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**ENVIRONMENTAL LICHENOLOGY:  
BIOMONITORING  
TRACE-ELEMENT AIR POLLUTION**

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**ENVIRONMENTAL LICHENOLOGY:  
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TRACE-ELEMENT AIR POLLUTION**

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Aan mijn vader

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- \* Sloof, J.E. and Wolterbeek, H.Th. (1991) A national monitoring survey using epiphytic lichens as biomonitors of trace-element air pollution. *Lichenologist* **23** (2), 139-165.
- \* Sloof, J.E. and Wolterbeek, H.Th. (1991) Patterns in trace elements in lichens. *Water, Air and Soil Pollution* **57-58**, 785-795.
- \* Sloof, J.E. and Wolterbeek, H.Th. (1992) Lichens as biomonitors for radiocesium following the Chernobyl accident. *Journal of Environmental Radioactivity* **16**: 229-242.
- \* Sloof, J.E. and Wolterbeek, H.Th. (1993) Interspecies comparison of lichens as biomonitors of trace-element air pollution. *Environmental Monitoring and Assessment* **25**, 149-157.
- \* Sloof, J.E. and Wolterbeek, H.Th. (1993) Substrate influence on trace elements in epiphytic lichens. *Environmental Monitoring and Assessment* **25**, 149-157.
- \* Kuik, P., Blaauw, M., Sloof, J.E. and Wolterbeek, H.Th. (1993) The use of Monte-Carlo methods in factor analysis. *Atmospheric Environment* (in press).
- \* Kuik, P., Sloof, J.E. and Wolterbeek, H.Th. (1993) Application of Monte-Carlo assisted factor analysis to large sets of environmental pollution data. *Atmospheric Environment* (in press).

## CHAPTER 1. INTRODUCTION

### 1.1 Air pollution

The environment is increasingly affected by a growing number of atmospheric pollutants other than the "traditional" air pollutants like sulphur oxides, ozone and nitrogen oxides. The concern about the growing list of heavy metals, polycyclic aromatic compounds and halogenated organic compounds, which are emitted from anthropogenic sources as air particulate matter and gasses, has reinforced efforts to establish control programmes in the industrialized countries.

In general, the policy is both source-orientated, using technology based emission management, and effect-orientated, using for example quantitative risk assessment. A number of regulatory instruments are used by the governments of industrialized countries, including The Netherlands, to reduce emissions of hazardous air pollutants (Wiederkehr 1991): 1) setting of emission standards for individual pollutants and source categories, 2) establishment of ambient air quality standards for individual hazardous air pollutants, 3) overall reduction goals for compound classes like volatile compounds and particulate matter, 4) restrictions on production, handling and product use, and 5) action programmes for individual pollutants or classes of pollutants. In most countries several of these regulatory instruments are combined to form a basis in coordinated control policy.

Air pollutants comprise a large number of compounds with widely different environmental and health impact properties. Therefore, several countries, e.g. the United States and The Netherlands, use priority substance lists (e.g. black list substances), to set priorities for how to focus necessary control. The criteria to list a pollutant include toxicity, carcinogenic potential, persistence, dispersion properties, and bio-accumulation as well as emission quantities and levels found in the environment.

Controlling air pollutants (from anthropogenic sources) in practice is a complex problem. The pollution sources and their emissions have to be identified, the analytical methods available have to be evaluated to measure and quantify the

emissions accurately, the risks which specific emissions pose on the environment (including human health) have to be investigated, the critical emissions have to be controlled and economical aspects have to be integrated. According to Wiederkehr (1991), priority action lists, based on international consensus do not exist and the legislation is not internationally uniform. All research necessary for managing air pollutants is therefore twofold in character, namely as a basis to contribute to legislation and as a tool to respond to existing legislation.

Information on air pollutants can be obtained (similar to the policy principles), either by pollutant fate and transport modelling, which is source emission orientated, or by field measurements of the immission, which is a receptor based approach.

Since air pollutants are emitted from various sources, such as fossil fuel combustion, industrial processes, traffic, agriculture, waste incineration and transshipment of substances and gasses, problems arise to measure the emissions from sources without a specific emission outlet. Emission measurements from industrial sources can be carried out in the stack and in- or outside the plume, with appropriate air sampling devices, equipped with air filters, denuders or impact collectors (Sverdrup *et al.* 1991). These measurements of air particulate matter and gasses give local and instantaneous information on emissions or atmospheric concentrations in air particulate matter.

Dispersion models are used to calculate the atmospheric concentrations in air particulate matter in the field. These models are based on meteorological observations, particle size distributions and deposition properties, information on surface conditions (roughness) and data obtained by emission registration (van Jaarsveld 1989). Estimates on basis of dispersion models are limited by the emission registration available and these estimates cannot account for the contribution of unknown sources. During the last two decades in The Netherlands, the approach of modelling and calculating the air quality and the environmental status has gained far more interest than actual field measurements, mainly for economical reasons, such as high costs for equipment and manpower (van Duijvenbooden 1992, van Ham 1992). It should be kept in mind, that validation of dispersion models requires field measurements at the receptor side.

Immission measurements, at the receptor side, can be carried out by means of

air sampling devices and deposition samplers (wet, dry and total deposition) to obtain information on actual atmospheric concentrations in air particulate matter and on deposition in the field. Immission measurements may be used to indicate unknown sources.

In order to ensure the temporal and spatial representativeness of immission measurements, continuous sampling on a long term at a large number of sites on a large scale is required. Measurements of air particulate matter and deposition with technical equipment over large areas have been few, mainly due to the lack of sufficiently sensitive and inexpensive techniques permitting simultaneous measurements of many air contaminants at a large number of stations (Puckett 1988). Growing concern about current and future levels of environmental pollution yet demand for experimental methods to study the environment. Biomonitoring is such an experimental receptor based approach.

## **1.2 What is biomonitoring?**

Biomonitoring, in a general sense, is the use of properties of an organism or a part of it to obtain information on a certain quantity in a certain part of the biosphere.

The relevant information in biomonitoring programmes using plants or animals is commonly deduced from either changes in the behaviour of the monitor organism (species composition, -richness, ecological performance, morphology) or from the concentrations of specific substances in the monitor tissues (Sloof *et al.* 1988).

The term monitor used in the context of this thesis refers to the second approach in which the concentrations in tissues or organisms are measured. The concentrations of trace elements in tissues of suitable biomonitors give information on the response of organisms to air pollution; they are used as a measure of the concentrations or availability of air particulate matter in certain parts of the environment. Monitors have the provision for regular surveillance and they quantify how much pollutant is present (Martin and Coughtrey 1982).

Application of biomonitors has several advantages compared to the use of air

filters and deposition collectors, related primarily to the permanent and common occurrence in the field, the ease of sampling and the degree of trace element accumulation. Furthermore, the biomonitor provides a measure of integrated exposure over a certain amount of time, it is present in remote areas and no expensive technical equipment is involved to use it (Sloof *et al.* 1988, Steinnes 1989).

To be suitable for application as monitor for air particulate matter, specific requirements have to be met by a biological tissue or monitor. General criteria of primary importance are:

- a) the response of the organism to the quantity of the elements to be monitored should be known
- b) the organisms should be common in the area of interest
- c) availability is required at any time or season
- d) the monitor should be tolerant to pollutants at relevant levels.

Additional requirements specific for monitoring atmospheric trace elements are:

- e) the element uptake should be independent of local conditions other than the levels of the elements to be monitored
- f) the element uptake should not be influenced by regulating biological mechanisms or antagonistic or synergistic effects
- g) the biomonitor should average the element concentrations over a suitable time period as a result of integrated exposure over a time period
- h) the organism should not take up appreciable amounts of elements from sources other than atmospheric
- i) the organisms should have low background concentrations
- j) the sampling and sample preparation should be easy and quick
- k) the accumulation should reach to concentration levels which are accessible by routine analytical techniques.

Suitable biomonitors, which meet all the requirements, make continuous monitoring and even retrospective monitoring air pollution possible at relatively low costs. When information on time averaged trace-element concentrations at specific sites in the environment is aimed at, the use of non-mobile monitors is preferred.

### 1.3 Why lichens?

Various monitor materials have been applied in trace-element air monitoring programmes, such as lichens, mosses, ferns, grass, tree bark, tree rings and pine-needles. Evaluation of the criteria mentioned above for the various biomonitor materials, leads to lichens and mosses as the best suitable monitors. For all biomonitors used, the mechanisms of trace element uptake and retention are still not sufficiently known. For grass, tree rings and ferns, substantial element contributions from other sources than atmospheric, such as the soil or the tree bole have to be taken into account (Robitaille 1981, Sloof *et al.* 1988, Zayed *et al.* 1992). Where comparisons have been made, lichens and mosses show consistently higher metal levels than higher plants (Puckett 1988). This characteristic allows for a more precise definition of deposition zones, easier trace-element analysis and early reflection of enhanced pollution levels. Material trapped by pine-needles has the same composition as soil and washing the waxy needles before analysis can remove over 80% of the trapped trace elements (Wyttenbach *et al.* 1985). According to Rasmussen (1978) differences between element concentrations in bark on different trees are often significant, but in an unpredictable way. Rough barks accumulated trace element more than smooth barks (Barnes *et al.* 1976). Of all biomonitors used, tolerant lichen species and mosses have the most common occurrence. Some species exist that have a large geographical range, occur in rural areas, as well as in urban and industrial areas. The morphology of lichens and mosses does not vary with seasons, thus accumulation can occur throughout the year. Lichens and mosses usually have considerable longevity, which led to their use as long-term integrators of atmospheric deposition (Puckett 1988). Thus, tolerant lichen species and mosses are likely to be the best suitable organisms.

Especially in industrialized and populated areas in The Netherlands, tolerant lichen species growing on trees (epiphytic) are more widely spread than mosses and lichens growing on litter layers and dead moss carpets (epigeic) or rocks (epilithic) and roofs (epigeic and epilithic). Substantial substrate influence on the element content in the biomonitor is less likely to occur in epiphytic lichens than in epigeic lichens, which grow directly on the (mineral containing) litter layer. In The



Netherlands, epiphytic lichens are likely the best suitable monitors of trace-element air pollution.

Lichens form a group of plants in which the plant body or thallus consists of fungal tissue. Microscopic photosynthetic algae are embedded in the fungal tissue, usually forming a distinct layer. In general, about 90% of the biomass is made up by the fungal partner (Tyler 1988). The two organisms are living in symbiosis. There are approximately 20,000 lichen species, consisting of several groups of fungi and algae. Approximately 560 species occur in The Netherlands (Brand *et al.* 1988). The majority of the lichen species form crusts on rocks or trees (the crustose lichens). The foliose (leaflike) lichens and fruticose (shrubby) lichens are less abundant. Lichens have a life span of tens to hundreds of years (Hale 1974). Unlike higher plants, lichens have no roots or a well developed cuticle. These morphological features emphasize the applicability for monitoring purposes in that they are strongly dependent for their mineral nutrients on material deposited through wet and dry deposition to the plant surface. In addition, the lichen surface, structure and roughness facilitate the interception and retention of particles.

The scope of this thesis was to get a better understanding of the applicability of lichens as biomonitors for trace-element air pollution. Starting from the receptor side in the field, the *aim* was to investigate the relationship between trace element concentrations in lichens and those in air particulate matter and/or deposition on a regional and national scale.

In the next section (1.4), the specific goals of this thesis will be formulated against the background of the progress and problems of certain fundamental aspects of trace-element biomonitoring with lichens as reported in literature.

#### **1.4 Environmental lichenology: Biomonitoring trace-element air pollution**

Lichens have been used as *monitors* for atmospheric particulate matter and deposition for about two decades. In 1972, Nieboer *et al.* showed a correlation between the nickel, copper and iron contents of lichens and the reciprocal distance from a nickel smelter in Ontario, Canada. Metal levels in lichens around a copper

mining area in Quebec, Canada, exhibited a similar relationship (LeBlanc *et al.* 1974). Several lichen species have been used to describe the distribution of air particulate emissions around a variety of point sources (e.g. copper-, zinc- and nickel smelters, steelworks, power plants) and line sources (e.g. road sides). A general decline in metal levels in the plants with increasing distance from the source was reported in literature, as reviewed by Nieboer and Richardson (1981) and Puckett (1988).

Comparisons of metal levels in lichens and air particulate matter and bulk precipitation have also shown empirical qualitative relationships. Saeki *et al.* (1977) observed parallelism between the geometric means of the element content of filter-trapped air particulate matter and the arithmetic means of lichen metal concentrations in the urban area of Sendai, Japan. Pilegaard (1979) showed that metal levels in bulk precipitation in the vicinity of a Danish steelworks followed a decreasing power curve when the distance from the source was increased; metal levels in transplanted lichens and naturally occurring lichens showed the same relationship.

In many biomonitoring studies, the concentrations of trace elements in lichens have been assumed to reflect the averaged concentrations as air particulate matter and wet and dry deposition. A few studies have been carried out at a large (national) scale to describe regional distribution patterns of various elemental concentrations in lichens and mosses in Scandinavia (Rühling and Tyler 1973, Pakarinen and Tolonen 1976, Pilegaard *et al.* 1979, Pakarinen 1981, Steinnes *et al.* 1992) and in North America (Groet 1976).

In this thesis, the questions "do element concentrations in lichens reflect atmospheric concentration gradients?" and "do element concentration patterns in lichens show temporal trends?" are addressed in Chapter 2. Thereby, the geographical concentration patterns of 15 trace elements in two lichen data sets are compared, which were obtained from two Dutch monitoring surveys on a national scale within a 5-year period (1982-1987), using one lichen species and a standardized sampling method. The patterns in lichens are also compared with data on air particulate matter and deposition and with geographical element gradients calculated with one of the dispersion models, that have been developed for application in The Netherlands.

Lichens accumulate particulate matter continuously from the atmosphere and a lichen sample represents some average value for dry and wet deposition over a

certain time period (Nieboer and Richardson 1981). The possibility to measure the response of atmospheric changes in a field study depends on the background concentrations in the lichens, the atmospheric pollution level and the chemical nature of the particles. The transport of the Chernobyl plume over The Netherlands provided an opportunity to obtain information about the accumulation efficiency and elimination processes in lichens. On a Euro-Asian scale, the fallout from Chernobyl has been measured in air particulate matter, deposition and biomonitors (e.g. Devell *et al.* 1986, de Vries and van der Kooy 1986, Papastefanou *et al.* 1988, Raes *et al.* 1990, Topcuoğlu *et al.* 1993). Significant  $^{137}\text{Cs}$  influx of short duration (4 days), mainly due to rainfall, resulted in  $^{137}\text{Cs}$  activity patterns in lichens, which had no measurable  $^{137}\text{Cs}$  background. Because of the small  $^{137}\text{Cs}$  mass involved no cesium saturation effects occurred in the lichens. In Chapter 3 a study is described of the short-term  $^{137}\text{Cs}$  supply originating from Chernobyl to lichens. The validation of biomonitoring with lichens was investigated by means of comparing the  $^{137}\text{Cs}$  activity gradients obtained from lichens with those from direct atmospheric measurements (air filters) and calculated values for wet and dry deposition on a national scale.

The element content in lichens will be influenced by natural as well as anthropogenic sources. The most widely used method in literature for appointing sources is the gradient method, using the fact that metal levels increase with increasing proximity to the suspected source (Puckett 1988). However, when several unknown sources are potential contributors, as is the case in many parts of The Netherlands, the geographical concentration patterns of trace elements obtained from lichen data sets alone do not give conclusive information on the character of all pollution sources. To identify likely pollution sources, their relative contribution and their localization, a multivariate statistical approach, namely factor analysis, has been used for interpretation of observed elemental composition. This study is described in Chapter 4. In air pollution studies, target transformation factor analysis has been used to resolve the composition of air particulate matter or element concentration patterns obtained from lichen data sets into factors, which represent the emission sources (Hopke *et al.* 1976, Alpert and Hopke 1980, Henry *et al.* 1984, Henry 1987, de Bruin *et al.* 1987, Pilegaard 1987, Hopke 1988, Sloof and Wolterbeek 1991). However, in these studies the reliability of the factor solution has not been investigated. These

aspects in particular are discussed in Chapter 4.

Natural sources include the marine aerosols, leachate from foliage and bark, and suspended particles derived from local and remote soils and bedrock. In source apportionment studies, such as those using factor analysis, generally one or two natural factors (especially soil factors) are derived from lichen data sets, characterized by elements which are typical for the crustal material (de Bruin and van Wijk 1988, Pilegaard 1987, Sloof and Wolterbeek 1991). This finding raises the question whether lichens growing on trees in areas with heavily polluted soil still reflect atmospheric concentrations in air particulate matter and deposition only, the local soil raised by the wind or perhaps the element concentrations derived from the substrate tree. Recent research on lichen element uptake suggests that direct uptake from the substrate by lichens may occur; it appears to vary among substrates, lichen species and elements (Goyal and Seaward 1981, de Bruin and Hackenitz 1986, Puckett 1988, Prussia and Killingbeck 1991). The substrate influence and the possible soil contribution to the element content is discussed in Chapter 5, on basis of the results of a field study. For this purpose lichens, substrate bark and tree rings were collected from an area with contaminated soil and compared with lichens and substrate bark from an area with unpolluted soil.

In conducting monitoring surveys on a large scale, a problem may arise in certain target sampling sites, especially near pollution sources or urban areas when the monitor species under study is lacking. The question arises if samples of other epiphytic lichen species can be used to include such sampling sites for determining regional elemental patterns. This question is discussed in Chapter 6. In the underlying field study, the interchangeability of two lichen species was investigated in a polluted area. Few studies have been carried out on interspecies calibration (Folkeson 1979, Boileau *et al.* 1982). In these studies the calibration factors were determined as the accumulation ratios between the mean concentration levels in various species; thereby no insight is obtained in the actual relationship between the concentrations in the lichen species. In Chapter 6 a method for the determination of the interspecies calibration factors is also evaluated.

If insight in the quantitative relationship between the emission and the receptor (immission) is aimed at, it is necessary to know the quantitative relationship

between a certain component of the emission (e.g. the concentration as air particulate matter or the wet and dry deposition) and the concentrations in lichens. Thereby, several questions arise, such as: Are concentrations in lichens related to concentrations of air particulate matter or to dry and wet deposition or both? Can these relationships be quantified? Are the elemental concentrations in lichens in equilibrium with the atmospheric concentrations in air particulate matter? These questions are addressed in Chapter 7. They are approached through a field study, in which lichen transplants, collected from a reference area, were exposed to the atmosphere at 8 stations spread over The Netherlands for 1, 2, 4 and 12 months, respectively. At the same stations air particulate matter and bulk precipitation were monthly collected during one year.

The use of lichens as quantitative biomonitors requires insight in the processes which are involved in the uptake and release mechanisms. From studies on uptake, retention and toxicity of heavy metals in lichens, as reviewed by Tyler (1989) and Brown (1991), the nature of the binding sites and the binding mechanisms in lichens are still insufficiently known: The uptake of heavy metal ions seems primarily a passive extracellular process of ion exchange and complex formation. Ligands are present in the cell walls of both symbionts, those of the fungal partner being quantitatively most important. Depending on the degree of affinity for the ligands and the total metal load, certain heavy metal ions, such as zinc, may penetrate the plasmalemma and occur in the cytoplasm (Tyler 1989, Brown 1991). To gain knowledge about whether passive or active processes are involved in the uptake mechanism of lichens, in Chapter 7 a field approach has been used, by comparing the response of lichens to that of a synthetic monitor (rag).

Both the algal and the fungal partner of the lichen may be responsible for element accumulation. Investigations of accumulation and release characteristics aimed at the quantification of kinetic parameters of element uptake can only be carried out in the laboratory, under as much as controlled conditions. Lichens have rough surfaces, which makes controlled uptake studies too complex. Algal suspensions can be better controlled compared to most fungal cultures to perform kinetic experiments. There are still gaps in the understanding of the kinetics of heavy metal uptake by algae, which has led to various models of metal uptake (Khummongkol *et*

*al.* 1982, Geisweid and Urbach 1983, Trevors *et al.* 1986, Ting *et al.* 1989). Chapter 8 describes a laboratory study of cadmium uptake in algae, in which experimental kinetic parameters of cadmium uptake were quantified by means of a model, analogous to a metal-ligand complex formation.

In Chapter 9 the results from all the studies described in this thesis are combined in general conclusions on the value of epiphytic lichens as biomonitors for trace-element air pollution.

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## CHAPTER 2. GEOGRAPHICAL CONCENTRATION PATTERNS OF TRACE ELEMENTS IN LICHENS

**Abstract.** *Two monitoring surveys on a national scale were carried out within 5 years, using *Parmelia sulcata* Taylor as a biomonitor for trace-element air pollution. The method of sampling was standardized. The lichen samples were analyzed by neutron activation analysis. Local variations in element concentrations in lichens from various deciduous tree species from several sampling sites were determined. Comparison of the two lichen data sets showed the relationship of the geographical concentration patterns with time. Combination of the available data gave insight into the possible locations of some pollution sources. The geographical concentration patterns obtained from the lichen data sets agreed with the element concentration gradients obtained from a dispersion model and measured data on atmospheric concentrations in air particulate matter and deposition.*

## CHAPTER 2. GEOGRAPHICAL CONCENTRATION PATTERNS OF TRACE ELEMENTS IN LICHENS

### 2.1 Introduction

In environmental biomonitoring programmes the relevant information is commonly deduced from the behaviour of the monitor organism or from the concentrations of specific substances in the monitor tissues (LeBlanc *et al.* 1974, LeBlanc and Rao 1974, Martin and Coughtrey 1982, Sloof *et al.* 1988). The first approach is followed in studies where data are obtained from characteristics of species composition, diversity and richness, ecological performance and morphology (Wielgolaski 1975, de Wit 1976, Seaward 1988). The second approach is followed in the present paper, where element concentrations in lichens were used to monitor trace elements in the atmosphere (cf. Goyal and Seaward 1981a,b, de Bruin *et al.* 1987, Pilegaard 1987, de Bruin and van Wijk 1988, Sloof *et al.* 1988).

The aim of the present research was to study epiphytic lichens as biomonitors of levels, geographical gradients and temporal changes of trace-element air pollution. For this purpose a monitoring programme on a national scale was set up, using *Parmelia sulcata* Taylor. Two monitoring surveys were carried out within five years, in 1982-1983 and 1986-1987. The lichen data sets were compared with data on air particulate matter and deposition, and with estimated geographical element gradients based on a dispersion model, which was developed for application in The Netherlands (van Jaarsveld and Onderdelinden 1986).

### 2.2 Materials and Methods

#### 2.2.1 Sampling

*Parmelia sulcata* Taylor was used as monitoring organism because: (1) it is one of the few species which is still commonly dispersed on various species of trees in The Netherlands, (2) it is a foliose lichen, and can be separated easily from the bark

substrate, and (3) it is tolerant to pollution ( $\text{SO}_2$ , heavy metals), which enables monitoring of high atmospheric element concentrations (Wielgolaski 1975, de Wit 1976, de Bruin *et al.* 1987).

In October 1982 to February 1983 a monitoring network on a national scale was set up using *P. sulcata* as a biomonitor for atmospheric concentration patterns. At 110 sites, samples of *P. sulcata* with adhering bark substrate were taken from various trees. In November 1986 to January 1987 the survey was repeated with the same lichen species using an intense network of 210 sampling sites based on a grid of 10 x 10 km, covering almost the entire geographical land area of The Netherlands (32,500 km<sup>2</sup>).

A uniform method of sampling was followed. Healthy samples were taken from pH neutral bark substrates such as alder, ash, elm, lime, oak, poplar and willow. Sampling positions were between 1.0 and 2.5 m above the ground. Samples of lichen material were only collected from trees in open light habitats, not exposed to extra nutrient supply (e.g. under fresh cut wounds). Sampling within a distance of 500 m of farms or motorways was avoided. At each sampling site, several lichen thalli of different sizes were taken from the same tree and likewise, if possible from 3 to 7 nearby trees. Where possible, the lichen material was taken from all around the tree to reduce the influence of the relative source positions.

### 2.2.2 Sample preparation

In the laboratory the lichens were separated from the bark substrate with nylon tweezers and washed with distilled water (30 ml) to remove adhering sand or dust. The samples were made brittle by immersion in liquid nitrogen, ground and homogenized in a mortar with a pestle and freeze dried for 24 h. Material from trees within one sampling site was combined to yield one representative sample. To establish the variation in element concentrations within one sampling site, material from different trees was ground separately. The lichen material (30 to 100 mg dry weight) was packed in polyethylene capsules for neutron activation analysis. For atomic absorption spectrophotometric analysis (AAS) the lichen material (30 mg dry weight) was subjected to acid digestion using a mixture of  $\text{HNO}_3/\text{HClO}_4/\text{HF}$  (10:1:1) in a Teflon-lined pressure vessel. The residue was diluted to 100 ml with deionized water and 1 ml of 1.4 M

lanthanum chloride solution (Bertenshaw and Gelsthorpe 1981). After centrifugation (6000 g) the supernatant was used for analysis, following procedures given by Dutch Norm (NEN 6429, 1983).

### 2.2.3 Analysis

The lichens were analyzed for metals and other trace elements by instrumental neutron activation analysis (INAA) (de Bruin *et al.* 1982, Bode and de Bruin 1990). The samples were irradiated twice in the reactor of the Interfaculty Reactor Institute (IRI) at a thermal neutron flux of  $5 \cdot 10^{16} \text{ m}^{-2} \cdot \text{s}^{-1}$ . A first irradiation of 30 s was followed, after 10 to 15 min decay time of the short-lived radionuclides, by a measurement of 5 min. After a second irradiation of 4 h, the samples were measured twice during 1 h, after decay times of 5 and 21 days, respectively. Measurements were carried out with semiconductor detectors (Ortec, Canberra) equipped with sample changers and connected to a PDP - 11/44 computer. The precision of the measurements as derived from counting statistics was 0.7-3% for antimony, arsenic, bromine, cobalt, chromium, iron, lanthanum, manganese, scandium, thorium, vanadium, zinc and 7-13% for cadmium, cesium, mercury, nickel, selenium and tungsten. Blanks were run with the same procedure (1 per 10 samples). Some detection limits typically obtained for lichens are  $0.1 \text{ mg} \cdot \text{kg}^{-1}$  for As and Sb,  $1 \text{ mg} \cdot \text{kg}^{-1}$  for Cd, Cr, Hg, Mn, Se, V and Zn, and  $30 \text{ mg} \cdot \text{kg}^{-1}$  for Fe (Bode and de Bruin 1990).

The lichens were analyzed for lead by AAS analysis, carried out by the Faculty of Civil Engineering (Delft University of Technology) using the graphite furnace technique. The spectrometer was a Perkin Elmer Model 3030B with a deuterium background correction facility. Standard stock solutions of  $1000 \text{ mg} \cdot \text{kg}^{-1}$  Pb were prepared daily by diluting a Merck Titrisol with a 0.1 N  $\text{HNO}_3$  solution. The detection limit, defined as three times the standard deviation in the background, was  $0.4 \times 10^{-3} \text{ mg} \cdot \text{kg}^{-1}$ . Blank solution concentrations amounted to  $2.8 \cdot 10^{-3} \text{ mg} \cdot \text{kg}^{-1}$  (N=9). The lead concentrations in the lichens ranged from 7.1 to  $130 \cdot 10^{-3} \text{ mg} \cdot \text{kg}^{-1}$ .

Quality control of the INAA and AAS results was based on the regular analysis of standard reference material SRM 1571 'Orchard Leaves' (National Bureau of Standards (NBS) 1971, Gladney *et al.* 1987) according to the same procedures as used

for the lichens. The INAA and AAS results for the reference material agreed with the NBS-certified values, and with the recommended values as given by Gladney *et al.* 1987 (Sloof and Wolterbeek 1991b).

#### 2.2.4 Mapping

The geographical distribution of the element concentrations in lichens were mapped by means of a computer programme (Plotfac, IRI 1988). For every grid cell of the map a plot value was calculated from the concentrations found for all sampling points as the average of these concentrations weighted with the third power of the distance between plot point and sampling point.

### 2.3 Results

#### 2.3.1 Local variation in element concentrations

The variation of the element concentrations in *P. sulcata* within one sampling site (local variation) was determined for As, Br, Cd, Cs, Fe, Hg, La, Ni, Sb, Se, V, W, and Zn for the 1986-1987 programme. At 40 sampling sites throughout The Netherlands, (selected out of the total 210 sites), the local variation in element concentrations was determined. This local variation was considered to comprise the variation in substrate, age and size of samples, and the individual variation in lichen specimens. At each of the 40 sampling sites, *P. sulcata* was collected from three to seven deciduous tree species and analyzed separately. The variances of the element concentrations were calculated per site. The local variation in element concentrations was expressed in relative variance (%) to compare the magnitudes of the variances for the 40 sampling sites. Table 2-I shows the range of relative variances (%) of element concentrations in *P. sulcata* in relation to tree species. A few relative variances are not presented in Table 2-I, due to the fact that in some of the samples collected the element concentrations were below the detection limit which could not be used in the statistical processing of the data.

In general, the relative variance ranged from 0.1% to about 40%. A few high values were observed for As (46%), Cs (65%), Fe (48%) for oaks. The smallest local

TABLE 2-I. The local variation of element concentrations in *Parmelia sulcata* per tree species. The ranges of the local variations are expressed as the relative variances (%), for each element and for each tree species. The local variation, determined at 40 sampling sites, out of 210 sites in the 1986-1987 programme, was not significantly related to tree species.

| Element | Poplars | Oaks   | Willows | Elms    | Mixed   |
|---------|---------|--------|---------|---------|---------|
| As      | 0.2-11  | 0.4-46 | 0.8-9.0 | 1.1-6.8 | 0.1-12  |
| Br      | 1.5-16  | 0.3-15 | 4.0-26  | 11-24   | 2.3-12  |
| Cd      | 2.0-29  | -      | -       | -       | -       |
| Cr      | 0.2-19  | 0.6-34 | 0.4-18  | 3.2-14  | 2.7-22  |
| Cs      | 0.2-11  | 0.1-65 | 0.3-11  | 0.1-5.0 | 5.8-28  |
| Fe      | 0.3-13  | 0.6-48 | 0.3-15  | 0.7-18  | 4.4-14  |
| Hg      | 1.0-38  | 0.1-17 | 12      | 4.4-10  | 0.4-13  |
| La      | 0.1-29  | 1.0-32 | 0.1-13  | 0.8-12  | 5.8-18  |
| Ni      | 1.7-21  | 0.1-36 | 0.4     | 8.4-9.3 | 4.2-7.8 |
| Pb      | 1.1-20  | 1.9-22 | 4.3     | 1.2     | 6.5-33  |
| Sb      | 0.8-41  | 3.2-22 | 0.4-3.2 | 1.2-14  | 1.4-18  |
| Se      | 1.4-22  | 0.8-14 | 6.8-43  | 2.8-16  | 6.3-15  |
| V       | 0.4-16  | 0.2-43 | 1.0-17  | 1.7-4.4 | 6.8-43  |
| W       | 1.3-32  | 1.4-36 | 3.2-4.6 | 4.0-13  | 15-16   |
| Zn      | 0.8-12  | 0.8-23 | 0.1-16  | 0.3-5.8 | 0.3-9.0 |

variation was found for Zn (0.1-23%). The mean local variance over all elements considered was 25%. The relative variance was not significantly related to the tree species under study.

### 2.3.2 Variation in element concentrations on a national scale

The variation of the element concentrations in lichens on a national scale was determined for As, Br, Cd, Cr, Cs, Fe, Hg, La, Ni, Sb, Se, V, W and Zn, in both surveys. The variation in Pb concentrations on a national scale (27%) was determined only in the second survey. The relative variance ranged from 16-570% for the 1982-1983 survey and from 19-2300% for the 1986-1987 survey (Table 2-II). For many elements in the 1986-1987 survey (As, Br, Cs, Fe, Ni, Pb, Sb, Se, V, Zn), the relative variance on a national

scale was of the same order of magnitude as on a local scale (Tables 2-I, 2-II). To determine whether the local variation in element concentrations is acceptable if compared to the variation on a national scale, information is needed on the geographical distribution of the element concentrations.

### 2.3.3 Geographical concentration patterns

The data sets of both surveys (1982-1983 and 1986-1987) are presented in plots of geographical concentration patterns for the elements As, Br, Cd, Cr, Cs, Fe, Hg, La, Ni, Pb, Sb, Se, V, W and Zn (Figures 2-1 to 2-15). The element concentrations were divided into 5 classes, according to the legends in Figures 2-1 to 2-15 and also given in Table 2-III. The geographical concentration patterns should be interpreted as geographical gradients on a national scale. The patterns obtained from the 1986-1987 data set show more structure than those obtained from the 1982-1983 data set, since the number of sampling points and thus the number of extreme values is much larger.

The local variation of element concentrations is not large enough to disturb the concentration patterns on a national scale, since the *range* of the element concentrations on a local scale is an order of magnitude lower than the *range* of concentrations on a national scale. The local variation was checked and found not to be significantly related to the pollution level of the sampling site (Table 2-III, Figures 2-1 to 2-15).

Comparison of the two data sets shows several pattern changes within 5 years. In the following paragraphs the situation in the period 1982-1983 will be taken as point of reference.

In the northern region of the country, only the geographical gradients of Se and W concentrations changed within 5 years: Areas with enhanced concentration classes expanded (Figures 2-12 and 2-14).

In the central region of The Netherlands, "new" areas with high concentration classes of Cd and Hg were found (Figures 2-3 and 2-7). In this region, areas with enhanced concentration classes of La, Ni, Se, V and W expanded (Figures 2-8, 2-9, 2-12, 2-13 and 2-14). The geographical distribution of As, Br, Cr, Cs, Fe, Sb and Zn concentrations did not change markedly within 5 years (Figures 2-1, 2-2, 2-4, 2-5, 2-6, 2-11 and 2-15).



TABLE 2-II. Metal concentrations in *Parmelia sulcata*, for the 1982-1983 and 1986-1987 studies.

| Element | Survey 1982-1983 |              |               |                   |  | Survey 1986-1987 |             |               |                   |  |
|---------|------------------|--------------|---------------|-------------------|--|------------------|-------------|---------------|-------------------|--|
|         | Mean             | Range        | Sample number | Relative variance |  | Mean             | Range       | Sample number | Relative variance |  |
| As      | 5.7*             | 1.7 - 17*    | 110           | 24%               |  | 5.7              | 0.5 - 17    | 329           | 24%               |  |
| Br      | 44               | 17 - 120     | 110           | 16%               |  | 56               | 15 - 170    | 337           | 19%               |  |
| Cd      | 2.0              | 0.8 - 5.8    | 72            | 19%               |  | 2.8              | 0.6 - 21    | 63            | 110%              |  |
| Cr      | 22               | 7.6 - 58     | 110           | 18%               |  | 26               | 4.0 - 270   | 337           | 97%               |  |
| Cs      | 0.9              | 0.3 - 21     | 108           | 570%              |  | 0.8              | 0.2 - 5.3   | 322           | 43%               |  |
| Fe      | 4100             | 1100 - 15000 | 110           | 43%               |  | 5800             | 720 - 30000 | 337           | 43%               |  |
| Hg      | 0.4              | 0.1 - 3.7    | 99            | 120%              |  | 0.5              | 0.1 - 36    | 316           | 2300%             |  |
| La      | 4.1              | 0.8 - 16     | 99            | 40%               |  | 6.2              | 0.8 - 64    | 316           | 110%              |  |
| Ni      | 14               | 3.0 - 53     | 69            | 47%               |  | 16               | 1.9 - 51    | 201           | 30%               |  |
| Pb      |                  |              |               |                   |  | 147              | 3.1 - 367   | 232           | 27%               |  |
| Sb      | 3.0              | 0.6 - 10     | 110           | 43%               |  | 3.3              | 0.3 - 12    | 334           | 46%               |  |
| Se      | 1.4              | 0.4 - 4.0    | 98            | 21%               |  | 1.8              | 0.4 - 7.5   | 332           | 25%               |  |
| V       | 29               | 8.8 - 130    | 102           | 35%               |  | 32               | 5.7 - 99    | 334           | 20%               |  |
| W       | 0.5              | 0.1 - 2.0    | 93            | 64%               |  | 0.9              | 0.1 - 10    | 248           | 92%               |  |
| Zn      | 90               | 80 - 630     | 110           | 19%               |  | 210              | 61 - 1100   | 327           | 36%               |  |

\*Values in mg.kg<sup>-1</sup>

In the southern region of the country, the geographical concentration patterns of almost all element concentrations changed within 5 years, except for Ni and V. The areas with high concentration classes for As, Cd, Cr, Cs, Fe, La, Sb, Se, W and Zn expanded, especially towards the south-eastern part of the country (Figures 2-1 to 2-15). In the south-western part, areas with enhanced Br and Hg concentration classes disappeared (Figures 2-2 and 2-7).

The relative variance increased for Cd, Cr, Hg, La, Ni, W and Zn, mainly due to the larger number of extreme values in the 1986-1987 data set (Table 2-II). Table 2-II also shows the means and ranges of element concentrations of both surveys. The mean concentrations of Cd, W and Zn increased, whereas the mean concentrations of the other elements studied remained the same.

#### *2.2.4 Comparison of lichen data with atmospheric data*

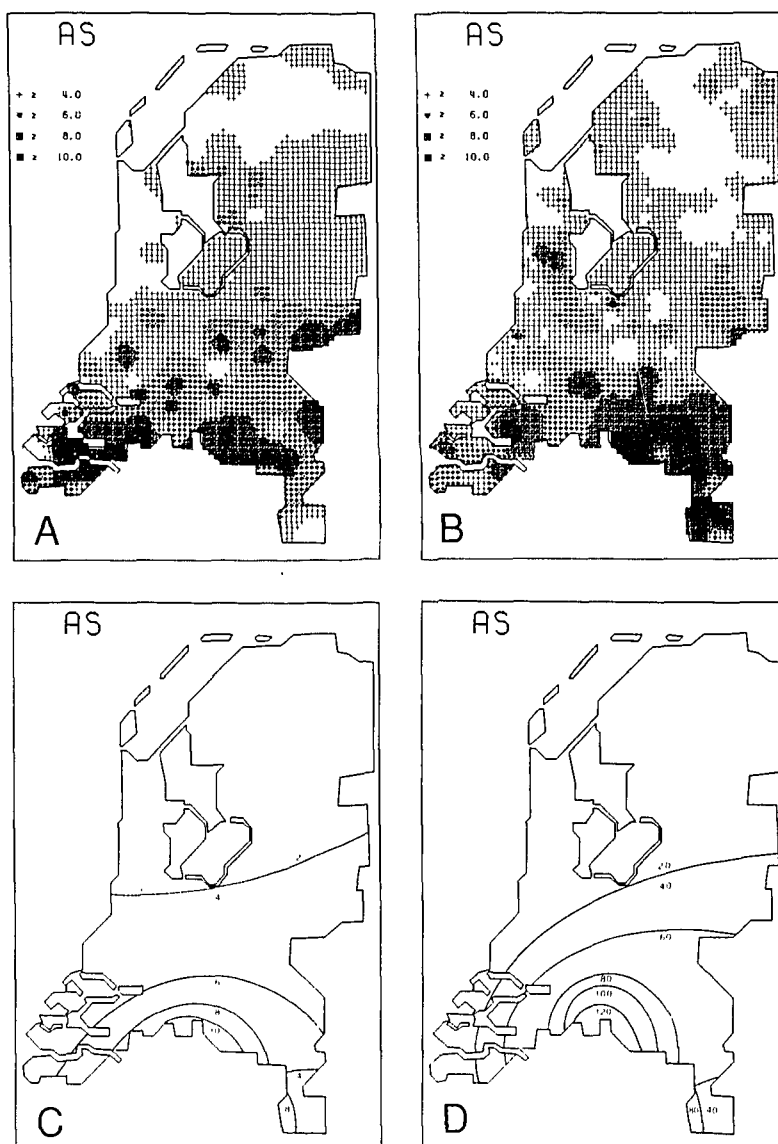
The geographical element concentration patterns obtained from the lichen data sets are considered to reflect the atmospheric element gradients. Verification (quantitative and qualitative) would have been possible when information on air particulate matter and deposition were available, from sampling sites and sampling periods similar to those of the lichen surveys. However, it was only possible to compare the 1982-1983 lichen data set with calculated gradients obtained from concentrations in air particulate matter in The Netherlands (1983), reported by van Jaarsveld and Onderdelinden (1986). The calculated annual averaged atmospheric concentrations in air particulate matter and deposition levels of trace elements were based on one dispersion model and an emission inventory of trace elements in The Netherlands (Van Jaarsveld and Onderdelinden 1986). Validation of the atmospheric data base was carried out by comparison of the calculated data with measured data on atmospheric concentrations in air particulate matter and depositions of trace elements. The measured data were gathered from 5 sampling sites, representing a number of regions in The Netherlands (Thijssen and Huygen 1985). According to Van Jaarsveld and Onderdelinden (1986) the measured data of the elements As, Cd, Cr, Ni, Pb, Sb, Se, V and Zn agreed with the estimates from the dispersion model.

On a national scale, the element gradients obtained from the lichen data set

(1982-1983) agreed with the calculated atmospheric element gradients (1983) for As, Cd, Cr, Ni, Sb, Se, V, and Zn as is illustrated in the Figures 2-1, 2-3, 2-4, 2-9, 2-11, 2-12, 2-13, 2-15). For Br, Cs, Fe, Hg, La, and W no data were available on atmospheric concentrations in air particulate matter and depositions (1983). In view of the agreement between concentration gradients obtained from the lichen data set (1983) and from the calculated atmospheric concentrations in air particulate matter and total depositions (1983) on a national scale, the geographical element concentration patterns obtained from lichens are considered to reflect the gradients of wet and dry deposition, as well as atmospheric concentrations in air particulate matter.

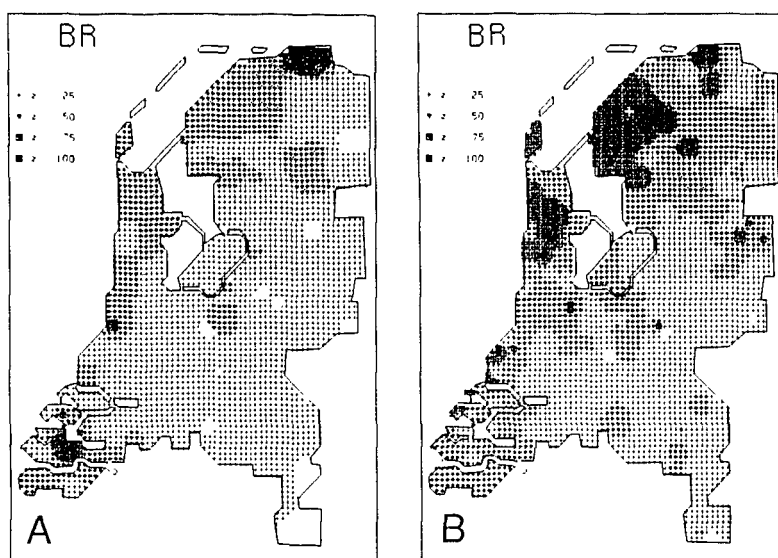
The geographical concentration patterns obtained from the 1986-1987 lichen data set could not be compared with atmospheric element concentration gradients, since no such calculated data were available. For 1987, measured data were available only for wet deposition and only for Cd, Pb, and Zn, gathered from 5 sampling sites (National Institute of Public Health and Environmental Protection (RIVM) 1988). Since the concentration gradients for Cd and Pb based on measured wet deposition in 1987 were markedly different from the concentration gradients based on calculated data on total deposition in 1983, the atmospheric concentrations in air particulate matter and depositions most probably have changed within 5 years.

Figures 2-3, 2-10 and 2-15 show the 1986-1987 element concentration gradients from the lichen data set in comparison with the measured 1987 wet deposition data. The geographical Cd concentration pattern obtained from the lichen data set in 1986-1987 agreed with the gradient based on wet deposition (1987) only in the southern parts of The Netherlands (Figures 2-3B,E). The high Cd concentrations in lichens in the middle and the north-western regions of the country were not reflected by the results of RIVM measurements on wet deposition of Cd. The high Pb concentrations in lichens (1986-1987) in the southern, eastern and northern regions of the country were not reflected by the RIVM (1988) results on wet deposition of Pb (Figures 2-10A,D). The Zn concentration gradient based on measured wet deposition in 1987 differed from the concentration based on calculated data for total deposition in 1983 only in the western and southern regions of the country. In these parts, enhanced Zn concentrations were found in wet deposition. Enhanced Zn concentrations in wet deposition in the western part of the Netherlands were not reflected in lichens (Figures 2-15B,D,E).

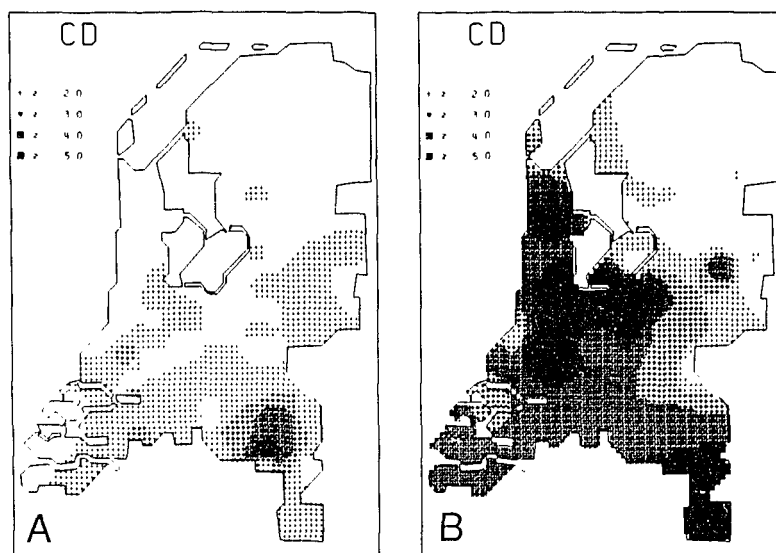


Figures 2-1A,B. Arsenic concentrations in *Parnelia sulcata* (in mg.kg<sup>-1</sup>) (A = 1982-1983; B = 1986-1987). The geographical concentration patterns contain five element concentration classes. Element concentration Class 1 (unshaded) ranges from the minimum concentrations determined to Class 2 (shaded with plus signs). Consequently, Class 5 (darkest shading) ranges up to the maximum concentration determined. Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 1.7 (0.5), 4.0, 6.0, 8.0, 10.0, 16.6 (17.0).

