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

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FOREWORD

Certain types of organisms integrate pollution over time, reducing the need for continuous chemical monitoring, thus avoiding the difficulty of interpreting "snapshot" measurements and offering the potential of retrospective monitoring. Such organisms enrich the substance to be determined so that the analytical accessibility is improved and the measurement uncertainty reduced. By observing and measuring the changes in an appropriately selected organism, a conclusion as to the kind of pollution, its source, and its intensity can be drawn.

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 on human health, and one of the main emphasis is on studying air contaminants. The IAEA has been systematically supporting biomonitoring atmospheric pollution for 10 years in the framework of its project on Environmental Pollution Monitoring and Research Using Nuclear and Related Analytical Techniques. The objective of this project is to identify the source and evaluate the fate of key non-radioactive environmental contaminants and provide the basis for improved health for human populations. The project has been implemented through a Co-ordinated Research Project on Validation and Application of Plants as Biomonitors of Trace Element Atmospheric Pollution Analysed by Nuclear and Related Techniques, several technical co-operation projects, and some dedicated analytical quality control activities.

Within the scope of these efforts, the Second International Workshop on Biomonitoring of Atmospheric Pollution (with Emphasis on Trace Elements) — BioMAP, was organized as a follow-up to the 1997 BioMAP workshop held in Lisbon, Portugal. The proceedings of the first workshop were published in IAEA-TECDOC-1152. The second workshop was held in Praia da Vitória, Azores Islands, Portugal, from 28 August to 3 September 2000. It was organized in co-operation with the Instituto Tecnológico e Nuclear (ITN), Sacavém, Portugal and the Universidade dos Açores, Azores Islands, Portugal. The workshop was attended by scientists dealing with general and specific (e.g. biomonitoring) issues of air pollution. Topics included goals and quality assessment of biomonitoring surveys, the applicability of bio-organisms in both qualitative and quantitative senses, response modelling, the use of multi-element analytical techniques, interpretation of results for specific pollutants, and the use of appropriate statistical tools for detailed data interpretation. This TECDOC contains the papers presented at the Workshop.

It is hoped that this publication will further 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.Th. Wolterbeek, Interfaculty Reactor Institute of the Delft University of Technology, Netherlands, B. Smodiš, IAEA, J.C. Soares and M.C. Freitas, ITN, Sacavém, Portugal.

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

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BACKGROUND

Air pollution is a serious problem in many parts of the world, affecting people and the economy of countries, leading to forest decline, loss in agricultural production and diminished status of the population. A wide array of substances including greenhouse gases, organic dust, and particulate matter is being emitted from natural and anthropogenic sources. The main global concern is on the climatic effects of greenhouse gases. However, airborne particulate matter (APM) is more directly affecting human health, leading to the need for strict governmental regulations on air pollutants. Namely, after emission, the pollutants are subjected to physical, chemical, and photochemical transformations, which ultimately decide their fate and atmospheric concentrations. Air pollutants do not remain confined near the source emission, but spread over distances, transcending natural and political boundaries depending upon topography and meteorological conditions, especially wind direction and speed, and vertical and horizontal thermal gradients.

There are two conceptual approaches for collecting samples relevant to air and atmospheric deposition related pollution studies: (1) the direct collection of APM, precipitation and total deposit, and (2) the use of air pollution biomonitors. The first approach is aimed at quantitative surveys at local, short-range, medium-range or global transport of pollutants, including health-related studies when collecting size-fractionated APM. It requires continuous sampling on a long-term basis at a large number of sites, in order to ensure the temporal and spatial representativeness of measurements. The application of such direct measurements on a large scale is extremely costly and person-power intensive. Furthermore, it is not possible, due to logistic problems, to install instrumental equipment at all needed locations. Therefore, the second approach is considered as a non-expensive, yet reliable means of air quality status assessment in a country or a region. Certain types of biological organisms provide a measure of integrated exposure over a certain amount of time and enrich the substance to be determined so that the analytical accessibility is improved and the measurement uncertainty reduced. Sampling is relatively simple and no expensive technical equipment is needed. In this context, biomonitoring is defined as a continuous observation of a geographical area with the help of suitable organisms that reflect changes over space and time (e.g. by their element content).

Biomonitoring may therefore be used in parallel to approaches and techniques based on dispersion modelling or air sampling. However, dispersion and deposition modelling ultimately depends on a priori knowledge of sources and meteorology, and air sampling requires expensive equipment, especially in larger-scale surveys. Biomonitoring relies on the common and permanent availability of bio-species in the field, the relative ease of sampling, the accumulative and time-integrative behaviour of selected bio-species and the absence of any need for in-field expensive equipment. Main topics in biomonitoring research concern the selection of appropriate biomonitor species, the dose-response relationships, and the possible interferences in using this tool.

THE WORKSHOP

The Second International Workshop on Biomonitoring of Atmospheric Pollution (BioMAP) was the follow-up to the 1997 BioMAP workshop held in Lisbon, Portugal. The second workshop was organized by the Instituto Tecnológico e Nuclear (ITN), Portugal, in collaboration with the International Atomic Energy Agency (IAEA), Austria, and the Universidade dos Açores, Azores Islands, Portugal. Central point of attention was biomonitoring air pollution, with emphasis on trace elements and on quantification of relationships between the concentrations of pollutants in the atmosphere and their levels in biomonitoring organisms.

The workshop addressed general survey topics such as the goals of (bio)monitoring, the use of analytical techniques, and the handling of the vast amounts of data obtained. In addition to the general and specific issues related to biomonitoring as a technique, this workshop specifically addressed the potential use of biomonitoring data in assessing the human exposure to toxic substances.

The workshop comprised oral sessions on (1) air quality management, (2) biomonitoring modelling, (3) international trends in biomonitoring, (4) biomonitoring of trace gases, (5) the use of mosses and other species, and (6) analytical aspects. In addition, the workshop comprised a poster session, and a round table discussion on the potential of biomonitoring data to be used in association with epidemiological databases. The workshop was attended by participants from 22 countries throughout the world.

The workshop addressed air quality management instruments, such as the EU Framework Directive (FWD), which requires an evaluation programme on environmental air quality, including the monitoring of air quality, the inventories of emissions and the modelling of pollution dispersion. Presentations emphasized the relationships between air pollution and diseases and mortality, thereby underlining the relevance of relating and linking (bio)monitoring data on air pollution to epidemiological information. A round table discussion on these issues pointed towards the necessity for interdisciplinary approaches: overall survey set-ups should include experts from environmental, biological, chemical-analytical, data-analytical, and medical fields. Moreover, from a medical "end-users" point of view, epidemiologists should be regarded as being of central importance in any set-up of joint research.

Various routinely used ways to obtain and interpret biomonitoring data were presented and discussed; most were intended to quantify relationships, to indicate emission source profiles, or to show first-ever results in various regions. Here, attention was not only focused on elements but also on radionuclides. In addition, attention was also given to more basic biochemical and physiological backgrounds for interelement relationships: the so-called Biological System of the Elements (BSE) and the Stoichiometric Network Analysis (SNA) were presented and discussed in the context of reducing background topologies of complex interactions into skeleton topologies around which the biosystem dynamics can be understood.

Biomonitor behaviour was modelled in simple-model approaches, thereby bridging the commonly detailed physiology-oriented laboratory studies and the more pragmatic approaches in fieldwork. In modelling, emphasis was on the dynamics of accumulation, thereby considering the simultaneous operation of processes of both influx and efflux. In explaining lichen's accumulation behaviour, rainwater was recognized as significantly affecting eventual element concentrations. Effects generally ranged from positive at light rainfall conditions to negative at heavy rainfall conditions; rain conditions may be specifically relevant for mobile elements such as sodium, potassium etc.

In parallel to the well-known moss-bags method, mosses were used while kept alive in a so-called moss-plate approach. Moss vitality was checked by the assessment of the chlorophyll fluorescence. Moss elemental data were compared to deposition data; furthermore, the in vivo distribution of accumulated elements were investigated by Synchotron induced X ray fluorescence analysis (SXRF). The results indicated element specific distributions along the shoots: Pb, Ti, Cl and Se were shown to short-term accumulate mainly in the old plant parts.

The workshop addressed the various ways to assess the impact of air pollution on biomonitor organisms. Underlying reasoning was that results could be used directly in dose-response relationships, and that they could also be used to ensure comparable organism vitalities over larger survey dimensions (e.g. geography, time, season). Methods used, reported and proposed, comprised ion leakage, photosynthetic activity, chlorophyll integrity, spectral reflectance, potential quantum yield, or the normalised difference vegetation index (NDVI). Comparable vitalities were felt as contributing to the comparability of biomonitor responses within larger survey scales.

Metal chemical speciation studies were discussed both on the basis aerosol measurements and on measurements of lichens. In aerosol samples sequential leaching was applied and in lichens metals were determined as associated to water-soluble metallothioneins. Both studies were aimed at pinpointing possible elemental pollution sources. In addition, LAMS (laser ablation/ionisation mass spectrometry) was applied for aerosol characterisation and source apportioning. By using LAMS in combination with TOF (time of flight) approaches, work could be performed involving both organic and inorganic constituents, and discriminating between particle sizes (classes) both in on-line and off-line modes. The workshop generally acknowledged the potential importance of metal speciation for more detailed clarification of monitor responses and for source profile assessment purposes.

Beside the presentations on metals and trace elements, a number of contributions dealt with the polycyclic aromatic hydrocarbons (PAHs), ozone, nitrogen and sulphur. Plant foliar injury symptoms could be used to indicate ozone. A Northern-Italy study comprised principal component analyses, which suggested poor correlations between PAHs and metals, in turn probably indicating differences in their emission patterns. In a feasibility study on biomonitoring atmospheric Mn due to addition of MMT (methylcyclopentadienyl manganese tricarbonyl) in gasoline, none of the investigated biomonitor species (tree species, oats, beans, dandelions, pigeons) had sufficient sensitivity to detect Mn at the levels arising from the combustion of MMT.

Finally the workshop underlined the necessity for harmonisation of sampling approaches and analytical procedures, although it acknowledged the reality of using different plants in geographically and climatically diverse parts of the world.

The main conclusions

- Data acquired during biomonitoring surveys offer the possibilities to pinpoint pollution sources. The obtained data on air pollution levels can be linked to epidemiological information. For this purpose, however, interdisciplinary approaches are necessary: overall survey set-ups should include experts from environmental, biological, chemical-analytical, data-analytical and medical-epidemiological fields. More efforts should be undertaken to implement such surveys.

- Biomonitor response modelling should receive ongoing attention, because modelling may be seen as bridging detailed physiological studies with pragmatic field approaches.
- The workshop generally acknowledged the potential importance of metal speciation assessment and the analyses of non-metals or (non-metal) organic compounds, both for more detailed clarification of monitor responses and for source profile purposes.
- More emphasis should be placed on the assessment of the biomonitor vitality, both for direct impact monitoring purposes and for the judgement of the comparability of biomonitor responses within larger-scale surveys.

THE ROLE OF NUCLEAR TECHNIQUES AND THE IAEA

Non-destructive nuclear and related analytical techniques such as neutron activation analysis (NAA) and X ray fluorescence analysis (XRF) have already proved to be well suited for the routine analysis of biomonitor samples. They provide multielement capability needed in creating databases to be used for assessing air pollution, source apportionment or time-trend assessments. These techniques, along with other multielement destructive ones such as inductively coupled plasma (ICP) — mass spectrometry (MS) or ICP — atomic emission spectrometry (AES), indeed provided the bulk of analytical data presented during the workshop.

The IAEA has been systematically supporting biomonitoring in its Member States since 10 years. It has been supporting a Research Co-ordination Project on “Validation and application of plants as biomonitors of trace element atmospheric pollution analysed by nuclear and related techniques” since 1997. The IAEA-336 lichen reference material was issued through a co-operation between ITN and IAEA, and another lichen material, coded IAEA-338, has been characterised and its assignment of reference values is in progress. Two analytical quality control exercises coded NAT –5 and NAT-6 were organised on lichen and moss materials. In addition, several Technical Co-operation projects involving biomonitoring of atmospheric pollution have been implemented including a regional one in Latin America. The relevant publications include:

NAHRES-45. Co-ordinated Research Project on "Validation And Application Of Plants As Biomonitors Of Trace Element Atmospheric Pollution, Analysed By Nuclear And Related Techniques"; Report on the First Research Co-ordination Meeting, Vienna, Austria, 28 September to 1 October 1998, Vienna, 1999
NAHRES-46. Report on the Intercomparison Run NAT-5 for the Determination of Trace and Minor Elements in two Lichen Samples; Prepared by A. Bleise and B. Smodis,Vienna, 1999
IAEA-TECDOC-1152. Proceedings of an International Workshop on “Biomonitoring of Atmospheric Pollution (With Emphasis on Trace Elements)” — BioMAP, held in Lisbon, Portugal, 21–24 September 1997. Vienna, June 2000
NAHRES-63. Report of the 2nd Research Co-ordination Meeting for the Co-ordinated Research Project on “Validation and Application of Plants as Biomonitors of Trace Element Atmospheric Pollution Analysed by Nuclear and Related Technique”, IAEA Headquarters, Vienna, Austria, 20–24 March 2000, Vienna, 2001
NAHRES-66. Report on the Quality Control Exercise NAT-6 for the Determination of Trace and Minor Elements in two Moss Samples; Prepared by A. Bleise and B. Smodis, Vienna 2001

PAPERS

THE BIOLOGICAL SYSTEM OF THE ELEMENTS (BSE) — A BRIEF INTRODUCTION INTO HISTORICAL AND APPLIED ASPECTS WITH SPECIAL REFERENCE ON "ECOTOXICOLOGICAL IDENTITY CARDS" FOR DIFFERENT ELEMENT SPECIES (F.E. AS AND SN)

[1]S. FRÄNZLE, [2]B. MARKERT

[1]Umweltforschungszentrum Leipzig/Halle (UFZ), Leipzig, Germany
[2]Internationales Hochschulinstitut (IHI) Zittau, Germany

Abstract

There are different methods to estimate and predict effects of chemical elements and corresponding speciation forms in biochemistry and toxicology, including statements on essentiality and antagonisms. Two approaches are given here:

a) "identity cards" describing biologically fundamental aspects of element chemistry and
b) qualitative discussions which assume the existence of (indirect ways into) chemical autocatalysis to be essential for maintaining life and permitting reproduction.

The latter method, developed by the present authors, draws upon Stoichiometric Network Analysis (a safe procedure for complexity reduction in feedback networks) and provides estimates of concentration regimes for different elements suitable for survival and reproduction. The biochemical hierarchy level considered here is that of (metallo-)proteins. Thermodynamic toxicity aspects are given in correlations with DMSO solvent affinities and thiocyanate bonding modes. Effects of antagonists and of ion substitution within metalloenzyms or of metabolic simplification can be dealt with, likewise increased sensitivities within symbiotic relationships and within carcinomas are explained which are relevant for environmental monitoring and tumor therapy, respectively.

Keywords: History of the Biological System of Elements; ecotoxicological identity cards; stoichiomet-ric network analysis; stability of biological autocatalysis; effects of antagonists and metal ion substitutions; derivation of generalized toxicological statements

1. INTRODUCTION

The development of knowledge concerning functions and roles of chemical elements (in biology) was always closely linked to progress in multielement (and later on speciation) analytics of biological samples. In the following a rough synthesis on milestones in Biological Trace Elements Research is given in Tab. 1 reflecting the personal European point of view by the authors. Multielement analytics in turn depend on the quality of data with respect to both precision and — which is even more impor-tant — to accuracy. In the beginning for geochemical purposes first larger sets of data and comprehensive papers were produced as far back as the 1950ies and –60ies [1–4]. Already then it became obvious that there are mainly two different ways to improve quality and reliability of analytic data, i.e. either by analyzing the same sample several times by mutually independent methods or in different laboratories or else by using standard reference materials with a well-defined chemical composition for comparison [5]. Starting with so-called in-house standards, standard reference materials were developed, both non-biological and biological ones. The first of the latter was conceived and developed by Bowen (1979) [3] and thus now is known as "Bowen´s kale". These "first-generation" reference materials meant to be used in chemical analytics solely then were replaced with "second-generation" ones produced by national and international organizations — materials which are closely similar to the original material but made suitable for standardization by methods applied in their preparation for avoiding contamination — and eventually with “third-generation” ones (integral standard reference materials to be used also with respect to an interdisciplinary extension of analytical procedures and of problem-related application fields). The corresponding organizations include f.e. the Bureau Community of Reference (BCR) at Brussels, the International Atomic Energy Commission (IAEA) at Vienna and the American National Institute of Standards and Technology (NIST) at Gaithersburg, MD.

Taking an ecosystems point of view distributions of elements and trace elements were first investigated in Belgium. Duvigneaud and Denaeyer-De Smet (1973) [6] first attempted to systematically classify the differences between plant species relative to their inorganic chemical composition. Garten (1976) [7] discovered high interelemental correlations for the elements P, N, K, Ca and Mg for 54 species growing in the field, resulting independently of the site of the respective plant species studied. The results of multi-element analyses in the 1980s permitted the element spectrum to be extended for correlation analyses of this type (f.e. [8–16]) In addition the publication of more general textbooks on trace element distributions and effects in the plant/soil/atmosphere/biosphere context has brought new insights into the complex dynamic of this field (f.e. [17–19]) including atmospheric and anthropogenic impact mechnisms [20]. The organization of conference series (f.e. the “Adriano” conferences in Orlando (1990), Taipeh (1993), Paris (1995), Berkley (1997), Wien (1999) and Guelph (2001)) espe-cially in the interdisciplinary field of Biogeochemistry brought scientists together in regular intervals to strenghten exchance of latest informations.

The interelement correlation found by Garten (1976) [7] could be extended first with accurate data from standard reference materials [21] and 5 years later with data from highly accurate research inves-tigations [22]. Using these data it appeared highly probable that besides the extent of supply of trace elements and parameters which influence bioavailability (solubility etc.) aspects from genetics and molecular biology have to be considered additionally, possibly even are more important than the former factors. Moreover, instead of the total abundance of some element the corresponding speciation forms must be taken into account. To systematize the “element behaviour” in form of a table leads first to the attemp of using the (chemical) Periodic System of Elements, but the (chemical) Periodic System of Elements itself has been long critized by chemists since it displays a wide range of confusing irregularities and the PSE basically only appears periodic upon superficial consideration (see in detail with Markert, 1996 [5]). Allen (1992) [23] comes to the conclusion that something fundamental is missing from the PSE as our basic instrument for organizing chemical phenomena.

To handle the large set of analytical data for f.e. plants and to produce a common comparibility first a so called "reference plant" comparable to reference man [24] has been established, so that different chemical fingerprints of different plant species could be derived [25]. Here the genetical influence on heavy metal distribution in living organisms becomes clear: Related plant species show similar distribution pattern! F.e. the Ericaceae Vaccinium vitis idea (red whortleberry) and Vaccinium myrtillus (blue whortleberry) shows similar element distributions, f.e. extrem accumulation of Mn, independent from the bioavailability!

On the 24th General Assembly of the International Union of Biological Sciences in Amsterdam IUBS co-sponsors, with its International Section for Ecology (INTECOL), a project titled “Element Concen-tration Cadasters in Ecosystems” which deals with a new area of ecologial chemistry, emphasizing the methods for the evaluation and assessment of element concentrations in the environment ([26]; for details see Markert, 1996 [5]).

Due to the inadequate chemical description of the biological and medical relations in the PSE, from the above mentioned findings a first Biological System of the Elements (BSE) has been established in 1994 (Fig. 1). The main factors for establishing this first "intuitive" system have been accurate and precise multielement data including representative sampling procedures, interelement relationships, physiological function of elements, uptake mechanisms and evolutionary aspects [27].

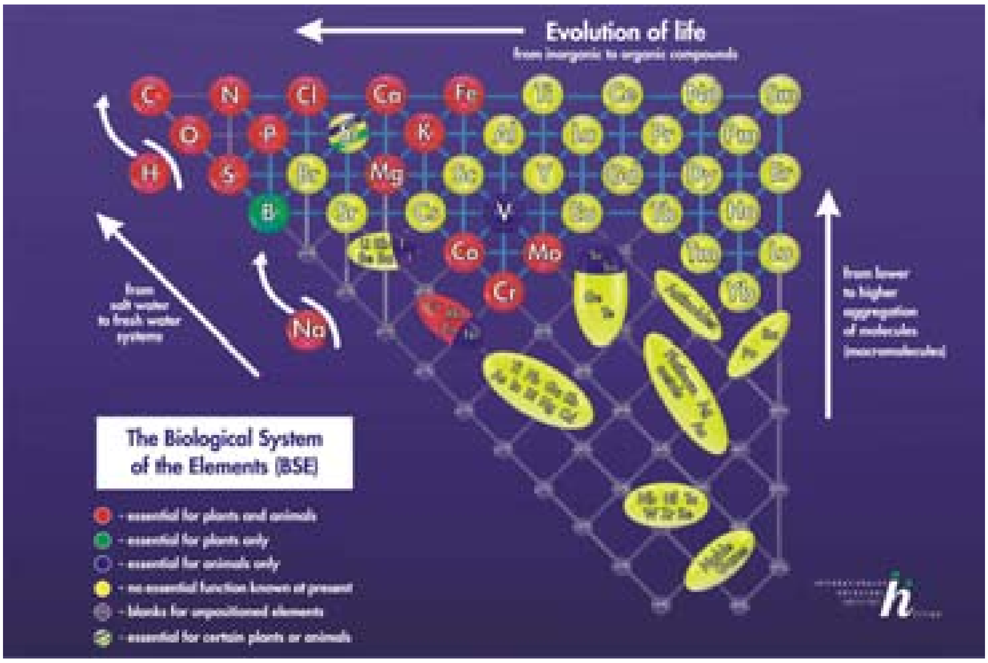

FIG. 1. The Biological System of the Elements (BSE) [27]

A theoretical fundament of abundance distributions and their causal analysis was achieved by using the so-called Stoichiometric Network Analysis [28–34] for the purpose of analyzing the biological roles of some species. This approach showed that deep-lying biochemical reasons and factors which give rise to the correlations among abundances of chemical elements in biological samples can be worked out. The next chapters and the ecotoxicological identity cards (Tabs. 2 and 3) give examples how SNA considerations and empirical correlations allow for conclusions whether some chemical element can be essential or toxic at all (for low doses) or in which speciation or binding form (f.e. coordinated or methylated) there will be biological activities, including pH and redox potential effects on speciation and biological activity. The scope of this work yet is far more comprehensive:

The chemical reasons of the above abundance correlations are traced back to the autocatalytic features of life itself. The limiting conditions then are given by the general character of biochemical processes and transformations and are eventually determined by the general definition of life, that is, by neces-sary properties of a system which links replication and internal autocatalysis to each other chemically, distinguishing it from both viruses and from mere growth of crystals. Nowadays we are concerned with applications for practical problems (Fig. 2) derived from this theoretical work. These include the development of biochips (interfacing semiconductor logics and biochemical signal sources), general bionic approaches, vision design, theoretical studies on medical uses of certain elements against tu-mors or parasites, the question whether land-dwelling green plants came from fresh- or seawater and complexity studies on development of organic life besides many other application fields which are going to get much more important in the near future [35].

TABLE I. MILESTONES OF MULTIELEMENT RESEARCH AND APPLICATIONS RELATED TO THE SCIENTIFIC DEVELOPMENT OF THE BIOLOGICAL SYSTEM OF THE ELEMENTS

Author	**Year**	**Results and publications on**
VERNADSKY	1951	Geochemical data mainly from Russia; development of the concept of increasing specific element transfer into the biosphere with ongoing evolution
BOWEN	1966 and 1979	Geochemical and biochemical data (worldwide)
WEDEPOHL	1969 – 1974	Geochemical data
	< 1965	“in-house” reference materials produced by geochemists
BOWEN	1965 – 1975	Production and use of BOWEN´s kale as first biological reference material for **quality control** of analytical data
EU AND OTHER INTERNATIONAL SOCIETIES, NATIONS OR INSTITUTES	1972	Foundation of the Bureau Community of Reference in Brussels for the professional development of standard reference materials (Similar Quality Control Centers grow up worldwide f.e. NIST in USA., BCR and IAEA in Europe)
DUVIGNEAUD and DENAEYER DE SMET	1965 – 1975	First systematic publications on element fluxes through forest ecosystems in Belgium (ecosystem research)
GARTEN (USA)	1976	Stated **“stable” correlations** of elements in plants
KABATA-PENDIAS and PENDIAS	1984	First more general textbook on trace element distribution in plants and soil
ADRIANO (USA)	1986	International conferences series directly relatet to trace element research
SANSONI	1985	First textbook on use and development of instrumental multielement techniques for environmental samples
NRIAGU and PACYNA (USA, CANADA)	1988	Worldwide estimation of contamination by heavy metals in the athmosphere, soils and water

LIETH and MARKERT	1988 a,b	determined quantitatively by various instrumental methods more than 60 elements in different plant species in Europe
LIETH and MARKERT ROSSBACH* JAYASERERA ZEISLER et al.*USA	1985 1986 1987 1988	Application and use of instrumental multielement methods on environmental samples in terms of highest quality from analytical point of view. * ESB: Environmental Specimen Bank-Development in Germany and the US
KOVACS DJINGOVA	1993 2000	Developing of instrumental multielement-techniques for environmental purposes
MARKERT	1988/1993	Interelement correlations found in different standard reference materials and highly representative research samples
IUBS	1991	The IUBS co-sponsors, with its Section for Ecology (INTECOL) a worldwide project titled “Element Concentration Cadasters in Ecosystems” (details in Markert, 1996)
ALLEN	1992	Proposed an extension and completion of the periodic table of elements
MARKERT	1992	Establishing of a “**reference plant**” for inorganic characterization of different plant species by **chemical fingerprinting**
MARKERT	1994	First publication for a „Biological System of the Elements“ (**BSE**) Analytical and biochemical aspects based on: - accurate and precise multielement data including representative sampling procedures - interelement relationships - physiological function of elements - uptake mechanisms and bioavailability - evolutionary aspects
FARAGO BARGAGLI	1994 1998	Further textbooks on trace elements in plants
FRÄNZLE and MARKERT	2000 a,b,c	Theoretical considerations and applications of Stoichiometric Network Analysis on the BSE

MARKERT and FRÄNZLE in MARKERT	2001	The Biological System of the Elements as a platform for technical and general application - Generation of Biochips - Creation of 'designer molecules' - Novel routes for and refinement of Bionics - Terrestrial colonization of green plants from freshwater - From single hypotheses to multiple theories - New paths in 'vision design'

2. MEANING AND SCOPE OF ELEMENT IDENTITY CARDS

The ecotoxicological identity cards (Tabs. 2 and 3) for an element are meant to give a first-hand description of properties relevant to biological and toxicological features of a certain chemical element and its geobiochemically plausible speciation forms. The focus of the identity cards and — of course — of the stoichiometric network analysis approach to the Biological System of Elements thus is beyond classical bioinorganic chemistry or toxicology though it embraces (broad bodies of information from) both. As an example, two such identity cards are given which discuss speciation and biological func-tions for arsenic and tin. The starting point are the environmental levels, modified by bioenrichment factors. Then functions are generally discussed in terms of bioinorganic chemistry insofar as such are known (f.e. gastrin complexes of Sn or the gross biochemical cycles of methylarsenic compounds [[36] in Xavier] in marine organisms).
(® Tabs. 2 and 3)

Arsenic is known to reduce the otherwise very high toxicity of (the essential element) selenium to-wards rats, dogs or poultry as well as in yeast except for SeO_4^{2-}; this holds for both inorganic and or-ganic As compounds. Corresponding work dates far back to the 1930ies: “Lardy & Moxon (1939) [37] showed that arsenite prevented inhibition of yeast fermentation caused by selenite. Arsenate and arsenite tended to increase the toxicity of selenate in this system. Moxon & DuBois (1939) [38] administered 5 ppm arsenic and several other elements in the drinking water of rats to study their effect on 11 ppm selenium as Se-wheat. Fluorine, molybdenum, chromium, vana-dium, cadmium, zinc, cobalt, nickel and uranium all increased the observed toxicity in the selenium-treated groups[1]. Tungsten gave some protection against liver damage and mortality, but complete pre-vention of selenium toxicity was only obtained with 5.0 ppm As as sodium arsenite. Other studies [39] demonstrated that arsenic in the diet would maintain liver glycogen stores in selenized rats at levels similar to the controls and much higher than in animals receiving just selenium. DuBois et al. (1940) [40] showed that administering 5.0 ppm As to rats, from either arsenite or arsenate, was effective in overcoming the effects of 11.0 ppm Se from Se-wheat, but arsenic sulfide was not effective” (insolu-ble and not bioavailable). “The authors (Moxon and his co-workers) stated (unpublished data) that arsenite was protective against Se from Se-wheat, selenite and selenocystine. This study also showed that arsenic would protect rats that had previously been fed selenium diets for 20 days”.

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[1] The toxicity increases with Mo, Cd, Zn or Ni show that Se is not reduced to HSe^- ion and thus cleaved as metal selenide but takes another biochemically active form the activity of which is increased by addition of „hard“ heavy metal ions.

TABLE II. ECOTOXICOLOGICAL “IDENTITY CARD” FOR SN SPECIES

	inorganic Sn (II)	**inorganic Sn (IV)**	**RSn^{3+}**	**R_2Sn^{2+}**	**$R_3Sn^{+/-}$**	**R_4Sn**
Environmental concentration (ocean)		4 pmol (all tin species combined)	(methylated tin species prevail in estuars)			
Environmental concentration (applies to fresh water)	low (undergoes rapid oxidation)	< 1 nM	?	≤ 100 pM	low	below 0.5 nM
Chemical aspects						
Strengths/preferences in complex formation	low	medium	probably bridging among sulfur centers		R_3Sn^+ binds to thio-groups; R_3Sn^- is a versatile soft anionic ligand	selective affinities (e.g. to fluoride)
Tendency for (cat-)ion formation	high	low	very low	very high	Restricted (ions form only if appropriate leaving groups are present, e.g. chloride or nitrate)	none
Lipophilic properties (used to estimate membrane toxicity and narcotic action)	low	absent	low	?	extremely high	very high
Model-theoretical considerations						
SNA: strong cycles possible?	no	yes				
Biological features						
Essentiality	no	probable	no	no	no	no
Toxicity	none	none	probable	high	very high	low
Binding to (as examples)		gastrine ?	$(S; Se)_x$ cluster		S species	unlikely
Reversible biomethylation?	Demethylation of $Sn(CH_3)_4$ to trimethyltin species is observed in mammal liver cells (entailing toxication), but generally photochemical degradation is more probable					
Terminal state of biomethylation in:					fungi	bacteria

TABLE III. ECOTOXICOLOGICAL “IDENTITY CARD” FOR AS SPECIES

Kind of speciation	**inorganic As (III)**	**inorganic As (V)**	**AsH_3**	**$RAsO_3H_2$**	**R_2AsO (OH)**	**R_3As**	**R_3As^+-CH_2X ~**
Environmental concentration (ocean)	16 nmol	70 pmol			presumably larger than that of inorganic As (R = CH_3)		Several nmol
Environmental concentration (applies to fresh water)		about 7 nM	extremely low		Occurs in rain water, rather stable	Low (undergoes rapid oxidation in air)	
Chemical aspects							
Preferences and strengths in complex formation	low	arsenate is a ligand possibly competing with phosphate	only as ligand (precipitation of metal arsenides M_xAs_n with soft metals)	poor ligands	as ligand	highly efficient ligands	none
Tendency for ion formation	Low (weak acid)	High (rather strong acid)	no	high (acid)	yes	no	is a cation
Lipophilic properties (used to describe membrane toxicity and narcotic action)	none	none	?	low (behaves as a tenside)	extremely high	high	depending on X
Model theoretical considerations							
SNA: strong cycles possible?	Yes	probably not	no		(As catalyses auto-catalytic cycles with C transfer in marine organisms)		
Biological considerations							
Essentiality	yes	if reduced?	No		?	no	yes
Toxicity	very high	yes	extremely high	no	?	yes	no
Binding to (as examples)						„soft“ metals	
Reversible biomethylation?	-		-	no	probably no	yes	yes
Maximum extent of biomethylation in					vertebrates	fungi	marine organisms

TABLE IV. RELATIONSHIP BETWEEN ORAL RODENT TOXICITIES OF METAL IONS AND THE MODE (DIRECTION) OF THIOCYANATOLIGAND COORDINATION: “PURE” S-BONDING IMPLIES VERY HIGH TOXICITY OF D-BLOCK IONS

Element	**mode of SCN^- coord.**	**LD$_{50(oral)}$** mouse or rat (mg/kg)	(µmol/kg)
Cu	mostly S	50	0.8
Ag	S	8 (rabbit)	0.074
Be	N ?	0.5 (disrupts phosphate metabol.)	0.055
Zn	N	2,000	30
Cd	S (BI in methanol)	27	0.24
Hg	S	27	0.13
Al	N	4,300	160
Ga	N	50	0.72
In	?	3	0.03
Tl	S	26	0.13
Sc	N	4,000	90
Sn	N (S in alkyl compounds)	160 (dog, inorg. tin (IV))	1.35
Pb	S	150	0.7
Ti	N	no data	
Zr	N	3,500	37
Hf	?*	140	0.8
Sb	S	600 (affects thiol-cont. enzymes)	4.9
Bi	BI	700	3.4
V	N in all common oxidation states	200 (rabbit)	4.0
Nb	N (BI in acetonitril)	3,000	32
Te	S	2	0.017
Cr	BI (+ III), N (+ VI oxid. state)	180	3.4
Mo	N (oxid.st. III to VI); S only in carbonyl compl.	190	2.0
W	like Mo (oxid. states + V and + VI)	240	1.35
Mn	mostly N; BI in carbonyl compl.	210	3.8
Re	BI (+ IV, + V); only N in carbonyl complexes	900	4.8
Fe	N (BI in certain solvents)	900	16
Ru	BI (+ III)	360	3.6
Os	BI (+ III; + IV), N (+ VI)	low (dog [do the data refer to OsO_4 ?])	
Co	N (+ II), BI (+ III)	500	8.5
Rh	like Ru	280	2.7
Ni	N, (rarely S)	800	14
Pd	S (BI with phosphine ligands)	20	0.19

* Interestingly, not only toxicities differ by a factor of about 45 between the otherwise very similar elements zirconium and hafnium. They can also be separated by liquid-liquid extraction from sulfuric or hydrochloric acid solutions into organic phases by means of thiocyanatocomplexes [69], the behaviour again suggesting S-binding in the more lipophilic hafnium complex. Moreover they do enrich in quite different tissues [66].

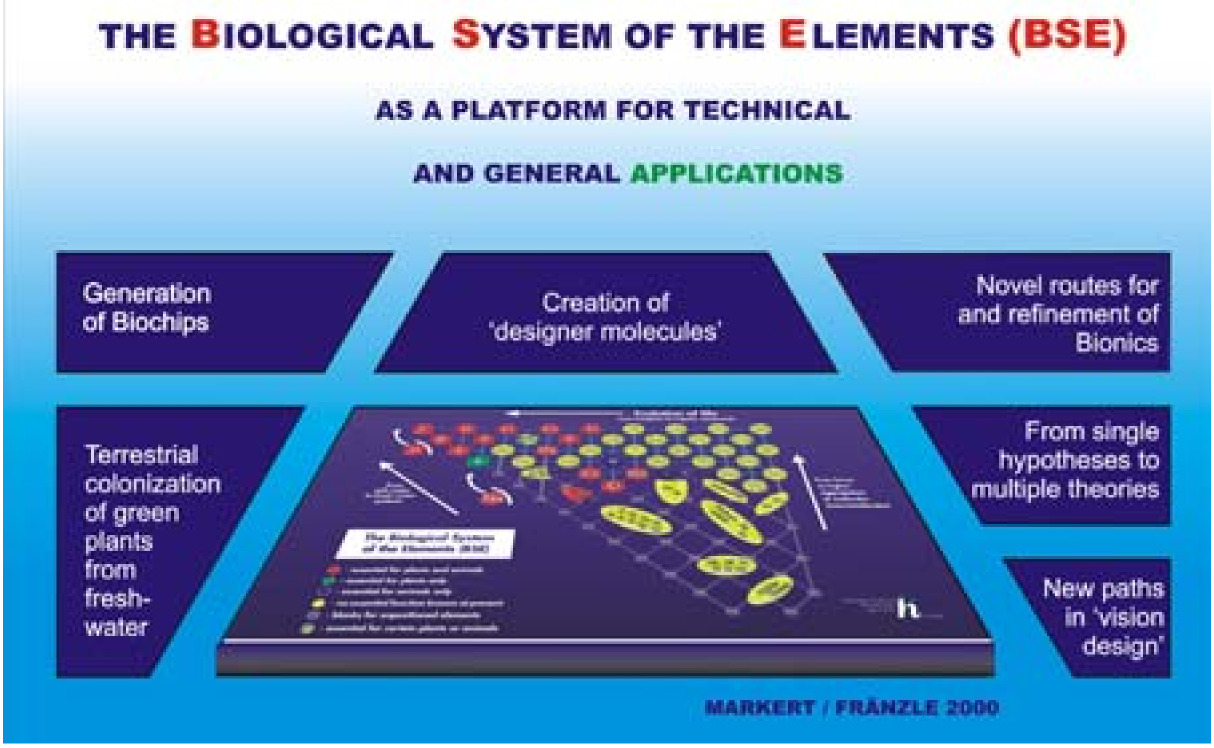

FIG. 2. The Biological System of the Elements (BSE) as a platform for technical and general applications by Markert & Fränzle in Markert [35].

Thus, there are complex interactions. These interactions and their effects on autocatalytic metabolic loops observed in biochemistry are the topic and focus of this application of stoichiometric network analysis (SNA) in biochemistry and toxicology.

3. INTRODUCTION TO SNA: AUTOCATALYSIS IN LIVING CREATURES

3.1. General Criteria: the Qualitative Approach

SNA generally is concerned with autocatalytic networks and their principal topological and dynamic features. In SNA different kinds of cycles are distinguished among which only the strong ones (see below) are capable of undergoing excitation by some chemical or photochemical perturbation etc.. SNA provides a method for safe and efficient reduction of complexity, reducing interaction feedback networks to the most simple ones which are still capable of the same and full dynamics of the structure to be modelled. Just comparing (formal kinetic) autocatalytic and exit reaction orders produces four categories, that is, strong, marginally strong, critical and weak cycles:

Strong	AC order > (or >>) exit order
Marginally strong	AC order is a little bit larger than exit order
Critical	AC order = exit order, highly susceptible to perturbations
Weak	AC order < exit order; autocatalysis breaking down over longer periods of time

Stable and efficient autocatalysis implies that the AC order be at least as large as that for exit. Main-tainance and efficiency of autocatalysis thus need sufficiently high autocatalytic orders which cannot be achieved by just a single and hardly by two functions accomplished by a single (kind of) autocata-lyst (i.e. by [species, f.e. enzymes which contain] an identical metal ion). Only strong cycles can “re-spond” to some “excitation” by chemical oscillations as are encountered in biochemistry which are used there to control and spatiotemporally organize biochemical transformations.

The SNA approach as used by the present authors is not at all concerned with such ions which are exclusively involved in regulation [41, 42] or signal transmission like the alkali metal Li through Rb; Ca may be a different case. Autocatalysis is an essential feature of living beings, and it is more than their ability to reproduce. It is also embedded in cell division of metazoa, and eventually in the reproduction of single enzymes (biocatalysts) included in either process. Of course hardly any molecules catalyze their own formation within cells directly (although self-replicating peptides first were isolated from common yeast [43, 44] but they rather do so via several intermediate steps.

In fact, which might be considered a delay en route towards reproduction is a necessary precondition for stability of the entire autocatalytic system once several such cycles become chemically coupled (like between the citric-acid cycle and amino acid biochemistries) and these cycles upon coupling turn into critical[2] ones (see Clarke, 1980 [29]). If there were molecules displaying autocatalysis in the very last reaction step before release of byproducts to whatever external sink, breakdown of either one of the coupled cycles could be caused even by a slight perturbation. In fact replication and autocatalysis on the metalloenzym level can escape this pitfall in each case because the metal ion would not be ac-tive as a simple aquaion but must be coordinated to proteins and/or cofactors for its function.

Tracing down (reproductional) hierarchy to the level of metalloproteins provides f.e. the following equation:

$$3\,[\mathrm{Mo(pterin)(protein)}]^{n-} + \text{substrates} + 3\,\mathrm{MoO_4}^{2-} + 3\ \text{pterin} \rightarrow$$
$$6\,[\mathrm{Mo(pterin)(protein)}]^{n-} + \text{products } (x\ \mathrm{CO_2} + y\ \mathrm{H_2O}\ \text{etc.});$$

assuming three unlike molybdoenzyms per cell (which is about average) which cannot fulfil functions of one another („chemical orthogonality“). Functional independence or chemical orthogonality mean the enzymes cannot be exchanged also with identical central ions, otherwise the function is lost even when substitution may work in vitro (Mo/W, Zn/Cd). The maximum autocatalytic order in the above Mo example would be 6 which appears to be extremely large in terms of usual (stoichiometric turnover) reaction orders but is a value frequently seen in AC-driven systems such as chemical oscillators.

The SNA line of reasoning explicitely deals with metal or non-metal elements or ions and their com-plexes directly involved in catalysis, allowing the organism to survive and consume/biochemically organize more of the same chemical species while reproducing. Given this, this approach is not meant to be a full-scale theoretical biology though the predictions and implications cover many areas and problems of biology, ecology and medicine likewise.

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[2] In order to maintain an element function, there must not be an autocatalytic last step placed directly before excretion to the environment in order to avoid instability in coupled critical cycles (cp. Clarke, 1980 [29], p. 162 ff.), that is some **linear** transformation must be intercalated. Metabolism (including the extraction of the nutrient metal ion by digestion from some matrix other than drinking water) provides some of the intermediate steps needed for stability.

The necessity for reproduction of a catalyst brings about additional conditions which are strongly disfavouring uses of complex mixtures of species each catalyzing but one step. The autocatalytic orders of such entities each would be low rendering strong cycles, if these exist, easily critical or even weak, thus causing breakdown of autocatalysis. When dealing with autocatalysis, the crucial question is no longer which species can catalyze some reaction (say protons or hydroxide ions or clay minerals or certain metals like Mo, Cu or Ir) but how stable is this „solution of a problem“ when subjected to the criterion that self-reproduction of some metasystem must be feasible using these catalysts? How many such different processes are then needed for a given catalytic center?

For sexual reproduction $n \geq 3$ functions are required, for an asexual one $n \geq 2$ suffices. The limiting AC orders then are 6 and 4, respectively. Hence „exotic“ elements can be used more straightforwardly in bacteria, one-cell algae and archeons (e.g. W [45] or Cd [46]). Generally speaking, AC systems are more sensitive and need a higher degree of homogeneity (a smaller extent of acceptable diversity) with respect to the catalytically active components.

The areas of acceptable concentration levels for maintaining biological activity between the two orders (see graphics below) must be positive for all essential species; this fact tends to limit the number of „essential“ species even though others would perhaps be more efficient in catalyzing certain single steps[3] . The number of some 24 „essential“ species for mammals (so far identified [47]) hence is pre-sumably close to the possible maximum. There are differences with respect to essentiality patterns between plants and animals; plants are autocatalytic with respect to carbon metabolism from inorganic sources, and the lists of essential elements differ considerably [48]. Symbiotic systems are inevitably critical cycles; the corresponding stability condition allow for an analysis of environmental perturbation effects f.e. in lichens (S. Fränzle, unpublished material). The level of molecules (in particular metalloproteines but not NAs) is the one we are analyzing here. What about the exact procedure?

3.2. Stoichiometric Network Analysis (SNA): A Comprehensive and Powerful Tool for Understand-ing Conditions and Criteria for Essentiality and Toxicity

3.2.1. Conditions for the SNA Formalism

First, let us repeat the preconditions for application of SNA to biology: living beings are

a) autocatalytic systems due to cell division, reproduction and other features of replication including internal processing of matter (excluding viruses); this effect is not limited to nucleic acids

b) these chemical processes necessarily include metal ion catalysis,

c) oscillations which occur in both biochemistry and population dynamics can formally and causally be dealt with much like with those present in chemical or physical oscillators.

There are both a formal and a topological (qualitative) procedure in SNA: The formal approach, sometimes applied to single oscillating biosystems like the glycolysis enzymes of yeast, means formulating both a stoichiometric and kinetic matrix of the corresponding set of reactions link to the vector of kinetic constants to find out stationary states; this approach is

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[3] This is not about bioavailability but more fundamental aspects which apply to any autocatalytic system are concerned; it need not even be a chemical one but also f.e. the replication of neutrons in a reactor (neutrons, not breeding of fissionable materials in the reactor !) can be considered in this manner.

complicated in terms of computation, presumably too complicated for most biological systems. The classification of networks depends on topological properties of the central (that is, autocatalyst-bearing) part of a cycle. These different types of cycles capable of causing sustained oscillation, clock-reactions or oligo-oscillations in batch systems include the „simple" patterns 1B[4] , 1CX, 1CW and 2, each bearing but one autocatalyst [ensemble]. For nomenclature and essential features of the feedback loops see Eiswirth et al. [49, 50]. Here X denotes the autocatalytic species, any Z causes negative feedback; Y and W are autocatalyst-consuming species within 1C-systems.

3.2.2. Comparison: "Simple" Autocatalytic Systems and their Biological or Ecological Counterparts

There are at least formal equivalents for these kinds of „simple" AC feedback loops realized in biology:
Antagonisms including precipitation reactions correspond to 1CX or 1CW patterns (f.e. the Mo/Cu-antagonism including metabolic pterine degradation = 1 CW), when any species Y will cause degradation of some cofactor which is required for biological function (ligand). For molybdopterin systems X = molybdopterin, Cu^{2+} = Z, Y = any catalyst or enzyme which degrades the pterine or its endithiol side chain to produce a thiophene (Mo-bound urothion). 1B feedback patterns are seen in biological systems with sulfide precipitation in sulfate-reducing microorganisms, producing solid ZnS and other products with biorelevant metal ions thereby consuming/binding part of these and thus increasing exit (loss). Z then is an intermediate of S(VI)-reduction which can form or release sulfide, f.e. thiosulfate.

Furthermore, one can compare (effects of) strong cycles in „purely chemical" and biochemical systems: in chemistry there are so-called clock reactions [51] in which AC entail suppression of one pathway until one substrate of the other was consumed completely, in biology we find diauxy after consumption of one more powerful biooxidant (even in eucaryotic organisms like yeasts). Diauxy takes place with O-containing acceptors (acc. = N(V), As(V) [52], S(VI), Se(IV or VI); with Oxygen transfer effected by molybdopterin catalysis; simple redox-active metal ions would not succeed as electrons could take too many different pathways. Also modified (that is, oscillating) clock reactions belong to the 1CX category [49, 50] which includes some assumptions on mechanisms and control patterns in metabolically flexible organisms. F.i. allostery can be effected within both the 1CX or 1CW frameworks.

As the corresponding redox potential values are substantially lower than those for man-made oscilla-tors Mn(II)catalyzed bromat/oxalic acid- oder –hypophosphite systems, the thermodynamics of the oxidant are not too important. Except for oxygen and oxyselenium species biology uses far weaker oxidants than in oscillatory or wave-front chemical systems (nitrate, halogenates, IO_{4-}, H_2O_2).

As many toxic species act as antagonists or competitors to essential ones, the comparison procedure can also be applied to „merely" toxic species such as alkylmercury ions. Then the physiological interval of concentrations is replaced with a tolerance region; of course there is

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[4] In 1B and 1C B refers to batch, C to continuous flow oscillator systems (an rather empirical distinction of network structures directly from experimental chemistry). As biological systems are open ones, it is straightforward to assign C-type systems to descriptions of the latter. There are also formal reasons for using these models (see above). When in-feeding of the autocatalyst X is required to sustain oscillations, we deal with 1CX, otherwise it would be 1CW.

no subphysiological (lack) region. Another case is hormesis [53], providing yet another dose-activity curve considerably differing from the colloquial one.

Going one more step down and focussing on the species which promote the catalyzed processes, that is, to metal centers in the reactive centers of enzymes, one can discuss catalytic features of the latter by a statistical point of view. This is to say that f.e. iron or zinc or copper or other multiply used metal ions in biology have many very different functions in which the same ion is subject to different binding/coordination patterns which determine the functions to an extent making it impossible to fulfil the same function by some other metal ion.

3.2.3. Doing it the Qualitative Way

The above kinds of so-called simple feedback patterns are derived from this formal, matrix-stability-analysis-based way of reasoning although substantial pieces of information about some autocatalytic (and thus feedback-) network can be obtained also from qualitative arguments on interactions and efficiencies of autocatalysis as brought about by certain key species, f.e. the centers of metallenzyms, control by allostery or by product and effects of metabolic intermediates. Hence the qualitative way is used for understanding more complex biochemical systems: Do a gross inspection of network structures and features (feedback modes and — species), then just estimate the loss (exit) orders and determine kind and character of cycles by comparison of orders. The crucial criterion is the multiplicity a certain metal ion or other catalytic entity is used with in a cell or organism; that is, there are primarily numeric criteria for essentiality, species which are used but once or twice in an organism will in fact no longer be used at all. Another case is **hormesis**, providing another dose-activity curve.

Given the dynamic features of strong or marginally strong cycles, the oscillations seen in Lotka-Volterra population kinetics allow for the conclusion that there are strong or at least critical cycles; the same must hold for the embedded single cells and metalloproteins. These must contain strong cycles, too; marginally strong ones are not a special category in dynamic terms but more sensitive and are also prone to regulation by antagonists: Mo/W is an example, while replacement of Mo or Fe by V defines and brings about yet another marginally strong cycle, that constructed around V. In the latter case, replacement can render a hitherto marginally strong cycle no longer strong, hence no longer capable of undergoing excitation.

Cooperative and antagonistic effects in toxicology (say between As and Se, both being essential ele-ments) are not only interesting in its own, but the more important when using the SNA model [28, 29, 32–34] since they are capable to modify either term of the conditions which combine to determine whether life can be maintained and sustained given a certain set of concentrations of various chemical elements and their speciation forms — be the latter essential or not.

The few identity cards so far available can just give an idea of what is feasible with this method; even though, sound descriptions and predictions have been worked out on different levels of hierarchy from metalloenzymes up to entire biocoenoses. The most interesting results and most straightforward ways of formal analysis are obtained for closely coupled symbiont systems like lichens and the factors which can affect or destroy them (S.Fränzle, unpublished work). Marine algae apparently can replace zinc with either cadmium[5] or cobalt [54], but there are so many Zn-dependent enzymes in each organ-ism that the AC cycle „running“ on zinc remains definitively strong anyway. The situation is different with more specifically used metals like molybdenum or nickel. Nickel metabolism in vertebrates interferes with several strong cycles like those of Mg, Ca, Fe and Zn, a situation which differs from effects of partial or complete liability to substitution.

Practically speaking from the viewpoint of biochemical research, marginally strong cycles are hard to evaluate since not all functions are already known. F.e., there are hints for essentiality of cadmium in goats [55] but no enzyme naturally containing Cd has so far been identified in mammals. On the other hand, selenium and molybdenum are known from „enzyme statistics“ to provide marginally strong cycles, Mo biochemistry being sensitive towards Cu or thiophilic heavy metals or substitution by W. For Co other functions than in cobalamine (B12) are known only for bacteria [56], and according to this theory, the various Se-glutathione-peroxidases can be predicted to be non-equivalent.

3.3. SNA and Evolution

For a new metal(-loid) which is (hypothetically) going to be established in biology, several functions must be implemented synchronously for the general dynamic reasons mentioned above (sufficiently high AC order); this has drawbacks throughout chemical and early biotic evolution, f.e. for the Pre-cambrian oxygen crisis and in access to new, metal-rich living sites (black smokers, archeons, plants and worms at highly metal-loaden ore excavation sites). For this, a substantial minimum concentration is needed. There should be traces from evolution which can be used to test this model. Interestingly, sometimes less efficient systems, f.e.V-nitrogenases oder Ni-containing hydrogenase persist until today besides the „better“ Mo- or Fe-enzymes even though these metals are rarer than the alternatives, hence it is unlikely that the organism will ever run short of the chemically better alternatives. Since the applications of both vanadium and nickel in biochemistry (but a few functions) are limited, one can conclude that marginally strong cycles are easier to control by allostery, expression of genes etc. than (even more) strong ones.

This provokes another question: is there an **optimum of multiplicity** for functions of a given element without taking too large risks of possible replacements in bioinorganic terms, f.e. for zinc (by cad-mium, cp. [46])? Chemical similarities may cause problems here, but the other cases (Fe, Cu) are less severe as replacement causes larger chemical changes. Metal admission to proteins is controlled, hence functional reconstitution of an apoenzym by means of some metal ion other than the biologically used one [57] does not imply replacement in biology is possible, too. As AC orders are controlled by the number of irreplacable and non-interchangeable (functions of metallo-)enzymes which share the same metal, and the uptake of metals to and by apoenzymes is biochemically controlled (selectivities of transfer proteins [transferrins and the like] etc.), a sufficient AC order may then be maintained but at the expense of chemical flexibility. This is a kind of specialization which might cause problems in further evolution.

