Introduction

Throughfall is defined as rainwater that after contacting the forest canopy falls to the forest floor. Bulk precipitation is defined as total (wet and dry) deposition in the open, generally a field or a clear-cut forest area [1, 2]. There is evidence of substantial buffering of precipitation chemistry, particularly by deciduous canopies and, hence, evolution of the chemistry during penetration of the canopy [3]. Leaves are seen as the primary interceptors of airborne metals in many forest ecosystems and contact with the canopy increases wet deposition [4]. The flux of elements in throughfall is an important pathway in the cycling of nutrients within forest stands [1, 5]. The effect of the tree canopy on element concentrations in throughfall varies between conifer needles and broad leaves, as well as between individual species. Furthermore, the composition of the precipitation falling on the canopy regulates the exchange processes taking place between the canopy and precipitation [5]. The chemical composition of throughfall results from the alteration of the composition of the rainwater as it passes through the forest canopy. Two mechanisms govern nutrient exchange at the canopy surface:

1. the dissolution and wash-off of deposits accumulated on the canopy between events, and
2. the exchange material occurring between plant tissues and external water [6-8].

Influence of Quercus robur Throughfall on Elemental Composition of Pleurozium schreberi (Brid.) Mitt. and Hypnum cupressiforme Hedw.

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Abstract

The aim of this paper was to estimate the influence of deposition of N, Mg, P, S, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb from under canopy in comparison with open space waters on the elemental composition of the moss layer to test the hypothesis that Pleurozium schreberi (Brid.) Mitt. and Hypnum cupressiforme Hedw. growing under the influence of canopy throughfall of Quercus robur contain higher amounts of elements than the same species growing in an open area. P. schreberi and H. cupressiforme growing under a Q. robur canopy contained higher amounts of N, Mg, P, S, K, Ca, Mn, Ni, Cu, Pb, and H. cupressiforme (in addition also Zn) than the same species growing in an open area. Principal component and classification analysis (PCCA) ordination revealed that P. schreberi and H. cupressiforme growing under throughfall and in open spaces were distinguished by a factor of 1 related to Mg, P, S, Mn, Fe, Zn, Pb (positive scores), and K, Ca (negative scores) and both species were distinguished by a factor of 2 related to Co, Cu, and Cd (negative scores).

Keywords: bioindication, elements, Pleurozium schreberi, Hypnum cupressiforme
Leaching from the canopy increases with rainfall acidity, particularly for pH < 3.3. This phenomenon is especially important for divalent cations such as Mg$^{2+}$ and Ca$^{2+}$, and can lead to nutrient loss in foliage [1, 8, 9]. Cation leaching could be explained by the exchange with H$^+$, whereas physical damage by the strong acidity to the foliar cuticle or to the underlying cells is also possible and could result in an increase in anion leaching [6]. In deciduous forests stemflow, throughfall and leaffall are the most important pathways for the input of nutrients and contaminants into the soil [10]. The chemistry of incident precipitation may be considerably altered after its passage through forest canopies. The resulting solution that reaches the forest floor may be enriched or depleted in certain ions, depending on ion reactivity and on the nature of the canopy [11]. Pollutants filtered from the atmosphere by the canopies will be transported with throughfall to mosses growing under the tree [10]. Therefore, it would be of interest to determine the influence of the deposition of elements by throughfall on the moss layer of *Hypnum cupressiforme* and *Pleurozium schreberi* growing under *Quercus robur*.

Throughfall measurements are also widely used to characterize and quantify the atmospheric pollution load in heavily contaminated forest ecosystems [5].

In this investigation, the hypothesis to be tested is: *Pleurozium schreberi* and *Hypnum cupressiforme* growing under a canopy of *Quercus robur* contain higher amounts of elements than the same species growing in open areas.

**Material and Methods**

The experiments were conducted during the growing season between May and September 2008 under and outside (open area) 60-year-old *Quercus robur* trees in 24 stands (1-12 under canopy and 13-24 in open area) located 4 km S from Strzelin (Fig. 1) for *P. schreberi*, and in 24 stands (25-36 under canopy and 37-48 in open area) located 4 km S from Środa Śląska (Fig. 2) for *H. cupressiforme*. Throughfall and rainfall in open space together with moss samples were collected at all sites in three replications and analyzed. Water samples were collected using a polyethylene funnel (10 cm in diameter) with nylon mesh at the bottom (to minimize contamination by plant debris and insects), which was connected to a dark polyethylene bottle (2L). The funnel was placed 1 m above the ground and about 50 cm aside the tree trunk under the canopy. Open space samples were collected near throughfall samples in open canopy sites in forest clearings of 30 m diameter to avoid interference by trees [12]. The rainwater samples were collected at irregular intervals of 2-3 weeks, depending on the quantity of rainfall during the period. The water volume was pooled before chemical analysis [7, 13, 14]. At each sampling time, plant debris and insects on the funnels were removed carefully [15]. Collectors contained the biocide thymol [8]. The locations of the individual samplers were not changed during the study.