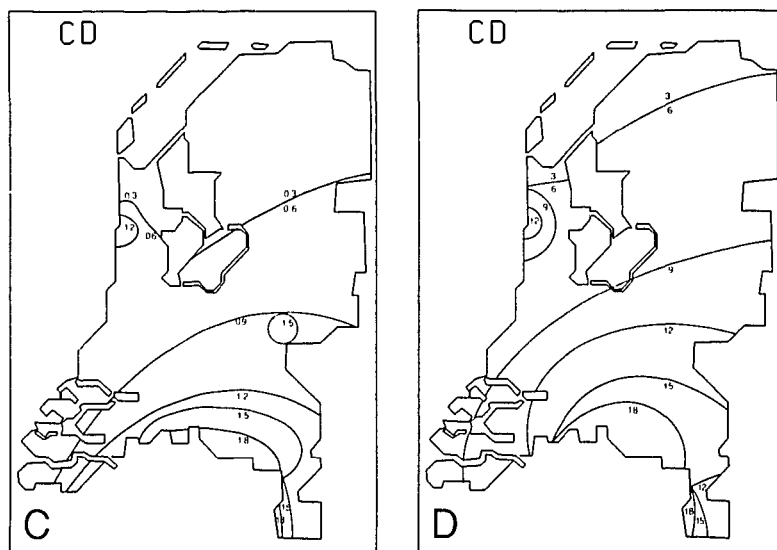
Figures 2-1C,D. Calculated 1983 atmospheric concentrations (in ng.m<sup>-3</sup>) for arsenic (C) and calculated 1983 total (wet and dry) deposition of arsenic (in mmol.ha<sup>-1</sup>.y<sup>-1</sup>) (D) (van Jaarsveld and Onderdelinden 1986).



Figures 2-2A,B. Bromine concentrations in *Parmelia sulcata* (in mg.kg<sup>-1</sup>) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 17 (15), 25, 50, 75, 100, 121 (165) (shading as in Fig. 2-1A,B).



Figures 2-3A,B. Cadmium concentrations in *Parmelia sulcata* (in mg.kg<sup>-1</sup>) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 0.8 (0.6), 2.0, 3.0, 4.0, 5.0, 5.8 (21).



Figures 2-3C,D. Calculated 1983 atmospheric concentrations for cadmium (in  $\text{ng.m}^{-3}$ ) (C) and calculated 1983 total (wet and dry) deposition of cadmium (in  $\text{mmol.ha}^{-1}.\text{y}^{-1}$ ) (D) (van Jaarsveld and Onderdelinden 1986). These concentration gradients agree with those obtained from the 1983 lichen data set (Fig. 2-3A).

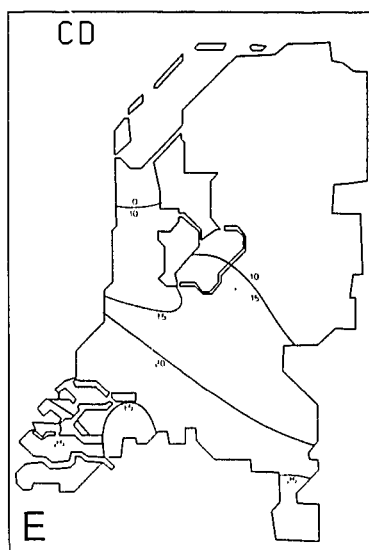
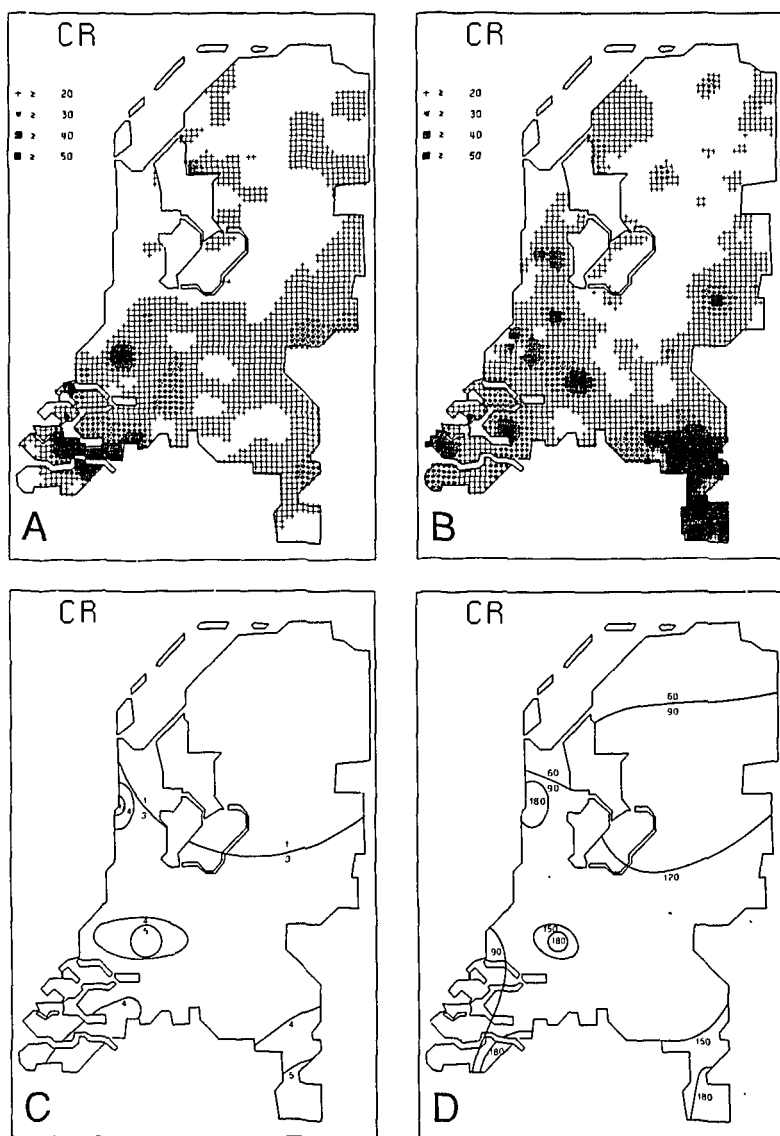
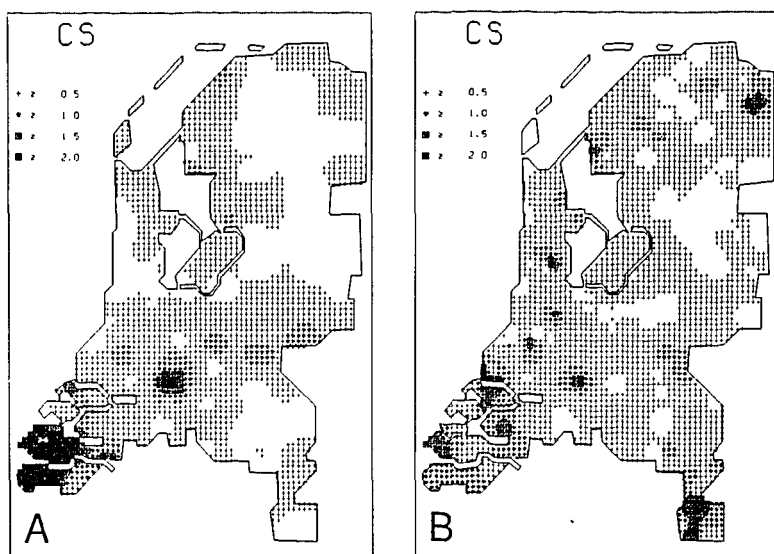


Figure 2-3E. Measured wet deposition of cadmium in 1987 (in  $\text{mmol.ha}^{-1}.\text{y}^{-1}$ ) (National Institute of Public Health and Environmental Protection 1988). The concentration gradient based on measured wet deposition in 1987 was different from those based on atmospheric concentrations and total deposition in 1983.

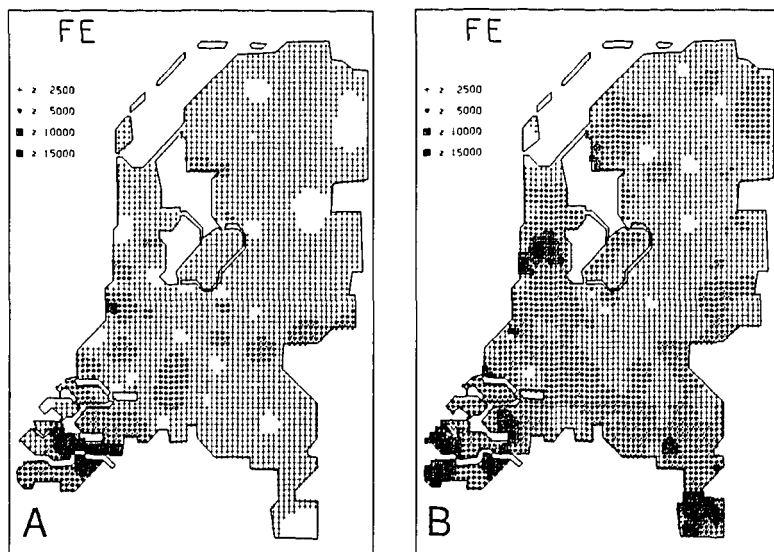


Figures 2-4A,B. Chromium concentrations in *Parnelia sulcata* (in  $\text{mg.kg}^{-1}$ ) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 8 (4), 20, 30, 40, 50, 58 (268) (shading as in Fig. 2-1A,B).

Figures 2-4C,D. Calculated 1983 atmospheric concentrations for chromium (in  $\text{ng.m}^{-3}$ ) (C) and calculated 1983 total (wet and dry) deposition of chromium (in  $\text{mmol.ha}^{-1}.\text{y}^{-1}$ ) (D) (van Jaarsveld and Onderdelinden 1986).

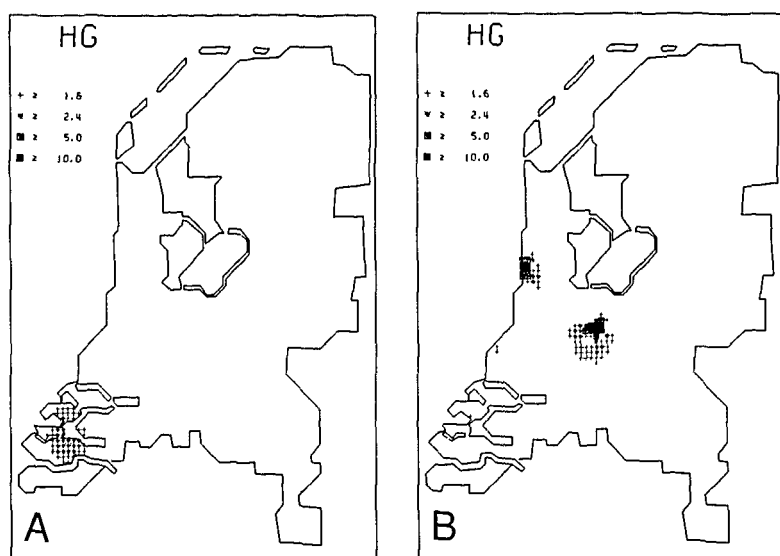


Figures 2-5A,B. Cesium concentrations in *Parmelia sulcata* (in  $\text{mg.kg}^{-1}$ ) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 0.3 (0.2), 0.5, 1.0, 1.5, 2.0, 21 (5.3) (shading as in Fig. 2-1A,B).

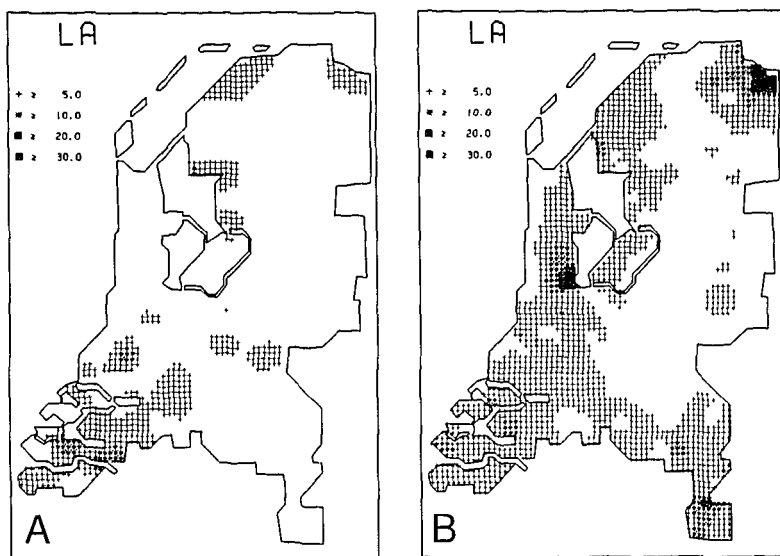


Figures 2-6A,B. Iron concentrations in *Parmelia sulcata* (in  $\text{mg.kg}^{-1}$ ) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 1050 (720), 2500, 5000, 10000, 15000, 15280 (29780) (shading as in Fig. 2-1A,B).

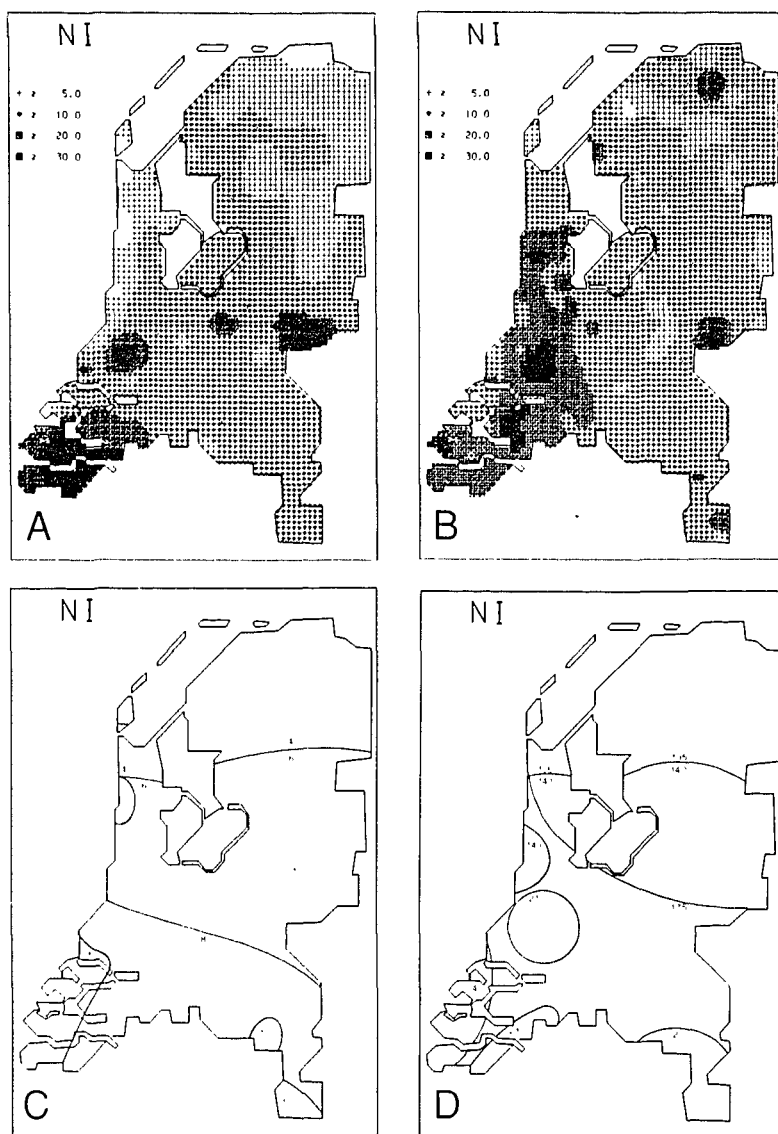




Figures 2-7A,B. Mercury concentrations in *Parmelia sulcata* (in mg.kg<sup>-1</sup>) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 0.1 (0.1), 1.6, 2.4, 5.0, 10, 3.7 (36.2) (shading as in Fig. 2-1A,B).

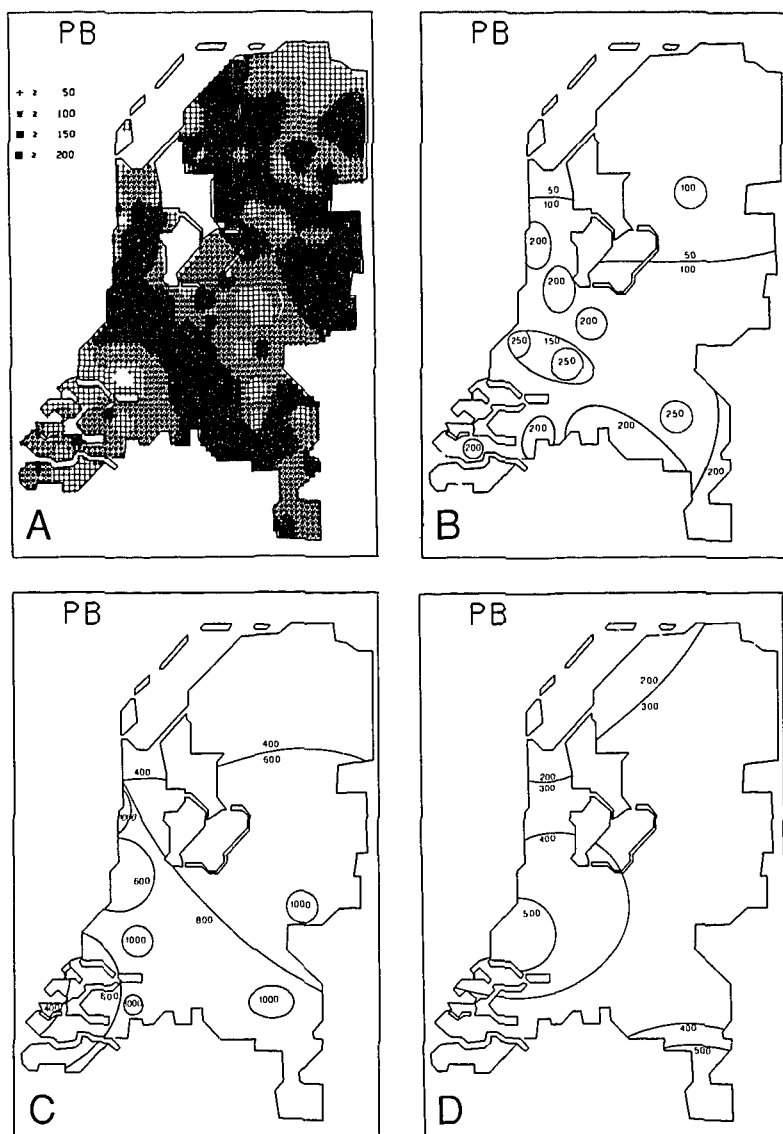


Figures 2-8A,B. Lanthanum concentrations in *Parmelia sulcata* (in mg.kg<sup>-1</sup>) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 0.8 (0.8), 5.0, 10, 20, 30, 16.1 (64.1) (shading as in Fig. 2-1A,B).



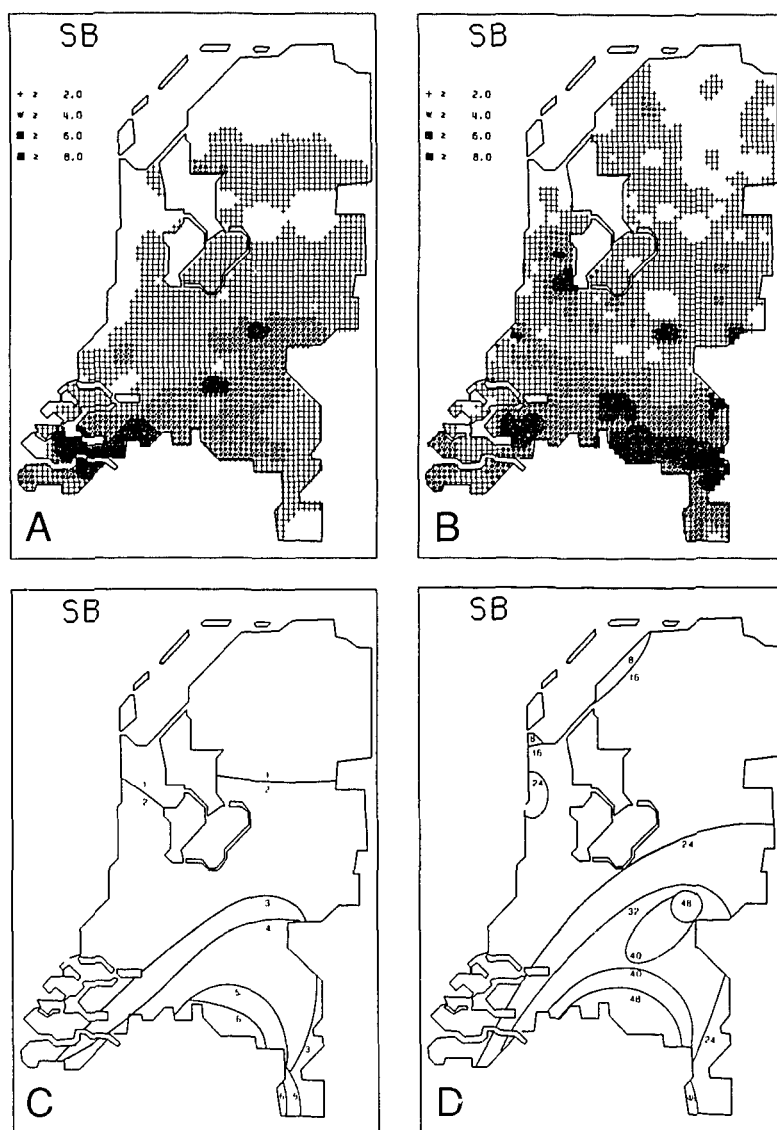
Figures 2-9A,B. Nickel concentrations in *Parnelia sulcata* (in mg.kg<sup>-1</sup>) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 3.0 (1.9), 5.0, 10, 20, 30, 52.5 (50.8) (shading as in Fig. 2-1A,B).

Figures 2-9C,D. Calculated 1983 atmospheric concentrations for nickel (in ng.m<sup>-3</sup>) (C) and calculated 1983 total (wet and dry) deposition of nickel (in mmol.ha<sup>-1</sup>.y<sup>-1</sup>) (D) (van Jaarsveld and Onderdelinden 1986).



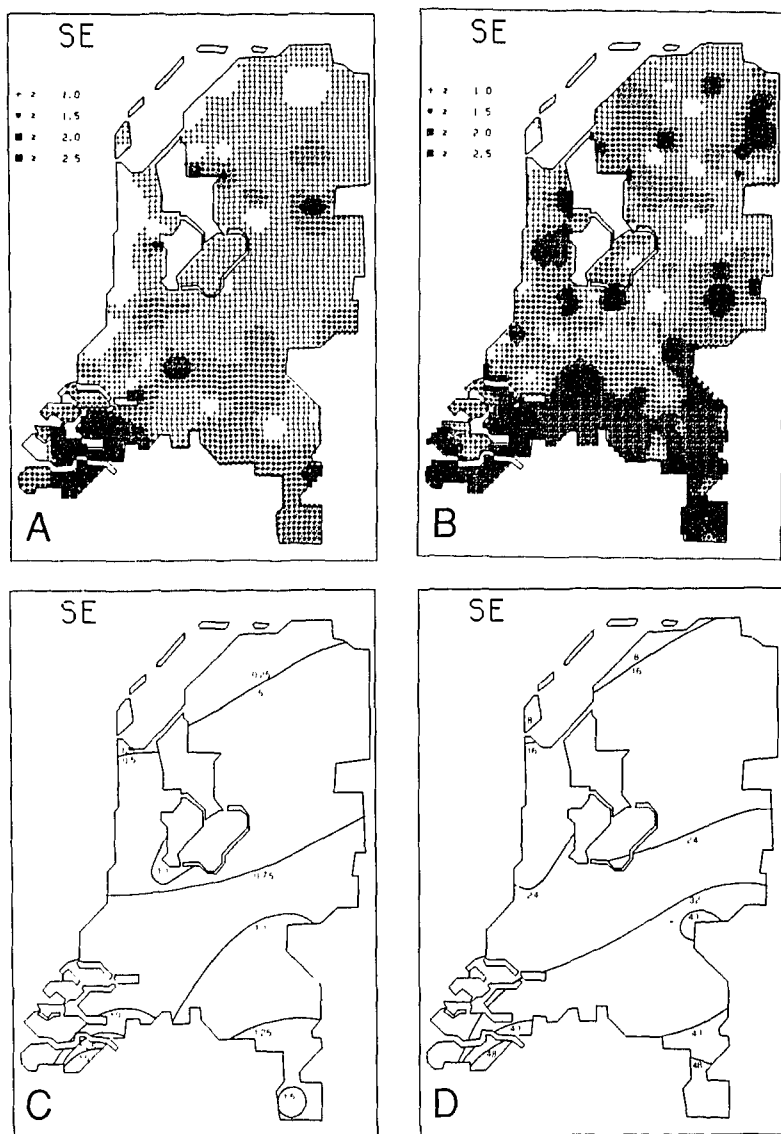
Figures 2-10A. Lead concentrations in *Parmelia sulcata* (in mg.kg<sup>-1</sup>) in 1986-1987. Classes 1-5 for 1986-1987: 3, 50, 100, 150, 200, 367 (shading as in Fig. 2-1A,B).

Figures 2-10B,C,D. Calculated 1983 atmospheric concentrations for lead (in ng.m<sup>-3</sup>) (B), calculated 1983 total (wet and dry) deposition of lead (in mmol.ha<sup>-1</sup>.y<sup>-1</sup>) (C) (van Jaarsveld and Onderdelinden 1986) and measured wet deposition of lead in 1987 (in mmol.ha<sup>-1</sup>.y<sup>-1</sup>) (D) (National Institute of Public Health and Environmental Protection 1988).



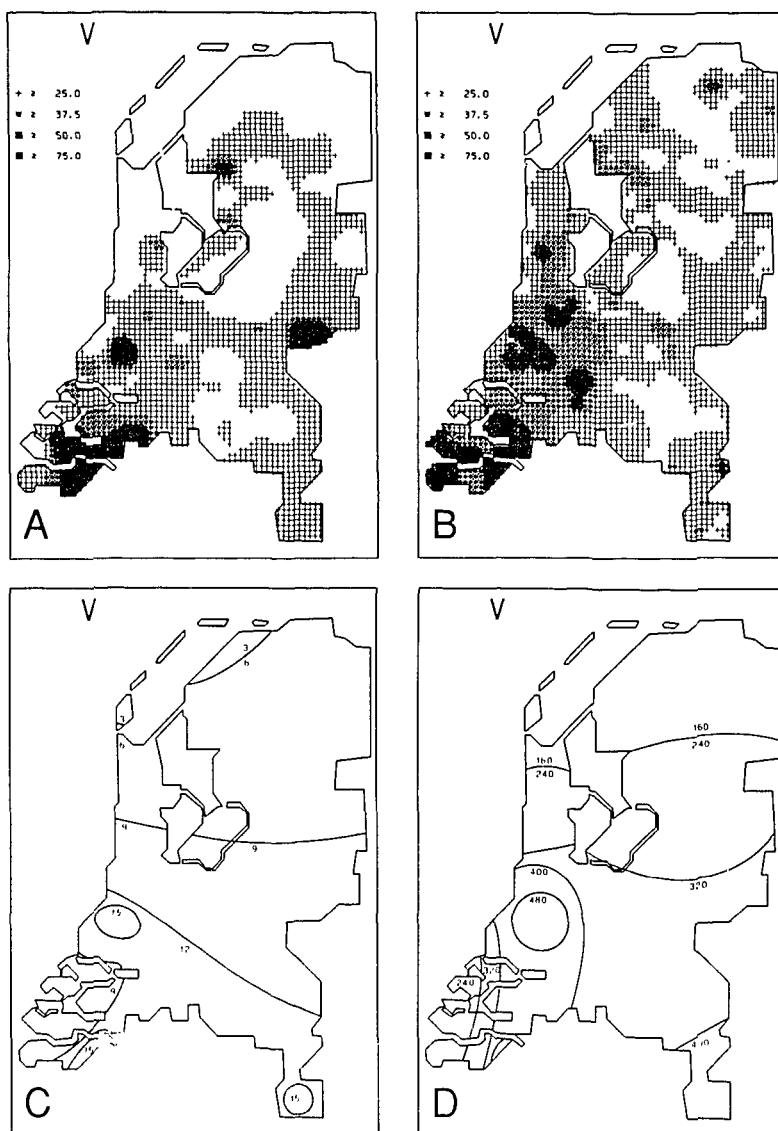
Figures 2-11A,B. Antimony concentrations in *Parmelia sulcata* (in mg.kg<sup>-1</sup>) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 0.6 (0.3), 2.0, 4.0, 6.0, 8.0, 10.2 (12.2) (shading as in Fig. 2-1A,B).

Figures 2-11C,D. Calculated 1983 atmospheric concentrations for antimony (in ng.m<sup>-3</sup>) (C) and calculated 1983 total (wet and dry) deposition of antimony (in mmol.ha<sup>-1</sup>.y<sup>-1</sup>) (D) (van Jaarsveld and Onderdelinden 1986).



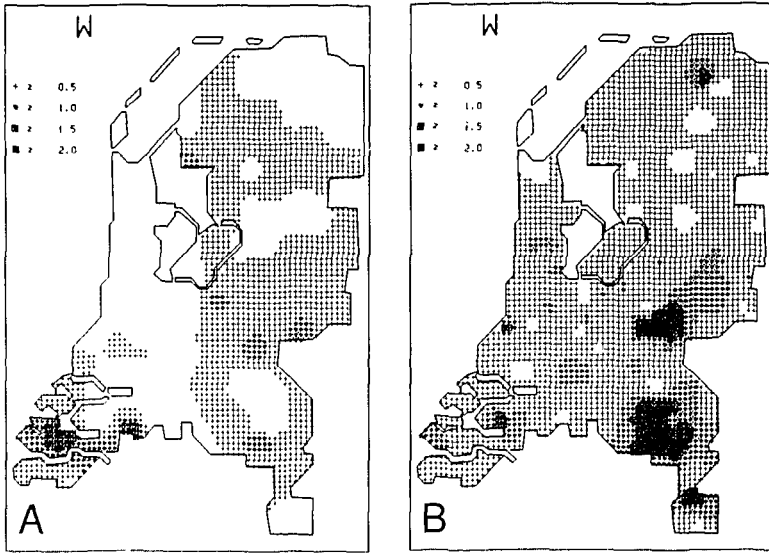
Figures 2-12A,B. Selenium concentrations in *Parnelia sulcata* (in  $\text{mg.kg}^{-1}$ ) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 0.4 (0.4), 1.0, 1.5, 2.0, 2.5, 4.0 (7.5) (shading as in Fig. 2-1A,B).

Figures 2-12C,D. Calculated 1983 atmospheric concentrations for selenium (in  $\text{ng.m}^{-3}$ ) (C) and calculated 1983 total (wet and dry) deposition of selenium (in  $\text{mmol.ha}^{-1}.\text{y}^{-1}$ ) (D) (van Jaarsveld and Onderdefinden 1986).

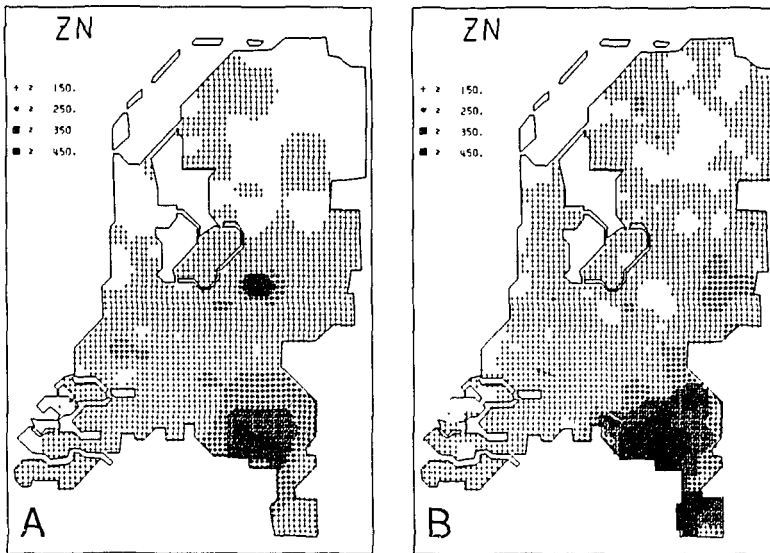


Figures 2-13A,B. Vanadium concentrations in *Parmelia sulcata* (in mg.kg<sup>-1</sup>) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 8.8 (5.7), 25, 37.5, 50, 75, 128 (98.6) (shading as in Fig. 2-1A,B).

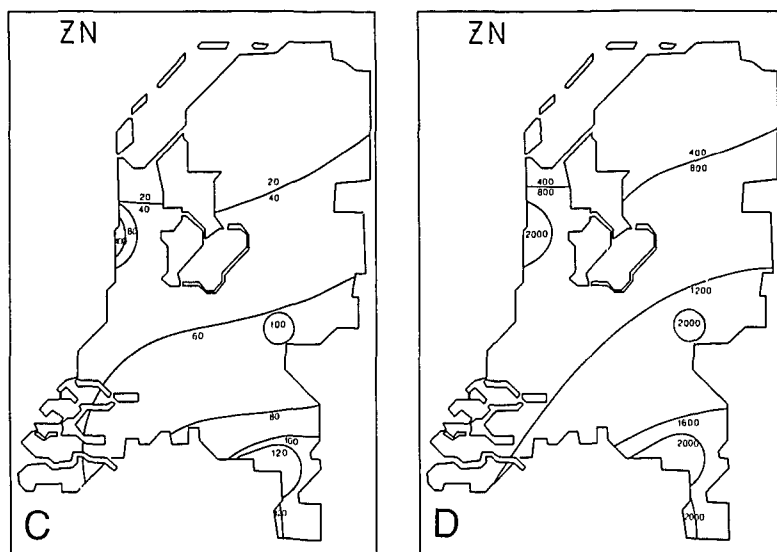
Figures 2-13C,D. Calculated 1983 atmospheric concentrations for vanadium (in ng.m<sup>-3</sup>) (C) and calculated 1983 total (wet and dry) deposition of vanadium (in mmol.ha<sup>-1</sup>.y<sup>-1</sup>) (D) (van Jaarsveld and Onderdelinden 1986).



Figures 2-14A,B. Tungsten concentrations in *Parmelia sulcata* (in  $\text{mg.kg}^{-1}$ ) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (values for 1986-1987 in brackets): 0.0 (0.2), 0.5, 1.0, 1.5, 2.0, 2.0 (10.1) (shading as in Fig. 2-1A,B).



Figures 2-15A,B. Zinc concentrations in *Parmelia sulcata* (in  $\text{mg.kg}^{-1}$ ) (A = 1982-1983; B = 1986-1987). Classes 1-5 for 1982-1983 (1986-1987): 80 (61), 150, 250, 350, 450, 634 (743) (shading as in Fig. 2-1A,B).



Figures 2-15C,D. Calculated 1983 atmospheric concentrations for zinc (in  $\text{ng.m}^{-3}$ ) (C) and calculated 1983 total (wet and dry) deposition of zinc (in  $\text{mmol.ha}^{-1}.\text{y}^{-1}$ ) (D) (van Jaarsveld and Onderdelinden 1986).

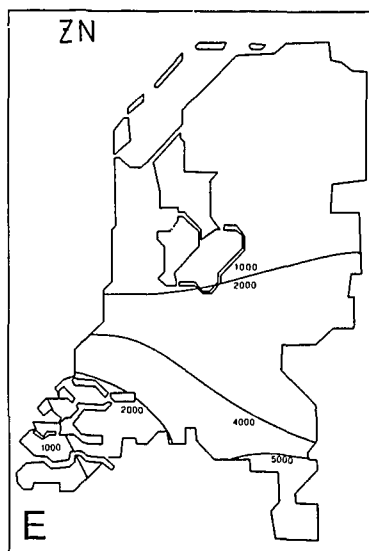


Figure 2-15E. Measured wet deposition of zinc in 1987 (in  $\text{mmol.ha}^{-1}.\text{y}^{-1}$ ) (National Institute of Public Health and Environmental Protection 1988).



TABLE 2-III. The local variation (in %) relative to pollution level for 15 elements in *Parmelia sulcata* in The Netherlands\*

| Element | Class 1 |        | Class 2 |         | Class 3 |         | Class 4 |          | Class 5 |          |
|---------|---------|--------|---------|---------|---------|---------|---------|----------|---------|----------|
| As      | 0.7-46  | (≥0.5) | 0.1-11  | (≥4.0)  | 0.5-22  | (≥6.0)  | 4.0     | (≥8.0)   | 0.8-7.0 | (≥10)    |
| Br      | -       | (≥15)  | 0.6-14  | (≥25)   | 2.0-16  | (≥50)   | 1.5-26  | (≥75)    | -       | (≥100)   |
| Cd      | 2.0     | (≥0.6) | -       | (≥2.0)  | -       | (≥3.0)  | -       | (≥4.0)   | 29      | (≥5.0)   |
| Cr      | 0.2-20  | (≥4.0) | 3.0-30  | (≥20)   | 0.6-18  | (≥30)   | 1.0-11  | (≥40)    | 3.5-6.2 | (≥50)    |
| Cs      | 0.2-65  | (≥0.2) | 0.1-28  | (≥0.5)  | 0.1-11  | (≥1.0)  | 6.0-11  | (≥1.5)   | 7.0     | (≥2.0)   |
| Fe      | 8.0     | (≥720) | 0.3-48  | (≥2500) | 0.7-18  | (≥5000) | 0.7-4.0 | (≥10000) | 3.1     | (≥15000) |
| Hg      | 0.1-29  | (≥0.1) | -       | (≥1.6)  | -       | (≥2.4)  | -       | (≥5.0)   | 4.4     | (≥10)    |
| La      | 0.1-32  | (≥0.8) | 0.1-13  | (≥5.0)  | 0.8-14  | (≥10)   | 29      | (≥20)    | 12      | (≥30)    |
| Ni      | 0.1     | (≥1.9) | -       | (≥5.0)  | 2.0-36  | (≥10)   | -       | (≥20)    | 0.1-21  | (≥30)    |
| Pb      | -       | (≥3.0) | 1.0-4.0 | (≥50)   | 1.1-22  | (≥100)  | 6.0-33  | (≥150)   | -       | (≥200)   |
| Sb      | 1.0-24  | (≥0.3) | 0.8-41  | (≥2.0)  | 0.4-22  | (≥4.0)  | 1.8-8.0 | (≥6.0)   | 1.2-8.0 | (≥8.0)   |
| Sc      | 6.0-8.6 | (≥0.4) | 4.0-13  | (≥1.0)  | 1.0-22  | (≥1.5)  | 1.0-14  | (≥2.0)   | 1.5-43  | (≥2.5)   |
| V       | 1.7-43  | (≥5.7) | 1.0-16  | (≥25)   | 0.4-11  | (≥38)   | 6.0-17  | (≥50)    | -       | (≥75)    |
| W       | 14      | (≥0.2) | 1.0-32  | (≥0.5)  | 3.3     | (≥1.0)  | -       | (≥1.5)   | 4.6-20  | (≥2.0)   |
| Zn      | 1.2-23  | (≥61)  | 0.1-12  | (≥150)  | 1.3-8.3 | (≥250)  | -       | (≥350)   | 0.3-16  | (≥450)   |

\*At 40 sampling sites, out of 210 sites in the 1986-1987 programme, the local variation, expressed as the relative variance (%), was calculated per site. Since the 40 sites were dispersed throughout the country, the pollution level at each site varied from element concentration Class 1 to Class 5, according to the legends of Figures 2-1 to 2-15. The range of the local variation for each element concentration class is presented. The minimum concentration (mg.kg<sup>-1</sup>) for each element concentration class, corresponding with the legends of Figures 2-1 to 2-15, are given in brackets.

The differences between the results from the 1986-1987 lichen data set and the measured 1987 wet deposition data, and the agreement between the 1982-1983 lichen data with the 1983 calculated data, indicate that the element concentration patterns in lichens reflect the total deposition and the atmospheric concentrations in air particulate matter, rather than represent wet deposition only. The differences between the 1982-1983 and the 1986-1987 lichen data indicate temporal changes in levels and geographical patterns of trace-element air pollution.

## 2.4 Discussion

The terms indicators and monitors have been used frequently in the context of employing lichens in air pollution studies. The term "indicator" refers to the ability of an organism to indicate the presence/absence or high/low level of any factor in air pollution. Where information is provided by the organism about pollutant gradients or changing pollution levels, the term monitor organisms should be applied (Sloof *et al.* 1988). *Parmelia sulcata* meets many of the requirements of suitability for atmospheric monitoring purposes (Sloof *et al.* 1988): it occurs throughout The Netherlands, is tolerant to pollution (Wielgolaski 1975, de Wit 1976), averages the element concentrations over a suitable time period, reflects atmospheric concentration gradients, and shows an acceptable local variation with respect to the variation in element concentrations and their geographical distributions on a national scale (Tables 2-I, 2-III and Figures 2-1 to 2-15).

In both programmes (1982-1983 and 1986-1987) the samples were collected from October - November to January - February to exclude differences in element concentrations, due to seasonal variations. It was not possible to collect all samples within one month, due to the extensive network of sampling sites. Markert and Weckert (1989) reported seasonal variation in element concentrations in moss species, in which highest concentrations were found in the winter season.

In the present study the local variation of element concentrations in *P. sulcata* was determined. The local variation in element concentrations is due to variation in substrate, age and size of the samples, the micro climate, and the individual variation in lichen specimen. From Table 2-I, 2-III and the geographical distribution of the element

concentrations (Figures 2-1 to 2-15) obtained from the lichen data set, it can be concluded that the local variation is not significantly related to tree species, nor to the trace-element air pollution level of the sampling site. According to Rasmussen (1978) differences between bark concentrations of elements on different trees are often significant, but in an unpredictable way. Rasmussen and Johnson (1976) found no differences in elemental concentrations in epiphytically growing mosses on two different tree species. For most of the trace elements, uptake from the bark into the lichen is not likely to occur, but for cadmium and zinc the possibility of such uptake can not be excluded (de Bruin and Hackenitz 1986).

The samples from all sampling sites were composed of lichen thalli of different sizes and ages. Although these differences of the thalli may cause large local variations, the local variations determined ranged from only 0.1% to about 40% for all elements (Table 2-I), independent of the trace element pollution level (Table 2-III). Furthermore, the range of the element concentrations on a local scale is of a lower order of magnitude than the range of element concentrations on a national scale. A major requirement for the use of epiphytes is that the gradients in atmospheric concentrations in air particulate matter and deposition over time and distance are so steep, that the local variations are without importance (Rasmussen 1978).

Orchard Leaves were used as a reference, because, unfortunately, standard lichen reference materials do not exist. SRM 1571 was considered a reasonable alternative, because it contains all elements of interest, in concentrations comparable with those in the lichen samples. Furthermore, both SRM 1571 and lichen materials contain appreciable amounts of silicon, making them comparable also with respect to AAS digestion procedures and to possible matrix effects on AAS and INAA results.

The element concentration patterns obtained from the 1982-1983 and 1986-1987 data sets differ. Most changes were enlargements of areas with high element concentration classes, especially in the southern regions of the country. The most striking change with time in concentration pattern was found for Cd in lichens (Sloof and Wolterbeek 1991a). Also the Cd concentration gradient based on measured wet deposition in 1987 was different from the concentration gradient based on calculated total deposition in 1983.

Since the element gradients obtained from the lichen 1982-1983 data set agreed

with the gradients as derived from a dispersion model and measured data on atmospheric concentrations in air particulate matter and depositions (1983), lichens may be considered as good qualitative monitors of trace-element air pollution, on the temporal and geographical scale considered.

Comparison of the element concentration patterns obtained from both lichen data sets, showed temporal and geographical changes. Though a complete data set on atmospheric concentrations in air particulate matter and depositions (1987) is not available, the change of the element gradients in measured wet deposition from 1983 to 1987 indicates that atmospheric levels changed within 5 years. The reflection of the atmospheric gradients (1983) in the 1982-1983 lichen data set, the change of patterns in both lichen data sets, and the change in gradients in wet measured deposition (1987), substantiate the conclusion that lichens are able to reflect changes in atmospheric concentration levels and their distribution within 5 years.

Except for Cd, W and Zn, mean element concentrations did not change with time over a 5 year period (Table 2-II). At the same time, areas with high concentration classes expanded (Figs 2-1 to 2-15), suggesting that (heavy) metal air pollution in The Netherlands changed from being a local problem into a national problem. Despite the efforts of the Dutch government policy, aimed at reduction of local emissions, the mean air pollution levels did not decline.

The element gradients obtained from a dispersion model, the emission inventory and measured data on atmospheric concentrations in air particulate matter and depositions gave insight in the localization and the emission levels of pollution sources of elements with a long range transport (van Jaarsveld and Onderdelinden 1986). High foreign contributions were calculated for As (97%), Cd (82%), Cr (64%), Ni (71%), Sb (87%), Se (82%) and Zn (83%). The Belgian and the West German contributions were especially high, due to the emissions of metallurgical industry in the northern region of Belgium and the western part of West Germany. According to van Jaarsveld and Onderdelinden (1986) the contribution of Dutch emissions to the atmospheric element levels occurring in The Netherlands is smaller than 45% for all elements considered. The highest domestic contributions were calculated for Hg (43%) and Pb (44%).

The element concentration patterns obtained from analysis of epiphytic lichens, gave more insight into the possible locations of pollution sources, due to the very dense

network of sampling sites. The element concentration patterns showed high concentration classes for As, Cd, Cr, Cs, Fe, Ni, Pb, Sb, Se, V, W and Zn along the Belgian and West German border. Probable Dutch local pollution sources of Br, Cs, La, Ni, Se, V and W were found in the northern region of the country. Other possible pollution sources were found in the central region of the country for Br, Cd, Cr, Cs, Fe, Hg, La, Ni, Se, V and W. More information about the localization and character of pollution sources may be obtained by application of factor analysis, which is described in Chapter 4.

