MOSS INTERSPECIES COMPARISONS IN TRACE ELEMENT CONCENTRATIONS

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Abstract. Within the framework of a European-scaled moss survey, various moss species were sampled throughout The Netherlands [NL], Germany [D], and Switserland [CH], and used in moss interspecies comparisons of elemental concentrations. Moss species considered were *Pleurozium schreberi* [NL,D,CH], *Brachythecium rutabulum* [NL], *Hypnum cupressiforme* [D,CH], *Hylocomium splendens* [D,CH], and *Scleropodium purum* [D]. Element analysis was carried out directly (The Netherlands, Instrumental Neutron Activation Analysis: As, Br, Ce, Cr, Cs, Fe, La, Mn, Na, Rb, Sc, Se, Sm, Th, Ti, V and Zn), or after sample digestion (The Netherlands, ICP-MS: Pb, Germany, AAS/ICP-AES: Cd, Cr, Cu, Fe, Ni, Pb, Ti, V, and Zn; Switzerland, ICP-AES/ICP-MS: Co, V, and Zn).

Local variations (= within sampling sites) in element concentrations were estimated, based upon in-site multiple sampling and analysis of *Pleurozium schreberi* species in The Netherlands. Element concentrations in moss species were compared in linear correlations, both in unweighted and weighted fits, with weighing factors based on the local variation data. Weighted fits were shown to generally improve the calibration characteristics, as tracked by χ^2 calculations.

The calibration data suggest the presence of previously unnoticed outliers in element concentrations. The absence of further information, however, may prescribe the use of all data in comparison procedures. These results indicate that interlaboratory analysis of replicate samples and the use of dedicated certified reference materials may help solving problems in the analysis of the sample series.

For several of the considered interspecies comparisons, weighted calibrations could be based on significant correlations ($P = 0.05$). Actual use, however, will remain arbitrarily decided upon, and may be based on decisions as to what to accept with respect to the levels of uncertainty in the calibration parameters. Furthermore, the use of calibrations in extrapolation modes is greatly restricted by the necessary reservations in geographically larger-scaled applications.

1. Introduction

In trace element biomonitoring studies, the selection of the species to be used is of primary importance, especially with respect to aspects like abundance, tolerance,

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ease of sampling, background values and accumulation factors (Sloof *et al.*, 1988; Markert, 1993a). Many investigations have been performed using a single species, in which the absence or presence made the selection of exact locations of sampling sites possible (Tyler, 1989; Sloof and Wolterbeek, 1991; Markert, 1993a, b, 1994). However, the tolerance of biomonitor species to elevated tissue concentrations of heavy metals may vary greatly between species as well as between elements (Puckett, 1988; Markert and Weckert, 1993). The latter makes it relatively difficult to set up any biomonitoring survey which is sufficiently large-scaled in both a geographical sense and in the number and concentrations of elements of interest, without getting problems with the selection of sampling sites and/or biomonitoring species.

One of the principal solutions to these problems may be the use in surveys of more than a single monitor species; in practice, however, any of these surveys should be accompanied by full calibrations on interspecies responses to variable element concentrations. Only a few studies have been carried out on interspecies calibration (Taylor and Witherspoon, 1972; Folkeson, 1979; Boileau *et al.*, 1982; Sloof and Wolterbeek, 1993), and all were restricted to relatively small geographical areas: Taylor and Witherspoon (1972) experimented locally with simulated ^{134}Cs fallout particles, Folkeson (1979) obtained biomaterials from the surroundings of a brass foundry, Boileau *et al.* (1982) collected lichens and mosses around two uranium mining sites, and Sloof and Wolterbeek (1993) exclusively used lichen species from a 1000 km^2 industrial area. As the interspecies correlations may depend on the range of element concentrations and chemo-geographical conditions, these aspects should be of similar scaling for both the interspecies calibration and the actual survey.

In the present paper, the biomonitoring species of interest were mosses, which were samples within the frame work of a European-scaled survey carried out in 1990–1992. Here, element analysis in moss samples was used to predict heavy metal atmospheric deposition. Since the early work of Rühling and Tyler (1970, 1973), mosses have been regularly used in larger-scaled monitoring surveys (Rühling *et al.*, 1987, 1992; Schaug *et al.*, 1990; Steinnes *et al.*, 1992); the account of the above-mentioned 1990–1992 survey has been published (Rühling, 1994) as part of a series of publications of the NORDIC Council of Ministers (see Rühling *et al.*, 1987, 1992).

The present data on mosses were obtained from chemical analysis of moss species which were sampled throughout The Netherlands (Kuik and Wolterbeek, 1994), Germany (Herpin *et al.*, 1994) and Switzerland (Schmid-Grob *et al.*, 1993). At each sampling site, various moss species were taken. to permit a large-scaled moss interspecies comparison on heavy metal concentrations.

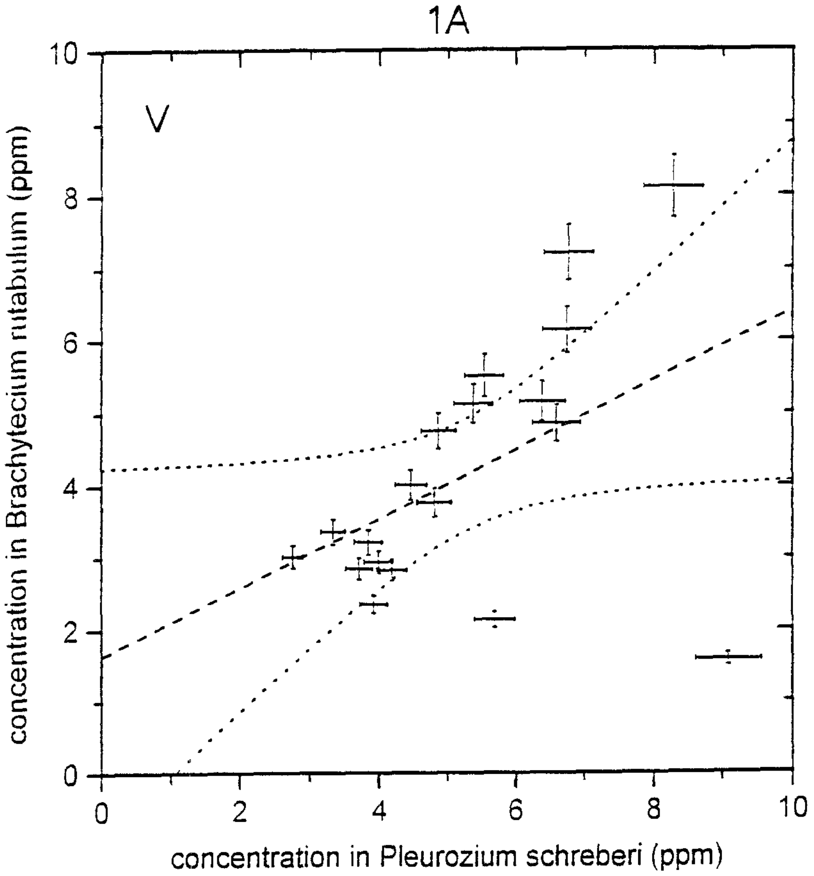

Fig. 1a.

Figs. 1(a)–(d). Comparisons of concentrations (ppm = mg kg^{-1}) of vanadium in *Pleurozium schreberi* and *Brachythecium rutabulum* (The Netherlands). Figure 1 A, B shows all data, Figure 1 C, D shows results after removal of two particular data. Figure 1A and 1C shows unweighted fits, Figure 1B and 1D present weighted results. The dashed lines represent the fitted $y = ax + b$ functions, the dots indicate the 95% probability areas. The uncertainties (*x,y directions*) in the individual data are expressed by error bars.

2. Materials and Methods

2.1. MOSS SPECIES COMPARISONS

Moss species sampled were *Pleurozium schreberi (P.s), Brachythecium rutabulum (B.r), Hypnum cupressiforme (H.c.), Hylocomium splendens (H.s.),* and *Scleropodium purum (S.p.).* Of these species, *P.s.* and *B.r.* (19 sampling sites) were compared in The Netherlands. In Germany, intercomparisons were carried out on *H.c.* and *P.s.* (25 sites), *H.s.* and *P.s.* (11 sites), and *S.p.* and *P.s.* (49 sites); in Switzerland *H.c.* and *H.s.* (45 sites) were compared.

