

# **PERSPECTIVES IN BIOMONITORING OF AIR POLLUTANTS WITH PLANTS**

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## **ABSTRACT**

European air quality policy aims at defining and establishing objectives for ambient air quality designed to avoid or reduce harmful effects on human health and the environment. In line with this goal, the European Commission has recently proposed the use of bioindicators to assess regional patterns of air pollutants and the development of an EU wide biomonitoring system to effectively address environment and health linkages. This has generally affirmed the use of biological methods in environmental monitoring and planning and has given incentive to improve the existing techniques. In many countries plants have long been used as reactive and accumulative indicators to confirm phytotoxic impacts of air pollutants and to demonstrate the discharge of toxic compounds into the environment and food chain. However, the methods have not been harmonised to date and the observed plant responses may not always be related to adverse effects of air pollutants on health and ecosystems. The paper focuses on recent developments and perspectives in biomonitoring of air pollutants. Examples for active and passive approaches are given for classical air pollutants, which continue to be a problem in many emerging economies, and for pollutants which have come to the environmental agenda more recently. International and national biomonitoring programmes are reviewed with the final suggestion to intensify the efforts to harmonise methodologies.

## **INTRODUCTION**

Foliar injuries caused by industrial fumes have long been used as the basic bioindication method for acute air pollution effects and visible plant or leaf damage still remains a widely-used meaningful criterion, e.g. in forest inventories and pictorial atlases, to describe adverse effects of environmental pollutants in the field [1, 2]. Other ecosystem oriented bioindication methods relate to the presence or absence of indicator species, e.g. lichens, and the evaluation of the environmental quality by so-called ecological indicator values [3], which are derived from vegetation relevés. Despite latter methods require special botanical knowledge such approaches are reasonable when effects of air pollutants on nature and biodiversity need to be assessed. A comprehensive overview on individual bioindication studies performed and approaches applied more widely throughout the 1990s was given in various WHO studies [4-6], in which also the need for international harmonisation of methods was urged. However, the German Association of Engineers (VDI) is currently the only organisation worldwide, which has initiated the national standardisation of bioindication methods [7]. Table 1 gives an overview on available VDI guidelines, which have all been translated into English. Using some of these highly standardised methods the EuroBionet network was the first EU funded project, in which co-ordinated biomonitoring activities were applied on a larger level [8]. Other pan-European biomonitoring programmes using standard protocols are being performed within the UN-ECE ICPs Vegetation and Forests. While in ICP Vegetation emphasis is laid

on the effects-based monitoring of tropospheric ozone, heavy metal and nitrogen deposition in crops and semi-natural vegetation at various sites in Europe [9], ICP Forests performs a monitoring of forest condition on a representative, systematic grid net throughout Europe [10].

As an important element within its integrated environmental policy the European Commission has recently proposed a European Environment and Health Strategy [11]. In chapter 5 of this document it reads that the “*community approach entails the collection and linking of data on environmental pollutants in all the different environmental compartments (including the cycle of pollutants) and in the whole ecosystem (bio-indicators) to health data (epidemiological, toxicological, morbidity).*” Also the recent EU daughter directive on air pollution by heavy metals and hydrocarbons [12] suggests the use of bioindicators in order to assess regional impacts of air pollutants on ecosystems. Both policy instruments will encourage the further use of biomonitoring in local, regional and international programmes. At the same time the demand is rising for the development of a unique and permanent harmonised European biomonitoring system.

In this paper we will review recent biomonitoring programmes and perspectives for the harmonisation of methods will be discussed. While most of the presented methods have originally been developed and applied in Europe, the reasonable use of these approaches in other regions of the world will be demonstrated. Namely, biomonitoring is meaningful especially in those countries, in which economic growth has reached a non-sustainable dimension. However, it may also be used for environmental reporting and policy evaluation studies in previously polluted regions, in which political measures have been introduced.

## BIOINDICATION AND BIOMONITORING METHODS

### Definitions

**Environmental indicators** refer to highly aggregated economical or political figures and have been derived to test whether the development in a region or a country stays within sustainable limits. They have been designed to identify driving forces in, pressures on, the state of, impacts on and responses to the environment. For environmental indicators relevant to Europe refer to [13]. In contrast, the use of **bioindicators** refers to the consideration of clear dose-response relationships in specific organisms. In order to describe differences between environmental indicators and bioindicators [14] states that the latter should be used in “*nature and biodiversity management, and in situations where organisms and their functions are a part of the solution to environmental problems*”.

