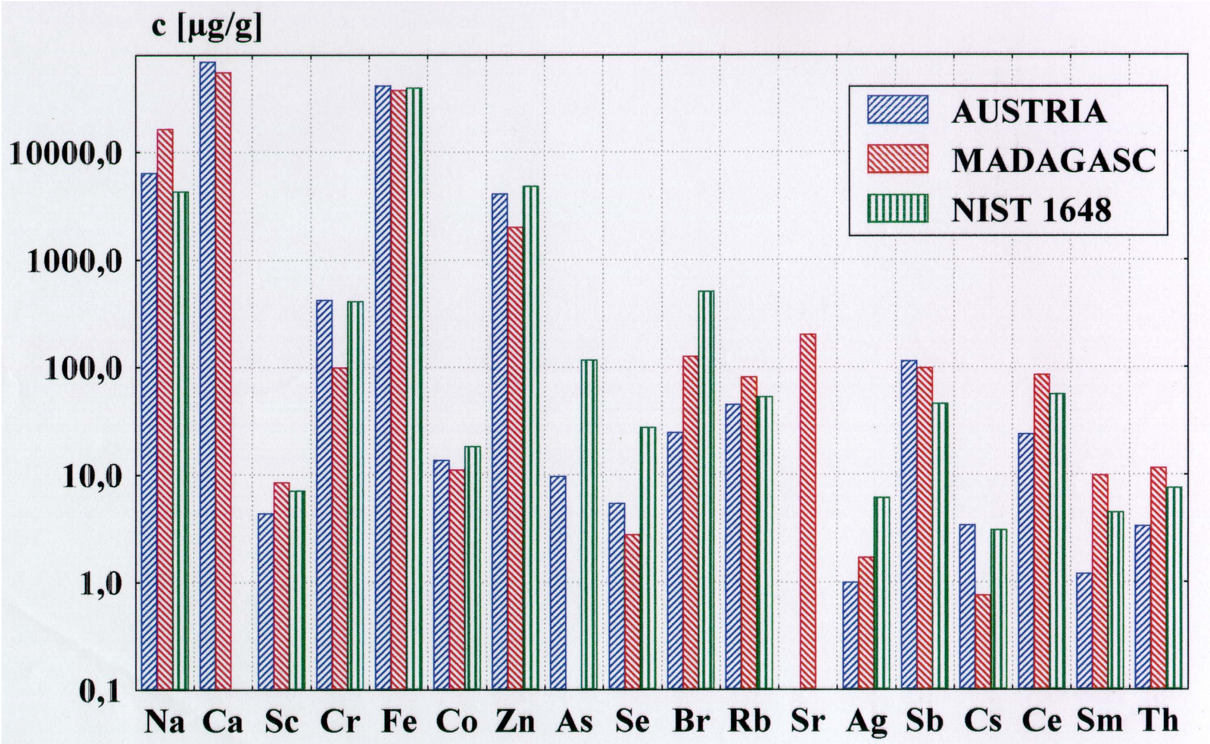

Figure 8: INAA results in large volume air dust samples from Austria, Madagascar and the USA.

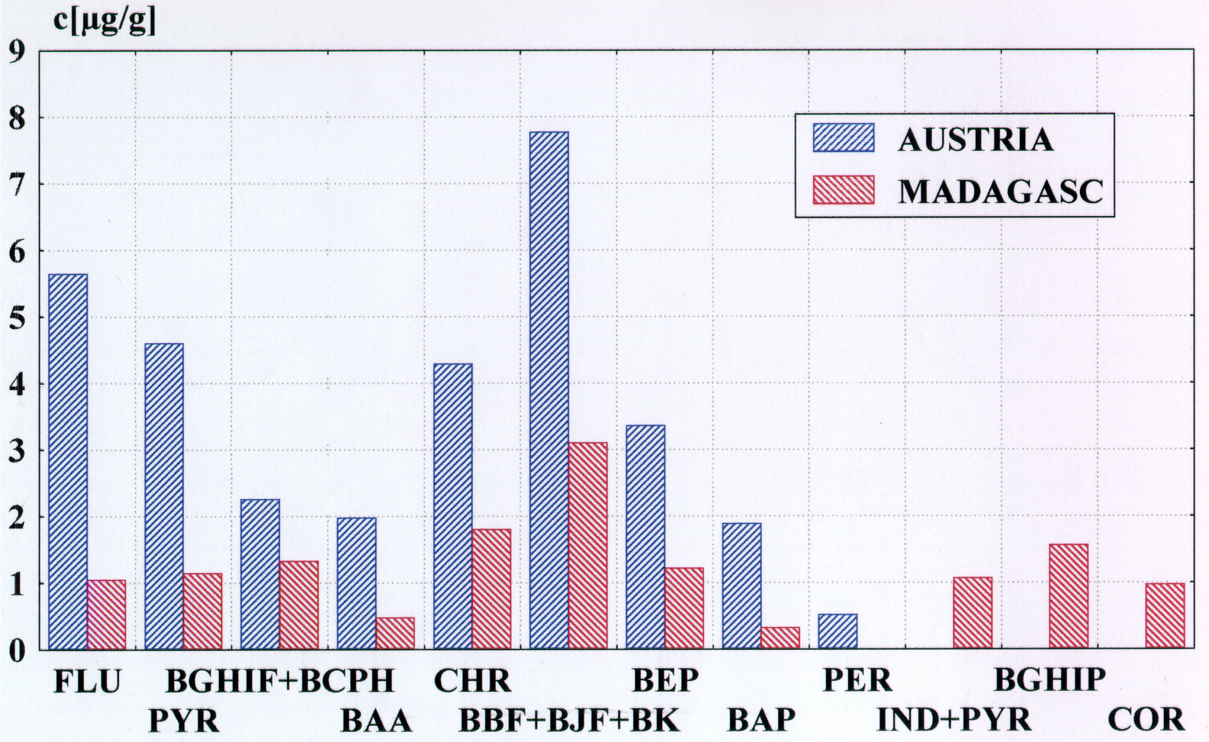

Figure 9: PAH analysis of large volume air dust samples from Austria and Madagascar. (FLU = Fluoranthen, PYR = Pyren, BGHIF+BCPH = Benzo[ghi]fluoranthen + Benzo[c]phenanthren, BAA = Benz[a]anthracen, CHR = Chrysen, BBF+BJF+BK = Benzofluoranthen[b+j+k], BEP = Benzo[e]pyren, BAP = Benzo[a]pyren, PER = Perylen, IND+PYR = Indol + Pyren, BGHIP = Benzo[ghi]perylen, COR = Coronen).

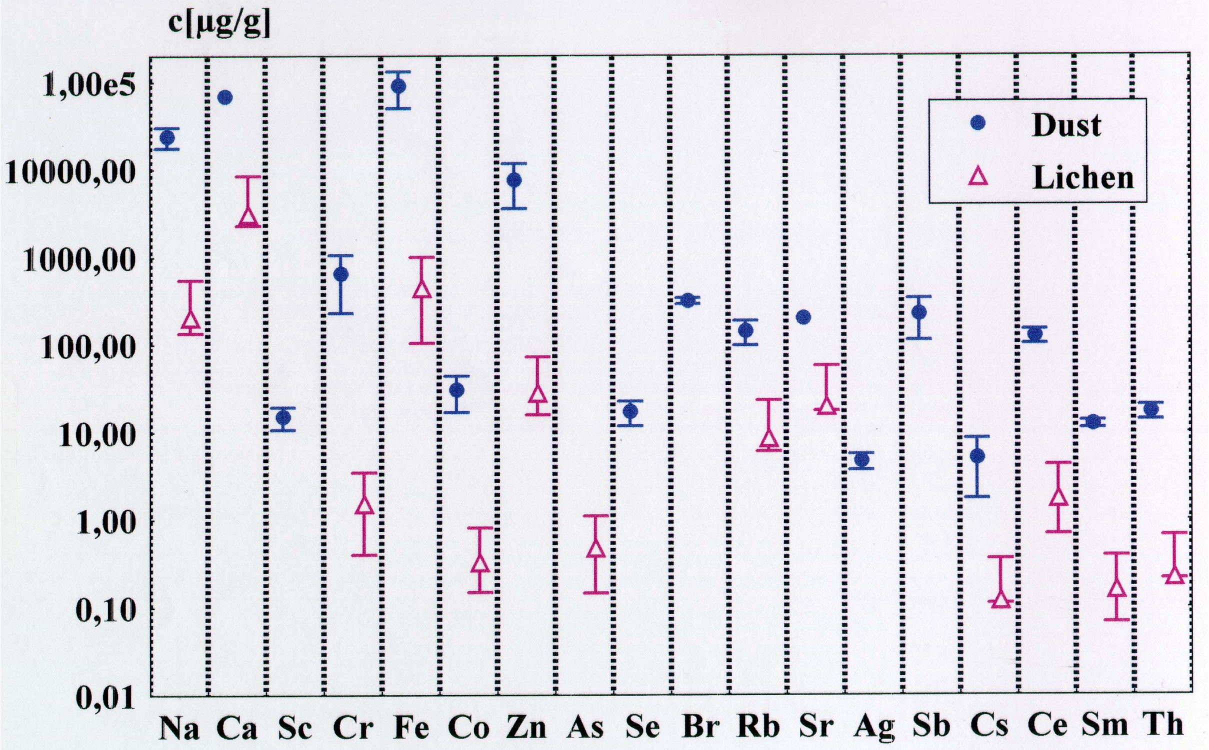

Figure 10: Range of element concentrations determined in lichen and dust samples in logarithmic scale.

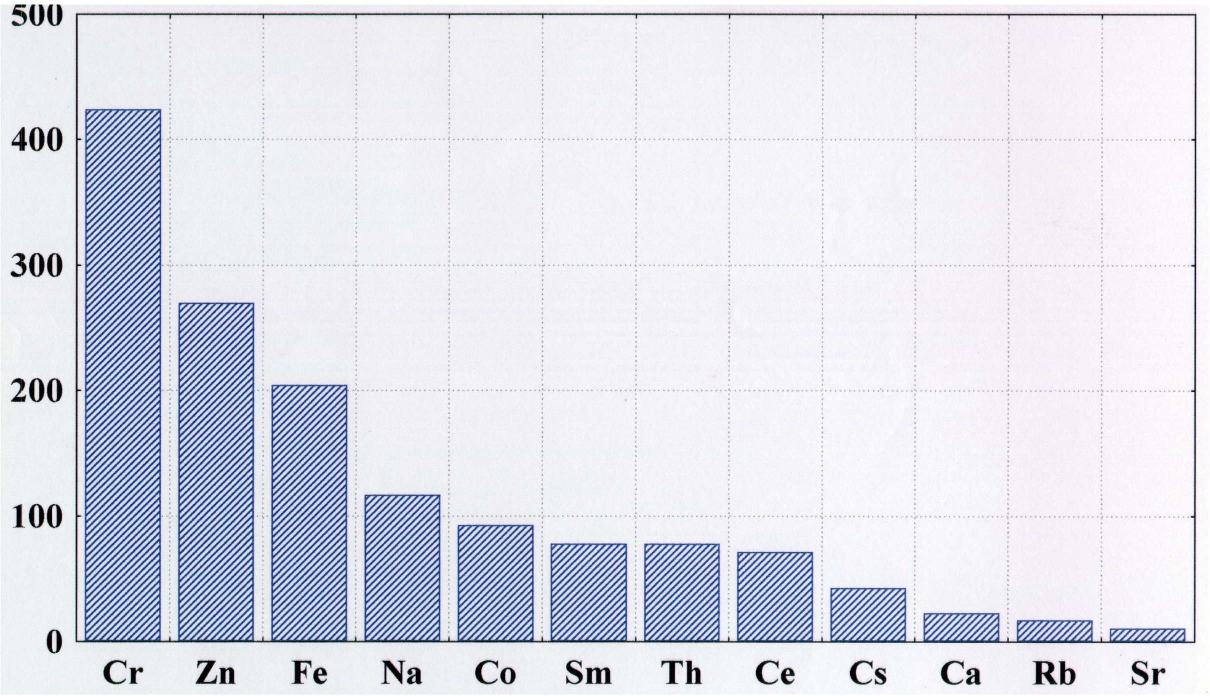

Figure 11: Ratio of mean concentrations in dust and lichen materials.

4. CONCLUSION

In a world of increasing communication and transportation facilities, collection of lichen samples from remote areas around the globe seems to be feasible. Sampling of air dust could be strongly facilitated by implementation of existing air conditioning installations from large hotels. These two materials seem to be complementary from several point of views:

- Lichen can be collected in remote, far from point source pollution areas only and, hence, represent air quality of natural reserve areas. Air dust from air conditioning systems of large hotels in the first place represent the air quality of large cities and is indicating more realistically what is inhaled by a large proportion of human population.
- Air dust as a mixture of soot, soil and aerosols in varying proportion is very much influenced from weather conditions and collection procedures whereas lichens absorb constituents from wet and dry precipitation more or less constantly during their entire life cycle. The contaminants and nutrients are embedded into a biological matrix. Excretion is negligible.
- Dust concentrations can be directly related to m^3 of air (using the capacity and throughput of the air conditioning system) whereas concentrations found in lichen can only be qualitatively related to the surrounding air masses. Although the argument of „the bioavailable fraction of trace elements determined in bioindicators“ is frequently cited, it seems somewhat questionable if this can be applied in the case of lichen.
- Lichen as well as air filter dust samples can be used for the assessment of organic and radionuclide air pollution. These two groups of contaminants are of increasing importance in air quality monitoring programs.

The combination of both, the biomonitoring approach as well as the use of air particulates for air quality monitoring opens the opportunity to assess and evaluate our findings from various locations and regions around the world. Whereas lichens seem to reflect the background levels from remote areas influenced only by long-range transport of trace contaminants, the dust samples, however, if taken from the city centers, reflect the highly contaminated, densely populated areas. Only the combination of these informations will give a full picture of the span of concentrations and the magnitude of burden in the vicinity of point source emission. Further experiments to expand the applicability of this approach are in progress.

ACKNOWLEDGEMENT

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MEAN ANNUAL RESPONSE OF LICHEN *PARMELIA SULCATA* TO ENVIRONMENTAL ELEMENTAL AVAILABILITY

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Abstract

Lichens collected in an area previously identified as unpolluted, were transplanted to six different places located in polluted areas near Power Plants (both fuel and coal powered). A total of 26 lichen transplants were made for each place, each transplant weighing about 2g. Two were analysed as zero or reference and the remain 24 were hanged in nylon net bags in order to be able to collect two transplants each month, out of every station during a one year period. Besides the 24 lichen samples, each station was provided with two total deposition collection 10 litter buckets (with 25 cm diameter funnels) and an aerosol sampler. Concentration in both lichens and aerosols were measured by PIXE and INAA at ITN. Total deposition residues were analysed by ICP-MS at the The Netherlands Geological Survey. On this work we present the results obtained by looking for correlation between lichens elemental concentrations and annual averages of elemental availability variables such as concentration in suspension in the atmosphere and concentration in total deposition samples, for a total of 40 elements. In order to access both the limitations and the reliability of the results a discussion on the details of handling this data set is presented. A mathematical function which tentatively represents the lichen up-take response to water availability is also proposed.

1. INTRODUCTION

The study of lichen response to transplant procedures to a new real environment condition is strongly related to the interpretation which might be made of big areas biomonitoring survey results. Nevertheless, lichen uptake characteristics are, most of the times, studied under laboratory conditions. In this work we followed the procedure used by Sloof and Wolterbeek [1] but tried to be as thorough as possible in what physical variables are concerned. We also tried to extend the study to as many elements as possible. We verified that most of the elements correlate better to deposition but a few like lead and sulphur correlate to other variables like the amount of element in suspended particulate matter. It is shown that simple linear models do not describe well enough the mean lichen concentration values over the whole year. A mathematical model was then introduced to describe the effect of rainfall on the lichens response to metal availability in environment. This model will also be presented and shortly discussed.

2. THE EXPERIMENT AND DATABASE

For this experiment 26x6 transplants of lichen *Parmelia sulcata* were prepared. Lichens were collected in one site previously identified as scarcely polluted [2, 3]. The selected lichen substrate was Olive tree and all lichens were picked-up at a distance between 1 and 2 meters above the ground. Lichens still on bark substrate were then mounted in nylon net bags and kept in the laboratory while waiting to be exposed in the polluted area. For logistic reasons, experimental sites were selected nearby power plants except for one which was *a priori* assumed as a remote clean air environment site. At each site 24 lichen transplants were suspended. The additional 2 lichen transplants from the set were at the same time separated and prepared for analysis to provide reference values. Afterwards, at intervals of approximately one month, two additional lichen samples were brought to laboratory and prepared for analysis. In Figure 1 we indicate the location of the six sites in the Portugal map: Tapada do Outeiro (TAP), Carregado (CAR), Palmela (PAL), Faralhão (FAR), Sines (SIN) and Monchique-Foia (MON). In each site two buckets with 25 cm diameter funnels were installed for total deposition collection. The month average of water volume collected in each station during the experiment was calculated and divided by the funnel inlet area. The month average of rain fall values thus obtained for each station are presented in brackets. Besides lichens and total deposition, also airborne particles were collected. A Gent Aerosol Sampling Unit [4] which allows the collection of particles in two separated filters by using NILU Stacked Filter Units was used. Air filtering conditions were set to 17 l/min thus providing a separation at 2.5µm E.A.D. (equivalent aerodynamic diameter). From this point forward we will refer to particles above 2.5µm E.A.D. as coarse particles and to those bellow 2.5µm E.A.D. as fine particles. The separation process allows for each of these types to be collected in a different filter.

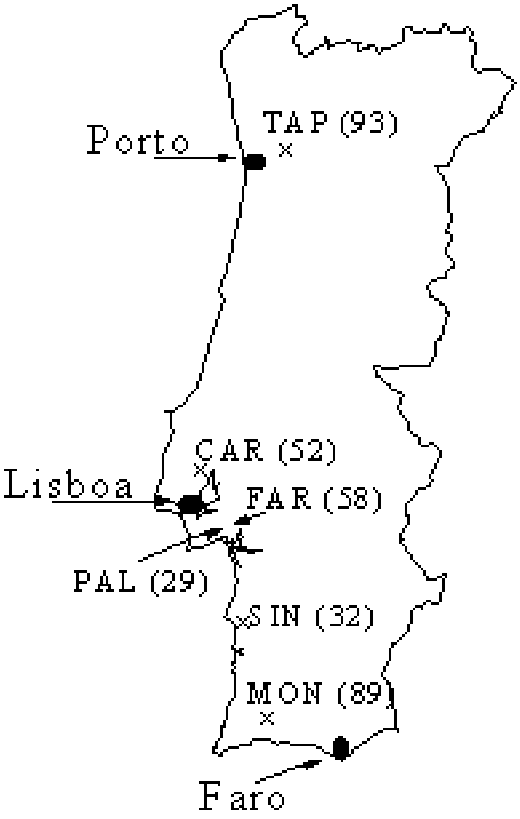


Figure 1. Portugal map. The location of the 6 sampling sites is shown. The first three letters of the location name were selected to identify the site. Thus TAP - Tapada do Outeiro, CAR - Carregado, PAL - Palmela, FAR - Faralhão, SIN - Sines and MON- Monchique (Foia). In brackets we present the month average amount of rain measured at each station in lm^{-2}.

The air was pumped through the system 10 minutes out of every two hours, thus making a total of 14 hour sampling during each week after which the filters were replaced by new ones. In a recently presented work [5] a comparison between this type of sampling and

TABLE I: ELEMENTS DETERMINED BY EACH ANALYTICAL TECHNIQUE AND DATA TYPE. THE LETTERS STAND FOR P - PIXE, I - K_0INAA, IC - ICP/MS. N.SP. IS THE NUMBER OF SAMPLES AND NON.0 THE NUMBER OF POSITIVE DETERMINATIONS OF ELEMENT CONCENTRATION IN THE SAMPLES. THE RIGHTMOST TWO COLLUMNS INDICATE THE ELEMENTS FOR WHICH MORE THAN 70% OF POSITIVE DETERMINATIONS WERE FOUND FOR TWO TYPES AND FOR ALL TYPES OF DATA, RESPECTIVELY. THE LETTER REFERS TO THE TECHNIQUE THAT PROVIDES THE RESULTS

	Lichens				Aerosol				Total		Two T	All T
Elem	Total	PIXE	Total	k_0INAA	Total	PIXE		k_0INAA		ICP		
	N.Sp	Non 0	N.Sp	Non 0	N.Sp	Non 0	N.Sp	Non 0	N.Sp	Non 0		
Li									136	116		
Be									136	129		
B									136	126		
Na			148	145			74	64	136	117	I,IC	
Mg	144	61	148	132	237	52			136	129	I,IC	
Al	144	121	148	146	237	214			136	129	P,I,IC	P,IC
Si	144	139			237	227					P	
P	144	139			237	64						
S	144	139			237	227					P	
Cl	144	139	148	144	237	227					P	
K	144	139	148	144	237	226			136	129	P,I,IC	P,IC
Ca	144	139	44	42	237	227			136	123	P,IC	P,IC
Sc			148	144			74	64			I	
Ti	144	139	148	144	237	226			136	105	P,I,IC	P,IC
V	144	84	148	144	237	202					ImP	
Cr	144	110	148	145	237	102	74	34	66	32		
Mn	144	139	148	145	237	186			136	129	P,I,IC	P,IC
Fe	144	139	148	145	237	225	74	65	136	129	P,I,IC	P,I,IC
Co			148	145			74	61	136	124	I,IC	I,IC
Ni	144	139			237	169			136	126	P,IC	P,IC
Cu	144	139			237	190			136	128	P,IC	P,IC
Zn	144	139	148	145	237	225	74	59	136	129	P,I,IC	P,I,IC
Ga									136	128		
As	144	61	148	145	237	49	74	19	84	79		
Se			44	42	237	14	74	15				
Br	144	139	148	145	237	91	74	49				
Rb	144	139	148	145	237	60						
Sr	144	139			237	56			136	129	P,IC	
Y									136	128		
Zr	144	132							136	129	P,IC	
Nb									136	129		
Mo									136	129		
Cd									136	128		
Sn									136	129		
Sb			148	144			74	64			I	
I			148	114								
Cs			148	142			74	47	136	128	I,IC	I,IC
Ba			148	144					136	128		
La			148	145			74	63	136	128	I,IC	I,IC
Ce			148	142			74	57	136	129	I,IC	I,IC
Pr									136	128		
Nd			126	113					136	128	I,IC	
Sm			148	144			74	59	110	84	I,IC	I,IC
Eu			148	145					136	104	I,IC	
Gd									136	128		
Tb			148	144					136	128	I,IC	
Dy									136	128		
Ho									136	128		
Er									136	128		
Tm									136	129		
Yb			148	126					136	129	I,IC	
Lu			148	143					136	128	I,IC	
Hf			148	145					136	129	I,IC	
Ta			148	145					136	129	I,IC	
W									136	129		
Hg			148	123			74	22				
Tl									84	74		
Pb	144	139			237	226			136	129	P,IC	P,IC
Bi									136	127		
Th			148	145			74	51	136	128	I,IC	I,IC
U			148	104			74	19	136	128	I,IC	
Total		2793		4695		3485		812		5964	34	16

24 hour period almost continuous sampling was presented showing that quite similar results can be achieved with both approaches.

The experiment now being reported provided 148 lichen samples (6 stations x 2 lichen per month per station x 13 month (12 + reference) - 8 lichen samples lost at Monchique due to a forest fire), and 136 total deposition samples (because the 12 lichen reference do not have any correspondence). For aerosol particle samples, 47 samples were collected at Tapada do Outeiro, 23 samples at Carregado, 54 samples at Palmela, 52 samples at Faralhão, 46 samples at Sines and 8 samples at Monchique. These provided a total of 230 fine particle samples and 230 coarse particle samples. The aerosol samples, due to logistic reasons were collected in a different way both at Carregado and Monchique. At Carregado, the samplers were set to 10 min. out of every four hours using a two weeks period before filter replacement, and at Monchique the filters were replaced once per month, instead of every week. This was possible at Monchique while keeping the 10 minutes out of every two hours because this was a remote station. The amount of suspended matter at this site was *a posteriori* verified to be a factor of 4 bellow any other station. Nevertheless it is interesting to note that this is not so for total deposition for which this station is not different from the others. The additional fluctuations in the number of samples is due to fortuitous reasons like samplers break down or samples loss.

Once at the laboratory the samples were dealt with in the way we now describe. Lichen samples were carefully removed from the substrate kept during transplant, rinsed for about 30 seconds, freeze dried and grind in a Teflon balls mill at LN temperature. Lichen sample powder was pressed into pellets both for analysis by k_0INAA and PIXE at ITN. For PIXE, radiation damage corrections were taken into account [6]. Short lived nuclides were determined at the facilities of the Hahn Meitner Institute, Berlin. For total deposition samples, the water volume was measured and samples were then dried by exposing them to an infrared lamp. Dry residue samples thus obtained were sent to The National Geological Survey of The Netherlands where they were acid digested and analysed by ICP-MS. The aerosol particle samples were weighed and cut in three parts in a clean laboratory environment. The three parts were obtained by dividing the filter first in two and afterwards dividing one of the halves in two parts. One of the ¼th of the filter was analysed by PIXE at ITN van de Graaff (providing weekly results), and the halves were grouped in month periods and analysed by k_0INAA.

After all these procedures we obtain a database where the worst problem is not the number of values itself. The way data combines, the eventual need to easily verify an individual value, the miscellaneous operations that must be performed and the fact that each individual value must be referenced by a lot of parameters are the relevant problems to be solved. Nevertheless, although the data set as a whole can be considered large, we are in fact dealing with several small sample statistics problems which increases the need for reliable individual data values.

To handle data in such a way that all these considerations are taken into account, and after several types of approaches we decided to create a database structure where each element data is stored in an individual file. These files are also different for different types of sample, for different sampling place reference and for different type of data (for instance, dry residue concentrations will be in a different file from total deposition concentrations). This approach provided a total of 2731 files.

A specific Turbo Pascal computer program under object oriented philosophy was developed so that a great deal of operations can be performed in many files only by writing a small command file which is then processed. The object oriented philosophy chosen for the program reveals itself very important mainly when increasing complexity arises and mixed file operations are required. Using this approach the process of obtaining results such as those presented in Table I, becomes easy and easily back traceable.

In Table I the number of determinations per each element is presented. From this table, it becomes clear the complexity of the data base. On the left column we can see that a total of

61 elements were measured in at least one type of sample and by one technique. On the two rightmost columns a short description on the types of measurement available for each element is presented.

The **two T** column resumes the elements for which there are enough measurements in more than one type of sample, and the **All T** column shows the elements for which there are enough measurements in all three types of samples. The letters are used to easily identify which techniques provide the results. We consider that there are enough measurements when we have more than 70% positive determinations.

In the lichen samples a total of 21 elements were measured by PIXE and 35 by k_0INAA. For aerosol samples 22 elements were measured by PIXE (Se was not measured by PIXE on lichen samples) and 17 elements were measured by k_0INAA. The much smaller mass of aerosol samples when compared to lichen samples is in the origin of this decrease in the number of elements measured by INAA. ICP-MS measured a total of 49 elements in total deposition samples.

It is interesting to note that out of the measured 61 elements only 34 have a number of determinations large enough for at least two types of samples. For all the three types of samples only 16 are in this condition, namely: Al, K, Ca, Ti, Mn, Fe, Co, Ni, Cu, Zn, Cs, La, Ce, Sm, Pb and Th. Of these elements 8 are based only on PIXE measurements (Al, K, Ca, Ti, Mn, Ni, Cu and Pb), 6 are based only on INAA measurements (Co, Cs, La, Ce, Sm and Th) and 2 can be based in both techniques (Fe and Zn). This result clearly shows the high level of complementarity between PIXE and k_0INAA techniques.

3. DATA AVERAGES AND CORRELATION

The first and most important aim of this experiment was to study the behaviour of lichens when facing a real (non-laboratorial) polluted environment, in a way similar to what was previously done by Sloof [1].

A priori we expected the lichens to evolve from a situation of equilibrium in a clean environment to a stage of equilibrium in a pollution environment. In another communication also presented at this workshop [7], we propose a model which shows that this is indeed the case. We want then to correlate the lichen concentration in this new state to the elements environmental availability. For that it is essential to have a measure of the concentration in the final state. Several approaches can be used. The final value, that is, the mean value of concentrations on the two lichen samples exposed for 12 months, is an hypothesis but it is expected to have a high associated error. The mean value for all the data is better in what concerns random errors reduction but has the drawback of being affected by the initial value. In order to optimise the balance between error and independence of the initial value, we chose to use the mean value of the samples exposed for more than five months.

The second problem we face is about element availability. This is assumed to be a measure of the dispersion of the element on the environment surrounding the lichen. To quantify it, two groups of variables can be used: intensive variables (concentrations) or extensive (total amounts). We also have data on both suspended particulate matter and deposition. Thus we defined nine variables that somehow measure the elements availability.

The extensive variables are: FAC, CAC and TAC (Fine, Coarse and Total Aerosol Concentration) for suspended mass in aerosols in fine, coarse and both types particle modes; and ATD (Absolute Total Deposition) for total mass deposited in the buckets. The intensive variables are: FPC, CPC and TPC (Fine, Coarse and Total Particle Concentration) the element concentration on fine, coarse and both types of airborne particles, obtained from the extensive variable by dividing by the suspended fine, coarse and total mass, respectively; DRC the elemental concentration in the dry residue; and WRC the concentration in the water samples, obtained by dividing the absolute total deposition by the amount of water collected (when there was no water collected there is no value for this variable).

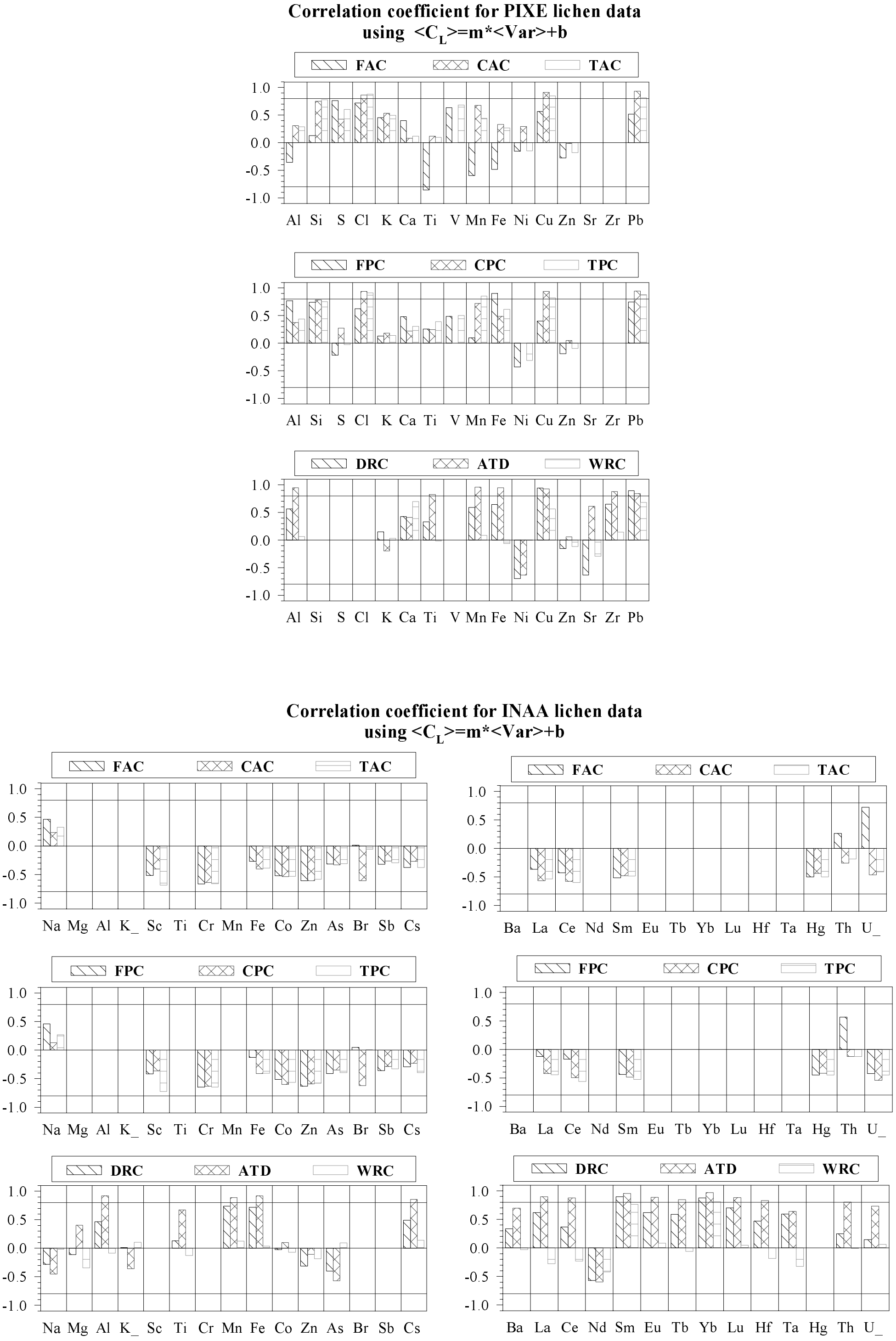


Figure 2. Correlation coefficients between concentration in lichens for 6 or more months exposure and the mean annual value of the defined environmental availability variables. A value above 0.8 represents a 95% confidence limit that the correlation is positive. Lichens and aerosol data from PIXE and k_0INAA are presented separately. See text for symbols.

Once defined these variables, we looked for correlation between the mean of the last six months of lichen concentrations and the annual mean values of each of these variables. The results are plotted Figure 2. From PIXE lichen and aerosol data we can see that Cl, Cu and Pb show correlation higher than 0.8 to aerosol concentrations. In our case this value of 0.8 for the correlation coefficient corresponds to a 95% confidence limit that the populations correlation is positive [8] When concentrations in the particles are considered, also Mn and Fe join the above group. For the PIXE data, correlations higher than 0.8 are still found relative do deposition variables, namely for: Al, Ti, Mn, Fe, Cu, Zr and Pb. Eight out the sixteen element concentrations determined by PIXE on the lichen samples are correlated to at least one availability variable.

When lichen and aerosol data determined by k_0INAA are used no correlation to aerosol variables is found. Attention must be drawn at this point to the fact that only K, Fe and Zn were determined by both INAA and PIXE in lichen and aerosol samples. The difference verified for Fe is nevertheless not understandable.

In what concerns deposition data, correlation higher than 0.8 is identified for Al, Mn, Fe, Cs, La Ce, Sm, Eu, Tb, Yb, Lu, Hf and Th. In this case 13 out of 24 elements do present significative correlation.

The comparison to PIXE data does not show any disagreement for deposition data, probably reflecting the fact that the discrepancy identified in the comparison of aerosol data is due to problems in measurements on the filters rather than on the lichen samples. Combining data from both techniques we get correlation for 17 elements, namely: Al, Cl, Mn, Fe, Cu, Zr, Cs, La, Ce, Sm, Eu, Tb, Yb, Lu, Hf, Pb and Th, even though we have not data for all types of samples for all of them. These results do not confirm those obtained by Sloof [1] for Co and Zn, and cannot be compared with the Sc results due to the lack of data on total deposition.

Still on this data set many of the elements do not show a straightforward correlation to availability variables.

A careful look into the way we are comparing lichen and availability data makes it clear that we are looking for correlation without considering other environmental variables. The mean value of water volume collected in the total deposition buckets provides both information on the amount of water available to the lichens as well as parameterises the environmental conditions. From figure 1 we can see that the amount of water that fell per month on average in each station varies by a factor of 3 from the minimum to the maximum thus showing that the environmental conditions to which lichens were exposed did vary significantly from station to station.

4. THE WATER FUNCTION

Based on the assumption generally accepted in relation to the water availability effect and rain effect on lichens, we searched for a mathematical function which could describe these effects. The first step towards this was to establish the conditions to which the function should obey. We decided it should be a multiplicative term. As a consequence of this, the condition of having value 1 for the absence of water is immediate. Because we saw no reason for a very small amount of water to have an effect much different from no water, we imposed also that the first derivative should be zero for zero value of water fall.

Now, water is assumed to be essential to both lichen growth and all other biological functions, thus it should also play an important role on the uptake of soluble elements and salts. Under this point of view, a small amount of rain should increase the uptake efficiency. The water function should be initially an increasing function of water availability. Nevertheless, after some threshold the water should start washing out the lichens; in fact if we take the limit at infinity for the amount of rain, the lichen concentration should go to zero due to the water washing effect.

The simplest function that complies to these conditions is:

$$e^{-(bx)^2 \ln(x)} = x^{-(bx)^2} \tag{1}$$

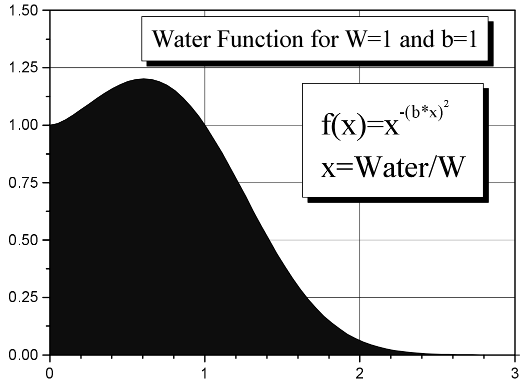


Figure 3. Shape of the mathematical function which is proposed to reproduce the effects of water on the lichens uptake.

The shape of these function is presented in Figure 3. If we set

$$x = \frac{\text{Mean water amount}}{C_W} \tag{2}$$

it means that C_W is the mean amount of water fall for which the increase in uptake and the washing effect balance out.

5. THE WATER EFFECT MODEL

Once established this water effect function, we looked for the correlation between data and the predictions that can be obtained using the model:

$$L_C = (a_0 + a_1 \cdot \text{var}) \cdot W_f$$

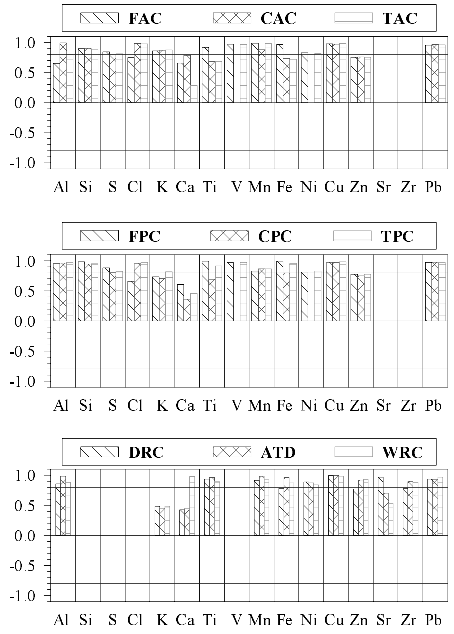
where L_C is the lichen concentration, var is one of the above defined variables and W_f is the Water effect function.

In order to obtain these correlation, the four parameters were selected by fitting data with a non-linear least squares Marquardt method (from SigmaPlot® package).

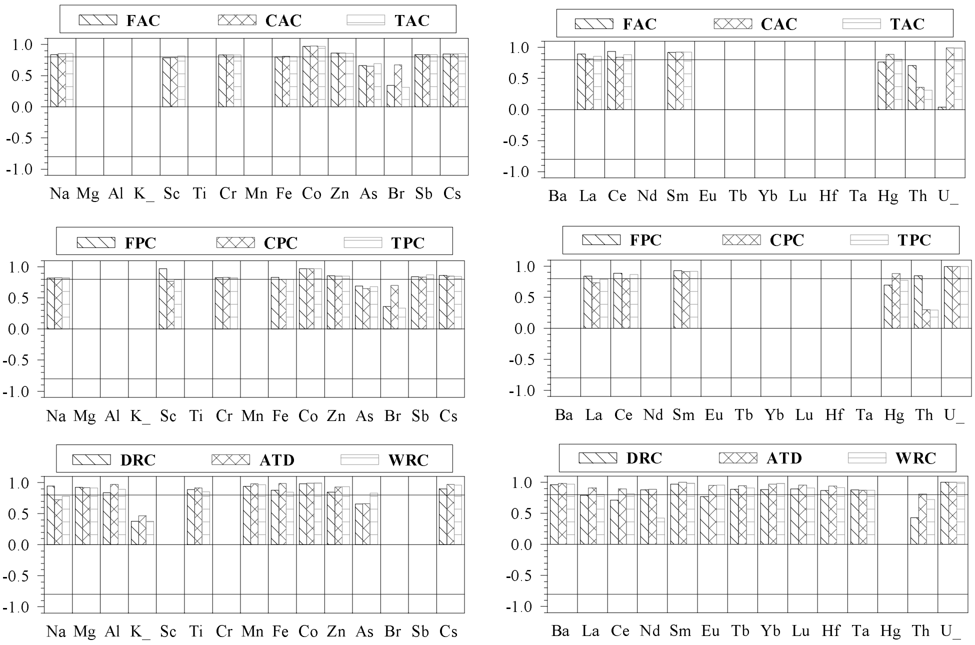
Once obtained the fitted coefficients, the correlation between predicted and measured values was calculated, the results are presented in figure 4.

For the PIXE measured elements, we see that for aerosol concentration variables it is now possible to obtain correlation larger than 0.8 for all elements except Zn. In the case of variables for concentration in particles, all elements except Ca and Zn present correlation coefficients above 0.8 and in the case of deposition variables, all elements except K present correlation larger than 0.8.

In the case of elements measured by INAA we have As, Br and Th which do not present correlation above 0.8 for aerosol concentration variables; only As and Br still do not present correlation above 0.8 in the case of variables for concentration in particles, and in the case of deposition variables only K does not show a correlation higher than 0.8.



Correlation coefficient for PIXE lichen data using
$C_L>=(a1*<Var>+a2)*x^{-(bx)^2}$; x=Water amount/C_w



Correlation coefficient for INAA lichen data using
$<C_L>=(a1*<Var>+a2)*x^{-(bx)^2}$; x=Water amount/C_w

Figure 4. Correlation coefficients between concentration in lichens for 6 or more moths exposure and the mean annual value of the defined environmental availability variables when the water function effect is considered. A value above 0.8 represents a 95% confidence limit that the correlation is positive. Lichens and aerosol data from PIXE and INAA are presented separately.

6. CONCLUSIONS

In this work, a measure of the final state concentration attained by lichens *Parmelia sulcata* was shown to: (a) for some elements, correlate to at least one environmental availability variable, (b) for all measured elements (Na, Mg, Al, Si, S, Cl, K, Ca, Sc, Ti,. V, Cr, Mn, Fe, Mn, Fe, Co, Ni, Cu, Zn, As, Br, Sr, Zr, Sb, Cs, Ba, La, Ce, Nd, Eu, Tb, Yb, Lu, Hf, Ta, Hg, Pb, Th and U), correlate to at least one environmental availability variable when a model of linear response affected by an *a priori* defined water function was fitted to data.

AKNOWLEDGEMENTS

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SOIL VERSUS LICHEN ANALYSIS ON ELEMENTAL DISPERSION STUDIES (NORTH OF PORTUGAL)

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Abstract

Chemical elements contents in topsoil and lichens sampled at regular intervals in the north of Portugal were obtained by using both comparative and k_0 methods of instrumental neutron activation analysis. The topsoil chemical composition was studied in an attempt to discriminate between natural background and anthropogenic inputs, as well as to eliminate the influence of soil on the lichen composition. Topsoil maps revealed the existence of areas with anomalous concentrations due: (1) to natural causes (inherited from the initial rock) especially Cr in Trás-os-Montes and (2) anthropogenic influence, namely Aveiro-Estarreja area for Cr and Co associated to high values of Fe. Br contents in topsoil decrease from the coast to the interior explained by the sea influence (wet deposition). Exceptional concentration values of As, Zn, and W were found in one particular topsoil (Nogueira do Cravo), probably due to aerial inputs originated in mining activities. The enrichment factors (E.F.) in *Parmelia sulcata* relative to topsoil clearly showed that lichens are fingerprints of atmospheric pollution, especially for Cr, Zn, As, Br, Sb, and W. The following areas with anomalous E.F. in lichens were detected: (1) Viana do Castelo – Braga for Cr, Sb, (Zn and Br), (2) Trás-os-Montes for Br, (3) Porto for As and Zn, and (4) Aveiro-Estarreja for As, Cr, Sb, and W. Identification of industrial pollution sources was possible.

1. INTRODUCTION

Trace element maps for application to environmental studies and agriculture should be based on systematic analysis of soils or plants. Anomalous trace elements levels in soils may be caused by natural causes (due to soil formation processes, including the weathering of mineralized materials) or anthropogenic inputs (industrial and urban pollution, agriculture and mining activities). The use of plants in air pollution studies is becoming more and more used in the last years. There is evidence that lichens may be peculiarly useful in studies of heavy metal pollution.

Several factors determine the form and quantities of atmospheric trace elements input and fate in the receiving soil or plant systems, such as particle size, solubility, distance and acidity of rainfall. The topsoil composition can be changed by aerial inputs, involving soluble forms of the elements, aerosols, particles from industrial pollution, domestic and vehicular fuel combustion, mine wastes and smelter. Industrial and domestic waste sludge, agricultural applications and irrigation waters may also modify the soil surface. Increasing attention has been given to the accumulation of trace elements by lichens, for the dependence of these plants on the atmosphere for their mineral supply can make them suitable biological instruments for measuring trace elements contamination of the atmosphere. In addition, the lichen use is easier and cheaper than the use of the classical methods demanding the analysis of aerosol filters and total deposition (rain water and air particulate). However, the relationship between the uptake of metals by lichen and metal concentrations in contaminated air, appears sometimes complex, needing further studies [1, 2].

The interface between natural sources and anthropogenic inputs of chemical elements into the soil is difficult to discern. Trace element concentrations in soils cannot be predicted through geological maps showing the spatial occurrence of different types of rocks. As a matter of fact, the chemical composition of the initial rock is not the only factor controlling the final composition of a soil, which is confirmed, by similar chemical compositions of soils derived from different types of rocks. Other major factors are the drainage conditions, climate, biological activity and time. In addition, the majority of the soils in Portugal have been removed by agriculture practices. However, especially in immature soils, the trace elements patterns in soils may traduce the chemical patterns of the initial rock. Thus, the evaluation of the chemical variations of soils due to natural sources is necessary in any study involving soil particulate composition interference, such as the use of lichens in air pollution control.

In this work the chemical spatial distributions of 28 elements are presented for the northern part of Portugal (north of Aveiro-Viseu) for superficial level of soils (0-20 cm depth) and for lichens (*Parmelia sulcata*) sampled at regular intervals. The chemical analyses were performed by instrumental neutron activation analysis using both comparative and k_0 methods.

The north of Portugal can be divided in two regions according to the climate. The Atlantic border has a maritime climate and the interior continental characteristics. The NE region has the highest aridity index of the area studied. The dominant winds near the coast are from N-NW and SW. In addition, the coast region has the highest urban population and industrial activity. For these reasons sampling was made according two different grids: 10x10 Km in the Atlantic border (up to 50 Km of the coast) and 50x50 Km in the interior part of the country.

In this work the relationship between the chemical composition of topsoil and the underlying type of rock is studied in order to differentiate natural and anthropogenic inputs of chemical elements, and locate areas with anomalous concentrations. The chemical composition of soils and lichens are compared and the enrichment of trace elements in lichens relative to topsoil is discussed, in an attempt to evaluate differences in the uptake of the chemical elements due to air pollution.

TABLE I. MEAN, STANDARD DEVIATION, NUMBER OF DETERMINATIONS (N), AND MINIMUM AND MAXIMUM OF THE MAJOR ELEMENTS IN SOILS (% WEIGHT OF OXIDES) AND TRACE ELEMENTS (EXPRESSED IN PPM) IN SOILS OF THE NORTH OF PORTUGAL

	Soils of the N of Portugal				
Element	n	Mean	Standard deviation	Minimum	Maximum
Na_2O	66	1.51	0.91	0.23	4.07
K_2O	66	4.10	2.04	0.556	15.5
Fe_2O_3	66	3.58	2.13	0.71	14.3
Sc	66	7.99	5.89	1.07	34.7
Cr	66	32.5	34.9	7.4	243
Co	66	6.99	7.35	0.72	43
Zn	66	91.3	37.66	5	187
Ga	48	22.9	12.4	4.09	71.3
As	66	21.6	30.7	1.84	208
Br	66	18.0	22.34	0.9	156
Rb	66	214	97	14.3	454
Sb	66	0.75	1.15	0.09	7.5
Cs	66	13.4	7.7	0.67	45.4
Ba	66	346	156	104	735
La	66	49.0	30.0	7	190
Ce	66	94.1	55.2	14.9	362
Nd	66	46.0	25.3	7.8	156
Sm	66	8.13	4.05	1.29	21.9
Eu	66	1.03	0.47	0.24	2.8
Tb	66	0.81	0.35	0.16	2.04
Yb	66	2.14	1.05	0.59	4.83
Lu	66	0.27	0.15	0.008	0.67
Hf	66	6.70	3.27	1.46	15.7
Ta	66	2.17	2.32	0.25	19.3
W	47	4.28	3.65	0.68	16.8
Th	66	22.2	14.0	0.92	57.5
U	50	6.8	4.2	0.01	22.3

2. EXPERIMENTAL

Regular sampling of soils and lichens (summer of 1993) was made in 67 different locations of the north of Portugal according to two sampling grids - 10x10 Km near the coast and 50x50 Km in the interior. The soils were collected between 0-20 cm depth and lichens from olive tree substrate at 1-2 m high. In each sampling locus, the lichens were collected from 5 different trees.

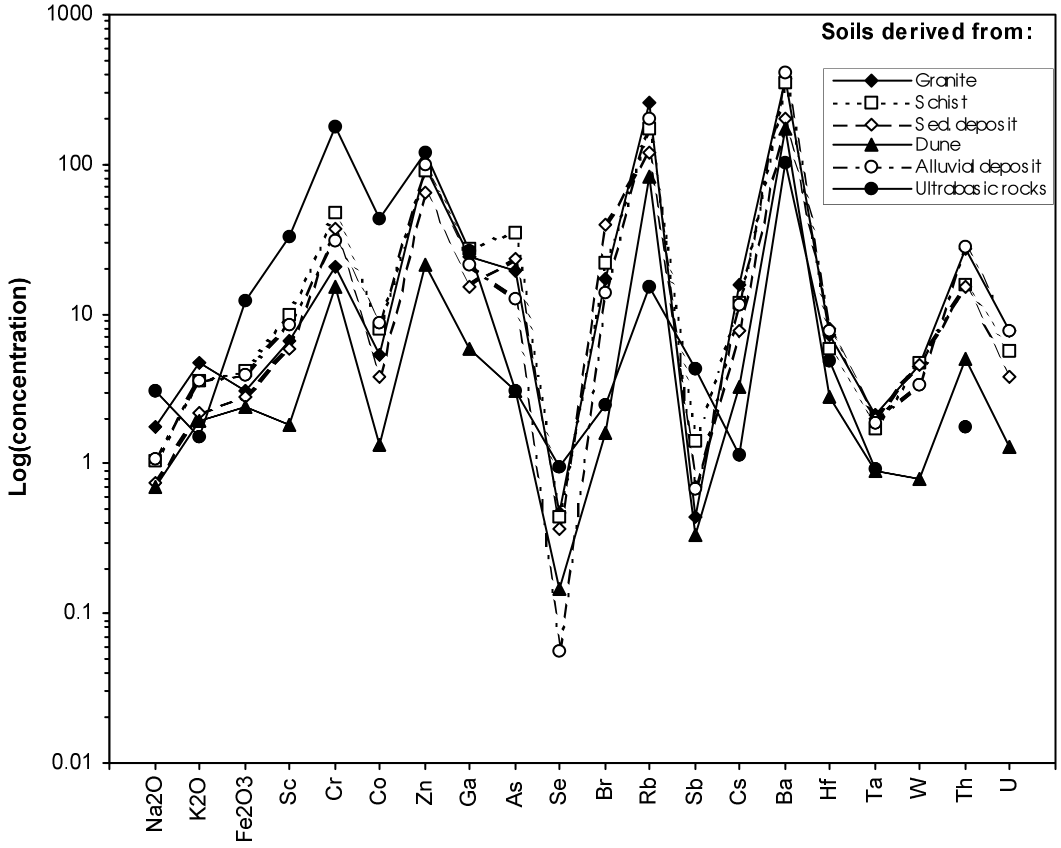

Fig. 1. Mean values of the chemical element concentrations in the different types of soils of north of Portugal.

Soils were dried at 30ºC for 24h, sieved to remove rock grains larger than 2 mm and plants residues. The samples obtained were ground and homogenized in an agate mortar and prepared for analysis. The organic matter (OM) proportion was also determined (800ºC ignition).

The substrate of the lichens was carefully removed, and the lichens were put on nylon sieves and washed for 30 sec., freeze-dried and ground in Teflon capsules.

Using the Portuguese Research Reactor (ITN) and the reactors of the Interfaculty Reactor Institute (Delft) and Hahn-Meitner Institute (Berlin), instrumental neutron activation analyses were performed (comparative and k_0 methods) [3, 4].

3. RESULTS AND DISCUSSION

3.1. Topsoil composition - underlying type of rock

The average values and respective standard deviations, minimum and maximum for the chemical elemental concentrations in the soils of the area studied, are given in Table I. These calculations did not include sample 58 (Nogueira do Cravo) due to exceptional concentrations of As, W, and Zn, which is discussed later on.

The soils were grouped according to the underlying type of rock (assuming that it refers to the initial rock from which the soil derived): granite rocks - 40 samples, metamorphic rocks (schist, greywacke and gneiss) - 16, ultrabasic rocks (Trás-os-Montes) - 2, sedimentary deposits - 3, alluvial deposits - 3

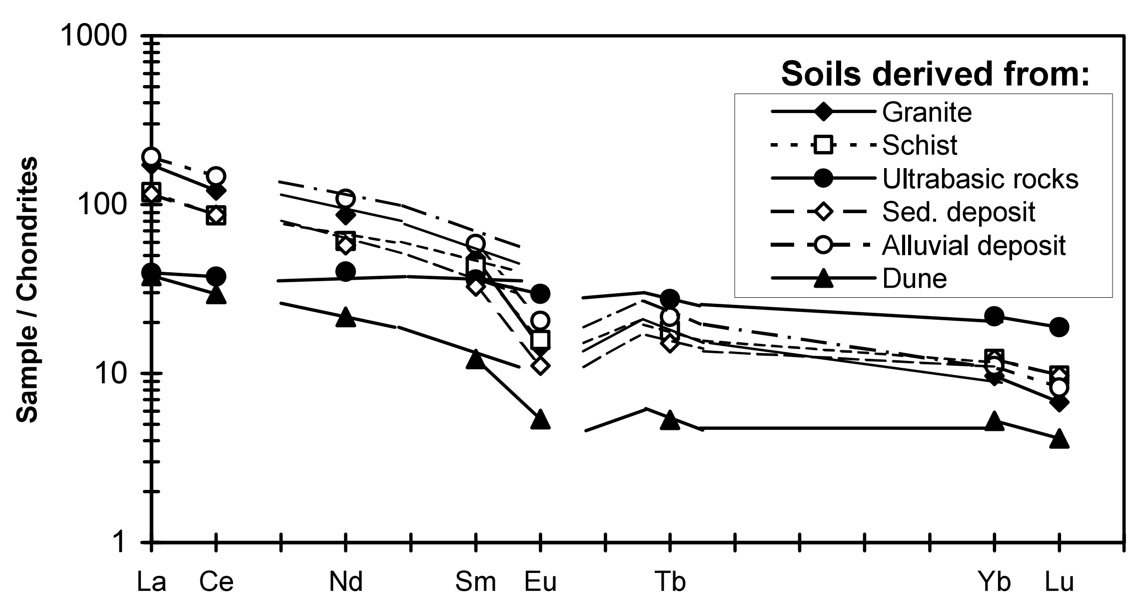

Fig. 2. Rare earth elements patterns relative to chondrites [5], of the different types of soils of north of Portugal (mean values).

and dunes (Atlantic coast) - 3. The number of each group of soils traduces the geology of the area. As a matter of fact, granites and metamorphic rocks dominate the north of Portugal. The elemental distribution patterns (using the mean values) of the different groups of soils are shown in Figs. 1 and 2.

The soils of Trás-os-Montes (ultrabasic complexes) are significantly different from all the others, due to higher contents of Na, Zn, and especially Fe, Sc, Cr, Co, Se, and Sb and lower concentrations of K, As, Rb, Cs, Ba, and Th. The higher proportions of ferromagnesian minerals in the underlying rocks greatly contribute to these differences. This type of soils also differ in the rare earth elements (REE) patterns, with a low proportion of light REE (LREE) relative to the heavy REE (HREE) and an insignificant Eu anomaly, inherited from the underlying rock.

Soils of granite environment differ from the metamorphic rock areas due to higher proportions of Na, K, Rb, Cs, and Ta and lower contents of Fe, Sc, Cr, Co, As, Br, and Sb. As far as the REE are concerned, soils derived from granites show a higher proportion LREE/HREE. These differences are probably mainly due to a higher proportion of feldspars, micas and heavy minerals like apatites and zircon, in soils derived from granites. It should be noted that, despite this tendency of differentiation, part of the soils from granites and metamorphic rocks could not be distinguished based only in the chemical elements determined in this work.

Soils from sedimentary and alluvial deposits are similar either to granite areas or metamorphic rock soils. The soils developed in coast dunes are in general poor in the elements determined in this work which is explained by silica (quartz) dilution.

It should be noted that within the groups of soil, the trace elements with the widest variations of concentration, i.e. greatly independent of the nature of the underlying rock, are Sb, W, As, Br, Se, and Zn.

3.2. Local anomalies in topsoil - Nogueira do Cravo

Anomalous concentrations of As (0.66 %), Zn (526 ppm), and W (88.3 ppm) associated to a high content of Fe (10 %) were found in the soil sample 58 collected near Nogueira do Cravo. These exceptional levels may be due to mineralized soil or aerially polluted soil. A further study in depth of this soil (to see if those elements are only accumulated in the surface horizons) and a detailed scheme of sampling of the soils in this area is necessary to discriminate the origin. However, an aerial contamination is for the moment the most probable cause, since there is a mine of As, W and other elements (Pintor mine) only 1.4 Km NW (dominant winds) of the sampling local. In addition this soil has a high content of organic matter (15 %) which is not common in a mineralized soil, and no lichens were found around this point.

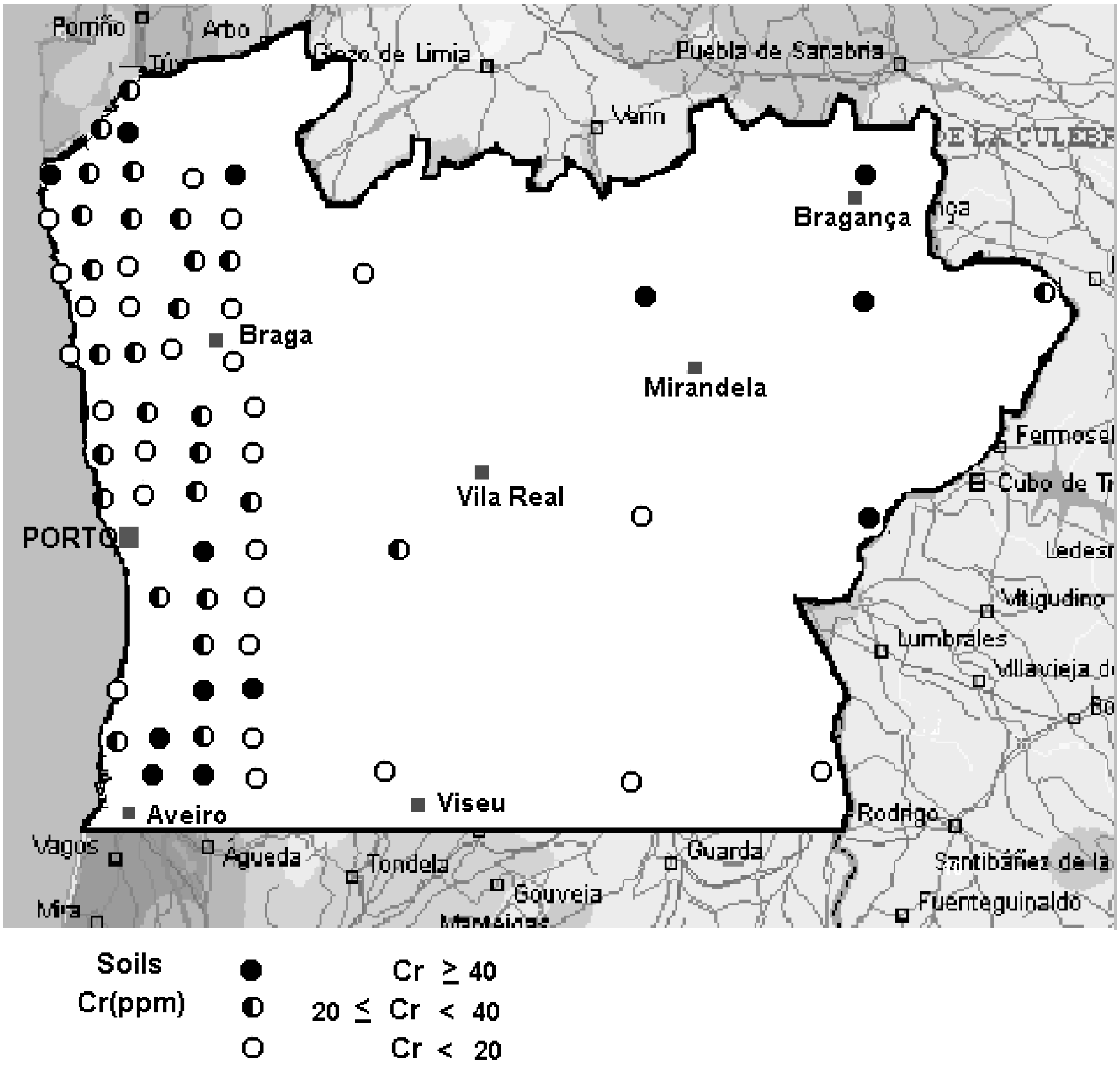

Fig. 3. Topsoil Cr map of north of Portugal.

