

Article

Estimating Background Values of Potentially Toxic Elements Accumulated in Moss: A Case Study from Switzerland

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Abstract: Although the use of moss as biomonitor of air pollution is relatively simple, the interpretation of the data needs reference values. Background values for Cd, Cu, Pb, and Zn accumulated in moss samples from Switzerland, collected every five years from 1995 to 2015 in the framework of the European Moss Survey, were statistically estimated. These background values can be used as reference for the assessment of spatial and temporal trends, to be expressed in terms of bioaccumulation ratios with actual values. The use of annual background values is of great importance to identify spatial trends, while period-wide background values identify temporal trends. The latter are consistent with those reported in other comprehensive similar biomonitoring studies in Europe and are required to be updated in time, possibly every five years. The use of cutoff values to be used as benchmark for bioaccumulation ratios is invaluable in having a scale for assessing ecological quality.

Keywords: biomonitors; PTE; temporal change; atmospheric pollution; deposition; heavy metal



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1. Introduction

The term “heavy metal”, although widely used in the literature, is now deprecated and it has been suggested to replace it with the term of potentially toxic element (PTE), especially in the case of environmental studies [1]. Having a wide variety of emission sources such as motor vehicles, heating systems, industrial plants, etc., PTEs are an important component of air pollution and have a great epidemiological concern because of their persistence in the environment and the negative effects on human health [2].

Since about 80% of the EU population lives in urban areas, urban air pollution affects the quality of life of most citizens. Consequently, urban air pollution has largely been investigated (see, e.g., [3]) and many urban areas have an air quality monitoring network to monitor whether the set environmental standards are met or not. However, studies on atmospheric deposition at remote areas to evaluate the impact of PTEs on ecosystems are less common [4,5] and an evaluation on background pollution in pristine areas is only very rarely accessed. This is often due to economic constraints related to the establishment and maintenance of sophisticated and costly equipment. In such cases, the use of living organisms may be very useful to complement the data obtained by physico-chemical measurements. Since any change taking place in the environment has a significant effect on the biota, biological monitoring (biomonitoring) is a very effective early warning system to detect environmental changes [6].

Carpet-forming moss species are among the most valuable biomonitors of atmospheric pollution as they are highly dependent on wet and dry atmospheric deposition for nutrients and lack a waxy cuticle and stomata, allowing the absorbance of contaminants over the whole moss surface [7]. Additionally, mosses can accumulate persistent pollutants, such as PTEs and are used to measure the amounts of pollutants in the ecosystem that are biologically available [8]. The abundance of these moss species in Scandinavia gave rise to the beginning of the moss monitoring survey making it possible to monitor PTE

pollution on a multicountry-wide scale [9]. Thanks to the standardized method and the relatively low costs as well as the ease of collection of samples, this well-established technique of moss monitoring has been adopted also in other European countries coordinated by an International Cooperative Program on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation) under the United Nations Economic Commission for Europe (UNECE) Geneva Air Convention. Through this program, mosses have been used to evaluate atmospheric deposition of PTEs at several European countries every five years since 1990 [10]. This enabled not only the comparison of spatial patterns of PTE deposition, but also the detection of temporal trends. Presently, also other pollutants have been added to the survey such as nitrogen, persistent organic pollutants (POPs), and, more recently, also microplastics. Although outside of Europe mosses have been sometimes used as biomonitors of air pollution, the wide use of the moss monitoring technique is mainly restricted to Europe, where ca. 80% of the studies have been performed [11].

Switzerland has been participating in the moss monitoring project since 1990 and the Swiss outcomes showed a general decline in element concentrations in time [12], likely determined by the closure of several small industries, mostly metallurgic, as well as improved abatement technology of waste incinerators, the ban of leaded fuel, and the wide use of catalytic converters in cars. This hypothesis is corroborated by the consistency of the temporal trends in moss with those of emission data for some elements [12]. The main aim of the European Moss Survey is to determine spatial differences and temporal changes in the atmospheric deposition of PTEs, estimated by their concentrations in moss. Therefore, it is of paramount importance that these concentrations are properly evaluated in terms of deviation from reference conditions, i.e., that the magnitude of pollution phenomena can be clearly depicted. As environmental quality standards (EQSs) set by legislation for the concentration of PTEs in biomonitors are missing, the interpretation of PTEs' contents in moss requires the estimation of deviation from an unaltered reference (background) condition. Therefore, the first step in any biomonitoring survey should be the definition of appropriate background values to be used as reference. In a second step, this reference can be used to calculate the extent of the deviation from this background condition.