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### CHAPTER 3. LICHENS AS BIOMONITORS FOR RADIOCESIUM FOLLOWING THE CHERNOBYL ACCIDENT

**Abstract.** *Cesium-137 resulting from the Chernobyl accident was monitored in lichens in The Netherlands. Cesium-137 activity in *Parmelia sulcata* ranged from 550 to 6100 (average 2500) Bq.kg<sup>-1</sup> dry weight. The ratio between the <sup>137</sup>Cs activity per unit lichen dry weight (kg) of *Parmelia sulcata* and the <sup>137</sup>Cs activity deposited per unit surface area (m<sup>2</sup>) was approximately 1.0 m<sup>2</sup>.kg<sup>-1</sup>. Measurements of <sup>137</sup>Cs accumulation in *Xanthoria parietina* show that the activity concentration could be expressed both on a dry weight and on a contour surface area basis. The determination of the biological half-life of <sup>137</sup>Cs in lichens was indicated to be subject to sources of error like growth and non-atmospheric / indirect <sup>137</sup>Cs influxes. The similarity between the geographical <sup>137</sup>Cs activity gradients and radioactivity values in *Parmelia sulcata* and data of wet and dry deposition, indicate the validity of lichen monitoring of atmospheric <sup>137</sup>Cs.*



## CHAPTER 3. LICHENS AS BIOMONITORS FOR RADIOCESIUM FOLLOWING THE CHERNOBYL ACCIDENT

### 3.1 Introduction

The accident at the Chernobyl nuclear reactor on 26 April 1986 resulted in the release and global dispersion of fission products. The radioactive plume reached The Netherlands on 2 May. Due to rainfall on 3 and 4 May, significant deposition on the ground took place. The maximum  $^{137}\text{Cs}$  concentration in the atmosphere was  $5.0 \text{ Bq.m}^{-3}$  on 2 May 1986. On 5 May 1986, the  $^{137}\text{Cs}$  concentration decreased from 0.15 to  $0.03 \text{ Bq.m}^{-3}$ . After 5 May, the contaminated air above The Netherlands was driven off by wind. As a result the concentrations of radionuclides in the atmosphere rapidly decreased to values around the limit of detection (ca.  $0.001 \text{ Bq.m}^{-3}$ ) (Coordinating Committee for the Monitoring of Radioactivity and Xenobiotic Substances (CCRX) 1986, de Vries and van der Kooy 1986), (6 - 28 May observations by de Vries and van der Kooy 1986).

The present paper deals with the validity of lichen monitoring of atmospheric Chernobyl  $^{137}\text{Cs}$ . Lichens depend on nutrients from the atmosphere, since they have no root system or cuticle like vascular plants. Lichens have been reported as efficient collectors of atmospheric trace-element pollution, owing to their relatively high accumulation capacity for heavy metals and radionuclides (Tuominen and Jaakkola 1973, Rao *et al.* 1977, Eckl *et al.* 1984, Kwapulinski *et al.* 1985, Papastefanou *et al.* 1989). The transport of the Chernobyl plume over The Netherlands provided an opportunity to get information about processes involved in the accumulation and elimination of atmospheric radionuclides by epiphytic and epilithic lichens. From the long-lived radionuclides,  $^{137}\text{Cs}$  was measured for its radiological importance.

Since significant  $^{137}\text{Cs}$  influx was of short duration (4 days, de Vries and van der Kooy 1986), mainly due to rainfall, it was expected that  $^{137}\text{Cs}$  activity patterns in lichen data should especially reflect radioactivity gradients due to wet and dry deposition. Deposition data are generally presented on a surface area basis (Svensson 1967, CCRX 1986, Fry *et al.* 1986, Vaas 1986), but determination of actual surface areas in lichens may give rise to serious problems: the thalli may be curly and may contain fruiting bodies

or soredia. The lichen species to be sampled may also vary with respect to its morphological features, related to geographical variations in the pollution levels ( $\text{SO}_2$  and heavy metal) in the sampling area. For this reason, apart from the species used for monitoring, a foliose lichen species was selected (*Xanthoria parietina*), in which the measured  $^{137}\text{Cs}$  activity was calculated both on a contour (macro) surface area and on a dry weight basis.

### 3.2 Materials and Methods

The presentation of  $^{137}\text{Cs}$  activity in lichens expressed on a dry weight basis and on a surface area basis were compared, using the species *Xanthoria parietina* (L.) Th. Fr. This foliose species was chosen for its morphological structure: the thallus shows clear gradients in fruiting body density. In July 1986, two specimens of *Xanthoria parietina* were collected in Delft, The Netherlands. In the field the lichens were divided in ring-shaped zones, resulting in samples containing fruiting bodies (in high or medium densities) and samples without fruiting bodies (foliose parts). The contours of each zone were drawn on a transparent sheet of paper in order to calculate the contour (macro) surface area of the sample. The samples were taken from the substrates with scalpels. In total nine samples (zones, 6 - 38  $\text{cm}^2$ ) differing in fruiting body densities were collected.

In the laboratory the lichen material was made brittle by immersion in liquid nitrogen, ground and homogenized in a mortar and freeze-dried for 24 h. The samples (0.5 - 2 g) were analyzed for  $^{137}\text{Cs}$  using a well-type germanium detector (resolution 1.95 keV at 1.33 MeV FWIIM) (Canberra).  $^{137}\text{Cs}$  was determined from the peak area at 661 KeV (absolute detector-efficiency: 9.8%, measurement uncertainty < 5%). A standard  $^{137}\text{Cs}$  source was used for calibration. All samples were measured for 10 h and data were corrected for physical decay.

In November 1986, 6 months after the Chernobyl accident, samples (200 mg) of *Parmelia sulcata* Taylor were collected at 44 sampling sites spread out over The Netherlands. Within one sampling site, lichens of various ages (estimated 2 - 40 y) were sampled from three to six nearby trees. Lichen material with adhering bark substrate from *Populus* species and *Salix* species was sampled between 1.0 and 2.0 meters above the ground, in order to reduce contamination of soil particulate matter.

In the laboratory the lichens were separated from the bark substrate with nylon tweezers. The samples were prepared and analyzed following the same procedure as described above.

To get information about the biological half-life of  $^{137}\text{Cs}$  in lichens, samples of *Xanthoria parietina* (L.) Th. Fr. (substrate: tile roof), *Lecanora conizaeoides* Nyl. (substrate: *Acer pseudoplatanus*) and *Parmelia sulcata* Taylor (substrate *Pyrus communis*) were periodically collected, during one and a half year after the Chernobyl accident, at three different sampling sites in the south-west of The Netherlands, at approximately monthly intervals. For each species samples (1 - 2 g) of several specimen were collected at the same site from the same substrate. All samples were prepared and analyzed for  $^{137}\text{Cs}$  as described above.

The Chernobyl-origin of the  $^{137}\text{Cs}$  activity was verified by the simultaneous measurement of  $^{134}\text{Cs}$  in 20 lichen samples (determined at 604.8 keV, using a well-type germanium detector). These samples were measured for 10 h (measurement uncertainty < 15%) and the  $^{134}\text{Cs}$  data were corrected for physical decay ( $T_{1/2} = 2.1 \text{ y}$ ). The  $^{137}\text{Cs} / ^{134}\text{Cs}$  activity ratio in lichens was  $1.65 \pm 0.34$ . This value is in accordance with the 2.0 ratio, which is generally associated with the Chernobyl release of radioactivity (Devell *et al.* 1986, Papastefanou *et al.* 1988, Seaward *et al.* 1988, Taylor *et al.* 1988, Raes *et al.* 1990).

### 3.3 Results and Discussion

#### 3.3.1 $^{137}\text{Cs}$ activity in lichens

Table 3-I shows the  $^{137}\text{Cs}$  activity in nine samples of *Xanthoria parietina*, expressed both on a dry weight basis and on a contour surface area basis, for three classes of fruiting body density. From Table 3-I it is evident that  $^{137}\text{Cs}$  uptake occurred in both the foliose parts (Class 1) and the fruiting body areas (Class 2 and 3). The results expressed on a dry weight basis are highly variable within a density class. Within the fruiting body medium density Class 2, the averaged activity expressed on dry weight basis was  $1050 \pm 508 \text{ Bq.kg}^{-1}$ . The averaged  $^{137}\text{Cs}$  activity per unit surface area in the intermediate zones (Class 2) was  $710 \pm 116 \text{ Bq.m}^{-2}$ .

TABLE 3-1. Comparison of the  $^{137}\text{Cs}$  activity expressed on a dry weight and on a contour surface area basis, for three classes of fruiting body density in *Xanthoria parietina* (July 1986). Class 1 represents zero fruiting bodies (foliose), Class 2 medium density and Class 3 high density of fruiting bodies. The activity results expressed on a surface area basis are rather constant per density class, whereas on a dry weight basis the activity results are highly variable within a density class.

| Sample no. | Class | Cs activity<br>$\text{Bq.kg}^{-1}$ | Cs activity<br>$\text{Bq.m}^{-2}$ |
|------------|-------|------------------------------------|-----------------------------------|
| 1          | 1     | 698                                | 390                               |
| 2          | 1     | 1290                               | 352                               |
| 3          | 2     | 1397                               | 715                               |
| 4          | 2     | 1408                               | 879                               |
| 5          | 2     | 1452                               | 753                               |
| 6          | 2     | 574                                | 596                               |
| 7          | 2     | 420                                | 607                               |
| 8          | 3     | 423                                | 985                               |
| 9          | 3     | 1387                               | 967                               |

The results expressed on a surface area basis seem to show an almost constant activity concentration per density class. Furthermore,  $^{137}\text{Cs}$  activity per unit surface area shows a gradient in relation to the density classes, likely due to an increase in the actual surface areas (resulting from the presence of fruiting bodies), which could not be accounted for in the contour surface area calculations. These results indicate that the activity in lichens can be best expressed on a surface area basis, provided that the samples are collected from a known fruiting body density class.

In general lichens have a high surface to mass ratio, especially foliose and fruticose species. This property is often reported as one of the main reasons for their relatively high capacity to accumulate heavy metals and radionuclides (Rao *et al.* 1977, Kwapulinski *et al.* 1985, Seaward *et al.* 1988, Papastefanou *et al.* 1989, Ellis and Smith 1987, Smith and Ellis 1990).

In cases when the accumulation capacity is high relative to the element concentration in the atmosphere, the element uptake by lichens will be directly related to their (actual) surface area. Concerning the carrier free  $^{137}\text{Cs}$  radionuclide, which mass

is very low, uptake was expected and it was shown to be related to the surface area. It is likely to assume that uptake of other elements or non carrier free radionuclides are not limited by the surface area only; the lichen weight and the thickness (number of cells) may gain importance in relation to element uptake with increasing atmospheric concentrations. Furthermore, elements may be translocated within the lichen thallus from the upper layer (the surface) into inner layers.

The influence of the texture and the relationships between the algal and the fungal components in the various thalli parts may also be important with respect to the mechanisms of the uptake of radionuclides (Rao *et al.* 1977). In *Cladonia* species, Hanson (1967) found the  $^{137}\text{Cs}$  content to be 2 to 14 times higher in the upper than in the lower cortex. Nevstrueva *et al.* (1967) observed in four 1-cm segments from apex to base of *Cladonia* thalli higher  $^{137}\text{Cs}$  contents in the upper than in the lower parts of thalli. The differences in activity in various parts of *Cladonia* thalli were not due to differences in surface/mass ratio's, but may be attributed to differences in texture and/or relationships of the symbionts.

Comparing the results, without giving notice to the fruiting body density, the variation of activity is 46% on dry weight basis and 33% on contour surface area basis. When sampling occurs in a survey area in which the total geographical variation in  $^{137}\text{Cs}$  activity is at least two orders of magnitude higher, results can be expressed on dry weight basis, which is in practice the most convenient option.

### 3.3.2 Validation of lichen monitoring

In *Parmelia sulcata* samples it was not possible to differentiate in foliose zones and zones with soredia. Therefore, samples of different size and age were collected in the field without measuring the surface area, and their dry weights were determined in the laboratory.

Figure 3-1 shows the geographical distribution pattern of  $^{137}\text{Cs}$  activity obtained from the *Parmelia sulcata* data set. The  $^{137}\text{Cs}$  activity in *P. sulcata* ranged from 550 to 6100 (average 2500)  $\text{Bq.kg}^{-1}$ . Unshaded parts on the map indicate activity levels lower than 1500  $\text{Bq.kg}^{-1}$  (Figure 3-1). The highest  $^{137}\text{Cs}$  activity was found in a zone from the south-eastern parts to the north-western parts of The Netherlands.

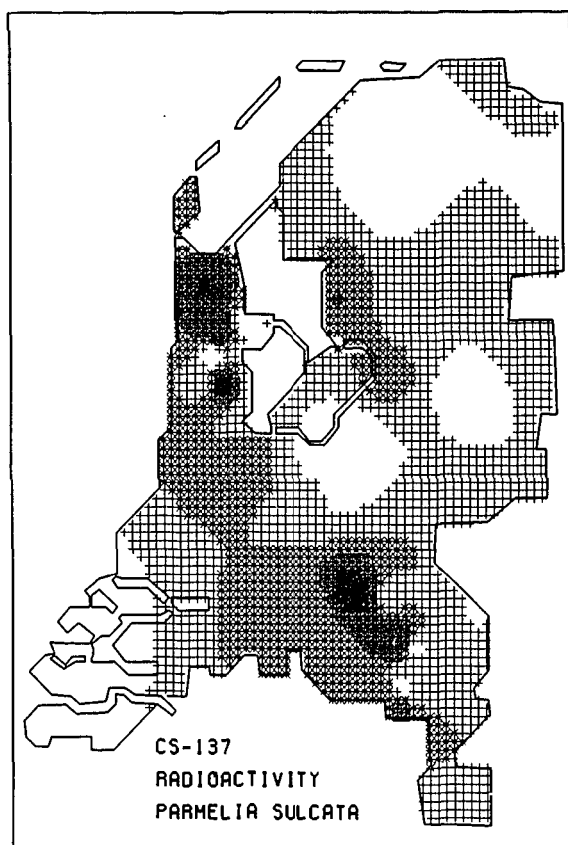


Figure 3-1. Geographical distribution pattern of cesium-137 activity in *Parmelia sulcata* in The Netherlands. The geographical distribution pattern contains five cesium-137 activity classes (in  $\text{Bq.kg}^{-1}$  dry weight). Class 1 (unshaded) ranges from the minimum activity determined to Class 2 (shaded with plus sign). Consequently, Class 5 (darkest shaded) ranges to the maximum activity concentration determined. Classes 1-5 values: 550-1500, 1500-2500, 2500-3500, 3500-4500, 4500-6100,  $\text{Bq.kg}^{-1}$  dry weight.

The  $^{137}\text{Cs}$  activity gradient obtained from the lichen data set was compared with the  $^{137}\text{Cs}$  gradient from direct atmospheric measurements (air filters) and calculated values for wet and dry deposition. Figure 3-2 shows the isodeposition lines for the estimated wet and dry deposition of  $^{137}\text{Cs}$  due to Chernobyl (CCRX 1986 Vaas 1986). Here, for each sampling site (total: 9), wet  $^{137}\text{Cs}$  deposition  $D_{\text{wet}}$  ( $\text{kBq.m}^{-2}$ ) was calculated following  $D_{\text{wet}} = F.C.h$  (Vaas 1986), with  $C$  as the mean  $^{137}\text{Cs}$  concentration in rainwater during the period 3-4 May 1986 ( $\text{kBq.m}^{-3}$ ),  $h$  as the amount of rainfall per unit surface



Figure 3-2 shows large geographical variations in  $^{137}\text{Cs}$  activity in wet deposition, due to differences in the amount of rainfall within The Netherlands during 3 and 4 May 1986 (CCRX 1986). The highest depositions were found in a small band from the south-eastern parts to the north-western parts of the country. This zone of relatively high total depositions (3 - 6  $\text{kBq.m}^{-2}$ ) is on both sides bordered by broad areas of lower depositions (1.5 - 3  $\text{kBq.m}^{-2}$ ).

Uptake and release of cesium in lichens may be affected by the chemically related and physiologically important elements calcium, potassium and sodium (Ellis and Smith 1987, Tuominen and Jaakkola 1973). Nevertheless, comparing the lichen data (Figure 3-1) with the data of the calculated atmospheric deposition (Figure 3-2) shows similar geographical distribution patterns of  $^{137}\text{Cs}$  activity. Moreover, it should be noted that the activity (dry weight basis) in *Parmelia sulcata* appears to be linearly related to the atmospheric deposition (surface area basis): The ratio between  $^{137}\text{Cs}$  activity accumulated per kg lichens and the  $^{137}\text{Cs}$  radioactivity deposited per  $\text{m}^2$  surface area is approximately 1.0, in  $\text{m}^2.\text{kg}^{-1}$ . The ratio suggests a specific surface area of *P. sulcata* of  $1 \text{ m}^2.\text{kg}^{-1}$ , its vertical position on tree stems, its rather curly thallus and the presence of soredia, indicate that this specific surface area should be considered as a contour surface area related value (cf. Table 3-I, *Xanthoria parietina*) or as an effective or apparent one.

The results indicate the validity of  $^{137}\text{Cs}$  monitoring using lichens. Based on the Cs-data, lichen monitoring of other atmospheric (heavy) metals may also be expected to yield reliable and interpretable results.

The average  $^{137}\text{Cs}$  activity in *Parmelia sulcata* in The Netherlands ( $2500 \text{ Bq.kg}^{-1}$ ) was lower than the average activity level in various lichen species in other countries. Seaward *et al.* (1988) reported  $^{137}\text{Cs}$  contents in *Umbilicaria* species in Poland, varying from 471 to  $36630 \text{ Bq.kg}^{-1}$ . The present results show that calculations on a surface area basis may have yielded significant differences between the lichen species. In northern Greece, Papastefanou *et al.* (1989) found no significant differences in  $^{137}\text{Cs}$  content (average  $5500 \text{ Bq.kg}^{-1}$ ) between fruticose species and foliose species studied. Sawidis (1988) observed  $^{137}\text{Cs}$  activity levels in three other foliose lichen species varying from 1100 to  $1700 \text{ Bq.kg}^{-1}$  in the same region in Greece. Since the dispersion pattern of  $^{137}\text{Cs}$  activity is related predominantly to total deposition (rainfall), the activity levels show large variations in countries with large geomorphological differences.



### 3.3.3 The biological half-life

The atmospheric influx of  $^{137}\text{Cs}$  was of short duration and had already become insignificant at the first sampling data (CCRX 1986, de Vries and van der Kooy 1986).

Decline of  $^{137}\text{Cs}$  activity in lichens is due to radioactive decay (rate constant  $\lambda$ ) and to biological removal processes, with a rate constant  $\lambda_b$  (Ellis and Smith 1987). Based on the assumed absence of any lichen growth and further  $^{137}\text{Cs}$  influx, the radioactivity per unit lichen dry weight  $A_t$  at any time  $t$  can be given as

$$A_t = A_0 \cdot e^{-(\lambda + \lambda_b)t}$$

where  $A_0$  is the activity per unit lichen dry weight at  $t = 0$ , set at the end of significant atmospheric  $^{137}\text{Cs}$  influx in The Netherlands (28 May 1986) (de Vries and van der Kooy 1986, CCRX 1986).

Figures 3-3 to 3-5 show the fitted curves of the  $^{137}\text{Cs}$  activity as a function of time after exposure to fallout for three lichen species (*Parmelia sulcata*, *Xanthoria parietina* and *Lecanora conizaeoides*). The activity in *P. sulcata* ranged from 340 to 600 Bq.kg<sup>-1</sup>, in *X. parietina* from 1200 to 2200 Bq.kg<sup>-1</sup>, and in *L. conizaeoides* from 170 to 390 Bq.kg<sup>-1</sup>. Samples were taken during June 1986 to September 1987. For each species the variations in the activity are due to variation in microclimate and possible genetic and/or morphological variability; the latter may in turn be related to different (local) atmospheric pollution (heavy metals, SO<sub>2</sub>) levels. Since each species was sampled at a different sampling site,  $^{137}\text{Cs}$  activity values could not be compared between species. In an earlier study the local variation in trace element concentrations, due to genetic variability, variation in size and age of lichen thalli and exposition within one sampling site, was found not to exceed about 30% (Sloof and Wolterbeek 1991). For the present results it is assumed that the variation in activity in samples from the same substrate for each species was of the same order of magnitude.

In *Parmelia sulcata* (Figure 3-3) the  $^{137}\text{Cs}$  activity concentration decreased with time (Chi sq. = 0.28), with  $A_0 = 518 \pm 103 \text{ Bq.kg}^{-1}$  and  $(\lambda + \lambda_b) = 7.73 \cdot 10^{-4} \text{ d}^{-1}$ . Since the physical half-life of  $^{137}\text{Cs}$  is 30 years, the biological half-life could be calculated as 977 days. This relatively short biological half-life (compared to 5-8 y: Ellis and Smith

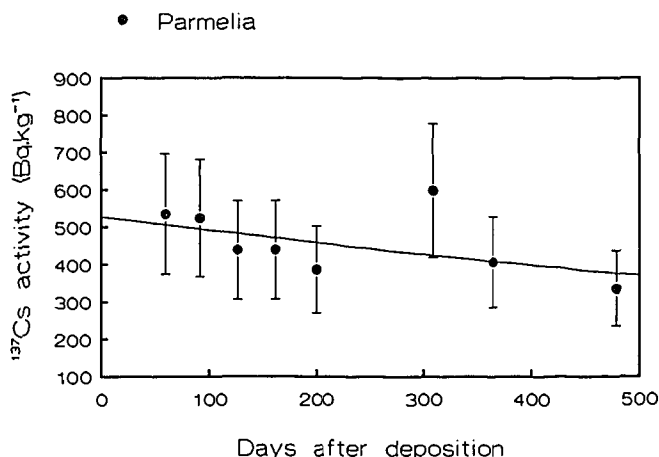


Figure 3-3. The cesium-137 activity in *Parmelia sulcata* (in Bq.kg<sup>-1</sup> dry weight) as a function of time after atmospheric deposition of cesium-137 ( $t = 0$  at 28 May 1986).

1987, 17 y: Liden and Gustafsson 1967) may be due to lichen growth: the growth rates for saxicolous species vary from 10 mm per year (foliose species) down to 0.1 mm per year (crustaceous species) (Jenkins 1987). Maximum growth rates for *P. sulcata* in The Netherlands are reported to be 4 mm per year (Richardson *et al.* 1982). Lichen growth is indicated to cause enhanced "dilution" of the activity concentration (Tuominen and Jaakkola 1973, cf. Ellis and Smith 1987), which will lead to increased  $\lambda_b$  values in the expression given above.

In *X. parietina* (Figure 3-4) and *L. conizaeoides* (Figure 3-5) the <sup>137</sup>Cs activity increased with time by a factor of almost 2, based on the fitted curves, apparently indicating negative ( $\lambda + \lambda_b$ ) values (Chi sq. = 0.33 and 0.27). Though the activity per unit lichen dry weight at  $t = 0$  ( $A_0$ ) was determined at the end of the atmospheric <sup>137</sup>Cs influx and the <sup>137</sup>Cs concentration in the atmosphere during the sampling period was below limits of detection, any further non-atmospheric <sup>137</sup>Cs influx during the measuring period may have led to decreased or even negative  $\lambda_b$  values, which may even yield negative values for ( $\lambda + \lambda_b$ ) rate constants. This further influx may have resulted from <sup>137</sup>Cs-leaching from the substrate and subsequent accumulation in lichens. Most likely, aeolian redistribution and washing out of <sup>137</sup>Cs from roofs and tree stems occurred.

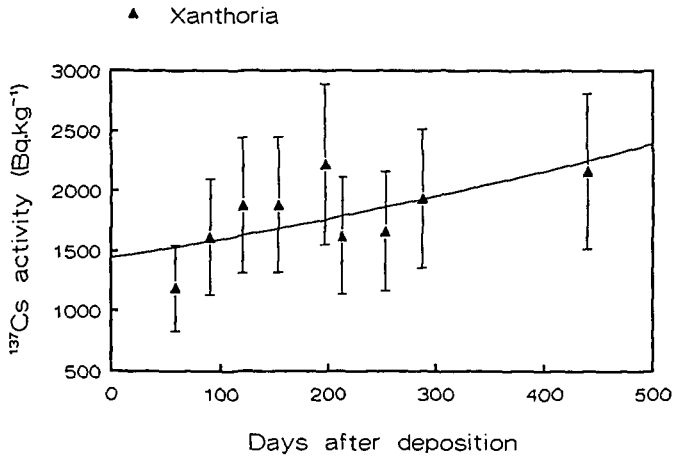


Figure 3-4. The cesium-137 activity in *Xanthoria parietina* (in Bq.kg $^{-1}$  dry weight) as a function of time after atmospheric deposition of cesium-137 ( $t = 0$  at 28 May 1986).

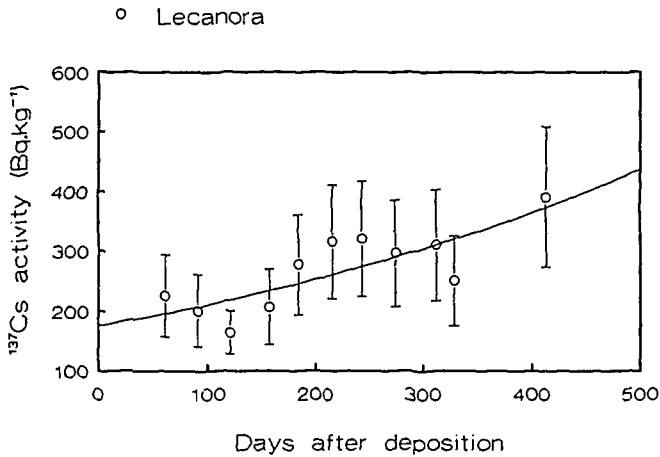


Figure 3-5. The cesium-137 activity in *Lecanora conizaeoides* (in Bq.kg $^{-1}$  dry weight) as a function of time after atmospheric deposition of cesium-137 ( $t = 0$  at 28 May 1986).

The results presented above (Figures 3-3 to 3-5) indicate the difficulties and uncertainties associated with the determination of biological half-lives *in situ*, and suggest that, unless all relevant processes are accounted for, no reliable values for biological half-lives can be established.

Based on  $^{137}\text{Cs}$  determinations in carpets of four lichen species in Sweden in 1962-1965, Liden and Gustafsson (1967), reported the biological half-life to be  $17 \pm 4$  years, using a similar equation as given in the present paper. They also reported that the sampling accuracy was a source of error and difficult to estimate. Ellis and Smith (1987) estimated the biological half-life of  $^{137}\text{Cs}$  in lichens to be 5 to 8 years, based on a radionuclide uptake model and measured data from the Chinese nuclear test fallout in 1980. Their lichen model was developed to predict lichen inventories based on atmospheric influx, including growth functions and bio-elimination rates, but they did not account for any leaching and subsequent input from the substrate.

The variation in values of biological half-lives given above (1 to 17 years) clearly stress the need for known relevant parameters in biological half-life determinations.

### 3.4 Conclusions

The  $^{137}\text{Cs}$  activity concentration in foliose lichens can be expressed on a dry weight basis and on a contour surface area basis. Without giving notice to the fruiting body density, the variation of activity is 46% on a dry weight basis and 33% on a contour surface area basis.

The  $^{137}\text{Cs}$  activity in *Parmelia sulcata* was linearly related to atmospheric deposition of this radionuclide. The ratio between the  $^{137}\text{Cs}$  activity per unit dry weight (kg) in lichens and the  $^{137}\text{Cs}$  activity per surface area unit ( $\text{m}^2$ ) in atmospheric deposition was approximately  $1.0 \text{ m}^2.\text{kg}^{-1}$ . These results indicate the validity of  $^{137}\text{Cs}$  monitoring using lichens.

The determination of the biological half-life of  $^{137}\text{Cs}$  in lichens may be subject to many sources of error: growth, genetic and microclimate variability,  $^{137}\text{Cs}$  leaching from the substrate leading to  $^{137}\text{Cs}$  influx not accounted for, and variations in density of fruiting bodies.

The Chernobyl accident providing carrier free  $^{137}\text{Cs}$ , makes the experiment unique, because of the very small absolute isotope mass involved. Notwithstanding the small  $^{137}\text{Cs}$  mass, lichens proved to be reliable biological monitors of  $^{137}\text{Cs}$ .

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## CHAPTER 4. PATTERN RECOGNITION IN LICHENS FOR SOURCE APPORTIONMENT

**Abstract.** *Monte-Carlo-assisted factor analysis has been applied to a lichen data set, obtained from a monitoring survey on a national scale in The Netherlands for source apportionment purposes. The Monte-Carlo method was used to estimate the uncertainties in the source profiles obtained. The factor analysis as applied revealed 10 factors, of which 8 could be interpreted in terms of realistic sources of pollution. Geographical distribution patterns of the factor values were used to facilitate and to support the identification of the associated sources.*



## CHAPTER 4. PATTERN RECOGNITION IN LICHENS FOR SOURCE APPORTIONMENT

### 4.1 Introduction

In many trace-element biomonitoring investigations in or near polluted areas, the element concentrations in lichens have been used to monitor atmospheric element concentrations. In general, the levels of metals and other trace elements in lichens decline with increasing distance from the pollution source (Rao *et al.* 1977, Addison and Puckett 1980, Nieboer and Richardson 1981, Puckett 1988). The geographical concentration patterns obtained from lichen data sets from two Dutch monitoring surveys, on a national scale, were consistent with the element concentration gradients obtained from a dispersion model and measured data on atmospheric concentrations in air particulate matter and depositions (see Chapter 2 of this thesis). The geographical concentration patterns obtained from lichens gave information on the possible location of pollution sources (see Chapter 2).

In order to obtain information on the character and the origin of pollution sources from the element concentration patterns obtained from the lichen data sets, a multivariate statistical analysis technique is used. Factor analysis is a technique suitable for simplifying large and complex data sets in such a way that it may create a limited number of factors, each representing a cluster of interrelated variables within a data set (e.g. geographically dependent correlations between element concentrations).

In air pollution studies Target Transformation Factor Analysis (TTFA) has been used to resolve the composition of atmospheric aerosols or element concentration patterns obtained from lichen data sets into factors, which may represent the emission sources (Hopke *et al.* 1976, Alpert and Hopke 1980, Henry *et al.* 1984, Henry 1987, de Bruin *et al.* 1987, Pilegaard 1987, Hopke 1988, Sloof and Wolterbeek 1991b). General goals of TTFA are the determination of the elemental source composition (profiles) of the independent sources (common factors) and the calculation of the contribution of each factor (source) to each sample.

It should be noted that the factor model is a receptor-based application of pattern recognition in trace elements in ambient samples, such as air particulate matter and lichens. The meteorological conditions, the transport characteristics of the emitted elements influenced by particle size and composition, the structure of the terrain over which the emitted elements are transported, the distance from the emission source and if present, the height of the stack, may be of influence on the size and the composition of particles collected with air filters or impactors and accumulated and/or trapped by lichens. Therefore, some factor loadings obtained from the immission measurements might not show all the characteristic elements or the element ratios for specific (types of) sources. Furthermore, some patterns found in for example, lichens, might reveal unknown sources that remain unidentified.

Contrary to the Chemical Mass Balance method (CMB) which requires both the number of sources and their composition at the receptor side to be known in advance (Hopke 1991), factor analysis is the most appropriate choice to obtain the desired information in cases where no a-priori information on these source properties at the receptor side is available.

The *aim* of the present study was to get insight in the potentials of the factor analysis approach for obtaining information on the character of pollution sources on basis of trace-element concentration patterns observed in lichens, using the factor analysis approach. The lichen data set was obtained from a monitoring survey in 1986-1987 on a large scale covering the entire land area of The Netherlands. A common occurring epiphytic species was used, namely *Parmelia sulcata* Taylor. From 40 sampling sites, out of 210 sites, 3 or more (up to 7) different samples were available, taken from adjacent trees within the sampling site. Apart from the counting statistics, the observed variation in the element concentrations on a local scale was referred to as "local variance" (see Chapter 2). The local variance comprised the effects of the variation in the substrate tree species, the age of the samples and individual (genetic and morphological) variation in lichen specimen. No significant correlation was observed between the *magnitudes* of the local errors and the *magnitudes* of the pollution level (see Chapter 2).

So far, most publications on factor analysis did not explicitly account for the uncertainties in the data set, nor did they facilitate the estimation of the uncertainties

in the factor solution. If no information on the uncertainties in the data set is available, principal components analysis is generally applied, in which the common factors must account for all variance in the data set. Hopke (1988) and Roscoe and Hopke (1981) discussed the so-called jack-knifing method, to obtain error estimates for the factor solution. However, the jack-knifing method does not take into account any knowledge (if present) about the uncertainties in the data set. Instead, it estimates error limits for the factor solution by subsequently eliminating a sample from the data set. It is doubtful whether these error estimates can be considered to be reasonable estimates of the true uncertainties.

Kuik *et al.* (1993a,b) reported a detailed study of TTFA and its applications to environmental studies, in which the reliability and the validation of the factor solution, as well as the choice of the number of factors, were investigated by using Monte Carlo techniques. The essence of the TTFA as developed by Kuik *et al.* is that the element concentration at each sampling site is considered as a Gaussian distribution, of which the width is determined by the uncertainty in each element concentration. In the Monte Carlo approach, a large number of modified data sets is generated, in which all element concentrations are randomly varied according to their associated Gaussian distribution. TTFA is applied on all the generated data sets in order to obtain the uncertainties of the factor loadings.

In the present study the Monte-Carlo assisted *principal factor* model as described by Kuik *et al.* is applied to the 1986-1987 lichen data set for source apportionment purposes.

## 4.2 Factor analysis

The mathematical details of the Monte-Carlo assisted factor analysis model have been outlined in another publication (Kuik *et al.* 1993a). Here, only the most essential steps in the factor model are presented to illustrate how element concentrations in lichens can be interpreted for source identification purposes. The main lines of the mathematical background are given in the Appendix.

A data set of  $n$  element concentrations is considered, determined at  $N$  sampling sites. The concentration of the  $j$ -th element ( $j = 1 \dots n$ ) at the  $i$ -th sampling

site ( $i = 1 \dots N$ ) is denoted by  $x_{ji}$ . In order to remove the different scales of measurement for the various chemical species, the data set is transformed to the standardized variables  $z_{ji}$  (see Appendix).

In the factor model each  $z_{ji}$  is assumed to be a linear sum of  $m$  common factors (to be interpreted as source profiles at the receptor side), with  $m \leq n$ , which account for the correlations between the variables, and a unique contribution which is specific for each individual sampling site:

$$z_{ji} = \sum_{k=1}^m a_{jk} f_{ki} + d_j u_{ji} \quad (1)$$

The components  $a_{ji}$  are called the *loadings* of the factors. They represent the *correlation* of element  $j$  with factor  $k$  and thus they are indicative for the relative element composition of the factor  $k$ . The  $m$  coefficients  $f_{ki}$  are usually called the *values* of the factors. They represent the contribution of factor  $k$  to sample  $i$ . The product  $d_j u_{ji}$  represents the residual error for element  $j$  in sample  $i$ , which is not accounted for by the  $m$  common factors. The so-called uniqueness  $d_j^2$  represents the fraction of the variance of variable  $j$  which is not explained by the common factors. Because this unexplained variance is reflected in measured variations on a local scale (as shown by multiple measurements within a single sampling site), the uniqueness is called the "local variance". The  $n$  coefficients  $u_{ji}$  indicate how each individual sample  $i$  contributes to the uniqueness (see Appendix).

For the complete data set, equation (1) can be expressed in matrix form:

$$Z = A F + D U \quad (2)$$

where  $Z$  is an  $n \times N$  matrix with components  $z_{ji}$ ,  $A$  an  $n \times m$  matrix with components  $a_{jk}$ ,  $F$  an  $m \times N$  matrix with components  $f_{ki}$ ,  $D$  an diagonal  $n \times n$  matrix with components  $d_j$  on the diagonal and  $U$  an  $n \times N$  matrix with components  $u_{ji}$ .

The initial step in TTFA is to transform the data into an  $n \times n$  correlation matrix, which contains the correlations between the elements. A first direct solution for  $A$  is obtained following the *principal factor* method, which mainly consists of the diagonalization of the reduced correlation matrix  $C$  (see Appendix). To obtain

physically meaningful solutions for  $A$ , the factor loadings should satisfy the following conditions:

- \* the factor loadings should not contain negative values (obviously a source cannot emit negative amounts of any element)
- \* the factor loadings must explain the original correlations in the data set.

Fulfilment of the conditions mentioned above is achieved by transformation of the direct solution  $A$  to a new solution  $B = AR$  by an iterative process called *target transformation*, with  $R$  the transformation matrix.

The uncertainties in the obtained factor loadings are estimated using a Monte-Carlo approach. The basic idea of this approach is to generate a large number (500) of modified data sets  $X'$ , with concentrations  $x'_{ji}$ , in which all element concentrations are slightly altered in a random way and subsequently perform factor analysis on these data sets. The magnitudes of the normally-distributed random deviations are chosen in accordance with the uncertainties in the original element concentrations (see Appendix).

Each modified data set  $X'$  is subject to factor analysis, resulting in a matrix of rotated factor loadings  $B'$ . It has been found that the arrangement of the  $m$  columns of the matrix  $B'$  may be different from the arrangement of the columns in the original matrix  $B$ . Hence it is necessary to identify corresponding columns of  $B$  and  $B'$ . Identification is accomplished by the method of geometrical correspondence: two vectors coincide if the angle between them is zero. In order to avoid scaling problems, the matrices  $B$  and  $B'$  are compared in the correlation domain, before back-transformation to the concentration domain. Sometimes two different columns of the matrix  $B'$  tended to be identified with the same column of the matrix  $B$ , resulting in an identification conflict. The number of identification conflicts was recorded, but the results for such a matrix  $B'$  was discarded for the Monte-Carlo process.

In practice, it has been found that the number of Monte-Carlo steps resulting in such a conflict is dependent on the number of factors. There is a sharp rise in the number of factor conflicts beyond the optimum number of factors, which can be used as a criterion for the determination of the optimum number of factors to explain the data set.

During the Monte-Carlo process, for each factor loading ( $b_{jk}$ ) the number of

zero values was recorded in order to enable the calculation of a probability for zero values  $P_{jk}(0)$ . The averaged loadings are considered to be significant if they are non-zero for more than 95% of the generated data sets. The final solutions for the factor loadings are determined by calculating means and standard deviations after completion of all Monte-Carlo steps.

After target transformation, each column of the obtained final solution is scaled to sum up to a total of 1,000,000 (arbitrary value). In the final step of the analysis, the matrix of factor values  $F$  is obtained by fitting the original data set  $X$  with the averaged solution  $B'$ , using a least squares method, which is not further discussed here.

### 4.3 The lichen data set

In 1986-1987 the lichen species *Parmelia sulcata* Taylor was sampled at 210 sampling sites from various tree species throughout the Netherlands. The sampling sites were approximately arranged in a grid network of about  $10 \times 10$  km. The lichens were collected according to a standardized sampling method, as described in Chapter 2 in paragraph 2.2.1. At each sampling site several lichen thalli of different sizes were taken from one tree and likewise, if possible, from 3 to 7 nearby trees. In some sampling sites, only one or two trees were appropriate for sampling.

In the laboratory the lichens were separated from the substrate with nylon tweezers and washed with 30 ml of distilled water to remove adhering sand or dust. Material from the same tree and from nearby trees within one sampling site was combined to yield one representative sample. The samples were made brittle by immersion with liquid nitrogen, ground and homogenized with a pestle and mortar and finally freeze dried for 24 h.

In order to establish the variation of element concentrations within one sampling site, the so called "local variation", for 40 sampling sites spread throughout The Netherlands, the samples from one tree were prepared separately.

The lichens were analyzed for aluminum, antimony, arsenic, bromine, cadmium, cesium, chromium, cobalt, iron, mercury, lanthanum, manganese, nickel, scandium, thorium, tungsten, vanadium and zinc by instrumental neutron activation

analysis (INAA) (de Bruin *et al.* 1982, Bode and de Bruin 1990). The samples were irradiated twice in the reactor of the Interfaculty Reactor Institute at a thermal neutron flux of  $5 \cdot 10^{16} \text{ m}^{-2} \cdot \text{s}^{-1}$ . A first irradiation of 30 s was followed, after 10-15 min decay time of the short lived radionuclides, by a measurement of 5 min. After a second irradiation of 4 h, the samples were measured twice during 1 h, after decay times of 5 and 21 days, respectively. Measurements were carried out using semiconductor detectors (Ortec, Canberra). The precision of the measurements was 0.7-3% for As, Br, Co, Cr, Fe, La, Mn, Sb, Sc, Th, V, Zn and 7-13% for Al, Cd, Cs, Hg, Ni, Se and W. Pb was determined with atomic absorption spectrometry (see Chapter 2, paragraph 2.2.3). Quality control of the INAA and AAS results was based on the regular analysis (1 per 10 samples) of standard reference material NBS SRM 1571 "Orchard Leaves" and the results agreed with the NBS-certified values and the recommended values by Gladney *et al.* (1987) (Sloof and Wolterbeek 1991a).

## 4.4 Results and Discussion

### 4.4.1 Estimation of the uncertainties in the data set

The uncertainty in an element concentration for each element at each sampling site was found to be determined mainly by local variations in the lichen data set. In most cases the local variations exceeded the instrumental errors in the concentrations by factors of 2 up to 10. The errors in the element concentrations based on the local variations, as determined for 40 sites, varied on the average between 13% (Zn) and 30% (Th). For each of these 40 sites, means and standard deviations for each element concentration have been determined and the standard error of the mean (SEM) was taken as the final error in the averaged concentration due to local variations. For the remaining sampling sites (from which only 1 or 2 samples were taken) estimates for the errors due to local variations were obtained by taking the "averaged" standard deviations found from the 40 multiple-sample sites mentioned above.

The values obtained for the local variance (uniqueness) ranged from 9% for Hg to 40% for Ni, the mean over all elements considered was 25%.

#### 4.4.2 Determination of the number of factors

There exists no mathematical formula to determine the correct number of factors to be retained from a data set. Several criteria for the determination of the number of factors have been used in literature, such as the observation of a significant drop in the magnitude between the  $m$ -th and  $(m + 1)$ -th eigenvalue of the reduced correlation matrix  $C$  (Alpert and Hopke 1980, Pilegaard 1987, Hopke 1988), but a certain amount of the modellers judgement remains necessary in choosing the number of factors.

Figure 4-1 shows the relation between the factor identification conflicts during the Monte-Carlo process and the number of factors to be retained in the data set.

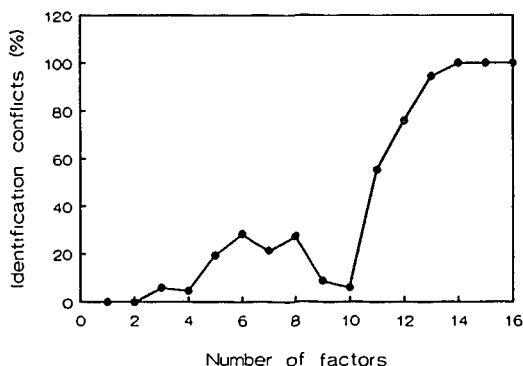


Figure 4-1. The relation between the identification conflicts and the number of factors.

There is a sharp rise in the percentage of identification conflicts as the number of factors becomes larger than 10. Addition of one more factor yielded an 11th factor, which almost perfectly corresponded with one of the previous set of 10 factors (cosine value of about 0.95). Thus it can be concluded that the increase from 10 to 11 factors does not provide new information. Factor 11 can be therefore be regarded as being superfluous and is omitted. In fact the 11th factor is similar to the first factor and responsible for the strong increase in factor-identification conflicts as shown in Figure 4-1. A similar explanation holds for the addition of subsequent factors. The small



increase in the number of identification conflicts around the 7th factor is not completely understood yet. However, repeated calculations have shown an excellent reproducibility, so there is little doubt that this structure is real.

Kuik *et al.* (1993a,b) compared the number of factors obtained when applying the present criterion (number of identification conflicts using the Monte-Carlo approach) and other criteria from literature for the 1986-1987 lichen data set. In all cases 10 factors were found to fit the lichen data set best.

#### 4.4.3 Factor loadings

The element of a profile with the largest loading in the *correlation* domain is called the *pilot* element. Such a pilot element of a factor has the strongest correlation with that specific factor. Consequently, it is the most characteristic element in the profile. Since the concentrations of several elements may differ orders of magnitude, the loading in the *concentration* domain of the pilot element may be much lower than the loading of a non-characteristic element in a profile. Therefore, the averaged factor loadings obtained are again normalized to a value of 100.0 for the *pilot* elements. Table 4-I shows the normalized factor loadings with their relative standard deviations in percent. In Table 4-I, only significant loadings are shown, according to the 95% probability criterion for the non-zero values. For the pilot elements, the relative uncertainties in the factor loadings varied from 6% (Br in factor 5) to 23% (W in factor 7). The non-significant loadings, for which the zero-value probability is larger than 5%, were found to have substantially larger errors than the significant ones. For example, the relative uncertainties in the non-significant factor loadings for Pb and Cr in factor 4 are 180-190%.

Table 4-II shows the average relative contributions  $S_{jk}$  of factor  $k$  to the total occurrence of element  $j$  in the data set. This quantity is defined by

$$S_{jk} = \sum_{i=1}^N (a_{jk} f_{ki}) M_i \bigg/ \sum_{i=1}^N x_{ji} M_i \quad (3)$$

with  $M_i$  the mass of sample  $i$ . In general, the significant factor loadings (Table 4-I) tend to have relatively large contributions to the elemental occurrences. For example

the relative contribution of Cd and Se in factor 4 are 56% and 34.1%, respectively of the total Cd and Se occurrence in the data set. For the non-significant loadings of factor 4, such as those of Cr and Pb, the relative contributions are only 1.3% and 2.1%, respectively, of the total Cr and Pb occurrence.

#### *4.4.4 Source apportionment*

The observed concentration patterns in lichens yielded 10 factors, giving rise to the interpretation in terms of 10 (types of) sources or processes.

The geographical distribution pattern of the factor values ( $f_{ki}$ ) may enable the possible location of the pollution sources. In Figures 4-2A to 4-2J the geographical patterns of the contributions (values) of all the factors (1 to 10) are shown. These factor values are dimensionless. For each factor contribution, a low-high gradient is presented by 4 classes, in which the darkest shaded class represents the highest contribution of the factor. In the Figures 4-2A to 4-2J, the unshaded parts represent factor values, which are lower than (approximately) the mean contribution of the factor. For all factors, the minimum contribution is zero. The numbers given in the legends (classes) can not be compared between the factors, since they were calculated independently after the final loadings were scaled to sum up to a total of 1,000,000.

