

Applying biological monitors and neutron activation analysis in studies of heavy-metal air pollution

Moss, tree bark, lichens and other materials can yield important information about environmental pollution and its likely sources

by M. de Bruin

Growing concerns about current and future levels of environmental pollution have led to an increased demand for experimental methods to study the environment. Although still under development, the application of biological monitors, or indicators, may provide important information on the levels, availability, and pathways of a variety of pollutants in the environment.

In such applications, relevant information is commonly deduced from studying an organism used for monitoring (the monitor organism). One approach involves analysis of changes in the organism's behaviour, such as abundance, diversity, ecological performance, or morphology. A second approach entails analysis of concentrations of specific substances that may accumulate in the organism's tissue. Typical examples of the first approach are the use of species-distributions of lichens as indicators for ambient sulphur dioxide levels and the behaviour of selected fish species in relation to water quality.

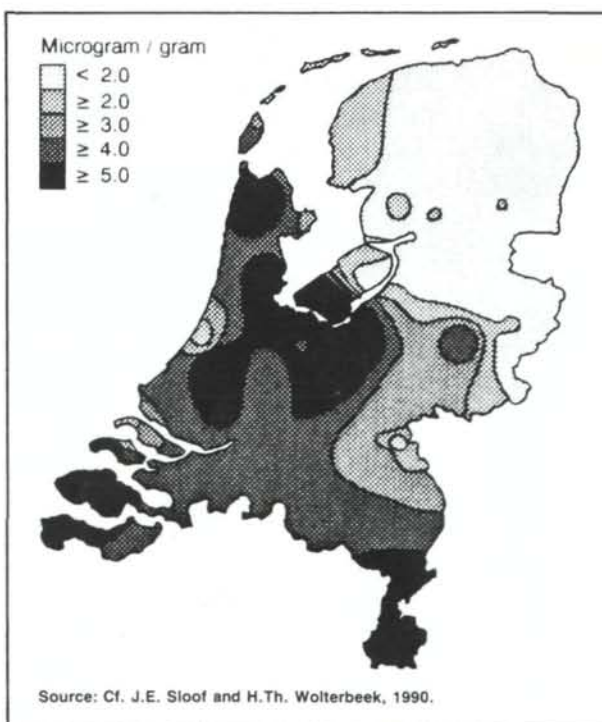
The second approach includes procedures in which the concentrations of trace substances in an organism are used as a measure of the concentrations or availability of these substances in certain parts of the environment. This approach will be discussed in more detail in this article. Attention will be focussed on the "biomonitoring" of heavy-metal air pollution and the role of instrumental neutron activation analysis (INAA) in such procedures. To illustrate the value of the combination of biomonitoring and INAA in air pollution studies, some results will be given that were obtained in a recent study in the Netherlands.

Biomonitoring of air pollution

The use of biological materials for monitoring heavy-metal air pollution was introduced about 30 years ago. Since then, a variety of materials and organisms has been proposed for biomonitoring purposes. These include mosses, lichens, tree bark, tree rings, pine needles, leaves, grass, ferns, or animal tissues such as feathers, hair, and liver.

A detailed comparison of the most commonly used materials leads to the conclusion that mosses and lichens

Figure 1. Concentrations of cadmium in *Parmelia sulcata* in the Netherlands, 1986-87.



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and, to a lesser extent, tree bark are the most suitable monitors of heavy metals in the atmosphere. However, for all biomonitors used, the mechanisms of metal uptake and retention are still not sufficiently known. Consequently, the *quantitative* relationships between the element's concentration in the monitor's tissue and its concentration in a relevant compartment of the atmosphere are not predictable. Therefore, without extensive calibration under all relevant conditions, the use of even one of the most suitable organisms will yield only *qualitative* information on atmospheric levels as a function of time or place. Probably for this reason, most publications on biomonitoring of air pollution refer to observed relationships between element concentrations in the biological monitor and the known levels or sources of heavy materials. Only in a few cases have biological monitors for air pollution been applied in realistic environmental situations, rather than in *a priori* known situations.