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[5] Quite recently a specific cadmium enzyme (a carboanhydrase) has been detected which differs also in terms of molecule size from its Zn counterpart [46].

Metals or metalloids which display **amphiphilic (ambidentate) bonding** can establish easier in biology though monofunctional enzyme centers are subject to rapid removal by evolution, because they can become and remain bound to quite different proteins and glycoproteins.

3.4. Graphical Determination of Limiting Conditions

The relationship between concentration and activity (essential, toxic etc.) of some element can be grossly described using the common curve which — apart from some differences for the low-concentration regimes for non-essential and/or hormesis-inducing elements (see below) — generally applies to biochemistry. Hence this generalizing relationship can be used to determine mathematically the crucial relationship between efficiency of autocatalysis and concentration, including toxicities of essential species at high levels (Se, Mn, Mo) and protective effects of yet other toxines (As vs. Se). These curves and their derivatives can be shown to give a unified description of the conditions which are necessary for or reconcilable with sustained life. For any organism, there are at least as many such curves as there are essential elements, and this corresponds to the number of limiting conditions.

3.4.1. The Formalism of Graphic Derivation of Sufficient Autocatalytic Activities: Superposition of Autocatalytic and Exit Orders as Depending on the Corresponding Concentrations

The graphics given in figures 3 a-d depict the mathematical transformation of the usual minimum/optimum/toxicity curve [59] for purposes of a discussion by SNA while 4–6 discuss the implications of increasing perturbances in biochemistry (by excess administration, by antagonists or by replacement with another similar ion).

The survival of an organism depends on the supply of all the essential elements in appropriate amounts and speciation forms (f.e. cobalamine rather than “useless“ $Co^{2+}aq$) while the extent of metabolic activity in turn is controlled by the specific amount of material available. For any given essential species, there is a minimum concentration for survival, to be followed by some usually fairly broad plateau and by an optimum and eventually a region in which the essential species becomes increasingly toxic regardless of the mechanisms used by the organism for regulating the uptake and concentration. Except for hormesis (cp. figures 6 a and 6 b), the qualitative shape of this curve is almost universal[6] . Within the framework of this qualitative discussion, the general pattern provides sufficient information. Unlike in “simple“ non-biological autocatalytic systems, the autocatalytic and exit orders in living beings sensitively depend on the applied concentrations. This very fact combined with the efficiency of excretion regardless of particular detoxification measures (f.e. methylation of Se up to $(CH_3)_3Se^+$) allows for a formal determination of tolerable intervals (which are narrow with Se). Since the above curve is close to universal, another one (fig. 3 b, see below) can be constructed by derivation vs. the concentration from this one for purposes of a general discussion.

On the ordinate of figure 3 a the concentration of the considered substance is given while the abscissa gives the metabolic activity as a first-order measure of autocatalytic activity. The lower line (survival minimum) means a critical cycle.
(® Fig. 3 a-d)

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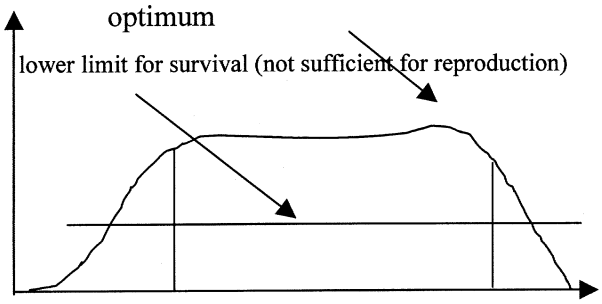
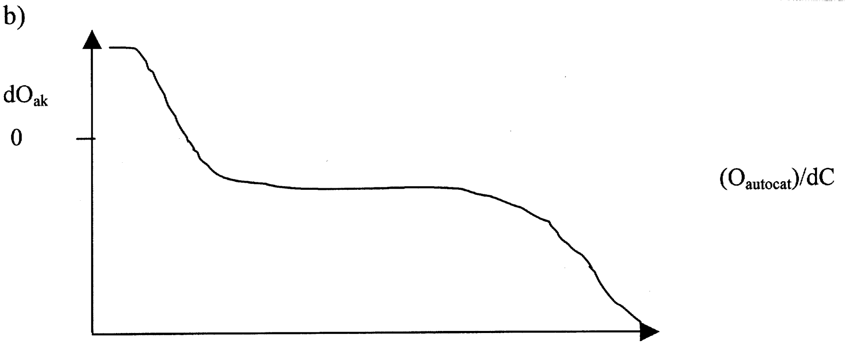
[6] This is no contradiction since, *by definition*, hormesis is (or may be) associated with low-amount administration of such species which are not essential but more or less toxic.

a)



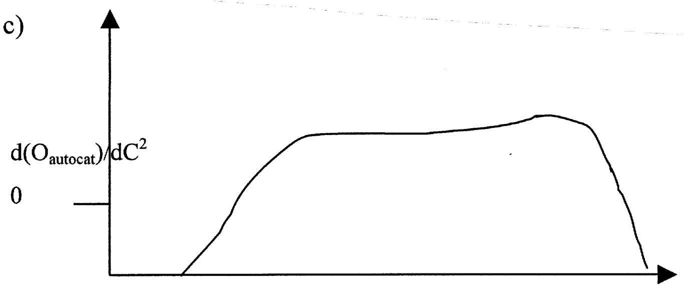
Metabolic activity vs. concentration

b)



First derivative of the above metabolic activity function:
autocatalytic order vs. concentration

c)



Variance of autokatalytic order vs. 2. derivative of concentration

d)

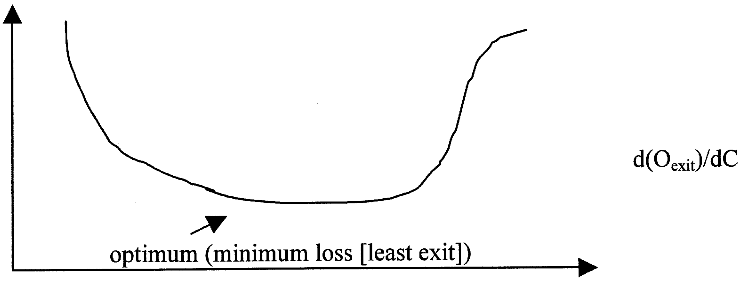


FIG. 3 a-d.
Loss (exit) order vs. concentration; assuming an increased excretion at high concentration levels.
The metabolic activity of an organism depends on the supply of all the essential elements in appropriate amounts and speciation forms (f.e. cobalamine rather than "useless" Co^{2+}_{aq}). Metabolic activity now is influenced by the extent of available material; for a given essential species, there is a minimum concentration for survival, than some usually fairly broad plateau followed by an optimum and eventually a region in which the essential species becomes increasingly toxic regardless of the mechanisms used by the organism for regulating the uptake and concentration.

Biochemical concentration-activity relationships can in this manner be derived for both essential and „generally“ toxic species and their mathematical derivatives. Loss extent and exit orders may depend on the redox potential (formation of insoluble sulfide in the digestive tract, breath or urine losses of biomethylation products etc.). Then the AC and exit diagrams are superposed to do qualitative SNA practically:
(® Fig. 4)

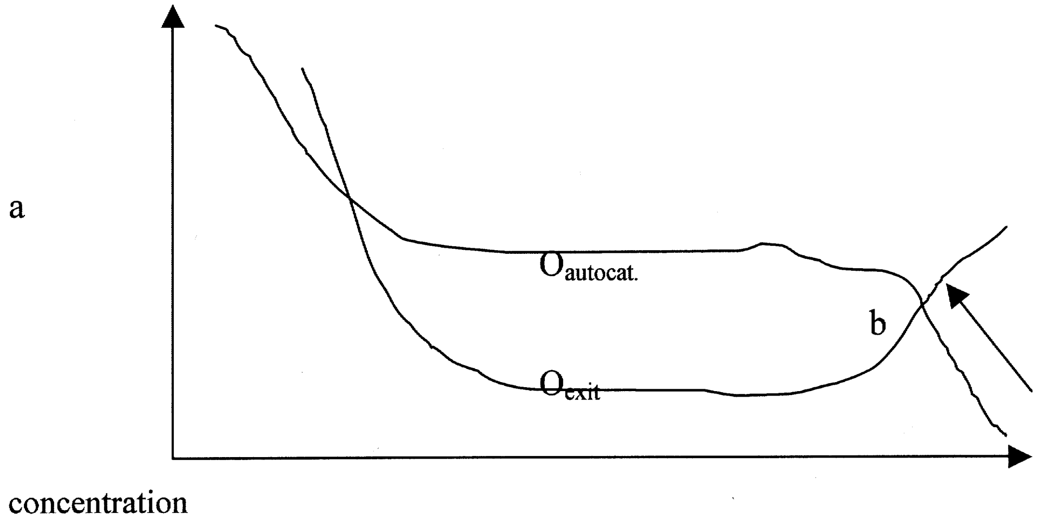


FIG. 4.

A und b are intersection points which denote conversion of strong into critical and eventually weak cycles. The arrow shows increasing loss orders by large-scale removal of excess essential material. The area between both curves is that in which life is possible owing to cycles being strong. Addition of antagonists will increase the exit order; the precise reduction of the interval of tolerable concentrations is determined by the dose-action relationship for the corresponding substance

It is important to understand that the autocatalytic order will be decreased also when the „original“ metal ion is replaced by some other in a metalloprotein or apoenzyme without loss of function. This even holds when the new central ion is more efficient in terms of mere catalysis. There should be at least three different functions for a single kind of element to keep it working in biology, that is, to maintain an AC order higher than conventional exit orders by excretion, loss of methylated volatiles etc. (® Fig. 5)

3.5. The Exception from the General Pattern: Hormesis and its Qualitative Treatment

For small concentrations, there may be another phenomenon with certain otherwise toxic species: **hormesis**, that is, a certain increase of metabolic activities and „vitality“ upon administration of small amounts or doses. The following picture is a representation of the hormesis function (f.e. for the activity of Ti in cereals):
(® Fig. 6)

Except for hormesis (cp. Fig. 6), the qualitative shape of this curve is almost universal. Within the framework of this qualitative discussion, the general pattern provides sufficient information. Unlike in “simple” non-biological autocatalytic systems, the autocatalytic and exit orders in living beings sensitively depent on the applied concentrations. Nevertheless, because the above curve is close to universal, another one (Fig. 3 b, see above) can be constructed from this one for a generalizing discussion by derivation vs. the concentration.

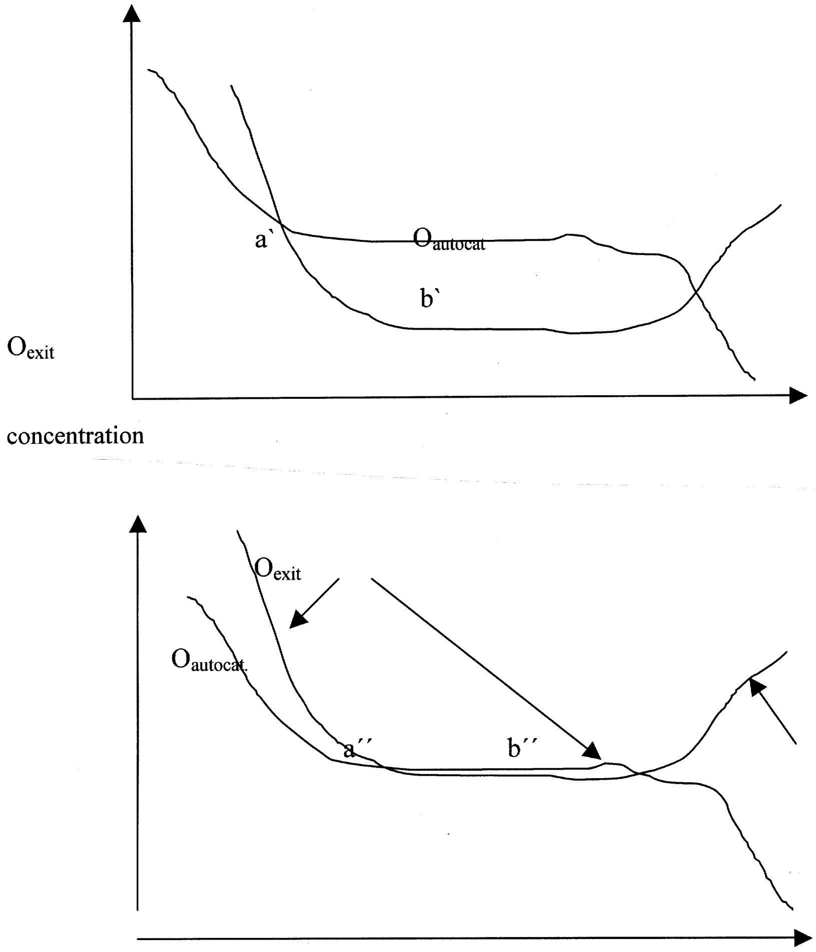


FIG. 5.
The diagrams above display increasing levels of perturbation: the interval [a`b`] is smaller than the original one [ab], [a``b``] being smaller once again. Very small circumscribed regions are not compatible with reproduction even though environmental levels may be efficiently buffered in water. Thus reproduction toxicity is reproduced by this method also for levels without acute toxicity, correctly depicting what is observed. With the intermediate area vanishing totally, sustaining life becomes impossible (lethal poisoning by f.e. an antagonist or by excess administration of an essential agent [say, Se or Mo]).

4. POSSIBLE MODELS FOR THE HIGH-CONCENTRATION REGIME TO CALIBRATE TOXICITY AND ECOTOXICITY IN SNA TERMS: TWO-PARAMETER TOXICITY FUNCTIONS FOR CAENORHABDITIS ELEGANS, RODENT TOXICITIES, LINKAGE ISOMERISM AND DIMETHYL SULFOXIDE AS A MODEL AGENT

4.1. Empiric Formulas for Toxicity of Metal(-oid)s in the Nematode Caenorhabditis elegans

Since the best correlation for metal ion toxicity in Caenorhabditis elegans is that one using hydrolysis constants of the corresponding metal ions combined with a covalent index [58], including affinities to both O and S donors, and also concerning toxicities of essential biocatalytic elements ([Cr], Mn, Fe, Cu, Zn, etc.), the first derivative of the corresponding equation vs. concentration can as well be used to describe the effects of various ions in the high-concentration, that is, the ecotoxicologically relevant [59] regime.

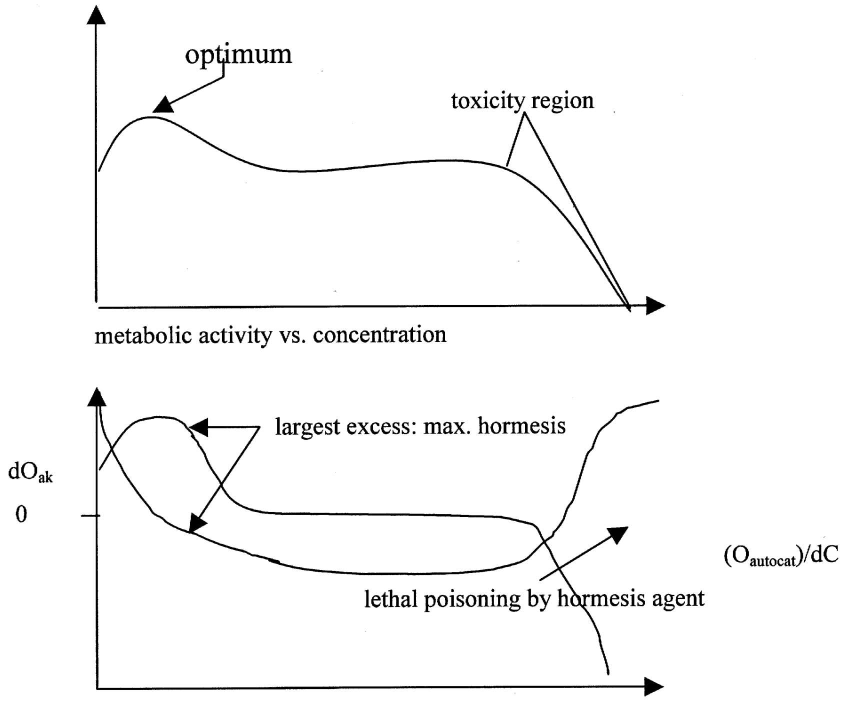


FIG. 6.
The lower graphic displays the first derivative of the above metabolic activity function: autocatalytic order vs. concentration.

The equation is:
$\text{Log } LC_{50} = 0.18\ [\log k_{OH}] - 0.36\ \chi^2_m r + 0.33;\ d\ [\log C]/dC = 1/C$

Hence this hydrolytic tendency should be one term in an identity card for a chemical element (for some metal at least). For single enzymes, toxicities can reasonably be described by one-variable mod-els using the hydrolytic constants, f.e. in lactic dehydrogenases or glutamate/oxalacetate transaminase [60], whereas for entire organisms two-variable models give far better representations of the effects even though one of the two parameters may be insignificant from a statistical point of view (ibid.).

Metal ion toxicity in the nematode Caenorhabditis elegans is most closely correlated with the hydrolysis/protolysis constant of the corresponding ions [58], except for barium which (like in mammals) is more toxic than predicted. Accordingly effects of oxidation and modifications of binding behaviour by methylation and by association with effectively irreversibly bound ligands (provided these are stable in the living organism) must be included, too.

4.2. General Arguments on Factors which affect Toxicities

For main-group elements at least, it is likely that lipophilic behaviour is far more influenced by (bio-)methylation than is the susceptibility to hydrolysis.

Hydrolysis of a metal ion in an oxidation state not higher than + III indicates some intrinsic oxophilic behaviour. The balance is required to maintain a stable state of multiple functions which are intrinsically unrelated but require different modes of bonding, that is, the corresponding metalloenzymes cannot replace each other even when allowing for small mutations.

As the softness parameter contributes more to toxicity, although with a negative sign, conclusions are possible with respect to „optimal“ properties of essential elements. The apparent reduction of toxicities (which is possibly more than compensated by hydrolytic tendencies of these very ions, f.e. for highly thiophilic ions like Hg, Pb, Cd etc.) upon increasing thiophily might represent larger stabilities of thiometal clusters. Corresponding data using some probe for both thiophily and oxophily would be optimal to represent the balance between the two ways of bonding excesses of either would bring about toxicity.

4.3. Solvent Transfer as a Model for the Diversity of possible Interactions between Metal Ions and a Multifunctional Medium

4.3.1. Multiple Sites of Interaction

For a more quantitative approach, it is straightforward to use data which refer to the fact that metal ions once passed into an organism may bind to a multitude of different sites and thus to different donor atoms even though there is a relationship between pronounced thiophilic behaviour and high toxicity at least in transition metals [plus Pb(II), In(III)]. Corresponding ligands which can serve as equivalent probes would be sulfoxides, such as DMSO, or sulfite anion. For both species linkage isomerism is well-established, f.e. for sulfite with Co(III), for sulfoxides f.e. with Ru(II) and Ru(III) [61]. Given the rather low charge density (positive polarization) of sulfur in sulfoxides, it can be expected that also some thiophilic metal ions will coordinate to sulfoxides via oxygen, and this is indeed observed for lead(II) (ibid.).

Like with thiocyanate, it is likely that this way of arguing is fully applicable only to transition metal ions and possibly to lanthanoides. This a priori undefined behaviour may be simulated by exposing the same metal ions (alkali metals, univalent Ag or Tl, divalent Zn, CD, Hg and Cu) to some solvent which is distinguished by two or more different potential donor sites and determine thermodynamics of solvation or phase transfer from water. Like with thiocyanate, it is likely that this way of arguing is fully applicable only to transition metal ions and possibly lanthanoides.

4.3.2. Intersolvent Transfer of Ions as a Toxicity Model: Regression Analysis and corresponding Arguments

In this model binding effectivities can be represented by phase transfer enthalpies from water to the corresponding solvent [62] with the metal ion “choosing” an optimal binding site in an ambidentate solvent. The corresponding solvents for which sufficient data are available include DMF and DMSO, others like N,N-dimethylthioformamide providing less exact correlations. In one case (DMF) the ion can „choose“ between nitrogen and oxygen, in the others between S and O or S and N, respectively.

The ideal solvent for representing the combined affinities towards different donors in the living being (oral toxicity in rodents) is dimethyl sulfoxide. A very large negative transfer enthalpy into some solvent which well represents the affinities towards biological materials implies that the ion be strongly bound there, and thus the considered metal ions are going to be highly toxic for reasons of competition. Even when correcting by simple division for the different charges, this holds for Ag, Cd and Hg in DMSO. Such transfer enthalpies corrected by charge correspond to potential shifts.

Dimethyl formamide does but give a very poor representation; apparently the situation in living beings is more similar to an S/O bias[7] than to an O/N bias given there are no net electric charges in the probe (see below). For S/N systems such as N-methylthiopyrrolidinone only data for monovalent ions are given. Both DMF and DMSO provide reasonable correlations for the oral toxicities of mono- and divalent ions in rodents (mice and rats) with very small scattering when weight toxicity data are used:

Log $LD_{50\ (\text{rodent; oral})} = 0.193\ \Delta G_{\text{water/DMF}} + 5.036$ (toxicity data in mg/kg) and

log $LD_{50\ (\text{rodent; oral})} = 0.0331\ \Delta G_{\text{water/DMSO}}$[8] $+ 3.464$

The (chemically more meaningful) corresponding data for **molar** quantities are:

log $LD_{50\ (\text{rodent; oral})} = 0.0924\ \Delta G_{\text{water/DMSO}}/q + 5.134$ (with concentrations given in µmol/kg),

while no meaningful linear or other correlation could be obtained for DMF. For N-methylated pyrrolidinone and –thiopyrrolidinone, respectively, there are only data for monovalent cations. This approach is indirectly linked to the Free Ion Activity Model (FIAM). For other solvents, there are too few data.

The exceptions (outliers) from such correlations refer to metal ions which are likely to use other binding sites; an outlier with both solvents is thallium(I), which is thus suggested to be linked to neither N nor S nor O. The DMF data cover the alkali metals (Li, Rb, Cs, while Na and K are not covered by toxicity data) and Ag besides Cu(II), while those for DMSO deal with the same alkali metals, Cd, Cu and Hg but not with Zn (toxicity presumably controlled by its essentiality), Ag and Tl.

4.3.3. Speciation and Toxicity: An Estimate from DMSO Data

The rather efficient detoxification of arsenic by biomethylation (a phenomenon which is limited to As and Sb only, all other elements becoming [often much] more toxic upon methylation) is reproduced in the DMSO transfer energy model by the fact that the transfer enthalpy of TMA^+ is close to zero [62] and that $(CH_3)_4As^+$ is only slightly larger and little more lipophilic an ion than $(CH_3)_4N^+$. Trivalent (inorganic) As toxicity is somewhat smaller than those of Cd or Hg (70 mg/kg = 0.93 µmol/kg), hence in this model $\Delta G_{\text{water/DMSO}}/q$ should be close to – 30 kJ/val. Detoxification by biomethylation can thus be estimated also in quantitative terms. Using the above regression equation, the detoxification factor should be about 100.

For sulfoxides some ambidentate behaviour is established. The relationship between the softness function and (mouse intraperitoneal) toxicities depends sensitively on the charge of the ion, except for trivalent ions [63] other than indium which is much more toxic (also when administered orally). For trivalent metal ions except of indium rodent toxicity differences are small. Notably the strongly coordinating Cr(III) ion (high activation barrier towards complex hydrolysis) does perfectly fit into this function, like those of Fe, Al and the group III b elements (Y, La, lanthanoides). If the softness parameter σp does not matter here with the toxicities being almost equal to each other though the hydrolytic activities (approximated by

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[7] Cp. the structure of phytochelatins which try to control a general metal burden by this very assembly of donor sites, represented by cystein (thiolate-*S*) and glutamic acid (carboxylate-*O*) residues being combined in a long repetitive sequence usually terminated by glycine or serine. This is the same S/O bias for biochemical response.

[8] $\Delta G_{\text{water/DMSO}}$ is given as a potential difference, dividing values for divalent ions by two. These corrected values are also used in the correlation.

the acidities of trivalent ions) do differ considerably, it can be concluded that the effects cancel each other for these trivalent ions.

4.4. Linkage Isomerism as a First-Approximation Model for Ambidentate Behaviour involved in ei-ther Balanced or Biased Behaviour of Metal Ions bringing about either high Toxicity or a sufficient Width of biological Uses

4.4.1. Why Thiocyanate?

When can a certain metal ion be introduced into quite different and „chemically orthogonal“ biological functions? Such diverse applications of certain metal(-loid)s in biochemistry are possible if there is diversified (coordination-) chemical bonding. This criterion can be tested by linkage isomerism [64, 65] by using ligands displaying two or more different of the biologically important donor sites. A selective, „unbalanced“ thiophilic behaviour of an ion with a bias towards S, including strong and possibly irreversible binding towards cysteine/selenocysteine or metal-sulfidoclusters obviously implies high vertebrate toxicities (Cd, Hg, Ag). There are two models for pronounced thiophilies and corresponding toxicities: the bonding direction of **thiocyanatoligands** and the phase transfer enthalpy into dimethylsulfoxide[9].

Cd, Hg, Pb(II), Ag, Tl(I) etc. which bind to SCN^- mostly or exclusively via S compete with biorelevant metals for „soft“ coordination sites in proteins; this will reduce AC orders also when enzyme activity is maintained. There are grave consequences in marginally strong cycles (Mo, Ni): once the AC order is depressed below the formal of reproduction or cell division, the now weak cycle is no longer excitable, and autocatalysis effectively ceases; signal processing will no longer occur. The data set on linkage isomerism of thiocyanate is given below, producing estimates of toxicity valid for transition metal ions:

The general pattern for mouse toxicity vs. linkage direction of thiocyanate is as follows [66-68], essential elements being denoted by fat letters (for [almost] all organisms) or italics (required by some organisms only):
(® Tab. 4)

As can be seen from these data, at least for mice and rats high oral toxicities are linked to thiophilic behaviour towards thiocyanate ligands; conversely, LD_{50} values of more than 10 µmol/kg correspond to exclusive N-bonding in simple complexes (Al, Sc, Zr, Nb, Fe, Ni, Zn). This criterion cannot be used for main-group elements exhibiting strong covalent bonding, like As. The extremely toxic (LD_{50} < 0.3 µmol/kg) transition metals Ag, Cd, Hg and Pd are S-bound throughout for simple complexes without phosphine or similar co-ligands.

For beryllium and indium, appropriate data are lacking which refer to the observed very high rodent toxicities but Be attacks polyphosphate bonds [66]. Interestingly, thiocyanate — being a charged species with fairly well-balanced charge distribution — thus provides a meaningful representation of metal toxicities like does the solvent affinity towards DMSO even though

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[9] A few ions are omitted from this analysis: Li because of many instances of anomal binding in quite different solvents and matrices („Li-bridges“), Zn for a high extent of both biological versatility and regulation, and Tl for an as yet unexplained anomaly in biological terms. Tl apparently binds to yet another kind of donors than O or S; moreover, it is a cumulative poison. The N/S selection in thiocyanate is somewhat related to the thermodynamics in sulfoxides. Charge effects compensate for the difference between O and N (there are almost no stable linkage isomers in cyanate).

the binding sites in the latter are different and negative charge cumulates on O. It is presumed that „softness“ effects are strongly involved in either system. Moreover, the thiocynate BI approach also holds for ions having larger oxidation states than + II. Sb is the only element besides As which is efficiently detoxified for vertebrates by methylation and undergoes methylation fairly easily; both trimethyl- and triphenyl Sb(V) thiocy-anates are N-bound.

5. TUMORS AND SPECIES WITH BIASED (INCREASED) SENSITIVITY: CHEMOTHERAPY FROM A SNA POINT OF VIEW OR MAKING STRONG CYCLES WEAKER

5.1. The General Approach from SNA

There are various possibilities to reduce the AC order with respect to a certain element:

1) the overall metabolism may be simplified, rendering some metalloenzymes futile,
2) one metal (f.e. Mo) may be replaced in a given apoenzyme by another (f.e. W in hydrogenases like formate oxidoreductase or V in nitrogenases) or
3) an antagonist might interfere (Cu/Mo), possibly enhancing excretion instead

5.2. Practical Implications: Tumors and Endoparasites

The first is realized in tumors or sometimes in (the evolution or ontogenesis of) endoparasites, the last is very common owing to non-specific ligation or the possibility of sulfur-bridging between different metals.

There are certain parasites which can no longer sustain their lifes on their own because the enzyme repertoires were reduced so much as to render them dependent on their hosts´ biochemistry. This is an extreme pathway of evolution but it can also be described in SNA terms: inevitably, for certain metals the range of „applications“ in biochemistry is thus thoroughly decreased, too. The same holds for a tumor in its individual development rather than evolution. Once strong cycles have turned marginally strong ones or chemical cooperation features (coupling among cycles) bring about critical cycles, they are now more susceptible to competition or antagonism just because the metabolism had become more simple.

This offers additional possibilities for therapy by antagonists or competitors: Ga^{3+} in tumor therapy, replacing Fe(III) when tumors have only marginally strong cycles with respect to Fe, and simple heavy-metal ions are or were used in various therapies (including some out-dated ones) of parasite diseases: Sb compounds against Leishmania [70], Te vs. Mycobacterium leprae. In these cases the exact way of action often is still uncertain but Te — like As — presumably interferes with selenium metabolism. The Se antagonism of As extending the tolerable dose of the former was already mentioned. These effects are connected with direct influences on autocatalytic (anabolic) metabolism, not with any binding of metal ions or complexes to DNA or mRNAs, notably.

A more precise paper on use of SNA for cancer therapy will be prepared soon.

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AIR QUALITY MANAGEMENT IN PORTUGAL: EXAMPLE OF NEEDS AND AVAILABLE TOOLS

C. BORREGO[1,2], A.I. MIRANDA[1], M. COUTINHO[2],
J. FERREIRA[2], A.C. CARVALHO[1]

[1]Department of Environment and Planning, University of Aveiro, Portugal
[2]IDAD — Institute for Environment and Development, Aveiro, Portugal

Abstract

The Framework Directive (FWD) and the proposed Daughter Directives for the different pollutants are the newest legislative instruments concerning a new political strategy and air quality management approach for Europe, conforming to sustainable development and taking into account the load capacity of natural ecosystems. In fact, the FWD will permit the control of a larger range of pollutants and includes not only the protection of public health but also that of ecosystems. In addition to the FWD, the member countries of the United Nations Economic Commission for Europe have included the concepts of critical load and level for planning air pollution abatement strategies and as a base of international agreements concerning limitation of the emissions of air pollutants. These concepts imply an accurate knowledge about pollutants deposition fluxes. Biomonitoring of atmospheric pollution could be a very useful and important tool either in the scope of the FWD or of the critical load concept. The paper describes the main needs and the tools available to define a strategy of air quality management in Portugal. Two study cases are presented: (i) development of an extensive monitoring plan aiming to assess the impact of an urban incinerator plant on the environment; and (ii) contribution to a methodology to estimate critical loads for a coastal region in Portugal. These different approaches allowed illustrating the complexity of the implementation of an air pollution management strategy.

1. INTRODUCTION

In the environment, air, water and soil are in a perfect equilibrium. An interaction is established between the air and the ground (animals and plants) by various processes like precipitation (which reaches the land surface and flows downhill from high places like mountais and hills into streams, then rivers, and finally into the ocean), infiltration, groundwater flow (where in the ground comes back to the land surface) and evapo-transpiration (liquid water on the land surface, in the soil, or in the ocean goes back into the air as water vapour). Figure 1 illustrates this complex interaction. When a pollution episode occurs, all this cycle is affected. Atmospheric pollutants are released to the atmosphere and can be transported and deposited at locations far from their sources. Wastewater generated will contaminate waters and soils, where the feed of animals and humans come from.

The food chain is the preferential contamination pathway by certain pollutants — ingestion of contaminated food and drinking water. Toxic air pollutants get into the body mainly through breathing. They can also be ingested or absorbed through the skin.

Once a pollutant enters the body it can stay in the lungs (like asbestos), be exhaled, or move into the blood from the lungs (like the oxygen we breathe) or from the digestive system or skin. In the blood it is carried to all parts of the body. As it moves around the body, a pollutant can undergo chemical changes, especially as it passes through the liver, becoming less, or more, toxic [1].

At certain levels, toxic air pollutants can cause human health effects ranging from nausea and difficulty in breathing to cancer. Health effects can also include birth defects, serious development delays in children, and reduced immunity to disease in adults and children.

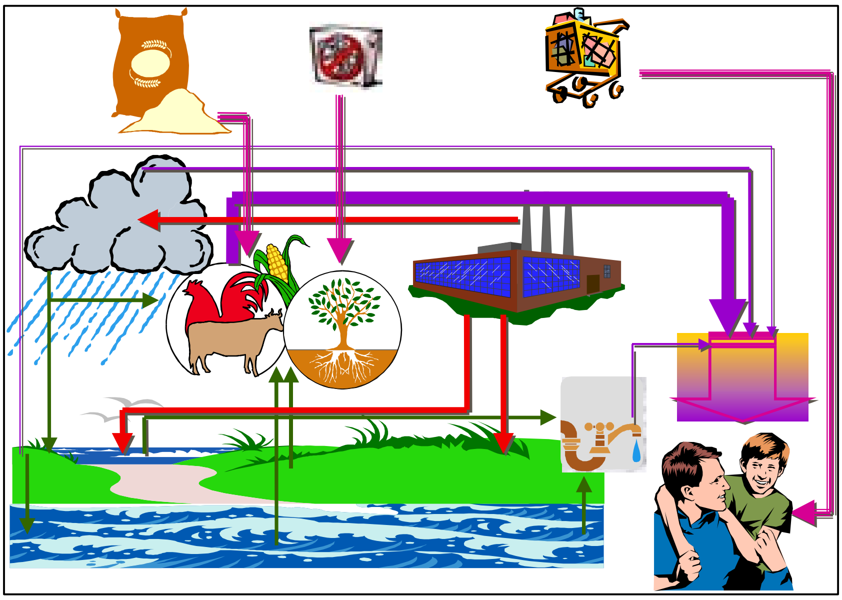

FIG. 1. Scheme illustrating the interaction established between the air and the ground (animals and plants).

Toxic air pollutants can also be deposited onto soil or into lakes or streams, where they affect ecological systems and can eventually affect human health when consumed in contaminated food [2]

The new FWD (96/62/EC) responds to this care with air pollutant effects on the human health and on the ecosystems, including mechanisms to assure their protection. A fundamental requisite of the FWD is the definition of an evaluation programme of the environmental air quality in the territories of the Member States, which should include three principal components: the monitoring of the air quality, emissions inventories, and atmospheric modelling.

In addition to the FWD, the member countries of the United Nations Economic Commission for Europe (UNECE) have included the concepts of critical load and level for planning air pollution abatement strategies and as a base of international agreements concerning limitation of the emissions of air pollutants. These concepts imply an accurate knowledge about pollutants deposition fluxes on surface, in order to estimate the ecosystems input for the chemical elements of interest. Data on deposition fluxes can be obtained by direct measurements or through model simulations.

In spite of the general idea that there are no problems of air pollution in Portugal, some difficulties can be predicted in achieving the new limits of the FWD [3], thus showing the urgent need for the definition of a clear and practicable strategy for air quality management. Biomonitoring of atmospheric pollution could be a very useful and important tool either in the scope of the FWD or of the critical load concept. The main purpose of this paper is to describe the main needs and the tools available to define a strategy of air quality management in Portugal. Two case studies are presented: (i) development of an extensive monitoring plan aiming to assess the environmental impact of an urban incinerator plant; and (ii) contribution to a methodology to estimate critical levels for a coastal region in Portugal. These different approaches allowed illustrating the complexity of the implementation of an air pollution management strategy.

In the first case, an External Monitoring Plan (EMP) has been designed with the objective of assessing the potential impact over the environment of the construction and operation of a municipal solid waste incinerator, the so-called LIPOR II. One of the main concerns of the EMP is to fulfil the lack of information about the environmental baseline levels of dioxins and furans prior to the operation of the incinerator. This information is essential to assess the actual environmental impact of LIPOR II. Under the EMP, samples of different environmental matrices (air, soil, water, vegetables, farm animals, and human fluids) were taken with the objective of assessing the migration cycle of organic pollutants.

Concerning the estimation of critical loads/levels, a coastal region was selected and maps of concentration levels of ozone were created aiming to evaluate the exceedance, or not, of the critical levels for sensitive plants, plant communities, and ecosystems. To accomplish this objective the development of a database covering different information (pollutant emissions, land cover and land use, etc.) was needed and a mesoscale system of models was applied to simulate the photochemical production, transport and deposition during a typical summer day. It was possible to evaluate the impact of photochemical pollution both in time and space. Comparison of the estimated values to the critical levels allowed verifying that this impact can be significant regarding sensitive plants, with some values surpassing the limit during the morning period.

2. PROGRESS OF AIR QUALITY MANAGEMENT IN PORTUGAL

Air quality management encompasses a set of measures and strategies whose purpose is, in the final analysis, the control of the sources and their emissions. Logically, the suppression of the problems of atmospheric pollution cannot be equated to a simple abolition of emissions, since such an extreme measure would imply the suspension of the majority of anthropogenic activities and the lifestyle of modern societies. On the other hand, the load capacity of natural ecosystems should not be underestimated, since through their inherent physical, chemical and biological processes, they are capable of eliminating significant quantities of pollutants and re-establishing the natural equilibrium to some degree.

It is evident from this analysis that the improvement of the environmental air quality is defined by a set of adequate measures and strategies, primarily with a view to the reduction of emissions, but that may and should also encompass the development of new technologies, be it gaseous depuration or ‘cleaner’ production, through urban and territory planning, or through a change in the lifestyles of the populations.

2.1. Legislative support

In historical terms, the European Union policy in relation to air quality has evolved from a command and control approach to a more integrated and holistic perspective. In fact, the first community policies (in the 1970s and 1980s) centred upon the definition of air quality and emission norms.

Although the adopted measures have contributed to an improvement in the environmental air quality all over Europe, the policy in what concerns the air quality has not seen new developments. On the other hand, the experience of the application of existing legislation has raised difficulties at the Community as well as the national level with respect to its applicability, equity, environmental efficiency and effectiveness, harmonisation and even at the information diffusion level [4].

A Community Directive on ozone adapted in this decade (92/72/EEC) introduced a significant advance, which translates into a new approach involving not only vigilance but also the interchange of data and information to the public. Simultaneously, the adoption of the 5th Environmental Action Programme introduces the concept of a sustainable development, which, in the atmospheric pollution area, translates into the necessity of altering the legislation with a view to the establishment of long-term objectives of air quality.

The new FWD is in response to this necessity, conforming to the orientation objectives of the 5th Action Programme, through a more encompassing and holistic approach to the problem. In particular, the new legislative map will permit the control of a larger range of pollutants and includes not only the protection of public health but also that of the ecosystems, as well as the establishment of a procedure aimed at the creation of an information circuit.

In accordance with the European Commission [5], the FWD is a political instrument which:

- Creates a consistent system at the EU level, for the settlement of binding objectives on the subject of air quality, and for a series of specific pollutants;
- Demands that Member States or their designated authorities create systems to evaluate the environmental air quality in their territories;
- Demands that the Member States or their designated authorities elaborate action plans and programmes which should be initiated when the environmental air quality does not satisfy the defined criteria;
- Establishes the formation of a complete and coherent system of gathering, transmitting and divulging information on air quality to the European Commission and the general public.

A fundamental requisite of the FWD is the definition of an evaluation programme of the environmental air quality in the territories of the Member States, which should include three principal components: the monitoring of the air quality, emissions inventory, and atmospheric modelling.

2.2. Available Tools

Emission inventories, modelling and monitoring of air quality are already amongst the most used tools to support and evaluate the air quality management.

Air quality management strategies clearly imply a deep knowledge of air pollutant emissions. This knowledge should temporally and spatially cover information about pollution sources, their quantity and dynamics. Developing estimates for how much of a pollutant is being emitted into the atmosphere is an essential step to determine air pollution control measures to protect human health and ecosystems.

Different methodologies could be applied to compile emission data. Direct measurements on a stack give accurate information but are not always available. In this case, emission estimates based on emission factors are useful tools that provide quantitative data, sometimes unique, for principal pollutants. Emission factors relate the mass of a pollutant released into the atmosphere with fuel consumed or product made. Their selection as well as the statistical information to be used in emission estimation have a significant impact on the final results and should be carefully processed.

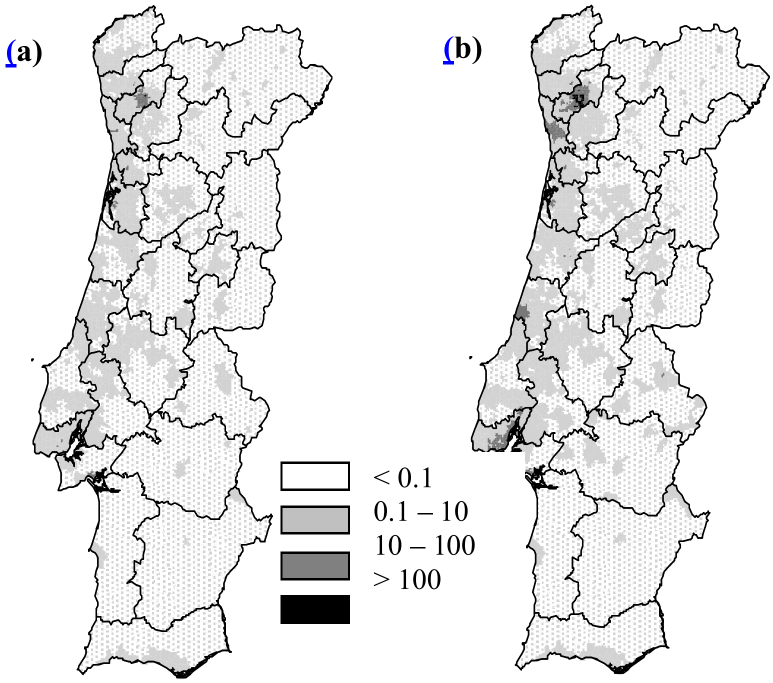

FIG. 2. Anthropogenic emissions (except road-traffic):(a) NOx, (b) SO2 (ton km-2 year-1).

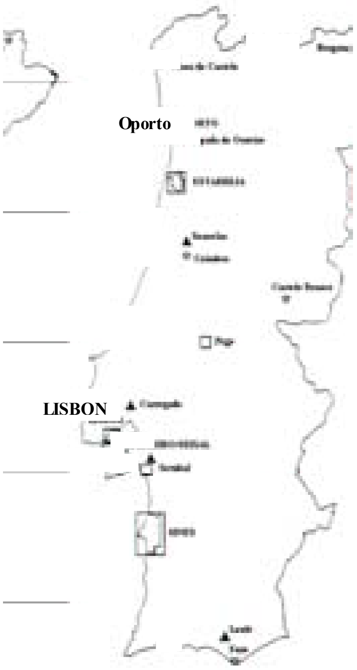

FIG. 3. Location of the air quality networks, reporting to Environment Regional Directorates.

The European Commission developed a methodology to estimate emissions in Europe that gave origin to the CORINAIR inventories. The CORINAIR inventory represents one of the possible bases for quantifying principal pollutants released annually into the atmosphere on the national scale. In Portugal, this is one of the principal reference databases about atmospheric emissions and is used as a supplementary tool for political decisions on the environment [6]. However, a larger spatial and temporal resolution is needed if one wants a deeper analysis. This downscaling exercise is based on specific factors related with source activities, such as fuel consumption and population statistical data [7]. For Portugal, the National CORINAIR 90 inventory was processed in order to obtain higher data resolution. Figure 2 presents the annual spatial distribution of NOx and SO2 anthropogenic emissions (excluding traffic emissions) disaggregated for the sub-municipal level [7].

Analysis of Figure 2 shows that major anthropogenic emissions, excluding those from traffic, are concentrated near the coastline corresponding to preferential distribution of population and economic activities, including large point sources, on the coast. Possible risk for human health by directly emitted or transported pollutants requires strict air quality control in this zone.

Monitoring all pollutants of interest, and using modelling as a complementary tool, as suggested in FWD, should contribute to the evaluation of the air quality in Portugal. The number of national operational air quality monitoring sites has varied in recent years as a result of a process of restructuring in some air quality sub-networks. In Figure 3, the general location of the air quality monitoring networks of Environment Regional Directorates is presented (some industries, like thermal power plants, cemeteries, pulp and paper mills, refineries, etc., have their own quality monitoring networks).

It is possible to verify from Figure 3 analysis that all the air quality monitoring stations are located along the coastline and close to the most industrialised and urbanised areas. Taking into account the poor spatial covering of monitoring data, the modelling approach becomes the most consistent tool for air quality management in Portugal.

Air pollution modelling can comprise and involve several temporal and spatial scales: from the global one until the local. Figure 4 illustrates a possible structure to use modelling techniques as a tool to air quality management.

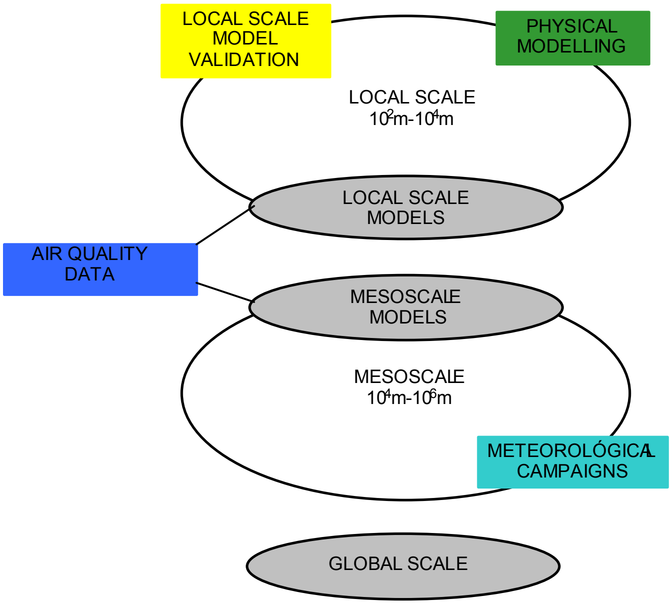

FIG. 4. A modelling structure for air pollution evaluation.

In fact, air pollution questions are not confined to a local area, as understood in the past, and available models reflect this new concept. Pollutants emitted in Portugal will contribute to the global greenhouse effect and climate change impact will affect the atmospheric circulation and even the Portuguese air quality [8]. In addition, mesoscale circulations, as sea breezes, are quite important concerning photochemical pollution, which has been affecting some areas of the Portuguese coast. [9] presents a study on the assessment of ozone control abatement strategies in Portugal through the application of mesoscale models.

3. CASE STUDIES

Taking into consideration the different available tools and processes to manage air quality, two case studies were selected to exemplify the application and development of air quality management in Portugal. The first one concerns an External Monitoring Plan (EMP) designed with the objective of assessing the potential impact over the environment of the construction and operation of a municipal solid waste incinerator, the so-called LIPOR II. The second case study is related to the estimation of critical loads/levels on a coastal region aiming to evaluate the exceeding, or not, of the critical levels for sensitive plants, plant communities, and ecosystems.

3.1. External Monitoring Plan for a municipal solid waste incinerator

One way of assessing the impact of a certain pollutant source in the environment and human health is the implementation of a monitoring plan involving not only an environmental program but also the inter-relation between various sectors. This kind of tool permits the evaluation of the direct relation between the potential environmental contamination and the public health as well as the appearance of physiological stress in some elements of the population caused by a negative perception of the project.

3.1.1. Description of the project

With the Environmental Impact Assessment study developed in 1995 for an Incineration Unit to treat 400,000 ton of municipal solid waste per year in Oporto region, the Institute for Environment and Development (IDAD) started a series of environmental framing studies of the construction project and operation of the referred industrial unit, commonly named as the LIPOR II project. LIPOR II is located in the North of Portugal, close to the Oporto City (Figure 5). The developed studies included the design of the EMP in 1997, its implementation and Pre-monitoring in 1998.

The objectives of EMP are the assessment of the actual environmental impact of the construction and operation of LIPOR II, the evolution of the public health, the evolution of the psychosocial reactions of the population, and a systematisation and integrated management of the obtained data.

During 1998 and 1999, an extensive monitoring program of environmental and biological levels of several pollutants was performed in the region of Oporto. A set of 26 monitoring stations in a radius of approximately 10 km from the incinerator was selected. The majority of the monitoring stations is located near the incinerator in the suburbs of Oporto [10].

One of the main concerns of the EMP was to fulfil the lack of information about the environmental baseline levels prior to the operation of the incinerator. This information is essential to assess the environmental Baseline State of the area. The collection of these data was extended until August 1999, when the operation of the incineration unit started. Trial burns were performed at LIPOR II during the summer and fall of 1999 and regular operation started in January 2000 [11].

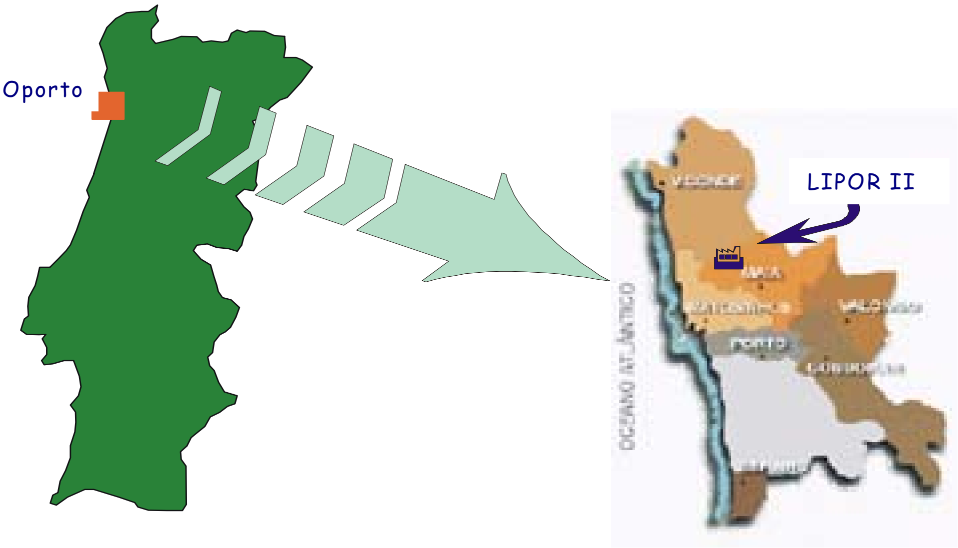

FIG. 5. Location of LIPOR II.

Three different and complementary media integrate the Monitoring Program: (i) Environment Monitoring Plan; (ii) Public Health Monitoring Plan; and (iii) Psychosocial Monitoring Plan.

The Environmental Monitoring Plan involves 4 sub-programs: air quality, water (surface and ground), biomonitoring (agriculture), and noise. The different collected information was grouped in two thematic areas: the environment area, with sampling collection of air, water, sediment, soil, and noise levels measurements, and the biological area which includes samples of cabbage leaves, corn, potato, chicken eggs and viscera, cow milk, and rye-grass.

The location of the monitoring stations for the 4 sub-programs was selected to permit the interrelation between the information collected in each type of sampling in order to identify possible cause-effect relations among the sub-programs. It has to be mentioned that some sampling stations are used for more than one sub-program, which allows the collection of various materials at the same station. Figure 6 shows the location of the sampling sites of the 4 sub-programs.

Regarding the air quality monitoring program, 5 monitoring stations were selected to characterise the baseline period (Crestins, Oporto, Agrela, V. N. da Telha, and Custóias), two of them, V. N. da Telha and Custóias, are integrated in the air quality monitoring network of Environment Regional Directorate of the North (DRA-Norte). In these two monitoring stations NO, NO_2, CO, O_3, SO_2, and PM_{10} are monitoring continuously. The other considered pollutants are heavy metals, acid compounds, particulate matter, non-methane volatile hydrocarbons, dioxins, and furans. During the operation of the incineration unit, the number of monitoring stations is reduced to four with a new station considered for the continuous monitoring.

The water sub-program considers the assessment of the quality of surface and underground waters in the area around LIPOR II. A total of 8 monitoring sites are considered: two in Leça river, one in the water line that surrounds LIPOR II, and five in existent wells close to the industrial units. It is foreseen the fitting of piezometers to control underground waters in the surround area of LIPOR II and in the particle deposition areas. Physico-chemical and microbiological parameters are measured in this sub-program, as well as metals and organic compounds.

