VALIDATION AND APPLICATION OF PLANTS AS BIOMONITORS FOR TRACE ELEMENT ATMOSPHERIC POLLUTION – A COORDINATED EFFORT IN 14 COUNTRIES

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ABSTRACT

The International Atomic Energy Agency (IAEA) organized a co-ordinated research project (CRP) on "Validation and application of plants as biomonitors of trace element atmospheric pollution analysed by nuclear and related techniques" involving 14 participating countries. The CRP's objective was to identify appropriate bioindicators for local and/or regional application and validate them for general air pollution monitoring. Activities included quantification studies, research into spatial and time resolution for particular organisms, and physiological studies. A number of suitable bioindicators were identified in

different parts of the globe and tested during the CRP. Sampling strategies were reviewed and the recommended approach adopted by the group. Appropriate sample preparation procedures were assessed and harmonised to the degree allowed by different geographic and climatic conditions in the participating countries. Two interlaboratory comparison exercises were carried out on lichen and moss materials. Results confirmed definite improvement in analytical performance of the participating laboratories, but also revealed possible inconsistencies due to different sample processing procedures. Several monitoring surveys were carried out and consequently pollution maps drawn for extended areas or countries. Overall results confirmed applicability of lower plants for assessing the degree of atmospheric pollution and provided several countries with effective monitoring tools not used before.

1   INTRODUCTION

The International Atomic Energy Agency (IAEA) is a specialized technical organization within the United Nations (UN) family that serves as the global focal point for nuclear cooperation. One of its goals, however is to encourage and assist research on, and development and practical application of, atomic energy for peaceful purposes throughout the world and to foster scientific and technical information, as well as the exchange of scientists in the field of peaceful uses of atomic energy. This activity includes support in utilizing research nuclear facilities such as research nuclear reactors, accelerators, and X-ray fluorescence equipment for environmental research.

The Research Contract Programme (RCP) is an IAEA tool in assisting its Member States in obtaining scientific results in an effective manner. It is focused on obtaining results that are usually expected to benefit society in the foreseeable future and is end-beneficiary oriented. Research efforts are normally carried out within the framework of Coordinated Research Projects (CRPs). These CRPs are developed around well-defined research topics on which research institutes are invited to collaborate in solving a problem of common interest. The research supported relates to the tasks included in the approved programme with the IAEA supporting and coordinating the research granting funded research contracts, cost-free research agreements, and organizing Research Coordination Meetings (RCMs). Once a CRP is formed, research teams from ten to twelve institutes, on average, are selected for participation in the project, which lasts up to five years. Institutes in developed countries work in close cooperation with those from developing countries and all participants are encouraged to conduct work that produces new research results and to apply those achievements to specific needs of their respective countries.

Biomonitoring using plants has been shown in several countries as a cost-effective tool for assessing trace element atmospheric deposition [1-4]. For assessing trace element levels and identifying their sources, databases containing 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 need for 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 the IAEA programmes. Indeed the IAEA has been systematically supporting these activities since 1992.

2   CRP ON VALIDATION AND APPLICATION OF PLANTS AS BIOMONITORS OF TRACE ELEMENT ATMOSPHERIC POLLUTION

In 1998, the IAEA initiated the CRP on "Validation and application of plants as biomonitors of trace element atmospheric pollution, analysed by nuclear and related

techniques". The project, concluded in 2002, was aimed at identifying suitable biomonitors of atmospheric pollution for local and/or regional application. Whenever possible the identified bioindicators should be validated for general environmental monitoring. The 14 countries participating in the CRP are listed in Table 1. The main activities carried out included quantification, time resolution, geographical resolution, survey, mapping and physiology. Each of these topics is explained and addressed in more detail in the next heading.

Table 1: Countries participating in the CRP and summary of their involvement. Abbreviations are explained in the text.