In this paper we aimed to estimate background values for some PTEs, namely Cd, Cu, Pb, and Zn accumulated in moss collected at remote sites of Switzerland in the framework of the European Moss Survey. These background values could be used as reference for the assessment of spatial and temporal pollution phenomena. Bioaccumulation differences between moss species, although sometimes reported as important [13], being also still unclear if this variation is species- or habit-specific, were outside the scope of this paper and were not considered.

2. Materials and Methods

2.1. Selection of Background Sites and Moss Sampling

Sampling sites for the European Moss Survey were evenly distributed across the five biogeographic regions of Switzerland: Jura, Plateau, and Northern, Central, and Southern Alps, which differ in elevation, geology, and meteorological conditions as well as in flora, fauna, and population density (Figure 1). Ten remote sites were selected as pristine areas in order to estimate the background deposition. All these sites were situated in alpine regions and were under the influence of only a very modest human activity. Therefore, these sites can be regarded as representative of the “natural” situation of Switzerland.

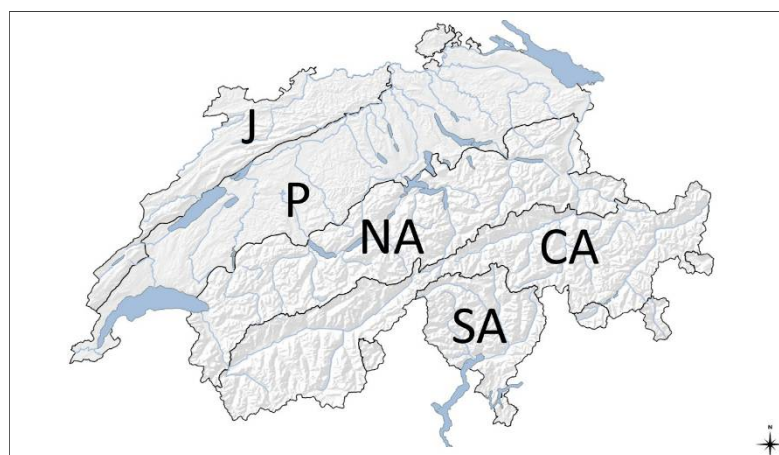


Figure 1. The five biogeographic regions of Switzerland: Jura (J), Plateau (P), Northern Alps (NA), Central Alps (CA), Southern Alps (SA). Adapted from Gutersohn [14].

The moss species *Hypnum cupressiforme* Hedw. and *Pleurozium schreberi* (Willd. Ex Brid) Mitt. were sampled, the former mostly at lowland sites and the latter mostly at Alpine sites. The moss samples were collected from tree trunks, in open areas such as forest clearings, at least 3 m away from the edge of the tree canopy, following the Moss Monitoring protocol [15]. At each site five subsamples were collected. Sampling took place every five years, namely 1990, 1995, 2000, 2005, 2010, and 2015, at the same sites and in the same period of the year (from April to October). According to the five-year cycle, the 2020 moss monitoring survey is currently ongoing, with samples being prepared for analysis and preliminary results expected by the end of 2021.

2.2. Chemical Analysis

In the laboratory, the samples were cleaned from dead or extraneous material such as litter, needles, soil, insects, etc., and only the green shoots roughly corresponding to the last three years of growth were cut for the chemical analyses. An equal amount of moss biomass was taken from each subsample and combined to form a single composite sample, taken as representative of the site following the Moss Monitoring protocol [15,16]. Samples were then dried at 40 °C. The chemical analyses were performed immediately after complete collection. Due to the time lags, the analyses were performed in different laboratories using the following analytical methods. Prior to the mineralization, each sample was pulverized in liquid nitrogen. Approximately 200 mg of moss powder was then mineralized in a microwave digestion system (Milestone Ethos 1) with 7 mL of HNO₃ and 3 mL of H₂O₂, using hermetic Teflon vessels at 130 °C and high pressure (100 bar). The mineralized and diluted solutions (up to 50 mL) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS Perkin Elmer—Sciex, Elan 6100). The results are expressed on a dry weight basis (µg g^{−1} dw). Analytical quality was checked by analyzing several Standard Reference Materials: the moss standards M2 and M3 (*Pleurozium schreberi* [17,18]), BCR 61 (*Platihypnidium raparioides*) and BCR 62 (*Olea europea*) [19], and LMS 2 (a laboratory internal moss reference material, [20]).