Principally, bioindicators may be differentiated into response and accumulation indicators. While the **response indicator** shows distinctive biological effects, e.g. foliar injury upon the exposure to a gas, **accumulation indicators** may show enhanced concentrations of a chemical compound without exhibiting a decreased vitality. A further differentiation of methods is made whether an organism is actively exposed in the field or whether a sample is taken from plants growing in the field (passive biomonitoring). Examples for bioindication methods and the differentiation between the different approaches may be followed in Table 1. Other references on general principles of bioindication and biomonitoring as well as on general plant responses to air pollution are given in [15, 16].

**Table 1.** Bioindication guidelines of the 3957 series (completed and in preparation) drawn up by the German Association of Engineers (VDI), Commission on Air Pollution Prevention of VDI and DIN - Standards Committee KRdL [7].

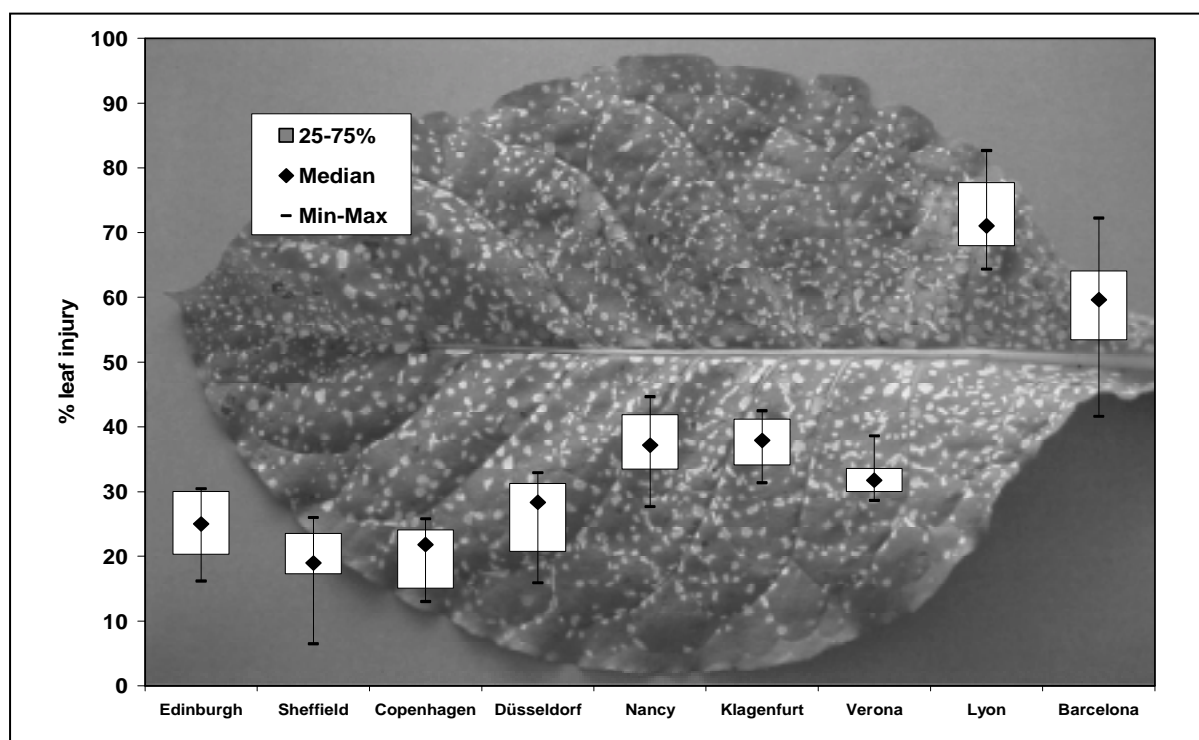
Guideline No.	Title	Status	Language	Method *
VDI 3957 part 1	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication) - fundamentals and aims	final 05.99	German/English	
VDI 3957 part 2	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication). Method of standardised grass exposure	final 01.2003	German/English	A, a
VDI 3957 part 3	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication). Standardised exposure of green cabbage	final 12.2000	German/English	A, a
VDI 3957 part 4	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication). Standardised exposure of green cabbage (food chain)	draft expected 2005	German (English due in 2006)	A, a
VDI 3957 part 5	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication). Standardised exposure of spruce	final 12.2001	German/English	A, a
VDI 3957 part 6	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication). Determination of the phytotoxic effects of ozone and other photooxidants. Standardised exposure of tobacco	final 04.2003	German/English	R, a
VDI 3957 part 8	Determination of the Growth Rate of Epiphytic Lichens for Ecological Long-Term Monitoring	final 01.2003	German/English	p
VDI 3957 part 10	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication). Recommendation for the use of bioindicators for monitoring air pollution effects of emission	Draft 11.02 guideline expected 2004	German (English due in 2004)	
VDI 3957 part 11	Standardisation of the sampling of leaves and needles for passive biomonitoring of ambient air pollutants	Draft expected 2004	German (English due in 2004)	A, p
VDI 3957 part 12	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication). Standardised mapping of moss diversity	Draft expected 2004	VDI 3957 part 12	p
VDI 3957 part 13	European standard for mapping lichen diversity as an indicator of environmental stress	Draft (German/English) due in 2004	VDI 3957 part 13	p
VDI 3957 part 14	Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication). Phytotoxic effect on inorganic fluorides in ambient air – method of standardised exposure of gladiolus	Draft 02.2004	German (English due in 2004)	R, a