Country	Bioindicator	Type of bioindication	Analytical method	Activity
Argentina	Lichens, Tillandsia	Passive	INAA, TXRF, AAS	Q, G, S, M, I
Brazil	Lichens, Tradescantia, tree bark	Active, passive	INAA	Q, G, S
Chile	Lichens	Active, passive	INAA	Q, G, S, M
China	Tree leaves	Passive	INAA	Q, T, G, S
Germany	Moss	Passive	ICP-MS	Q, T, G, S, M
Ghana	Lichens	Passive	INAA, XRF	G, S
India	Mosses, lichens, weeds, shrubs	Passive	PIGE, ICP-MS	Q, G, S
Israel	Lichens	Active	ICP-AES	T, G, S, I
Jamaica	Lichens, mosses, Tillandsia	Passive	INAA, XRF	Q, T, G, S, M
The Netherlands	Tree bark, mosses	Passive	INAA	Q, T, G, S, M
Norway	Mosses	Passive	ICP-MS, INAA	Q, T, S, G, M
Portugal	Lichens	Active	INAA, PIXE	Q, T, G, S, M
Romania	Mosses	Passive	INAA, AAS	G, S, M
Russian Fed.	Mosses	Passive	INAA, AAS	T, G, S, M

Note: INAA – instrumental neutron activation analysis, XRF – X-ray fluorescence analysis, TXRF – total reflection XRF, AAS – atomic absorption spectrometry, ICP-MS – inductively coupled plasma-mass spectrometry, ICP-AES – inductively coupled plasma-atomic emission spectrometry, PIGE – particle induced gamma-ray emission, PIXE – particle induced X-ray emission; Q – quantification, T – time resolution, G – geographical resolution, S – survey, M – mapping, I – impact

The group agreed upon the following terminology [4]:

A **bioindicator** is an organism (or part of an organism or a community of organisms) that contains information on the **quality** of the environment (or a part of the environment).

A **biomonitor** is an organism (or a part of organism or a community of organisms) that contains information on the **quantitative** aspects of the quality of the environment. A biomonitor is always also a bioindicator, but a bioindicator does not necessarily meet the requirements for a biomonitor.

Biomonitoring is a continuous observation of an area with the help of bioindicators, e.g., by repeated measurement of their responses in a manner that reveals changes over space and time (by measuring of the xenobiotics taken up, for example). **Active** bioindication (biomonitoring) is when bioindicators (biomonitors) bred in a laboratory or collected at a

selected site are exposed in a standardised manner in an area of investigation for a defined period of time. **Passive** biomonitoring is when *in-situ* occurring organisms are examined for their reactions.

2.1 Selection of bioindicators

In the initial phase of the project implementation, the participating scientists selected appropriate bioindicators. They focused on lichens and mosses, having in mind that they both (1) lack roots and therefore obtain most of their nutrient supply directly from precipitation and dry deposition of airborne particles with mostly insignificant risk of taking up metals from the substrate, and (2) have no or only a very reduced cuticle so that ions retained on their surface have direct access to exchange sites on the cell walls [2,4,5]. The final selection was made according to individual specific project objectives of individual participants and the related work plans. In several cases lower plants were not available at selected areas of investigation, so either active biomonitoring or higher plants as bioindicators were selected. The selected bioindicators in the participating countries are presented in Table 1.

2.2 Analytical quality assurance and control (QA/QC)

Adequate provision of QA/QC in its Member States is one of important and continuous activities of the IAEA. Two interlaboratory comparison exercises were carried out within the project: the first one, denoted as NAT − 5 in 1998 on two lichen samples, and the second one (NAT − 6) on two moss samples in the year 2000 [6, 7]. The initial selection comprised 23 elements. However, due to variety of problems tackled and analytical methods used in individual countries, each participant decided the suite of elements considered. The first exercise revealed systematic discrepancies found for some elements between non-destructive methods (e.g. INAA, PIXE, XRF) and some analytical methods, where sample dissolution is needed before the final measurement. The discrepancies were attributed to different sample digestion procedures and after their harmonisation they were not observed in the second exercise. Overall, only about 5 % of all submitted results were rejected as outliers after thorough statistical evaluation. The participants showed their ability in providing analytical results at up to about 20 % uncertainty level for most trace elements, which was found to be adequate for biomonitoring purpose. All the participants also developed written protocols for sample collection and preparation.