Biomonitoring programme in the Netherlands

In 1986 in the Netherlands, a biomonitoring programme was started using the epiphytic lichen *Parmelia sulcata* as monitor for heavy-metal air pollution. Full details and results of the study, which followed an earlier programme in 1982, are being published.

Samples were collected at 250 sampling sites that covered the whole country. Lichen samples were separated from the bark substratum, washed, dried, and homogenized. Aliquots of 30-100 milligrams dry weight were analysed using the Interfaculty Research Institute (IRI) system for routine INAA. A standard protocol was used comprising an irradiation of 30 seconds followed by a measurement after 15 minutes, and a second irradiation of 4 hours followed by two measurements after 5 days and 20 days, respectively. The irradiations were carried out in the institute's 2 megawatt research reactor. In a separate sample aliquot, lead was determined by graphite-furnace atomic absorption spectroscopy, after acid destruction.

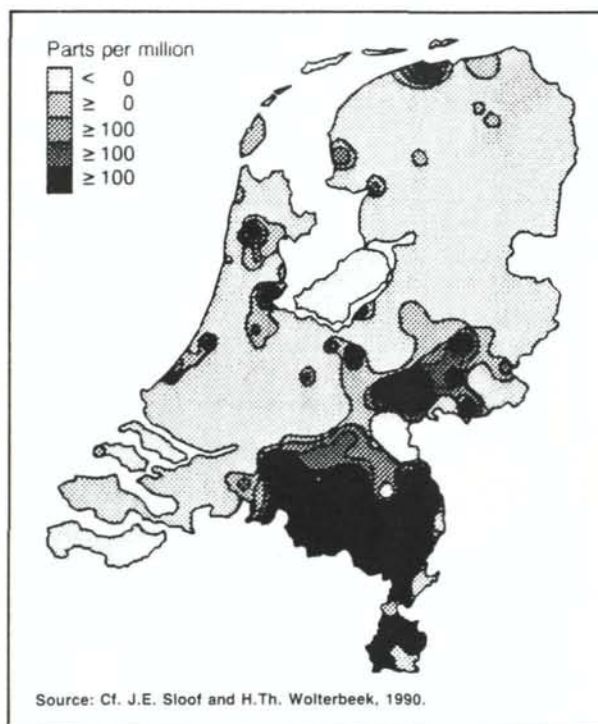
For the interpretation of the data, two methods were applied. In a straightforward approach, concentrations of the individual trace element were plotted geographically (See Figure 1 for an example.) Such plots give direct insight into regional variations of the heavy-metal air pollution. Moreover, combining these plots with information on prevailing wind direction may provide information on the geographical positions of individual sources of air pollution. But, as can be expected, this method of source appointment yields adequate results only in uncomplicated situations with only a few dominant sources. Both the 1986 study and the earlier 1982 study show that this approach alone is not successful in the Dutch situation, which is characterized by numerous sources of pollution inside and outside the country.

In another approach, additional information was derived from correlations existing between the concen-

trations of different elements. For most sources, the emissions are not limited to only one element. They comprise a mixture of toxic and non-toxic elements with a concentration pattern characteristic of specific sources. Therefore, the observed correlations and ratios between concentrations of different elements may provide significant information on the nature of the contributing sources.

The relevant information can be deduced from the analytical data by a specific multi-variate statistical procedure known as "Target Transformation Factor Analysis" (TTFA). From the concentration patterns observed in the set of samples, sets of correlated elements and associated concentration profiles (factors) are deduced. These can be regarded as (hypothetical) emissions from pollution sources. On the basis of the nature of the constituting elements and their ratios, the hypothetical emissions can often be identified as materials from specific realistic sources. The identification can further be improved by plotting geographically the calculated contributions from the different hypothetical emissions for each sampling point. (See Figure 2 for an example of such a plot.) This combination of element concentration patterns and geographical distributions allows for the unambiguous identification of pollution sources even in a very complex environment.

Figure 2. Calculated contribution from zinc-smelters to the cadmium concentrations in the Netherlands.



This computer generated map of the Netherlands shows the geographical distribution of the calculated contribution to the total concentration in *Parmelia sulcata* of assumed cadmium emissions from zinc smelters and/or the electronics industry. The observed maxima nicely coincide with known concentrations of non-ferrous metal industries in the southeastern part of the country.