TABLE 4-I. Normalized averaged factor loadings obtained after 500 Monte-Carlo variations of the 1986-1987 data set. Indicated loadings were found to be significantly positive ( $P > 95\%$ ). Relative errors in percent are given in parentheses.

|    | F1    |      | F2   |      | F3     |      | F4  |      | F5   |      |
|----|-------|------|------|------|--------|------|-----|------|------|------|
| Al | 13000 | (46) | -    |      | 320000 | (16) | -   |      | -    |      |
| As | 13    | (23) | 2.0  | (11) | 100    | (36) | 19  | (53) | -    |      |
| Br | -     |      | -    |      | -      |      | -   |      | 100  | (6)  |
| Cd | -     |      | -    |      | -      |      | 100 | (20) | -    |      |
| Co | 7.3   | (21) | 0.60 | (15) | 59     | (25) | 6.2 | (58) | -    |      |
| Cr | 59    | (51) | 13   | (20) | 1900   | (20) | -   |      | -    |      |
| Cs | 1.8   | (39) | -    |      | 51     | (13) | 2.2 | (60) | 0.39 | (43) |
| Fe | 31000 | (20) | 860  | (37) | 300000 | (19) | -   |      | 4900 | (32) |
| Hg | -     |      | -    |      | -      |      | -   |      | -    |      |
| La | -     |      | -    |      | 350    | (28) | -   |      | 2.3  | (59) |
| Mn | -     |      | -    |      | -      |      | -   |      | -    |      |
| Ni | 100   | (12) | -    |      | -      |      | -   |      | -    |      |
| Pb | -     |      | -    |      | -      |      | -   |      | -    |      |
| Sb | 13    | (26) | 1.0  | (23) | -      |      | 14  | (54) | -    |      |
| Sc | 2.1   | (46) | 0.16 | (47) | 75     | (11) | 2.9 | (56) | 0.56 | (38) |
| Se | 4.1   | (31) | 0.34 | (24) | 44     | (25) | 23  | (22) | -    |      |
| Th | -     |      | 0.21 | (49) | 100    | (12) | -   |      | -    |      |
| V  | 160   | (13) | -    |      | 660    | (43) | -   |      | 13   | (45) |
| W  | -     |      | -    |      | -      |      | -   |      | -    |      |
| Zn | -     |      | 100  | (9)  | -      |      | -   |      | -    |      |

|    | F6    |      | F7  |      | F8   |      | F9   |      | F10 |      |
|----|-------|------|-----|------|------|------|------|------|-----|------|
| Al | -     |      | -   |      | 1600 | (42) | -    |      | -   |      |
| As | -     |      | -   |      | 0.93 | (47) | -    |      | 13  | (44) |
| Br | -     |      | -   |      | -    |      | -    |      | -   |      |
| Cd | -     |      | 59  | (68) | -    |      | -    |      | -   |      |
| Co | -     |      | -   |      | 0.49 | (35) | 0.28 | (46) | -   |      |
| Cr | -     |      | -   |      | -    |      | -    |      | -   |      |
| Cs | -     |      | -   |      | -    |      | -    |      | -   |      |
| Fe | 33000 | (46) | -   |      | 1400 | (44) | -    |      | -   |      |
| Hg | 100   | (21) | -   |      | -    |      | -    |      | -   |      |
| La | -     |      | -   |      | -    |      | -    |      | 100 | (15) |
| Mn | -     |      | -   |      | 100  | (7)  | -    |      | -   |      |
| Ni | 65    | (48) | -   |      | -    |      | -    |      | -   |      |
| Pb | -     |      | -   |      | -    |      | 100  | (7)  | -   |      |
| Sb | -     |      | 120 | (34) | 0.73 | (45) | 0.60 | (43) | 16  | (48) |
| Sc | -     |      | -   |      | 0.15 | (62) | -    |      | 2.4 | (75) |
| Se | -     |      | -   |      | 0.22 | (47) | -    |      | -   |      |
| Th | -     |      | -   |      | -    |      | -    |      | -   |      |
| V  | -     |      | 390 | (50) | -    |      | 4.7  | (48) | -   |      |
| W  | -     |      | 100 | (23) | -    |      | -    |      | -   |      |
| Zn | -     |      | -   |      | -    |      | -    |      | -   |      |

TABLE 4-II. Averaged relative contributions (in percent) of each factor to the total elemental occurrences, obtained after 500 Monte-Carlo variations of the 1986-1987 data set. Values associated with significant loadings (see Table 4-I) are printed bold face.

|    | F1          | F2          | F3          | F4          | F5          | F6          | F7          | F8          | F9          | F10         |
|----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Al | <b>8.4</b>  | 2.4         | <b>19.0</b> | 4.7         | 10.8        | 0.3         | 3.4         | <b>19.2</b> | 4.0         | 4.8         |
| As | <b>8.0</b>  | <b>32.4</b> | <b>5.4</b>  | <b>9.5</b>  | 0.5         | 0.4         | 2.6         | <b>10.6</b> | 1.5         | <b>4.0</b>  |
| Br | 0.1         | 0.0         | 0.2         | 1.7         | <b>68.6</b> | 0.1         | 0.0         | 0.1         | 4.8         | 0.2         |
| Cd | 1.7         | 10.2        | 0.3         | <b>56.0</b> | 14.2        | 0.0         | <b>7.5</b>  | 0.3         | 7.4         | 1.8         |
| Co | <b>12.4</b> | <b>28.1</b> | <b>8.9</b>  | <b>8.5</b>  | 8.1         | 0.4         | 1.8         | <b>15.5</b> | <b>10.0</b> | 1.9         |
| Cr | <b>7.6</b>  | <b>48.9</b> | <b>22.5</b> | 1.3         | 7.2         | 0.7         | 2.6         | 9.7         | 12.0        | 3.3         |
| Cs | <b>8.7</b>  | 8.6         | <b>21.5</b> | <b>8.3</b>  | <b>19.5</b> | 0.4         | 1.6         | 2.9         | 0.5         | 2.3         |
| Fe | <b>18.1</b> | <b>13.4</b> | <b>15.5</b> | 4.1         | <b>29.8</b> | <b>1.9</b>  | 2.1         | <b>14.9</b> | 5.3         | 3.1         |
| Hg | 1.3         | 0.9         | 0.2         | 1.8         | 10.2        | <b>50.6</b> | 3.4         | 3.3         | 10.1        | 0.5         |
| La | 5.0         | 6.1         | <b>19.0</b> | 8.0         | <b>14.9</b> | 0.2         | 1.8         | 3.7         | 1.6         | <b>28.8</b> |
| Mn | 2.8         | 7.1         | 3.0         | 0.3         | 1.2         | 0.4         | 0.3         | <b>58.4</b> | 3.4         | 0.9         |
| Ni | <b>26.0</b> | 6.2         | 3.3         | 4.5         | 15.7        | <b>1.8</b>  | 0.7         | 5.5         | 1.6         | 4.2         |
| Pb | 0.4         | 9.1         | 0.0         | 2.1         | 8.8         | 0.1         | 1.6         | 2.2         | <b>51.7</b> | 0.2         |
| Sb | <b>13.3</b> | <b>29.7</b> | 0.8         | <b>11.5</b> | 0.8         | 0.6         | <b>12.0</b> | <b>14.3</b> | <b>12.7</b> | <b>7.7</b>  |
| Se | <b>6.7</b>  | <b>13.2</b> | <b>21.2</b> | <b>7.2</b>  | <b>19.0</b> | 0.4         | 1.8         | <b>8.8</b>  | 4.9         | 3.7         |
| Sc | <b>7.9</b>  | <b>18.3</b> | 7.11        | <b>34.1</b> | 8.0         | 0.3         | 1.1         | <b>8.0</b>  | 5.3         | 1.9         |
| Th | 1.9         | <b>16.4</b> | <b>26.2</b> | 4.7         | 8.1         | 0.1         | 1.6         | 8.2         | 6.3         | 4.9         |
| V  | <b>16.6</b> | 0.0         | 6.1         | 7.2         | <b>15.0</b> | 0.2         | <b>4.0</b>  | 8.5         | <b>10.6</b> | 1.8         |
| W  | 1.1         | 23.9        | 1.5         | 6.6         | 2.2         | 0.3         | <b>39.3</b> | 1.3         | 6.7         | 0.6         |
| Zn | 0.0         | <b>46.7</b> | 3.8         | 6.0         | 1.2         | 0.1         | 2.0         | 3.4         | 8.9         | 2.0         |

Factor 1 comprises the elements Ni, V, Fe, Sb, Co, Cs, Al, As, Se, Cr and Sc. Ni and V are the most characteristic elements in this factor with large relative occurrences and relatively small errors in the loadings (12-13%). Mainly based on the significant loadings of Ni and V, factor 1 is considered to represent oil combustion processes. The ratio Ni/V in heavy residual fuel oil ranges from 1/1 to 1/3, depending on the type of chemical state and/or trace-element composition of the crudes (Kleinman *et al.* 1980, Olmez and Gordon 1985, Pacyna 1984). Data for crude oils are limited; it is known that, compared to coal and crustal material, V is high

relative to La (Olmez and Gordon 1985). Figure 4-2A shows the distribution of factor 1. The mean contribution is 14.6, the maximum contribution is 143.6. High contributions were found in the western part of The Netherlands. The presence of industries, boat-traffic and oil refineries in the surroundings of IJmuiden, Rotterdam (Pernis), Moerdijk and Antwerp (Belgium) (Hoste *et al.* 1974) and the prevailing south-western wind supports the source apportionment of factor 1. It should be noted that the areas with high contributions of factor 1 do not indicate the relative contributions of each oil-related source type. For example, 95% of the oil refinery activities in The Netherlands occur in Pernis.

Factor 2, characterized by the elements Zn, Cr, Co, As, Sb, and Se, most likely represents zinc smelters (de Bruin *et al.* 1987, Sloof and Wolterbeek 1991 *a,b*). Though Fe, Sc and Th have significant loadings in factor 2, the loadings have large uncertainties (37-49%). These elements are not characteristic for zinc industry, but may originate from the soil (see factor 3). Since zinc ores contain cadmium, both elements used to be emitted by zinc smelters; the Zn/Cd-ratio of 200:1 is typical for the commonly used zinc ores (de Bruin *et al.* 1987). However, new processes have been used recently, leading to reduced emission (if any) of Cd from zinc smelters. In factor 2 the loading of Cd was not significant. Figure 4-2B shows the distribution of factor 2, having a mean contribution of 11.9. High contributions were found in the southern part of the country. The presence of zinc industries in this area ("The Kempen", an area on both sides of the borderline between The Netherlands and Belgium) supports the source identification given for factor 2. This factor also shows a high contribution in a small area in the eastern part of the country; it is unknown whether zinc emitting industry is present in this area. On basis of the lichen data it would be interesting to search for such sources in this area.

Factor 3 strongly depends on concentrations of Th, Sc, Al, Cr, Fe, and Cs, which are all elements typical for crustal material (Edelman and de Bruin 1986, Hopke 1988). Other significant loadings in this factor are found for La, Co, As, Se and V, which are common trace elements in uncontaminated Dutch topsoils. For most trace elements, the Dutch top-soil background values are significantly ( $P = 99.9\%$ ) linearly related with the clay content, which may be ascribed to high element concentrations in minerals in the clay fraction in comparison with concentrations in

the loam and sand fraction (Edelman and de Bruin 1986). Factor 3 is interpreted as representative for the soil. Unlike factor 1, La has a significant loading in this factor and the La/V ratio is 0.53. This ratio is a reasonable value according to the background values of elements of Dutch top-soils reported by Edelman and de Bruin (1986). Figure 4-2C shows the distribution of the soil factor. The mean contribution is 20.0. High contributions of factor 3 correspond geographically with areas of sea clay (the south-western- and northern parts of the country and the drained polders around The IJsselmeer), areas of river clay (central parts along the rivers Meuse and Rhine) and areas of loess (southern part of the country).

Factor 4 is related to Cd, Co, Cs, Sc and the volatile elements As, Sb and Se and represents the major source of Cd. Possible sources are waste incineration and the production of Cd containing alloys (Hutton 1983, Hoffman and Hoffman 1991, Olmez *et al.* 1988, van Jaarsveld and Onderdelinden 1989, Bode and Wolterbeek 1992). Figure 4-2D shows the distribution of factor 4. The mean value of the factor is 5.91. The largest areas with high contributions were found in the southern parts of the country, suggesting a significant contribution from metallurgical industries and waste incinerations in the northern part of Belgium, where large non-ferrous metallurgical industries and many waste incinerations are situated (Hoste *et al.* 1974). In the western and north-western parts of The Netherlands, three waste incinerations with large throughput capacities are situated (van Jaarsveld and Onderdelinden 1989). Also in the northern part of the country a waste incineration is present, with relatively small throughput capacity. Waste incineration as an important source for atmospheric cadmium pollution might be related to the fact that storage of wastes, containing plastics with cadmium compounds, is increasingly being replaced by incineration (Bode and Wolterbeek 1992). Emitted products from the main sources might be deposited in the western, central and northern parts of the country (Figure 4-2D), taking the prevailing south-western wind direction into account. Moreover, the height of the stacks from the waste incinerations are variable, and with variable meteorological conditions, this leads to different deposition patterns of the particles (van Jaarsveld and Onderdelinden 1989). At the receptor side, thus in the biomonitor, the trace-element pattern and its geographical distribution might be different from the pattern and the location of the pollution source, due to transport over a large

distance.

Factor 5 contains Br as pilot element. Fe, Sc, Cs, V and La are other elements with significant loadings in this factor, which is likely to represent sources of coal combustion. Although coal fly ash has a composition similar to that of the crustal material, trace elements like Br, Cd, Hg, Ni, Pb, Sb, Se, V, W and Zn are enriched in emissions from coal fired plants (Mey *et al.* 1984, Olmez and Gordon 1985, van der Sloot *et al.* 1985, Olmez *et al.* 1988). The ratios between the elements are variable due to the various types of processes and coal composition (Institute for Nuclear Sciences, Gent 1972, van der Sloot *et al.* 1985). Figure 4-2E shows the distribution of factor 5. The mean contribution is 29.3. The geographical pattern shows high contributions along the coast line, in the western and northern parts of the country, indicating a marine origin. Luten (1977) determined high contributions of Br in coastal rainwater, originating from sea aerosols. Steinnes *et al.* (1992) also found high levels of Br in mosses along the coast line of Norway and suggested Br to originate from the marine environment. Long range transport of coal fly ash, especially from Great Britain, is likely to occur. Steinnes *et al.* (1992) reported excess deposition of various trace-elements, as derived from moss data, in southern Norway, which was predominantly due to long range atmospheric transport from other parts of Europe.

Factor 6 is characterized by Hg concentrations. The relative contribution of Hg to the total Hg occurrence is 50.6%. Only Fe and Ni are significant in factor 6, with large uncertainties in the mean loadings (46-48%). In Figure 4-2F the distribution of factor 6 is shown. The mean contribution is 0.79, the maximum contribution is 75. High values of this factor are found locally in 5 spots. Possible sources of Hg are various, such as waste incineration, steel producing industry, battery industry, coal combustion and cinnabar assimilation. It is possible that this factor represents a variety of sources. In the western part of the country iron and ferroalloys producing industries are located (IJmuiden). The local distribution of this factor might be an indication for the location of the Hg sources. It would be interesting to search for sources in these local areas. So far, the type of source associated with this factor is unknown.

Factor 7 comprises W, Cd, V and Sb. A possible source is the non-ferrous metal producing industry. In Figure 4-2G the distribution of factor 7 is given, which value

has a mean value of 2.85. In the central-southern part of the country, non-ferrous metal industries are known to be present, which locations correspond with the high contribution of factor 7 in this area. De Bruin *et al.* (1987) suggested the preparation or handling of phosphors as a possible source of W and Cd. The presence of electronic industries (TV screens, TL tubes) in this area supports this suggestion. Factor 7 may also represent glass industries, comprising the production of TV screens, tubes, packing material, isolation material and crystal glassware. The fact that air particulate matter collected on air filters in the surroundings of these industries, which were located in the areas corresponding with the high contribution of factor 7 contained Cd, W and V (Brasser, personal communication), supports this source apportionment.

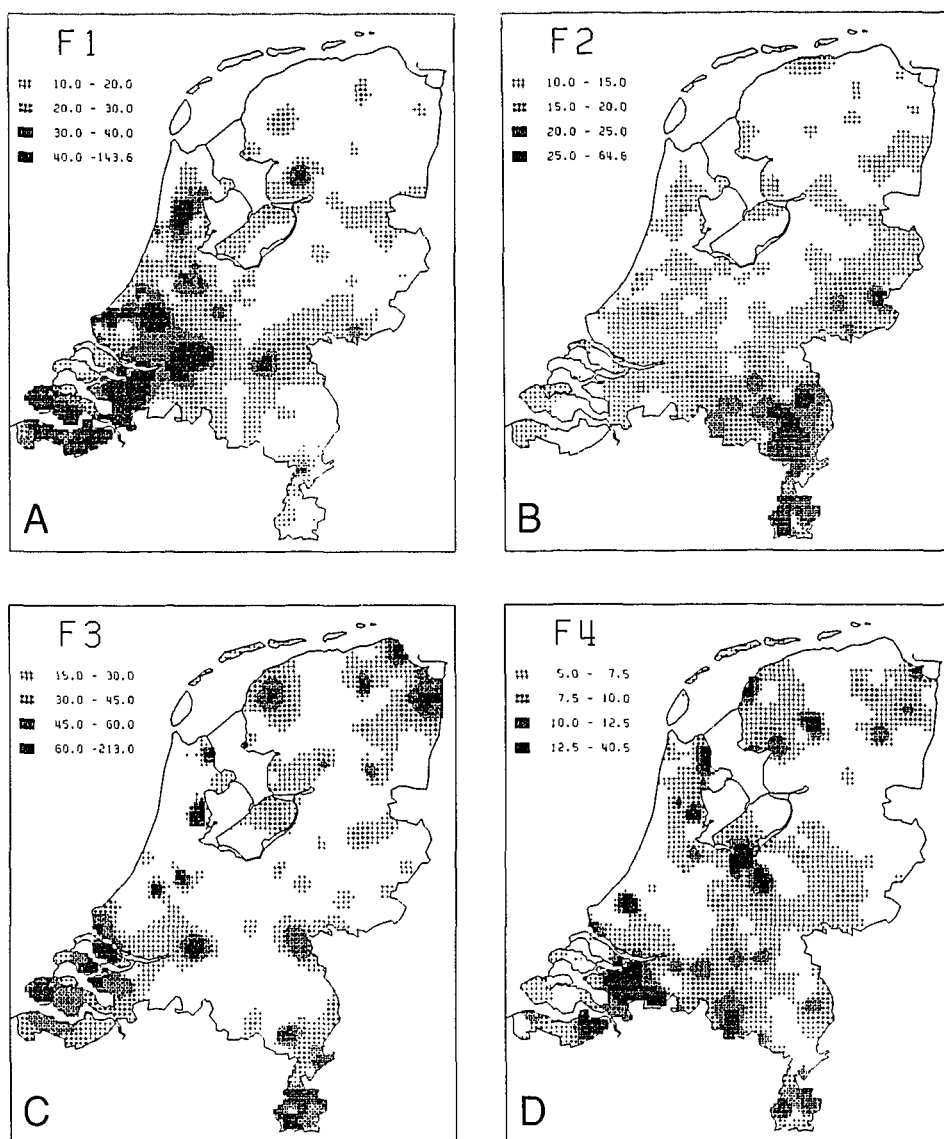
Factor 8 is related to Mn, Al, Co, Sb, Fe, As, Sc and Se, which may be indicative for high-temperature processes, such as iron and ferroalloys producing industry (Hampel 1968). Mn is the most widely used element in ferroalloys (Pacyna 1984). The distribution of factor 8 is given in Figure 4-2H. The mean value of this factor is 23.8. In the north-western part of The Netherlands iron and ferroalloys producing industries are located (IJmuiden), which may account for the high contributions of factor 8 in this area. Long range transport of trace-elements from steel producing industries located in Belgium (Gent and Genk) and Germany (Ruhr area) is also likely to occur. Also the wear and corrosion processes of iron and steel constructions (e.g. railway tracks, breaking-up yards) throughout the country, lead to a wide dispersion of particles and may be an important source represented by factor 8.

Factor 9 is related to Pb, V, Sb and Co. Pb is a tracer for particles associated with automobiles (Kleinman *et al.* 1980). Although Br-containing compounds are known to be used as additives in gasoline, no correlation with Pb is evident from the present data. The chemical composition of particles containing Pb and Br may be changed during transport in the atmosphere. Br is likely to be exchanged by Cl and subsequently by SO<sub>4</sub>, which has also been observed in marine aerosols. In the lichens as receptors, the element composition and the elemental ratios may be different from those in the particles emitted; alterations may occur during transport or in the lichens. Furthermore, sampling of lichens near motor ways was avoided. Figure 4-2I shows the distribution of the factor 9, the mean value being 6.32. Factor 9 may represent motor

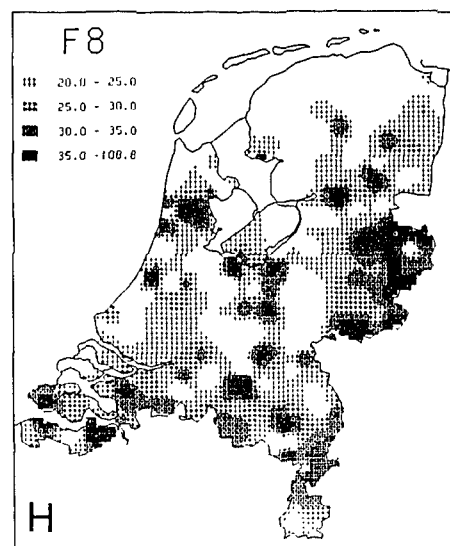
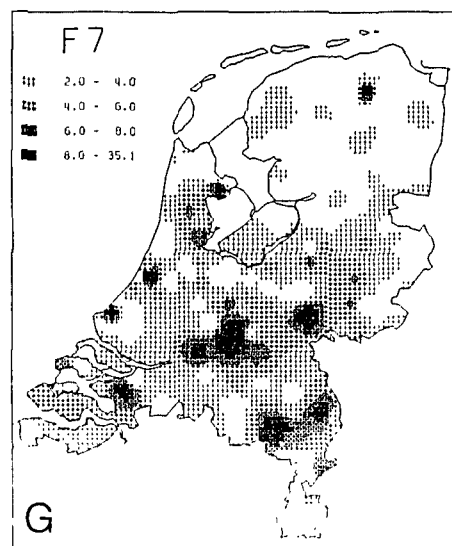
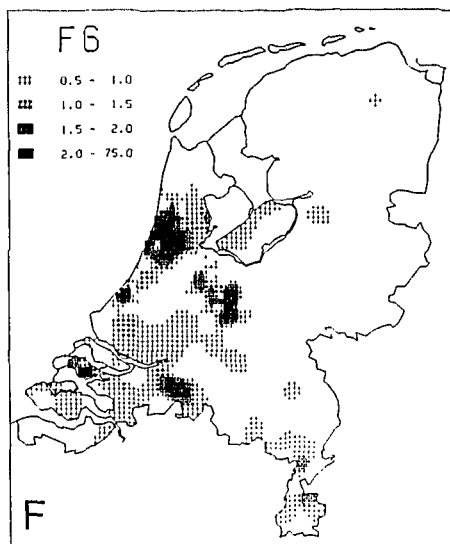
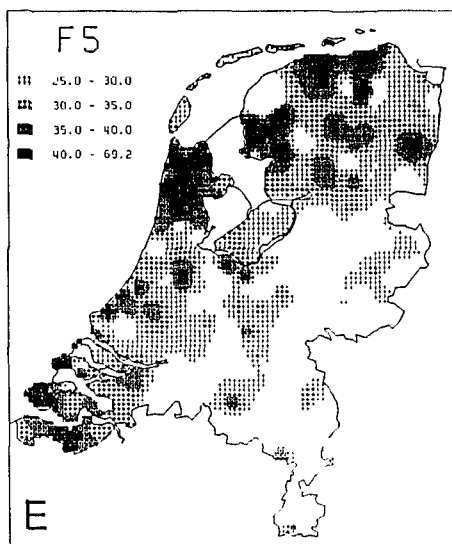


traffic, though the distribution of this factor does not support this source apportionment. The high contributions in the northern part of the country cannot be explained, and in the western part of the country (especially the Rotterdam area) the low contribution of this factor is not in agreement with the local heavy traffic. Possibly other (unknown) sources than motor traffic may be represented by factor 9.

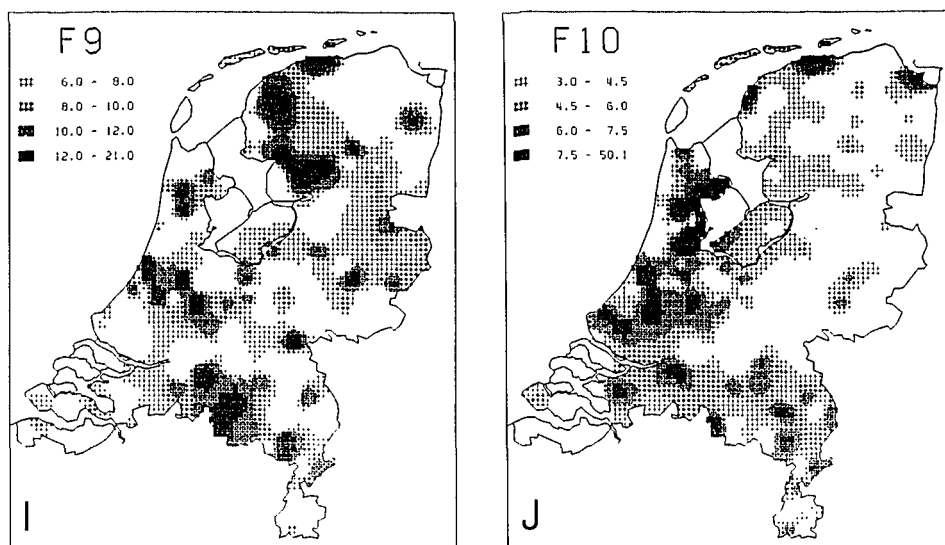
Factor 10 is characterized by La, As, Sb and Sc. Rare earth compounds are used in industrial catalysts, but knowledge about quantity, emission to the air and composition of these sources in our study area was not available. La and As are also used in the glass industry (Lide 1991). Figure 4-2J shows that the largest areas with high contributions of factor 10 are situated in the northern, western and central-southern parts of the country. The mean value of factor 10 is 3.76. Generally, the geographical distribution of this factor is in agreement with the locations of industrial activities: In the northern and southern parts of the country two glass fibre industries are located (Hoogezand and Etten Leur, respectively) and in the western part glassware producing industries are present in Schiedam and Leerdam. Furthermore, in the southern-and northern -western parts high-temperature processing industries (Moerdijk, Bergen op Zoom and IJmuiden) are located, which support the source apportionment. The sources associated with this factor are speculative and it is unknown whether one or more source types are involved.



Figures 4-2A-D. The geographical patterns of the contributions (values) of factor 1, "oil combustion processes" (A), factor 2, "zinc smelters" (B), factor 3, "soil" (C) and factor 4, "waste incineration and metallurgical industries" (D).

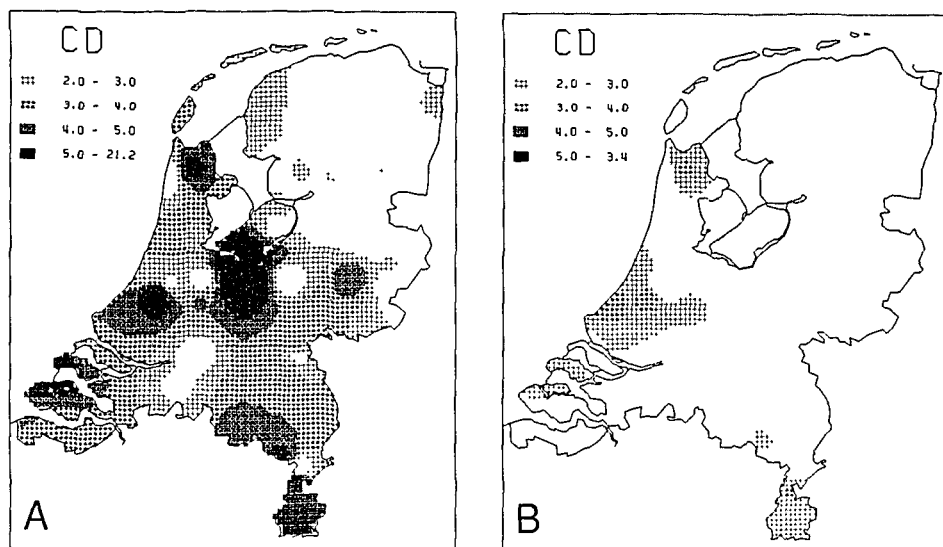


Figures 4-2E-H. The geographical patterns of the contributions (values) of factor 5, "coal combustion processes" (E), factor 6, "mercury sources" (F), factor 7, "electronic and glass industries" (G) and factor 8, "high-temperature processes" (H).



Figures 4-2I,J. The geographical patterns of the contributions (values) of factor 9, "motor traffic and other lead sources" (I), and factor 10, "lanthanum sources" (J).

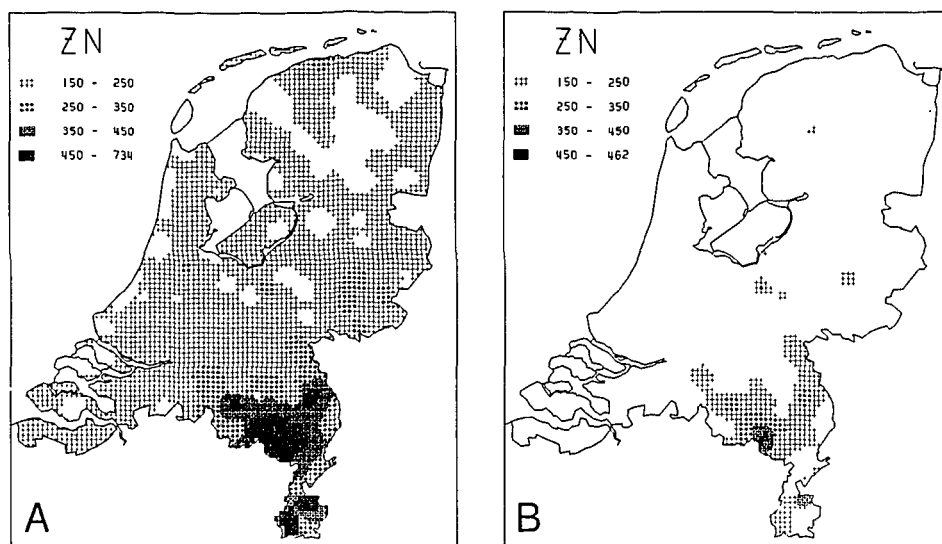
How well the lichen data set is modeled by the 10 common factors can be visualized by filtering the element concentrations in lichens for one or more of the common factors and investigating the remaining geographical distribution of the concentrations. For example, the Cd concentration pattern in lichens was filtered for factor 4, of which the relative contribution to the total Cd occurrence was 56%. The original Cd concentrations ranged 0.65 to 21  $\text{mg.kg}^{-1}$  (Figure 4-3A). After filtering for factor 4, the remaining Cd concentrations ranged from -0.5 to 3.4  $\text{mg.kg}^{-1}$  (Figure 4-3B), indicating that the majority of the Cd pattern was explained by this factor. Filtering for all 10 factors yielded an almost blank chart, except for a small spot with a maximum concentration of 2.5  $\text{mg.kg}^{-1}$ . The effect of filtering for all factors was expected to be a random geographical distribution, representing the local variance and the individual analytical uncertainties. The observed remaining Cd concentrations after filtering for 10 factors are lower than 2  $\text{mg.kg}^{-1}$ , (except for one small spot), indicating that all the geographical patterns have been explained by the 10 factors.



Figures 4-3A,B. The cadmium concentration pattern in lichens observed (A) and filtered for factor 4, "waste incineration and metallurgical industries" (B).

Due to the statistical treatment, the minimum remaining Cd concentration was found to be slightly negative, which should be seen as an indication that the factors fully explained the variance of the data set.

Another example is given in Figures 4-4A and 4-4B for Zn. The Zn concentration pattern in lichens (Figure 4-4A) was filtered for factor 2, of which the relative contribution to the total Zn occurrence was 46.7% (Figure 4-4B). The original Zn concentrations ranged from 74 to 734  $\text{mg.kg}^{-1}$ . After filtering for factor 2, the remaining Zn concentrations ranged from -21 to 462  $\text{mg.kg}^{-1}$ . After filtering for all factors, the remaining Zn concentrations in lichens are lower than 150  $\text{mg.kg}^{-1}$  in almost the whole country, except for a small spot in The Kempen area. From these results it can be concluded that the majority of the Zn variance in the data set was explained by factor 2.



Figures 4-4A,B. The zinc concentration pattern in lichens observed (A) and filtered for factor 2, "zinc smelters" (B).

#### 4.5 Concluding remarks

Validation of the factor loadings at the receptor side and assignment to real sources, requires extensive information on the location and character of the pollution sources (element composition, particle size, element ratios), as well as the meteorological conditions, the distance from the source, and the deposition velocities. So far, not all the required information on the pollution sources for validation is available. The geographical patterns of the factor values may be useful for further investigation, to search for local pollution sources. Further research is needed to obtain information on the ratios of the element composition of the raw materials and those from the emitted components and their transport characteristics.

However, the factor analysis revealed 10 factors from the experimental data set, of which 8 factors could be interpreted in terms of realistic sources, namely oil combustion, zinc industry, soil, non-ferrous industry, waste incineration, coal

combustion, steel producing industry and motor traffic. The factor depending on lead, probably represents motor traffic and other (unknown) lead sources. The factors depending on Hg and La, respectively, are still unknown. Notwithstanding the possible differences between the elemental ratios in particles and their composition in the emission and those in lichens, at the receptor side, lichens may be used as a tool for source apportionment. So far, the interpretation of the factors should be regarded as indicative.

Validation of the applied factor model with artificial data sets has been carried out by Kuik *et al.* (1993a,b). For all investigated test-data sets it was found that the final solution, obtained after completion of a large number of Monte-Carlo iterations of the data set, appeared to be by far more accurate than the single solution.

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## CHAPTER 5. SUBSTRATE INFLUENCE ON EPIPHYTIC LICHENS

**Abstract.** *The origin of soil elements in the epiphytic lichen *Lecanora conizaeoides* Nyl. was investigated in an area with heavily polluted soil (The Broekpolder, The Netherlands). Analysis results of lichens, bark and tree rings of the supporting trees indicated that for As, Co, Cr, Cu, Fe, Hg, Sc and V the levels in lichens may be considered to originate from wet and dry deposition. The Cd, Mn and Zn levels in lichens might have originated from both wet and dry deposition and the substrate bark. Especially for these three elements, accumulation characteristics in lichens should be further investigated.*

## CHAPTER 5. SUBSTRATE INFLUENCE ON EPIPHYTIC LICHENS

### 5.1 Introduction

Although extensive literature data are available on the use of lichens as biomonitors of trace-element air pollution (Nash and Wirth 1988, Sloof and Wolterbeek 1991<sup>a,b</sup>, Tyler 1989), there is no consensus yet regarding the contribution of substrate-derived elements to the element content in epiphytic lichens. Recent research on element uptake by lichens suggested that uptake from the substrate by lichens may occur; it appears to vary among substrates, lichen species and elements. Goyal and Seaward (1981) demonstrated metal uptake by rhizines of *Peltigera* species, Prussia and Killingbeck (1991) found significant differences between the concentrations of P, K, Ca and Zn in *Flavoparmelia baltimorensis* thalli growing on granite boulders and in those lichens growing on adjacent *Quercus* bark, which suggests that substrate type can exert an important influence on lichen element content. De Bruin and Hackenitz (1986) found concentrations of Ba, Ca, Cd, Mn and Zn in *Lecanora conizaeoides*, which were similar to the levels in the supporting tree bark, in The Kempen area (The Netherlands).

In source apportionment studies, using statistical analysis methods such as factor analysis, generally, one or two soil factors are derived from lichen data sets (Chapter 4 of this thesis, De Bruin and van Wijk 1988, Pilegaard 1987, Sloof and Wolterbeek 1991<sup>b</sup>). The soil factor(s) are characterized by Al, Cr, Fe, Mn, Sc and Th, which are all typical elements for the crustal material (Edelman and de Bruin 1986, Hopke 1988). This finding raises the question whether lichens still reflect atmospheric concentrations in air particulate matter and/or depositions in an area with heavily polluted soil or that those lichens reflect soil raised by the wind.

In general the presence of soil elements in epiphytic lichens may originate from three main element pools: 1) contamination of local soil raised by the wind, 2) substrates and 3) wet and dry deposition. The second pool might result in direct uptake from substrate elements and/or in indirect atmospheric input: Elements may be taken up from the atmosphere by the bark surface, followed by uptake into the

lichen through the rhizines and/or after weathering of the bark surface. In the latter case, the origin of the element content in lichens is still atmospheric, and the concentrations in lichens and outer bark are expected to be of comparable magnitude.

The aim of the present study was to estimate the contribution of the three pathways to the element content in lichens, for some of the soil elements. For this purpose, lichens, substrate bark and tree rings were collected from an area with contaminated soil and compared with lichens and substrate bark from an area with unpolluted soil.

## 5.2 Experimental

### 5.2.1 Sampling

The area selected for the collection of lichens and tree rings from trees growing on polluted soil is the Broekpolder, which lies at the North East of Rotterdam, The Netherlands. The Broekpolder is composed of dredged sludges from the eastern Rotterdam harbour area. These materials, composed of sediments from the rivers Meuse and Rhine, and the ports of Rotterdam, are mixed with marine sediments from the North Sea, and are disposed in adjacent polders. Disposal of the dredged sludges was done with layers of 0.60 to 1.5 m high. This was followed by physical and chemical ripening for three years, rotatilling and deep dewatering before the next layer was applied, in order to enhance the leaching of marine salts (Kay *et al.* 1989). The Broekpolder (ca. 6 km<sup>2</sup>) was utilised for disposal until 1975, but was not covered by a clean top soil layer (van Driel *et al.* 1977, van Lindt personal communication 1992). Table 5-I shows the enhanced element concentrations in the Broekpolder soil (van Driel 1980) compared to Dutch reference clay-soil (van Driel 1980, van Driel and Smilde 1981, Edelman and de Bruin 1986). Due to a preferred occurrence of the heavy metals in the finest grain-size fractions, (linear relationships are always found between the contents of the heavy metals and the clay (< 16 microns) fractions) (de Groot *et al.* 1971, de Bruin and Edelman 1986), the element contents in Table 5-I were normalized to a constant particle size composition (50% < 16 microns) and concerned the CaCO<sub>3</sub>-free mineral fractions.

TABLE 5-1. Element concentration ranges in soil (mg.kg<sup>-1</sup>).

|    | Broekpolder soil<br>range | Dutch reference clay soil <i>a,b,c</i><br>range |
|----|---------------------------|---|
| As | 41 - 61                   | 12 - 21   |
| Cd | 5.8 - 21                  | 0.40 - 0.50                                     |
| Co | n.d.                      | 14 - 16   |
| Cr | 160 - 290                 | 78 - 117  |
| Cu | 110 - 370                 | 23  |
| Fe | n.d.                      | 33000 - 39000                                   |
| Hg | 3.8 - 7.5                 | 0.18 - 0.20                                     |
| Mn | 490 - 880                 | 470 - 651                                       |
| Sc | n.d.                      | 12 - 16   |
| V  | n.d.                      | 124 - 126                                       |
| Zn | 590 - 1500                | 97 - 120  |

*a* van Driel 1980; *b* van Driel and Smilde 1981; *c* Edelman and de Bruin 1986.

n.d. denotes not determined.

Furthermore, the presented concentrations concern the averaged values over 20 cm soil depth.

In November 1987, samples (100 mg) of *Lecanora conizaeoides* Nyl. together with the substrate bark (*Populus* species) were collected from 15 poplars inside the Broekpolder and from 13 poplars in the surroundings of the Broekpolder, at a height between 1.0 and 2.5 m above the ground. From each tree, samples of lichen material were taken from the tree at the four wind directions and combined to yield one representative sample. From three trees in the Broekpolder, one core per tree was also taken, diametrically through the whole trunk. The diameter of the cores was 8 mm.

### 5.2.2 Sample preparation

The lichens were separated from the substrate bark with a stainless steel scalpel. The remaining bark was separated into two fractions: the 2-3 mm thick dark-

brown coloured and weathered outer layer and the light-brown coloured inner layer. Lichen and bark samples were washed with distilled water to remove adhering dust, freeze dried and ground in an agate ball mill. The tree cores were separated in growth increments (tree rings) of two years and freeze dried for 24 h.

All samples (100 - 200 mg) were packed in polyethylene capsules for analysis.

### 5.2.3 Analysis

The samples were analyzed for the elements arsenic, cadmium, chromium, cobalt, copper, iron, mercury, manganese, scandium, vanadium and zinc by instrumental neutron activation analysis (INAA) (de Bruin *et al.* 1982, Bode and de Bruin 1990). The samples were irradiated in the reactor at the Interfaculty Reactor Institute (IRI) at a thermal neutron flux of  $5 \times 10^{16} \text{ m}^{-2} \cdot \text{s}^{-1}$ . A first irradiation of 30 s was followed, after 5-15 min decay time, by a 5 min measurement of the short-lived radionuclides. After a second irradiation of 4 h, the samples were measured twice during 1 h, after decay times of 5 and 21 days, respectively. Measurements of the gamma-ray spectra of the irradiated samples were carried out with Germanium-Lithium semiconductor detectors (Ortec, Canberra).

## 5.3 Results and discussion

### 5.3.1 Lichens and outer bark

Table 5-II shows the means, standard deviations and ranges of the element concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Sc, V and Zn in *Lecanora conizaeoides* Nyl. and outer bark from trees inside (polluted soil) and outside (unpolluted soil) the Broekpolder. To detect possible significant differences between the means of the lichen and bark populations inside respectively outside The Broekpolder, Student's t-test was applied (Zijp 1980). Regarding the large ranges, the high standard deviations and the small sample size of the lichen and bark data sets, the meaning of the statistical results should be interpreted with care.

The mean element contents in lichens inside the Broekpolder were similar or

TABLE 5-II. Element concentrations in lichens and outer bark ( in mg.kg<sup>-1</sup>).

|                | Lichens |      |            |      | Outer bark |      |             |      |
|----------------|---------|------|------------|------|------------|------|-------------|------|
|                | mean    | SD   | range      | (n)  | mean       | SD   | range       | (n)  |
| <i>Inside</i>  |         |      |            |      |            |      |             |      |
| As             | 3.3     | 1.5  | 1.6-5.9    | (11) | 0.74       | 1.1  | 0.055-3.5   | (9)  |
| Cd             | 3.7     | 1.5  | 2.3-6.2    | (5)  | 4.1        | 1.1  | 2.9-6.7     | (10) |
| Co             | 3.0     | 1.7  | 0.89-6.3   | (11) | 0.62       | 0.45 | 0.10-1.4    | (10) |
| Cr             | 120     | 63   | 36-240     | (11) | 16         | 13   | 0.97-38     | (10) |
| Cu             | 84      | 87   | 14-180     | (3)  | 15         | 13   | 5.0-37      | (6)  |
| Fe             | 5800    | 2900 | 3200-11000 | (11) | 1000       | 790  | 62-2400     | (10) |
| Hg             | 0.88    | 0.31 | 0.54-1.2   | (5)  | 0.17       | 0.23 | 0.034-0.59  | (5)  |
| Mn             | 84      | 28   | 42-140     | (11) | 28         | 31   | 12-110      | (10) |
| Sc             | 1.3     | 0.70 | 0.50-2.6   | (11) | 0.14       | 0.10 | 0.0079-0.32 | (10) |
| V              | 47      | 22   | 19-87      | (11) | 13         | 8.7  | 1.7-28      | (10) |
| Zn             | 180     | 96   | 85-360     | (11) | 190        | 30   | 130-230     | (10) |
| <i>Outside</i> |         |      |            |      |            |      |             |      |
| As             | 4.1     | 2.1  | 1.8-8.9    | (10) | 0.79       | 0.54 | 0.28-1.9    | (7)  |
| Cd             | 4.7     | 3.8  | 1.2-8.8    | (3)  | 3.0        | 3.0  | 1.3-9.8     | (7)  |
| Co             | 4.8     | 3.6  | 1.1-13     | (10) | 1.5        | 0.96 | 0.60-3.3    | (9)  |
| Cr             | 170     | 120  | 36-360     | (10) | 35         | 33   | 5.2-99      | (9)  |
| Cu             | 36      | 34   | 27-110     | (3)  | 45         | 34   | 23-110      | (6)  |
| Fe             | 9200    | 6700 | 3000-23000 | (10) | 2600       | 2600 | 303-8700    | (9)  |
| Hg             | 0.75    | 0.54 | 0.37-1.4   | (3)  | 0.16       | 0.04 | 0.12-0.2    | (3)  |
| Mn             | 130     | 130  | 38-410     | (10) | 41         | 33   | 19-110      | (9)  |
| Sc             | 1.3     | 1.3  | 0.47-4.6   | (10) | 0.32       | 0.31 | 0.025-1.0   | (9)  |
| V              | 80      | 45   | 23-160     | (10) | 44         | 29   | 5.0-81      | (9)  |
| Zn             | 230     | 140  | 88-470     | (10) | 160        | 63   | 74-260      | (9)  |

even lower than outside the polluted area (Table 5-II). At the 90% probability level, the mean V concentration in lichens outside The Broekpolder was significantly higher than inside the soil-polluted area. No significant differences were found for all other elements at the 90, 95 and 99% probability levels.

Taking into account the enhanced element levels of the Broekpolder soil, for example the Cd, Hg and Zn concentrations exceed those in reference soil by a factor 10 (Table 5-I), and the fact that the metals are generally adsorbed at the surface of clay minerals (de Groot *et al.* 1971), thus available for lichen uptake, it is unlikely that the element levels present in the lichens samples originate from local soil raised by the wind. Though the atmosphere may contain soil particles, apparently, the lichens do not directly reflect the local differences in soil trace-element levels. These data either indicate that the local top layer soil particles dispersed by the wind may not reflect average 20 cm depth soil element concentrations, or show that the local soil contribution, if any, is too small compared with the other contributions to lichens to become manifest. The present results indicate, that the presence of soil elements in lichens must originate from two remaining element pools: 1) substrates and/or 2) wet and dry deposition.

To gain insight in the possible substrate-derived contribution to the element content in lichens, the elemental content of outer bark was compared with those in lichens. Both on *polluted* and *unpolluted* soil, the concentrations of Cd and Zn ( $P = 0.99$ ) were similar in lichens and outer bark; for As, Co, Cr, Cu, Fe, Hg, Mn, Sc and V the mean concentrations in lichens were significantly higher than in outer bark (Table 5-II) ( $P = 0.99$ ), although the ranges of the As, Co, Cu, Hg, Mn and V concentrations partly overlapped. Although the higher concentrations in lichens may be expected to result primarily from atmospheric input (de Bruin and Hackenitz 1986), the similarity of Cd and Zn concentrations in outer bark and lichens might suggest some kind of interaction. To study the possibility of an interaction in more detail, for trees inside The Broekpolder, also the elemental content of phloem and xylem zones were examined.