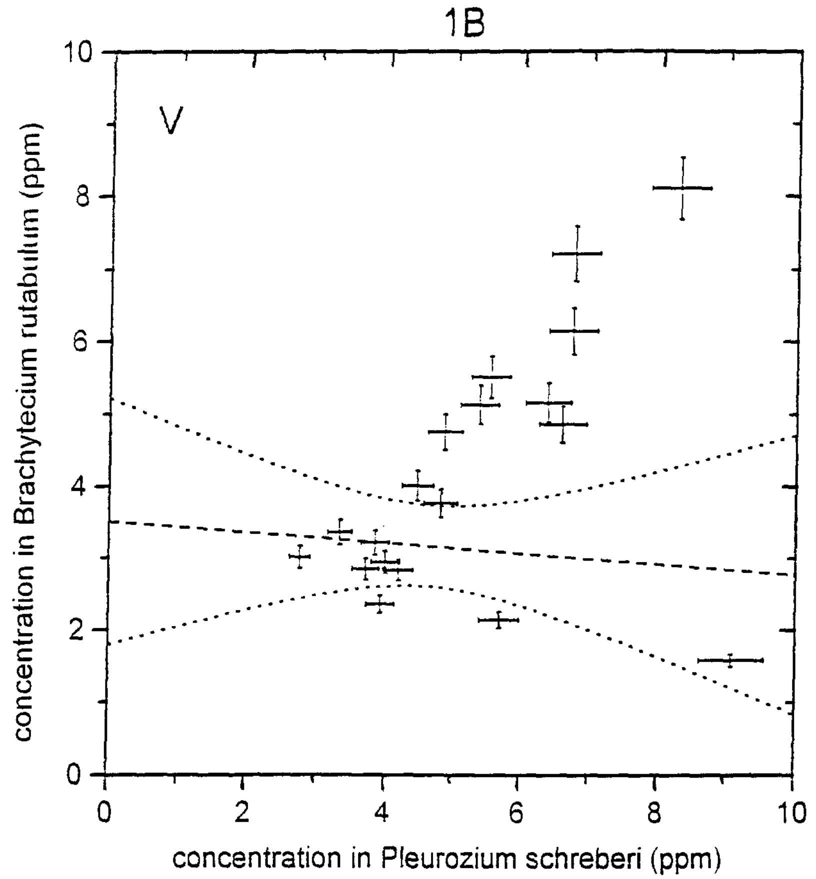

Fig. 1b.

2.2. SAMPLING SITE CHARACTERISTICS

Moss samples were mainly taken in forests. Sampling spots were chosen as not directly exposed to throughfall precipitation, which, in practice, means that mosses were gathered at least 5 m away from the nearest tree. Sampling sites were located at least 300 m away from the nearest highway, village or industrial settlement, and at least 100 m away from any road or single house. At each sampling site, 5 to 10 subsamples were taken within an area of 50 × 50 m. These subsamples were mixed after cleaning, or analyzed separately (6 sites in The Netherlands), in the latter case to permit determination of local (= within sampling sites) variations.

2.3. SAMPLING, AND SAMPLE HANDLING

Sampling and sample handling was performed using polyethylene gloves; samples were stored in paper/polyethylene bags. Throughout the study, paired moss species

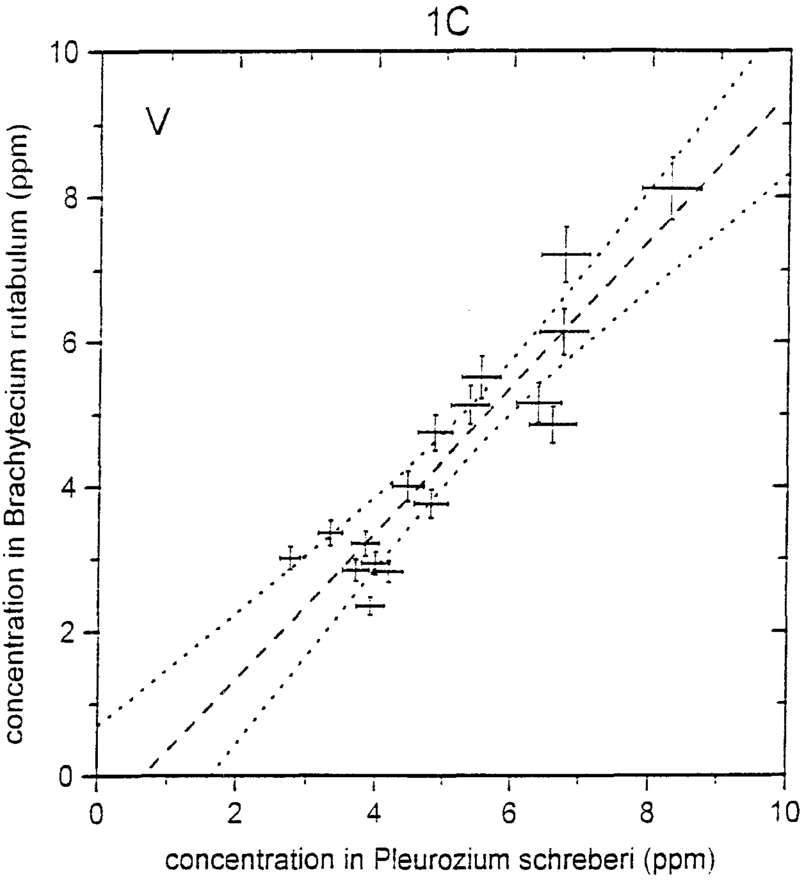

Fig. 1c.

were sampled at each selected location. In the laboratory, the upper three segments of *Hylocomium splendens*, or the green-greenbrown parts of *Pleurozium schreberi, Hypnum cupressiforme, Scleropodium purum* or *Brachythecium rutabulum* were separated and further processed.

2.3.1. *Germany/Switzerland*

The samples obtained were dried at 40 °C for 24 h, homogenized in an agate ball mill (Germany), or used completely (Switzerland). The sample were digested in concentrated HNO_3 under pressure in closed quartz (Germany, about 400 mg portions) or Teflon vessels (Switzerland, about 1.0–1.5 g portions). After digestion, samples were diluted to about 40 ml in bidistilled water (Germany) or Millipore water (Switzerland). The Swiss samples were vacuum-filtered over porous polyethylene filters, the German samples were subjected to analysis directly.

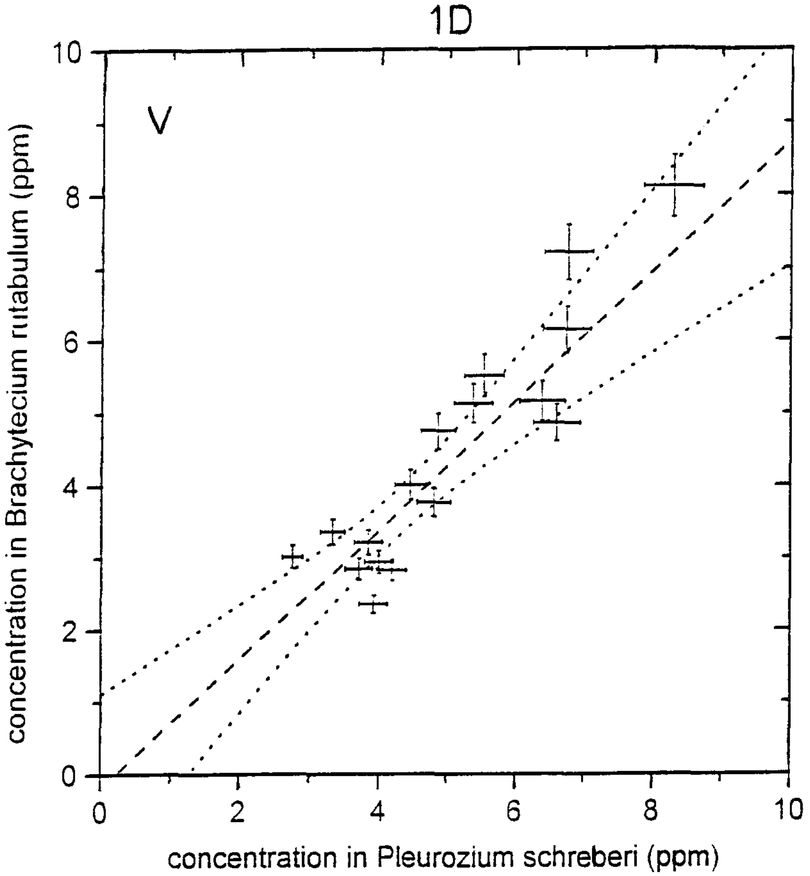

Fig. 1d.