3.3. Topsoil chemical maps

The spatial distribution of the chemical elements in topsoil, and its relationship with the nature of the underlying type of rocks mainly show that: (1) Na, K, Rb, Cs, Ba, Fe, Sc, Cr, Co, Th, U, and REE depend principally on the nature of the underlying type of rock; (2) anomalous levels of Cr (up to 240 ppm) and high concentrations of Fe, Sc, and Co were found in soils of the northeastern region of Portugal mainly due to natural sources; (3) Br and Sb are correlated with the organic matter content; and (4) among the trace elements determined, As, W, and Zn appear to be the most influenced by anthropogenic activities.

Br concentration in topsoil is correlated with the organic matter content and decrease from the coast to the interior, which is explained by sea influence (wet deposition as washout by precipitation). Thus some of the Br, which is retained in the humic portion of the soils is derived from Br in atmospheric precipitation and from organic detritus such as fallen leaves.

The high values of Fe, Co, and especially Cr (Fig. 3) in the topsoil of the Aveiro-Estarreja area, independent of the underlying type of rocks, suggest an anthropogenic input. An exceptional value of Cr was also found east of Porto. High values of Sb occur between Porto and Aveiro.

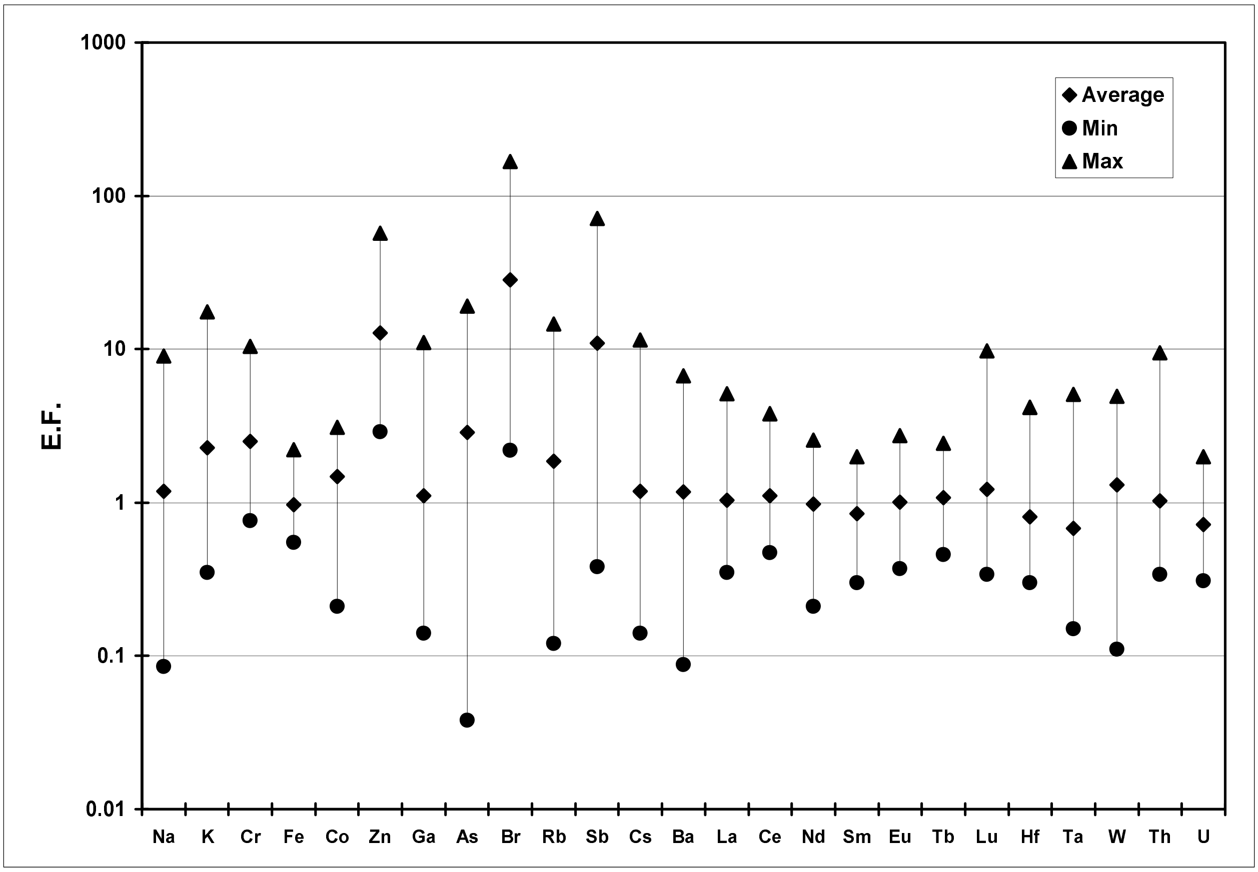

Fig. 4. Mean, minimum and maximum values of the enrichment factors of lichen relative to topsoil.

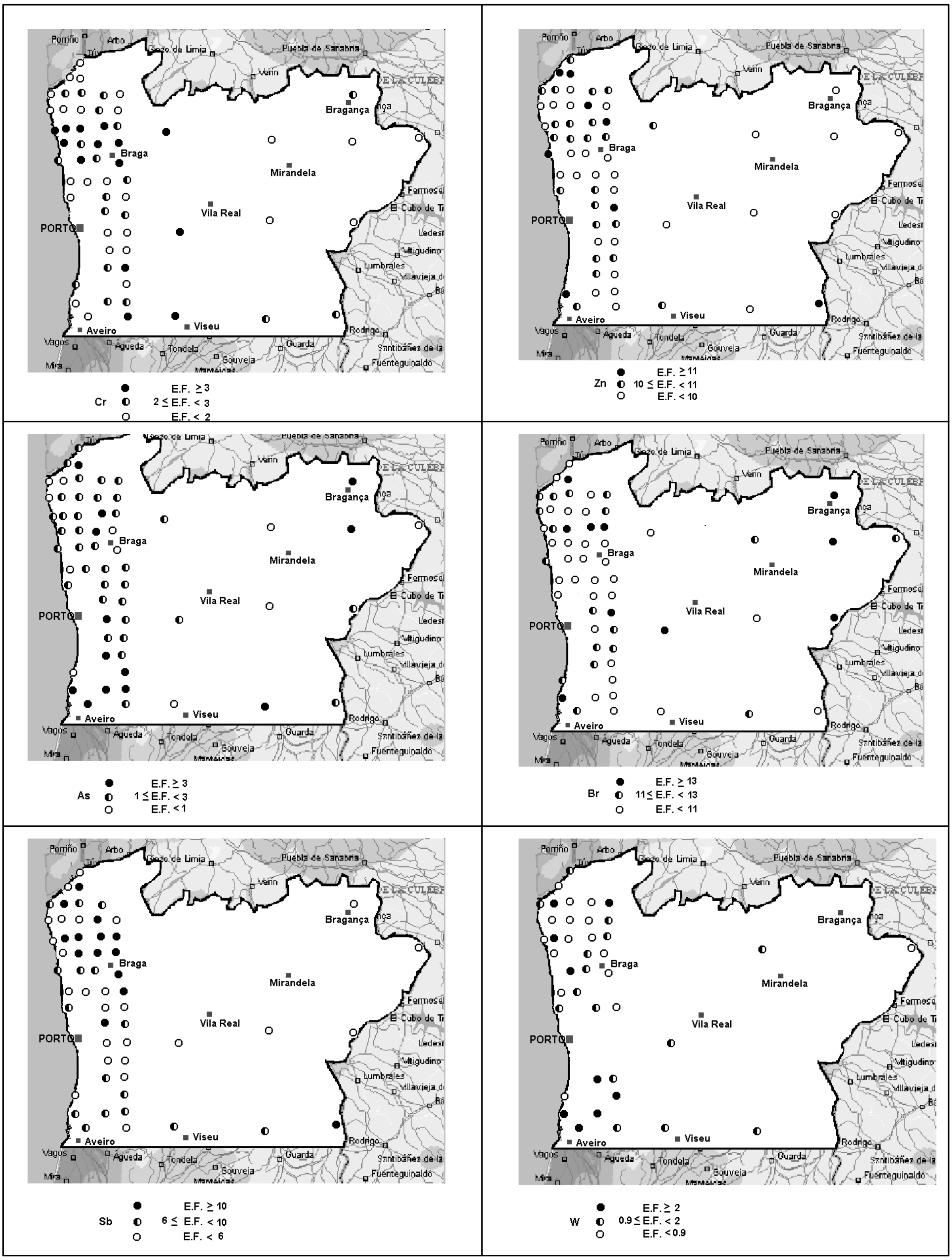

Fig. 5. E.F. maps of Cr, Zn, As, Br, Sb and W in lichens relative to topsoil of north of Portugal.

3.4. Enrichment factor – lichen / soil

The enrichment factor (E.F.) of the chemical elements in the lichen compared to topsoil was calculated relatively to Sc:

$$E.F. = [X_{lichen}] / [Sc_{lichen}] \times [X_{soil}] / Sc_{soil}]$$

The E.F. means, minimum and maximum for each element are represented in Fig. 4.

The results obtained show that some elements are much more enriched in the P*armelia sulcata* samples analyzed than others. The chemical analyses of plant species have been shown that, amongst the chemical elements, biological systems concentrate certain elements, some of them being essential. Besides, an element that is to be used by a given species must be in an easily extractable form in the environment [6]. In this work we are not concerned with the concentration of the chemical elements itself but mainly with significant variations of their concentration in the lichen analyzed after normalization to topsoil, especially with regional anomalies.

In the NW part of the region studied an enrichment of K and the related elements Rb, Cs, and Ba, in lichens was observed. This may be explained by the nature of the soils (granites) with higher contents in these elements than the other types of soils, and probably in an easier extraction form.

Among the elements studied, the E.F. maps of Cr, Zn, As, Br, Sb, and W appear to indicate atmospheric pollution of the following areas: (1) Viana do Castelo-Braga: Cr and Sb (Fig. 5), and also high values of Zn and Br; (2) Trás-os-Montes: Br; (3) Porto: As and Zn; and (4) Aveiro-Estarreja: As, Cr, Sb, and W. Comparing to previous studies carried on [7], we identify as possible pollution sources coal combustion (3), electric power plant and paper industry (1), and industrial complexes (4). Cl was found in large amounts in Trás-os-Montes, where Br was now also identified. These two elements may be somewhat correlated.

4. CONCLUSIONS

The topsoil elemental distribution is partially correlated with the composition of the initial type of rock and/or influenced by anthropogenic activities. Anomalous concentrations inherited from the parent rocks were clearly found in Trás-os Montes (NE) for the first-row transition metal elements. Anomalies probably due to anthropogenic activities were found in the Aveiro-Estarreja region for Fe, Co, and especially Cr. An exceptional Cr content was also detected near Porto, and high values of Sb between Porto and Aveiro were also found. The most anomalous topsoil analyzed (especially for As, Zn, and W) is located near Nogueira do Cravo, certainly due to mining activity.

Enrichment factors of the elements in lichens related to topsoil showed that mining activities only detected in one topsoil (Nogueira do Cravo), appear to influence lichens in an widespread area, especially for As and W. Besides, atmospheric pollution is also responsible for high contents in Cr and Sb in the Viana do Castelo – Braga region, As and Zn in Porto area, and As, Cr, Sb, and W in the Aveiro –Estarreja region. Br anomalies were also detected in Trás-os-Montes. Correlation to industrial pollution sources was identified.

Further detailed sampling schemes for areas of particular interest are needed, including especially those indicated as containing exceptional levels of more than one element on the basis of topsoil and lichens survey.

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USE OF MOSSES AS BIOMONITORS OF ATMOSPHERIC DEPOSITION OF TRACE ELEMENTS

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Abstract

Some basic facts about the use of mosses as biomonitors of atmospheric trace element deposition are reviewed, and advantages and limitations of this approach are discussed, largely on the basis of experience from regular use of this technique in Norway over the last 20 years. Topics discussed include different versions of the moss technique, mechanisms and efficiences of trace element uptake, conversion of concentrations in moss to bulk deposition rates, and contribution from sources other than air pollution to the elemental composition of different elements. Suggestions are presented for further work in order to extend the use of mosses as biomonitors.

1. INTRODUCTION

Mosses have no root system, and pick up nutrients and other chemical substances mainly from wet and dry deposition. Moreover they have a high capacity to retain many trace elements. Mosses, and particularly species growing on the ground, have therefore shown to be suitable biomonitors of atmospheric deposition of trace elements. Since this approach was first proposed 30 years ago to monitor the deposition of lead [1] it has come into use in more than 15 countries in Europe [2], and the original applications focusing on a single or a few elements have been succeded by multi-element surveys where more than 30 elements have been studied simultaneously by techniques such as inductively-coupled plasma mass spectrometry [3] or epithermal neutron activation analysis [4]. In Norway the moss technique has been used regularly for the last 20 years in deposition surveys on a national scale [5-9]. In the present paper experience from this monitoring activity will be reviewed, and typical results for trace elements will be presented and discussed. Emphasis will be put on the possibility to calibrate moss concentration values versus bulk deposition data from precipitation analyses in order to convert relative deposition data, as normally obtained by moss analysis, to absolute deposition rates. Furthermore sources of trace element supply to mosses other than atmospheric deposition of pollutants will be discussed, and the significance of these sources for various elements will be assessed.

2. APPROACHES TO STUDY THE SUPPLY OF AIRBORNE TRACE ELEMENTS

The conventional way of studying rates of atmospheric deposition is to analyse precipitation samples collected over a given period of time and calculate deposition figures on the basis of the results of the chemical analyses. The samplers may be of the "bulk deposition" type, sampling all the time and thus also collecting dry-deposited aerosols during dry periods, or "wet-only" samplers which open automatically when a precipitation event starts and thus collect only wet deposition. These measurements are difficult because of the generally low levels of trace elements in precipitation. This means that a) the analyses are difficult and b) contamination problems during sampling and analysis may often be serious unless extreme care is taken to avoid them. Moreover the running of a precipitation sampling network will normally be restricted to a few stations. Thus the possibility of spatial distribution studies will be very limited.

Some of these problems can be overcome by the use of plants as biomonitors. Mosses and lichens, which absorb aerosols as well as substances dissolved in precipitation quite efficiently, have been most frequently used for this purpose. These biomonitors are easily collected, and the concentrations of trace elements in the moss or lichen is normally several orders of magnitude higher correspondingly smaller, and the sampling and analysis much easier and cheaper. The use of biomonitors therefore allows deposition surveys to be carried out simultaneously over large areas with a high spatial resolution. On the other hand the results are at best a relative measure of the atmospheric deposition of the elements in question, but this may be adequate for the study of temporal and spatial trends. What the results indicate is an integrated exposure over a certain period of time.

In Norway the moss *Hylocomium splendens* is used for trace element deposition monitoring on the national scale every 5 years. This moss species has a growth pattern that makes it easy to distinguish the annual incremental growth. The last 3 years´growth is taken for analysis, which means that the exposure period is well defined. Several other moss species also grow in a way that makes it possible to estimste the age of the sub-sample taken for analysis. Lichens absorb trace elements from the atmosphere with similar efficiency as mosses [10] but the age determination is often difficult or impossible. Lichens are therefore generally inferior to mosses as biomonitors of atmospheric deposition of trace elements.

Vascular plants have also been suggested as trace element biomonitors. In particular conifer needles or leaves of deciduous trees shed annually might seem convenient, since the exposure period in this case would be extremely well defined. A major drawback however is that it is normally not possible to distinguish beween fractions of an element in the sample supplied from the soil by root uptake and absorbed from the air - unless the surface layer is washed off and analysed separately [11]. Moreover comparative studies have shown that the retention of trace elements in conifer needles from the air is sometimes less than 10 % of that observed in moss growing at the same site [12]. It seems reasonable to assume that the waxy surface of needles (and some deciduous leaves) is a better retention surface for aerosols than for ions dissolved in precipitation.

Organic surface soils concentrate many trace elements from the atmosphere very efficiently, and can therefore be used to monitor long time integrated exposure of these elements. Peats [13, 14] and natural surface soils from boreal forests and other ecosystems in temperate regions [15, 16] have been used quite successfully for this purpose. A main interfering problem in the latter case is contribution from mineral matter in the soil, but there are ways of overcoming this problem [17], in particular if the underlying subsoil is analysed simultaneously. Peat cores can be conveniently used to monitor temporal trends of atmospheric deposition of trace elements [18], and is an interesting alternative to snow and ice cores and lake sediments, neither of which are generally applicable.

3. MOSSES AS MONITORS OF ATMOSPHERIC DEPOSITION

3.1. Classification of mosses for biomonitoring

Mosses have been used for biomonitoring in a number of different ways which may lead to rather different results, and some kind of classification seems necessary at this point.

The large-scale mapping excercises are normally done by means of *indigenous* (i.e. naturally growing) mosses. An alternative possibility is to use moss *transplants*, e.g. in the form of "moss bags" [19]. In this approach a sample of "clean" moss, either collected from a very clean area or cleaned with dilute acid before use, is left at the sampling site for a given exposure period. This approach has been mainly used in local studies with rather high pollution levels. In cases with high exposure of either heavy metals or gaseous pollutants the indigenous moss populations may have been exterminated, and the use of transplants may be the only possibility.

Epigeic mosses (growing on the ground) are preferred in the regional surveys in northern and central Europe. Where mosses grow on the top of a substrate mainly consisting of organic soil and decaying plant debris, this is clearly preferable to using *epiphytic* mosses growing on trees. In the case of mosses collected from trunks or branches of trees it is very likely that the incoming flux of trace elements from the atmosphere - wet or dry deposition - will be substantially modified by the canopy before reaching the moss, and it is also conceivable that trace elements supplied to the tree from the soil through the root system and eventually leached e.g. from leaf surfaces will form part of the exposure of the moss. In warmer climates however the use of epigeic mosses may be rendered impossible because they grow more or less directly on the mineral soil and hence pick up soil particles to a great extent. In such cases other solutions must be found, and one possibility would be to use epiphytic species growing on stumps. In any case, when collecting mosses in forest sampling under the canopy of trees should be avoided as far as possible.

Two groups of epigeic mosses have been most commonly used for moss surveys:_*Feather mosses* such as *Pleurozium schreberi* and *Hylocomium splendens,* and peat mosses such as *Sphagnum fuscum.* Peat mosses possess the advantage of growing on a pure organic substrate, minimising the problem of soil contamination, but the habitat of a given species of *Sphagnum* moss normally does not show the kind of even geographical distribution necessary for a regional survey. The two above epigeic species are both very common in the boreal zone and can be used interchangeably in monitoring studies because they absorb many trace elements with very similar efficiency [20].

Lichens to be used for trace element monitoring can be classified in as similar way as done above for mosses. The advantages and problems associated with the various classes are likely to be similar for lichens as for mosses.

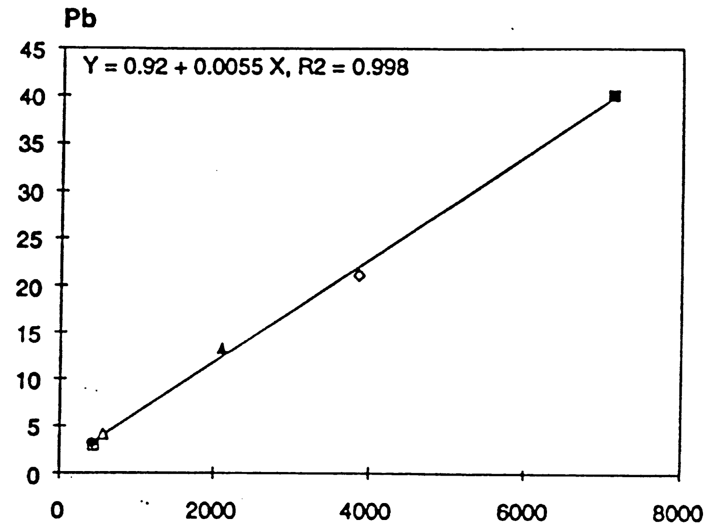
3.2. Uptake efficiency for trace elements in mosses

Trace elements may be absorbed on the moss from the atmosphere either as soluble chemical species in wet deposition or contained in particles from dry deposition. Part of the trace element content of particulates may eventually be released by weathering and reabsorbed by the moss. The uptake efficiency for a given element differs between different moss species [21], and large differences in uptake efficiency are evident for different element in the same moss species [20]. Presumably the "uptake efficiency" is also different for the same element from wet and dry deposition, and it seems reasonable to assume that the particle size distribution is another significant factor. Whereas uptake efficiencies for particulate-bound trace elements are generally poorly known, the knowledge is better regarding the uptake of dissolved elements in ionic form. Ions may be subject to active uptake into cells or attached on the moss surface by physical and chemical forces. Methods are available to distinguish between intracellular and surface-bound fractions of elements [22]. As far as the surface bound fraction is concerned, little is known about the binding mechanisms, but the fact that different metals show rather large differences in their retention capacities [20] indicates that both simple cation exchange on negative surface charges and complex formation with ligands on the moss surface are involved. Laboratory studies with uptake of selected metals to moss from artificially made precipitation samples, and at realistic metal concentrations in the solution, indicated that the moss behaves somewhat similar to a commercial cation exchanger [23]. The sorption of Pb and Cu was stronger than of Zn and Cd. The uptake was less from precipitation with significant content of marine salts, which may be due to competition from marine cations such as Mg, or possibly partial metal complexing by the chloride ion. Uptake was significantly reduced when the precipitation pH dropped appreciably below 4.0.

In a recent study [20] of relative uptake efficiencies for different air-pollution associated trace elements in *Hylocomium splendens* and *Pleurozium schreberi,* Pb and Tl were found to be most strongly bound among the elements studied. Relative to a Pb uptake defined as 1.00, the following approximate factors were observed: Mo, 0.75; V, Cd, 0.60; Sb, 0.25; As, Se, 0.15. The above elements showing low relative uptake are probably present mainly as anions in wet deposition. Experience from the national monitoring work in Norway however clearly indicates that the moss technique works well even for these elements with respect to temporal and spatial trends [6-9].

3.3. Conversion from relative to absolute deposition rates

Comparison of trace element concentrations in moss and bulk precipitation samples at six different stations in Norway [24] showed that calibration of concentrations in moss to bulk deposition could be done very well for some elements, thus converting relative deposition figures from moss surveys to absolute deposition rates. Examples are shown in Fig. 1 for Pb and Cu. For Pb the calibration curve goes through the origin, indicating that essentially all Pb in the moss was derived from atmospheric deposition. In the case of Cu the intercept with the ordinate axis indicates a "baseline" concentration of about 3 ppm in the moss not related to deposition. More recently the study



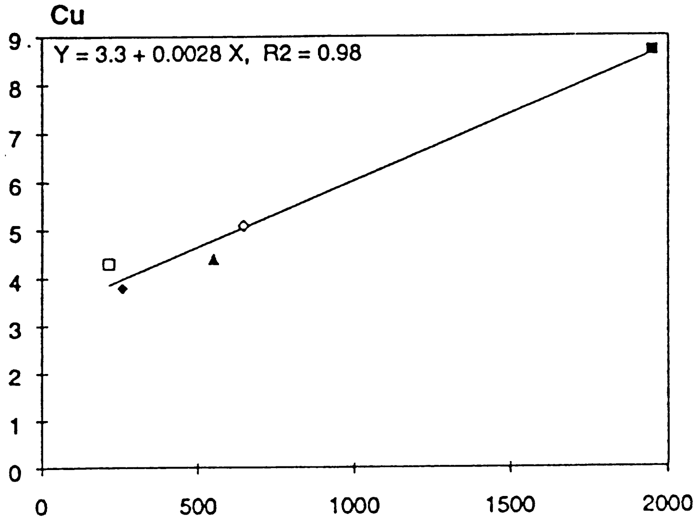


Fig.1. Calibration plots of trace element concentrations in Hylocomium splendens moss ($\mu g\ g^{-1}$) versus atmospheric bulk deposition ($\mu g\ m^{-2}$) from precipitation analysis. Modified from Berg et al. [24].

was extended to 13 stations, and satisfactory calibration relations were obtained also for elements such as As and Sb [20] earlier shown to have a low relative uptake in the moss.

4. PROBLEMS WITH THE USE OF MOSSES AS BIOMONITORS

Although the moss method has had considerable success in the past and the areas of application are currently extending, there are certain limitations that should not be overlooked when this method is used for biomonitoring of trace element deposition from the atmosphere. Some of these problems are discussed in the following. The discussion is specifically related to the application of naturally growing epigeic mosses. Many of the problems discussed are also relevant and may be even more serious when other biomonitoring approaches are used.

4.1. Contribution from sources other than air pollution

As discussed more in detail elsewhere [25] there are several other sources for mineral elements in mosses than the deposition of air pollutants from local and distant sources, which is the target of the biomonitoring work:

TABLE I. RELATIVE INFLUENCE FROM DIFFERENT FACTORS TO THE ELEMENTAL COMPOSITION OF MOSSES GROWING IN RURAL AND REMOTE AREAS (Cfr. Section 4.1).
+, ++, +++ : positive contribution, increasing importance.
-, - -, - - - : negative contribution (removal), increasing importance.

Element	Air pollution	Marine factor	Vegetation factor	Soil factor
Al				+++
V	++			+
Cr	+			++
Mn		- -	+++	
Fe	+			+++
Ni	++			++
Cu	++		+	
Zn	++	-	++	
As	+++	-		+
Se	++	++		
Br		+++		
Sr		+++		+
Cd	++		+	
Sb	+++			
Hg	++			+*
Pb	+++			

* Gaseous emission of Hg^0 from the soil may be absorbed in the moss.

a. Atmospheric supply of marine elements and other components from natural cycling processes. In coastal areas far from local pollution sources e.g. elements such as Br and Se in moss are likely to be derived mainly from this source [6].

b. The "Vascular pump", i.e. root uptake of elements into higher plants, in particular trees, and subsequent leaching onto the moss from living or dead plant tissue.

c. Windblown mineral dust from local soil. This can be a serious problem, in particular in areas with sparse vegetation such as the polar regions [26, 27].

d. Transport of soluble compounds from the soil into moss tissue, particularly during periods with excessive soil/water contact such as during snowmelt. Although mosses do not have a root system, influence from this source cannot be disregarded, in praticular in areas with low atmospheric deposition [28].

The relative contribution from these additional sources will differ substantially among the elements. In Table I experience from the Norwegian moss surveys with respect to the relative influence from different source categories is presented for 15 of the elements most frequently studied in connection with environmental pollution. From the table and the evidence it is based on it is obvious that e.g. the deposition of Mn cannot be studied by the moss method. Also for elements such as Fe and Zn there are considerable problems, unless the contamination level is very high.

4.2. Other disturbing factors

Although the contribution from other sources to the elemental composition of the moss is the main problem to be considered when evaluating the results of a moss survey, there are also other factors that should not be overlooked, such as:

- Differences in growth rate of the moss within the region.

- Re-distribution of snow by wind, contributing to irregular snowmelt patterns.

- Variable forest density, influencing the dry deposition to the moss.

- Variations in precipitation chemistry during the exposure period.

- Sensitivity of the moss to air pollutants (SO_2, metals, - -).

5. CONCLUDING REMARKS AND SUGGESTIONS FOR FURTHER WORK

Even though the problems discussed above to some extent influence the precision and accuracy of the moss technique to depict the atmospheric deposition and suggest some limitations to the use of this approach, it is clear that biomonitoring of trace elements has come to stay for the foreseeable future. It is hardly possible by any other approach to obtain such a detailed picture of variations in time and space within reasonable limits of cost. The calibration of the moss technique versus bulk deposition adds futher to the usefulness of the method.

A discussion of the feasibility of different analytical techniques for moss surveys is beyond the scope of the present paper. Still it is clear that nuclear and nuclear-like methods have played, and are presently playing, an important role in this work, in regular surveys as well as in the calibration of reference materials to be used in this work [29].

The use of the two epigeic feather mosses *Hylocomium splendens* and *Pleurozium schreberi* in large-scale biomonitoring studies has in most cases worked very well in the northern half of Europe because one species or the other can be easily found in most places. When moving this activity farther south, and eventually to other continents, these mosses may have to be replaced by other species of moss or lichen, and epigeic species may not any longer be the best choice. In order to extend the existing European deposition monitoring network beyond its present borders, it will be necessary to calibrate new species versus the present ones with respect to collection efficiency of important trace elements. It also appears advantageous to carry out calibrations against bulk deposition wherever a new biomonitor species is introduced for local or regional studies, in order to be able to estimate absolute deposition rates.

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ATMOSPHERIC METAL DEPOSITION IN FRANCE: ESTIMATION BASED ON MOSS ANALYSIS. FIRST RESULTS

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Abstract

The aim of this programme set up by University Pierre and Marie Curie-Paris VI and ADEME (French Agency for the Environment and Energy Management) is to obtain information on the atmospheric deposition of 36 elements (most being heavy metals) all over France, using 5 common mosses as bioaccumulators : *Pleurozium schreberi, Hylocomium splendens, Hypnum cupressiforme, Scleropodium purum* and *Thuidium tamariscinum*. Sampling was performed in 1996 from April to November thanks to 43 collectors. One sample of moss at least has been collected in 512 sites distributed over France, with an average density of one site each 1000 km^2. Procedures for sampling, drying, cleaning, sorting are strictly codified based on Scandinavian guidelines. Analyses are performed according to two procedures : ICP-MS (Inductively Coupled Plasma, Mass Spectrometry) for Pb, Ni specially and INAA (Instrumental Neutron Activation Analysis for other elements. Data concerning As, Cd, Cr, Cu, Fe, Hg, Pb, Ni, V, Zn will be incorporated into the 1995-1996 European Programme "*Atmospheric Heavy Metal Deposition in Europe - estimation based on moss analysis*" coordinated by the Nordic Council. The analyses are in progress, but preliminary results from Ile-de-France have been achieved for 34 elements in INAA. A preliminary study has shown that interspecies calibration could be possible for some heavy metals and that saturation effects in one species could be present when the intercalibration between species is not possible. Such a programme is made possible thanks to the financial support of the French Ministry of Environment and ADEME and with the active cooperation of several national organisations, especially the Laboratory Pierre Süe (CNRS-CEA). Key-words : Moss, Air pollution, Intercalibration, France, INAA, ICP-MS, Al, As, Au, Ba, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Eu, Fe, Hg, I, K, La, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Sc, Se, Sm, Th, Ti, V, W, Zn, Zr.

1. INTRODUCTION

A number of studies showed the ability of Bryophytes to intercept, retain and accumulate pollutants including metals. They also have the ability to accumulate metals to levels far greater than their expected physiological needs. Thus, mosses seem to be suitable long-term integrators of atmospheric pollution, and since the 1980's some moss species have been used to monitor atmospheric metal deposition. But, the level of metal in mosses depends on many features. Certain morphological features modify the ability to accumulate pollutant particulates. Consequently, the sampling of numerous moss species to monitor atmospheric pollution on a large scale seems to be a main difficulty to analyze data.

The primary purpose of this paper is to present French data concerning the atmospheric heavy metal depositions, using common mosses as biomonitors. For the first time in 1996, France participated in the second campaign of the recent european programme on atmospheric heavy metal deposition measured from mosses [1]. This article also investigates the intercalibration between moss species using our preliminary data.

2. MATERIALS AND METHODS

The methods used in this study are largely based on the Scandinavian recommendations [2], but they were adapted to French ecological conditions which largely vary and are supplemented where necessary.

2.1. Moss species and Sampling

Five common mosses were collected all over France in order of preference as following, *Pleurozium schreberi, Hylocomium splendens*, *Hypnum cupressiforme*, *Scleropodium purum* and *Thuidium tamariscinum*. These species grow on soil and also for *Hypnum cupressiforme* on the surface of decaying stumps. Such a large choice of species was made necessary because none of them is commonly present everywhere, due to the great number of types of soils as well as geographical and climatic conditions.

Sampling was performed in 1996 from April to November thanks to 43 collectors, either professionals (for example, forest rangers working with French National Forest Office in French permanent Plot network for the Monitoring of Forest Ecosystems) or persons with high competence in bryology. The sampling sites were selected according a reference grid made of squares (33 km x 33 km), excepted in one region (Ile-de-France, where the squares were 20 km x 20 km) and, were given in Greenwich coordinates. The sampling points were located at sites representative of non-urban areas at least 300 meters from highways and at least 100 meters from any road or single house. On each site, 5 to 10 subsamples were collected within a 50 x 50 m area. The samples were taken in small gaps in forest as much as possible, but always located 3 meters away from the nearest trees, so not to be directly exposed to throughfall precipitation.

2.2. Preparation of samples, cleaning, pelleting (INAA) and digestion (ICP-MS)

The samples were cleaned in the same way according to the Scandinavian guidelines and were not subjected to any further washing processes. Shoots of moss corresponding to the last 3 years growth, excepting the current year, were taken for analysis. For example, the results from the 1996 campaign represent the average deposition situation during the years 1993-1995 for elements retained in the moss. In other words, results give an integrated exposure over 3 years. After drying at 80°C the sample was homogenized. Three accurately weighted portions of each sample (about 50-80 mg) were specifically prepared according to the analytical methods used : two portions were used for the INAA (Instrumental Neutron Activation Analysis) and the third one was used for the ICP-MS (Inductively Coupled Plasma-Mass Spectrometry).

For the INAA method, the samples were prepared in the form of pellets. About 50-80 mg of moss were mixed with 20 mg of ultra-pure cellulose powder (Whatman CF11). A pressure of 5 tons was applied to make the pellet. Two pellets were made from each sample, corresponding at both analyses performed after short and long irradiations.

For the ICP-MS technique, the homogenized samples were mineralized by the micro-wave method. Successive additions of ultra-pure reagents (HNO_3, H_2O_2, HF, HNO_3, H_2O) were used to induce digestion of samples. Then, the solutions were diluted with high purity water (18.2 MΩ/cm) to 20 ml.

Quality control of the results (INAA, ICP-MS) was ensured by comparison with the standard reference materials Lichen 336 IAEA (International Atomic Energy Agency).

2.3. Analysis of samples

In order to analyze the largest possible number of elements into moss samples, two analytical procedures were applied in this work. 36 elements were determined up to µg/g levels or lower. Analyses are performed at the Saclay Nuclear Research Center (Pierre Süe Laboratory, Saclay/Paris, France) which already participated in several intercomparison studies organised by the IAEA to authenticate certified material results (among which Lichen Material 336 IAEA).

On the one hand, 34 elements were analyzed by the INAA quasi-absolute k_o-method with detection limits around 1 µg/g for non-treated samples. The samples were irradiated in Saclay's ORPHEE reactor with a neutrons flux of 1.37×10^{13} n. $cm^{-2}.s^{-1}$ for short irradiations and 2.3×10^{13} n. $cm^{-2}.s^{-1}$ for long irradiations. Two kinds of analysis were made : short irradiations of 1 minute in which short lived radionuclides were analyzed (Al, Ca, Cl, Cu, K, I, Mg, Mn, V, Ti) and long irradiations of 17 hours were used to analyze long lived radionuclides (As, Au, Ba, Br, Cd, Ce, Co, Cr, Cs, Eu, Fe, Hg, La, Mo, Na, Rb, Sb, Sc, Se, Sm, Th, W, Zn, Zr). The gamma-activities of samples were measured three times after decay times of 2 min, 15 min and 6 d, 7 d, 15 to 21 d, respectively. The γ-spectra of samples were determined using a Ge-Li detector.

On the other hand, the ICP-MS is a multi-element technique and uses the digestion of samples in a solution. We used it to analyze especially lead (Pb), and nickel (Ni) which was never measured with our INAA procedure. We also analyzed cadmium (Cd) and copper (Cu) with the ICP-MS method because the detection limits were better than INAA.

We could also have to compare results obtained by both analytical methods for arsenic (As), cobalt (Co), cesium (Cs) and vanadium (V).

3. RESULTS

557 moss samples were collected all over France at 512 sites, in decreasing order : *Hypnum cupressiforme* (199), *Scleropodium purum* (159), *Pleurozium schreberi* (111), *Hylocomium splendens* (60), and *Thuidium tamariscinum* (28). We used 48 of 102 sites in French permanent Plot for the Monitoring of Forest Ecosystems coordinated by French National Forest Office. The sampling of moss species in 22 administratives regions was located as shown in Table 1. One sample of moss at least was collected at each of 512 sites, with a density of almost one site for every 1000 km^2. Some regions like Brittany, the North and the Pays-de-Loire were not heavily sampled (0.6 sites/1000 km^2). In 45 sites, two species of moss were collected together on each site (Table II). We found often

TABLE I. MOSS SPECIES SAMPLED IN FRANCE

Regions (administratives)	P.s.	H.s.	H.c.	S.p.	T.t.	total samples	samples/ 1000 km^2	sites	sites/ 1000 km^2
Alsace	6	-	2	2	-	10	1.2	8	1
Aquitaine	10	2	14	14	1	41	1	41	1
Auvergne	13	6	4	4	3	30	1.2	27	1
Bourgogne	3	10	3	10	6	32	1	31	1
Basse-Normandie	2	-	11	7	-	20	1.1	20	1.1
Bretagne	3	-	6	8	-	17	0.6	17	0.6
Centre	18	4	8	11	1	42	1.1	39	1
Champagne Ardenne	-	4	15	9	2	30	1.2	25	1
Corse	-	-	-	3	-	3	0.3	3	0.3
Franche-Comté	1	2	5	3	7	18	1.1	17	1
Haute-Normandie	4	-	4	3	2	13	1.1	10	0.8
Ile-de-France	8	1	28	9	-	46	3.8	30	2.5
Limousin	8	2	3	-	-	13	0.8	12	0.7
Lorraine	2	1	4	13	-	20	0.8	20	0.8
Languedoc Roussillon	7	1	14	3	-	25	0.9	25	0.9
Midi-Pyrénées	8	8	19	5	1	41	0.9	41	0.9
Nord	1	1	4	3	-	9	0.7	8	0.6
PACA	3	6	16	5	-	30	0.9	28	0.9
Poitou Charentes	2	1	6	23	2	34	1.3	33	1.2
Picardie	1	1	13	7	1	23	1.2	21	1.1
Pays-de-Loire	3	-	8	11	-	22	0.7	19	0.6
Rhône-Alpes	8	10	12	6	2	38	0.9	37	0.8
TOTAL FRANCE	111	60	199	159	28	**557**	46	**512**	0.9

PACA = Provence-Alpes Côte d'Azur ;
P.s. = *Pleurozium schreberi* ; H.s. = *Hylocomium splendens* ; H.c. = *Hypnum cupressiforme* ;
S.p. = *Scleropodium purum* ; T.t. = *Thuidium tamariscinum* ;
(-) = no sampling

TABLE II. SAMPLING OF BOTH SPECIE ON SITE (ALL OVER FRANCE)

Mosses	P.s./H.s.	P.s./H.c.	P.s./S.p.	P.s./T.t.	H.s./H.c	H.s./S.p	H.s./T.t	H.c./S.p.	H.c./T.t.
Sites	3	10	3	1	7	2	3	15	1

TABLE III. ELEMENTAL CONCENTRATIONS IN MOSSES (μg/g MOSS DRY WEIGHT)/H*ypnum c. - Pleurozium s*

		Hypnum cupressiforme			***Pleurozium schreberi***			
El.	n_{Hc}	Mean	SD	Range	Mean	SD	Range	n_{Ps}
As	10	0.3	0.1	0.2-0.5	0.4	0.3	0.2-1	10
Cd	10[e]	-	-	<0.2-<1	-	-	<0.7-<1	10[d]
Cr	10	3.0	2.0	1.4-8	3.5	2.4	1.9-9.5	10
Cu	10[b]	8	2.8	4-<16	-	-	<11-<38	10[e]
Fe	10	534	261	265-1050	637	420	330-1640	10
Hg	10[a]	0.05	0.02	0.03-0.07	0.04	0.03	0.02-0.1	10[c]
V	10	2.2	0.6	1.4-3.5	2.7	0.9	1.9-4.7	10
Zn	10	42	9	29-55	34	6	26-43	10

a,b,c,d,e = 1,2,4,9 or 10 data below or close to detection limits in INAA

TABLE IV. ELEMENTAL CONCENTRATIONS IN MOSSES (μg/g MOSS DRY WEIGHT)/H*ypnum c. - Scleropodium p.*

		Hypnum cupressiforme			***Scleropodium purum***			
El.	n_{Hc}	Mean	SD	Range	Mean	SD	Range	n_{Sp}
As	10	0.4	0.1	0.3-0.6	0.6	0.05	0.2-0.9	10
Cd	10[c]	-	-	0.2-<1	-	-	0.6-<1	10[c]
Cr	10	3.8	1.5	2.3-6.5	7.2	2.5	3.1-21.3	10
Cu	10[a]	11	1.9	<5-<30	-	-	<16-<38	10[c]
Fe	10	708	309	380-1160	1529	336	543.5-3115	10
Hg	10	0.06	0.02	0.03-0.1	0.09	0.02	0.05-0.2	10[b]
V	10	3.1	0.8	2-4.5	4.1	0.6	1.95-6.7	10
Zn	10	55	16	35-85	52	3	16-99	10

a,b,c = 4,7 or 10 data below or close to detection limits in INAA

sampled *Hypnum cupressiforme* and *Pleurozium schreberi* together and *Hypnum cupressiforme* and *Scleropodium purum* together, on 10 sites and 15 sites respectively. In the future, we'll be able to obtain some information on species-specific accumulation.

At present, results are in progress. 557 mosses were cleaned, sorted and, 150 samples among which 68 from the Ile-de-France area (including also the nearest sites from Ile-de-France) were analyzed by INAA up to july 1997. The analyses were not yet started in ICP-MS. We used our data on 34 elements analyzed by INAA to map element deposits in the Ile-de-France area, thanks to IAURIF (Institute for Regional and Urban planning of the Ile-de-France). One map was drawn for each element, identifying moss species and analysis results from each samples. The interpretation of maps to distinguish local emissions and long distance deposits is in progress. The sampling of 4 moss species as *Hypnum c.* (41), *Scleropodium p.* (14), *Pleurozium s.* (12), *Hylocomium s.* (1), poses problems in interpretation of these maps because differences in the elemental concentrations exist between moss species collected at the same site.

In the Ile-de-France area, we collected *Hypnum c.* and *Pleurozium s.* samples from 10 sampling sites where both moss species occurr together and, also *Hypnum c.* and *Scleropodium p.* samples from 10 other sampling sites where both moss species occurr together. We tried to investigate

TABLE V. PEARSON'S CORRELATION COEFFICIENTS (r) AND THE PARAMETERS (A, B) FOR THE FONCTION Hc = a.Ps + b, WITH Hc AND Ps BEING THE ELEMENTAL CONTENT IN H*ypnum cupressiforme* AND P*leurozium schreberi*, RESPECTIVELY. (ILE-DE-FRANCE)

	Hypnum cupressiforme / Pleurozium schreberi						
El.	n	r	a	confidence limits (95%)	b	confidence limits (95%)	SD
As*	10	**0.73**	0.34	0.07 - 0.59	0.22	0.11 - 0.33	0.09
Cr	10	0.60	0.49	-0.04 - 1.01	1.31	-0.9 - 3.52	1.66
Fe*	10	**0.72**	0.45	0.09 - 0.8	249	-15.75 - 514	193
V*	10	**0.72**	0.48	0.09 - 0.87	0.94	-0.14 - 2.02	0.45
Zn	10	0.51	0.79	-0.31 - 1.89	14.99	-22.72 - 52.69	8.32

Significant (*) at P = 95%

TABLE VI. PEARSON'S CORRELATION COEFFICIENTS (r) AND THE PARAMETERS (a, b) FOR THE FONCTION Hc = a.Sp + b, WITH Hc AND Sp BEING THE ELEMENTAL CONTENT IN *Hypnum cupressiforme* AND S*cleropodium purum*, RESPECTIVELY. (ILE-DE-FRANCE)

	Hypnum cupressiforme / Scleropodium purum						
El.	n	r	a	confidence limits (95%)	b	confidence limits (95%)	SD
As	10	-0.12	-0.05	-0.38 - 0.29	0.42	0.22 - 0.63	0.11
Cr	10	-0.28	-0.07	-0.28 - 0.13	4.36	2.52 - 6.21	1.5
Fe	10	0.02	0.009	-0.29 - 0.30	694	183 - 1206	328
V	10	0.07	0.03	-0.34 - 0.40	2.94	1.29 - 4.59	0.87
Zn**	10	**0.84**	0.61	0.29 - 0.92	22.9	4.7 - 41.0	8.9

Significant (**) at P = 99%

the interchangeability of moss species (*Hypnum c.* with *Pleurozium s.* and *Hypnum c.* with *Scleropodium p.*) in a polluted area and to evaluate the method for the determination of the calibration factors. This preliminary study is part of work which should carry out information on **the inter-species calibration** in France [3].

Tables III and IV show the means, standard deviations and the ranges of the elemental concentrations (As, Cd, Cr, Cu, Fe, Hg, V, Zn) in both moss species occurring together.

The mean elemental concentrations in both species, *Hypnum c.* and *Pleurozium s.* (Table III) were of comparable magnitudes, except for Cu and Cd no calculated. For these elements, most of the elemental concentrations data set are below detection limits by INAA. In a futur study, we shall accept Cu and Cd for discussion because the ICP-MS method will be used which go down these detection limits. Comparing the ranges in Table III, *Pleurozium s.* showed markedly higher extreme concentrations than *Hypnum c.* for As and Fe. On the contrary, the highest concentrations were found in *Hypnum c.* for Zn.

The mean elemental concentrations in both species, *Hypnum c.* and *Scleropodium p.* (Table IV) were of comparable magnitudes, except for Cr and Fe. For these elements the averages in the *Scleropodium purum* data set were 1.9 to 2.15 times higher than in the *Hypnum c.* data set. Comparing the ranges in Table IV, *Scleropodium p.* showed markedly higher extreme concentrations than *Hypnum c.* for Cr and Fe.

We focussed our attention on the linear relationships between the elemental concentrations which were of comparable magnitude, in both moss species. The interspecies relations employing Pearson's correlation coefficient are given in Table V (*Hypnum c.* and *Pleurozium s.*) and Table VI

FIGURE 1. Pleurozium / Hypnum (x/y) linear regression analysis, illustrating that interspecies calibration is possible for arsenic

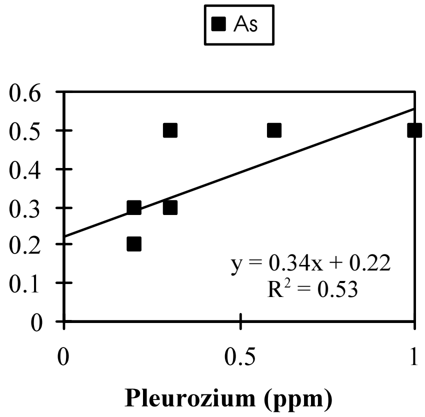


FIGURE 2. Scleropodium / Hypnum (x/y) linear regression analysis, illustrating that interspecies calibration is possible for zinc

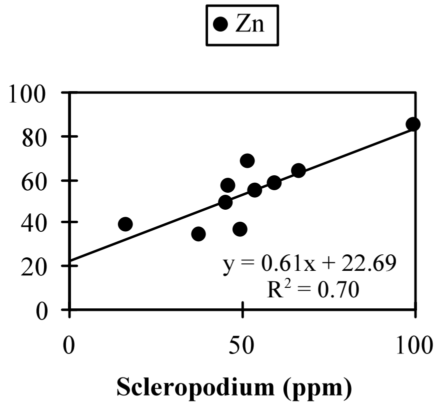


FIGURE 3. Pleurozium / Hypnum zinc concentration ratios (Ps.Hc^{-1}) as a function of the inverse Hypnum concentrations (Hc^{-1}), illustrating that interspecies calibration is not possible due to saturation effects in Pleurozium

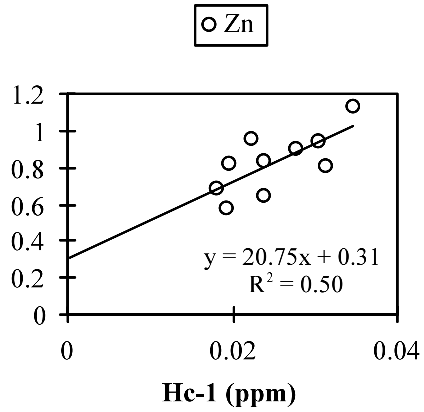
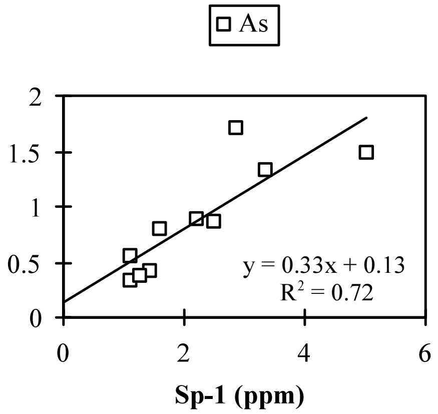


FIGURE 4. Hypnum / Scleropodium arsenic concentration ratios (Hc.Sp^{-1}) as a function of the inverse Scleropodium concentrations (Sp^{-1}), illustrating that interspecies calibration is not possible due to saturation effects in Hypnum



(*Hypnum c.* and *Scleropodium p.*), respectively. No correlation results for Hg are determined in Tables V and VI because much more data are given with limits or close to detection limits.

Hypnum c. and *Pleurozium s.* are significantly correlated for 3 elements among the 8 studied. For As, Fe and V the correlation is significant at the 95% probability level. The correlation coefficients range from 0.72 to 0.73. For the other elements, no significant correlations could be determined. But, a correlation could be significant for chromium (Cr) at the 90% probability level. For example, Figure 1 shows the good correlation between *Hypnum c.* and *Pleurozium s.* for arsenic.

Hypnum cupressiforme and *Scleropodium purum* are significantly correlated for Zn at the 99% probability level (Figure 2). The Pearson's correlation coefficient is 0.84. No significant correlations could be found for the other elements studied.

The constants a and b mean the slope and the intercept, respectively and their 95% confidence limits are also given in Tables V and VI, derived from suitable fonction $y = a.x + b$ for the data. For Zn and As, the a values (Table V and Table VI, respectively) did not significantly differ from zero, whereas the b values were significantly positives, suggesting a saturation effect in one species. If the concentrations in one species (x) have reached the saturation level (c value), then the ratio (y/x) as a function of the inverse concentrations (1/x), would be a straight line going through the origin and c being the value for the slope. Figures 3 and 4 show for Zn and As, respectively that the ratios (Ps/Hc and Hc/Sp) were significantly correlated with 1/Hc (P=95%) and 1/Sp (P=99%), respectively and, the

TABLE VII. PRELIMINARY STUDY ON INTERSPECIES CALIBRATION USING ILE-DE-FRANCE DATA ($n_{Ps/Hc}$=10, $n_{Sp/Hc}$=10), FOR ARSENIC (As), CHROMIUM (Cr), IRON (Fe), VANADIUM (V) AND ZINC (Zn)

	Correlation	
	Significant	No significant
Pleurozium schreberi *Hypnum cupressiforme*	As, Fe, V	Cr, Zn^{Ps}
Scleropodium purum *Hypnum cupressiforme*	Zn	As^{Hc}, Cr^{Hc}, Fe^{Hc}, V^{Hc}

^{Hc} = no significant correlation for the element due to saturation effects in *Hypnum cupressiforme*
^{Ps} = no significant correlation for the element due to saturation effects in *Pleurozium schreberi*

intercepts were nearly zero, suggesting a saturation effect in *Pleurozium s.* and *Hypnum c.*, respectively. We can briefly summarize preliminary results on interspecies calibration as shown in Table VII.

4. DISCUSSION

The difficulty of thorough sampling for a single species has often been mentioned in the literature [4]. At the scale of a country and even of a region it is not always possible to collect one specie in line with a high density of sampling [5]; this is only too true at the scale of the Europe [2].

In France, it was not possible to sample the main species *Pleurozium schreberi* and *Hylocomium splendens* throughout the country according to the Scandinavian specifications. Therefore, in absence of both these species, *Scleropodium p.* and *Hypnum c.* were supplemented where necessary. *Pleurozium schreberi* and *Hylocomium splendens* are widespread, carpet-forming species abundant on acid and organic substrates in coniferous forests throughout mountains and many French regions. In France, it was difficult to collect these species because the substratum of so many areas (for example, in the Ile-de-France area) is mainly limestone. Thus, we sampled *Scleropodium p.* which is a widespread species on siliceous and calcareous substrate. In addition, *Scleropodium p.* is very similar to *Pleurozium s.* in shape. *Hypnum c.* was the most sampled species because it also grows on the surface of decaying stumps. We collected a large diversity of moss species because French ecological, geographical and climatic conditions vary largely. The differences between species in response to pollution levels may be due to differences in accumulation characteristics, possibly related to morphological differences in moss features [6]. Results cannot be combined if interspecies calibration is not possible. Significative correlations have been shown in the accumulation of some trace elements in lichens [7] and mosses [8] concerning largely studied mosses as *Pleurozium s.* and *Hylocomium s.*. In the literature, there is a lack of information about interspecies calibration with *Hypnum c.* and *Scleropodium p.*. Our preliminary study has shown that interspecies calibration could be possible for arsenic, iron and vanadium with *Hypnum c.* and *Pleurozium s.* ,and for zinc with *Hypnum c.* and *Scleropodium p.*. But, in many cases interspecies calibration should be not possible due to saturation effects in one species. The problem of saturation level has already been mentioned in the lichenology literature [7]. The saturation effect is a parameter which should be defined for the calibration factor between moss species.

5. CONCLUSION

Our project applied to France followed the Sandinavian guidelines for the 1995-1996 European programme "*Atmospheric Heavy Metal Deposition in Europe - estimation based on moss analysis*" coordinated by the Nordic Council. A large diversity of moss species were collected which poses problems for interspecies calibration, more particularly between *Hypnum c.* and

Scleropodium p.. A preliminary work of interspecies calibration in the Ile-de-France area focussed on both pairs of moss species, *Hypnum c.* and *Pleurozium s.* and, also *Hypnum c.* and *Scleropodium p.*. Significant correlations were shown for some heavy metals and, when the intercalibration was not possible, saturation effects in one species were sometimes observed in data set. However, those conclusions being based on very small number of moss couples, we plan to extend this study to other pairs collected in France. In the future, all of the results will be used to map element deposits in France, including various statistical approaches and a calibration factor between moss species. Local emissions and long distance deposits will be distinguished.

ACKNOWLEDGEMENTS

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VERTICAL PROFILES OF $^{239(240)}$Pu, ^{238}Pu AND ^{241}Am IN SOME PECULIAR ITALIAN MOSSES

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Abstract

During the last two years the Urbino University and the Padua ICTIMA CNR were working on a special radioecological program having the aim to study the Pu and Am retention behaviour in different species of mosses growing in two Italian regions (Urbino, Central Italy, 450 m a.s.l. and Alps region, Northern Italy, 1500 m a.s.l.). ^{239,240}Pu, ^{238}Pu and ^{241}Am were separated and determined by extraction chromatography, electroplating and alpha spectrometry; ^{242}Pu and ^{243}Am were used as the yield tracers. The paper summarizes the results dealing with the vertical profiles of the radionuclides in three different species of mosses. Several 1-2 cm high sections were obtained and dated by ^{210}Pb determination. Typical concentration peaks for Pu and Am were found for very old moss species ("Sphagnum Compactum" and "Sphagnum Nemoreum") at a depth corresponding to the early 1960's which is the period characterized by the maximum nuclear weapon tests. In more recent moss species ("Neckeria Crispa") no peak was observed and the regression curves showed that Am is more mobile than Pu.

1. INTRODUCTION

The use of bioindicators to assess the occurence of airborne pollutants is receiving an ever increasing attention. In fact, they can provide basic information on the pollution levels of airborne chemical species of present and past times. As is well known mosses present a particular interest as bioaccumulators [1-3]. Furthermore some peculiar old mosses appear to be particularly suitable to investigate atmospheric contamination and they may provide a record of the history of the atmospheric fallout, especially when collected in appropriate sampling sites (open areas, absence of trees and vascular plants).

The present paper deals with the concentration vertical profiles of plutonium and ^{241}Am (daughter of ^{241}Pu) in three different species of Italian mosses .

2. EXPERIMENTAL

2.1. Sampling

Fig. 1 shows the two sites where the samples were collected: the first site was at 450 m a. s. l. in Central Italy where some "Neckeria Crispa" species mosses were collected; the second sampling site was located in the Dolomitic Alps (North eastern Italy at 1500 m a.s.l.) were "Sphagnum Compactum" and "Sphagnum Nemoreum" species were collected. To get the vertical distribution the samples were cut into 1-2 cm sections and analyzed separately.

2.2. Radioanalytical methods

The analytical procedure used in this investigation can be described as follows. All the samples were dried to constant weight at 105°C for 24 h and then ground into dust for homogenization. Known activities (10-30 mBq) of ^{242}Pu and ^{243}Am as the yield tracers were added to the sample which was then dry-ashed in muffle at 450°C. Two leachings were carried out with 6 M HCl.