### 5.3.2 Outer bark, inner bark, youngest wood ring

In woody stems the tissues external to the cambium are commonly referred to as bark (Greulach 1973). Here, the inner bark is defined as consisting of the younger layers of the secondary phloem. These layers have living sieve tubes, companion cells and parenchyma cells and they function in the transport of solutes.



The outer bark consists of the older dead and non-functional phloem intermixed with cork.

When comparing the element concentrations in outer bark with inner bark from trees growing on polluted soil, the mean concentrations of Mn and Zn do not differ significantly at the 99% probability level (Tables 5-II and 5-III); for all other elements studied, the mean concentrations in outer bark were significantly higher than in inner bark ( $P = 0.99$ ). Only for Cd, the concentration ranges were of comparable magnitudes. De Bruin and Hackenitz (1986) also found comparable concentrations between lichens (*Parmelia sulcata*) and substrate (inner and outer) bark from various tree species for the elements Cd, Mn and Zn in a polluted area (The Kempen) in The Netherlands.

Upward translocation of ions from the roots and the soil toward the leaves is through the xylem. Downward translocation is through the phloem, but the phloem translocation may also be upward and both into and out of leaves. Comparison of the element concentrations in inner bark with the youngest annual cylinders (1986-1987) of xylem (sapwood) from trees inside the polluted area yielded similar concentrations for all elements, except for Fe and Zn (Table 5-III). Fe and Zn concentrations in tree rings were a factor 15 respectively 10 higher than in inner bark.

For As, Co, Cr, Cu, Hg, Sc and V, with concentrations in inner bark similar to the concentrations in the 1986-1987 tree rings, but both very low compared to the concentrations in lichens, outer bark and soil, a contribution to the element concentrations in lichens and outer bark from the element content in xylem or phloem of the supporting tree seems to be of minor importance.

For Cd, Fe, Mn and Zn, a substrate-derived contribution to the content in lichens can not be excluded. Figures 5-1 to 5-4 illustrate the Cd, Fe, Mn and Zn contents in the four adjacent compartments (wood 1986-1987, inner bark, outer bark and lichens). For Fe (Figure 5-2) the contribution of the supporting tree to the content in lichens is assumed to be negligible, because the concentrations in inner bark are a factor 30 lower than those in wood (1986-1987) and outer bark. It is likely that the Fe content in outer bark originated from atmospheric input and not from the tree bole. Consequently, if outer bark influenced the Fe content in lichens, the origin of such a contribution may be regarded as atmospheric as well. Fe is a typical soil

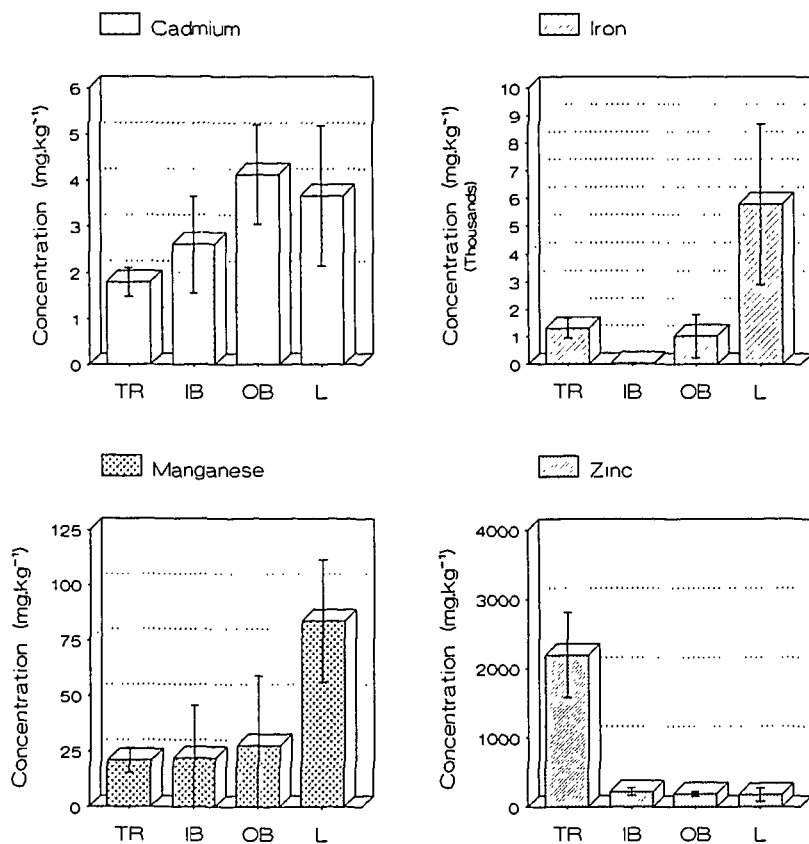
TABLE 5-III. Element concentrations in inner bark and tree rings 1986-1987 (in mg.kg<sup>-1</sup>), inside The Broekpolder. In case only detection limits were found, the highest detection limit was given.

|    | Inner bark |        |               |      | Tree rings 1986-1987 |       |              |     |
|----|------------|--------|---------------|------|----------------------|-------|--------------|-----|
|    | mean       | SD     | range         | (n)  | mean                 | SD    | range        | (n) |
| As | 0.056      | 0.03   | 0.037-0.13    | (10) | 0.071                | 0.017 | <0.046-0.095 | (4) |
| Cd | 2.6        | 1.04   | 1.2-4.9       | (11) | 1.8                  | 0.31  | 1.4-2.1      | (5) |
| Co | 0.11       | 0.15   | 0.031-0.56    | (11) | 0.18                 | 0.03  | 0.14-0.23    | (5) |
| Cr | 1.3        | 0.76   | 0.52-2.7      | (7)  | 1.8                  | 0.81  | 0.84-3.0     | (5) |
| Cu | 2.8        | 1.4    | 1.1-4.2       | (5)  | 5.0                  | 2.7   | 2.8-9.0      | (4) |
| Fe | 36         | 23     | 10-61         | (7)  | 1300                 | 360   | 970-1900     | (5) |
| Hg | <0.033     | -      | -             | -    | <0.32                | -     | -            | (5) |
| Mn | 22         | 24     | 6.1-90        | (11) | 21                   | 5.5   | 14-26        | (5) |
| Se | 0.0038     | 0.0029 | 0.0019-0.0059 | (4)  | <0.013               | -     | -            | (5) |
| V  | 0.32       | 0.43   | 0.0033-0.42   | (7)  | 0.18                 | 0.044 | <0.05-0.22   | (4) |
| Zn | 220        | 54     | 150-300       | (11) | 2200                 | 620   | 1700-3200    | (5) |

element, as is Se, for which the route from soil via the tree bole towards the lichen is considered to be far less likely than atmospheric deposition on the lichens, since the Se content in the tree tissues responsible for the transport was 2 orders of magnitude lower than in the lichens. For Cd, Mn and Zn (Figures 5-1, 5-3 and 5-4) lateral exchange between the living xylem and phloem might have taken place.

### 5.3.3 Tree rings

The relation between age of tree rings and element accumulation in the xylem might give insight in the possible pathways and mobilities of the elements within the tree. Table 5-IV shows the mean element concentrations of tree ring samples of three poplars (planted in 1975 inside The Broekpolder) per two-year intervals from 1975 to 1987. Since the cores were sampled diametrically, the element concentrations of the tree ring samples formed in the same years were averaged, in accordance with the representative samples of lichens and bark, both combined of material from all



Figures 5-1, 5-2, 5-3, 5-4. Mean concentrations of cadmium, iron, manganese and zinc, respectively, in tree rings 1986-1987 (TR), inner bark (IB), outer bark (OB) and lichens (L) from trees inside The Broekpolder.

around the tree. In Table 5-IV it is shown, that high mean concentrations were found in the 1980-1981 interval for As, Co, Cr, Fe and Zn. For some elements (As, Co, Fe, Mn and Zn) the mean concentrations increased with time, whereas other elements (Cd, Cr, Cu, V) showed rather constant concentrations.

The element concentrations in two individual tree ring samples of the same two-year interval on either side of the tree often showed large differences. Figures 5-5 to 5-8 show the ranges of Cd, Fe, Mn and Zn concentrations in tree rings from 1975 to 1987 for one tree. The asymmetry in the wood is shown by the large range per

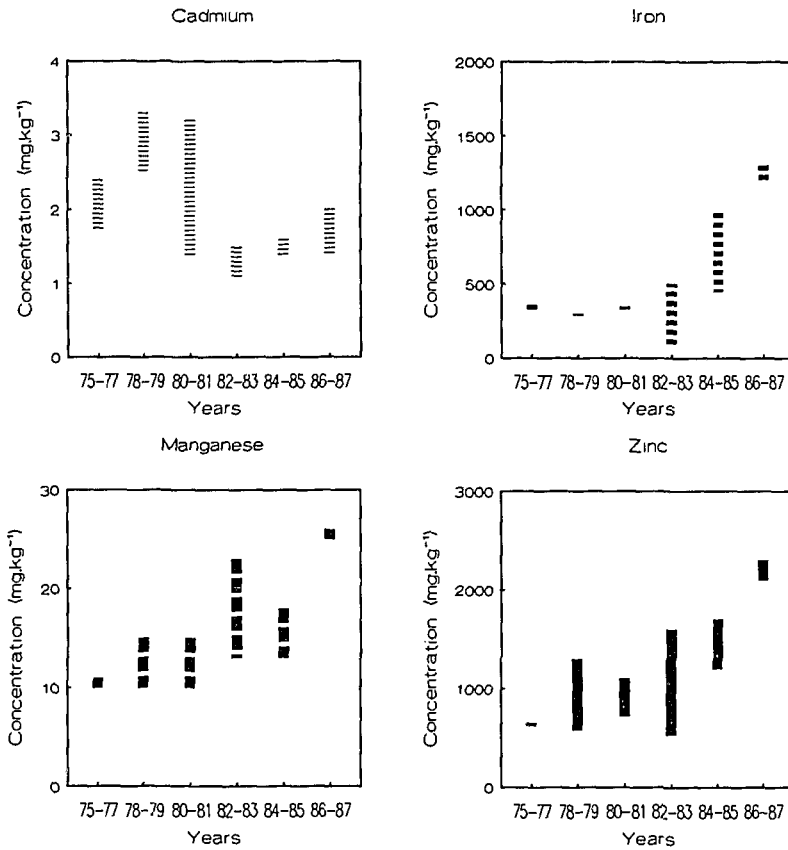
TABLE 5-IV. Mean element concentrations (mg.kg<sup>-1</sup>) in tree rings inside The Broekpolder.

| (n) | 1975-1977<br>2 | 1978-1974<br>4 | 1980-1981<br>3 | 1982-1983<br>5 | 1984-1985<br>6 | 1986-1987<br>5 |
|-----|----------------|----------------|----------------|----------------|----------------|----------------|
| As  | <0.030         | 0.027          | 0.060          | 0.046          | 0.049          | 0.071          |
| Cd  | 2.1            | 2.4            | 2.5            | 2.0            | 2.1            | 1.8            |
| Co  | 0.065          | 0.071          | 0.12           | 0.087          | 0.086          | 0.18           |
| Cr  | <0.78          | 1.4            | 2.1            | 1.1            | 1.0            | 1.8            |
| Cu  | 4.0            | 3.9            | 4.0            | 3.8            | 3.6            | 5.0            |
| Fe  | 330            | 240            | 930            | 520            | 490            | 1300           |
| Hg  | <0.13          | <0.15          | <0.18          | <0.44          | <0.18          | <0.32          |
| Mn  | 11             | 7.0            | 11             | 15             | 9.9            | 21             |
| Sc  | <0.0078        | <0.0053        | <0.0088        | <0.0086        | <0.0051        | <0.013         |
| V   | 0.088          | 0.075          | 0.085          | 0.14           | 0.074          | 0.18           |
| Zn  | 640            | 720            | 1900           | 610            | 950            | 2200           |

sample interval, consisting of two samples of two annual rings. For Cr and Cu asymmetry was also found in the wood, whereas As, Co and V concentrations were similar on either side of the pith of the tree.

Radial translocation of elements (the movement from pith to bark or the reverse) within the tree bole is a common occurrence in many tree species (Guyette *et al.* 1991). This radial movement, whether by diffusion or mass flow can greatly complicate or invalidate elemental histories from tree rings. The present tree ring results showed an increase in mean concentrations with time for As, Co, Fe, Mn and Zn, suggesting radial translocation of these elements from older wood to younger wood, if no chemical, depth and/or time related variations in soil element concentrations are considered.

Lövestam *et al.* (1990) found for Mn a strong discontinuity in the concentration at the boundary between bark and wood, while for other elements a more gradual change was observed. According to Baes III and Mc. Laughlin (1984), translocation in years following formation of historical rings would smooth out year to year variability in trace metal content and any correlation between growth and xylem accumulation would be lost. They found translocation from younger to older tree rings in *Pinus*



Figures 5-5, 5-6, 5-7, 5-8. Concentration ranges of cadmium, iron, manganese and zinc, respectively, in one core of a poplar inside The Broekpolder

species, but the serial correlations between growth and xylem accumulation argued against across-ring translocation of Al, Ca, Cd, Cu, Mn and Zn. Robitaille (1981) found no evidence for translocation of Co and Zn in *Abies balsamea*.

The present results showed that for Cd, Mn and Zn translocation might have taken place from the wood through the bark to the lichens; here, accumulation characteristics in lichens should be further investigated. For all other elements, (As, Co, Cr, Cu, Fe, Hg, Sc and V) wet and dry deposition may be considered as the main element pool responsible for the content in lichens.

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## CHAPTER 6. INTERSPECIES COMPARISON OF LICHENS AS BIOMONITORS FOR TRACE-ELEMENT AIR POLLUTION

**Abstract.** *The linearity of the interspecies relation between the elemental contents in *Parmelia sulcata* Taylor and *Lecanora conizaeoides* Nyl., sampled in a polluted area, was examined by the functions  $y = a.z + b$  and  $y.z^{-1} = a + b.z^{-1}$ , with  $y$  and  $z$  being the elemental content in *Parmelia* respectively *Lecanora*. For As, Co, V and Zn significant positive  $a$  and  $b$  values were found, allowing interspecies calibration. For Br, Cd, Cr, Fe, La, Sb, Se and W the  $a$  values were zero and the  $b$  values were positive, indicating saturation of *Parmelia* and thus prohibiting interspecies calibration. For Cu a part of the data set showed saturation effects in *Lecanora*. The results showed that an average concentration ratio could not be used for intercalibration purposes, because the concentration ratios were dependent on the concentrations.*



## CHAPTER 6. INTERSPECIES COMPARISON OF LICHENS AS BIOMONITORS FOR TRACE-ELEMENT AIR POLLUTION

### 6.1 Introduction

The use of lichens as biomonitors for air pollution offers means for achieving preliminary estimates of the extend of such pollution, the location and identification of the pollution sources and also a method for the comprehensive mapping of the pollution situation in an area (Kauppi 1980, Rasmussen *et al.* 1980, de Bruin *et al.* 1987, Sloof and Wolterbeek 1991*a,b*).

Many investigations have been reported on only one monitor species (Tyler 1989), which conditioned the locations of the sampling sites by its presence. In biomonitoring studies, the selection of species used is of prime importance; species tolerance levels often restrict the available number of relevant species. The tolerance of lichens to elevated tissue concentrations of heavy metals varies greatly between species and elements (Puckett 1988). As the information on species-specific accumulation characteristics is only scarcely available, it is in practice not possible to replace one lichen species by another, when the monitor species is not present in a chosen sampling site. Interspecies calibration permits the inclusion of sampling sites that lack the monitor species under study.

Few studies have been carried out on interspecies calibration (Folkeson 1979, Boileau *et al.* 1982). In these studies the calibration factors were determined as the element accumulation ratios between the mean concentration levels in different species, whereby insight in the actual relationship between the concentrations in the individual lichen species is lost.

The aims of the present study were to investigate the interchangeability of lichen species in a polluted area and to evaluate the method for the determination of the calibration factors.

## 6.2 Experimental

### 6.2.1 Sampling

The lichen species *Lecanora conizaeoides* Nyl. and *Parmelia sulcata* Taylor were chosen for their common occurrence in The Netherlands. *Lecanora conizaeoides* is highly tolerant to pollutants and is the most SO<sub>2</sub> resistant species occurring on trees (Wielgolaski 1975), whereas *Parmelia sulcata* is less tolerant to pollutants (Wielgolaski 1975, de Wit 1976). The sampling area was The Kempen, an area of approximately 1000 km<sup>2</sup>, situated along both sides of the border between Belgium and The Netherlands. This area is known to be heavily polluted by metals released from industries inside and outside the region (Lemmens and Roos 1982, van Luit and Smilde 1983, de Bruin *et al.* 1987). *Parmelia* and *Lecanora* samples were collected simultaneously at one hundred sampling sites, according to a sampling location grid of 3 x 3 km<sup>2</sup>. Samples were taken from the tree species *Populus* and *Salix*, between 1 m and 2 m above the ground. If possible, the lichens were sampled from all around the tree, to reduce the influence of the prevailing wind direction.

### 6.2.2 Sample preparation and analysis

In the laboratory the lichens were separated from the substrate, using nylon tweezers for *Parmelia* and a stainless steel scalpel for *Lecanora* and rinsed with distilled water (30 ml) to remove adhering dust. After grinding and homogenizing in an agate ball-mill the samples were freeze-dried for 24 h. Samples of 50-100 mg were packed for analysis in polyethylene capsules.

The elements antimony, arsenic, bromine, cadmium, chromium, cobalt, copper, iron, lanthanum, selenium, tungsten, vanadium and zinc in the samples were determined by instrumental neutron activation analysis (INAA) (Bode and de Bruin 1990). The samples were irradiated in the reactor at the Interfaculty Reactor Institute (IRI) at a thermal neutron flux of  $5 \times 10^{16} \text{ m}^{-2}\text{s}^{-1}$ . A first irradiation of 30 s was followed, after 10-15 min decay time, by a measurement during 5 min of the short-lived radionuclides. After a second irradiation of 4 h, the samples were measured twice during 1 h, after decay

times of 5 and 21 days, respectively. Measurements were carried out with semiconductor detectors (Ortec, Canberra). The precision of the measurements was 1 - 5% for As, Br, Co, Cr, Fe, La, Sb, V, Zn, and 5 - 15% for Cd, Cu, Se and W. Quality control of the INAA results was based on the regular analysis (1 per 10 samples) of the NIST standard reference material SRM 1571 (Orchard Leaves) according to the same procedures as used for the samples.

### 6.3 Results and Discussion

Table 6-I shows the means, standard deviations and the ranges of the elemental concentrations in the *Parmelia* and *Lecanora* samples from those sampling sites, where both lichen species occurred together. The mean elemental concentrations in both species were of comparable magnitudes, except for Fe, Sb and W. For these elements the averages in the *Lecanora* data set were 1.5 to 2.3 times higher than in the *Parmelia* data set. Comparing the ranges in Table 6-I, *Lecanora* showed markedly higher extreme concentrations than *Parmelia* for Br, Cd, Co, Fe, Sb and W. For Cu, the highest concentrations were found in *Parmelia*.

From a previous study it was concluded that the variation in the elemental content in several *Parmelia* samples collected from one sampling site, did not exceed approximately 40 %, independent of the pollution level of the sampling location or the phorophyte species (see Chapters 2 and 4 of this thesis). In the present study, within an area of 1000 km<sup>2</sup> with local pollution sources, the elemental contents in the lichens showed much larger ranges than to be expected from local variations, suggesting good responses to variable levels of trace-element air pollution. The differences between species in response to pollution levels may be due to differences in accumulation characteristics, possibly related to morphological differences in lichen features (cf. Sloof and Wolterbeek 1992).

Though the means of the *Parmelia* and *Lecanora* data sets were of comparable magnitude (Table 6-I), the relationships between the elemental concentrations in the two species have to be examined in more detail so as to exclude a possible dependence of the ratios on the concentrations. The interspecies relations employing Pearson's correlation coefficient are shown in Table 6-II.

TABLE 6-I. Elemental concentrations in lichens (in mg.kg<sup>-1</sup>).

| El. | <i>Parmelia</i> |      |      |           | <i>Lecanora</i> |      |            |
|-----|-----------------|------|------|-----------|-----------------|------|------------|
|     | (n)             | mean | SD   | range     | mean            | SD   | range      |
| As  | 50              | 10.9 | 4.5  | 4.8-19.9  | 9.5             | 3.9  | 5.1-24     |
| Br  | 50              | 43   | 17.8 | 19.7-91   | 43              | 21   | 19.5-138   |
| Cd  | 42              | 5.0  | 1.7  | 2.3-9.3   | 1.5             | 2.8  | 1.9-18     |
| Co  | 50              | 3.5  | 1.5  | 1.0-7.3   | 4.7             | 2.8  | 1.5-20     |
| Cr  | 50              | 29   | 16.6 | 8.8-80    | 30              | 9.6  | 10.7-54    |
| Cu  | 34              | 49   | 56   | 12-320    | 40              | 23   | 16-110     |
| Fe  | 50              | 4000 | 1510 | 1610-7500 | 6200            | 2000 | 3000-12000 |
| La  | 47              | 3.8  | 1.34 | 1.56-6.9  | 4.6             | 1.45 | 1.75-8.0   |
| Sb  | 50              | 4.6  | 2.0  | 1.5-9.3   | 10.7            | 5.5  | 4.1-31     |
| Se  | 47              | 2.0  | 0.67 | 1.1-5.4   | 2.3             | 0.84 | 0.80-4.2   |
| V   | 50              | 29   | 8.3  | 11-46     | 32              | 9.3  | 12.4-51    |
| W   | 46              | 0.80 | 0.29 | 0.27-1.5  | 1.2             | 0.50 | 0.60-3.1   |
| Zn  | 50              | 540  | 240  | 210-1090  | 430             | 190  | 162-1070   |

Both species are significantly correlated for As, Co, Cu, V and Zn at the 95% probability level. The correlation coefficients were very low (0.36 - 0.45), due to the large variation in the data sets and possibly the species were not linearly related in the full range of concentrations. No significant correlations could be determined for Br, Cd, Cr, Fe, La, Ni, Sb, Se and W.

Table 6-II also presents the constants  $a$  and  $b$  and their 95% confidence limits, derived from fitting the function  $y = ax + b$  to the data obtained ( $y$  and  $z$  being the elemental concentrations in *Parmelia* and *Lecanora*, respectively). This linear function was expected, based on the assumption that both species were linearly related with the atmospheric concentrations or the wet and dry deposition:

$y = a_1x + b_1$  and  $z = a_2x + b_2$ ,  $y$  and  $z$  being the elemental content in *Parmelia*, respectively *Lecanora* and  $x$  being the atmospheric concentrations or deposition. Table 6-II shows that for As, Co, V and Zn the slopes ( $a = a_1/a_2$ ) and the intercepts ( $b = b_1 - a.b_2$ ) were significantly positive, whereas for Cu the intercept was not significantly different from zero.

TABLE 6-II. Pearson's correlation coefficients ( $r$ ) and the parameters ( $a$ ,  $b$ ) for the function  $y = a.z + b$ , with  $y$  and  $z$  being the elemental content in *Parmelia* respectively *Lecanora*.

| El. | $r$   | $a$   | confidence limits (95%) | $b$  | confidence limits (95%) | ratio $y/x$ | SD   |
|-----|-------|-------|-------------------------|------|-------------------------|-------------|------|
| As  | 0.36* | 0.42  | 0.11 - 0.73             | 6.9  | 3.8 - 10                | 1.23        | 0.52 |
| Br  | 0.16  | -0.14 | -0.39 - 0.11            | 49   | 37 - 61                 | 1.21        | 0.82 |
| Cd  | 0.10  | 0.06  | -0.13 - 0.24            | 4.7  | 3.7 - 5.7               | 1.41        | 0.83 |
| Co  | 0.44* | 0.24  | 0.10 - 0.38             | 2.4  | 1.6 - 3.2               | 0.86        | 0.46 |
| Cr  | 0.19  | -0.34 | -0.83 - 0.16            | 39   | 23 - 54                 | 1.14        | 0.91 |
| Cu  | 0.45* | 1.12  | 0.33 - 1.92             | 49   | -32 - 41                | 1.25        | 0.84 |
| Fe  | 0.27  | 0.19  | -0.02 - 0.41            | 2900 | 1470 - 4200             | 0.70        | 0.31 |
| La  | 0.23  | 0.21  | -0.06 - 0.48            | 2.8  | 1.5 - 4.1               | 0.90        | 0.40 |
| Sb  | 0.26  | 0.09  | -0.08 - 0.20            | 3.6  | 2.3 - 4.8               | 0.50        | 0.29 |
| Se  | 0.15  | -0.12 | -0.35 - 0.12            | 2.3  | 1.7 - 2.9               | 1.5         | 0.68 |
| V   | 0.38* | 0.34  | 0.10 - 0.58             | 18   | 10 - 26                 | 0.95        | 0.30 |
| W   | 0.04  | -0.02 | -0.20 - 0.16            | 0.82 | 0.59 - 1.1              | 0.77        | 0.36 |
| Zn  | 0.37* | 0.48  | 0.13 - 0.83             | 340  | 176 - 500               | 1.37        | 0.64 |

Significant (\*) at  $P = 95\%$

If the element concentrations in both species were linearly related in the full range of concentrations, then the ratio ( $y/z$ ) is linearly related with the inverse concentrations in *Lecanora*, the slope being ( $b_1 - a.b_2$ ) and the intercept being  $a$ , which is shown in Figure 6-1 for Zn as an example. The ratio was significantly correlated ( $P = 95\%$ ) with the inverse concentrations in *Lecanora*, the slope being 245 and the intercept 0.7. The correlation coefficient (0.44) of this function was not much improved with respect of the value given in Table 6-II (0.37), due to the large scatter in the data sets. From the parameters it can be deduced that the starting concentration in *Parmelia* was 245  $\text{mg.kg}^{-1}$  ( $a$ ) and that *Lecanora* accumulates Zn 1.4 ( $1/b$ ) times faster than *Parmelia*. Also for As, Co and V, the species could be intercalibrated using the parameters of the function between the concentration ratios and the inverse concentrations in *Lecanora*.

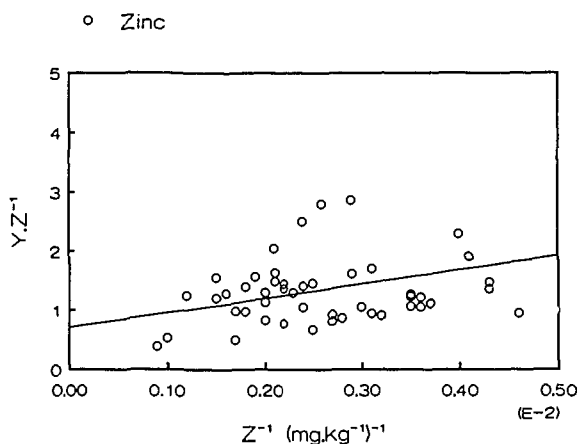


Figure 6-1. *Parmelia/Lecanora* zinc concentration ratios ( $Y.Z^{-1}$ ) as a function of the inverse *Lecanora* concentrations ( $\text{mg.kg}^{-1}$ ) $^{-1}$  ( $Z^{-1}$ ), illustrating that interspecies calibration is possible.

Figure 6-2 shows that the ratio ( $y/z$ ) for Cu was not significantly correlated with the inverse concentrations in *Lecanora* (correlation coefficient = 0.25), probably due to saturation effects in *Lecanora* for some points in the data set.

For Br, Cd, Cr, Fe, La, Sb, Se and W, the  $a$  values (Table 6-II) did not significantly differ from zero, whereas the  $b$  values were significantly positive, suggesting saturation effects in *Parmelia*. If the concentrations in *Parmelia* have reached the saturation level ( $c$  value), then the ratio ( $y/z$ ) as a function of the inverse concentrations in *Lecanora* would be a straight line, going through the origin, and  $c$  being the value for the slope. For example, Figures 6-3 and 6-4 show that the ratios for Cd and W were significantly correlated ( $P = 99\%$ ) with  $1/Z$  and the intercepts were nearly zero. The relative high correlation coefficients of these functions (0.70 for Cd and 0.76 for W) support the conclusion that *Parmelia* has reached saturation levels. This information can not be deduced from the function  $y = a.z + b$ , since the correlation coefficients of this function were 0.10 for Cd and 0.04 for W.

Intercalibration studies for different cryptogam species have been reported by Folkesson (1979) and Boileau *et al.* (1982). These studies did not present levels of significance of the interspecies correlations. The calibration factors given were invariably determined as the element accumulation ratios between species.

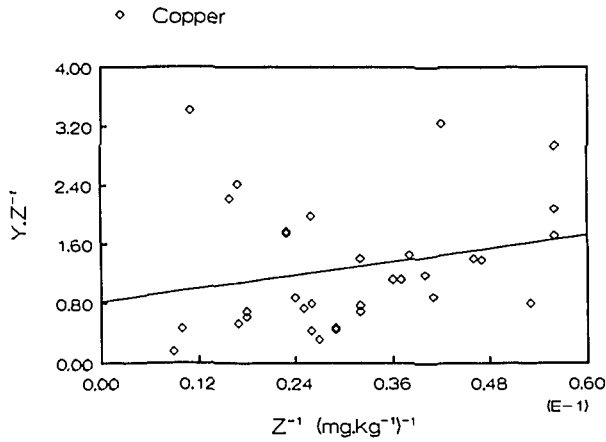


Figure 6-2. *Parmelia/Lecanora* copper concentration ratios ( $Y.Z^{-1}$ ) as a function of the inverse *Lecanora* concentrations ( $\text{mg.kg}^{-1}$ ) ( $Z^{-1}$ ), illustrating that intercalibration is not possible.

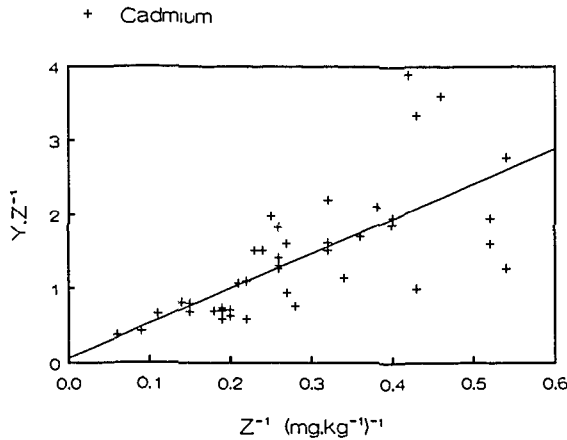


Figure 6-3. *Parmelia/Lecanora* cadmium concentration ratios ( $Y.Z^{-1}$ ) as a function of the inverse *Lecanora* concentrations ( $\text{mg.kg}^{-1}$ ) ( $Z^{-1}$ ), illustrating that intercalibration is not possible.

Folkesson (1979) reported ratios varying from 0.35 to 3.2 for four moss species and four lichen species against the corresponding Fe, Cu, Zn, Pb, Ni and Cd content in *Hylocomium splendens* and *Pleurozium schreberi*. The ratios Boileau *et al.* (1982) computed for three lichen species and four moss species against the corresponding Fe, Ni, Pb, Ti and U content of *Cladonia rangiferina* varied from 0.76 to 3.43.

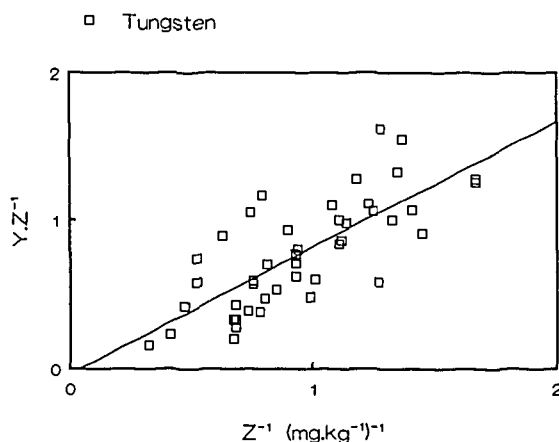


Figure 6-4. *Parmelia/Lecanora* tungsten concentration ratios ( $Y.Z^{-1}$ ) as a function of the inverse *Lecanora* concentrations ( $(\text{mg.kg}^{-1})^{-1}$  ( $Z^{-1}$ ), illustrating that intercalibration is not possible.

Table 6-II also shows the average *Parmelia/Lecanora* ratios and their standard deviations for different elements. The ratios varied from 0.50 for Sb to 1.41 for Cd. One difference between the present ratios and the ones reported by Folkesson (1979) is that the present mean ratios are based on individually determined ratio values, whereas the others were determined from mean concentrations. The latter values yield error estimates for ratio values, which may be suspected to be related to both concentration ranges and to the variation in ratios themselves. Another difference between the present data and those from Folkesson (1979) consists of the markedly higher standard deviations of the present ratios: Folkesson (1979) reported standard deviations of about 5%, whereas the SD values given in Table 6-II range from 32 to 80% (cf. Boileau *et al.* 1982: ratio SD values of 5-30%). As the variances in elemental concentrations of our data set differed about a factor 2-3 with the ones given by Folkesson (1979), the latter data were probably obtained from a uniform set of concentrations. Although average ratios of about unit value (Table 6-II) suggest similar accumulation characteristics and full interchangeability (Boileau *et al.* 1982), our results show that analysis of the ratios should indicate whether or not any conclusions on relations between species can be drawn.

The present results indicate that the concentration ratios will change with



increasing or decreasing concentrations and therefore averaged or even unit ratios may not be used for intercalibration, unless the actual concentration relationship values ( $a$ ,  $b$ ) or trends in ratio values have been examined. In any case, the use of interspecies parameters should be strictly confined to the calibrated concentration intervals only: The significant presence of  $b$  values may indicate the non-linearity of the actual concentration relationship between lichen species.

To gain knowledge about the differences in metal accumulation characteristics between various lichen species, further research should be done. Metal accumulation by lichens is influenced by many factors such as (micro)climate, pH, rainfall, chemical forms of pollutants, tolerance, metal load, age, growth rate, and chemical and/or morphological properties of the lichen species (cf. Sloof and Wolterbeek 1992). Therefore, it is difficult if not impossible to define these characteristics in the field.

It should be noted, that *Lecanora* and *Parmelia* may be significantly correlated for other elements (e.g. Pb, Ni) in larger scale sampling networks or in other survey areas showing different element levels. Therefore, separate calibration parameters should be established in different biomonitoring studies. In biomonitoring studies in remote areas, there may be no problems regarding saturation effects.

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## CHAPTER 7. LICHENS AS QUANTITATIVE BIOMONITORS FOR ATMOSPHERIC ELEMENT DEPOSITION

**Abstract.** *At 8 stations spread over The Netherlands transplants of *Parmelia sulcata* Taylor and impregnated cloth as a non-biological monitor were exposed to the atmosphere for periods up to 12 months. At the same stations air particulate matter and total (wet and dry) deposition were collected on a monthly basis. Quantitative linear relationships between the concentrations in the 12 months exposed lichens and the cumulative total deposition during the same period, yielded accumulation factors for cobalt, scandium and zinc. The lichens reflected the total deposition, but not the concentration ratios in the total deposition. No correlations were found between the cobalt, scandium and zinc concentrations in lichens and air particulate matter, as measured with air filters, or between these element concentrations in impregnated cloth (rag) and air particulate matter.*

*The comparable ratios between the accumulation factors in lichens and in rag, for cobalt, scandium and zinc gave some insight in the uptake mechanism, probably mainly passive, such as particulate trapping, adsorption/desorption and/or ion-exchange.*

## CHAPTER 7. LICHENS AS QUANTITATIVE BIOMONITORS FOR ATMOSPHERIC ELEMENT DEPOSITION

### 7.1 Introduction

Information on atmospheric elemental concentrations and depositions can be obtained by physical and chemical methods. Generally, elemental concentrations in the environment are calculated using models for the dispersion of pollutants in the atmosphere and for deposition. Dispersion models are based on meteorological observations, particle size distributions and their deposition properties, surface conditions (roughness) and emission registrations (van Jaarsveld 1989).

The combined use of lichens as biomonitors and the results of dispersion models in trace-element air pollution monitoring yielded *qualitative* relationships between the concentrations in lichens and atmospheric concentrations in air particulate matter and depositions (see Chapters 2 and 3). On a national scale in The Netherlands, the geographical concentration patterns of trace elements obtained from lichen data sets agreed with the elemental concentration gradients obtained from a dispersion model and measured data of atmospheric concentrations and depositions (see Chapter 2). Also the geographical  $^{137}\text{Cs}$  activity gradients and radioactivity values obtained from lichen data sets, as a result from the Chernobyl accident, agreed with a dispersion model and data on wet and dry deposition of  $^{137}\text{Cs}$  on a national scale in The Netherlands (see Chapter 3).

So far, only a few *quantitative* comparisons of biomonitoring methods with the physical and chemical measurements of atmospheric deposition have been published. Pilegaard (1979) showed that the amounts of Cr, Cu, Fe, Pb and Zn accumulated in cryptogam transplants were linearly related with the concentrations in total deposition in the vicinity of Danish steelworks. However, in a follow-up study from Vestergaard *et al.* (1986), it was found that the uptake in lichen transplants was not directly proportional to the concentrations in total deposition, using the same species and methodology as Pilegaard (1979). Hanssen *et al.* (1980) assumed linear relationships between trace-element concentrations in moss samples from 510 localities in Norway

and estimated deposition from precipitation samples at only four stations, based on a log-log scaled diagram. Andersen *et al.* (1980) inferred linear relationships between total deposition and heavy-metal concentrations in indigenous lichens (for Pb and Zn) and mosses (for Cu, Pb, Ni and Zn) near metal-processing industry in the Copenhagen area. These relationships were based on correlation coefficients varying from 0.28 to 0.61 in a selected part of the data set, which was not specified. Kansanen and Venetvaara (1991) inferred a log-log linear relationship between the metal content of moss and deposition from snow samples near ferrochrome and steel works in Finland.

Trace elements are deposited to the lichen surface either as dry particulates or as material dissolved and/or suspended in wet precipitation. Trace elements may be retained by particulate entrapment, physio-chemical processes such as ion exchange, as well as by passive and active intracellular uptake (Nieboer and Richardson 1981, Puckett 1988, Tyler 1989). But there is still a lack of information on mechanisms of element uptake in lichens, element release from lichens under field conditions and the corresponding rates.

In order to get insight in actual responses of lichens to trace-element air pollution, the aims of this paper were (1) to investigate whether simple quantitative relationships exist between trace-element concentrations in lichens and those in the atmosphere, as measured by air filters and the total deposition under varying field conditions, (2) to elucidate whether passive or active processes are involved in the uptake mechanism, by comparing the response of the biomonitor to that of a synthetic monitor. The determination of the dry deposition flux, which depends on meteorological conditions, nature of the surface, properties of the deposited particle and dry deposition velocity (Davidson and Wu 1989, Ottley and Harrison 1991), is beyond the scope of this study.

## 7.2 Experimental

### 7.2.1 Sampling area

The fieldwork was carried out at 8 stations in The Netherlands, where identical

portacabins were situated. These stations form part of the national air quality monitoring network, which is under the management of the National Institute of Public Health and Environmental Protection (Elskamp 1989). The criteria for the choice of these stations, -representative for a spatial area with a diameter of tens of kilometres- were the different trace-element air pollution levels and the various types of surroundings.

Station 1, Witteveen ( $52^{\circ}48'49''\text{N}$   $06^{\circ}40'11''\text{E}$ ) is representative for the north-eastern part of the country and is surrounded by arable land, woods and heath. At this station, the trace-element pollution levels were the lowest for all stations involved (van Jaarsveld and Onderdelinden 1986, Sloof and Wolterbeek 1991).

Station 2, Bilthoven ( $52^{\circ}07'15''\text{N}$   $05^{\circ}11'44''\text{E}$ ) is representative for the centre of the country and it is surrounded by meadows and woods. The trace-element air pollution levels are regarded as relatively low.

Station 3, Leiduin ( $52^{\circ}20'44''\text{N}$   $04^{\circ}35'52''\text{E}$ ) is representative for the western coastal area of the country, with comparable pollution levels to station 2. The surroundings of this station are bulb arable land, dunes and woods.

Station 4, Vlaardingen ( $51^{\circ}55'03''\text{N}$   $04^{\circ}21'26''\text{E}$ ) represents an urban and industrial area of the Rotterdam harbour industries.

Station 5, Huybergen ( $51^{\circ}26'06''\text{N}$   $04^{\circ}21'37''\text{E}$ ), station 6, Houtakker ( $51^{\circ}31'10''\text{N}$   $05^{\circ}08'56''\text{E}$ ), station 7, Vredepeel ( $51^{\circ}32'28''\text{N}$   $05^{\circ}51'13''\text{E}$ ) and station 8) Wijnandsrade ( $50^{\circ}54'11''\text{N}$   $05^{\circ}52'56''\text{E}$ ) are representative for the southern parts of the country. The pollution levels at these stations vary from high to very high, due to the influence of industries situated in the Dutch and Belgian Kempen area and the German Ruhr area. The surroundings of the stations are woods and heath (station 5), arable land, woods and heath (stations 6 and 7) and arable land (station 8). The geographical distribution of the 8 stations is given in Figure 7-1.

### *7.2.2 Sampling and sample preparation*

In this study, four types of samples were collected at each station and prepared for analysis, namely air particulate matter, total deposition, lichens and impregnated cloth.

1) At each station air particulate matter was collected on a monthly basis, from March 1988 to March 1989. A low-volume aerosol sampler (Romega type 070) was used with a flow rate of  $0.46 \pm 10\% \text{ m}^3\text{h}^{-1}$ . A polycarbonate filter holder (Nuclepore, diameter 47 mm) for serial filtration was mounted on the top of the portacabin at a height of 3.5 m above ground level. The filter holder contained two polycarbonate membrane filters (Nuclepore), with different pore sizes, namely  $8.0 \mu\text{m}$  (outer filter) and  $0.4 \mu\text{m}$  (inner filter). The filter holder was protected against rainfall by means of a polyethylene funnel and the inlet was faced downwards. Monthly, the filter holder was cleaned with distilled water and alcohol, before new filters were applied. The filters were handled with nylon tweezers and kept in sealed polyethylene bags until analysis. In the laboratory the whole filters were packed in polyethylene capsules for analysis.

2) Total deposition was collected also monthly at each station, in an open 5 L high-density polyethylene sampler with a funnel diameter of 20 cm (according to NEN 1984). The sampler was mounted on the top of the portacabin at a height of 3.5 m above ground level. Before exposure, 5 ml concentrated nitric acid (Suprapur Merck) was added to the sampler, in order to prevent retention of metals to the container wall (Barrie and Schemenauer 1989, De Boer and Fortezza 1989). For every exposure period, cleaned bulk samplers were used (NPR 1987). After collection, ammonium-pyrrolidine-dithiocarbamate (APDC,  $50 \text{ mg}\cdot\text{L}^{-1}$ ) was added to a 200 ml aliquot sample as complexing agent at a pH of  $7.8 \pm 0.5$  and the sample aliquot was preconcentrated on activated carbon (van der Sloot 1976). A suspension of 20 mg activated carbon in 20 ml distilled water was led through a  $8.0 \mu\text{m}$  polycarbonate membrane filter (Nuclepore) to form a homogeneous carbon layer. Then the sample aliquot was filtered over the carbon layer. After washing the carbon three times with 10 ml distilled water, the filter was dried and transferred to a polyethylene capsule. Blanks were run with the same procedure (1 per 8 samples). The carbon had been prepared by The Netherlands Energy Research Foundation.

For the determination of the total amount of wet precipitation during the exposure period, data from The Royal Dutch Institute of Meteorology (KNMI) were available, obtained from daily measurements, using wet only samplers. These wet only samplers were located within 2.5 km from the stations of the exposure experiment, in



an open terrain at a height of 0.4 m above ground level.

The total (wet and dry) elemental deposition was calculated from the product of the concentration in total deposition and the amount of wet precipitation measured with the official KNMI rain gauge.

3) Samples of the lichen species *Parmelia sulcata* Taylor with adhering bark substrate (transplants) were collected from an area with a low trace-element pollution level, in the north-west of The Netherlands, as indicated in Figure 7-1. The choice of this reference area was based on the earlier analysis results obtained for *Parmelia sulcata* Taylor in the framework of a monitoring study for determining trace-element concentrations in epiphytic lichens on a national scale in 1982-1983. In November/December 1987, healthy lichen transplants were collected from *Populus* sp. and *Salix* sp. at sampling positions between 1 and 2.5 m above the ground and where possible, from all around the tree, in order to reduce influences from prevailing wind directions. The untreated samples were stored in the laboratory at room temperature until exposure.

During March 1988 until March 1989, the lichens transplants were exposed to the atmosphere at each station for 1, 2, 4 and 12 months, respectively. The macro contour surface area of the lichen transplants was approximately 9 cm<sup>2</sup>. For each exposure period, new transplants were used, yielding 22 samples per station, consisting of 12, 6, 3 and 1 samples of 1, 2, 4 and 12 months exposure periods, respectively. The lichen samples were exposed with their adhering bark substrate in bags of polyethylene netting (1 x 1 mm) tied on a nylon rope on the top of the portacabin, at a height of 3.5 m above ground level.

In the laboratory, the exposed samples were separated from the bark substrate with nylon tweezers, transferred into a polyethylene sieve and rinsed with 20 ml distilled water to remove adhering sand or dust. The samples were made brittle by immersion in liquid nitrogen, ground and homogenized in a mortar with a pestle and freeze-dried for 24 h. The lichen material (100 mg dry weight) was packed in polyethylene capsules for neutron activation analysis.

4) At each station, also a synthetic non-biological monitor was exposed. The monitor, called "rag", consisted of muslin-like cloth impregnated with a resinous material (Royal Unirag type GTI 33204, Unichem B.V.). A rectangle (7 x 6 cm) of

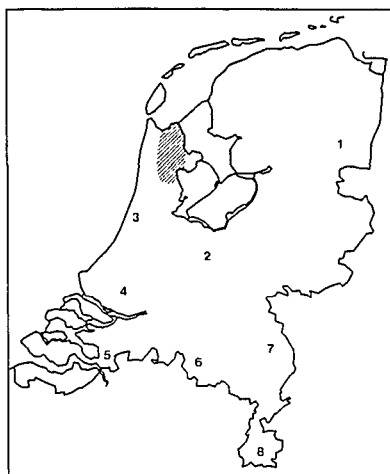


Figure 7-1. Location of stations 1 to 8 and the reference area (shaded) from which the lichen transplants were collected.

rag was wrapped around a plastic hair roller (cf. Gailey and Lloyd 1986) to allow the entire surface area of one side of the fabric to be exposed to the atmosphere. The sample was held in position by a thin nylon line. The samples, tied on a nylon rope next to the lichen samples, were exposed for 1 month. After the period of exposure, each roller was transferred into a polyethylene bag. In the laboratory, the rag sample (210 mg) was air dried and packed in polyethylene capsules for analysis.