2.3.2. *The Netherlands*

The samples obtained were dried 40 °C for 24 h, homogenized in an agate ball mill, and weighed-in directly in dried form (about 200 mg portions) in polyethylene capsules. For Pb determinations, about 200 mg sample was digested in a 7 ml 2 : 5 mixture of HNO_3 and H_2SO_4 for 14 h at 140 °C. After digestion, samples were diluted in 100 ml deionized water, and subjected to analysis directly.

2.4. ANALYSIS

2.4.1. *Germany*

The analysis of Cd and Pb was carried out by AAS. For Cr, Cu, Fe, Ni, V, Ti and Zn measurements were performed by ICP–AES. Analytical performance was checked by regular analysis of the reference materials Citrus Leaves (NBS-1572) and Pine Needles (NBS-1575).

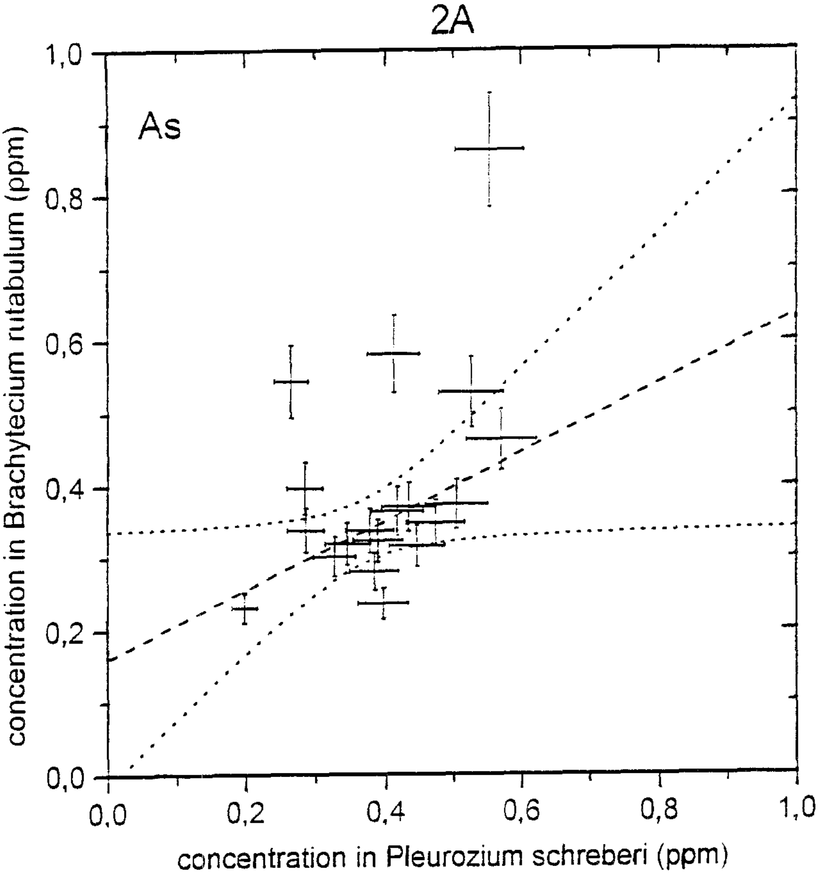

Fig. 2a.

Figs. 2(a)–(d). Comparisons of concentrations (ppm = mg kg^{-1}) of arsenic (As, Figure 2A), chromium (Cr, Figure 2B), vanadium (V, Figure 2C) and zinc (Zn, Figure 2D) in *Pleurozium schreberi* and *Brachythecium rutabulum* (The Netherlands). All lines were derived from weighted fits. For further details see Figure 1.

2.4.2. *Switzerland*

The analysis of Co, V, and Zn was performed by ICP–MS. Analytical performance was checked by regular analysis of the reference materials Olive Leaves (BCR-62) and Platihypnidium Riparioides (BCR-61).

2.4.3. *The Netherlands*

The analysis of As, Br, Ce, Co, Cr, Cs, Fe, La, Mn, Na, Rb, Sb, Sc, Se, Sm, Th, V, and Zn was carried out by Instrumental Neutron Activation. For Pb, measurements were performed by ICP–MS. Analytical performance was checked by the regular analysis of the reference materials Citrus Leaves (NBS-1572) and Orchard Leaves (NBS-1571).

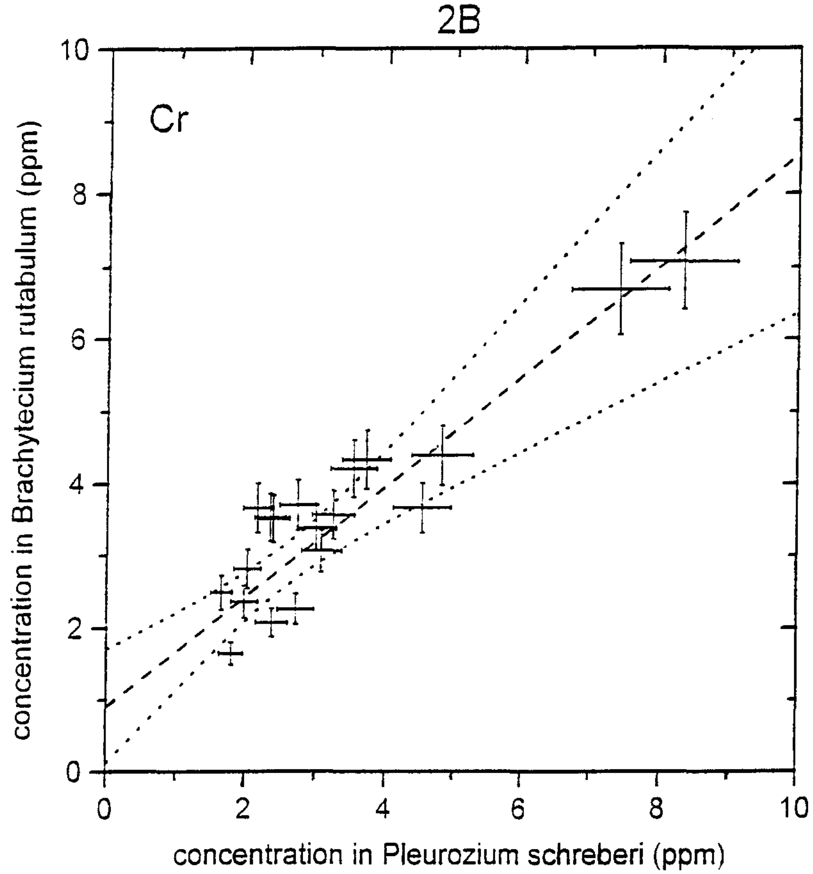

Fig. 2b.

2.5. STATISTICS

The interspecies comparisons were performed using the linear relationship $y = ax + b$, where x and y are the element concentrations of two different moss species in corresponding sampling locations, and a and b constants. For all fits, constants a and b were calculated, as well as the linear correlation coefficient, the average concentration ratio (y/x) and the reduced χ^2. Furthermore, for each element, both a weighted and an unweighted fit was carried out.