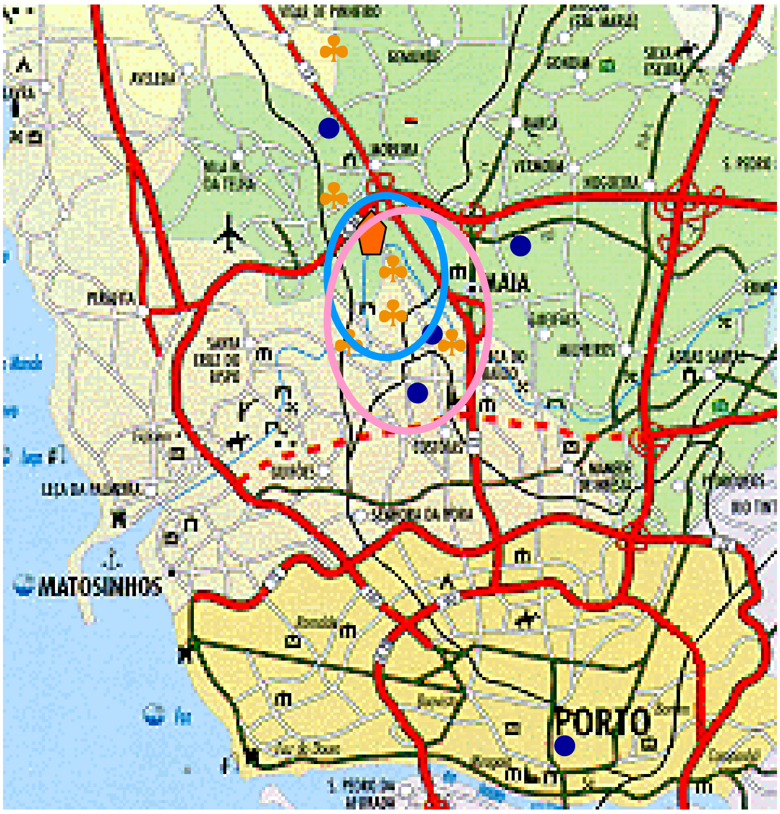

FIG. 6. Location of the monitoring stations considered in the Environmental Plan of LIPPOR II.

Concerning the biomonitoring sub-program, the selection of the sampling areas for the terrestrial biota program was based on the identification of the local main cultures and agriculture and stock raising production and on considering the possible contamination by the deposition of atmospheric pollutants or by surface and underground water. This led to the selection of six monitoring stations in a radius of 5 km from LIPOR II, where samples of cabbage leaves, corn, chicken eggs and viscera, cow milk, and potato and rye-grass soil are collected for the analysis of chemical parameters, metals, dioxins and furans. For the monitoring of aquatic biota, the three sampling sites selected are located in Leça river, two of them upstream and one downstream.

The scope of the Public Health Monitoring Plan is the assessment of the possible risks to the public health associated to the normal operation of LIPOR II, in the population of its area of influence. The plan integrates two complementary measures, biological surveillance and risk factors surveillance. The biological surveillance plan includes the analytical determination of dioxins and furans levels in human milk and blood and the analysis of lead and other heavy metals levels in blood. Risk factors surveillance plan consists of studies based on inquire and on the actualisation of data in identified sources, aspects related to smoking habits, fertility and mortality.

The Psychosocial Monitoring Plan involves a data research that guarantees the life quality of the populations during the construction and the operation of the incineration unit. This plan focuses on the answers of an inquire by the habitants of three different regions: Crestins/ Araújo, a residential area in Oporto and a residential area in Maia. The selection of the habitants takes into account the distance from the house to LIPOR II, the sex, and the qualifications of people inquired. Stress, anxiety, and depression were considered as psycho-sociologic impact indicators.

3.1.2. Presentation of some results

Dioxins and Furans group of compounds is one of the most important analysed parameter in the environmental monitoring plan because the combustion of solid waste is one of the main sources of these compounds, they are lipophilic substances (tend to accumulate in life tissues) and they are considered toxic pollutants.

It should be mentioned that the commonly denominated "dioxins" are compounds with 75 isomers, and the most toxic is 2,3,7,8-Tetrachlorinated dibenzo-*p*-dioxin (TCDD). The same is true for "furans", with 135 isomers, being the 2,3,7,8-Tetrachlorinated dibenzo-*p*-furan (TCDF) normally considered. The toxicity depends of the number and position of the chlorine atoms. In order to make easier the interpretation of analytic results and to aggregate the toxic effects of isomer mixtures, the International Toxicity Equivalent factor (I-TEQ), related to TCDD, is used. The most common measured dioxin/furan is PCDD/PCDF.

As referred in the EMP, the PCDD/PCDF levels were measured in ambient air of Oporto region (air quality sub-program), and in samples of soil and vegetables (biomonitoring sub-program)

As regards the PCDD/PCDF air concentrations, the results obtained were treated statistically, the mean value and the upper and lower limits with a confidence interval of 95% were calculated for the baseline and tests periods and for winter and summer seasons, and are represented in Figure 7.

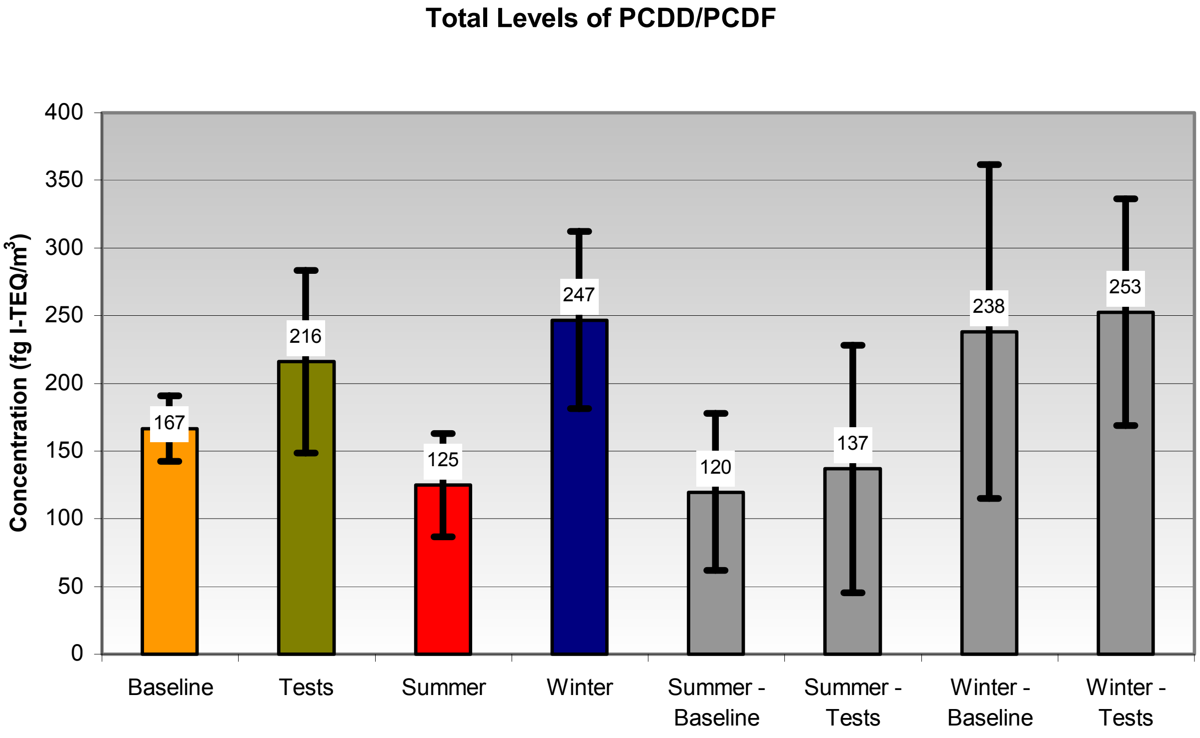

FIG. 7. Environmental levels of dioxin and furans for specific periods (fg I-TEQ m^{-3}).

The average PCDD/PCDF concentration increased with the start up of the Municipal Solid Waste incinerator (from 167 fg I-TEQm^{-3} to 216 fg I-TEQm^{-3}, but this increase is not statistically significant. When comparing the results obtained for summer (125 fg I-TEQm^{-3}) with winter (247 fg I-TEQm^{-3}) it can be said that the increase is more noticeable and, it is statistically significant at 95%.

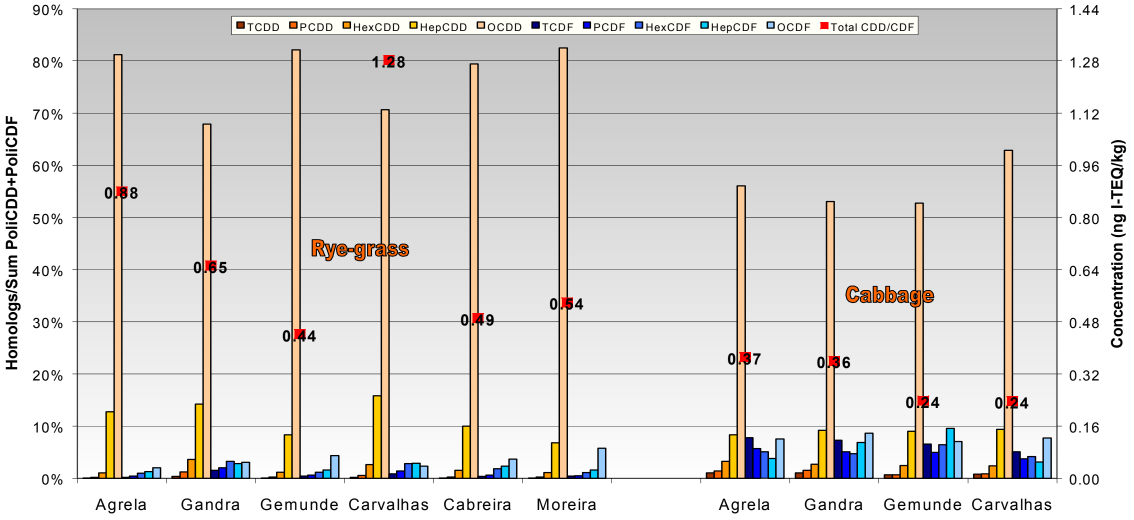

FIG. 8. Levels of dioxin and furans in soils.

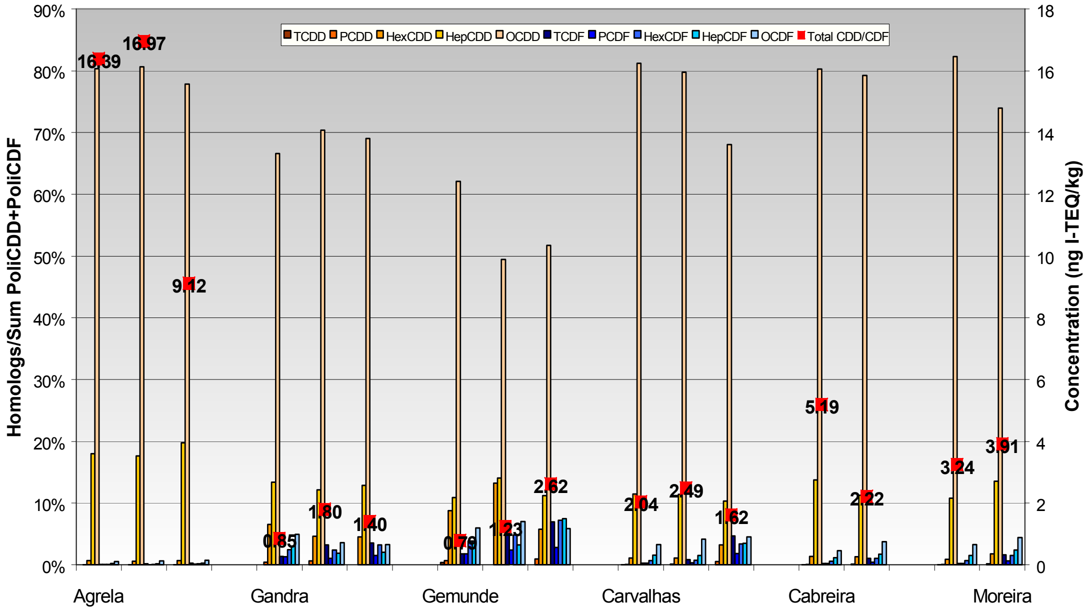

FIG. 9. Levels of dioxin and furans in vegetable species.

Figure 8 shows the results obtained in the analysis of soil samples collected in different sites.

Levels obtained are low except in Agrela. Although, it should be referred that the structure of the samples differ from site to site as it can be observed in the figure. Cabbage leaves samples (Figure 9) showed similar levels and congener patterns among the monitoring stations. Rye-grass samples indicate spatial variability of the PCDD/PCDF levels, but there were no significant differences as regards the congener patterns.

Data obtained through this study was fundamental to know dioxin and furan levels in Portugal The levels obtained during the Baseline phase of the project showed significant levels indicating some hot spots that require additional investigation.

3.2. Estimation of Critical Loads for a coastal region in Portugal

The member countries of the UNECE pointed out the critical load concept for planning air pollution abatement strategies and as a base of international agreements concerning limitation of the emissions of air pollutants. Critical load is defined as the “highest load that will not cause chemical changes leading to long term harmful effects in the most sensitive ecological systems” [12]. Critical loads can be calculated for acidification and/or eutrophication, depending on the pollutant, over a specific ecosystem. For gaseous pollutants, the critical level concept is used instead of critical load. “The concentrations of pollutants in the atmosphere above which direct adverse effects on the receptors, such as plants, ecosystem or materials, may occur, according to the present knowledge” is the definition of critical levels proposed by the UNECE [13].

The methodology to estimate critical loads/levels involves the application of knowledge from different areas, from the modelling of atmospheric transport and deposition until the geochemistry, the hydrology, and the biology. Table I presents the sequence suggested by the UNECE [14] to estimate critical loads aiming to reduce atmospheric pollution

TABLE I. SEQUENCE TO APPLY CRITICAL LOADS [14]

Key-Phases	**Description**
I	Pollutant selection
II	Selection of the appropriate receptor
III	Determination of the critical chemical level
IV	Method selection
V	Delimitation of areas presenting receptors with similar sensitivities
VI	Collection of the needed data
VII	Calculation of the critical loads
VIII	Use of deposition data to verify exceeding values in relation to the critical loads
IX	Application of the critical loads values in emission-deposition models

One of the main purposes of the work to be developed is the presentation of an European map showing the places where natural limits to pollutants are exceeded. To accomplish that task, a data base of different information type is needed (Figure 10), namely: pollutant emissions, photochemical production and dispersion, long range transport, amount of deposition, land cover, land use, relative vulnerability of the different ecosystems, meteorology, among others.

The total deposition concept is deeply involved in the estimation of critical levels and loads. Its definition takes into account the contribution of three depositions processes: the dry, wet and occult deposition [15]. The wet and dry depositions are susceptible of measure as well as of numerical calculation and give the amount of pollutants entering in the ecosystems, per unit area and time.

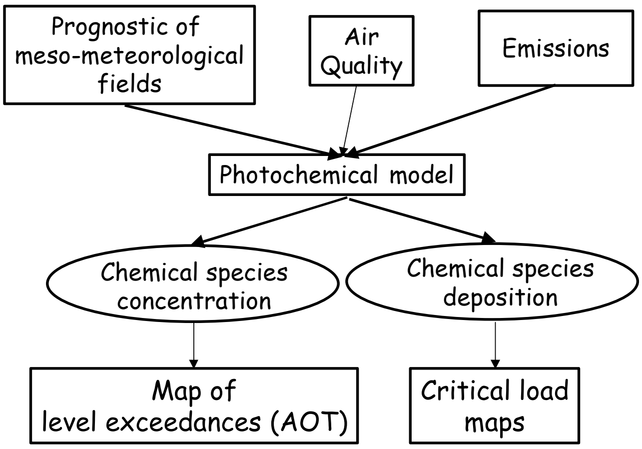

FIG. 10. Scheme for numerical calculations of critical levels and loads (AOT-Accumulated Ozone Threshold).

The development of dry deposition processes for atmospheric chemical models requires parameterisations in their formulation. This parameterisation is based on measurements performed in laboratory, using enclosure methods, as well as in pollutants flux measurements field campaigns, namely in Northern Europe and Northern America, above their specific ecosystems and climatology. Nevertheless, the application of photochemical transport models for air pollutant concentration and dry depositions maps over Mediterranean areas requires tests on the dry deposition parameterisations. This relies on the fact that these areas are at lower latitudes and cloudiness, when compared to the Northern Europe and North America. More incoming radiation is observed which leads to a different growing season period, from early spring to early autumn, and also favours the development of strong mesoscale circulation. In Portugal, measurements of ozone concentration, ozone dry deposition flux, and micrometeorological, variables were taken within the framework of an European Commission Project (MEDFLUX — Quantification of Pollutant Dry Deposition Fluxes over Mediterranean Type Ecosystems), by the University of Aveiro [16], allowing to obtain fundamental data to South European countries.

TABLE II. OZONE CRITICAL LEVELS FOR SENSITIVE PLANTS, PLANT COMMUNITIES, AND ECOSYSTEM [17]

Exposure duration	***Concentration***	
(h)	***$\mu g.m^{-3}$***	***Parts per million by volume***
0,5	***300***	***0,150***
1,0	***150***	***0,075***
2,0	***110***	***0,055***
4,0	***80***	***0,040***
8,0	***60***	***0,040***
Vegetation/growing period (average of 7 h (mean/day)	***50***	***0,025***

Aiming to contribute to the European air pollutants exceeding values map, dry deposition calculations were performed for a coastal area, with the mesoscale numerical model system MAR-IV. The air pollutant under consideration was ozone and therefore critical level thresholds for this pollutant were considered. The UNECE has established ozone critical levels for sensitive plants, plant communities, and ecosystems, according to the exposure duration to ozone air concentrations (see Table II).

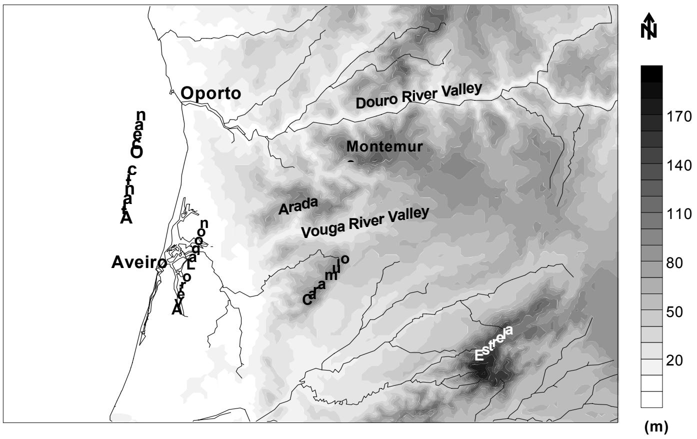

FIG. 11.Topography of the study domain.

Aveiro region was chosen, as a coastal area, to apply the photochemical modelling system in order to estimate ozone concentration values during a typical summer day. Aveiro's domain covers an area of 140 × 200 km^2, including Oporto and Aveiro city's. Figure 11 presents the topography of the Aveiro region.

The western part of the study region is dominated by the Aveiro lagoon (about 80 km^2), which is a long and branched lagoon extended in the North-South direction following the coastline. In opposition, the eastern part of the domain is characterised by mountain chains that can reach 2,000 m in high. The topography is also determined by the presence of the Vouga and Douro River valleys. The meteorological conditions in this region are strongly influenced by the nearby Atlantic Ocean, with the frequent occurrence of sea breeze winds in spring and summer periods. The main anthropogenic emission sources are located at the urban and industrialised areas of Oporto, in the North, with about 2 million inhabitants and the second biggest refinery of Portugal (Matosinhos refinery), and Aveiro, in the South, with about 80 thousands inhabitants and an important industrial chemical complex (Estarreja). To the inland, the anthropogenic pressure is replaced by the presence of large extensions of forest and agriculture fields.

The numerical simulation was carried out by the application of the model system MAR IV [18]. Two mesoscale models, the Colorado State University (CSU) model and the photochemical Urban Airshed Model (UAM), compose this system. The CSU model is a hydrostatic, terrain-following model, which produces three-dimensional fields of meteorological variables. These fields are introduced as input to the UAM model, together with an emission grid file. The UAM is a grid model that contains a photochemical mechanism, the version IV of the Carbon Bond Mechanism (CB-IV), covering 85 reactions and 23 species [19] and treating the isoprene explicitly. In the UAM, dry deposition is assumed to occur in a two-step process: the transfer of pollutants through the atmosphere to the surface and the uptake of the pollutants by vegetation and other materials at the surface. The MAR IV system was applied with a horizontal resolution of 5 × 5 km^2. In the vertical direction the grid consisted of 28 non-equidistant layers until 8 km for the mesometeorological system module and 7 layers up to 3 km for the photochemical transport system module. Simulations were performed for both domains using a typical summer synoptical circulation [20], characterised by an extension of the Azores anticyclone over the northern part of the Iberian Peninsula and to the location of a low pressure system to the west of the British Islands. A thermal low was created at the high and arid central plateau of the peninsula, producing a weak N-NW wind over Portugal. Strong insulation promoted the formation of mesoscale circulation.

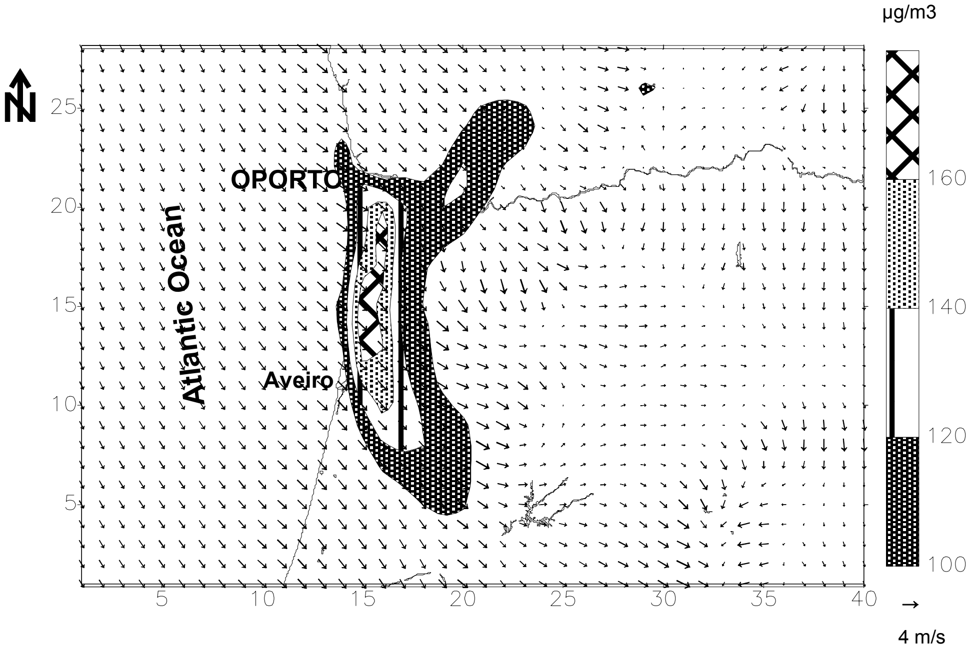

FIG. 12. Ozone concentration field over Aveiro region at 10H00 UT.

The simulated ozone concentration field at 10H00 (Universal Time – UT), presented in Figure 12, shows the presence of the ozone Oporto urban plume, which is, during the morning, transported to South and advected inland with the sea breeze circulation during the afternoon.

The ozone concentration impact was predicted both in time and space, considering the ozone concentration value for one hour exposure duration (see Table III), and the ozone concentration values pointed in the current 92–72 EEC directive. In Table III it is shown the numbers of cells that registered concentration levels above the following thresholds: 150, 180, and 360 μm^{-3}. As it can be seen, several grid cells of the domain were exposed to ozone concentration levels above 150 μm^{-3}, between 10H00 and 15H00, which indicates a potential critical situation for the sensitive plants and ecosystems of the affected region. None of the domain's cells is exposed to ozone concentrations higher than 360 μg.m^{-3}. In order to verify the performance of the simulations, a preliminary comparison between the system predictions and the deposition and concentration data recorded on a measurement station located in the study area was performed. Figures 13 and 14 present, respectively, these comparisons between concentration and deposition values.

TABLE III. NUMBER OF CELLS EXPOSED TO OZONE CONCENTRATION VALUES ABOVE OZONE THRESHOLDS.

Time	**≥ 150 μg.m^{-3}**	**≥ 180 μg.m^{-3}**	**≥ 360 μg.m^{-3}**
10	16	-	-
11	9	3	-
12	13	4	-
13	16	1	-
14	7	-	-
15	2	-	-

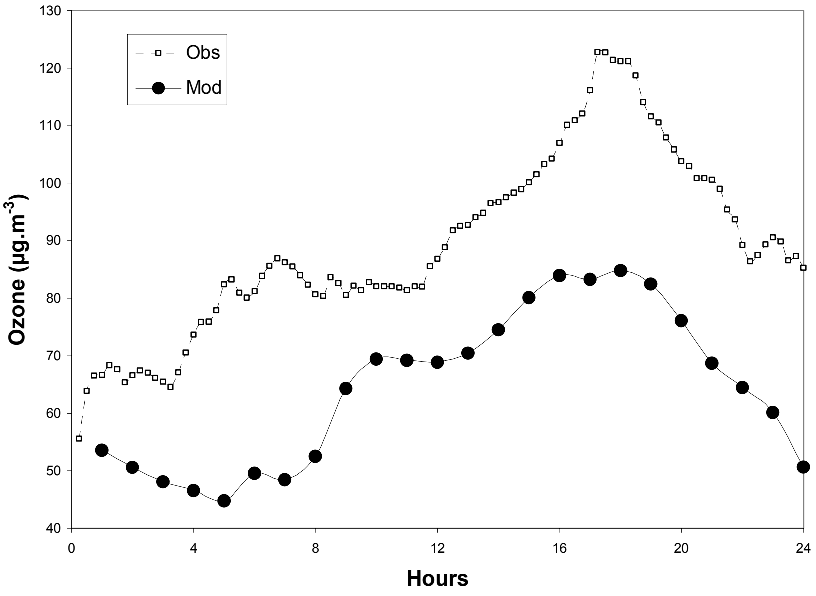

FIG. 13. Daily evolution of ozone concentration values at the measurement station site

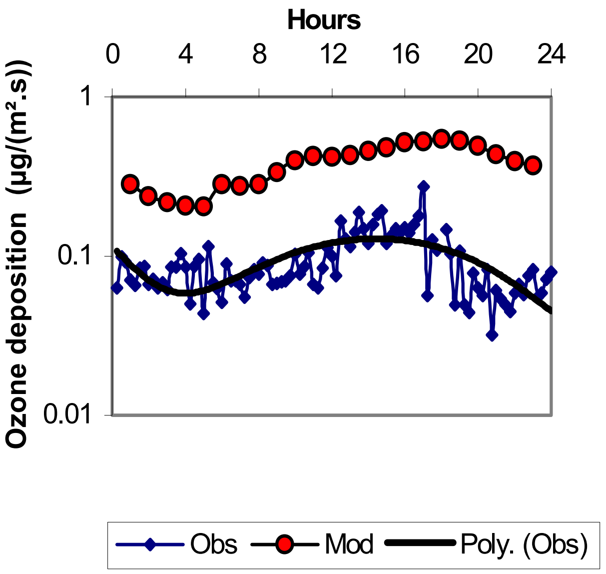

FIG. 14. Daily evolution of ozone deposition values at the measurement station site

Data obtained in the measurement station show a quite reasonable evolution of concentration and deposition parameters with a compensatory shift between them, i.e. higher ozone concentration values are related with higher values for deposition fluxes. However, underestimated values were simulated during all the simulation period and on the contrary, higher deposition values were measured. This could be related to an over estimation of the deposition velocity, or to an input deviation on the land use.

A sensitivity study of the deposition module allowed verifying its strong dependency of input values, like the land use and the roughness estimate [16]. Nevertheless, more field experimental data are required in order to proceed with the evaluation of the MAR IV system, in particular with the evaluation of the deposition module concerning vertical wind profile, for a better estimation of friction velocity, and the default parameters of the resistance to surface removal.

4. CONCLUSIONS

In this paper two different case studies are presented concerning the monitoring of the complex interactions involving the exposure to atmospheric pollutants. More traditional studies are limited to the measurement of atmospheric concentrations in the air and the comparison of the atmospheric levels with limit values published either in the legislation or in the scientific literature. Nevertheless, if it is pretended to assess how the population is exposed to these contaminants, it is fundamental to understand, in one hand how these pollutants are transferred between the different environmental media, and in the other hand, to estimate the environmental critical loads.

In Portugal's mainland, the forest areas and the agriculture fields occupy a significant part of the country. On other hand, relatively extensive zones are considered as being of ecological interest, and are legally protected. Monitoring all pollutants of interest, and using modelling as a complementary tool, as suggested in FWD, should contribute to the evaluation of total exposure in these zones.

Monitoring of biological matter such as soils, vegetables, and animal tissues, represents also an appropriate way to determine the exposure paths of the human population to atmospheric pollutants. These studies require a good description of the atmospheric pollutants deposition process and provide a cost-effective mechanism to perform a long-term sampling.

To improve the air quality in Portugal requires an integrated abatement strategy, cost-effective ceiling, and effect-oriented and source-based solutions to achieve a set of atmospheric concentrations for the protection of human health, vegetation, and ecosystems.

In the future, this research strategy could be applied to the traditional pollutants in order to assess if the limit values defined in the legislation correspond to sound environmental levels in several media, distant from the critical loads that cause long term harmful effects in the environment.

It is not surprising that the selection of a solution for an environmental problem is a complex process and that the number of possible solutions is high, because these problems are very complicated and the translation from emission to effect is very intricate. How many descriptions are needed to give a proper coverage of a very complex environmental issue? The answer is probably many.

This enormous "needs" and their great complexities of "tools" may explain why only a few of the many environmental problems that have so far been identified have been solved. Past solutions have not considered all aspects of the problems. The optimum solutions have not been found, because there was no attempt to overview the complexity of these problems and the systems they concern.

Entering in the 21st Century, it should be applied this to towards making environmental management more multidisciplinary and holistic, and develop a broader use of environmental models. However, models should be understood as more than just computer models used to find "optimum" solutions based on good mathematical description. They should instead be seen as a tool to overview complex systems and problems, and to facilitate communications among the various disciplines, which today sometimes hardly understand each other's language.

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LICHENS AS INTEGRATING AIR POLLUTION MONITORS

Z. JERAN[1], R. JAĆIMOVIĆ[1], F. BATIC[2], R. MAVSAR[3]

[1] Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
[2] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia
[3] Slovenian Forestry Institute, Ljubljana, Slovenia

Abstract

Bioindication using epiphytic lichens has developed rapidly in the last decades. Both mapping of lichen vegetation and multielement analysis of Hypogymnia physodes collected on a national scale were used. A simple method based on the assessment of the cover and frequency of crustose, foliose and fruticose lichens on different tree species was performed in 1991 and 1995 and the so-called Index of Atmospheric Purity (IAP) was calculated, providing a picture of the general air quality in the country. However, by analysis of lichen material using k0-instrumental neutron activation analysis detailed information about its composition and levels of pollutants was obtained. In the present work an attempt to combine the IAP results with the quantitative levels of certain trace elements together with available results for SO2 in the atmosphere using multivariate statistical methods is presented and discussed.

1. INTRODUCTION

Epiphytic lichens can be used in different ways as air-quality bioindicators. The first group of methods is floristic, based mainly on lichen-specific sensitivity to gaseous pollutants, especially SO2, fluorides and strongly oxidizing compounds, such as ozone. The classical methods of these groups are species distribution mapping and the phytosociological approach, which studies different lichen communities [1,2]. Using these methods the changes in lichen species, especially the sensitive ones or community distribution, can be a measure of air pollution. However, nothing can be said about the origin and type of pollutants. One demand in the use of these methods is a well-trained lichenologist, and if possible, good historical records about the lichen flora of the area under consideration. By adding numerical values to phytosociological characteristics of lichens and through a mathematical formula, the so-called Index of Atmospheric Purity (IAP) can be obtained for each site. Using this method which was pioneered by De Sloover & Le Blanc [3] and was then widely used in other investigations [4], the gradient of an effect can be obtained, before a species is completely eliminated because of severe pollution.

The second group of methods is based on physical, chemical and biological properties of lichens which enable them to be used as monitors of metal deposition from the atmosphere, since they can accumulate trace elements to levels far greater than their expected physiological needs [5]. Passive as well as active biomonitoring using epiphytic lichens can be used on a national scale as well as around certain pollution sources to obtain the information about the levels of particular trace elements in the atmosphere. Since the sources of elements that cause elevated levels in lichens are both natural (crustal material, marine aerosols) and anthropogenic (industry, traffic, etc.), there are at least two ways of identifying the contributions of elements from different source terms: (i) by calculating the enrichment factor (EF) for each individual element, and (ii) by applying multivariate statistical techniques to the data set.

Both kinds of methods are used in Slovenia. Initial observations on the disappearance of lichens from urban and industrialised areas were recorded about forty years ago by schoolteachers. Due to the lack of trained lichenologists, the first mapping of lichens around polluted areas was carried out in the late seventies [6,7] when comparisons were made in virgin forest reserves [8]. As the lichen flora was poorly known, only lichen thalli types (crustose, foliose, fructicose) were mapped throughout Slovenia in several school projects [9] and later this method, the so-called IAP-method, was adopted and modified for studies connected with forest decline inventories. Since 1985 it was used regularly as an air quality indicator on more than 500 plots used for forest decline studies [10]. The results of observations in 1990 and 1991 showed that the epiphytic lichen flora look quite poor with more than 70% of the territory in the fourth and third classes, which represent highly polluted and moderately polluted air [9,10].

In order to obtain information about atmospheric trace element levels in Slovenia, a monitoring survey has been performed in 1992 at 86 sampling sites on the national scale using the epiphytic lichen Hypogymnia physodes, and by the application of multivariate statistical method the possible pollution sources were identified [11].

The main aim of the present work is an attempt to combine the IAP results with the quantitative levels of certain trace elements together with available results for SO2 in the atmosphere.

2. MATERIALS AND METHODS

2.1. Determination of the Index of Atmospheric Purity (IAP)

In the period from July to September 1991 a lichen survey was performed on 546 inventory plots of the 4 × 4 km grid as a part of a forest die-back inventory (Fig. 1). All lichen observations were performed exclusively on those plots, which fall in forests, and none of the plots was in the close vicinity of any emission source. It is worth mentioning that forests in Slovenia represent more than 50% of the territory.

In the group of six marked trees on each of the inventory plots the abundance (a) and coverage (c) of three of the most important epiphytic lichen thallus types, crustose (C), foliose (F) and fructicose (R), were mapped at three heights (h) (1 — from 0 to 0.5 m, 2 — from 0.5 to 2.5, 3 — above 3 m). If possible, climatozonal forest tree species were chosen for observation. From the data collected an index of atmospheric purity was calculated for each inventory plot (IAPt) for each stratum of observation separately (IAP_1 = observations on the tree trunks up to 0.5 m, IAP_2 = 0.5–2.5 m; IAP_3 = above 3 m), using the following formulas:

$$IAP_{1,2,3} = C(a + c) + F(a + c) + R(a + c)$$
$$IAP_t = IAP_1 + IAP_2 + IAP_3$$

where the symbols represent the above mentioned observed lichen parameters. The IAP index has a span between 0–54 where the value 0 means a plot without lichens and very polluted air, and the value 54 means very rich lichen vegetation and very clean air. The details of the IAP method are given elsewhere [9].

2.2. Determination of trace elements in lichens

In the period from September to November 1992, the epiphytic lichen Hypogymnia physodes was collected at 86 sampling locations of the national 16 × 16 km bioindication grid. In contrast to the above mentioned biomonitoring survey in 1991, all sampling in 1992 was made on open habitats or in the nearest forest glade of the plot, sometimes a few hundred metres away from the well defined plots of the forest decline inventory. In each lichen sample more than 30 elements, namely As, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hf, Hg, K, La, Mo, Na, Rb, Sb, Sc, Se, Sm, Sr, Th, U, W and Zn. were determined using k_0-INAA. Total concentrations of sulphur, P and Pb in lichen tablets were determined by X ray fluorescence spectrometry (Philips PW 1410 with rhodium tube). The details of sampling, sample preparation and determination of trace elements were described previously [11].

For routine quality control NIST SRM Citrus leaves (CL-1572) was analysed according to the same procedures as lichens.

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3. RESULTS AND DISCUSSION

A direct comparison between IAP values obtained from forest decline inventories and trace element composition of lichens was possible for 61 sampling plots of the 86 set from the 16 × 16 km bioindication grid. If at a certain plot of the 16 × 16 km bioindication grid the IAP value was missing, the average value of the surrounding plots of the 4 × 4 km bioindication grid was used for evaluation (Fig 1).

FIG. 1. A map of inventory plots (4 × 4 km grid) for IAP determination (small spots), 16 × 16 km grid for H. physodes collection (big spots) and SO_2 air measurement sampling locations (open triangle).

In the first step the IAP results for 61 sampling locations from the observation of lichen flora in 1991 were ranked, similarly to damage to forest trees, in 5 classes. Then as presented in Table I, a mean value with its standard deviation was calculated for 30 trace elements measured in *Hypogymnia physodes* for each IAP class. As evident from Table I (line 3), none of the 61 sampling points was in the first and fifth class; however 88.5% of sampling points were included in classes 3 and 4, suggesting that the air is moderately to highly polluted and only 11.5% of locations belong to class 2 of low pollution. As is further seen from Table 1 for a group of elements, namely Cr, P, Ba, Zn, Sr, Hf, As, Mo, and K there was a distinct tendency to lower values with increasing IAP values; however, the standard deviations were rather high. For other elements relevant to pollution like Na, S, Pb, Sb, Co, W, and Cd the highest values were found in classes 4 and 3. An explanation for the rather high variability of trace element content in lichens collected within a certain pollution class might be that only a part of the total content could be attributed to pollution, the other part representing the natural origin of the elements, where the geology of the territory could not be neglected. Namely, Slovenia is known for its very heterogeneous geology, which is manifested by higher contents of certain elements in mineral particles suspended in air. And further, as described earlier, the sampling locations of both monitoring surveys were not exactly the same.

Nevertheless, comparing our results with the results of the more sophisticated study of Herzig et al [12], similar trends (decreasing contents with decreasing total pollution) of environmentally significant elements (Ba, Cr, P, Zn, Fe, Pb, Cd, S) were found. Also, the mean values of common elements in H. physodes in correspondent pollution zones agreed well in both studies, as well the background levels.

TABLE I. MEAN ELEMENTAL LEVELS WITH THEIR STANDARD DEVIATION (mg/kg) IN HYPOGYMNIA PHYSODES COLLECTED AT 61 SAMPLING LOCATIONS OF THE 16 × 16 km BIOINDICATION GRID CALCULATED FOR FIVE IAP — CLASSES.

Class	**5**	4		**3**		**2**		1	background	
IAP-range		1.0–13.5		13.6–27		27.1–40		40.6–54		
%	0	38		51		11		0		
Elements		mean	std	mean	std	Mean	std		mean	std
IAP91		9.38	2.91	19.31	3.58	31.64	2.47			
As		1.43	0.53	1.21	0.57	1.14	0.42		0.51	0.17
Ba		38.51	26.07	30.89	37.57	14.82	3.57		23.7	8.21
Br		13.26	5.39	16.99	6.46	14.49	3.26		10.1	2.58
Ca		23257	14562	24172	12179	16693	14739		22266	3745
Cd		1.26	1.04	0.97	0.43	1.09	0.44		0.76	0.19
Ce		2.45	1.24	2.75	1.25	2.06	0.86		1.53	0.43
Co		0.55	0.23	0.63	0.32	0.43	0.13		0.28	0.16
Cr		6.69	4.19	5.83	3.95	4.82	2.04		2.62	0.82
Cs		0.33	0.17	0.36	0.24	0.26	0.09		0.17	0.03
Fe		1175	567	1363	634	1220	749		676	231
Ga		0.59	0.28	0.66	0.43	0.42	0.14		0.3	0.1
Hf		0.19	0.10	0.17	0.09	0.12	0.05		1.38	2.75
Hg		0.10	0.04	0.10	0.05	0.11	0.04		0.08	0.03
K		4158	1191	4138	1366	3469	947		3205	893
La		1.12	0.53	1.25	0.59	0.89	0.38		0.67	0.17
Mo		1.03	1.86	0.57	0.23	0.43	0.22		0.26	0.17
Na		182	91	190	95	126	48		101	27
P		1298	83	1264	157	1236	48		1200	
Pb		33.42	50.81	27.25	7.77	28.18	12.34		26.5	9.19
Rb		13.26	6.45	16.75	12.49	12.73	7.61		20.5	12.2
S		1865	492	1902	559	1730	510		980	14.1
Sb		0.50	0.67	0.31	0.13	0.35	0.22		0.19	0.04
Sc		0.36	0.19	0.38	0.21	0.29	0.10		0.2	0.07
Se		0.27	0.11	0.29	0.14	0.27	0.11		0.21	0.02
Sm		0.18	0.09	0.21	0.10	0.14	0.06		0.11	0.03
Sr		26.5	19.3	22.6	15.9	12.0	5.6		31.6	10.6
Th		0.28	0.15	0.31	0.15	0.24	0.09		0.16	0.06
U		0.12	0.05	0.13	0.07	0.09	0.03		0.07	0.04
W		0.23	0.21	0.15	0.11	0.17	0.11		0.1	0.04
Zn		104.0	23.8	92.6	28.4	85.1	24.0		57.3	12.1

On the basis of observations of lichen vegetation on different tree species combined together a lichen map was drawn which visualises through lichen vegetation the regions where air quality was bad. Comparing lichen map (Fig.2) with the map of SO2 levels (Fig.3) as obtained from the National Air-Pollution Monitoring Network [13], it is seen that maps correspond quite well, especially in the central and northern parts of Slovenia. However, the basic Monitoring Network in Slovenia comprised 49 sampling location for 24-hour measurement of SO2 concentrations, which are located mainly in urban centres or close to the main pollution sources (Fig.1). This was the reason for the SO2 results not being used in statistical evaluation of lichen results (see further).

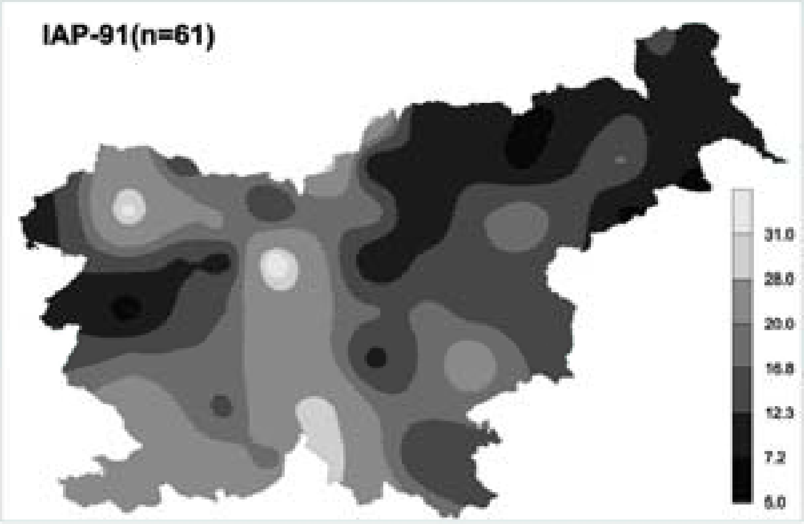

FIG. 2. Lichen map drawn on the basis of the epiphytic lichen vegetation assessment in the forest decline inventory of 1991 calculated for 61 sampling points. Observations were on all three species. IAP values have a span between 0–54 and are divided into 7 classes to the percentile values (10, 30, 50, 70, 90, 95%). Dark areas represent very poor vegetation and presumably highly polluted air.

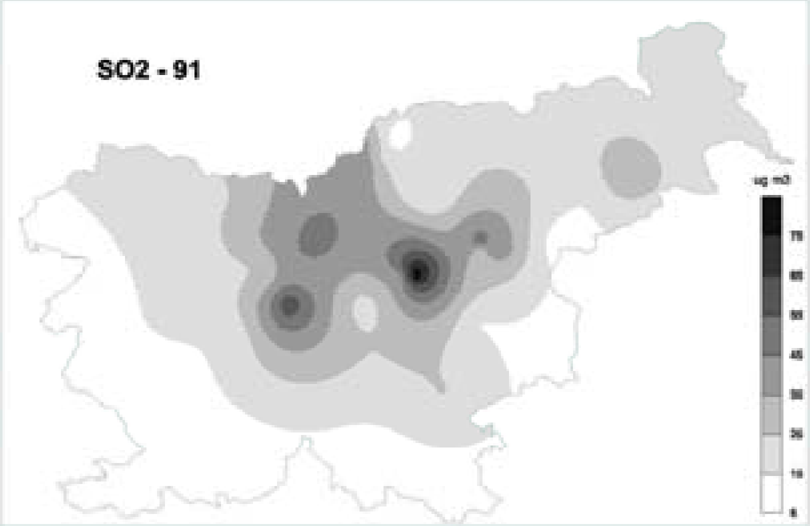

FIG. 3. The geographical concentration pattern of the annual mean levels of SO_2 in air, measured in 1991 in the framework of the national air monitoring programme. The concentrations are divided into 7 classes according to percentile values.

TABLE II. FACTOR LOADING MATRIX AFTER VARIMAX ROTATION FOR 61 SAMPLES OF *HYPOGYMNIA PHYSODES*

Component	F1	F2	F3	F4	F5	F6	F7
IAP91	-0.039	**0.407**	-0.104	**-0.508**	0.016	**-0.222**	**-0.391**
As	**0.680**	0.035	**0.370**	**0.327**	**0.366**	0.101	-0.015
Ba	-0.011	**-0.639**	0.056	0.168	0.006	**-0.400**	-0.103
Br	**0.604**	**0.551**	0.072	-0.133	0.153	-0.158	0.132
Cd	0.015	-0.134	0.210	-0.124	0.009	0.059	**0.786**
Ce	**0.961**	0.060	0.026	-0.043	0.042	-0.040	0.087
Co	**0.783**	0.074	0.016	0.028	0.265	0.014	0.192
Cr	**0.362**	-0.009	-0.014	**0.643**	0.255	-0.036	0.145
Cs	**0.372**	0.126	-0.008	0.178	**0.593**	-0.200	0.026
Fe	**0.870**	0.098	0.035	0.103	0.290	-0.043	0.029
Ga	**0.850**	-0.046	0.050	0.173	0.179	0.037	-0.134
Hf	**0.723**	-0.151	-0.040	0.080	0.162	0.189	0.155
Hg	0.190	**0.328**	0.103	**0.349**	**0.620**	-0.236	-0.071
K	**0.442**	**-0.409**	-0.064	-0.097	**0.459**	**0.392**	0.036
La	**0.962**	0.061	0.023	-0.029	0.057	-0.062	0.082
Mo	0.027	0.042	0.076	0.065	-0.022	**0.810**	0.024
Na	**0.892**	-0.170	-0.045	0.052	0.181	0.030	0.066
P	**0.418**	-0.232	-0.015	-0.002	**0.712**	**0.343**	-0.030
Pb	0.045	0.005	**0.960**	-0.069	-0.101	-0.045	0.130
Rb	0.187	0.104	-0.084	-0.155	**0.740**	0.035	0.237
S	**0.723**	0.108	-0.050	0.165	**0.366**	0.190	-0.101
Sb	0.094	-0.003	**0.968**	0.087	0.041	0.115	0.097
Sc	**0.931**	-0.004	0.048	0.102	0.291	0.032	-0.002
Se	**0.733**	**0.375**	0.177	-0.048	0.164	0.024	0.163
Sm	**0.961**	0.080	0.023	-0.037	-0.054	-0.058	0.063
Sr	-0.107	**-0.843**	-0.013	-0.120	-0.107	0.063	0.123
Th	**0.956**	0.104	0.060	0.085	0.177	-0.035	0.010
U	**0.862**	0.119	0.063	0.149	0.086	0.119	-0.110
W	-0.017	0.026	-0.007	**0.836**	-0.075	0.017	-0.130
Zn	0.218	0.292	0.009	0.294	**0.320**	-0.068	**0.632**
Explained variance (%)	38	8	7	7	10	5	5

It was found earlier [11] that using factor analysis, the concentration levels of 28 selected elements in lichens collected at 86 sampling locations can be explained by 9 factors or source types in Slovenia. To find out how the two biomonitoring surveys (IAP and quantitative determination of trace elements in lichens) can explain the pollution situation in our country, a slightly modified data set compared to the above mentioned study was prepared since some additional trace elements (P, Pb, S) were included in the data set. All together 30 elements (As, Ba, Br, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hf, Hg, K, La, Mo, Na, P, Pb, Rb, S, Sb, Sc, Se, Sm, Sr, Th, U, W, and Zn) together with IAP values for 61 sampling locations were used in factor analysis. By using the Statistica for Windows 5.0 software package in the present study, seven factors were extracted which explain 78.6% of the total variance of the data set. In Table II those loadings after varimax rotation that were equal or greater than 0.3 are presented in bold print. Although a slightly different data set for trace elements than that published before [11] was used, similar factors were extracted. There were 3 factors, namely F4, F2, and F7 in which IAP values were included with loadings greater than 0.3; however in the other factors, loadings for IAP were much lower, with the exception of F6 where a loading of 0.2 was found for IAP. Again, as in previous work [11], the so-called soil factor (F1) explained the highest percentage (37%) of the total variance in the data set, and from its composition it is evident (Table II) that IAP was not included.

A detailed insight into the composition of F4 which has the highest loadings of IAP among the factors, showed that it contains the elements W, Cr, Hg, As, and Zn which are likely to represent the metal producing or steel industry since the geographical distribution also supports its origin (Fig. 4) [11]. The highest contribution of this factor as presented in Fig. 4 coincides with the lower IAP levels in the N, NE and W parts (near the Italian border) of Slovenia (Fig. 4).

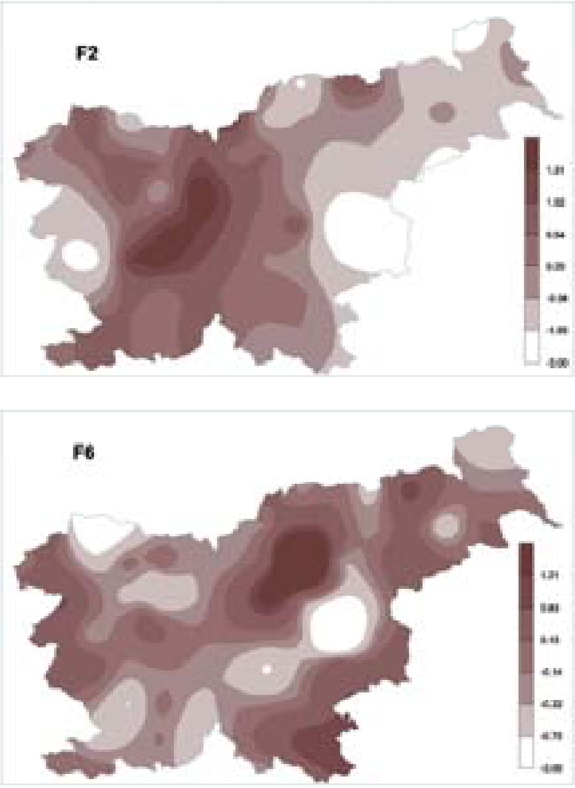

FIG.4. Geographical distribution patterns of the factors F2, F4, F6, and F7. Increasing depth of shading represents higher factor scores as shown in scale.

Factor 2 is characterised by the elements Sr, Ba, Br, K, Hg, and Se, with relatively high loadings (0.4) for IAP. It can be considered as a complex factor, representing a marine component (high loadings for Br) on the one hand, and on the other hand the high loadings for Ba and Sr could suggest a Ba-source. Namely, the geographical distribution of F2 is very similar to F5 [11] defined as a Ba-source and was explained by formation of insoluble (Ba and Sr) compounds in the atmosphere due to elevated levels of SO_2. It appears rather surprising that S is not included in F2. Higher loadings of S (0.4) were found in factor 5 with elements such as Rb, P, Hg, K, Cs, and in the soil factor (0.7) where no correlation with IAP was found.

The third factor with higher loadings for IAP is factor 7, which contains Cd and Zn, and as follows from its geographical distribution (Fig. 4) and also from a comparison of the composition of this factor with that of F2 from the previous work [11], reflected the steel industry. The higher loadings of this factor coincide with the location of a steel factory at Jesenice (NW Slovenia), the industrial region around Trieste (Italy), the steel factory at Ravne (northern Slovenia) and some other metal industry in the eastern part. The higher scores of F7 are also in agreement with the poor lichen vegetation in NW and central parts of Slovenia (Fig. 4).

Factor 6 has relatively low loadings (0.2) of IAP; however its composition and especially its high loadings for Mo could suggest emissions from coal fired power plants since the highest contribution of F6 (Fig. 4) coincides with the location of the largest Slovenian power plant (central part of Slovenia), while in the western part the influence of atmospheric transport from Italy can be recognised. Again, this kind of pollution can be responsible for lower IAP values in these regions (Fig. 2).