*Fig.1. Sampling sites: ** ***Urbino****, Central Italy, 450 m a.s.l., (Neckeria Crispa)* ◊ ***Dolomitic Alps****, Northern Italy, 1500 m a.s.l. ("Sphagnum Compactum" and "Sphagnum Nemoreum").*

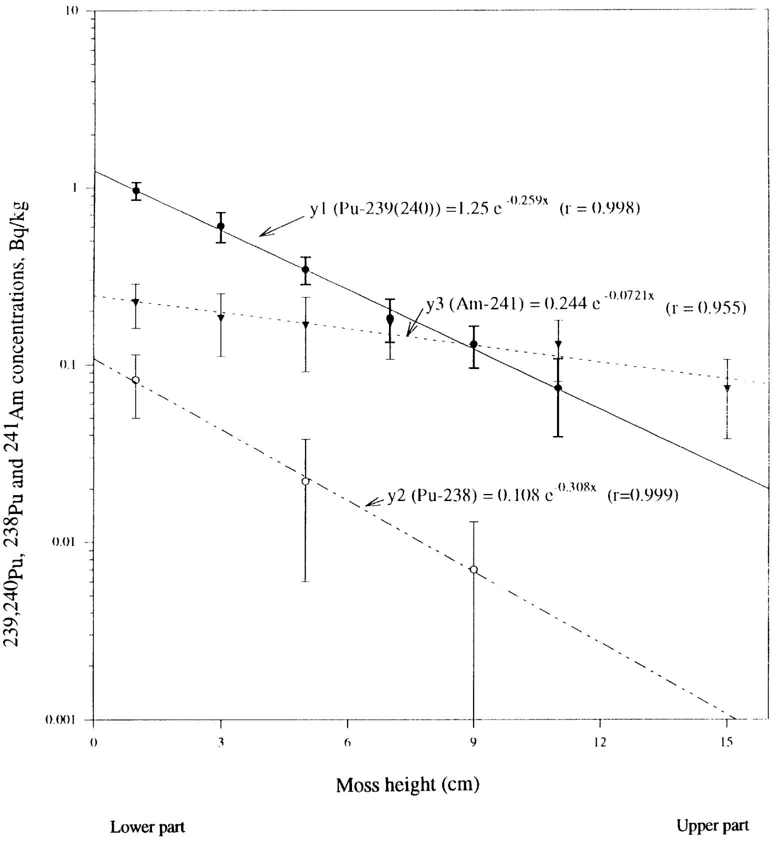

Fig. 2. ^{239,240}*Pu (•),* ^{238}*Pu (o) and* ^{241}*Am (♦) vertical distribution in "Neckeria Crispa" mosses.*

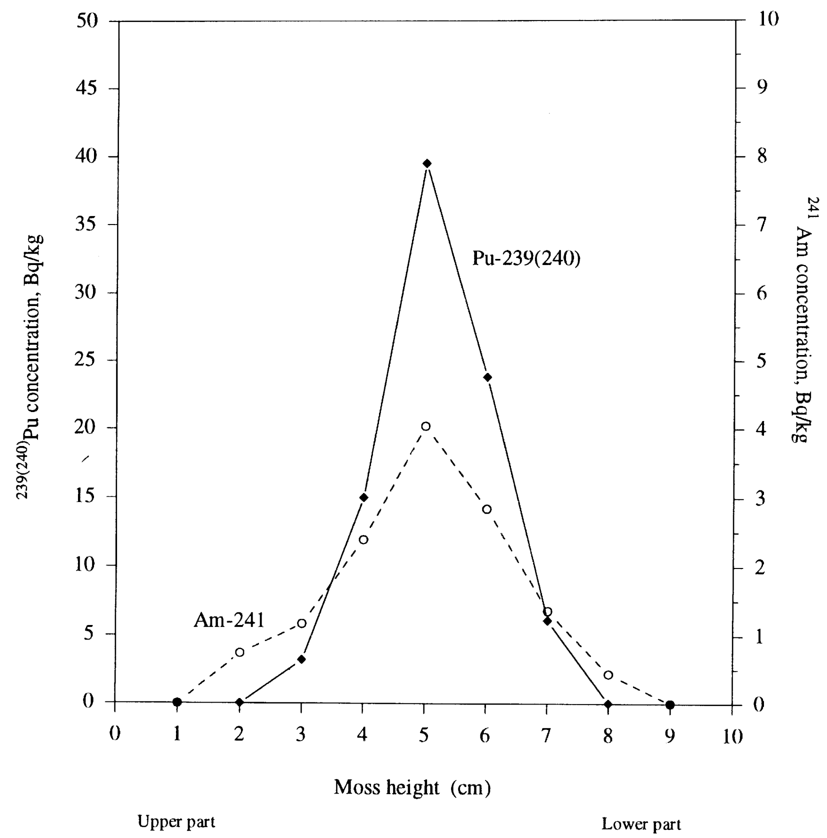

Fig. 3. ^{239,240}*Pu and* ^{241}*Am vertical distribution in "Sphagnum Compactum" mosses.*

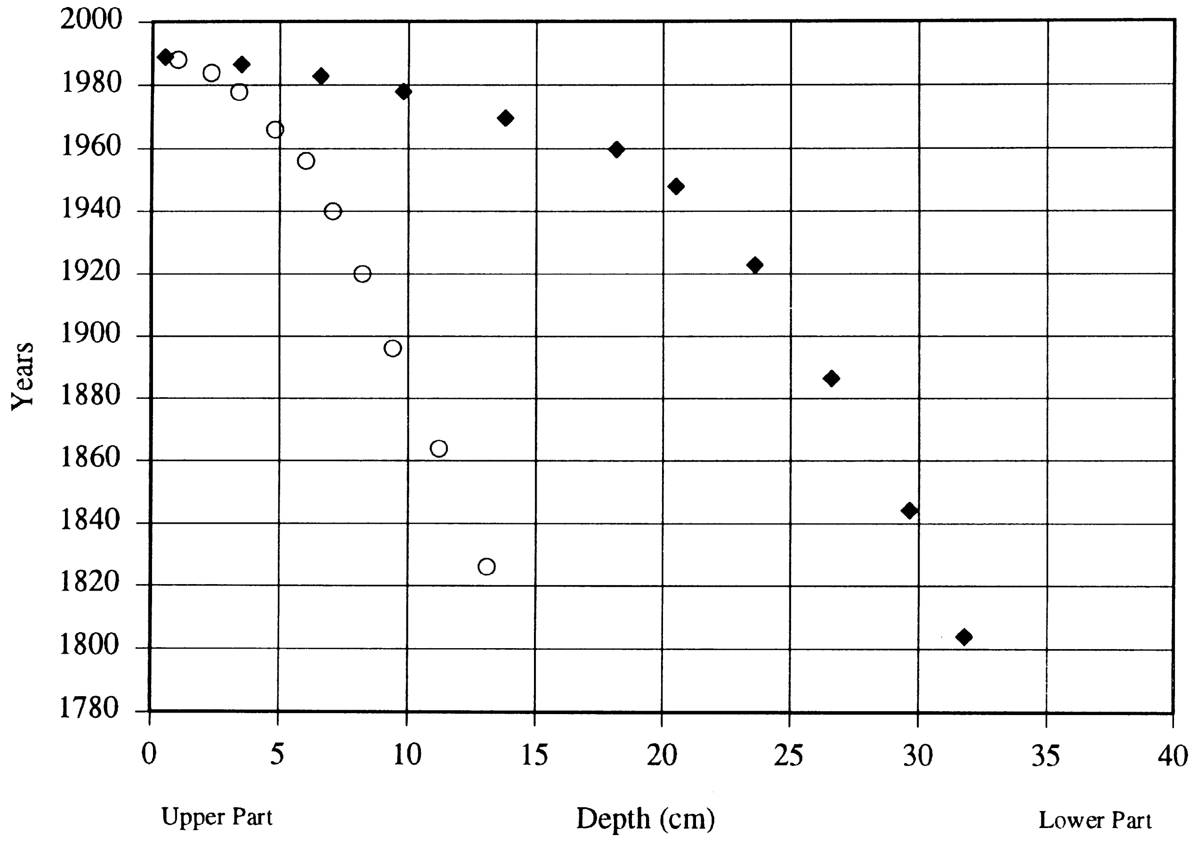

Fig. 4. "Sphagnum Compactum" (o) and "Sphagnum Nemoreum" (♦) sections datation by the ^{210}*Pb method.*

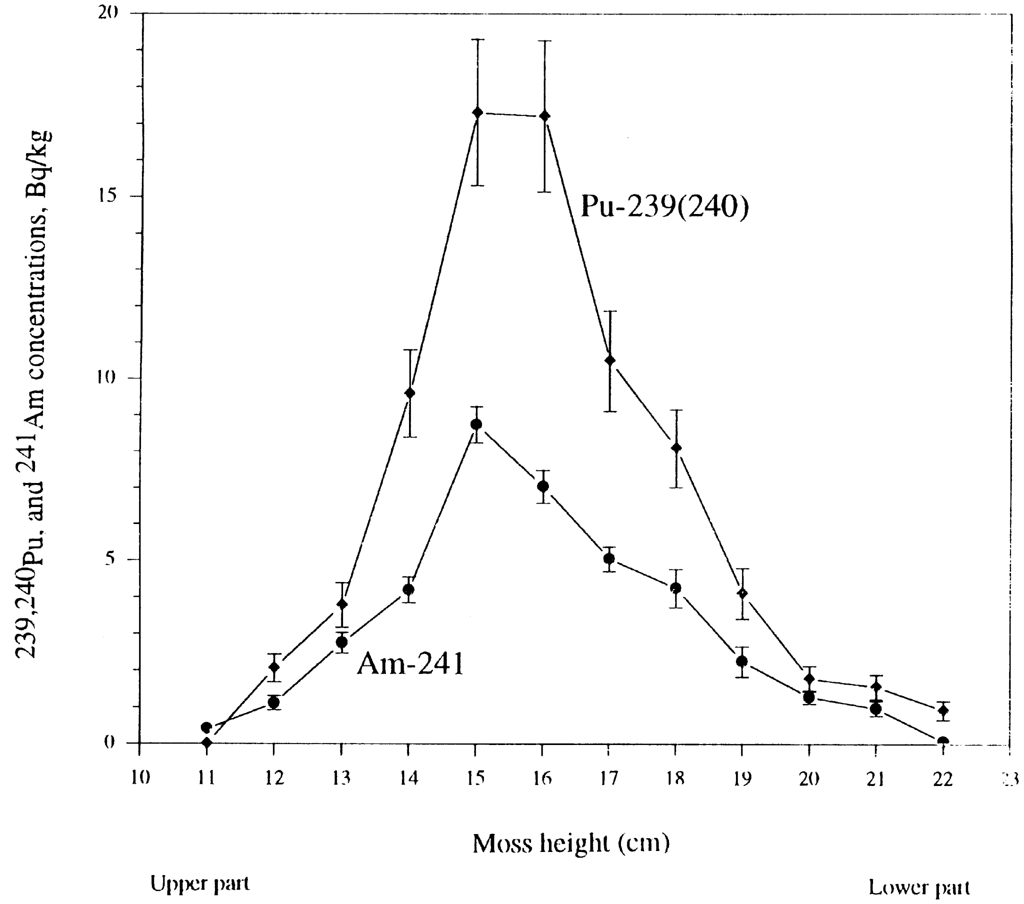

Fig. 5. ^{239,240}*Pu and* ^{241}*Am vertcal distribution in "Sphagnum Nemoreum" mosses.*

Plutonium was adsorbed in HCl medium by a Microthene (microporous polyethylene)-TNOA (tri-n-octyl-amine) column, eluted by a 0.025 M $H_2C_2O_4$/0.15 M HNO_3 solution, electroplated on a stainless steel disk at pH 1.5 and determined by α spectrometry. The column effluent was kept for the americium analysis. Americium was separated by a Microthene-HDEHP [di(2-ethylhexyl)phosphoric acid] column, purified by a PMBP (1-phenyl-3-methyl-4-benzoyl-5-pyrazolone)-TOPO (tri-octyl-phosphine oxide) extraction, electroplated from a 0.025 M $H_2C_2O_4$/0.1 M HNO_3 solution at pH 1.5 and counted by α spectrometry. The detection limits for 2 g moss samples were 28 mBq/kg for ^{238}Pu and ^{239,240}Pu and 34 mBq/kg for ^{241}Am. For more details see Ref. [4].

3. RESULTS

3.1. "Neckeria Crispa"

^{239,240}Pu, ^{238}Pu and ^{241}Am vertical distribution in the "Neckeria Crispa" moss species (Fig. 2) indicates that all the three radionuclides are present in the lower part of the plant and that they are undetectable above a 16 cm height. As expected the regression curve slope for ^{238}Pu is very close to that of ^{239,240}Pu; on the contrary the ^{241}Am regression curve shows a lower slope indicating that Am is more transferable than Pu in this kind of mosses.

3.2. "Sphagnum Compactum"

^{239,240}Pu and ^{241}Am vertical distribution [5] in this very peculiar and old terrestrial moss is shown in Fig.3. An interesting peak at 5÷6 cm from the top is observed for both radionuclides.

A dating of the different sections was performed by using the Pb-210 measure [6]: this method can be correctly applied as the lead concentration profiles showed a negligible mobility of this element in *Sphagnum* mosses. The curve shown in Fig.4 was obtained and it was possible to calculate that this peak corresponds to the period 1960-1965 which was characterized by the heavy radioactive pollution due to the several nuclear weapon tests in the open air. This fact demonstrates also that the mobility of Plutonium and of Americium-241 along the plant is negligible due to the chemical features of these two transuranic elements. As a matter of fact it was not possible to find a similar correlation with radionuclides (^{137}Cs, ^{134}Cs, ^{90}Sr, etc.) of more mobile elements for nuclear accidents occurred long ago.

3.3. "Sphagnum Nemoreum"

The ^{239,240}Pu and ^{241}Am vertical distribution obtained for the "Sphagnum Nemoreum" species is shown in Fig.5. A concentration peak for both radionuclides was found at 15-16 cm from the top. The relevant dating curve (Fig.4) shows that this peak corresponds to the same period obtained for the “Sphagnum Compactum” moss (1960-1965). This trend means also that the growing factor for the “Sphagnum Nemoreum” mosses is higher than for the “Sphagnum Compactum” mosses.

4. CONCLUSIONS

The results show that mosses are very effective accumulators of Plutonium and Americium. They may act as efficient filters in trapping airborne radioactive contaminants as well as natural reservoirs from which these radionuclides are periodically released. Therefore mosses can be recommended as very good biological accumulators of Pu and Am fallout caused by nuclear facilities accidents and from nuclear weapon tests. When they are long-life plants, they can play an important role in cycling naturally or artificially enhanced radionuclides in the atmosphere over long time scales. Moreover, they are easily available in any region and at any season.

The results also show that ^{241}Am contents in these plants are high enough to be determined by a sensitive radiochemical method. From the radiation protection point of view, ^{241}Am, which is one of the most toxic transuranic nuclides, is as much important as ^{239,240}Pu, and therefore it should be included in any future environmental monitoring program, due also to its concentration increase with time as it takes place from its parent ^{241}Pu ($T_{1/2}$ = 14.4 y).

Moreover these studies are important to predict the level and distribution of the radionuclides in the environment, to understand their biological and ecological behaviour in a given contamination condition and to evaluate the relevant possible hazard to human beings.

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QUALITATIVE ASPECTS OF BIOMONITORING: *SPHAGNUM AURICULATUM* RESPONSE VS. AEROSOL METAL CONCENTRATIONS (Pb Ca, Cr, Cu, Fe, Mn, Ni and Zn) IN THE PORTO URBAN ATMOSPHERE[*]

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Abstract

Bags of *S. auriculatum* and a low-volume aerosol sampler provided with 0.8 µm pore size filters were exposed, in parallel, to the atmosphere of Porto, at different sampling points and in different periods of time, between 1991 and 1997. The levels of lead in the moss (weekly samples) and in the filters (daily samples) were determined by atomic absorption spectrophotometry and the results were compared. Living *S. auriculatum* exposed in bags to the Porto atmosphere died in several weeks (about a month), but continued to sorb metals from the atmosphere for about another month. In dry weather periods (relative humidity ≤ 76 %) the rate of lead uptake by moss was approximately constant and proportional to the levels of the metal in atmospheric aerosols. A converting factor [$CF = |Pb|_{moss}$ (µg/g.day)/ $|Pb|_{air}$ ($\mu g/m^3$)] allowed conversion of the lead levels in *S. auriculatum* to those in the atmospheric aerosols. Because the moss fixed lead from gas, aerosol and particulate matter, the rate of sorption depends markedly on the distance to the lead sources (mainly traffic) and on surrounding obstacles which retain particles. Therefore, specific calibration by mechanic monitoring, at each sampling point is required in a first stage of biomonitoring, when moss bag samplers are used to provide quantitative information about lead levels in the atmosphere. The mean Pb levels were ≤ 0.5 $\mu g/m^3$ and approximately constants at each sample point up to January 1996. After that date it decreased about 50 %, in consequence of the reduction of the Pb concentration in leaded gasoline. In wet weather periods, higher but irregular rate of lead uptake was observed. In contrast, the lead levels in atmospheric aerosols decreased when the humidity increased due to wet deposition. Therefore, no proportionality between lead levels in the moss and in air were found. For about two months, in 1994, during a dry weather period, the levels of Ca, Cr, Cu, Fe, Mn, Ni and Zn were also biomonitored and monitored in parallel. For all the heavy metals, the rate of metal uptake by moss was significantly correlated with the metal concentration in atmospheric aerosols. The results indicated that moss bags of *S. auriculatum* can provide quantitative estimation of the concentration of different heavy metals in urban atmosphere since the present methodology is used. *S. auriculatum* showed not to be a good quantitative bioindicator for Ca. The mean aerosol metal concentrations found in Porto atmosphere were similar to those observed in other urban atmospheres in different countries. The relative order of the mean metal concentrations was Fe (1.8 $\mu g/m^3$) > Ca > Zn> Pb > Cu > Cr > Mn > Ni (20 ng/m^3). **Key words**: Urban atmosphere, Heavy metals, Aerosols, Moss bags, Low-volume sampler, Atomic absorption spectrometry.

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[*] Only an abstract appears here since parts of this paper have been published in Toxicol. Environ. Chem. **54** (1996) 195–209.

THE USE OF BIOMONITORS AND NEUTRON ACTIVATION ANALYSIS IN THE STUDY OF AIR POLLUTION OF BUENOS AIRES CITY

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Abstract

Biomonitors were used as part of a pollution study of Buenos Aires city atmosphere under the International Atomic Energy Agency Research Contract ARG 7251, from the Co-ordinated Research Programme on Applied Research on Air Pollution using Nuclear Related Analytical Techniques. Lichens were primarily selected as indicators. Two different approaches were conducted, direct sampling of *Parmotrema reticulatum*, at a few places and the use of lichen bags, filled with *Usnea sulcata* from a northern national park, and hung at different sites. Simultaneously, tree bark was tried as biomonitor. *Platanus acerifolia* and *Melia azedarach* were selected as candidates, for being the most common trees in the city, but only *P.acerifolia* was analyzed. All the samples were analyzed using instrumental neutron activation analysis at the Ezeiza Atomic Centre of the National Atomic Energy Commission. RA-3 reactor was used for the irradiations, determining: As, Ba, Br, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Na, Rb, Sb, Sc, Sm, Ta, Tb, Th, U, Yb and Zn. Concentration values for *P. reticulatum* compared well with values from literature. For *U. sulcata* differences were found among the tested sites and also, for some elements an increasing trend with time was observed. Enrichment factors calculated using Sc as reference and Mason´s crustal average concentrations showed vehicules and refuse incineration as contributing sources to the aerosol. Tree bark from Buenos Aires and from a smaller city with mainly agricultural activities were analyzed and the results are coincident with those from lichens. This work is the first and preliminar contribution to the study of Buenos Aires aerosol using biomonitors.

1. INTRODUCTION

Air pollution studies using biomonitors are an interesting and economic alternative to those performed by means of direct measurements, especially when large areas have to be covered [1-3]. Buenos Aires urban area has aproximately 11 million inhabitants and 2.7 million vehicules, numerous industries and workshops, and varied traffic conditions and building profiles. With these characteristics it could present many of the pollution problems typical of a mega city. Its principal emission source has been considered to be vehicule circulation but, until recently, there has been no information about other emission sources and the city has no air monitoring network. Several years ago, a municipal sampling network for SO_2, NO_x, CO_2 and particles, was deactivated and since then, only isolated efforts had been done on this subject. Previous to an aerosol study conducted between 1993 and 1996, at the Neutron Activation Analysis Laboratory of the National Atomic Energy Commission, there were no trace-element data available for atmospheric aerosols. This study was conducted within an IAEA Co-ordinated Research Programme on Applied Research on Air Pollution using Nuclear Related Analytical Techniques (Research Contract ARG 7251) and it not only allowed to gather information about the aerosol elemental profile but also to know the most important types of pollution sources for the city area.

As a supplement of the direct sampling conducted at this programme, a study was began to explore the possibility of the application of biomonitors to the study of air pollution in Buenos Aires. Its results though preliminar, are the first ones for this kind of approach to aerosol studies, for the city.

Parmotrema reticulatum was suggested as a suitable biomonitor, but a survey showed that although this lichen is present at different locations of the country, it is scarce in the city. As another approach, lichen transplants were tried, using *Usnea sulcata* from a clean area at a National Park in the north of the country.

As an alternative biomonitor tree bark was considered, as tree-lined streets are very common in Buenos Aires. The municipal inventory of tree species showed *Platanus acerifolia* and *Melia azedarach* as the most common ones. *M. azedarach* has a very rough bark which offers more difficulties for sampling and sample preparation, than that of *P. acerifolia*, so this last tree species was selected.

2. SAMPLING AND SAMPLE PREPARATION

2.1. Lichens

For *Parmotrema reticulatum,* some direct sampling was conducted at different places. These samples were taken from trees at a height between 1.5 and 2 m, put into clean plastic bags and transported to the laboratory where the lichen was separated from adhering bark with plastic tweezers. Special care was taken in selecting trees of similar age, not sampling from fallen, decayed or young ones. A first cleaning operation was done to separate soil, bark particles and other lichen species, then the samples were washed with deionized water with gentle agitation and dried in oven at 40 °C for 24 hours [1]. The dried material was ground in an agate mortar with the help of liquid nitrogen to get it brittle and the material was kept refrigerated until its analysis [4].

Usnea sulcata was collected at a clean area at “El Copo” National Park (Santiago del Estero province). Nylon-mesh bags filled with the lichen were hung from trees at five sites of Buenos Aires area, but they were recovered only from three of them. Three bags were used at each site, to try exposure times of three, six and nine months.

Once the bags were collected and carried to the laboratory, the lichen filling was lyophilized and ground for the analysis.

2.2. Tree bark

Tree bark samples [1, 3] were collected from trees at a height between 1.5 and 2 m. At the laboratory the samples were gently brushed to remove soil, insects or any other solid pollutants and then treated as *P. reticulatum* samples.

3. ANALYTICAL METHODS

Masses of about 150 mg were used for instrumental neutron activation analysis, doing three replicates of each sample. The samples were sealed in quartz ampoules and irradiated for five hours at the RA-3 reactor (thermal flux 3.10^{13} $cm^{-2}.s^{-1}$ and 4.5 MW) located at the Ezeiza Atomic Centre of the National Atomic Energy Commission. Once the irradiation was concluded, the ampoule contents were transferred to fresh plastic vials for counting. Two measurements were done, with decay times of 6 days and 4 weeks, for the determination of As, Ba, Br, Ce, Co, Cr, Cs, Eu, Fe, Hf, La, Lu, Na, Rb, Sb, Sc, Sm, Ta, Th, U, Yb and Zn [5,6]. They were performed using a HP Ge detector (resolution 1.9 keV for the 1332.5 keV ^{60}Co) coupled to a Series 85 Canberra Multichannel and the elemental concentrations were calculated using a software developped at the laboratory. The accuracy of the analytical technique was tested by the analysis of standard reference materials from NIST: SRM-1572 Citrus Leaves and IAEA: V-10 Hay Powder, SL-1 and Soil -7, finding good agreement with literature values.

4. RESULTS AND DISCUSSION

P. reticulatum growing at eight different locations was analyzed and enrichment factors (EF), using Sc and Mason´s crustal abundances [7] as reference, were calculated. Enrichment factor values from three representative locations figure in Table I. These are: Longchamps (L_1), a residential

TABLE I. ENRICHMENT FACTORS FOR *Parmotrema reticulatum*

L_1	Buenos Aires suburb, Longchamps
L_5	El Copo National Park, Santiago del Estero
L_6	Martín García Island Reserve

	L_1	**L_5**	**L_6**
Ba	3.4	2.3	2.9
Br	160	49	64
Ce	1.4	5.0	5.3
Cr	1.7	1.0	0.9
Cs	2.6	8.5	9.9
Eu	0.5	3.5	3.3
Fe	1.1	1.4	1.3
Hf	---	5.3	4.1
K	1.4	4.2	2.2
La	3.5	2.5	4.2
Lu	---	0.9	1.8
Na	1.2	0.9	0.8
Nd	---	4.0	4.6
Rb	2.8	2.5	4.0
Sb	63	15	17
Sm	2.9	4.0	4.4
Ta	---	1.7	1.8
Th	---	4.5	3.7
Yb	---	2.6	2.8
Zn	51	13	20

Sc was used as reference.

The absence of a value is due to the missing of the corresponding concentration.

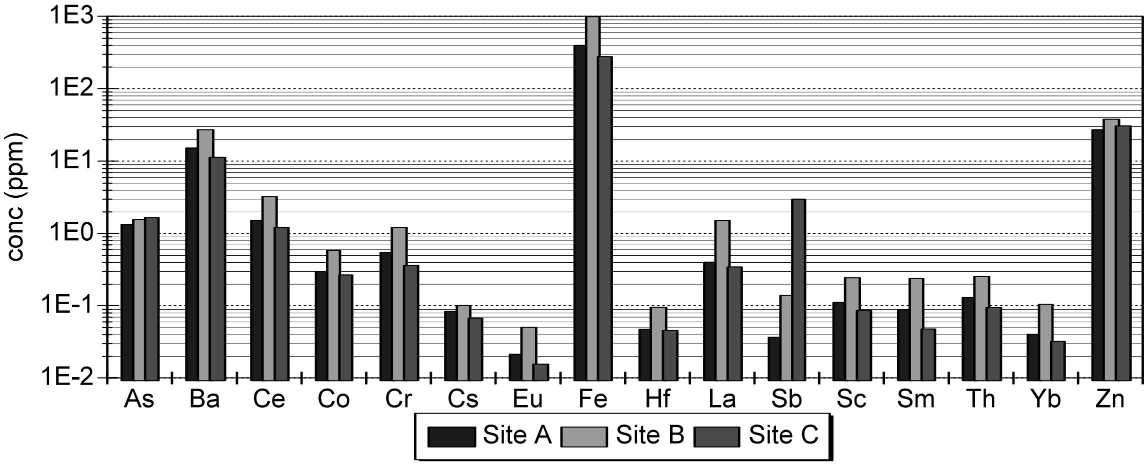

Fig 1. Usnea sulcata *concentrations for 3 months exposure.*

TABLE II. ENRICHMENT FACTORS FOR *Usnea sulcata*

site A	Buenos Aires suburb, on-storey houses, medium-low traffic
site B	Buenos Aires downtown, high buildings, high traffic
site C	Buenos Aires downtown, workshops, medium traffic

	Site A			**Site B**			**Site C**		
	A_3	**A_6**	**A_9**	**B_3**	**B_6**	**B_9**	**C_3**	**C_6**	**C_9**
Ba	7.0	9.8	10.5	5.7	6.5	4.8	6.8	0.8	0.9
Br	292	412	407	182	109	191	314	295	370
Ce	5.1	3.5	3.1	4.9	3.1	3.3	5.1	3.5	3.0
Co	2.3	2.5	2.5	2.0	2.0	2.0	2.7	2.6	2.6
Cr	1.1	2.0	2.8	1.1	1.8	1.4	0.9	1.2	1.8
Cs	3.9	4.6	4.4	2.2	2.9	2.8	5.7	7.5	8.2
Eu	3.5	3.9	2.6	3.7	2.4	2.6	3.3	3.5	4.4
Fe	1.6	1.3	1.2	1.8	1.3	1.3	1.5	1.2	1.5
Hf	3.1	4.6	3.6	2.8	5.0	3.6	3.9	3.8	5.5
K	4.6	8.4	5.3	5.7	2.3	3.3	16	30	62
La	2.5	3.2	3.5	4.4	3.0	3.2	3.0	3.0	2.8
Lu	1.5	1.9	2.3	0.2	2.1	2.3	3.8	1.9	2.8
Na	1.4	3.0	2.9	1.1	1.2	0.7	3.9	2.6	9.5
Rb	3.1	6.3	3.4	2.5	1.8	1.4	2.9	4.8	5.2
Sb	36	118	152	63	99	77	3773	5381	5918
Sm	2.9	2.4	2.4	3.6	2.6	2.8	2.1	2.1	1.9
Ta	2.3	2.0	1.3	1.3	0.9	1.3	2.4	4.0	2.9
Th	3.6	2.3	2.4	3.1	3.4	2.9	3.4	3.6	2.3
Yb	2.4	1.5	1.7	2.8	2.2	2.0	2.4	2.0	1.7
Zn	76	197	179	48	44	42	112	166	430

Sc was used as reference.
For each site, subindices 3, 6 and 9 are for three, six and nine month of exposure.

neighborhood of Buenos Aires southwestern suburbs, El Copo National Park (L_5) in Santiago del Estero province (1200 km north of Buenos Aires) and Martín García Island (L6), a reservation area on the Río de la Plata river. Three elements were found to be enriched with respect to the average crust composition (considering as enriched those values higher than 10). The higher values were those of the Buenos Aires sample and the enrichment of Br, Sb and Zn, suggests vehicule exhausts and refuse incineration as pollution sources [6]. These results are in agreement with what was expected on potential sources, but local soils will have to be analyzed in order to recalculate EF. The obtained elemental concentrations, compared well with other values from literature [1, 8].

The *Usnea sulcata* bags were recovered at three places: site A: a southwestern suburb of Buenos Aires, with one storey-houses with gardens and medium to low traffic; site B: a downtown location with extense park areas and high buildings and high vehicule circulation and site C, also downtown, but by a printing workshop and with medium traffic. For nearly all the determined elements, site B showed greater concentration values than the other two sites and also, looking at the results of 3, 6 and 9 months of exposure, a marked increasing tendency with time. For Zn, the three sites exhibit a similar performance and incresing tendency. As for Sb, site C has a much higher value than the other two locations. The differences obtained for site B were expected, in accordance to its characteristics. As for site C, some elements exhibit higher values than for site B, and this can be

TABLE III. *Platanus acerifolia* BARK ELEMENTAL CONCENTRATIONS (JUNIN SAMPLES)

	Range	**Mean (n=8)**	**Std. Dev.**
As	0.044 - 0.54	0.28	0.20
Ba	65 - 116	88	17
Br	1.3 - 6.5	3.1	1.7
Ce	0.44 - 1.57	0.70	0.38
Co	0.226 - 0.368	0.28	0.06
Cr	0.12 - 0.96	0.49	0.24
Cs	0.008 - 0.13	0.06	0.04
Eu	0.011 - 0.026	0.017	0.005
Fe	97 - 770	340	190
Hf	0.011 - 0.16	0.072	0.043
K	1674 - 3907	2668	652
La	0.11 - 0.72	0.34	0.19
Na	98 - 574	246	143
Rb	1.12 - 4.27	2.08	0.99
Sb	0.004 - 0.12	0.72	0.04
Sc	0.03 - 0.244	0.110	0.061
Sm	0.023 - 0.132	0.064	0.031
Th	0.032 - 0.24	0.113	0.057
Yb	0.012 - 0.069	0.030	0.018
Zn	6.3 - 18.7	12.4	3.9

Concentrations in ppm.

related to the presence of workshops in the area. Figure 1 shows the elemental concentrations for the three sites, for the bags corresponding to three months of exposure.

For all the results, enrichment factors were calculated to explore the source of the elements that had been determined, specially those showing rapid increase with time of exposure. The results are in Table II and it can be seen that those elements related to soil, as Fe and rare earths, are not enriched; Br, Sb and Zn appeared enriched as in the *P. reticulatum* experiment, signaling the same polluting sources.

The analytical methodology for tree bark was adjusted analyzing eleven samples of *Platanus acerifolia* from Junín, a city with mainly agricultural and cattle raising activities, 250 km from Buenos Aires. The elemental concentration ranges, average concentration and standard deviation are in Table III. Some samples from Buenos Aires were also analyzed and the results are in Table IV, together with those of a Junín sample. These bark samples were taken at sites with different characteristics: B$_3$: downtown with heavy traffic and high buildings; B$_4$: downtown, with medium traffic and low buildings; B$_8$: suburbs, with high vehicule circulation and low buildings and B$_9$: similar to B$_8$ but with medium traffic; the Junín sample (B$_{11A}$) represents a site with low buildings and medium traffic. The values for B$_9$ are, for nearly all the elements, out of Junín concentration ranges (Table III); for Cr, Sb and Zn, also B$_3$ and B$_4$ concentrations, exceed the upper limit of the range. Enrichment factors were calculated for these concentrations finding similar results to those obtained with lichens.

5. CONCLUSIONS

The results for the two lichen species are coincident on pointing to soil, vehicules and refuse incineration as contributors to atmospheric aerosol and similar results were achieved by a more extensive study with aerosol direct sampling. Vehicles were already suggested to be the most

TABLE IV. *Platanus acerifolia* BARK CONCENTRATIONS

B_3	Buenos Aires downtown, high buildings, high traffic
B_4	Buenos Aires downtown, low buildings, medium traffic
B_8	Buenos Aires suburbs, low buildings, high traffic
B_9	Buenos Aires suburbs, low buildings, medium traffic
B_{11A}	Junín, low buildings, medium traffic

	B_3	**B_4**	**B_8**	**B_9**	**B_{11A}**
As	0.201	0.348	0.160	0.630	0.152
Ba	111	57	110	208	80
Br	2.337	3.843	1.366	7.587	1.692
Ce	0.733	1.473	0.299	4.21	0.605
Co	0.390	0.501	0.342	0.936	0.326
Cr	1.33	1.67	0.341	3.72	0.398
Cs	0.045	0.081	0.019	0.267	0.063
Eu	0.0128	0.0217	0.0086	0.068	0.0151
Fe	430.3	630.3	122.1	1628.1	320.5
Hf	0.055	0.187	0.027	0.426	0.065
K	995	1332	2473	------	2595
La	0.515	1.006	0.171	0.0050	0.408
Na	156.3	380.7	108.6	907.1	196.3
Rb	1.55	2.26	1.55	5.58	1.49
Sb	0.209	0.148	0.039	0.276	0.065
Sc	0.0772	0.1744	0.0362	0.0318	0.1008
Sm	0.0539	0.1217	0.0028	0.0858	0.0586
Th	0.0752	0.1574	0.0404	0.570	0.1120
Yb	0.0246	0.0507	0.0139	0.140	0.0220
Zn	45.4	46.2	7.17	39.0	12.9

Concentrations in ppm

polluting source for the city which has daily values of CO concentration up to 18 ppm. With respect to refuse incineration, although it is prohibited within the city limits, more than 100 dumpsites have been reported at the suburbs, in what is called Great Buenos Aires area.

This work is only a preliminar one and more samples as well as local soils, have to be analyzed, but its results allowed to evaluate potential biomonitors, for a future monitoring network for Buenos Aires, or any of the other cities in the country with pollution problems. Also, through this study, it has been possible to obtain the first results on Buenos Aires aerosol using biomonitors.

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BIOMONITORING OF HEAVY METALS: DEFINITIONS, POSSIBILITIES AND LIMITATIONS[*]

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Abstract

Increasing attention given to heavy metals as components of the pollutant load in ecosystems makes it necessary to find reliable biological indicators. Fundamental investigations into the effect of heavy metals on organisms are therefore required. Different organisms (mosses, snails, etc.) were chosen as indicator organisms to optimize the indication of heavy metal loads at the physiological and biochemical level. All current programmes are designed to observe and measure pollutant inputs on a short or long-term basis. However, the changes in the environment of a phenological, physiological, sociological, genetic and physiological/biochemical nature have been investigated by biologists since the beginning of biological scientific research. So far excellent scientific results have been produced by qualification of the heavy metal status in ecosystems. Until now, the quantification of the results with regard to pollutant inputs in ecosystems (mass balances) and their action in these ecosystems have been investigated inadequately.

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DENDROANALYSIS: A TOOL FOR BIOMONITORING ENVIRONMENTAL POLLUTION?

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Abstract

Trees of temperate regions usually form visible annual growth rings, which can be dated accurately. It is then possible to collect wood samples of different age and analyse its heavy metal content in order to get a chronological record of trace element pollution in the tree environment. A basic assumption of dendroanalysis is the stability of the mineral distribution patterns, i.e., no significant mobility of the elements should occur once storaged. Additionally, neighbouring trees growing in the same environment should present a similar radial pattern. While some studies showed a good correlation between radial distribution of heavy metals in tree rings and temporal records of pollution from industry and traffic, others failed in using dendroanalysis as a chronological record of pollution. Probably some elements can move at a certain rate in radial direction through the ray parenchyma cells. Growth rates of tree rings can also influence the concentration of elements in wood. During periods of declining growth higher concentrations of elements can be found. Radial distribution patterns of heavy metals in wood rings should be carefully used as a tool for chronological record of environmental pollution.

1. DENDROANALYSIS: BASIC ASSUMPTIONS

In temperate regions trees usually produce one growth ring every year. It is then possible to accurately date the rings. When the annual ring ceases to function stores the information of the xylem sap, which is connected with the environment of the tree. It is a tempting idea to collect growth rings of different ages and analyse several trace elements in order to get a chronological record of the element composition of the environment of the tree [1]. It is assumed that the elements absorbed from the environment should be deposited only in currently growing parts of the tree, and that there is no significant movement of elements between the growth rings.

Some authors were apparently successful in showing a correlation between environmental pollution and the concentration of heavy metals in the growth rings [2-4]. The increasing concentrations of Al in growth rings of *Fagus sylvatica*, in recent years, was explained due to the acid depositions, which makes that element more available in the soil (Fig. 1). In *Pinus silvestris* the concentration of sulphur in the wood follows the increasing sulphur dioxide in the atmosphere (Fig. 2).

2. WEAK ASPECTS OF DENDROANALYSIS

2.1. Seasonal variations

Are elements present in stem wood really immobile? Some authors found seasonal variations of the elements (Fig. 3). Highest levels of trace elements were observed in the dormant season (December) and before bud break (April). In spring and summer, metal concentrations were lower [5]. Fluctuations in trace element concentrations in wood can be linked to changes in quantity and composition of the xylem sap during the year [5]. An increment of amino acids content in the xylem

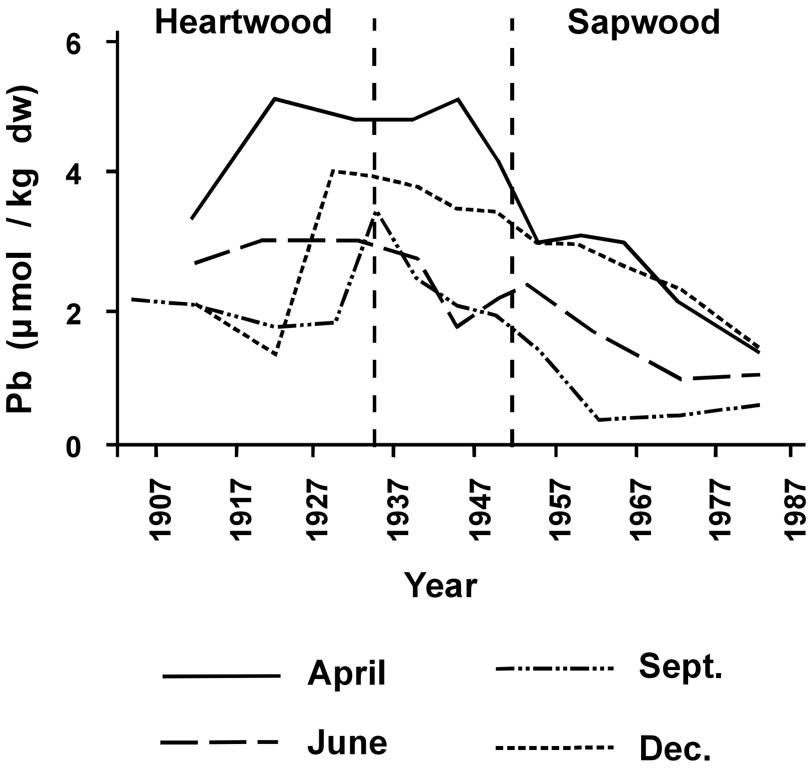

Fig. 1. Radial distribution of Al in tree rings of Fagus sylvatica L. [2].

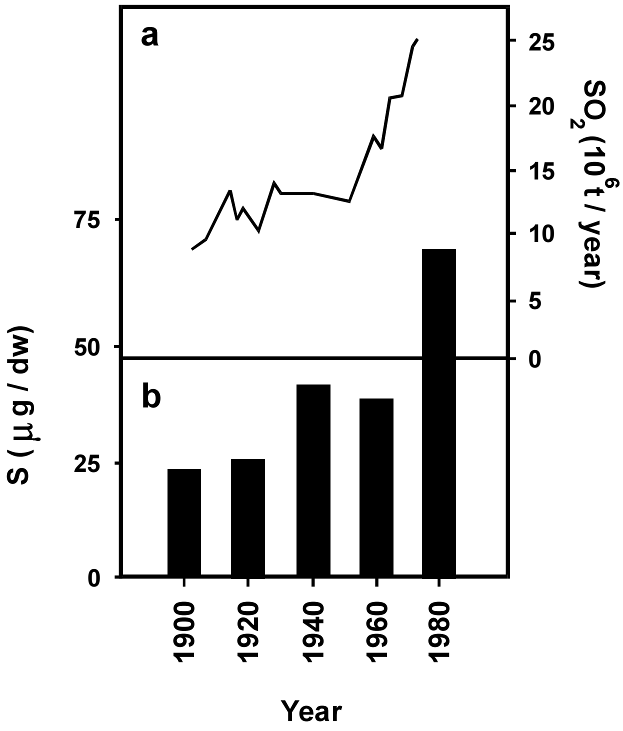

Fig. 2. Comparison between emission of sulphur dioxide in Europe (curve on top) and concentration of sulphur in the wood of Pinus silvestris [3].

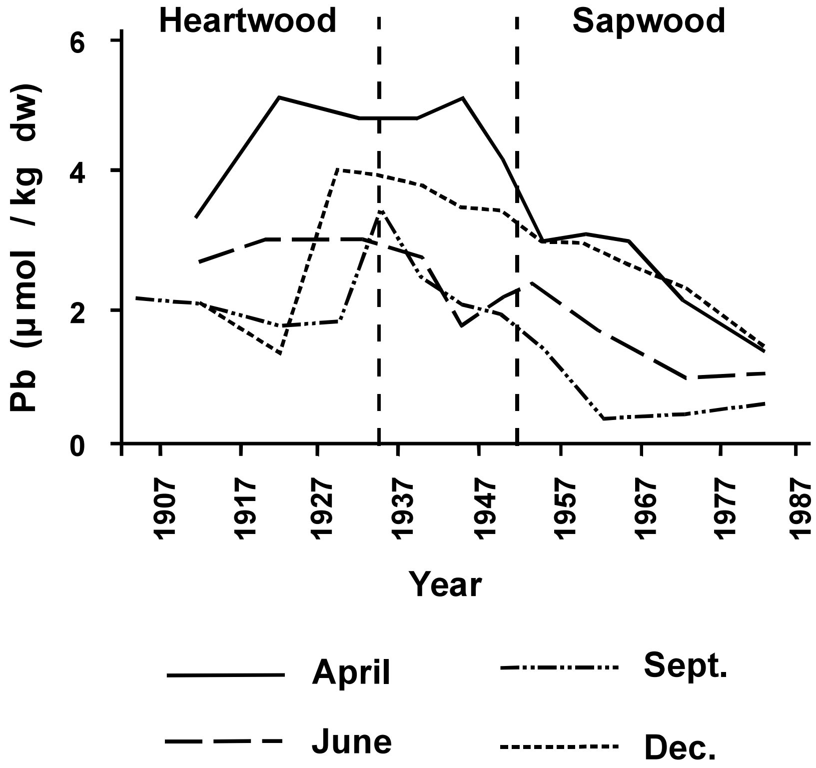

Fig. 3. Seasonal variations in concentrations and radial distribution patterns of Pb in stem wood of Fagus sylvatica L. [5].

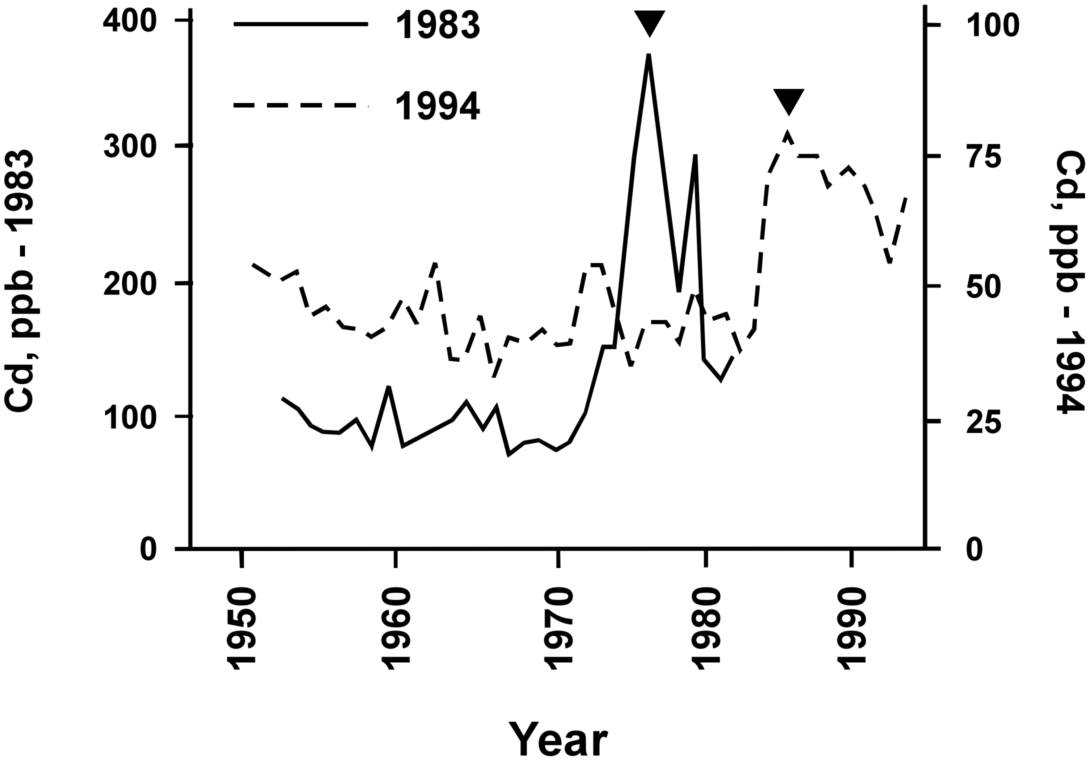

Fig. 4. Radial distribution of Cd in stem wood of oak trees (Quercus robur L.) sampled in January 1984 and in February 1994. The arrows indicate the transition between sapwood and heartwood at the time of sampling [5].

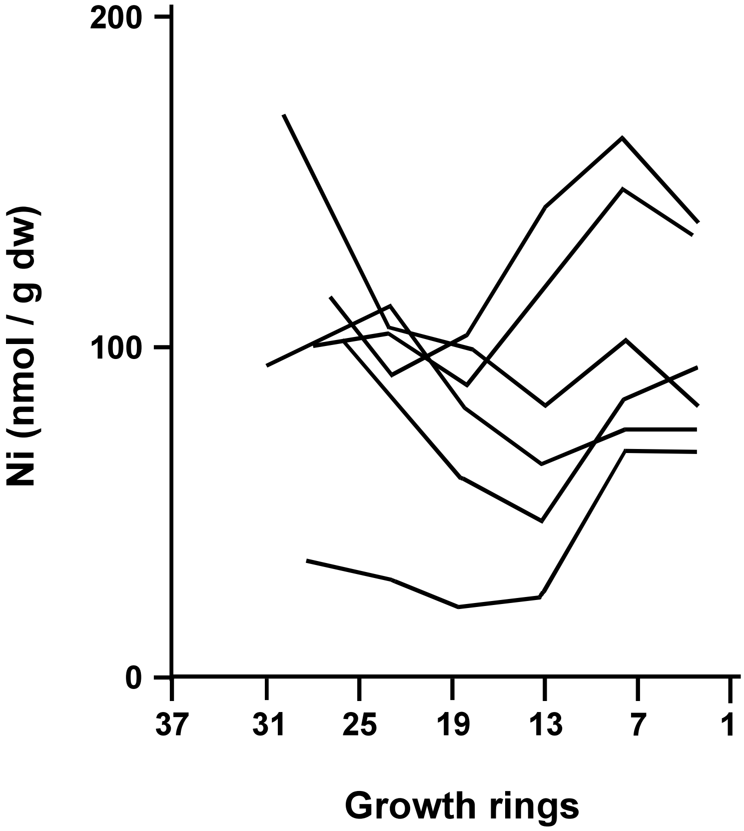

Fig. 5. Radial distributions of Ni in stem wood of 6 Quercus ilex L. trees growing on serpentine soil [12].

sap [6] and a consequent increase in the capacity to complex and translocate elements is usually observed in spring. Additionally, the higher velocity of the transpiration stream in spring, increases the depletion of mobile elements in stem wood that are not rapidly replaced by root uptake.

2.2. Heartwood/Sapwood

Many authors observed that the radial distribution patterns are influenced by the location of sapwood and heartwood [7, 8]. Toxic elements like Cd and Pb showed low concentrations near the cambium and higher towards the stem center. A translocation of toxic substances through the rays into the heartwood was suggested as a possible mechanism of detoxification of the sapwood. The transport of elements is restricted to the sapwood and ceases at the sapwood-heartwood boundary, where the ray cells die in the conversion process of sapwood to heartwood [9]. The wood rays are the more important storage tissue in trees. Their function is to connect phloem and xylem in the exchange of several substances, but also an exchange of solutes between the rays and the xylem vessels, through specialized cells named contact cells [10]. These cells have enlarged pits in contact with the xylem vessels, and they also present an increase respiratory and phosphatase activity during spring.

In Ref. [5] it is described peaks of Cd at the sapwood-heartwood boundaries in stems of oak trees that were apparently mobile (Fig. 4) [11]. The locations of these peaks are no reliable markers of pollution events in the trees environment. The distribution of trace elements seems to be more affected by endogenic processes.

2.3. *Quercus ilex* in serpentine soils. A case study.

Most of the studied cases in dendroanalysis involved trees that at some point of their growth suffered an increase of the trace elements present in the environment. In a situation where the tree is always exposed to high levels of heavy metals, how is the radial distribution?

Serpentine soils are naturally rich in the trace elements Ni, Cr and Co. *Quercus ilex* L. is the only tree that grows in the serpentine soils of north-east Portugal [12]. Assuming that the availability of Ni does not change significantly with time, concentrations of Ni in growth rings should not vary much with time, i.e. with radial position in the stem. However, the distribution of Ni in the stem wood was shown to be variable, and different between trees (Fig. 5).

Growth rates can probably influence the concentrations of elements [13] and thus explain the variability of Ni concentrations, i.e, if growth is uniform, the concentrations of Ni in growth rings are equal, assuming that the availability of Ni does not change significantly with time. In some *Q. ilex* trees it was observed that during periods of declining growth there was an increasing concentration of the trace elements. However, other trees did not showed that relation. The diluting effect of wood production on the concentration of elements cannot *per se* explain the radial patterns of Ni found in some *Q. ilex* trees.

3. CONCLUSIONS

Peaks of heavy metals are apparently mobile in the stem wood. Therefore the current locations of these peaks are no reliable markers for the dating of pollution events in the tree environment.

Seasonal changes in element concentrations in stem wood are probably linked to the physiological activity of trees.

The heartwood/sapwood boundary seems to play an important role in the redistribution of some trace elements.

Radial distribution patterns of heavy metals in wood rings should be carefully used as a tool for chronological record of environmental pollution.

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A BRAZILIAN TREE COLLECTION ANALYZED BY X RAY FLUORESCENCE

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Abstract

The analysis of the inorganic components of wood is of great interest for several reasons, including the acquisition of basic data creating a data base of values for individual species. Knowing the wide variability in matrix composition (lignin, oil, resin, silica) and densities (0.39-1.09 g cm^{-3}), 40 species of trees were analyzed by X ray fluorescence (XRF) to determine the concentrations of Br, Ca, Cu, K, Mn, Pb, Rb, Sr, and Zn. This technique is widely used because of its accuracy and simplicity of sample preparation, normally complex for this type of biological material. This multi-elemental analysis has proven suitable for wood, a material in which a wide range for each element was encountered in the different species studied: 0.3-5.2 for Br, 126-9074 for Ca, 2.2-11 for Cu, 108-5873 for K, 3.1-134 for Mn, 0.5-4.7 for Pb, 0.3-20 for Rb, 1.2-120 for Sr, and 1.1-20 for Zn (values given in μg g^{-1}).

1. INTRODUCTION

Forests are the principal form for vegetation covering the planet surface, which has been modified and removed by man, and fall into three basic types: deciduous, evergreen and tropical. Tropical forests are made up for many biologically diverse species in contrast to the evergreen forests which contain a limited number of species. The value of a forest should not only be measured by commercial values, such as wood, resins, oils, latex, medical and industrial extracts and fruits, but also by virtue of its direct influence on the equilibrium of ecosystems, local climate, soil conservation and local biodiversity, all of which are involved in the quality of human lifestyle.

About 30% of the world's tropical forests lie within Brazilian territory and are divided into three main areas: Amazônica (Terra Firme, Várzea, Igapó, Cocais, and Canarana), Mata Atlântica (Floresta Atlântica, Floresta dos Tabuleiros and Estacional, and ecosystems associated with restinga, mangue and brejo) and Matas Ciliares which are found on riverbanks. The largest of these forests, the Amazon forest, covers an area of 6.5 million km^2, with approximately 2,500 catalogued species, representing 20% of the world's tropical forest and 80% of the Brazilian forest resources [1]. Other forest types, albeit almost severely reduced in area due to their destruction during the advance of civilization, show an exceptional level of biodiversity, with indices among the highest in the world. These forests lie in the south and southeast of Brazil occupying only 10% of their original habitat [2]. Due to the human occupation of these areas, with sometimes serious ecological and social consequences, many of these arboreal species are endangered with extinction even before their physicochemical properties have been fully characterized.

Environmental studies have increasingly focused on trees since they represent a biomaterial which has the ability to absorb and accumulate inorganic components into the plant tissue, making them valuable biomonitors [3]. Wood is an organic material formed basically from lignin and cellulose with a large number of macro- and micronutrients and other chemical elements incorporated into this matrix. Depending on various factors, including the tree species, soil nutritional status and the local pollution levels in soil, air and water, the inorganic components may be present in variable quantities [4].

The X ray fluorescence has already been used to analyze wood produced in temperate climates [5]. The study was carried out on conifers within managed forests and showed that elemental levels

TABLE I.DESCRIPTION OF THE POPULAR AND SCIENTIFIC NAMES, AND DENSITY (D, IN g cm^{-3}) OF THE WOOD SAMPLES ANALYZED

Sample	Popular Name	Scientific Name	D
# 1	Timburi	***Enterolobium contortisiliquum*** (Vell.)	0.39
# 2	Castanheira	***Bertholletia excelsa*** H.B.K.	0.93
# 3	Aroeira	***Myracrodruon urundeuva*** Fr. All.	1.09
# 4	Canelão-amarelo	***Ocotea velutina*** (Nees) Rohwer	0.94
# 5	Cabreúva	***Myroxylon peruiferum*** L.f.	0.90
# 6	Óleo de copaíba	***Copaifera langsdorffii*** Desf	0.76
# 7	Imbuia	***Ocotea porosa*** (Nees) L. Barroso	0.72
# 8	Gonçalo Alves	***Astronium fraxinifolium*** Schott	1.09
# 9	Peroba	***Aspidosperma discolor*** A. DC.	0.76
#10	Pau d' alho	***Gallesia integrifolia*** (Spreng.) Harms	0.67
#11	Louro-preto	***Cordia glabrata*** (Mart.) DC.	0.84
#12	Cerejeira	***Eugenia involucrata*** DC.	0.56
#13	Jequitibá-branco	***Cariniana estrellensis*** (Raddi) Kuntze	0.65
#14	Canela-fedida	***Ocotea corymbosa*** (Meissn.) Mez	0.73
#15	Caxicanhém	***Roupala brasiliensis*** Klotz.	0.61
#16	Guarantã	***Esenbeckia leiocarpa*** Engl.	1.03
#17	Caixeta	***Simarouba amara*** Aubl.	0.48
#18	Pinho	***Araucaria angustifolia*** (Bert.) Kuntze	0.58
#19	Jacarandá da Bahia	***Dalbergia miscolobium*** Benth.	0.91
#20	Cedro	***Cedrela fissilis*** Vell.	0.58
#21	Guaiuvira	***Patagonula americana*** L.	0.87
#22	Jatobá	***Hymenaea courbaril*** L.var. ***stilbocarpa*** Lee et Lang.	0.80
#23	Amendoim	***Pterogyne nitens*** Tul.	0.86
#24	Freijó	***Cordia trichotoma*** (Vell.) Arrab. Ex steud.	0.57
#25	Perobinha	***Paratecoma peroba*** (Rec.) Kuhlm.	0.76
#26	Araruva	***Centrolobium tomentosum*** Guill. ex Benth.	0.81
#27	Pau-marfim	***Calycophyllum spruceanum*** Benth.	0.77
#28	Mogno	***Swietenia macrophylla*** King.	0.67
#29	Ipê	***Tabebuia chrysotricha*** (Mart. ex DC.) Standl.	0.95
#30	Caviúna	***Machaerium scleroxylon*** Tul.	0.86
#31	Angico	***Anadenanthera macrocarpa*** (Benth.) Brenan	0.91
#32	Jacarandá Paulista	***Machaerium villosum*** Vog.	0.86
#33	Pinho	***Pinus caribea***	0.56
#34	Guatambu	***Aspidosperma parvifolium*** A. DC.	0.63
#35	Sucupira	***Diplopis incexis*** Rizz.&Matt.	1.05
#36	Cabreutinga	***Cyclolobium vecchi*** A. Samp. ex Hoehne	0.75
#37	Eucalipto	***Eucalyptus grandis***	0.99
#38	Eucalipto	***Eucalyptus saligna***	0.94
#39	Jequitibá-rosa	***Cariniana legalis*** (Mart.) Kuntze	0.52
#40	Pinho	***Pinus*** sp.	-

were lower than those encountered in natural forests [5-7]. Brazilian natural forests, in an area of 8.5 million km^2 with a great diversity in soil and climate conditions, give rise to wood with much more varied matrices.

Due to the large natural variation in multi-elemental levels expected within the wood taken from those Brazilian natural forests, this work was conceived to test the analytical capability of XRF in the analysis of wood samples with divergent physical and chemical characteristics.

TABLE II. ELEMENTAL CONCENTRATIONS (IN $\mu g\ g^{-1}$) FOR EACH OF THE 40 WOOD SAMPLES.

	Br	Ca	Cu	K	Mn	Pb	Rb	Sr	Zn
# 1	BDL	129±27	3.4±.03	3028±182	BDL	1.0±0.2	6.3±0.2	4.6±0.2	1.5±0.2
# 2	0.9±0.1[a]	3078±107	3.3±0.3	205±53	BDL	BDL	1.4±0.1	67±1.0	1.5±0.2
# 3	BDL	2295±73	3.4±0.3	BDL	BDL	BDL	0.3±0.1	33±0.5	1.4±0.2
# 4	1.0±0.1	261±22	2.4±0.2	334±54	26±1.6	BDL	1.3±0.1	7.8±0.2	1.4±0.1
# 5	BDL	1058±41	4.0±0.3	BDL	BDL	BDL	0.3±0.1	21±0.3	2.6±0.1
# 6	1.9±0.1	1423±61	3.4±0.3	382±56	30±1.7	BDL	1.4±0.1	46±0.8	12.3±0.4
# 7	BDL	BDL	3.2±0.2	BDL	3.2±0.9	BDL	0.4±0.1	3.1±0.2	1.3±0.1
# 8	BDL	1375±46	3.3±0.3	444±51	BDL	BDL	2.6±0.1	46±0.6	1.7±0.2
# 9	2.4±0.1	738±40	2.9±0.2	121±41	BDL	BDL	0.3±0.1	26±0.4	1.1±0.1
#10	5.2±0.1	9074±250	2.9±0.3	5873±192	109±3.2	0.5±0.2	8.4±0.2	39±0.6	2.2±0.2
#11	BDL	1540±59	11±0.4	BDL	BDL	BDL	BDL	12±0.2	1.1±0.2
#12	BDL	4258±197	3.9±0.4	BDL	BDL	2.1±0.3	1.3±0.1	68±1.0	1.8±0.2
#13	0.9±0.1	908±45	2.9±0.2	1383±94	15±1.3	BDL	0.9±0.1	9.6±0.2	2.7±0.2
#14	BDL	126±18	3.2±0.2	1298±42	19±1.3	BDL	1.7±0.1	5.0±0.1	1.4±0.1
#15	1.3±0.1	838±39	2.8±0.2	478±49	93±3.3	0.7±0.1	1.9±0.1	8.4±0.2	6.2±0.2
#16	BDL	512±29	3.0±0.2	BDL	3.9±0.9	BDL	BDL	14±0.3	1.3±0.1
#17	1.8±0.1	2735±141	3.6±0.3	4195±225	BDL	0.5±0.2	20±0.3	20±0.3	2.0±0.2
#18	BDL	319±22	2.2±0.2	733±57	130±4.9	0.5±0.1	1.7±0.1	17±0.3	4.1±0.2
#19	BDL	1338±55	8.2±0.3	180±53	14±1.4	0.5±0.2	1.0±0.1	31±0.5	2.7±0.2
#20	BDL	735±54	2.5±0.3	BDL	BDL	BDL	0.8±0.1	38±0.6	3.3±0.2
#21	BDL	3090±104	3.5±0.2	1360±81	5.9±1.1	BDL	4.7±0.1	45±0.7	1.9±0.2
#22	BDL	671±35	4.2±0.2	648±57	27±1.5	BDL	4.1±0.1	19±0.3	6.3±0.2
#23	0.3±0.1	428±28	3.0±0.3	BDL	BDL	0.5±0.1	BDL	9.4±0.2	1.3±0.2
#24	0.5±0.1	2675±149	3.7±0.3	108±73	BDL	0.5±0.1	0.6±0.1	33±0.6	1.3±0.2
#25	BDL	2047±77	4.3±0.3	BDL	BDL	BDL	0.3±0.1	26±0.4	1.4±0.1
#26	BDL	1573±59	3.0±0.2	114±45	36±1.6	BDL	0.5±0.1	46±0.7	7.4±0.3
#27	BDL	247±23	3.3±0.2	253±50	15±1.2	0.6±0.1	1.1±0.1	9.9±0.2	1.9±0.1
#28	BDL	884±38	2.2±0.2	BDL	BDL	BDL	BDL	22±0.3	1.2±0.2
#29	BDL	447±26	5.1±0.3	BDL	BDL	BDL	BDL	7.2±0.2	1.2±0.1
#30	BDL	3684±132	4.8±0.3	146±52	7.7±1.1	0.6±0.2	0.3±0.1	120±1.8	4.7±0.2
#31	BDL	2759±98	3.4±0.3	119±67	BDL	BDL	0.5±0.1	36±0.6	1.7±0.2
#32	0.5±0.1	441±30	4.8±0.3	117±58	10±1.2	0.5±0.2	0.8±0.1	10±0.2	1.6±0.2
#33	BDL	330±30	2.5±0.3	151±48	12±1.3	BDL	0.6±0.1	3.8±0.2	3.7±0.2
#34	BDL	1275±79	4.2±0.3	1644±122	5.5±1.2	BDL	3.8±0.1	19±0.4	2.3±0.3
#35	0.3±0.1	580±34	3.6±0.3	163±55	BDL	BDL	0.8±0.1	11±0.2	1.6±0.2
#36	1.4±0.1	509±31	3.9±0.3	308±67	134±4.7	0.8±0.2	1.5±0.1	11±0.3	20±0.5
#37	1.1±0.1	BDL	2.6±0.3	BDL	BDL	BDL	BDL	1.2±0.1	1.3±0.1
#38	0.5±0.1	134±24	3.8±0.2	BDL	3.5±1.0	BDL	BDL	1.5±0.1	1.8±0.1
#39	2.0±0.1	1750±89	4.2±0.3	1204±94	3.1±1.2	4.7±0.2	1.3±0.1	36±0.5	2.4±0.2
#40	BDL	402±35	2.8±0.3	150±44	97±4.6	0.6±0.2	1.1±0.1	2.4±0.2	9.1±0.3
DL	0.2	44	0.7	36	2.6	0.4	0.2	0.2	0.5
Mean	1.3	1491	3.7	888	36.3	0.96	2.2	24.6	3.2
STD	1.2	1642	1.5	1360	43.1	1.1	3.6	23.0	3.6
C.V.	0.87	1.1	0.42	1.5	1.2	1.12	1.63	0.93	1.14

BDL = Below detection limit
DL = Detection limit
[a] Uncertainty (one standard deviation) due to counting statistics
STD = Standard deviation
C.V. = Coefficient of variation

2. EXPERIMENTAL

Wood samples taken from 39 Brazilian tree species [8] and one *Pinus* (collected in Vienna, Austria) were used as the basis of this study (Table I). Samples were prepared in a power lathe as "thick target samples" in duplicate discs of 25 mm in diameter and 3 mm in thickness from wood blocks of 250x570x1080 mm. In order to test the homogeneity of the samples taken from each block, 5 blocks were randomly selected (wood #02, #12, #19, #35 and #39) and six disk samples cut, at equidistant positions, from each block. The wood density was measured by attenuation of gamma radiation from ^{241}Am [9] after the humidity was adjusted to approximately 8%.