In the weighted fit, weighted factors were determined by the uncertainties in both x and y, using an iterative process. In the first step, only the uncertainties Δy_i in the y-values y_i ($i = 1, ..., N$, with N the number of data points) are taken into account, using the standard approach for a weighted linear least squares fit with weighing factors $w_i = (\Delta y_i)^{-2}$. This fit yields a first approximation $a^{(1)}$, $b^{(1)}$ of the constants a and b, which are then used in the next step to convert the uncertainties

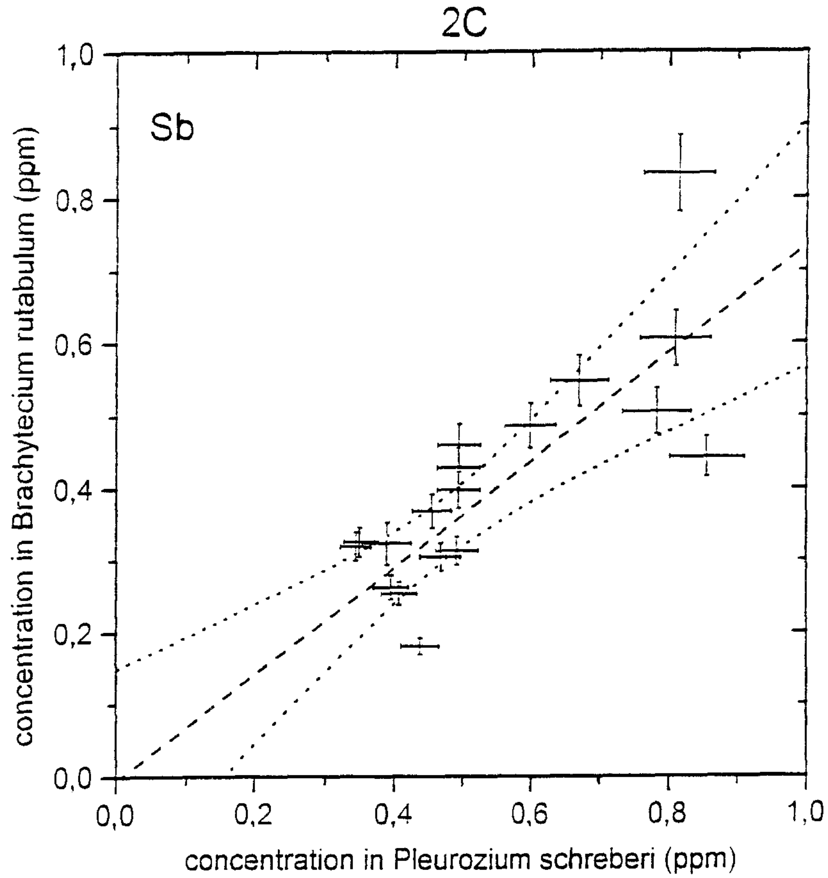

Fig. 2c.

Δx_i in the x-values x_i to virtual uncertainties in the y-values by multiplication with the slope $a^{(1)}$. Thus the total incertainty in y_i becomes

$$\Delta y_i^{\text{tot}} = [(\Delta y_i)^2 + (a^{(1)} \cdot \Delta y_i)^2]^{1/2}, \tag{1}$$

resulting in new weighing factors $w_i' = (\Delta y_i^{\text{tot}})^{-2}$. The fit is then repeated with these new weighing factors, yielding a second – more refined – approximation $a^{(2)}$, $b^{(2)}$ of the constants a and b. Thus the iterative process consists of repeated calculations of the weighing factors as outlined above, each time using the estimated value of the constant a from the previous iteration step. The iterations should be continued until the relative changes in subsequent estimations of a and b have become sufficiently small. In practice, two or three steps were found to suffice in most cases. The applied uncertainties were based on an average relative standard error of the mean (SEM) e_r (in percent), as obtained by 5-fold analysis of *Pleurozium schreberi* samples from 6 sites in the Netherlands: $\Delta x_i = e_r \cdot x_i/100$; $\Delta y_i = e_r \cdot y_i/100$.

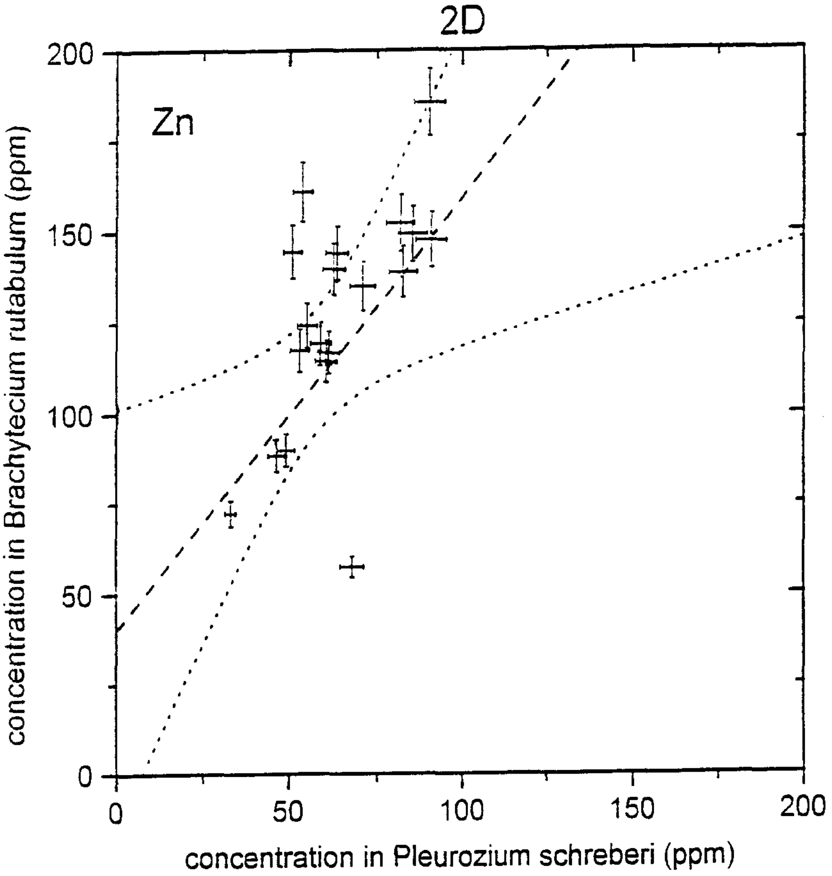

Fig. 2d.

In the unweighted fit, weighing factors were equal for all data points: here all concentrations were assigned an identical 'average' error, which was determined as follows. Again assuming a relative uncertainty e_r (in percent), all x-values were assigned an identical absolute uncertainty $\Delta x = e_r \cdot \bar{x}/100$, with $\bar{x}$ the average x-value. Likewise, all y-values were assigned an uncertainty $\Delta y = e_r \cdot \bar{y}/100$, with $\bar{y}$ the average y-value.

3. Results and Discussion

For 6 locations in The Netherlands, the 5-fold sampled *Pleurozium schreberi* species were analyzed separately, yielding an estimate of the local variance for a number of elements (Table I). As may be clear, these local variances comprise the uncertainties arising from sampling, sample handling as well as element analysis itself. In all further calculations, and for all species considered, local variances

TABLE I

Relative values (%) for standard errors of the means (SEM) of element concentrations in *Pleurozium schreberi* species, obtained from 5-fold sampling and analysis of samples in 6 selected sites in The Netherlands

Element	SEM (%)	Element	SEM (%)
As	9.1	Pb	6.1
Br	8.0	Rb	9.7
Ce	8.8	Sb	6.3
Co	5.9	Sc	6.4
Cr	9.4	Se	12.3
Cs	13.5	Sm	6.7
Fe	7.8	Th	6.6
La	6.3	V	5.2
Mn	15.0	Zn	5.0
Na	8.1		

were assumed to be like the values given in Table I. The unweighted fits were carried out with weighing factors equal for all data points; their magnitudes were based on the relative errors e_r directly taken from Table I.

Table II presents intercomparison data for *Pleurozium schreberi* and *Brachythecium rutabulum*, obtained from the survey in The Netherlands. In general, the concentration ranges in both species were of comparable order of magnitude (except for Zn, see Figure 2), but y/x ratios regularly differed from unit values. The absence of differences in y/x values for unweighted means and median values may indicate the absence of effects of severely outlying data; apart from *V*, the latter is also indicated by the absence of differences between values for weighted and inweighted slopes and y/x ratios. For *V* (Figure 1A, B), both weighted and unweighted comparisons are hampered by the presence of two particular data points (note the differences in slopes of weighted and unweighted fits), without which comparisons were obtained as indicated in Figure 1C, D. Generally comparing slopes with y/x values shows the slopes to be somewhat smaller than the y/x values, meaning that *Brachythecium rutabulum* may be a better accumulator at relatively low concentrations. The presence of significant intercept (*b*) values should be interpreted as indicating that the value of the y/x ratio may depend on concentration (Sloof and Wolterbeek, 1993), with the more severe effects shown by the larger differences between slope and y/x values. The reduced χ^2 values (Table II) demonstrate the effects of weighted fits; their absolute values also suggest that the use of SEM values may give an underestimate of the local variation by a factor of 2 to 5. Possibly, the use of SD values (larger by about a factor of 2) may have been a better measure