4. CONCLUSIONS

The evidence of our work is that by using factor analysis it is possible to identify possible pollution sources and elements which might influence the deterioration of lichen vegetation as expressed by IAP values. The relatively low loadings for IAP in factor analysis pointed that there are different effects which cause poor lichen vegetation and only part can be explained by trace elements. Although the IAP-method used in the present study was very simple, based on observation of coverage and abundance of lichen thallus types, where mistakes are possible due to subjective judgement and also the estimation of crustose thallus types at heights above 3 m is sometimes difficult, reasonably good results were obtained. The comparison between lichen element concentration levels and IAP values was difficult, since as evident from the IAP results the two extreme IAP classes were missing. Nevertheless, the list of elements (As, Cr, Cd, Mo, Zn) which defined particular factors and could have negative influence on lichen vegetation is very similar to those of Herzig et al. [12].

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MAPPING OF PARTICULATE AIR POLLUTION IN BEIJING BY PLANT BIO-MONITORS

J.P. GARREC[1], S. HE[2], C. ROSE[1], F. RADNAI[1]

[1] INRA — Centre de Recherche Forestière de Nancy, Laboratoire Pollution atmosphérique, Champenoux, France

[2] Chinese Academy of Forestry, Research Institute of Forest Ecology and Environmental Science, Beijing, China

Abstract

Beijing is known for its high level of air pollution, but the town is only equipped with a small air monitoring network and for this reason nothing is known about the location of the different polluted areas, their surface, and their level of pollutants. In a first step, Beijing was divided in a 211 grid plots system. During summer 1998, leaves have been sampled on one poplar in each plot (Populus tomentosa). Among the different elements analysed in the particles collected by the leaves, two elements Fe and S, are particularly interesting: Fe is a tracer of the air pollution linked to road or railway traffic, and S is a tracer of the air pollution linked to domestic or industrial use of energy from coal or fuel. From the identification of 3 classes of iron and sulphur levels, the first air pollution maps of Beijing have been drawn up. Concerning S pollution, the analysis and the air pollution map show that this pollution is mainly present downtown, area of old habitat where coal is still largely used as an energy source, but also on some places, just at the periphery of the town of Beijing. Concerning Fe pollution, the analysis and the air pollution map show that now, on Beijing, as on all megacities of the world, the air pollution coming from the road traffic is widespreading. This pollution is detected everywhere on the town of Beijing, but particularly near and between the two main circle boulevards of the town.

1. INTRODUCTION

In urban or sub-urban areas, the majority of air pollution sources (road or railway traffic, industrial activity, individual or collective heating, etc…) emit at the same time gaseous and particulate pollutants.

Plant bio-monitoring is an attractive alternative method to detect and to monitor particles present in the atmosphere, owing to the fact that they are well collected by leaf surfaces due to their particular properties: presence of waxes and trichomes [1–3].

Study of the particles collected by vegetation is finally a simple and cheap method to obtain rapidly a good estimation of the relative levels of the "global air pollution" present on urban or sub-urban areas.

Beijing is known for its high level of air pollution [4–6], but the town is only equipped with a small air monitoring network and, for this reason, nothing is known about the location of the different polluted areas, their surface, and their level of pollutants.

The Franco-Chinese cooperative programme of advanced research (PRA), using the new plant bio-watching methods developed in the Air Pollution Laboratory at INRA — Nancy, drew up the first air pollution maps of Beijing.

2. METHODS

In a first step, Beijing was divided fallowing a 211 grid plots system: one hundred and twenty 750 × 750m grid plots downtown, and ninety one 1500 × 1500m grid plots in the rest of the town.

During summer 1998, leaves have been sampled on one poplar (between 2 to 3 meters above ground by using a tree-pruner) in each plot. Poplar (Populus tomentosa) is the tree which is the more frequent on Beijing, and the tree selected in each plot was a tree representative of the local mean pollution level (not too close to a main street of a factory, for example). After drying of the leaves (60°C during 5 days), particles have been extracted with the cuticular waxes from the surface of the polluted leaves by chloroform, and then collected on a filter (Fig. 1) [3].

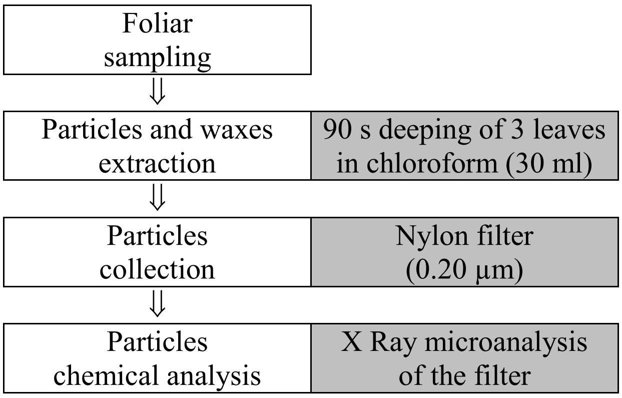

FIG. 1. Experimental protocol used for particle analysis.

After the filters have been carbon coated, the particles (shape, size, quantity, mineral composition) are studied and analysed with the filter by an X ray micro-analyser (Stereoscan 90 electron microprobe fitted with an AN 1000 10/25 energy-dispersive-analyser [Cambridge Instruments]). On each filter, three areas have been analysed. Spectra have been treated with the program ZAF 4 — FLS [Cambridge Instruments].

The concentration of 9 elements have been determined: Mg, Al, Si, P, S, Cl, K, Ca, F. Results are expressed in% of the mineral part of the particles only.

3. RESULTS

Among the different elements analysed in the particles, two elements, Fe and S, are particularly interesting:

- Fe is an element characteristic or a tracer of the air pollution linked to road or railway traffic,
- S is an element characteristic or a tracer of the air pollution linked to domestic or industrial use of energy from coal or fuel.

From this analysis, it is possible to know the different relative levels of particulate pollution in each area, but it is always very difficult to go back to its absolute concentration in the air. Before to drawing up the air pollution maps, the different concentrations of each element in the particles have been regrouped in three large classes of pollution by the statistical method of clusters (dendogram).

The identification of these 3 classes of iron or sulphur pollution on the map of Beijing permits us to determine the position and the surface of the different polluted areas with their levels, and also to identify the main sources of pollution and their impacts.

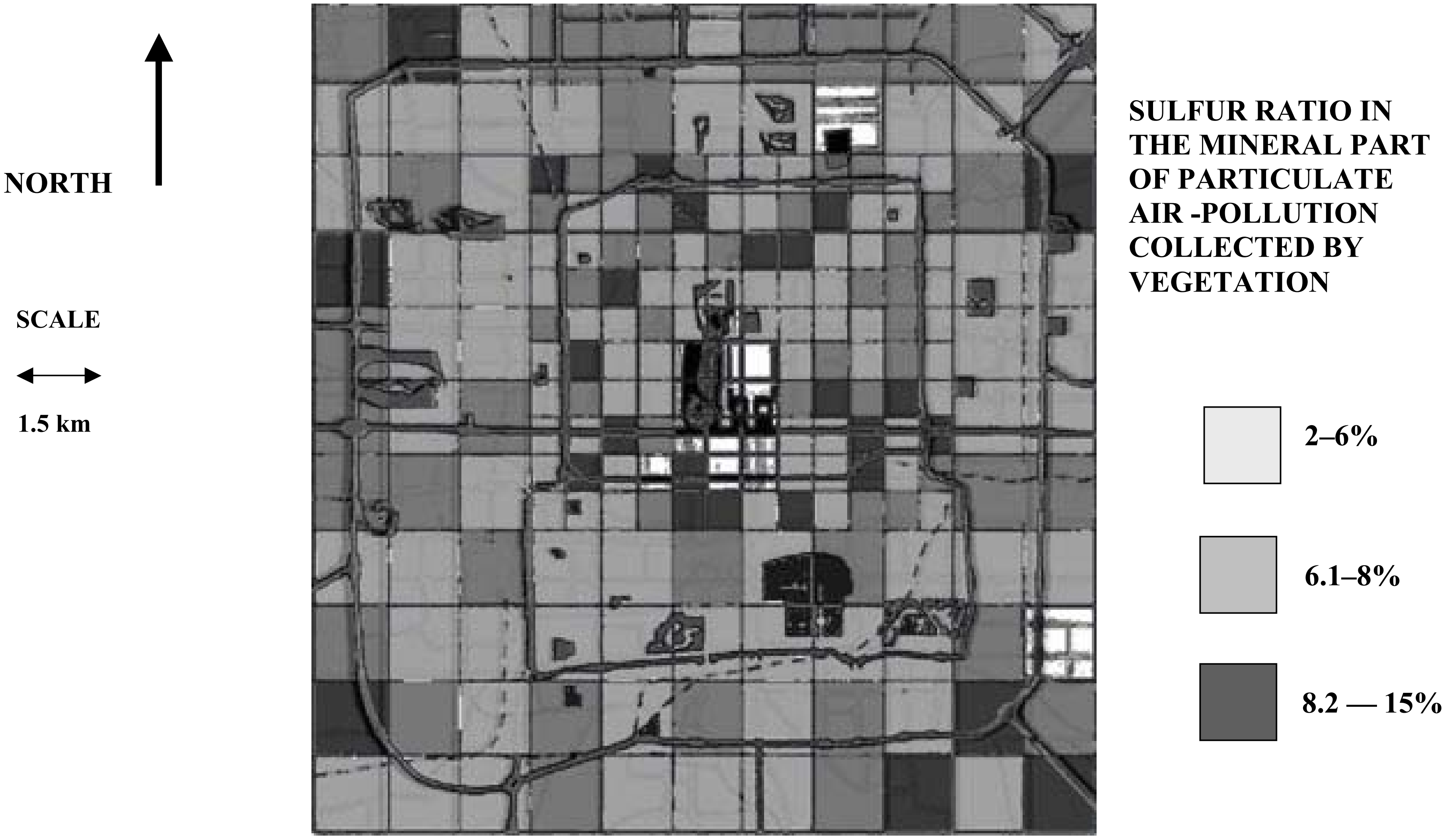

FIG.2. Localization and levels of sulfur particulate air –pollution on Beijing.

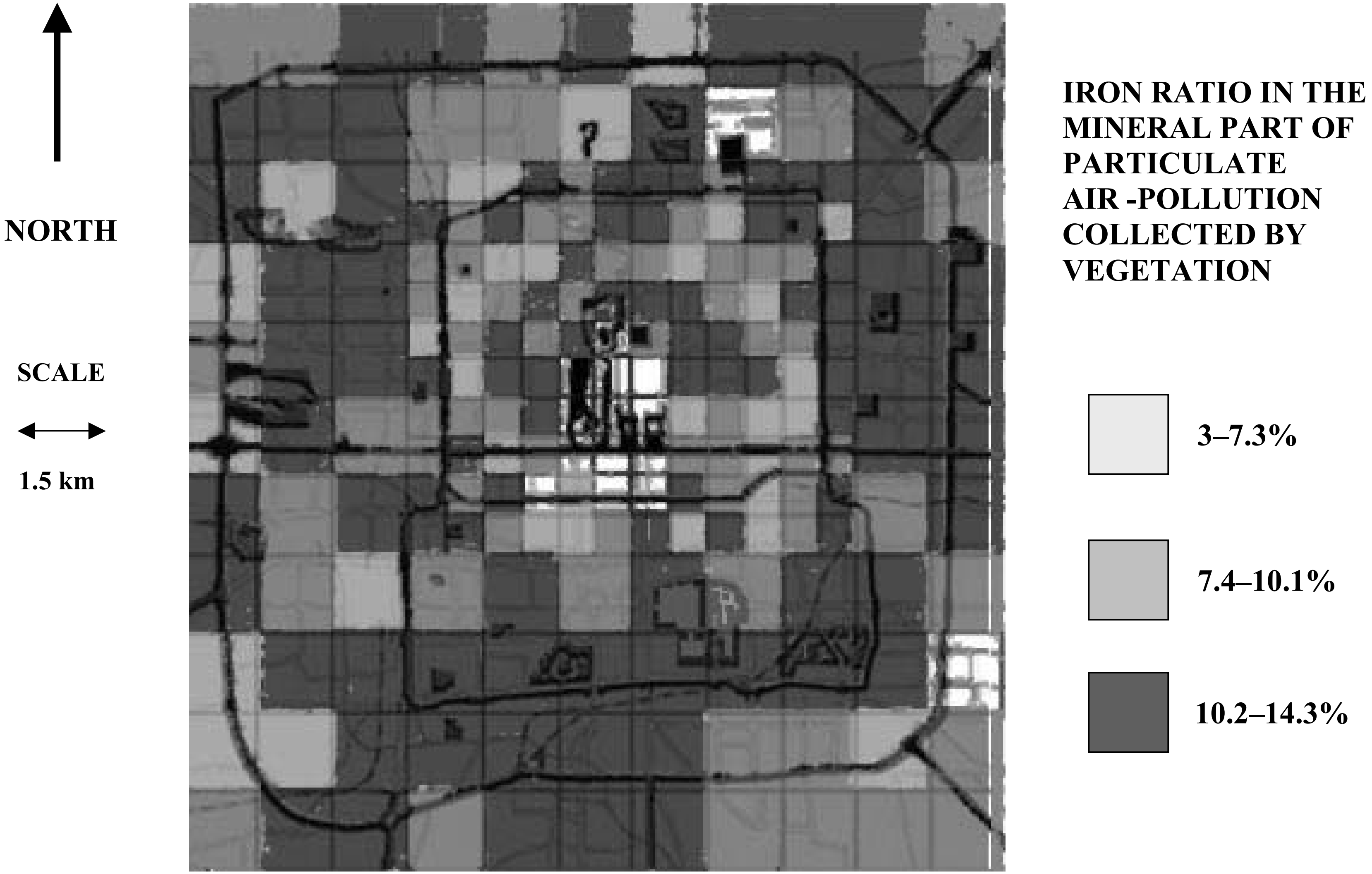

FIG.3 Localization and levels of iron particulate air –pollution on Beijing.

Air pollution on the town of Beijing.

- Concerning sulphur pollution, Fig. 2 shows that this pollution is mainly present downtown, area of old habitat where coal is still largely used as an energy source, but also on some places, just at the periphery of town of Beijing (outside factories).
- Concerning the iron pollution, Fig. 3 shows that now on Beijing, as on all the megacities of the world, the air pollution coming from the road traffic is widespreading. This pollution is detected everywhere on the town of Beijing, but particularly near and between the 2 main circle boulevards of the town.

4. CONCLUSION

Maps on Figs. 2 and 3 show for the first time the trend of air pollution localisation and level on Beijing and on its suburbs, and, at the same time they confirm the validity of this new method using plant bio-indicators to study air pollution.

More details and information on air pollution of Beijing area could be obtained by working at a smaller scale.

ACKNOWLEDGEMENTS

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AIR PARTICULATE MATTER CHARACTERIZATION IN THE NORTH OF LISBON (COMPARISON WITH REFERENCE VALUES)

S.M. ALMEIDA, M.C. FREITAS, M.A. REIS, M.M. FARINHA

ITN, EN10 Sacavém, Sacavém, Portugal

Abstract

Since 25th January 1999 airborne particulate matter samples are being collected in three stations located at the North outskirts of Lisbon. The samples are analysed by two nuclear analytical techniques: Neutron Activation Analysis (INAA) and Particle Induced X ray Emission (PIXE). The multielemental characteristics of the two techniques used and its complementarity makes it possible to determine concentration for 37 elements. The main point of this paper is to present results for PM10, PM2.5, Pb, Ni, As, Cd, Hg, for comparison, with the existing legislation, with values from other regions, and with existing health guidelines.

1. INTRODUCTION

In the atmosphere, particles occur with diameters from nanometers up to 50 µm. Particulate matter with diameter less than 2.5 µm (PM2.5) is generally referred as fine particulate matter. Medical researchers have defined the respirable fraction as those particles with diameters less than 10 mm (PM10) and the total suspended particulate (TSP) fraction is all suspended matter, usually diameters less than about 50 mm [1].

Suspended particulate matter (SPM) is the most important pollutant worldwide, both indoors and outdoors, in terms of human health effects. Particulate, especially fine particles, contain large amounts of inorganic and organic toxic materials, such as heavy metals and polycyclic aromatic hydrocarbons. Fine particles are small enough to penetrate deep into the lungs and hence have significant health implications for humans [2]. The weight of evidence from numerous epidemiological studies on short-term effect points clearly to associations between concentrations of particulate matter and adverse effects on human health at low levels of exposure commonly encountered in developed countries. Effects on mortality, respiratory, and cardiovascular hospital admissions have been observed at daily average PM10 levels well below 100 µg/m^3 [3].

The European Council Directive 96/62/CE from 27th September 1996 establishes a stepwise process for adoption of limit values for PM10, Pb, Cd, As, Ni, and Hg in order to protect the human health, especially in the more vulnerable population. The European Council Directive 1999/30/CE from 22nd April 1999 defines the limit values for PM10 and Pb and introduces the need for PM2.5 sampling into EU regulation. The PM10 limit value for human health protection is settled in 50 µg/m^3 for 24 hours average and 40 µg/m^3 for year average. The Pb human health protection limit value for annual average is settled in 0.5 µg/m^3.

The Environmental Protection Agency — EPA (USA) proposes values for PM2.5 and PM10. The PM2.5 limit value is settled in 15 µg/m^3 for a year average (based on the arithmetic annual average of three years) and in 65 µg/m^3 for a 24 hours period (based on the 24-hour 98th percentile average of three years). The PM10 limit value is settled in 50 µg/m^3 (based on the 24-hour 99th percentile of three years).

The reference method establishes gravimetry for PM10 measurement and atomic absorption spectroscopy (AAS) for Pb determinations. At ITN, PM10 and PM2.5 gravimetric measurements as well as multielement characterisation of airborne particles have been made systematically since 1993. Alves et al. presents the first set of multielement characterisation and gravimetric measurements and reports a campaign held in Lisbon from October 1993 to January 1995 [4]. PIXE and INAA results obtained at ITN have been compared to AAS data in several intercomparison runs and a specific comparison between Pb measurements of PIXE and a certified AAS laboratory was made using about 40 vegetable samples, including some certified reference materials [5].

In this work, data for PM10, PM2.5, and some important elements are presented for three measuring stations located up to 10 km North from Lisbon (Bobadela, S. João da Talha and Quinta da Piedade). The sampler situated in Bobadela has been working since 25th January 1999, the S. João da Talha (SJT) and Quinta da Piedade (Piedade) started working seasonally in 7th March 1999.

The area at North of Lisbon is a very industrialised and populated area. This makes more dangerous the degradation of the air quality. Last year a new domestic urban waste incinerator started working in this area. This project is associated with the monitoring studies of this structure and it is being made under contract with ValorSul (responsible for the domestic solid waste incinerator). The elements monitored by this contract are Pb, Cr, Cu, Mn, Ni, As, Cd, Hg, K, Sb, V, and Zn.

2. METHODOLOGY

Air collection is made with Gent samplers [6]. This kind of sampler is equipped with a SFU (stacked filter unit), which carries two 47 mm diameter Nuclepore® polycarbonate filters. Filters of 8 and 0.4 μm pore sizes are used in the first and second stages, respectively. Air is sampled at a rate of 15 l/min, which allows the collection of PM2.5 in the second stage. The sampler inlet is a PM10 separator so that the first filter collects only coarse particles (PM10–2.5) having aerodynamic diameter between 2.5 and 10 μm. The samplers work during a 24 hours period. Air pumping is made only 40 minutes in each hour in a total of 16 hours per day. Sampling is made twice a week: on Sunday and on a midweek day.

Filter loads are measured by gravimetry using a balance with 0.1 mg sensitivity and elemental analyses, are carried out using INAA [7] and PIXE, [8] techniques. For elemental analysis the filters are cut into three parts: one half is analysed by INAA, one quarter is analysed by PIXE and the other quarter is kept for other possible measurements of replicates.

3. RESULTS AND DISCUSSION

In Figures 1 and 2 the concentrations for PM2.5 and PM10 in SJT, Bobadela, and Piedade are shown. The grey points are an average, calculated for each point, of the 24 neighbour points. Figure 1 shows an increase in PM2.5 since October 1999 in SJT and Bobadela. The average of the concentrations in Piedade, Bobadela, and SJT is 13.8 μg/m^3, 15.8 μg/m^3, and 20.9 μg/m^3, respectively. The annual EPA limit value is exceeded in SJT and Bobadela. The 24 hour EPA limit value is not exceeded in the three stations. SJT station is placed between the others two stations; therefore it seems to exist a particle source near SJT because the concentrations there are higher. The 24-hour EC limit value was exceeded 20 times in SJT and Bobadela. In Piedade 24,-hour limit value was exceeded 6 times.

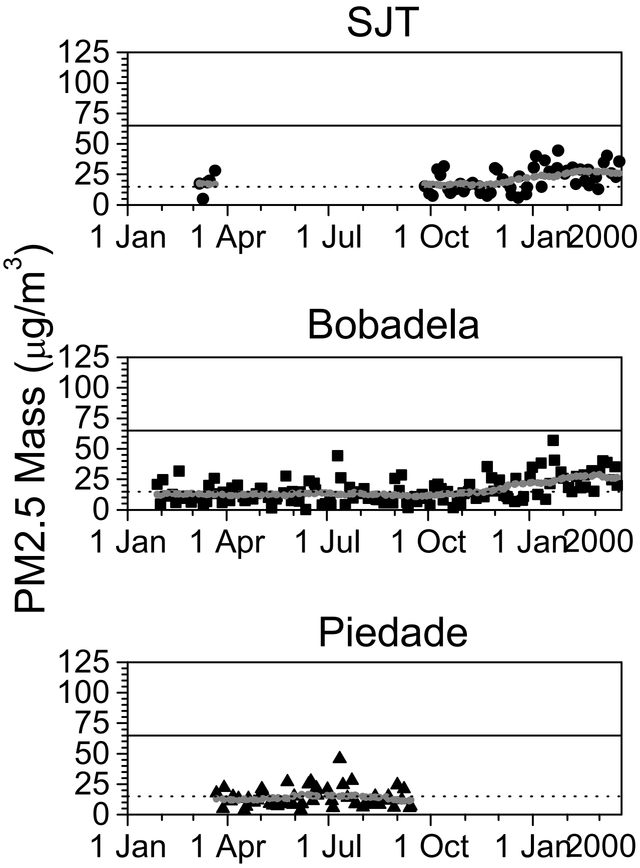

FIG. 1. PM2.5 concentration in SJT, Bobadela, and Piedade (values in µg/m^3).Full line: 24h EPA limit value – 65 µg/m^3; pointed line: annual EPA limit value – 15 µg/m^3.

The annual average for Bobadela is 31.1 µg/m^3 which is inferior comparing with the annual EC limit value, in Piedade and SJT we do not have a year of values but the average for the values determinate is 32.2 µg/m^3 and 46.3 µg/m^3, respectively. SJT exceeds the legislated limit value.

The 99th percentile is 106 µg/m^3 in SJT, 76 µg/m^3 in Bobadela, and 71 µg/m^3 in Piedade. Therefore, the EPA PM10 limit value is exceeded in the three stations.

In Western Europe, North America, and Western Pacific, except China, annual mean SPM concentration range between 20 and 80 µg/m^3, and PM10 levels are between 10 and 55 µg/m^3. High SPM and PM10 annual mean concentrations are found in South East Asia ranging between 100–400 µg/m^3 for SPM and 100–300 µg/m^3 for PM10. However, the highest recorded annual SPM concentrations of 300–500 µg/m^3 are observed in the larger cities of China [3]. Our values are in the same range of values of Europe.

In Table I statistics is shown for Pb to PM2.5, PM10-2.5, and PM10, for the three stations.
Pb annual mean concentration is below 0.1 µg/m^3 in most cities of Western Europe and between 0.2 and 0.6 µg/m^3 in Eastern European cities. In North American cities, annual mean Pb concentration is below 0.05 µg/m^3. Pb concentrations in the North of Lisbon are much lower than in the other countries of Europe [3].

Table I shows higher PM2.5 concentrations in Bobadela. For PM10-2.5 SJT have higher concentrations than in Bobadela. These two stations are close TO important automobile accesses to Lisbon (A1 and EN10).

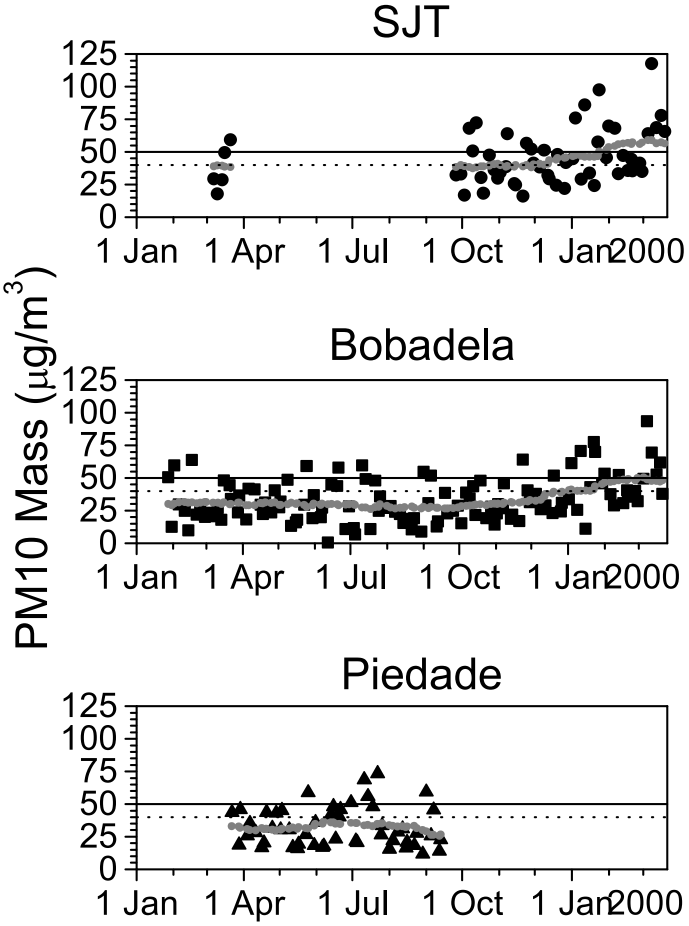

FIG. 2. PM10 concentration in SJT, Bobadela and Piedade.(values in µg/m^3). Full line: 24h EC and EPA limit value — 50 µg/m^3; pointed line: annual limit value — 40 µg/m^3

TABLE I. STATISTICS FOR Pb (VALUES IN ng/m^3).

Pb	Station	Average	SD	Maximum	Minimum	Count no
PM2.5	Bobadela	29	55	379	5	97
	SJT	23	19	94	6	40
	Piedade	12.5	7.4	35.8	2.9	48
PM10-2.5	Bobadela	30	55	290	3	76
	SJT	30	42	202	5	39
	Piedade	9.8	8.4	44.7	2.9	38
PM10	Bobadela	49	98	621	5	98
	SJT	48	48	202	7	38
	Piedade	19	14	73	3	51

The central nervous system is the primary target organ for Pb toxicity in children. Exposure of children to even low concentrations may produce neurophysiological damage, including impairment of learning abilities, behaviour, intelligence, and motor coordination. Prenatal exposure to Pb produces toxic effects on the human foetus including reductions in gestational age, birth weight, and mental development [3].

In fig. 3 the Pb values determined for SJT, Bobadela, and Piedade are shown.

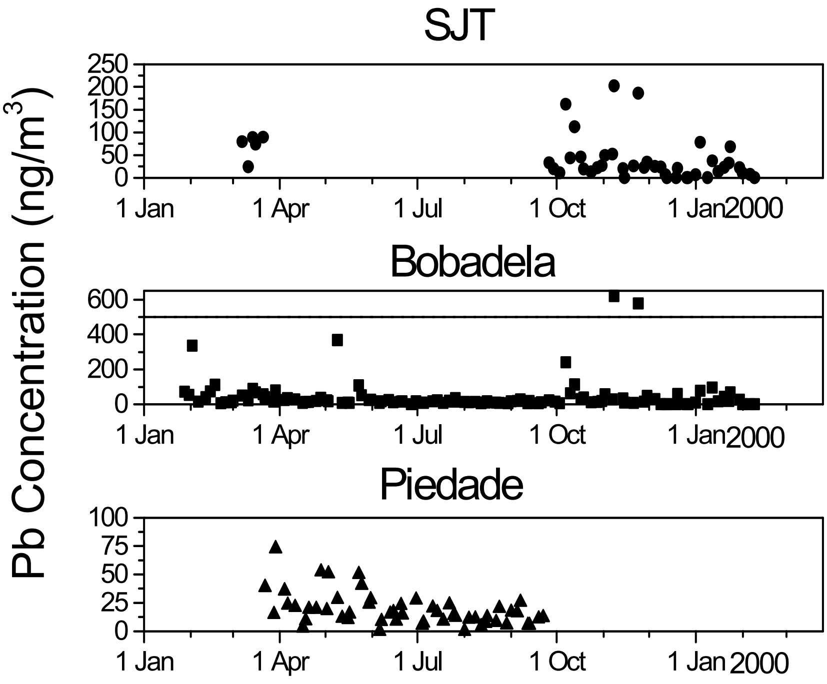

Fig. 3. PM10 Pb concentrations in SJT, Bobadela, and SJT (values in ng/m^3). Full line: annual limit value- 500 ng/m^3

In Bobadela, Pb limit value was exceeded twice. in the other stations this value was not overpassed.

In Table II, statistics for Ni, As, Cd, and Hg PM2.5, PM10-2.5, and PM10 for the three stations are shown.

Ni

In the ambient air, Ni levels are in the range of 1–10ng/m^3 in urban areas [3], although much higher levels (120–170 ng/m^3) have been recorded in heavily industrialized areas and large cities [9]. A study made in the suburb of Boston shows Ni concentrations of 8.57 ng/m^3 for PM2.5 and 2.44 ng/m^3 for PM15-2.5 [10]. The PM2.5 value determined in Boston is similar to the concentration found in SJT. Moreover, in Boston the concentration of the fine fraction is also higher than in the coarse fraction. Ni concentrations in the North of Lisbon are in the same range of values of the urban areas. However, Ni concentrations in SJT present higher values than in the other stations, exceeding the upper limit of this range of values.

The more usual Ni effect is skin allergies. Some studies show that 2.5 to 5% of the population is sensible to Ni. It is proved that concentrations between 0.1 and 100 mg/m^3 are mortal for animals, concentrations between 0.1 and 1 mg/m^3 provoke negative lung effects on animals, and concentrations of 10 µg/m^3 are responsible for effects on the immune system on animals. Concentrations of 1 µg/m^3 have a minimum risk except for cancer on human beings [11]. Ni concentrations determined in the North of Lisbon are much lower than these guidelines.

TABLE II. STATISTICS FOR Ni, As, Cd, AND Hg (VALUES IN ng/m^3).

		Station	Average	SD	Maximum	Minimum	Count nr.
Ni	PM2.5	Bobadela	4.1	3.3	17.3	0.3	100
		SJT	8.4	8.0	42.9	0.9	41
		Piedade	2.5	1.3	5.7	0.8	40
	PM10-2.5	Bobadela	3.1	2.2	10	0.8	83
		SJT	5.6	5.5	31.3	0.6	43
		Piedade	1.9	1.4	6.1	0.5	35
	PM10	Bobadela	6.4	5.2	22.0	0.9	104
		SJT	13	12	54	0.65	44
		Piedade	3.8	2.6	11.2	0.7	43
As	PM2.5	Bobadela	0.57	0.74	3.95	0.04	82
		SJT	0.67	0.39	1.83	0.11	25
		Piedade	0.22	0.11	0.42	0.06	22
	PM10-2.5	Bobadela	0.24	0.24	1.54	0.04	57
		SJT	0.8	1.7	8.5	0.07	23
		Piedade	0.19	0.10	0.43	0.06	20
	PM10	Bobadela	0.67	0.83	3.95	0.04	90
		SJT	1.3	1.6	8.5	0.1	26
		Piedade	0.36	0.19	0.70	0.11	24
Cd	PM2.5	Bobadela	2.5	2.9	8.8	0.8	7
		SJT	1.6	1.7	6.2	0.4	14
		Piedade	1.16	0.59	1.71	0.49	4
	PM10-2.5	Bobadela	1.01	-	1.01	1.01	1
		SJT	1.21	0.27	1.36	0.90	3
		Piedade	-	-	-	-	0
	PM10	Bobadela	2.7	3.2	9.8	0.8	7
		SJT	1.7	1.6	6.2	0.4	15
		Piedade	1.16	0.59	1.71	0.49	5
Hg	PM2.5	Bobadela	0.18	0.19	1.07	0.0028	69
		SJT	0.21	0.17	0.64	0.025	18
		Piedade	0.116	0.083	0.421	0.0265	23
	PM10-2.5	Bobadela	0.4	1.7	12.7	0.01	55
		SJT	0.27	0.20	0.70	0.03	17
		Piedade	0.080	0.048	0.208	0.020	21
	PM10	Bobadela	0.4	1.5	13.3	0.003	81
		SJT	0.38	0.34	0.97	0.03	20
		Piedade	0.17	0.11	0.49	0.02	26

As

Airborne concentrations of arsenic range from 1 to 10 ng/m^3 in rural areas and typically from 10 to 200 ng/m^3 in urban areas. Near emission sources, such as non-ferrous metal smelters and power plants burning arsenic-rich coal, concentrations of airborne arsenic can exceed 1 µg/m^3 [3]. Average contents in Germany are 5–20 ng/m^3 [12]. A study made by Pringle [13] shows concentrations for As in three places: Toronto, Washington, and Chicago. The mean concentrations are 5.3 ng/m^3, 3.25 ng/m^3 and 4.3 ng/m^3, respectively. The concentrations in North of Lisbon are lower comparing with these concentrations of reference.

In Piedade, As has a lower concentration. The other stations have similar concentrations in PM2.5. In PM10 the concentrations are higher in SJT.

EPA studies show that the inhalation of 1 mg/m^3 during the life period has a risk of lung cancer of 0.4%. The concentrations determined in this work are more than 1,000 times lower.

Cd

Mean annual air Cd levels for the period 1980–1988 in the Northern European countries were approximately 0.1 ng/m^3 in remote areas, 0.1–0.5 ng/m^3 in rural areas, 1–10 ng/m^3 in industrial areas (with levels up to 100 ng/m^3 near emission sources) [3]. In, cities Cd ranged from 6 to 360 ng/m^3 in the USA, from 2 to 50 ng/m^3 in Europe, and from 10–53 ng/m^3 in Japan. In remote areas these values are a factor of 10–1000 lower, whereas in polluted areas much higher values may be found [14]. In the North of Lisbon, Cd was detected few times. In SJT and in PM2.5, Cd is detected more frequently than in the other stations.

Cd atmospheric concentrations of 1 mg/m^3 provoke strong irritation on lungs. Long expositions on environment with 0.1 mg/m^3 could provoke an increase on pulmonary disease namely efiseme. These levels are also associated with the development of kidney lesion. A long exposition (during a life time) in a concentration of 1µg/m^3 Cd is associated with a lung cancer risk of 2 people in 1000. The values recommended by the WHO are 5 ng/m^3 in rural areas and 10 ng/m^3 in urban and industrialised areas. The concentrations in the North of Lisbon are lower.

Hg

Atmospheric Hg concentrations levels in member nations of the European Community have been reported to be 0.001 to 1 ng/m^3 in urban areas and up to 20 ng/m^3 in industrial areas [3]. A long exposition on an environment with Hg provokes diseases on kidney, brain and in the development of foetus.

The estimated guideline from direct inhalation effects of mercury is 1 µg/m^3. However, present knowledge suggests that effects on the immune system at lower exposures cannot be excluded [3]. Our levels are marginal compared with these values.

Hg concentrations for PM2.5 are similar in the three stations. However, for PM10, Piedade has lower concentrations and Bobadela has higher. Bobadela had a significant maximum for PM10 in 16th March 1999.

4. CONCLUSIONS

The element concentrations in the North of Lisbon are systematically lower when compared with values from other places. The fact that Portugal is geographically near the ocean, having only Spain as a neighbour, could be responsible for these lower concentrations. Furthermore, PM10 and PM2.5 concentrations sometimes exceed the limit values proposed by the legislation and EPA.

Concentrations in SJT are systematically higher than in the other stations. SJT, is placed between Piedade and Bobadela and it is near the incinerator, Covina (glass factory) and the main automobile access to Lisbon (A1). Despite the concentrations having increased since October, which correspond to the date when the incinerator started working effectively, this study has still results to conclude the contribution of this infrastructure to the air pollution in this place.

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LAMS, LASER ABLATION/IONIZATION MASS SPECTROMETRY FOR AEROSOL CHARACTERIZATION AND SOURCE APPORTIONING

S. OWEGA, G.J. EVANS, R.E. JERVIS, J. TSAI, E. KREMER, P.V. TAN

Facility for Aerosol LAMS,
Department of Chemical Engineering and Applied Chemistry,
University of Toronto, Ontario, Canada

Abstract

Laser ablation/ionization mass spectrometry (LAMS) of particulate matter (PM) was undertaken on-line in order to extend PM source identification and source apportionment studies reported previously. Qualitative LAMS results for urban/industrial and selected contributory PM sources from Toronto demonstrated the scope of the technique and its suitability and future potential for PM physical and chemical characterization, emission source identification and receptor modeling. Organic and inorganic constituents of certified materials were successfully observed with an in-house on-line LAMS, and verified its proper working condition. Organic and inorganic constituents of several source materials were also observed with the on-line LAMS. Common components identified from both types of sources were Na, Al, Ca, Fe, and K. Additional peaks were also observed but not identified. Recognized components were compared with marker elements reported for some common PM emission sources. Marker elements that were identified for the certified materials were Mn, Mg, Cr, Li, Sr, Bi, Hg, As, Rb and Tl, and marker elements that were identified for the source materials were Ba, Mo, Cu, Cr, Co, Pb, Li, Tl, V, Cl and Cd. Further, an in-house off-line LAMS analyzed urban Toronto PM deposited on glass substrates, while the on-line LAMS analyzed individual urban Toronto PM that were introduced directly into the instrument. Scanning electron microscopy (SEM) X ray spectrometry and inductively coupled plasma atomic emission spectrometry (ICP-AES) were used for confirmatory purposes. Organic and inorganic constituents of PM at their typical ng/m–3 concentrations were successfully observed in mass spectra using an off-line LAMS and an on-line LAMS instrument. The ability to detect and analyze individual respirable PM (viz. < 2 microns), both for inorganic TE's and for organic constituents, greatly extended our capability to characterize PM emission sources and also to achieve estimates of source concentration contributions for each component.

1. INTRODUCTION

Particulate matter (PM) is an increasing field of governmental [1], industrial [2] and academic [3] research because its impact on health is tremendous [4–7]. Its size and chemical composition can be used to identify sources (incinerators, smelters, power plants, motor vehicles, etc.), a major goal of PM studies [8,9]. Techniques used to characterize PM include inductively coupled plasma atomic emission spectrometry/mass spectrometry (ICP-AES/MS) [10,11], voltametry [12,13], direct current glow discharge mass spectrometry (dc-GDMS) [7], total-reflection X ray fluorescence (TXRF) [14], particle-induced X ray emission (PIXE), neutron activation analysis (NAA), and even Photochemical Activity and Solar Ultraviolet Radiation (PAUR) [15]. Unfortunately, many of these instruments cannot measure PM size, an important physical attribute because PM can be separated into different sources based on size[16]. Another inherent problem is a lack of knowledge of the chemical speciation of PM from different sources since most of these techniques provide only elemental analysis. Marker elements, or signatures that identify specific PM sources, are commonly used to achieve source apportionment of the PM. Table I lists marker elements that correspond to some atmospheric emission source(s).

By using lasers to ionize solids in a mass spectrometer [17], the first off-line laser microprobe mass spectrometer (LAMMS) was constructed to characterize PM [18]. Focusing the laser beam to a small laser beam diameter later enhanced the off-line LAMMS [19]. Friedlander recognized the next development when he described the ideal aerosol instrument as one that sizes and analyzes individual PM in real-time [21]. The first on-line laser ablation mass spectrometer (LAMS) was constructed by accelerating a particle beam from atmosphere into a mass spectrometer. Both on-line and off-line LAMS have since evolved, as evidenced by reviews[2,23] and papers[24,25], but real-time single particle measurements, introduced first by Sinha [26] and further developed by Prather et al. [27–29], provided the superior capabilities of on-line PM analysis. The dual-laser aerodynamic particle sizing/tracking configuration is currently the most precise sizing method that has been successfully coupled to the time-of-flight mass spectrometer (TOFMS) [9]. Examples of mass spectra for automobile, ambient/urban, organic/inorganic oxide, fireworks' smoke, river/canal sediment, marine, and soil particles using on-line LAMS are shown in recent reports [9,30,31].

TABLE I. TABLE OF POSSIBLE PM SOURCES AND CORRESPONDING MARKER ELEMENTS.

Possible PM Sources [20]	Marker Elements
Soil Dust	O, Al, Si, K, Ca, Ti, Mn, Fe, Sc
Coal Combustion	H, C, N, O, Na, Al, Si, P, S, K, Ca, Fe, Mn
Motor Vehicles	H, C, N, O, Na, Al, Si, S, Cl, Fe, Zn, Br, Pb, Mn
Construction/Cement	Ca, Fe, S, Sc
Smoke	H, C, N, O, Cl, K, Ca
Sea Spray	Na, S, Cl, K, Ca
Base Metal Smelter	Cd, Pb, Sb, Zn, As
Hospital Incinerator	Ag, Cd, Cl, Cr, Sb, Zn
Iron/Steel Smelter	Co, Cr, Fe, Mn
Refuse Incinerator	Se, Zn, I, Cl, Ag, Cr
Sewage Incinerator	Ag, Cr, Zn, Ti, Pb
Industry	H, C, N, O, P, S, V, Cr, Cu, Pb
Oil Combustion	S, V, Ni, In, Se

In this paper, we present and compare preliminary results obtained from an in-house on-line LAMS to results obtained from complementary methods, including a commercial scanning electron microscope (SEM), a commercial ICP-AES and an in-house off-line LAMS. Elemental analysis of standard reference materials (SRMs) is contrasted with mass spectral analysis using the in-house on-line LAMS to evaluate its performance. SRM elemental analysis also provides possible source profiles when compared to urban Toronto PM by recognizing their marker elements. On-line LAMS was also performed on several source materials collected from Toronto, and identification of marker elements was attempted. Lastly, urban Toronto PM was analyzed via on-line LAMS, off-line LAMS, ICP-AES, and SEM. Identification of Toronto PM sources was attempted by comparing the elements observed in their LAMS mass spectra with the marker elements observed in SRMs and several source materials.

2. MATERIALS AND METHODOLOGY

2.1. University of Toronto Facility for Aerosol Characterization

Over the last three years, a new facility has been developed containing a range of instruments for characterizing PM:

(1) PM calibration equipment, including a TSI Model 3320 Aerodynamic Particle Sizer (APS), a TSI Model 3075/3076 Constant Output Atomizer, a TSI Model 3012 Aerosol Nebulizer, a TSI Model 3433 Small-Scale Powder Disperser, TSI Model 3062 Diffusion Dryer, a TSI Model 3071 Electrostatic Classifier, a TSI Model 3020 Condensation Nucleus Counter, and a TSI Model 390077 Differential Mobility Particle Sizer (DMPS) Interface Box (St. Paul, USA).

(2) PM equipment for elemental analysis, consisting of an in-house off-line LAMS, and an in-house on-line LAMS.

(3) PM sampling equipment, including an in-house PM10 collection system that distributes urban Toronto PM to the instruments within the lab, a Sierra Instruments Model 353 High Volume Air Sampler (Berkerly, USA), a Gent dichotomous sampler (Potsdam, USA), a URG–2000–30EHB Cyclone (Chapel Hill, USA), and a MIE personalDataRAM Model pDR-1200 (Burlington, Canada).

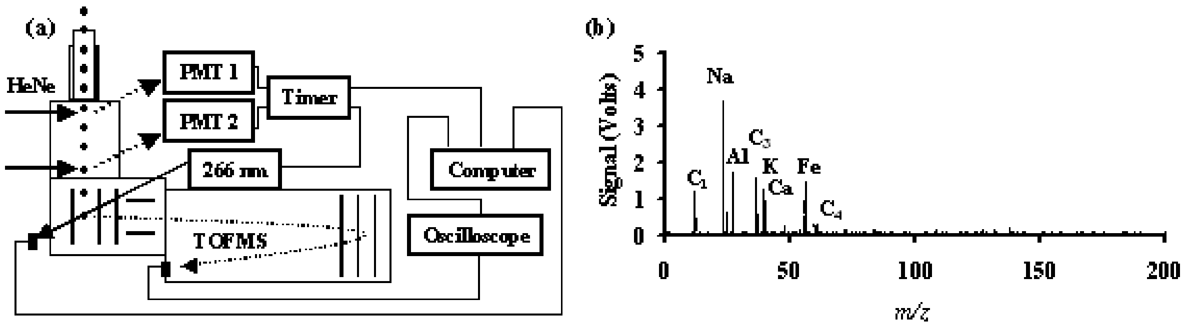

FIG. 1. Diagram of: (a) the in-house on-line LAMS, and (b) a Vehicle Exhaust positive mass spectrum using the in-house on-line LAMS.

Urban Toronto PM was collected without size discrimination on microscope glass slides and glass vials using the in-house PM10 collection system at the Wallberg Building (University of Toronto). The cyclone was also used to collect urban Toronto PM >1.0 mm-aerodynamic diameter. After each day, all samples were transferred into a glass vial. This procedure continued for 14 days.

All materials, each collected in a glass vial, were introduced into a differentially-pumped inlet nozzle (0.5 mm) of the on-line LAMS instrument and next ablated using a 266-nm laser beam from a Big Sky Nd:YAG laser (Bozeman, USA). Particle sizing was not performed. Ablation and ionization of the PM occurred in the ion source region of a linear TOFMS, equipped with a reflectron for improved resolution. Ions were extracted with an electric field, separated according to their m/z ratios, and detected with a multi-channel plate (MCP) detector. The MCP signal was fed into an amplifier, then into a 500 MHz LeCroy Model LC334A digital storage oscilloscope (DSO) (Chestnut Ridge, USA), and finally into a computer that displayed the resulting mass spectrum. The mass spectra were calibrated with known-mass peaks from the Na+, K+, Fe+, and the Pb+ isotopes using the empirical equation of $m/z = ((t-a)/b)^2$, where a was an initial delay time, b was a calibration constant, and t was the time-of-flight observed in the mass spectrum. The on-line LAMS can generate either a positive or a negative mass spectrum. A schematic diagram of the in-house on-line LAMS is shown in Figure 1., with an example of a Vehicle Exhaust sample mass spectrum taken in the positive mode.

Urban Toronto PM, that was introduced into the off-line LAMS as sample deposited on a microscope glass slide, was back-illuminated using a 532-nm laser beam from a Quantel Nd:YAG laser (Santa Clara, USA). After ablation and ionization of the PM, the cations were extracted into a TOFMS with an electric field, separated according to their m/z ratios, and detected with a MCP detector. The MCP signal was fed into an amplifier, and then into a 500 MHz LeCroy Model LC334A DSO. The resulting mass spectra were calibrated with known-mass peaks from Na+, K+, and Fe+ using the theoretical equation of $m/z = (kt)^2$, where k was a calibration constant, t was the time-of-flight observed in the mass spectrum. SEM analysis of the urban Toronto PM sample was next conducted.

2.2. University of Toronto ANALEST Facility

Urban Toronto PM10 was collected for 24 hrs. on Whatman 41 cellulose filter paper using an in-house PM10 collection system at the Wallberg building. Urban Toronto PM2.5 was also collected for 24 hrs on Whatman 41 cellulose filter paper using the Sierra High Volume Air Sampler at the Gage Institute (University of Toronto). The Whatman 41 cellulose filter paper was acid digested, microwave digested, diluted with deionized water (18 MΩ), then analyzed with a Perkin Elmer Optima Model 3000 DV, AS90 ICP-AES instrument (Norwalk, USA) at the University of Toronto ANALEST facility.

The ICP-AES measured at least two emission wavelengths for each element of interest. For each sample solution, the monitored elements, whose measured concentrations had a precision of 5–10%, were Al, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sb, Sr, Tl, V, and Zn. A known concentration of a multi-element standard solution was analyzed intermittently to verify the continued proper operation of the ICP-AES.

2.3. SRMs and Other Materials

Montana II soil # 2711, Urban Dust (organics) # 1649a, Urban PM # 1648, and Coal Fly Ash # 1633b (SRMs) were purchased from National Institute of Standards and Technology (NIST) (Gaithersburg, MD). Non-Ferrous Dust PD-1 (CRM) was purchased from Canadian Certified Reference Materials Project (Ottawa, Canada) and Vehicle Exhaust PM was obtained as NIES #8 (Tsukuba, Japan). Light grade Portland cement (Toronto, Canada) was purchased. Lakeview Generator Fly Ash (Lakeview, Canada) and Commissioner Street Waste Incinerator Fly Ash (Toronto, Canada) were collected. Toronto soil and urban Toronto PM were collected at the Wallberg Building and Gage Institute (University of Toronto, Canada). A multi-element standard solution was purchased from High-Purity Standards (Charleston, USA).

3. RESULTS AND DISCUSSION

3.1. Marker Elements from SRMs

In Table II are listed the certified elements from standard materials and the ions identified using the on-line LAMS. In the positive mode, on-line LAMS results (%) are listed for occurrences greater than 7.5% (the number of times the element is observed per total number of mass spectra taken for that source). In the negative mode, on-line LAMS results (%) are listed for occurrences greater than 35%. Identified mass spectral peaks were confirmed with their isotopic abundance ratios. Clearly many certified elements are observed in the mass spectra, but other mass spectral peaks that could not be identified with certainty are not listed. Additional non-certified elements are also observed in the mass spectra. Observation of these elements confirms the proper working condition of the on-line LAMS.

Regarding source apportionment, many elements were observed for multiple sources. For example, Na, Al, Ca, Fe, Ti, and K were observed frequently in all mass spectra from different sources, and hence cannot be used as marker elements. Their observation was expected, since many of these elements originate from common sources and are crustal components (see Table I.). However, other elements like Mn, Mg, Cr, Li, Sr, Bi, Hg, As, Rb and Tl were only seen in three or less sources, even though some of these elements were not frequently observed.

TABLE II. TABLE OF CERTIFIED MATERIALS ANALYZED WITH THE ON-LINE LAMS.