Prior to analysis, all water samples were filtered using a Whatman glass microfibre filter (GF/F). The three replicate plant samples collected at one time at the end of the experimental period were analyzed separately.

The following parameters were determined in the water samples (detection limits indicated between brackets Mg (0.1 µg L$^{-1}$), K (10 µg L$^{-1}$), Ca (0.03 µg L$^{-1}$), Mn (0.3 µg L$^{-1}$), Fe (1.5 µg L$^{-1}$), Co (4 µg L$^{-1}$), and Zn (0.9 µg L$^{-1}$) with an ICP OES Spectroflame SIMSEQ; Cd (0.5 pg), Ni (5 pg), Cu (1.8 pg), and Pb (2 pg) using an AAS with graphite fur-
nace (Philips PU 9200X); nitrate (2 µg L⁻¹), ammonium (2 µg L⁻¹), phosphate (5 µg L⁻¹, colorimetric), and sulphate (2500 µg L⁻¹, nephelometric) using a Technicon Autoanalyzer System II TRAAC 800; and pH (potentiometric). Concentrations of Co, Ni, Cd, and Pb in the water samples were below the detection limit.

Plant material was dried at 50°C for 48 h and homogenized in a laboratory mill. Samples (200 mg, in triplicate) were digested with nitric acid (pro analysi, 67%) and hydrogen peroxide (pro analysi, 35%), during which temperatures were raised to about 95°C, until the evolution of nitrous gas stopped and the digest became clear. After dilution to 10 mL, the plant digests were analyzed for Mg, K, Ca, Mn, Fe, Co, Ni, Zn, and Pb using simultaneous sequential inductively coupled plasma emission spectrophotometry, and for Cu and Cd using furnace atomic absorption spectrophotometry. Phosphorus in the plant digest was determined using a Technicon AutoAnalyser II. The dried and pulverized plant samples were used for total N and S (detection limit 10 mg kg⁻¹, Carlo Erba NA-1500 CNS Analyzer).

All elements were determined against standards (BDH Chemicals, pro analysi quality) and blanks prepared in 0.5 M nitric acid. Blanks and standards contained the same matrix as the samples. All results for plants were calculated on a dry-weight basis.

The recovery rates, relative to the results of an interlaboratory study on digesting and analyzing reference materials (Wageningen Evaluating Programmes for Analytical Laboratories, WEPAL), were as follows for each of the investigated elements (percentages with SD): N (99±3), Mg (97±4), P (101±2), S (96±3), K (96±4), Ca (98±3), Mn (98±4), Fe (99±2), Co (101±4), Ni (99±4), Cu (97±4), Zn (101±3), Cd (95±3), and Pb (95±4). The reference material consisted of pine needles (IPE 761) and leaves of Nymphaea alba (not coded).

Statistical Analysis

Differences between sampling sites in terms of concentrations of elements in water and mosses were evaluated by ANOVA on log-transformed data to obtain a normal distribution of features according to Zar [16]. The normality of the analyzed features was checked by means of Shapiro-Wilk’s W test and the homogeneity of variances was checked by means of Bartlett's test [16, 17].

The t-test [17, 16] was applied on log-transformed data to compare the metal concentrations in water and mosses between under canopy and open areas.

The matrix of concentrations of 14 elements in mosses from 48 sampling sites under canopy and in open areas was used for a factor analysis by means of a principal component (PCA) and classification analysis (CA) to detect groups of samples with similar patterns of element concentrations. PCA is often used in ecology to reduce the amount of data and stabilize subsequent statistical analyses [18, 19]. PCCA was earlier applied in environmental sciences [20, 21]. A PCA plot of scores (coordinates of objects for the new variables) gives information about similarities between samples, and the plot of loadings shows correlations between the original variables and the first two factors [22]. Plotting of PCCA (Principal Component and Classification Analysis) accumulatively presents similarities between samples and correlations between the investigated variables and selected factors. The calculations were done with Statistica 8.0 [23].

Table 1. Concentrations of elements (mg L⁻¹) in under canopy of Quercus robur and open space waters in Pleurozium schreberi sites; \( t_{0.05} \) tab=2.013.