TABLE II

Interspecies calibration between *Pleurozium schreberi* (x variable) and *Brachythecium rutabulum* (y variable), obtained from 19 sites in The Netherlands, except for Pb ($n = 18$), Sb ($n = 18$) and Ti ($n = 14$). For all elements, weighted (W) and unweighted (U) mean y/x ratios and fits have been calculated. [1] Mean or median ratio y/x differ significantly from unit value ($P = 0.05$). [2] Significant ($P = 0.05$) correlation. Absolute uncertainties in the determined quantities are given between parentheses

Element	Concentration range x (μg/g)	Concentration range y (μg/g)	Median ratio y/x	Mean ratio coefficient	Correlation	Slope	Intercept	Reduced chi square
As	0.198–0.570	0.232–0.863	0.90	1.02 (U)	0.469[2]	0.69 (0.32)	0.12 (0.13)	2.1
				0.87 (W)[1]	0.465[2]	0.48 (0.22)	0.16 (0.08)	6.6
Br	1.597–4.57	1.721–5.844	1.18	1.19[1]	0.653[2]	0.82 (0.23)	0.91 (0.66)	7.7
				1.04	0.592[2]	0.70 (0.23)	0.99 (0.58)	7.0
Ce	0.598–2.217	0.700–1.822	1.13	1.13	0.597[2]	0.56 (0.18)	0.54 (0.20)	6.4
				1.01	0.623[2]	0.59 (0.18)	0.43 (0.17)	6.8
Co	0.163–0.501	0.193–0.660	1.23[1]	1.28[1]	0.741[2]	0.98 (0.22)	0.08 (0.07)	8.5
				1.18[1]	0.766[2]	1.10 (0.22)	0.03 (0.06)	7.8
Cr	1.653–8.323	1.654–7.08	1.13	1.15[1]	0.919[2]	0.71 (0.07)	1.25 (0.28)	2.0
				1.04[1]	0.803[2]	0.76 (0.14)	0.92 (0.38)	3.1
Cs	0.125–0.684	0.095–1.609	0.85	1.22	0.462[2]	1.32 (0.61)	−0.02 (0.17)	27
				0.85[1]	0.663[2]	1.06 (0.29)	−0.03 (0.06)	4.1
Fe	330.8–1174	409.0–965.6	1.04	1.03	0.675[2]	0.58 (0.15)	269 (100)	4.7
				0.94[1]	0.653[2]	0.60 (0.17)	223 (100)	4.5
La	0.321–0.973	0.349–0.964	1.07	1.09	0.606[2]	0.64 (0.20)	0.23 (0.11)	9.5
				0.99	0.655[2]	0.70 (0.20)	0.16 (0.10)	7.6
Mn	110.4–794.5	125.8–1085	1.61[1]	1.66[1]	0.751[2]	0.95 (0.20)	149 (60)	4.7
				1.32[1]	0.675[2]	1.06 (0.28)	80 (54)	3.1
Na	129.8–254.5	125.9–377.6	1.05[1]	1.17[1]	0.494[2]	1.00 (0.43)	33 (84)	9.1
				1.02	0.542[2]	0.91 (0.34)	29 (62)	7.5
Pb	11–29	11.4–27	1.14	1.20 (U)[1]	0.607[2]	0.49 (0.16)	11.6 (2.9)	6.2
				1.08 (W)[1]	0.578[2]	0.57 (0.20)	9.3 (3.4)	7.8

TABLE II

(Continued)

Element	Concentration range X (μg/g)	Concentration range Y (μg/g)	Median ratio Y/X	Mean ratio coefficient	Correlation	Slope	Intercept	Reduced chi square
Rb	11.99–29.5	15.67–48.81	1.33^1	1.40^1	0.521^2	0.97 (0.38)	8.6 (8.3)	5.4
				1.23^1	0.605^2	0.99 (0.32)	5.9 (6.3)	4.5
Sb	0.344–1.066	0.181–0.974	0.81^1	0.77^1	0.866^2	0.84 (0.12)	–0.043 (0.08)	6.2
				0.68^1	0.785^2	0.74 (0.15)	–0.005 (0.07)	7.0
Sc	0.062–0.233	0.073–0.211	1.02	1.04	0.558^2	0.53 (0.19)	0.06 (0.02)	14
				0.93^1	0.633^2	0.58 (0.17)	0.04 (0.02)	8.6
Se	0.340–1.092	0.200–0.831	0.65^1	0.73^1	0.759^2	0.79 (0.16)	–0.03 (0.09)	3.5
				0.64^1	0.710^2	0.71 (0.17)	–0.02 (0.08)	2.5
Sm	0.038–0.148	0.048–0.131	1.09	1.12	0.677^2	0.64 (0.17)	0.03 (0.01)	9.2
				1.01	0.680^2	0.65 (0.17)	0.03 (0.01)	6.8
Th	0.069–0.233	0.068–0.198	1.02	1.09	0.591^2	0.64 (0.21)	0.05 (0.03)	17
				0.92^1	0.599^2	0.66 (0.21)	0.03 (0.02)	12
Ti	33.48–103.6	29.7–106.1	1.05	1.15	0.188	–0.21 (0.32)	74 (19)	11
				0.78^1	0.205	–0.20 (0.28)	64 (17)	11
V	2.75–0.076	1.586–8.114	0.84^1	0.81^1	0.460^2	0.48 (0.22)	1.7 (1.2)	40
				0.44	–0.109	–0.07 (0.16)	3.5 (0.9)	53
Zn	33.01–90.76	57.52–185.7	1.91^1	2.02^1	0.609^2	1.21 (0.38)	49 (25)	12
				1.72^1	0.554^2	1.19 (0.17)	40 (26)	21

TABLE III

Interspecies calibration between *Pleurozium schreberi* (*x* variable) and *Hypnum cupressiforme* (*y* variable), obtained from 25 sites in Germany. For all elements, weighted (W) and unweighted (U) mean *y/x* ratios and fits have been calculated. [1] Mean or median ratio *y/x* differ significantly from unit value ($P = 0.05$). [2] Significant ($P = 0.05$) correlation. Absolute uncertainties in the determined quantities are given between parentheses (uncertainties of cadmium levels are unknown)

Element	Concentration range *x* (μg/g)	Concentration range *y* (μg/g)	Median ratio *y/x*	Mean ratio coefficient	Correlation	Slope	Intercept	Reduced chi square
Cd	0.155–0.777	0.202–1.7	1.04	1.14 (U)^1	0.849^2	1.86 (0.24)	–0.22 (0.09)	–
Cr	0.618–3.28	0.607–3.59	1.09	1.07 (U)	0.774^2	0.89 (0.15)	0.23 (0.27)	7.5
				0.86 (W)^1	0.709^2	0.70 (0.15)	0.32 (0.18)	7.8
Cu	4.46–12.9	4.58–14.8	1.01	1.00	0.705^2	0.79 (0.17)	1.54 (1.41)	0.9
				0.89^1	0.611^2	0.59 (0.16)	2.54 (1.20)	0.9
Fe	149–1327	180–1340	0.98	0.98	0.691^2	0.71 (0.16)	124 (89)	19
				0.82^1	0.761^2	0.70 (0.12)	76 (48)	9.3
Ni	0.59–4.07	0.65–5.53	1.25	1.37^1	0.697^2	0.92 (0.20)	0.68 (0.45)	1.5
				1.04	0.757^2	0.84 (0.15)	0.51 (0.23)	1.4
Pb	5.72–80.5	5.33–145	1.05	1.09	0.938^2	1.71 (0.13)	–9.0 (2.9)	21
				0.89^1	0.757^2	0.79 (0.14)	2.1 (1.6)	17
Ti	3.92–48	6–68.2	0.94	1.00	0.708^2	0.81 (0.17)	2.3 (3.2)	26
				0.77^1	0.672^2	0.57 (0.13)	3.1 (1.5)	8.8
V	1.32–6.09	1.15–5.44	0.92	0.98	0.546^2	0.51 (0.16)	1.3 (0.5)	30
				0.77^1	0.633^2	0.56 (0.14)	0.79 (0.39)	28
Zn	23.9–61.8	30.4–81.4	1.15^1	1.16^1	0.739^2	1.24 (0.22)	0.63 (9.3)	9.2
				1.07^1	0.687^2	0.90 (0.20)	8.0 (7.8)	9.5