\* denotes “A” accumulation indicator, “R” response indicator, “a” active biomonitoring and “p” passive biomonitoring.

## Response indicators

Tropospheric **ozone** is one of the most important air pollutants, which is known to have adverse effects on plants. Although research on ozone was already initiated in the US in the 1950s, there is still much uncertainty and debate on air quality criteria for this phytotoxic compound. In Europe, critical levels and threshold concentrations for the photo-oxidant ozone have recently been reviewed and updated by [17-19].

The classical response bioindicator tobacco (Bel-W3 cultivar) has been employed in numerous studies worldwide to provide evidence for adverse effects of tropospheric ozone. The extent of necrotic leaf area is used in this method to derive dose-response relationships. A recent example from EuroBionet based on a highly standardised protocol is presented in Figure 1.

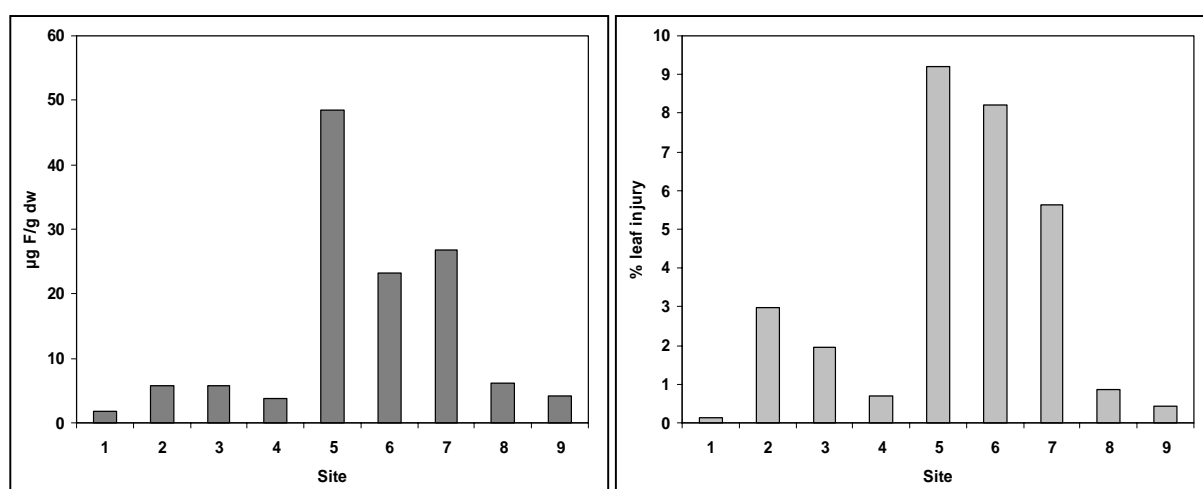


**Figure 1** Results of the exposure of tobacco Bel-W3 within the EuroBionet during eight consecutive bi-weekly periods in 2001 showing the distribution of site means [20]. Plant cultivation and exposure was performed according to the standardised method VDI 3957 part 6 (see Table 1).