2.3 Quantification

Quantification studies were defined as assessment of a quantitative relationship between the elemental content of the biomonitor species and the (wet or bulk) deposition of the atmospheric concentrations. This topic included initial studies with regard to sample preparation, i.e. if and, if yes, how to wash the biomonitor prior to analysis. The essence of biomonitoring is to provide data on xenobiotics contained within the organism and not on adsorbed on its surface. Therefore, there is evident need to clean the organism surface prior to its analysis, in particular in countries having high proportion of dry deposition. However, cleaning procedure should be confronted with the possibility of leaching trace elements from the biomonitor if washed for an extended period of time. It was confirmed by several experiments that rinsing with distilled water for less than a minute is the best compromise. Only in countries with almost exclusive wet deposition of contaminants (expected to be present mostly in a dissolved form) such rinsing may be avoided.

Quantification studies were carried out under very different climatic conditions ranging from prevailing wet to prevailing dry deposition patterns. In Norway, for instance, significant

positive correlations between biomonitor content and precipitation data for two different moss species were found for As, Cd, Co, Fe, Mo, Pb, Sb, Tl, V, and Y. Extensive quantification experiments on lichens and airborne particulate matter were carried out in Portugal in places with prevailing dry deposition. Although sophisticated models taking into account lichen surface, wet and dry deposition, and wind direction were developed, reliable calibration relationships could not yet be derived.

All participants tested several bioindicators from the same area thus allowing for interspecies calibration. This is important for two reasons: (1) to select the most appropriate biomonitor available at certain area, and (2) to apply in the areas where the most appropriate biomonitor is not available another one knowing his response in relation to the previous one.

2.4 Time resolution

Assessments of the element accumulation rate in the biomonitor allowing estimating the time needed for the monitor to reflect new element atmospheric/deposition conditions were made. Experiments carried out in Norway on mosses showed that the uptake rate from wet depositions is minute range, i.e. in less than one hour for the elements studied. Studies also showed that some moss species could provide data on temporal trends, on a yearly basis. The picture obtained in dry areas such as in Portugal on exposed lichens revealed more complicated situation where both wet and dry deposition should be accounted for. Nevertheless, exposed lichens might reflect changes at a monthly scale. Some kinds of biomonitors such as poplar leaves may provide only seasonable data during their growing period.

2.5 Geographical resolution

Geographical resolution is the resolution strength of the biomoinitor in a spatial sense. This means that local variability in biomonitor responses, survey design (grid density) and spatial variability in deposition and atmospheric concentrations of trace elements should be considered simultaneously. In many surveys, a sampling site is simply selected or defined as a small spot relative to the dimensions of the total survey area. However, in carrying out meaningful surveys, attention should be focused not only to analytical uncertainty in determining particular trace elements, but also on local variances due to biological and other variances at a sampling site. To account for this, pooled samples should be taken from an area rather than a spot (e.g. from 2000 m^2), and at least 10-20% of samples should be tested for intra-site variability by analyzing at least 5 local individual sub-samples. Local sites and sampling strategies should be carefully examined before surveys, aimed at assessing local distributions, and expressed in number of samples to be taken, sample handling and analysis. During a survey, strict procedures and control should be agreed upon with respect to sample handling in all cases where multiple samples are to be processed into single analysis procedures [8,9].