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>S.D.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>S.D.</th>
<th>t-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>6.8</td>
<td>6.6</td>
<td>0.1</td>
<td>6.7</td>
<td>6.9</td>
<td>6.8</td>
<td>0.1</td>
<td>-4.67</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NO₃</td>
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<td>2.3</td>
<td>1.4</td>
<td>0.3</td>
<td>0.24</td>
<td>0.30</td>
<td>0.26</td>
<td>0.01</td>
<td>9.49</td>
<td>&lt;0.001</td>
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<tr>
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<td>0.64</td>
<td>0.33</td>
<td>0.11</td>
<td>0.16</td>
<td>0.29</td>
<td>0.23</td>
<td>0.02</td>
<td>4.95</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mg</td>
<td>1.8</td>
<td>4.7</td>
<td>2.6</td>
<td>0.95</td>
<td>1.1</td>
<td>2.0</td>
<td>1.6</td>
<td>0.3</td>
<td>4.49</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PO₄</td>
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<td>0.76</td>
<td>0.21</td>
<td>0.32</td>
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</tr>
<tr>
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<td>2.3</td>
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<td>5.3</td>
<td>1.5</td>
<td>3.40</td>
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</tr>
<tr>
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</tr>
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<tr>
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<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
<td>6.12</td>
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</tr>
<tr>
<td>Fe</td>
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<td>0.23</td>
<td>0.15</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
<td>7.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cu</td>
<td>0.015</td>
<td>0.026</td>
<td>0.02</td>
<td>0.005</td>
<td>0.01</td>
<td>0.016</td>
<td>0.013</td>
<td>0.003</td>
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</tr>
<tr>
<td>Zn</td>
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<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.13</td>
<td>0.07</td>
<td>0.02</td>
<td>2.15</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
### Results and Discussion

The ranges of concentrations of elements in water and mosses subjected to the canopy throughfall and from open space are displayed in Tables 1-4. Water and mosses differed significantly in terms of the concentrations of the elements assessed (ANOVA, p<0.05).

According to Lawson [24], rainwater can leach a wide variety of elements from tree foliage and wash off dry deposited elements. Throughfall fluxes increased relative to open space deposition for all elements in *P. schreberi* sites and, except for sulphates and Zn in *H. cupressiforme* sites (Tables 1 and 2). Sulphates and Zn in *H. cupressiforme* sites were not different between under canopy and open space deposition. Concentrations of nitrate, ammonium, and phosphate in rainwater was higher in throughfall than in open space (Tables 1 and 2). According to [3], ammonium (and to a lesser extent nitrate) are frequently observed to be leached by the foliage. Results indicate that leaching was the main enrichment process for Mn. Concentration of this element in throughfall water was significantly higher than in rain from open space (Tables 1 and 2), which is in agreement with Avila and Rodrigo [14]. The cations known to increase most in throughfall are Mg, K, and Ca [1, 25, 26, 11, 27]. This statement was confirmed also in this investigation (Tables 1 and 2). All of these elements were significantly lower in rainwater from the open space. Also, Stachurski and Zimka [28] confirm that Mg and K are known to be leached from foliage. These authors state that potassium is a main element detected in throughfall rapidly leached from foliage during rainwater passage [28]. Sulphur flux in throughfall beneath a given canopy is an excellent indicator of total loading, including inputs of aerosols and gaseous forms of the element. This is because canopies hardly modify the pool of sulphur derived from the atmosphere in a sense of uptake or leaching and sulphur passes through the canopies with minor interactions [28]. In this investigation there was no difference in concentration of sulphate between rainwater from open space and throughfall in *H. cupressiforme*. There was a significantly higher concentration of sulphate in throughfall in *P. schreberi* sites, which may suggest, according to Stachurski and Zimka [28], higher sulphate pollution in the area where *P. schreberi* was growing. According to Avila and Rodrigo [14] no difference in concentration of sulphate in *H. cupressiforme* under canopy and in open space rainwater could indicate that canopy uptake was higher than deposition. According to Avila and Rodrigo [14], metal concentrations were higher in throughfall than in open space water except for Zn and Cd, where negative throughfall fluxes indicated higher uptake than deposition. This mechanism was also found in this investigation in the case of Zn in *P. schreberi* sites (Table 1). There was a significantly higher concentration of Zn in open space water than in throughfall in the *P. schreberi* sites [14].

