# ACCUMULATION OF INORGANIC CONTAMINANTS

R. BARGAGLI[1] and I. MIKHAILOVA[2]
[1]*Department of Environmental Biology, via P.A. Mattioli 4, I-53100 Siena, Italy (bargagli@unisi.it)*
[2]*Institute of Plant and Animal Ecology UD RAS, 8 Marta Str. 202, 620144 Ekaterinburg, Russia (mikhailova@ipae.uran.ru)*

## 1. Introduction

Human activities release large amounts of exchangeable elements into the environment and have become a major factor in altering biogeochemical cycles. This alteration is progressively affecting the long-established steady equilibrium between the Earth's processes and biological evolution [50]. Increasing body burdens of potentially toxic elements in organisms, even from remote regions of the Northern Hemisphere far from significant sources of local pollution, have underlined the importance of establishing reliable monitoring systems at different scales. A reliable appraisal of pollutant concentrations in such an extremely variable compartment as the atmosphere needs a statistical approach based on a large number of samples in both time and space (see chapter 9, this volume). The high costs of establishing and managing automatic monitoring networks often limit the number of sampling stations and/or the number of pollutants considered. Thus, although very reliable, data from instrumental recording may be statistically weak and their integration with diffusion models cannot give reliable information about the deposition and impact of atmospheric pollutants on terrestrial ecosystems.

Biological accumulators are organisms that reflect the chemical content of their environment. Biological monitoring with accumulator organisms provides an essential adjunct to instrumental recording. Hundreds of studies carried out over the last 30 years confirm that lichens are among the most reliable accumulators of airborne inorganic contaminants. The use of bioaccumulators is comparatively cheap, enabling coverage of large and remote areas, and provides current and retrospective information on the integrated effects of atmospheric pollutants and other environmental factors.

Lichens are ectohydric organisms which lack specialised structures for water and gas exchange and hence absorb gases and water with dissolved substances (including many pollutants) over much of their outer surface. The rich thallus branching of many lichen species and the large intercellular spaces within thalli facilitate trapping of particulate pollutants. The chemical composition of lichens therefore largely reflects the availability of elements in the environment. Lichens thus behave as long-living collectors of atmospheric pollutants, especially those associated with airborne particles such as trace metals, fluorine and radionuclides. In polar and alpine ecosystems where

*P.L. Nimis, C. Scheidegger and P.A. Wolseley (eds.), Monitoring with Lichens – Monitoring Lichens, 65-84.*

lichens constitute the bulk of the biomass, knowledge of their role in environmental biogeochemistry is essential to establish the path of element cycling, the transfer of pollutants to herbivores, and to predict possible effects of environmental and climate changes. Ecophysiological studies of lichens subject to increased levels of potentially toxic elements can shed valuable new insights into the adaptation of living organisms to environmental stress.

## 2. Sources of contaminants in lichen thalli

Atmospheric deposition (wet and dry) is the main source of elements to lichen thalli. Wet deposition includes precipitation (rainfall, wash-out and snowfall) and occult precipitation such as fog and dew. Under some conditions, occult precipitation may be an important source of water and element supply for lichens. Concentrations of elements such as Pb are sometimes higher in fog than in rainwater [52]. Gaseous or particulate elements in the atmosphere are also scavenged through dry deposition. Larger particles settle by gravitation (especially around cement factories, mining, smelting and metallurgical plants); fine particles (from about 0.01 to 100 µm in diameter) are removed continuously from air, accumulating on exposed surfaces. The aerial input of elements into ecosystems depends on element concentration and chemical form but is also affected by surface effects (e.g. interception) [95]. Interception increases sharply on moist plant surfaces and cannot be simulated by inert or surrogate surfaces in automatic collectors. To evaluate the impact of atmospheric deposition on terrestrial ecosystems and the transfer of potentially toxic elements to herbivores and ultimately to man it is therefore necessary to collect and analyse suitable species of lichens, mosses and vascular plants [6].

Owing to their slow growth rate, longevity, high surface-to-mass ratio and lack of protective outer structures, the more resistant lichen species concentrate persistent atmospheric pollutants to readily detectable levels. For instance, an uptake of sulphur dioxide by lichens 50 times higher than in vascular plants has been reported [94]. Isotopic studies demonstrate a close relationship between the sulphur isotope composition in lichens and that in the atmosphere whereas soil was found to be the main source of sulphur in pine needles [43]. Despite these and other results since the 1970s, which have failed to show significant transfer of elements from bark to epiphytic lichens, the possible role of the substratum in lichen nutrition is still largely unknown.

The high specificity of most lichens for definite chemical types of substrate suggests such interactions. The higher element content found in the lower thallus surface and rhizinae of terricolous lichens [38] was assumed to indicate element exchange between the substrate and lichens. This possibility has also been postulated for epiphytic lichens [24, 81]. Brown *et al.* [21] showed that particulates trapped between the lichen thallus and bark may play a role in supplying elements to lichens, e.g. *Parmelia sulcata* growing directly on bark contained higher concentrations of Al, Cr, Fe and Mg than did samples growing over epiphytic mosses. Moreover, levels of the same elements were much lower in adjacent surface bark uncolonised by lichens than in debris entrapped between the analysed thallus and the underlying bark. These results indicated that the presence of the lichen leads to the accumulation of particulate elements beneath the thallus (i.e. lichens act as a filter for particles washed down the tree-trunk by stem flow). The role of rhizinae and of the lower surface of the lichen thalli in the uptake of elements has yet to be fully investigated; however it seems likely that a fraction of elements in water and/or particles in stem flow are directly accessible to lichens. These elements may contaminate samples and cannot be ignored when using epiphytic lichens for biomonitoring purposes. Barkman [10] found it more important to investigate the chemical composition of the water in contact with the bark than the bark itself. The chemical composition of precipitation is changed after contact with tree leaves and bark and in turn the chemistry of these substrates is modified by precipitation (e.g. by altering acidity, adding or leaching elements). Atmospheric deposition, tree leaves and bark should therefore be considered as a system affecting the elemental composition of epiphytic lichens.