2.6 Survey studies

Survey is an assessment of geographical differences and/or time trends in deposition and/or atmospheric concentrations by the determination of elemental content of the sampled biomonitor species. The surveys carried out comprised areas within the participating countries from Africa, Asia, the Caribbean, Europe and Latin America. In some countries biomonitoring for assessing trace element deposition data was used for the first time, whilst in other countries, mainly the developed ones, it has been already a long-year practice. The case studies included: (1) single emission sources such as coal-fired power plant (Israel),

thermometer factory (India), various industrial complexes (China, Portugal), mining areas (Ghana), (2) urban areas (Argentina, Brazil, Chile, China, India, and (3) large-scale surveys (Argentina, Germany, Israel, Jamaica, The Netherlands, Norway, Romania, Russian Federation). The areas studied ranged from about 1,000 km^2 up to about 300,000 km^2, proving that biomonitoring could be used at a regional scale. In many cases soil samples were assayed simultaneously to add value to the surveys by accounting for possible soil re-suspension contribution. The sampling network in Romania is shown in Figure 1.

Figure 1: Sampling network in Romania

Participants from India, Jamaica and Russian Federation included in their survey scanning electron microscopy analysis of individual particles trapped on the biomonitor surface, thus obtaining additional information regarding discrimination of local pollution sources from long-range transport. An example of trapped particles on moss collected in Russian Federation is shown in Figure 2. The first three particles are consisting mostly of iron, whilst the last one shows diatomic alga. Organism *Tillandsia recurvata* forming composite balls proved to be a dual monitor of air pollution; besides acting as bioindicators it traps large amounts of airborne particulate matter within its form allowing for analysis of both materials from the same sampling site.

An important part of transforming data into information is data evaluation and interpretation, and pollution source apportionment. Variety of tools were applied for this purpose starting from simple correlation plots, calculation of enrichment factors, applying descriptive statistics, regression and analysis of variance, up to more advanced multivariate analysis, cluster analysis, principal component analysis and factor analysis. Bootstrap procedures and Monte Carlo aided target transformation analysis proved to be useful tools in

accounting for local variability. German and Dutch participants were able to compare deposition data with human health indices showing future application of the accumulated deposition data linked with epidemiological evidence.
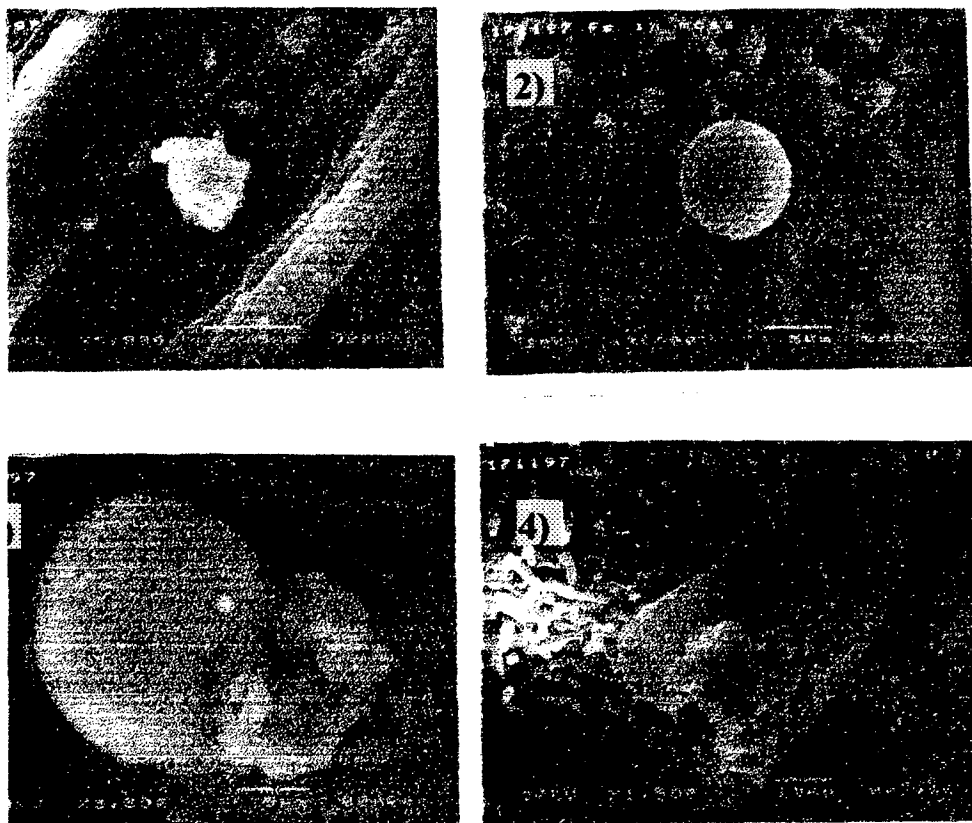