Analysis of samples in biomonitoring

Analysis of collected samples constitutes a large part of the total effort required in biomonitoring programmes. A number of analysis techniques currently are used for studying heavy-metal air pollution. These techniques include induction coupled plasma emission spectroscopy (ICPES); atomic absorption spectroscopy (AAS); X-ray fluorescence analysis (XRF); charged particle induced X-ray emission analysis (PIXE); and instrumental neutron activation analysis (INAA).

The success of the biomonitoring programme as a whole fully depends on the quality of the analytical data. When selecting the proper analysis method, a number of aspects has to be taken into account. Particularly relevant are multi-element capability; sensitivity; accuracy and precision; analysis costs; sample characteristics; and availability.

Multi-element capability. It is clear that in heavy-metal air pollution studies the analysis procedure applied to the monitor material will be focussed primarily on the concentrations of these heavy-metal elements. The spectrum of elements should be as broad as possible to minimize the chance of overlooking any unexpected pollutant. When possible, the analysis procedure should also provide information on accompanying non-toxic elements. The concentration patterns of these additional elements may provide information on the nature and origin of certain pollutants, and for discriminating between individual natural and anthropogenic sources.

Sensitivity. The detection limits for heavy metals must be low enough to enable study of these elements in very early stages of pollution. In addition, a broad spectrum of non-toxic trace elements should be detectable in the majority of the samples if trace element concentration patterns are to be used in source appointment procedures. The required sensitivities can be deduced from data of the concentrations of 34 elements determined by INAA in Dutch lichens. (See Table 2.)

Accuracy and precision. Results of environmental monitoring programmes may establish the basis for often very expensive measures for environmental protection. Therefore, high demands are made on the accuracy of the analytical techniques employed. However, when using biological monitors, the precision of the whole procedure is limited by biological variations in the response of the monitor organism. For the analytical procedure, no extreme precision is required.

Analysis costs. Biomonitoring programmes generally comprise several hundreds to several thousands of samples. Therefore, the analysis method should be applicable on a routine scale and the costs of the analysis should be acceptable when compared to the total costs of the monitoring programme. The high costs associated with collecting and preparing samples, coupled with the administrative procedures and data interpretation, make it worthwhile to apply an analytical procedure that provides maximum information from the samples. Economizing too much on the analysis procedure easily becomes a matter of "penny-wise and pound-foolish".

Sample characteristics. Some materials commonly used for biomonitoring air pollution can be easily digested (or dissolved) prior to analysis. As a result, they can be analysed by techniques that require solid samples just as well as by techniques that are only suitable for solutions. For plant materials, however, a careful and time-consuming procedure is required to assure the sample's complete destruction. Moreover, lichens usually contain mineral particles, which further complicates the destruction procedure. Therefore, for plant materials, a method of non-destructive analysis has to be preferred.

Availability. The analytical method to be employed in a biomonitoring programme should be well developed and mature, so that the analytical activities can be properly planned in advance together with other project activities. The available analysis capacity should be sufficient to absorb the significant workload associated with a monitoring programme. Moreover, contact is required between field workers who collect the samples and the analyst, to assure proper sampling and sample preparation procedures.

Comparison of analytical techniques

There are many pitfalls associated with making a comparison between analytical techniques. Moreover, local conditions, such as availability or easy access to a certain facility, will strongly determine the choice of a proper analytical method. The comparison presented here (see Table 1) should be regarded as a personal view; it does not pretend to give a detailed, absolute, or generally valid rating of techniques.

For this comparison, ICPES seems to be the method of choice, provided the matrix can easily

be dissolved. But INAA is preferred for difficult materials, such as lichens, that require laborious destruction procedures with the associated consequences for costs and accuracy. AAS is basically a single-element method and therefore only applicable in well-established situations where the monitoring programme can be focussed on only

one or two elements. If information on a larger number of elements is needed, consecutive analyses have to be carried out which will easily lead to prohibitive costs per sample. The major limitation of XRF is its limited sensitivity. PIXE is primarily a micro-beam technique and less suited for accurate bulk analysis.