In general, the possible influence of substrata on the elemental composition of lichens depends on lichen species, bark properties, levels of environmental pollution and the element in question. To minimise these effects in biomonitoring surveys it is necessary to select species loosely attached to the substrate and to collect samples in areas of the tree relatively unaffected by stem flow. The lower surface of the thallus needs careful cleaning (not washing) and whenever possible, the outermost part of the thallus, which has scanty or no connection with tree bark, should be selected for chemical analyses [3].

## 3. Element accumulation

Inorganic contaminants may occur in lichens as 1) particles (adsorbed onto the thallus surface or within intercellular spaces), 2) ions bound to extra- or intracellular exchange sites, and 3) soluble intracellular ions.

### 3.1. PARTICULATE MATERIAL

Particulate material within lichen thalli includes unaltered particles ranging from sub μm to > 100 μm trapped directly from the environment, and various metabolic products including metal compounds and complexes of certain metals (especially Ca, Mg and Cu) with organic acids produced by the mycobiont.

Lichens effectively trap airborne particulate matter (Figure 1) both from natural sources (e.g. soil, volcanic eruptions, sea salt aerosols, wild forest fires and biogenic volatile non-methane hydrocarbons) and anthropogenic sources (mining, smelting and metallurgical activities, combustion of fossil fuels, incineration of refuse, cement production, etc.).

There is much direct and indirect evidence for particle trapping by lichen thalli. Indirect evidence includes an increase in ash and metal content in samples growing near particle emission sources [8, 13, 58]. The presence of metal-rich particles on the thallus surface and in intercellular spaces of the medulla has been demonstrated by SEM and electron microprobes [33, 34, 73] (Figure 1). How particles reach these spaces is not clear. However, features of the thallus surface such as the absence or presence of a cortex, roughness of epicortex, and size of pores could play a significant role in the amount and size of trapped particles. Comparing the chemical composition of airborne particulates and that of *Parmelia caperata* and *P. rudecta* collected around a coal-fired power plant, Olmez *et al.* [63] found that the thalli did not collect all size particles with equal probability. Larger particles, probably deposited by impaction and/or dry deposition, were preferentially accumulated. While it is clear that trapping of large-sized particles allows the accumulation of extremely high concentrations of potentially toxic elements in lichen thalli, the significance of PM10's remains largely unquantified. Garty [33] suggested that the coefficient of variation of lichen metal content in a given locality may be due to different sizes of metal particles. A low coefficient of variation indicates a homogeneous spatial distribution of small particles whereas a high coefficient indicates localized deposition of big-sized particles.

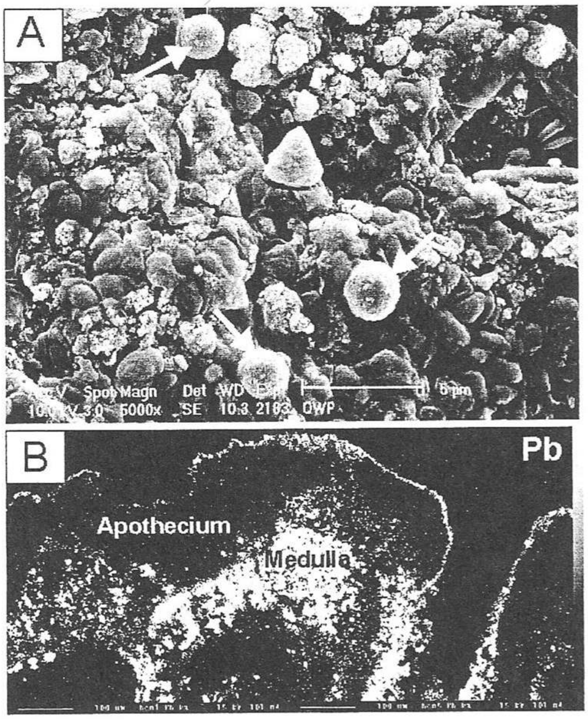

*Figure 1. Accumulation of aerial particulates from smelter emissions by the crustose lichen* Acarospora smaragdula, *Zlatna, Romania. A) Pb-rich PM10s on thallus surface (arrowed). B) Pb X-ray map of section of* A. smaragdula *apothecium (after [73]).*

Higher concentrations of elements are mostly found in older central parts of foliose lichen thalli [8, 39]. This accumulation pattern could be at least partly due to the progressive uptake of particles. This has important implications for biomonitoring because it means that thalli (or parts of thalli) of the same age must be used if element concentrations are to be compared in different lichen samples [3].

Many large-scale surveys have found that most particles trapped by lichens are simply soil and rock dust especially in so-called 'clean' control and remote areas (Figure 2). This is readily demonstrated by the high, intercorrelated concentrations of Al, Ti, Si and other lithophilic elements. Bargagli [5] showed that irrespective of macrolichen species, average regional background concentrations of various elements quoted in the literature increased in proportion to Al, Ti or Fe content, from forest to more open environments such as farmland, upland prairies and tundra. Thus in order to estimate concentrations of elements derived from anthropogenic sources in samples collected in different environments, or in the same site but at different times, the effect of soil contamination must be minimized. This may be accomplished by normalising raw concentrations of elements in each lichen sample to residual ash content or to concentrations of soil tracers such as Al, Ti, Si, Zr and Sc [5].