Blew et al. [29], Fernández and Carballeira [30] and Krawczyk et al. [31] found increases in nutrient concentration in throughfall by washing and leaching of foliage, contributing to a substantial portion of yearly nutrient fall. Čeburnis and Steinnes [32] reported that certain trace metals are found in higher concentrations in mosses under canopy than in open areas. In this investigation, concentrations of elements in *P. schreberi* and *H. cupressiforme* growing under *Q. robur* canopy contained significantly higher concentrations of all elements except Fe, Co, Cd, and Fe; Co, Zn, and Cd were not significantly different in

### Table 2. Concentrations of elements (mg L⁻¹) in under canopy of *Quercus robur* and open space waters in *Hypnum cupressiforme* sites; t₀.₀⁵ tab= 2.013.

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>S.D.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>S.D.</th>
<th>t-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.4</td>
<td>6.7</td>
<td>6.6</td>
<td>0.1</td>
<td>6.5</td>
<td>6.8</td>
<td>6.7</td>
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<tr>
<td>NO₃</td>
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<td>0.3</td>
<td>0.02</td>
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<tr>
<td>NH₄</td>
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<td>0.12</td>
<td>0.84</td>
<td>0.39</td>
<td>0.29</td>
<td>-5.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mg</td>
<td>2.1</td>
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<td>2.6</td>
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<td>0.08</td>
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</tr>
<tr>
<td>PO₄</td>
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<td>0.01</td>
<td>0.03</td>
<td>0.02</td>
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</tr>
<tr>
<td>SO₄</td>
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<td>Ca</td>
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<tr>
<td>Mn</td>
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<td>0.01</td>
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<td>0.01</td>
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<tr>
<td>Fe</td>
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<td>0.022</td>
<td>0.005</td>
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<td>Cu</td>
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<td>Zn</td>
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<td>&gt;0.05</td>
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### Table 3. Concentrations of elements (mg kg⁻¹) in *Pleurozium schreberi* under the canopy of *Quercus robur* and from open space; \(t_{0.05 \, \text{tab}}=2.013\).

<table>
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<th>Under canopy</th>
<th>Open space</th>
</tr>
</thead>
<tbody>
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<td>N</td>
<td>18,645</td>
<td>16,841</td>
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<tr>
<td>Mg</td>
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<tr>
<td>P</td>
<td>2,546</td>
<td>1,959</td>
</tr>
<tr>
<td>S</td>
<td>3,851</td>
<td>2,295</td>
</tr>
<tr>
<td>K</td>
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<td>8,352</td>
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<td>Co</td>
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<td>Ni</td>
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<td>Zn</td>
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<td>129</td>
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<tr>
<td>Cd</td>
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</tr>
<tr>
<td>Pb</td>
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<td>9.2</td>
</tr>
</tbody>
</table>

### Table 4. Concentrations of elements (mg kg⁻¹) in *Hypnum cupressiforme* under the canopy of *Quercus robur* and from open space; \(t_{0.05 \, \text{tab}}=2.013\).

<table>
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<th>Element</th>
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<th>Open space</th>
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<td>P</td>
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<td>43</td>
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<tr>
<td>S</td>
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</tbody>
</table>
mosses growing under and outside the canopy. The ordination of samples based on the concentration of elements in *P. schreberi* and *H. cupressiforme* are shown in Fig. 3. Groups of both species are distinguished by the second principal component and groups from under and outside the tree canopy by the first principal component. All *H. cupressiforme* sites characterize positive scores of factor 2, and all *P. schreberi* sites score negative scores of this factor. Both species from under the canopy are characterized by positive scores of factor 1 and from open space by negative scores of this factor. Projection of the variables on the factor plane indicated that factor 1 was related to Mg, P, S, Mn, Fe, Zn, and Pb (positive scores) and K and Ca (negative scores). Factor 2 was related to Co, Cu, and Cd (negative scores). So both moss species from under the canopy and from open spaces were distinguished by accumulated elements: the toxic Pb and the essential elements for plants Mg, P, S, K, Ca, Fe and Zn. Between-species accumulation was related to a not yet explained mechanism influenced by concentrations of Co, Cu, and Cd in mosses.

**Conclusions**

1. *Pleurozium schreberi* and *Hypnum cupressiforme* growing under the influence of canopy throughfall of *Quercus robur* contain higher amounts of the elements N, Mg, P, S, K, Ca, Mn, Ni, Cu, and Pb, and in addition Zn, only in *H. cupressiforme* than the same species growing in open areas.

2. *P. schreberi* and *H. cupressiforme* growing under canopy and in open space were distinguished by a factor of 1 related to Mg, P, S, Mn, Fe Zn, and Pb (positive scores), and K and Ca (negative scores), and both species were distinguished by a factor of 2 related to Co, Cu, and Cd (negative scores).

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