TABLE IV

Interspecies calibration between *Pleurozium schreberi* (x variable) and *Hylocomium splendens* (y variable), obtained from 11 sites in Germany. For all elements, weighted (W) and unweighted (U) mean y/x ratios and fits have been calculated. [1] Mean or median ratio y/x differ significantly from unit value ($P = 0.05$). [2] Significant ($P = 0.05$) correlation. Absolute uncertainties in the determined quantities are given between parentheses (uncertainties of cadmium levels are unknown)

Element	Concentration range x (μg/g)	Concentration range y (μg/g)	Median ratio y/x	Mean ratio coefficient	Correlation	Slope	Intercept	Reduced chi square
Cd	0.200–0.315	0.155–0.31	0.88	0.89 (U)[1]	0.828[2]	1.15 (0.26)	–0.06 (0.07)	–
Cr	0.975–2.11	1.07–4.51	1.06	1.18 (U)	0.709[2]	1.97 (0.65)	–1.1 (1.0)	6.1
				0.97 (W)	0.591	1.06 (0.48)	–0.04 (0.65)	8.2
Cu	4.12–11.5	4.88–14.4	1.07	1.13	0.780[2]	1.03 (0.28)	0.77 (2.30)	0.6
				1.06[1]	0.853[2]	1.01 (0.21)	0.62 (1.47)	0.5
Fe	202–622	210–1583	1.16	1.33	0.369	1.01 (0.84)	132 (370)	43
				1.05	0.687[2]	0.88 (0.31)	86 (110)	13
Ni	1.07–2.77	1.39–5.1	1.28	1.34[1]	0.788[2]	1.39 (0.36)	–0.12 (0.71)	0.8
				1.18[1]	0.709[2]	0.97 (0.32)	0.45 (0.52)	0.7
Pb	9.78–12.8	9.19–18.7	1.16	1.14[1]	0.696[2]	1.82 (0.63)	–7.6 (7.1)	2.5
				1.08[1]	0.669[2]	1.66 (0.62)	–6.1 (6.8)	3.2
Ti	4.64–33.8	4.52–47	1.20	1.36	0.136	0.21 (0.50)	12.8 (7.6)	81
				0.59[1]	0.427	0.30 (0.21)	4.9 (2.4)	27
V	1.54–4.12	1.75–6.1	1.16	1.12	0.764[2]	1.25 (0.35)	–0.34 (0.92)	13
				1.01	0.514	0.63 (0.35)	0.99 (0.39)	16
Zn	27.2–52.4	26.5-81.6	1.00	1.09	0.369	0.67 (0.52)	17 (22)	29
				1.00	0.639[2]	0.79 (0.32)	9.0 (12)	13

TABLE V

Interspecies calibration between *Pleurozium schreberi* (x variable) and *Scleropodium purum* (y variable), obtained from 49 sites in Germany. For all elements, weighted (W) and unweighted (U) mean y/x ratios and fits have been calculated. [1] Mean or median ratio y/x differ significantly from unit value ($P = 0.05$). [2] Significant ($P = 0.05$) correlation. Absolute uncertainties in the determined quantities are given between parentheses (uncertainties for Cd are unknown)

Element	Concentration range x (μg/g)	Concentration range y (μg/g)	Median ratio y/x	Mean ratio coefficient	Correlation	Slope	Intercept	Reduced chi square
Cd	0.155–0.707	0.159–0.681	1.12	1.11 (U)[1]	0.800[2]	0.87 (0.10)	0.06 (0.03)	–
Cr	0.618–4.95	0.607–4.08	0.94	0.98 (U)	0.749[2]	0.79 (0.10)	0.25 (0.18)	8.2
				0.79 (W)[1]	0.653[2]	0.61 (0.10)	0.33 (0.14)	7.1
Cu	4.32–12.91	4.03–17.2	0.98	1.02	0.693[2]	0.86 (0.13)	1.12 (1.04)	0.9
				0.90[1]	0.635[2]	0.71 (0.13)	1.69 (0.90)	0.9
Fe	149–1177	142–1555	0.88	0.93	0.729[2]	0.83 (0.11)	47 (61)	19
				0.76[1]	0.763[2]	0.72 (0.09)	41 (31)	9.6
Ni	0.59–4.75	0.53–5.32	0.92	0.99	0.547[2]	0.60 (0.14)	0.66 (0.30)	2.4
				0.62[1]	0.231	0.19 (0.12)	0.96 (0.22)	2.6
Pb	5.72–25.5	5.95–20.51	0.86[1]	0.88[1]	0.869[2]	0.76 (0.06)	1.2 (0.8)	4.8
				0.83[1]	0.778[2]	0.66 (0.08)	2.1 (0.8)	4.8
Ti	3.53–33.8	2.62–43.2	0.88	0.95	0.624[2]	0.69 (0.13)	2.6 (1.8)	20
				0.55[1]	0.436[2]	0.33 (0.10)	2.6 (1.0)	20
V	0.92–6.14	1.01–4.92	0.81[1]	0.84[1]	0.741[2]	0.62 (0.08)	0.49 (0.25)	21
				0.67[1]	0.572	0.40 (0.08)	0.80 (0.20)	25
Zn	23.9–117	27.2-135	1.16[1]	1.26[1]	0.766[2]	0.90 (0.11)	15 (6)	15
				1.12[1]	0.650[2]	0.77 (0.13)	17 (6)	14

TABLE VI

Interspecies calibration between *Pleurozium schreberi* (x variable) and *Hypnum cupressiforme* (y variable), obtained from 45 sites in Switzerland. For all elements, weighted (W) and unweighted (U) mean y/x ratios and fits have been calculated. [1] Mean or median ratio y/x differ significantly from unit value ($P = 0.05$). [2] Significant ($P = 0.05$) correlation. Absolute uncertainties in the determined quantities are given between parentheses

Element	Concentration range x (μg/g)	Concentration range y (μg/g)	Median ratio y/x	Mean ratio coefficient	Correlation	Slope	Intercept	Reduced chi square
Co	0.129–1.19	0.126–2.28	0.96	1.37 (U)	0.183	0.38 (0.31)	0.32 (0.14)	83
				0.54 (W)[1]	0.042	0.03 (0.11)	0.23 (0.05)	66
V	0.986–6.039	0.508–4.314	0.66[1]	0.81[1]	0.243	0.15 (0.09)	1.58 (0.29)	61
				0.48[1]	0.467[2]	0.30 (0.09)	0.64 (0.22)	55
Zn	13.8–61.2	17.1–92.7	1.08	1.15[1]	0.290	0.43 (0.21)	21.3 (7.3)	63
				0.75[1]	0.058	0.05 (0.13)	24.8 (4.3)	51

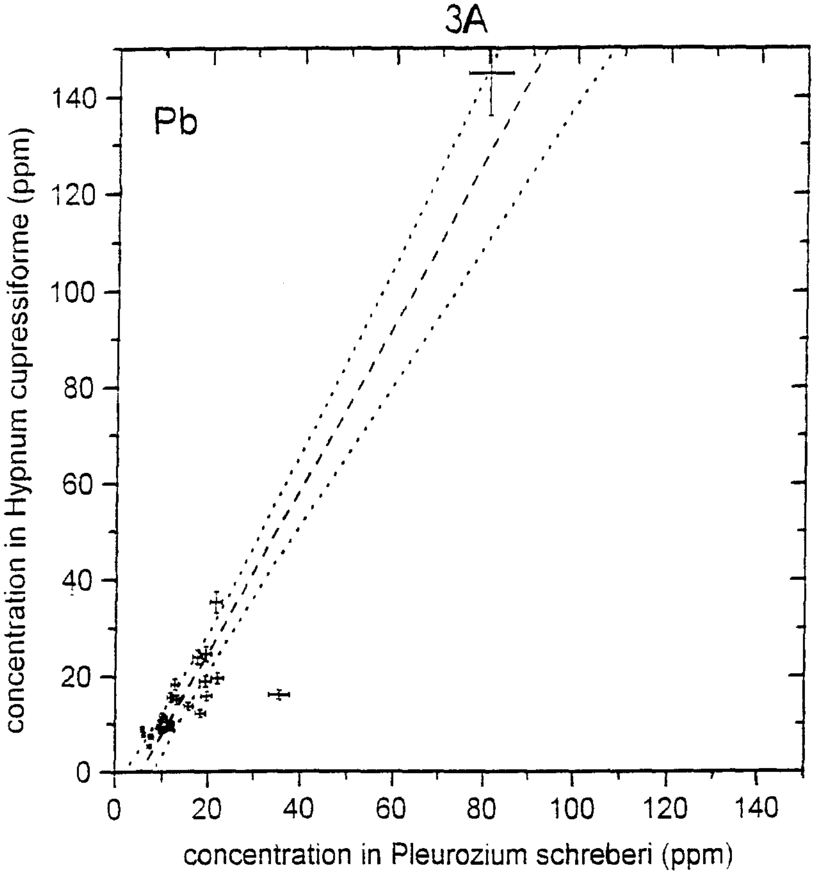

Fig. 3a.