2.3. Statistical Analysis

For each element, the background data set was first checked for outliers using the Tukey test; in case an outlier emerged, its value was replaced by the median value of the remaining data set [21]. Based on this data set, for each element and for each year, median values and confidence limits were estimated (note that the confidence limits are not necessarily symmetric around the sample estimate, as is the case when standard errors are used to construct the confidence intervals) by bootstrapping [22]. For each element, the sig-

nificance of differences between years was evaluated by means of a pairwise permutation test [23], correcting for multiple testing according to Benjamini and Hochberg [24].

3. Results and Discussion

Based on our estimates of background concentrations (Table 1), very different trends emerged for the four elements investigated. The background value of Cd showed a continuous and constant decrease, with differences requiring 10 years (two moss monitoring project sampling campaigns) to become significant. Lead showed a sharp decrease in the first two periods and from the year 2000 differences became insignificant. Copper, although with some higher values, did not show any temporal trend. Values for Zn, although decreased from 1990 to 2010, remained, overall, quite constant, without any significant difference through years.

Table 1. Median and 95% confidence limits (c.l.) of Cd, Cu, Pb, and Zn concentrations ($\mu\text{g g}^{-1}$ dw) in moss at background sites of Switzerland. Different letters in a column indicate statistically significant ($p < 0.05$) differences.

	Cd		Cu		Pb		Zn	
	Median	c.l.	Median	c.l.	Median	c.l.	Median	c.l.
1990	0.20 a	0.13–0.36	4.5 ad	3.7–6.4	14.4 a	9.5–22.2	34.6	22.1–38.1
1995	0.18 a	0.12–0.28	4.8 a	4.0–6.4	5.6 b	3.2–7.3	30.9	23.0–48.6
2000	0.12 bc	0.09–0.14	7.5 bc	5.3–9.6	1.7 c	1.3–1.8	29.9	23.5–38.6
2005	0.10 cd	0.07–0.15	5.9 abcd	4.8–7.1	1.7 c	0.9–1.8	31.4	25.6–36.6
2010	0.08 de	0.06–0.10	6.4 cd	5.4–7.1	1.3 c	0.8–1.5	22.8	19.9–30.4
2015	0.07 e	0.04–0.10	4.9 ad	4.1–6.5	1.2 c	0.7–1.9	23.2	19.8–32.4
whole period	0.11	0.09–0.13	5.6	5.0–6.4	1.7	1.5–2.6	25.6	24.3–32

An important consequence of this methodological approach for estimating background element concentrations in moss is that, in addition to a proper selection of background sites, background values may change in time due to efforts to reduce emissions. Also, legal reference values for environmental pollutants measured instrumentally are updated in time, with the progress of measurement techniques, increase of knowledge, and decreasing environmental concentrations. In this light, the assessment of background values of PTEs should be intended as a dynamic process, with periodically updating with the most recent data.

To detect a significant accumulation for a given PTE, values must be significantly different from those measured at background areas. A commonly adopted approach in this sense (see, e.g., [25]) is to use the upper limit of the confidence interval as threshold. After having assessed this background threshold, a very simple and reliable method to estimate the degree of deviation from background conditions is to calculate the ratio between the concentration of a given element to its background value. This approach has the great advantage of allowing element- and species-specific differences to be overcome, thus permitting the use of a single interpretative scale in any circumstance. This method has been implemented, e.g., in the European Water Framework Directive (WFD), using so-called ecological quality ratios (EQRs), and is now successfully used in many studies [26]. Following this approach, Cecconi et al. [27] suggested an interpretative scale for Italian foliose lichens based on bioaccumulation ratios (B ratios), i.e., ratios of actual values to background values, established according to the 25th, 75th, 90th, and 95th percentiles of the frequency distributions as follows: <1 no bioaccumulation, 1–2.1 low bioaccumulation, 2.1–3.4 moderate bioaccumulation, 3.4–4.9 high bioaccumulation, >4.9 severe bioaccumulation.