Certified Materials	Identified Ions	Certified Components
Montana II Soil NIST SRM # 2711	+’ve mode: Al^a, K^a, Ca^a, Fe^a, C_1^b, Na^b, Mg^b, C_2^b, AlO^b, SiO^b, Si^c, C_3^c, C_3H^c, Ti^c, C_4^c, Mn^c, $CaOH^c$, Cu^c, Zn^c, Cd^c, Fe_2O^c, Ba^c, Fe_3O^c, Fe_3OH^c, Pb^c -‘ve mode: H^a, CH_3^a, O^a, OH^a, $C_2H_2^a$, $C_2H_2O^a$, $C_2H_3O^a$, CH_3COO^a, SiO_2^a, PO_2^a, SiO_3^a, $HSiO_3^a$, PO_3^a, C_2^b, C_2H^b, $COOH^b$, $H_2PO_4^b$	Al, Ca, Fe, Mg, P, K, Si, Na, Ti, S, As, Sb, Ba, Cd, Cu, Pb, Mn, Ni, Sr, Tl, V, Zn, Hg, Se, Ag
Vehicle Exhaust NIES SRM # 8	+’ve mode: C_1^a, Na^a, C_2^a, Al^a, C_3^a, C_3H^a, K^a, Ca^a, C_4^a, Ti^a, Fe^a, C_5^a, C_5H^b, C_6^b, C_7^b, $C_2H_5^c$, $C_3H_2^c$, C_4H^c, $C_4H_2^c$, V^c, Cu^c, C_6H^c, C_7H^c, C_8^c, C_9^c, Fe_2O^c, Ba^c, Fe_3OH^c -‘ve mode: H^a, C_2^a, C_2H^a, $C_2H_2^a$, C_3^a, C_4^a, C_4H^a, C_5^a, C_6^a, C_7^a, C_8^a, C_8H^a, C_9^a, $C_4H_2^b$, C_6H^b	Ca, Al, Na, K, Zn, Mg, Pb, Sr, Cu, Cr, Ni, V, Sb, Co, As, Cd
Non-Ferrous Dust CRM PD-1	+’ve mode: Na^a, K^a, Fe^a, Cu^a, Zn^a, Pb^a, AsO^a, Tl^b, C_1^c, C_2^c, Al^c, Ca^c, Ti^c, FeO^c, Cd^c, Fe_2O^c, CdO^c, Fe_3O^c, Hg^c, Bi^c -‘ve mode: H^a, O^a, S^a, SO^a, SO_2^a, SO_3^a, SO_4^a, HSO_4^a	Pb, As, Hg
Urban Dust (Organics) NIST SRM # 1649a	+’ve mode: C_1^a, Na^a, Al^a, C_3^a, K^a, Fe^a, C_2^b, C_3H^b, Ca^b, C_4^b, Ti^b, V^b, Pb^b, $C_2H_4^c$, AlO^c, Mn^c, C_5^c, C_5H^c, Cu^c, Zn^c, VO^c, C_6^c, $Fe(OH)^c$, C_7^c, C_7H^c, Fe_2O^c, Ba^c, BaO^c, Fe_3O^c, Fe_3OH^c -‘ve mode: H^a, O^a, C_2^a, C_2H^a, $C_2H_2^a$, Cl^a, C_3^a, $C_2H_2O^a$, NO_2^a, C_4^a, HNO_3^a, Br^a, SO_3^a, SO_4^a, HSO_4^a, C^b, CH_3^b, OH^b, C_3H^b, C_4H^b, $C_4H_2^b$, C_5^b	PAHs PCBs
Urban PM NIST SRM # 1648	+’ve mode: C_1^a, Na^a, Al^a, C_3^a, K^a, Ca^a, Fe^a, CaO^a, C_2^b, C_3H^b, AlO^b, $AlOH^b$, Ti^b, C_4^b, V^b, $CaOH^b$, C_5^b, C_5H^b, Cu^b, Fe_3OH^b, Pb^b, Si^c, $C_3H_2^c$, C_4H^c, Cr^c, Mn^c, Zn^c, VO^c, As^c, $Na[NaCl]^c$, $Ti(OH)_2^c$, VO_2^c, Rb^c, Sr^c, VO_3^c, Fe_2O^c, Ba^c, $K[KBr]^c$, Fe_3O^c, Tl^c -‘ve mode: H^a, O^a, C_2^a, C_2H^a, $C_2H_2^a$, Cl^a, C_3^a, C_3H^a, $C_2H_2O^a$, $C_2H_3O^a$, NO_2^a, C_4^a, $C_4H_2^a$, HNO_3^a, Br^a, SO_3^a, SO_4^a, HSO_4^a, C_1^b, CH_3^b, OH^b, C_4H^b, C_5^b, NO_3^b	Al, Fe, K, Pb, Na, Zn, As, Cd, Cr, Cu, Mn, Ni, U, V
Coal Fly Ash NIST SRM #1633b	+’ve mode: Na^a, Al^a, K^a, Ca^a, Fe^a, Ti^b, Zn^b, Li^c, C_1^c, C_2^c, Mg^c, Si^c, AlO^c, V^c, FeO^c, Sr^c, Ba^c, Fe_2O^c, Fe_3O^c, Pb^c -‘ve mode: H^a, O^a, C_2^a, $C_2H_3O^a$, $C_2H_3O_2^a$, SiO_2^a, PO_2^a, SiO_3^a, $HSiO_3^a$, PO_3^a, C_1^b, $C_2H_2O^b$	Al, Ca, Fe, Mg, K, Na, Si, S, As, Ba, Cd, Cr, Cu, Pb, Mn, Ni, Sr, V, Hg, Se, Th, U

[a] Occurrences greater than 50%.

[b] Occurrences less than 50% and greater than 25%.

[c] Occurrences less than 25%.

3.2. Toronto Sampling

Figure 2 illustrates a mass spectrum for Toronto soil. In Table III are listed elements and ions observed through on-line LAMS analysis of samples from several sources including urban Toronto PM. The positive mode results (%) for sources are listed for occurrences greater than 7.5%, while those for urban Toronto PM are listed for occurrences greater than 35%. The negative mode results (%) are listed for occurrences greater than 35%. Identified mass spectral peaks were confirmed using their isotopic abundance ratios.

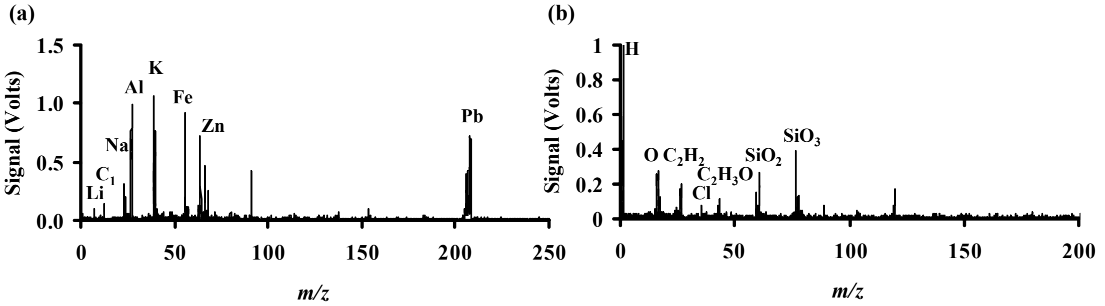

FIG. 2. On-line LAMS spectra of Toronto soil: (a) in the positive mode, and (b) in the negative mode.

Mass spectral analysis of Lakeview generator fly ash, Commissioner Street incinerator fly ash, Toronto soil, and Light Grade Portland Cement revealed five common elements that may contribute significant concentrations to airborne urban Toronto PM. These were Na, Al, Ca, Fe, and K. Again, their observation was expected since many of these elements are crustal in origin and originate from common sources (see Table I.). Note that Ti was not observed in Light Grade Portland Cement, even though it was frequently observed in all the certified materials analyzed (see Table 2.). The composition of each source also corresponded well with cited literature, as evidenced by several papers [32–39]. Elements that were not observed in many sources, and could be used as marker elements, were Ba, Mo, Cu, Cr, Co, Pb, Li, Tl, V, Cl and Cd.

The preliminary analysis of urban Toronto PM indicates many distinct features. According to the APS, the number concentration of urban Toronto PM samples is largest for PM diameters below 1.0 mm. However, the larger diameter contributes significantly to the total mass of urban Toronto PM according to mass concentration analysis. SEM images of urban Toronto PM on a glass substrate indicate that the PM diameters range from 1–10 mm. This is expected since larger urban Toronto PM will strike the glass slide and smaller PM will not. X ray analysis from the SEM indicates ten different elements that comprise the urban Toronto PM sample. These are Na, Mg, Al, S, Cl, K, Ca, Ti, Mn and Fe. Referring to Table 1, many sources have these elements, which makes source apportionment difficult. Note, however, that Pb, Al, Ca, Fe, K, and Mg are observed in significant concentrations in the ICP-AES analysis. Small concentrations of Ba, Cu, Mn, Na and Zn are also detected. Most of the ICP-AES results correlate qualitatively with the SEM X ray results. The appearance in the off-line LAMS mass spectral peaks at Al, Ca, Mg, Na, K, Fe, Zn, and Ti is consistent with the results from the previous two methods. Although preliminary on-line LAMS results are reported in this paper, all of the elements observed using the previous three techniques are also observed in the on-line mass spectra. Support for the presence of chlorine is provided by its detection in both the on-line LAMS analysis and the SEM X ray analysis. The elements observed from this analysis are consistent with other reports, as evidenced by recent articles [40–44].

TABLE III. TABLE OF IDENTIFIED ELEMENTS AND IONS ON-LINE LAMS ANALYSIS OF VARIOUS SOURCE MATERIALS AND COMPREHENSIVE CHARACTERIZATION OF URBAN TORONTO PM BY ICP-AES (ng/m^3), SEM X ray, OFF-LINE LAMS, AND ON-LINE LAMS.

Source Materials	Identified Ions
Lakeview generator fly ash	+’ve mode: Na[a], Al[a], K[a], Ca[a], Fe[a], C_1[b], C_2[b], Mg[b], Si[b], AlO[b], SiO[b], Ti[b], CaO[b], TiO[b], Fe_2O[b], Ba[b], Fe_3OH[b], Li[c], C_3[c], V[c], Mn[c], CaOH[c], Al_2O[c], FeO[c], VO_2[c], BaOH[c], Fe_3O[c] -‘ve mode: H[a], CH_3[a], O[a], OH[a], C_2[a], C_2H_2O[a], CH_3CO[a], C_4[a], CH_3COO[a], SiO_2[a], PO_2[a], HPO_2[a], SiO_3[a], $HSiO_3$[a], PO_3[a], HPO_3[a], C_1[b]
Commissioner Street waste incinerator fly ash	+’ve mode: Na[a], Al[a], K[a], Ca[a], Fe[a], Pb[a], Mg[b], Zn[b], Fe_3O[b], C_1[c], AlO[c], SiO[c], Ti[c], Cr[c], Mn[c], Cu[c], CrO[c], Na[NaCl] [c], Cd[c], CdO[c], Fe_2O[c], Fe_3OH[c], Tl[c] -‘ve mode: H[a], CH_3[a], O[a], C_2[a], Cl[a], C_4[a], PO_2[a], SiO_3[a], PO_3[a], SO_3[a], C_2H_3O[b]
Toronto Soil	+’ve mode: Na[a], Al[a], K[a], Ca[a], Fe[a], Mg[b], Ti[b], CaOH[b], Zn[b], TiO[b], Fe_3O[b], C_1[c], Si[c], AlO[c], SiO[c], Mn[c], Cu[c], Al_2O[c], Fe_2O[c], Ba[c], Fe_3OH[c], Pb[c] -‘ve mode: H[a], O[a], OH[a], C_2[a], C_2H[a], C_2H_2[a], Cl[a], C_3[a], C_3H[a], C_2HO[a], C_2H_2O[a], C_2H_3O[a], C_4[a], C_4H[a], C_4H_2[a], CH_3COO[a], SiO_2[a], PO_2[a], SiO_3[a], $HSiO_3$[a], PO_3[a], C_1[b], CH_3[b], $HSiO_2$[b]
Light Grade Portland Cement	+’ve mode: Na[a], Al[a], K[a], Ca[a], Fe[a], Mo[a], C_1[b], C_2[c], C_3[c], C_3H[c], AlO[c], C_4[c], Co[c], Zn[c], Na[NaCl] [c], K[KCl] [c], Fe_2O[c] -‘ve mode: H[a], C[a], CH_3[a], O[a], H_2O[a], C_2[a], Cl[a], C_4[a], HSO_4[a]
Urban Toronto PM	ICP-AES: Al[d], Ca[d], Fe[d], K[d], Mg[d], Pb[d], Ba[e], Cu[e], Mn[e], Na[e], Zn[e] SEM X-Ray[f]: Na, Mg, Al, Si, S, Cl, K, Ca, Ti, Mn, Fe +’ve mode off-line[f]: Al, Ca, Mg, Na, K, Fe, Zn, Ti, C_1, C_2, C_3 +’ve mode on-line: C_1[a], Na[a], C_2[a], Al[a], K[a], Ca[a], Fe[a], CaOH[a], FeO[a], Fe_2O[a], C_3H[b], K[b], Ca[b], Na[NaCl] [b] -‘ve mode on-line: H[a], CH_3[a], O[a], OH[a], C_2[a], C_2H_2[a], Cl[a], NO_2[a]

[a] Occurrences greater than 50%.

[b] Occurrences less than 50% and greater than 25%.

[c] Occurrences less than 25%.

[d] Concentrations found are greater than 50 ng/m^3 sample.

[e] Concentrations found are less than 50 ng/m^3 sample.

[f] Qualitative results are presented.

4. CONCLUSION

Certified materials, source materials and urban Toronto PM were analyzed with ICP-AES, SEM, off-line LAMS and on-line LAMS. Many certified elements were observed in the mass spectra of certified materials, confirming the proper working condition of the in-house on-line LAMS. Marker elements that were identified were Mn, Mg, Cr, Li, Sr, Bi, Hg, As, Rb and Tl. Mass spectral analysis of four source materials was next performed, and Ba, Mo, Cu, Cr, Co, Pb, Li, Tl, V, Cl and Cd were identified as marker elements. Many of these elements are verified using additional techniques reported in articles. Lastly, ICP-AES, SEM X ray, off-line and on-line analysis of urban Toronto PM was performed and demonstrated consistency among these techniques.

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BIOMONITORING OF TRACE ELEMENT AIR POLLUTION: PRINCIPLES, POSSIBILITIES AND PERSPECTIVES

B. WOLTERBEEK
Delft University of Technology, Interfaculty Reactor Institute (IRI),
Department of Radiochemistry, Nuclear Environmental Studies,
Delft, Netherlands

Abstract

The present paper discusses the biomonitoring of trace element air pollution. Much attention is given to both lichens and mosses as the dominant plant species used in biomonitoring surveys. Biomonitoring is regarded as a means to assess trace element concentrations in aerosols and deposition. This implies that the monitor should concentrate the elements of interest and quantitatively reflect its elemental ambient conditions. Environmental impact on the biomonitor's behaviour is viewed as resulting in changes in the dose-response relationships. The current literature is briefly reviewed, for plant's behaviour modelling, for laboratory studies on physiological processes responsible for accumulation, retention, and release, and for fieldwork on quantification of dose-response relationships. Monitoring of elemental atmospheric availability is presented as deriving its relevance from presumed impact on both ecosystem performance and human health; source apportionment is regarded as an important parallel result for purposes of emission regulatory management. For source apportionment, the paper argues in favour of multi-elemental determinations, supplemented by information on organic compounds and elemental chemical forms. Furthermore, the discussion points towards more explicit coupling of biomonitoring data to knowledge and databases on emission registration, ecosystem performance, and human health. This means that multidisciplinary programs should be set up, which accommodate expert inputs from biomonitoring, emission control programs, analytical chemistry, ecology, and epidemiology.

1. INTRODUCTION

Concern about atmospheric pollutants underlies the efforts to establish control programmes in many countries. Policies may be 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 new controlled, and economical aspects have to be integrated [1,2].

The necessary information on air pollutants can be obtained by dispersion modelling (source-orientation, a priori known emission sources) and by field measurements of the immission (receptor/effect orientation). In many countries, dispersion modelling has gained more and more interest, also based on economic reasons: technical field measurements require equipment and manpower and are generally associated with high costs [3,4]. Immission 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, which are not known or registered [2]. Immission measurements require long-term sampling at large numbers of sampling sites. Such measurements using technical equipment have been few, mainly due to the high costs, and the lack of sufficiently sensitive and inexpensive techniques, which permit the simultanuous measurement of many air contaminants [5]. It is here that biomonitoring comes in.

The present paper addresses biomonitoring as a technique used in the context of programmes on trace element air pollution. Attention is given to principles, to possibilities, and eventually, to the biomonitoring technique's perspectives in future studies. Throughout the paper, emphasis is on lichens and mosses, since they are the dominant plant species used in environmental surveys on trace element air pollution.

2. OUR INTEREST IN TRACE ELEMENT AIR POLLUTION

2.1. Ecosystem performance

Longest standing attention for widespread air pollution stems from our concern over its ecological effects. The environmental impact of atmospheric deposition has been studied for more than a century; probably the first effect that was described on a scientific basis was the decline of epiphytic lichens in areas with high levels of atmospheric pollution. Ever since Nylander's (1886) classical report [41] on the epiphytic lichens of Paris and its surroundings, extensive studies have been performed in many areas [26,28,30]. The decline of forest tree vitality caused by atmospheric pollution has been known for a long time [29,40], and the occurrence of forest decline over large parts of Europe is now well documented [36]. In analogy to the lake- and stream water acidification, first described for Scandinavia [35], wide-spread effects of atmospheric deposition ("acid-rain") were described for ecosystems such as freshwater bodies, forest tree layers, heath land, chalk grass- and moor lands [25,27,32,39], and for the decline of many plant species [33,38].

Although the research effort has led to a greatly improved scientific understanding of the abiotic effects of atmospheric deposition, especially in the fields of atmospheric chemistry [24], soil chemistry [34], and water chemistry [37], many of the biotic effects are still poorly understood, particularly in the terrestrial environment: although many changes in vegetation are now generally attributed to atmospheric deposition, dose-effect relationships are usually poorly known [31]. Reviews on monitoring, behaviour, and impact of terrestrial trace element pollution can be found in [6–12].

2.2. Human health

In addition to the ongoing concern for ecosystem performance as such, attention has been and becomes increasingly more directly focused on human health. This may be ascribed to the generally recognized impact of ecosystem performance on human well-being; furthermore, health-care has also been progressively developing towards approaches which include our nutrition and our social and environmental surroundings [13].

As a result, throughout the world, epidemiological studies were set up on air pollution and mortality rates and respiratory health effects, initially mostly on air particulates, ozone, acid rain, NOx, and sulphur oxides [14,16,17,22]. For The Netherlands, the latter may be illustrated by the steady increase in clinical-epidemiological medical services from the 1980's onwards [13], and by current Dutch epidemiological studies, which also focus on the relationships between atmospheric black smoke (diesel engines) and nitrogen dioxide (all motorized vehicles) on the one hand and both total mortality and cancer incidence on the other ([17]; Bausch-Goldbohm, personal communication).

Today, epidemiological studies progressively include evaluations of possible relationships between health effects and the (soluble) elemental concentrations in particulates [15,18–21]. Wappelhorst et al. [23], in a study in the German-Czech-Polish Euro region Neisse, are among the first to relate disease incidences with elemental biomonitoring outcomes. This growing attention to atmospheric pollutants other than the "traditional" air pollutants (sulphur oxides, ozone, nitrogen oxides), such as heavy metals, polycyclic aromatic compounds and halogenated organic compounds, all differing widely with respect to their environmental and health impact properties, is also shown by their presence as "black list substances" in the priority action lists in a large number of countries (see Wiederkehr [42]).

3. ASSESSING ATMOSPHERIC TRACE ELEMENTS

Information on atmospheric trace elements can be obtained by modelling of their atmospheric dispersion and deposition, based on a-priori known emission sources (source orientation), and by measurements of actual atmospheric occurrences and/or deposition (receptor-orientation) [2,43]. In many countries, the dispersion modelling approach is attracting more and more interest, also due to economical reasons: the receptor-oriented measurements require expensive equipment and manpower, and are generally associated with high costs. Generally, dispersion modelling is based on meteorological observations, particle size distributions and their deposition properties, surface roughness conditions and emission registrations [44]. 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 the temporal and spatial representativeness of in-field measurements, sampling is required on a long-term basis, and at a large number of sites [45]. Such measurements of air particulate matter and deposition, 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. Measurements are mostly performed on restricted geographical scales [46,47], or are dedicated to the assessment of the impact of specific sources [48]. In a restricted set-up in the Dutch Rotterdam harbour area, De Bruin and Wolterbeek [49] tried to identify geographical source positions, making use of a wind trajectory approach, in which elemental filter data were obtained and correlated to wind direction.

Providing that certain criteria are fulfilled, biomonitoring can be an efficient supplement or even replacement for the above-mentioned type of investigations, and also permits larger-scaled multiple-sites programs, the latter shown by the running series of NORDIC moss surveys [50].

4. BIOMONITORS

4.1. The biomonitoring concept

Biomonitoring, in a general sense, may be defined as the use of bio-organisms/materials to obtain (quantitative) 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 (impact: species composition and/or richness, physiological and/or ecological performance, morphology) or from the concentrations of specific substances in the monitor tissues. With proper selection of organisms, the general advantage of the biomonitoring approach is related primarily to the permanent and common occurrence of the organism in the field, even in remote areas, the ease of sampling, and the absence of any necessary expensive technical equipment.

4.2. Selection criteria

Referring to the determination of the biomonitor's elemental content, organisms may be further selected on basis of their accumulative and time-integrative behaviour (see also Wolterbeek et al. [51,52]). In the literature, biomonitoring species for trace element air pollution are often selected on basis of criteria such as specificity [157] (which means that accumulation is considered to occur from the atmosphere only), accumulation ratio's [1,5], or a well-defined representation of a sampling site [51,52]. Wolterbeek et al. [52] reasoned that selection should be considered on basis of the differences between local and survey variances. Of course, the almost implicit criterion for selection is the biomonitors common occurrence. In earlier work, air pollution was indexed by geographical variances in biodiversity and biomonitor's species richness [6,26,28]; more recent work is aimed at clarification of the impact of variable levels of atmospheric pollution on the biomonitor's responses. In this sense, much study is devoted to processes such as photosynthesis, respiration, transpiration, element accumulation etc. [53,54]. The latter studies share two different objectives: first, knowledge is gathered on the (ecological/physiological) impact of atmospheric air pollution, and second, by relating impact to response in terms of elemental accumulation, knowledge is gathered on the dose-response relationships for the biomonitor of interest.

5. BIOMONITORING: DOSE-RESPONSE RELATIONSHIPS

5.1. The dose and the response

To avoid any lengthy discussion on terminology of what is meant by monitors, indicators, collectors etc. (see Puckett [5], Garty [53] or Markert et al. [55] for clear overviews), in the present paper the term biomonitoring is handled in a more general way: "we use the biomonitor organism to get information on elemental deposition and/or atmospheric levels, thereby including impact information, because we have to try and quantify the dose-response relationships as far as we are able to". The latter means that, although the information on impact also serves its own additional purposes [55], if we regard the elemental levels in the biomonitor as a response to ambient elemental levels (air, deposition = the dose), and if we restrict ourselves to the context of the dose-response relationship, impact on biomonitor physiology should be seen as relevant because it may cause changes in the nature of this dose-response relationship (see Garty [53] for a review on the impact on lichen physiology of metals such as Pb, Fe, Cu, Zn, Cd, Ni, Cr, Hg). Throughout the years, the dose-response relationship has received considerable attention, initially in a rather implicit manner, but in later years research efforts have become more and more specifically aimed at the precise characterization of biomonitor's responses.

5.2. The quantification problem

Apart from the reported regional distribution patterns of elemental concentrations in biomonitor materials [1,57,68,69] with implicitly presumed positive correlations between dose and response, the early work on dose-responses was mostly on the correlations between elemental levels in biomonitors and their distance to metal point sources [5,70–72]. In later work, averaged elemental contents of filter-trapped air particulate materials or deposition were compared with biomonitor's averaged metal concentrations: Saeki et al. [73], Andersen et al. [61], and Pilegaard [62] reported parallelisms and linear relations with lichens, Sloof [1] and Jeran et al. [56] found positive correlations between metals in air particulates and transplanted lichens, Hanssen et al. [74], Ross [75], Berg et al. [76], and Berg and Steinnes [77] observed significant correlations between wet deposition and moss metal concentrations.

Parallel to these phenomenological approaches was the work carried out to gain knowledge on the processes responsible for accumulation, retention and releases of elements in the biomonitor materials. These studies have included much laboratory investigations, and have shown that metal retention may be by particulate entrapment, physio-chemical processes such as ion exchange, as well as by passive and active cellular uptake [58–60,71,5,78,79,103]. In addition, the Chernobyl accident has provided much information on accumulation efficiencies and release rates in biomonitors: fall out has been measured in air particulate matter, deposition, and biomonitors [1,64,80–83,92]. Retention efficiencies and release rates could be calculated from the time course of radioactivity levels in biomonitors after the short-term atmospheric influx [65,66,93].

So far, however, hardly any work has been done to bridge the laboratory findings and the results obtained in field work: Ellis and Smith [65] tried to interpret ^{137}Cs lichen data by dynamic flux modelling, Schwartzman et al. [67] applied ion-exchange models for explaining ^{210}Pb and Pb uptake in lichens, and Garty [53] wrote a review on metal accumulation in lichens under both laboratory and field conditions. Sloof [43] compared metal accumulation in lichen transplants with that in synthetic rags, and compared transplant outcomes with data obtained with in situ growing lichens, and Reis et al. [63] modelled lichen behaviour towards ambient air particulate, and deposition metal availability, thereby including a memory loss concept, based on uptake and release processes, which describes the lichen transplant’s progressive reflection of its new ambient conditions.

6. BIOMONITOR BEHAVIOUR

6.1. General

Lichens and mosses may be considered as the most commonly applied biomonitor organisms. This is largely based on their lack of any roots comparable to higher plants, which makes that both are thought to obtain their mineral supplies from aerial sources and not from the substratum [6,50,84,90]. However, uptake or other contributions from the substrate may occur. For lichens, Goyal and Seaward [86] demonstrated possible metal uptake by rhizines, Prussia and Killingbeck [87] found differences in lichen metal content associated to differences in substrata, De Bruin and Hackenitz [88] found metal concentrations which did not differ between lichens and their bark substrata, and Sloof and Wolterbeek [89] suggested further study on possible bark-lichen interferences for Cd, Mn, and Zn. For mosses, Kuik and Wolterbeek [91] found relatively high crustal contributions to the moss levels of elements such as Al, Sc, La and further lanthanides, and Brown and Brown [84] suggested that the increase in cation exchange capacity from moss apex to base is part of its natural balance of elements, which in turn is affected by the proximity of the soil.

Both lichen and moss species are suggested to accumulate elements largely by passive processes [43,79]. Furthermore, biomonitor’s field data are often interpreted as related to the entrapment of relatively insoluble metal-rich particulates rather than by the retention of soluble elements [84], which supports their reported high retention efficiencies [50,85]. The finding that older lichen and moss parts show the higher metal concentrations has led to the additional assumption that the plants provide an historical and interactive recording of the metal supply in the environment. For lichens, the central older parts of the thallus, carrying most fruiting bodies, have been shown to have higher metal concentrations [84,93]. For mosses, the carpets are build up during a period of 3–5 years, and their metal content is generally considered to reflect the atmospheric deposition during that period [50].

However, much more research emphasis has been placed on metal acquisition by biomonitors than on the possible metal redistribution within or losses from the plants. For mosses, Tyler [116], Brown and Wells [117] and Wells and Brown [118] indicated metal transfer into the cell protoplasts, and Brown and Brown [84] demonstrated metal-specific rates of intracellular transport from old to newly grown tissues. Taylor and Witherspoon [60] reported rainfall-related losses of deposited 137Cs carrying particles, and eventually calculated particles "weathering" half-lives for both mosses and lichens of about 50 days. For lichens, Goyal and Seaward [119] reported free metal movement from rhizinae to the upper thallial surfaces and vice versa. Losses were mostly indicated on basis of (Chernobyl) radio caesium data [60,65,93,120,121]; uptake and release processes are also part of models on lichen behaviour used by Schwartzmann et al. [67] and Reis et al. [63].

Since we need to understand the monitor's behaviour before we will be able to reliably exploit them in air pollution surveillance, the above data suggest that more research effort is needed on the dynamics of their metal accumulation and release processes.

6.2. Impacts on biomonitors

The ongoing attention for trace element air pollution stems from the interests we have in ecosystem performance and human health. The consequence is that we need means to assess trace element occurrences and levels in air particulates and deposition. We argue that we can use biomonitor organisms to do this on large scales, and we like to develop biomonitoring into a fully accepted quantitative tool in air pollution programs. Here, one of the problems encountered is the biomonitor performance. The impact of trace element air pollution may be discussed in terms of effects on ecosystems, or on human health (see § 3), but it may also have effects on the biomonitor's behaviour (see Lepp [8]; Pignata et al. [95]; Gonzalez and Pignata [94]; Garty [53]). Moreover, natural variability in ambient macro- and microclimate conditions, such as acidity, temperature, humidity, light, altitude, or ambient elemental (nutritional) occurrences may cause the biomonitor to exhibit variable behaviour (see Seaward et al. [96] for altitude effects on lichen responses). Part of this variance is shown as local variance [52], but it may be clear that this variable behaviour becomes a problem when it seriously affects the biomonitor in its accumulative responses.

Seasonal effects in plant elemental concentrations have been described by Markert and Weckert [97], Ernst [98], and Markert [99], which, in general terms may be ascribed to both elemental leaching and increased availability by rainfall (Reis, unpublished), and to seasonally varying degrees of dilution by mass increments, the latter due to seasonal variations in growth rates [99]. At this point, metal phytotoxic effects on plant physiology should be regarded. Growth is often used as striking marker for strong physiological disorder [100], but effects on metal accumulation already occur when this visible growth symptom is less pronounced or even absent. One of the most direct effects on the cellular level is the alteration of the plasma membrane permeability, which may cause excess leakage of ions [101], and may have effects on metal accumulation characteristics [102].

For moss, although deposition rates have been estimated on presumed quantitative metal retention in moss top segments [104], uptake efficiencies were shown to be strictly ordered for a number of metals [103]. However, competition effects may significantly influence uptake and retention: high sea salt input showed effects even on the retention of strongly absorbed metals such as Pb and Cu [76,77,79]. Furthermore, also strongly acidic precipitation, generally largely associated to atmospheric SO_2 [105,106], may yield lower moss metal concentrations [79]. In this context it should be noted that toxic action in plants is indicated especially for SO_2 and NO_2 [107–109].

For lichens, Demon et al. [110,111] studied effects by acidity, temperature, and calcium ions on metal accumulation in both algal and fungal components. For both components, hardly any effect from temperature could be determined for Cu and La, indicating the absence of metabolically controlled uptake, but the reverse was shown for As, W, Zn, and Cd in the algal component (see Gjengedal and Steinnes [79] for mosses). Metal uptake in the algae was generally faster at pH 7, in the fungus uptake rates were higher at pH 5. In intact lichens, pH dependent uptake was reported by Puckett et al. [112] and Puckett and Burton [90].

Ambient SO2 has been considered in initial studies with bark [113,114], estimations were based on measurements of bark S and bark acidity. In a later bark study, Wolterbeek et al. [158] examined relationships between sulphate, ammonia, nitrate, acidity, and trace metals, and the distance to the sea. Bark sulphate, ammonia, and nitrate were interpreted as not significantly affecting bark metal retention, Ca and Hg were affected by acidity. For bark, the Ca loading in particular may determine the buffering capacity with respect to incoming acidic precipitation [115]; further neutralization may be brought about by alkalising effects from atmospheric NH3 [109]. Based on moss, lichen, and bark data, Wolterbeek and Bode [51] proposed to supplement the trace metal analysis in biomonitors with the determination of pH, NH_4, NO_3, and SO_4.

7. BESIDES TRACE ELEMENTS

Today, lichens and mosses are mostly used as biomonitor plants for atmospheric trace elements. In addition, and especially after the Chernobyl accident, much literature has emerged on the use of both plant species for the assessment of the atmospheric levels and deposition of radionuclides. Apart from these two applications, and already in the 1950’s, lichens have also been studied extensively in the context of the deposition of nitrogen and acidity [26,28,122]. In these studies, species diversity and richness of occurrence was compared to variability in N and S atmospheric levels, both for impact assessments and for monitoring purposes [123]. It may also be noted here that mosses have been used for the measurement of industrially emitted fluorine as early as 1950 (MacIntyre et al. [124]). From the 1960’s onwards, lichens have been used in both laboratory and field fumigation experiments on the effects of gaseous pollutants such as SO_2, NO_2, HF, or O_3 [28,125–128]. In current research a large variety of plant species, such as wheat, barley, maize, grass, or tobacco, is investigated for their responses towards gaseous pollutants [129–131], both for impact and monitoring purposes. Mosses were applied as monitors of the deposition of organic micropollutants from the 1980’s onwards [132]. Most of the early work concentrated on the deposition of organochlorine compounds such as pesticides and polychlorobiphenyls (PCBs). However, in later work, both lichens and mosses have been applied also in monitoring of atmospheric polycyclic aromatic hydrocarbons (PAHs), which are emitted in association with human activities such as the production of coke from coal, the combustion of fossil fuels, or the aluminium and carbon electrode industries [132–137,159].

Based on the above, and in the context of the use of both lichens and mosses, combined determinations of trace elements, PAHs, and S and N compounds may be considered: combined approaches in field work and analyses will facilitate the interpretation of individual elements and compounds and may permit a more reliable recognition of source fingerprints [51].

8. MULTI-ELEMENTAL LARGER-SCALED (BIO)MONITORING DATA

When multi-parameter analysis is applied, especially in larger-scaled surveys, the information which is obtained from (bio)monitoring may consist of many thousands of data points [50,138]. Apart from being taken into straightforward statistics, geographical mapping or direct plotting in series of time or distance, these data may be processed in a variety of further mathematical routines to permit a condensed and strongly smoothed presentation of results and conclusions.

In air pollution studies, graphical techniques are used to pinpoint elemental origins (Rahn and Huang [139]; Factor Analytical (FA) techniques are used to resolve the elemental composition of aerosols or biomonitor organisms into a set of factors, which are interpreted as representing source elemental profiles [1,140–149]. The Monte Carlo aided FA routines developed by Kuik et al. [140,141] permit the assessment of the reliability of the factor solutions, which offer the opportunity to manipulate the data with full consideration for progression of uncertainties. FA routines are thus used in data clean up procedures (e.g. the removal of the soil dust factor) and source isolation procedures (e.g. the isolation of a specific source profile); these procedures are shown to give higher-quality survey outcomes [52].

As may be clear, any combination of biomonitoring data on elements and organic compounds may increase the resolving strength of source profiling routines. The underlying reasoning is that a source emits an array of compounds and elements. The chemistry of emitted substances and its impact on accumulation specifics [150–152] makes that the progressive increase in analytical efforts on the determination of elemental chemical forms in aerosols, particulates and biomonitor tissues [153–156] should be considered as similarly improving our means for assessing source fingerprints from biomonitoring.

9. BIOMONITORING BETWEEN SOURCE AND IMPACT: PERSPECTIVES

9.1. Status Quo

In the present paper, biomonitoring is regarded as a means to assess trace element concentrations in aerosols and deposition. This means that the biomonitor should concentrate the elements of interest, and that it should quantitatively reflect its ambient elemental conditions. Seen from this point of view, the relevance of elemental impact is primarily important in terms of changes in the dose-response relationships. Any significant impact on the behaviour of the monitor may lead to changes in the way the monitor reflects elemental availability, thus disturbing the assumed relationships.

In current literature, reports are on the impact term, that is, changes in plant physiological parameters, are studied extensively, without however, notation on the consequences for the dose-response relations. In parallel, reports, based on laboratory studies, are on physiological processes, which are operating in elemental accumulation, here without full exploitation of gained knowledge in field approaches of biomonitoring. In field situations, most quantification work is of a purely phenomenological nature.

Essentially, biomonitoring of trace element air pollution is regarded as relevant, because of the pollution's impact on ecosystem performance and human health. To make the assessment even more significant, much research effort is devoted to the extraction of source terms (emission profiles) from the data obtained.

9.2. Perspectives

The relative ease of sampling, the absence of any need for complicated and expensive technical equipment, and the accumulative and time-integrative behaviour of the monitor organisms make that biomonitoring of atmospheric trace elements will be continued for the foreseeable future, especially in larger-scaled surveys. The necessary quantitative assessment of elemental availability asks for well-defined dose-response relationships, and knowledge on disturbances by impacts on the plant parameters on accumulation, retention and release processes. This implies that much effort should be devoted to bridge laboratory findings and field results.

Biomonitoring may gain importance when the source-terms are reliably extracted from the data obtained. For this, it seems that determinations should be multi-elemental, and supplemented by any additional information on emissions. Source apportionment may get more specific when data on total concentrations of elements are supplemented by information on organic compounds and elemental chemical forms.

The assessment of atmospheric elemental availability derives its relevance from presumed impact on both ecosystem performance and human health; in this context source apportionment should be regarded as an important parallel result for emission regulatory management. Biomonitoring data should be coupled more explicitly to knowledge and data bases on emission registration, ecosystem performance, and human health. This means that multidisciplinary programs should be set up, which accommodate expert inputs from biomonitoring, emission control programs, analytical chemistry, ecology, and epidemiology.

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CALIBRATION OF LICHEN TRANSPLANTS CONSIDERING FAINT MEMORY EFFECTS

M.A. REIS[1], L.C. ALVES[1], M.C. FREITAS[1], B. VAN OS[2],
J. DE GOEIJ[3], H.TH. WOLTERBEEK[3]

[1]ITN, EN10 Sacavém, Sacavém, Portugal
[2]National Geological Survey, Haarlen, Netherlands
[3]IRI-TUDelft, Delft, Netherlands

Abstract

Biomonitoring is for more than 20 years now, a tool to study the dispersion of trace elements through the atmosphere and back to earth surface again. A qualitative improvement upon the use of biomonitors is their calibration against more traditional element availability variables like deposition or airborne concentration. Usually it is assumed that a linear regression between biomonitors concentration and availability average values provides a good calibration. In this work, an uptake experiment using transplants of lichen *Parmelia sulcata* carried out in Portugal during a two years period (1994/96) is described. Nuclear analytical techniques PIXE and INAA were used to analyse lichen and aerosol samples, and ICP-MS was used to analyse total deposition dry residue samples. A database of roughly 70,000 values was created and biomonitor calibration done considering also effects due to non-permanent memory. Data allowed to show, that if annual surveys are made by sampling lichens four times with a period of 3 months in-between sampling campaigns, availability mean, maxima and standard deviation, can be recovered from lichen data for: Al, Si, Cl, Ca, Ti, Fe, Co, Cu, Zn, Zr, Sm, Tb, Hf, Pb, and Th.

1. INTRODUCTION

Modern biomonitoring, according to [1] can be considered to start with the work of Rühling and Taylor in 1968, nevertheless a method to convert biomonitoring data into an organism (the biomonitor) independent data, was not yet proposed in a generally accepted approach. In the European deposition maps [2,3] a linear response is assumed to exist between the annual average of deposition and the moss content for the elements used in that study. Nevertheless, studies involving a big number of elements and biomonitor response to their variation in natural conditions are not common in literature, thus the validity of the linear response approach from an experimental point of view for a great number of elements is not yet shown. From a theoretical point of view, the assumption of linear response to the average cannot be easily sustained if it is assumed that the uptake and release channels are unidirectional and have different working rates. In fact, considering this unidirectional character immediately leads to the introduction of the concept of remembrance time as the time during which the biomonitor preserves the memory of a given availability episode. To speak in a precise manner relative to these episodes, and associated remembrance time, the concept of availability must also be specified. In this work, the term availability will be used instead of airborne concentration, composition of the airborne particles, total deposition or any other physical way of measuring the amount of element that is made available to the biomonitor, and thus to any entity (living or not) that cohabits with the biomonitor.

In the models that will be used in this work, remembrance time for low availability conditions will be assumed to be zero. This assumption is based on experimental uptake results obtained by Taylor [4] and Sloof [5] both pointing out to a very fast response of biomonitors to the increase in availability, at least in wet environment conditions. Non-infinity remembrance times of conditions of high availability will be thus the basis of discussion.

For the work now being presented, a lichen transplant uptake experiment was carried out between 1994 and 1996 in Portugal. For the calibration process, data were gathered in such a way that each experimental station provided one value per element and per variable being studied.

2. MATERIALS AND METHODS

2.1. Experimental

For the experiment, 26 × 6 transplants of lichen *Parmelia sulcata* were prepared. Lichens were collected at one site previously identified as scarcely polluted [6,7]. 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 as reference set. Afterwards, at intervals of approximately one month, two additional lichen samples were brought to laboratory and prepared for analysis. Lichen samples were prepared by removing lichens from any remaining Olive tree substrate used during transplantation, rinsing in 18 MΩ·cm water for about 30 seconds for cleaning out dust, freeze drying, and grinding in a Teflon balls mill cooled to LN temperature. The powder obtained in this way was pressed into different pellets for PIXE and for INAA analysis.

At each site, two buckets with 25 cm diameter funnels were also installed for total deposition collection. These were replaced every time a new set of lichens was recollected. Besides lichens and total deposition, also a Gent Aerosol Sampling Unit [8] was installed at each site for PM2.5 and PM10-PM2.5 airborne particles collection.

Lichen and filters used for particle collection were analysed by both PIXE and INAA at ITN Van de Graaff and Portuguese Nuclear Research Reactor (RPI) facilities. Short-lived isotopes INAA analysis was carried out at the Hahn-Meitner Institute, Berlin.

Total deposition samples were dried under an infrared lamp at ITN and the residues were acid digested and analysed by ICP-MS at The Netherlands National Geological Survey facilities.

2.2. Calibration Methods

Calibration methods tested and compared can be grouped in four types. In each type of methods, calibration relative to averages and to maxima of availability was tested. The four different types consist of: (1) a simple approach using linear regression, (2) an approach using a model that considers faint memory effects by weighing availability by the time between the availability event and the lichen recollection, (3) a bilinear regression using besides average or maxima, also the standard deviation of availability, (4) an approach where lichen content average and standard deviation are used to infer availability average or maxima, and availability standard deviation.

2.2.1. Direct calibration methods

This is the group of methods referred in (1). Calibration of lichen content versus availability average was coded DC. Calibration of lichen content versus availability maxima were coded DM.

Calibrating biomonitors out of an uptake experiment such as the one just described requires some care in calculating averages because lichens exposed for say two months cannot have any information of what will append afterwards, and lichens exposed for long periods are exposed to a mean availability value different from the average value of the month period just before its collection from the experiment site. To proceed with a straightforward linear regression calibration the following expressions were used:

$$\langle LEC\rangle_{DC,\lambda} = a \cdot \bar{A}_{DC,\lambda} + b \quad \text{being} \quad \bar{A}_{DC,\lambda} = \frac{1}{N}\sum_{i=1}^{N}\langle A\rangle_i = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{\sum_{j=1}^{i}\left(\Delta T_j^{\lambda}\cdot A_j^{\lambda}\right)}{\sum_{j=1}^{i}\Delta T_j^{\lambda}}\right) \tag{1}$$

$$\langle LEC\rangle_{DM,\lambda} = a \cdot \bar{A}_{DM,\lambda} + b \quad \text{being} \quad \bar{A}_{DM,\lambda} = \frac{1}{N}\sum_{i=1}^{N}\langle A\rangle_{DM,i} = \frac{1}{N}\sum_{i=1}^{N}\left(Max\left\{A_j^{\lambda}\right\}_{j=1}^{i}\right) \tag{2}$$

where $\langle LEC\rangle_{*,\lambda}$ are the algebric mean values of element contents of the 24 lichen transplants exposed during the experiment, $\bar{A}_{*,\lambda}$ the year average availability, and ΔT_j^{λ} the time interval for the j^{th} month period, all at station λ. The calibration parameters were obtained by interpolation based on the minimization of the sum of squares of the difference between prediction and measurement. Both missing data and values below detection limit were not replaced by zero but overlooked in the calculations.

2.2.2. Weighing availability methods

In the direct calibration methods above, the invertion of lichen calibration is immediate. Once obtained the values for a and b, the determination of the average (DC method) or the maxima (DM method) of availability from lichen elemental content is straightforward, namely, $\langle A\rangle = (LEC - b)/a$. In such case, the availability average refers to a time average since the beginning of times:

$$\langle A\rangle = \int_{-\infty}^{0} A(t)\,dt \Big/ \int_{-\infty}^{0} dt\,.$$

Nevertheless, it is evident that this expression can only be understood in a formal way because the denominator is an improper integral.

If a faint memory correction term is introduced, the average availability becomes:

$$\langle A\rangle = \int_{-\infty}^{0} e^{\frac{t}{\lambda}} A(t)\,dt \Big/ \int_{-\infty}^{0} e^{\frac{t}{\lambda}}\,dt$$

which is meaningful even in a strict mathematical sense, as long as λ, the remembrance time (or memory decay time), is not zero or infinity.

In order to accommodate such a concept into the simple calibration procedure, only a weighing term must be added to availability prior to averaging. Lichen concentration averages can now be written as:

$$\langle LEC\rangle_{MC,\lambda} = a\cdot \bar{A}_{MC,\lambda} + b \quad \text{being} \quad \bar{A}_{MC,\lambda} = \frac{1}{N}\sum_{i=1}^{N}\langle A\rangle_{MC,i} = \frac{1}{N}\sum_{i=1}^{N}\left[\frac{\sum_{j=1}^{i}\left(e^{-\frac{T_i - Tj}{\lambda}} A_i\, \Delta T_i\right)}{\sum_{j=1}^{i}\left(e^{-\frac{T_i - Tj}{\lambda}}\, \Delta T_i\right)}\right] \tag{3}$$

$$\langle LEC\rangle_{MM,\lambda} = a\cdot \bar{A}_{MM,\lambda} + b \quad \text{being} \quad \bar{A}_{MM,\lambda} = \frac{1}{N}\sum_{i=1}^{N}\langle A\rangle_{MM,i} = \frac{1}{N}\sum_{i=1}^{N}\left[Max\left(e^{-\frac{T_i - Tj}{\lambda}} A_j\right)_{j=1}^{i}\right] \tag{4}$$

MC being the code for faint **M**emory **C**orrected calibration to averages, and MM the code for faint **M**emory corrected calibration to **M**axima.

It was shown that, by minimizing the sum of squares of the difference between model and observation using expressions (3) and (4), the remembrance time values, λ, can be determined prior to the knowledge of the values of the regression coefficients [9].

2.2.3. Standard deviation methods

Introducing the above faint memory effects considering methods, a well-defined behaviour of the memory faiding process is imposed, namely that memory should decay exponentially. This may not be the correct mathematical description.

Faint memory effects put an emphasis on high availability events. On the other side, the standard deviation of availability is a measure of the frequency of these high availability events, and also their size relative to the average. It should then be possible to use it to account for faint memory effects on biomonitors element content. To do so, expressions (5) and (6) were used. In these, DS is the code for **D**irect calibration using the average and the **S**tandard deviation of availability, and MS the code for the calibration using the **M**axima and the **S**tandard deviation of availability.

$$\langle LEC\rangle_{DS,\lambda} = a_1 \cdot \bar{A}_{DS,\lambda} + a_2 \cdot \overline{StDev(A)}_{DS,\lambda} + b$$

$$\text{being} \quad \bar{A}_{DS,\lambda} = \frac{1}{N}\sum_{i=1}^{N}\left\langle A^{\lambda}\right\rangle_{DS,i} = \frac{1}{N}\sum_{i=1}^{N}\left[\frac{\sum\limits_{j=1}^{i}\left(A^{\lambda}{}_{j}\,\Delta T_{j}^{\lambda}\right)}{\sum\limits_{j=1}^{i}\Delta T_{j}^{\lambda}}\right] \tag{5}$$

$$\text{and} \quad \overline{StDev(A)}_{DS,\lambda} = \frac{1}{N}\sum_{i=1}^{N} StDev\left(A_{i}^{\lambda}\right)_{DS} = \frac{1}{N}\sum_{i=1}^{N}\left\{\sqrt{\frac{\sum\limits_{j=1}^{i}\left[\Delta T_{j}^{\lambda}\cdot\left(A^{\lambda}{}_{j}-\left\langle A^{\lambda}\right\rangle_{i}\right)\right]^{2}}{\sum\limits_{j=1}^{i}\left(\Delta T_{j}^{\lambda}\right)^{2}}}\right\}$$

$$\langle LEC\rangle_{MS,\lambda} = a_1 \cdot \bar{A}_{MS,\lambda} + a_2 \cdot \overline{StDev(A)}_{MS,\lambda} + b$$

$$\text{being} \quad \bar{A}_{MS,\lambda} = \frac{1}{N}\sum_{i=1}^{N}\left\langle A^{\lambda}\right\rangle_{MS,i} = \frac{1}{N}\sum_{i=1}^{N}\left[Max\left(A_{j}\right)_{j=1}^{i}\right] \tag{6}$$

$$\text{and} \quad \overline{StDev(A)}_{MS,\lambda} = \frac{1}{N}\sum_{i=1}^{N} StDev\left(A_{i}^{\lambda}\right)_{MS} = \frac{1}{N}\sum_{i=1}^{N}\left[\sqrt{\sum_{j=1}^{i}\left(A^{\lambda}{}_{j}-\left\langle A^{\lambda}\right\rangle_{i}\right)^{2}}\right]$$

2.2.4. Inversion Methods with Standard Deviation

Once it is recognised that the standard deviation of availability can be used to quantify simultaneously the difference between maxima levels and mean levels of availability, and the frequency of occurrence of these maxima, a solution to the inversion question: "Is it possible to infer both the average and standard deviation of availability out of the average and standard deviation of lichen element content?", was searched based on the following equations:

$$\begin{aligned}
\bar{A}_{DS,\lambda} &= a_{1}^{IS}\cdot\langle LEC\rangle_{\lambda} + a_{2}^{IS}\cdot StDev\langle LEC\rangle_{\lambda} + b^{IS} \\
\bar{A}_{MS,\lambda} &= a_{1}^{IM}\cdot\langle LEC\rangle_{\lambda} + a_{2}^{IM}\cdot StDev\langle LEC\rangle_{\lambda} + b^{IM} \\
\overline{StDev(A)}_{MS,\lambda} &= a_{1}^{VM}\cdot\langle LEC\rangle_{\lambda} + a_{2}^{VM}\cdot StDev\langle LEC\rangle_{\lambda} + b^{VM}
\end{aligned} \tag{7}$$

In these equations, *V* stands for the **V**ariability component, M is a reference to the method providing the **M**axima values of availability, *I* is a reference to the main part of availability (average or maxima), and *S* is used to make the connection to standard deviation methods.

3. RESULTS AND DISCUSSION

To explore the uptake experiment up to its limits, nine availability variables were defined. Lichen data were then tentatively calibrated against these variables using the first three methods described above. Table I describes the availability variables codification and their definition. Prior to its use for calibration, lichen data were checked to verify if the variability within there was explainable by random changes within the error limits of the reference values. Elements for which this was the case for two or more stations were not used for calibration. Sets of data presenting less than 70% of positive measurements were also discarded. A list of 25 elements: Na, Al, Si, S, Cl, Ca, Sc, Ti, Fe, Co, Ni, Cu, Zn, Zr, Sb, Ba, Ce, Tb, Lu, Hf, Ta, Pb, Th, and U thus remained to proceed with calibration methods. In this list, aerosol data is non-existing or did not comply to the conditions for selection for 7 elements: Zr, Ba, Tb, Lu, Hf, Ta, and U. In the same conditions but for the deposition variables, 6 elements were found: Si, S, Cl, Sc, Sb, and Sm.

Each calibration was considered a good calibration if a correlation coefficient better than or equal to 0.8 was found between method predictions and observations. As this should not be seen as a sharp cut-off, data will be presented for correlation coefficients above 0.7.

For the direct calibration methods, good calibration conditions were found for Al, Si, Cl, Fe, Cu, and Pb when only aerosol availability variables were considered. Relative to absolute total deposition variable (ATD), good calibration conditions were determined for: Al, Ti, Fe, Cu, Zr, Ce, Tb, Lu, Pb, Th, and U. Therefore, a total of 13 elements (nearly 50%) were calibrated using these simple models. Lowering the cut-off correlation coefficient to 0.7, S and Hf list are added to this list.

The test made using the weighted availability models did not alter much the number of elements for which a good calibration was found but it provided values for the remembrance time for various elements presented in Table II. It can be seen that remembrance times are larger for the occurrence of high mean availability conditions then for high maxima conditions. In the cases of Ti, Fe, Ce, Tb, and Lu, no remembrance time could be determined for mean values of availability. It is important to realise that most values for remembrance time for maxima events (MM method) are within 10 to 100 days, implying that, for many of the elements, after a period of 3 months, a given high availability event will contribute to the lichen content with less than 37% of its value. After one year, it can thus be stated that there is no longer any significant effect.

In spite of this additional information, the fact that it was not possible to calibrate more elements using weighing availability methods is curious. One of the reasons might be that these methods imply a well defined response from the lichen, namely an exponentially fayding remembrance. A way to overcome this limitation was the use of the standard deviation methods described in equations (5) and (6). Upon doing this, good calibration conditions were found for: Al, Si, Cl, Ca, Ti, Fe, Co, Ni, Cu, Zn, Zr, Ce, Sm, Tb, Lu, Hf, Ta, Pb, Th, and U. When the cut-off-condition for good calibration is dropped for a correlation coefficient of 0.7, then only Ba remains out of the list; it should also be noticed that, for Ba, only deposition data were considered in this study.

TABLE I. AVAILABILITY VARIABLES DEFINITION

	Description	Units	Calculation method
FAC	Fine Aerosol Concentration	ng/m^3	Direct measurement
CAC	Coarse Aerosol Concentration	ng/m^3	Direct measurement
TAC	Total Aerosol Concentration	ng/m^3	Sum of FAC and CAC
FPC	Fine Particle Content	µg/g	FAC times 1000 divided by Fine particles mass concentration (in µg/m^3)
CPC	Coarse Particle Content	µg/g	CAC times 1000 divided by Coarse particles mass concentration (in µg/m^3)
TPC	Total Particle Content	µg/g	TAC times 1000 divided by the sum of Fine and Coarse particles mass concentration
DRC	Dry Residue Content	µg/g	Direct measurement
ATD	Absolute Total Deposition	mg	DRC times 1x10^{-6} multiplied by the Dry Residue Mass in mg Data was stored in mg per month period but divided by the funnel inlet area and by the exposure time before use in other calculation providing final results in (ng/cm^2)/day
WRC	Water Residue Concentration	mg/λ	ATD devided by the collected water volume in liters

The main interest of calibrating lichens or any other biomonitor is, nevertheless the possibility to infer values about the availability data or about its effect based on the biomonitor data. These calibration approaches all were carried out trying to reproduce lichen data out of availability data. If it is immediate that it is indifferent which is the dependent and the independent variable when linear regression is used, this is no longer the case for the remaining methods. Due to this reason, and in face of the results obtained for the standard deviation methods, the calibration of availability data using lichen data as independent variables was attempted. This was done using the invertion methods with standard deviation described above.