The XRF measurements were carried out by using an X ray tube excited energy-dispersive spectrometer (Ag X ray tube, 50 kV, 20 mA, measurement time 3000 s) with a Si(Li) solid-state detector and a Canberra MCA. Spectrum evaluation was carried out using the AXIL program (Analysis of X-Ray Spectra by Iterative Least Squares Fitting) and the emission-transmission (E-T) method was used for quantification [10-12].

A quality control test for XRF analysis of wood was performed by analyzing pellets of reference materials NBS 1572 (citrus leaves), BCR 62 (olive leaves) and IAEA V-10 (hay powder). The measuring time for the reference materials and samples was fixed in order to obtain significant number of counts to minimize the uncertainty of the area under each of the characteristic X ray peaks. For the reference samples the measuring time was fixed at 1000 s.

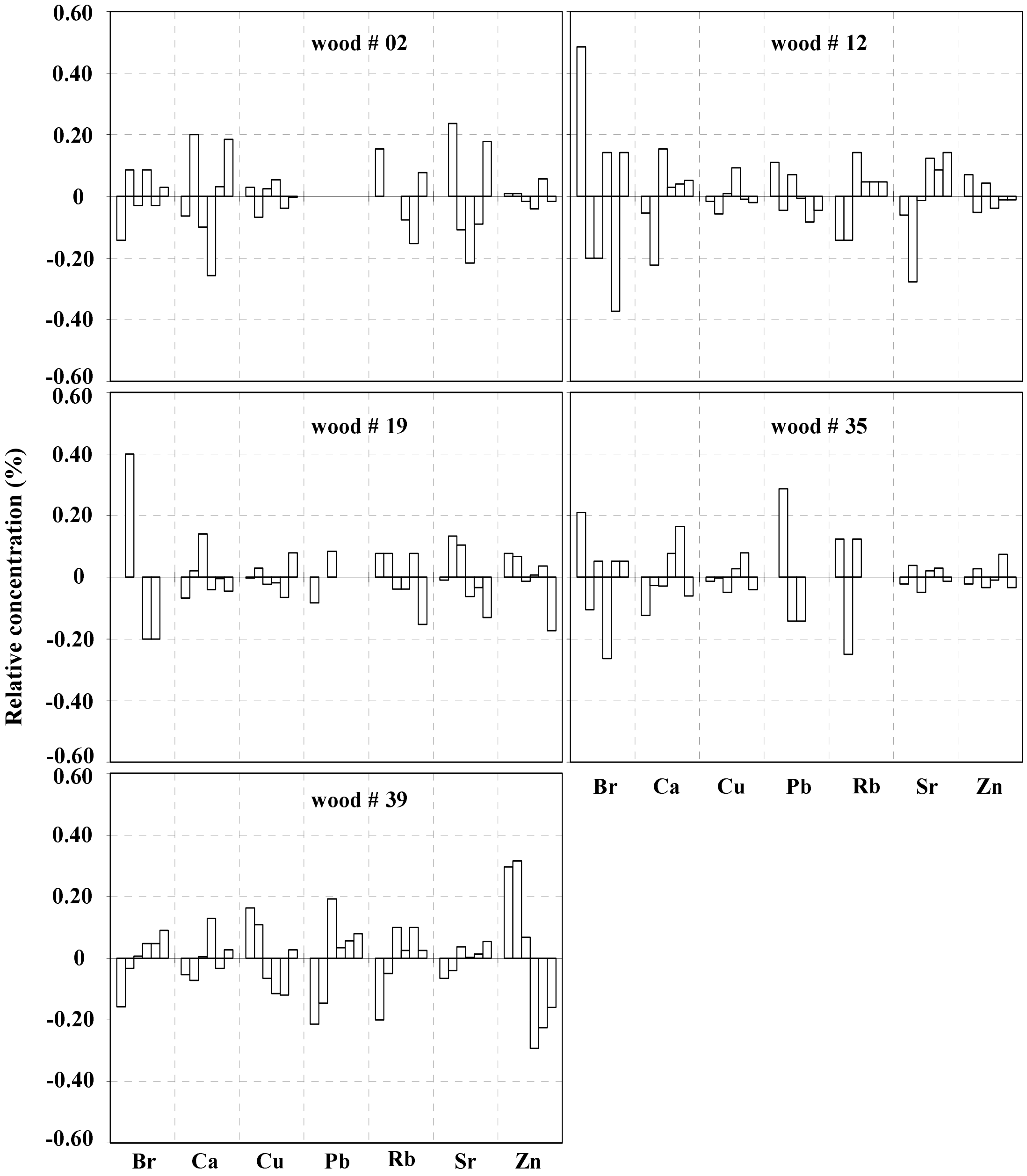

Figure 1. Results from the homogeneity tests on five wood blocks; the relative concentration (%) for each element resulted from the analysis of 6 samples per block.

TABLE III. RESULTS OF THE XRF ANALYSIS VS. CERTIFIED VALUES FOR REFERENCE MATERIALS, IN μg g^{-1}. CA AND K ARE EXPRESSED IN %.

	Citrus leaves NBS 1572			Olive leaves BCR N°. 62			Hay (powder) IAEA V-10		
Elements	Measured value	Certified value (± 1σ)	Error (%)	Measured value	Certified value (± 1σ)	Error (%)	Measured value	Certified value (± 1σ)/confidence interval	Error (%)
Br	7.6 ± 0.4 [a]	8.2 [b]	- 7	7.9 ± 0.4	-		7.2 ± 0.4	8 (7-11)	- 10
Ca	2.6 ± 0.11	3.15 ± 0.10	- 17	1.47 ± 0.08	-		1.72 ± 0.07	2.16 (2.10-2.22)	- 20
Cu	15.7 ± 1.0	16.5 ± 1.0	- 5	45.6 ± 1.8	46.6 ± 1.8	- 2	11 ± 1.0	9.4 (8.8-9.7)	+ 17
Fe	71.8 ± 7	90 ± 10	- 20	284 ± 12.5	-		162 ± 7.1	185 (177-190)	- 12
K	1.8 ± 0.08	1.82 ± 0.06	- 1	4112 ± 273	-		2.1 ± 0.09	2.1 (1.9-2.3) [b]	0
Mn	16.5 ± 4.9	23 ± 2	- 28	48.7 ± 4.6	57 ± 2.4	- 15	41.8 ± 4.2	47 (32-52) [b]	- 11
Pb	16.9 ± 0.9	13.3	+ 27	25 ± 0.8	25 ± 1.5	0	1.9 ± 0.6	1.6 (0.8-1.9)	+ 19
Rb	4.7 ± 0.4	4.8 ± 0.06	- 2	2.5 ± 0.3	-		6.4 ± 0.3	7.6 (7.3-7.8)	- 16
Sr	93.2 ± 1.5	100 ± 2	- 7	35.9 ± 0.8	-		40 ± 0.8	40 (37-44)	0
Zn	26.3 ± 1.1	29 ± 2	- 9	18.5 ± 0.8	16 ± 0.7	+ 16	22.3 ± 0.9	24 (21-27)	- 7

[a] Uncertainty (one standard deviation) due to counting statistics

[b] Not certified, values for information purposes

\- Not reported

3. RESULTS AND DISCUSSION

The concentrations of the elements encountered in the 40 different wood samples are shown in Table II, together with the mean values (μ), standard deviations (σ), and the coefficients of variation (μ/σ) of the analyses. All the 9 elements showed different concentration among the samples. The wide range obtained for the coefficients of variation (from 0.42 to 1.63) indicates that there is indeed a difference in the concentration of many of elements among the wood samples taken from different species. Elements such as Ca and K, occurring in greater concentrations than other elements because of their macroelement status, generally show larger variations in concentration. This variation trend was also found in wood samples taken from *Mimosa acutistipula* Benth and *Jatropha Curcas* Linné, trees which are characteristic of the northeast region of Brazil [13].

TABLE IV. INTERELEMENTAL RELATIONSHIPS, r

	K	Sr	Ca
Br	0.78[a] (N=13)	0.39[a] (N=16)	0.81[a] (N=16)
Ca	0.62[a] (N=27)	0.61[a] (N=40)	
Rb	0.79[a] (N=27)		

[a] Correlations were significant at 99.5% confidence level
N = Casewise deletion of missing data

The results obtained for the relative concentrations of Br, Ca, Cu, Pb, Rb, Sr, and Zn for each of the six samples are shown in Figure 1. The elemental composition showed significant variation for Ca and Sr in sample # 02; Br, Ca, Rb, and Sr for sample # 12; Br, Ca, Rb, Sr, and Zn for sample #19; Br, Ca, Pb, Rb for sample #35; Br, Ca, Cu, Pb, Rb, and Zn for sample #39. Differences in wood elemental levels can be attributed to species variation and biological conditions at the site of growth [14-15]. These aspects will not be discussed further here, but it is clear that a strict control of all pertinent parameters is very important in interpreting the results obtained during physiological/nutritional studies. Variations in wood levels, equal or higher than those presented in this work, are principally found in studies which utilize growth rings of *Pinus*. These results suggest that micro-analysis by X-Ray or PIXE could be used in the study of the radial distribution of these elements in tree rings during the various phases of tree growth [16-18].

Table III presents the results of the elemental concentrations and the deviations from the certified values found in the quality control test. For the standard sample NBS 1572, the elements presenting a significant error were Mn and Pb. The error was equal to or less than 20% for all the other elements and standard samples. These errors are attributed to the calculation of the net area under the peak which consequently affects the accuracy of the elemental concentration. This method presented an average relative error of about 9% which is considerably lower than the errors cited in the literature (close to 20%) [19].

Due to the great interest in dendrochemistry, the relationship between the elements contained in the trees, expressed as the linear correlation coefficients, were computed between all possible pairs of elements determined. The best correlations were observed between the Br-Ca, Br-K, Ca-Sr and K-Rb, as shown in Table IV.

The results presented indicate that wood samples, with widely contrasting physical and chemical properties, can be characterized by XRF with adequate levels of the detection limits and reproducibility. This method is suitable for routine use because of its speed, low analysis cost and simplicity of sample preparation, without need for pre-treatment or pre-concentration.

One of the limitations of this work was the lack of information about climatic history, tree age and details of the sample collection, which could be useful in the investigations related to the accumulation, migration and retention of trace elements in plant tissues, and in eventual use of this type of data in establishing background levels for future environmental monitoring.

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ANALYSIS OF *CANOPARMELIA TEXANA* LICHENS COLLECTED IN BRAZIL BY NEUTRON ACTIVATION ANALYSIS

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Abstract

The accumulation of elements by lichens is well known. Therefore analyses of lichen samples have been carried out in order to use this kind of material in environmental studies. In this work, the epiphytic lichen *Canoparmelia texana* collected from the bark of trees was analyzed by instrumental neutron activation analysis. The preparation of samples consisted of cleaning, washing with water, lyophilization and grinding. Elements Al, As, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Eu, Fe, K, La, Lu, Mg, Mn, Mo, Na, Rb, Sb, Sc, Se, Sm, Tb, Th, Ti, U, V, Yb, and Zn were determined quantitatively by using short and long irradiations carried out under a thermal neutron flux from 10^{11} to 10^{13} n cm^{-2} s^{-1} in the IEA-R1 nuclear reactor. Radioactivity measurements were performed by using a hyperpure Ge detector. Results obtained by analyzing a sample of *Canoparmelia texana* species in replicates revealed a good precision with relative standard deviations varying from 0.5 to 15%. Also the precision and the accuracy of the method were evaluated by analyzing reference materials IAEA 336 Lichen, NIST 1575 Pine Needles and 1572 Citrus Leaves. In order to study the influence of age of the lichen, in the collection, the center and outer fraction of each sample were collected separately. For some elements the center of the lichen sample presented slightly higher concentrations than the outer fraction, indicating that the accumulation of the elements increases with the lichen age or with the length of exposure. Results obtained for lichens collected from different trees from the same sampling area showed relative standard deviations varying from 20 to about 60%.

1. INTRODUCTION

In the last years, studies on environmental pollution by trace elements have gained increasing concern and lichens have been analyzed for assessing the baseline levels of pollutants and for identifying contamination sources.

However in Brazil analytical data of pollutants obtained using biomonitors are very scarce. Most of our environmental data have been obtained by application of direct measurements by analyzing air, water or soil and this fact is requiring enormous efforts due to the great extension of the country and to the serious problems of pollution encountered, specially in big cities like São Paulo.

Consequently studies concerning trace element determinations in lichens are of great interest due to several advantages related to ease of sampling and to the particular capability of metal accumulation presented by this monitor.

Following our studies [1] of determination of trace elements in lichens, by applying neutron activation analysis, this paper presents results obtained in *Canoparmelia texana* (Tuck.) Elix & Hale collected in Brazil. The purpose of this work is to contribute for establishing adequate conditions for sampling and analysis of lichens in a further biomonitoring programme.

2. EXPERIMENTAL

2.1. Selection of lichen *Canoparmelia texana*

In biomonitoring studies, the selection of the species to be analyzed is of primordial importance [2, 3]. The *Canoparmelia texana* (Tuck.) Elix & Hale species selected in this work is an epiphytic lichen from the family of *Parmeliaceae*, very abundant in several parts of Brazilian territory, except the coast. This lichen species presents similar behaviour to *Leucanora conizeoides* (L. pityrea) well known in Europe and it grows in regions relatively polluted where other species are not present due to

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the pollution. These species which show tolerance to air pollution are of great interest to environmental studies. *Canoparmelia texana* species grows on bark of the trees and it is formed of circular thalli with radial growth. Its growth rate depends on the environmental conditions. In general, for foliose species, the growth rate is in the order of 0.5 to 5.0 mm per year [4].

2.2. Sample collection

Samples of *Canoparmelia texana* were collected from the bark of trees, at about 1.5 m from the soil and stored in paper bags. To remove the sample from the bark, a titanium knife was used.

The following samples were collected:

Sample S: About 1.0 g of lichen was collected from a same tree. This sample was used to evaluate the precision of the results that can be obtained in lichen sample analysis.

Samples CF: These samples were collected in order to study the influence of age of the lichens in their contents of the elements. In this case, in the collection, each sample was divided, in two fractions, constituted of center and peripheral parts and their analyses were performed separately.

Samples LQ: Four samples were collected on the same day from four individual trees in the surroundings of IPEN-CNEN/SP, located at the Campus of São Paulo University, SP, within an area of about 350 m^2. These samples were analyzed to study the variation in the element concentrations within one sampling site.

2.3. Preparation of the samples for analysis

In order to remove eventual bark substrates or other extraneous materials, the lichen samples were cleaned by using an Olympus zoom stereo microscope model SZ 4045. Then, they were washed in distilled water for about 5 min and placed on filter papers for drying at room temperature. The samples were also freeze-dried for about 8 hours under a pressure of about 4x10^{-2} mbar and in this process a mean weight loss of about 13% was found. The fine powder of lichen sample was obtained by grinding, manually, in an agate mortar.

2.4. Preparation of synthetic standards of elements

Stock solutions of the elements were individually prepared by dissolving metals, oxides or salts of the elements with adequate reagents and then diluted using distilled water in a quartz apparatus. High purity or spectroscopically pure reagents were used to prepare these standard solutions. Single or multielement solutions were prepared by mixing appropriate amounts of stock solutions. These solutions were then pipetted onto sheets of 35 x 12 mm Whatman n° 40 filter paper using an Eppendorf pipette. After drying in a dessicator at room temperature, filter papers were folded, placed into polyethylene bags previously cleaned using diluted HNO_3 solution and distilled water, being these bags heat-sealed.

2.5. Procedure used for neutron activation analysis

About 150 mg of each sample were weighted in polyethylene bags and irradiated together with the standards. Two separate irradiations were performed to determine a large number of elements. Irradiations of 5 minutes under a thermal neutron flux of 4.25 x 10^{11} n cm^{-2} s^{-1} from the IEA-R1 nuclear reactor were carried out to determine Al, Br, Cl, K, Mg, Mn, Na, Ti, and V. Longer irradiations of 16 hours under a neutron flux of 10^{13} n cm^{-2} s^{-1} were done to determine As, Ca, Cd, Ce, Co, Cr, Cs, Cu, Eu, Fe, La, Lu, Mo, Rb, Sb, Sc, Se, Sm, Tb, Th, U, Yb, and Zn.

Irradiated samples and standards were placed on stainless steel planchets and after adequate decay times, they were measured using an EG & G Ortec Model GMX20190 Ge detector with a resolution (FWHM) of 0.80 keV at 122 keV ^{57}Co and 1.80 keV at 1332 keV ^{60}Co. The detector was coupled to an EG & G Ortec 918A Multichannel Buffer and this one to a microcomputer. Samples and standards were measured at least twice after different decay times. Analyses of gamma spectra were carried out by using VISPECT [5] software and the concentrations of the elements were calculated by comparative method.

TABLE I. ELEMENTAL CONCENTRATIONS IN *CANOPARMELIA TEXANA*
(Concentrations in µg/g unless otherwise indicated)

Element	Sample S (r = 1)		Sample LQ (r = 4)		
	Mean ± s	$(n)^b$	Mean ± s	s_r (%)	Range
Al	2120 ± 125	(3)	3433 ± 415	12.1	3140 - 3727
As (µg/kg)	769 ± 7	(3)	904 ± 496	54.9	272 - 1347
Br	16.3 ± 2.9	(5)	10.2 ± 6.7	65.6	2.5 - 14.8
Ca (%)	2.96 ± 0.25	(3)	4.5 ± 2.5	56.9	3.5 -17.7
Cd (µg/kg)	1486 ± 95	(3)	2495 ± 1326	52.0	978 - 3727
Cl	520 ± 15	(5)	243 ± 81	33.0	181 - 362
Co (µg/kg)	1745 ± 238	(3)	1720 ± 558	32.4	1003 - 2257
Cr	4.6 ± 0.2	(2)	5.3 ± 2.9	54.5	2.1 - 7.7
Cs (µg/kg)	193 ± 4	(3)	202 ±127	62.8	75 - 340
Cu	11.1 ± 1.9	(3)	19.2 ± 8.1	42.2	14 - 25
Eu (µg/kg)	68 ± 3	(3)	61 ± 32	52.0	31 - 90
Fe	1598 ± 44	(3)	1732 ± 1089	62.9	971 - 2747
K	1666 ± 51	(3)	1477 ± 484	32.8	846 - 1926
La	3.41 ± 0.05	(3)	3.0 ± 1.4	48.0	1.7 – 4.4
Lu(µg/kg)	17.9 ± 2.1	(3)	16.4 ± 10.7	65.2	8.2 - 26.5
Mg	1419 ± 204	(5)	1122 ± 694	61.2	283 - 1946
Mn	70 ± 4	(5)	103.9 ± 56.3	54.4	41.6 - 148.1
Mo	1.07 ± 0.12	(3)	0.94 ± 0.26	27.2	0.68 - 1.17
Na	127 ± 7	(5)	61.6 ± 19.3	31.3	35 - 76
Nd	2.21 ± 0.01	(3)	2.2 ± 1.0	46.9	1.2 - 3.2
Rb	9.37 ± 0.03	(2)	6.7 ± 3.1	46.0	3.8 - 9.9
Sb (µg/kg)	455 ± 21	(3)	536 ± 243	45.3	245 - 774
Sc (µg/kg)	277 ± 26	(3)	324 ± 208	64.2	118 - 515
Se (µg/kg)	245 ± 32	(3)	322 ± 175	54.4	116 - 487
Sm (µg/kg)	339.6 ± 3.3	(3)	310 ± 165	53.1	164 - 477
Tb (µg/kg)	44.3 ± 3.2	(3)	43.6 ± 23.1	52.9	22 - 66
Th (µg/kg)	525 ± 27	(3)	535 ± 358	66.9	194 - 922
Ti	179 ± 11	(5)			
V	5.5 ± 0.4	(5)	5.0 ± 2.7	53.1	2.2 - 8.0
Yb (µg/kg)	96 ± 14	(3)	96.3 ± 51.9	53.9	49 - 152
Zn	98.0 ± 1.2	(3)	99.6 ± 32.4	32.5	68 - 135

a - r indicates number of samples analyzed
b - n indicates number of determinations

2.6. Analysis of certified reference materials

Three certified reference materials, IAEA 336 Lichen, NIST 1572 Citrus Leaves and NIST 1575 Pine Needles were analyzed for the evaluation of the accuracy of the method. They were analyzed by applying the same experimental conditions used in lichen analyses. The moisture content in these reference materials was ascertained by drying in accordance with the producer's recommendations. The following values (in percent) of the weight loss were found and used for correcting the final results: 7.1 for Lichen, 4.9 for Citrus Leaves and 5.2 for Pine Needles.

3. RESULTS AND DISCUSSION

Table I presents results obtained by analyzing sample S, in replicates, as well as the results found in four samples codified LQ. Results obtained for sample S show a good precision for most of elements with relative standard deviations varying from 0.3 to 14.6%. These findings indicate that the procedure adopted for lichen sample preparation and homogenization was adequate. The results

TABLE II - ELEMENTAL CONCENTRATIONS IN TWO FRACTIONS OF SAMPLES CF (C- CENTRAL FRACTION, F- PERIPHERAL FRACTION)
(Results in µg/kg unless otherwise indicated)

Elements	CF 1		CF 2		CF 3		CF 4		CF 5	
	C 1	F 1	C 2	F 2	C 3	F 3	C 4	F 4	C 5	F 5
Al (µg/g)			3410 ± 63	2056 ± 28	1431 ± 56	631 ± 24	2544 ± 49	1393 ± 20	3187 ± 54	927 ± 14
As	1099 ± 10(a)	914 ± 11	982 ± 9	846 ± 10	563 ± 9	304 ± 7	879 ± 7	607 ± 7	876 ± 10	684 ± 10
Ca (%)	8.99 ± 0.07	6.23 ± 0.05	12.31 ± 0.09	9.07 ± 0.06	13.13 ± 0.09	9.27 ± 0.07	5.26 ± 0.04	4.01 ± 0.03	4.80 ± 0.03	2.03 ± 0.01
Cd (µg/g)	3.3 ± 0.2	2.3 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	1.4 ± 0.1	3.1 ± 0.2	2.1 ± 0.1	2.5 ± 0.2	1.2 ± 0.1
Ce	8367 ± 24	5616 ± 16	7003 ± 20	5156 ± 19	3532 ±16	1817 ± 13	6935 ± 25	3996 ± 14	7469 ± 21	2521 ± 14
Cl (µg/g)	208 ± 1	176 ± 5	265 ± 7	166 ±4	143 ± 4	172 ± 4	210 ± 9	186 ± 4	224 ± 5	158 ± 3
Co	1570 ± 6	1739 ± 22	639 ± 10	1793 ± 27	1949 ± 30	1258 ± 18	1452 ± 21	1452 ± 21	1465 ± 21	1314 ± 19
Cr	8691 ± 70	5186 ± 45	7410± 56	6379 ± 51	4371 ± 40	2116 ± 24	6975 ± 56	4604 ± 40	7490 ± 60	3711 ± 32
Cs	260 ± 5	159 ± 4	405 ± 6	358 ± 5	139 ± 4	128 ± 4	188 ± 4	127 ± 3	308 ± 4	192 ± 3
Eu	100 ± 1	66 ± 1	81 ± 1	55 ± 1	40 ± 1	39 ± 1	71 ± 1	43 ± 1	84 ± 1	32 ± 1
Fe (µg/g)	3007 ± 22	1844 ± 13	2616 ± 17	1893 ± 13	1027 ± 7	480 ± 4	1656 ± 11	974 ± 7	1865 ± 13	675 ± 5
K (µg/g)	1497 ± 10	1249 ± 12	2380 ± 12	3611 ± 21	4411 ± 176	4116 ± 17	2538 ± 11	3623 ± 15	3516 ± 21	4888 ± 24
La	4647 ± 21	2935 ± 15	3640 ± 16	2657 ± 13	2011 ± 10	942 ± 6	3355 ± 12	2062 ± 9	4161 ± 15	1378 ± 7
Lu	28.3 ± 0.3	17.8 ± 0.2	24.8 ± 0.2	16.1 ± 0.3	12.4 ± 0.3	5.0 ± 0.3	20.4 ± 0.2	10.5 ± 0.2	18.7 ± 0.3	10.4 ± 0.3
Mg (µg/g)	1372 ± 177	1197 ± 149	1617 ± 172	1413 ± 148	796 ± 112	778 ± 89	1736 ± 149	1161 ±119	1703 ± 165	1078 ± 104
Mn (µg/g)	108 ± 1	124 ± 2	116 ± 1	130 ± 2	54.3 ± 0.6	49.1 ± 0.6	85 ± 1	117 ± 1	103 ± 1	96 ± 1
Mo	1630 ± 101	1119 ± 79	1394 ± 85	1102 ± 74	757 ± 71	449 ± 62	829 ± 69	631 ± 63	893 ± 82	408 ± 63
Nd	3368 ± 41	2237 ± 30	2464 ± 32	1801 ± 40	1225 ± 43	670 ± 43	2525 ± 33	1399 ± 28	2664 ± 39	1019 ± 61
Rb (µg/g)	7.9 ± 0.1	6.4 ± 0.1	14.5 ± 0.2	18.9 ± 0.2	8.2 ± 0.1	8.4 ± 0.1	11.9 ± 0.2	14.3 ± 0.2	10.7 ± 0.1	11.4 ± 0.12
Sb	763 ± 4	525 ± 3	574 ± 3	490 ±3	284 ± 2	161 ± 3	466 ± 2	352 ± 2	480 ± 3	244 ± 2
Sc	539 ± 2	331 ± 1	460 ± 2	321 ± 1	195 ± 1	190.4 ± 0.9	359 ± 2	194 ± 1	416 ± 2	136.8 ± 0.7
Se	435 ± 20	316 ± 16	379 ± 20	317 ± 17	245 ± 17	202 ± 15	331 ± 17	210 ± 11	311 ± 17	183 ± 13
Sm	544.7 ± 0.8	376.8 ± 0.8	403.4 ± 0.9	291.8 ± 0.7	206.2 ± 0.7	105.6 ± 0.5	347.2 ± 0.8	208.1 ± 0.5	413.1 ± 0.9	149.5 ± 0.6
Tb	78 ± 3	49 ± 3	57 ± 3	41 ± 3	32 ± 3	29 ± 3	54 ± 3	24 ± 2	63 ± 3	20 ± 2
Th	971 ± 3	561 ± 2	760 ± 3	593 ± 3	315 ± 2	137 ± 2	601 ± 3	334 ± 2	693 ± 3	210 ± 2
Ti (µg/g)	220 ± 37	132 ± 22	302 ±36	163 ± 21	200 ± 16	39 ±8	178 ± 28	131 ± 19	220 ± 29	81 ± 17
U	197 ± 14	195 ± 17	179 ± 15	135 ± 13	128 ± 13	71 ± 10	150 ± 13	107 ± 12	180 ± 15	69 ± 11
V (µg/g)	2.9 ± 0.2	2.1 ± 0.1	7.6 ± 0.4	4.5 ± 0.2	2.6 ± 0.2	1.12 ± 0.08	6.2 ± 0.4	3.8 ± 0.2	5.9 ± 0.4	2.5 ± 0.2
Yb	203 ± 6	119 ± 4	168 ± 4	116 ± 5	85 ± 6	57 ± 5	150 ± 6	82 ± 4	130 ± 5	148 ± 5
Zn (µg/g)	113.7 ± 0.5	117.2 ± 0.5	131.9 ± 0.6	165.8 ± 0.8	115.5 ± 0.5	106.3 ± 0.5	121.8 ± 0.4	137.5 ± 0.5	111.4 ± 0.5	97.3 ± 0.4

a- uncertainty calculated using statistical counting errors of sample and standard.

TABLE III: ELEMENTAL CONCENTRATIONS IN REFERENCE MATERIALS

Element	Reference material (a)	This work(c)	Ref. [6-7]	Element	Reference material(a)	This work(c)	Ref. [6-7]
Al (μg/g)	1	702 ± 18	680(570 - 780)(b)	Tb (μg/kg)	1	17 ± 3	7-13(b)
	2	112 ± 11	92 ± 15		2	9.5 ± 0.1	
					3	4.4 ± 0.4	(5)
As(μg/kg)	1	699 ± 25	640(650-720)	Yb(μg/kg)	1	37.1 ± 1.8	
	2	3174 ± 44	3100 ± 300		2	8.37 ± 0.06	
	3	196 ± 14	210 ± 40		3	11.9 ± 1.4	12(b)
Br (μg/g)	1	11± 1	12.9(11.2-14.6)	Lu (μg/kg)	1	6.5 ± 0.7	
	2	7.7 ± 1.3	8.2(b)		2	1.3 ± 0.4	
	3	6.9 ± 0.3	9 (b)		3	1.85 ± 0.26	2(b)
Ca (μg/g)	1	2895 ± 100	2600(2400-3300)(b)	Mg (μg/g)	1	721 ± 104	610(500-710)(b)
	2	36179 ±751	31500 ± 1008		2	6249 ± 269	5800 ± 301
	3	4037 ± 134	4100 ± 201				
Cd(μg/kg)	1	172 ± 72	117(100-134)	Mn (μg/g)	1	64.7 ± 2.7	64(57-71)
	3	279 ± 21	<500		2	21 ± 2	23 ± 2
					3	607 ± 47	675 ± 15
Cl (μg/g)	1	1805 ± 77	1900(1650-2200)(b)	Mo(μg/kg)	1	480 ± 42	
	2	395 ± 12	414(b)				
Co(μg/kg)	1	285 ± 28	290(250-330)	Na (μg/g)	1	323 ± 7	320(280-360)
	2	30.2 ± 2.1	20(b)		2	173 ± 4	160 ± 21
	3	177 ± 31	100(b)		3	16.0 ± 1.2	
Cr (μg/g)	1	1100 ± 400	1030(800-1170)(b)	Rb (μg/g)	1	1.80 ± 0.01	1.72(1.52-1.92)
	2	762 ± 26	800 ± 200		2	4.8 ± 0.2	4.84 ± 0.06
	3	2658 ± 152	2600 ± 200		3	11.2 ± 0.3	11.7 ± 0.1
Cs(μg/kg)	1	121 ± 7	110(97-123)	Sb (μg/kg)	1	86 ± 3	73(63-83)
	2	99.0 ± 1.4	98(b)		2	56 ± 9	40(b)
	3	132 ± 5			3	209 ± 14	200(b)
Fe (μg/g)	1	518 ± 23	425(380-470)	Sc (μg/kg)	1	168 ± 11	170(148-192)
	2	99 ± 13	90 ± 9		2	9.8 ± 0.4	10(b)
	3	192 ± 16	200 ± 10		3	38.1 ± 2.3	30(b)
K (μg/g)	1	1940 ± 362	1840(1640-2040)	Se (μg/kg)	1	243 ± 25	220(180-250)
	2	20947± 1454	18200 ± 600		2	51.1 ± 6.5	25(b)
	3	4112 ± 476	3700 ± 200		3	73.6 ± 5.1	
La(μg/kg)	1	630 ± 31	660(550-760)(b)	Th (μg/kg)	1	145 ± 9	140(120-160)(b)
	2	182 ± 27	190(b)		2	13.6 ± 1.3	
	3	190 ± 15	200(b)		3	38.5 ± 2.9	37 ± 3
Ce(μg/kg)	1	127 ± 5	127(109-144)	Ti (μg/g)	1	69.7 ± 8.7	
	2	388 ± 46	280(b)				
Nd(μg/kg)	1	762 ± 68	(500-1000)(b)	U (μg/g)	3	19.1 ± 1.2	20 ±4
	2	279 ± 10					
Sm(μg/k)	1	110 ± 8	106(92-120)	V (μg/g)	1	1.42 ± 0.08	1.5(1.2-1.7)(b)
	2	52.1 ± 3.4	52(b)				
	3	28 ± 1	30(b)				
Eu(μg/kg)	1	24.7 ± 1.8	23(19-27)(b)	Zn (μg/g)	1	34.3 ± 2.1	31.5(28-35)
	2	11.7 ± 1.2	10(b)		2	30.6 ± 1.1	29 ± 2
	3	6.4 ± 0.8	6(b)		3	65.6 ± 9.9	

a – 1- IAEA 336 Lichen; 2- NIST 1572 Citrus Leaves; 3- NIST 1575 Pine Needles.

b - indicates information values

c – mean and standard deviation obtained in n determinations ($3 \leq n \leq 5$)

obtained for sample LQ exhibit considerable variability of the elemental concentrations within one sampling area. The relative deviations of these results varied from 12 to about 60%.

Analytical results obtained for samples CF presented in Table II show that most the elements present slightly higher concentrations in the central fraction than in the peripheral part. For the elements Co, K, Mn, Rb, and Zn both of the fractions presented very close concentrations. These results indicate that the accumulation of the elements in *Canoparmelia texana* increases with the lichen age or with the length of exposure. Therefore the variation of the element concentrations in *Canoparmelia texana* within one sampling site may probably be reduced by collecting samples with the same age or the same length of exposure.

The analytical results for IAEA 336 Lichen, NIST 1572 Citrus Leaves and NIST 1575 Pine Needles are given in Table III together with literature values for comparison. Most of our results are in good agreement with these certified values with relative errors lower than 15%. The less accurate result was obtained for Al in NIST 1572 Citrus Leaves reference material. For some elements there are no certified values and in these cases, results presented here constitute a contribution for their certification. Also the relative standard deviations obtained for most elements in reference materials were lower than 15% which is generally considered a good result in trace analysis.

Results obtained in this work indicated the feasibility in using *Canoparmelia texana* as bioindicator of environmental pollution. From the results obtained for precision and accuracy it was confirmed that instrumental neutron activation analysis is one of the most adequate method for environmental studies.

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BIOMONITORING OF AIRBORNE INORGANIC AND ORGANIC POLLUTANTS BY MEANS OF PINE TREE BARKS.

I. TEMPORAL AND SPATIAL VARIATIONS

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Abstract

Scots pine (*Pinus sylvestris* L.) bark samples were collected at two field sites (Neuglobsow, Rösa) and in different years between 1987 and 1996 in the east of Germany. The barks were analyzed with respect to the following inorganic and organic substances: Al, As, B, Ca, Cd, Ce, Cr, Cu, Fe, Hg, Mo, NH_4^+, Ni, NO_3^-, PO_4^{3-}, Pb, Sr, SO_4^{2-}, Ti, V, W, Zr, Zn, benzo(a)pyrene, fluoranthene, pyrene, a-hexachlorocyclohexane (a-HCH) and dichlorodiphenyltrichloroethane (DDT). In addition to bark samples from the site Rösa, 53 test sites were investigated in the Nature Park Dübener Heide. Here, the analysis of the barks aimed at discovering spatial patterns of the above-mentioned substances. Since 1991, most of the determined substances (e.g. sulfate, nitrate, calcium, lead, benzo(a)pyrene, a-HCH) show decreased concentration values in bark samples from both sites. Temporal variations reflect substantial infra-structural changes in eastern Germany, especially at Rösa and in the industrial region around the cities Leipzig, Halle, and Bitterfeld. Moreover, nitrate concentrations in barks are increasing since 1995. The trend can be explained with increased nitrogen emissions from motor traffic and livestock farms. Spatial patterns of sulphate and ammonia reflect inputs from power plants and agriculture in pine stands of the Nature Park Dübener Heide. The results show that barks of pine trees can be used as biomonitoring tools to indicate and characterize depositions of airborne organic and inorganic pollutants.

1. INTRODUCTION

Pine tree barks are excellent adsorbents of airborne pollutants, including anthropogenic heavy metals [1-2]. The bark surface is very porous, and the absence of metabolic processes makes it almost inert in the presence of inorganic and organic substances. Additionally, pine stands are widespread in Central, Northern and Eastern Europe. Therefore pine barks have proven useful as passive bioindicators for airborne pollutants [3-8].

Monitoring with pine barks supplies low-cost information on the composition and quantity of the deposition of pollutants. As shown recently, pine tree barks can also be used to quantify throughfall rates in pine stands [9]. While pine tree barks have often served as biomonitoring tool for airborne sulphate, calcium and heavy metals, there are only few studies with airborne organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and semivolatile organic compounds (SOCs). Moreover, there are only few field studies with pine barks on regional and supra-regional scales, which contrasts with the situation with mosses and lichens as bioindicators.

In the present study, pine barks are used to characterize temporal variations and spatial patterns of selected inorganic and organic substances, and the suitability of this biomonitoring tool is demonstrated by comparing contaminant loads in the bark of pine stands at two test sites with known low and high airborne pollution.

2. MATERIAL AND METHODS

2.1. Sampling and sites

Sampling was carried out in different years between October 1987 and October 1996. Bark samples were taken from 60 to 80 years old Scots pine trees (*Pinus sylvestris* L.) in the following areas: Neuglobsow near lake Stechlin about 70 km north of Berlin (Brandenburg, Germany), Rösa (Nature Park Dübener Heide) in the northeast of the industrial region Leipzig-Halle (Sachsen-Anhalt, Germany), and in different background or reference areas of South Norway (National Park Jostetalsbreen), Russia (Syktywkar) and Poland (Bialowieska). Additionally, the area of Nature Park Dübener Heide (Fig.1) was subdivided in a 2.5 x 2.5 km grid of 56 test sites to analyze spatial patterns of airborne pollutants. Using a hard steel knife, the external surface of barks was collected from the non-weather area at a height of 1.50-1.70 m above the ground. Samples from at least 25 pine stems from each test site were mixed, dried at 30°C,and milled in a contamination-free manner for further cleanup and analysis. For statistical calculations, each of the test areas was subdivided into five test sites. Each test site was represented by one mix sample of pine barks.

Fig.1 Map of Germany with location of test areas with sites for bark sampling in Scots pine stands. 1 - Neuglobsow (near lake Stechlin); 2 - Rösa (Dübener Heide).

2.2. Chemical Analyses

Anion concentrations (chloride, sulphate, nitrate, phosphate) were determined using an ion chromatograph (DX 500, Dionex). At ambient temperature, 0.5 g of bark samples were extracted with 25 mL deionized water for 45 min. A 50 mL sample was injected into the chromatographic system. The separation was performed on a IONPAC AS12A column (Dionex). The mobile phase consisted of 2.7 mM CO_3^{2-}/0.3 mM HCO_3^- solution. The flow rate was fixed at 1.5 mL/min. Ammonium concentration was measured using bark extracts (6.25 g bark/25 mL 1% K_2SO_4 w/w) with a gas-sensitive ammonia electrode (Type NH 500/2, WTW).

For the determination of heavy metals, 0.5 g of bark samples were digested with 5 mL concentrated nitric acid at 150°C and 1.2 MPa in a microwave digester (MDS 2000, CEM). Elemental contents in the digestion solution were analyzed using inductive coupled plasma atom emission spectrometry (JY24, Jobin Yvon) and inductive coupled plasma mass spectrometry (ELAN 5000, Perkin Elmer, Norwalk, CT, USA).

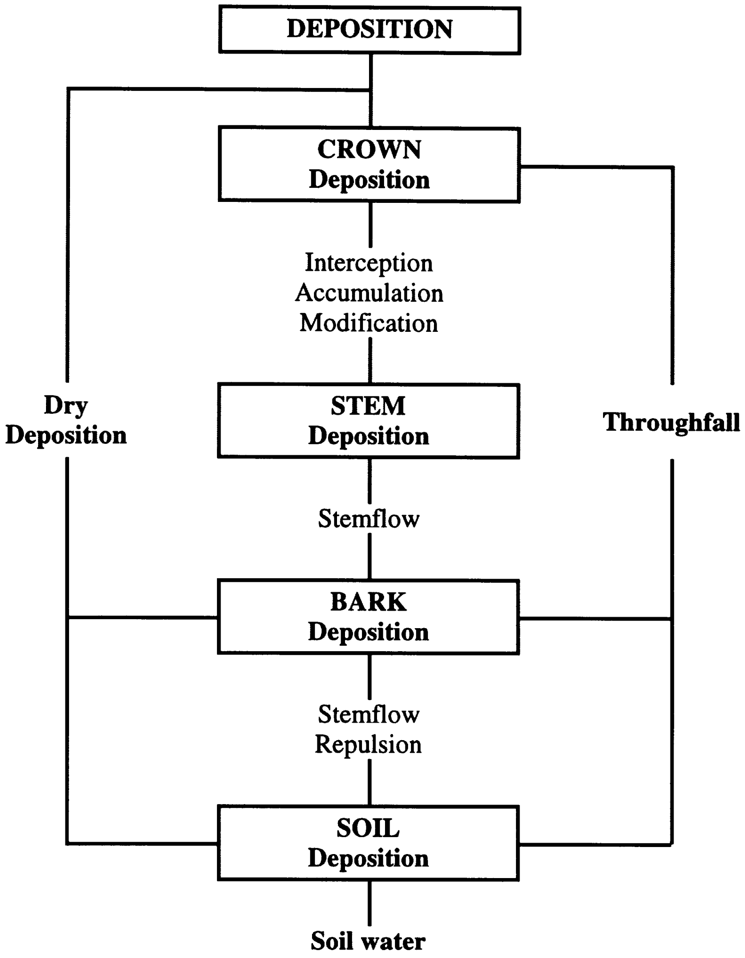

Fig.2 Survey of accumulation process in pine bark.

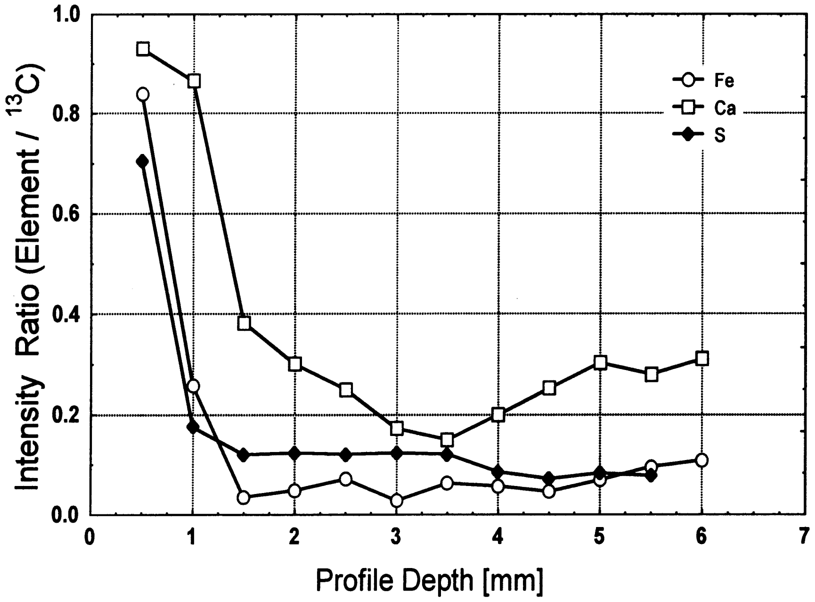

Fig.3 Result of a bark profile analysis that was performed with a Laser ICP-MS. The intensities of sulphur, iron and calcium were measured in 0.5 mm steps and were applied to ^{13}C as the inner standard. The 0.1 mm depth corresponds to the outer layer of tree (bark surface). The measure points represent mean values of three determinations.

TABLE I. CONCENTRATION OF INORGANIC AND ORGANIC SUBSTANCES IN BARK SAMPLES TAKEN IN THE EURO-PEAN COUNTRIES GERMANY, NORWAY, POLAND AND RUSSIA. THE CONTENTS ARE SHOWN AS MINIMA, MAXIMA AND BIOINDICATION INDEXES (MIN/MAX).

Element/ Compound	Minimum	Maximum	Bioindication Index
	(μg g^{-1} d.w.)		
Al	1	1500	1500
As	0.01	3.9	386
B	0.8	24	30
Ca	1800	27000	15
Cd	0.1	1.2	12
Ce	0.1	13.8	125
Cr	0.5	10.4	20
Cu	1	36	36
Fe	51	4794	94
Hg	0.001	1.180	1180
Mo	0.01	4.70	470
NH_4^+	20	3800	190
Ni	0.01	8.2	820
NO_3^-	1	800	800
Pb	1.7	61.2	36
PO_4^{3-}	25	150	6
Sr	4	92	23
SO_4^{2-}	30	57000	1900
Ti	3.2	372	118
V	0.04	13	325
W	0.01	1	100
Zn	4.50	189	42
Zr	0.03	6	200
Benzo(a)pyrene	0.0004	0.0210	50
Fluoranthene	0.0074	0.1332	18
Pyrene	0.005	0.083	17
α-HCH	0.008	0.120	15
DDT	0.019	0.304	16

The profile analyses were determined with a laser inductive coupled plasma mass spectrometer (LS 320-ELAN 5000, Perkin-Elmer). A nitrogen purged Laser-ICP-AES combination was used to measure the sulfur profile. A rubin laser (LMA 10, 1 694 nm, Carl Zeiss Jena) for volatilization of the bark sample was coupled with a Spectroflame ICP-AES spectrometer (Spectro). The element distribution in the bark layers was measured in 0.5-mm steps starting from the surface. The amount of ^{13}C was used as an inner standard for the different bark layers.

TABLE II. MEAN CONCENTRATIONS (± STANDARD ERROR) OF ELEMENTS AND COMPOUNDS IN BARK SAMPLES (N=5) FROM BACKGROUND AREAS (NATIONAL PARK JOSTE-DALSBREEN, NORWAY; SYKTYWKAR, RUSSIA), LOW POLLUTED AREAS (BIALOWIESKA, POLAND; NEUGLOBSOW, GERMANY) AND HIGH POLLUTED AREA (RÖSA, GERMANY).B(A)P - BENZO(A)PYRENE; α-HCH - α-HEXACHLOROCYCLOHEXANE.

Element/ Compound	**Jostedalsbreen**	**Syktywkar**	**Bialowieska**	**Neuglobsow**	**Rösa**
SO_4-S (mg g^{-1})	0.08 ± 0.01	0.07 ± 0.05	0.23 ± 0.03	0.95 ± 0.07	15.77 ± 0.77
NH_4-N (mg g^{-1})	0.049 ± 0.034	0.036 ± 0.007	0.078 ± 0.011	0.077 ± 0.003	0.68 ± 0.13
NO_3-N (mg g^{-1})	0.02 ± 0.0001	0.001± 0.0001	0.08 ± 0.001	0.019 ± 0.003	0.024 ± 0.002
Ca (mg g^{-1})	3.49 ± 0.34	2.98 ± 0.17	6.11 ± 0.36	3.01 ± 0.31	21.03 ± 0.86
Fe (mg g^{-1})	0.10 ± 0.04	0.11 ± 0.02	0.24 ± 0.05	0.40 ± 0.04	3.49 ± 0.21
Al (μg g^{-1})	0.39 ± 0.06	0.31 ± 0.03	0.48 ± 0.02	0.28 ± 0.02	1.09 ± 0.05
Ti (μg g^{-1})	6.3 ± 2.2	7.2 ± 0.4	16.1 ± 1.4	9.1 ± 1.1	241 ± 14.9
Pb (μg g^{-1})	1.9 ± 0.1	2.2 ± 0.1	4.5 ± 0.3	15.2 ± 1.7	20.9 ± 1.3
Cd (μg g^{-1})	0.2 ± 0.001	0.2 ± 0.001	0.4 ± 0.001	0.2 ± 0.001	0.3 ± 0.001
Cr (μg g^{-1})	1.1 ± O.4	0.8 ± 0.1	3.1 ± 0.2	1.4 ± 0.2	7.1 ± 0.4
V (μg g^{-1})	0.3 ± 0.1	0.8 ± 0.1	1.5 ± 0.2	2.4 ± 0.2	10.7 ± 0.6
B(a)p (ng g^{-1})	1.18 ±0.36	2.6 ± 0.42	1.42 ± 0.59	3.87 ± 0.21	9.14 ± 1.08
α-HCH (ng g^{-1})	-	-	-	27.0 ± 3.2	100.3 ± 9.8

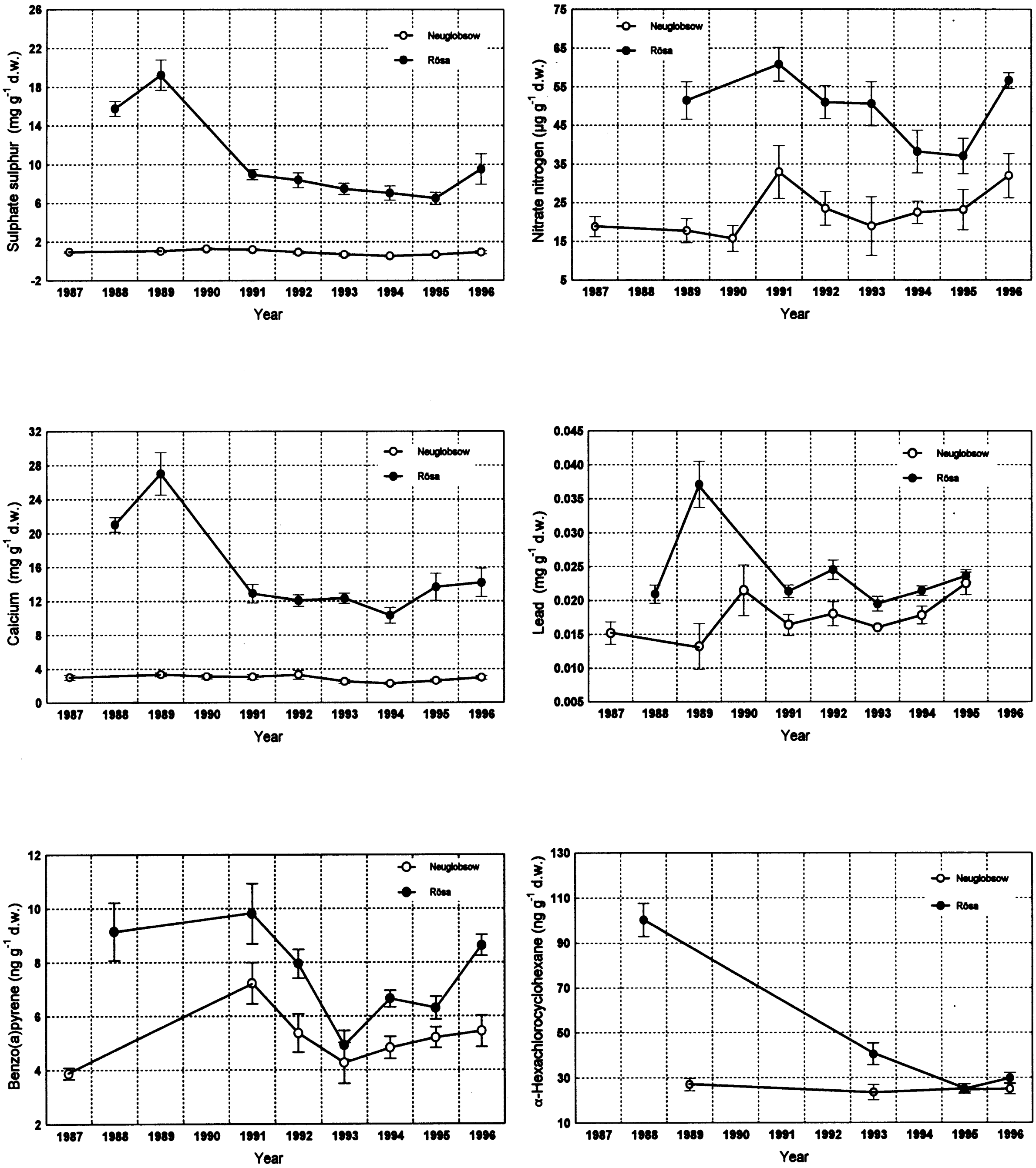

Fig.4 Saisonal variations of sulphate sulphur, nitrate nitrogen, calcium, lead, benzo(a)pyrene and α-hexachlorocyclohexane in pine barks from the sites Rösa and Neuglobsow between 1987 and 1996. The measure points represent mean values of 5 mix samples per test area with standard errors.

For the PAH analysis 5 g of bark samples were extracted with toluene and a solvent exchange to cyclohexane was carried out. After the samples were subjected to a silica gel clean-up and a final solvent exange (acetonitrile). The determination was carried out with a HPLC system (System Gold, Beckmann) equipped with a programmable fluorescence detector (Shimadzu RF-551). The PAHs were separated on a Bakerbond PAH 16 Plus column (250x3 mm I.D.) with a pre-column at a temperature of 25°C. Eluent (A) was water, eluent (B) acetonitrile and the eluent programme started with 50% A and 50% B for 5 min, then the content of B was increased to 100% in 30 min with a linear gradient. This concentration was held constant for 10 min until the end of the analysis.

HCHs and DDX-compounds (SOCs) were determined with a HP 5890 II device (Hewlett Packard) with electron capture detection (ECD). The extraction of 3 g of bark samples was performed using an accelerated solvent extraction device (ASE 200, Dionex) with the following conditions: acetone/hexane (1/1, v/v), 100°C, 10 MPa, static extraction time 10 min. The extracts were concentrated to 1 ml and subjected to a florisil clean-up. For the analysis a 30m x 0.32mm I.D. HP5 column was used. The samples were injected in the splitless mode at 220°C and the temperature programme was as follows: initial temperature 50°C (held for 8 min), increased at 6°C/min to 250°C and held at the final temperature for 5 min. The detector temperature was 300°C. The carrier gas was hydrogen, and the make-up gas ECD nitrogen.

2.3. Statistical and computer-aided data analysis

Maps of airborne pollutants in pine barks were calculated for the Nature Park Dübener Heide area using the surface mapping system Surfer ver. 6.0 (Golden Software Inc., Golden, Colorado, USA). The contour lines were interpolated by the simple Kriging method. The data were subjected to analysis of statistical parameters by using the statistical package SPSSPC+4.0 (SPSS Inc. Chicago, USA).

3. RESULTS AND DISCUSSION

3.1. Characteristics of contaminant accumulation in pine barks

Deposition from the atmosphere forms the input of airborne pollutants to the pine stand (Fig.2). Deposition can be divided into dry deposition and wet deposition. In pine forests, part of the precipitation (wet deposition) falls through the canopy without being intercepted, while another part is intercepted. The sum of both parts is called the crown throughfall. The part running down the tree trunk is called stemflow. The stem deposition as well as parts of the dry deposition and throughfall forms the bark deposition. According to element profiles of sulphur, iron and calcium in a piece of bark as shown in Fig.3, the accumulation process of airborne substances takes place on the outer porous layer of bark surface. For all three elements, a significant concentration gradient is observed between 0.5 and 1.5 mm bark depth, with only low concentration levels at deeper bark layers [10]. It follows that sampling of the outer 2 mm bark will be sufficient for the analysis of pollutants taken up from the air.

The accumulation process of airborne substances is closed with the regeneration of the bark surface (repulsion). After ca. two years, the external bark sheet is rejected, and a new bark layer is formed by sheets (approximately 1-2 mm thick) growing from inside. Correspondingly, a new accumulation process starts after each regeneration of the external bark layer. Due to the small size of the accumulation layer of the bark, only limited time will be necessary to reach equilibrium between the accumulated compound contents and the part of crown throughfall running down the tree trunk.

Table I shows minimum and maximum concentrations (mg g^{-1} d.w.) of various inorganic and organic substances in pine barks from differently polluted areas in Northern, Central and Eastern Europe. The highest concentrations were determined for sulphate, calcium, iron and ammonium. Organic substances are found in relatively low concentrations. Here, the bioindication index (maximal content/minimal content) ranges from 15 to 50. Much larger differences up to bioindication indexes of 1900 are found for inorganic pollutants such as sulphate (1900) and aluminium (1500) as well as mercury (1200). These concentration ranges indicate, that differences in airborne pollution can be reasonably well reflected by corresponding differences in accumulated contamination loads in the barks of pine stands.

In Table II are summarized the deteremined mean concentrations of selected elements and compounds in bark samples, which were collected in various European background and impact areas. All concentration values show a clear differentiation between background areas (National Park Jostedalsbreen, Syktywkar) and impact areas (Bialowieska, Neuglobsow, Rösa). For example, the sulphur values of Jostedalsbreen (Norway) and Syktywkar (Russia) are 200-10 times lower than those in the impact areas in East Germany (e.g. Neuglobsow, Rösa). A similar trend is found for the concentrations of all trace elements, e.g. cadmium that increases from 0.2 ppm in the background area of Norway and Russia to 20.9 ppm in Rösa. High concentrations of benzo(a)pyrene and a-hexachlorocyclohexane were measured in pine barks from Rösa. Pine barks reflect the atmospheric depositions of nutrient and heavy elements as well as polycyclic aromatic hydrocarbons and organochlorine compounds. The results are in agreement with findings of

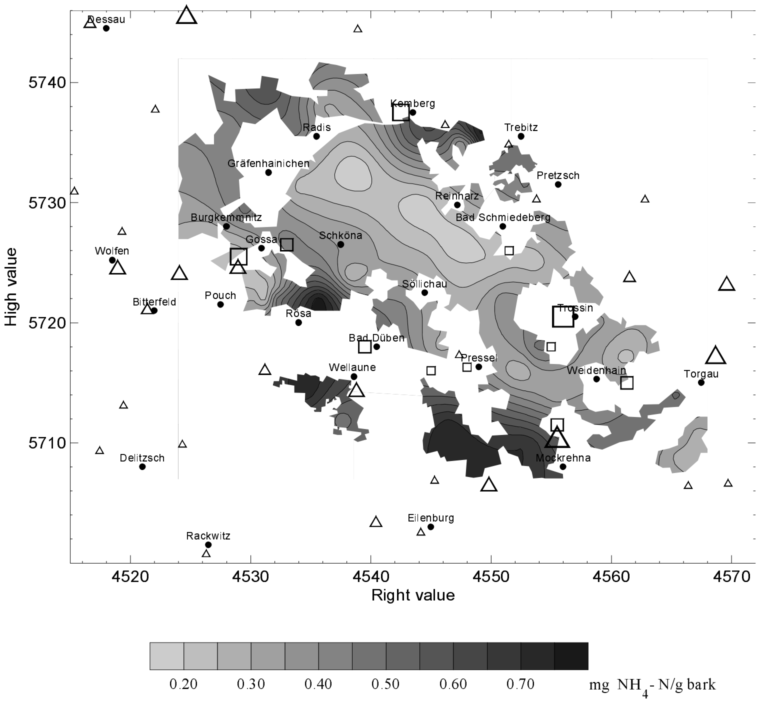

Fig.5a Spatial distribution of sulphate sulphur in pine barks from site Nature Park Dübener Heide (2.5 x 2.5 km grid of 56 test sites in Gauss-Krüger coordinates) in 1994 on the basis of mix samples. The open triangles represent locations of industry and power plants and yearly mean Deposition of sulphur (SO_2-S + SO_3-S) in tonnes (10-50, 50-100, 100-250, 250-1000,1000-20000).

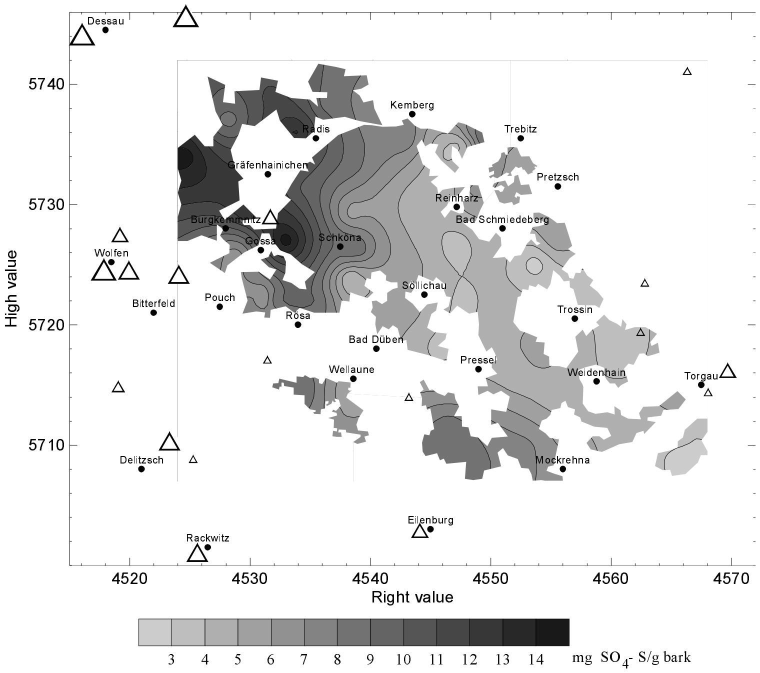

Fig.5b Spatial distribution of ammonium nitrogen in pine barks from site Nature Park Dübener Heide (2.5 x 2.5 km grid of 56 test sites in Gauss-Krüger coordinates) in 1994 on the basis of mix samples. The open triangles represent locations of industry and power plants with a yearly mean Deposition of nitrogen (NH_4-N+ NO_x-N) in tonnes (10-50, 50-100, 100-250, 250-1000, 1000-20000). The open squares represents locations of agricultural sources with a yearly mean Deposition of nitrogen (liquid manure) in tonnes (16-40, 40-80, 80-120, > 120).

Poikolainen [11]. The raw concentrations of several deposited elements and compounds in pine bark of background areas were lowly variable and allow a reliable assessment of baseline concentrations.