Figs. 3(a)–(c). Comparisons of concentrations (ppm = mg kg^{-1}) of lead (Pb) in *Pleurozium schreberi* and *Hypnum cupressiforme* (Germany). Figure 3A, B gives the respective unweighted and weighted fit of all data, Figure 3C presents the weighted relations after removal of the single high Pb concentration observed in *Hypnum cupressiforme*. For all further details see Figure 1.

(cf. Ross, 1990). The present use of SEM values, however, was essentially based on the presence in each of the sample of multiple moss plants taken from adjacent sampling positions.

Figure 2 shows graphs of weighted fits for the elements As, Cr, Sb, and Zn, for which Dutch data were taken as represented in Table II. The graphs clearly indicate the differences between elements in 95% reliability areas; they further show the differences in element concentration ranges between moss species, the latter most clearly for Zn.

Tables III–V present interspecies calibrations for various species sampled in Germany: *Pleurozium schreberi* was compared with *Hypnum cupressiforme* (Table III), *Hylocomium splendens* (Table IV), and *Scleropodium purum* (Table V). As in

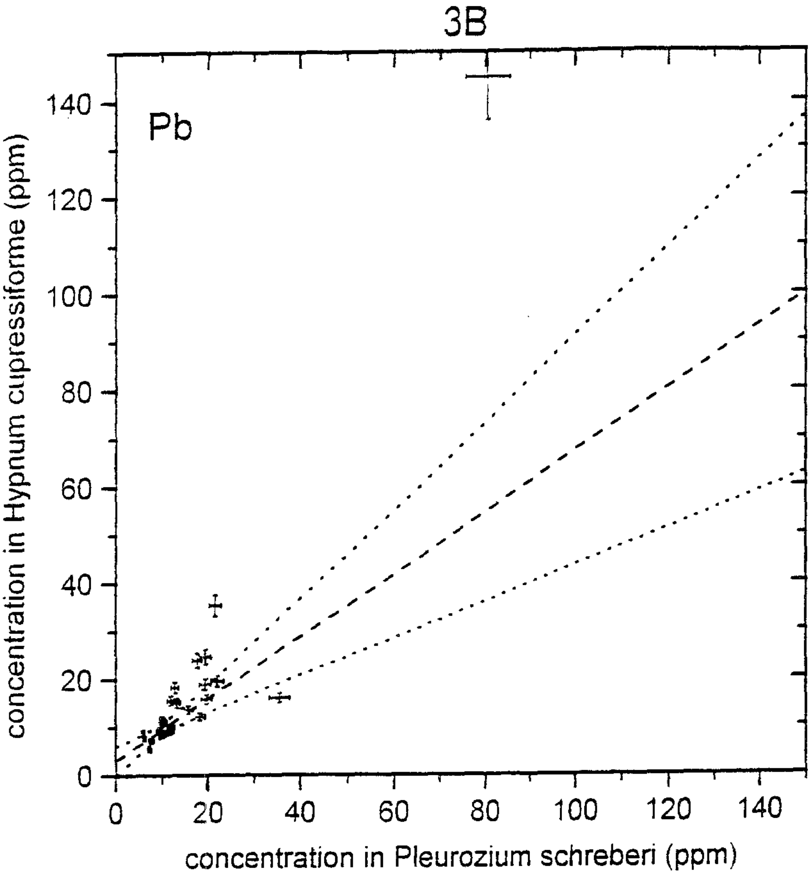

Fig. 3b.

The Netherlands, the data do not show marked differences between median and unweighted mean y/x ratios, but slopes were markedly affected in weighted fits for Pb (Table III), V and Cr (Table IV), and Ni and Ti (Table V). Figure 3A–C shows the Pb-relation between *Pleurozium schreberi* and *Hypnum cupressiforme*, for which a single 140 mg kg^{-1} observation in *Hypnum cupressiforme* seems to make out the difference between weighted (Figure 3A) and unweighted (Figure 3B) calibrations. Figure 3C shows the Pb-relations after removal of the above-mentioned Pb-data: here, the slope could be determined as 0.65 ± 0.13, independent of weighing (see also Figure 1, for Dutch V determinations). Figure 4 presents the differences in graphs for V between weighted and unweighted calibrations for *Pleurozium schreberi* and *Hylocomium splendens*. Here, the unweighted fit seems 'affected' by a single observation of a relatively high V concentration in *Hylocomium splendens*: the weighted fir shows a markedly reduced slope and associated higher intercept value (see Table IV).

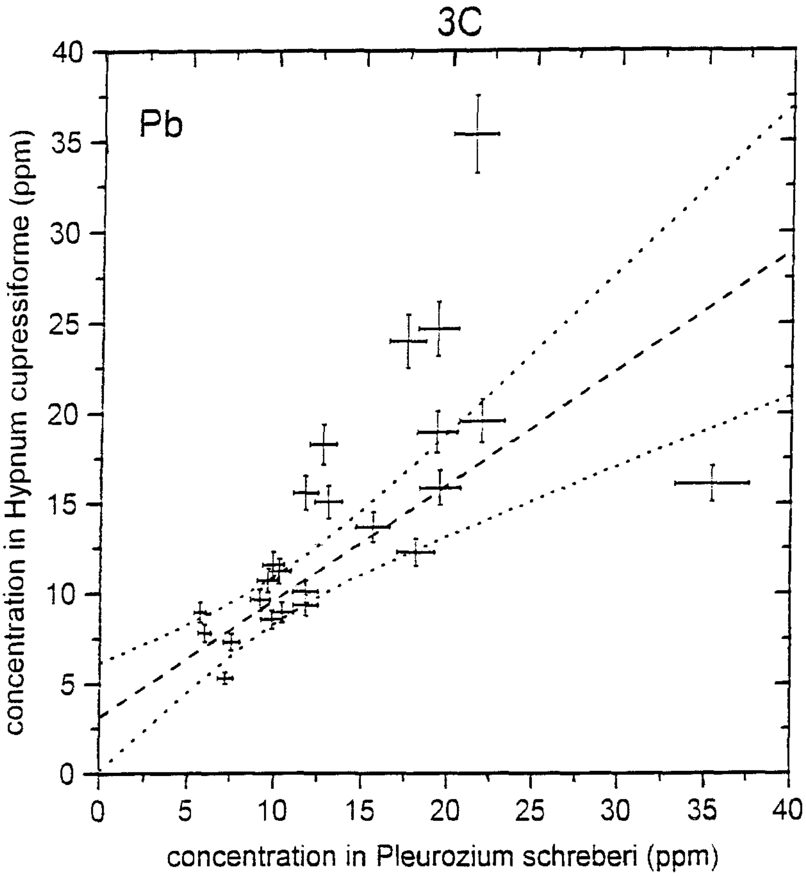

Fig. 3c.

Considering the data in the Tables, of which some are visualized in Figures 1–4, the question may arise as to whether some particular data should be regarded as (analytical ?) outliers: the consequence may be that (a) the use of certified reference materials which differ in (matrix) composition from the mosses do not always give full indication of analytical reliability, (b) particular samples suffer from unnoticed elemental contamination or loss, or (c) particular samples may be incompletely digested (see below). The above suggests that in all series of samples analyses, ourliers may have gone unnoticed, irrespective of the reference materials used (see Materials and Methods Section) or the moss species under consideration. A possible solution for these problems may be the performance of replicate interlaboratory sampling/analyses and the issue of moss certified reference materials.