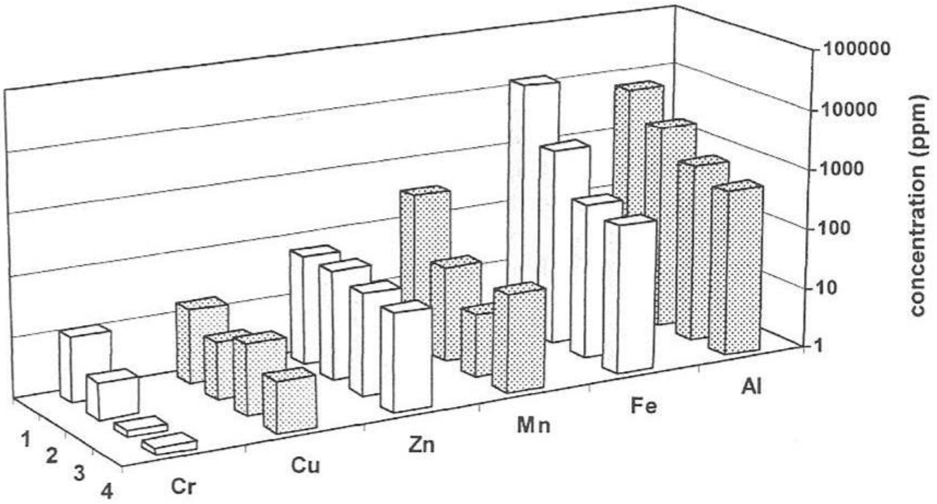

*Figure 2. Average concentrations of elements in* Parmelia sulcata *thalli from background areas, differently affected by soil contamination of samples (data from [37, 46, 8] for control areas 1, 2, and 3-4, respectively).*

Entrapped particles may remain unaltered within lichen thalli for long periods. However, even relatively insoluble compounds may over time be partly dissolved by organic acids, or in polluted regions possibly by acid deposition, and may affect metabolic processes [20]. A wide range of particles may also be formed within or on lichens, particularly by crustose lichens growing on metal-rich rocks, where a wide range of metal-rich crystals occur, including relatively insoluble extracellular substances such as metal oxalates (e.g. Ca, Cu, Mn, Mg and Fe oxalates) and metal - lichen acid complexes (e.g. Cu-norstictic acid and Cu-psoromic acid [26, 72, 77]). Though lichens are known to alter rock chemistry, the quantitative significance of element uptake and release from the substrate by acid lichen compounds is unclear. Formation of copper oxalate was attributed to absorption through run-off rather than extraction from the rock itself [71].

## 3.2. EXTRACELLULAR BINDING OF CATIONS

Cations may bind to extracellular exchange sites in the cell wall and the outer surface of the plasma membrane. The process is accompanied by a release of protons. Cation-binding is a rapid, passive physico-chemical process [18, 53] and may increase with cell death, presumably by exposing exchange sites previously protected by the cell membrane [75]. Few studies have attempted to identify the precise binding sites and cations are assumed to bind to a variety of functional groups including hydroxyl, phosphate, amine and sulphydryl groups [18]. Both the photobiont and mycobiont may be involved in cation binding; however, since the fungal partner constitutes the bulk of the thallus, most cations bind extracellularly to fungal cell walls [18]. Many free-living fungi, especially filamentous species, are known to be effective biosorbents of metals and are attracting the attention of biotechnologists [31]. The photobiont presumably plays a proportional role in the binding of cations, because algal cells also have a high accumulation capacity [59].

Extracellular binding is a reversible process and cations bound to exchange sites are displaced by cations with a higher binding affinity or higher concentrations but lower affinity. Metal ions are classified according to their ligand binding affinities and polarizing power (charge/radius ratio) since such properties can determine biological activity [55]. 'Class A' metals have a preference for O>N>S donors, 'Class B', having a preference for S>N>O and borderline metals fall in between [55]:

- Class A metals: $Al^{3+}$, $Ba^{2+}$, $Ca^{2+}$, $K^{+}$, $Mg^{2+}$, $Na^{+}$ and $Sr^{2+}$
- Class B metals: $Ag^{+}$, $Cu^{+}$, $Hg^{2+}$ and $Pb^{2+}$
- Borderline: $As^{2+}$, $Co^{2+}$, $Cr^{2+}$, $Fe^{3+}$, $Mn^{2+}$, $Ni^{2+}$, $Sn^{2+}$, $Ti^{3+}$, $V^{3+}$ and $Zn^{2+}$

Competition experiments have established a sequence of ion affinities for exchange sites: monovalent Class A< divalent Class A< borderline divalent < divalent Class B [53]. The reversibility of cation binding has important implications for bioaccumulation studies because the content of elements bound to exchange sites mainly reflects recent atmospheric deposition and may be readily exchanged by cations dissolved in rain or by short-term exposure to pollutants. The predictable replacement of one element by another has been used to study movements of metals from cell wall to cell interior and the effects of desiccation on intracellular metal concentration [20].

The significance of passive extracellular binding in uptake and/or release of elements in lichens is indirectly shown by comparable ratios between the accumulation factors for Co, Sc and Zn in transplanted thalli of *Parmelia sulcata* and in "rag" (a muslin-like cloth impregnated with a resinous material) exposed at the same site [79].

## 3.3. INTRACELLULAR UPTAKE

In contrast to extracellular uptake, intracellular uptake is a slow and selective, energy-dependent, plasma membrane-controlled process [18]. Despite the greater biological significance of intracellular elements, few data are available on their uptake by lichens under field conditions. Most results are from laboratory experiments and their significance for field studies is unclear. Beckett and Brown [12] supplied *Peltigera* thalli with Cd and found that intracellular uptake increased linearly over time, substantially dependent on temperature and stimulated by light. Because the Cd uptake was competitively inhibited by other cations, particularly Mg, it was postulated that Cd utilised the carrier transporting Mg into the cell. These results were obtained using a sequential elution procedure involving washing lichens with deionized water (to remove intercellular soluble elements), displacement of exchangeable elements by a divalent cation and release of intracellular elements with total digestion in a concentrated acid. A new step was subsequently introduced to release soluble intracellular elements and those exchangeably bound at intracellular sites [20].