The origin of the ozone sensitive tobacco cultivar is the Eastern US. It has often been argued to use plant species for bioindication, which are native to the region under study. Within the UN-ECE ICP Vegetation ozone biomonitoring, clones of white clover (*Trifolium repens*) are applied, which originate from the US as well. However, white clover is native to the Northern hemisphere and has been introduced as a fodder plant to the Southern hemisphere. The clover bioindicator system makes use of a sensitive (NC-S) and a resistant line (NC-R). Although the sensitive clones may respond with specific foliar injuries to ozone, it is the biomass ratio NC-S / NC-R, which is used for the bioindication of ozone. Over the past five years, many institutions performed experiments with the clover clone biomonitoring system across Europe

and a relationship between cumulative ozone doses (AOT40) and biomass ratios could be derived [9]. However, future approaches will take into account the flux concept to derive flux-response relationships in crops, wild plant species, semi-natural vegetation and forests [17].

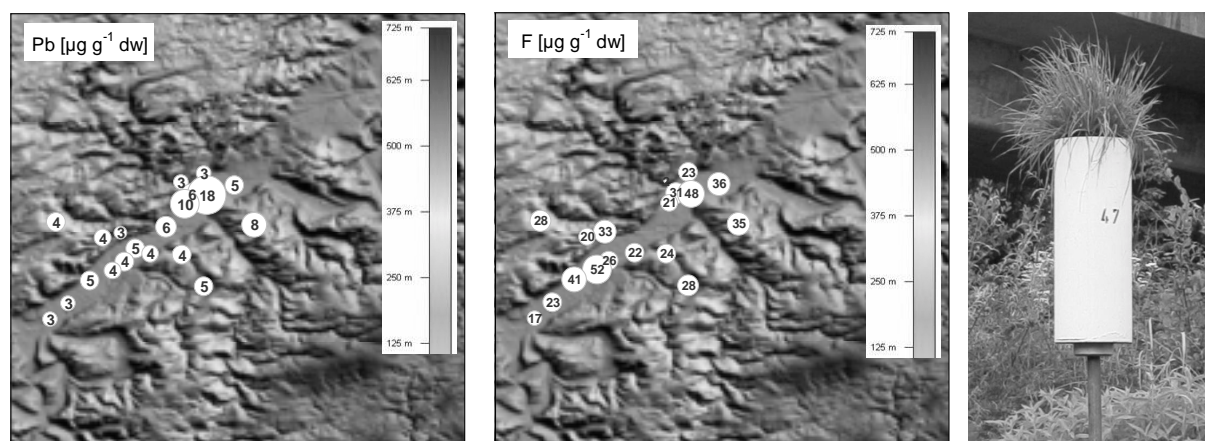
In many regions, **fluoride** from industrial sources causes damage in adjacent vegetation and bioindicator methods may be used to address the severity of such emissions. The extent of necrotic lesions on *Gladiolus* leaves has been employed as an effect criterion in many studies [21, 22]. Recently, the results of single studies have been evaluated and a standard protocol for the use of *Gladiolus* as fluoride bioindicator has been laid down in VDI 3957 part 14 (see Table 1). Figure 2 gives an example of bioindication of fluoride emissions at an industrial site in Brazil. Because fluoride accumulates in the plant, the concentration of this compound can also be analysed in active or passive accumulation indicators (see below).



**Figure 2.** Results of the exposure of *Gladiolus* cv. ‘White Friendship’ in an industrial area in SE-Brazil showing fluoride contents of leaf tips (left) and percentage of fluoride-induced leaf injury (right) as means of 26 periods of four weeks each.