Table 1. Applicability of analytical techniques in biomonitoring heavy-metal air pollution

Technique	Aspect					
	Multi-element capability	Sensitivity	Accuracy and precision	Costs	Sample characteristics	Availability
ICPES	+	+	+	+	+/-	+
AAS	-	+	+	+	+/-	+
XRF	+	-	+	+	+	+
PIXE	+	+/-	+/-	-	+	+/-
INAA	+	+	+	+/-	+	+/-

ICPES = Induction Coupled Plasma Emission Spectroscopy.

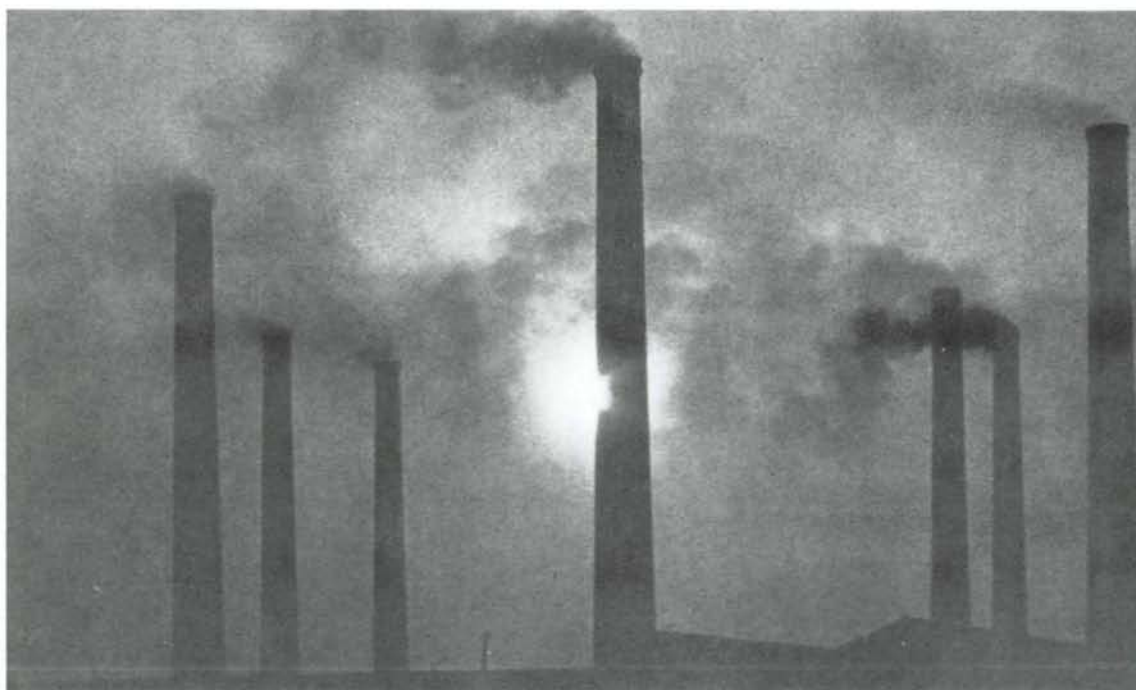
AAS = Atomic Absorption Spectroscopy.

XRF = X-ray Fluorescence Analysis.

PIXE = Charged Particle Induced X-ray Emission Analysis.

INAA = Instrumental Neutron Activation Analysis.

Studies of air pollution sources and pathways are increasingly being done in many countries.



Features

Selected results

In the 1986 Dutch study, 34 elements were commonly observed in the lichen samples. (See Table 2.) The statistical procedure (TTFA) was applied to the concentration data of 15 selected elements and it yielded 10 "factors" of hypothetical emissions. (See Table 3.) On the basis of the element concentration patterns, most of these hypothetical emissions can be assigned to specific sources:

- **Factor 1:** crustal material;
- **Factor 2:** zinc smelters and/or electronic industry;
- **Factor 3:** oil processing or combustion;
- **Factor 4:** waste incineration or non-ferrous metal industry;
- **Factor 5:** probably coal combustion;

- **Factor 6:** unknown;
- **Factor 7:** probably waste incineration;
- **Factor 8:** traffic;
- **Factor 9:** unknown;
- **Factor 10:** unknown.