It is important to understand the relative proportions of extra- and intracellular elements in lichens to evaluate the potential toxicity of accumulated elements under field conditions.

### 3.4. UPTAKE OF ANIONS AND NITROGENOUS COMPOUNDS

Few studies on anion uptake by lichens have been carried out compared with those on cation uptake. Only phosphate [27], sulphite [28] and arsenate [57] uptake have been studied in any detail owing to the difficulty in obtaining reliable measurements. Mechanisms of arsenate uptake by fungi, bacteria and algae have been found to be similar to those of phosphate [76]. The uptake of these two anions by lichens is an active process and hence their accumulation by dead thalli is negligible. The existence of at least two independent systems for arsenate uptake has been suggested, which function at different arsenate concentrations [76]. Studies of anion uptake from complex solutions have shown competition by sulfite and phosphate on arsenate uptake and enhancement by sulphate [57]. These results may have ecological implications, because the environmental bioavailability of phosphates may reduce accumulation of toxic sulphite and arsenate ions. Alternatively, increased sulphate content in industrial areas may favour phosphate uptake [76].

Both active and passive sulphite uptake has been suggested for lichens with prevalently passive adsorption [28]. Extracellular anion exchange has been proposed with proteins as possible candidates for binding sites in fungal cell walls. Comparison of uptake of cationic, neutral and anionic complexes of the uranyl ion by *Cladina rangiferina* suggested that the cationic form is most readily taken up from solution, whereas the neutral and anionic forms are absorbed with intermediate and very low uptake capacities [15]. The cationic form behaves like metal cations binding extracellularly. Like arsenate, the uptake of the anionic form of the uranyl ion shows a biphasic pattern and occurs intracellularly. Solutions containing the anionic form of the uranyl ion have therefore been considered to be the most toxic for lichens [16].

Nitrogen compounds as $NH_3$ (or $NH_4^+$ ions) and nitrates may be deposited on lichen surfaces by wet and dry deposition. Lichens assimilate both forms of N from solution, but the mechanisms and rates of transport are not well understood. $NH_3$ is usually more easily absorbed than nitrate. Ammonium ions are initially bound to the cell wall and then transferred to the cytoplasm [22].

## 4. Factors affecting accumulation rate

### 4.1. INTERSPECIFIC VARIATION

Both particle trapping and ion uptake from solutions depend to a large extent on physical aspects such as thallus type (fruticose, foliose or crustose) and other morphological features such as branching, wrinkling and roughness. Anatomical features such as the size of pores in the epicortex and density of hyphae in the medulla may also influence the size of trapped particles and their localisation and redistribution in the thallus. However, there have been few field studies of the comparative uptake ability of different lichen species living in the same habitat. Differences in the content of certain elements in species groups (*Cladina* vs. *Thamnolia*) were found in pristine locations in Canada [54]. Addison and Puckett [1] reported significantly higher S-levels in *Evernia mesomorpha* than in *Hypogymnia physodes*. Although morphological features of *E. mesomorpha* may enable more efficient trapping of gaseous and particulate forms of S, differences in habitat (twigs and trunks) could also play a role. Comparison of element uptake by morphologically similar species of *Parmelia* did not reveal any clear trend [8]. In the genus *Cladina*, small but statistically significant interspecies differences in element concentrations have been reported in several studies [47, 64, 70]. These differences may reflect differences in thallus surface structure such as the presence or absence of an epicortex.

In addition to species morphology, the photobiont species appears to play a role in accumulation ability. In general, cyanolichens have higher concentrations of many elements than do lichens which contain green algae. The latter usually have more homogeneous element spectra than cyanolichens [54]. Many cyanolichens have a gelatinous thallus and their ability to reach higher levels of saturation may facilitate enhanced deposition of aerosols.

## 4.2. INTRASPECIFIC VARIATION

The best way to avoid species-specific variations in element uptake and accumulation is to select the same foliose or fruticose species throughout the study area for a particular study. Important criteria to consider when selecting species are: (a) wide distribution throughout the region; (b) relative resistance to gaseous pollutants, (c) capacity to accumulate and retain metals (d) sufficient material should be available for replicate analyses.

However, even selecting a single species does not necessarily guarantee reproducible results unless a rigorous sampling procedure is adopted. A recommended method is given in chapter 23, this volume.

## 4.3. TEMPORAL VARIATION

The typical occurrence of higher element concentrations in older parts of thalli suggests that lichens retain elements efficiently and behave as time-integrators of persistent atmospheric pollutants. However, retrospective studies of element deposition by using this zonation pattern are premature [6] as an understanding of the dynamics of uptake and leaching processes for each element in different-aged parts of the thallus is required. In reality, the thallus cannot be regarded as a medium where acquired elements remain unchanged and cannot be released. Although some data exists on rates of element transformation and leaching, lichens are known to modify the chemistry of rainfall as it passes over them. Pilegaard [66] found a significant negative correlation between rainfall and the content of the several heavy metals in the thalli of *Hypogymnia physodes* and *Dicranoweisia cirrata*. Puckett [68] explained a seasonal variation in mineral content of lichens by dynamics of snow melting. The initial melt water is enriched with mineral substances and causes a sharp increase in element concentrations in thalli, but subsequent melt water may leach out more elements.

In monitoring studies, it is important to know how quickly a lichen species reflects changes in metal deposition patterns. Most studies dealing with temporal changes in the element concentrations of lichens rely on background values in herbarium material e.g. lead following introduction of lead-free petrol. However, pre-operational surveys of element concentrations in lichens have become quite common in recent years, as the result of a requirement for environmental impact statements prior to plant construction [69]. Temporal trends of element concentrations in lichens from industrial [32, 40] and remote areas have been reported [14].