### 7.2.3 Choice of elements

The choice of elements in this study was based on four criteria: 1) the elements had to be suitable for analysis by neutron activation, 2) the elemental levels in the exposed samples had to be significantly higher than the concentrations in the blanks, 3) the elements should have similar and limited number of oxidation states, so as to reduce the diversity of the number of possible chemical forms in advance, and 4) the environmental relevance had to be clear, preferably representative for soil and different types of air pollution.

The elements cobalt, scandium and zinc sufficiently met the requirements mentioned above. Cobalt (oxidation state 2 or 3), alloyed with chromium and tungsten is used for high-speed, heavy duty, high temperature cutting tools and for dies. Cobalt is also used in magnet steels and stainless steels (including electroplating) and in alloys used in jet turbines and gas turbine generators. Cobalt from anthropogenic sources occurs also in the crustal material. Scandium (oxidation state 3) is a typical element for crustal material. It is widely distributed on earth and occurs in very minute quantities in over 800 mineral species. Zinc (oxidation state 2) is employed to form numerous alloys with other metals (brass, nickel silver, soft solder). Large quantities of zinc are used to produce die castings, used extensively by the automotive, electrical and hardware industries. Zinc is also used to galvanize other metals such as iron to prevent corrosion.

#### *7.2.4 Sample analysis*

The elements cobalt, scandium and zinc in the samples were determined by the single comparator method of instrumental neutron activation analysis (INAA) (Bode and de Bruin 1990). The samples were irradiated in the reactor at the Interfaculty Reactor Institute at a thermal neutron flux of  $5 \cdot 10^{16} \text{ m}^{-2} \cdot \text{s}^{-1}$ . After irradiation of 4 h the samples were measured twice during 1 h, after decay times of 5 and 21 days, respectively. Measurements were carried out with Ge(Li)-semiconductor detectors (Ortec, Canberra).

The precision of the analysis, expressed as relative standard deviations, for Co, Sc and Zn in air particulate matter were 4-9%, 2-9% and 1-3%, respectively, in total deposition 2-5%, 2-6% and 2-5%, respectively, in lichens 2-4%, 1% and 2%, respectively, and in rag 2-8%, 2-7% and 2-7%, respectively. Quality control of the INAA results was based on the regular analysis (1 per 8 samples) of NIST standard reference materials SRM 1572 (Citrus Leaves) and SRM 3172 (Multielement Mix B Standard Solutions) according to the same procedures as used for the samples. The analysis results obtained from the standard reference materials agreed with the certified values. In the blank air filters (Nuclepore, 17.3 mg) only Zn ( $6.0 \mu\text{g} \cdot \text{g}^{-1}$ ) could be detected.

The blank concentrations of the total deposition samples, set by the preconcentration step, were  $20.5 \pm 6.3 \text{ ng.L}^{-1}$  for Co,  $1.20 \pm 0.30 \text{ ng.L}^{-1}$  for Sc and  $2.79 \pm 0.60 \text{ } \mu\text{g.L}^{-1}$  for Zn. For the preconcentration method a constant chemical yield of  $97 \pm 3\%$  was assumed for Zn, Co and Sc (cf. van der Sloot 1976).

### 7.3 Results and Discussion

For two stations (1 and 4) the results are based on data obtained from half the exposure period (6 months). For station 1 the data from the period March 1988 until September 1988 were omitted, because of irregular collection times and loss of rag samples. The irregular collection times resulted also in loss of material of air particulate matter and total deposition. For station 4 no samples of any type were collected from the period March 1988 until September 1988, due to the absence of mounting and electricity facilities in this period.

#### 7.3.1 Air

Table 7-1 shows the monthly mean Co, Sc and Zn concentrations in air particulate matter on both filters ( $8.0 \text{ } \mu\text{m}$  and  $0.4 \text{ } \mu\text{m}$ ) together expressed in  $\text{ng.m}^{-3}$  (or  $\text{pg.m}^{-3}$ ). For Co, Sc and Zn the ratio between the concentrations in air particulate matter on the  $8.0 \text{ } \mu\text{m}$  and those on the  $0.4 \text{ } \mu\text{m}$  filters varied per month and per station.

For Co, the monthly mean concentration in air particulate matter on both filter types, varied from 64 to  $320 \text{ pg.m}^{-3}$  at 8 stations, as calculated from the data over a period of 12 months (Table 7-1). Seasonal trends were not observed, except that in November 1988, at the stations 1, 2, 3, 4 and 5 the concentrations in air particulate matter on the coarse filters were remarkably higher than those on the fine filters. The mean ratio between the monthly mean concentrations in air particulate matter on the  $8.0$  and  $0.4 \text{ } \mu\text{m}$  filters was 1.8, with a standard deviation of 0.6.

For Sc, the monthly mean concentrations in air particulate matter on both filter types together varied from 8.1 to  $24 \text{ pg.m}^{-3}$ , depending on the station (Table 7-1). Station 4 showed a relatively high mean concentration ( $24 \text{ pg.m}^{-3}$ ) over a period of

TABLE 7-I. Monthly mean element concentrations in air particulate matter on both fractions (8.0  $\mu\text{m}$  and 0.4  $\mu\text{m}$ ) together and the mean ratios between the 8.0 and the 0.4  $\mu\text{m}$  fractions for March 1988 to March 1989.

| station | Cobalt                         |                                 |                  | Scandium                       |                                 |                  | Zinc                           |                                 |                  |
|---------|--------------------------------|---------------------------------|------------------|--------------------------------|---------------------------------|------------------|--------------------------------|---------------------------------|------------------|
|         | mean<br>( $\text{pg.m}^{-3}$ ) | range<br>( $\text{pg.m}^{-3}$ ) | ratio<br>8.0/0.4 | mean<br>( $\text{pg.m}^{-3}$ ) | range<br>( $\text{pg.m}^{-3}$ ) | ratio<br>8.0/0.4 | mean<br>( $\text{ng.m}^{-3}$ ) | range<br>( $\text{ng.m}^{-3}$ ) | ratio<br>8.0/0.4 |
| 1*      | 134                            | 42-510                          | 2.5              | 8.9                            | 5.5-15                          | 3.7              | 48                             | 23-66                           | 1.7              |
| 2       | 134                            | 64-560                          | 2.5              | 14.9                           | 8.0-31                          | 6.7              | 34                             | 18-51                           | 1.1              |
| 3       | 150                            | 60-640                          | 2.3              | 14.1                           | 5.9-31                          | 3.7              | 58                             | 25-81                           | 0.5              |
| 4*      | 320                            | 115-980                         | 2.2              | 24                             | 19-29                           | 5.2              | 39                             | 34-50                           | 1.0              |
| 5       | 120                            | 38-260                          | 1.0              | 14.1                           | 6.8-31                          | 5.6              | 40                             | 22-56                           | 1.0              |
| 6       | 64                             | 27-140                          | 1.1              | 8.1                            | 3.1-21                          | 4.6              | 32                             | 14-59                           | 0.6              |
| 7       | 88                             | 18.9-210                        | 1.2              | 12.7                           | 2.0-36                          | 6.5              | 36                             | 5.3-86                          | 1.3              |
| 8       | 116                            | 49-180                          | 1.6              | 21                             | 11-50                           | 7.4              | 136                            | 54-295                          | 1.1              |

The asterisks denote the period September 1988 to March 1989.

6 months. At station 8, an extreme concentration in the  $8.0\ \mu\text{m}$  fraction was found in April 1988 ( $50\ \text{pg.m}^{-3}$ ). At the other 7 stations the monthly mean concentrations of both fractions varied from 2.0 to  $36\ \text{pg.m}^{-3}$ . The highest concentrations were found in the spring at all stations. The concentrations in the coarse fractions were always higher than those in the fine fractions. The mean ratio between the monthly mean concentrations in air particulate matter on the  $8.0\ \mu\text{m}$  and  $0.4\ \mu\text{m}$  filters was higher than for Co, namely 5.4, with a standard deviation of 1.4.

For Zn, the monthly mean concentrations in air particulate matter (both fractions) varied from 32 to  $136\ \text{ng.m}^{-3}$ , depending on the station (Table 7-I). At station 8 the monthly concentration was a factor 3-4 higher than at the other stations. The concentrations in both fractions were higher in the spring/summer seasons than in the autumn/winter seasons at the stations 1, 3, 6 and 7. At the stations 3, 4, 5, 6 and 7 the lowest concentrations were found in December 1988 in both fractions. The mean ratio between the monthly mean concentrations in the coarse and the fine fractions was  $1.0 \pm 0.4$ , which is lower than for Co and Sc.

The explanation for the different mean ratios between the monthly mean concentrations over a period of 12 months in the coarse and the fine fractions for Sc (5.4), Co (1.8) and Zn (1.0) is the size distribution of the particles related to these elements. According to Milford and Davidson (1985) the aerodynamic mass median diameters of particles related to Sc, Co and Zn are  $4.39\ \mu\text{m}$ ,  $2.63\ \mu\text{m}$  and  $1.13\ \mu\text{m}$  respectively, based on a large number of independent distributions obtained with different techniques and at different sample locations. The mean ratios of coarse to fine fractions for Sc, Co and Zn (5.4, 1.8 and 1.0) indicate that particles with an aerodynamic mass diameter smaller than  $8.0\ \mu\text{m}$  are also collected on the  $8.0\ \mu\text{m}$  filter. However, the largest particles are associated with Sc, which are more retained by the coarse filters than by the fine filters; the finest particles associated with Zn are equally retained (on the average) by both filter types. Particles associated with Co are of intermediate size and agree with an intermediate mean ratio between the two fractions.

The retention efficiency of Nuclepore capillary pore membranes is dependent on particle size and concentration, pore size and length, porosity and flow rate (Nuclepore Corporation 1980, Heidam 1981). For particles larger and much smaller

than the pore size, the retention efficiency is 100%. Large particles that are smaller than the pore size are captured to a certain extent by direct interception and by inertial impaction, due to the bending of the air streamlines near the pore walls (Heidam 1981). The retention of particles much smaller than the pore size takes place by diffusion to the pore walls (Heidam 1981).

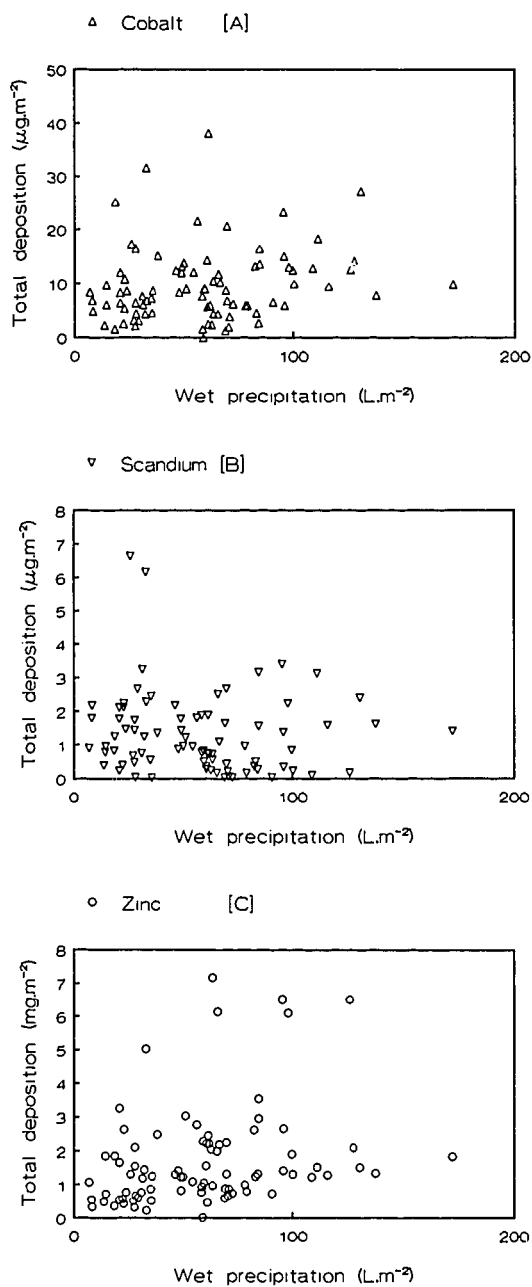
Milford and Davidson (1985) reported that those elements with aerodynamic mass median diameter larger than  $2\text{ }\mu\text{m}$ , (e.g. Sc and Co) generally have a unimodal distribution, with relatively little mass below  $1\text{ }\mu\text{m}$ . Elements with aerodynamic mass median diameter less than  $2\text{ }\mu\text{m}$ , (e.g. Zn) generally have bimodal distributions: the dominant peak occurs in the range  $0.5\text{--}1\text{ }\mu\text{m}$ , with a smaller peak at about  $3\text{--}5\text{ }\mu\text{m}$ . These figures indicate that the bulk mass of Sc, Co and Zn could be sampled with at least the  $0.4\text{ }\mu\text{m}$  filter.

If the impactor inlet is faced directly into the wind and the inlet air speed is identical to the ambient wind speed (isokinetic sampling), the particulates can be separated according to their size. In the present study, the amounts of air particulate matter in the  $0.4$  and  $8.0\text{ }\mu\text{m}$  filters were not representative for two separated particle size fractions, because the impactor inlet was faced downwards to prevent wetting from rainwater and the ambient wind speed and its variation were unknown.

### 7.3.2 Total deposition

The total (wet and dry) elemental deposition was calculated from the product of the amount of wet precipitation measured with the official KNMI rain gauge and the concentration in total deposition, thereby assuming that evaporation losses during the sampling period could be neglected.

Figures 7-2A-C show the scattergrams of the relation between the total deposition and the volume of wet precipitation. The correlation coefficients were very low, for Co 0.21, for Sc 0.017 and for Zn 0.31, respectively. The total deposition was not significantly positively related with the volume of wet precipitation, probably due to contributions of dry deposition and washout effects. The contribution of dry deposition in the bulk samples may have been in the order of magnitude of 10 to 40% (Ridder *et al.* 1984).



Figures 7-2A,B,C. The relation between total deposition of cobalt ( $\mu\text{g.m}^{-2}$ ) (A), scandium ( $\mu\text{g.m}^{-2}$ ) (B) and zinc ( $\text{mg.m}^{-2}$ ) (C), respectively, and the volume of wet precipitation ( $\text{L.m}^{-2}$ ).



TABLE 7-II. Monthly mean total deposition for March 1988 to March 1989.

| station | Cobalt                           |                                   | Scandium                         |                                   | Zinc                           |                                 |
|---------|----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|--------------------------------|---------------------------------|
|         | mean<br>( $\mu\text{g.m}^{-2}$ ) | range<br>( $\mu\text{g.m}^{-2}$ ) | mean<br>( $\mu\text{g.m}^{-2}$ ) | range<br>( $\mu\text{g.m}^{-2}$ ) | mean<br>( $\text{mg.m}^{-2}$ ) | range<br>( $\text{mg.m}^{-2}$ ) |
| 1*      | 3.0                              | 1.3-6.2                           | 0.38                             | 0.03-0.74                         | 0.57                           | 0.49-0.75                       |
| 2       | 6.6                              | 1.9-15                            | 1.0                              | 0.03-2.5                          | 0.94                           | 0.23-2.7                        |
| 3       | 5.3                              | <0.5-13.1                         | 0.64                             | 0.03-1.6                          | 0.92                           | 0.011-2.3                       |
| 4*      | 17.0                             | 9.0-32                            | 2.2                              | 0.17-6.2                          | 3.9                            | 2.7-6.5                         |
| 5       | 8.9                              | 2.6-18.3                          | 1.5                              | 0.02-3.1                          | 1.6                            | 0.55-6.1                        |
| 6       | 15.1                             | 4.9-27                            | 1.6                              | 0.23-6.7                          | 1.9                            | 0.33-7.2                        |
| 7       | 12.6                             | 4.4-38                            | 1.2                              | 0.17-2.1                          | 1.7                            | 0.43-2.5                        |
| 8       | 9.4                              | 1.6-23                            | 1.7                              | 0.35-3.4                          | 2.8                            | 0.94-6.5                        |

The asterisks denote the period September 1988 to March 1989.

To some extent the dry periods corresponded with periods in which high concentrations in total deposition were measured.

The monthly mean volume of wet precipitation as measured with the wet only samplers (KNMI) was  $58 \text{ L.m}^{-2}$  (58 mm). Heavy rainfall occurred in several months during the year; over  $95 \text{ L.m}^{-2}$  per month was measured in March (near stations 6, 7), July (near stations 2, 5, 6, 7, 8), August (near stations 1, 3), October (near stations 5, 6, 7) and December 1988 (near stations 2 and 8). The spring of 1988 was a relatively dry season, as were November 1988 and February 1989 dry months. In February less than  $32 \text{ L.m}^{-2}$  was measured with the wet only samplers (KNMI) at all collection sites, which corresponded with less than 1 L per bulk sampler at the 8 stations.

The concentrations of Co, Sc and Zn in total deposition were high at all stations during February 1989. High concentrations in total deposition were also found in April for Sc, and in January for Zn (except at stations 7 and 8). Low concentrations in total deposition were found in March 1988 for Co (except station 7), Sc and Zn (except station 3), in September for Sc (except station 7) and in December for Co and Sc (except station 8). The mean concentrations and associated standard deviations over 12 months in total deposition of Co was  $0.24 \pm 0.12 \mu\text{g.L}^{-1}$

(range  $<0.01$  to  $1.35 \mu\text{g.L}^{-1}$ ), of Sc  $38 \pm 19 \text{ ng.L}^{-1}$  (range  $0.38$  to  $265 \text{ ng.L}^{-1}$ ) and of Zn  $39 \pm 21 \mu\text{g.L}^{-1}$  (range  $0.19$  to  $158 \mu\text{g.L}^{-1}$ ).

Table 7-II shows the monthly mean total deposition of Co, Sc and Zn for 8 stations as calculated from the data over a period of 12 months. The data from the stations 1 and 4 were given from the period September 1988 until March 1989. From March 1988 to March 1989 the monthly mean total deposition of Co varied from  $3.0$  to  $17.0 \mu\text{g.m}^{-2}$ , of Sc from  $0.38$  to  $2.2 \mu\text{g.m}^{-2}$  and of Zn from  $0.57$  to  $3.9 \text{ mg.m}^{-2}$ .

### 7.3.3 Lichens

Tables 7-III, 7-IV and 7-V show the mean Co, Sc and Zn concentrations and their standard deviations in lichen samples per exposure period for each station. The mean Co, Sc and Zn concentrations in the lichens of 1, 2 and 4 months exposure, based on 12, 6 and 3 samples per station and the 12 months exposed samples were all uncorrected for the starting concentrations in the lichen material. Unlike rag, the starting concentrations of the lichen transplants are not fixed values, because the lichen transplants have been exposed for many years in the reference area. The mean starting concentration and standard deviation per element in the transplants was determined on basis of 15 samples from the reference area. Since the concentrations in the exposed samples are of the same order of magnitude as the starting concentrations, the mean values of the measured concentrations in the exposed samples (uncorrected for the starting values) are given in Tables 7-III, 7-IV and 7-V. The relative variation of the concentrations in lichens of 1, 2 and 4 months exposure was of comparable magnitude per element, which comprises genetic, morphological, sampling and seasonal variation (Tables 7-III, 7-IV and 7-V). The error in the elemental concentrations in the 12 months exposed samples is assumed to be of comparable magnitude as the standard deviations of the starting concentrations, since the seasonal variations are probably averaged out.

For cobalt, the concentrations in the lichens exposed for one year, varied from  $1.71$  to  $3.45 \text{ mg.kg}^{-1}$  (Table 7-III). At stations 1, 6 and 7 the Co concentrations in these samples were significantly higher (over two standard deviations of the starting concentration) than the concentrations in samples of shorter exposure periods.

TABLE 7-III. Mean cobalt concentrations (in  $\text{mg.kg}^{-1}$ ) and standard deviations (SD) in exposed lichens (N samples) for March 1988 to March 1989.

| station | Exposure time |      |                    |      |                   |      |                   |      |               |
|---------|---------------|------|--------------------|------|-------------------|------|-------------------|------|---------------|
|         | 0 m<br>N = 15 |      | 1 m<br>N = 12 (6*) |      | 2 m<br>N = 6 (3*) |      | 4 m<br>N = 3 (1*) |      | 12 m<br>N = 1 |
|         | mean          | SD   | mean               | SD   | mean              | SD   | mean              | SD   |               |
| 1*      | 1.37          | 0.55 | 1.92               | 0.58 | 1.95              | 0.67 | 2.01              | -    | 3.45          |
| 2       | 1.37          | 0.55 | 1.31               | 0.59 | 1.72              | 0.49 | 2.22              | 1.27 | 1.74          |
| 3       | 1.37          | 0.55 | 1.37               | 0.59 | 1.42              | 0.70 | 2.07              | 1.03 | 1.71          |
| 4*      | 1.37          | 0.55 | 1.53               | 0.64 | 1.93              | 1.06 | 1.95              | 0.62 | 2.34          |
| 5       | 1.37          | 0.55 | 1.35               | 0.72 | 1.54              | 0.40 | 1.73              | 0.65 | 2.00          |
| 6       | 1.37          | 0.55 | 1.40               | 0.78 | 1.77              | 0.93 | 2.00              | 0.68 | 3.27          |
| 7       | 1.37          | 0.55 | 1.37               | 0.64 | 1.60              | 0.94 | 1.84              | 0.64 | 3.45          |
| 8       | 1.37          | 0.55 | 1.32               | 0.58 | 1.28              | 0.55 | 1.48              | 0.85 | 1.83          |

The asterisks denote the period September 1988 to March 1989.

Generally, the exposed samples showed an increasing Co uptake with increasing exposure time. However, the mean concentrations in samples of 1, 2 and 4 months exposure and the 12 months exposed samples from stations 2, 3, 4, 5 and 8 were not significantly different from the starting concentrations.

For scandium, the concentrations in the lichens exposed for one year, varied from 1.22 to 3.09  $\text{mg.kg}^{-1}$  (Table 7-IV). Only at station 1, the Sc concentration in the 12 months exposed sample was significantly higher than those in samples of shorter exposure periods. Generally, the Sc uptake increased with increasing exposure time. However, the concentrations in the exposed samples were not significantly different from the starting concentrations (except for station 1).

For zinc, the concentrations in the lichens exposed for one year, varied from 132 to 381  $\text{mg.kg}^{-1}$  (Table 7-V). At the stations 4, 6, 7 and 8, the Zn concentrations in the 12 months exposed samples were significantly higher than the concentrations in samples of shorter exposure periods. Though not significantly, the Zn concentration in the 12 months exposed samples were higher than the starting concentration at the stations 1, 2 and 5, but lower at station 3.

TABLE 7-IV. Mean scandium concentrations (in  $\text{mg.kg}^{-1}$ ) and standard deviations (SD) in exposed lichens ( $N$  samples) for March 1988 to March 1989.

| station | Exposure time |      |               |      |              |      |              |      |         |
|---------|---------------|------|---------------|------|--------------|------|--------------|------|---------|
|         | 0 m           |      | 1 m           |      | 2 m          |      | 4 m          |      | 12 m    |
|         | $N = 15$      |      | $N = 12$ (6*) |      | $N = 6$ (3*) |      | $N = 3$ (1*) |      | $N = 1$ |
|         | mean          | SD   | mean          | SD   | mean         | SD   | mean         | SD   |         |
| 1*      | 1.06          | 0.41 | 1.24          | 0.38 | 1.56         | 0.66 | 1.42         | -    | 3.09    |
| 2       | 1.06          | 0.41 | 0.85          | 0.41 | 1.16         | 0.43 | 1.48         | 0.99 | 1.22    |
| 3       | 1.06          | 0.41 | 0.90          | 0.38 | 0.89         | 0.41 | 1.23         | 0.28 | 1.36    |
| 4*      | 1.06          | 0.41 | 0.84          | 0.38 | 1.14         | 0.81 | 1.30         | 0.75 | 1.71    |
| 5       | 1.06          | 0.41 | 0.85          | 0.52 | 1.10         | 0.35 | 1.11         | 0.54 | 1.41    |
| 6       | 1.06          | 0.41 | 0.95          | 0.63 | 1.14         | 0.63 | 1.13         | 0.63 | 1.84    |
| 7       | 1.06          | 0.41 | 0.81          | 0.40 | 0.97         | 0.83 | 1.08         | 0.43 | 1.51    |
| 8       | 1.06          | 0.41 | 0.83          | 0.34 | 0.89         | 0.33 | 0.96         | 0.50 | 1.28    |

The asterisks denote the period September 1988 to March 1989.

TABLE 7-V. Mean zinc concentrations (in  $\text{mg.kg}^{-1}$ ) and standard deviations (SD) in exposed lichens ( $N$  samples) for March 1988 to March 1989.

| station | Exposure time |    |               |      |              |      |              |      |         |
|---------|---------------|----|---------------|------|--------------|------|--------------|------|---------|
|         | 0 m           |    | 1 m           |      | 2 m          |      | 4 m          |      | 12 m    |
|         | $N = 15$      |    | $N = 12$ (6*) |      | $N = 6$ (3*) |      | $N = 3$ (1*) |      | $N = 1$ |
|         | mean          | SD | mean          | SD   | mean         | SD   | mean         | SD   |         |
| 1*      | 143           | 24 | 148           | 19.8 | 164          | 4.36 | 127          | -    | 164     |
| 2       | 143           | 24 | 160           | 30.1 | 160          | 38.2 | 174          | 75.8 | 175     |
| 3       | 143           | 24 | 136           | 33.9 | 151          | 16.9 | 166          | 38.2 | 132     |
| 4*      | 143           | 24 | 177           | 33.0 | 164          | 46.4 | 170          | 20.6 | 206     |
| 5       | 143           | 24 | 134           | 35.7 | 151          | 34.3 | 138          | 33.7 | 163     |
| 6       | 143           | 24 | 144           | 26.5 | 138          | 37.2 | 170          | 16.3 | 231     |
| 7       | 143           | 24 | 162           | 34.8 | 164          | 48.6 | 173          | 46.1 | 268     |
| 8       | 143           | 24 | 156           | 33.9 | 177          | 16.2 | 173          | 21.1 | 381     |

The asterisks denote the period September 1988 to March 1989.

For all three elements the lichen transplants showed quantitative differences between geographical locations. Only the 12 months exposed samples showed significantly higher concentrations than the starting concentrations at some stations (Tables 7-III, 7-IV, 7-V). These results indicate that at least one year of exposure time is required to distinguish the concentration of the exposed lichen from the starting concentration of the material used in this study.

#### 7.3.4 Rag

Table 7-VI shows the monthly mean uptake for March 1988 to March 1989 and the standard deviations in the rag samples for Co, Sc and Zn per station, corrected for the blank values. The mean blank values, based on 16 samples were  $18.7 \pm 1.6 \mu\text{g.kg}^{-1}$  for Co,  $0.77 \pm 0.29 \mu\text{g.kg}^{-1}$  for Sc and  $5.2 \pm 1.0 \text{ mg.kg}^{-1}$  for Zn. The monthly uptake in the rag samples, averaged over one year, varied from 90 to  $500 \mu\text{g.kg}^{-1}$  for Co, from 12.3 to  $164 \mu\text{g.kg}^{-1}$  for Sc and from 11.7 to  $44 \text{ mg.kg}^{-1}$  for Zn (Table 7-VI). At stations 1 and 4, the mean concentrations were based on 6 exposure months. For all elements the concentrations were highly variable during the year, resulting in relative variances up to 103%. The data did not show a seasonal trend.

TABLE 7-VI. Monthly mean element concentrations (corrected for blanks) and standard deviations (SD) in rag samples for March 1988 to March 1989.

| station | Cobalt ( $\mu\text{g.kg}^{-1}$ ) |     | Scandium ( $\mu\text{g.kg}^{-1}$ ) |     | Zinc ( $\text{mg.kg}^{-1}$ ) |     |
|---------|----------------------------------|-----|------------------------------------|-----|------------------------------|-----|
|         | mean                             | SD  | mean                               | SD  | mean                         | SD  |
| 1*      | 90                               | 49  | 12.3                               | 6.3 | 16.9                         | 8.7 |
| 2       | 142                              | 64  | 42                                 | 22  | 11.7                         | 4.6 |
| 3       | 230                              | 103 | 64                                 | 50  | 17.4                         | 9.5 |
| 4*      | 500                              | 210 | 164                                | 31  | 44                           | 16  |
| 5       | 189                              | 61  | 47                                 | 18  | 15.6                         | 7.9 |
| 6       | 490                              | 240 | 145                                | 149 | 23                           | 8.6 |
| 7       | 470                              | 290 | 87                                 | 71  | 37                           | 22  |
| 8       | 210                              | 121 | 68                                 | 44  | 41                           | 28  |

The asterisks denote the period September 1988 to March 1989.

### 7.3.5 Quantification of the relationships between element concentrations in lichens, rag and atmospheric concentrations in air particulate matter and/or depositions.

With the high starting concentrations in the lichens under study, it is not possible to examine quantitative relationships between air particulate matter or deposition versus lichens, on a monthly basis. In the lichens used in this study, significant element accumulation occurred only after 12 months exposure. Thus, examination of the 12 months exposed lichens for their relationship with air particulate matter ( $8.0 + 0.4 \mu\text{m}$  fractions) and with the total deposition can be carried out on a yearly basis.

The approach in the present pilot field study was to compare the concentrations of the 12 months exposed lichens with the mean concentrations in air particulate matter collected over a period of 12 months (Figures 7-3A,C,E). In this way, the seasonal trend in the concentrations of air particulate matter was averaged out. The concentrations of the 12 months exposed lichens were also compared with the total deposition on a yearly basis. The total (wet and dry) deposition, monthly collected with bulk collectors, was calculated cumulative over a period of 12 months (Figures 7-3B,D,F).

For *cobalt*, the scattergram of the relation between the concentrations in the 12 months exposed lichens and the mean concentrations in air particulate matter (on both filters) in one year, yielded two clusters, as shown in Figure 7-3A. At the stations 2, 3, 5 and 8, the concentrations in lichens remained constant with increasing concentrations in air particulate matter. At the stations 6 and 7, high concentrations in the lichens corresponded with low mean concentrations in air particulate matter (Figure 7-3A). The concentrations in lichens were not significantly correlated with the annual mean concentrations in air particulate matter of both fractions together, nor with those of the coarse and the fine fraction separately.

The concentrations in the 12 months exposed lichens were significantly correlated with the cumulative total deposition in one year (Figure 7-3B, Table 7-VII). The quantitative relationship can be described by the following equation:

$$L_{\text{Co}} = (997 \pm 339) + (12.1 \pm 3.0) D_{\text{Co}}$$

where  $L_{\text{Co}}$  is the Co concentration in the 12 month exposed lichens (in  $\mu\text{g.kg}^{-1}$ ) and

$D_{Co}$  is the cumulative Co total deposition in one year (in  $\mu\text{g.m}^{-2}$ ).

Assuming that the observed concentration in the exposed lichens represents the starting concentration plus the Co uptake, the intercept of this equation is indeed comparable to the Co starting concentration ( $1370 \mu\text{g.kg}^{-1}$ ). The starting concentration is high compared to the net Co uptake (Table 7-III).

A general relationship for the net Co uptake in lichens, using the starting concentration of  $1370 \mu\text{g.kg}^{-1}$  as blank to correct for, is given by the equation:

$$L_{\text{net, Co}} = (9.2 \pm 1.5) D_{Co}$$

where  $L_{\text{net, Co}}$  is the net Co uptake by lichens in one year (in  $\mu\text{g.kg}^{-1}$ ).

The slope of this equation ( $9.2 \text{ m}^2.\text{kg}^{-1}$ ) is called the "Accumulation Factor (AF)".

For *scandium*, the scattergram of the relation between the concentrations in the 12 months exposed lichens and the mean concentrations in air particulate matter (on both filters) in one year, is shown in Figure 7-3C. The concentrations in lichens were not significantly correlated with the annual mean concentrations in air particulate matter of both fractions together, nor with those of the coarse and the fine fraction separately.

However, the concentrations in the 12 months exposed lichens did correlate with the cumulative total deposition in one year, though the correlation coefficient was not significant at the 90% probability level (Figure 7-3D, Table 7-VII). At all stations, the concentrations in the lichens were quite similar. The quantitative relationship between the concentrations in lichens and the annual total deposition, is given by the equation:

$$L_{Sc} = (1097 \pm 172) + (22 \pm 12) D_{Sc}$$

where  $L_{Sc}$  is the Sc concentration in the 12 month exposed lichens (in  $\mu\text{g.kg}^{-1}$ ) and

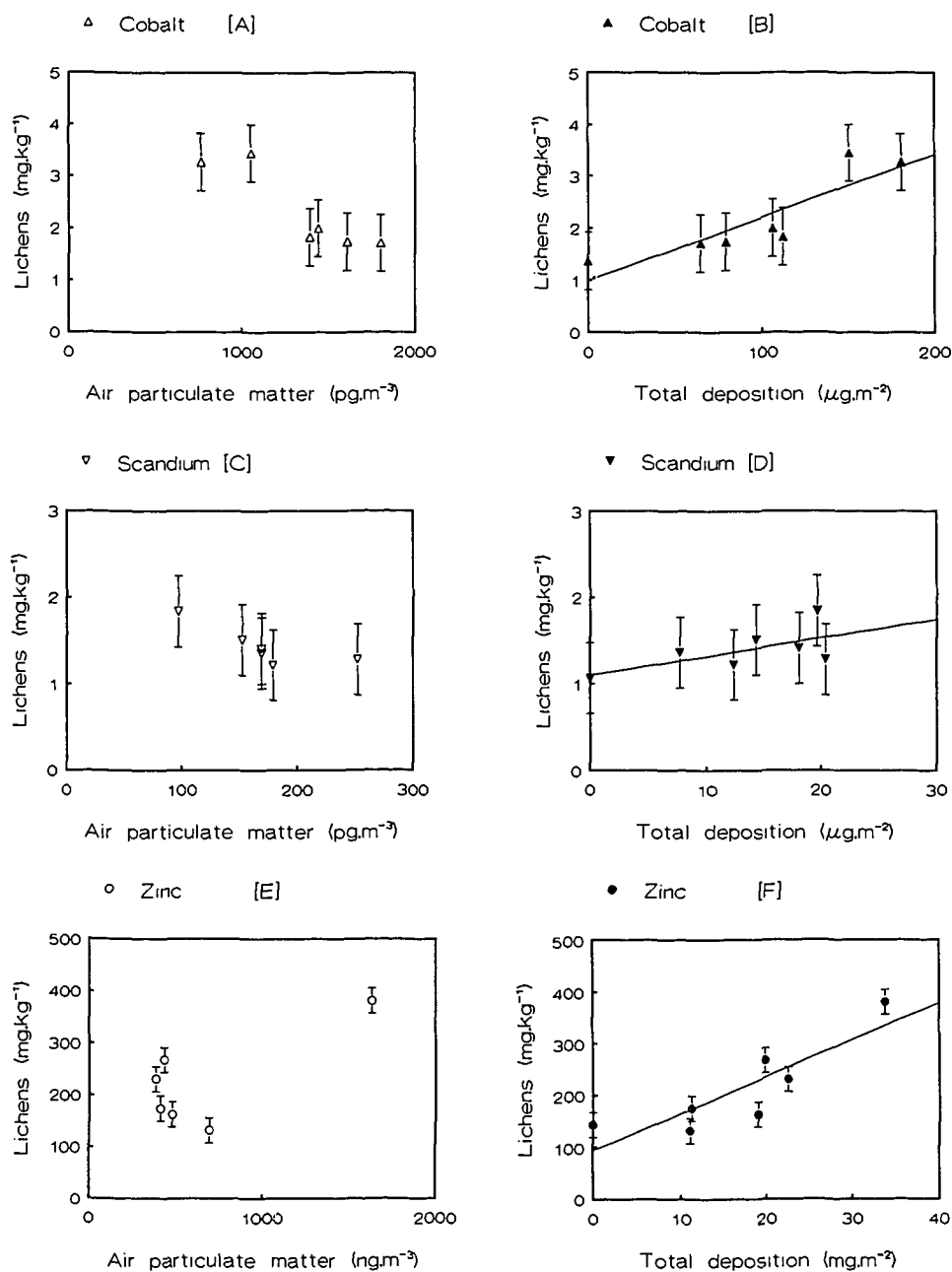
$D_{Sc}$  is the cumulative Sc total deposition in one year (in  $\mu\text{g.m}^{-2}$ ).

The intercept of this equation is similar to the Sc starting concentration in lichens ( $1060 \mu\text{g.kg}^{-1}$ ). Also for Sc, the starting concentration is high compared to the net Sc uptake (Table 7-IV). A general relationship for the net uptake in lichens, using the starting concentration of  $1060 \mu\text{g.kg}^{-1}$  as blank to correct for, is given by the equation:

$$L_{\text{net, Sc}} = (24 \pm 5) D_{Sc}$$

where  $L_{\text{net, Sc}}$  is the net Sc uptake by lichens in one year (in  $\mu\text{g.kg}^{-1}$ ).

The accumulation factor for Sc is  $24 \text{ m}^2.\text{kg}^{-1}$ .



Figures 7-3A-F. The relation between the concentrations in lichens and those as air particulate matter ( $8.0 \pm 0.4 \mu\text{m}$ ), and the cumulative total deposition, respectively, over a period of 12 months, for cobalt (A,B), scandium (C,D) and zinc (E,F).



For *zinc*, a high concentration in the 12 months exposed lichen sample corresponded with a high annual mean concentration in air particulate matter, only at station 8 (Figure 7-3E). The concentrations in lichens were not significantly correlated with the annual mean concentrations in air particulate matter of both fractions together, nor with those of the coarse and the fine fraction separately.

The concentrations in the 12 months exposed lichens were highly significantly correlated with the cumulative total deposition in one year (Figure 7-3F, Table 7-VII). The quantitative relationship between the Zn concentrations in the 12 months exposed lichens and the total deposition is given by the equation:

$$L_{Zn} = (93 \pm 37) + (7.1 \pm 1.9) D_{Zn}$$

where  $L_{Zn}$  is the Zn concentration in the 12 months exposed lichens (in mg.kg<sup>-1</sup>) and

$D_{Zn}$  is the cumulative Zn total deposition in one year (in mg.m<sup>-2</sup>).

*The intercept of this equation is comparable to the Zn starting concentration in lichens (143 mg.kg<sup>-1</sup>) within the error margins. The starting concentration is high compared to the net Zn uptake, except for station 6, 7 and 8 (Table 7-V). The relationship for the net uptake, using the starting concentration of 143 mg.kg<sup>-1</sup>, is given by the equation:*

$$L_{net, Zn} = (5.0 \pm 1.0) D_{Zn}$$

where  $L_{net, Zn}$  is the net Zn uptake by lichens in one year (in mg.kg<sup>-1</sup>).

The accumulation factor for Zn is 5.0 m<sup>2</sup>.kg<sup>-1</sup>.

As was mentioned before, no significant correlations were found between concentrations in lichens and in air particulate matter. However, it would be premature to draw the conclusion that lichens cannot be related at all with air particulate matter collected on air filters. A number of factors influencing such a relation, should be further investigated, such as the high starting concentration in the lichen, the influence of the polyethylene netting and the surface of the transplant, the exposition direction of the transplant, the meteorological conditions and the dry deposition velocity.

The use of rag samples might give insight in the uptake mechanisms in lichens, by comparing the response of the rag samples, which have a known passive uptake mechanism (ion-exchange, adsorption of dissolved trace elements and/or trapping of

particulate material) with that of lichens.

The relation between concentrations in rag samples and those in air particulate matter was studied. Identical results as for lichens were obtained, namely that the concentrations in rag were not significantly correlated with those in air particulate matter of both fractions together, nor with those of the coarse and the fine fractions separately.

For Co and Zn, the uptake in one year in rag, was significantly correlated with the cumulative total (wet and dry) deposition, a low correlation was observed for Sc (Table 7-VII). The starting concentrations in the rag are low compared to the net elemental uptake. The quantitative relationships between the net elemental uptake in rag and the total deposition are given by:

Co:  $R_{\text{net, Co}} = (31 \pm 2.6) D_{\text{Co}}$ , where

$R_{\text{net, Co}}$  is the Co uptake in rag in one year (in  $\mu\text{g.kg}^{-1}$ ) and

$D_{\text{Co}}$  is the cumulative Co total deposition in one year (in  $\mu\text{g.m}^{-2}$ ).

Sc:  $R_{\text{net, Sc}} = (57 \pm 10) D_{\text{Sc}}$ , where

$R_{\text{net, Sc}}$  is the Sc uptake in rag in one year (in  $\mu\text{g.kg}^{-1}$ ) and

$D_{\text{Sc}}$  is the cumulative Sc total deposition in one year (in  $\mu\text{g.m}^{-2}$ ).

Zn:  $R_{\text{net, Zn}} = (15 \pm 1.5) D_{\text{Zn}}$ , where

$R_{\text{net, Zn}}$  is the Zn uptake in rag in one year (in  $\text{mg.kg}^{-1}$ ) and

$D_{\text{Zn}}$  is the cumulative Zn total deposition in one year (in  $\text{mg.m}^{-2}$ ).

For rag, the Co, Sc and Zn accumulation factors are 31, 57 and 15  $\text{m}^2.\text{kg}^{-1}$ , respectively.

Investigation of the relationships between the annual mean Co, Sc and Zn concentrations in rag and the concentrations in the 12 months exposed lichens, yielded high correlation coefficients (Table 7-VII) and they are quantified by the following equations:

Co:  $L_{\text{net, Co}} = (0.30 \pm 0.03) R_{\text{net, Co}}$

Sc:  $L_{\text{net, Sc}} = (0.42 \pm 0.03) R_{\text{net, Sc}}$

Zn:  $L_{\text{net, Zn}} = (0.33 \pm 0.06) R_{\text{net, Zn}}$

The slopes of these equations are the ratios of the accumulation factors for lichens to those for rag and the ratios are comparable for all three elements.

TABLE 7-VII. Correlation coefficients of the relation between concentrations in lichens or rag and the cumulative total deposition for zinc, cobalt and scandium.

|          |   | Zinc |       |       | Cobalt |       |       | Scandium |       |       |
|----------|---|------|-------|-------|--------|-------|-------|----------|-------|-------|
|          |   | L    | R     | D     | L      | R     | D     | L        | R     | D     |
| Zinc     | L | 1    | 0.877 | 0.861 | 0.347  |       |       | 0.207    |       |       |
|          | R |      | 1     | 0.889 |        | 0.620 |       |          | 0.599 |       |
|          | D |      |       | 1     |        |       | 0.752 |          |       | 0.917 |
| Cobalt   | L |      |       |       | 1      | 0.953 | 0.875 | 0.851    |       |       |
|          | R |      |       |       |        | 1     | 0.927 |          | 0.926 |       |
|          | D |      |       |       |        |       | 1     |          |       | 0.855 |
| Scandium | L |      |       |       |        |       |       | 1        | 0.955 | 0.642 |
|          | R |      |       |       |        |       |       |          | 1     | 0.681 |
|          | D |      |       |       |        |       |       |          |       | 1     |

L, R and D denote the type of sample, respectively lichens (12 months exposed), rag (12 months) and the cumulative total deposition of one year. The critical values for the correlation coefficients were 0.729 ( $P = 90\%$ ), 0.811 ( $P = 95\%$ ) and 0.917 ( $P = 99\%$ ) ( $N = 6$ ).

On basis of the relatively low blank values of Co, Sc and Zn in the rag, this material can thus be used for quantification purposes, for shorter periods than one year. The uptake in the rag was significantly correlated with the cumulative total deposition of at least 6 months. For shorter periods, the correlations between the rag and total deposition were not significant, possibly due to the variation in meteorological conditions. For example for Zn, at least 6 months uptake in rag was needed to show a significant correlation with the cumulative total deposition of 6 months (correlation coefficient = 0.794,  $P = 95\%$ ).

### 7.3.6 Concentration ratios

To get insight in the relationship between all sample types (air particulate matter, total deposition, lichens and rag), the elemental ratios between the different types of samples were calculated (Table 7-VIII). Though the elemental ratios between total deposition, lichens and rag can also be derived from the accumulation factor ratios, Table 7-VIII is more illustrative and also includes air particulate matter. The ratios of the annual mean concentrations, as given in Table 7-VIII, were normalized

TABLE 7-VIII. Ratios between the annual mean concentrations of Co, Sc and Zn in air particulate matter, total deposition, lichens and rag for 6 stations (2, 3, 5, 6, 7 and 8).

| Type of sample                   | Sc/Sc | Co/Sc         | Zn/Sc            |
|----------------------------------|-------|---------------|------------------|
| aerosol, 8.0 + 0.4 $\mu\text{m}$ | 1     | $8.1 \pm 1.8$ | $3800 \pm 1500$  |
| aerosol, 8.0 $\mu\text{m}$       | 1     | $6.0 \pm 2.0$ | $2100 \pm 860$   |
| aerosol, 0.4 $\mu\text{m}$       | 1     | $21 \pm 5.0$  | $13800 \pm 6600$ |
| total deposition                 | 1     | $7.6 \pm 2.0$ | $1300 \pm 280$   |
| net uptake lichens               | 1     | $2.4 \pm 1.2$ | $290 \pm 400$    |
| net uptake rag                   | 1     | $4.0 \pm 0.8$ | $340 \pm 150$    |

to the soil element Sc, to obtain information on possible enrichment of Co and Zn with respect to Sc in the samples.

The ratios of the annual mean concentrations in aerosol samples of both fractions together, of Sc : Co : Zn are in the proportion of 1.0 : 8.1 : 3800; for the aerosol samples collected on the 8.0  $\mu\text{m}$  filters and the 0.4  $\mu\text{m}$  filters these ratios are in the proportion of 1 : 6.0 : 2100 and 1 : 21 : 13800, respectively (Table 7-VIII). In the 0.4  $\mu\text{m}$  fraction the Co/Sc and the Zn/Sc ratios are approximately 3.5 times, respectively 7 times higher than in the coarse fraction. These results may suggest that Co partly and Zn mainly occur as fine particles in the aerosol, which are emitted by high-temperature processes. This suggestion agrees with the aerodynamic mass median diameters as reported by Milford and Davidson (1985).