3.2. Temporal variations

In Fig.4, seasonal variations are shown for sulphate sulphur, nitrate nitrogen, calcium, lead, benzo(a)pyrene and a-hexachlorocyclohexane. The data are based on bark samples collected from five low and five high polluted sites in the surroundings of Neuglobsow near lake Stechlin in the north of Berlin as well as in the surroundings of Rösa in the Dübener Heide. In comparison to the reference area Neuglobsow, bark samples from the test site Rösa indicate significantly higher depositions of airborne pollutants. The concentrations of inorganic and organic substances in barks reflect substantial infrastructural changes in former Eastern Germany since 1990: Both sulphur dioxide emissions and alkaline fly dust have been reduced significantly as compared to former times in the industrial region around the cities Leipzig, Halle, and Bitterfeld [12]. Consequently, there is a particular decrease of the concentrations of sulphate sulphur and calcium in pine barks at the site Rösa.

Similarly, the decrease of nitrate nitrogen in pine barks at both sites reflects an overall reduction of nitrogen emissions in this region. The recent increase of nitrate nitrogen concentrations in barks since 1995 can be traced back to the fact that NO_x losses due to reduced coal combustion are balanced by an increase of automobile traffic in both areas. The latter can also be seen by temporal variations of airborne lead and benzo(a)pyrene.

A further interesting fact is the general decrease of a-hexachlorocyclohexane (a-HCH) concentrations in pine barks in these areas. The semivolatile organic compound a-HCH is a waste product of the production of the insecticide lindane (g-hexachlorocyclohexane), which was stopped in the 1980s in former Eastern Germany. It is thus likely that the formerly increased a-HCH contents in barks of pine stands were due to evaporation from regional sources associated with the lindane production.

3.3. Spatial patterns

In the pine stands of the Nature Park Dübener Heide, spatial patterns of sulphate sulphur and ammonium nitrogen are studied using pine barks at 53 test sites, which were selected using statistical criteria to cover an area of 20 * 30 km in a systematic way. Application of the Kriging method for linear interpolation leads to the contour maps of Fig. 4a and b, showing the spatial patterns of SO_4-S and NH_4-N in pine stands of the Nature Park Dübener Heide.

As can be seen from Fig.5a, high contents of SO_4-S in pine barks are mainly found in the north-west around Gräfenhainichen. Here, mean concentrations of sulphate sulphur (14 mg SO_4-S g^{-1} bark) are substantially higher than in the periphery with typical values of 3-4 mg SO_4-S g^{-1} bark. Analysis of the local infrastructure reveals combustion processes, e.g. from brown coal power plants and private house heating, as major emission sources. Furthermore, the data in Fig.4a show a general decrease in SO_4-S from west to east. Additional hot spots are given in the north and south of the Nature Park Dübener Heide as well as in the industrial area from Bitterfeld and Leipzig.

Fig.5b shows corresponding spatial patterns of NH_4-N. The overall pictures indicate significant differences in the local deposition structure. In contrast to SO_4-S with one major emission area, nitrogen emission is apparently caused by a number of agricultural sources mainly in the periphery of the Nature Park Dübener Heide. The NH_3 deposition is likely to result mainly from livestock management and partially also from fertilizer application. This would be in agreement with an earlier suggestion, that the contribution from agriculture to total NH_3 emission in East Germany amounts to more than 90% [13].

4. SUMMARY AND CONCLUSIONS

The results show how pine tree barks can be used as a biomonitoring tool to indicate depositions of airborne pollutants in pine stands. In particular, this bark technique offers a new way to scale up measuring results from individual sites to the area in the field. Barks of pine trees appear to be well suited for monitoring of various inorganic and organic airborne pollutants, which are deposited in pine stands. Pine barks are inert, show good accumulation properties in relatively short times and are easy to handle.

Furthermore, the bark method offers a simple way to study temporal and spatial patterns of airborne pollutants on regional and supra-regional scales. In the Nature Park Dübener Heide, the results of sulphate sulphur and ammonium nitrogen reflect inputs from power plants, house heating and agriculture. It is suggested that on the basis of a statistically selected network of measuring sites, analysis of contaminant profiles in pine barks yields useful information for the evaluation of possible ecotoxicological effects of airborne pollutants on forest ecosystems.

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BIOMONITORING OF AIRBORNE INORGANIC AND ORGANIC POLLUTANTS BY MEANS OF PINE TREE BARKS.
II. DEPOSITION TYPES AND IMPACT LEVELS

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Abstract

A total of 273 pine bark samples collected from various pine stands in Central and East Germany, South Norway, Poland, and Russia was analyzed with respect to 20 inorganic and organic substances (sulphate, nitrate, ammonia, calcium, 3 PAHs, 5 heavy metals, 9 other elements). Multivariate statistics were applied to characterize the multiple exposure of airborne pollutants in terms of major sources, deposition types and impact levels. The former was studied with factor analysis, whilst the latter two were addressed by applying cluster and discrimination analysis. Factor analysis of the concentration values suggest separation into three factors with the following characteristics: Factor 1 shows higher contributions from sulphate and calcium, factor 2 from fluoranthene, benzo(a)pyrene as well as from pyrene, and factor 3 from nitrate and ammonia, respectively. According to results from the cluster analysis, three major deposition types can be identified: "Industry and House heating", "Motor traffic", and "Agriculture". The first deposition type is characterized by high contents of sulphate and calcium. The other two deposition types contain specific composition profiles for nitrogen-containing components and PAHs. Impact levels are separately classified with the characteristic variables of main deposition types. Finally, discriminant analysis is used to allocate new bark samples to the classified deposition types and impact levels. The results demonstrate the usefulness of multivariate statistical techniques to characterize and evaluate multiple exposure patterns of airborne pollutants in forest ecosystems.

1. INTRODUCTION

Tree barks have proven useful as bioindicators for atmospheric depositions of inorganic and organic pollutants and trace elements [1-8]. A more detailed analysis of the accumulation process in pine barks revealed, that most of the inorganic and organic substances taken up from precipitation and from dry deposition of airborne particles is stored in the outer layer of the bark, being in a depth of up to 2 mm [9]. Apart from airborne pollutants, pine barks have also a capacity to retain trace elements [8]. It follows that chemical analysis of pine barks using inductive coupled plasma mass spectrometry (ICP-MS), ion chromatography (IC), gas chromatography (GC), and high performance liquid chromatography (HPLC) tend to produce large data sets that may be difficult to interpret. Multivariate analysis, e.g. factor analysis, cluster analysis and discriminant analysis, can be used in such cases to obtain a more condensed overview of the data. Recent applications of these statistical methods can be found in the literature [3, 12-16].

In this paper, biomonitoring results using pine barks from various sites in Europe and times between 1987 and 1996 are presented and discussed. Factor analysis, cluster analysis and discriminant analysis are applied to identify and characterize deposition types and impact levels for a wide range of airborne pollutants. The results are compared with recent investigations of actual deposition conditions in pine forests of the Nature Park Dübener Heide in Sachsen-Anhalt in eastern Germany.

2. MATERIAL AND METHODS

Sampling was carried out in different years between October 1987 and October 1996. Bark samples were taken from 60 to 80 years old Scots pine trees (*Pinus sylvestris* L.) in various regions of eastern Germany including test sites at Rösa near Bitterfeld (Nature Park Dübener Heide, Sachsen-Anhalt) and reference areas in Europe (Denmark, Norway, Poland, Russia). For more details about the geographical location of the sites, the sampling procedure and the chemical analyses the reader is referred to [10]. Factor analysis, cluster analysis and discriminant analysis were performed using the statistical package SPSSPC+4.0 (SPSS Inc., Chicago, USA).

3. RESULTS AND DISCUSSION

In the first part of our investigations in the field it was shown how measurements of inorganic and organic substances in pine tree barks can be evaluated with univariate statistical methods [10]. The results were restricted to elucidating trends of individual compound contents in barks. The current analysis focuses on the more complex question, whether and how concentration profiles of compound groups can be traced back to certain emission sources. To this end, multivariate statistical techniques are used to derive parameters (latent variables) that allow a distinction between different deposition types. The respective mathematical procedure is outlined in Fig.1.

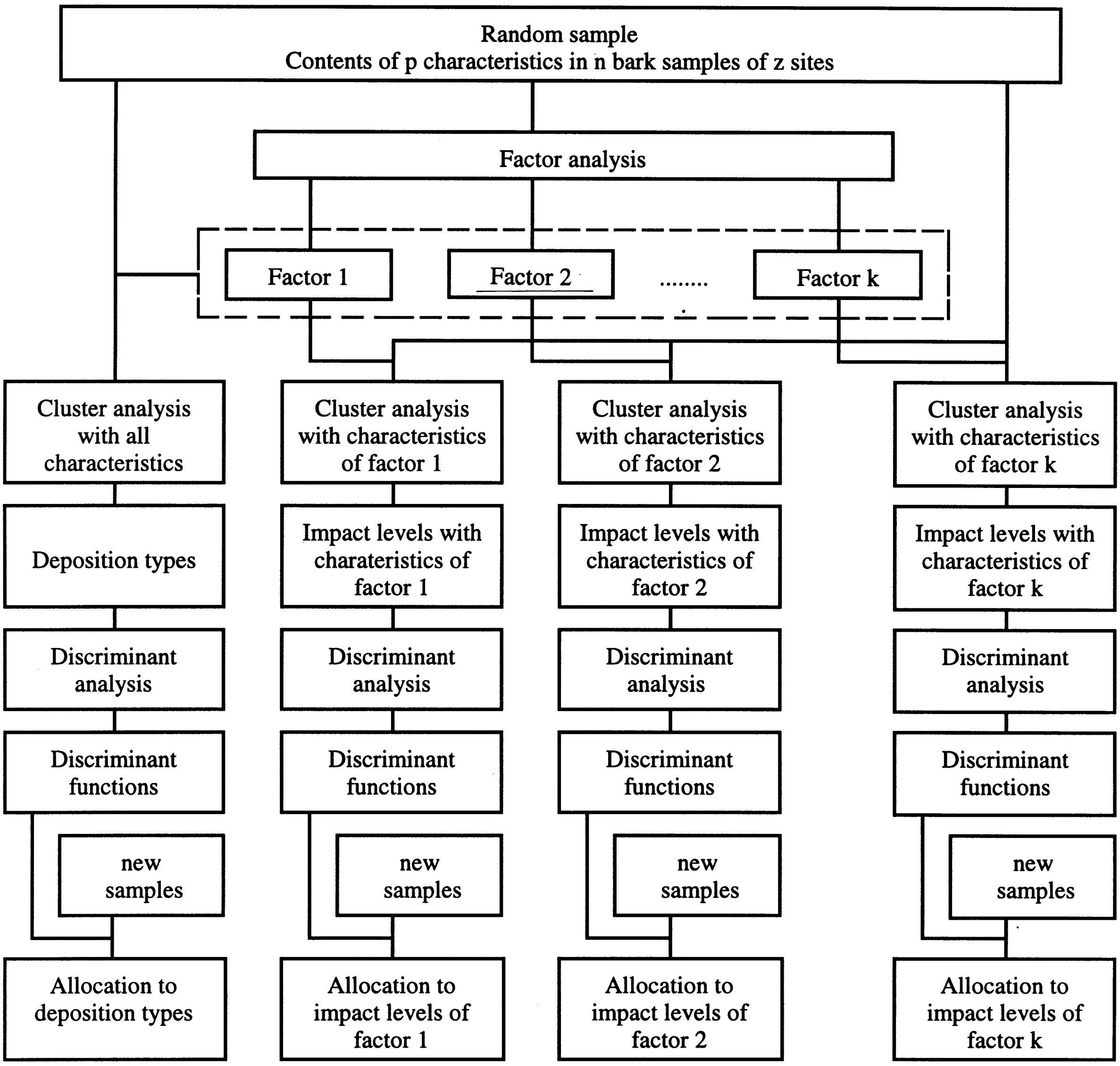

FIG. 1. General scheme of the procedure of multivariate analysis.

3.1. Factor analysis

Factor analysis allows to condense the information contained in a set of variables into a smaller set of new composite variables called factors, which are derived from the correlation structure of the initial (observed) variables. For the current study, principal component analysis was used to generate the factors, which were then subjected to orthogonal rotation with the varimax method (cf. textbooks about chemometrics). Application of this approach to the observed concentration values of 20 inorganic and

TABLE I. FACTOR LOADINGS OF THE ROTATED MATRIX FOR 20 BARK CHARACTERISTICS AND 273 BARK SAMPLES

Characteristics	Factor 1	Factor 2	Factor 3
Ti	0.96	0.20	0.09
Ce	0.95	0.09	0.07
La	0.95	0.13	0.08
Nd	0.92	-0.01	0.03
Zr	0.91	0.29	0.16
Sr	0.90	0.27	0.25
V	0.87	0.06	0.14
Fe	**0.83**	0.38	0.15
Cr	0.80	0.28	0.17
Cu	0.79	0.30	0.16
Ca	**0.75**	0.30	0.22
SO_4	**0.74**	0.35	0.30
As	0.67	0.24	0.36
Al	0.66	0.39	0.19
B	0.62	0.31	0.54
Pyrene	0.12	**0.93**	0.15
Fluoranthene	0.18	**0.92**	0.23
Benzo(a)pyrene	0.40	**0.72**	0.14
NH_4	0.08	0.18	**0.88**
NO_3	0.18	0.16	**0.88**

organic substances in pine barks shows, that 82% of the overall variation in the data can be explained by three latent variables. The resultant factor loadings after varimax rotation are given in Table I and correspond to the following grouping of elements and compounds: V, Ce, La, Nd, Ti, Zr, Sr, As, Cu, Cr, Ca, Al, SO_4, Fe, B (factor 1), fluoranthene, pyrene, benzo(a)pyrene (factor 2), and NO_3 as well as NH_4 (factor 3).

Variation of the number of sites and selection of different rotation methods yields always three factors accounting for more than 80% of the total variation, which indicates that the results can be considered as being statistically robust. The factors are suggested to represent the following emission sources: “Industry and House heating” (factor 1), “Motor traffic” (factor 2), and “Agriculture” (factor 3). As regards “Industry and House heating”, a more detailed analysis (which is not given here) leads to a further differentiation between the fraction of airborne particles mainly loaded with Ca, Al, SO_4,and Fe, and a fraction of particles mainly loaded with the remaining 11 elements. In any case, the 15 major elements and substances of factor 1 are mainly released through combustion of brown coal or fossil fuels in the form of industrial dust and fly ashes, and that their deposition occurs often together with acidic and alkaline components as discussed earlier [3]. On the other hand, it is difficult to explain the origin of both particle fraction. The adsorbed elements and compounds in barks (factor 1) probably consist of particles from fly ashes associated with mineral particles, mainly windblown dust. According to [4], iron from pine bark is mostly in a soluble form, so it is assumed that it is generally adsorbed with SO_4 from rainwater. The soluble fraction of iron in precipitation has an average value of 60% [5]. In dry depositions, iron is mainly in an insoluble form. It is the dominant component of emitted fly ash particles from power plants and house

TABLE II. MEANS OF DEPOSITION TYPES AND STANDARD DEVIATIONS (□< 0.05) BASED ON THE CLUSTER ANALYSIS WITH EIGHT SELECTED CHARACTERISTICS AND A RANDOM SAMPLE WITH 273 CASES. B(A)P - BENZO(A)PYRENE, FLUO - FLUORANTHENE, PYR - PYRENE

	Bark contents							
Deposition type	Ca	Fe	SO_4	NH_4	NO_3	B(a)p	Fluo	Pyr
	(mg g^{-1} d.w.)					(ng g^{-1} d.w.)		
Background 1 (n = 68)	3.31 ±1.29	0.41 ± 0.23	1.78 ± 1.46	0.18 ± 0.14	0.08 ± 0.06	3.7 ± 1.9	26.9 ± 11.3	15.7 ± 7.1
Background 2 (n = 107)	6.25 ± 1.98	1.59 ± 0.65	9.37 ± 4.37	0.44 ± 0.19	0.17 ± 0.06	8.5 ± 2.4	52.4 ± 12.5	31.3 ± 9.4
Agriculture (n = 43)	9.25 ± 3.77	1.84 ± 0.76	18.21 ± 9.49	1.45 ± 0.57	0.39 ± 0.14	8.2 ± 3.0	61.8 ± 20.9	36.5 ± 12.9
Motor traffic (n = 15)	13.42 ± 5.62	2.87 ± 0.76	26.76 ± 13.55	0.78 ± 0.31	0.20 ± 0.08	12.9 ± 3.1	80.1 ± 25.1	49.5 ± 17.2
Industry and House heating (n = 40)	14.16 ± 3.50	3.03 ± 0.39	26.06 ± 8.50	0.53 ± 0.17	0.19 ± 0.05	7.5 ± 2.2	45.0 ± 11.6	26.3 ± 6.5

TABLE III. MEANS OF IMPACT LEVELS AND STANDARD DEVIATIONS (□< 0.05) BASED ON THE EXTRACTED FACTORS WITH THEIR TYPICAL CHARACTERISTICS (RANDOM SAMPLE WITH 273 CASES). B(A)P - BENZO(A)PYRENE, FLUO - FLUORANTHENE, PYR - PYRENE

Impact level	Factor 1			Factor 2		Factor 3		
	Bark contents							
	Ca	Fe	SO_4	NH_4	NO_3	B(a)p	Fluo	Pyr
		(mg g^{-1} d.w.)		(mg g^{-1} d.w.)			(ng g^{-1} d.w.)	
1	3.49 ± 1.18	0.48 ± 0.24	2.40 ± 1.66	0.14 ± 0.09	0.05 ± 0.03	2.3 ± 1.5	17.9 ± 7.5	9.7 ± 3.6
2	6.82 ± 1.33	1.91 ± 0.41	10.71 ± 3.02	0.44 ± 0.15	0.16 ± 0.04	6.5 ± 1.9	41.6 ± 8.6	24.3 ± 5.5
3	10.56 ± 1.65	2.35 ± 0.39	20.94 ± 4.00	0.97 ± 0.33	0.28 ± 0.06	10.6 ± 2.9	61.3 ± 8.7	36.6 ± 6.2
4	14.32 ± 2.24	3.07 ± 0.46	26.58 ± 6.31	1.69 ± 0.69	0.48 ± 0.16	9.3 ±1.8	79.6 ± 15.5	49.7 ± 7.3
5	21.31 ± 2.32	3.60 ± 0.58	44.88 ± 5.81			13.9 ± 1.7	105.0 ± 16.3	67.4 ± 9.1

TABLE IV. COEFFICIENTS OF STANDARDIZED CANONICAL DISCRIMINANT FUNCTIONS OF DEPOSITIONS TYPES AFTER VARIABLE REDUCTION

Characteristic	Coefficients			
	Function 1	Function 2	Function 3	Function 4
Calcium	0.35968	- 0.13303	- 0.00022	0.53245
Iron	0.50480	- 0.49288	- 0.20320	- 0.88381
Sulphate	- 0.24362	- 0.03642	- 0.51276	0.48926
Benzo(a)pyrene	0.17443	- 0.28152	0.72626	0.00992
Pyrene	0.29870	0.06342	0.43766	0.24888
Ammonium	0.58145	0.42131	0.07028	0.18476
Nitrate	0.23272	0.52759	- 0.22954	- 0.39901

TABLE V. RECLASSIFICATION OF CLUSTER ANALYSIS. DEPOSITION TYPES WERE CHECKED BY STANDARDIZED CANONICAL DISCRIMINANT FUNCTIONS (94.14% CORRECT GROUPED).

Prior deposition typ (number of cases)		Predicted deposition type				
		Background 1	Background 2	Agriculture	Motor traffic	Industry and House heating
Background 1	Number	68	1	0	0	0
(69)	Percent	98.6%	1.4%	0%	0%	0%
Background 2	Number	7	90	0	0	0
(97)	Percent	7.2%	92.8%	0%	0.0%	0%
Agriculture	Number	0	1	36	1	0
(38)	Percent	0%	2.6%	94.7%	2.6%	0%
Motor traffic	Number	0	2	1	25	2
(30)	Percent	0%	6.7%	3.3%	83.3%	6.7%
Industry and House heating	Number	0	0	0	1	38
(39)	Percent	0%	0%	0%	2.6%	97.4%

heating, where the soluble fraction is about 10%. Insoluble compounds exist in the form of oxides and hydroxides. Iron-rich particles can contain a high level of titanium [5]. The highly significant correlations between the iron and sulphate contents on the one side and the high factor loadings of Ti, Ce, La, Nd, Zr, Sr, V, Cr, Cu, Ca, As, Al, and B on the other hand indicate the frequent occurrence of these elements together with different particles in the air. Due to variable solubility of particles, it is difficult to determine

TABLE VI. ASSIGNMENT OF NEW BARK SAMPLES FROM RÖSA (1996) TO IMPACT LEVELS OF EXTRACTED FACTORS, COMPARISON 1996 TO CLASSIFIED SAMPLES FROM 1988.

Test sites	Impact levels											
	Factor 1				Factor 2				Factor 3			
	Individual		Mean		Individual		Mean		Individual		Mean	
	88	96	88	96	88	96	88	96	88	96	88	96
Rösa 1	5	4			5	2			3	2		
Rösa 2	5	4			4	2			3	3		
Rösa 3	5	4			4	2			2	3		
Rösa 4	5	5			3	2			2	3		
Rösa 5	5	3	5	4	3	2	3.8	2	2	2	2.4	2.6

exactly what fraction of the total element content is present in a nonparticulate form in pine barks. B, Al, and As show no significant loading separation which could be a hint for soilborne element inputs, but a separate soil factor with Al and Fe as main elements could not produced. Therefore, the effects of soil contamination, as observed in bark samples of oaks (*Quercus cerris* and *Q. pubescens*) from a background area, should be minimized [6]. The PAHs benzo(a)pyrene, fluoranthene and pyrene form the main components of the "Motor traffic" factor, whilst benzo(a)pyrene contributes also to factor 1. The factor "Agriculture" is only loaded by ammonium and nitrate.

Among the substances with large loadings in factor 1, the largest absolute contents in the bark samples are provided by Fe, Ca and SO_4. From this viewpoint, factor 1 may also be represented by these three analytes, which allows a more condensed description of the main features behind the deposition patterns. Consequently, the subsequent analyses were restricted to Fe, Ca, and SO_4 representing factor 1, pyrene, fluoranthene and benzo(a)pyrene representing factor 2, and NH_4 and NO_3 representing factor 3.

The question is now, whether and how the selected chemical characteristics can be used for classification of deposition types and impact levels to characterize and quantify certain atmospheric depositions. The cluster analysis is a useful tool for such model investigations.

3.2. Cluster analysis

Cluster analyses were performed using the quadratic Euclidean distance measure and the Ward criterion. They were used to address the following two questions: How can the total of 273 measuring sites be grouped into clusters representing the deposition types as suggested by factor analysis, and how can the samples be classified according to different impact levels?

For the first question, a cluster analysis was performed based on the concentration values of the eight analytes selected from the factor analysis as mentioned above. The results are summarized in Table II and reveal five clusters with distinct average contents of the eight bark characteristics. Inspection of the average concentration values leads to the interpretation in terms of the deposition types listed in the first column, which is now demonstrated for the cluster representing mainly depositions from agriculture. As can be seen from the table, this cluster contains the greatest average contents of NH_4 (1.45 mg/g d.w.) and NO_3 (0.39 mg/g d.w.), which in turn had already been found as major loadings of factor 2 (agriculture) according to the previous factor analysis. The other characterizations of clusters in terms of deposition types have been performed in a similar way, following the specific composition profiles and maximum average contents of elements and compounds as outlined before. Three of the five clusters listed in Table II represent the previously defined deposition types "Industry and House heating", "Motor traffic" and "Agriculture", and in addition two clusters are assigned to different background levels.

The deposition types "Industry and House heating" and "Motor traffic" are characterized by extremely high contents of sulphate in pine barks, but differ as regards their average PAH contents. For ammonium and nitrate, "Industry and House heating" as well as "Motor traffic" yield only half of the contents as compared to "Agriculture". For the sake of brevity, the assignment of individual sites to clusters is not shown.

The second question of identifying impact levels is analyzed separately for the three groups of contaminants that were used to define the deposition types. The results of these three calculations are listed in Table III. As can be seen from the left data block in the table, the cluster analysis based on Ca, Fe and SO_4 (typical characteristics of factor 1) yields five clusters representing distinct impact levels of these three analytes, where the highest impact level is characterized by average concentration values of 21.31 mg/g d.w. Ca, 3.60 mg/g d.w. Fe and 44.88 mg/g d.w. SO_4. The corresponding analysis applied to NH_4 and NO_3 (characteristics of factor 3) yields only four impact levels covering ca. one order of magnitude for the contents of both compounds (central data block in Table IV), and for the three PAHs (characteristics of factor 2) there are again five different impact levels as shown in the right data block of Table III.

The next question is whether deposition types and impact levels can be described by a system of mathematical functions that allow to allocate new cases or bark samples to the existing cluster scheme.

3.3. Discriminant analysis

In the previous section, two different cluster schemes were derived that allow to unravel systematic features behind the deposition patterns as characterized by the total of 273 pine bark samples: five clusters representing different deposition types, and clusters representing various impact levels (Tables II and III). A mathematical means to test the statistical significance of the occurrence of these clusters is given by deriving sets of discriminant functions, which allow to evaluate the degree of separability of the data in terms of these pre-defined clusters as well as to apply the classification scheme to newly tested bark samples.

In the first step of the discriminant analyses based on the medium Mahalanobis distances, the statistical significance of the four sets of clusters were subjected to F tests. The results showed that both the previously derived deposition types and impact levels are significant on the $p < 0.5$ level (details not given). In the next step of discriminant analysis, the diagnostic relevant independent characteristics were established because it is not necessary to have all by factor analysis preselected elements in order to characterize the five deposition types. Thus it is possible to determine the diagnostic optimum number of characteristics p^* where $p^* < p$, by means of the method of maximization of the smallest F-ratio between pairs of types. To separate the five types of deposition, we used four discriminant functions. Deleting fluoranthene gave the highest statistical significance. In Table IV the four functions discriminating between the five deposition types are given in terms of linear combinations of the remaining seven analytes Ca, Fe, SO_4, benzo(a)pyrene, pyrene, NH_4 and NO_3. As result by removing of fluoranthene it was contain a set of seven characteristics (SO_4, Ca, Fe, benzo(a)pyrene, pyrene, NH_4, NO_3). Thus each deposition type can be described by four discriminant functions using the calculated coefficients in Table III and the mean values for the bark characteristics in Table II. The calculation of discriminant functions (F1, F2, F3, and F4) gives the coordinates (discriminant values of F1, F2, ... Fn) for each deposition type (group centroid) in the discriminant vector space. The dimension of discriminant vector space is determined by eigenvalues, which correspond to discrimination functions, Wilks with chisquare test and other statistical quantities.

As indicated above, application of these functions allows to classify analytical results of new samples with regard to the deposition types. Furthermore, these functions can be applied to the present data set to derive a second classification, which can be compared with the classification according to the previously performed cluster analysis. The corresponding results are listed in Table V. As can be seen from the table, the deposition types "background 1" and "Industry and House heating" yield excellent degrees of agreement between both classification schemes (98.6% and 97.4%, respectively). The greatest portion of different classifications was observed for the deposition type "Motor Traffic", where five samples corresponding to 17% were allocated to other clusters. However, the overall agreement of ca. 94% is quite satisfactory and confirms the previous classification developed with cluster analysis. Similar results were obtained for the second cluster scheme representing different impact levels.

Finally, the standardized canonical discriminant functions were used to allocate new bark samples collected at Rösa (Nature Park Dübener Heide, industrial region Leipzig-Bitterfeld-Halle) in 1996. The coordinates for the new cases are calculated using the analyzed bark contents of eight typical characteristics and the calculated coefficients of standardized canonical discriminant functions. The resulting classifications are compared with corresponding data from 1988 and summarized in Table VI. As can be seen from the table, the two sets of data reveal some interesting differences between the emission situations of 1988 and 1996: The mean impact levels of the deposition types "Industry and House heating" and "Motor traffic" have decreased since 1988, while the impact level of deposition type "Agriculture" shows no significant differences between 1988 and 1996. These results confirm previously observed changes in the composition of atmospheric pollutants as well as in the temporal variations of individual airborne depositions since the economic changes of 1989, which can be explained by shutdowns of chemical and power plants as well as by a more efficient use of energy sources [8]. Moreover, this analysis demonstrates how changes in composition of atmospheric pollutants can be detected by means of creating complex "dependent variables" composed of a selection of inorganic and organic pine bark characteristics. Thus, the computer-aided diagnose with pine barks is a useful biomonitoring tool for the characterization and evaluation of multiple exposure patterns of airborne pollutants in forest ecosystems.

4. SUMMARY AND CONCLUSIONS

The results show how the combination of factor analysis, cluster analysis and discriminant analysis can be used to characterize and evaluate depositions of airborne pollutants in terms of both deposition types and impact levels. In particular, the deposition types and impact levels as derived by factor analysis and cluster analysis can be used as a differential diagnosis tool for characterization of complex airborne pollutants and the analysis of emission sources. With the calculated canonical discriminant functions, new bark samples can be allocated to the classified deposition types and impact levels. Therefore, the multivariate results are useful for the interpretation and quantification of multiple exposure patterns of airborne pollutants in forest ecosystems. Moreover, the data already collected can serve as valuable information for the short-term and long-term monitoring of atmospheric depositions in forests.

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LICHENS AS BIOMONITORS OF ATMOSPHERIC AMMONIUM/AMMONIA DEPOSITION IN PORTUGAL

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Abstract

The aim of the present work was to evaluate the potentiality of lichens as biomonitors of NH_4^+/NH_3 (ammonium/ammonia) and NO_3^- (nitrate) atmospheric deposition. For that, we used as a field station a rice plantation which is submitted, once a year, to air spraying fertilization with a misture of nitrogen sources. Samples of an epiphytic lichen, *Ramalina fastigiata*, were collected from an ash-tree bordering the rice-plantation by the Sorraia River Valley (Central Portugal). The study started one month before fertilization and sampling was carried out for five months. The concentration of ammonium in the lichen was highly and significantly correlated with the number of days without precipitation before sampling, and had an inverse correlation with fluorescence values. Under these conditions, the amount of NH_4^+ found in the lichen appers to reflect ammonium/ammonia dry deposition.

1. INTRODUCTION

Since the last century, occurred an increase in atmospheric pollution, being NO_x and NH_x the most common atmospheric sources of nitrogen. In comparison with NH_3, NO_x has a higher relative abundance in industrial and urban areas and also a higher toxicity. The oxidized species are mostly produced by the motor vehicle industry, while the sources of reduced species are essentially of agricultural origin.

In Portugal the monitoring network for atmospheric nitrogen compounds has some flaws mainly related to the small number of sampling stations. Estimated N atmospheric deposition in Portugal through the CORINAIR program showed values similar to those found in the rest of the European Union.

Lichens are extremely sensitive symbiotic organisms consisting of fungi and algae. There are several characteristics which make lichens ideal biological monitors [1, 2]: (1) wide geographical distribuition; (2) no morphological changes with seasons; (3) absence of a well developed cuticle, which results in little control of the uptake of water and solutes from the atmosphere; (4) close dependence on the atmospheric deposition for nutrition; (5) ability to accumulate pollutants througout the year [3]. Sudies of nitrogen metabolism and biomonitoring in lichens were reported by Brown, Bruteig and Crittenden [3–5].

The deposition of contaminants in lichens occurs by dry deposition as well as by wet deposition. The latter results from both precipitation and occult precipitation, mainly fog and dew. The dry deposition includes interception of particles and gas absorption [6].

The chlorophyll fluorescence parameter, Fv/Fm (variable fluorescence to maximal fluorescence) has been studied in different photossintetic organisms, including lichens [7], to access the efficiency of the photosynthetic apparatus and to reflect the efficiency of the primary photochemical reactions in the PSII. Branquinho *et al* [8] have also used this parameter to evaluate the physiological effects of contaminants in lichens.

The objective of this work is to evaluate the ability of lichens as biomonitors of N deposition, their physiological response (Fv/Fm parameter) will be taken into account.

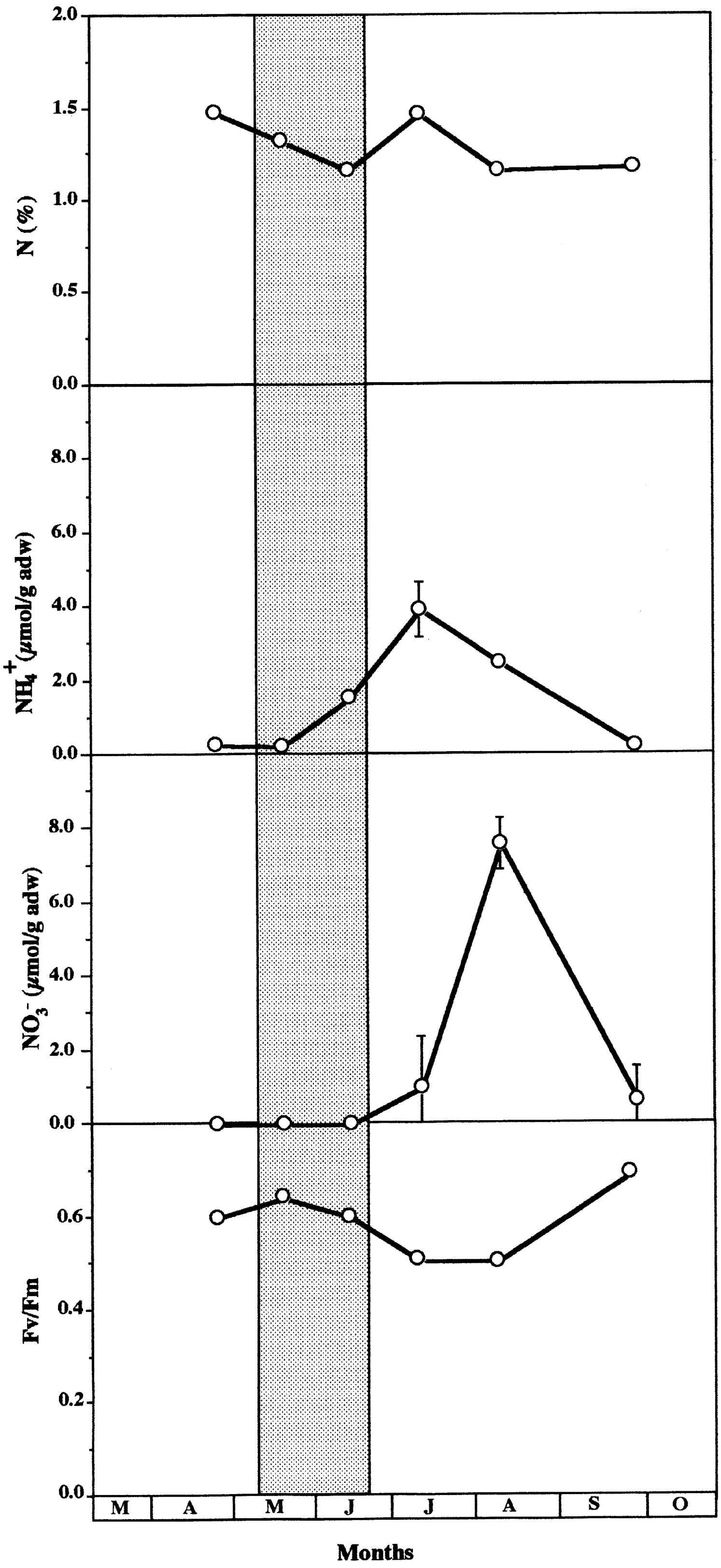

FIG. 1. Monthly variation of the total N (A), NH_4^+ (B) and NO_3^- (C) concentrations in Ramalina fastigiata in 1996. Each symbol and the bars represent the mean and standard deviation (respectively) of 3 samples (A, B and C). In D each point corresponds to the measurement of one sample of the fluorescence ratio of variable fluorescence to maximal fluorescence (Fv/Fm). The fertilization period is indicated by shaded area.

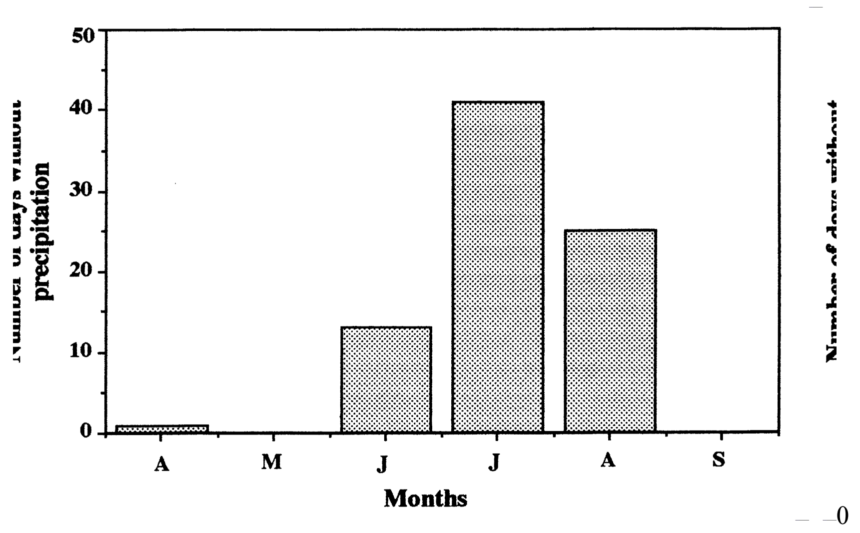

FIG. 2. Cumulative number of days without precipitation before each sampling.

2. MATERIALS AND METHODS

2.1. Study area

The Sorraia River Valley (38°56'N, 8°35'W) is a rice plantation area. It receives an annual input of nitrogenous compounds as fertilisers sprayed by aeroplane, from April until mid-June. Besides this agricultural activity, grazing may also represent an important contribution to the rise of N levels in the atmosphere.

2.2. Sampling

The experiment was carried out for 6 months (April to September) in 1996. Once a month, samples of the fruticose lichen *Ramalina fastigiata* were collected from a *Fraxinus angustifolia* tree bordering the cultivated area. During transport to the laboratory the lichen material was kept in plastic bags. The samples were kept at room temperature until analysis.

2.3. Total Nitrogen Analyses

After being air dried, the samples were ground to powder in a mill (Glen Creston Lt. MM 200). Subsequently they were analysed in an EA1108 CHNS-O Fisson Instruments Analyser.

2.4. Inorganic Nitrogen Analyses

Prior to analysis, 50 mg of air dried samples were extracted in 5 ml of destilled water at 100°C. The extracts were filled up to 10 ml, filtered and used for determination of the concentration of NO_3^- and NH_4^+ and kept cool (4°C).

For the determination of NH_4^+, the extracts were analysed by the Indophenol-Blue method [9]. This method measures all the ammonia N (NH_4^+ and NH_3).

For the determination of NO_3^-, the extracts were analysed according to a colorimetric method in which the NAS reagent (Diphenylamine Sulfonic Acid Cromogene) is added to the solution to be analyzed.

The absorbance of the samples was read at 630 nm (NH_4^+)[9] and 570 nm (NO_3^-) in a Philips PU8620 Spectrophotometer. All the results were expressed in µmol/g adw (air dried weight).

2.5. Chlorophyll fluorescence

A PAM 101 Chlorophyll Fluorometer (Walz, Effeltrich, Germany) was used to measure chlorophyll fluorescence of *Ramalina fastigiata*. Lichen samples were dark adapted for 10 min before the measurements. The minimum and maximum fluorescence (Fo and Fm, respectively) were determined. Variable fluorescence, Fv, is the difference between Fm and Fo, and it was calculated in order to obtain the parameter Fv/Fm.

3. RESULTS AND DISCUSSION

A small variation in the total N concentration (Fig.1A) of the lichen samples after the fertilization period was observed (1 - 1.5%). These values are within the range of those obtained by Crittenden [10] for other lichen species (0.51 - 4.01 %). Considering that 90% of a lichen mass is fungi, lichen N concentration should reflect fungal N concentration rather than algal N concentration. Concentrations of N in fungi under optimal nitrogen supply range between 0.23 and 5.3 % [11]. Therefore the concentrations found in *Ramalina fastigiata* seem to be whitin the normal values.

The total N concentration showed no substancial increase, NH_4^+ (Fig.1B) and NO_3^- (Fig.1C) concentrations increased 4 and 7 times respectively, after the fertilization period. This observation agrees with the results of Boonpragob *et al* [12] who obtained the maximum NH_4^+ and NO_3^- concentration during Summer, also in lichen samples.

The concentration of ammonium in the lichen was highly and significantly correlated ($p \leq 0.01$) with the number days without precipitation (Fig.2). The agreement between the variation patterns in Figs.1B and 2 suggests that the accumulation of NH_4^+ in lichen talli is determined by the dry deposition. We suggest that within the precipitation events the lichen surface is washed, leading to lower NH_4^+ concentration values.

The Fv/Fm values in *Ramalina fastigiata* (Fig.1D), used to indicate the physiological state of the lichen, decreased after the fertilization period. The lowest values were obtained in July-August, followed by a recovery in September. A significant negative correlation was observed between NH_4^+ concentration and Fv/Fm values ($p \leq 0.01$). Similar results were obtained by Boonpragob *et al* [13] using the chlorophyll and net photossyntesis parameters. Our results might indicate a physiological effect of the fertilizers in the lichen talli or just a seasonal variation of fluorescence, with lower Fv/Fm values during Summer (drought season).

This work shows that lichens can be used as biomonitors of N dry deposition.

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USE OF NUCLEAR AND RELATED ANALYTICAL TECHNIQUES IN ENVIRONMENTAL RESEARCH AS EXEMPLIFIED BY SELECTED AIR POLLUTION STUDIES

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Abstract

Among nuclear and nuclear related analytical techniques, neutron activation analysis and X-ray fluorescence spectrometry proved to be particularly useful for environmental studies owing to their nondestructive character and multi element capability. This paper emphasizes their importance among other multielement analytical methods by discussing their specific role due to specific physics basis, quite different to other destructive non-nuclear methods, and by summarizing results obtained in several studies related to air pollution research, including analyses of airborne particulate matter, water samples, lichens and mosses.

1. INTRODUCTION

1.1 Air pollution

The environment is increasingly affected by a growing number of various atmospheric pollutants. The pollutants may include any natural or artificial composition of matter capable of being airborne. They may occur as solid particles, liquid droplets, gases, or in various admixtures of these forms. Natural sources include volcanic emissions, accidental fires in forests and on prairies, dust storms, soil particles, salt particles emitted from the oceans, and various products given off by plants. Anthropogenic sources of air pollution comprise emissions due to industrial activities (e.g. manufacturing products from raw materials, industries that convert products to other products, etc.), power generation (e.g. fossil fuel combustion), traffic, agriculture, waste incineration, residential heating, and many others. These pollutants, if present in high concentrations, are contaminating the environment close to the emission sources, posing a threat to human health, and also contribute to regional and global atmospheric pollution problems. Main sinks of airborne pollutants include the soil, vegetation and water bodies. Due to the diversity of contaminants and materials to be analyzed, a large variety of analytical techniques should be available in studies related to air pollution problems.

Toxic and heavy metals represent an important group of air pollutants to be considered, and are therefore taken into the consideration in this contribution.

1.2. Multielement analytical techniques

For the assessment of pollutant elemental levels and identification of their sources, which are also prerequisite for studying effects of contaminants on the environment and human health, a multivariate data base containing as many pollutant elements should be generated. Therefore, multielement methods are usually used for such studies. The analysis of samples for their bulk minor and trace element content is governed by the sample type, the elements of interest, the sensitivity, precision and accuracy needed and the availability of (or access to) the technique. The choice of multielement methods available includes inductively coupled plasma atomic emission spectrometry (ICPAES), inductively coupled plasma mass spectrometry (ICPMS), X-ray fluorescence spectrometry (XRF), ion beam analysis (IBA) [i.e. particle induced X-ray emission (PIXE) and proton induced

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gamma-ray emission (PIGE)] and nuclear activation analysis [instrumental neutron activation analysis (INAA), prompt gamma neutron activation analysis (PGNAA), charged particle activation analysis (CPAA)], and several other methods, which are seldom used on a routine basis. Some of these methods can be complemented by the use of single-element techniques such as anodic stripping voltammetry (ASV) or atomic absorption spectrometry (AAS).

1.3. The role of nuclear and nuclear related analytical techniques

For handling solid samples, nuclear activation analysis, XRF and PIXE are the most appropriate as they are nondestructive methods, so the probability of contaminating a sample with the element to be determined is negligible, and eventual losses during the sample destruction and preparation are excluded. Among the three methods, neutron activation analysis (NAA), in its instrumental or radiochemical mode, is often used as one of the analytical methods in the process of certifying new reference materials. This is due to its good accuracy, selectivity and sensitivity for many elements.

Environmental samples such as soils, airborne particulate matter (APM) or plant materials, often contain an insoluble component, e.g. a mineral fraction, which could be only partially soluble in acids routinely used for sample destruction applied in many laboratories. Therefore the accuracy and comparability of measurements obtained using different procedures and/or methods for determination of total elemental content should be validated and taken into account. It has been shown, for instance, that several elements in National Institute of Standards and Technology (NIST) leaf standard reference materials (SRMs) are only partially soluble in $HNO_3/HClO_4$ [1], that about 20% of the uranium in NIST SRMs 1571, 1575, and 1572 (Orchard Leaves, Pine Needles, and Citrus Leaves, respectively) resides in a phase that is not made soluble by conventional wet-ashing procedures [2], and that even in NIST SRMs of the new generation such as 1515 Apple Leaves and 1547 Peach Leaves, which were prepared using dedicated equipment and modern technology, several percent of the total element content is contained in the mineral grit phase [3]. It was also shown [4] that incomplete solubilization can occur even when well-accepted, vigorous regimens are used. So, the analysis of any botanical sample (this is also valid for soil and APM material) by solution-based analytical techniques for the total element content must ensure that the mineral is dissolved as well as the bulk material.

Methods intercomparisons using different multielement analytical techniques [5-9] confirm the conclusions drawn in the previous section. The quality of destructive analysis of biomonitors is influenced by specific matrix effects that may not be matched by the matrix of other plant reference materials. The level of the mineral fraction contained in a biomonitor may depend on the level of pollution, so the recovery of an element during the dissolution may not be the same for the same type of material analyzed. One should also be careful when using reference materials or samples, since in some cases the values are reported on an acid-soluble basis, so misleading conclusions may be drawn by their analysis [9].

These facts and problems encountered by using destructive methods make nuclear and nuclear-related analytical techniques (NATs) indispensable in laboratory intercomparisons to improve the quality of trace element determinations in environmental materials. NATs also proved to be highly appropriate for the analysis of various materials used in studying trace element air pollution. NAA has been extensively used for such studies and numerous references and review papers can be found in the literature [e.g. 10-23]. The k_0 -based INAA [24] has been found to be a particularly useful tool, offering the possibility of determining all the radionuclides appearing in the accumulated gamma spectra of the neutron-irradiated sample. It has also been shown [e.g. 22, 23, 25-28] that relatively simple energy dispersive X-ray fluorescence spectrometry (EDXRF) using radionuclide excitation sources can be efficiently used in air pollution research. In addition, the samples analyzed by this technique can subsequently be submitted to analysis by other techniques, which is not the case when using other methods. Some examples of the use of INAA and EDXRF in atmospheric pollution studies in Slovenia are presented in the following sections.

2. SAMPLING AND SAMPLE PREPARATION

Various types of samples are collected and analyzed for studies aimed at determining the levels of trace elements contaminating the environment and at better understanding the fate and cycling of contaminants, both on local and national scale. The samples include the following types of materials: airborne particulate matter, bulk deposition, soil leachate water, spring water, soil, and rocks.

2.1. Airborne particulate matter (APM)

APM samples are collected either by the "Gent" stacked filter unit [29] for NAA analysis or by an in-house constructed single-jet APM separator [27] offering the possibility of collecting higher filter loadings (up to 1 mg cm^{-2}) needed for EDXRF analysis due to its lower sensitivity. In both cases two fractions, from approximately 2.5 to 10 μm and finer than 2.5 μm, are collected on polycarbonate membranes. Loaded filters are weighed after neutralizing the charge with a static eliminator. Filters are kept in Petri dishes prior to analysis. APM-loaded filters are pelletized with a manual press in a pellet die of 5 mm when analyzed by INAA.

2.2. Liquids

Water samples are usually not considered as appropriate to be analyzed by NAA. Moreover, in many reactors it is even not allowed to irradiate liquid samples. However, if a suitable neutron source is available, NAA can efficiently be used for water analysis, providing information on the same elements as determined in other types of samples from the same study. Typical detection limits for some elements in a typical water sample are outlined in Table I.

TABLE I. COMPARISON OF NAA DATA WITH CERTIFIED VALUES FOR NIST SRM 1643C TRACE ELEMENTS IN WATER

Element	NAA data	Certified values	Detection limit
Ag / μg L^{-1}	2.4 ± 0.4	2.21 ± 0.3	0.3
Al / μg L^{-1}	124 ± 8	114.6 ± 5.1	4
As / μg L^{-1}	88.6 ± 3.3	82.1 ± 1.2	0.2
Ba / μg L^{-1}	54 ± 8	49.6 ± 3.1	11
Ca / mg L^{-1}	38.2 ± 1.1	36.8 ± 1.4	0.6
Cd / μg L^{-1}	14.4 ± 1.9	12.2 ± 1.0	0.05
Co / μg L^{-1}	24 ± 4	23.5 ± 0.8	0.03
Cr / μg L^{-1}	20.9 ± 0.6	19.0 ± 0.6	0.5
Fe / μg L^{-1}	124 ± 28	106.9 ± 3.0	50
K / mg L^{-1}	2.43 ± 0.16	2.30	0.04
Mg / mg L^{-1}	10.0 ± 0.4	9.45 ± 0.27	0.1
Mn / μg L^{-1}	35.0 ± 0.5	35.1 ± 2.2	0.07
Mo / μg L^{-1}	104 ± 5	104.3 ± 1.9	0.1
Na / mg L^{-1}	12.4 ± 0.2	12.19 ± 0.36	0.7
Rb / μg L^{-1}	12.1 ± 1.4	11.4 ± 0.2	1
Se / μg L^{-1}	14.2 ± 0.5	12.7 ± 0.7	0.1
Sr / μg L^{-1}	278 ± 14	264.0 ± 2.6	18
Ta / μg L^{-1}	0.061 ± 0.017	0.079	0.01
V / μg L^{-1}	31.1 ± 3.1	31.4 ± 2.8	0.3
Zn / μg L^{-1}	83.9 ± 2.5	73.9 ± 0.9	2

Bulk deposition measurements are used in developing input budgets of water and chemical constituents into the area under investigation. The bulk precipitation is collected by continuously open collectors and comprises only elements and substances which are deposited by sedimentation under the influence of gravity and washout. The collection system consists of a polyethylene funnel (diameter

26 cm) and a polyethylene bottle (5 L). The funnel-bottle system, having an orifice of 452 cm^2, is mounted inside a stainless steel cylinder placed on a concrete plate, keeping the upper edge of the funnel 1.3 m above ground level. The opening of the funnel is protected by a coarse filter mesh made of polyethylene (with mesh diameter 2 mm) and a nylon net (pore size 250 μm) in order to prevent an ingress of coarse particles (e.g. leaves) and insects. Before each sampling period (one month), 10 ml conc. HNO_3 (suprapure) is added to the bottle to preserve the precipitation collected, achieving a final pH of about ~2.

Soil leachate water is collected to monitor dissolved and colloidal elements and substances. For this purpose, lysimeters are used. They are in form of an open-topped square bodied drainage vessel (45 cm x 35 cm) made of PVC with an outlet at the bottom. The lysimeter is inserted intact into the soil at 50 cm depth to collect the vertical water flow. The vessel is filled with silicate sand (2 mm diameter) and connected to a 5 L sampling bottle. The collection bottle contains nitric acid prior to collection, to achieve a final pH of about ~ 2.

Spring water measurements are used to estimate output budgets and chemical constituents of the watershed under investigation. Water is collected directly in a polyethylene bottle.

All water samples are filtered (0.45 μm polycarbonate membrane filters of Nuclepore) and analyzed by INAA, measuring the filtrate and the filters with suspended particles separately. A 5 ml aliquot of filtered water is transferred to an acid washed polyethylene vial, and the filter is pelletized with a manual press in a pellet die of 5 mm.

2.3. Solids

Soil samples are taken with a plastic shovel, usually from four different depths: 0-5 cm, 5-10 cm, 10-20 cm, and 40-50 cm, about 1 kg each. The average soil sample from each site is prepared as a composite of three subsamples. In the laboratory the samples are homogenized, dried at 30 ^{0}C and passed through 2 mm plastic sieve. The dried soil samples are then ground in an agate mill to a suitable size (100-mesh).

Rocks: approximately 5 kg is collected at a chosen sampling site. An appropriate subsample is taken and crushed by a hammer. A suitable subsample is taken then and ground in an agate mill to an appropriate size (~ 1 μm).

Both types of samples are pelletized with a manual press.

2.4. Biomonitors

The sampling sites for collecting lichens are located at least 300 m away from main roads and at least 100-200 m away from any road or dwelling, mainly in forest glades or open habitats. Several healthy lichen thalli of different sizes are taken from the same tree and likewise from 3-5 nearby trees, 1.5-2 m above the ground, and if possible the lichen material is taken from all around the tree to reduce the influence of relative source position. In the laboratory lichens are moistened with distilled water and then the adhering bark particles removed from the lichen using nylon tweezers. The samples are made brittle by immersion in liquid nitrogen, and then crushed and ground in a zirconium mortar with a Zr ball in a Fritch vibration micro-pulverizer. About 100-200 mg of dry lichen powder is then used to make tablets for INAA.

The moss sampling is carried out according to the recommendations for the European moss survey [30]. The three youngest fully developed segments of plant or corresponding green or green-brown parts are taken for analysis. The samples are prepared in a similar way as lichens.

About 100-200 mg of dry lichen or moss powder is used to press tablets for INAA.

3. ANALYSIS

3.1. INAA

Samples are irradiated, together with an Al-0.1% Au alloy wire of 1.0 mm and a 0.125 mm Zr foil (serving as comparator and fluence rate monitors), in the 250 kW TRIGA Mark II reactor of the

Jozef Stefan Institute (IJS), using different irradiation-decay-measurement schemes [31, 32]. After irradiation, the samples are transferred into clean polypropylene mini scintillation vials for measurement on an HP Ge detector connected to a Canberra Series 90 multi-channel analyzer. The radionuclides used in the determination of 53 elements in each sample, their half-lives and gamma energies measured are given elsewhere [32]. Spectra are processed by the SAMPO program [33] and elemental content determined by the KAYZERO/SOLCOI software package [34].

3.2. EDXRF analysis

Samples are placed on an in-house constructed automatic sample changer carousel facility with a capacity of ten specimens. Two different setups are used for EDXRF analysis: (1) a 1 Gbq ^{109}Cd excitation source and a Si(Li) detector; and (2) a 1 Gbq ^{241}Am excitation source or a 1.8 Gbq ^{55}Fe source and a low-energy Ge detector, both coupled to a Canberra S 100 multi-channel analyzer. Spectra are processed and quantitative analysis performed using AXIL-PC software [35]. Using the described setups, up to 20 elements having $Z > 13$ are usually sought: (i) Si, P, S, K, Ca, Ti and V using a ^{55}Fe exciting source, (ii) Cr, Mn, Fe, Ni, Cu, Zn, Pb, Br, Sr and Zn using a ^{109}Cd exciting source, and (iii) Cd, Sb and Ba using a ^{241}Am exciting source.

3.3. Analytical quality assurance and control (QA/QC)

Continuous emphasis is placed in developing and enforcing appropriate QA/QC procedures for the analysis. These procedures comprise careful verification of any new procedures used, participation in the relevant laboratory intercomparisons, and the inclusion of reference materials as “blind” samples in each batch of samples analyzed. Environmentally related reference materials of different origin and matrices are used, including the following ones which have been found appropriate for routine quality control:

Institute for Reference Materials and Measurements (IRMM) Certified Reference Materials (CRMs) 038 Fly Ash from Pulverised Coal, 101 Spruce Needles, 128 Fly Ash on Artificial Filter, 277 Estuarine Sediment;

International Atomic Energy Agency (IAEA) Reference Materials (RMs) SL-1 Lake Sediment, Soil 7, 336 Lichen;

National Institute for Standards and Technology (NIST) standard reference materials (SRMs) 1515 Apple Leaves, 1547 Peach Leaves, 1570 Spinach, 1571 Orchard Leaves, 1572 Citrus Leaves, 1573 Tomato Leaves, 1573a Tomato Leaves, 1633a Coal Fly Ash, 1643c Trace Elements in Water, 1646 Estuarine Sediment, 2704 Buffalo River Sediment, 3087 Metals on Filter Media.

The results have been reported in the literature [13, 20, 27, 31, 36-39], and only two examples are presented in this paper: NAA results for NIST SRM 1643c Trace Elements in Water (Table 1), and EDXRF results for NIST SRM 3087 Metals on Filter Media (Table II).

TABLE II. EDXRF DATA FOR NIST SRM 3087 METALS ON FILTER MEDIA

Element	EDXRF data [μg per filter]	Certified values [μg per filter]
Ba	25 ± 4	25.88 ± 0.29
Cd	16 ± 4	15.5 ± 0.17
Cr	10.0 ± 2.0	10.33 ± 0.12
Fe	28 ± 4	25.84 ± 0.29
Mg	< 1000	25.83 ± 0.29
Ni	27 ± 4	25.86 ± 0.29
Pb	39 ± 6	41.33 ± 0.46
Se	23 ± 4	25.84 ± 0.29
Zn	109 ± 14	103 ± 1.2

4. EXAMPLES OF AIR POLLUTION STUDIES

4.1. Air pollution study using NAA

As part of the IAEA research co-ordination project on "Applied research on air pollution using nuclear related techniques", APM samples were collected, using the "Gent" stacked filter unit (SFU) and analyzed by INAA. Two sampling stations were chosen: one at the urban residential area located at the Hydrometeorological Institute of Slovenia (HIS) situated in Ljubljana city, and other one at the rural site at Iskrba. At both sites, the samples were collected for the period of 24 hours, twice per week, during one year. The aim of the study was to determine elemental concentrations in both inhallable and respirable fractions of APM in order to find out the levels and possible sources of pollutants, including their seasonal trends. Some findings are summarized in Table 3. Average annual APM concentrations found were 47 μgm^{-3} and 21 μgm^{-3} for Ljubljana and Iskrba, respectively. Higher values for the site in Ljubljana are mostly due to higher concentrations found in winter (up to 200 μgm^{-3}), caused by coal burning.

Principal component analysis, applied to the data set, yields similar patterns for each APM fraction at both sampling sites. Therefore, similar pollution sources for both sampling sites may be assumed. High enrichment factors for As, Cd, Cu, Mo, Sb, Se, and Zn were found in the fine APM fractions; and for As, Cd, Cu, Mo, Sb, and Se, in the coarse fractions, at both sites, respectively (Table III). Additional measurements are being carried out to identify the potential sources.

TABLE III. AVERAGE ANNUAL ELEMENTAL CONCENTRATIONS IN APM FRACTIONS AND ENRICHMENT FACTORS FOR ABOVE TWO STATIONS

	Concentration (ng/m^3)				Enrichment factor			
Element	HIS Coarse	Iskrba Coarse	HIS Fine	Iskrba Fine	HIS Coarse	Iskrba Coarse	HIS Fine	Iskrba Fine
Al	229	56	40	26	1	1	1	1
As	0.47	0.40	1.8	1.6	92	323	2030	2780
Ca	1083	242	350	350	11	10	19	30
Cd	0.71	1.3	1.4	0.95	1270	9440	14200	14900
Cr	12	9.3	2.6	0.11	42	135	53	3
Cu	12	5.4	13	6.1	76	143	473	347
Fe	334	75	163	48	2	2	7	3
K	114	51	223	133	2	3	17	16
La	0.18	0.08	0.14	0.09	2	4	9	9
Mn	6.7	1.5	7.8	2.1	3	2	17	7
Mo	0.54	0.46	0.48	0.22	128	445	650	460
Sb	1.3	0.13	1.26	0.42	2310	940	12800	6570
Se	0.39	0.14	1.14	0.53	2770	4070	46300	33150
Th	0.06	0.03	0.03	0.02	3	6	8	9
V	2.4	0.46	7.4	2.6	6	5	111	60
Zn	17	1.9	48	22	87	40	1380	980

4.2. Environmental contamination caused by polluted air in the Salek valley

The Šalek valley and its surroundings, comprising an area of about 40 km^2 is one of the most polluted areas in Slovenia. The coal fired Šovtanj thermal power plant (TPP), is a large regional SO_2,

NO_x, dust, and trace elements emission source, and the main reason for the pollution of the valley. Its annual emissions during last years reached 90000 tons of SO_2, 12000 tons of NO_x, 5000 tons of escaping fly ash, and unknown quantities of other pollutants. It was calculated [38] that the total annual release of volatile elements from the TPP operation estimated as the emission on stack fly ash plus the vapour phase release is about 6 t for As, 2.7 t for Cd, 36 t for Cr, 0.6 t for Hg, 134 t for Pb, and 122 t for Zn. Comprehensive studies have been carried out, aimed at studying environmental impacts of the TPP and other sources on the environment, including analyses of coal, electrostatically precipitated (ESP) fly ash, size fractionated escaping fly ash particles, APM, bulk precipitation deposition, leachate water, spring water, soil, rocks, and biomonitors.

The composition of coal and ash particles from the TPP using INAA and atomic absorption spectrometry (AAS) for Cd and Pb was studied to determine the elemental composition of size fractionated ash particles emitted by the stack, and those trapped by the ESP, in comparison to the original coal composition. 39 elements were analyzed by INAA. Enrichment ratios (leading to a detailed source characterization) and overall mass balance during operation of the power plant were calculated [38].