From transplant studies, we know that many lichen species respond to an increase in atmospheric pollution within a few months. With regard to falling environmental pollution levels, several studies have reported a decrease in element concentrations in lichens [78, 89, 92]; however it is not clear whether this decrease reflected loss of previously accumulated elements or reduced content in tissues formed after the reduction of emissions. Deruelle [25] reported that Pb accumulated by lichen transplants near a road was lost in several months when the thalli were returned to the background site. According to Brown and Brown [20], this loss may be due to rainwater washing particles from the thallus surface.

In general, field studies of temporal changes in metal levels in lichens show that values decrease after 1-2 years once emission ceases and the residence time of many elements in thalli is 2-5 years [92]. However, these estimates are only indicative because the residence time is affected by many internal and external factors, such as growth rates of thalli, element speciation and location in cell fractions, as well as meteorological and environmental conditions.

## 4.4. SPATIAL VARIATION

Spatial variations in the elemental composition of lichens may reflect spatial changes in pollutant deposition (at different scales) as well as variations in the accumulation capacity of thalli (for a given element load). These variations are mainly due to climatic and environmental conditions, which affect metabolism, growth rate and desiccation of thalli, deposition of elements and their availability. In background areas the composition of lichens may change with altitude [4, 42] probably because the amount of precipitation and deposition of long-range transported elements such as Pb, Cd, and Zn increases with altitude.

Local topography and vegetation structure also significantly affect pollutant deposition [82]. Single trees or trees in open woodland obviously obtain more particles than those in high dense stands. Takala *et al.* [87] measured higher F concentrations in lichens from pines and birches than spruce and suggested that samples from spruce trunks were sheltered from particulate F by twigs. Mechanical sheltering from pollutants was also described by Perkins *et al.* [65] for epilithic lichens growing on different parts of a wall.

Element content of epiphytes may also depend on their height on the host tree (with higher concentrations lower down [84, 87]) or on one side of tree trunk [86]. In addition to the increasing availability of moisture and soil particles near the base of tree trunks, these variations are due to the pattern and elemental composition of stemflow. In fact, concentrations of elements in precipitation increase as they move through the tree canopy, in the order crowndrip<throughfall<stemflow [2]. Tree inclination is also important for stemflow pattern. The upper part of a tree gets a large portion of rainwater but drying or desiccation is also faster. On strongly inclined trees with smooth bark, rainwater may collect in drops on the underside. Different microclimatic conditions on different parts of the trunk (faster desiccation on the southern side, lower evaporation rates, sheltering by grass or epiphytic moss and long periods of snow cover near the base) may determine spatial variations in the elemental composition of lichens.

Thus, standardized sampling procedures should include forest type, species of phorophyte, degree of tree inclination, position on trunk, bark morphology, microclimatic conditions and health of thalli. As snow, rainfall and drought affect the elemental composition of thalli, the sampling period should be harmonised to make results of time series studies or between different environments more reliable. Despite standardized sampling procedures, even in homogeneous lichen populations, element concentrations may be quite variable ("biological variability"). As the frequency distribution of measured values is close to normal [61], this variation is stochastic and the representativeness of the sampling can easily be improved by collecting a number of individual thalli in each sampling site [60].

## 5. Transplant studies

Lichen transplants are used for monitoring air pollution especially in areas where no suitable indigenous lichens exist. Relatively large foliose or fruticose lichens are generally used for trace element biomonitoring. Foliose species are preferable because they are generally more tolerant of gaseous pollutants. The general scheme of this biomonitoring approach is as follows: pieces of bark or twigs covered by healthy lichen thalli are transferred from unpolluted to polluted areas. Progressive accumulation of elements is measured after definite exposure periods. Alternatively, lichens packed loosely in a fine nylon net ("lichen bags") were also exposed in several urban and industrial environments (see [23, 36, 49] and chapter 24, this volume).

The main advantage of the transplant technique over collection and analysis of naturally growing thalli is that this procedure can be standardized to a high extent. Standardization includes collecting transplanted material from the same type of substrate with the same thallus size, and placing transplants in sites with similar meteorological and environmental conditions (including position, orientation and exposure time).

A major problem of transplant surveys is establishing an appropriate exposure period. In heavily polluted environments, transplanted lichens may become saturated with elements and their surface structure and physiological performance may be significantly altered. In addition to the accumulation of persistent atmospheric pollutants, many parameters can be measured for an indirect estimate of relative levels and biological effects of atmospheric pollutants. These parameters include: chlorophyll content and degradation, ATP decrease, respiration and photosynthesis rate, increase in membrane leakage, and peroxidation of membrane lipids [36]. Cell membrane damage in exposed lichens has been detected long before any indication of damage becomes apparent in the photobiont chlorophyll [35]. Since this damage may cause loss of elements during rain, it is not surprising that concentrations of some elements in exposed lichens may decrease. Thus, the exposure time should be established according to the purpose of the survey, climatic and environmental conditions of the study area and the expected degree of stress for the selected species. In general, a nearly linear increase in element bioaccumulation occurs in the first few weeks and most surveys are based on one to three month exposure periods. However, under suitable environmental and climatic conditions, longer periods of exposure have also been recommended [85].