On the basis of this scale, we compared the background values estimated for each period with those measured at the remaining 65 sites [12] distributed among the five Swiss biogeographic regions sampled in the corresponding year (Table 2). This comparison shows an overall absence of bioaccumulation or low bioaccumulation for all elements at all regions, with the notable exception of Pb at the Southern Alps, which showed values up to severe bioaccumulation. On an average basis, the Southern Alps were always the

region with the highest bioaccumulation. However, maximum values of B ratios (data not shown) indicated a very wide array of variation, with some values reaching severe bioaccumulation for all the four investigated elements. The Southern Alps are exposed to winds from the South and, therefore, also to pollution coming from that direction (e.g., from the Po Plain, a nearby and highly polluted area of Italy), and at the same time they act as a barrier to air pollutants for the other Swiss regions.

Table 2. Bioaccumulation ratios (median values) at the five Swiss biogeographic regions during the six European moss surveys, using annual background values and the remaining 65 sampling sites. See Figure 1 for explanation of regions.

PTE	Region	1990	1995	2000	2005	2010	2015
Cd	J	1.0	0.9	1.5	1.0	1.3	1.2
	P	0.9	0.9	1.6	1.2	1.3	1.4
	NA	0.9	0.9	1.0	1.0	1.2	1.0
	CA	0.6	0.5	0.6	0.6	0.6	0.7
	SA	1.6	1.4	1.6	1.7	1.8	1.4
Cu	J	0.7	0.7	0.4	0.7	0.6	0.7
	P	0.6	0.6	0.4	0.6	0.5	0.7
	NA	0.6	0.6	0.6	0.9	0.7	0.7
	CA	0.7	0.8	0.5	0.7	0.8	0.7
	SA	1.4	1.2	0.8	1.1	0.8	1.0
Pb	J	0.7	0.8	1.6	1.8	1.6	1.2
	P	0.7	0.8	2.0	1.9	1.3	1.3
	NA	0.7	0.9	1.3	1.3	1.7	1.0
	CA	0.7	0.7	1.0	1.0	0.8	0.6
	SA	2.0	3.7	5.0	5.1	2.9	2.1
Zn	J	0.8	0.5	0.7	0.7	0.8	0.7
	P	0.9	0.6	0.6	1.0	0.7	0.9
	NA	0.8	0.7	0.8	0.9	0.8	0.7
	CA	0.9	0.6	0.6	0.8	0.8	0.8
	SA	1.8	1.2	1.2	1.5	1.3	1.2

Estimated element background values in moss for the whole period 1990–2015 (Table 1) are consistent with those estimated for the lichen *Flavoparmelia capera* (a species with ecological requirements similar to those of *H. cupressiforme* and *P. schreberi*) from Italy (Cecconi et al. 2019): Cd = 0.18 $\mu\text{g g}^{-1}$ dw, Cu = 6.2 $\mu\text{g g}^{-1}$ dw, Pb = 2.4 $\mu\text{g g}^{-1}$ dw, and Zn = 35.3 $\mu\text{g g}^{-1}$ dw. Additionally, these data also match background values estimated with a Bayesian approach in the lichen *Evernia prunastri* from Tuscany, Italy [28]: Cd = 0.13 $\mu\text{g g}^{-1}$ dw, Cu = 5.1 $\mu\text{g g}^{-1}$ dw, Pb = 2.4 $\mu\text{g g}^{-1}$ dw, and Zn = 22.5 $\mu\text{g g}^{-1}$ dw. Overall background values are also in line with the lowest values of the scales adopted to map element bioaccumulation in the European moss surveys [10]: Cd = 0.1 $\mu\text{g g}^{-1}$ dw, Cu = 4 $\mu\text{g g}^{-1}$ dw, Pb = 2 $\mu\text{g g}^{-1}$ dw, and Zn = 20 $\mu\text{g g}^{-1}$ dw.

Using these whole-period background values (upper limit of 95% confidence limits), ratios with values measured at the five Swiss biogeographic regions during the six surveys (Table 3) are indicative of temporal changes. At all regions, a gradual decrease can be seen for Cd and a marked drop for Pb; values of Zn also indicated a modest decreasing trend from 1995 to 2015, while Cu values remained quite constant. Also, in the case of temporal changes, the Southern Alps were the Swiss region with the highest bioaccumulation ratios.

Table 3. Bioaccumulation ratios (median values) at the five Swiss biogeographic regions during the six European moss surveys, using whole-period background values. See Figure 1 for explanation of regions.