The coefficients for the invertion methods with standard deviation described in equations (7) were determined using the whole 12 month lichen data set and are presented in Table III.

To test the possibility of applying the inversion methods with standard deviation in survey conditions (where monthly recollections are not possible), availability parameters calculated from the mean and standard deviation of lichen content determined using only the pairs of transplants exposed for 3, 6, 9, and 12 months, were compared to the experiment results. The correlation coefficients obtained for all cases are presented in Table IV. It can be seen that, for mean, maxima, and standard deviation values of availability, simultaneous correlation coefficients above 0.8 are found for 15 elements, namely: Al, Si, Cl, Ca, Ti, Fe, Co, Cu, Zn, Zr, Sm, Tb, Hf, Pb, and Th. Restricting to average and maxima of availability, and considering correlation coefficients down to 0.7, it is seen that only S, Sb, Ba, and Ta, do not show at least a good inversion calibration method.

TABLE II. REMEMBRANCE TIME, IN DAYS, FOR HIGH AVAILABILITY CONDITIONS FOR THE CASES WHERE A CORRELATION COEFFICIENT ABOVE 0.7 WAS FOUND BETWEEN PREDICTION AND MEASUREMENTS (FOR METHODS DESCRIBED IN EQUATIONS (3) AND (4)). WHEN NO REMEMBRANCE VALUE COULD BE CALCULATED, A VALUE OF 1.00E+14 IS PRESENTED

	MC Remembrance time					MM Remembrance time		
	FAC	FPC	ATD	WRC		FAC	FPC	ATD
Na								
Al		1.00E+14	4.79E+01				1.21E+02	1.95E+01
Si		4.67E+01					8.72E+01	
S	2.34E+04					1.39E+02		
Cl	4.00E+00	9.04E+01					6.88E+01	
Ca								
Sc								
Ti			1.00E+14			3.94E+01		1.68E+02
Fe		2.71E+01	1.00E+14					7.62E+01
Co								
Ni								
Cu			2.13E+01	4.52E+01				3.92E+01
Zn								
Zr			3.38E+01					8.34E+01
Sb								
Ba								
Ce			1.00E+14					4.19E+01
Sm								
Tb			1.00E+14					4.18E+01
Lu			1.00E+14					3.59E+01
Hf								3.78E+01
Ta								
Pb			4.42E+01	4.71E+01				3.14E+01
Th			2.66E+01				1.00E+14	1.58E+02
U			2.28E+02					5.82E+01

4. NEW CONCEPTS

An important point has now been reached. The predictions of the calibration methods proposed present good correlation to measured data. The performance of the methods being improved at the same time as the physical understanding of the meaning of the methods gets more and more distant. A point has been reached where this understanding must be recovered. (1) What is the meaning of the especially averaged availability calculated by the MC and MM methods? (2) What is the meaning of calibrating against availability and standard deviation of availability? (3) What type of availability information is obtained using the inversion methods with standard deviation?

Fig.1 shows four simulated availability time series (line) and the maximum weighted availability (circles) for the MM method, equation (4).

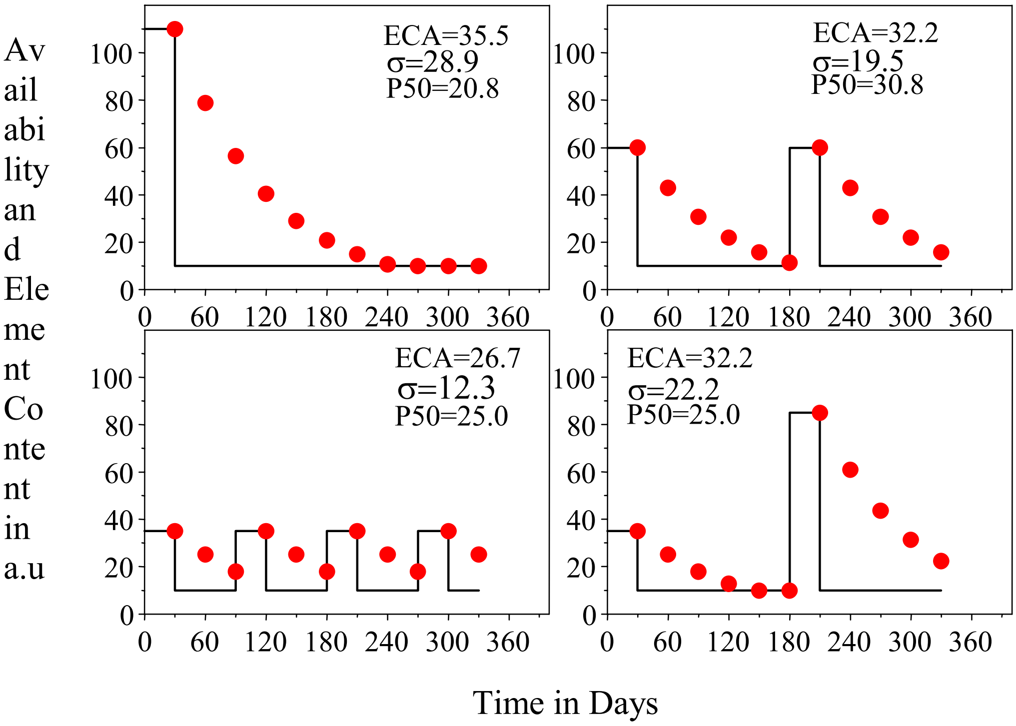

FIG. 1. Simulated availability time series (line), and lichen response with unit calibration coeficient, in the case of Memory method with Maxima, MM, using a remembrance time constant of 90 days. Availability mean values are, by construction, the same for every case. The availability standard deviation and the median, or 50% percentile, of the weighted availability are also presented (P50). ECA: equivalent constant availability; a. u.: arbitrary units.

If, for simplicity of explanation, the calibration coefficient is assumed to be 1, the circles will also represent the lichen element content expected by the MM method.

Calculating the year average lichen content for these simulated availability time sequences, it can be seen that it is identical to the value that would be achieved if the availability was constant and equal to its year average.

Whatever information there is in the lichens relative to availability, it can, therefore, only be described as an ***equivalent constant availability***, **ECA**, once faint memory effects are accepted.

Time series in Fig. 1 were constructed such that the average availability is the same for all four cases. Nevertheless, ECA, which is just the average of the weighted availability, differs, being higher for the sequence presenting the single very high maxima in the beginning.
In ecotoxicology, it is assumed that effects are a function of both exposure level and exposure time [10]. This implies that a higher exposure, although in a shorter time, is expected to have a bigger effect than that of the same amount of exposure in a longer time. ECA being higher for the single peak case, shows a better compatibility to ecotoxicological assumptions than average availability does. ECA is therefore a measure of the biological effect of the environment element dispersion.

TABLE III. CALIBRATION COEFFICIENTS FOR THE MEAN (IS), MAXIMA (IM), AND STANDARD DEVIATIONS (VM) OF AVAILABILITY, ACCORDING TO EQUATIONS (7). THE WHOLE 12 MONTHS LICHENS DATA SET WAS USED FOR THE DETERMINATION OF THESE COEFFICIENTS. VALUES ARE PRESENTED FOR THE AVAILABILITY VARIABLE FOR SHOWING BETTER PERFORMANCE. THE CASES OMITTED ARE THOSE FOR WHICH NO CORRELATION COEFFICIENT ABOVE 0.7 WAS FOUND. NEGATIVE COEFFICIENTS ARE PRESENTED IN BOLD OVER GREY.

		IS				IM				VM	
	a1	a2	b		a1	a2	b		a1	a2	b
Na	**-2.293E+02**	4.508E+02	4.382E+04		**-3.604E+02**	7.331E+02	9.298E+04		**-1.088E+02**	2.523E+02	3.929E+04
Al	2.353E-02	6.152E-02	**-1.534E+01**		1.563E-02	2.054E-01	**-4.299E+01**		8.185E-04	1.992E-01	**-1.145E+02**
Si	3.331E+00	1.035E-01	9.395E+03		1.785E-01	9.706E+00	5.516E+04		6.352E+00	**-4.020E+00**	**-4.033E+03**
S											
Cl	2.161E+01	**-3.777E+00**	1.525E+03		1.917E+02	**-1.847E+01**	**-1.293E+05**		3.883E+01	3.547E-02	**-2.378E+04**
Ca	1.121E-03	**-8.469E-04**	**-1.610E+00**		4.163E-03	**-2.321E-03**	**-2.449E+01**		3.547E-03	**-1.866E-03**	**-2.728E+01**
Sc	**-1.183E+02**	1.687E+02	3.373E+01								
Ti	3.467E-02	5.025E-02	**-3.652E+00**		5.453E-02	1.715E-01	**-1.374E+01**		3.523E-02	1.955E-01	**-2.020E+01**
Fe	6.055E-02	**-5.057E-03**	**-2.678E+01**		6.462E-02	1.160E-01	**-9.967E+01**		3.415E-02	1.617E-01	**-1.663E+02**
Co	**-3.705E+00**	5.000E+00	1.703E+00		**-6.910E+00**	9.290E+00	3.233E+00		**-3.421E+00**	4.655E+00	1.565E+00
Ni					**-4.445E-03**	1.658E-03	6.860E-02				
Cu	1.174E-01	**-6.202E-02**	**-4.510E-02**		1.829E-01	**-8.834E-02**	**-1.904E-01**		8.271E-02	**-3.786E-02**	**-1.659E-01**
Zn	9.009E-01	**-7.388E-01**	**-4.953E+01**		1.205E+00	**-9.468E-01**	**-6.033E+01**		5.594E-01	**-4.537E-01**	**-2.792E+01**
Zr	2.962E-02	2.505E-02	**-1.921E-01**		4.648E-02	6.703E-02	**-3.750E-01**		**-2.446E-03**	9.446E-02	**-2.848E-01**
Sb											
Ba											
Ce					**-5.834E-02**	2.797E-01	6.717E-02		**-3.162E-02**	1.823E-01	**-5.504E-02**
Sm	**-2.501E-01**	2.587E-01	8.647E-02		**-2.770E-01**	4.511E-01	9.767E-02		**-2.102E-01**	2.271E-01	7.919E-02
Tb	1.536E-02	4.127E-02	9.783E-03		2.426E-02	2.494E-02	7.463E-02		**-1.563E-03**	4.524E-02	2.783E-02
Lu	4.321E-03	1.736E-02	**-3.252E-05**						1.224E-02	3.478E-02	**-2.812E-03**
Hf	**-6.852E-02**	2.100E-01	2.717E-04		**-7.054E-02**	3.631E-01	**-6.256E-04**		**-2.477E-02**	2.742E-01	**-1.536E-03**
Ta											
Pb	**-1.767E+01**	4.230E+01	6.711E+02		**-7.861E+01**	1.997E+02	2.540E+03		**-2.319E+01**	7.282E+01	4.358E+02
Th	**-1.305E-01**	3.252E-01	2.762E-03		**-1.660E-01**	5.579E-01	9.656E-04		**-6.837E-02**	3.734E-01	**-3.399E-03**
U									3.277E-02	9.866E-02	**-1.797E-02**

TABLE IV. CORRELATION COEFFICIENTS BETWEEN THE MEAN AND STANDARD DEVIATIONS OF AVAILABILITY, PREDICTED BY APPLYING THE INVERSION METHODS USING STANDARD DEVIATION WITH CALIBRATION COEFFICIENTS OBTAINED FROM THE WHOLE 12 MONTHS LICHENS DATA SET.(THE AVERAGE AND STANDARD DEVIATION OF LICHEN DATA ESTIMATED FROM THE PAIRS EXPOSED FOR 3, 6, 9, AND 12 MONTHS).

IS	Na	Al	Si	S	Cl	Ca	Sc	Ti	Fe	Co	Ni	Cu	Zn	Zr	Sb	Ba	Ce	Sm	Tb	Lu	Hf	Ta	Pb	Th
FAC	**0.83**	0.62	0.43	0.51	0.39	**0.96**	0.44	**0.93**	**0.91**	**0.75**	0.47	**0.78**	0.46		-0.27		-0.86	**0.83**					0.56	0.38
CAC	**0.78**	0.13	**0.73**	-0.07	**0.85**	0.66	0.49	0.43	0.57	**0.85**	-0.30	**0.92**	0.45		-0.21		0.61	0.65					**0.95**	0.12
TAC	**0.80**	0.52	**0.78**	0.25	**0.83**	**0.73**	0.69	0.40	0.57	**0.81**	0.30	**0.89**	0.44		-0.24		0.67	0.67					**0.85**	0.17
FPC	**0.84**	0.56	**0.83**	0.65	0.48	**0.82**	0.47	-0.11	**0.77**	0.67	0.27	**0.81**	0.29		-0.30		-0.10	**0.88**					**0.76**	0.64
CPC	**0.76**	0.45	**0.84**	0.47	**0.88**	**0.77**	0.48	-0.02	-0.05	**0.76**	-0.32	**0.94**	0.25		-0.18		0.52	0.63					**0.97**	0.10
TPC	**0.81**	0.38	**0.80**	0.50	**0.96**	**0.77**	**0.74**	0.64	-0.10	**0.76**	0.10	**0.86**	0.25		-0.25		0.64	0.67					**0.89**	0.24
DRC	**0.78**	**0.87**				0.55		0.08	0.41	0.69	0.64	**0.95**	**0.94**	0.11		0.59	-0.25		**0.82**	**0.70**	0.03	0.49	**0.77**	0.52
ATD	**0.73**	**0.90**				0.29		**0.97**	**0.94**	**0.78**	0.55	**0.97**	**0.95**	**0.92**		0.60	0.63		**0.88**	**0.75**	**0.87**	0.48	**0.87**	**0.97**
WRC	0.53	-0.18				**0.96**		0.58	0.43	**0.71**	0.18	0.37	0.36	0.02		-0.04	0.17		-0.07	0.08	0.25	0.44	0.65	0.35
IM																								
FAC	0.44	0.41	0.48	0.27	0.32	0.19	0.13	**0.84**	0.62	**0.75**	0.43	0.34	0.50		-0.20		0.00	**0.91**					0.02	0.52
CAC	**0.77**	-0.25	0.31	-0.09	**0.76**	0.37	0.45	0.11	0.35	**0.86**	0.00	**0.83**	0.47		-0.20		0.48	0.60					**0.85**	0.11
TAC	**0.71**	0.34	0.40	0.04	**0.79**	0.17	0.43	0.17	0.40	**0.84**	0.43	0.50	0.50		-0.21		0.58	0.67					0.35	0.19
FPC	**0.72**	**0.88**	**0.88**	-0.09	0.60	-0.11	0.12	0.55	0.33	**0.72**	0.67	0.20	0.47		-0.19		0.32	**0.92**					0.00	0.70
CPC	**0.73**	0.55	**0.87**	0.42	**0.84**	-0.29	0.48	0.35	**0.71**	**0.83**	0.03	**0.95**	0.30		-0.20		0.45	0.61					**0.97**	0.10
TPC	0.61	0.38	0.64	0.07	**0.92**	0.00	0.33	0.39	0.43	**0.82**	0.66	0.13	0.45		-0.21		0.58	**0.75**					0.06	0.25
DRC	0.39	**0.73**				0.67		-0.02	0.48	0.69	0.64	**0.97**	**0.82**	0.07		0.63	0.25		0.35	-0.12	0.19	0.46	**0.70**	0.23
ATD	0.48	**0.89**				-0.16		**0.95**	**0.92**	**0.78**	0.63	**0.94**	**0.94**	**0.86**		0.65	**0.82**		**0.84**	0.70	**0.93**	0.50	0.49	**0.96**
WRC	0.32	-0.12				**0.95**		0.63	0.48	0.69	**0.71**	0.24	0.36	-0.25		-0.32	0.35		0.26	-0.06	0.33	0.51	0.21	0.42
VM																								
FAC	0.70	0.35	0.34	0.40	0.58	0.62	0.12	**0.78**	0.49	**0.77**	0.39	0.34	0.47		-0.17		0.05	**0.94**					-0.11	0.62
CAC	**0.74**	-0.07	0.10	0.26	**0.82**	0.36	0.50	-0.01	0.23	**0.85**	0.02	**0.83**	0.46		-0.25		0.54	0.62					**0.94**	0.12
TAC	0.69	0.36	0.29	0.29	**0.86**	0.34	0.47	-0.08	0.15	**0.83**	0.41	0.53	0.45		-0.20		0.61	0.69					0.68	0.18
FPC	**0.76**	**0.87**	**0.88**	0.30	0.60	-0.03	0.12	0.35	0.58	**0.76**	0.68	0.31	0.44		-0.19		0.41	**0.90**					-0.09	**0.78**
CPC	0.68	0.62	**0.97**	0.44	**0.80**	**0.84**	0.48	0.12	0.28	**0.81**	0.06	**0.92**	0.41		-0.24		0.47	0.67					**0.99**	0.29
TPC	0.59	0.15	**0.87**	0.34	**0.94**	0.26	0.39	0.20	0.28	**0.81**	0.67	0.53	0.42		-0.19		0.58	**0.72**					0.49	0.24
DRC	0.05	0.37				**0.80**		0.00	0.43	**0.72**	0.64	**0.95**	**0.93**	0.52		0.58	0.41		0.42	-0.08	0.22	0.61	**0.94**	-0.17
ATD	0.41	**0.87**				0.09		**0.95**	**0.89**	**0.77**	0.62	**0.93**	**0.96**	**0.92**		0.59	**0.81**		**0.84**	**0.73**	**0.93**	0.51	**0.78**	**0.96**
WRC	0.47	0.42				**0.94**		0.62	0.50	**0.75**	0.62	0.10	0.31	-0.10		-0.04	0.41		0.19	0.32	0.35	0.56	0.14	0.41

ECA concept allows the grouping of different types of availability time series into classes, much like EAD (equivalent aerodynamic diameter) groups aerosol particles in classes of aerodynamic behaviour in a given system. The group property of aerodynamic behaviour is now replaced by the group property of the effect upon the living organism.

In Fig. 1 the standard deviation of availability is also presented. The correlation coefficient between the standard deviation and ECA for this case is 0.975. Although theses are just build up cases, the result was not *a priori* introduced into the building conditions. This result is but a consequence of the fact that, using the standard deviation, is another way to account for faint memory effects. Nevertheless, using the standard deviation does not impose any restriction to the type of process controlling memory fading, therefore equivalent constant availability data obtained by using the standard deviation of availability must be understood in a different manner.

The fact that these methods introduce a probabilistic character into the process leads to the definition of a ***generalised equivalent constant availability*** ECAg, equivalent to the previous one but embedded now in a probabilistic nature. To better understand this fact, consider the 50% percentile, P50, of weighted availability, also shown in Fig.1. If a bilinear regression is made considering the P50 as dependent variable and assuming maxima and the standard deviation as independent variables, the following result is obtained for this built case:

$$P50 = \left(3.83 * \frac{StDev}{Max} - 0.894\right) * Max + 9.79 \tag{8}$$

only valid if the coefficient of the independent variable is positive, that is:

$$\frac{StDev}{Max} > \frac{0.894}{3.93} = 0.23$$

It is thus seen that a statistical interpretation of ECAg both accounts for negative coefficients, found in Table IV, and points limitations on the applicability of methods with standard deviation. Out of a frequency of high availability occurrences, to be determined for each calibration case individually, the methods stop to be representative. Out of that range, the response of the organism can no longer be modelled by these methods.

5. CONCLUSIONS

In this work a lichen response calibration experiment was carried out and several calibration methods were tested. The hypothesis of lichens memory fading permitted to find methods with better performance than linear regression calibration. Nevertheless, imposing an exponential decaying memory does not improve much. The approaches, which do not restrict the memory fading process, on the other side, provide a very big improvement.

Once these results were achieved, it becomes clear that lichens do not act like a measuring instrument. Instead they present information on availability, already biased by biological effects. The modified availability results were named Equivalent Constant

Availability, ECA, and represent a class characteristic similar to Aerodynamic Diameter for airborne particles size.

Finally, the importance of the statistical description is such that by using it, a calibration method was achieved which allows the determination of average, maxima, and standard deviation of availability variables out of a lichen survey data. For this, only a significative number of time separated samplings (say 3 or four sampling campaigns within a year period) must be made.

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LICHENS AND NEW POSSIBILITIES OF BIO-INDICATION IN FRANCE

O. DAILLANT[1], , G. KIRCHNER[2], , J.-L. BELTRAMO[3], C. GUEIDAN[1], L. JACQUIOT[1], E. JOYEUX[1], M.N. LOIZEAU[4], A. PARIS[1], POGNAT[1], C. TILLIER[4]

[1] Observatoire Mycologique, Néronde, Mazille, France
[2] Universität BREMEN, Fachbereich Physik, Germany
[3] Université de Bourgogne, Csaab, Dijon, France
[4] Cité Administrative, Macon, France

Abstract

In France, lichens had mainly been used, until recent years, for general bio-indication or for the bio-accumulation of lead. In Burgundy, the authors have nevertheless begun to study the accumulation of other metals and trace-elements; They have been monitored in potentially contaminated sites and in many rural areas for comparisons. As, Cd, Co, Cr, Cu, Mg, Ni, Pb, and Zn have been monitored since 1992 in the Saône-et-Loire Department; this environmental follow-up was recently completed with analyses of lichen samples from 45 sites in 1998 and 60 other sites in 1999. When the industrial environment seemed to justify it, other elements were also looked for, such as Ag, Sn, and V. The lichens involved were mainly Parmelia sulcata, but also Xanthoria parietina, X. polycarpa and a few other species. A statistical treatment has been done for some of the elements in P. sulcata and X. parietina. The regional backgroud for each element was estimated and it appeared that for several elements (Ag, Cr, Ni, Pb...) the highest values near sensitive sites exceeded this background by two orders of magnitude. For others such as Cd or As, the differences were usually only of one order of magnitude. The results are now numerous enough to envisage the use of lichens as a real tool kit to monitor and follow up the presence of heavy metals and other trace elements in the environment: 1)The ratio between different elements can be used to follow evolutions; 2)The biological period of some elements in lichens can be estimated more accurately; 3)In some cases (Pb), we have noted that in sites with lead problems in humans (more than 100 µg Pb per litre blood), samples of Parmelia sulcata showed an average concentration above 500 mg/kg; 4)The dating and evolution of contaminations can be approached using the different ages and the different biological half lives of different lichen species; thus the "youth" and the rapid development of Xanthoria polycarpa has been used to make comparisons with other species.

1. INTRODUCTION

In France the use of lichens as bio-monitors of trace-elements is not very widespread. Apart from the quite exhaustive works, which concentrate on lead [1,2], data are quite scarce. This was regrettable since the French lichen landscapes are often very rich and it is easier to sample suitable species for further analyses than in some neighbouring countries. Nowadays lichen presence spreads in peri-urban and some industrial areas, due to the fact that general pollution patterns have changed: sulphur pollution decreases as nitrogen deposition increases, creating a favourable environment for lichen colonisation and the trace-element deposition does not seem to have an immediate impact on them.

This availability led the authors to make the choice of lichen bio-monitoring in Burgundy, the main area of investigation. Another consideration, which should by no means be underestimated, is the fact that lichens have sometimes concentrations of trace-elements which are very spectacular indeed: for decision makers, this can be an incentive for action. The investigations were made partly on a systematic way, with a certain number of sampling points in each Department and partly to monitor the emissions due to present or past activities: ceramic production plants, smelters, steel industries, battery processing facilities, a photographic industry, a coal power plant, plastic industries, incineration plants, big motorways, land fillings, vineyards, and even a pilgrimage site, were taken in consideration.

2. METHODS

2.1. The areas under investigation

Burgundy is located in the centre east of France and has areas with a continental climate as well as Atlantic influences in its south-western part. The sampling sites were at heights between 150 and 600 meters approximately. Some areas in the centre and the south are famous for their wine production, but parts of Burgundy have also a very ancient industrial tradition. It ranges from metal working before the roman conquest to glass manufacturing in the XVII century and to the discovery of autunite (1805), the first uranium ore identified as such near Autun. In the XIXth and the beginning of the XXth century, le Creusot had the most famous steel industry in France. The invention of photography near Chalon-sur-Saône led to the tradition of photographic industry in this town. Nowadays, the activities are more diversified, but the steel industry is still well represented in different towns.

The samples of lichens were collected in the four Departments of Burgundy in 1998 and 1999, in the context of a more general survey of air quality using lichens, which was decided by the health authorities of the four Departments. 45 samples were analysed in 1998 and 60 in 1999, coming from 31 different sites. These 105 samples, being collected and measured in homogenous conditions in a well-defined period, have been statistically treated when possible. For silver, tin, and vanadium, the number of samples was lower.

In addition, we have sampled and analysed lichens on two sites in "emergency" situations with potential health problems: in one case a factory of electronic components in the Côte d'Or Department had a breakdown of its air filters, associated with massive release of some elements, lead in particular. In the second case a battery recycling plant had released for several years high amounts of lead and cadmium in the environment.
This site is in the Ardennes Department, outside Burgundy, but the geographical conditions are not very different and the results are interesting enough to be presented here.

2.2. Sampling

All lichen samples were taken from bark substrate: if possible, they were taken from three different trees at least and the number of thalli (between 30 and 100) was high enough to be considered representative of the site. The trees had to have a minimum diameter of 15 to 20 cm and the sampling surface had to be vertical. Juvenile or depleted lichens were avoided; the whole thallus was sampled.

The lichens were soaked with distilled water and then separated from their substrate using a plastic knife and transported in plastic bags. The same day, they were sorted to remove carefully remaining bark pieces or other impurities before being air dried on a plastic mesh above a warm air source. On their arrival at the laboratory, they were oven dried to remove residual water content.

2.3. Analyses

The analyses were made at “The Cellule de Spectrométrie d'Absorption Atomique” (CSAAB) of the “Université de Bourgogne” in Dijon. The concentration of all elements was measured by atomic absorption spectrometry. Lichens were digested in a microwave digestor (MLS — 1200 mega, Milestone, Italy). After dilution in ultrapure water, Ag, As, Cd, Co, Cr, Cu, Ni, Pb, Sn, and V concentrations of digested samples were measured using a Zeeman electrothermal atomic absorption spectrometer (SpectrAA-300, VARIAN, France). Mg and Zn concentrations were determined using a flame atomic spectrometer (deuterium correction GBC 203).

3. RESULTS

The results are expressed in mg/kg (or ppm) on the dry matter. The most characteristic values are reported on table I: the two species Parmelia sulcata and Xanthoria parietina, supposed to have a similar behaviour, are pooled together; all the other species have been excluded.

The background concentrations are given as the average of the 3 less contaminated samples. We have also indicated the maximal regional value for each element as well as the second regional record. For cadmium, lead, and tin, we also indicate the minimal and maximal concentrations found on particularly contaminated sites. On the last line, we indicate the ratio between the background values and the maximal concentrations.

Table II presents a statistical treatment of the results for arsenic, copper, manganese, lead, tin, zinc, and vanadium. The results for the other elements had neither a normal nor a lognormal distribution (p < 001) and are therefore not included in this statistics.

Table III gives the order of magnitude of the ratio between lichen concentrations in the most contaminated areas and the background values. The origins of the contamination could very often be made out and are indicated in the right column.

TABLE I. MAIN DATA RESULTING FROM THE ANALYSIS OF 105 SAMPLES OF LICHENS COLLECTED IN 1998 AND 1999 IN BURGUNDY, FRANCE. BACKGROUND VALUES ARE THE AVERAGES OF THE THREE LOWEST LEVELS. THE LAST LINE PRESENTS THE RATIO BETWEEN THE MOST CONTAMINATED LICHENS AND THE BACKGROUND. THE RESULTS ARE EXPRESSED IN ppm DRY MATTER.

		Ag*	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Sn**	**Zn**	**V**
Background		2.9	0.11	0.22	O,1	1.0	8.31	11.1	0.7	5.67	0.47	45	0.76
Maximal regional value		101	8.2	3.39	103	256	183	507	690	515	9.6	725	20.1
2nd highest regional value		19.6	7.81	3.34	60	254	95.5	304	417	195	8.3	389	19.4
Heavily polluted sites with health problems	max			12.40						1158	12.0		
	min			1.20						232	3.8		
Highest value/ background		35	75	56	1030	255	22	46	985	206	26	16	26

TABLE II. PERCENTILES, BACKGROUND VALUES, AND MAXIMUM LEVELS OF THE ELEMENTS ANALYSED IN *PARMELIA SULCATA* AND *XANTHORIA PARIETINA*. RESULTS EXPRESSED IN ppm ON DRY MATTER.

		As	Cu	Mn	Pb	Sn	Zn	V
Background		0.22	8.31	11.13	5.67	0.47	45	0.76
Percentile								
10%		0.22	8.9	14.5	9.2	0.47	54	1.05
25%		0.44	13.5	24.3	15.8	0.79	76	1.75
50%		0.90	21.6	43.1	28.8	1.39	111	3.10
75%		1.85	34.3	76.2	52.4	2.43	163	5.47
90%		3.55	52.2	127.5	89.9	4.04	230	9.14
95%		5.23	67.1	173.3	124.1	5.48	282	12.41
99%		10.83	107.4	308.6	227.2	9.68	414	22.05
Maximal regional value		8.2	183.2	507	515.4	9.60	725	20.10
2nd highest regional value		7.81	95.5	304	194.8	8.32	389	19.80
Heavily polluted sites with health problems	max				1158	12.0	688	
	min				232	3.8	189	

TABLE III. ORDER OF MAGNITUDE OF THE RATIO BETWEEN THE HIGHEST VALUES AND THE BACKGROUND VALUES. THE KNOWN ORIGINS OF THE HIGHEST VALUES ARE REPORTED IN THE RIGHT COLUMN AND INCLUDE THE TWO SITES WITH SANITARY PROBLEMS.

Ratio Maxi./background	Elements	Known origins of the highest concentrations in lichens
10 - 100	Ag	Photographic industry, film processing
	As	Industrial areas
	Cd	Recycling of batteries; electric components
	Cu	Vinyards
	Mn	No obvious origin
	Sn	Welding of electronic components
	Zn	(Ancient) Industries; Transport
	V	Manufacturing of frying pans; Transport
> 100	Co	Ancient industries
	Cr	Special steels
	Ni	Special steels
	Pb	Recycling of car batteries; welding of electronic components

4. DISCUSSION

4.1. Biological half life

Estimations of the residence time of some trace elements already exist in the literature. The ideal situation for such estimation is the analyses of lichens several years after a source of pollution has completely ceased. However it is not possible to exclude other residual local sources, or the depositions resulting from long range transport. For some elements we can define at least a maximum residence time, which might be shorter in some circumstances. This residence time is expressed as biological period, i.e. the time necessary for the concentration of an element to be halved. These elements are silver, chromium, and lead. The data on which the estimates are based range from 1992 to 2000 for Chalon-sur-Saône, from 1994 to 1996 for Mâcon, and from 1995 to 2000 for le Creusot (only for chromium and lead in the later cases).

- Lead: The biological period was estimated as equal or inferior to 2 years in Parmelia sulcata and ranged from 2.7 to 3.9 years in Xanthoria parietina.

- Chromium: The biological period was remarquably similar in P. sulcata and X. parietina: 3 years in the first case and from 2.7 to 2.9 in the second.

- Silver: 3.3 years in X. parietina. No analyses were made on P. sulcata, but a sample of Physcia tenella was analysed very near to the source (less than 100 meters) in 1992 and again in 2000; in the first case the sample showed a concentration of 1934 ppm and in the second 95 ppm, due to a new filtering system set up in 1993; the biological half life was 1.8 years, but this species was not used in other circumstances and is mainly mentioned because of its spectacular silver content.

4.2. Problem areas and statistical use of the data

When possible, the data gathered in homogeneous conditions were statistically treated. It appears that, with the exception of copper and zinc, all data above the 95% percentile are higher by one order of magnitude than the data below the 10% percentile. Copper and zinc are precisely the elements, which are widespread in the environment and are more representative of a general pollution rather than a specific contamination.

For some elements however, the data showed neither a normal nor a lognormal distribution; in these cases, we still have to refer to more empiric reference values. The average of the 3 smallest concentrations was calculated in order to be used as background concentration of an element. For having an idea of the range of values, the ratio of the highest results and background values is shown on table III. This ratio is superior to 100 for cobalt, chromium, nickel and lead. It lies between 10 and 100 for the other elements. Except for manganese (another element which is not necessarily representative of a specific contamination but the presence of which may be due to geogenic circumstances or a general dust burden), the origin of all the heavy contaminations of lichens could clearly be identified (see also table III).

4.3. Specific surveys

In two cases, the “Observatoire Mycologique”, has had the opportunity to sample lichens and evaluate the results in situations of emergency.

In the first case, the plant producing electronic components had a filter breakdown and caused massive releases of lead, tin, copper, antimony, and zinc. This release lasted for an unknown but limited period of time, only in one direction. It has to be noted that the surroundings of the plant were already chronically contaminated in all directions (though to a lesser extent) by regular deposition of these metals.

A survey showed that many spots in the most contaminated area had a lead concentration in soil superior to 1000 ppm. It also showed that 20 children (out of 94 and a total of 296 in the town) going to the school on the other side of the road had lead concentrations in blood superior to 100 µg/l, which is a guideline value.

In Burgundy, monitoring of lead in blood has been done in some other cases and concentrations approaching that guideline value of 100 µg/l have never been found, except in two specific cases which could clearly be attributed to old indoor paintings.

In the second case, a recycling unit for batteries in the Ardennes Department, chronic atmospheric releases of lead and cadmium in the environment took place: 140 kg/year between 1989 and 1995 and 70 kg/year after 1995. Concentrations of lead in the soil of up to 1,050 ppm have been found. A soil sample at 300 meters showed a lead contamination of 677 ppm and another soil sample, at 700 meters, showed a concentration of 367 ppm [3]. These are also the distances at which the lichens were sampled.

In July 1998, 22 children (out of 96) and 9 adults (out of a total of 750 inhabitants) had a lead concentration in blood superior to 100 µg/l. One year before, in 1997, 4 adults had a lead concentration in blood between 100 and 200 µg/l and 3 above 200 µg/l.

In both cases lichens have been sampled and analysed.

In the first case, 8 lichen samples were collected:

2 P. sulcata samples in the direction of the recent strong contamination,
2 P. sulcata samples in the area submitted to chronic deposition,
1 X. polycarpa samples in each location, and
2 P. acetabulum samples in the direction of the strong contamination.

In the direction of the strong contamination, P. sulcata showed concentrations of 563 to 942 ppm. In the other direction, the concentrations were 402 and 484 ppm. These values are all well above the 99% percentile of the 1998–1999 survey, and are equivalent to166 times the background lead concentration in lichens.

In the second case, 9 lichen samples (5 P. sulcata) have been sampled and analysed:

The lead concentrations ranged from 232 to 1158 ppm (average 622) in P. sulcata. The cadmium concentrations ranged from 1.19 to 12.4 (average: 5.5) in the same species. For lead, the lowest result was just above the 99% percentile calculated in the 1998 –1999 surveys in Burgundy. The highest result was 2,066 times the background value. For cadmium, the results ranged from 5,5 times to 5.6 times the background concentration in lichens.

According to these results it appears that if lead concentration in one single sample of P. sulcata is superior to 227 ppm (or say 200 to be conservative) further investigations should be done. If these further analyses show that one or several samples have concentrations as high as 500 ppm or more, the possibility of occurring a health problem should be looked at.

4.4. Use of other species

In some cases, other species were tested: Parmeia acetabulum, Evernia prunastri, Hypogymnia physodes, and Xanthoria polycarpa. As regards the 3 first species, the results did not radically differ and the averages were even quite similar to the averages of P. sulcata; there were nevertheless significant individual differences for which no explanation was at hand: we thus think that comparisons with those species should be avoided.

X. polycarpa on the other hand is a species colonizing new substrates and could potentially be an indicator of recent deposition - or recent recovery. It was undoubtedly the case near the plant in case study nr.1 (welding of electronic equipment, see 4.3.), where the lead contamination in the direction of the recent deposition (305 ppm) was quite high (not much lower than P. sulcata: 563 ppm), whereas on the other side, where pollution had ceased for some time, it showed only a contamination of 38.4 ppm, i.e. considerably less than P. sulcata (484 ppm). The same principle applies in case study nr. 2 (Ardennes) where the deposition has been continuous: X. polycarpa showed a contamination only slightly below that of P. sulcata: 806 ppm instead of 1158 ppm. In two other towns under investigation in Burgundy, in which classic lead pollution is believed to have diminished in recent years, Digoin and Mâcon, X. polycarpa showed much lower concentrations than X. parietina or P. sulcata.

4.5. General evolution as observed using lichens

The recent results presented here can be compared with more ancient ones: in general, lead concentrations due to traffic have dramatically decreased; this is not the case with other elements usually associated with road traffic, i.e. zinc and cadmium, which have remained constant over the years, or are even increasing. As regards elements associated to metallurgical activities, such as chromium, nickel, arsenic, and cobalt, they tend to be still present in high concentrations, but mainly in the neighbourhood of smelters or processing plants. The concentrations in other urban areas tend to diminish. Along these lines, it will be interesting to follow the evolution of the ratios Pb/Cd, Zn/Pb, as well as the ratios of elements considered characteristic for metallurgy and those resulting from road traffic.

5. CONCLUSION AND SUGGESTIONS

The concentrations of the trace-elements in lichens may vary by one or two orders of magnitude according to pollution sources, which makes them particularly suitable for environmental bio-monitoring.

The corticolous lichens normally used have no contact with the soil and can be used to demonstrate whether or not a contamination problem is due to atmospheric deposition.

Knowing the biological half-life of elements in lichens and the species which are indicators of recent or past deposition, it will soon be easier to draw up the historic evolution of emissions and deposition.

It should be kept in mind that the objective of surveys is the protection of the environment and of human health. We therefore suggest the think over the potential of lichens to monitor health risks: this has already been done for the general air quality in relation with lung cancer [4]; for lead, it is a method much less traumatic than many others; we can already suggest concentrations of 200 ppm and 500 ppm in Parmelia sulcata: the first value should justify further lichen analyses and the second should justify a health screening.

ACKNOWLEDGEMENTS

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DEVELOPMENT AND CALIBRATION OF EPIPHYTIC LICHENS AS SALTFALL BIOMONITORS

R. FIGUEIRA[1,2], A.M.G. PACHECO[2,3], A.J. SOUSA[2], F. CATARINO[1]

[1] University of Lisbon, Lisbon, Portugal
[2]CVRM, Instituto Superior Técnico, Technical University of Lisbon, Lisbon, Portugal
[3]DEQ, Instituto Superior Técnico, Technical University of Lisbon, Lisbon, Portugal

Abstract

Lichen transplants (*Ramalina calicaris* and *Usnea* sp.) were investigated as biomonitors of the atmospheric deposition of marine salt, and a calibration model was set up to predict the dry deposition of saline elements from the concentration of salt tracers in lichens. The study was performed in the Portuguese Atlantic coast, where a monthly transplantation program was run in two stations that show clear differences in terms of precipitation regimes. At both stations, dry deposition and precipitation records were kept for the whole duration of the program. General trends in results have indicated that rainwater may wash saline elements out of lichens, though such an effect does not appear to be linear. A multiple-regression approach was taken to look for a calibration between dry-deposition fluxes and lichen concentrations through a stepwise technique. The calibration model for data obtained in both stations features two break points that define precipitation ranges for low, moderate, and heavy rain conditions. The results show that lichens can really be used to indicate the dry deposition of sea salt.

1. INTRODUCTION

Lichen biomonitoring is an interesting tool for environmental assessment, with plenty of applications in the evaluation of most notorious contaminants, such as heavy metals, trace elements, radionuclides, and nitrogen/sulphur oxides. Some of these contaminants result from anthropogenic activities, which the majority of publications in the field are devoted to. Compared to this, little attention has been paid to the study of sea-salt deposition inland — a natural vector of contamination — despite its hazardous consequences on terrestrial areas, ranging from materials failure to soil and water impoverishment and desertification at large [1].

The accumulation of airborne elements by lichens — particularly, cations — is known to be dependent on the cation-exchange capacity of the cell wall, in which negatively-charged, binding sites establish an interaction with cations. Anionic uptake is less understood, though it is generally accepted that the anionic-exchange capacity of lichens is much lower [2]. Nonetheless, dissimilar cations show different affinities to the exchange sites. In general terms, such an affinity can be ordered in the sequence found by Nieboer and Richardson [3], based on competitive experiments: monovalent Class A < divalent Class A < Borderline divalent < divalent Class B.

According to this order, sodium and magnesium, which are among the most abundant elements in sea water, are classified as monovalent class A and divalent class A, respectively. This means that the capacity of retention of such elements is limited if other cations are present in plentiful supply in the environment, as they are likely to be displaced through competition for binding sites. Also, the bulk of the elements present in extracellular compartments of the lichen thalli can be simply deposited on the surface or into intercellular spaces [4]. Similarly, most airborne chloride should be deposited on the surface as well. The mobilisation of these elements, that may be in particulate form, can be greatly dependent on climatic conditions, therefore all these factors should be considered when the use of lichens as saltfall biomonitors is attempted.

The aim of this study is to assess and improve the use of lichens as biomonitors of the atmospheric deposition of sea salt, by means of a calibration between lichen accumulation and dry deposition of saline elements. The work was developed using lichen transplants collected from a remote site and exposed during one-month period at two meteorological stations near the coast. The calibration model was implemented through a stepwise-regression procedure.

2. MATERIAL AND METHODS

Two atmospheric stations with strong differences between their annual precipitation regimes were selected in the Portuguese east coast. The first one is located in Monte Velho (MV), south of Lisbon, and features an annual precipitation below 600 mm; its distance from the shore is about one kilometre. The second station is located in Meadela (ME), north of Porto, some four kilometres from the coast, and it shows rainfall averages above 1400 mm per year. Samples of the lichens *Ramalina calicaris* and *Usnea* spp. were collected at a remote, clean site in southern Portugal, approximately 18 km from the coast, and transplanted into both stations on a monthly-exposure basis. The first species was sampled on olive trees (*Olea europaea*), and the second one on cork oaks (*Quercus suber*). Transplants were exposed by attaching the original-phorophyte branches to a wood stand installed in either station, using a nylon thread. After one month, the lichen thalli were removed to the laboratory, cleared of wood and other alien materials, and then processed for the determination of element concentrations.

Surface Cl^- was determined by shaking *ca* 50 mg of lichen samples in plastic flasks twice, with 10 ml of deionised water each time. The leachates were assessed by mercurimetric titration [5]. The weight of each sample was determined after drying at 80 C for 16 h.

The determination of extracellular, cationic (Na^+ and Mg^{2+}) loads generally followed the sequential-elution technique by Brown and Wells [6], with some modifications though. Prior to elution, each sample of approximately 50 mg of intact thalli had been stored for 24 h at 100% relative humidity. The total extracellular fraction, that includes all ions over the surface, within intercellular spaces and exchangeably bound to cell walls, was obtained by shaking samples in plastic flasks with 10 and 5 ml of $NiCl_2$ (20 mM) for 40 min and 30 min, respectively. Samples were dried at 80 C for 16h before weighing. $[Na^+]$ and $[Mg^{2+}]$ were measured by atomic absorption spectrometry.

All results are given in micromole per gram of dry weight ($\mu mol \cdot g^{-1}$ d.w.) of lichen, and they are based on ten independent samples for each transplantation site. In addition to biological data, dry deposition and precipitation data were supplied for each station by the Portuguese Institute of Meteorology and an official, environmental agency (DRARN-Alentejo), for the whole extent of the exposure program.

3. RESULTS AND DISCUSSION

Chloride, sodium, and magnesium were selected as the sea-salt tracers to be determined on lichens and then compared to the dry deposition. The biological values correspond to differences between pre- and post-exposure concentrations, therefore any negative value indicates loss of an element. The reason for this loss can be related to the washout effect of precipitation, giving an indication of the importance of such factor in the process of accumulation of airborne elements by lichens. Because different washout effects should result from different rain intensities, values of precipitation were weighted by the time length of rain events (in days), on a monthly basis, in order to produce a rain intensity measurement, which was called Reduced Average Precipitation (RAP, in mm/day). The transplant program first started at MV station, in September 1993, where it was run until the end of 1997: these results are presented in Figure 1. It can be observed that *R. calicaris* and *Usnea* spp. clearly show some different ability to accumulate saline elements, but the temporal variation is consistent between the two species, as proved by the significant correlation coefficients for all elements (0.61 for Cl^-, 0.67 for $Na^{+,}$ and 0.55 for Mg^{2+}). Such variation in time also seems to match the change in rainfall intensity observed throughout the study. Generally speaking, periods with higher precipitation rates correspond to lower extracellular concentrations of saline elements on lichens, and the contrary seems to apply for drier months.

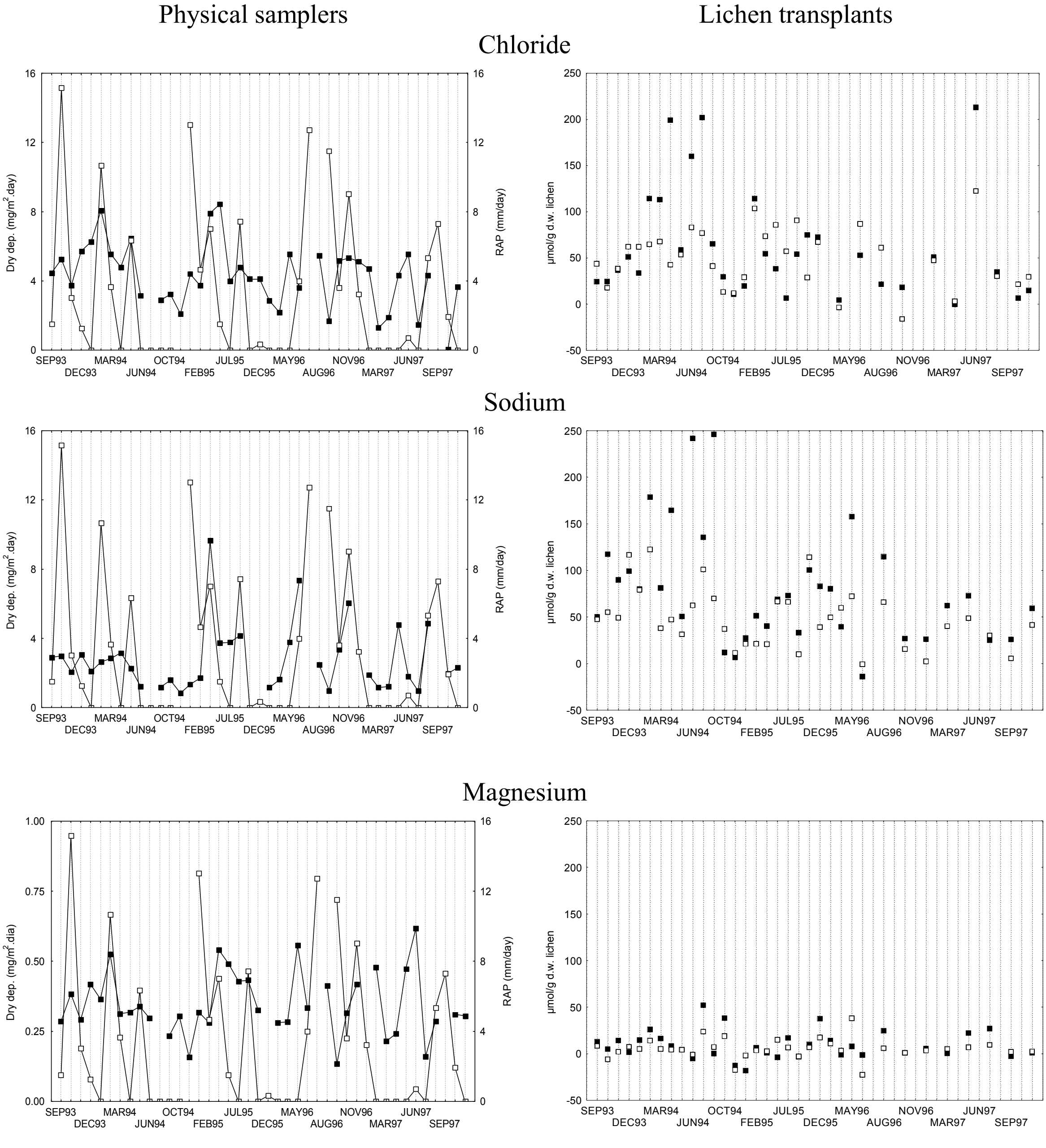
The precipitation events may still promote the accumulation of saline elements by lichens, for low amounts of rain that fail to leach the lichen thalli thoroughly. In such cases, elements in rainwater may be accumulated by lichens, as well as particulates that have been trapped on the surface and into intercellular spaces. All of them may become available for the establishment of van der Waals interactions with the exchange sites over the cell wall, or even for an uptake by the cell itself.

However, there is no definite linear relationship between the amount of rain and the concentration of elements in lichens, as demonstrated by the low and non-significant correlation coefficients (Table I). This way mean that such a relationship features some discontinuity, resulting from the action of different intensities of rain on extracellular elements deposited on lichens. For high precipitation rates, the leaching and washout effects of rainwater should act more like removing elements from the lichen surface, and to some extent, from exchangeable sites, at least for elements with lower ionic affinities with such sites in the cell wall. About 80% of the sodium in the extracellular fraction is deposited on the surface or trapped into intercellular spaces [4], and it can be easily leached away from lichens by distilled water [7]. The washout of nutrients from lichen thalli has already been observed in former experiments [8]. The effect of long periods of rain could be similar to the effect of distilled water, at least when the rain events last for long enough to cut down on the element concentrations in rainwater.

These considerations indicate that precipitation is an important factor to be accounted for in putting together a calibration model that estimates atmospheric deposition of saline elements from lichen data, and that such relationship may indeed display non-linear features. Still, the relationship can be linearly modelled in the ranges of precipitation where the effect is only of one type. The parametric definition of the trigger value that switches the effect of rain from an increase to a decrease in element uptake by lichens is quite difficult, and, arguably, it can only be done for specific conditions, as other ecological and environmental factors may also influence the uptake process — for instance, air humidity, wind regime and lichen morphology, to mention only a few. Therefore, a stepwise regression approach was taken, which includes the biological concentrations of Cl^-, $Na^{+,}$ and Mg^{2+}, and RAP. The break point of RAP found for the model is 5.5 mm day^{-1}, as given in Equation 1, and the corresponding coefficients are presented in Table II. The model seems to yield acceptable results, in particular for *R. calicaris*. The biplots of predicted *vs* observed values of dry deposition for all three elements can be found in Figure 2, from where it can be concluded that no systematic bias is evident in the results of the model.

$$[X]_{atm} = (a_0 + a_1[Cl^-]_{bio} + a_2[Na^+]_{bio} + a_3[Mg^{2+}]_{bio} + a_4RAP) \times (RAP < 5.5) + \\ + (b_0 + b_1[Cl^-]_{bio} + b_2[Na^+]_{bio} + b_3[Mg^{2+}]_{bio} + b_4RAP) \times (RAP \geq 5.5) \tag{1}$$

In the above equation, $[X]_{atm}$ stands for the dry deposition of salt tracer X, estimated in mg m^{-2} d^{-1} and $[X]_{bio}$ stands for the concentration of element X in the saltfall biomonitor, measured in µmol·g^{-1} d.w.


Physical samplers

Lichen transplants

Chloride

Sodium

Magnesium

FIG. 1. Time profiles of physical (left) and biological (right) data obtained at MV station. In physical-data patterns, solid symbols are for dry deposition and open ones are for RAP. In biological-data patterns, solid symbols are for Usnea spp. and open ones are for R. calicaris.

TABLE I. CORRELATION COEFFICIENTS BETWEEN RAP AND THE CONCENTRATION OF EACH ELEMENT ON LICHENS.