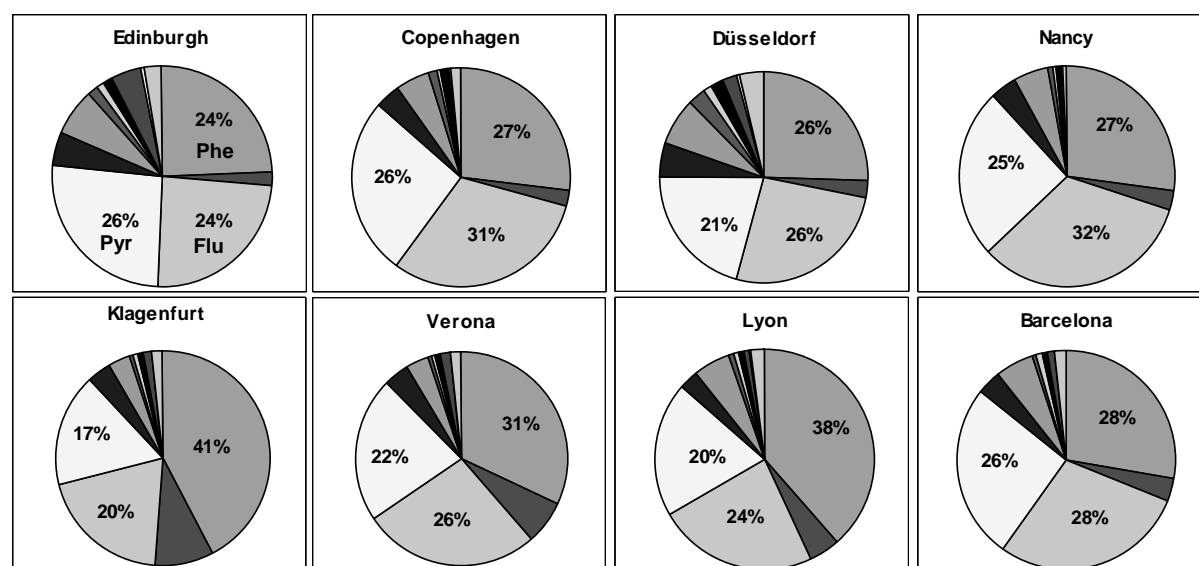
### Accumulation indicators

Analysing environmental pollutants in plant leaves is generally regarded as a suited biomonitoring method, especially when the plants under study are an important part of the food chain and when legally binding limit values of certain chemical compounds in food or feed stuff have been defined. A common approach is the use of **active biomonitoring** with **standardised grass cultures** or **curly kale**, which are exposed in the vicinity of pollution sources during fixed time intervals (e.g. four weeks). Both approaches have extensively been applied in Germany and recently within the EuroBionet project, which resulted in the highly standardised guidelines VDI 3957 parts 2 to 4 (see Table 1). Examples for results using the standardised grass culture method are given in Figure 3. The study was part of a local air quality investigation and provided important evidence for the spatial impact of local heavy metal and fluoride emissions. Within the EuroBionet project [20], analyses of the standardised grass culture proved the significant reduction of lead in the European environment following the reduction of lead levels in petrol.



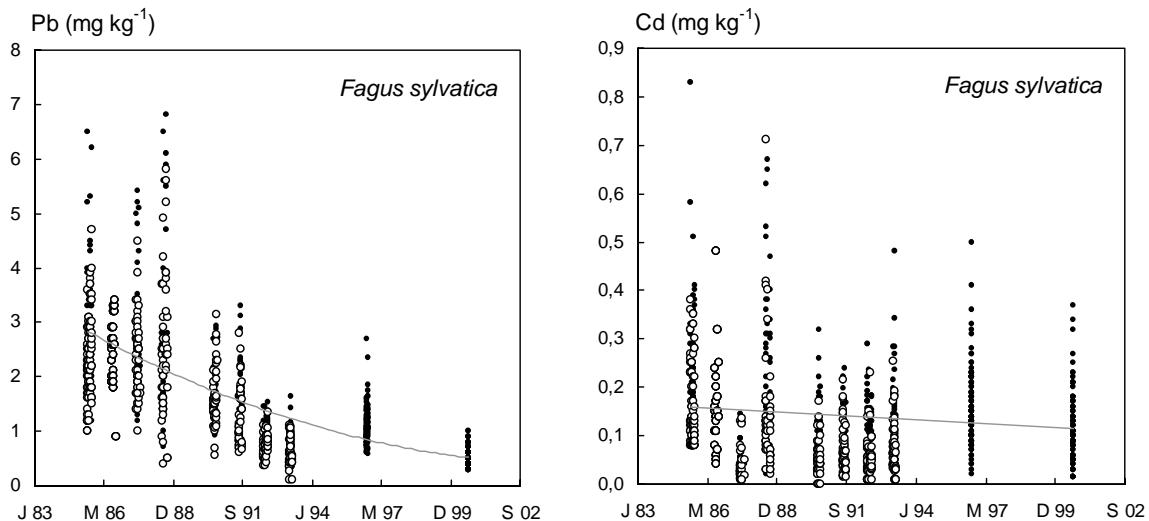
**Figure 3.** Mean concentrations of lead (left) and maximum concentrations of fluoride (centre) in standardised grass cultures (right) exposed in the Moselle valley (SW Germany) in the summer of the year 1993. The map represents a section of 30 x 30 km. Data based on 12 fortnightly exposure periods after [23]. The German Feedingstuff Regulation (FuttMV 2000, Anlage 5) and Council Directive 1999/29/EC define maximum concentrations in fodder of 40 mg kg<sup>-1</sup> for lead and 50 mg kg<sup>-1</sup> for fluoride. In the case of fluoride fodder from the vicinity to emission sources might have posed a problem for ruminants.