A monitoring system comprising six sampling sites has been established to: (1) follow the concentrations of metals in APM, (2) determine the participation of local emitters of APM in Šalek valley, and (3) determine the exposure of the population to emission of APM, including possible health-effects assessment. Due to large number of the collected samples EDXRF technique is applied, and the in-house constructed APM samplers are used. It has been found that elemental concentrations can be measured for Ca, Fe, Pb, S, Si, Ti, and Zn in all ambient APM samples, while levels of the elements Ba, Br, Cd, Cr, Cu, K, Mn, Ni, Sr, V, and Zr are sometimes below the detection limit [27]. The TPP, however, was found to be the main source of pollution, although other local sources were also identified [27].

To identify the causal relationships between forest damage, atmospheric deposition, and the consequences in runoff and soil water, investigations on the deposition of inorganic substances in forest areas have been initiated [13, 20]. An ecosystem unit in a study carried out was a watershed or drainage area, with vertical and horizontal boundaries defined functionally by its biological activity and the drainage of water. Three small watersheds with similar vegetation and geology and subject to the same climate, were used for the study. Samples of bulk deposition, soil leachate water, spring water, soil, and rocks were collected and analyzed by INAA. Overall, the analytical results showed: (1) an increased input flux and accumulation trend for some elements including As, Ba, Br, Cd, Co, Cr, Cu, Sb, Se, U, and Zn in the Šalek valley, and (2) increased input fluxes of trace elements close to TPP, being this plant the main pollution source [20].

4.3. Biomonitoring atmospheric trace element deposition

It is well known that direct measurements of airborne pollutants require enormous efforts as to investments in infrastructure and manpower. Application of direct measurements in air, e.g. APM, on a large scale is extremely costly and labor intensive, therefore impractical and almost impossible. Airborne pollutants can be transported over large distances, raising a high public interest also in remote areas, and biomonitoring is an appropriate tool for assessing the levels of air pollution. In several countries biomonitoring is used on a regular basis for such surveys [30, 40]. Application of biomonitors has several advantages compared with the use of direct measurements of contaminants, related primarily to the permanent and common occurrence in the field, the ease of sampling and trace element accumulation. Furthermore, biomonitors provide a measure of integrated exposure over a certain amount of time, they are present in remote areas and no expensive technical equipment is involved in collecting them. Suitably chosen biomonitors accumulate contaminants over certain periods of time, concentrate them, thus allowing more reliable analytical measurements. Simple and cheap sampling procedures (in contrast to direct measurements) allow a very large number of sites to be included in the same survey, permitting detailed geographical patterns to be drawn. Nuclear and related analytical techniques have been shown to be particularly appropriate for the analysis of air pollution biomonitors, such as moss and lichen [10, 12, 15, 17, 18], being multielement, reliable, extremely sensitive for many toxic elements, matrix independent and suitable for all concentration ranges (i.e. from nanogram to percent levels). The efficacy of these sampling and analysis techniques has been amply shown in several national and international programmes. In combination with the specimen banking (long-term storage) of selected samples, biomonitoring can be an effective tool for pollutant mapping and trend monitoring by real time and retrospective analysis. By application of appropriate statistical tools, information can also be obtained on the type and location of pollution sources as well as on the short, medium and long range trans-boundary transport of environmental pollutants.

A monitoring survey on the national scale using the epiphytic lichen *Hypogymnia physodes* (L.) Nyl. was carried out, using the existing 16 × 16 km bioindication grid which had previously been introduced for some regular studies connected with forest decline [41]. Samples were collected as described in section 2.4. 36 elements were determined using INAA, in each lichen sample from 86 grid points. Application of Monte Carlo-assisted factor analysis [42, 43] on the 28 elements with environmental relevance resulted in nine factors (possible types of sources), that explained 84% of the total variance in the data set. Detailed results can be found elsewhere [19].

The survey using lichens has been supplemented with the nationwide monitoring using mosses [44]. Samples of the moss species *Pleurozium schreberi* and *Hypnum cupressiforme* were collected from 30 sites, uniformly selected from the same grid, so as to comply with the recommendations for European moss surveys [30]. 40 elements were determined using INAA. Comparison of both surveys is summarized in Table IV. The mean elemental contents are evidently comparable for both biomonitors. The mean values for Ag, As, Br, Hg, Sb, and Zn are significantly higher in lichens, and the mean values for Ba, Co, Cr, Fe, K, Sc, Th, and U are significantly higher in mosses. It can only be concluded, however, that both organisms used for biomonitoring are suitable; the content of metals in lichen reflects an average exposure of at least ten years period of time, and in mosses an average exposure of three years period of time [45]. Observed differences may therefore be attributed to yearly variations in deposition rates, and not to different accumulation properties of the biomonitors used.

TABLE IV. COMPARISON OF NATIONWIDE BIOMONITORING SURVEYS USING LICHENS AND MOSSES. RESULTS ARE IN mg kg^{-1}

	Lichens		Mosses	
Element	Range	Mean	Range	Mean
Ag	0.03 - 0.21	0.087	0.06 - 0.22	0.007
As	0.57 - 2.97	1.24	0.14 - 5.8	0.8
Ba	7.1 - 212	28	11 - 131	40
Br	6.0 - 32	15	1.1 - 4.9	2.0
Cd	0.3 - 5.4	1.1	0.24 - 3.0	1.0
Co	0.24 - 1.74	0.55	0.13 - 7.8	1.0
Cr	2.3 - 22	5.8	1.4 - 50	8.0
Fe	492 - 3760	1250	206 - 14800	1940
Hg	0.04 - 1.46	0.11	0.01 - 0.13	0.04
K	1650 - 8640	4090	3540 - 12200	7200
Mo	0.19 - 9.2	0.72	0.14 - 1.7	0.6
Sb	0.14 - 3.5	0.35	0.07 - 0.58	0.2
Sc	0.15 - 1.2	0.37	0.05 - 5.9	0.7
Se	0.05 - 0.68	0.27	0.06 - 0.4	0.2
Th	0.11 - 0.82	0.29	0.04 - 6.4	0.7
U	0.04 - 0.31	0.12	0.015 - 1.8	0.2
Zn	47 - 151	90	25 - 74	41

5. CONCLUSIONS

Although it is considered that nuclear methods are in a continuous decline due to the emergence of new competitive analytical techniques, they definitely have an important role in environmental studies related to trace element pollution and cycling. Examples shown in this paper strongly support this conclusion.

Intercomparisons aimed at certifying new reference materials have amply shown that the results obtained by NAA are reliable and accurate. Therefore, it is highly appropriate for validating other methods and checking procedures used by destructive analytical techniques. NAA is particularly suitable for analyzing samples where low detection limits are needed, having in mind that the detection limits are largely element-dependent. Using the k_0 -based of INAA as implemented in the above studies, more than 60 elements can in principle be determined from double irradiation. When analyzing environmental samples, quantitative data for 30 - 40 elements can realistically be expected. It is well known, however, that Pb cannot be determined by NAA at $\mu g.g^{-1}$ level, and another technique (including ED XRF) should be applied for analysis of this heavy metal. Since the throughput of samples analyzed by INAA in the Laboratory for Radiochemistry of the IJS has been found to be approximately twelve samples per day for short irradiations and approximately twenty samples per week for long irradiations, the method is more usable for the analysis of biomonitors, where the number of samples is relatively low, rather than for APM collected on filters, where the demand exceeds the analytical capacity. In the latter case INAA can best be used as a reference and control method for a limited number of samples analyzed by ED XRF.

This method is much faster than INAA, requiring less than two hours per measurement, thus allowing a throughput of about sixty samples per week when using a sample changer and two detectors. It is suitable for processing a large number of samples of similar matrix composition such as APM collected on filters; however, the quantity of air sampled should be such as to allow elemental surface densities of approximately 0.1 $\mu g.cm^{-2}$ in order to ensure acceptable quantitative analysis.

Both techniques are extensively used at the IJS for the analysis of bulk precipitation, biomonitors of atmospheric deposition, and for the analysis of APM. Based on experience obtained in implementing both methods, EDXRF was chosen for the analysis of APM, and the INAA procedure as a control method for the APM, as well as for the analysis of bulk precipitation and biomonitors. The in-house constructed APM separator collects up to 1 mg APM cm^{-2} of filter, which allows quantitative determination of 15 elements by EDXRF, on average, including Si, S, Ni, and Pb, thus representing elements complementary to the INAA data.

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PRELIMINARY ANALYSIS OF A NEW IAEA LICHEN AQCS MATERIAL

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Abstract

Lichen with a higher content on interesting trace elements were analyzed by activation analysis and by X-RF measurements on pressed lichen samples. The activation analyses were performed in three different ways: Short-time AA in the Fast Irradiation & Measurement System. Up to 580mg of lichen were irradiated 5-300s in polyethylene containers. Single spectra and spectra of 6 samples were summed up and evaluated. Longer irradiation at the ASTRA-Reactor: 2h at 8E13/s cm^2. 100-150mg of lichen were irradiated in quartz suprasil vials. Longer irradiation at the Institute`s TRIGA-Reactor: 6-7h at 1.8E12/s cm^2, sample size: 7-48g of lichen were irradiated in polyethylene containers and after irradiation transferred to new measurement containers and measured in a device constructed by Gwozdz. The X-RF analysis was performed with a Spectrace 5000 energy dispersive X-ray fluorescence analyzer with a rhodium anode tube for excitation. From the activation analyses, the following elements were determined: Ag, Al, As, Au, Ba, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Dy, Eu, Fe, Hf, Hg, I, K, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, Rb, Sb, Sc, Se, Sr, Ta, Tb, Th, Ti, U, V, Yb, Zn. From the X-RF measurements, the elements Ag, Al, Ba, Br, Ca, Cd, Cu, Fe, I, K, Mg, Mn, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, Y, Zn, and Zr were evaluated. From the X-RF data as well as from the AA-data of samples of different weight it is apparent that milling to a particle size of 200m is not sufficient for all elements, especially not for gold, cadmium, and cobalt which may be present as nuggets or accessory heavy minerals. It is therefore advisable to mill the sample to a particle size which is an order of magnitude smaller and remove the not adhering dust, even if this lowers the content of these elements.

1. INTRODUCTION

Lichen can be used as a monitor for aerosols, as it integrates dust over a longer period of time. Therefore, the IAEA has introduced a standard reference material IAEA 336. For the preparation of a more contaminated lichen standard material, the region near Bleiberg/Carinthia seemed a suitable lichen source, as lead was mined and smeltered there for a long time. In autumn 1995 a preliminary search showed that lichen (mainly *pseudevernia furfuracea*) is available in sufficient quantity in this region. Analyses of several samples by short-time activation analysis indeed showed higher contents of interesting elements. So 24 kg of lichen were collected there in September 1996. A representative sample of lichen and its dust was crushed after drying in an agate mill to a particle size of about 200 m. At least parts of the lichen sample must have had a residence time of more than 10 years, as measured by the $^{137}Cs/^{134}Cs$ ratio, which showed a surplus of ^{137}Cs.

2. SAMPLE

2.1 Collection of lichen

About 24kg lichen (mainly pseudevernia furfuracea) were collected at different places around Bleiberg: 1) On Dobratsch in a height of 1400-1600m in the main wind direction of the lead mining tower. 2) On Dobratsch near the lower cable car station at about 1000m, in the main wind direction. 3)Facing Dobratsch, at a distance of 2km from the mining tower at about 1400m. 4) South of Kreuth, on the western part of Dobratsch exposed to the south at 1300m. 5) Southeast of Arnoldstein near the Austrian/Slovenian border in the main wind direction from the lead processing plant at a height of about 1000 m.

2.2 Preparation

The lichen was manually sorted by the IAEA crew in Seibersdorf and the lichen containing adhearing bark particles were separated. This procedure was performed on all the collected lichen. The lichen with adhering bark particles was selected for a preliminary analysis. The lichen was manually selected from bark particles, needles, and dust. **Lichen and dust** were combined and milled in an agate mill until it passed a 200mm sieve, and mixed by shaking 6h in a plastic container for analysis. Bark particles and needles were separated for an activation analysis.

2.3 Residence time evaluation

A rough residence time determination of materials exposed to aerosols is possible by measurement of the activity of the Cs-radionuclides which were emitted from the Cernobyl accident: The Cs-137 activity (half life of 30y) is compared with Cs-134 activity (half life 2.07y). If Cs-134 is found in the sample it must stem partly from the Cernobyl accident on 1-May-86. If a surplus of Cs-137 appears , a remaining activtiy from the bomb-test series must be present, so the mean age of the measured sample must date back to at least 1986. Milkpowder is a suitable reference material to obtain a good approximation for the Cs-isotope ratio at the time of the Cernobyl accident as milk is produced by cows fed with grass of the same year, in which a memory effect of Cs-137 is very unlikely. 500g corresponding to 800ml of the milk powder was measured in a Marinelli beaker on a 30% Ge(Li)-detector. The calculated Cs-137/134 ratio at the Cernobyl accident time 1-MAY-86 was **1.702 +/- 0.018**.

To verify that the mean age of the lichen sample is older than 1986 its activity was measured: 500g corresponding to 800ml were measured in the Marinelli beaker. The Cs-137 / Cs-134 ratio calculated for 1-MAY-86 was found to be **1.808 +/- 0.058**. The small surplus of Cs-137 showed that some amount of Cs-137 was left from the atomic bomb test series, so that some of the measured lichen must be more than 10 years old . This is important as the lead mine was closed down around this date.

3. LICHEN ANALYSIS

The samples were analyzed by activation analyses and XRF-measurements on pressed lichen samples. Bark and needle samples were only measured by activation analysis.

3.1 Activation analysis

These analyses were performed in three different ways:

1) Short-time AA in the **F**ast **I**rradiation & **M**easurement **S**ystem **FIMS.**
Up to 580mg of sample were irradiated 5-300s in polyethylene containers. Single spectra and spectra of 6 samples were summed up and evaluated.

2) Longer irradiation at the ASTRA-Reactor: 2h at 8E13/s cm^2. 100-150mg of the sample were irradiated in quartz suprasil vials (lichen, bark and needles).

3) Longer irradiation at the Institute´s TRIGA Reactor: 6-7h at 1.8E12/s cm^2 sample size: 7-48g of lichen samples were irradiated in polyethylene containers and transfered to new measurement containers. Five 10g-samples were measured to test the homogeneity. The measurements were performed on different HP-Ge detectors: in FIMS, a 15% detector with resetting preamplifier was used, which feeds the signal into a newly constructed digital preloaded filter amplifier [1] . The signals pass a dual LFC system for deadtime and pile up correction, and are then stored by an ACCUSPEC B chart on the hard disk of a 386PC. Three spectra with successively increasing measurement time were registered.

TABLE Ia. ASNALYSIS OF LICHEN COLLECTED AT BLEIBERG, CARINTHIA, AUSTRIA

					Comparator Orchard/Citrus l.	Comparator Pine Needles
					XRF	XRF
ELEMENT/UNIT	Short time AA	ASTRA	TRIGA	TRIGA	2.2-5.5g pellets	2.2-5.5g pellets
SAMPLE SIZE	0.3-0.5g	0.10-0.14 g	5.5-8.8g	47.9g	865+/-18	
P mg/kg					107.7+/-15.2	
Pb mg/kg					25.0+/-2.0	18.2+/-1.4
Rb mg/kg		17.9+/-1.9	22.2+/-1.0	24.2+/-0.2	2213+/-26	
S mg/kg					1.52+/-0.34	
Sb mg/kg		0.685+/-0.011	1.97+/-021	1.73+/-0.02		
Sc mg/kg		0.436+/-0.010	0.398+/-0.002	0.415+/-0.001		
Se mg/kg			1.60+/-0.36	1.55+/-0.06	4974+/-122	
Si mg/kg					3.19+/-0.58	
Sn mg/kg					10.5+/-0.65	7.56+/-0.47
Sr mg/kg		11.9+/-0.67				
Ta mg/kg		0.048+/-0.05				
Tb ng/kg		33+/-8				
Th mg/kg		0.36+/-0.07			161+/-20	111+/-14
Ti mg/kg	193+/-12					
Tm mg/kg						
U mg/kg			(0.122)Np239			
V mg/kg	7.80+/-0.02				2.45+/-0.58	1.69+/-0.40
Y mg/kg						
Yb ng/kg			105+/-10	97.7+/-2.1	176.2+/-4.6	120.4+/-3.1
Zn mg/kg		121+/-2	112+/-2	118+/-1	8.96+/-2.3	5.91+/-1.52
Zr mg/kg						
Ag mg/kg		0.11+/-0.03	0.17+/-0.02	0.13+/-0.01	0.57+/-0.14	
Al mg/kg	2024+/-10				1360+/-85	
As mg/kg			1.44+/-0.10	1.24+/-0.03		
Au ng/kg		18+/-2	118+/-5	12.0+/-0.1		
Ba mg/kg	72+/-13	65.3+/-7.1	77.1+/-0.9	75.8+/-0.6	40.4+/-6.2	
Br mg/kg	29.4+/-2.1	24.3+/-1.1	29.2+/-0.1	27.3+/-1.1	32.5+/-1.9	24.0+/-1.4
Ca mg/kg	6250+/-150	6480+/-100	5000+/-300	6500+/-300	8750+/-78	5651+/-51
Cd mg/kg			1.3+/-0.4	0.72+/-0.09	0.62+/-0.19	
Ce mg/kg		2.00+/-0.06	2.20+/-0.30	2.22+/-0.01		
Cl mg/kg	2940+/-30					
Co mg/kg		0.485+/-0.11	1.07+/-0.04	1.18+/-0.03		
Cr mg/kg		9.80+/-0.12	11.9+/-0.65	11.7+/-0.01		
Cs mg/kg		0.55+/-0.05	0.62+/-0.05	0.72+/-0.02		
Cu mg/kg	26.3+/-3.8				33.5+/-19.2	22.4+/-12.8
Dy mg/kg	0.135+/-014					
Eu ng/kg		46+/-4	43+/-3	46.1+/-0.8		
Fe mg/kg		1846+/-40	1888+/-55	1912+/-39	1957+/-20	1267+/-13
Hf mg/kg	0.242+/-0.002	0.214+/-0.020	0.240+/-0.006	0.236+/-0.002		
Hg mg/kg		0.82+/-0.02	0.70+/-0.05	0.936+/-0.009		
I mg/kg	6.49+/-0.49				4.97+/-0.85	
K mg/kg			3650+/-300	3110+/-200	3447+/-29	2842+/-24
La mg/kg		2.10+/-0.42	2.13+/-0.01	2.10+/-0.01		
Lu ng/kg			8.62+/-0.26	8.66+/-0.08		
Mg mg/kg	1335+/-152				540+/-51	
Mn mg/kg	69.3+/-7.7				106.8+/-7.9	69.2+/-5.2
Mo mg/kg			16.2+/-1.0	12.0+/-3.0		
Na mg/kg	225+/-10		262+/-10	232.4+/-5.0		
Nd mg/kg		1.43+/-0.08				
Ni mg/kg		11.7+/-2.7	15.8+/-1.2	11.9+/-1.0		

The measurements of the samples irradiated at the ASTRA-reactor were measured at least twice after appropriate decay time, by an electronic equipment corresponding to that described above, but stored on 486 PC. Some measurements were performed on a well-type detector [2].

The large samples irradiated at the Institute`s TRIGA-reactor were measured with the same system. The peak areas of all spectra were evaluated by a modified PEAK program, developed for the dual LFC- systems [3].

3.2 XRF-analysis

The measurements were performed with a Spectrace 5000 energy dispersive X-ray fluorescence analyzer with a rhodium anode tube for excitation. 10 samples, dried at 6O°C for 24h, were pressed at about 10 t to pellets having an area density between 0.316 and 0.769 g/cm^2. Assuming similar properties of the matrices, NBS SRM 1572 (Citrus Leaves) and NBS SRM 1571 (Orchard Leaves) were used for one set of calibration data. Another set was based on NBS SRM 1575 (Pine Needles). The elements Ti, Mn, Fe, Cu, Zn, Br, Rb, Sr, Y, Zr and Pb were measured at 40 kV, 0.06mA, with a thick Pd-filter in the primary beam, for 1000 s life time, the elements Mg, Al, Si, P, S, K, and Ca at 7kV and 0.35mA under vacuum conditions, for 1000 s life time, the elements Ag, Cd, Sn, Sb, I, and Ba at 50kV, 0.35mA, and a Cu-filter in the primary beam, for 2000 s life time. The net peak area of the fluorescence lines was extracted from the measured spectra by the AXIL-peak fitting routine. For the conversion to concentrations the program "Direct comparison of count rates " was used (software package QXAS, Version 3.5.).

4. RESULTS

The data of the lichen analyses are summarized in Table Ia and Ib. The results of the analysis of small lichen-, bark-, and needle- samples are given in Table II.

TABLE II. LICHEN, BARK, NEEDLES COLLECTED AT BLEIBERG, KÄRNTEN, AUSTRIA

ELEMENT/UNIT	LICHEN	BARK	NEEDLES	COMPARATOR	DATA USED
SAMPLE SIZE	0.136	0.101	0.113	[g]	
Au ng/kg	18+/-2	0.26	7.3	IAEA 336	3.31+/-0.03
Ba mg/kg	65.3+/-7.1	28.6	11.8	NBS 1632a	130.
Br mg/kg	24,3+/-1.1	15.4	6.1	IAEA 336	13.04+/-0.52
Ca mg/kg	6686+/-285	9558.	13330.	- " -	2870+/-124
Ce mg/kg	2.00+/-0.06	2.17	0.33	- " -	1.28+/-29.4
Co mg/kg	0.48+/-11	0.33	1.09.	- " -	0.295+/-0.014
Cr mg/kg	9.80+/-0.12	6.25	1.97	BCR 176	863 +/-30
Cs mg/kg	0.55+/-0.05	0.22	0.11	IAEA 336	109.7+/-2.2
Eu ng/kg	46+/-4	24.9	<6	- " -	22.5+/-1.2
Fe mg/kg	1846+/-40	878.	1607.5	- " -	430.4+/-10.1
Hf mg/kg	0.214+/-0.020	0.107	0.026	- " -	57.6+/-1.8
Hg mg/kg	0.82+/-0.02	0.46	0.20	Hg-Std	50.
La mg/kg	2.10+/-0.42	1.2	0.29	IAEA 336	0.671+/-0.034
Lu ng/kg	19.7+/-0.99	13.8	1.8	- " -	6.71 +/- 1.01
Nd mg/kg	1.43+/-0.08	3.14	1.04	- " -	524.5+/-0.50
Rb mg/kg	17.9+/-1.9	11.4	17.5	BCR 1	133+/-23
Sb mg/kg	0.69+/-0.01	0.87+/-0.04	<0.05	IAEA 336	0.0736+/-0.003
Sc mg/kg	0.436+/-0.009	0.223+/-0.029	0.029	- " -	0.172+/-0.005
Sr mg/kg	11.9+/-0.67	27.9	20.6	- " -	9.59+/-0.33
Th mg/kg	0.36+/-0.07	0.193	0.024	- " -	0.142+/-0.005
Zn mg/kg	121+/-2	134.	61.9	- " -	30.25+/-0.75

5. DISCUSSION OF RESULTS

The values +/- correspond to the error of the mean values of at least two measurements of the same or of different samples. Some of the results by short-time AA were obtained by evaluation of six summed spectra. The comparators for the data evaluation were synthetic multi-element standards as well as NBS- and IAEA- standards: NBS1632a (Bituminous Coal), NBS1575 (Pine Needles), NBS1570 (Spinach), IAEA336 (Lichen), Animal Bone, and BCR176 (City Waste Incineration Ash). Most data correspond to GLADNEY´s compilation listed in [4]. However, the value of chromium in IAEA336 is about a factor 3 too low compared with single element standards, BCR176, NBS 1632a, NBS 1575, and NBS 1575.

The homogeneity of the sample for some elements is not sufficient as seen from the results of gold, cadmium, and cobalt with samples of different size. These elements may be present as nuggets or accessory heavy minerals. The 47.9g sample, however, can be used as a good approximation for the total content of these elements.

Some major divergent data of Mg and Al between activation analysis and XRF-fluorescnce measurements may due to the fact that these lines are just on the lower Z-limit of the method. The differences between the data obtained with different comparators show that even NBS SRM would require harmonizing.

6. CONCLUSIONS

With few exceptions (Na, Mn, Sr) the content of elements in the lichen collected at Bleiberg is a factor of about 2-10 higher than in the lichen standard IAEA 336. Provided the Bleiberg-lichen is milled to a smaller particle size, it will supplement the IAEA 336 lichen standard for higher element content, so that a lichen standard for strongly contaminated areas is available.

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BIOMONITORING AIR POLLUTION IN THE CZECH REPUBLIC BY MEANS OF TREE BARK

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Abstract

From the point of view of atmospheric pollution some parts of the Czech Republic rank among the most devastated areas in Europe. Heavy industry is the source of exhausts which, especially in North-West Bohemia, have made large pieces of the country nearly dead. Therefore, monitoring air pollution is one of the key questions in environmental studies in the country. Our survey intended to use similar methods like those used in the Netherlands at the end of the 80's, i.e., activation analysis of lichen *Parmelia sulcata*. However, preliminary investigations have shown that the proper lichens have disappeared in the most polluted areas. Therefore, tree bark has been chosen as a biomonitor. Both activation analysis in the IRI TUDelft and radionuclide X-ray fluorescence in the FNSPE CTU Prague have been used as the methods of trace element analysis. Some methodological remarks are summarised in the first part of the paper. The effort was directed towards optimising the method in the relatively complicated height profile of the Czech landscape. Finally, oak bark was chosen as the biomonitor; investigations of disturbing effects led to the conclusion that they were within the error of measurement. The second part of the paper is devoted to the results of application of the method to a specified area of the Czech Republic. This survey covered the area of nearly 40,000 square kilometres. It included the most important parts of the country from the point of view of atmospheric pollution. The evaluation of both the INAA and RXRFA results is still in progress. Nevertheless, some maps of relative distribution of air pollution over the monitored area can now be presented. They show that for some elements (sulphur, titanium) the range of the concentrations measured is extraordinarily high and that the situation in North-West Bohemia is really alarming.

1. INTRODUCTION

The historical development of the Czech industry, especially after World War II, has resulted in high energy consumption by heavy industry. This concept has led to the fact that from the point of view of atmospheric pollution and the corresponding impact on the plant kingdom some parts of the Czech Republic rank among the most devastated areas in Europe. In particular open-cast coal mining and burning it in relatively obsolete power plants, as well as production in some chemical plants, are sources of various harmful exhausts which, especially in North-West Bohemia, have made large pieces of the country nearly dead. Sulphur and nitrogen oxides, as well as traces of various metals are the main environmentally dangerous products of these activities.

Therefore, monitoring air pollution is one of the key questions in environmental studies in the Czech Republic. Good knowledge of environmental pollution distribution can contribute substantially to identification of sources of contamination and consequently to proposing measures for mitigation of the harm. We have carried out a wide project of mapping the relative levels of various harmful chemical elements throughout the area of the country, inspired by the project carried out in the Netherlands at the end of the 80' [1-3], which used lichen *Parmelia sulcata* as a biomonitor and instrumental neutron activation analysis (INAA) as an analytical tool for monitoring the area of about 21,000 km^2, representing about 2/3 of the country. In addition to activation analysis used in the Dutch study, radionuclide X-ray fluorescence analysis (RXRFA) has also been applied in the Czech survey. The first of the two methods has been provided by the IRI research reactor and laboratories, the second by the laboratories of CTU-FNSPE.

2. CONCLUSIONS FROM PRELIMINARY INVESTIGATIONS

A preliminary study was carried out, monitoring the approximate range of concentrations of various elements in the Czech Republic. Samples of lichens, tree bark and soil were collected for this purpose at 12 selected places, estimated to be typical from the point of view of the possible contamination. The first set of samples covered the severely damaged landscape of North-West Bohemia (Chomutov, Most), which was thought to be one of the most polluted places of all. The second set was collected south of Prague (Tábor, Orlík). This is not a heavily industrialized area and is assumed to be relatively clean. Finally, the third set of samples represented an anticipated intermediate situation between the previous two extremes (the area east of Prague - Kutná Hora, Podebrady). As mentioned above, the method of monitoring applied in the Netherlands was based on bioaccumulation in lichens. Unfortunately, even this introductory study has shown that proper lichens cannot be found in some parts of the Czech Republic (they are quite sensitive to air pollution and sometimes even their appearance serves as a monitor of the air cleanness). This conclusion was soon confirmed by an independent wider survey [4], which found that even the relatively resistant species *Hypogymnia physodes* had disappeared from some industrial regions and the same was valid for *Parmelia sulcata* and other species. Therefore, it became necessary to base the study on some other biomonitor, e.g., tree bark, which is less sensitive, yet available everywhere, and to use lichens only for comparison in those places where they are easily obtainable. Moreover, we found that concentrations of most elements in the bark and lichen from the same tree were of the same order of magnitude and they differed only by a factor of two or three; the possibility of obtaining much larger bark than lichen samples cannot be also neglected. Oak was chosen as the „reference“ tree, but a few samples from the other species (poplar, maple, false acacia) were also collected for comparison.

This first methodological study led to some conclusions important for further research [5, 6]:

1) Both radioanalytical methods are proper for samples of biomonitors and can identify concentrations of more than 30 elements ranging from tenths of ppm (e.g., some rare earths) up to about 1 % (especially iron).

2) Well observable differences exist in concentrations of many elements in the suspected „clean“ and „dirty“ areas.

3) Very high local differences are measured even between various samples from one place, taken from trees growing ten or twenty meters apart.

4) Generally, the mean concentrations of pollutants do not differ significantly between the Netherlands and the Czech Republic, the most marked exceptions being, e.g., arsenic, whose presence is higher in the Czech Republic (probably due to extensive burning of coal with high arsenic content), or bromine, whose presence is higher in the Netherlands (probably due to the vicinity of the sea).

Therefore it was decided to provide a more detailed and systematic testing of the possible reasons for the high local variations which had been found (conclusion 3). This was done by selecting very typical locations for sampling and measurement and taking large sets of samples [7] to confirm or exclude the possibility that the effect was caused by some strong shielding effects (e.g. by buildings) or by the broken altitude profile of the country. The sampling sites were selected as follows:

1) Various sides of an isolated hill in a flat country. Because of prevailing westerly winds (blowing from the very polluted North-West Bohemia), some shielding of the trees growing on the eastern side could be expected. Ríp, a hill about 30 km west of the North-West Bohemian coal basin, was the ideal choice.

2) Various trees from a small area (within 100 m) in a very flat country. The samples were taken from oaks, pines and false acacias to show the difference in absorption ability of various species.

3) Randomly distributed places in a 10 x 10 km square in a moderately hilly area. This sampling represents a typical situation and is intended to give information about the anticipated uncertainty of results, if the square grid is used as a basis for monitoring large areas.

4) Several places in very hilly areas. Comparison with the previous case gives information about the influence of the landscape profile onto the reliability of results.

In all these places soil samples were also taken to enable finding a possible correlation of the contaminating elements in soil and bark. The result was negative, no measurable contribution from element content in soil to bark was found. Thus, the main contribution to the element content in the bark came from the air.

No results of measurement confirmed the influence of the landscape profile or shielding by hills. This means that the method is well applicable even in the Czech conditions of a very complicated and hilly territory. The concentrations of some trace elements differed substantially in various tree species, for certain elements (Ca, Sc, Cr, Se, Rb, Sr, Ba, Hf, and others) even by a factor of four. False acacias appeared to be the most sensitive to nearly all the measured elements, and concentrations of most elements were the lowest in pine. However, the sensitivity of all tree species is satisfactory for application as a biomonitor (the same was valid also for poplars and maples, as was seen from the first preliminary study). Finally, oak was chosen as the very widely occurring in most parts of the Czech Republic and easily distinguishable tree with proper thickness and structure of the bark. Some other species in regions without oaks (oaks like warmer climate and cannot be found in higher altitudes) can be used only after recalibration, taking into consideration their different absorption capability (i.e., normalised to oak).

A rather unpleasant fact for the application of such a biomonitoring method lies in the relatively high local differences in concentrations (i.e., concentrations in samples taken from various trees growing in the area serving as a single point of the monitoring network). The mean quadratic deviation is of the order of tens of percentage points, in the worst cases being even up to 50 % for any element analysed. This fact was found in both phases of these preliminary investigations and cannot be avoided. For representative results it is therefore necessary to take the mean value from several samples as a single point in the final mapping.

3. METHODS OF MEASUREMENT

Samples of oak bark for the large area survey were collected in the squares of the standard Mid-European co-ordinate grid MTB with a field of 10x6 geographic minutes (approx. 132 km^2). The mean value of concentration in at least 6 samples (i.e., 6 oaks) forms a single point of the system of results. The oak bark was cut from all four sides of a tree and mixed so as to avoid the influence of directional flows of contaminating elements with the winds directed from their sources. Then the bark was cleaned and only the outer 3 mm thick layer was used. The material from each oak was milled and sieved to a maximum grain size of 0.2 mm. These samples were analysed using both INAA and RXRFA.

Instrumental neutron activation analysis was carried out at the 2 MW reactor of the IRI TU Delft (thermal neutron flux approx. 5.10^{16} m^{2}s^{-1}). The irradiation time was 4 hours, measurements for medium and long-living radionuclides were carried out after 5 and 21 days. In this standard method applied at IRI 53 various elements are taken into consideration. However, in most of our samples some of them have concentrations below the detection limit. Therefore, the elements claimed to be routinely quantitatively measurable well over the detection limit are as follows: Na, K, Sc, Cr, Fe, Co, Zn, Ga, As, Se, Br, Rb, Sr, Sb, Cs, Ba, La, Ce, Nd, Sm, Eu, Tb, Yb, Lu, Hf, Ta, W, Au, Hg, Th, and U. The evaluation of these results is still in progress and we will not deal with them here in more detail. We will turn our attention to XRFXA.

Our RXRFA system consists of the measuring head with the ring photon source (^{55}Fe with the activity of approx. 7.10^8 Bq is the suitable choice for light elements, especially sulphur measurement, ^{238}Pu and ^{241}Am are also at our disposal for measurements of heavier elements), the sample holder and the ORTEC Si/Li semiconductor detector with an effective diameter of 6 mm and thickness of 5 mm. FWHM of the detector for the line of 5.9 keV (^{55}Fe) is 170 keV. The detector is connected to the multichannel analyser Canberra 35. The spectra measured are processed by the AXIL-QXAS code for determining peak areas, which has been obtained from the IAEA Vienna, and by our own code for suppressing the matrix effect, based on the method of empirical coefficients. The measured material was filled into the holder of the measuring head in such a way that a saturated layer was reached. Rather long times of measurement - 3600 s - were used to obtain low detection limits (of the order of ppm) for trace elements. ^{55}Fe allows to detect elements up to Cr; in our environmental samples S, K, Ca, Ti, V, and Cr were measurable. ^{238}Pu gives spectra with well distinguished peaks of Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, and Ga (K series), however, also Pb can be measured using L-lines (some difficulties are caused by the interference with the K-lines of As) [8].

4. RESULTS

The concentration maps of sulphur, titanium and potassium serving as examples of results are presented on Figs. 1 - 3. The area covered by the survey is about 40,000 km^2, i.e. approx. one half of the total area of the Czech Republic. Sulphur can be considered the most important and dangerous contaminant in the region, forming SO_2 in the air and finally resulting in acid rains heavily damaging forests, as we can see especially in the mountains along the north and west border of the country. Summarizing the results for sulphur biomonitoring, we obtain the differences between the most contaminated and cleanest regions as high as two orders of magnitude (Fig. 1): we have measured concentrations of more than 5000 ppm in the industrial country around the town of Most in North-West Bohemia, on the other hand, the lowest concentrations in relatively clean areas (as, e.g., around the town of Slaný) are below 100 ppm. This result is not surprising, if we take into consideration the usual concentrations of sulphur in the coal from the North-West Bohemian open-cast mines, which can reach even 5 % (see, e.g., [9]). A similar picture can be obtained for some other trace elements, e.g., titanium (Fig. 2). Concentrations exceeding 700 ppm can be measured in the larger part of the North-West Bohemian coal basin; on the other hand, the lowest concentration in the area covered by our survey is only 28 ppm. In both maps some influence of large cities (Prague) on the contamination is also evident, but it is in no case equal to the influence of heavy industry. Nevertheless, the concentration of sulphur exceeds 1000 ppm, of titanium 300 ppm. There are also some local „hot spots“ with extremely elevated concentrations in the air, surrounded by relatively clean areas. They can usually be attributed to the influence of industrial activities in the neighbourhood (e.g., very high concentration of sulphur near Beroun is probably due to a cement factory or ironworks in the place). Potassium, being a naturally occurring element in biological materials, does not have so wide range of concentrations and cannot be correlated with the air pollution (Fig. 3).

5. CONCLUSIONS

Practical results of our investigation give evidence of an excellent analytical potential of both RXRFA and INAA for similar environmental studies using various biomonitors. The high information content of such multielemental studies is without any doubts. Applied specifically to the Czech Republic, they also show quantitatively that environmental problems, especially those connected with traditional coal-fuelled power production, are even higher than fixed in the general opinion. The differences as high as two orders of magnitude in the contents of harmful elements between various parts of the country are really alarming. Therefore, the running and planned desulphurization of the coal fuelled power plants or their replacing by alternative sources (including the nuclear power plant Temelín under construction in South Bohemia) is an activity of the first order of importance. This fact is even more apparent, if we take into consideration the extreme landscape changes caused by the open-cast mining, and the costs of the revitalization of the destroyed biosphere. Though a lot has been done in the recent years for improving the environmental conditions in the country, we are still quite far from the end of this way.

Results for some other elements are still being processed (especially INAA measurements) and further studies are under preparation.

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NON DESTRUCTIVE SXRF ANALYSIS OF HEAVY ELEMENTS IN LICHENS[*]

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Abstract

Various species incrusting granitic and calcareous rocks were collected at different places covering a large span of atmospheric conditions. They were chemically studied by synchrotron-induced X-ray fluorescence (SXRF), a non-destructive technique of outmost importance in the field, allowing for a future use of the same lichen fragments in further tests. This study proved that lichens - at least the saxicolous species - concentrate heavy elements recognized as strong pollutants, like lead and bromine, irrespective the environmental availability of these elements, either in the atmosphere or in the rock substrate, partially frustrating the use of saxicolous lichens as pollution monitors.

1. INTRODUCTION

Lichens display a remarkable capacity of surviving under environmental conditions of high toxicity, accumulating heavy metals in concentrations that would be mortal for most living species. Accordingly, these organisms have been quoted as biomonitors and pollution tracers [1-3].

On the other hand, lichen weathering of rock minerals is important in pedogenesis and many studies published in the last decade on lichen chemistry have focused on the biochemical and geochemical processes involved in soil formation [4]. Even so, the mechanism(s) of mineral weathering by lichens are not yet well understood.

The unique properties of synchrotron radiation (SR) - particularly, high-brilliance, linear polarization and coherence - provide significant analytical advantages like the possibility of tunning the excitation energy by using a suitable monochromator, thus enhancing sensitivity (minimum detection limits, MDL's) for most chemical elements. Furthermore, the very low angular divergence of the SR beam enables the non-destructive, multi-elemental X-ray fluorescence microanalysis, suitable for the chemical study of minute probes and for the mapping of an analyte over heterogeneous samples. Indeed, this technique is nowadays a powerful tool for *in-situ* microanalysis, allowing for the non-destrutive determination of sub-trace contents of heavy chemical elements in light matrices - minerals or organic materials.

The usefulness of synchrotron-induced X-ray fluorescence (SXRF) for the study of bio-mineralization and mineral weathering by lichens was already emphasized [5]. In this work, we report the results of a chemical study of lichen species colonizing granitic and calcareous stones in Portuguese monuments located in different atmospheric conditions (salinity and pollution).

2. EXPERIMENTAL WORK

Various fragments of *Xanthoria parietina* - a saxicolous lichen species developing well over silicate and calcareous rocks - were collected at different places in Portugal:

(a) three samples of the lichen incrusting granites in the Castle walls at Monsanto, a village inside the country, Beira Baixa province, in a non-polluted rural area;

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[*] The experimental work was done at the LURE with the financial support of European Union through the TMR Programme.

(b) six samples of the lichen incrusting limestones at the Monastery of Batalha, a village in central Portugal, nowadays close to a highway with intense traffic;
(c) three samples at the Palace of Queluz (limestone), inside a large garden located in an urban area nearby Lisbon;
(d) six samples at the Tower of Belém (limestone), in Lisbon nearby the river Tagus, not far from a large avenue.

SXRF spectra were collected at the LURE (Laboratoire pour l'Utilization du Rayonnement Eléctromagnetique) in Orsay, France, at the D-15A station of the DCI storage ring using the photon microprobe [6] equiped with a Si(Li) detector and a multichannel analiser. An excitation energy of 18.9 keV was used throughout the study. The samples were fixed over a special adhesive tape to reduce interferences from the support. The area to be irradiated (0.05 $mm^{2)}$ was positioned with the aid of a laser beam using a computer-controlled micrometer stage.

Peak assignment is based on the diagnostic lines, usually Kα lines in the X-ray emission spectrum of elements with medium atomic number and Lα in the case of heavy elements. Data handling and processing programs developed at the LURE were used in spectra deconvolution and analysis [7, 8].

Fig. 1 compares the SXRF spectra obtained for lichens collected at the castle of Monsanto.

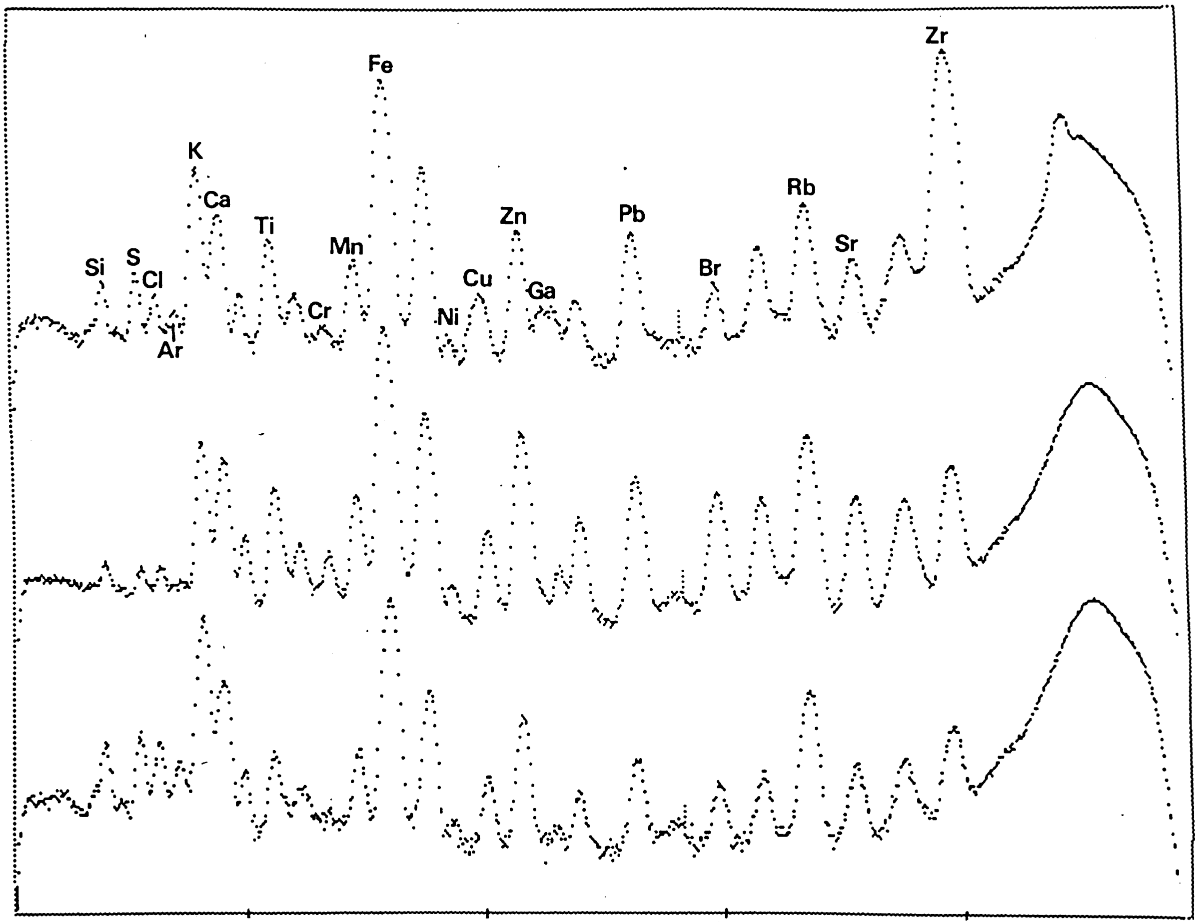

Fig. 1. SXRF spectra of fragments of Xanthoria parietina collected over granites at Monsanto Castle.

3. RESULTS

Elemental concentrations are roughly proportional to diagnostic peak areas, but the correct conversion factors can only be determined if the concentration of at least one element is known, which is not the case. Because comparative results are already useful to establish chemical trends, numerical data are based on the areas ratio with respect to a common element, which is independent from the sample itself - argon in the atmosphere separating the irradiated sample from the detector window, assuming that it is placed at a fixed distance and also, that the iron content does not vary significantly from sample to sample. Accordingly, M/Ar ratios (M = analyte) were used for comparing chemical data.

TABLE I. PEAK AREAS RATIOS M/Ar FOR LEAD AND SULPHUR

Location	Lead, **Pb**				Sulphur, **S**			
Of sampling	Mean	Max.	Min.	Δ	Mean	Max.	Min.	Δ
MONSANTO Castle	15.3	33.6	1.4	32.2	4.6	6.6	3.4	3.2
BELÉM Tower	11.6	39.6	2.5	37.1	5.3	7.8	1.8	6.0
BATALHA Monastery	8.4	23.1	0.5	22.6	4.4	13.6	0.4	13.2
QUELUZ Palace	7.2	15.9	1.9	14.0	3.9	5.6	2.5	2.9

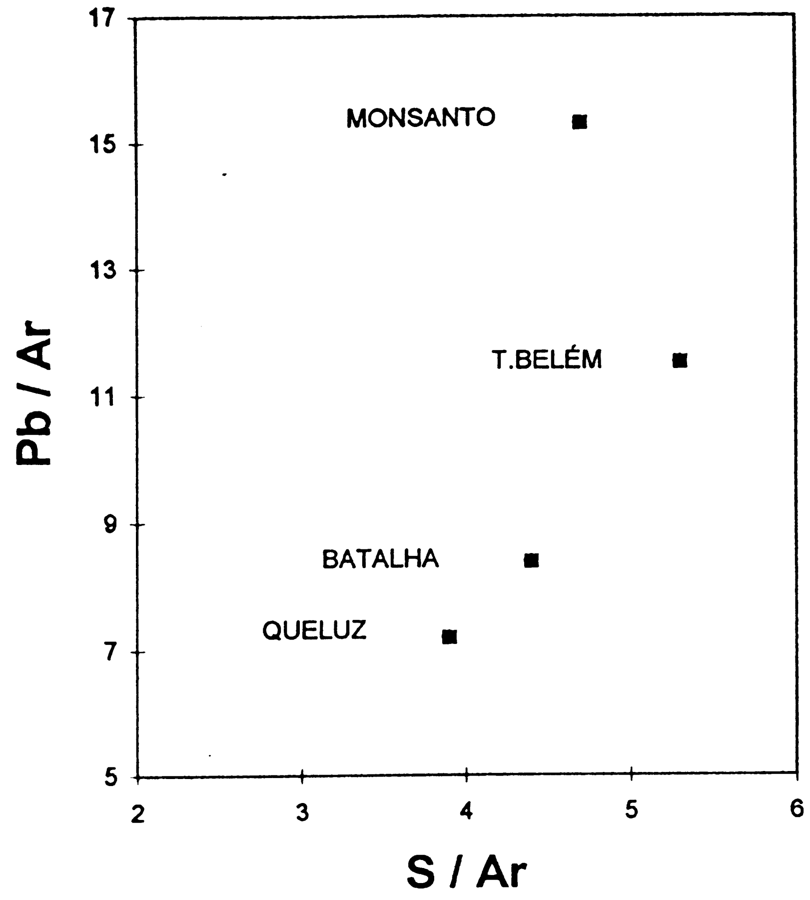

Fig. 2. Correlation of Pb vs. S contents as represented by the mean peak areas ratio M/Ar in lichen samples collected on limestone and granite substrates.

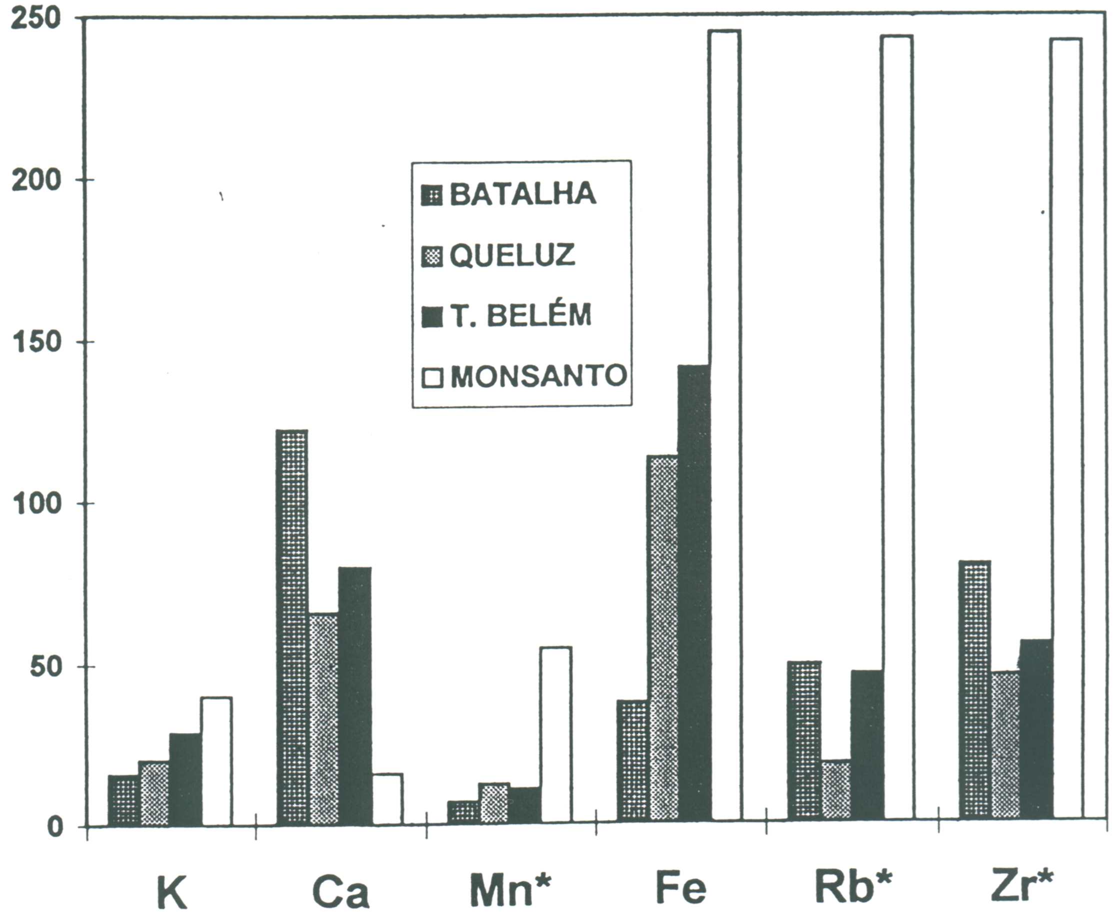

*Fig. 3. Role of the rock substrate in elemental uptake. Y-axis represents peak areas ratios (M/Ar). The levels of Mn, Rb, Zr (assigned *) are incremented by a factor of ten.*

Elements usually related to environmental pollution (S, Pb, Br) are present in all samples, irrespective the rock-type substrate.

As previously reported, bromine contents in incrusting lichen species do not correlate with the pollution level of the environment [5]. Indeed, Br-contents vary significantly from sample to sample at the same place and, for the same rock-type substrate, the mean value of Br/Ar ratios does not increase in polluted zones. These results suggest that bromine may be inherent to the metabolic processes in the living organism of the lichen.

Consequently, lead and sulphur were the only elements taken into account regarding the possible influence of environmental pollution. Table I lists the mean , maximum and minimum values plus the range of variation of M/Ar intensity ratios for these elements. From listed values it becomes apparent that the range of variation (Δ) of sulphur increases with the pollution level, being larger for Batalha samples. Lead contents are more dispersed, presenting much higher Δ values.

Mean values of M/Ar intensity ratios present a positive correlation in lichen samples collected over limestones (fig. 2), the concentrations of both lead and sulphur increasing from Queluz Palace (lower pollution degree) through Batalha Monastery close to a highway to Belém Tower, located within the urban area of Lisbon close to the Tagus river border.

The representative point of *Xanthoria parietina* incrusting granites in Monsanto Castle deviates significantly from this correlation line, a feature possibly related with variations in the sulphur level inherent to the incrusted rock. Thus, the contents of Pb and S in the lichen seem to respond to the pollution degree of the environment for the same substrate, at least for limestones.

Chlorine contents appear to depend on the atmospheric availability of this halogen, higher values being found in limestones close to the seaside.

As expected from the mineral composition of the rock, the mean values of M/Ar ratios for K, Mn, Fe, Rb, Zr are higher for *Xanthoria* samples collected over granites compared to limestones. Conversely, corresponding values for Ca are significantly increased in samples incrusting calcareous rocks (fig. 3).

The levels of the remaining elements present an aleatory variation.

3. FINAL COMMENTS

The present results rise some doubts over the use of lichens to monitor the pollution degree of the environment.

Indeed, in view of the clear dependence of the contents of most inorganic components on the mineral nature of the substrate, it can be concluded that elemental concentrations in lichens - at least in saxicolous species - do not respond directly to the environmental levels. A more careful selection of the lichen species is advisory in pollution studies, along with the complementary use of pollution biomonitoring features - such as, the species vitality and coverage capability [9] - other than the chemical concentrations of polluting elements alone.

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SPATIO-TEMPORAL VARIATIONS OF ATMOSPHERIC HEAVY METAL DEPOSITION IN THE LISBON AREA BY MOSS MONITORING

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Abstract

The use of mosses as biomonitors of heavy metal deposition has been extensively applied in numerous studies in the last three decades. This type of organisms acquires almost their nutrients from the atmosphere, by dry or wet deposition. In Portugal, the first biomonitoring survey using mosses was done in 1992, which covered all national area. A new study is presently being implemented, where samples are collected in the same sampling points of the previous ones, so a comparison can be made between the results of the two campaigns. This work presents the results obtained for the Lisbon region. Samples of *Hypnum cupressiforme,* or whenever unavailable, *Scleropodium touretii* were collected in 27 sites in this area, and the concentration of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn was determined for each sample. Maps for each element were drawn after geostatistical estimation of the metal concentration in the moss. The spatial and temporal variations in the distribution of metal concentration are discussed.

1. INTRODUCTION

Mosses are likely to acquire almost their nutrients from the atmosphere, by means of dry or wet deposition. Field and laboratory data showed that these plants have the ability to retain the elements in ionic form, bound to the anionic sites of the cell wall [1]. They can also capture the atmospheric deposition of particles originated not only by air pollution caused by human activity, but also of mineral origin. Also, for some essential elements like zinc, there can be some natural recycling in the moss carpet [2]. These factors should be considered when interpreting element concentration in mosses for identification of air pollution sources.

The background concentration of heavy metals has been followed in the European northern countries by moss biomonitoring since the eighties, by repeated surveys done in more or less five years intervals. This program has expanded to other countries of central and south Europe in the beginning of the nineties, producing a general map for the majority of the European countries. In Portugal, the second survey is now taking place, which covers all sampling points first collected in 1991/1992.

Surveys of biomonitored heavy metals in the northern countries indicate a general depletion in the concentration in mosses. This may be due to better emission control legislation, closure of old industries and better emission control techniques. For lead, the emission was reduced also by the generalisation in use of unleaded petrol by automobile vehicles [3]. In Portugal, the adoption of the European Community legislation in relation to emission control, associated with the implementation of better emission control technology also should conduct to some reduction in deposition rates. However, other sources than the anthropogenic ones may account for an important part of total element concentration determined in mosses. Previous studies [4] indicate that for eight heavy metals measured in mosses, only five of them show relation to the geological properties of the sampling sites. Also high erosion rates in some areas of Portugal may have great importance as element source for mosses [5].

The main aim of the 1996/1997 survey presently implemented is the qualification and quantification of the actual deposition of heavy metals in Portugal, and the determination of its regional background patterns. Another goal is to follow the evolution of this pattern in the period between the two surveys. This paper presents the preliminary results for the Lisbon area, after a geostatistical estimation of values. The maps obtained are compared with the maps of the previous campaign, also estimated by kriging.

2. MATERIAL AND METHODS

Two field surveys took place in the Lisbon region in different dates: from June 1991 to March 1992 and from October 1996 to March 1997. Samples of *Hypnum cupressiforme* Hedw. or, whenever unavailable, *Scleropodium touretii* (Brid.) L. Kock were collected in 27 sites in the Lisbon area in the first campaign. In the second date, 25 of these sites were visited for collection of new samples. *H. cupressiforme* is a subcosmopolite and ubiquous moss, relatively common in the region, usually found in pine forests, and *S. touretii* is a submediterranean species.

The collection, handle and cleaning of samples was done with plastic gloves and pincers, avoiding any contact with hand of steel materials. Subsamples of approximately 2.5 g went through a wet digestion with 30 ml HNO_3 at 55-65 ºC for 70-74 hours, until almost complete evaporation. The residuals were then diluted in HNO_3 (1M) and filtered to a final volume of 25 ml. The concentration of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn was determined by atomic absorption spectroscopy. Results are expressed in µg/g dry weight (d.w.) of moss.

Basic statistics parameters were calculated for all elements. The cartographic analysis was done applying geostatistical methods. Omnidirectional variograms were calculated for all variables and spherical models were applied in the ordinary kriging estimation [6]. Maps were drawn for all elements with the estimated values.

3. RESULTS AND DISCUSSION

A general increase in the overall mean is observed between the 1992 and 1997 survey for almost all elements analysed (Table I). This increase is of a factor of two for Fe and Zn, three for Cr and Ni, and ten for Cd. Only Cu and Pb is out of this pattern, showing a slight decrease in the average between the two campaigns.

TABLE I. STATISTICAL PARAMETERS CALCULATED FOR THE SAMPLES COLLECTED IN 1992 AND 1997. VALUES ARE EXPRESSED IN µg/g d.w

Metal	1992				1997			
	Mean	Min	Max	Std.Dev.	Mean	Min	Max	Std.Dev.
Cd	0.14	0.04	0.54	0.12	1.20	0.06	3.00	0.83
Cr	1.51	0.48	4.30	0.87	4.11	0.06	16.04	3.62
Cu	12.08	5.20	31.00	6.11	10.96	3.34	41.62	8.39
Fe	731.93	215.00	2360.00	570.87	1431.05	193.19	4986.05	1171.60
Mn	82.48	16.00	231.00	60.91	98.04	15.48	354.26	82.20
Ni	3.56	0.47	25.00	4.70	9.31	2.38	22.57	5.70
Pb	29.19	6.00	172.00	31.17	29.08	2.38	177.77	33.32
Zn	67.93	18.00	660.00	119.71	101.56	33.60	709.79	134.21

TABLE II. CORRELATION COEFFICIENTS CALCULATED BETWEEN 1992 AND 1997 SAMPLES.SIGNIFICANT VALUES (P< 0.05) ARE INDICATED BY BOLD FONT.

	Metals							
	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
R	0.16	0.24	0.20	0.05	**0.66**	**0.47**	**0.96**	**0.95**

However, these variations in the overall mean may result from an alteration of the datum position of the low and high values, or it may result from an increase or decreased observed in the whole area. The first situation could represent the presence of new contamination sources in the area that were not identified in the first campaign. The second indicates that the contamination sources for the metal are the same, although it can be an alteration in their magnitude. To find out which situation is observed, it was calculated the correlation coefficient between the two campaigns for all elements (Table II).

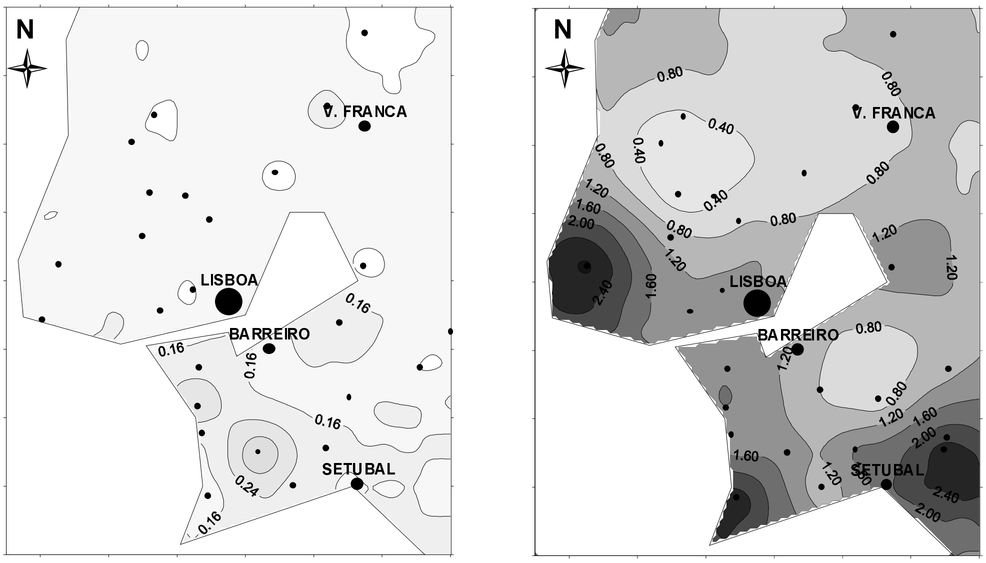

Fig.1. Cd concentration (μg/g d.w.) in mosses collected in Lisbon area. In the left are presented 1992 results and in the right the 1997 results. Sampling points are also shown.