	Usnea spp.			*R. calicaris*		
	Cl^-	Na^+	Mg^{2+}	Cl^-	Na^+	Mg^{2+}
RAP	-0.35	-0.31	-0.51	-0.28	-0.21	-0.21

TABLE II. REGRESSION COEFFICIENTS (a_I, b_I) AND CORRELATION COEFFICIENTS (r) OF THE CALIBRATION MODEL FOR LICHEN SPECIES IN MV STATION.

	a_0	a_1	a_2	a_3	a_4	b_0	b_1	b_2	b_3	b_4	R
R. calicars											
Chloride	1.719	0.007	0.019	0.057	0.443	6.875	-0.007	0.019	0.103	-0.153	0.74
Sodium	1.669	0.001	0.004	0.038	0.082	2.456	0.022	0.001	-0.022	-0.075	0.46
Magnesium	0.214	0.000	0.001	0.006	0.014	0.389	0.001	0.000	0.007	-0.003	0.72
Usnea spp.											
Chloride	3.158	0.007	0.000	0.008	0.255	7.179	-0.008	0.014	0.063	-0.218	0.63
Sodium	1.669	0.001	0.004	0.038	0.082	2.456	0.022	0.001	-0.022	-0.075	0.40
Magnesium	0.214	0.000	0.001	0.006	0.014	0.389	0.001	0.000	0.007	-0.003	0.54

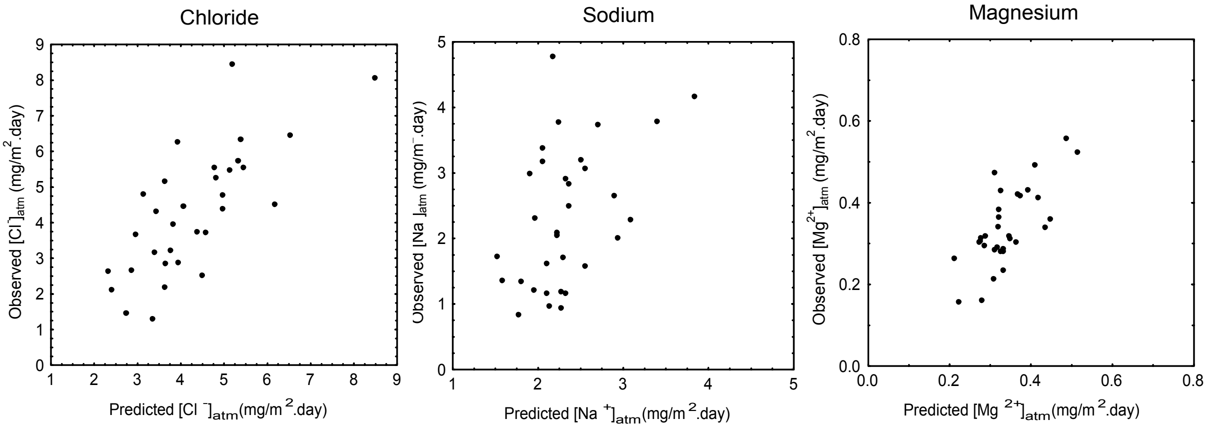
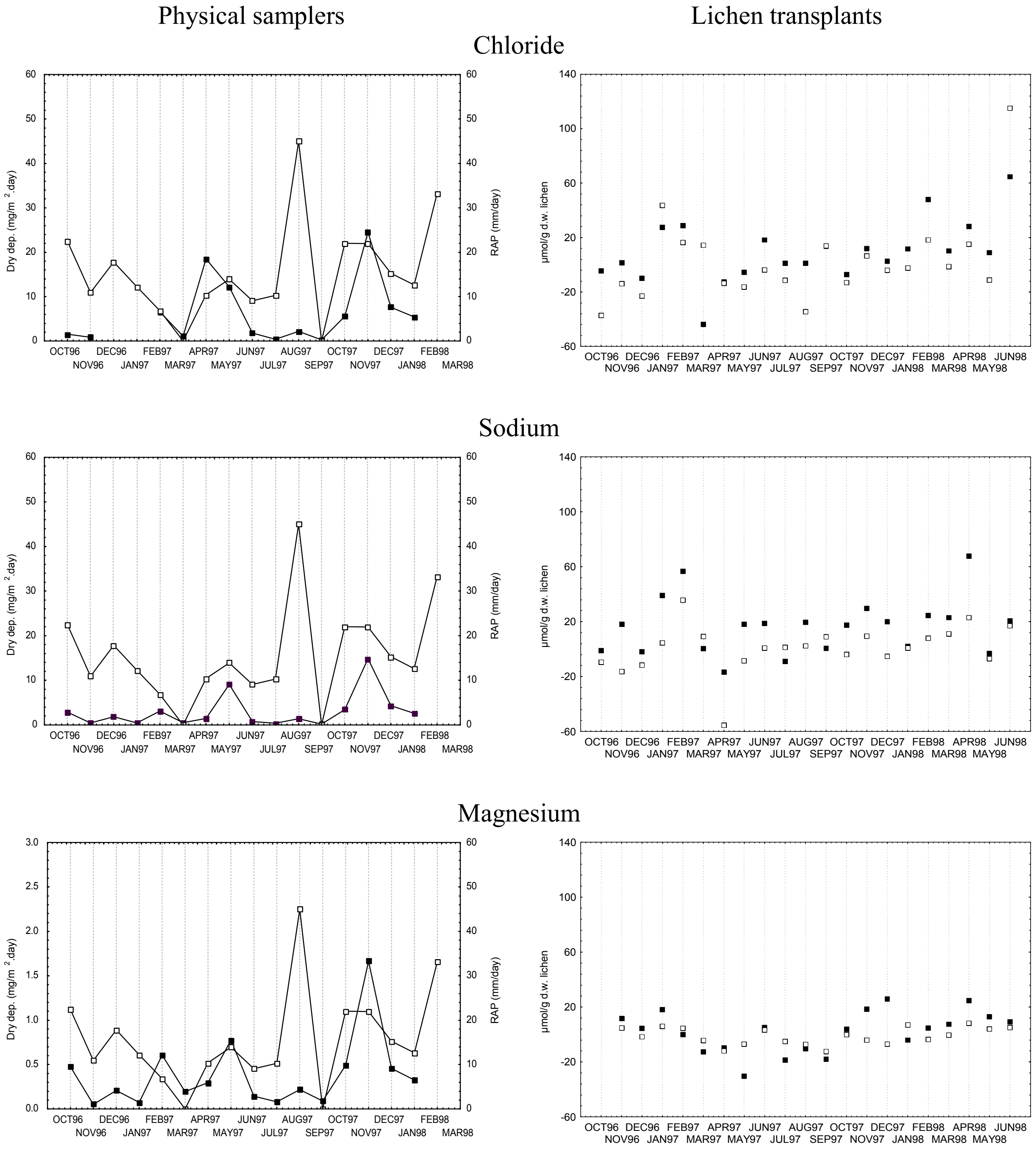


FIG. 2. Results of the calibration model of R. calicaris for Cl^-, Na^+ and Mg^{2+} at the MV station.

The study was also run at the ME meteorological station, where it was possible to test the lichens as saltfall biomonitors in very different conditions, in what concerns precipitation regimes. This station is located in the northernmost section of the Portuguese (west) Atlantic coast, which presents clear differences in terms of the annual precipitation average (about 1,400 mm in ME, as opposed to 600 mm in MV). The transplantation program started in October 1996 and lasted until June 1998; its *modus operandi* was the same as before (MV station). Figure 3 shows the corresponding values for physical and biological data. Now, *Usnea* spp. seems to have a superior ability to accumulate saline elements than *R. calicaris*. This may be a consequence of a higher availability of elements in ionic form, due to the frequent occurrence of rain events that make elements readily available for uptake by cell walls. Laboratory observations of cation uptake by these species, when incubated into artificial seawater, have already pointed to a higher cation-exchange capacity of *Usnea* spp. as regards Na^+ and Mg^{2+} [9].


Physical samplers

Lichen transplants

Chloride

Sodium

Magnesium

FIG. 3. Time profiles of physical (left) and biological (right) data obtained at ME station. In physical-data patterns, solid symbols are for dry deposition and open ones are for RAP. In biological-data patterns, solid symbols are for Usnea spp. and open ones are for R. calicaris.

It was also found that the difference between original and terminal concentrations was often negative, confirming the mobilising action of rain on the extracellular elements. The type of calibration model tested for MV was also implemented for ME, and the analysis of results showed that a different break point had to be established, in order to adjust the model to the higher RAP values observed in this station. The value of the break point that came out is 15 mm day^{-1}, and the coefficients of the model described in Equation 2 are displayed in Table III.

$$\begin{aligned}[X]_{atm} = {} & (a_0 + a_1[Cl^-]_{bio} + a_2[Na^+]_{bio} + a_3[Mg^{2+}]_{bio} + a_4RAP) \times (RAP < 15) + \quad (2)\\ & + (b_0 + b_1[Cl^-]_{bio} + b_2[Na^+]_{bio} + b_3[Mg^{2+}]_{bio} + b_4RAP) \times (RAP \geq 15)\end{aligned}$$

In the above equation, $[X]_{atm}$ stands for the dry deposition of salt tracer X, estimated in mg m^{-2} d^{-1} and $[X]_{bio}$ stands for the concentration of element X in the saltfall biomonitor, measured in $\mu mol \cdot g^{-1}$ d.w.

The model seems to fit well the experimental values, producing good estimates as confirmed by the correlation coefficient, generally above 0.9, and this result could be obtained for both lichen species. Biplots of predicted *vs* observed values for *R. calicaris* also confirm that no systematic bias underlies the calibration results — Figure 4.

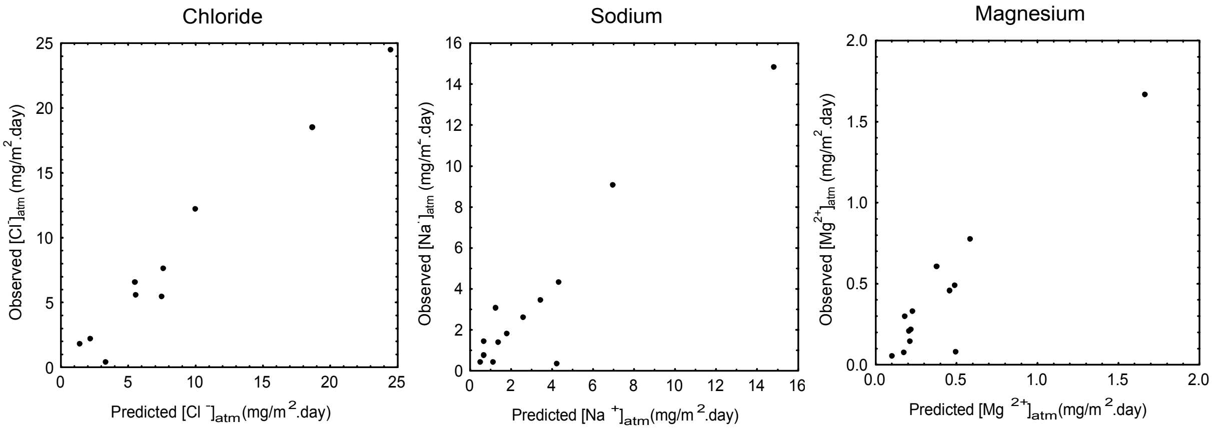


FIG. 4. Results of the calibration model of R. calicaris *for Cl^-, Na^+, and Mg^{2+} at the ME station.*

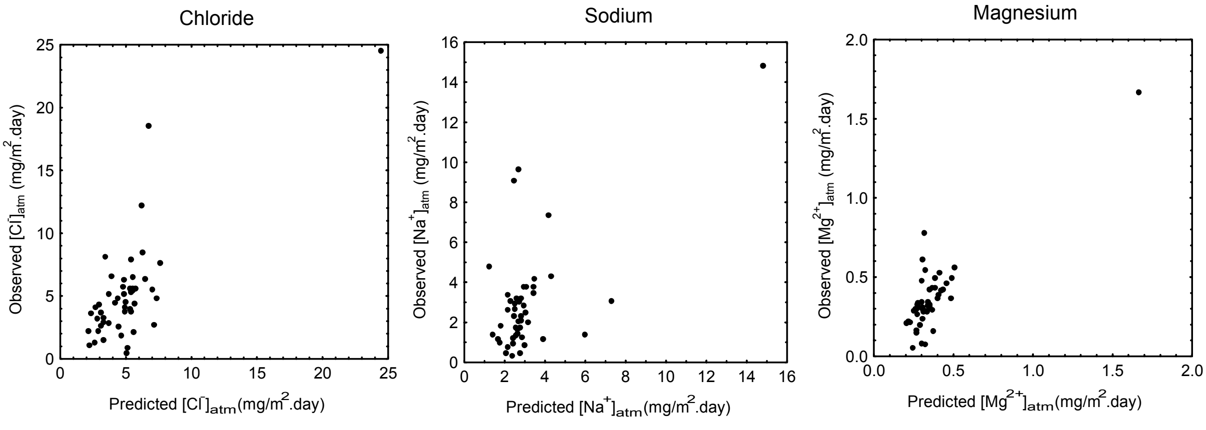


FIG. 5. Results of the global calibration model obtained for R. calicaris at both stations (MV, ME).

The manifest difference in rainfall between stations allows the study of two different situations that more or less make the extremes of what can be found in Portugal, in terms of precipitation regimes. This enables the calculation of a global model that covers the whole range of precipitation conditions, by joining the two data sets and using a calibration model with break points for both low and high precipitation rates, calculated for each of the stations. A general-purpose model was calculated using the break points previously found (Equation 3), and its coefficients are listed in Table IV. This model was set up for *R. calicaris* only, owing to the better results given by this species in the MV calibration.

Results of the global model are acceptable, as can be observed by the correlation coefficient (Table IV) and biplots between predicted and observed values (Figure 5), but it requires the definition of three intervals dependent on the precipitation intensity. This points to an open subject still needing research, in order to describe the relationship between precipitation and lichen accumulation of saline elements.

TABLE III. REGRESSION COEFFICIENTS (a_I, b_I) AND CORRELATION COEFFICIENTS (r) OF THE CALIBRATION MODEL FOR LICHEN SPECIES IN ME STATION.

	a_0	a_1	a_2	a_3	a_4	b_0	b_1	b_2	b_3	b_4	r
R. calicaris											
Chloride	-11.07	0.845	-0.217	-0.699	2.018	0.29	0.996	0.017	0.182	0.831	0.98
Sodium	-5.97	-0.038	0.098	-0.264	0.810	30.00	-0.722	1.866	-0.144	-1.309	0.94
Magnesium	0.00	-0.002	0.009	-0.026	0.031	3.01	-0.065	0.184	0.004	-0.121	0.93
Usnea spp.											
Chloride	15.65	-0.372	0.090	-0.147	-0.841	0.29	0.404	1.502	-0.484	-0.735	0.86
Sodium	-4.74	-0.063	0.107	-0.145	0.497	33.57	1.097	0.116	-0.762	-0.968	0.99
Magnesium	0.09	-0.005	0.010	-0.014	0.004	3.83	0.118	0.020	-0.091	-0.113	0.96

$$\begin{aligned}[X]_{atm} = {} & (a_0 + a_1[Cl^-]_{bio} + a_2[Na^+]_{bio} + a_3[Mg^{2+}]_{bio} + a_4RAP) \times (RAP < 5) + \\ & + (b_0 + b_1[Cl^-]_{bio} + b_2[Na^+]_{bio} + b_3[Mg^{2+}]_{bio} + b_4RAP) \times (RAP \geq 5) \times (RAP < 15) + \\ & + (c_0 + c_1[Cl^-]_{bio} + c_2[Na^+]_{bio} + c_3[Mg^{2+}]_{bio} + c_4RAP) \times (RAP \geq 15)\end{aligned} \tag{3}$$

$[X]_{atm}$ stands for the dry deposition of salt tracer X, estimated in mg m^{-2} day^{-1} and $[X]_{bio}$ stands for the concentration of element X in the saltfall biomonitor, measured in $\mu mol \cdot g^{-1}$ d.w.

The understanding of such phenomena is of paramount importance not only for calibration purposes, but also for time modelling of airborne deposition of marine elements, since it was recognised that precipitation plays an important role in the spatio-temporal estimation of salt levels in lichens [10].

TABLE IV. REGRESSION COEFFICIENTS (a_I, b_I, c_I) AND CORRELATION COEFFICIENTS (r) OF THE GLOBAL CALIBRATION MODEL FOR *R. CALICARIS* IN THE MV AND ME STATIONS.

	Chloride	Sodium	Magnesium
a_1	2.316	-1.635	0.315
a_2	–0.006	0.025	–0.002
a_3	0.017	0.038	0.002
a_4	0.033	–0.070	0.003
a_5	0.266	1.508	0.006
b_1	2.496	2.136	0.232
b_2	0.041	0.014	0.001
b_3	–0.025	–0.002	0.001
b_4	–0.046	–0.031	–0.004
b_5	0.278	0.021	0.006
c_1	0.271	30.004	3.011
c_2	0.997	–0.722	–0.065
c_3	0.016	1.866	0.184
c_4	0.182	–0.144	0.004
c_5	0.833	-1.309	–0.121
r	0.77	0.73	0.86

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THE POTENTIAL OF LICHENS AS LONG TERM BIOMONITORS OF NATURAL AND ARTIFICIAL RADIONUCLIDES

G. KIRCHNER
University of Bremen, Bremen. Germany

O. DAILLANT
Observatoire Mycologique, Mazille. France

Abstract

Lichens were used as biomonitors of Chernobyl fallout ^{137}Cs, of cosrnogenic ^{7}Be and of radioactive members of the natural uranium and thorium decay chains. Samples were taken from two locations in France, including lichens sampled at, different distances of a coal fired power plant, and close to a uranium ore processing waste disposal site. All samples were analyzed gamma-spectrometrically after equilibrium concentrations of short-lived isotopes had been attained. Activity concentrations in Parmelia sulcata, sampled 1994 decrease with distance from the plant. whereas in lichens taken at the waste disposal site a decrease with time was observed. Retention half-lives calculated with a simple one-compartment model are 2.6±1.2 a for cesium, which was detected in all samples even more than a decade after the Chernobyl accident, and of 0.7 (± 0.1) a to 3.3 (± 0.7) a for lead. Consequences of our results for model identifiability and parameter estimation of a two-compartment model arc discussed.

1. INTRODUCTION

The ability of lichen to accumulate trace substances present in the lower atmosphere is well known and their potential as bioindicators of environmental pollution by heavy metals has been documented by numerous studies (see [1] for a review). Compared to heavy metals, the use of lichen as biomonitor of atmospheric pollution by radionuclides has not attracted much interest. although their capability to indicate atmospheric depositions has been shown for atomic weapons [2,3] as well as Chernobyl fallout [4,b] and for emissions from an abandoned uranium mine [6].

The objectives of the present, study are (i) to test and compare the suitability of lichen as biomonitor for radionuclides of different origin (Chernobyl fallout 137Cs. cosrnogenic 7 Be and members of the natural uranium and thorium decay chains); and (ii) to discuss implications of our experimental data for modelling accumulation of trace metals by lichen.

2. MATERIALS AND METHODS

2.1. Sites

Two sampling sites — Montceau-les-Mines and Gueugnon, were chosen in the Departement Saône-et-Loire (France). At Montceau-les-Mines samples were taken in winter 1994 at increasing distances from a coal power plant along the Canal du Centre. Sampling was repeated at the hand of 1999 with some additional samples taken west of the plant. At Gueugnon samples were taken in 1994, 1999, and 2000 adjacent to a disposal site of radioactive wastes which originated from uranium ore processing (yellow cake production). These wastes had been covered by a layer of tmcontarr1inated soil in 1980 which was improved ten years later [7].

2.2. Sampling

If present the foliose lichen Parmelia sulcata was collected, since the suitability of this common species as a quantitative biomonitor of atmospheric deposition has been proven [4,8]. If no specimen of P. sulcata was found, other epiphytic. species (Xanthoria parietina, Hypogymnia physodes, and Evernia prunastri) were sampled. Lichens growing at heights between 0.7 and 2 m above ground on trunks of 20 cm minimum diameter were selected. Usually 30 to 100 thalli were taken. Remaining bark was removed; and after rinsing them with distilled water the lichens were air-dried.

2.3. Analytical procedures

All samples were analyzed γ-spectrometrically using a large volume Reversed Electrode High-Purity Germanium detector (Canberra, 55% relative efficiency). Unlike conventional coaxial detectors, this detector has a usable energy range which extends down to 5 keV and thus enables to determine ^{210}Pb by measuring its 46.5 keV gamma line.

All samples were analyzed in a stove at 380 °C. As the intensity attenuation of gamma radiation in matter considerably varies with its density, the ashed samples were mixed with appropriate amounts of wax and pressed to pellets of standard geometry (70 mm diameter. 5 mm height) and density (1.0 g cm^{-3}), for which the detector was calibrated using a multi-isotope standard. All samples were sealed prior to the measurements using an aluminium foil coated with plastic (thickness 0.1 mm) and stored for more than three weeks to ensure that, ^{226}Ra and ^{228}Ra were equilibrated with their decay products ^{214}Pb and ^{212}Pb, respectively. Activity concentrations of ^{226}Ra were determined indirectly by ^{214}Pb. since the gamma decay energy of ^{226}Ra (186 keV) interferes with a γ-line of ^{235}U which may also be present in the lichens. As time between sampling and analyses usually exceeded two months; the short-lived γ-emitting isotopes ^{228}Ac and ^{234}Th were used to determine concentrations of its long-lived precursers (^{228}Ra and ^{238}U respectively).

3. RESULTS AND DISCUSSION

3.1. Radionuclide concentrations of lichens from Montceau-les-Mines

Activity concentrations of the radionuclides monitored are listed in Table I. For the lichens sampled in 1994 small amounts of ^{134}CS (half-life 2.1 a) were detected with ^{134}Cs: ^{137}Cs activity ratios varying between (3.7 ± 0.2) × 10^{-2} and (4.6 ± 0.3) × 10^{-2}. The ^{134}Cs:^{137}Cs activity ratio of the Chernobyl fallout of 0.528 ± 0.008 at the time of deposition [10] corresponds to an activity ratio of these two isotopes of (4.5 ± 0.1) × 10^{-2} at the time of our sampling, indicating that the ^{137}Cs almost exclusively originates from the Chernobyl accident. Activity concentrations of ^{210}Pb are consistently higher — often by more than one order of magnitude — than those of its long-lived precursor nuclide ^{226}Ra. Analysis of a sample of the coal fueled to the power plant showed activity concentrations of ca. 40 Bq kg^{-1} of ^{226}Ra and its decay products (^{228}Ra decay chain: approx. 10 Bq. kg^{-1}). This clearly indicates that ^{210}Pb present in the lichens mainly originates from nuclides produced in the atmosphere by the decay of gaseous ^{222}Rn.

Activity concentrations of ^{226}Ra, ^{238}U, ^{228}Th and ^{228}Ra detected in P. sulcata in samples collected in January 1994 along the transect decrease with distance from the power station and reflect their activity ratios present in the coal. Thus it seems to be reasonable to assign the source of these radionuclides to emissions of the local power plant. Lichens sampled in autumn 1999 along the transect show considerably lower concentrations of these radionuclides no longer related to the distance from the plant. This was expected, since in 1994 a desulphurization treatment was put into operation which is known to precipitate radium with the gypsum produced [11]. As these members of' the natural decay chains are present in soils, their concentrations detected in 1999 are likely to reflect loading of the lichen with soil-derived dust. Consequently, any of these members of the natural decay chains may be used to estimate soil-derived fractions of other pollutants (e.g. heavy metals) accumulated by a lichen.

Activity concentrations of cosmic, ray produced ^{7}Be (half-life 53 d) in P. sulcata are normally distributed with 242 ± 150 Bq kg^{-1} (mean ± s.d.). Equilibrium inventories of 230–330 Bq m^{-2}, 370–740 Bq m^{-2} and 140–340 Bq.M^{-2} have been measured (Ref. 12], and references therein). Since it is reasonable to assume that removal of this short-lived isotope trapped by lichens is dominated by radioactive; decay, our data generalize the observation made by Sloof and Wolterbeek [4] that P. sulcata, per kg (dry weight) accumulated approximately the activity of Chernobyl ^{137}CS deposited per m^{2} surface area. As atmospheric ^{7}Be is regionally uniform, variations in its concentrations in lichens reflect differing local rainfall intensities and trapping effectivities of individual samples and thus they may be used to quantify these factors.

3.2. Radionuclide concentrations of lichens from Gueugnon

Activity concentrations of the radionuclides monitored are listed in Table 11. For ^{7}Be, ^{210}Pb, and ^{137}Cs, the data are in good agreement, with the results given in Table 1. For the long-lived members of the natural decay chains, however, a marked decrease with time of the activity concentrations present in lichens is apparent. This is most likely to reflect the washout curve of radionuclides resuspended from the wastes and deposited before the repository had been completely covered by uncontaminated soil.

TABLE I. ACTIVITY CONCENTRATIONS OF NATURAL RADIONUCLIDES AND OF ^{137}Cs DETECTED IN *PARMELIA SULCATA* NEAR THE COAL POWER STATION OF MONTCEAU-LES-MINES; FOR THE 1994 SAMPLES DATA OF THE NATURAL DECAY CHAIN NUCLIDES WERE TAKEN FROM REF. [9]

No.	Location[a]	Sampling date (Y-M)	Activity concentration (Bq/kg)[b]						
			^{7}Be	^{137}Cs	^{210}Pb	^{226}Ra[c]	^{238}U[c]	^{228}Th[c]	^{228}Ra[c]
1	1.0 km NE	94-01	334 ± 7	50.4 ± 0.6	299 ± 4	17.2 ± 0.9	9.1 ± 1.0	10.3 ± 0.7	6.1 ± 0.8
2	2.5 km NE	94-01	146 ± 5	95.4 ± 0.7	204 ± 3	23.4 ± 0.8	8.7 ± 1.0	6.9 ± 0.6	7.2 ± 0.8
3	4.5 km NE	94-01	415 ± 10	65.3 ± 0.9	246 ± 5	13.3 ± 1.2	4.8 ± 1.5	1.8 ± 0.9	3.4 ± 1.1
4	10.0 km NE	94-01	112 ± 5	154.8 ± 0.9	239 ± 6	5.8 ± 0.7	< 1.9	2.0 ± 0.6	2.4 ± 0.6
5	4.0 km NE	99-12	513 ± 22	1.3 ± 0.3	297 ± 9	< 1.7	< 4.9	1.3 ± 0.5	2.9 ± 0.8
6	6.0 km NE	99-12	150 ± 16	22.9 ± 1.0	310 ± 11	< 1.9	< 7.6	1.9 ± 0.5	4.2 ± 0.9
7	7.5 km NE	99-11	245 ± 73	14.8 ± 1.5	367 ± 18	< 6.3	< 18.0	< 6.0	< 11.5
8	8.5 km NE	99-12	85 ± 9	15.5 ± 0.6	235 ± 7	2.6 ± 0.4	< 4.2	1.5 ± 0.3	2.4 ± 0.5
9	9.5 km NE	99-12	80 ± 1	30.6 ± 0.9	314 ± 9	1.8 ± 0.4	< 5.2	1.0 ± 0.3	1.7 ± 0.5
10	10.5 km NE	99-12	208 ± 13	17.3 ± 0.6	391 ± 10	3.1 ± 0.3	4.1 ± 0.7	2.0 ± 0.2	3.1 ± 0.4
11	2.5 km W	99-11	448 ± 52	< 1.7	350 ± 15	< 25.1	< 10.2	< 2.4	< 6.7
12	5.0 km W	99-12	164 ± 11	11.6 ± 0.4	407 ± 11	< 1.1	< 3.5	1.0 ± 0.3	2.0 ± 0.5
13	5.3 km W	99-11	< 151	18.1 ± 1.4	338 ± 17	74.8 ± 3.1	< 14.8	< 3.3	< 6.9

[a] relative to the power station
[b] dry weight, referred to the sampling date
[c] derived from short-lived decay products (see text)

TABLE II. ACTIVITY CONCENTRATIONS OF NATURAL RADIONUCLIDES AND OF ^{137}Cs DETECTED IN VARIOUS LICHEN SPECIES NEAR THE RADIOACTIVE WASTE REPOSITORY OF GUEGNON; FOR THE 1994 SAMPLES DATA OF THE NATURAL DECAY CHAIN NUCLIDES WERE TAKEN FROM REF. [9]

Species	Sampling date (Y-M)	Activity concentration (Bq/kg)[a]						
		^{7}Be	^{137}Cs	^{210}Pb	^{226}Ra[b]	^{238}U[b]	^{228}Th[b]	^{228}Ra[b]
Parmelia sulcata	94-02	264 ± 31	51.6 ± 2.0	241 ± 17	114. ± 5.4	91. ± 9.0	72. ± 4.6	38.0 ± 5.1
Xanthoria parietina	94-02	220 ± 96	177. ± 8.0	400 ± 44	388. ± 17.0	92. ± 24.0	63. ± 9.8	< 23.0
Parmelia sulcata	99-07	216 ± 36	8.6 ± 1.4	409 ± 20	< 5.2	18.5 ± 5.5	< 3.6	12.6 ± 2.2
Xanthoria parietina	99-07	68 ± 19	7.7 ± 0.9	94 ± 8	18.0 ± 1.7	< 17.2	7.7 ± 1.2	< 9.4
Hypogymnia physodes	00-06	246 ± 11	18.1 ± 0.8	298 ± 11	< 2.4	< 7.1	< 1.7	4.5 ± 1.0
Evernia prunastri	00-06	321 ± 9	2.6 ± 0.2	234 ± 7	2.0 ± 0.4	7.0 ± 0.9	2.8 ± 0.3	3.3 ± 0.5

[a] dry weight, referred to the sampling date

[b] derived from short-lived decay products (see text)

4. DYNAMIC MODELS

In the following the two compartmental models shown in Fig. 1 will be considered for describing the dynamics of the accumulation of trace substances by lichens. Model (1) represents the simplest approach possible, whereas model (2) which is a slight modification of a model recently proposed by Reis and coworkers [13] takes into account knowledge on mineral cycling in lichens [14]. We shall confine our discussion on the two limiting cases of a single pulse-like input and of a constant input rate.

4.1. Model (1)

Time-dependent activity concentrations on a dry weight basis; c(t), are given as

$$c(t) \;=\; I_p \,\frac{A}{m}\, e^{-(k_{01}+\lambda)\,t} \tag{1}$$

for a pulse-linke input I$_p$ (B(I-rn^{-2}) and as

$$c(t) \;=\; \frac{I_c}{(k_{01}+\lambda)}\,\frac{A}{m}\,\left(1 \;-\; e^{-(k_{01}+\lambda)\,t}\right) \tag{2}$$

for a constant input rate I$_c$ (Bq m^{-2} s^{-1}). The ratio A/m can be interpreted as the effective surface area of fallout, trapped per lichen mass. As discussed above, the ^{7}Be concentrations of Table I indicate that for P. sulcata A/m ≃ 1 m^{2} kg^{-1}. This value is used in the following calculations.

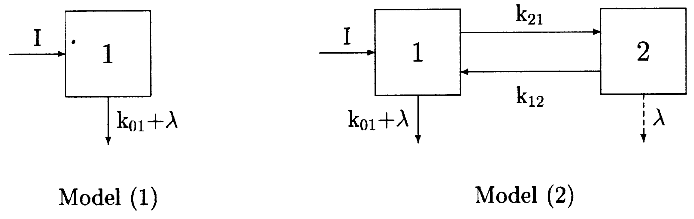

FIG. 1. Structure, of models for metal accumulation. by lichens; I denotes input, kij are fractional transfer rates from comportment j to i (0: environment), and λ is the radioactive decay constant.

The 1994 and 1999 radiocesium data can be used to estimate ko1. Both data sets are normally distributed with means (± s.d.) of 91.5 ± 46.2 Bq kg^{-1} and 16.5 ± 8.5 Bq kg^{-1} respectively. With eqn. (1) a retention half-life of ^{137}Cs of 2.6 ± 1.2 a is calculated from these data. This result compares well with values derived from Chernobyl fallout cesium of 2.7 a (P. sulcata, [4]), 4 a (H. physodes, [15]) and 4.9y (X. parietina. [16]). However, our value may be biased, since the mean of a function nonlinear in a stochastic parameter is not generally equal to the value the function takes on with the parameter mean.

For the activity concentrations of ^{210}Pb given in Table I a mean (± s.d.) of 307 ± 63 Bq.kg^{-1} is calculated. Assuming that concentrations of this long-lived radioisotope (half-life 22.3 a) in the analyzed lichens approximate equilibrium, eqn. (2) simplifies to

$$c_{eq} = \frac{I_c}{(k_{01} + \lambda)} \frac{A}{m} \quad . \tag{3}$$

If in the absence of data on regional ^{210}Pb deposition rates the range of 75–333 Bq m^{-2} a^{-1} which is given for average fallout rates in the Northern Hemisphere [17] is used, a retention half-life of ^{210}Pb in the range of 0.7 (± O.l) a to 3.3 (± 0.7) a is calculated from our observations. This result is in excellent agreement with biological half-lives reported in the literature for stable and radioactive lead of 1–2 a (Cladonia rangiferina. [3]), 0.14–1. 7 a (Pseudoparmelia baltimorensis. [18]) and 1.6 a (P. sulcata, [13]).

4.2 Model (2)

Measured activity concentrations record the total activity present in the system. With the solutions given by Jacquez [19] for the concentrations in the individual compartments, we obtain for a pulse-linke input

$$c(t) = I_p \frac{A}{m} \left[\frac{r_1 + k_{12} + k_{21} + \lambda}{r_1 - r_2} \mathrm{e}^{r_1 t} - \frac{r_2 + k_{12} + k_{21} + \lambda}{r_1 - r_2} \mathrm{e}^{r_2 t} \right] \tag{4}$$

and for a constant input rate

$$c(t) = I_c \frac{A}{m} \left[\frac{r_2 + k_{12} + k_{21} + \lambda}{r_2 (r_1 - r_2)} \left(1 - \mathrm{e}^{r_2 t}\right) - \frac{r_1 + k_{12} + k_{21} + \lambda}{r_1 (r_1 - r_2)} \left(1 - \mathrm{e}^{r_1 t}\right) \right] \tag{5}$$

where r_1, r_2 are the eigenvalues (with $r_1 \neq r_2$)

$$r_{1,2} = -\frac{1}{2} (k_{01} + k_{21} + k_{21}) - \lambda \pm \frac{1}{2} \sqrt{(k_{01} + k_{21} + k_{21})^2 - 4 k_{01} k_{12}} \quad . \tag{6}$$

The three unknown parameters k_{0l}, k_{21}, k_{21} can be uniquely identified from eqn. (4) or (5) as we checked using the Laplace transform method [19]. Although parameter values could not be calculated from the data given in Tables I and 11, our measurements are helpful to derive information on experimental designs appropriate to estimate numerical values of these parameters from concentration measurements, as will be discussed for lead in the following.

From eqns (1), (2), (4) and (5) it becomes obvious that the two models differ by the number of exponential terms present in time series data of concentrations. For data with statistical errors two exponential terms usually can be resolved only if the exponential constants differ by a factor of two or more [20]. On the other hand, lead has been found in lichens to accumulate mainly at the extracellular matrix and the cell wall [1]. Assuming, therefore; (i) that $2 \left| r_1 \right| \leq \left| r_2 \right|$, (ii) that at equilibrium the ratio of lead concentrations in the individual compartments

$$\frac{c_1^{eq}}{c_2^{eq}} = \frac{k_{12} + \lambda}{k_{21}} \tag{7}$$

is 10:1, and (iii) that $k_{01} = 1.5$ y as estimated using model (1) (since $c_2^{eq} \ll c_1^{eq}$, taking this parameter induces a small error only), we obtain $k_{12} > 0.599$ y^{-1}, $k_{21} > 0.063$ y^{-1} or $k_{12} < 0.287$ y^{-1}, $k_{21} < 0.038$ y^{-1}. This result indicates that observations of lead concentrations in lichens which cover a time period of some years may be needed to check whether model (3) more adequately represents lead dynamics in lichens than model (1) and to estimate parameter values.

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INTERNATIONALLY HARMONISED APPROACH TO BIOMONITORING TRACE ELEMENT ATMOSPHERIC DEPOSITION

SMODIŠ, B., BLEISE, A.
Division of Human Health, International Atomic Energy Agency, Vienna

Abstract

The International Atomic Energy Agency (IAEA) has been supporting work on airborne particulate matter since 1992 through various co-ordinated research and technical co-operation projects. In 1997, biomonitoring air pollution using plants, became officially a part of the IAEA project on environmental pollution monitoring and research. Based on: (1) positive experience in using biomonitors, especially lower plants such as mosses and lichens in several developed countries; (2) the continuous use of biomonitors in several Member States; and (3) he fact that nuclear and related analytical techniques have been shown to be particularly appropriate for the analysis of air pollution biomonitors, such studies are now being supported by the IAEA in 14 countries within a co-ordinated research project. The main emphasis of this project is on (1) identification of suitable biomonitors of atmospheric pollution for local and/or regional application, and (2) their validation for general environmental monitoring, whenever possible. Although the participants are using different plants as biomonitors in their research in geographically and climatically diverse parts of the world, they are harmonising sampling approaches and analytical procedures. In this paper, an overview of these activities is given, along with the details, where possible. In all of these activities, proficiency testing and analytical quality assurance are important issue, which merits special attention. To this end, a variety of analytical quality control materials have been used in intercomparison exercises and proficiency testing. Within the scope of the NAT-5 intercomparison exercise, two lichen materials were distributed among 15 participating laboratories and a proficiency test was organised. The results obtained proved satisfactory performance for most participating laboratories.

1. INTRODUCTION

Air pollution is a serious problem in many parts of the world, particularly in developing countries. The significant contribution of air pollution to the diminished health status of the exposed human populations, forest decline, loss of agricultural productivity, etc., has been a cause of increasing public concern on this issue throughout the world. Therefore, it has become a topic of intense scientific, governmental, and also industrial interest.

A wide array of air pollutants including particulates, liquids, and gasses is being emitted both from natural and anthropogenic sources. The main components include sulphur dioxide, airborne particulate matter (APM), carbon monoxide, reactive hydrocarbon compounds, nitrogen oxides, and ozone. Among them, heavy metals and other toxic elements, mostly associated with APM, represent an important group to be considered. After emission, the pollutants are subjected to physical, chemical, and photochemical transformations, which ultimately decide the their fate and atmospheric concentrations.

Increased urbanization and industrialization, the rapid growth of transportation, in connection with population growth, has altered the nature of air quality problems and the focus of air pollution research during recent years. In addition to air pollution found around large, uncontrolled point sources, problems associated with regional-scale elevated levels of pollutants have been identified. Air pollutants do not remain confined near the source of emission, but spread over distances, transcending natural and political boundaries depending upon topography and meteorological conditions, especially wind direction and speed, and vertical and horizontal thermal gradients.

There are two conceptual approaches for collecting samples relevant for air and atmospheric deposition related pollution studies: (1) the direct collection of APM, precipitation, and total deposit, and (2) the use of suitable air pollution biomonitors. The first approach is aimed at quantitative surveys of local, short-range, medium-range or global transport of pollutants, including human health-related studies when collecting size fractionated APM. It requires continuous sampling on a long-term basis at a large number of sites, in order to ensure the temporal and spatial representativeness of measurements. The application of such direct measurements on a large scale is extremely costly and person-power intensive. Furthermore, it is often not possible, due to logistic problems, to install instrumental equipment at all needed locations. Therefore, the second approach is considered as a non-expensive but yet reliable means of air quality status assessment in a country or a region. In this context, biomonitoring is defined as a continuous observation of a geographical area with the help of suitable organisms that reflect changes over space and time (e.g. by their elemental content). Certain types of biological organisms provide a measure of integrated exposure over a certain amount of time and enrich the substance to be determined so that the analytical accessibility is improved and the measurement uncertainty reduced. Sampling is relatively simple and no expensive technical equipment is needed.

For assessing trace element levels and identifying their sources, a database containing as many elements should be generated. Therefore, multielement analytical methods are usually used for such studies. The ability of nuclear and related analytical techniques (neutron activation analysis, ion beam analysis, and X ray fluorescence analysis) to analyse solid phase samples for many elements without needing sample dissolution or digestion with the high degree of sensitivity and selectivity, makes them particularly suitable for the elemental analysis of biomonitor samples. This fact brings the issue of biomonitoring trace element atmospheric deposition within the scope of programmes of the International Atomic Energy Agency (IAEA). The IAEA has been supporting these activities since 1992 through its mechanisms described in more detail in the following paragraphs. A critical component of these activities has been harmonisation of sampling and data interpretation procedures, and analytical quality control. Consequently, several reference materials have been produced and analytical quality control exercises carried out.

2. IAEA’S SUPPORT MECHANISMS

2.1. The Technical Co-operation Programme

The IAEA is actively involved in technical co-operation as part of its promotional activities. Within the framework of its Technical Co-operation Programme (TCP), the IAEA assists developing Member States in solving technical problems, at the same time providing the necessary know-how. Technical Co-operation (TC) projects are carried out with a view to increasing the recipient institution’s self-reliance.

- The projects provide expertise through scientists who are already well versed in particular techniques (experts) and who visit institutions that are carrying out such projects in order to train, advise or otherwise assist local scientists in conducting the work plan of the project.
- TC projects can support the transfer of know-how to scientists from developing countries through training on specific techniques by providing training fellowships and scientific visits, and organising training courses, meetings, and workshops.

- Provision is made to supply larger items of equipment (e.g. a gamma ray spectrometry system).

By these means, TC projects aim at building up the research capability of institutions in developing countries to the point where scientists are able to conduct such work more-or-less independently and without further substantial outside funding when the project terminates.

The TCP comprises national projects, regional and interregional projects.

- A national project derives from a formal request for co-operation by a Member State.
- For administrative purposes, Member States engaged in technical cooperation with the IAEA are grouped in geographical areas or regions. Regional projects are (1) those proposed by the IAEA or a group of Member States in response to the expressed needs of several Member States of a region; and (2) those proposed by Member States of a region collaborating within a Regional Co-operative Agreement. These projects seek to achieve greater implementation efficiency or to improve effectiveness by consolidating common objectives and modalities between multiple Member States. Such projects employ greater use of group activities, distance learning materials, technical networking, integrated expert missions, technical co-operation between developing countries and in-kind contributions from host facilities.
- Interregional projects are established by the IAEA to serve the common needs of several Member States in different geographical regions. They use similar mechanisms as those used for regional projects.

Over the past four decades, the IAEA has planned and delivered projects worth more than US $800 million in many fields, including energy, safety, agriculture, industry, medicine, water and environmental studies. In its early stage, the TCP was mostly aimed at building up scientific and technical capacity and the supporting infrastructure. In that period, many countries were keen on establishing the foundation for using the new technology in a variety of areas, both power and non-power. At the same time, most countries faced a major constraint: the lack of infrastructure, particularly the lack of human resources trained in the field of peaceful uses of nuclear energy. Therefore, a key during this first phase was the emphasis on human resources development.

In the early 1990s, after decades of capacity building, the TCP became less supply-oriented and more demand-driven, focused on using built capacity in meeting needs for sustainable development. The core concept of this approach was embodied in the motto “Partners in Development”. Because the new strategy was meant to guide the transition from a programme of technology-driven assistance to one aimed at problem solving, new partnerships had to be forged with actual “problem-holders”. These are the end-users of nuclear technologies, the main link in the chain connecting national counterparts with the ultimate beneficiaries, the public at large. In the second half of the 1990s a great deal of effort went into helping the nuclear research establishment reach out to mainstream development ministries such as health, agriculture, natural resources, and environment. The focus on problem solving led logically to the need to collaborate with other parties, such as development financing and development co-operation organizations, working on the same problems from their own perspective. A third type of partnership is between the more advanced developing countries having know-how and establishments that equal those of developed ones, and the least developed countries.

On the turn of the centuries, the TCP is oriented towards targeted programming, where projects are results-based and focused on specific problems. Due to funding constraints, the emphasis is on establishing priorities. TC projects are being linked to existing programmes of national investments in sustainable development. Evidence-based priority setting is becoming a key means of targeting programming by finding good opportunities and avoiding dead-ends, providing that TC projects deliver real impact. Priority-setting is widely based on forty years of experience; where this experience is not available, the priority-setting process is based on careful feasibility and cost-effectiveness analyses under local conditions. The TCP is now on the way of playing more of a facilitating and monitoring role, as well as strengthening the management of nuclear technology for development.

2.2. The Research Contract Programme

The Research Contract Programme (RCP) has been designed to fulfil Article III of the IAEA Statute which authorizes the Agency to encourage and assist research on, and development and practical application of, atomic energy for peaceful purposes throughout the world and to foster the exchange of scientific and technical information, as well as the exchange of scientists in the field of peaceful uses of atomic energy. Therefore, the RCP is one of the scientific arms of the IAEA. Its primary function is to support research of interest to the IAEA's programme of work and its principle aim is to obtain scientific results in an effective manner. However, the Agency does not support purely basic research (which aims to improve knowledge *per se* rather than to generate results or technologies which are likely to benefit society in the foreseeable future) unless this can provide a stimulus or support for more "downstream" or end-beneficiary oriented research. Research Contract activities provide opportunities for scientists and institutions in Member States to conduct the more "upstream" types of research in which strategic and applied issues with their subsequent opportunities for spillovers drive the research agenda. Technical Co-operation, on the other hand, focuses on the transfer of well-tried and tested technologies which nevertheless often require significant adaptive research in the process of adoption by end-users or end-beneficiaries, as well as the involvement of an often complex array of stakeholders.

Research efforts supported by the Agency are normally carried out within the framework of Co-ordinated Research Projects (CRPs). These CRPs are developed in relation to well defined research topics on which an appropriate number of institutes are invited to collaborate and represent an effective means of bringing together researchers in both developing and industrialized countries to solve a problem of common interest. The research supported by the Agency relates to the tasks included in the approved Programme, with the Agency supporting and co-ordinating the research through research contracts and cost-free research agreements and Research Co-ordination Meetings (RCMs).

Once a CRP is formed, research teams from an average of ten to twelve institutes are normally selected for participation in the programme, which lasts three to five years. Regardless of the field of research, each CRP involves certain common aspects. Institutes in developed countries work in close co-ordination with those in developing countries and all participants are encouraged to conduct work which will produce new research results and to apply these results to needs in their countries. Co-operation between institutes is strongly encouraged and supported by RCMs which are financed by the IAEA for contractors and agreement holders and held at appropriate intervals for each CRP. At these meetings, the progress of the CRP is reviewed in detail and the future direction of work is established. At the final RCM, results are reviewed and evaluated by all participants and, where appropriate, recommendations are made for future work in the field.

The results of research supported under the RCP are shared by all Member States, and are disseminated through national, international and Agency scientific and technical publications, and other communications media. Where feasible, the knowledge gained is also used to enhance the quality of projects delivered to Member States through the Agency's Technical Co-operation Programme.

3. HARMONISED APPROACH TO BIOMONITORING TRACE ELEMENT ATMOSPHERIC DEPOSITION

In the two IAEA CRPs on "Applied research on air pollution using nuclear-related analytical techniques" (1992–1998), participants from 29 countries focused mainly on harmonized collection on airborne particulate matter. However, participants from six countries had included the collection and analysis of various bioindicators as a supplementary part of their studies, using both passive and active monitoring around point sources as well as larger areas. These countries were: Argentina, Bangladesh, Jamaica, Portugal, Slovenia, and Viet Nam. With the exception of Bangladesh, all of them were applying lichens, and the main analytical tool was neutron activation analysis [1]. Building on positive experience of this work, a CRP on "Validation and application of plants as biomonitors of trace element atmospheric pollution, analysed by nuclear and related techniques" was initiated in 1998. The countries, participating in this CRP, are listed in Table I. There have been two RCMs held for the project: in September 1998 and in March 2000, both in Vienna. During these meetings, progress was reviewed and plans for future investigations were presented. Particular emphasis was on harmonising research approaches and biomonitoring techniques. The report of the first meeting is already available [2].

TABLE I. COUNTRIES PARTICIPATING IN THE CRP ON "VALIDATION AND APPLICATION OF PLANTS AS BIOMONITORS OF TRACE ELEMENT ATMOSPHERIC POLLUTION, ANALYSED BY NUCLEAR AND RELATED TECHNIQUES"

Country	Bioindicator	Type of bioindication	Analytical method
Argentina	lichens	passive, active	INAA[a]
Brazil	lichens	passive	INAA
Chile	lichens	passive	INAA
China	Lichens, leaves	passive	INAA
Germany	mosses	passive, active	ICP-AES[b], ICP-MS[c]
Ghana	Lichens, leaves	passive	INAA, XRF[d]
India	Mosses, shrubs	passive	ICP-MS, INAA, PIXE[e]
Israel	lichens	active	ICP-MS
Jamaica	Lichens, mosses, tillandsia	passive	INAA, XRF
Netherlands	Lichens, mosses, bark	passive	INAA
Norway	mosses, pits	passive	ICP-MS
Portugal	lichens, bark	active	INAA, PIXE
Romania	mosses	passive	AAS[f], INAA
Russian Fed.	mosses, pits	passive	AAS, INAA

[a] Instrumental neutron activation analysis.
[b] Inductively coupled plasma — atomic emission spectrometry.
[c] Inductively coupled plasma — mass spectrometry.
[d] X ray fluorescence spectrometry.
[e] Particle induced X ray emission spectrometry.
[f] Atomic absorption spectrometry.

The specific objective of the CRP is to identify suitable biomonitors of atmospheric pollution for local and/or regional application (e.g. moss and/or lichen). Whenever possible these bioindicators should be validated for general environmental monitoring. In order to achieve this objective, the participants are:

- Developing sampling design and Guidelines for sample collection, sample preparation and analysis, including quality control;
- Collecting, preparing, and analysing samples in accordance with the Guidelines;
- Carrying out statistical analysis and creating graphical plots showing the geographical distributions of the elements, the levels of selected environmental pollutants, identifying pollution sources and the data on time trends;
- Studying calibration of elemental content in biomonitoring species against absolute data for bulk deposition or air concentrations, whichever approach, or against another biomonitoring species already validated.

During the two RCMs the participants agreed to focus on the following scientific aspects:

- Assessment of a quantitative relationship between the elemental content of the biomonitor species and the (wet or bulk) deposition or the atmospheric concentrations (quantification aspects);
- Assessment of the element accumulation rate in the biomonitor to permit estimates of the time needed for the monitor to reflect new elemental atmospheric/deposition conditions (time resolution aspects);
- Resolution-strength of the biomonitor in a spatial sense involving local variability in biomonitor responses, survey design (grid density), and spatial variabilities in deposition and atmospheric concentrations should be considered simultaneously (geographical resolution aspects);
- Assessment of geographical differences and/or time trends in deposition and/or atmospheric concentrations by the determination of the elemental content of the sampled biomonitor species (biomonitoring survey aspects);
- Graphical presentation of the biomonitoring results, when local and/or regional surveys are carried out (mapping);
- Assessment of the changes in biomonitor parameters as a result of ambient and/or internal conditions. This means that selected physiological/biochemical parameters are quantified in relationship with varying extent of deposition and/or atmospheric concentrations. Furthermore, changes in selected parameter values are studied in order to get insight in the consequences: changed values may lead to changed relationships between monitor and deposition and/or atmospheric concentrations (impact aspects).

Within the CRP the participants had prepared: (1) written protocols on sample collection procedures, (2) written protocols on sample preparation procedures, and (3) written protocols on sample analysis. All protocols were prepared on the basis of harmonised guidelines agreed upon and adopted during the RCMs [2]. For instance, they recommended the following sample preparation procedure for lichens and mosses: cleaning, separation of samples from substrate (lichens), rinsing in distilled water for 5–10 seconds, drying at 40^{o}C for 48 hours or freeze-drying, grinding using liquid nitrogen if available. The participants had also agreed upon harmonised guidelines for the selection of sampling sites and types of samples to be collected, sampling techniques and equipment to be used, data processing and interpretation, as well as quality assurance and quality control procedures in all phases of the studies concerned.

All the participants have already tested at least one biomonitor species with respect to sample collection, preparation and analysis. Many participants have already carried out monitoring surveys and presented maps showing geographical distribution of selected toxic elements. Some participants are performing various studies to get additional knowledge on the role of the physiological processes in biomonitors.

One of the conclusions to-date is an experience that due to diverse meteorological and other environmental conditions in different parts of the world (e.g. temperature, amount of dust in air, amount of precipitation, etc.), the possibility of harmonising operating procedures is limited. If the full potential of biomonitoring approach is to be utilised, the procedures optimised to the local or regional conditions are to be applied.

Another important finding is that several epiphytic species from the genus *Tillandsia* (family Bromeliaceae) have a potential of becoming a regional biomonitor for tropical and subtropical Americas. Studies of its applicability are presently carried out in several Latin American and Caribbean countries.

FIG 1. Tillandsia recurvata: composite ball aged 1–3 years.

4. ANALYTICAL QUALITY ASSURANCE AND CONTROL

In all of these activities, the IAEA is giving special attention and high priority to analytical quality assurance and quality control. Besides preparing written analytical protocols, participants in the CRP on "Validation and application of plants as biomonitors of trace element atmospheric pollution, analysed by nuclear and related techniques" were requested to participate in two analytical quality control exercises, coded NAT – 5 and NAT – 6.

The NAT –5 interlaboratory comparison (IC) comprised two lichen materials, one from unpolluted area in Portugal, the other from a mining area in Austria. The one from Portugal was actually the IAEA reference material IAEA-336 Lichen, under different code name, so that the participants could not have revealed its origin. The results for the Austrian lichen sample were evaluated in the standard way for IAEA intercomparisons, whereas the results for IAEA-336 were evaluated as a proficiency test [3]. A brief conclusion is that all the participants showed satisfactory qualifications as far as chemical analyses are concerned. For both materials, only about 5% of the results were detected as outliers by statistical data evaluation. No laboratory having all results out of the acceptable uncertainty range was found [3]. In Fig. 3, the IC results for 22 elements in the IAEA-336 Lichen are summarised. Both reference (adopted from [4]) and IC values are normalised to unity. With the exception of Hg and Sb, all IC mean values lie within the assigned confidence intervals.