Figure 2: Electron microscope images of particles trapped on moss, collected in Russian Federation.

2.7 Mapping

Mapping is graphical representation of the biomonitoring results, mostly referred to cases where local and/or regional surveys are carried out. It is an efficient way of presenting survey data to general public. Within the CRP, the presentations varied from simple lines connecting points with equal element content found in bioindicators, to much more sophisticated coloured plots based on geographical information systems and advanced graphical tools. It turned out very useful to present distribution maps in terms of factors obtained after applying multivariate statistics to the measurement database; in such approach a particular pollution source is usually better visualised. Another useful approach for general public presentation is creation of simple "pollution coefficients" by combining individual components into one single value broadly describing the pollution level at a certain sampling site, and creating a map containing those values.

2.8 Impact studies

Impact studies were defined as assessment of changes in biomonitor parameters as a result of ambient and/or internal conditions, by quantifying selected physiological/ biochemical parameters with respect to varying extent of deposition and/or atmospheric concentrations. Changes in the selected parameter values might lead to changed relationship between (1) monitor and (2) deposition and/or atmospheric concentration data. Studies carried out mainly in Argentina and Israel included determination of several parameters aimed at evaluating vitality and degree of stress induced in lichens and *Tillandsia* species due to environmental conditions. The proposed parameters include measurements of cell membranes integrity by electrical conductivity, ethylene production, photosynthetic parameters (chlorophyll), and lipid peroxidation product estimation. Measurement results could be presented in form of pollution index, foliar damage index, or parameters of lichen vitality, and included as separate parameter in subsequent data evaluation by multivariate statistics.

3   CONCLUSIONS

-    This paper presents an overview of work carried out and summarises conclusions found within the IAEA CRP on "Validation and application of plants as biomonitors of trace element atmospheric pollution analysed by nuclear and related techniques". More details obtained within each of 14 participating countries may be found in their individual papers published in this issue.

The results reconfirmed applicability of lower plants, and in some cases higher plants, for assessing: (1) the degree of atmospheric pollution around point sources and (2) trace element atmospheric deposition on large geographical areas.

It was revealed that there is no universal bioindicator, but several regional ones, depending on climatic conditions within investigated areas. Where several organisms are used within a survey, they should be intercalibrated to yield comparable results. Furthermore, sampling, sample preparation and analysis procedures should be harmonized before surveys if they are to be quantitatively compared afterwards.

Epiphytic plants from the genus *Tillandsia* have been identified as potentially highly appropriate biomonitor for tropical and subtropical Americas, due to its physiological characteristics and wide geographical availability. It is presently being extensively tested in 12 Latin American countries within an IAEA technical cooperation project.

Physiological parameters should be measured in addition to trace element content whenever possible. Changes in those parameters might lead to different element uptake and consequently to deterioration of survey quality.

With the growing body of high quality biomonitoring data new research areas are expected to be explored, in particular in linking deposition data with epidemiological evidence, e.g. mortality, disease and other health indices. Such correlations would add precious value to other health-related studies and contribute significantly to identifying possible sources of disease at large geographical areas.

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