Geographical distributions also were done of the contributions from the (assumed) emissions from specific sources.

Only a small part of the study's results have been referred to in this article. Nonetheless, they clearly show the power of the application of lichens as biological monitors, when combined with INAA as a multi-element analysis procedure and with TTFA techniques to interpret observed trace element patterns for purposes of establishing and identifying sources of heavy-metal air pollution.

Table 2. Trace elements in the lichen *Parmelia sulcata* in the Netherlands, determined by INAA (1986)

Element	Concentration in mg/kg		
	Mean	Range	INAA detection limit
Aluminum	5800	230 - 21000	100
Arsenic	5.7	0.5 - 17	0.1
Bromine	56	15 - 170	0.1
Calcium	4700	1100 - 21000	300
Cadmium	2.8	0.6 - 21	1
Cobalt	2.0	0.20 - 6.3	0.1
Chromium	26	4.0 - 270	1
Caesium	0.8	0.2 - 5	0.3
Copper	33	1.4 - 120	10
Iron	5800	720 - 30000	30
Gallium	47	2.3 - 180	3
Hafnium	1.4	0.09 - 8	0.1
Mercury	0.5	0.1 - 36	0.1
Iodine	15	5.3 - 32	3
Potassium	5200	1300 - 16000	100
Lanthanum	6.2	0.8 - 64	1
Lutetium	0.071	0.009 - 0.46	0.01
Magnesium	1800	490 - 3800	100
Manganese	110	16 - 400	1
Sodium	1000	260 - 5400	1
Nickel	16	1.9 - 51	3
(Lead)	(150)	(3.1 - 370)	
Rubidium	13	1.8 - 62	0.3
Antimony	3.3	0.3 - 12	0.1
Scandium	1.1	0.16 - 5.2	0.3
Selenium	1.8	0.4 - 7.5	1
Strontium	50	8.5 - 400	30
Thorium	1.2	0.14 - 8.9	0.1
Thallium	490	78 - 2000	100
Uranium	0.57	0.089 - 2.7	0.3
Vanadium	32	5.7 - 99	1
Tungsten	0.9	0.1 - 10	0.3
Ytterbium	0.4	0.078 - 3	0.03
Zinc	210	61 - 1100	1

Features

Future directions

Biological monitors, combined with an analytical technique that provides information on concentrations of a broad pattern of elements, have proved to be a powerful tool for detecting and identifying sources of heavy-metal pollution. If lichens are used as monitor organisms, INAA is the analytical method of choice. It provides accurate data on a broad range of elements. As no labourious sample preparation or destructive steps are required, INAA can be economically competitive with other multi-element analysis techniques.

Application of INAA on a large scale, as relevant in environmental monitoring programmes, requires certain measures to assure or improve its practical usefulness or applicability. Research reactor facilities should be willing to carry out a large number of routine analyses and

to take the necessary infrastructural measures to avoid unwanted interference between routine analysis and scientific research. Moreover, quality assurance procedures have to be developed so that the intrinsic accuracy of INAA is indeed reflected in the quality of the results of large-scale routine analysis.

For obvious reasons, INAA requires direct access to a research reactor. At present, the potential use of activation analysis is already very strongly limited, since only a small number of such reactors is presently in operation. Further reduction of this number will gradually lead to the end of the many important applications of activation analysis in environmental research.

Table 3. Results of the Target Transformation Factor Analysis

Element	Normalized factor compositions									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Aluminium	420 000				12 000			370	98 000	
Arsenic		1.8		2.3						
Bromine					100					
Cadmium		0.36		100			5.8			
Cobalt			6.6							
Chromium	2 300	15								
Caesium	72			0.28	0.26				11	
Iron	380 000				3 200	29 000				
Mercury						100				
Lanthanum							100			
Manganese								9.8		100
Nickel			100		4.0	34				
Lead					43			100		
Antimony		1.1					13			
Scandium	100				0.44					
Selenium				22						
Thorium	140									
Vanadium			150		9.4			4.3	380	
Tungsten		0.35							100	
Zinc		100								

Note: Concentrations are normalized to a concentration equalling 100 for the element regarded as characteristic of each factor.