## 6. Lichen tolerance to inorganic contaminants

Exposure of natural vegetation communities to increasing metal bioavailability initiates selective processes among the species and only some will be able to adapt and survive. In cryptogams and higher plants, adaptation to metal pollution is quite rapid as it has been observed at sites of recent (last 30 years) contamination. This tolerance is a genetically based phenomenon; probably, it is not metal-specific and in higher plants shows a continuous gradation (from weak to strong) among ecotypes, physiotypes or races [48]. In lichens metal tolerance has been demonstrated by field and laboratory investigations. One example is the existence of natural undamaged populations near emission sources where transplants showed signs of injury after several months of exposure [83]. Saxicolous lichens growing on metalliferous rocks have several biochemical mechanisms for metal detoxification, mainly consisting in the complexation of Cu, Zn, Fe, Mn, Mg and other cations by lichen substances and deposition of the resulting compounds on the thallus surface and in the upper cortex [72]. Sarret *et al.* [77] studied the speciation of Pb and Zn by powder X-ray diffraction (XRD) and extended X-ray absorption fine structure (EXAFS) spectroscopy and found that hyperaccumulation of metals in *Diploschistes muscorum* was achieved by the production of oxalate. Besides lichen acids and oxalate, other ligands such as citrate, malate and malonate, which have also been proposed as metal ligands for fungi [31], may also be involved in the immobilization and excretion of metals by lichens. However, not all lichen species growing in the same environment contain similar compounds within their thalli. Therefore, this way of exclusion of potentially toxic cations is not a universal tolerance mechanism [18, 71].

Comparative laboratory investigations in *Peltigera* revealed reduced intracellular Cd uptake by thalli from contaminated localities compared to those from background sites, whereas the rate of extracellular binding was similar [12]. However, the reasons for reduced intracellular uptake are not well understood. Metal exclusion mechanisms such as an altered transmembrane carrier system have been proposed, but were not confirmed by following investigations [93]. Extracellular binding of large quantities of native mineral ions has been hypothesized as a mechanism limiting input of toxic cations to cell [93]. Decreased intracellular uptake was supposed as a reason for higher resistance of photosynthesis in *Peltigera* collected from contaminated sites than in thalli collected in unpolluted areas [19]. Resistance was assessed as the ratio of photosynthetic rates after metal and after water treatment under laboratory conditions.

Levels of Zn tolerance were proportional to the initial (field) Zn content of the thalli. Resistance of photosynthesis in samples from uncontaminated localities was artificially raised by Zn pre-treatment one week before an experiment [19].

These results suggest that at least part of the metal resistance in lichens may be phenotypically acquired. Different species or communities of lichens may develop different detoxification mechanisms. However, in most species of foliose lichens used in biomonitoring surveys these mechanisms essentially consist of accumulation of potentially toxic elements in the fungal cell wall and decreased intracellular metal uptake.

Goyal and Seaward [38] described some morphological and histological alterations in *Peltigera* thalli from highly contaminated localities and interpreted them in terms of adaptations. An increase in the thickness of the outer cortex and medulla, and higher density of rhizines may provide effective accumulation of metals in the mycobiont preventing damage to the more sensitive photobiont. However, according to Beckett and Brown [11] these modifications could also be due to water regime, because *Peltigera* thalli were collected from moss cover under background conditions and from naked soil at the mine site. Studies on *Hypogymnia physodes* from urban sites [41] revealed the opposite, namely a decrease in cortex thickness and an increase in thickness of the photobiont layer.

## 7. Metal toxicity and typical concentrations of trace elements in lichens

Toxic effects of metals on lichens under field conditions have been reported as sudden or gradual changes in lichen morphology [38], or as an increase in species abundance or biodiversity with increasing distance from emission sources [17, 29]. Along a metal pollution gradient, several hundred ppm of Cu, Pb and Zn were reported as thresholds for the survival of *Hypogymnia physodes* and *Parmelia squarrosa* [74]. However, most studies give rather ambiguous examples of metal toxicity because they are based on total metal concentrations and disregard other environmental factors. Changes in lichen morphology, biodiversity and element accumulation capacity are almost certainly also affected by gaseous pollutants, especially $SO_2$, acidification and climatic factors. The only reports of lichen death which can convincingly be ascribed to metal toxicity are those in extreme environmental conditions such as beneath copper or barbed wire or galvanised supports [6].

Field studies on metal toxicity require a detailed knowledge of physico-chemical forms and cellular location of metals in the lichen thalli. Branquinho *et al.* [17] for instance, studied physiological effects of Cu-dust in *Ramalina fastigiata* near a mine and they found that although the intracellular Cu uptake was much smaller (6%) than the extracellular, it explained the physiological changes and the survival of the species in the surroundings of the copper-mine.

In general, owing to the uptake of particulate elements and the immobilization of soluble forms in fungal cell walls, several species of epiphytic lichens are relatively tolerant to atmospheric pollutants and can be used as biomonitors in urban and industrial environments. As a rule, species having a wide ecological range such as *Hypogymnia physodes*, *Evernia prunastri*, *Pseudevernia furfuracea*, *Xanthoria* *parietina* and several species of the genus *Parmelia s. lat.* are among the most frequently used. In northern boreal forests and tundra, epilithic and epigeic lichens of the genera *Cladonia* and *Cetraria* have been extensively used and some species of *Umbilicaria*, *Xanthoria* and *Usnea* may even enable biomonitoring surveys in remote and extreme environments of the Arctic and Antarctic [9, 30].

The introduction of very sensitive instruments for trace and ultratrace analysis and the progressive standardization of sample collection and preparation procedures make it possible to establish contemporary background element concentrations in lichens from different regions (Table 1). In general, samples of epigeic and epiphytic lichens relatively unaffected by soil contamination have concentrations of elements in the range of Table 1. Baseline concentrations of trace elements are necessary in order to assess pollution levels. As a rule, in most biomonitoring surveys baseline concentrations of elements in the selected lichen species are estimated indirectly by analysing samples from control areas. However, the values in Table 1 may be useful to establish whether values measured in a biomonitor species are clearly indicative of environmental pollution (e.g. being orders of magnitude higher) or whether correction for soil contamination of samples is necessary (see chapter 23, this volume).