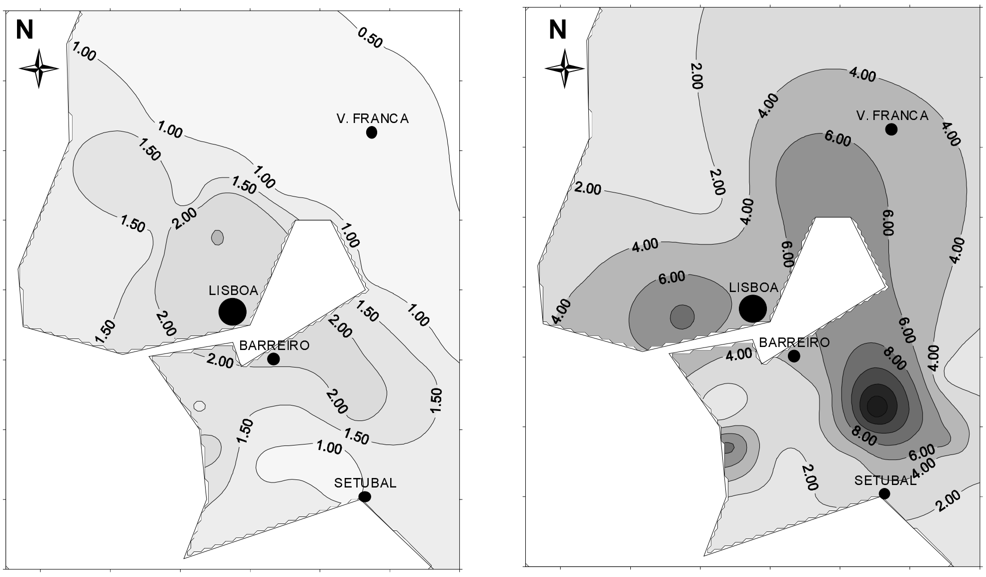

Fig.2. Cr concentration (μg/g d.w.) in mosses in Lisbon area. For details see legend of Fig. 1.

As it can be observed from the coefficients, only Pb and Zn show high correlation between the two sampling dates, showing that the spatial pattern must be similar in the two campaigns. Mn and Ni also show significant coefficients, which means that some matching should occur between surveys, however the same cannot be said for the other elements.

The estimated maps for Cd show a significant alteration in the pattern between the two campaigns (Fig. 1). There is a substantial increase in the concentration of this element in the total area, both in the Setubal peninsula, where the values were initially high, and also in the west side of Lisbon, where a new area with high values emerged. This increase may be related with the terrestrial transport of tailings from mines. The implementation of a new exhibition complex (EXPO98), which involves the increase of traffic way and an intensive urbanisation in the surrounding areas of Lisbon has

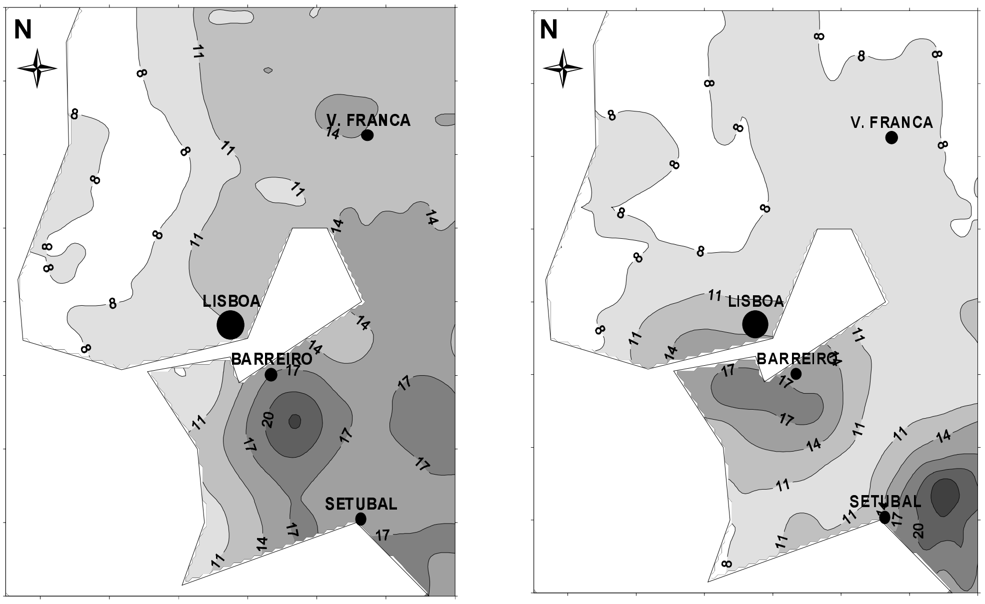

Fig.3. Cu concentration (μg/g d.w.) in mosses in Lisbon area. For details see legend of Fig. 1.

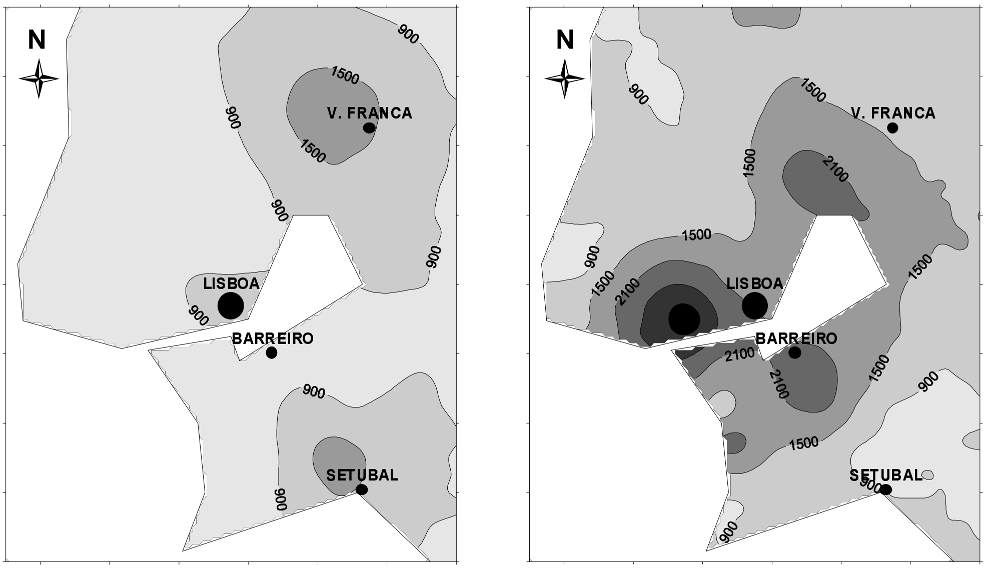

Fig.4. Fe concentration (μg/g d.w.) in mosses in Lisbon area. For details see legend of Fig. 1.

induced an intensive stone mine exploitation of sedimentary rocks, which is one main contamination source of Cd [7]. The Cr maps also show a significant change, with the high values in areas north and east from Lisbon (Fig. 2), which can be related, as Cd, to the important urban development observed in the region, inducing the construction of new urban areas and motorways.

Cu shows a general decrease in the concentration, except for the Setubal region, where it was found the highest value in 1997 survey (Fig. 3). The contamination caused by industry are clearly defined by the distribution of this metal in both campaigns, indicating that this activity is the most important source of pollution in the area.

The Fe values show an important increase, with high values surrounding the Tagus estuary (Fig. 4). Location with value higher than 3000 μg/g has appeared west from Lisbon, in a station, in which vicinity a new motorway was constructed. However, the high values previously observed in the Setubal area decreased.

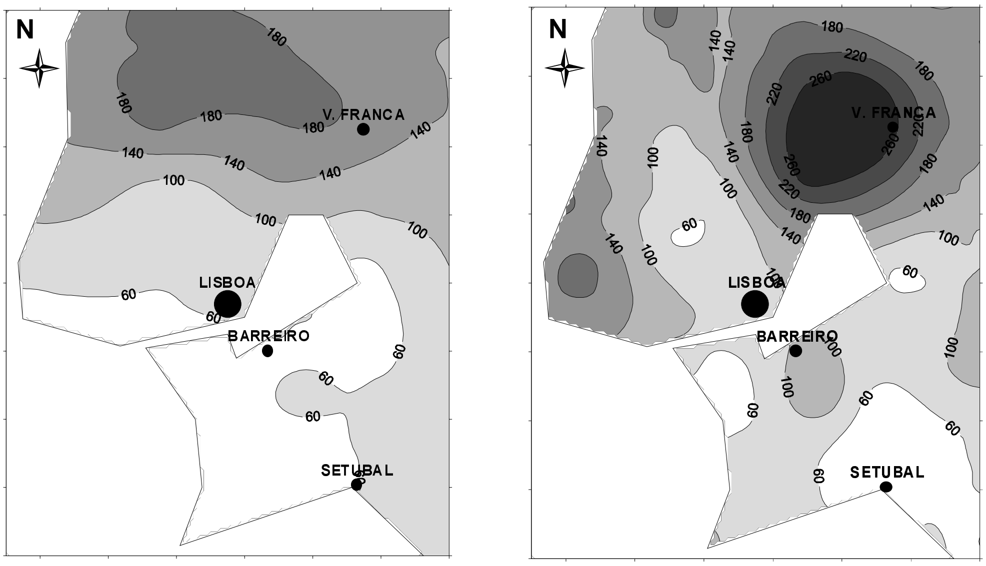

Fig.5. Mn concentration (µg/g d.w.) in mosses in Lisbon area. For details see legend of Fig. 1.

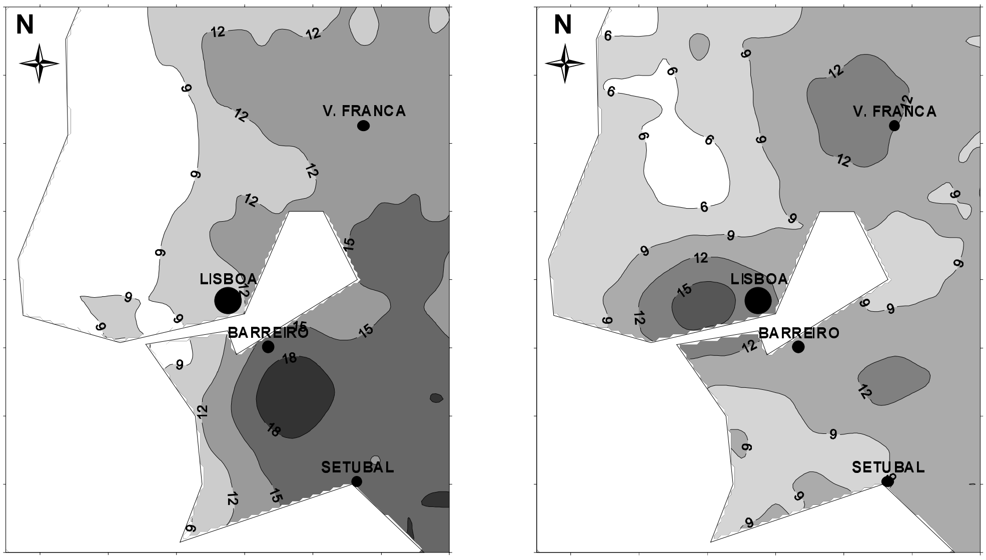

Fig.6. Ni concentration (µg/g d.w.) in mosses in Lisbon area. For details see legend of Fig. 1.

Mn also shows an increase in concentration, generalised to all area (Fig. 5). In both campaigns, the higher values are located in the region north from Lisbon, where an important contamination source exists near Vila Franca. The lowest values of the area are observed in the Setubal peninsula.

For Ni, there was a concentration increase in the areas initially with lower values, in the north-western region, and the inverse in the areas with higher values, in Setubal peninsula (Fig. 6). However, the general balance is of an increase in the overall mean.

Pb (Fig. 7) and Zn (Fig. 8) show a very similar pattern between both surveys. For the first element, the concentrations are nearly the same, and a small decrease is observed in north vicinity from Lisbon. The reduction can be due to the decrease in leaded gasoline consumption, however as the mean for both surveys is near the same, it is possible that there was some increase in other sources. The zinc concentration suffered depletion in the Lisbon/Barreiro zone, but an increase is observed in

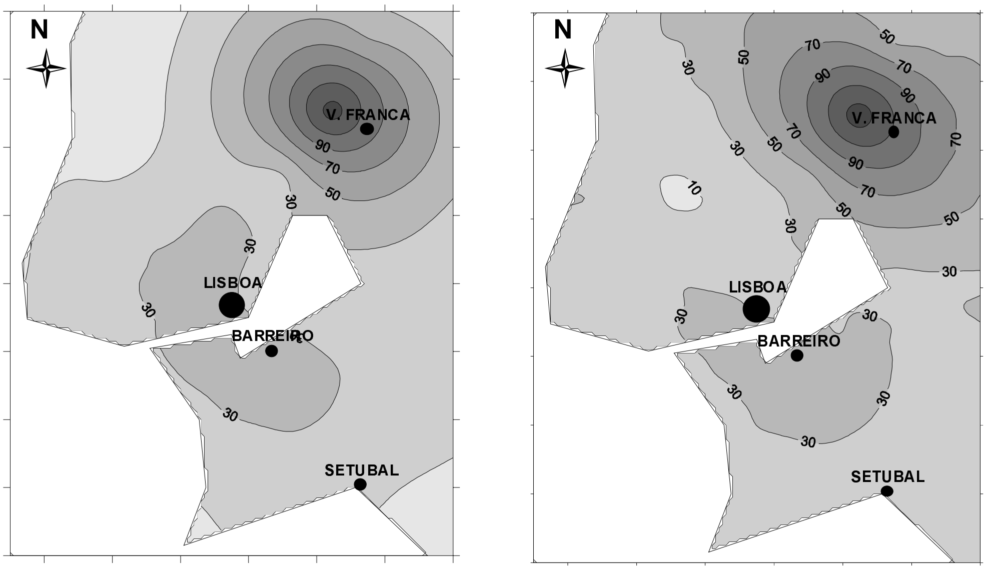

Fig.7. Pb concentration (µg/g d.w.) in mosses in Lisbon area. For details see legend of Fig. 1.

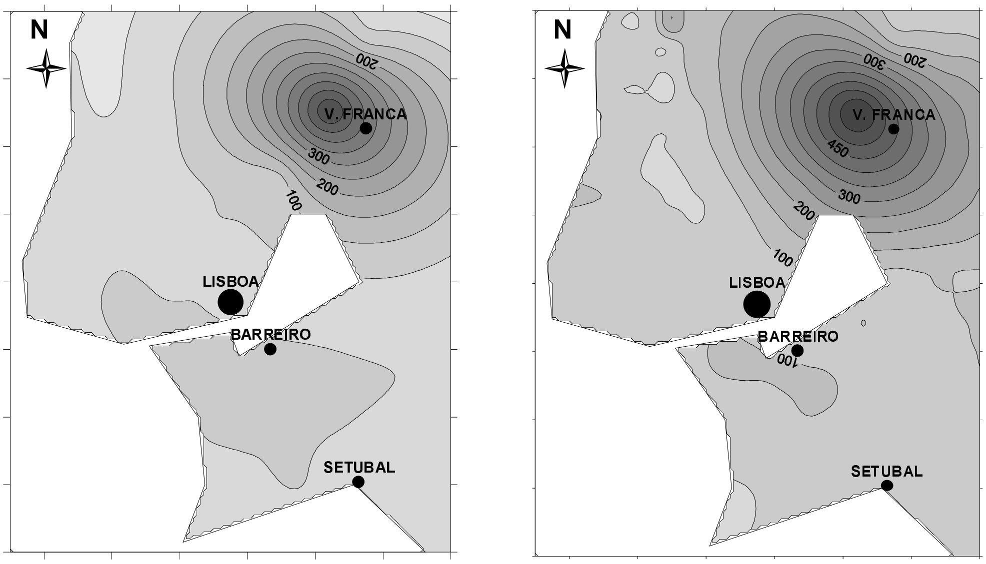

Fig.8. Zn concentration (µg/g d.w.) in mosses in Lisbon area. For details see legend of Fig. 1.

the site near Vila Franca. In this location an important power plant and a battery industry are installed, which may contribute for the high values obtained in the area.

Although some considerations are presented about possible contamination sources, with these preliminary results it is not possible to use more powerful analysis, like factorial analysis, in the identification of contamination factors, because of the small number of samples collected in the area.

4. CONCLUSIONS

Results show that for the majority of analysed metals there was an increase in heavy metal deposition in Lisbon area in the period between 1992 and 1997. The most important increase observed was for Cd, which can be a result of large urbanisation activity, stone mine exploitation and transport verified in the area for the last five years. Also the installation of new motorways have to be accounted as new pollution sources. Some metals – Pb and Zn – preserved the distribution patterns between surveys, while in others a considerable change was observed in their pattern.

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ANALYSIS OF AEROSOL SAMPLES IN MEXICO CITY

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Abstract

Total solid particles and 9 metals pontentially hazardous for health were determined in Mexico City dwellings by gravimetry and X-ray fluorescence techniques, respectively . Monitoring was performed in spring and winter, on districts covering center, northeast, southeast and southwest of the City. Results show that, in general, the average concentration of metallic contaminants have increased with time and, in the particular case of lead it is higher than the indicative WHO maximum level. The total solid particles figures are also above the U.S. norm of 75 $\mu g.m^{-3}$. Results obtained in samples taken in the same dwelling at different seasons show that lead was not present in a measurable concentration all the time. It seems to exist a correlation between the increase in lead and the increase in total solid particles whose distribution pattern in Mexico City follows wind directions. Another relevant correlation seems to exist between lead concentration and the rate of combustion of fuel in the transport system, which varies with the zone and the season.

1. INTRODUCTION

Metals differ from other toxic substances in that they are neither created nor destroyed by humans. Nevertheless, utilization by humans influences the potential for health effects in at least two major ways: first, by environmental transport, that is, by human or anthropogenic contribution to air, water, soil, and food, and second by altering the speciation or biochemical form of the element. The geologic and biological cycles naturally redistribute metals by physical process in the first case or bioconcentration in second case. However, human industrial activity has demonstrated a major role in metals redistribution. Therefore, metals contamination of environment reflects both natural sources and contribution from human industrial activities [1].

Metals are transported in ambient air by particles and in Mexico City, one of the seven cities in the world with major pollution and one of the thirteen most crowded (roughly 20, 000, 000 inhabitants) [2], the total solid particles (TSP) have been found to be mainly soil derived (roughly 50 % of the total average of 214.1 $\mu g.m^{-3}$) [3, 4]. The most dangerous, respirable particles (RP) represent a substantial percentage, between 40 and 50%, with an average of 96.4 $\mu g.m^{-3}$.

The particular objective of this work is to estimate the concentration of TSP as well as that of 9 metals by means of X-ray fluorescence spectroscopy. Some of them are considered essential metals like copper, iron, manganese, and zinc, but all of them potentially hazardous to health. This study is a part of the environmental program of the Faculty of Chemistry, that includes the determination of radon and gamma radiation levels [3, 5].

2. EXPERIMENTAL

The air survey was carried out in winter 96-97 and spring 97, both indoors in ten single family dwellings with normal occupancy conditions in the main living area, living-dining room or bedroom and outdoors. Monitored dwelling were situated in differents districts of Mexico City: Cuauhtémoc, Milpa Alta, Magdalena Contreras, Alvaro Obregón, Coyoacán, Xochimilco, Benito Juarez, Gustavo A. Madero, Tláhuac and Ecatepec in the neighborhood state of Mexico. These districts cover the center, northest, southeast and souhtwest of the City. Samples were taken during 120-hour periods

TABLE I. CONCENTRATION OF CONTAMINANTS IN INDOOR AIR (SPRING) DETERMINED BY X-RAY FLUORESCENCE ($\mu g.m^{-3}$)

Sample	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Pb	TSP
1	7.18±1.22	1.21±0.33	*	0.71±0.15	1.5±0.23	0.65±0.19	0.56±0.18	0.61±0.2	5.08±0.91	311.79
2	6.1±1.93	*	0.86±0.21	*	1.57±0.25	0.78±0.3	0.73±0.16	0.61±0.37	*	228.75
3	9.26±1.17	*	1.23±0.24	*	1.72±0.32	0.9±0.15	0.82±0.13	0.67±0.41	*	391.4
4	8.57±0.2	*	0.89±0.23	*	1.79±0.22	0.79±0.81	1.0±0.15	0.88±0.81	*	41.43
5	14.23±2.83	2.24±0.82	1.02±0.58	0.98±0.06	1.66±0.17	1.28±0.42	0.92±0.11	0.62±0.1	*	207.86
6	16.14±2.52	1.88±0.49	0.94±0.58	*	1.8±0.16	0.82±0.39	0.71±0.06	0.28±0.11	*	122.87
7	15.51±2.5	2.12±0.78	0.95±0.2	*	2.43±0.39	0.77±0.24	0.74±0.23	0.5±0.23	*	188.96
8	12.18±0.97	1.48±0.73	1.31±0.3	*	2.24±0.11	0.82±0.056	0.85±0.25	0.56±0.39	5.94±2.39	236.21
9	10.07±2.27	1.63±0.95	0.84±0.19	*	1.63±0.2	0.78±0.13	1.04±0.1	0.42±0.15	4.49±1.35	141.72
Mean	11.03	1.76	1.01	0.84	1.82	0.83	0.82	0.57	5.17	207.67
S.D.**	3.76	0.39	0.18	0.19	0.31	0.13	0.15	0.17	0.73	103.26

* under the minimum detection level

** standard deviation

TABLE II. CONCENTRATION OF CONTAMINANTS IN INDOOR AIR (WINTER) DETERMINED BY X-RAY FLUORESCENCE (μg.m^{-3})

Sample	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Pb	TSP
1	8.96±0.27	*	*	*	2.15±0.25	0.81±0.26	0.68±0.21	1.35±0.06	*	207.14
2	14.67	1.9	*	0.9	2.46	0.51	1.02	*	5.47	538.57
3	10.26±1.45	1.54±0.29	0.92±0.14	0.92±0.2	2.04±0.09	0.83±0.17	0.91±0.16	0.44±0.02	*	207.14
4	13.33±1.21	1.72±0.68	*	*	2.08±0.25	0.92±0.13	0.89±0.15	0.82±0.07	*	165.71
5	9.66±1.07	*	*	1.15±0.13	1.34±0.58	0.56±0.08	0.91±0.09	0.53±0.07	*	62.14
6	7.86±0.72	*	*	0.96±0.2	1.62±0.16	0.9±0.37	1.15±0.2	1.16±0.18	3.48±0.55	321.07
7	9.07±2.14	*	1.20±0.31	1.06±0.07	1.37±0.52	0.91±0.11	0.87±0.05	0.94±0.11	*	207.14
8	8.83±2.94	*	0.72±0.16	0.73±0.4	1.4±0.05	0.82±0.01	0.76±0.2	0.5±0.18	*	100.36
9	8.96±1.71	1.14±0.4	*	*	1.65±0.11	0.75±0.14	1.04±0.11	*	*	155.36
Mean	10.18	1.58	0.95	0.95	1.79	0.78	0.92	0.82	4.48	218.29
S.D.**	2.29	0.33	0.24	0.14	0.4	0.15	0.14	0.35	1.4	140.77

* under the minimum detection level

** standard deviation

TABLE III. CONCENTRATION OF CONTAMINANTS IN INDOOR AIR (SPRING AND WINTER) DETERMINED BY X-RAY FLUORESCENCE (μg.m^{-3})

Sample	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Pb	TSP
1	7.18±1.22	1.21±0.33	*	0.71±0.15	1.5±0.23	0.65±0.19	0.56±0.18	0.61±0.2	5.08±0.91	311.79
2	6.1±1.93	*	0.86±0.21	*	1.57±0.25	0.78±0.3	0.73±0.16	0.61±0.37	*	228.75
3	9.26±1.17	*	1.23±0.24	*	1.72±0.32	0.9±0.15	0.82±0.13	0.67±0.41	*	391.4
4	8.57±0.2	*	0.89±0.23	*	1.79±0.22	0.79±0.81	1.0±0.15	0.88±0.081	*	41.43
5	14.23±2.83	2.24±0.82	1.02±0.58	0.98±0.06	1.66±0.17	1.28±0.42	0.92±0.11	0.62±0.1	*	207.86
6	16.14±2.52	1.88±0.49	0.94±0.58	*	1.8±0.16	0.82±0.39	0.71±0.06	0.28±0.11	*	122.87
7	15.51±2.5	2.12±0.78	0.95±0.2	*	2.43±0.39	0.77±0.24	0.74±0.23	0.5±0.23	*	188.96
8	12.18±0.97	1.48±0.73	1.31±0.3	*	2.24±0.11	0.82±0.056	0.85±0.25	0.56±0.39	5.94±2.39	236.21
9	10.07±2.27	1.63±0.95	0.84±0.19	*	1.63±0.2	0.78±0.13	1.04±0.1	0.42±0.15	4.49±1.35	141.72
10	8.96±0.27	*	*	*	2.15±0.25	0.81±0.26	0.68±0.21	1.35±0.06	*	207.14
11	14.67	1.9	*	0.9	2.46	0.51	1.02	*	5.47	538.57
12	10.26±1.45	1.54±0.29	0.92±0.14	0.92±0.2	2.04±0.09	0.83±0.17	0.91±0.16	0.44±0.02	*	207.14
13	13.33±1.21	1.72±0.68	*	*	2.08±0.25	0.92±0.13	0.89±0.15	0.82±0.07	*	165.71
14	9.66±1.07	*	*	1.15±0.13	1.34±0.58	0.56±0.08	0.91±0.09	0.53±0.07	*	62.14
15	7.86±0.72	*	*	0.96±0.2	1.62±0.16	0.9±0.37	1.15±0.2	1.16±0.18	3.48±0.55	321.07
16	9.07±2.14	*	1.20±0.31	1.06±0.07	1.37±0.52	0.91±0.11	0.87±0.05	0.94±0.11	*	207.14
17	8.83±2.94	*	0.72±0.16	0.73±0.4	1.4±0.05	0.82±0.01	0.76±0.2	0.5±0.18	*	100.36
18	8.96±1.71	1.14±0.4	*	*	1.65±0.11	0.75±0.14	1.04±0.11	*	*	155.36
Mean	10.60	1.69	0.99	0.93	1.80	0.80	0.87	0.68	4.89	212.98
S.D.**	2.99	0.36	0.18	0.15	0.35	0.14	0.15	0.29	0.95	119.89

* under the minimum detection level

** standard deviation

TABLE IV. CONCENTRATION OF CONTAMINANTS IN OUTDOOR AIR (SPRING AND WINTER) DETERMINED BY X-RAY FLUORESCENCE ($\mu g.m^{-3}$)

Sample	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Pb	TSP
Spring	10.9	*	*	*	1.72	0.79	0.66	1.04	*	310.71
Winter	14.0	*	*	*	2.96	0.92	1.18	3.62	*	279.61
Mean	12.44	*	*	*	2.34	0.85	0.92	2.32	*	295.16
S.D.**	2.18	*	*	*	0.88	0.093	0.37	1.82	*	21.99

* under the minimum detection level

** standard deviation

using a monitor WLM-1A with an air sampler pump, 0.13 $L.min^{-1}$ flow rate on a Millipore filter 0.80 μm. In some dwellings samples were taken in both seasons, two to four times.

The filters were analyzed by X-ray fluorescence spectroscopy in a detection system consisting of a Si(Li) detector coupled to a Norland Inotech 5400 channel analyzer. The excitation source was ^{239}Pu. Quantification was carried out utilizing the AXIL program [6] and based on standard references. Total solid particles were determined by gravimetry.

3. RESULTS AND DISCUSSION

Tables I and II show the concentration in indoor air samples obtained in spring and winter, of Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Pb, and total solid particles. As it can be seen the average in each season is more or less the same; therefore Table III includes all samples and the total average.

Table IV shows the average outdoor concentration of two samples of the same dwelling in Tláhuac.

The very high concentration of calcium in all samples is well correlated with salty soil conditions and low moisture content. Essential metals concentrations (indoor and outdoor) : Cu, Fe, Mn, and Zn and minor toxic metal, Ti, are higher than those reported for some cities in the U.S. [7], higher than those obtained in other study for the same City in 1974 [8] but some of them lower than other performed in 1995 in district of Cuajimalpa [9], Figure 1. Such values are lower than prevailing standards for the Threshold Limit Values (TLV) for eight-hours occupational exposure in fumes and dust or, in the Ni case, in solution [10]. Major toxic metals like Cr (firstly in oxidation state VI), Ni, and Pb (either in solid or vapors form) have multiple effects but primarily carcinogenic the first two and the last one antagonistic to normal central nervous system function and inhibitor of macrophage alveolar activity. Such contaminants are fixed to respirable particles (less than 10 μm) and then deposited on the low respiratory system, primarily on alveola [11]. The indoor average concentration of chromium 0.99±0.18 $\mu g.m^{-3}$ and Ni 0.80± 0.14 $\mu g.m^{-3}$ are much higher than the values of 0.01 to 0.03 $\mu g.m^{-3}$ and 0.02 $\mu g.m^{-3}$ respectively for industrial cities [7], but lower than those obtained in the 1995 study.

The indoor average concentration of lead 4.89±0.95 $\mu g.m^{-3}$ is higher than obtained values in other studies for the same City [4, 9], above the indicative maximum average value WHO [2], EPA [12], and Mexican Technical Norm [13] (averaging 1.5 $\mu g.m^{-3}$ over three months) but lower than TLV value of 150 $\mu g.m^{-3}$ [7].

The average of TSP was 213± 140.8 $\mu g.m^{-3}$ equal to reported value in previous papers. As it can be seen this value is higher than the U.S. Norm of 75 μg.m^{-3}/y, lower than the denominated IMECA 100 (Metropolitan Index Air Quality) of 275 μg.m^{-3} [14, 15]. Some dwellings have shown values above IMECA 200 (456 μg.m^{-3}).

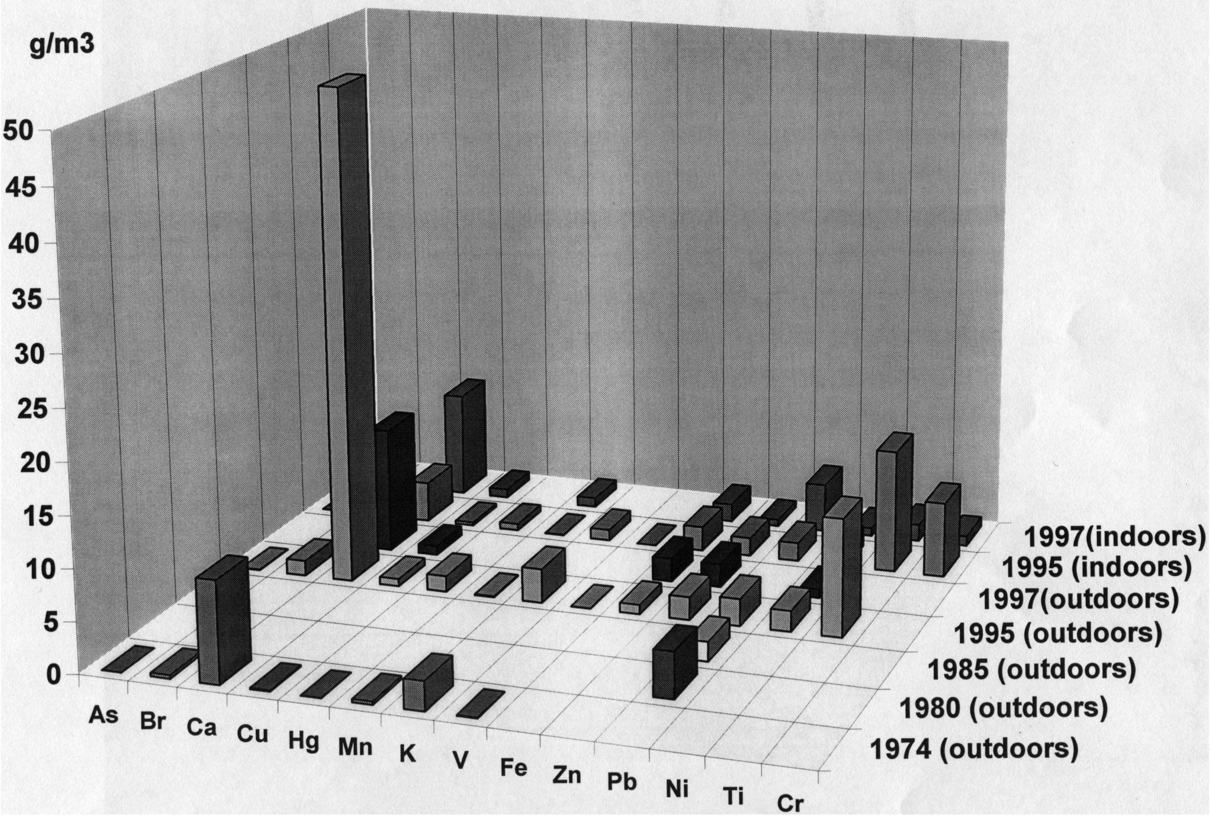

Figure 1. Pollutants evolution in Mexico City.

Results obtained in samples taken in the same dwelling in different seasons show that lead was not present at elevated concentration all the time. It seems to exist a correlation between the increase in TSP and the increase in lead.

4. CONCLUSIONS

Results show that in general, metallic pollutants have increased with time and some zones are more contaminated than others because of industrial activities, indicating that human behavior still remains as the most important component of biogeochemical cycle of metals. In the particular case of lead it seems to be, in addition, two correlations: the concentration of TSP whose distribution pattern in Mexico City follows wind directions, mainly from northwest and northeast; and the rate of combustion of fuel in the transport system, which varies with the zone, and the season.

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REGIONAL MONITORING OF METALS IN THE MUNICH METROPOLITAN AREA: COMPARISON OF BIOMONITORING (STANDARDIZED GRASS CULTURE) WITH DEPOSITION AND AIRBORNE PARTICLES

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Abstract

In the Munich metropolitan area a close association of lead (Pb) and antimony (Sb) impacts with traffic was observed in 1992 and 1993 [1, 2]. The intercorrelation of both metals was found by samples of standardised grass cultures and was reflected by deposition sampling, too. With respect to location-specific variations, however, both methods revealed differing gradients of Pb and Sb concentrations with increasing distance from traffic. It appeared that Sb variations according to traffic implications were particularly well indicated by means of biomonitoring, while Pb variations were not indicated adequately. As a result, a special qualification of grass to selectively collect metals on airborne dust according to particle sizes was suggested. Further investigations on the correlations between metal biomonitoring, metal deposition and airborne metals in 1994 - 1996 corroborated method-specific sampling features. They in turn showed that one interference is the individual prevalence of the metals on different particle sizes.

1. INTRODUCTION

According to the Federal Law of Protection Against Emissions (Bundes-Immissionsschutzgesetz), metal depositions [3] and the possible consequences for bioaccumulation in standardised grass cultures [4] are routinely monitored in Bavaria. Both methods have been successfully established to indicate metal pollutions. Active biomonitoring with *Lolium multiflorum, var. italicum, subspec. Lema* is supposed to reveal possible impacts of air pollutants in terms of bioaccumulation. The term 'bioaccumulation' in this context is taken to denote the amount of pollutant found in the plant after impact to the grass surface and/ or subsequent incorporation into the grass foliage. By contrast, the sampling of depositions allows the evaluation of airborne metal pollutions with respect to time and area units.

The survey of metal impacts in Munich revealed differing qualities of the monitoring methods with regard to indication of traffic-derived metal pollution [1, 2]. As far as location-specific variations were concerned, bioaccumulation of Pb in grass was moderate compared to Pb depositions. Bioaccumulation of Sb, however, showed stronger concentration gradients compared to Sb depositions. Nevertheless, impacts of both metals indicated association with traffic impacts by decreasing concentrations with increasing distance to traffic. On account of the brushlike structure of the grass culture a special qualification to selectively collect metals on fine particles was suggested.

Thus, the correlation between metal impacts in grass and depositions vs. metals on airborne particulate matter was examined 1994 - 1996. Suspended particles were fractionated by a virtual impactor (dichotomous sampler) equipped with a PM10 inlet head. In order to study potential health damage arising from inhalation, fractionation of the airborne particles complied with the thoracic and respirable conventions of the International Organisation for Standardisation (ISO). According to [5] the site of particle deposition in the respiratory tract essentially depends among others on the size of inhaled particles. In accordance with these findings, air quality guidelines define conventions for size-selective sampling of airborne particles when the purpose of sampling is health-related [6]. As stated by ISO conventions, the particle fraction, which is inhaled in a human body (thoracic fraction), is defined as the mass fraction of inhaled particles which penetrate beyond the larynx. The respirable fraction in turn is defined as the percentage of the inhaled particle mass which penetrates the unciliated airways.

2. METHODS

Details of sampling, exposure and analytical methods have been elaborately described in [7].

Metal monitoring was performed by **standardised grass exposure** [3] and by **deposition** sampling [4]. Measurements were conducted in consecutive two weeks' intervals during the vegetation periods from May to October. Observations were made at nineteen locations situated in varying distance to traffic in 1992 and 1993. At two locations (I and II) out of these nineteen locations observations were made in 1994 to 1996, too. Location I was situated in the south-west of Munich, at a 3-m distance to a crossing of roads with a high traffic density (about 120.000 cars/ d). Location II was situated in the north-east of Munich with modest traffic influence and was considered to supply low control values.

Parallel to biomonitoring and deposition sampling intervals, **size-selective sampling of airborne dust** was conducted during the vegetation periods of 1994 to 1996. Particles were fractionated by a dichotomous sampler (Ser. 245, Andersen, Atlanta) with a sampling flow rate of $1 m^3/ h$ (16.7 l/ min). The sampler was equipped with a PM10 (**p**articulate **m**atter < 10 µm) sampling inlet head of the EPA-Norm, which is designed to mimic the inhalation characteristics of human beings by separating suspended particles according to the thoracic particle size convention ($d_{ae} < 10$ µm). Fractions of particles with aerodynamical diameters (d_{ae}) < 2.5 µm (df-mode) and 2.5 µm < d_{ae} < 10 µm (dc-mode) were separated.

3. RESULTS AND DISCUSSION

A strong intercorrelation of Pb and Sb depositions 1992-1996 is found all over the Munich metropolitan area (see Fig. 1., Table I). Association of both metals with traffic emissions is suggested.

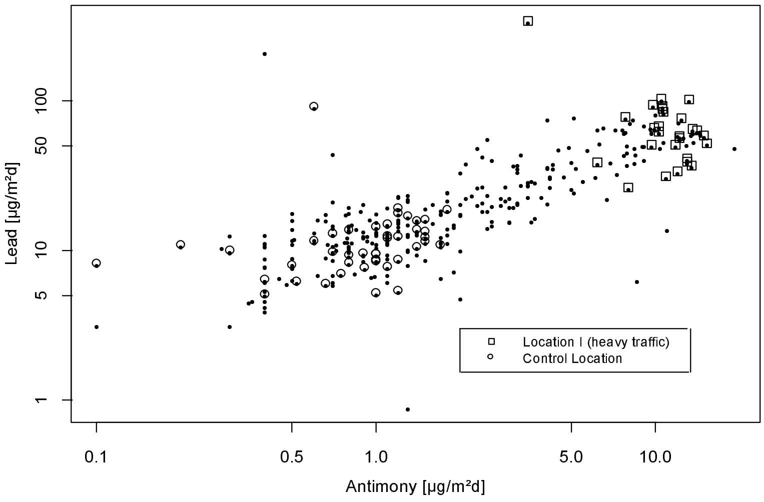

*Fig. 1. Intercorrelation of Pb and Sb depositions (µg/ m²*d) in the whole Munich metropolitan area, 1992 - 1996.*

TABLE I. INTERCORRELATION OF PB AND SB DEPOSITION IN THE WHOLE MUNICH METROPOLITAN AREA , 1992-1996

	n	Pearson	Spearman	intercept (a)	stand. error of interc. (s(a))	slope (b)	stand. error of slope (s(b))
LocationI (heavy traffic)	27	-0.47	-0.20	5.98	0.71	-0.78	0.30
Control Location	43	0.24	0.49	2.41	0.08	0.20	0.13
whole Munich metr.area	334	0.79	0.80	2.59	0.03	0.58	0.03

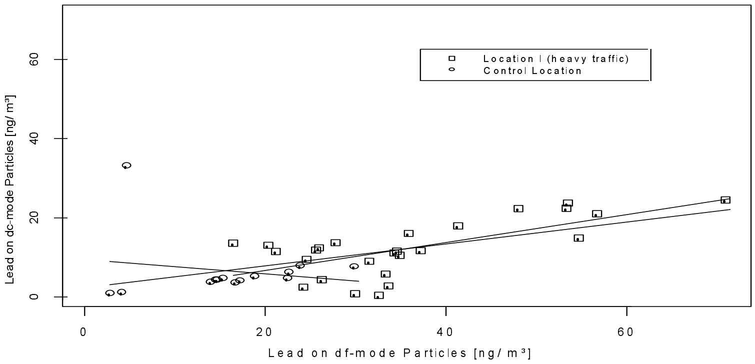

Fig. 2. Common distribution of airborne Pb on particles of the df-mode and the dc-mode (ng/m³), 1994-1996.

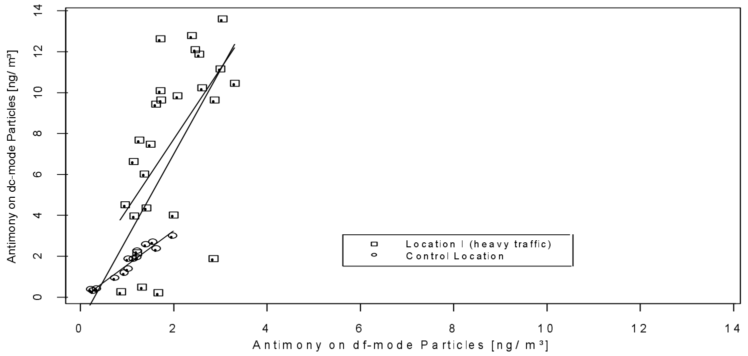

Fig. 3. Common distribution of airborne Sb on particles of the df-mode and the dc-mode (ng/m³), 1994-1996.

TABLE II. COMMON DISTRIBUTION OF AIRBORNE PB AND SB ON DF-MODE AND DC-MODE PARTICLES (ng/ m³), 1994 - 1996

	n	**Pearson**	**Spearman**	**intercept (a)**	**stand. error of interc. (s(a))**	**slope (b)**	**stand. error of slope (s(b))**
Pb							
Location I (heavy traffic)	26	0.67	0.56	-0.37	3.02	0.35	0.08
Control Location	14	-0.18	0.61	9.50	5.02	-0.18	0.29
pooled data	40	0.54	0.54	2.32	2.27	0.28	0.07
Sb							
Location I (heavy traffic)	26	0.59	0.64	0.86	1.98	3.43	0.97
Control Location	14	0.97	0.98	-0.10	0.14	1.68	0.12
pooled data	40	0.72	0.75	-1.22	1.15	4.11	0.64

Analysis of the data of airborne particle sampling (see Figs. 2. and 3., TABLE II.) shows a predominance of Pb on df-mode particles ($Pb_{df\text{-}mode} : Pb_{dc\text{-}mode} = 3 : 1$), while Sb is found to be mainly associated to particles of the dc-mode ($Sb_{df\text{-}mode} : Sb_{dc\text{-}mode} = 1 : 3$). The Pb correlation is only found to be significantly positive near traffic ($r_{Location\ I} = 0.67$). By contrast, the Sb correlation is found to be significantly positive at both locations ($r_{Location\ I} = 0.59$, $r_{Control} = 0.97$).

Fig. 4.a. Correlation coefficient: Pearson = 0.18, Spearman = 0.27
Regression equation: y = 26.08 + 2.503 log(x)

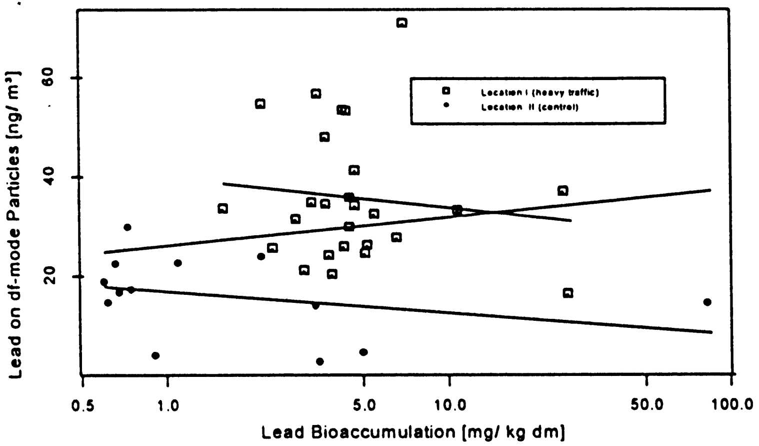

Fig. 4.b. Correlation coefficient: Pearson = 0.18, Spearman = 0.05
Regression equation: y = 6.34 + 0.481 log(x)

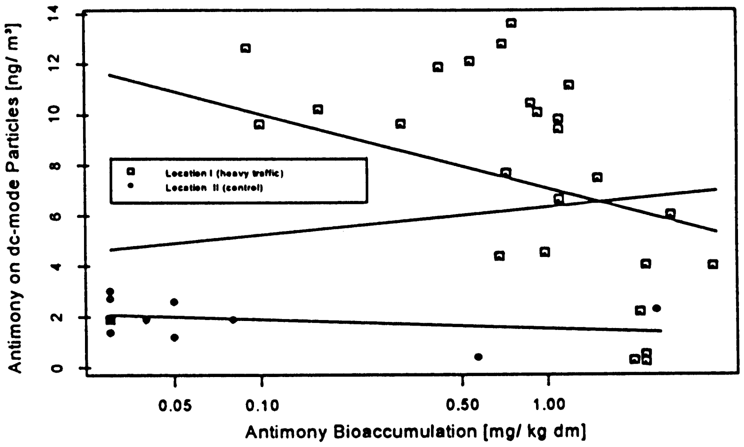

Fig. 4.c. Correlation coefficient: Pearson = 0.78, Spearman = 0.84
Regression equation: y = -18.814 + 14.542 log(x)

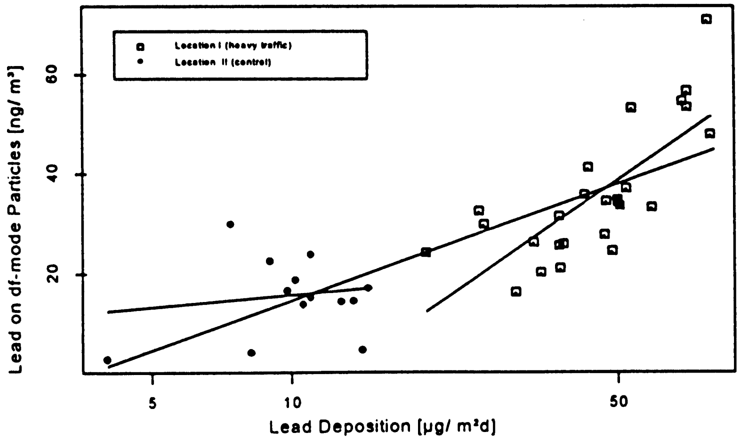

Fig. 4.d Correlation coefficient: Pearson = 0.63, Spearman = 0.57
Regression equation: y = 1.267 + 2.516 log(x)

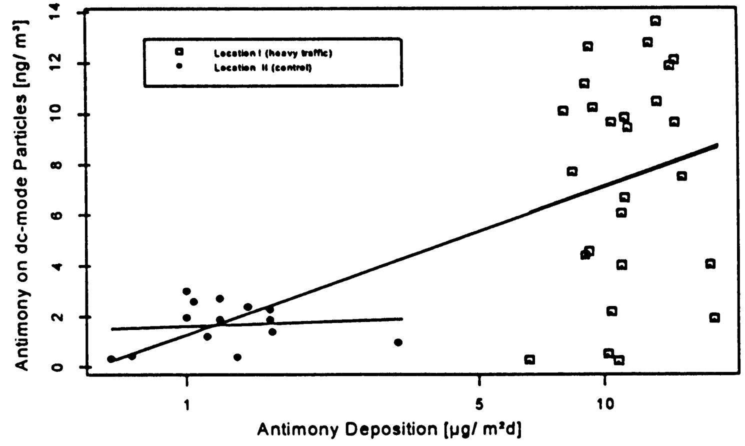

*Fig. 4. Validation of Pb/ Sb bioaccumulation (mg/ kg dm) and Pb/ Sb deposition (µg/ m^2*d) vs. Pb/ Sb on their predominant airborne particle fraction (ng/ m^3).*

Fig. 5.a.

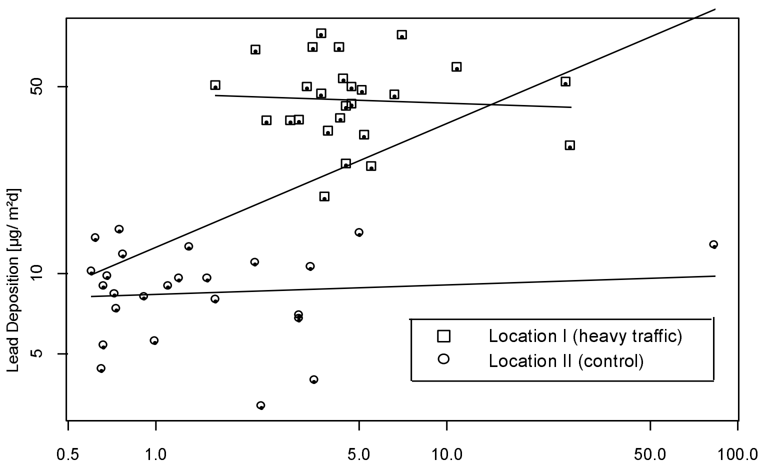

Fig. 5.b.

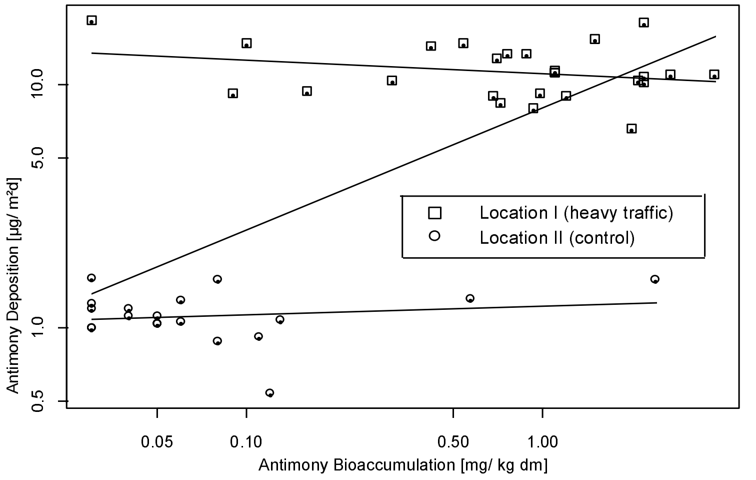

*Fig. 5. Validation of Pb/ Sb bioaccumulation (mg/ kg dm) vs. Pb/ Sb deposition (µg/ m²*d).*

Location-specific variations of Pb and Sb impacts are reflected by all types of samples (see Fig. 4). They are most distinctly revealed by depositions (see Figs. 4.c. and d.). Only at Location I (heavy traffic) a strong correlation of Pb deposition (µg/ m^2*d) vs. Pb on df-mode particles (ng/ m^3) is discovered (see Fig. 4.c., r = 0.75). The correlations of the pooled data arise from a strong heterogeneity of the results and reflect the sufficient qualification of all methods to reveal large data variations.

No location-specific correlations between bioaccumulation and deposition data are found (see Fig. 5.). The correlation of the pooled data arises from a strong heterogeneity of the results (see also Fig. 4). Sb bioaccumulation shows more variation compared to Sb deposition, while variations of Pb impacts are found by both, deposition and bioaccumulation.

Time trends of metal impacts in Munich show a decline of Pb impacts, while Sb impacts are mostly pronounced found to be increasing at the location with heavy traffic (see Table III.).

4. CONCLUSIONS

According to the location-specific variations (Fig. 4), traffic association of Pb and Sb impacts is well indicated by all monitoring methods. Nonetheless, method-specific sampling qualities are found, which appear to reflect different aspects of emission-derived metal impacts.

TABLE III. TIME TRENDS OF PB AND SB DEPOSITION IN MUNICH, 1992-1996

	1992	**1993**	**1994**	**1995**	**1996**
Pb					
Location I (heavy traffic)	81.3	84.3	58.0	39.2	36.9
Control Location	10.9	12.5	9.6	7.7	7.0
Sb					
Location I (heavy traffic)	10.1	11.2	12.2	13	13.0
Control Location	0.7	1.1	1.0	1	0.75

On account of the shielding function of the vessel's side the **deposition** is mainly limited to particle deposition from above, which requires that particles remain suspended long enough for a subsequent wet and dry deposition into the vessel. This conclusion is corroborated by the finding of a strong correlation of Pb deposition vs. Pb on its predominant df-mode particle fraction (Fig. 4.c.). Once established in the Bergerhoff instrument, deposition is to a large degree unaffected by meteorological influences.

By contrast, the brushlike shape makes the **grass** a type of air filter, which easily takes up particle-associated metals from all sides. In addition to collecting depositions from above, it seems that the grass grasps coarser particles from lateral road dust entrainment, too. Caused by an increased dust raising near traffic combined with the susceptibility of grass to both particle fractions, median values show that Sb bioaccumulation indicates traffic impacts by a factor of 20 compared to 10 (depositions) and 3 (airborne particles).

Measurements of **airborne dust** reveal different origins of the metal impacts. The predominance of Pb on the df-mode (see Fig. 2.) probably results from main derivation of exhaust emissions. Due to an increased use of unleaded motor fuel Pb depositions appear to decline (see TABLE III). By contrast, Sb is found to be prevalent on dc-mode particles (see. Fig. 3.). Sb data probably bring to light emissions dating from abrasions of brake linings or tyres, which obviously augment (see TABLE III). Furthermore, toxicological aspects of the impacts are considered by measurements of airborne dust. For Sb, which is comparable in its toxicological behaviour to As, chronic respiratory intoxication is known from exposure to airborne particles in the workplace [8]. With respect to our findings, most of the traffic-associated Sb impacts in terms of the ISO thoracic convention belong to the fraction, that might penetrate beyond the larynx (d_{ae} <10 μm), but only a minor proportion to the fraction that reaches the alveolar region (d_{ae}<2.5 μm, df-mode). For further assessment of the toxicological relevance of the Sb impacts in Munich, however, elucidation on the physico-chemical behaviour of the Sb compounds monitored is necessary.

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TIME TRENDS OF METAL CONTENTS OF BAVARIAN MOSSES *HYPNUM CUPRESSIFORME*

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Abstract

Since 1981, the Bavarian State Office for Environmental Protection has been operating a biomonitoring network of epiphytic mosses H*ypnum cupressiforme* which is located on a regular grid with distances 16 km. About 300 moss specimens are collected yearly (since 1991 every second year) at the end of the vegetation period, and the content of trace metals in the dry substance is measured. In this paper Ti, V, Cr, As, Cd, Hg, and Pb are treated in detail. The overall temporal trends of metal contents are described by quantile curves; the spatial distributions and their change over time are visualized by consecutive maps. The Ti, V, and Cr loads have not changed substantially since the early eighties; their spatial distributions show a clear gradient with high values in North-Eastern Bavaria. With respect to the As values, a similar spatial gradient has diminished, and the concentration quantiles have decreased up to 1995. For the rest of the metals, no clear and consistent spatial trends were found. Since the early eighties, Hg and Sb levels have been remaining constant, whereas Pb and Cd values have decreased. The temporal and spatial distributions of metals in Bavarian mosses indicate that the transport of metal-containing particles from adjacent areas in the North-East is the major common source for several metals; other known single emission sources are also reflected by the metal-specific maps.

1. MATERIAL AND METHODS

The biomonitoring network of epiphytic mosses *Hypnum cupressiforme* is located on a regular grid of 16 km x 16 km and has been operated by the Bavarian State Office for Environmental Protection since 1981. Up to 1988, the distance of sampling locations was 8 km in highly populated regions. Moss samples have been yearly collected, since 1991 every second year, at the end of the vegetation period. In 1989 no moss sampling took place. The number of locations varied between 201 and 391.

Moss sampling and analysis. At each location, four trees are selected, and moss is sampled from at least two sides of each trunk up to 1.8 m height; the annual growth increment of all location-specific specimens is combined and dried. The contents of the metals Al, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb are determined. Sb was only measured 1982-1984 and 1995, As was not measured in 1981. In this paper, we focus on the metals Ti, V, Cr, As, Cd, Hg, and Pb.

2. DESCRIPTION OF THE TEMPORAL AND SPATIAL DISTRIBUTIONS

Overall **time trends** in Bavaria are described by plotting the 5%-, 25%-, 50%-, 75%-, and 95%-quantiles of the metal concentrations against time. The quantile curves are restricted to those 121 locations at which moss specimens were taken every year, in order to avoid bias resulting from differing spatial patterns of sampling locations.

The year-specific **spatial distributions** were plotted using a color scheme which differentiates values between the overall 5%- and 95%-quantiles. Neither interpolation nor outlier elimination were performed. The temporal development of the spatial distributions is illustrated by consecutive plots of the 1984, 1986, 1988, 1990, 1993, and 1995 data.

3. RESULTS AND DISCUSSION

Titanium. Since 1985, there has been little change in titanium concentrations, with median levels of about 20-40 µg/g (Fig. 1). Higher values occurred in 1982-1984. A strong spatial gradient with high values in North-Eastern Bavaria has remained throughout the observation period (Fig. 2). Relatively high values also occur at certain locations in the East.

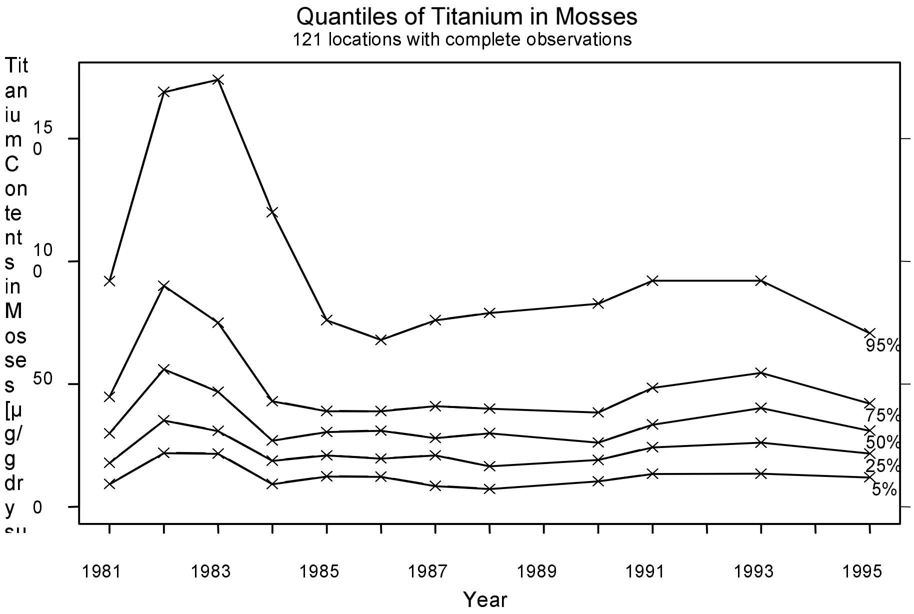

Fig. 1. Time trend of titanium.

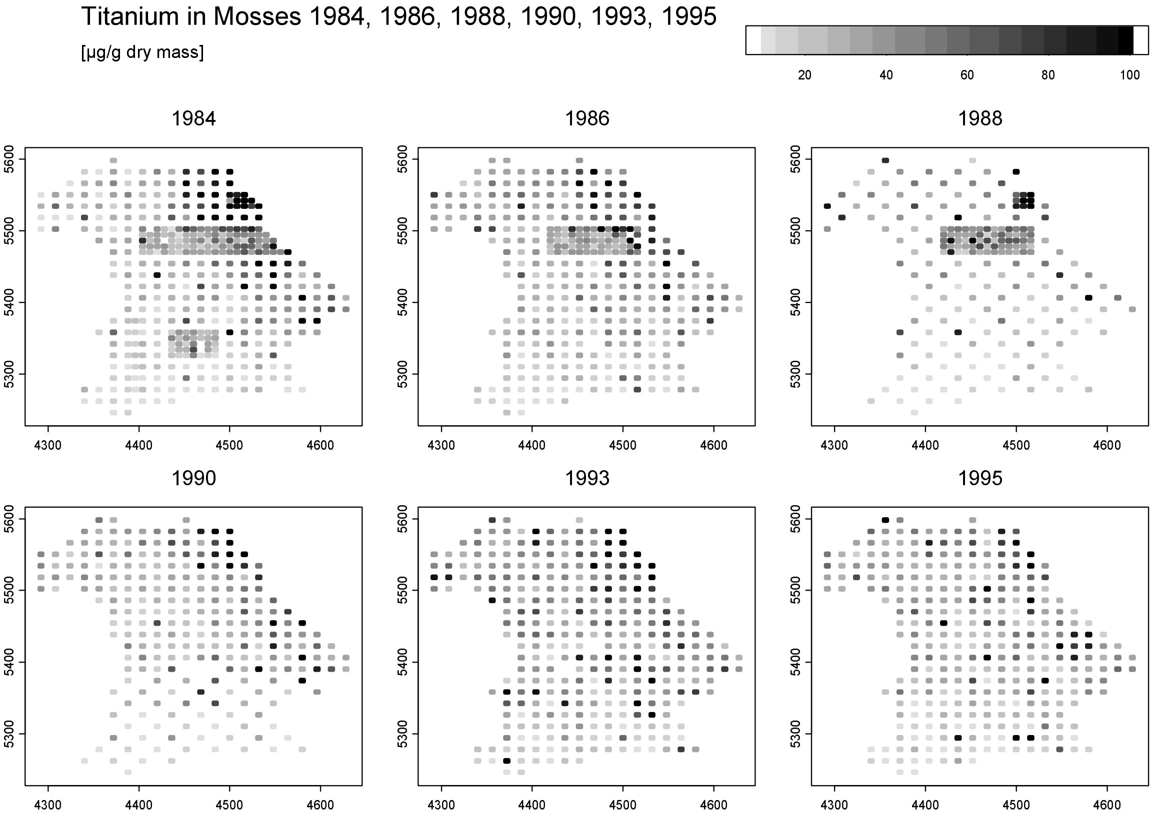

Fig. 2. Year-specific spatial distributions of titanium.