The ratios of the annual mean total deposition of Sc : Co : Zn are in the proportion of 1.0 : 7.6 : 1300 (Table 7-VIII). The Co/Sc ratio in the total deposition (7.6) was similar to the elemental ratios of the annual mean concentrations in air particulate matter on both filters (8.1) and the 8.0  $\mu\text{m}$  fraction (6.0), but approximately a factor 3 lower than in air particulate matter of the 0.4  $\mu\text{m}$  fraction (21). The Zn/Sc ratio in the total deposition (1300) was comparable to the one in the 8.0  $\mu\text{m}$  fraction (2100), but an order of magnitude lower than in the 0.4  $\mu\text{m}$  fraction (13800). These results indicate that the 0.4  $\mu\text{m}$  fraction was depleted in the total deposition with regard to the 8.0  $\mu\text{m}$  fraction, which might be explained by the aerodynamic behaviour of fine particles.

The ratios of the net uptake (concentrations corrected for their starting

concentrations) in the 12 months exposed lichens are in the proportion of 1 : 2.3 : 290 (Table 7-VIII). The Co/Sc and the Zn/Sc ratios of the net uptake in lichens are a factor 3, respectively a factor 4 lower than in the total deposition. Compared to the aerosol samples from the 8.0  $\mu\text{m}$  and the 0.4  $\mu\text{m}$  air filters, the depletion of Zn in lichens covers a factor 7 and 48, respectively. These results indicate that the lichens tend to reflect the total deposition more than air particulate matter collected on air filters.

The ratios of the annual mean concentrations in rag samples of Sc, Co and Zn are in the proportion of 1.0 : 4.0 : 345, which are comparable to those of the net uptake in lichen samples, namely 1.0 : 2.4 : 290.

### *7.3.7 Uptake mechanisms in lichens*

The use of rag has given some insight in the uptake mechanisms in lichens. The concentrations in lichens were significantly correlated with those in rag samples for Co, Sc and Zn. The ratios of the accumulation factors for lichens to those for rag for Co, Sc and Zn were comparable (0.30, 0.42 and 0.33, respectively), indicating a similar response of lichens and rag to the total deposition. These results indicate that, as for rag, mainly passive processes are involved in the uptake and/or release mechanisms, such as particulate trapping, ion-exchange, adsorption and/or desorption for all three elements. The extracellular binding sites in the resin (rag) and the cell walls (lichens) are pectic compounds, which are polymers of alpha-D-galacturonic acid, containing free carboxylic groups (Greulach 1973). Supportive data for the importance of particulate trapping as an uptake mechanism in lichens were reported by Nieboer and Richardson (1981), including laboratory metal elution studies and electron microscopic evidence.

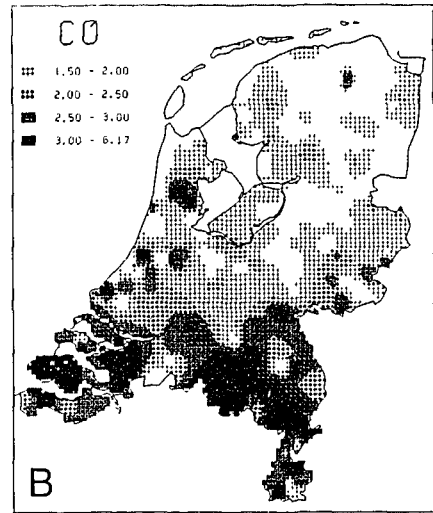
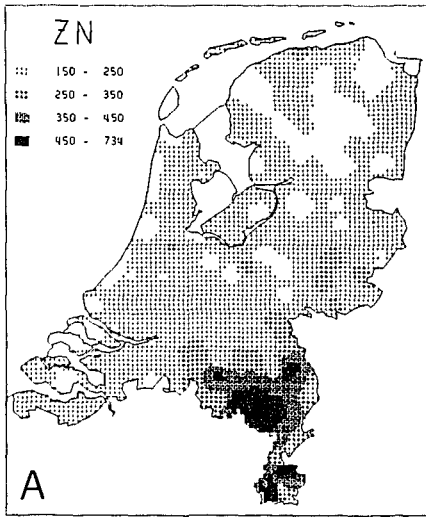
Both in lichens and in rag the accumulation factor for Sc was markedly higher than for Co and Zn. This result suggests a different uptake mechanism for Sc than for Co and Zn. It is likely that particles containing the soil element scandium are large and insoluble in water. These particles might be trapped by lichens and stuck to rag. The cobalt and zinc containing particles, resulting from high temperature processes are likely to be small and soluble in water. Cobalt occurs in the soil as well and may

therefore be present partly in coarse and less soluble particles. For Zn and to some extent Co the main processes in the uptake- and/or release mechanisms may be adsorption and/or desorption and ion-exchange and, to a minor extent, metabolic activated mechanisms. The results of the concentration ratios (Table 7-VIII) support the conclusion that accumulation is related to the aerodynamic mass diameter of the particles.

The Zn and Co concentrations in lichens are assumed to reach a state of equilibrium with atmospheric concentrations. This assumption is supported by the corresponding Zn and Co concentrations in the exposed lichens and the concentrations in *P. sulcata* growing *in situ*, exposed for many years. The lichen data set exposed for many years was obtained from a previous monitoring study on a national scale (see Chapter 2).

From the present study, the starting Zn concentration of *P. sulcata* ( $143 \pm 24$  mg.kg<sup>-1</sup>, Table 7-V), in the northern region of the country and the concentrations in samples exposed at stations 1, 2, 3, 4 and 5 agreed with the concentrations classes 1 and 2 (80 - 150 mg.kg<sup>-1</sup> and 150 - 250 mg.kg<sup>-1</sup>, respectively) obtained from the lichens growing *in situ* (Figure 7-4A). The Zn concentrations in the 12 months exposed samples at stations 6, 7 and 8 (Table 7-V) corresponded with concentration classes 2 (150 - 250 mg.kg<sup>-1</sup>), 3 (250 - 350 mg.kg<sup>-1</sup>) and 4 (350 - 450 mg.kg<sup>-1</sup>), which was for each station one class lower than observed in the monitoring survey. Probably the exposure period of 12 months was too short to reach the concentrations as high as measured in the lichens growing *in situ* in the southern region of the country. Also Pilegaard (1979) concluded that an exposure time of 7 months was too short to achieve equilibrium between the fallout of metals from the atmosphere and the metal content in transplanted *Hypogymnia physodes* to stations within 3 km from a steel works.

The Co concentrations in lichens exposed at stations 2, 3, 4, 5, 6 and 7 corresponded with the concentration classes based on lichens growing *in situ*, exposed for many years (Figures 7-1 and 7-4B); the Co concentrations in the 12 months exposed lichens were relatively high at station 1 compared to the values obtained from the lichen data set from the monitoring study (Table 7-III, Figure 7-4B).



Figures 7-4A and 7-4B. Concentration pattern in lichens growing *in situ* (1987) for zinc and cobalt, respectively.

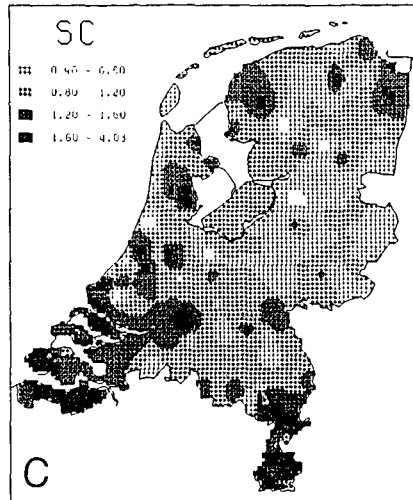


Figure 7-4C. Concentration pattern in lichens growing *in situ* (1987) for scandium.

The fact that the Sc concentrations in the 12 months exposed lichens did not correspond with the concentrations observed in lichens, growing *in situ*, exposed for many years (Table 7-IV and Figures 7-1, 7-4C), supported the assumption that the

uptake mechanism for Sc was different than for Co and Zn.

## 7.4 Conclusions

For cobalt, scandium and zinc, the concentrations in the 12 months exposed lichens were significantly correlated with the annual total deposition, but not with the annual mean concentrations in air particulate matter, indicating that lichens tend to reflect total deposition more than air particulate matter.

Lichens and rag are good monitors for the total deposition of cobalt, scandium and zinc. The element specific accumulation factors in lichens, were in the proportion of about 5 : 2 : 1 for scandium, cobalt and zinc, respectively, indicating that the accumulation of an element by the monitor is related to its chemical properties and the properties of the associated particles (size, composition, solubility).

From the comparable ratios between the accumulation factors in lichens and in rag for cobalt, scandium and zinc, it is concluded, that mainly passive processes are involved in the uptake- and release mechanisms. The higher accumulation factor for scandium compared to cobalt and zinc in lichens and rag suggests a different uptake mechanism for these three elements. Most likely, coarse and insoluble particles associated with scandium are trapped by lichens and stuck to rag; fine and soluble particles associated with zinc and to some extent with cobalt, may be accumulated by lichens and rag by ion-exchange and/or adsorption and/or desorption.

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## CHAPTER 8. KINETICS OF CADMIUM UPTAKE BY GREEN ALGAE

**Abstract.** *The kinetics of cadmium uptake by *Selenastrum capricornutum* Printz was studied at 21° C and 0° C, at four different concentrations of free cadmium ( $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$  and  $10^{-3}$  M). The free cadmium concentrations in the medium were kept constant through buffering by a chelating agent (NTA).*

*Initial uptake occurred within 10 min at both temperatures, indicating a fast process. At 21° C, the cadmium uptake increased during the experimental sampling times from 0.12 h to 50 h, whereas at 0° C, the cadmium uptake remained constant after the first sampling time. Apparently, two different processes were involved in cadmium uptake, a first fast, probably passive process and a second slower, possibly physiological process.*

*The cadmium uptake processes were described with a generalized metal-ligand complex formation model. The experimental dissociation constants for the fast ( $K_{R,f}$ ) and the slow process ( $K_{R,s}$ ) were reproducible for different experiments and they were both in the order of  $5 \cdot 10^{-6}$  mol.L<sup>-1</sup>. For the slow process, the uptake- and release rate constants ( $k_1$  and  $k_2$ ) were quantified; for two experiments, the mean value for  $k_1$  was  $111 \pm 28$  L.mol<sup>-1</sup>.h<sup>-1</sup> and the mean value for  $k_2$  was  $(5.1 \pm 1.8) \cdot 10^{-4}$  h<sup>-1</sup>.*

## CHAPTER 8. KINETICS OF CADMIUM UPTAKE BY GREEN ALGAE

### 8.1 Introduction

From the literature on uptake and accumulation of heavy metals in algae it is known, that the uptake processes are dependent on the type and chemical form of the metal ions, the biological system under study and the environmental (or experimental) conditions, such as light, temperature, aeration and the composition of the surface water or medium (latest reviews by Rai *et al.* 1981, Wood and Wang 1983, Whitton 1984, Trevors *et al.* 1986). However, there are still gaps in the understanding of the kinetics of heavy metal uptake by algal cells, which has led to various models of metal uptake.

In general, two stages are thought to be involved in the kinetics of metal uptake (Khummongkol *et al.* 1982, Geisweid and Urbach 1983, Trevors *et al.* 1986, Ting *et al.* 1989). The first is very rapid, assumed to be passive (i.e. physical and/or chemical adsorption or ion exchange at the cell surface), and occurs immediately after initial contact with the metal. The second stage is slow and possibly active, being related to some type of metabolic activity. The relative importance of the two stages may vary with algae species and metal ion (Trevors *et al.* 1986).

As shown by Trevors *et al.* (1986), much attention has been focused upon cadmium uptake by microorganisms. Cadmium is widespread in the environment. It is a potent toxicant (inhibiting photosynthesis and damaging mitochondria), even at low levels (Rai *et al.* 1981). Its toxicity is a function of the free cadmium ion concentration (Allen *et al.* 1980). Cadmium has no known biological function (from Trevors *et al.* 1986).

Yet, only in a few papers the cadmium uptake kinetics have been quantified. Geisweid and Urbach 1983 have interpreted their results quantitatively by using the Freundlich- and Langmuir adsorption isotherm. However, these isotherms show only equilibrium conditions for varying concentrations and do not provide information on the rates of uptake. Khummongkol *et al.* (1982) developed a model for metal uptake based on surface adsorption. Long-term uptake experiments (over 11 h) in growing

cultures showed a greater cadmium accumulation than predicted by the adsorption model, while short-term experiments (10 min) under the same conditions, showed less uptake than predicted, indicating that the experiments could not be described by one adsorption isotherm. Ting *et al.* (1982) proposed a model analogous to an enzyme-substrate coupling scheme, to explain their experimental findings. This model may be valid to describe the uptake mechanism, but the main assumptions of this model could not be validated by experimental measurements. For instance, it is assumed that carriers are present on both sides of the cell membrane and that the diffusion of the metal-carrier complex through the cell membrane is very rapid. Furthermore, the concentrations of the carrier and the metal-carrier complex are assumed to be small compared with both the extracellular and the intracellular metal concentrations.

The aim of the present study was to quantify kinetic parameters of cadmium uptake by green algae. Here, the cadmium uptake is defined as the concentration of cadmium bound to the algae, comprising all the possible locations of cadmium on or in the algae cells, i.e. the cell wall, the cell membrane, the space between the cell wall and the cell membrane and the intracellular space within the cell membrane. In the cadmium-uptake-experiments in the present study, the time, algae mass, temperature and a range of free cadmium concentrations ( $10^{-9}$  to  $10^{-3}$  M) were variables. At low cadmium concentration levels, without a chelating agent, cadmium depletion may occur in dense algae suspensions and significant cadmium loss due to cadmium-nutrient complexation or adsorption to the glass wall is possible. Thus, during each time series, the free cadmium concentrations were kept constant through buffering by a chelating agent (NTA), to prevent uncontrolled disturbances in the cadmium-algae equilibria.

## 8.2 Model description

In the present study, the cadmium uptake is regarded as a reaction between cadmium ions and algae leading to the formation of a cadmium-algae complex. Possibly, several processes are involved in cadmium uptake, which may take place in different compartments, having specific parameters. However, the concentration of cadmium bound to the algae in the possible compartments, could not be measured

separately. Thus, the cadmium-algae complex measured, is the concentration of cadmium bound to the algae, as a result of all the processes, such as adsorption (binding to the cell surface), diffusion, ion exchange and physiological processes.

From literature two stages of uptake are thought to be involved, a first rapid uptake, occurring immediately after initial contact with the ions and a second slower uptake. The approach in the present study was to start from a generalized metal-ligand complex formation model for each of the two (i.e. fast and slow) individual processes, which can be represented as:



The equilibrium expression corresponding to this relation is:

$$K_C = \frac{[CdA]}{[Cd]_f \cdot [A]_a} \quad (2)$$

where,

$K_C$  = the complex forming constant in this heterogenic equilibrium between cadmium and algae cells in medium, valid for the experimental conditions, analogous to the formation constant in homogenous equilibria (Kruyt and Overbeck 1969). The inverse of  $K_C$  is the dissociation constant  $K_R$  (release of cadmium from the cadmium-algae complex), both  $K_C$  and  $K_R$  differentiated to fast or slow process,

$[Cd]_f$  = the free Cd concentration (in moles Cd per litre algae suspension),

$[A]_a$  = the concentration of free binding sites in algae available for binding free cadmium (in moles per litre algae suspension), differentiated to fast or slow process and

$[CdA]$  = the concentration of cadmium bound to the algae (in moles per litre algae suspension), differentiated to fast, slow or both processes.

The concentration of free binding sites in algae available for binding free cadmium is dependent on the biomass concentration. In the present study, the algae cultures were in the exponential growth phase (cf. Monod 1949) at the beginning of

the batch experiments. During the batch experiments the biomass concentration ( $W$ ) increases with time according to  $W_t = W_0 \cdot e^{\mu t}$ , where  $W_t$  is the biomass concentration of the algae population at time  $t$  and  $\mu$  is the specific growth rate constant. The specific growth rate constant  $\mu$  is a measure of the efficiency of growth.

Assuming that the capacity of binding sites in the algae available for each individual process for binding free cadmium ( $A_F$  for the fast process and  $A_S$  for the slow process), is linearly related with the biomass concentration ( $W_t$ ), the fast and slow process can be described by  $A_{F,t} = A_{F,0} \cdot e^{\mu t}$  and  $A_{S,t} = A_{S,0} \cdot e^{\mu t}$ , respectively. Furthermore, the fast process  $[A]_{F,t}$  can be expressed as:

$$[A]_{F,t} = [A]_{F,a,t} + [CdA]_{F,t} \quad (3)$$

The subscripts  $f$  and  $a$  denote free and available, the subscripts  $t$ ,  $0$  denote time and time zero, respectively and the subscripts  $F$  and  $S$  denote the fast and the slow process, respectively.

Substitution in equation 2 gives for the fast process:

$$[CdA]_{F,t} = \frac{[Cd]_f \cdot [A]_{F,0} \cdot e^{\mu t}}{K_{R,F} + [Cd]_f} \quad (4)$$

Analogous to the fast process, the equilibrium expression for the slow process can be expressed as:

$$[CdA]_{S,t} = \frac{[Cd]_f \cdot [A]_{S,0} \cdot e^{\mu t}}{K_{R,S} + [Cd]_f} \quad (5)$$

The free cadmium concentration was kept constant during the experiment and the change in the concentration of algae material available for complex formation with cadmium was unknown ( $[A]_{F,a}$  and  $[A]_{S,a}$ ). As mentioned above, the measured concentration of cadmium bound to the algae ( $[CdA]_{T,t}$ ), comprises the total concentration of CdA of both processes ( $[CdA]_{F,t} + [CdA]_{S,t}$ ).

If the equilibrium state of the complex formation has not yet been reached, as can be expected for the slow process, the net change of the concentration of CdA



with time, can be expressed by the differential equation:

$$\frac{d[CdA]_{s,t}}{dt} = k_1 \cdot [Cd]_f \cdot ([A]_{s,0} \cdot e^{\mu t} - [CdA]_{s,t}) - k_2 \cdot [CdA]_{s,t} \quad (6)$$

The formation- and dissociation rate constant of the complex  $CdA_s$ ,  $k_1$  and  $k_2$ , respectively, can be calculated from the solution of this differential equation:

$$[CdA]_{s,t} = \frac{k_1 \cdot [Cd]_f \cdot [A]_{s,0}}{\mu + k_2 + k_1 \cdot [Cd]_f} \cdot (e^{\mu t} - e^{-(k_2 + k_1 \cdot [Cd]_f) \cdot t}) \quad (7)$$

The kinetic parameters of cadmium uptake in growing cultures, with a known growth rate constant, can thus be calculated from the equations 4, 5 and 7, only under the assumption that  $A_f$  and  $A_s$  are linearly related with the biomass concentration ( $W_t$ ).

### 8.3 Experimental

#### 8.3.1 Algae cultures

Three batch experiments were carried out using the unicellular green algae *Selenastrum capricornutum* Printz. The algae cultures were obtained from *S. capricornutum* populations grown in CITIRI medium in a chemostat, continuously illuminated, aerated and stirred at 16° C (Department of Civil Engineering, Delft University of Technology). In a chemostat, the culture volume is held constant by means of a constant level overflow (Gons 1977). The composition of CITIRI medium, based on modifications on Z-8 medium (Skulberg 1964) and EPA medium (Miller 1978), consisted of 1.37 mM  $NaNO_3$ , 0.25 mM  $NaHCO_3$ , 0.22 mM  $FeCl_3 \cdot 6H_2O$ , 62.5  $\mu M$   $Ca(NO_3)_2 \cdot 4H_2O$ , 25.4  $\mu M$   $MgSO_4 \cdot 7H_2O$ , 17.8  $\mu M$   $KH_2PO_4$ , 0.75  $\mu M$   $H_3BO_3$ , 0.52  $\mu M$   $MnCl_2 \cdot 4H_2O$ , 7.5 nM  $Na_2MoO_4 \cdot 2H_2O$ , 6 nM  $ZnCl_2$ , 1.5 nM  $CoCl_2 \cdot 6H_2O$ , 18 pM  $CuCl_2 \cdot 2H_2O$ , 25 mM of the pH buffer 2-(N-morpholino)ethanesulphonic acid (MES) and 1.8 mM  $Na_2NTA$  (nitrilo-triacetic-acid). The pH was set at 6.15 with HCl. All chemicals were of *pro analyse* quality. During the experiments of 55 hours each, 400 ml of the algae cultures were grown in 1 L Erlenmeyer flasks, placed on a white magnetic stirring plate immersed in a thermostated water bath, under constant

lighting (TL, 'cool white', intensity at the bottom level of the flasks under water:  $150 \mu\text{E m}^{-2}\text{s}^{-1}$ ). The culture flasks were closed with sponge buttons to allow aeration. Experiment 1 and 2 were carried out at  $21^\circ\text{C}$ , experiment 3 at  $0^\circ\text{C}$ .

During the experiments, the biomass in all batch cultures was measured as the optical density at 750 nm (Perkin Elmer 552) of a 1 ml sub-sample volume, taken at 5 moments a day. Few and small samples were taken to prevent any disturbance of the experimental conditions in the remaining solution. The relation between the optical density and the biomass was determined by calibration of the concentration of dry biomass and the optical density at 750 nm of a larger volume of algae suspension. To construct the calibration function, the concentration of dry biomass was determined by filtration of 50 ml algae suspension from the synchronous culture. The calibration function was:

$$W \text{ (mg.L}^{-1}\text{)} = (186 \pm 6) \cdot \text{OD}_{750} - (6 \pm 4). \quad (N = 3, r = 0.98).$$

The precision of the concentration of dry biomass determination was 5-10%.

Per batch culture the growth was determined by fitting the biomass measurements with the function  $W_t = W_0 \cdot e^{\mu t}$ , where  $W_t$  is the concentration of dry biomass ( $\text{mg.L}^{-1}$ ) at time  $t$  and  $\mu$  is the growth rate constant (in  $\text{h}^{-1}$ ) (cf. Monod 1949). It should be noted that the growth rate constant ( $\mu$ ) is dependent on the experimental conditions, such as light, temperature, medium composition and the concentration of toxic substances (e.g. free cadmium) (Gonçalves *et al.* 1988). The biomass of each experimental sample taken at time  $t$  was calculated from the "growth curve" of the corresponding batch culture and expressed in concentration of dry biomass using the calibration curve.

### 8.3.2 Spiking with cadmium

The algae suspensions were spiked with  $^{115}\text{Cd}$  as radiotracer (in the order of  $140 \text{ MBq.g}^{-1}$ ). In each of the 3 experiments, the tracer was added at  $t = 0$  to all the batch cultures, except to the blank. Each experiment consisted of four batch cultures each with a different cadmium concentration. The blank was grown simultaneously with the cadmium containing batch cultures. For all 3 experiments the concentration ranges of cadmium was similar. The ratio between the concentrations of total

cadmium ( $\text{Cd}_T$ ) and the free cadmium ( $\text{Cd}_f$ ) in the solution was calculated by means of the computer programme Soilchem (Sposito *et al.* 1988). In the experiments, the free cadmium concentrations were targeted at respectively 1)  $10^{-3}$  M, 2)  $10^{-7}$  M, 3)  $10^{-8}$  M, 4)  $10^{-9}$  M and 5) 0 M (the blank).

The  $^{115}\text{Cd}$  tracer was made as follows; cadmium chloride (Merck, *pa*) as a residue from an evaporated aqueous solution, sealed in a polyethylene capsule, was irradiated in the reactor at the Interfaculty Reactor Institute at a thermal neutron flux of  $5 \cdot 10^{16} \text{ m}^{-2} \cdot \text{s}^{-1}$  for 4 h. After irradiation, the residue was dissolved in an aliquot of CITIRI medium and stirred for 30 min. Since the tracer was prepared in a calibrated irradiation facility, the specific activity of the  $^{115}\text{Cd}$  tracer was known. As a check, the  $^{115}\text{Cd}$  carrier solution aliquots were measured in a calibrated well-type semi conductor detector (Canberra) at 528 keV. From the activities measured, absolute cadmium masses were calculated. Quality control of the results was based on the analysis of a reference  $\text{CdCl}_2$  solution (Merck, Titrisol 9960) and on the comparison with the expected amount. The ratio of the results from the reference solution and the expected value was  $1.00 \pm 0.02$ , indicating no significant systematic errors in the analysis. The precision of the measurements was 1-2%.

### 8.3.3 Sample analysis

From every batch culture, 10 ml samples were taken at 12-14 sampling times during 3 days. All samples, except the blanks, were centrifuged at 2700 g for 10 min (Dijkstra, swing out rotor) in 10 ml glass tubes (Kimble). No centrifuge brake was used in order to prevent resuspension of the algal pellet. To wash the medium containing  $\text{Cd}_T$  from the algae, 9.5 ml of the supernatant was removed with a Pasteur pipette and the pellet was resuspended in 10 ml demineralized ice water and centrifuged again as described above. After the second centrifugation and removal of 9.5 ml of the supernatant, the pellet was resuspended in 0.5 ml demineralized ice water and brought into an eppendorf tube. To remove as much as possible any remaining medium containing  $\text{Cd}_T$ , the algae were finally centrifuged through silicon oil (100  $\mu\text{l}$ ) at 11000 g at 0° C for 3 min (Sigma 2K15, angular rotor). The densities of the CITIRI medium and the silicon oil were respectively 1.001 and 1.007  $\text{g} \cdot \text{cm}^{-3}$ .

(DMA 48 AP). After centrifugation, the pellets were separated from the supernatant by the oil layer. The eppendorf tubes were immersed in liquid nitrogen for 10 s to freeze both the pellet and the supernatant. The tips of the tubes, containing the pellet, were cut off. The pellets were measured in a calibrated well type semiconductor detector (Ge-Li Canberra) at 528 keV for 1 h. The precision of the measurements was 2% for the pellets from the batches 1 and 2 containing the two highest cadmium concentrations (1, 2) and 5% and 12-15%, respectively for the pellets from batches 3 and 4 containing the two lowest the cadmium concentrations. The detection limit for cadmium in the algae pellets under the experimental conditions described in section 8.3.2, was  $2.15 \text{ mg.kg}^{-1}$ . The concentration of  $\text{Cd}_T$  in the batch culture was determined by measuring 1 ml samples of the supernatant after the first centrifugation. In the oil samples and the blank algae cultures, no cadmium could be detected.

## 8.4 Results and Discussion

### 8.4.1 Cadmium uptake at $21^\circ \text{C}$

In the present study, a constant sample volume was taken with time, namely 10 ml algae suspension, with a variable concentration of algae biomass ( $W_t$ ). The cadmium uptake data ( $[\text{CdA}]_{T,t}$ ) were expressed in moles per litre algae suspension and normalized to a dry algae biomass concentration of 100 mg per L suspension. This value is close to the average biomass concentration for all three experiments. Thus  $x \text{ } \mu\text{g}$  cadmium measured as  $\text{CdA}_{T,t}$  in a pellet, containing  $W_t$  mg algae from 10 ml suspension, was transformed to

$$x \cdot 1/112.40 \cdot 10^{-6} \cdot 100/W_t \text{ moles CdA}_{T,t} \text{ per L suspension.}$$

The normalization step ( $100/W_t$ ) can also be expressed as  $100/W_0 \cdot e^{\mu t}$ .

Table 8-I shows the calculated  $[\text{Cd}]_f$  and measured ( $\text{Cd}_T$ ) as used in the experiments I and II and the corresponding range of cadmium uptake with time.

In the experiments, it was not possible to measure  $[\text{CdA}]_T$  within 7 minutes.

TABLE 8-I. The cadmium concentrations ( $[Cd]_f$  and  $[Cd]_T$ ) in experiment I and II (in  $\text{mol.L}^{-1}$ ). The corresponding range of cadmium uptake (in  $\text{mol.L}^{-1}$ ) from  $t = 1$  (0.12 h in exp. I and 0.17 h in exp. II) to  $t = 12$  (48.90 h in exp. I and 53.74 h in exp. II) was normalized to a dry algae biomass concentration of  $100 \text{ mg.L}^{-1}$ .

| Exp. | Batch | $[Cd]_f$             | $[Cd]_T$             | $[CdA]_{T, t=1}$     | $[CdA]_{T, t=12}$    |
|------|-------|----------------------|----------------------|----------------------|----------------------|
| I    | 1     | $1.15 \cdot 10^{-3}$ | $3.76 \cdot 10^{-3}$ | $9.87 \cdot 10^{-6}$ | $2.39 \cdot 10^{-5}$ |
| II   | 1     | $9.04 \cdot 10^{-4}$ | $3.39 \cdot 10^{-3}$ | $3.92 \cdot 10^{-6}$ | $1.08 \cdot 10^{-5}$ |
| I    | 2     | $5.05 \cdot 10^{-7}$ | $1.23 \cdot 10^{-3}$ | $7.97 \cdot 10^{-7}$ | $2.43 \cdot 10^{-6}$ |
| II   | 2     | $4.40 \cdot 10^{-7}$ | $1.17 \cdot 10^{-3}$ | $4.75 \cdot 10^{-7}$ | $1.06 \cdot 10^{-6}$ |
| I    | 3     | $2.71 \cdot 10^{-8}$ | $1.88 \cdot 10^{-4}$ | $1.14 \cdot 10^{-7}$ | $4.20 \cdot 10^{-7}$ |
| II   | 3     | $2.64 \cdot 10^{-8}$ | $1.80 \cdot 10^{-4}$ | $6.41 \cdot 10^{-8}$ | $1.83 \cdot 10^{-7}$ |
| I    | 4     | $1.77 \cdot 10^{-9}$ | $1.36 \cdot 10^{-5}$ | $1.33 \cdot 10^{-8}$ | $5.68 \cdot 10^{-8}$ |
| II   | 4     | $2.39 \cdot 10^{-9}$ | $1.80 \cdot 10^{-5}$ | $1.02 \cdot 10^{-8}$ | $2.62 \cdot 10^{-8}$ |

The first sampling time ( $t_1$ ) was 0.12 h in experiment I and 0.17 h in experiment II. The last sampling time ( $t_{12}$ ) was 48.90 h and 53.74 h, respectively for experiment I and II. In Table 8-I it is shown, that the highest cadmium uptake occurred in batch 1 in both experiments and that the cadmium uptake decreased one order of magnitude per experiment with decreasing cadmium concentrations. The concentration of free cadmium ( $[Cd]_f$ ) was assumed to be the parameter primarily responsible for the order of magnitude difference of cadmium uptake by the algae (Allen *et al.* 1980, Rai *et al.* 1981).

The concentration of dry biomass of the algae from the blank batches increased from  $57 \pm 6$  to  $140 \pm 15 \text{ mg.L}^{-1}$ , in experiment I and from  $120 \pm 12$  to  $154 \pm 15 \text{ mg.L}^{-1}$  in experiment II. For both experiments, no growth was observed in algae from batch 1 with the highest cadmium concentration. The growth rate constants in experiment I were  $0.012 \text{ h}^{-1}$  (batch 2),  $0.016 \text{ h}^{-1}$  (batch 3), and  $0.010 \text{ h}^{-1}$  (batch 4), based on 15 measurements. In experiment II, the growth rate constants were  $0.004 \text{ h}^{-1}$  (batch 2),  $0.006 \text{ h}^{-1}$  (batch 3) and  $0.005 \text{ h}^{-1}$  (batch 4).

Figure 8-1 shows the cadmium uptake by the algae in experiment I as a function of time at  $21^\circ \text{ C}$ . This figure shows, that each  $Cd_f$  concentration corresponded with a different level of cadmium uptake. For the growing cultures, at the lower cadmium concentrations in batch 2, 3 and 4, peaks in the cadmium uptake

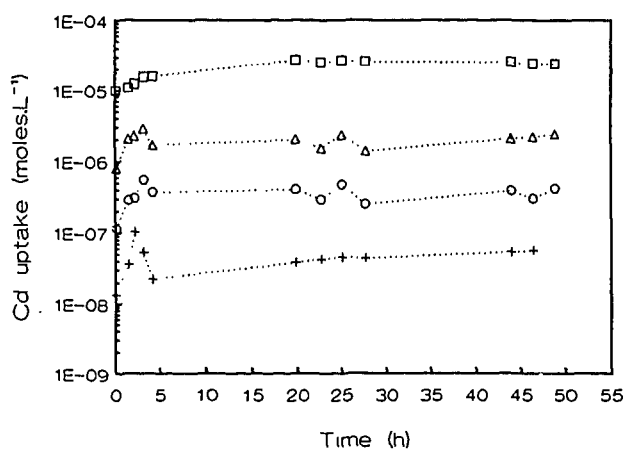


Figure 8-1. The cadmium uptake (normalized to a dry algae biomass concentration of  $100 \text{ mg.L}^{-1}$ ) at  $21^\circ \text{C}$  (exp. I) in relation with time. The  $[\text{Cd}]_i$  in the batches were  $10^{-3}$  M (squares),  $10^{-7}$  M (triangles),  $10^{-8}$  M (circles) and  $10^{-9}$  M (plus signs), respectively.

are present at  $t = 2-3$  h and dips at  $t = 4.20$  h (Figure 8-1). These peaks and dips could not be explained by known factors, such as corrections in the concentration of dry biomass, counting statistics, remaining medium containing  $\text{Cd}_T$  in the pellet, because the possible errors made by such factors were much lower than the differences in cadmium uptake at the different sampling times.

Figure 8-2 shows the cadmium uptake by the algae in experiment II as a function of time, also at  $21^\circ \text{C}$ . As for experiment I, each  $\text{Cd}_i$  concentration corresponded with a different level of cadmium uptake. At the lower cadmium concentrations in batch 2, 3 and 4, in experiment II, peaks in cadmium uptake are present at  $t = 1$  h and dips at  $t = 2$  h (Figure 8-2). Also these peaks and dips could not be explained. The peaks and dips in cadmium uptake for the growing cultures were observed at the same time for all batches per experiment, but they did not correspond in time between experiment I and II. Further research on the number and the surface area of the cells might give insight the different response of the algae in experiment I and II.

The cadmium uptake in experiment I was higher than in experiment II for the batches 1, 2 and 3, probably due to the slightly higher free cadmium concentrations in

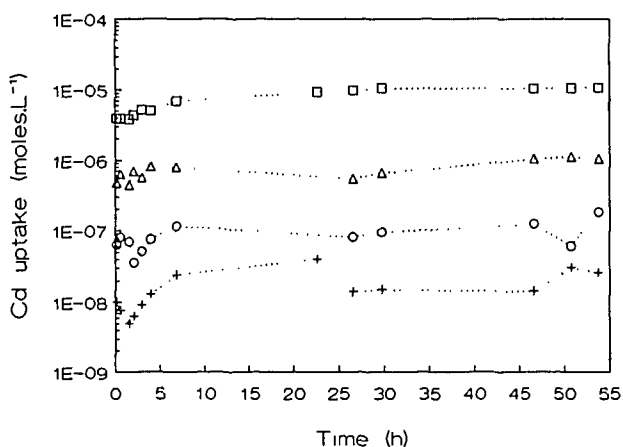
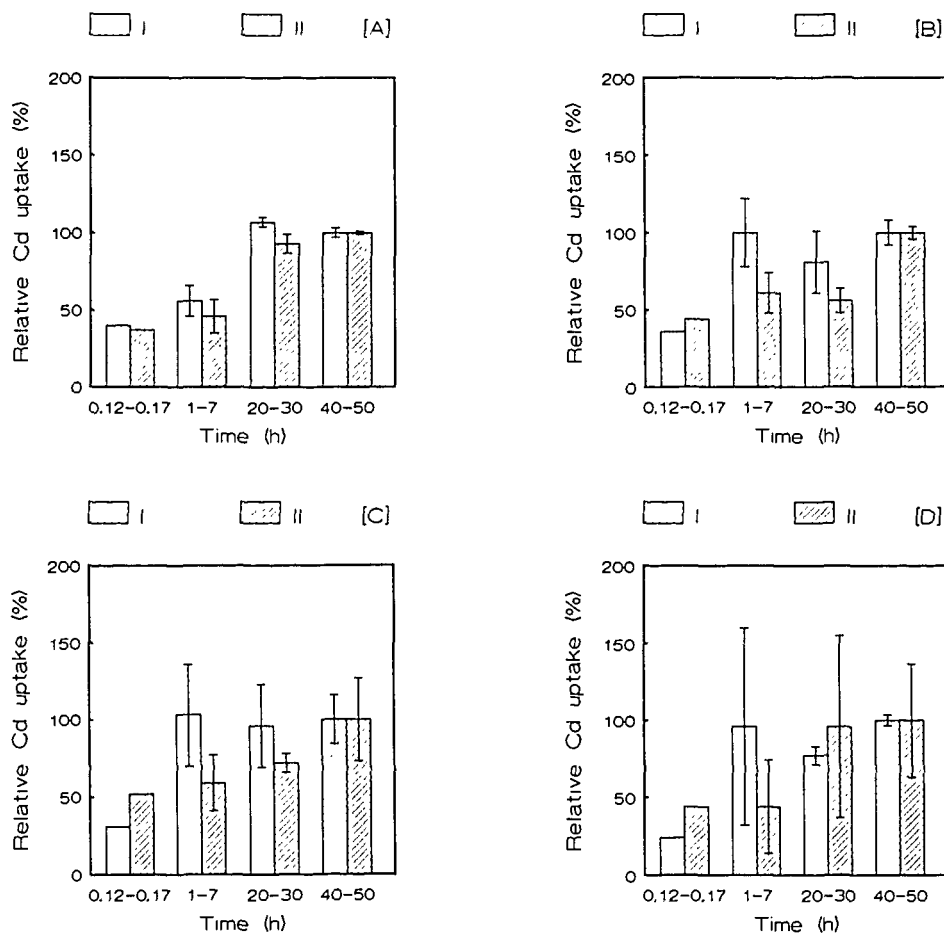


Figure 8-2. The cadmium uptake (normalized to a dry algae biomass concentration of  $100 \text{ mg.L}^{-1}$ ) at  $21^\circ \text{C}$  (exp. II) in relation with time. The  $[\text{Cd}]_f$  in the batches were  $10^{-3} \text{ M}$  (squares),  $10^{-7} \text{ M}$  (triangles),  $10^{-8} \text{ M}$  (circles) and  $10^{-9} \text{ M}$  (plus signs), respectively.

experiment I (Table 8-I, Figures 8-1 and 8-2). However, for the lowest free cadmium concentration, a lower  $[\text{Cd}]_f$  in experiment I corresponded with a higher cadmium uptake compared to experiment II.

To indicate the reproducibility of the method the relative cadmium uptake of both experiments was compared. Thereby, averages were calculated for the groups of data points from the 0.12 - 0.17, 1 - 7, 20 - 30 and 40 - 50 h interval and normalized to 100% for the average of the 40 - 50 h interval.

Figures 8-3A to 8-3D show the cadmium uptake relative to the uptake in the period 40 - 50 h, as a function of time intervals for all  $\text{Cd}_f$  concentrations in the solution. The standard deviations of the mean relative cadmium uptake, calculated from several measurements per time interval, increased with decreasing cadmium concentrations in the solution for both experiments (Figures 8-3A to 8-3D). For the batches 1 and 2, the mean cadmium uptake at the first sampling time (0.12 - 0.17 h) was in the order of 40% of the final equilibrium level, for both experiments. For the lower  $\text{Cd}_f$  concentrations (batches 3 and 4), the cadmium uptake at the first sampling time was in the order of 27% and 48% of the final equilibrium level for experiment I and II, respectively.



Figures 8-3A to 8-3D. The cadmium uptake relative to the uptake in the interval 40 - 50 h in experiment I (dotted bars) and experiment II (striped bars) as a function of time for the free cadmium concentrations  $10^{-3}$  M (A),  $10^{-7}$  M (B),  $10^{-8}$  M (C) and  $10^{-9}$  M (D); normalized data.

For the highest cadmium concentration the equilibrium level was not reached after 7 h, but only in the third sampling time period, in both experiments.

For the time interval 40 - 50 h, the ratios between the cadmium uptake for the  $Cd_f$  concentrations in batch 1, 2, 3 and 4, respectively, were in the proportion of  $(1.00 \pm 0.04) : (0.090 \pm 0.008) : (0.015 \pm 0.003) : (0.002 \pm 0.0001)$  for experiment I,



$(1.00 \pm 0.02) : (0.100 \pm 0.004) : (0.012 \pm 0.006) : (0.002 \pm 0.0008)$  for experiment II. These results indicate that the ratios between the equilibrium concentrations of CdA for different free cadmium concentrations were reproducible for algae from two different experiments.

#### 8.4.2 Cadmium uptake at 0° C

Figure 8-4 shows the cadmium uptake in the algae as a function of time in experiment III, at 0° C.

As for experiment I and II, each  $Cd_f$  concentration in experiment III corresponded with a different level of cadmium uptake (Figure 8-4, Table 8-II). In batch 1 of experiment III, having the highest  $Cd_f$  concentration, the cadmium uptake remained constant with time, indicating that the equilibrium concentration of CdA has been reached at the first sampling time (0.17 h). Also for the lower  $[Cd]_f$  in the batches 2, 3 and 4, the cadmium uptake remained rather constant with time, though more variable than in batch 1, and the equilibrium concentration was reached quickly. These results show that even at 0° C, cadmium uptake occurred within 10 minutes.

The concentration of dry biomass of the algae from the blank batch remained constant at  $104 \pm 10 \text{ mg.L}^{-1}$  during the experiment. No growth was observed in the algae for all 4 cadmium concentrations in the solution.

Table 8-II shows the cadmium concentrations in experiment III at 0° C and the corresponding mean cadmium uptake per batch. Comparison of the experiments I, II and III show that initial uptake occurred within 0.12 h, both at 21° C and at 0° C. The mean normalized  $[CdA]_{T,1}$  for all free cadmium concentrations at 0° C are within the range of the normalized CdA concentrations at 21° C of the first sampling time (Tables 8-I and 8-II). These results indicate that the initial uptake occurred rather independent of the temperature. Probably a passive process is involved in the cadmium uptake in the first minutes of contact between cadmium and algae.

From experiment I and II, in which cadmium uptake increased with time, and from experiment III, in which the cadmium uptake remained constant with time, it is indicated that a second slower process was involved in the uptake, which was temperature dependent.

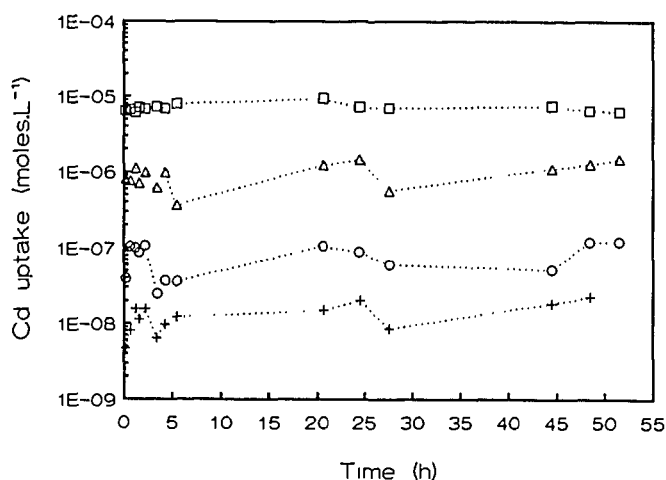


Figure 8-4. The cadmium uptake (normalized to a dry algae biomass concentration of  $100 \text{ mg.L}^{-1}$ ) at  $0^\circ \text{C}$  (experiment III) in relation with time. The  $[\text{Cd}]_i$  in the batches were  $10^{-3} \text{ M}$  (squares),  $10^{-7} \text{ M}$  (triangles),  $10^{-8} \text{ M}$  (circles) and  $10^{-9} \text{ M}$  (plus signs), respectively.

TABLE 8-II. The cadmium concentrations ( $[\text{Cd}]_i$  and  $[\text{Cd}]_T$ ) and the corresponding mean cadmium uptake (normalized) with standard deviation (in  $\text{mol.L}^{-1}$ ) from  $t = 1$  (0.17 h) to  $t = 12$  (51.53 h) in experiment III.

| Batch | $[\text{Cd}]_i$      | $[\text{Cd}]_T$      | mean $[\text{CdA}]_{T,1}$ | SD                   |
|-------|----------------------|----------------------|---------------------------|----------------------|
| 1     | $1.15 \cdot 10^{-3}$ | $3.81 \cdot 10^{-3}$ | $7.08 \cdot 10^{-6}$      | $0.84 \cdot 10^{-6}$ |
| 2     | $9.40 \cdot 10^{-7}$ | $1.44 \cdot 10^{-3}$ | $9.60 \cdot 10^{-7}$      | $3.38 \cdot 10^{-7}$ |
| 3     | $2.85 \cdot 10^{-8}$ | $1.96 \cdot 10^{-4}$ | $7.74 \cdot 10^{-8}$      | $3.46 \cdot 10^{-8}$ |
| 4     | $2.57 \cdot 10^{-9}$ | $1.94 \cdot 10^{-5}$ | $1.29 \cdot 10^{-8}$      | $0.55 \cdot 10^{-8}$ |

Physiological processes are more sensitive to temperature changes from  $0^\circ \text{C}$  to  $21^\circ \text{C}$  than physical or chemical processes. Therefore it is likely that a physiological process is involved in the second and slower uptake  $21^\circ \text{C}$ . The two stages involved in the kinetics of metal uptake in the present study are in accordance with literature (cf. Khummongkol *et al.* 1982, Geisweid and Urbach 1983, Trevors *et al.* 1986, Ting *et al.*

1989).

However, Sakaguchi *et al.* (1979) reported that the amount of cadmium absorbed by living cells of *Chlorella vulgaris* scarcely varied with the temperature range observed (0° C to 30° C). This characteristic was also demonstrated by heat-killed cells. The heat-killed cells absorbed twice the amount of cadmium in the temperature range studied. Hence, the cadmium uptake was presumably not directly mediated by any metabolic processes. Furthermore, Sakaguchi *et al.* (1979) demonstrated that the cadmium uptake by *Chlorella* cells was not significantly affected by the treatment with metabolic inhibitors.

#### 8.4.3 Estimation of the experimental kinetic parameters

For the normalized data set, the measured  $[CdA]_{T,t}$  becomes:

$$\frac{100}{W_0 \cdot e^{\mu t}} \cdot [CdA]_{T,t} = \frac{100}{W_0 \cdot e^{\mu t}} \cdot [CdA]_{F,t} + \frac{100}{W_0 \cdot e^{\mu t}} \cdot [CdA]_{S,t} \quad (8)$$

Referring to equations 4 and 5, for the normalized data set the equilibrium concentrations of  $[CdA]_{F,t}$  and  $[CdA]_{S,t}$  become constant values.

To indicate that the concentrations of CdA and A are normalized, they are printed bold face in the next paragraphs.