*TABLE 1. Background concentrations of elements (μg $g^{-1}$ dry wt.) in lichens (* = slightly contaminated by soil particles).*

| | Italy [60] Epiphytic (*Parmelia, Xanthoria*) | Hungary [87] Epigeic (*Cladonia convoluta*) | Antarctica [9] Epilithic (*Umbilicaria decussata*) | Europe and America [14] Epiphytic/epilithic (*Hypogymnia physodes*) | European part of Russia [89]* Epiphytic (*Hypogymnia physodes*) |
|---|---|---|---|---|---|
| Al | 350.0 | 185.0 | 180.0 | 363.0 | 638,0 |
| As | 0.3 | - | - | 1.4 | 1,3 |
| Ba | 3.3 | - | 2.5 | - | 23,0 |
| Cd | 0.2 | 0.3 | 0.1 | 0.2 | 0,2 |
| Cr | 1.2 | 0.4 | 0.5 | 1.7 | 2,2 |
| Cu | 7.0 | 7.3 | 3.5 | 4.3 | 10,9 |
| Fe | 290.0 | 197.0 | 200.0 | 480.0 | 664,0 |
| Hg | 0.1 | - | 0.1 | - | 0,3 |
| Mn | 20.0 | 9.4 | 9.0 | 35.0 | 149,0 |
| Ni | 1.0 | 1.1 | 0.9 | 12.0 | 3,4 |
| Pb | 4.0 | 7.4 | 0.3 | 28.0 | 8,6 |
| Ti | 10.0 | 3.0 | 7.0 | - | - |
| V | 0.6 | 1.1 | 0.7 | - | 1,5 |
| Zn | 30.0 | 31.0 | 10.0 | 44.0 | 72,0 |

## 8. Differentiating sources of elements found in lichens

As previously discussed, the elemental composition of lichens depends not only on concentrations of airborne pollutants, but also on climatic and environmental conditions, which affect the metabolism and growth rate of lichens, and the deposition and availability of trace elements. The variability of results is therefore a problem inherent to biomonitoring studies and it is difficult to establish whether slightly elevated levels of one or more elements in lichens are due to intrinsic biological variability, natural sources of elements or local or remote human activities. Unfortunately, element concentrations in wet and dry deposition may only be of limited help in interpreting the results of biomonitoring surveys. In fact, the accumulation of airborne particles in lichens through interception, impaction or sedimentation differs from that in surrogate surfaces in automatic samplers. Element pools in lichens and artificial collectors also have different temporal significance. Instrumental data often reflect element deposition rates over short or irregular time periods, whereas lichens are time-integrators of persistent atmospheric deposition and the age (exposure time) of the thalli analysed is often unknown. Besides, elements may be washed away by rain and snow and in polluted environments saturation of extracellular binding sites or physiological impairment may prevent further uptake of elements by lichens.

Isolated point emission sources are the easiest to identify by lichen monitoring, but in urban and industrial areas or in large-scale surveys there is a range of possible sources of elements. In general, these sources are identified on the basis of element distribution patterns in the study area and by comparing the elemental composition of lichens with emissions from potential sources.

Enrichment Factors (EF) with respect to crustal composition [70] or to the composition of surface soils of the study area (analyzed by the same procedures used for lichens [3]), have often been used to estimate anthropogenic contributions to the elemental composition of lichens. The general formula of EF for element $x$ is:

$$\mathrm{EF} = \frac{([\mathrm{x}]/[\text{reference element}])\ \textit{in lichens}}{([\mathrm{x}]/[\text{reference element}])\ \textit{in crust}} \tag{1}$$

where Al, Ti, Sc and other lithophilic elements may be the reference element. As a rule, the more the EF values approximate unity, the more the element of concern derives its source from soil particles, rather than from anthropogenic sources.

Besides determining the correlation coefficients between pairs of element concentrations in the thalli, multivariate techniques such as factor analysis and principal component analysis have been applied to lichen monitoring data to identify sources of elements (e.g. [7, 45, 56, 67, 80]).

Isotope studies are another method of differentiating of element sources in lichens. The impact of a point source of S has been detected over a distance of several kilometres [44] and relative inputs of natural and anthropogenic sources of S distinguished in lichens from regions with naturally high atmospheric S concentrations [91]. On the basis of Pb isotope composition, Monna *et al.* [51] recognized two main anthropogenic sources and "natural" Pb contamination from active volcanoes in lichens from eastern Sicily.

Some sources of the most widespread inorganic contaminants are summarized in Table 2. The major source types have been classified in different ways and possible secondary associations between elements may change their profile under different environmental conditions. The impact of oil combustion and processing of oil products, for instance, are usually assumed to be reflected in lichen thalli by an association between V and Ni concentrations. However, both elements may also be of crustal origin. Associations between Br, Hg, Cd, Fe, Se and other elements are generally considered to indicate emission from coal-fired plants, but coal fly ash has a chemical composition quite similar to crustal materials and the bioaccumulation of elements such as Hg, Cd, Zn or Pb in lichens may also come from long-range atmospheric transport.

Many "new" elements are increasingly entering our environment. Some lanthanoids are components of alloys for magnetic materials and new high-temperature superconductors. The installation of catalytic converters in motor vehicles is expected to result in emission of elements such as Pt, Pd, Ru, Rh and Ce [50]. The possible biological effects of increased environmental availability of these elements are unknown. Lichen biomonitoring will be a valuable adjunct to instrumental monitoring for evaluating the spatio-temporal deposition patterns of these pollutants providing an early warning of their biological effects.