Table VI shows the results obtained in a Co, V and Zn calibration for *Hylocomium splendens* and *Hypnum cupressiforme*, based on samples from 45 sites in Switzerland. For all three elements, unweighted and weighted slopes were marked-

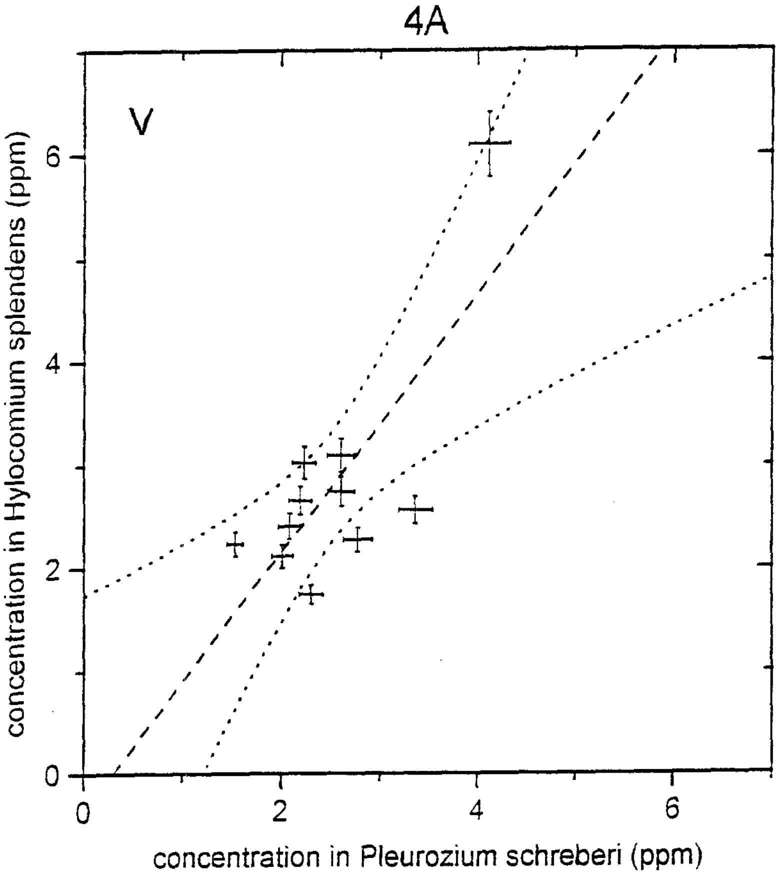

Fig. 4a.

Figs. 4(a)–(b). Comparisons of concentrations (ppm = mg kg^{-1}) of vanadium (V) in *Pleurozium schreberi* and *Hylocomium splendens* (Germany). Figure 4A, B gives the respective unweighted and weighted fits of all data. For all further details see Figure 1.

ly different, as were the differences between weighted and unweighted mean *y*/*x* ratios. Correlation coefficients were relatively low, indicating the data variability; significant correlations are absent especially for the weighted data on Co and Zn.

Comparing unweighted mean *y*/*x* ratios for the Swiss *H.c.*/*H.s.* and the German (*H.c.*/*P.s.*)/(*H.s.*/*P.s.*) data gives German to Swiss ratios of 1.09, and 0.92 for V and Zn respectively; recalculations based on weighted mean *y*/*x* ratios yielded German to Swiss ratios of 1.58 and 1.43 for V and Zn respectively. Due to the extent of variability in both German (*H.c.*/*P.s.*)/(*H.s.*/*P.s.*) (see Figures 3 and 4) and Swiss data (*H.c.*/*H.s.*) (Table VI) with respect to the weighted and unweighted slopes for Co, V and Zn, clear comparisons between the two countries in *H.c.*/*H.s.* ratios seem hardly possible. Considering these observations, interspecies calibrations

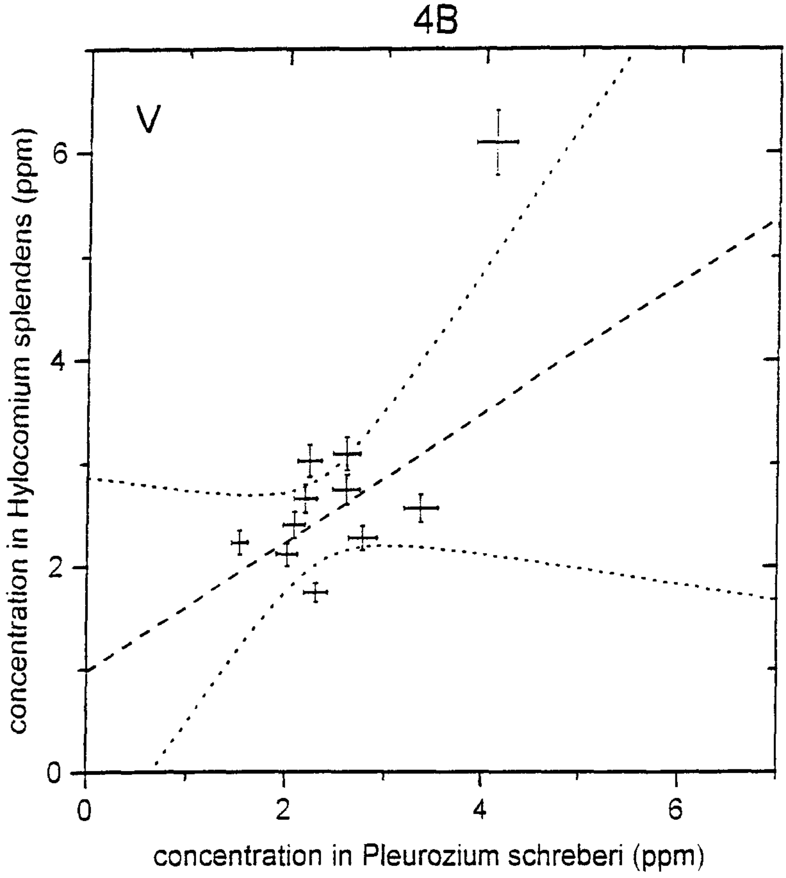

Fig. 4b.

based on values for mean element y/x ratios only (Folkeson, 1979), may easily lead to misinterpretations of the actual presence or absence of significant correlations (cf. Sloof and Wolterbeek, 1993).

For the Swiss data on *Hylocomium splendens* and *Hypnum cupressiforme*, significant correlations could be calculated for V (weighted relations) only. The possible reasons for the absence of correlations between the other elements remain obscured; the element concentration ranges are similar to the ones found in The Netherlands and Germany.

The Swiss data are obtained after filtering of the sample solutions (see Materials and Methods section), but although in principle any less than complete digestion of the sample may have led to variable retention of residues in the filters, Swiss data on Co, V and Zn certified reference materials and on interlaboratory analysis of Scandinavian moss samples (Schmid-Grob *et al.*, 1993) did not indicate such problems.

Another underlying point relevance for the differences between German and Swiss outcomes may be the possible differences in behaviour between reference materials and moss samples: these may be due to variable (silicon) matrices. Furthermore, the moss silicon matrix may not only depend on species, but also on variable soil parameters. However, these aspects of analytical comparisons are of a general character, and are not limited to filtering only: matrix effects may also occur in analytical instruments/procedures like AAS, or ICP/MS (Markert, 1993b). Here, again, one of the possible solutions may be the set-up of interlaboratory analysis of actual samples and the issue of moss certified standard materials.

Based on the above, however, the differences between the German and Swiss analytical outcomes may be related to so far unknown differences in field situations: the data should be interpreted as indicating the necessary reservations in geographically large-scaled applications of interspecies calibrations.

4. Conclusions

The present data show that the calibration of elemental concentrations in various moss species may not simply lead to a controlled use of more than a single species within the frame work of any survey. Moreover, the concentration data suggest the presence of essentially unnoticed outliers, possibly in all of the individual species series. Without further information, however, all data should be used in calibration processes. Here, the question may be raised as to whether interlaboratory analyses of replicate samples and the issue of dedicated cerified reference materials will help solve the problems indicated.

The determination and use in interspecies comparisons of local variations are shown to improve the calibration parameters relative to unweighted processing of data. Furthermore, calibration using mean or median ratios only is presented as easily leading to erroneous interspecies comparisons. The most severe effects may be expected when one of the species is exhibiting saturation effects in element accumulation. It may be clear that progressive or the more sudden onset of saturation effects mean that the calibration data should never be used in extrapolation modes.

Although the present data indicate that several of the weighted interspecies calibrations may be successfully employed, their actual use will remain arbitrarily decided upon, possibly based on decisions as to what to accept with respect to the levels of uncertainty in the calibration parameters. Moreover, the use of calibrations is severely restricted by the necessary reservations in larger-scaled applications: the comparisons of outcomes of Germany and Switzerland show that extrapolations in a geographical sense may easily lead to unreliable results.

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