PTE	Region	1990	1995	2000	2005	2010	2015
Cd	J	1.7	1.0	0.8	0.7	0.5	0.5
	P	2.6	1.8	1.8	1.4	1.0	1.1
	NA	2.6	1.9	1.1	1.1	0.9	0.8
	CA	1.7	1.0	0.7	0.7	0.5	0.5
	SA	4.4	2.9	1.8	1.9	1.4	1.1
Cu	J	0.7	0.7	0.6	0.8	0.7	0.7
	P	0.6	0.6	0.6	0.7	0.6	0.7
	NA	0.6	0.6	0.9	1.0	0.8	0.7
	CA	0.7	0.8	0.8	0.8	0.8	0.7
	SA	1.4	1.2	1.2	1.3	0.9	1.0
Pb	J	6.2	2.3	1.1	1.2	0.9	0.8
	P	5.7	2.3	1.4	1.3	0.8	0.9
	NA	5.8	2.5	0.9	0.9	1.0	0.7
	CA	5.7	2.1	0.7	0.7	0.5	0.4
	SA	17.3	10.3	3.5	3.5	1.7	1.5
Zn	J	0.9	0.8	0.8	0.7	0.8	0.7
	P	1.1	0.9	0.8	1.2	0.6	0.9
	NA	1.0	1.0	0.9	1.0	0.7	0.7
	CA	1.1	0.8	0.8	0.9	0.7	0.8
	SA	2.1	1.9	1.4	1.7	1.3	1.3

The concentrations of trace elements (C_{el}) accumulated by biomonitors can be converted into estimates of PTE deposition rates (D) [3–5,29] according to the formula:

$$D = C_{el} \cdot R \cdot t^{-1} \quad (1)$$

where R is the weight/area ratio and is the time period the sample is covering. Assuming that the final concentrations in moss represent an average equilibrium of three years with the environmental conditions at the site and knowing that pleurocarpous moss species have a weight/area ratio of 175 g m^{-2} [30], we were able to estimate annual element deposition rates at background Swiss sites as follows: $\text{Cd} = 8 \text{ g km}^{-2} \text{ y}^{-1}$, $\text{Cu} = 0.4 \text{ kg km}^{-2} \text{ y}^{-1}$, $\text{Pb} = 0.2 \text{ kg km}^{-2} \text{ y}^{-1}$, and $\text{Zn} = 1.9 \text{ kg km}^{-2} \text{ y}^{-1}$. Estimated values of Cd and Pb were consistent with the lowest annual total deposition of these elements modeled for Switzerland ($\text{Cd} < 10 \text{ g km}^{-2} \text{ y}^{-1}$, $\text{Pb} < 0.28 \text{ kg km}^{-2} \text{ y}^{-1}$) in the framework of the EMEP (European Monitoring and Evaluation Program) project, a co-operative program for monitoring and evaluation of the long-range transmission of air pollutants in Europe [31].

According to Aboal et al. [32], estimation of bulk atmospheric deposition from moss data is possible only for some PTEs such as Cd and Pb, because these elements are almost exclusively of atmospheric origin. However, for other elements this relationship is more labile since element concentrations in moss represent a steady state of non-equilibrium with the surrounding environment, rather than a time-integrated measure of element deposition [33]. Nevertheless, despite these limitations, which are intrinsic of a biological surrogate method, moss monitoring remains a very useful and efficient way of determining spatial and temporal patterns of PTEs, allowing the detection of relevant element sources and temporal trends. From this perspective, small- as well as large-scale biomonitoring surveys have, thus, their own value in regional, national, and international projects aiming at evaluating biological effects of airborne PTEs.

4. Conclusions

We statistically estimated background values for Cd, Cu, Pb, and Zn accumulated in moss from Switzerland using 10 remote sites. Our data showed that background values

change over time and can be used as reference, when assessing spatial and temporal trends expressed in terms of bioaccumulation ratios with values at other sites. The use of annual background values is of great importance to identify spatial trends, while the background value over all sampling periods helps to identify temporal trends. The latter are consistent with those reported in other comprehensive, similar biomonitoring studies and require to be updated in time, possibly every five years. The use of cutoff values to be used as benchmark for bioaccumulation ratios is invaluable in having a scale for assessing ecological quality.

Author Contributions: S.L. conceived and designed the study; M.M. provided the data; Z.K. managed and reprocessed the raw data; S.L. and Z.K. analyzed the data; S.L. wrote the paper; Z.K. and M.M. supervised the text. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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