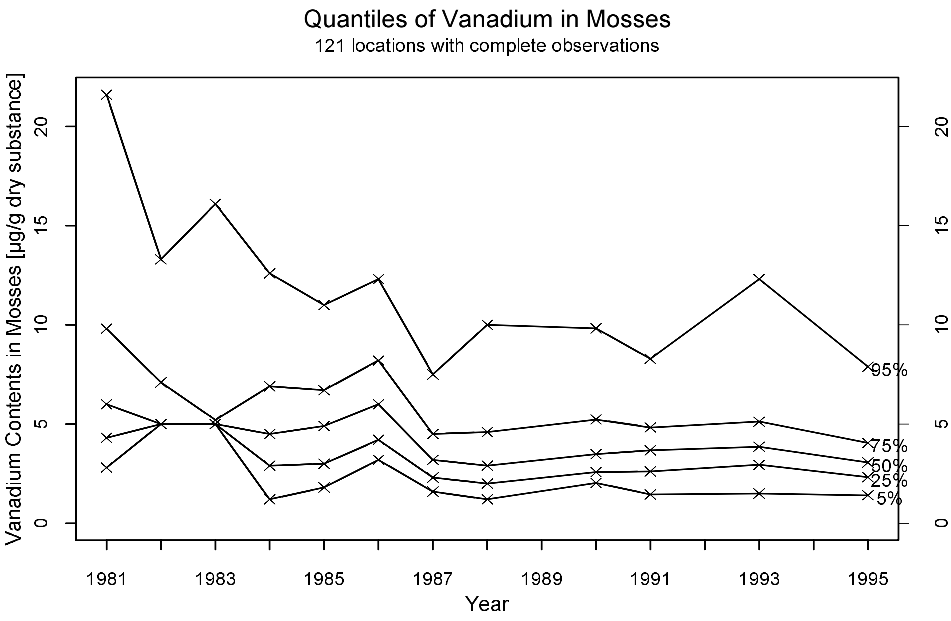

Fig. 3. Time trend of vanadium.

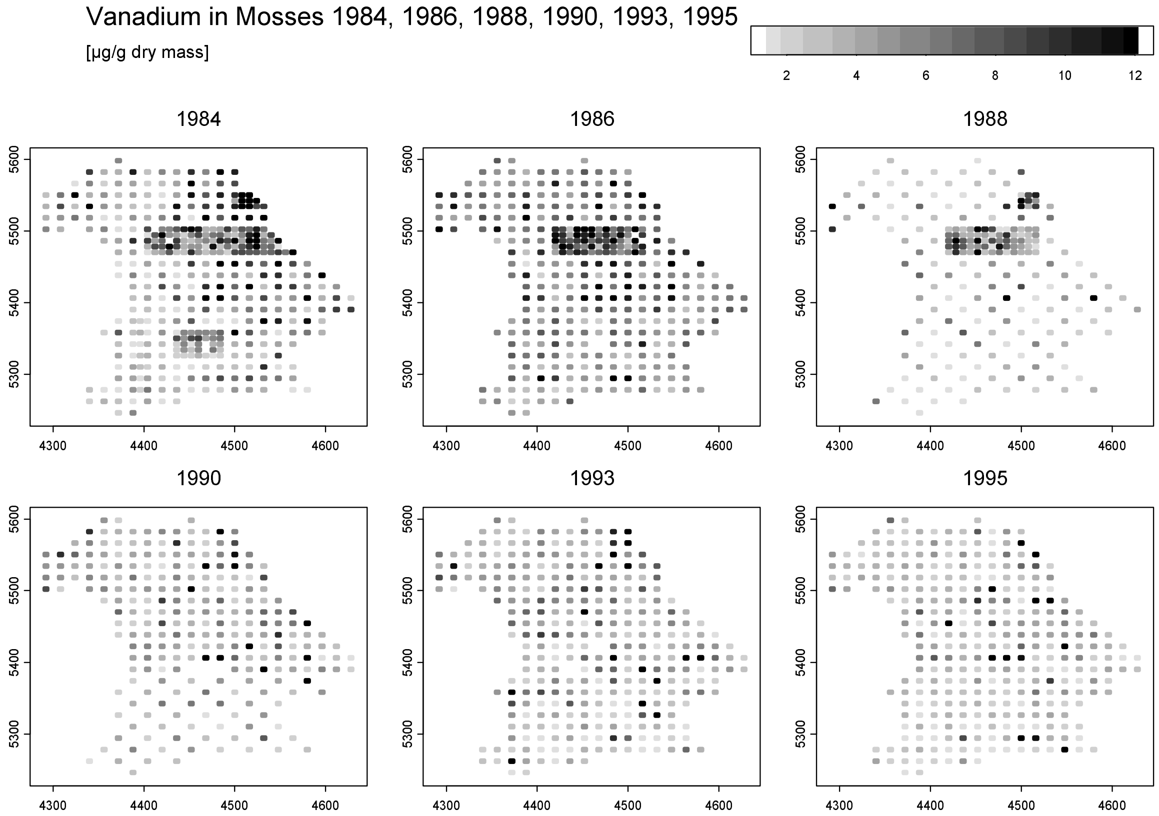

Fig. 4. Year-specific spatial distributions of vanadium.

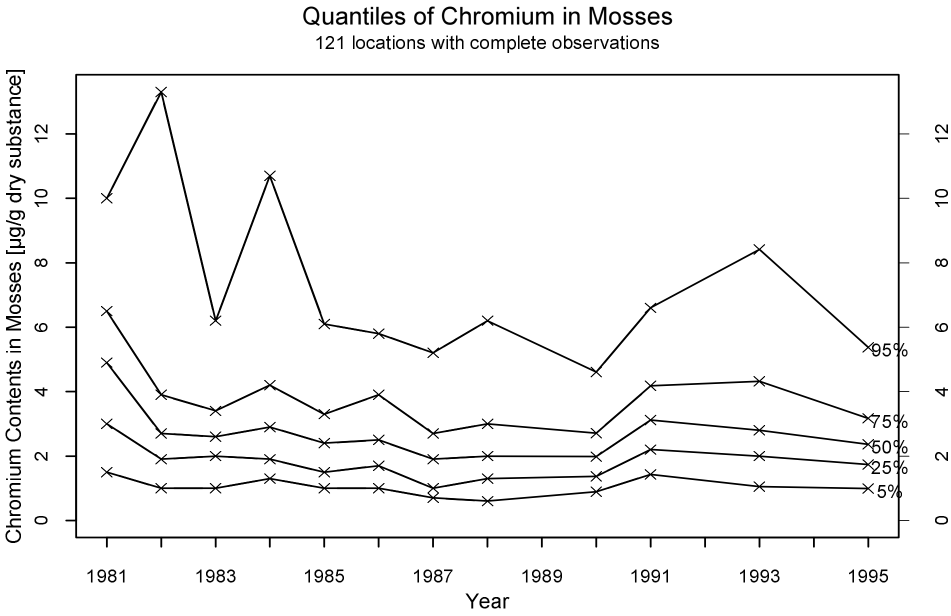

Fig. 5. Time trend of chromium.

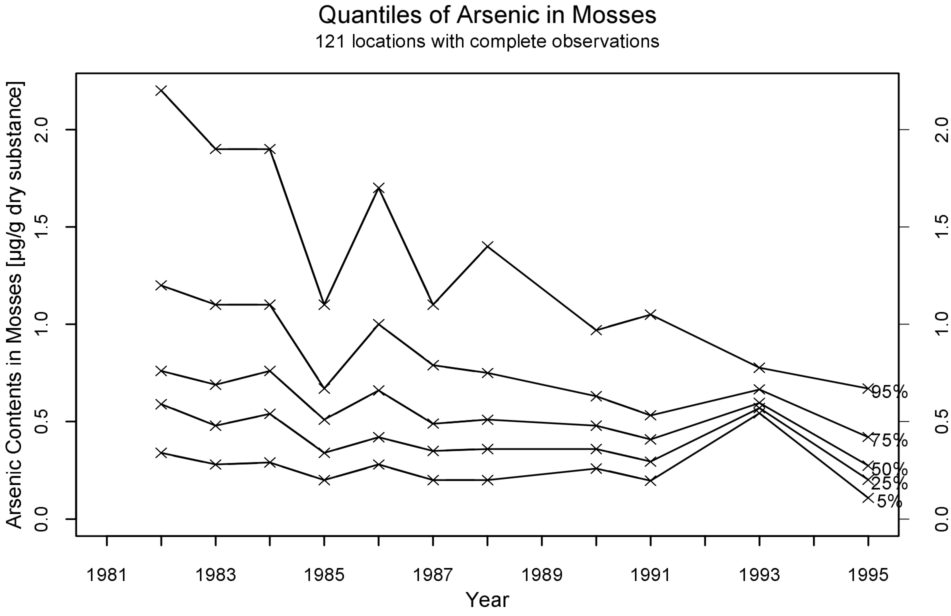

Fig. 6. Time trends of arsenic.

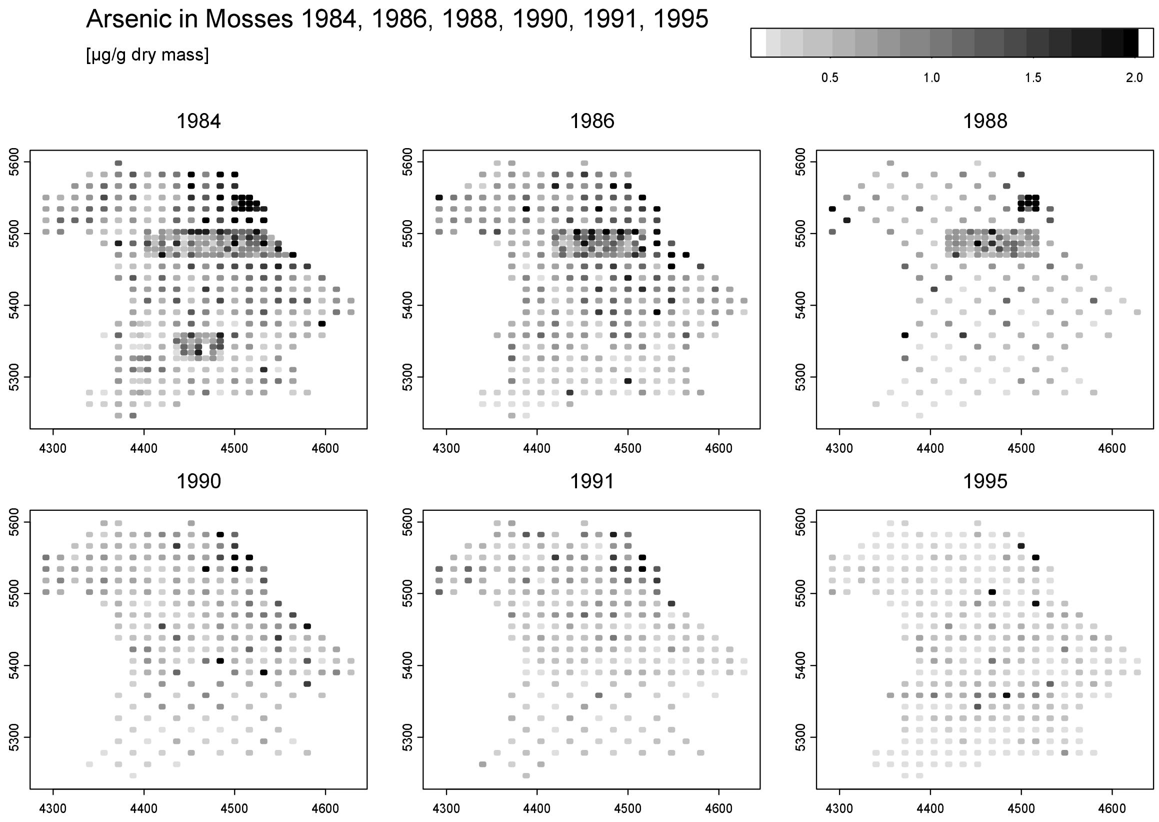

Fig. 7. Year-specific spatial distributions of arsenic.

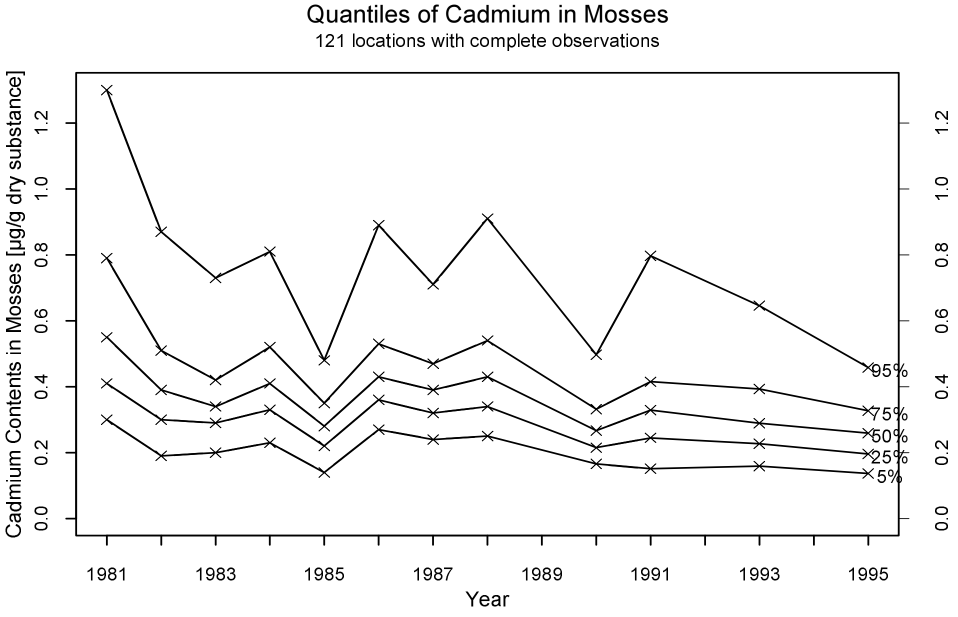

Fig. 8. Time trend of cadmium.

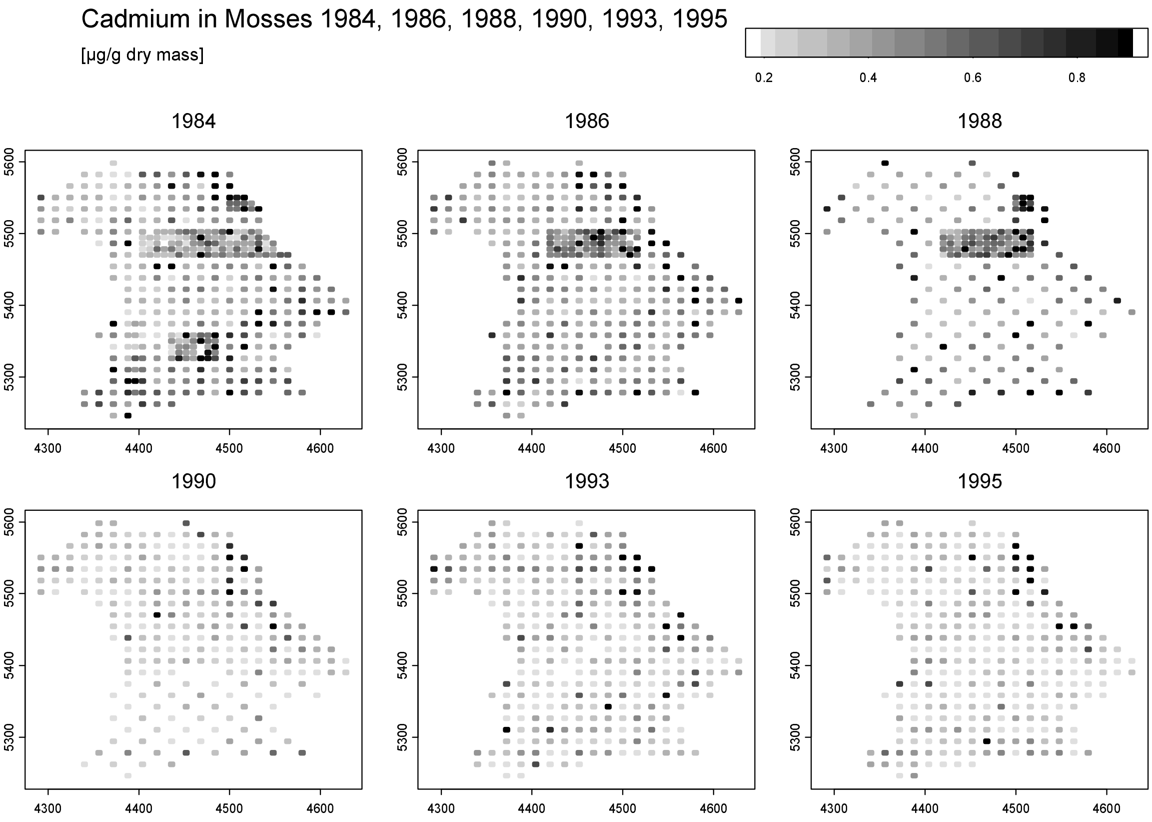

Fig. 9. Year-specific spatial distributions of cadmium.

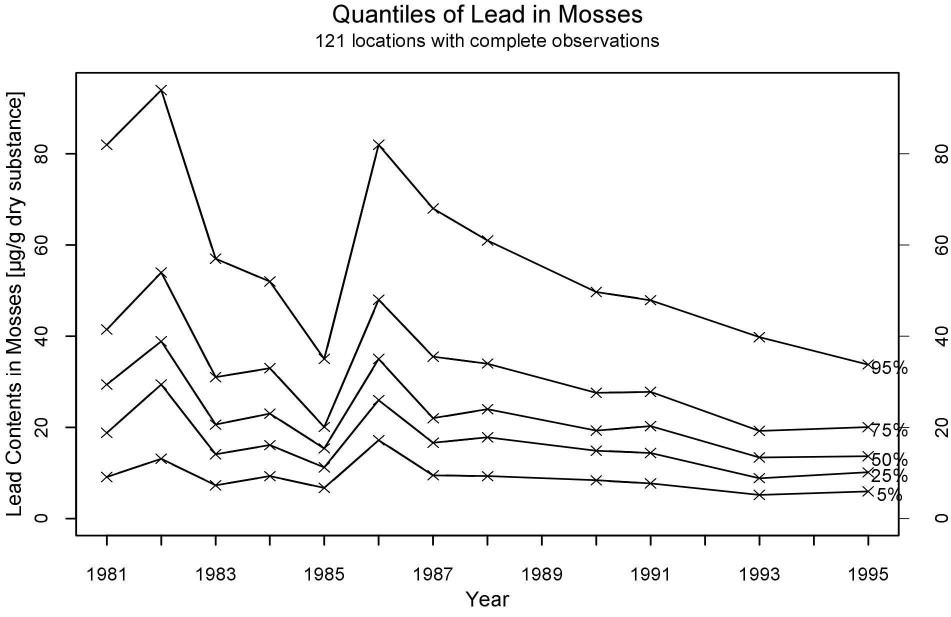

Fig. 10. Time trend of lead.

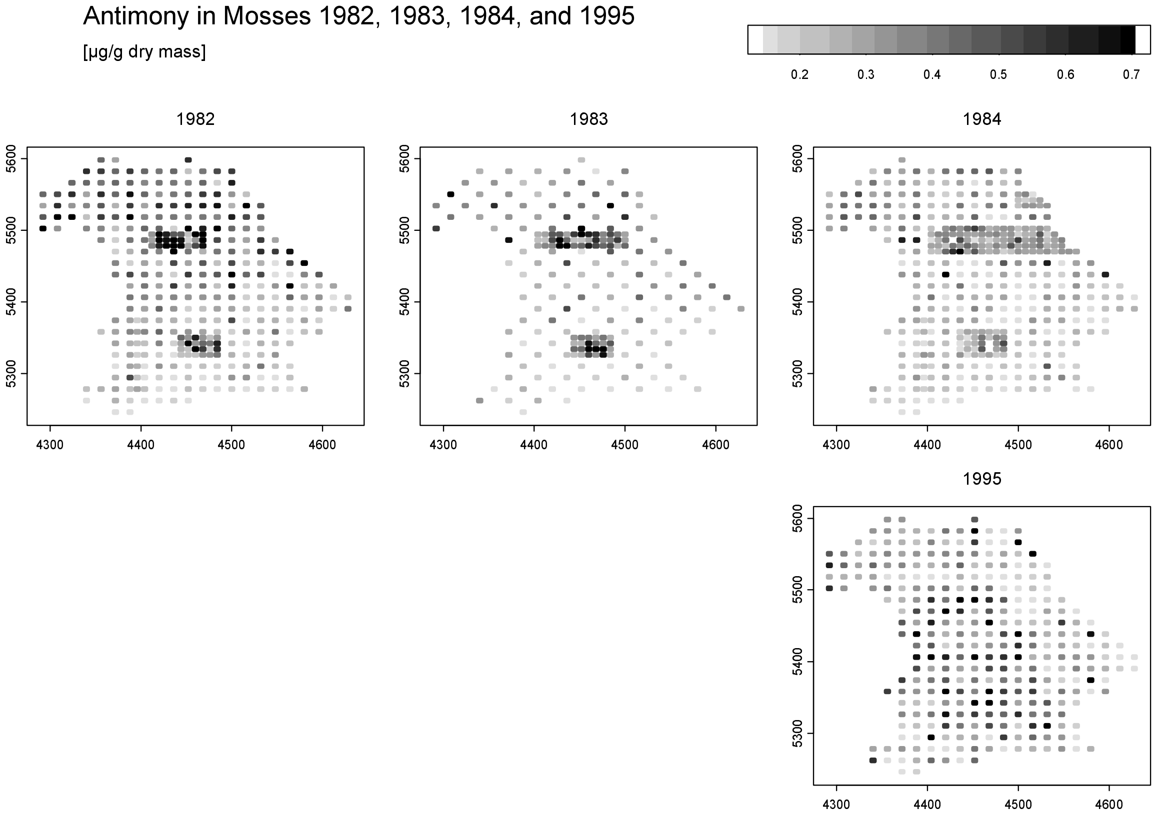

Fig. 11. Year-specific spatial distributions of antimony.

Ti is particle-associated and frequently considered as an indicator of particle pollution. The high Ti contents in mosses from the North-East clearly coincide with the generally high air pollution being drifted to Bavaria from the adjacent strongly industrialized areas in the Czech Republic and Saxony.

Vanadium. The 1982 and 1983 V values are not informative. Median concentrations decrease from about 5 µg/g before 1987 to 3-4 µg/g; since then, fluctuations have been small (Fig. 3). The North-East is higher polluted with V (Fig. 4), but with smaller contrasts as compared to Ti, and the differences have become less pronounced in the nineties. Large values have also been found repeatedly in the surroundings of Ingolstadt (coordinates 4484/5406) and Erlangen (coordinates 4452/5502).

The spatial distribution of V clearly reflects emissions from lignite (in North-Eastern Bavaria) and oil combustion (oil-processing plants near Ingolstadt).

Chromium. The percentile curves do not reveal a clear trend; throughout the observation period (except 1981), median concentrations vary between 2 and 3 µg/g (Fig. 5). The spatial distribution and its change are similar to that of V. Consistently high Cr contents were observed in mosses from Sulzbach-Rosenberg (coordinates 4484/5486).

Sulzbach-Rosenberg is the location of a large steel-processing plant, which explains the consistently high Cr values. Apart from that, the similarity to the spatial distribution of V reflects common sources of both metals.

Arsenic was not observed in 1981. In 1993, the majority of As measurements were below the analytical detection limit because most specimens had very low weight. Hence, informative data only exist for the years 1982-1991 and 1995; therefore, the map of 1991 instead of 1993 is shown. Up to 1991, the As concentrations decreased with respect to the median values (from about 0.7 to about 0.4 µg/g), this trend was especially pronounced with respect to the 95%-quantiles (from 2.2 to 1.1 µg/g) (Fig. 6). Extraordinarily high values of more than 2.5 µg/g were almost exclusively found in mosses from the North-East and occurred less frequently in later years (Fig. 7). The isolated 1995 data further support the impression of a decreasing trend and a diminishing spatial gradient.

The main source of the high As loads in the North-East is industrial and domestic lignite combustion in the Czech Republic and the former DDR. The influence of the As-containing lignite was also demonstrated by [1] and [2] in 1991 measurements of *Pleurozium schreberi*, where the highest values were found in Saxony and the Czech Republic themselves, but no gradient within Bavaria was visible in their maps. Our *Hypnum cupressiforme* measurements from the same year, however, were considerably higher (overall Bavarian median twice as high), even taking into account the regression coefficients for interspecies comparison given by [1]. Soil mineral is another possible source of As contamination in the most eastern part of Bavaria, but the concentrations reached there are considerably lower than those in the North-East influenced by lignite combustion emissions.

Cadmium. Regarding the years 1982-1995, the quantile curves reveal a tendency to lower values, with a median decrease from 0.4 µg/g to 0.25 µg/g (Fig. 8). Especially high values were observed in 1981, low values in 1985. A slight spatial trend to higher values in the North-East is visible in the maps (Fig. 9). In the rest of Bavaria, high Cd loads were found at varying locations.

Because Cd is easily water-soluble, precipitation is a strong source of variation of Cd deposition, but as well of washing off the foliage. Hence, atmospheric Cd pollution is imprecisely reflected by biomonitoring. Nevertheless, the intensive industrial activity and combustion in the adjacent Czech and Saxonian regions are indicated by the higher loads in North-Eastern Bavaria.

Mercury. After a sharp decline 1981-1983, the quantiles of Hg have remained almost constant. The spatial distribution is characterized by a considerable small-scale variation and differs strongly between years; no consistant spatial trend is visible.

This high variability might be due to the high volatility of Hg which influences the concentration not only during the vegetation period but also during sample preparation for the chemical analysis.

Lead. After a sharp decrease between 1981 and 1985 and a subsequent increase in 1986, the quantile curves follow a constant trend to lower Pb values, from median values of about 35 to about 17 µg/g (Fig. 10). By contrast to the metals considered before, the spatial distributions do not show consistent patterns over time.

Whereas it is difficult to explain the course of the quantile curves before 1986, the clear trend exhibited by the second part of the curves reflects the decreasing use of leaded gasoline, presumably the main source of lead deposition in Bavaria.

Antimony. As only data from 1982-1984 and from 1995 are available, a time trend can only tentatively be described. The 1995 quantiles reach the same levels as in the early eighties (medians of 0.3-0.4 µg/g). However, the spatial distributions differ strongly between the eighties and 1995 (Fig. 11): whereas in 1982-1984 Sb concentrations tended to be lower in Southern Bavaria (except Munich), in 1995 high values are spread all over Bavaria and seem to concentrate on the metropolitan areas of Munich and Nürnberg/Erlangen.

There is no clear interpretation of the spatial trend observed in the eighties. The change in the spatial distribution observed in 1995 might be explained by an increasing predominance of traffic impact all over Bavaria, reflecting higher traffic intensity combined with increasing use of Sb as asbestos surrogate in brake linings.

4. CONCLUDING REMARKS

Compared to published 1991 data on metal contents of *Pleurozium schreberi* in Bavaria ([1-2]), the concentrations of metals in *Hypnum cupressiforme* are generally higher and reveal stronger spatial differences. These discrepancies cannot fully be explained by interspecies differences in metal accumulation, as they remain present after correction by calibration equations given by [1].

The temporal and spatial distributions of metal contents of mosses in Bavaria indicate that the transport of metal-containing particles from adjacent areas in the North-East is the major common source for several metals. The metal-specific maps also reflect other known single emission sources. Further insight on possible sources results from the study of correlation patterns (presented in [3]).

ACKNOWLEDGEMENT

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CORRELATION PATTERNS OF Ti, V, Cr, As, Cd, Sb AND Pb IN BAVARIAN MOSSES *HYPNUM CUPRESSIFORME*

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Abstract

Since 1981, the Bavarian State Office for Environmental Protection has been operating a biomonitoring network of epiphytic mosses H*ypnum cupressiforme* which is located on a regular grid with distances 16 km. About 300 moss specimens are collected yearly (since 1991 every second year) at the end of the vegetation period, and the content of trace metals in the dry substance is measured. In order to gain insight on main sources of metal pollution in Bavaria, correlation patterns between the metals Ti, V, Cr, As, Cd, Sb, Hg, and Pb were analyzed. The year-specific correlation coefficients for each pair of metals reveal the following behavior consistently over years: Within each pair out of the metals Ti, V, Cr, and As there is a strong and consistent linear relationship; Hg and Cd do not correlate with any other metal; Pb and Sb show moderate correlations to the rest of the metals except Hg and Cd. Higher-dimensional correlation patterns were studied by means of principal component analysis with varimax rotation. Results are presented for the 1984 and 1995 data with the Hg variable excluded, results from the rest of the years were similar. At least 82% of the variance could be explained by three principal components. The following three factors were extracted by varimax rotation from both data sets: one factor with similar loadings for Ti, V, Cr, and As; one factor loading highly on both Sb and Pb; one factor representing mainly Cd. The spatial distribution of the first factor integrates the common gradient with high values in the North-East of Bavaria, indicating that industrial and domestic lignite combustion in the adjacent regions is an important source of those metals.

1. INTRODUCTION

This work presents further results on the metal contents of Bavarian mosses *Hypnum cupressiforme* introduced in Ref. [1]. Details on moss sampling and analysis are given there.

Correlations persisting in space and time are likely to reflect common sources of the metals involved. In order to gain insight into the main origins of metal pollution in Bavaria, correlation patterns between pollutants of interest – Ti, V, Cr, As, Cd, Sb, Hg, and Pb – were analyzed. Higher-dimensional correlations were studied by principal component analysis.

Factor-analytical methods have already been applied to element measurements in mosses and lichens. Refs. [2] and [3] report on principal component analyses with varimax rotation applied to 26 elements (not including Ti) in *Hylocomium splendens* in Norway. Most of the metals considered in this paper (V, As, Cd, Sb, Hg, Pb) load highly on the first factor found in the Norwegian study. This factor revealed the highest score values in the South and was interpreted as combination of pollutants emitted from different sources in adjacent regions and drifted to Norway by long-range atmospheric transport.

Highly sophisticated factor analyses with *Pleurozium schreberi* data were conducted in the Netherlands [4-5]. As the data included repeated observations from selected locations, local variation of the mosses' metal content could be estimated. These estimates allowed inference on uncertainties of the factor model by applying Monte Carlo methods.

For all analyses reported here, a logarithmic transformation was applied to the data because most metal- and year-specific distributions were skewed.

2. CORRELATIONS BETWEEN PAIRS OF METALS

In Figures 1-6, year-specific scatterplots and PEARSON correlation coefficients are given for each pair of the metals Ti, V, Cr, As, Cd, Sb, Hg, and Pb and for the selected years 1984, 1986, 1988, 1990, 1993 and 1995. Sb measurements were only available from 1984 and 1995; the 1993 As measurements were not informative.

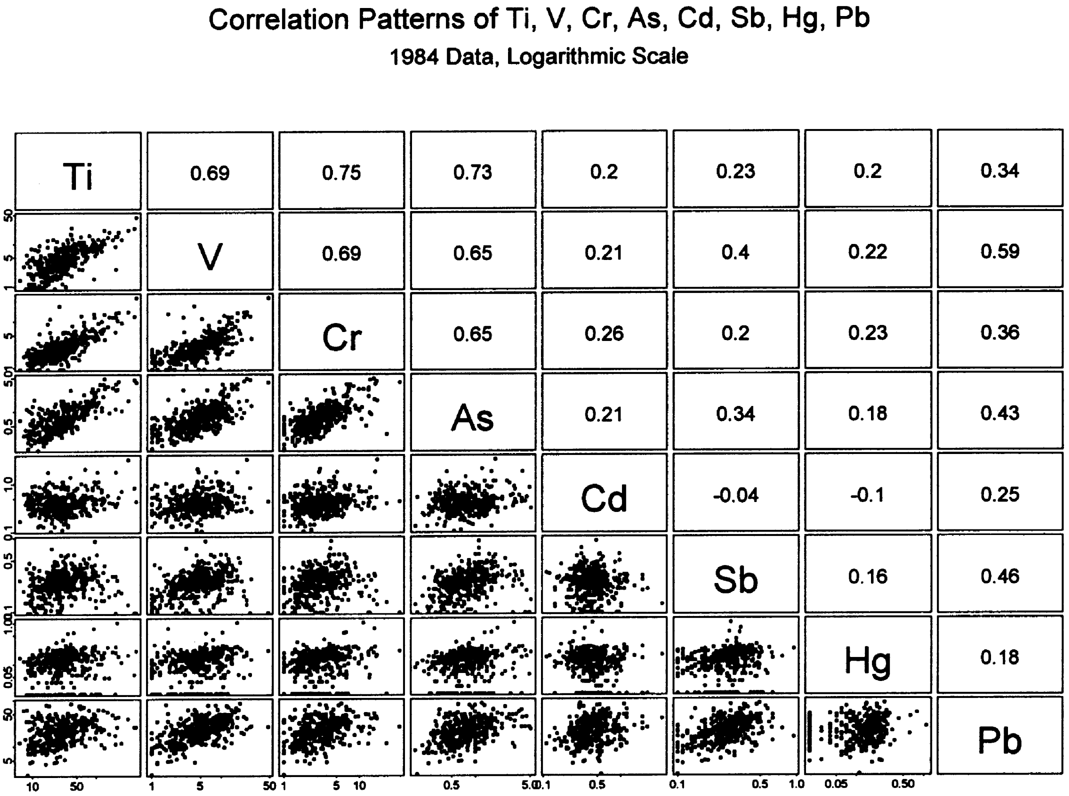

Fig. 1. 1984 correlation patterns.

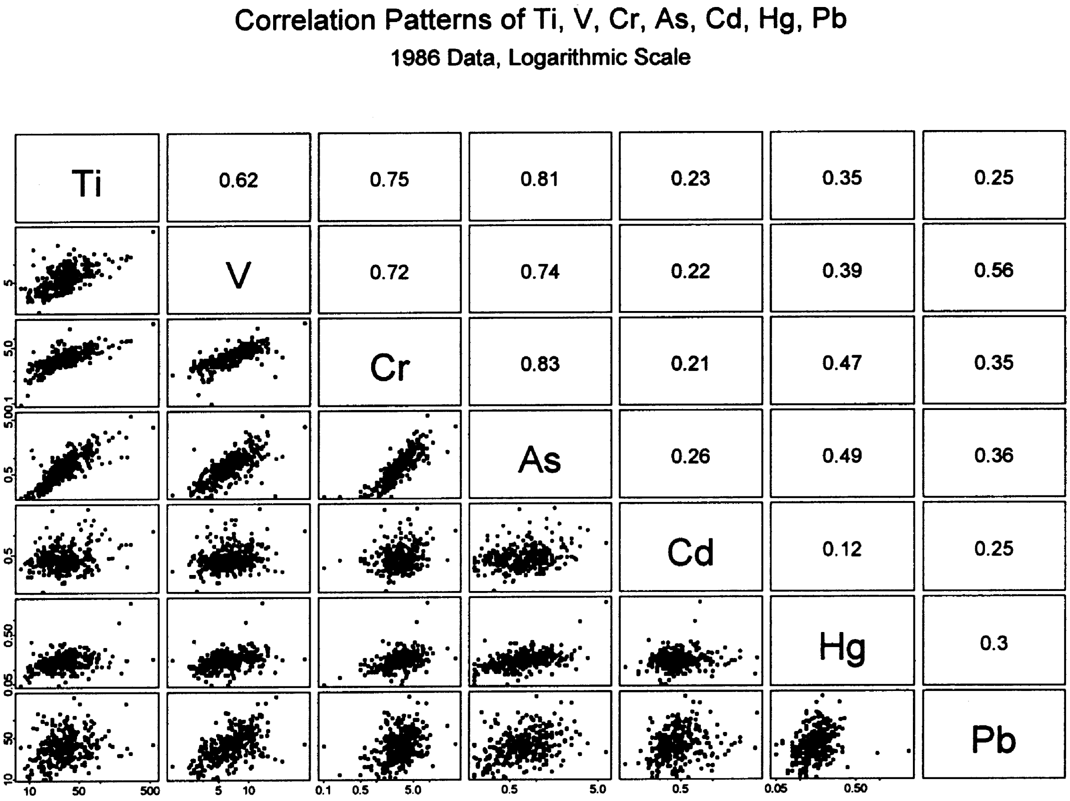

Fig. 2. 1986 correlation patterns.

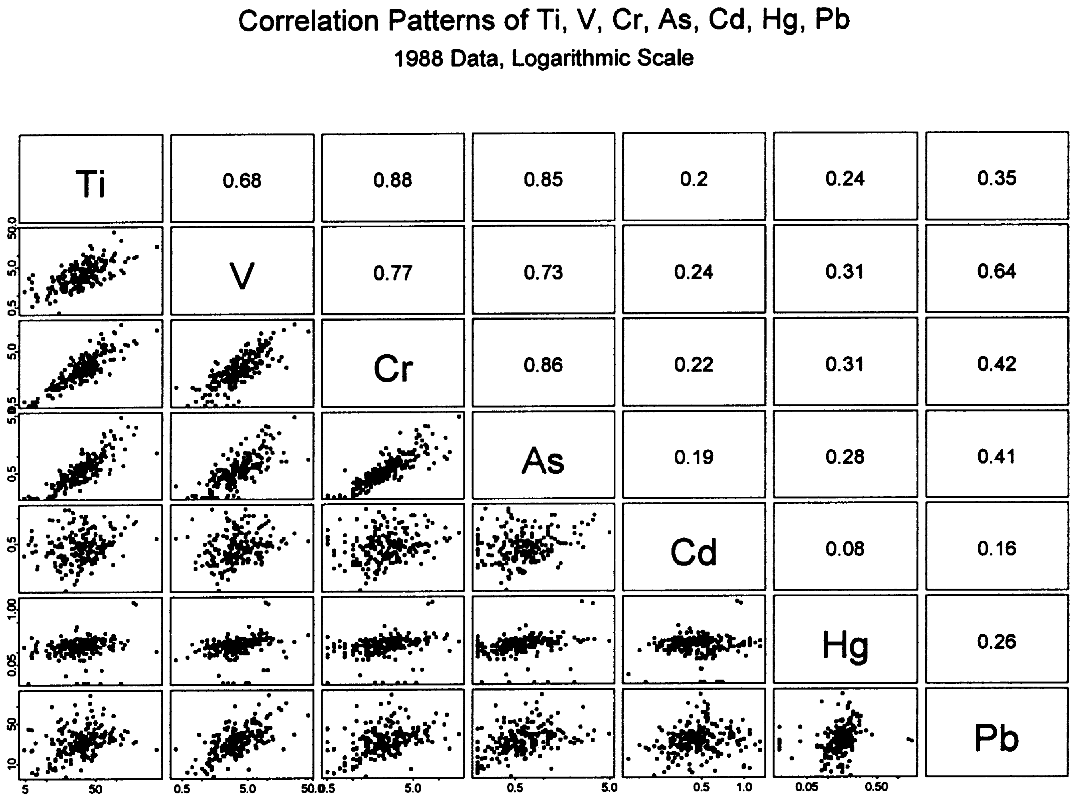

Fig. 3. 1988 correlation patterns.

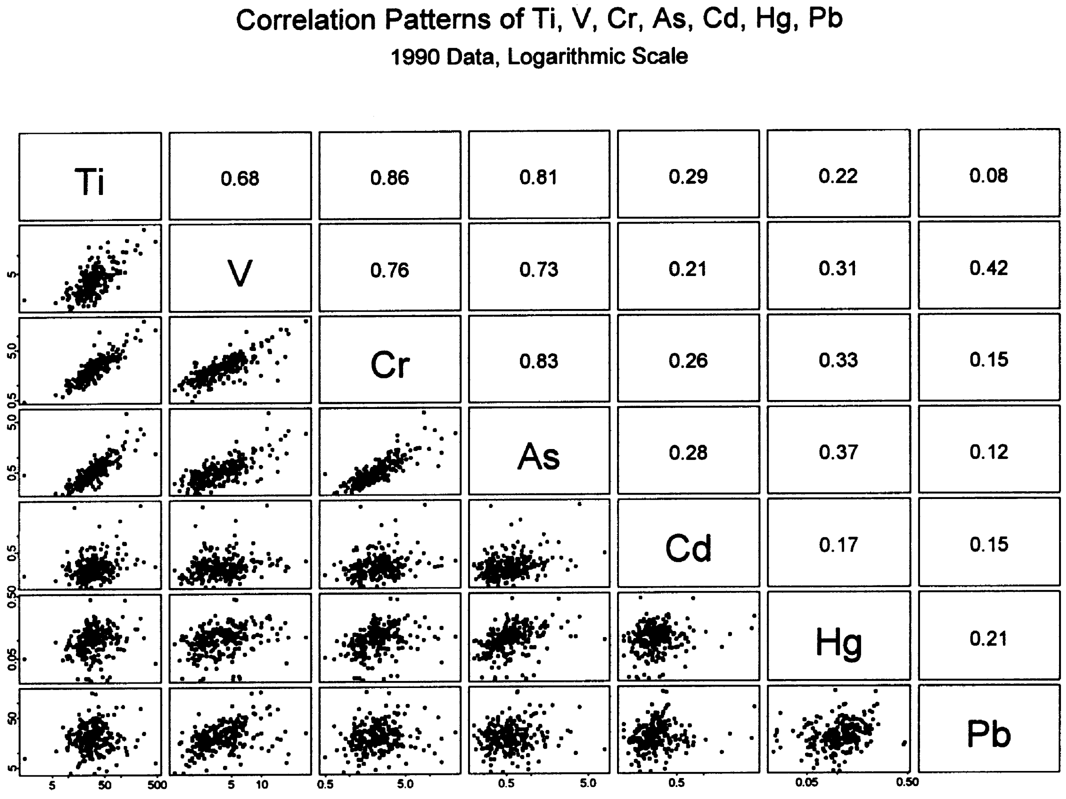

Fig. 4. 1990 correlation patterns.

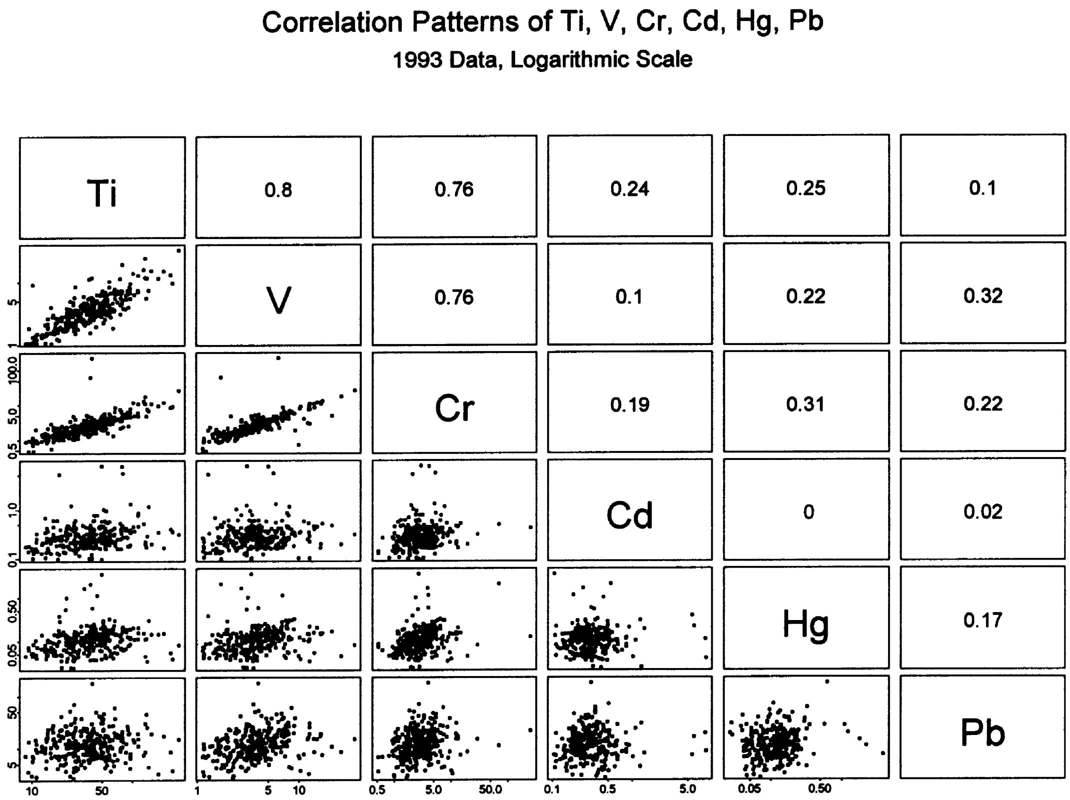

Fig. 5. 1993 correlation patterns.

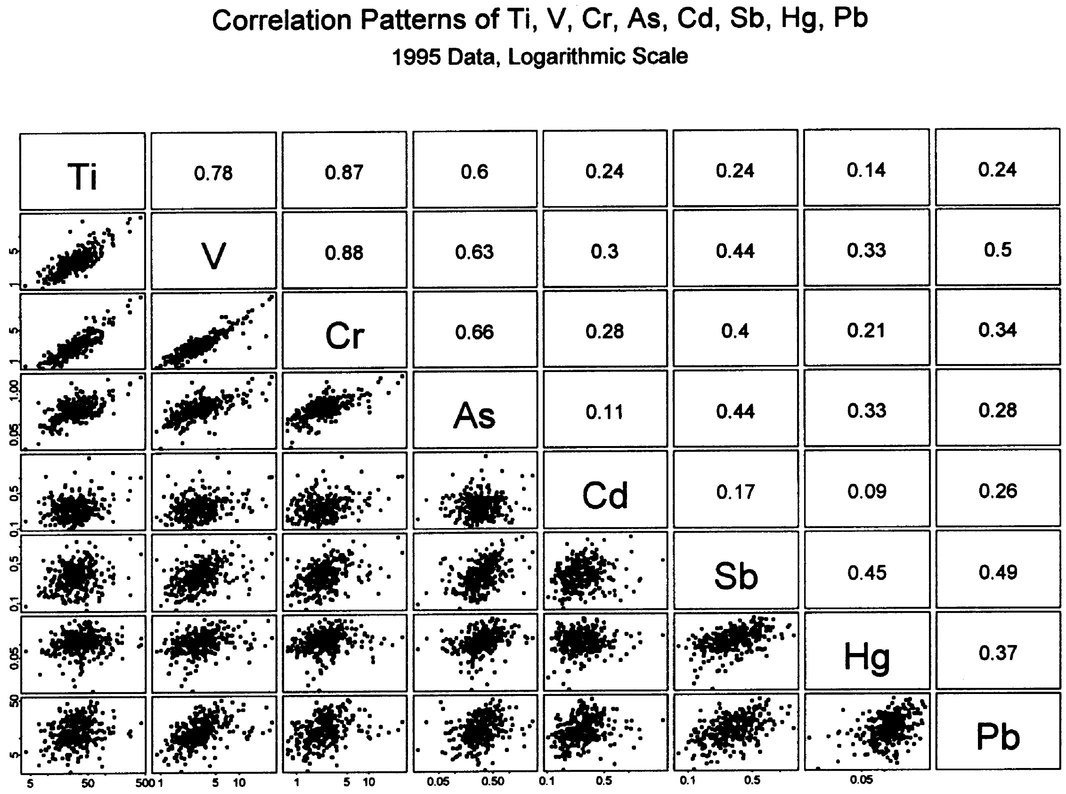

Fig. 6. 1995 correlation patterns.

TABLE 1. COEFFICIENTS OF PRINCIPAL COMPONENTS, 1984 DATA

	Principal Component						
Elements	1	2	3	4	5	6	7
Ti	0.44	-0.22	-0.31			-0.17	0.79
V	0.46			0.28	0.34	-0.68	-0.36
Cr	0.43	-0.26	-0.22		0.45	0.66	-0.24
As	0.44		-0.18	-0.25	-0.75		-0.38
Cd	0.18	-0.50	0.77	-0.34			
Sb	0.25	0.71	0.13	-0.60	0.22		
Pb	0.35	0.35	0.46	0.62	-0.24	0.27	0.21
eigenvalue	1.92	1.05	0.96	0.70	0.59	0.49	0.46
cumulative proportion of variance	0.52	0.68	0.82	0.88	0.93	0.97	1

TABLE 2. LOADINGS OF THE FIRST THREE VARIMAX ROTATED COMPONENTS, 1984 DATA

	Factor		
Elements	1	2	3
Ti	0.57	-0.10	
V	0.40	0.24	
Cr	0.54		
As	0.47		
Cd			0.94
Sb		0.73	-0.24
Pb		0.62	0.25
cumulative proportion of variance			0.82

For most pairs of variables, the correlation coefficients behave rather consistently over years. The following groups of variables can be distinguished:

- **Ti, V, Cr, As.** Each metal in this group shows a strong and consistent linear relationship with one another, with correlation coefficients of 0.7 and more. The 1995 correlations of As with the other metals are slightly weaker than before.
- **Hg and Cd with other metals.** For pairs of metals involving Hg or Cd, low correlation coefficients between 0 and 0.3 were found predominantly, with a zero correlation for the pair Hg/Cd itself.
- **Pb and Sb with other metals except Hg and Cd.** The correlations between these pairs of variables were low to moderate (0.3-0.5). The strongest linear relationships occurred for the pairs V/Pb and Sb/Pb.

3. PRINCIPAL COMPONENT ANALYSES

Correlation patterns of metals in Bavarian mosses were further studied by year-specific principal component analyses (PCA) for each of the years when moss sampling took place. PCA was performed for the correlation matrix, i.e. the variables were transformed to unit variance. Components with eigenvalues greater than or near to 1 – representing a variance at least as large as the average variance – were selected and subject to a varimax rotation.

TABLE 3. COEFFICIENTS OF PRINCIPAL COMPONENTS, 1995 DATA

	Principal Component						
Elements	1	2	3	4	5	6	7
Ti	0.43	-0.40	0.11	-0.11	0.20	0.68	0.37
V	0.48			-0.24	0.13	-0.69	0.47
Cr	0.47	-0.24			0.26	-0.11	-0.80
As	0.40	-0.15	-0.29	0.36	-0.78		
Cd	0.19	0.30	0.88	0.29	-0.15		
Sb	0.30	0.51	-0.36	0.56	0.44		
Pb	0.28	0.63	0.11	-0.64	-0.23	0.22	
eigenvalue	1.92	1.04	1.00	0.77	0.62	0.40	0.29
cumulative proportion of variance	0.54	0.70	0.83	0.91	0.96	0.99	1

TABLE 4. LOADINGS OF THE FIRST THREE VARIMAX ROTATED COMPONENTS, 1995 DATA

	Factor		
Elements	1	2	3
Ti	0.57	-0.19	
V	0.47	0.12	
Cr	0.53		
As	0.41	0.14	0.27
Cd			-0.94
Sb		0.68	0.13
Pb		0.68	-0.13
cumulative proportion of variance			0.83

For this presentation, we focus on the results of 1984 and 1995. These years were selected because Sb measurements were available and because by 1984 certain irregularities occurring in the first years of moss biomonitoring had disappeared. The results from the rest of the years did not differ substantially from those presented here.

In a first run of PCA, Hg was included in the variable set. The high variability of Hg measurements mentioned in the other communication to this workshop [1] and the low correlation with any other variable lead to a common feature of all year-specific analyses: One of the rotated principal components turned out to be almost identical to the variable Hg itself. Because of this orthogonality of Hg to the rest of the data we excluded it from the PCA runs presented here.

4. RESULTS AND DISCUSSION OF THE PRINCIPAL COMPONENT ANALYSES

In Tables 1-2, the results of the PCA and subsequent varimax rotation are given for the 1984 data, in Tables 3-4 for the 1995 data. Only loadings >0.1 are reported. For each year, the first three principal components belong to eigenvalues larger than or near to 1 and they explain at least 82% of the variance in the data. The varimax rotations exhibit the following common features of both data sets:

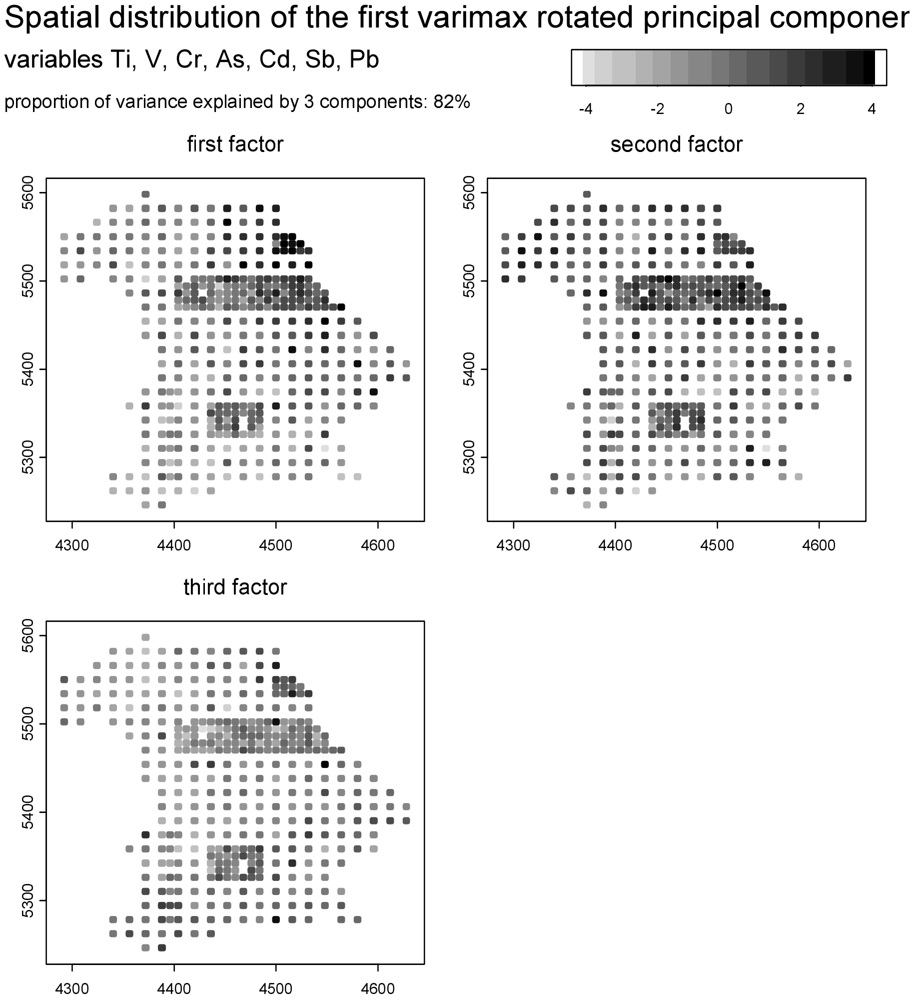

Fig. 7. Spatial distribution of the first three varimax rotated principal components 1984.

– one factor with similar loadings for Ti, V, Cr, and As;
– one factor loading highly on both Sb and Pb;
– one factor representing mainly Cd.

Figures 7-8 show the spatial distributions of the varimax rotated principal components for the years 1984 and 1995, respectively. The most important features of the maps are:

The **first factor** integrates the similar spatial behavior of Ti, V, Cr, and As, as demonstrated by the element-specific maps in [1]. The 1984 data reveal a strong spatial gradient with high values in the North-East. This gradient is less pronounced in the 1995 data, which in turn reveal more local variation of the first factor. This result further supports the impression that in the North-East of Bavaria, where neither industry nor dense population are located, deposition of Ti, V, Cr, and As results from a mixture of pollutants emitted by lignite combustion and other industrial processes in the adjacent Saxonian and Czech regions, whereas in the rest of Bavaria various local sources account for high values of this factor. Three of the elements involved – V, Cr, and As – are known to be emitted by combustion of oil and coal, especially lignite with its well-known high As contents. The lower

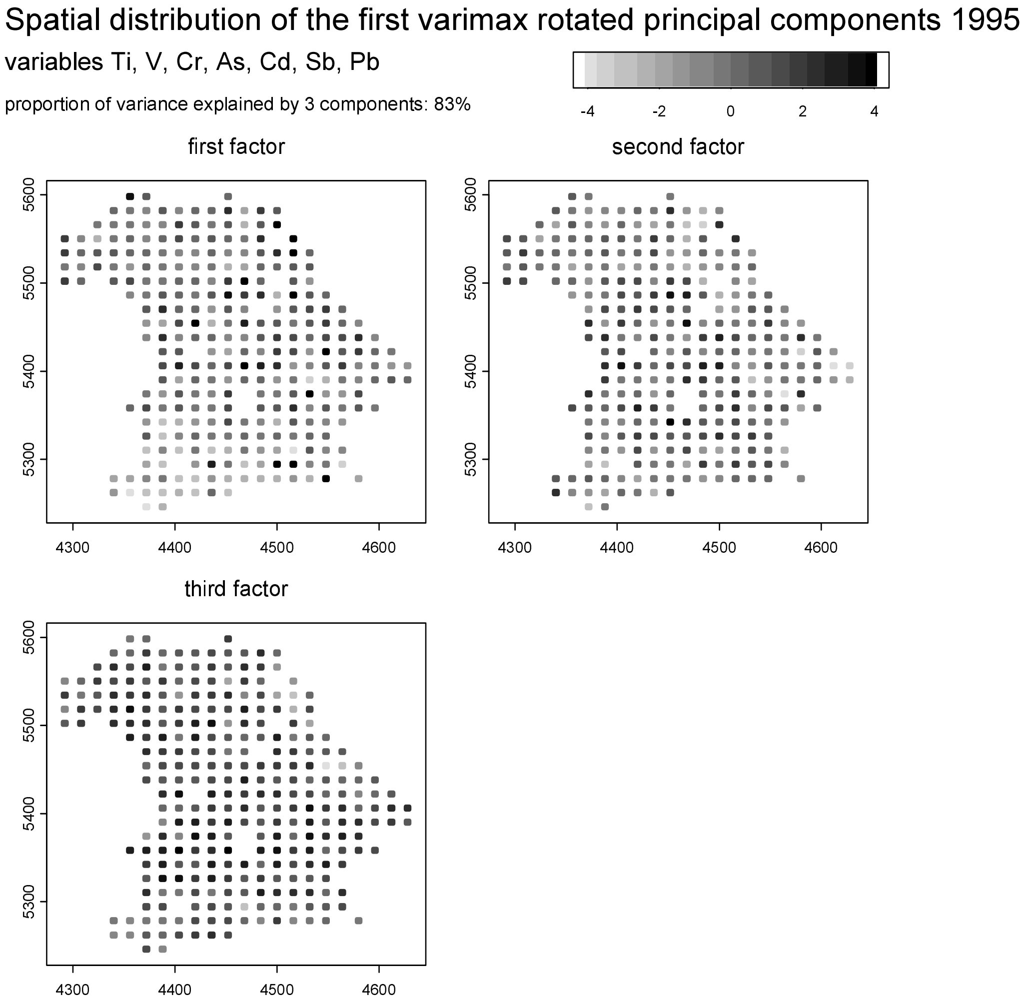

Fig. 8. Spatial distribution of the first three varimax rotated principal components 1995.

1995 correlations of As with V and Cr – coinciding with considerably lower As concentrations in 1995 as compared to the eighties – possibly result from a decrease of lignite use in the nineties. The high correlations of those combustion-associated pollutants with Ti, which has frequently been considered as indicator of soil particles, suggest that there might be in fact common emission sources of all four metals.

The spatial distribution of the second factor, representing the intercorrelated metals Sb and Pb, differs considerably between 1984 and 1995 and does not correspond to known differences neither in population density nor in the intensity of industrial processes. Despite the fact that moss sampling was performed at a minimum distance of 300 m from main roads, this factor might reflect a contribution of traffic emissions to general pollution.

The third factor mainly represents the Cd deposition and therefore is similar to the respective Cd maps in Ref. [1]. As pointed out there, the Cd content in mosses is subject to high variability due to the physico-chemical properties of this metal. This might have led to very low correlations with other metals and consequently to Cd being represented by a separate factor, although there are in fact common sources.

5. SUMMARIZING DISCUSSION

In [6], correlation matrices of the 1991 moss survey in Western Germany and in the Southern part of the former DDR are reported. Comparison to our 1991 results exhibits remarkable differences in all correlations involving elements of the first factor (Ti, Cr, V, As): The correlations from "Southern DDR" are substantially higher than those from Western Germany, but only slightly higher than those from Bavaria. As the results from the former DDR apparently reflect the predominance of industrial and domestic lignite combustion as emission source in those regions adjacent to North-East Bavaria, the similarity to Bavarian correlation patterns further supports the interpretation that transport of lignite combustion products is an important source of those metals in Bavaria.

ACKNOWLEDGEMENT

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