*TABLE 2. Some potentially toxic elements and their anthropogenic sources.*

| | Toxicity | Sources |
|---|---|---|
| Al | Affects root growth and element uptake in plants, P metabolism in animals, implicated in the Alzheimer's disease | Non-ferrous metal-works, sheet metal, wires and alloys |
| As | Phytotoxic, believed to be carcinogenic, Black-foot disease | Mining and smelting, coal-fired power plants, fertilizers, alloys, insecticide, fungicide |
| Be | Highly toxic to plants and animals, allergenic, some forms are carcinogenic, may cause rickets | Electrical insulators, aerospace applications, clock springs, X-ray windows, alloys. |
| Cd | Interferes with photosynthesis, uptake and transport of elements in plants. Carcinogenic, Itai-Itai disease | Metallurgical plants, refuse incineration, mineral fertilizers, coal combustion, accumulators |
| Cr | $Cr^{6+}$ may cross cell membrane and is toxic to plants, animals and man, carcinogenic | Metallurgy, production of paints, catalysts, tanneries |
| Hg | Highly toxic to plants, methylated Hg is biomagnified in food chains, Minamata disease | Chlor-alkali plants, coal combustion, smelting, some types of pesticides |
| Pb | Cumulative poison, carcinogenic and teratogenic, may affect the intellectual development of young children | Automobile exhaust (especially in the past), metallurgy, accumulators, cable coating, die castings |
| Pd, Pt, Rh, Ru | Toxicity is not exactly known | Automobile three-way catalytic converters |
| Se | Some plant species are accumulators of Se and can pose a danger to grazing animals, white muscle disease | Electroplating, glass industry |
| Tl | Hinders seed germination, affects photosynthesis and transpiration. Tachycardia and hypertension | Mineral processing, cement factories |
| V | Phytotoxic, in animals and man can be toxic when entering by way of respiratory system, carcinogenic | Smelters, steel production, coal- and oil-burning plants |

## 9. Conclusions

The ability for lichens to accumulate high levels of elements far in excess of their physiological requirements closely correlated with atmospheric levels has led to their wide-scale application as practical biomonitors of metal contamination. In large-scale surveys and remote regions where few recording gauges are present, lichen biomonitoring is one of the only ways to determine the deposition patterns of trace elements (Figure 3). As a rule, this approach is based on the analytical determination of total concentrations of elements in lichen thalli and is successfully applied throughout the world.

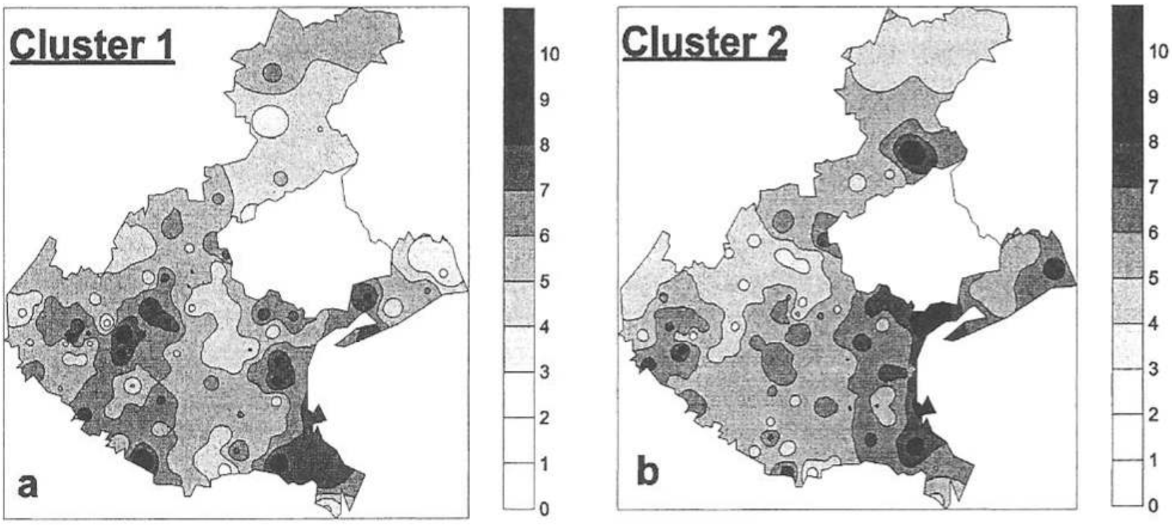

*Figure 3. Mapping element data in Veneto (NE Italy), showing spatial variation of element distribution according to two principal clusters of metals in a dendrogram. For each cluster, values were normalised and expressed on a 0-10 scale (after [62]).*

Particle trapping and the passive complexation of cations to fungal cell walls seem to be the most frequent uptake mechanisms in epiphytic species most frequently used in biomonitoring surveys.

Particles and elements bound to exchange sites may be lost (especially in desiccated thalli) when climatic and/or other environmental conditions change. Environmental factors may also contribute to the variability of element concentrations within thalli because they may affect lichen growth rate, element availability and the trapping of airborne soil particles. Thus, the selection and analysis of thalli (or portions of thalli) of a similar age and according to strict sampling protocols is a prerequisite to their successful use as predictive quantitative biomonitors of inorganic contaminants.

As particles and soluble elements may also be derived from a variety of natural sources, it is essential to distinguish man-made perturbations from intrinsic biological variability and natural sources of inorganic contaminants.

Modern analytical systems for multielement determination at trace and ultratrace levels, in parallel with SEM/electron probe microanalysis and other techniques, provide valuable new opportunities to understand mechanisms of aerial particle uptake and possible release under different environmental conditions.

Particulate characterisation, localisation, quantification, redistribution and/or transformation (including element complexation by organic substances) may be studied in small samples. An understanding of the effects of different metal compounds, their physico-chemical forms and/or cellular location on lichen physiological processes will enhance the role of lichens as sensitive biomonitors of the atmospheric deposition of inorganic contaminants. It will also provide an early warning system of their biological effects in terrestrial ecosystems.