Active biomonitoring using edible curly kale (*Brassica oleracea* var. *acephala*) is a suited method to address spatial and temporal effects of polycyclic aromatic hydrocarbons (PAH), of which some are mutagenic and others show similar behaviour as persistent organic pollutants. Examples for this method are [24, 20], the results of latter are presented in Figure 4.



**Figure 4.** Results of the exposure of curly kale in the EuroBionet during eight weeks in 2001 showing the component profile at the sites with PAH maximum values. Data represent the concentrations of phenanthrene (Phe), fluorene (Flu), pyrene (Pyr) and other components as percentage of the sum of 12 less volatile PAH compounds [20]. Plant cultivation and exposure was performed according to the standardised method VDI 3957 part 3 (see Table 1).

The most prominent examples of **passive biomonitoring** using repeated plant collections from geo-referenced sites are the German **Environmental Specimen Banking** (Umweltprobenbank) and the **European Moss Monitoring programme** [9, 25, 26]. Latter programme was established in the 1980s and has given evidence on the general reduction of heavy metal deposition over much of Europe. It will be continued within the framework of UN-ECE ICP Vegetation to follow the efficiency of air pollution reduction policies. As an example for policy evaluation studies, results from a regional monitoring programme from SW-Germany are shown in Figure 5, confirming the significant reduction of lead deposition in the 1990s.



**Figure 5.** Results of a passive biomonitoring study in the German Federal Land Baden-Württemberg confirming the reductions of lead (left) and cadmium (right) in environmental samples. Fully expanded leaves of European Beech were sampled at reference plots in remote forest regions of SW Germany. Data after Landesanstalt für Umweltschutz (Ökologisches Wirkungskataster), LfU Karlsruhe (personal communication).

### New pollutants – new methods

While heavy metal discharges into the environment have been reduced significantly in Europe and elsewhere, environmental concentrations of other pollutants have increased in biota and soils. An outstanding example is the increased concentration in environmental samples of noble metals (Pt group), which are released as wearout from catalytic converters [27]. To study such discharges in traffic-related situations standardised grass cultures may be used [28]. Other compounds of current interest are a variety of organic substances, including POPs, chemicals and endocrine disruptors, which must be (re)evaluated according to the forthcoming EU REACH legislation [29]. Environmental monitoring of such compounds may be achieved by using accumulation indicators (e.g. standardised grass culture) or through monitoring of the associated mutagenic effects using e.g. the newly developed *Tradescantia* method [30, 31]. Finally, the continuously high deposition rates of nutrient nitrogen as NO<sub>x</sub> and NH<sub>y</sub> require modified bioindication methods, which can be employed in local or national monitoring programmes. Atmospheric nitrogen pollution in Europe is also a priority of UN-ECE ICP Vegetation and Forests, which both make use of biomonitoring methods.

## OUTLOOK

Pollutant transport via the atmosphere has a global impact and even in the remoter areas deposition will further increase levels of environmental contaminants until eventually toxic levels are reached. Hence, there is great demand for environmental monitoring using bioindicators as these methods integrate between ambient levels of compounds and their associated biological effects. The growing body of literature on biomonitoring of air pollutants especially outside Europe proves acceptance of such methods but at the same time it points up that these methods need to be internationally harmonised. Within the European Environment and Health Action Plan 2004-2010 [32] a reliable “*cause-effect framework*” will be established, which will be reached by the involvement of environment, food, health and research experts. Biomonitoring of air pollutants with plants will probably play an important role within this programme and the development of standardised protocols and the derivation of coherent assessment criteria may result from this. At the same time the EU has called within its LIFE and 6<sup>th</sup> Framework Programmes for RTD projects and coordinated actions in the field of environmental analysis and monitoring of emerging environmental pollutants. Biomonitoring could also become an important component in these initiatives to strengthen the European Research Area. But also outside Europe, the standardised application of plant biomonitoring tools has some potential, e.g. within the international ecosystem assessment initiatives, global change and health research programmes.

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