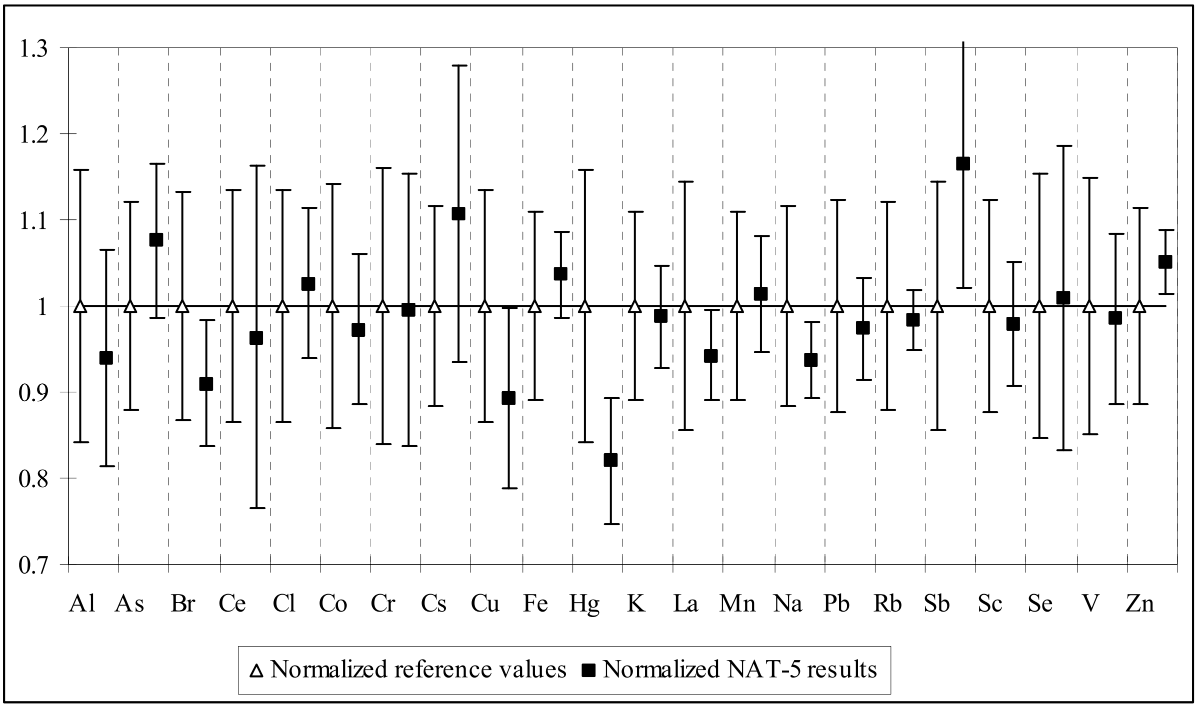

FIG 2. Comparison of elemental reference values with NAT - 5 results for IAEA-336

The NAT – 6 IC comprised two moss samples supplied by the Finnish Forest Research Institute. Analogically to the NAT – 6 exercise, one moss sample was from a contaminated site and the other from an unpolluted area. Although all the CRP participants had already submitted the measurement results, the evaluation is still in progress. Preliminary results, however, suggest that similar outcome as in the NAT – 5 may be expected.

The results of the analytical quality control exercises pertaining to bioindicators are described in much more detail in another paper presented at this Workshop [5].

5. CONCLUSIONS

Applications of nuclear and related analytical techniques in the health-related environmental science continue to be an important research activity in many of the IAEA's Member States. In response to theses activities and needs, the IAEA will continue encouraging such studies with providing assistance by its support mechanisms.

The accomplishments of the projects and activities described emphasise the importance of using biomonitors in studying atmospheric pollution, and the significance of the relevant studies in the IAEA projects has been demonstrated.

One of the important challenges in the biomonitoring studies remains further harmonisation of sampling design, sample collection, and elemental analysis, in order to obtain comparable results on at least regional scale. It is hoped and expected that the IAEA will be able to make a significant contribution to environmental studies of this kind, through its co-coordinative and supportive role to its Member States.

It is also evident that nuclear and related analytical techniques have played, and are continuing to play, an important role in this work, both in regular surveys as well as in the interlaboratory comparisons of types of materials to be used in this research.

ACKNOWLEDGEMENTS

The authors wish to acknowledge all the numerous participants in the concerned projects and interlaboratory comparisons, especially from the countries mentioned in Table I, without whom this contribution would not be possible.

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SURFACE-LAYER MODEL OF LICHEN UPTAKE, MODELLING Na RESPONSE

M.A. REIS[1], M.C. FREITAS[1], J. DE GOEIJ[2], H.Th. WOLTERBEEK[2]

[1]ITN, EN10 Sacavém, Sacavém, Portugal
[2]IRI-TUDelft, Delft, Netherlands

Abstract

In the period of 1994 to 1996, a transplant experiment was carried out in Portugal yielding a database of roughly 70,000 values of lichen element contents, total deposition, and aerosol measurements. This database was used to establish and test a physical model for lichen uptake of chemical elements discarding as much as possible information on biological functions. In a previous work, the model was presented in particular cases corresponding to limit conditions for the parameter values. In this work, the whole model is presented and applied to the case of Na uptake. It will be shown that, considering the model parameters as constants is not enough to reproduce the high variability found in the Na lichen content time series measured. A revised version of the model, where some of the parameters are made dependent of water availability, is presented here and shown to be able to solve the problem.

1. INTRODUCTION

Biomonitoring is an approach within environmental studies being applied for more than 20 years ([1], and references there in). However, there is not yet a physical or mathematical model reported about the processes involved in elemental accumulation or general lichen responses to elemental availability, the latter comprising both total deposition and atmospheric concentrations. Although several laboratory studies were performed in the context of these questions ([2,3], and references in [3]), hardly any answers have been found under natural environment conditions [1,4,5].

One of the problems with studies under real environmental conditions is the virtual absence of data on response-times and elemental interferences [6]. A possible approach to these problems is the development and subsequent validation of a generalized model, which will permit an improved assessment of the relevance of real environment data. In the present study an "as simple as possible" model for the lichen elemental uptake is proposed. The model presents two limiting cases, which were shown in a previous work [7] to reproduce measured data for different, and non-compatible, behaviour conditions. The model is an adjustable parameters model for which a pure mathematical approach was taken. This means that the model was required to prove its explaining capacities prior to its physical or biological interpretation. This made it impossible to define initial values for the parameters, which then lead to the need for a new fitting algorithm. The results obtained from fitting the sodium lichen content data are here presented. It is shown that a constant parameters values model is not capable of reproducing the whole variability of data found. The physical meaning of the parameters is then discussed and the water availability effects are introduced into the model.

2. MATERIALS AND METHODS

2.1. Experimental

For the experiment, 26 × 6 transplants of lichen *Parmelia sulcata* were prepared. Lichens were collected at one site previously identified as scarcely polluted [8,9]. 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 two lichen samples from the set were at the same time separated and prepared for analysis as reference set. Afterwards, at intervals of approximately one month, two additional lichen samples were brought to laboratory and prepared for analysis. Lichen samples were prepared by removing lichens from any remaining Olive tree substrate used during transplantation, rinsing in 18 MΩ·cm water for about 30 seconds for cleaning out dust, freeze drying, and grinding in a Teflon balls mill cooled to LN temperature. The powder obtained in this way was pressed into different pellets for PIXE and for INAA analysis.

At each site, two buckets with 25 cm diameter funnels were also installed for total deposition collection. These were replaced every time a new set of lichens was recollected. Besides lichens and total deposition, also a Gent Aerosol Sampling Unit [10] was installed at each site for PM2.5 and PM10-PM2.5 airborne particles collection.

Lichen and filters used for particle collection were analysed by both PIXE and INAA at ITN Van de Graaff and Portuguese Nuclear Research Reactor (RPI) facilities. Short-lived isotopes INAA analyses were carried out at the Hahn-Meitner Institute, Berlin.
Total deposition samples were dried under an infrared lamp at ITN and the residues were acid digested and analysed by ICP-MS at The Netherlands National Geological Survey facilities. Data obtained in this way were grouped in the availability variables described in Table I. Airborne particle data were divided into concentration in the atmosphere and content in the particles data. The fine and coarse modes correspond to particles having the aerodynamic diameter below and above 2.5 µm, respectively.

TABLE I: AVAILABILITY VARIABLES DEFINITION

	Description	Units	Calculation method
FAC	Fine Aerosol Concentration	ng/m^3	Direct measurement
CAC	Coarse Aerosol Concentration	ng/m^3	Direct measurement
TAC	Total Aerosol Concentration	ng/m^3	Sum of FAC and CAC
FPC	Fine Particle Content	µg/g	FAC times 1000 divided by Fine particles mass concentration (in µg/m^3)
CPC	Coarse Particle Content	µg/g	CAC times 1000 divided by Coarse particles mass concentration (in µg/m^3)
TPC	Total Particle Content	µg/g	TAC times 1000 divided by the sum of Fine and Coarse particles mass concentration
DRC	Dry Residue Content	µg/g	Direct measurement
ATD	Absolute Total Deposition	mg	DRC times 1x10^{-6} multiplied by the Dry Residue Mass in mg Data was stored in mg per month period but divided by the funnel inlet area and by the exposure time before use in other calculation providing final results in (ng/cm^2)/day
WRC	Water Residue Concentration	mg/λ	ATD divided by the collected water volume in litres

2.2 The Surface Layer Model

In a previous work [7], the Surface Layer Model for lichen uptake was introduced. Shortly it consists of idealising a lichen interior I (see Fig.1) which does not communicate with the outside except through a buffer called Surface. The mathematical expression describing such a system is:

$$\begin{cases} \dfrac{d\Sigma_S^i}{dt} = \dfrac{D_S}{A_S} N_E^i - U\Sigma_S^i - R_S \Sigma_S^i + L_r \dfrac{M_L}{A_S} C_L^i \\ \dfrac{dC_L^i}{dt} = U\Sigma_S^i - \left(L_r + \dfrac{d\ln\left(M_L\right)}{dt} \right) C_L^i \end{cases} \tag{1}$$

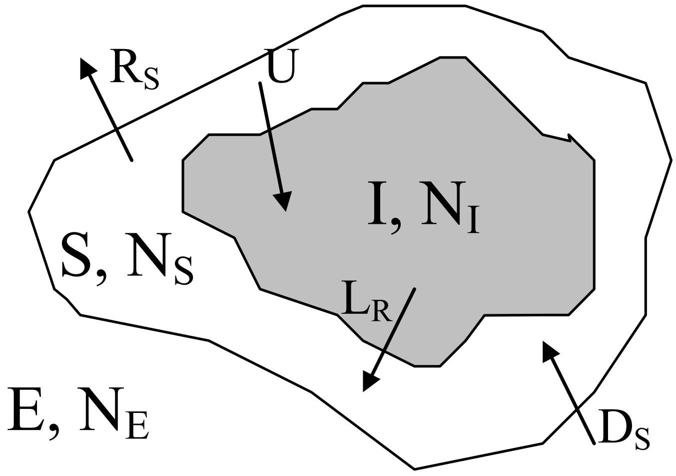

FIG.1. Scheme of the physical structure of the lichen assumed in the model. Four time rates define the model: D_S, the deposition rate, U, the uptake rate, R_S and L_r the rates at which the material is lost. Three compartments are defined, the exterior, E, where an amount N_E of element is available; the lichen surface, S, with an amount N_S of element; and the lichen interior, I, containing an amount N_I of element. All exchanges between the interior and the exterior must be mediated by the surface. The lichen surface is defined by the sample preparation procedure as the lichen part, which is fully depleted, of elements during rinsing procedures.

Solving analytically considering that the variability of availability is much smaller then the availability itself, provides the solution below. These are slightly different from those presented in [7] because in those a small error in the surface concentration expression was found which is now corrected.

$$\begin{cases} \Sigma_S^i\left(t\right) = \dfrac{D_s}{A_s} \cdot N_E^i \cdot \eta(t) + \Sigma_s^i\left(0\right)\zeta(t) + L_r \left[\dfrac{M_L C_L^i(0)}{A_s} + \Sigma_s^i(0) \right] \theta(t) \\ C_L^i\left(t\right) = \dfrac{UD_S}{L_r R_S} \cdot \dfrac{N_E^i}{M_L} \cdot \left(1 - \phi(t)\right) + C_L^i\left(0\right) \cdot \phi\left(t\right) + \left[\dfrac{U A_S}{M_L} \Sigma_s^i(0) - L_r C_L^i(0) \right] \theta(t) \end{cases} \tag{2}$$

In eq. (2), $C_L^i(0)$ and $\Sigma_S^i(0)$ are the initial bulk and surface concentrations of the lichen, and:

$$
\begin{aligned}
&\eta(t) = 1 - e^{-\frac{A_1}{2}t}\left[Cosh\left(\frac{B_1}{2}t\right) + \frac{A_1+2R_S}{B_1}Sinh\left(\frac{B_1}{2}t\right)\right] = 1-\phi(t)-R_S\theta(t) \\
&\zeta(t) = e^{-\frac{A_1}{2}t}\left[Cosh\left(\frac{B_1 t}{2}\right) - \frac{A_1}{B_1}Sinh\left(\frac{B_1 t}{2}\right)\right] = \phi(t) - A_1\theta(t) \\
&\theta(t) = \frac{2}{B_1}e^{-\frac{A_1}{2}t}Sinh\left(\frac{B_1 t}{2}\right) \\
&\phi(t) = e^{-\frac{A_1}{2}t}\left[Cosh\left(\frac{B_1 t}{2}\right) + \frac{A_1}{B_1}Sinh\left(\frac{B_1 t}{2}\right)\right] = \frac{A_1+B_1}{2B_1}e^{-\frac{A_1-B_1}{2}t}\left(1-\frac{A_1-B_1}{A_1+B_1}e^{-B_1 t}\right)
\end{aligned}
\tag{3}
$$

being:

$$A_1 = U + R_s + L_r = U\cdot\left(1+\frac{R_s+L_r}{U}\right)$$

$$B_1 = \sqrt{\left(A_1{}^2 - 4L_r R_s\right)} = U\cdot\sqrt{1+2\frac{R_s+L_r}{U}+\left(\frac{R_s-L_r}{U}\right)^2}$$

As it will be shown, it was not possible to obtain a single set of parameter values for this model, which allow the reproduction of all Na lichen content variability. In order to achieve that, the effect of water upon elements uptake and release, had to be considered. The model was then renamed as Surface Layer and Water model: SLAW-model, and the water function already presented [11] was used for this purpose, as a factor in the definition of water dependent uptake U, and release, Lr, coefficients:

$$U = U0*\left(x^{-(Ub\cdot x)^2}\right); x = \frac{Water}{U_{W0}} \quad ; \quad Lr = Lrr0*\left(x^{-(Lrb\cdot x)^2}\right); x = \frac{Water}{Lr_{W0}} \tag{4}$$

3. RESULTS AND DISCUSSION

The determination of the model parameter values was made by fitting a simulated

Markov chain to the time series data using an algorithm presented elsewhere [12]. The results obtained for each station individually and for all of them simultaneously (Overall) using the model with constant parameters are presented in Table II. It can be seen that for the worst case, that of Carregado station (CAR), the correlation found is not good. Introducing the water dependent parameters described in equation (4), and repeating the fitting, provides the results presented in Table III. It can be seen that, now, very good correlations between simulation and measurement can be found, mainly when using total deposition (ATD) as availability variable.

TABLE II. PEARSON CORRELATION COEFFICIENTS BETWEEN THE BEST-FIT LICHEN CONTENT TIME SERIES AND THE MEAN VALUES OF THE MEASURED LICHEN CONCENTRATIONS. RESULTS ARE PRESENTED FOR EACH STATION INDIVIDUALLY, AND FOR THE WHOLE 6 SIMULTANEOUSLY (OVERALL)

Corr	TAP	CAR	PAL	FAR	SIN	MON	Overall
FAC	0.81	0.17	0.45	0.68	0.61		0.42
CAC	0.77	0.48	0.53	0.34	0.58	0.79	0.42
TAC	0.78	0.14	0.54	0.62	0.58	0.87	0.42
FPC	0.77	0.55	0.59	0.49	0.59	0.84	0.42
CPC	0.78	0.20	0.42	0.25	0.59	0.60	0.41
TPC	0.77	0.29	0.52	0.26	0.60	0.70	0.42
DRC	0.86	0.30	0.36	0.49	0.69	0.69	0.47
ATD	0.91	0.17	0.39	0.50	0.75	0.69	0.48
WRC	0.92	0.29	0.45	0.46	0.64	0.56	0.07

TABLE III. PEARSON CORRELATION COEFFICIENTS BETWEEN THE BEST FIT LICHEN CONTENT TIME SERIES AND THE MEAN VALUES OF THE MEASURED LICHEN CONCENTRATIONS WHEN USING THE WATER DEPENDENT U AND LR PARAMETERS. RESULTS ARE PRESENTED FOR EACH STATION INDIVIDUALLY, AND FOR THE WHOLE 6 SIMULTANEOUSLY (OVERALL)

Corr	TAP	CAR	PAL	FAR	SIN	MON	Overall
FAC	0.95	0.90	0.86	0.90	0.95	0.98	0.65
CAC	0.95	0.90	0.90	0.77	0.94	0.98	0.61
TAC	0.94	0.88	0.88	0.92	0.95	1.00	0.59
FPC	0.94	0.87	0.92	0.89	0.93	0.88	0.63
CPC	0.95	0.92	0.91	0.80	0.93	0.99	0.59
TPC	0.94	0.87	0.93	0.84	0.93	0.99	0.60
DRC	0.92	0.76	0.70	0.73	0.92	0.95	0.55
ATD	0.94	0.96	0.83	0.73	0.93	0.98	0.69
WRC	0.93	0.30	0.18	0.46	0.63	0.73	0.17

The values determined for the coefficients in the SLAW-model are presented in Table IV, and the comparison between measured data and best-fitted simulated Markov chain for the overall case is presented in Fig. 2.

It can be seen from Table IV that the values of the parameters vary from station to station by one or more orders of magnitude. In Table V, the proper interpretation of the Ds parameter for the various variables used is presented. When coupling with the ATD availability variable, Ds represent the operational area of the lichen. From Table V, the Ds values are quite high at Carregado and Sines, but not so at Tapada, Palmela, Faralhão and Monchique.

If the lichen samples are assumed as square sheets with 10 × 10 cm2 and a thickness of 0.01cm, composed of a set of spheres 1µm in diameter, all closely packed, then they can be thought as a set of 10^{12} of such spheres. Because one sphere has an outer surface of 3.14×10^{-12} m^2, then a total surface of 3 m^2 or 3×10^4 cm^2 can be found. If, in addition, the smaller structure of the cell walls is considered, assuming that it corresponds to stems of 100 Å rising from a spherical surface, an additional gain of 100 in surface is determined.

The values found for the Ds parameter thus show that, for some cases, the ***SLAW model lichen interior is the interior of the lichen individual elements, algae cells or fungi stems***, making that, in these cases, the operational surface is the cell walls including eventually its more fine details. In the case of Sines, the parameter value is too big and so not understandable. From the biological point of view, it is not possible to accept without strong proof that some structure from inside of the cells may be acting like surface and be cleaned out with a 30 seconds rinsing. In this case it may happen that the fitting routine is unable to cope with very large values of the Ds parameter. Nevertheless, for all the remaining values, the operational area of the lichen is seen to vary significantly from one place to another. A possible explanation for this is a different nature of the pollutants in each area. Lichens may therefore be responding as an instrument to assess differences in chemical and/or physical characteristics of the media carrying the element under study.

TABLE IV. PARAMETER VALUES FOR THE FITS USING ATD AVAILABILITY VARIABLE, FOR THE INDIVIDUAL STATIONS FITS, FOR THE SIMULTANEOUS SIX STATIONS FIT (OVERALL), AND THE NATURAL LOGARITHM AVERAGE AND NORMAL AVERAGE OF THE INDIVIDUAL STATIONS FIT PARAMETERS

Param	**TAP**	**CAR**	**PAL**	**FAR**	**SIN**	**MON**	**Ln Average**	**Average**	**Overall**
Ds	2.30E+05	1.67E+04	3.00E+02	5.81E+02	9.29E+05	2.70E+04	1.60E+04	2.00E+05	6.95E+09
Rs	4.96E-04	6.69E-05	2.17E-03	2.21E-05	1.05E+02	4.33E-02	4.39E-03	1.75E+01	1.27E-05
U r0	1.05E-05	1.27E-03	2.71E-01	1.26E-02	5.05E-02	2.17E-04	2.82E-03	5.59E-02	3.61E-09
U W0	4.47E-02	1.45E-01	1.46E-03	5.68E-02	1.33E-03	1.17E-01	2.09E-02	6.10E-02	1.00E-03
U b	2.42E-03	1.36E-01	1.00E-03	1.58E-03	1.02E-02	9.26E-03	6.05E-03	2.68E-02	7.79E-03
Lr r0	3.53E-01	8.62E-03	2.86E-02	4.86E-02	1.53E-09	3.13E-02	2.42E-03	7.83E-02	1.25E-02
Lr W0	2.50E+01	1.22E+00	3.48E+03	4.46E+01	4.22E+00	5.90E+00	2.21E+01	5.94E+02	6.40E+02
Lr b	1.00E-03	3.21E+00	9.93E-02	6.06E+00	2.27E-02	2.88E+00	2.24E-01	2.04E+00	2.80E-01

TABLE V. UNITS AND MEANING OF THE DS PARAMETER FOR THE VARIOUS AVAILABILITY VARIABLES

Availability variable	**Availability units**	**D_S units**	**D_S meaning**
FAC, CAC, TAC	ng/m^3	m^3/day	Pumping capacity
FPC, CPC, TPC, DRC	ng/mg	mg/day	Uptake capacity
ATD	(ng/cm^2)/day	cm^2	Real lichen operational area
WRC	ng/ml	ml/day	Filtering capacity

4. CONCLUSIONS

After the first results for Na, it was verified that the model based on constant rates could not explain all the variability found in Na data. In a preliminary work [11], water effects were seen to be able to account for unexplained variability in calibration data. The same mathematical expression for the effect of water was then used to convert the constant rates of transfer between surface and lichen interior into water availability dependent rates. Both uptake and release biological processes were assumed boosted under low water availability (when compared to no water availability). Under too much water conditions the rates are expected to become null. The amount of water needed to change from one condition to another is an adjustable parameter of the model. The model obtained by considering the impact of water availability in this way was named SLAW-model (Surface Layer And Water model). The unexplained variances in the Na data were then explained. Furthermore, the different values obtained for the lichen operational surface dimension, one of the parameters, allowed the recognition of the physical/biological counterpart of the model mathematical surface concept.

In particular, differences verified between the values of this parameter determined for different stations, were interpreted as an indication of difference in environmental impact and/or a different response of the lichen to diverse chemical or physical forms of the element available. Further study is needed to establish which is the case or if both cases are verified.

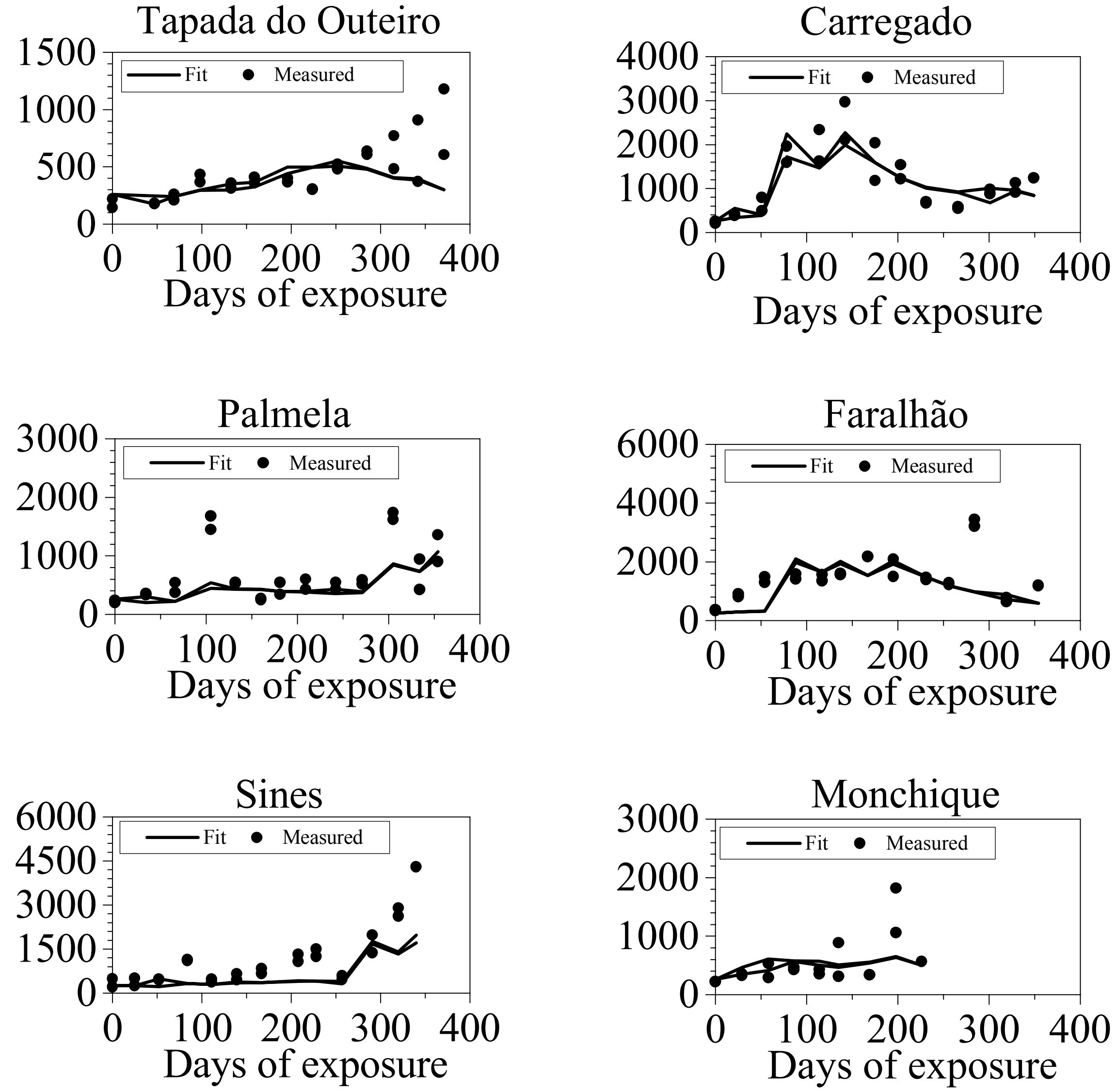

FIG. 2: Lichen transplants content time series, fitted and measured for Na for all six experimental stations. Fit was made for all stations simultaneously.

ACKNOWLEDGEMENTS

This work was partially funded by the International Atomic Energy Agency and Portuguese Electricity Production Company (CPPE). The authors also want to thank all the CPPE personnel that helped in making all the data collection possible. A special word of thanks is also expressed to Mrs Isabel Dionísio and Mrs Rute Pinheiro for all the careful lab work and sample preparation needed to carry such an experiment to a good end.

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BIOMONITORING WITH EPIPHYTIC LICHENS AROUND GAS TREATMENT PLANTS

Z. JERAN[1], M. HORVAT[1], R. JAĆIMOVIĆ[1], Z. SPIRIC[2]

[1] Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
[2] INA- Naftaplin, Zagreb, Croatia

Abstract

This paper describes the use of passive and active biomonitoring with epiphytic lichens for spatial monitoring of mercury and other elements in air at 9 different locations around three natural gas pumping sites in Croatia. It is well known that at the Molve station the concentration of mercury in natural gas is very high and it has to be removed before further processing in order to prevent technological and environmental problems. To monitor the efficiency of the industrial facility for removal of mercury from natural gas, besides a conventional monitoring programme which includes Hg measurement in air, biomonitoring was also applied as an alternative methodology. Various *in-situ* lichen species, namely *Parmelia caperata*, *Evernia sp.*, *Hypogymnia physodes* and *Xanthoria parietina* were collected during 1997–2000 and analysed. For transplantation experiments *H. physodes* was used. For Hg determination in lichens cold vapour atomic absorption spectrometry was used, while for trace elements k_0-neutron activation analysis was performed. A good correlation between mercury concentrations in air and lichens was found. The preliminary results also indicate that Hg levels in *in-situ* and transplanted lichens were relatively low, with somewhat higher levels only in the immediate vicinity of the facility for Hg removal. The results of multielement analysis showed that there were source-related high levels of some elements, which could be explained by technological processes or geological factors.

1. INTRODUCTION

Epiphytic lichens are among the best known and extensively used biomonitors for air pollution. Passive as well as active biomonitoring using lichens has been applied in different countries to study the distribution and impact of various trace elements due to anthropogenic or natural emissions [1–2]. Namely, it was found that surveys of a large territory using *in-situ* lichens (passive biomonitoring) allows the identification of atmospheric pollution sources [3–5]. However, the transplantation method (active biomonitoring) using tolerant lichen species like *H. physodes* or *P. sulcata* around known pollution sources or in mixed industrial environment enables the study of pollution in a more detailed manner [6–8].

At Molve, and some other places near Ivanič Grad, Croatia, rather large natural gas treatment facilities are located. The natural gas at Molve is known for its elevated levels of mercury but it is removed from the raw gas during the recovery of liquid constituents and also during the removal of hydrogen sulfide. In order to monitor the efficiency of an industrial facility for removal of Hg from natural gas at the INA Naftaplin gas treatment plant, Molve, Croatia, a comprehensive environmental monitoring programme, including Hg measurement in air, was established [9]. Generally, total Hg concentrations in air are very low (e.g. below 10 ng m^{-3}) and therefore, potentially subject to analytical errors. In the present study, an alternative methodology using lichens as passive and active biomonitors was initiated for monitoring Hg and also other trace elements, which might be emitted there.

2. MATERIALS AND METHODS

2.1. Sampling and transplantation of lichens

From 1996 to 1999 four different sampling campaigns using in-situ lichens of various species (*Hypogymnia physodes, Parmelia sulcata, Parmelia caperata, Xanthoria parieta)* around gas pumping sites in Croatia were performed. The first three were made in November 1996, April

1997 and September 1997 at several locations around the gas treatment plant in Molve, Croatia [10] (Fig.1). However, in 1999 besides these locations two new locations with gas pumping facilities close to Zagreb, namely Žutica and Etan (at Ivanič grad), were included (Fig.1). At each location lichens were carefully removed from the trunks of dominant tree species, 1.5 to 2 m above the ground, stored in paper bags and transported to the laboratory at the Jožef Stefan Institute for further preparation.

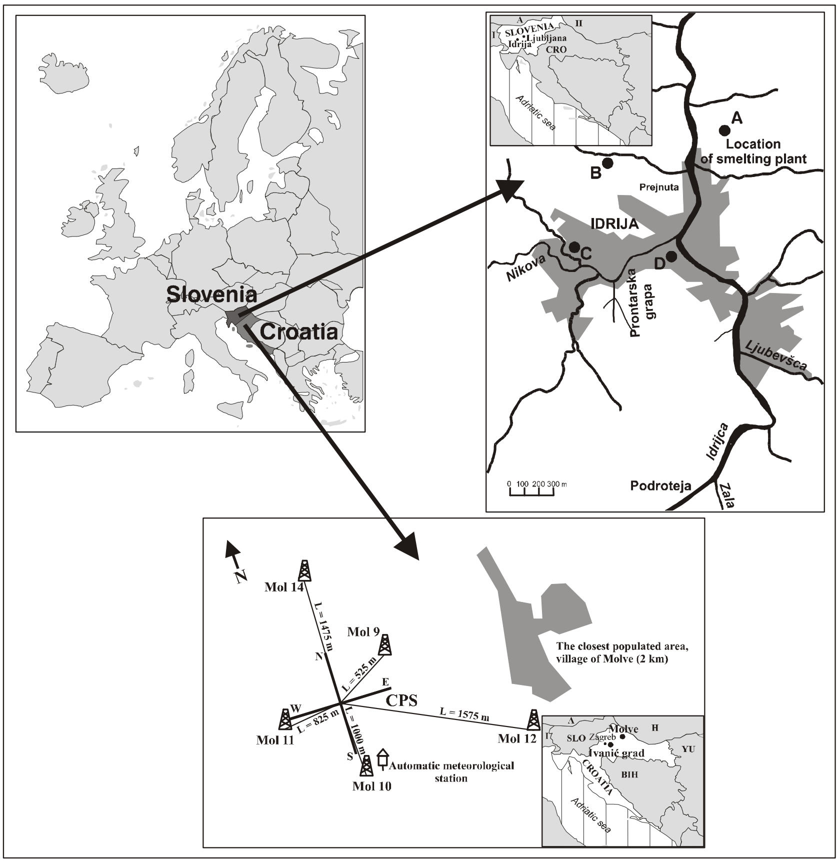

FIG. 1. Sampling locations

Two experiments with transplanted lichens were also performed. In the first experiment the lichen species *H. physodes* was exposed for 5 and 8 months in the period between April 1997 to December 1997 [10] at 5 locations around the Molve gas treatment plant and at 4 locations in the Idrija mining area, Slovenia (known for elevated mercury concentrations in air) (Fig.1). In the second experiment, *H. physodes* was transplanted to 9 locations in Croatia (Etan (2), Žutica (3), Molve (4)) for 6 and 12 months between April 1999 and April 2000. The reference locations were in Slovenia (Idrija area and the Reactor Centre near Ljubljana). *H. physodes* used for transplantation was collected from apple trees at the remote locations of Mislinjski jarek (1997) and in Tuhinjska dolina (1999), Slovenia. In the first experiment (1997), branches covered with lichens were used for transplantation, which were then tied with nylon thread on to branches of local trees at each exposure location. However, in the second experiment (1999–2000) lichens were first carefully removed from the branches and were then packed into polyethylene nets, transported to the sampling locations and fastened to branches of local trees or on an artificial rod about 1.5–2 m above the ground. Two polyethylene nets were exposed at each sampling location. For determination of the initial concentration of Hg and other trace elements some lichen material was kept in the laboratory in a closed container. At the end of each sampling period lichens were removed from trees and transported to the Jozef Stefan Institute (JSI).

2.2. Sample preparation

In the laboratory the lichen samples were moistened with distilled water and carefully separated from the bark substratum using nylon tweezers, and subsequently lyophilised. 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 walls. 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.

2.3. Analytical methods

k_0-instrumental neutron activation analysis — (INAA) was performed [11] for multielemental determination of lichens. The details of sample preparation, irradiation, measurement and elemental concentration calculation are given elsewhere [5]. For determination of Hg cold vapour atomic absorption spectrometry was used [12,13,10]. Namely, it was found that due to the highly volatile nature of Hg the neutron activation techniques used, using plastic irradiation vials, gave about 30% lower values.

TABLE I. CONCENTRATIONS FOR ELEMENTS DETERMINED BY INAA AND CV AAS IN IAEA-336.

Element	***Conc. found (mg/kg); (n)***	**Recommended (and information) value (95% Conf. Int.)**
Al	*785 ± 14 (3)*	680 (570–780)i
As	*0.69 ± 0.05 (3)*	0.639 (0.562–0.716)
Ba	*6.90 ± 0.34 (3)*	6.4 (5.3–7.5)i
Br	*12.3 ± 0.3 (3)*	12.9 (11.2–14.6)
Ca	*2600 ± 110 (3)*	2600 (2400–3300)i
Ce	*1.34 ± 0.02 (3)*	1.27 (1.09–1.44)
Co	*0.29 ± 0.01 (3)*	0.287 (0.248–0.326)
Cr	*1.17 ± 0.07 (3)*	1.03 (0.80 –1.17)i
Cs	*0.116 ± 0.001 (3)*	0.110 (0.097–0.123)
Fe	*438 ± 19 (3)*	426 (380–472)
Hg*	*0.194 ± 0.011 (3)**	0.200 (0.167–0.233)*
K	*1830 ± 60 (3)*	1840 (1640–2040)
La	*0.61 ± 0.01 (3)*	0.66 (0.55 –0.76)i
Mg	*684 (2)*	610 (500 –710)i
Mn	*65.4 (2)*	64.0 (57.0–71.0)
Na	*312 ± 5 (3)*	320 (280–360)i
Rb	*1.80 ± 0.07 (3)*	1.72 (1.52–1.92)
Sb	*0.079 ± 0.005 (3)*	0.073 (0.063 –0.083)
Sc	*0.183 ± 0.004 (3)*	0.170 (0.148 –0.192)
Se	*0.229 ± 0.016 (3)*	0.216 (0.185–0.247)
Sm	*0.110 ± 0.009 (3)*	0.106 (0.092 –0.120)
Sr	*9.1 ± 0.3 (3)*	9.2 (8.1 –10.3)i
Th	*0.145 ± 0.008 (3)*	0.142 (0.123–0.161)i
U	*0.051 ± 0.004 (3)*	0.041 (0.031 –0.051)i
V	*1.73 (2)*	1.5 (1.25 –1.69)i
Zn	*31.3 ± 1.5 (3)*	31.6 (28.1–35.1)

CV AAS; i - information value; n - number of samples analysed.

TABLE II. RESULTS FOR TOTAL MERCURY IN IN-SITU LICHENS (mg/kg, DRY WEIGHT) COLLECTED IN 1996, 1997, AND 1999 AT INA NAFTAPLIN GAS TREATMENT PLANT MOLVE AND AT ETAN AND ŽUTICA.

Sampling location	Lichen specie	Nov 96	Apr 97	Sept 97	Apr 99
Etan	*H.physodes*				0,18
Žutica	*H.physodes*				0,10
	P.caperata				0,16
	Evernia sp.				0,07
Mol 9	*Parmelia sulcata*	0,53	0,20	0,20	
	P.caperata				0,33
	X.parietina		0,29	0,27	0,43
Mol 10	*H.physodes*	0,20	0,20		
	P.sulcata	0,15	0,16		
	X.parietina			0,16	0,40
Mol 11	*P. sulcata*	0,34	0,23	0,28	
	X.parietina	0,20	0,21		0,24
Mol 12	*P.sulcata*	0,11	0,17	0,12	
	X.parietina			0,12	0,24
Mol 14	*P. sulcata*	0,54	1,17	0,70	
	X.parietina	2,80	2,27	0,53	

2.4. Analytical Quality Control

The accuracy of the results was checked by the use of the certified reference material IAEA-336, Trace and Minor Elements in Lichens. Comparison of the results obtained by INAA and CV AAS with certified values is shown in Table I.

3. RESULTS AND DISCUSSION

3.1. Mercury in *in-situ* samples

Concentrations of Hg in *in-situ* lichens collected in the period between 1996–1999 at three natural gas treatment locations of INA-Naftaplin, Croatia, are presented in Table II. Due to the different environmental characteristics at the three sampling locations of Etan, Žutica and Molve the abundance of lichen species was different. Therefore, it was impossible to collect the same lichen species at all the investigated areas, which makes direct comparison difficult. Hg levels in lichens (*H. physodes, P.caperata, Evernia sp.)* at Etan and Žutica, collected mostly in the forest, varied between 0.07 and 0.18 mg.kg^{-1}. As evident from Table II somewhat higher values were found from the area at the Molve treatment plant, but these levels can also be considered as low. The only exception was the location MOL 14 where in *Xanthoria parietina* a concentration higher than 2 mg/kg was found. However, since environmental measurements of Hg in air performed by Špirić and McNamara [9] show no significant increase during Dec. 1995 and June 1997, the higher values observed in *in-situ* lichens at this location are most probably related to some past events.

3.2. Mercury in transplanted samples

Hg concentrations in transplanted lichens of the second experiment performed between April 1999 and April 2000, their initial values (before exposure) and mean values of in-situ lichens are presented in Fig 2. Evidently only a very slight increases in Hg concentrations after six and twelve months of exposure were observed at all locations, except at the Reactor centre in Ljubljana where Hg concentrations in lichens remained the same throughout the year. This is probably in response to the stable and low Hg air concentrations in Ljubljana (average between 2–3 ng g^{-1}). Similar to the experiment performed in 1997 [10], the highest Hg levels were found at station CPS (0.39 and 0.34 mg kg^{-1}) which lies close to gas treatment facilities for elimination of Hg from the original gas. It is further seen that the Hg levels in *H. physodes* after 12 months of exposure differ somewhat from the mean *in-situ* lichen concentrations. As expected, the highest Hg concentrations (0.7 mg/kg) were recorded in samples from Idrija after six months of exposure (the sample after 12 months was not available). The enrichment factor for Hg of about 6 in Idrija compares well with data obtained in previous studies [10,14]. Namely, in the Idrija area Hg concentrations in the air were measured several times in 1990 [14] and during the first study period in 1997 and varied between 10 and up to 3000 ng.m^{-3} at the most exposed site near the mine ventilation system [10,14]

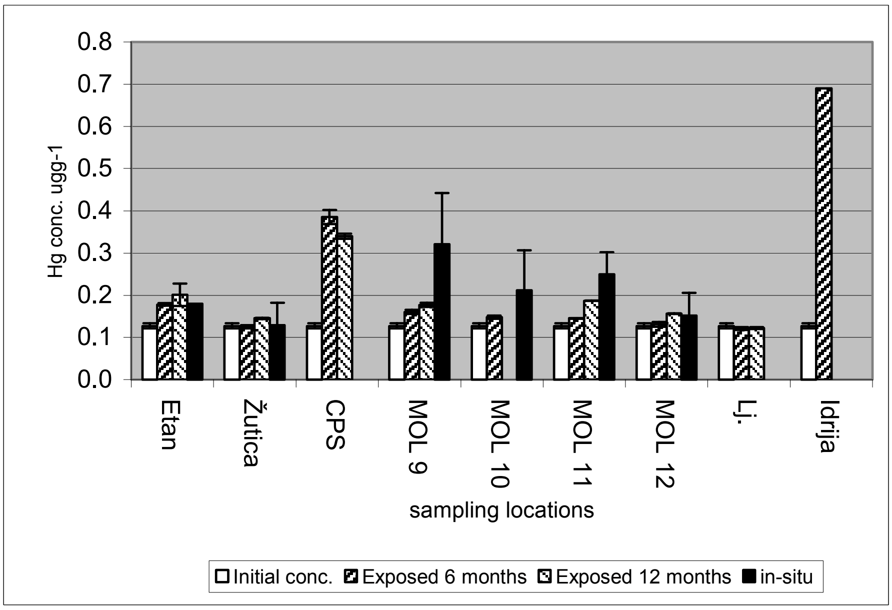

FIG. 2. Mercury concentrations in transplanted Hypogymnia physodes and in-situ lichens (mean values for different lichen species) around gas treatment plants in Croatia and reference locations at the Reactor Centre and Idrija in Slovenia.

3.3. Multielement analyses in *in-situ* lichens

Table 3 gives the results for some selected trace elements (Ba, Br, Cd, Co, Cr, Sb and Zn) that differ significantly in *in-situ* lichens collected in the period between 1997-1999 at different locations in the Molve area as well as at two other gas pumping sites in Croatia. For comparison the elemental composition of *H. physodes* used for transplantation is included in Table III. Results showed that on the average the lowest values for the above mentioned elements were found at Žutica, were slightly higher at Etan and were highest at Molve stations. Results for lichens collected at Žutica agreed well with the levels found in *H.physodes* from two remote locations in Slovenia. It was further seen that the levels of the selected elements, with some exceptions, do not differ significantly between different lichen species. Among the Molve stations significantly higher Ba levels (103 and 600 mg kg^{-1}) were found in *P.sulcata* from MOL-9 and in *X. parietina* collected in 1999 at MOL-12 as well in both lichen species at MOL-14 (217 and 163 mg kg^{-1}). At MOL-14 higher Br was found in *X.parietina*. Also, significantly higher was Sb in *X.parietina* collected at Mol-12 in 1999.

TABLE III. ELEMENT CONCENTRATIONS IN IN-SITU LICHENS (mg kg^{-1}) AT VARIOUS LOCATIONS AROUND GAS PUMPING STATIONS IN CROATIA AND TWO REMOTE LOCATIONS IN SLOVENIA

Sample:		sampling year	Ba	Br	Cd	Co	Cr	Sb	Zn
Etan 1	H.physodes	1999	28.3	10.3	< 0.6	0.61	5.83	0.35	136
Etan 2	*H.physodes*		35.3	9.79	0.48	0.60	5.78	0.41	115
Žutica	*H.physodes*	1999	34.7	6,15	0.50	0.41	2.76	0,14	46.2
	P.caperata		50.7	6.05	0.48	0.19	1.96	0.17	41.5
	Everina sp.		13.0	3.31	< 0.4	0.13	0.76	0.07	36.0
MOL-9	*X. parietina*	1997	59.8	35.3	1.67	0.50	4.56	0.17	46.0
	P. sulcata		103	17.8	2.09	0.61	5.38	0.26	143
	P.saxatilis	1999	42.1	19.8	2.87	0.62	5.04	0.26	184
	X. parietina		62.0	29.5	1.20	0.83	6.88	0.30	67.1
MOL-10	*X. parietina*	1997	31.3	16.7	2.36	0.53	3.93	0.38	56.0
		1999	58.3	12.3	1.16	0.49	4.49	0.21	51.9
MOL-11	*P. sulcata*	1997	51.4	60.2	0.28	0.45	4.89	0.27	86.0
	X. parietina	1999	38.7	12.7	0.71	0.76	5.40	0.23	50.8
MOL 12	*P. sulcata*	1997	55.5	19.1	1.25	0.43	3.59	0.25	89.1
	X. parietina		26.4	15.6	2.17	0.43	3.41	0.30	52.3
	X. parietina	1999	600	28.5	2.46	0.78	5.95	1.98	90.7
MOL 14	*X. parietina*	1997	217	244	1.14	0.61	4.35	0.27	54.8
	P. sulcata		163	29.5	0.36	0.73	6.29	0.30	74.4
Mislinjski jarek	*H. physodes*	1997	24.2	10.1	0.30	0.30	3.00	0.10	49.1
Tuhinjska dolina	*H. physodes*	1999	27.9	8.57	0.5	0.14	1.62	0.16	40.5

3.4. Multielement analysis of transplanted lichens

In Fig. 3 the results normalised to their initial concentrations for lichens exposed in the period between April 1999–April 2000, for six and twelve months at selected locations in Croatia are presented. Due to strong winds which occurred in the autumn of 1999, one polyethylene net filled with lichens at location MOL 10 was lost, so there are no results for this location after one year. As evident from Fig. 3 the concentration levels of practically all elements increased with time. It is further seen that for the lanthanide and some typical so-called soil elements (As, Fe, Th, U) the increase is similar to that of Sc and can suggest the deposition of local soil dust. Sc is namely, an excellent normalizing element to assess the soil loading of lichen or moss samples as well as to other plants [15,16]. Results of the second experiment are also in agreement with a similar investigation performed in 1997 [10].

Evidently, the results at the Molve gas treatment plant (CPS) show a significant increase of Hg over time as was discussed before. Additional elements that need to be mentioned are Ba, Na, Sb, and Cr. For Ba the enrichment factors in the second experiment at stations MOL-12 increased from 6 to 10 after six and twelve months of exposure, respectively. At MOL-11, Ba concentrations also increased by a factor of 2 after 6 months of exposure, and by a factor of

13 after twelve months of exposure. Antimony (Sb) significantly increased at station MOL-12 (enrichment factors from 5.5 to 23). Cr concentrations at CPS and MOL-9 increased 3 times after six months and 5.5 after twelve months of exposure. At CPS an increase of Na by a factor of 6 and 8 was also observed.

It is interesting to note that in the *in-situ* lichens collected in 1999 around MOL 12 the highest Sb concentration was also found (1.98 mg kg^{-1}) which is almost one order of magnitude higher than at the other locations where levels between 0.2–0.3 mg kg^{-1} were measured (Table III).

High levels of Ba and Sb at certain locations (MOL 11 and MOL 12) most probably originate from technological processes at the gas treatment plant facilities.

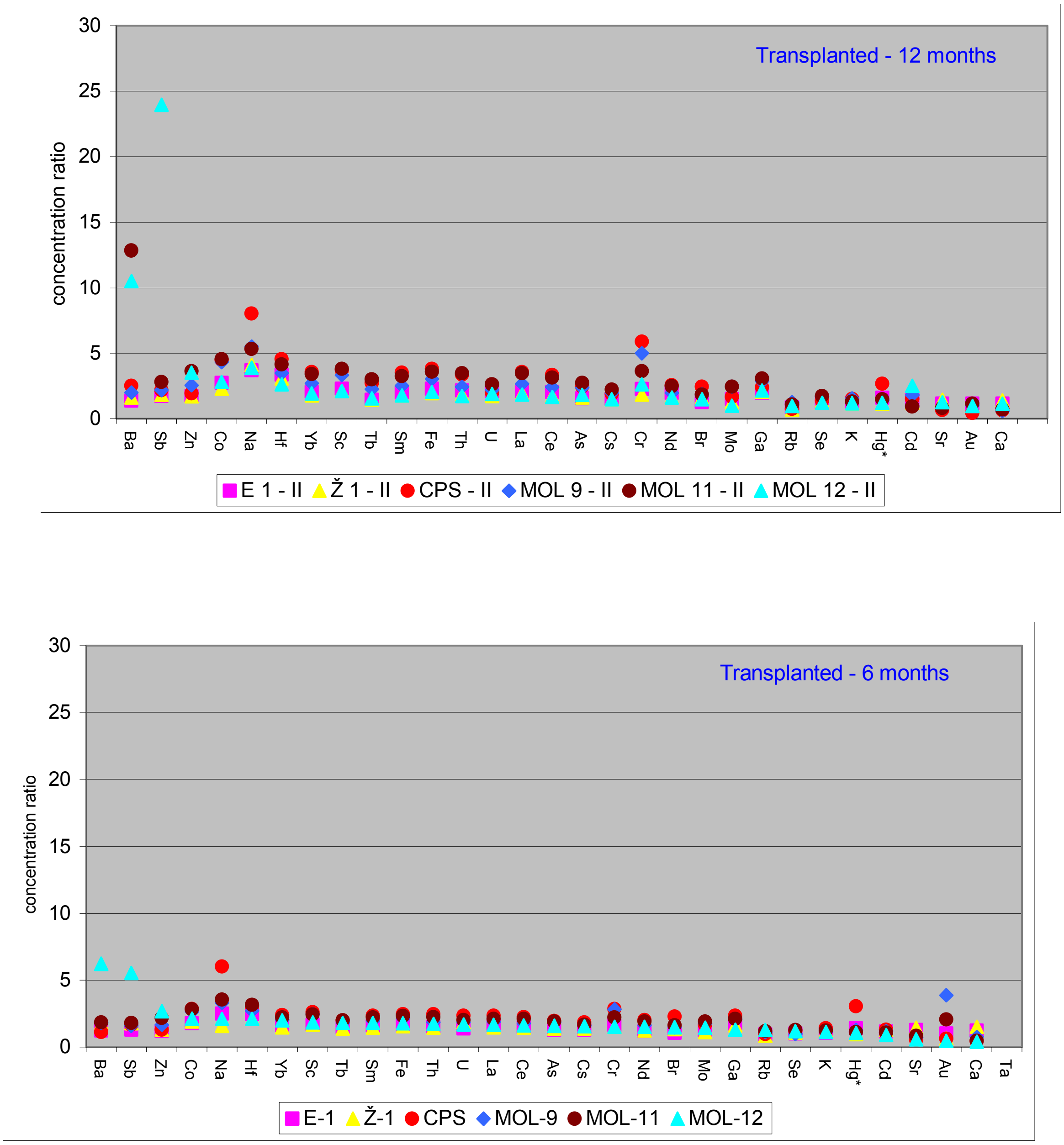

FIG 3. Concentration ratio of trace elements to initial concentration levels in transplanted lichens after 6 and 12 months of exposure.

4. CONCLUSION

These two short term experiments with transplanted lichens around the gas treatment plants confirm that lichens can successfully be used as biomonitors of Hg air pollution. Using both passive as well as active biomonitoring, it was confirmed that during the study periods the air at Molve as well as around Žutica and Etan was not significantly polluted with Hg. However, high Hg levels of *in-situ* lichens at Mol-14 pointed to some past event.

Multielemental analysis of *in-situ* as well as of transplanted lichens revealed some other elements (Ba, Br, Cr, Na and Sb) that are emitted from the Gas Treatment Plant, Molve, which might be related either to technological processes at the gas Treatment Plant and/or other geological factors of the area. For other trace elements similar behaviour as for Sc was observed, pointing to local dust deposition. It is worth mentioning that careful selection of sampling sites is needed since as seen at Molve some elements were systematically higher at certain microlocations.

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