The results of experiment I, II and III showed that initial cadmium uptake could be distinguished from the slower uptake phase.

Assuming that the first, fast process has reached the equilibrium state before the first sampling time (0.12 h),  $[CdA]_{F,t}$  becomes a constant value.

The dissociation constant  $K_R$  for the fast process can be determined from experiment III, where the cadmium uptake remained constant after 0.17 h, indicating that only the fast process occurred. Using the mean **CdA** concentrations for each  $[Cd]_t$  at 0° C, the  $K_{R,F}$  for the fast process was determined using equation 4:

$$[CdA]_{T,t} = [Cd]_t \cdot [A]_{F,0} / (K_{R,F} + [Cd]_t).$$

The  $K_{R,F}$  for the fast process from experiment III was  $(5.4 \pm 0.8) \cdot 10^{-6} \text{ mol.L}^{-1}$ .

The  $K_{R,F}$  for the fast process can also be estimated from experiment I and II, using the **CdA** concentrations at the first sampling times. Since these values are comparable to the mean **CdA** concentrations from experiment III, comparable values of  $K_{R,F}$  are obtained for the fast process from experiment I and II  $(5.9 \pm 0.5) \cdot 10^{-6} \text{ mol.L}^{-1}$  and  $(3.5 \pm 0.4) \cdot 10^{-6} \text{ mol.L}^{-1}$ , respectively.

Previously (Figure 8-3A to 8-3D) it was shown that the equilibrium level for the slow process was not reached after 6 h for the highest cadmium concentration in both experiment I and II. Furthermore, the equilibrium level in the slow process was not reached after 30 h in experiment II in the batches 2 and 3 with lower  $\text{Cd}_f$  concentrations. Therefore  $K_{R,S}$  for the slow process has to be determined from the mean  $[\text{CdA}]_{S,t}$  values from the 40 - 50 h interval, in which the  $\text{CdA}_f$  concentrations have become the equilibrium concentrations in both experiments. The  $K_{R,S}$  for the slow process was determined using equations 5 and 8 for  $t = 40 - 50 \text{ h}$ :

$$[\text{CdA}]_{T,t} = [\text{CdA}]_{F,t} + \frac{[\text{Cd}]_f \cdot [\text{A}]_{S,0}}{K_{R,S} + [\text{Cd}]_f} \quad (9)$$

There are two different possibilities for inserting the value of  $[\text{CdA}]_{F,t}$ , being a constant for  $t > 0.12 \text{ h}$ , namely

- 1) the fitted value for  $[\text{CdA}]_{F,t}$  from equation 9 (for  $t = 40 - 50 \text{ h}$ ) and
- 2) the **CdA** concentrations of the first sampling time ( $t = 1$ ) at  $21^\circ \text{C}$  (Table 8-I).

The fitted value from equation 9 yielded large standard deviations of  $[\text{CdA}]_{F,t}$ , namely 80% in experiment I and 780% in experiment II, due to the large range of the four  $\text{Cd}_f$  concentrations ( $10^{-9}$  to  $10^{-3} \text{ mol.L}^{-1}$ ). The fit ( $R^2$ ) was 0.994. The values for  $K_{R,S}$  for both experiments were not different within the error margins, namely  $(5.1 \pm 0.5) \cdot 10^{-6} \text{ mol.L}^{-1}$  for experiment I and  $(3.9 \pm 3.4) \cdot 10^{-6} \text{ mol.L}^{-1}$  for experiment II.

Using the **CdA** concentrations of the first sampling time in experiment I and II for each  $[\text{Cd}]_f$  as values for  $[\text{CdA}]_{F,t}$ , yielded the best results for  $K_{R,S}$ . For experiment I, the  $K_{R,S}$  was  $(4.6 \pm 0.6) \cdot 10^{-6} \text{ mol.L}^{-1}$  and for experiment II, the  $K_{R,S}$  was  $(4.6 \pm 0.3) \cdot 10^{-6} \text{ mol.L}^{-1}$ . The fit ( $R^2$ ) was 0.999.

The dissociation constants  $K_R$  of the cadmium-algae complex for the slow process of both experiments appeared to be constant ( $4.6 \cdot 10^{-6} \text{ mol.L}^{-1}$ ) under the same experimental conditions in which the biomass of the algae and the free cadmium concentrations were variables. These results indicate that it was possible with the experimental method to reproduce the dissociation constant for the slow process with different experiments, using the equilibrium model.

In other experiments, with another algae species and/or other medium, light conditions, temperature, aeration conditions, the dissociation and formation constant of the cadmium-algae complexes will be different, making comparisons to these values in literature quite difficult. Geisweid and Urbach (1983) reported binding constants (at  $21^\circ \text{C}$ ) of cadmium for the fast process for three green algae species (*Chlorella vulgaris*, *Ankistrodesmus braunii* and *Eremosphaera viridis*), calculated from the Langmuir isotherm, which were  $2 \cdot 10^{-5}$ ,  $2 \cdot 10^{-6}$  and  $1 \cdot 10^{-5} \text{ mol.L}^{-1}$ , respectively. The dissociation constants of the present study for the fast and the slow process were comparable to the values for the binding constants for the fast process, reported by Geisweid and Urbach (1983). Ting *et al.* (1989) reported an adsorption constant of cadmium for the fast process for *Chlorella vulgaris* at  $25^\circ \text{C}$  of  $3.74 \cdot 10^{-7} \text{ mol.L}^{-1}$ , which is lower than the  $K_{R,F}$  of the present study by a factor of approximately 15.

For the slow process, the kinetic parameters  $k_1$  and  $k_2$  can be calculated using the equations 7 and 8, for the normalized data set:

$$[\text{CdA}]_{T,t} = [\text{CdA}]_{F,t} + \frac{k_1 \cdot [\text{Cd}]_f \cdot [\text{A}]_{S,0}}{\mu + k_2 + k_1 \cdot [\text{Cd}]_f} \cdot (1 - e^{-(\mu + k_2 + k_1 \cdot [\text{Cd}]_f) \cdot t}) \quad (10)$$

Equation 10 is in the form of the function  $[\text{CdA}]_{T,t} = [\text{CdA}]_{F,t} + a \cdot (1 - e^{-b \cdot t})$  where  $a = k_1 \cdot [\text{Cd}]_f \cdot [\text{A}]_{S,0} / (\mu + k_2 + k_1 \cdot [\text{Cd}]_f)$  and

$$b = \mu + k_2 + k_1 \cdot [\text{Cd}]_f$$

As mentioned above, the value of  $[\text{CdA}]_{F,t}$  can be chosen as the fitted value from equation 10 or the **CdA** concentrations of the first sampling time at  $21^\circ \text{C}$ .

The fitted values for  $[\text{CdA}]_{F,t}$  ( $8.1 \cdot 10^{-6}$  and  $3.5 \cdot 10^{-6} \text{ mol.L}^{-1}$ , respectively for

experiment I and II) are comparable to the values of the **CdA** concentrations of the first sampling time at 21° C (Table 8-III).

Using  $K_{R,S} = 4.6 \cdot 10^{-6} \text{ mol.L}^{-1}$ , the parameter  $k_1$  can be calculated from  $b = \mu + k_1 \cdot (K_R + [Cd]_f)$ , since  $K_R = k_2 / k_1$ .

Application of the model to the experimental data for the growing algae cultures in the batches 2, 3 and 4, resulted in very poor fits ( $R^2$  varied from 0.03 to 0.5). With these fit results, it is not meaningful to quantify the uptake rate constant ( $k_1$ ) and the release rate constant ( $k_2$ ), since both will be accompanied by errors over 100%. However, the results indicated that a slower uptake phase was present in the batches 2, 3 and 4, but the kinetic parameters could not be quantified.

For the batches 1 in experiment I and II, with the highest  $[Cd]_f$  ( $10^{-3} \text{ M}$ ), the uptake- and release rate constants could be calculated. For these cultures the growth rate ( $\mu$ ) was zero. Figure 8-5 shows the fit results for cadmium uptake in experiment I and II at the highest  $[Cd]_f$  ( $10^{-3} \text{ M}$ ), whereby  $[CdA]_{E,1}$  was fitted.

In Table 8-III the kinetic parameters  $k_1$  and  $k_2$  for the slow process of experiment I and II are given for the possible values for  $[CdA]_{E,1}$ . The model fitted well to the experimental data of batch 1 in both experiment I and II (Table 8-III).

In experiment I,  $k_1$  was  $134 \pm 34 \text{ L.mol}^{-1}.\text{h}^{-1}$  and  $k_2$  was  $(6.2 \pm 1.7) \cdot 10^{-4} \text{ h}^{-1}$ . In experiment II,  $k_1$  was  $87 \pm 16 \text{ L.mol}^{-1}.\text{h}^{-1}$  and  $k_2$  was  $(4.0 \pm 0.9) \cdot 10^{-4} \text{ h}^{-1}$ . The values of  $k_1$  and  $k_2$ , respectively originating from both experiments do not differ significantly within the error margins. However, real differences in the experimental conditions cannot be excluded. Literature data on k-values suitable for comparison have not been found.

Knowing the values of  $k_1$  and  $k_2$ , the contribution of the slow process at the first sampling time with respect to the equilibrium level of the slow process can be calculated, using the term  $(1 - \exp(-\mu - k_2 - k_1[Cd]_f \cdot t))$  from equation 10. For the highest  $Cd_f$  concentration, the cadmium uptake in the fast process is in the order of 40% of the final equilibrium level (Figure 8-3A), thus the cadmium uptake in the slow process is in the order of 60% of the final (measured) equilibrium level.

TABLE 8-III. Values of  $[\text{CdA}]_{\text{F},t}$  and the uptake-and release rate constants for the batches 1 with the highest  $[\text{Cd}]_t$  in experiment I and II.

| Exp. | $[\text{CdA}]_{\text{F},t}$<br>$\text{mol.L}^{-1}$ | $k_1$<br>$\text{L.mol}^{-1}.\text{h}^{-1}$ | $k_2$<br>$\text{h}^{-1}$      | fit<br>$R^2$ |
|------|--|--|-------------------------------|--------------|
| I    | $[\text{CdA}]_{0.12\text{ h}} (9.9 \cdot 10^{-6})$ | $112 \pm 25$                               | $(5.2 \pm 1.4) \cdot 10^{-4}$ | 0.958        |
| II   | $[\text{CdA}]_{0.17\text{ h}} (3.9 \cdot 10^{-6})$ | $66 \pm 14$                                | $(3.1 \pm 0.7) \cdot 10^{-4}$ | 0.982        |
| I    | fitted $(8.1 \cdot 10^{-6})$                       | $134 \pm 34$                               | $(6.2 \pm 1.7) \cdot 10^{-4}$ | 0.967        |
| II   | fitted $(3.5 \cdot 10^{-6})$                       | $87 \pm 16$                                | $(4.0 \pm 0.9) \cdot 10^{-4}$ | 0.988        |

For example, in experiment I, at  $t = 0.12\text{ h}$ , 1.8% of the equilibrium concentration of the slow process was reached. This amount corresponds to a contribution of 1.1% of  $[\text{CdA}]_{\text{T}, t = 40-50\text{ h}}$ , or a contribution of 3% of the cadmium uptake of the fast process. These results indicate that the contribution of the slow process at  $t = 0.12\text{ h}$  is indeed negligible compared to contribution of the fast process at  $t = 0.12\text{ h}$ .

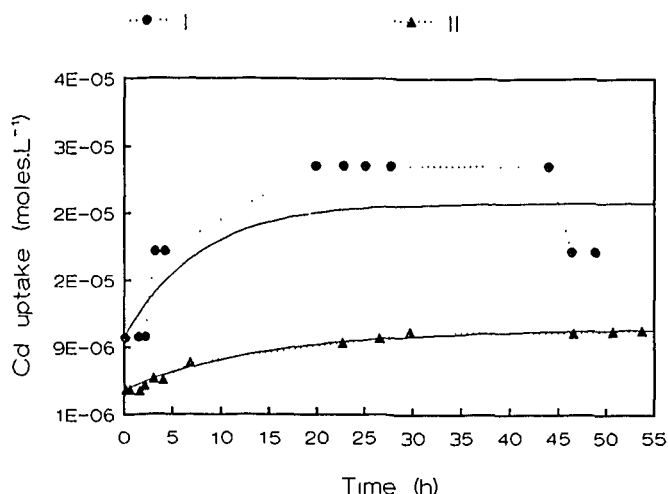


Figure 8-5. Cadmium uptake at  $21^\circ\text{C}$  in experiment I (circles) and II (triangles) for the highest free cadmium concentrations ( $1.15 \cdot 10^{-3}\text{ M}$  and  $9.0 \cdot 10^{-4}\text{ M}$ , respectively).

## 8.5 Concluding remarks

The cadmium uptake in algae at 21° C showed a rapid first phase and a slower second phase. At 0° C the cadmium uptake also showed the rapid first phase, but no second phase was observed. Apparently, two different processes are involved in cadmium uptake. A first, rather temperature independent process, probably passive, reaches an equilibrium concentration after a few seconds or minutes. The second, a temperature dependent process, possibly physiological, reaches an equilibrium concentration after 40 h. The equilibrium concentration of the cadmium-algae complex of the fast process is in the order of 40% of the final equilibrium concentration of the complex at  $t = 40 - 50$  h. The contribution of the slow process at  $t = 0.12$  h is negligible compared to the contribution of the fast process at this time.

With the experimental method used, the dissociation constants for the fast and the slow process could be reproduced with different experiments. The value of  $K_{R, F}$  for the fast process was comparable to the value of  $K_{R, S}$  for the slow process. Using the equilibrium model the kinetic parameters could be quantified. The values of the uptake- and release rate constants for experiment I and II do not differ within the error margins.

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## CHAPTER 9. GENERAL CONCLUSIONS

In this chapter main conclusions are presented on the applicability of epiphytic lichens as biomonitors for trace-element air pollution, resulting from the experiments described.

### 1. Geographical and temporal concentration patterns of trace-elements in lichens

Strong indications were obtained that the trace-element levels in *Parmelia sulcata* originate primarily from wet and dry deposition and not from the soil through the supporting tree.

On a national scale, geographical trace-element patterns in *Parmelia sulcata* generally agreed with the patterns as derived from a dispersion model applicable for The Netherlands and measured data on concentrations in air particulate matter and depositions. Within a 5-year period the geographical trace-element patterns in lichens followed a trend of changing patterns of concentrations in air particulate matter and depositions.

On a local scale, the relative variation of trace-element concentrations in lichens was in the order of 25%, comprising the effects of different substrate tree species, the age of the samples and individual (genetic and morphological) variation in lichen specimens. The concentration patterns on a national scale were not disturbed significantly by these local variations, because the range of the element concentrations on a local scale was an order of magnitude lower than the range of concentrations on a national scale.

The cesium-137 activity in the atmosphere as a result of the Chernobyl accident, was also reflected by lichens. The geographical pattern of  $^{137}\text{Cs}$  in lichens and in total (wet and dry) deposition were similar.

Combination of the results from the studies on the geographical patterns of trace elements and  $^{137}\text{Cs}$  activity in lichens leads to the conclusion that lichens reflect at least in a qualitative way concentrations in air particulate matter and depositions and  $^{137}\text{Cs}$  activity.

Two tolerant lichen species, *Parmelia sulcata* and *Lecanora conizaeoides*, as

measured in The Kempen area showed comparable mean element concentrations. However, the concentration ratios were dependent on the concentration levels and indications were obtained for saturation effects in both species for different elements. Only for a few elements a linear relationship between the element concentrations in both lichen species was found, allowing a concentration independent interspecies calibration.

## **2. Pattern recognition in lichens**

The concentration patterns of aluminum, antimony, arsenic, bromine, cadmium, cesium, chromium, cobalt, iron, mercury, lanthanum, lead, manganese, nickel, scandium, selenium, thorium, tungsten, vanadium, and zinc in *Parmelia sulcata* from 210 sampling sites spread over The Netherlands, could be used for source apportionment. Application of Monte Carlo-assisted factor analysis to these trace-element patterns in lichens resulted in 10 factors, of which most could be interpreted in terms of emission sources, such as oil combustion, zinc industry, soil, waste incineration, coal combustion, electronic industry and high temperature processes such as iron and ferroalloy industry. The reliability of the factor solution could be estimated by the Monte Carlo technique, using the known uncertainties in the lichen data set (local variations). Generally, the source apportionment was supported by the geographical distributions of the factor contributions.

## **3. Quantitative biomonitoring**

For cobalt, scandium and zinc, the concentrations in *Parmelia sulcata* transplants, exposed for one year at different stations spread over the country, were significantly correlated with the annual total deposition, but not with the annual mean atmospheric concentrations in air particulate matter, indicating that lichens tend to reflect the total deposition rather than air particulate matter.

Lichens are good quantitative monitors for the total deposition of cobalt, scandium and zinc. The accumulation factors in lichens were element specific. The accumulation factors were in the proportion of about 5 : 2 : 1 for scandium, cobalt

and zinc, respectively, indicating that the accumulation of an element by the monitor is related to its chemical properties and the properties of the associated particles (size, composition, solubility).

#### 4. Aspects of uptake-and release mechanisms of trace elements

For cobalt, zinc and scandium, the ratios between the accumulation factors in *Parmelia sulcata* and in synthetic rag, exposed at the same locations, were comparable, indicating that mainly passive processes were involved in the uptake-and release mechanisms, such as particulate trapping, ion-exchange, adsorption and/or desorption.

The fact that a  $^{137}\text{Cs}$  efflux from lichens was observed, after a short term  $^{137}\text{Cs}$  influx in lichens as a result of the Chernobyl accident, led to the assumption that the element concentrations in lichens reach a state of equilibrium with atmospheric conditions. This assumption was also supported by the fact that the cobalt and zinc concentrations in *Parmelia sulcata* transplants from a reference area, exposed for one year at different stations, adapted their starting concentrations to element levels corresponding with those in *Parmelia sulcata* growing *in situ*, exposed for many years.

Lichens are composed of an algae symbiont (for approximately 10%) and a fungal symbiont (for approximately 90%), which are together responsible for the accumulation characteristics of the whole plant. In a laboratory study on the uptake kinetics of cadmium in a green algae species, the cadmium concentration in algae was a factor 120-200 higher than the free cadmium concentration in the solution. The high accumulation capacity for cadmium suggested that algae play an important role in the uptake of cadmium and similarly behaving elements by lichens. In the cadmium uptake by algae two processes were involved. The first process is fast (within 10 min.), highly temperature independent and probably passive. In the second, slower process, the concentrations of the cadmium-algae complexes at different free cadmium concentrations reached an equilibrium state after 40 - 50 h. The second process is temperature dependent and is therefore likely to be physiological.

Lichens are able to accumulate  $^{137}\text{Cs}$  within four days from atmospheric input,

but retain  $^{137}\text{Cs}$  for more than two years. On the other hand, at least one year is required for lichens to accumulate a significantly higher amount of cobalt and zinc than the relatively high starting concentrations of these elements in the lichens obtained from a reference area in The Netherlands. However, within a 5-year period, lichens are able to reflect changes of increasing and decreasing atmospheric concentration levels and their geographical distribution.

Combination of the results from the study on algae and the field experiments with lichens leads to the conclusion that the uptake- and release processes in algae are much faster than those in lichens observed, indicating that other processes and/or factors determine the rate of the uptake- and release processes in lichens, such as the chemical characteristics of the elements, the chemical composition and size of the particles associated with elements, one or both symbionts involved, and the influence of other elements present in the lichen.

## **5. The value of biomonitoring**

For the lichen species, elements and conditions as described, the central conclusions of this thesis are:

- Trace-element concentrations in lichens reflect at least qualitatively atmospheric trace-element concentrations and depositions. Lichens reflect also  $^{137}\text{Cs}$  activity, resulting from the Chernobyl accident, in total (wet and dry) deposition;
- Trace-element concentration patterns in lichens provide information on the types of sources and localization of local sources;
- Lichens are quantitative monitors for trace elements in the total deposition rather than amounts present in air particulate matter. The element specific accumulation factors are related to the chemical properties of the elements and the properties of the associated particles (size, composition, solubility);
- The establishment of an equilibrium state between trace-element concentrations in lichens and atmospheric element levels occurs in the order of years. Therefore, lichens reflect atmospheric element levels averaged over a certain period of time, rather than instantaneous atmospheric conditions. In

- the uptake- and release mechanisms mainly passive processes are involved, such as particulate trapping, ion-exchange, adsorption and/or desorption;
- Algae have a high accumulation capacity for cadmium, indicating that algae play an important role in the accumulation of cadmium and similarly behaving elements in lichens.

Next to emission measurements and modelling, biomonitoring using lichens is a valuable receptor-based approach to contribute to the assessment of atmospheric pollution levels and the origin of responsible sources and may therefore be supportive for environmental policy.



## SUMMARY

In this thesis several experiments have been described on the applicability of lichens as biomonitors for trace-element air pollution. The aim of the thesis was to gain knowledge about the relationships between trace-element concentrations in lichens and concentrations in air particulate matter and/or depositions of trace elements on a regional and national scale, and about the processes involved in the element uptake- and release by lichens.

Chapter 1 describes the possibilities to study trace-element air pollution in order to get insight in the character and element levels of such pollution. This information is useful for environmental policy. There are three ways to study trace-element air pollution, namely measuring the emission, modelling, or measuring the immission at the receptor side. Measurements in the field (immission) are needed to verify dispersion models. Immission measurements have to be carried out on a large scale for a long time in order to guarantee the spatial and temporal representativeness of the measurements. Biomonitoring is an experimental method to measure the response of organisms to air pollution. From literature it is known that the element concentrations in lichens decrease with the distance of the source. Several studies with different lichen species have been published, which describe the dispersion of air particulate matter and deposition around a variety of sources, such as metal producing industries and roads. From literature it is known that lichens may be a valuable tool to get insight in high or low levels of trace-element air pollution. Specific questions, formulated in the appropriate chapters, are raised and discussed in the context of literature results. These questions comprise trace-element concentration patterns in lichens (geographical distributions, temporal changes as well as fingerprints of source characteristics), source apportionment, interspecies calibration, substrate influence, and qualitative and quantitative relations between element concentrations in lichens and air particulate matter and/or total deposition. Also questions about the processes involved in the element uptake- and release by lichens are presented in this chapter.

Chapter 2 describes two monitoring surveys using *Parmelia sulcata* Taylor on a national scale, in which spatial and temporal concentration patterns of antimony;-



arsenic, bromine, cadmium, chromium, iron, mercury, lanthanum, lead, nickel, selenium, tungsten, vanadium, and zinc were investigated. The surveys were carried out in 1982-1983 at 110 sampling sites and in 1986-1987 at 210 sampling sites.

Comparison of both data sets showed that the areas with high concentration classes expanded for most elements, whereas the mean element concentrations remained similar. For a few elements (bromine and mercury) a decrease of areas with enhanced element levels was observed. The geographical concentration patterns obtained from the 1982-1983 lichen data set generally agreed with the patterns as derived from a dispersion model applicable for The Netherlands and measured data on concentrations in air particulate matter and depositions. The trace-element concentration pattern obtained from the 1986-1987 lichen data set showed temporal and geographical changes compared to those from the 1982-1983 lichen data set. Though a complete data set on air particulate matter and total deposition in 1986-1987 was not available, the change of the element gradients obtained from measured wet deposition from 1983 to 1987 indicate that atmospheric levels changed within 5 years. The concentration patterns in lichens on a national scale were not disturbed significantly by local variations, because the range of the element concentrations on a local scale was an order of magnitude lower than the range of concentrations on a national scale. From these studies it was concluded that lichens are at least good qualitative biomonitors for atmospheric trace-element levels.

Chapter 3 describes the response of lichens to the cesium-137 activity as a result of the Chernobyl accident, deposited by rainfall in The Netherlands. The geographical distribution of cesium-137 activity in lichens was similar to that of total (wet and dry) deposition of this radionuclide. From this study it was concluded that lichens are good biomonitors for atmospheric cesium-137 activity<sup>too</sup>. The averaged retention time of this radionuclide in lichens, after uptake, appeared to be longer than two years.

Chapter 4 describes the application of factor analysis to a lichen data set from a monitoring survey on a national scale (1986-1987), for source apportionment. The concentration patterns of 20 trace elements in lichens, based on 210 sampling sites, gave rise to the identification of several emission sources, such as oil combustion, zinc industry, soil, waste incineration, coal combustion, electronic industry and high

temperature processes such as iron and ferroalloys industry. The elements under study were aluminum, antimony, arsenic, bromine, cadmium, cesium, chromium, cobalt, iron, mercury, lanthanum, lead, manganese, nickel, scandium, selenium, thorium, tungsten, vanadium and zinc. This chapter also describes how the reliability of the factor solution was estimated.

In Chapter 5 a field study is described on the contribution of a possible influence from the soil to element concentrations in *Parmelia sulcata* Taylor growing on trees in an area with polluted soil (The Broekpolder). From the analytical results for lichens, bark and tree rings from the supporting trees, it was concluded that for arsenic, chromium, cobalt, copper, iron, mercury, scandium and vanadium, the levels in lichens originate only from wet and dry deposition and not from the soil through the supporting tree. For cadmium, manganese and zinc, this field study showed that besides the atmospheric uptake, a contribution to the element contents in lichens from the substrate bark might be possible.

Chapter 6 describes a field study on the interchangeability of two tolerant lichen species (*Parmelia sulcata* Taylor and *Lecanora conizaeoides* Nyl.) in a polluted area (The Kempen area). The mean concentrations of antimony, arsenic, bromine, cadmium, chromium, cobalt, copper, iron, lanthanum, selenium, tungsten, vanadium and zinc were of comparable magnitude. Only for arsenic, cobalt and zinc it was possible to determine a linear relationship between the concentrations in both species, allowing a concentration-independent interspecies calibration. For the other elements saturation effects in one of the two species were indicated.

In Chapter 7 a field study is described in which the quantitative relationships between concentrations of cobalt, scandium and zinc in lichens and concentrations in air particulate matter and total deposition (wet and dry) were investigated. From the results obtained from lichens, exposed for one year at 8 stations spread over the country, it appeared that lichens are good biomonitors for the local total deposition, but not for concentrations in air particulate matter. The linear relationships between the concentrations of cobalt, scandium and zinc in lichens and total deposition were quantified by means of accumulation factors. The accumulation factors were in the proportion of about 5 : 2 : 1 for scandium, cobalt and zinc, respectively. These results indicated that the quantitative relationships between concentrations in lichens and

total deposition is related to the chemical properties of the elements and the properties of the associated particles (size, composition, solubility). Next to the lichen transplants, impregnated cloth (rag) samples were exposed at the same stations, in order to get insight in the uptake- and release mechanisms by comparing the responses of rag and lichens. For cobalt, scandium and zinc the ratios of the accumulation factors in lichens to those in rag were comparable, indicating that mainly passive processes were involved in the uptake mechanisms, such as particulate trapping, ion-exchange, adsorption and/or desorption.

Chapter 8 describes a laboratory study on the kinetics of the uptake-and release of cadmium in a green algae species (*Selenastrum capricornutum* Printz), which is regarded to be representative for the algal symbiont in the lichens used in this thesis. From the high accumulation capacity of the algae it was concluded that algae may play an important role in the uptake of cadmium and related elements in lichens. The uptake-and release rate constants were quantified by means of a metal-ligand complex formation analogous model. It was concluded, that two processes were involved in the cadmium uptake. The first process is fast (within 10 min.), highly temperature independent and therefore probably passive. In the second, slower process, the concentrations of the cadmium-algae complexes reached an equilibrium state after approximately 50 h at different free Cd concentrations. The second process is temperature dependent and is therefore likely to be physiological.

Chapter 9 presents the central conclusions of this thesis for the lichen species, elements and conditions under study.

- Trace-element concentrations in lichens reflect at least qualitatively atmospheric trace-element concentrations and depositions. Lichens reflect also cesium-137 activity, resulting from the Chernobyl accident, in total (wet and dry) deposition.
- Trace-element concentration patterns in lichens provide information on the types of sources and localization of local sources.
- Lichens are quantitative monitors for trace elements in the total deposition rather than for the amounts present in air particulate matter. The element specific accumulation factors, are related to the chemical properties of the elements and the properties of the associated particles (size, composition,

solubility).

- The establishment of an equilibrium state between trace-element concentrations in lichens and atmospheric element levels occurs in the order of years. Therefore, lichens reflect atmospheric element levels averaged over a certain period of time, rather than instantaneous atmospheric conditions. In the uptake- and release mechanisms mainly passive processes are involved, such as particulate trapping, ion-exchange, adsorption and/or desorption.
- Algae have a high accumulation capacity for cadmium, indicating that algae may play an important role in the accumulation of cadmium and similarly behaving elements in lichens.

In this thesis selected aspects of the applicability of lichens as biomonitors for the determination of atmospheric pollution levels and the origin of responsible sources have been studied. Next to emission measurements and modelling, biomonitoring using lichens may be supportive for environmental policy.



## SAMENVATTING

In dit proefschrift wordt een aantal experimenten beschreven betreffende de toepasbaarheid van korstmossen als biomonitoren voor luchtverontreiniging met zware metalen en andere spooorelementen. Het doel van het onderzoek is inzicht te verkrijgen in de relatie tussen spooorelementconcentraties in korstmossen en atmosferische concentraties en/of deposities van spooorelementen op regionale en nationale schaal en de processen die een rol spelen bij de opname en afgifte van spooorelementen in korstmossen.

In hoofdstuk 1 worden de mogelijkheden voor onderzoek naar luchtverontreiniging met spooorelementen uiteengezet, om inzicht in de aard en de omvang van luchtverontreiniging met deze stoffen te verkrijgen. Deze informatie is van belang voor het milieubeleid. Er zijn drie mogelijke benaderingen: metingen aan de emissie, modellering van de immissie en metingen van de immissie. Metingen in het veld (immissie) zijn nodig om de modellen te verifiëren. Immissiemetingen moeten op grote schaal en gedurende lange tijd worden uitgevoerd om de representativiteit van de metingen in ruimte en tijd te garanderen. Biomonitoring is een experimentele methode om de respons van organismen op milieuverontreiniging te gebruiken als maat voor die verontreiniging. Uit de literatuur is bekend dat de elementconcentraties in korstmossen afnemen met de afstand van de bron. Verschillende studies met verschillende korstmossoorten zijn gepubliceerd, die de verspreiding van luchtstofemissies beschrijven rondom verschillende soorten van bronnen, zoals metaal- producerende industrieën en snelwegen. Uit de literatuur is gebleken dat korstmossen een waardevol instrument kunnen zijn bij het verkrijgen van inzicht omtrent de hoogte van de niveaus van luchtverontreiniging. Specifieke vragen, die in de beschreven experimenten onderzocht zijn, worden gepresenteerd tegen de achtergrond van wat er in de literatuur over bekend is. Dit betreft vragen over patronen van spooorelementconcentraties in korstmossen (zowel de geografische verspreiding en tijdsafhankelijke veranderingen van de concentraties per element, alsook de combinatie van verschillende elementconcentraties per monsterpunt), brontoewijzing, uitwisselbaarheid van soorten, substraatinvloed, kwalitatieve en kwantitatieve relaties tussen spooorelementen in korstmossen en in luchtstof en/of

totale (droge en natte) depositie. Tevens worden vragen over de aard van de processen die een rol spelen bij de opname en afgifte van spooorelementen in korstmossen aan de orde gesteld.

In hoofdstuk 2 worden twee veldstudies op nationale schaal aan geografische en tijdsafhankelijke concentratiepatronen van antimoon, arseen, broom, cadmium, chroom, ijzer, kwik, lanthaan, lood, nikkel, seleen, vanadium, wolfram en zink in korstmossen beschreven. De studies werden uitgevoerd in 1982-1983 en 1986-1987. Het aantal monsterpunten bedroeg in de eerste studie 110, in de tweede 210. Uit vergelijking van de resultaten van beide studies is gebleken, dat de gemiddelde concentraties voor de meeste spooorelementen gelijk zijn gebleven, maar dat de gebieden met hoge concentraties zich hebben uitgebreid. Voor een paar elementen (broom en kwik) is echter een afname van gebieden met hoge concentraties waargenomen. De verspreidingspatronen op nationale schaal in korstmossen bleken niet verstoord te worden door variaties in concentraties op lokale schaal, omdat deze laatste verhoudingsgewijs slechts van beperkte omvang zijn. De concentratiepatronen in korstmossen, verzameld in 1982-1983, bleken goed overeen te komen met concentratiepatronen geschat op basis van een verspreidingsmodel dat toepasbaar is voor Nederland. Uit de beschikbare gegevens over atmosferische concentraties en deposities voor 1987 is gebleken, dat de verspreidingspatronen van de natte depositie van enkele spooorelementen een vergelijkbare trend vertonen als in korstmossen. Uit deze studies is geconcludeerd dat korstmossen op zijn minst goede kwalitatieve biomonitoren zijn voor luchtverontreiniging van spooorelementen.

Hoofdstuk 3 beschrijft de respons van korstmossen op de cesium-137 activiteit, afkomstig van het Tsjernobyl ongeval en door regenval in Nederland gedeponneerd. De geografische verspreiding van de cesium-137 activiteit in korstmossen bleken goed overeen te komen met de geschatte en gemeten cesium-137 activiteit in de totale (droge en natte) depositie in Nederland. Uit deze studie is geconcludeerd dat korstmossen goede biomonitoren zijn voor cesium-137 activiteit in de totale depositie. De gemiddelde verblijftijd van dit radionuclide in de korstmos na opname, bleek meer dan twee jaar te bedragen.

Hoofdstuk 4 geeft de toepassing van een statistische methode (factor analyse) op concentratiepatronen van spooorelementen in korstmossen (*Parmelia sulcata*

*Taylor*), die in 1986-1987 op nationale schaal zijn verzameld, om de aard van emissiebronnen vast te stellen. Uit de concentratiepatronen van 20 spoorelementen in korstmossen, afkomstig van 210 monterpunten, zijn een aantal bronnen geïdentificeerd, zoals oliestook, ijzer- en staalindustrie, grondbijdrage, zinkindustrie, steenkoolverbranding, elektronische industrie en vuilverbranding. De in dit onderzoek betrokken elementen zijn aluminium, antimoon, arseen, broom, cadmium, cesium, chroom, cobalt, ijzer, kwik, lanthaan, lood, mangaan, nikkel, scandium, seleen, thorium, vanadium, wolfram en zink. In dit hoofdstuk wordt tevens beschreven hoe de betrouwbaarheid van de factoroplossing is bepaald.

In hoofdstuk 5 wordt een veldstudie beschreven over de omvang van de eventuele bijdrage van de bodem aan elementconcentraties in korstmossen (*Parmelia sulcata Taylor*) op bomen in een gebied waar de bodem sterk verontreinigd is (Broekpolder). Uit analyseresultaten van korstmossen, boombasten en hout-jaarringen van de betreffende bomen is geconcludeerd, dat de concentraties van arseen, chroom, cobalt, ijzer, koper, kwik, scandium en vanadium in korstmossen niet afkomstig is van opname via het substraat uit de bodem. Uit deze studie kon voor cadmium, mangaan en zink een bijdrage van de boom en/of bodem aan de concentraties in korstmossen niet worden uitgesloten.

In hoofdstuk 6 wordt de uitwisselbaarheid van twee tolerante korstmossoorten (*Parmelia sulcata Taylor* en *Lecanora conizaeoides Nyl.*) beschreven in een gebied met een hoge graad van luchtverontreiniging (De Kempen). De gemiddelde concentraties van 13 spoorelementen bleken in de twee korstmossoorten vergelijkbaar te zijn. De onderzochte elementen zijn antimoon, arseen, broom, cadmium, chroom, cobalt, ijzer, koper, lanthaan, seleen, vanadium, wolfram en zink. Alleen voor arseen, cobalt en zink is het echter mogelijk gebleken een significante lineaire relatie tussen de concentraties in beide korstmossoorten vast te stellen, die een concentratie-onafhankelijke calibratie tussen beide soorten mogelijk maakt. Voor de overige elementen zijn er aanwijzingen, dat er verzadiging is opgetreden in een van beide soorten.

In hoofdstuk 7 wordt een veldstudie beschreven waarin de kwantitatieve relaties tussen concentraties van cobalt, scandium en zink in korstmossen (*Parmelia sulcata Taylor*) en die in luchtstof en totale (droge en natte) depositie van deze



elementen zijn onderzocht. Uit de resultaten verkregen met korstmossen, gedurende een jaar blootgesteld aan de atmosfeer op acht lokaties verspreid over Nederland, is gebleken dat deze korstmossen wel goede kwantitatieve biomonitoren zijn voor de plaatselijke totale depositie, maar niet voor de atmosferische concentraties van elementen aanwezig in luchtstof. De kwantitatieve relaties tussen cobalt, scandium en zink concentraties in korstmossen en de totale depositie van deze elementen zijn uitgedrukt in accumulatiefactoren. De accumulatiefactoren bleken niet gelijk te zijn, maar zich te verhouden als 5 : 2 : 1, respectievelijk voor scandium, cobalt en zink. Hieruit is geconcludeerd, dat de kwantitatieve relatie tussen concentraties in korstmossen en de totale depositie gerelateerd is aan de eigenschappen van de elementen en de geassocieerde deeltjes (grootte, samenstelling en oplosbaarheid). Naast de korstmossen, zijn met lijm geïmpregneerde doekjes op dezelfde lokaties, gedurende dezelfde tijd blootgesteld aan de atmosfeer, om via vergelijking inzicht te verkrijgen in het opname- en afgifte mechanisme van spoorelementen in korstmossen. Voor cobalt, scandium en zink zijn de verhoudingen van de accumulatie factoren in korstmossen ten opzichte van die in doekjes ongeveer gelijk, waaruit geconcludeerd is, dat voornamelijk passieve processen een rol spelen bij het opname mechanisme, zoals invangst van vaste deeltjes, adsorptie/desorptie en ionen uitwisseling.

In hoofdstuk 8 wordt een laboratoriumstudie beschreven betreffende kinetiek van de opname en afgifte van cadmium in een groene algensoort, die representatief wordt geacht voor de algcomponent in de korstmossen die in dit proefschrift zijn genoemd. De algen zijn in staat cadmium in hoge mate te accumuleren, hetgeen wellicht betekent dat algen een belangrijke rol spelen in het accumulatie gedrag van korstmossen voor cadmium en verwante elementen. De snelheidsconstanten van cadmium opname-en afgifte zijn gekwantificeerd met behulp van een model, analoog aan een algemeen model voor metaal-ligandcomplex vorming. Uit deze studie is geconcludeerd, dat er twee processen betrokken zijn bij de opname van cadmium. Het eerste proces speelt zich af in de eerst tien minuten, is vrijwel temperatuur ongevoelig en daarom waarschijnlijk passief van aard. In het tweede, langzamer proces, bereiken de cadmium-algen complexen bij verschillende vrije cadmium concentraties een evenwichtstoestand na ongeveer 50 uur. Dit langzamer proces is sterk gevoelig voor een temperatuurverandering van 0° C naar 21° C en daardoor

wellicht fysiologisch van aard.

In hoofdstuk 9 worden de belangrijkste conclusies uit de resultaten van de experimenten gepresenteerd. De waarde van biomonitoring met korstmossen blijkt uit de vijf kernpunten die in dit proefschrift zijn beschreven, namelijk

- de korstmossen weerspiegelen kwalitatief atmosferische concentraties en deposities van spooelementen. Korstmossen reflecteren ook de activiteit van het splijtingsprodukt cesium-137, afkomstig van Tsjernobyl, in de totale depositie;
- patronen van spooelementconcentraties in korstmossen geven informatie over brontypen en ligging van lokale bronnen;
- de korstmossen zijn kwantitatieve monitoren voor de totale depositie van spooelementen. De relaties tussen spooelementconcentraties van de bestudeerde elementen in korstmossen en totale depositie is verschillend en gerelateerd aan de eigenschappen van de elementen en de geassocieerde deeltjes (grootte, samenstelling en oplosbaarheid);
- de evenwichtsinstelling tussen concentraties in korstmossen en atmosferische niveaus verloopt langzaam, in de orde van jaren. Dit betekent dat korstmossen gemiddelde atmosferische concentraties en deposities weerspiegelen over een periode van een paar jaar, in plaats van momentane atmosferische condities. In de opname- en afgifte mechanismen zijn voornamelijk passieve processen betrokken, zoals invangen van vaste deeltjes, adsorptie/desorptie en ionen-uitwisseling;
- algen hebben een hoge accumulatiecapaciteit voor cadmium, hetgeen wellicht betekent dat algen een belangrijke bijdrage hebben in de opname-en afgifte-karakteristieken voor cadmium en verwante elementen in korstmossen.

In dit proefschrift zijn een aantal aspecten omtrent de bruikbaarheid van korstmossen als biomonitoren voor het bepalen van de aard en de omvang van lucht-verontreiniging met zware metalen en andere spooelementen beschreven. Naast emissiemetingen en modellering kan biomonitoring met behulp van korstmossen een bijdrage leveren aan het milieubeleid.



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## CURRICULUM VITAE

De schrijfster van dit proefschrift werd op 27 februari 1959 geboren te Enschede. Het Gymnasium  $\beta$  diploma werd in 1978 behaald aan het Kottenpark College te Enschede. In datzelfde jaar begon zij haar biologiestudie aan de Rijksuniversiteit Utrecht. In 1981 werd het kandidaatsexamen in de specialisatierichting geologie afgelegd. De doctoraalfase, opgebouwd uit de hoofdvakken vergelijkende dierfysiologie en botanische ecologie en de bijvakken milieurecht en milieukunde, werd afgesloten met het behalen van het diploma in 1985. Vanaf juli 1986 was zij als tijdelijk wetenschappelijk medewerkster verbonden aan het Interfacultair Reactor Instituut van de Technische Universiteit Delft bij de vakgroep Radiochemie, waar dit proefschrift werd bewerkt.



## APPENDIX

### Main lines of the mathematical background of factor analysis

A data set of  $n$  elemental concentrations is considered, determined at  $N$  sampling sites. The concentration of the  $j$ -th element ( $j = 1 \dots n$ ) at the  $i$ -th sampling site ( $i = 1 \dots N$ ) is denoted by  $x_{ji}$ . In order to remove the different scale of measurement for the various chemical species, the data set is transformed to the standardized variables  $z_{ji}$  by

$$z_{ji} = (x_{ji} - \bar{x}_j) / \sigma_j \quad (1)$$

with the mean of element  $j$  given by

$$\bar{x}_j = \sum_{i=1}^N x_{ji} / N \quad (2)$$

and its standard deviation by

$$\sigma_j = \left( \sum_{i=1}^N (x_{ji} - \bar{x}_j)^2 / (N-1) \right)^{1/2} \quad (3)$$

As a result, the  $z_{ji}$  have a mean of zero and a variance of unity. In the factor model the  $z_{ji}$  are assumed to be a linear sum of  $m$  common factors, with  $m \leq n$ , which account for the correlations between the variables, and a unique contribution which is specific for each individual sampling site:

$$z_{ji} = \left( \sum_{k=1}^m a_{jk} f_{ki} \right) + d_j u_{ji} \quad (4)$$

Similar to the standardized variables  $z_{ji}$ , the  $f_{ki}$  and the  $u_{ji}$  have variances of unity, giving the following relation:



$$\sum_{k=1}^m a_{jk}^2 + d_j^2 = 1 \quad (j = 1 \dots n) \quad (5)$$

The uniqueness can be determined in advance from the uncertainties in the individual elemental concentrations. From Equations (2) and (3) it follows that the total amount of variance of element  $j$ , denoted by  $V_{TOT,j}$ , is given by:

$$V_{TOT,j} = \sigma_j^2 = \sum_{i=1}^N (x_{ji} - \bar{x}_j)^2 / (N - 1) \quad (6)$$

The amount of variance due to local variations in the data set can be expressed by

$$V_{LOC,j} = \sum_{i=1}^N (\Delta x_{ji})^2 / N \quad (7)$$

where  $\Delta x_{ji}$  is the total, absolute uncertainty in the concentration  $x_{ji}$  of element  $j$  at sampling site  $i$ . Thus the uniqueness  $d_j^2$  is given by

$$d_j^2 = V_{LOC,j} / V_{TOT,j} \approx \sum_{i=1}^N (\Delta x_{ji})^2 / \sum_{i=1}^N (x_{ji} - \bar{x}_j)^2 \quad (8)$$

in which use has been made of the approximation  $(N - 1) \approx N$  for sufficiently large  $N$ .

The matrix notation for the factor model can be expressed by  $Z = AF + DU$ , where  $Z$  is an  $n \times N$  matrix with components  $z_{ji}$ ,  $A$  an  $n \times m$  matrix with components  $a_{jk}$ ,  $F$  an  $m \times N$  matrix with components  $f_{ki}$ ,  $D$  an diagonal  $n \times n$  matrix with components  $d_j$  on the diagonal and  $U$  an  $n \times N$  matrix with components  $u_{ji}$  ( $j = 1..n$  elements,  $i = 1..N$  sampling sites and  $k = 1..m$  factors).

Assuming the existence of  $m$  common factors, a first direct solution for  $A$  is obtained from the *principal factor* method, which mainly consists of the diagonalisation of the  $n \times n$  matrix  $C$  of reduced correlations between variables (here: elements), with components

$$c_{jk} = \frac{1}{N} \sum_{i=1}^N z_{ji} z_{ki} - \delta_{jk} d_j^2 \quad (9)$$

where  $\delta_{jk} = 1$  for  $j = k$  and  $\delta_{jk} = 0$  for  $j \neq k$ .

If  $Q$  is the  $n \times m$  matrix of eigenvectors of  $C$ , it can be shown that a possible solution of  $A$  is given by

$$a_{jk} = q_{jk} (\lambda_k)^{\frac{1}{2}} \quad (10)$$

satisfying the relationship

$$C = Q \Lambda Q^{-1} = A A^T \quad (11)$$

where  $\Lambda$  is the diagonal matrix of eigenvalues  $\lambda_k$  of  $C$ . It should be noted that if  $A$  is a solution of Equation (11), then every matrix  $A' = AT$ , with  $T$  an orthogonal transformation satisfying the relationship  $T = T^{-1}$ , will be a solution too.

In the Monte-Carlo approach a large number (500) of modified data sets  $X'$  are generated, with concentrations  $x'_{ji}$ , in which all element concentrations are slightly altered in a random way. The magnitudes of the normally-distributed random deviations are chosen in accordance with the uncertainties in the original element concentrations:

$$x'_{ji} = x_{ji} + \Delta x_{ji} N(0; 1) \quad (12)$$

in which  $\Delta x_{ji}$  is the total absolute uncertainty in the original concentration  $x_{ji}$  and  $N(0; 1)$  is a normally-distributed random deviate with a mean of zero and a standard deviation of unity.