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NATURAL ISOTOPIC VARIATION IN SPECIES OF LICHENS ON AN ALTITUDE GRADIENT IN THE EASTERN CENTRAL ALPS

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Zusammenfassung

Stabile Isotope biologisch bedeutender Elemente werden zunehmend wichtiger als ökotoxikologische Indikatoren in der ökologischen Forschung. In der vorliegenden Arbeit werden die natürlichen Varianten von $^{15}N/^{14}N$ and $^{13}C/^{12}C$, die in der Blattflechte *Hypogymnia physodes* (sie bevorzugt saure und nur wenig eutrophierte Substrate) und in der Strauchflechte *Pseudevernia furfuracea* (sie ist empfindlich gegenüber dem Einfluß von Luftverunreinigungen) auftreten, in Abhängigkeit von einem Höhengradienten in den östlichen Zentralalpen (Austria, Salzburg, Lungau, 1.080 – 1.940 msm, Katschberg) untersucht. Der Vergleich der entsprechenden Isotopenvariation des Substrates (Borke von *Picea abies* oder *Larix decidua*) mit jener der epiphytischen Flechten und zusätzlich dem Bodensubstrat von *Cetraria islandica* soll Rückschlüsse auf die Fähigkeit der Flechten, Substanzen aus der Luft zu absorbieren und sie in ihren Metabolismus einzubauen, zulassen. Die Eignung der natürlichen Isotopenvariationen von $^{15}N/^{14}N$ und $^{13}C/^{12}C$ als ökologische Indikatoren wird dargestellt.

Abstract

Stable isotopes of biologically significant elements are becoming more and more important as ecotoxicological indicators in ecological research. In the present work, the natural isotopic variants $^{15}N/^{14}N$ and $^{13}C/^{12}C$ present in the foliose lichen *Hypogymnia physodes* (which prefers an acid and only slightly eutrophic substrate) and in the fruticose lichen *Pseudevernia furfuracea* (which is particularly sensitive to air pollutants) are analysed in relation to an altitude gradient in the eastern Central Alps (Austria, Salzburg, Lungau, 1.080 – 1.940 msm, Mt. Katschberg). By comparing the corresponding isotopic variation of the substrate (bark of *Picea abies*

or *Larix decidua*) with that of the epiphytic lichens and additionally the soil substrate of *Cetraria islandica* should allow conclusions to be drawn concerning the capacity of the lichens to absorb substances from the air and to integrate them into their metabolism. The suitability of the natural isotopic variations $^{15}N/^{14}N$ and $^{13}C/^{12}C$ as ecological indicators is subsequently discussed.

Key words: Lichens, $^{15}N/^{14}N$ ratio, $^{13}C/^{12}C$ ratio, altitude gradient, ecological indicators

1 Introduction

Many lichens grow in microhabitats which provide completely different conditions for the development of plant life. Lichen vegetation along an altitude gradient is exposed to numerous natural and man-made stress factors and reacts sensitively to any pollution. Measurements of pollutants (SO_2, 0_3, NO_x) on the altitude gradient at Ziller Valley (BOLHAR-NORDENKAMPF 1989) have shown that in areas far away from emitters, e.g. at higher elevations, the secondary air pollutant ozone is of particular importance (SMIDT 1989a). Different degrees of pollution also occur with respect to proton and element assimilation (SMIDT 1989b).

Most of the above-mentioned stress factors have a direct or indirect effect on the photosynthetic apparatus of the lichens. According to investigations by TEERI (1981), some species of the genus *Cladonia* and *Stereocaulon* feature the C_3 photosynthesis type of CO_2 fixation catalyzed by ribulose-5-phosphate carboxylase.

The photosynthetic activity of *Pseudevernia furfuracea* from the eastern Central Alps varies considerably depending on various environmental factors (TÜRK 1981; 1983). Such differences in CO_2 metabolism also occur among lichens in relation to altitude (EICKMEIER and ADAMS 1973). Morphological and physiological differences on a single altitude gradient have been described for the lichens *Dictyonema pavonium* in the Andes (Venezuela, LARCHER and VARESCHI 1988) and the Alps (Austria, GUTTENBERGER et al., 1991). The thiole content of lichens increases as a function of altitude – something which could be interpreted as a protective mechanism against the greater quantity of radicals which appear as a result of more intensive exposure to UV and ozone (GUTTENBERGER et al., 1991).

Natural variation of stable isotopes of important bioelements (N, C) are increasingly being used in connection with the ecophysiological and ecobiochemical analysis of the impact of environmental factors on plant metabolism (RUNDEL et al., 1989; GEBAUER 1991; FAUST 1993). The reasons for the fluctuation of the $\delta^{15}N$ and $\delta^{13}C$ isotope levels in plants include the kinetic isotopic effects which occur in diffusion processes, intermolecular interaction and enzyme reactions in the metabolism, in all of which the heavier isotope is fundamentally discriminated against in favour of the lighter one. In *Pinus sylvestris* needles from regions with different degrees of air pollution, significant differences can be observed in the natural isotopic variants $^{15}N/^{14}N$ accompanied by differential inhibition among the ammonia-fixing enzymes glutamine synthetase and glutamate dehydrogenase (JUNG et al., 1993; SCHLEE et al. submitted for publication).

The natural variation of the carbon isotopes ^{12}C and ^{13}C is also based on the preference for ^{12}C in metabolism and the partial exclusion of ^{13}C by plants (BERRY 1989). C_3 plants ($\delta^{13}C$ values between approx. -23 and -32 ‰) discriminate more than C_4 plants ($\delta^{13}C$ values between approx. -9 and -16 ‰).

In the present study the natural isotopic variation $^{15}N/^{14}N$ and $^{13}C/^{12}C$ present in the foliose lichen *Hypogymnia physodes* (which prefers an acid and only slightly eutrophic substrate) and in the fruticose lichen *Pseudevernia furfuracea* (which is particularly sensitive to air pollutants) is analysed in relation to an altitude gradient in the eastern Central Alps (1,080–1,940 msm, Katschberg, Salzburg, Austria). By comparing the corresponding isotopic variation of the substrate (bark of *Picea abies* or *Larix decidua* and soil as the substrate of *Cetraria islandica*) should allow to draw conclusions concerning the capacity of the lichens to absorb substances from the air and to integrate them into their metabolism. The suitability of the natural isotopic variants $^{15}N/^{14}N$ and $^{13}C/^{12}C$ as ecological indicators is subsequently discussed.

2 Material and methods

Area of investigation

The lichens were collected on an altitude gradient from St. Michael in the Lungau via Mt. Katschberg to Aineck (Salzburg, Austria) at 1.080 to 1.940 m above sea level.

Plant material

Investigations were carried out on the fruticose lichen *Pseudevernia furfuracea* (L.) ZOPF var. *furfuracea* and the foliose lichen *Hypogymnia physodes* (L.) NYL. (representing epiphytic lichens), as well as the common *Cetraria islandica* (L.) ACH. (representing an epigeic lichen).

Sample preparation and isotopic analysis

The complete lichens including the base matter were carefully washed with distilled water and dried at 75 °C until their weight remained constant. One hundred mg of the dried material was sufficient for N determination according to Kjeldahl and ^{15}N isotopic analysis. Because of the low N content of the samples, isotopic ratio mass-spectrometry analysis (IRMS) was performed not on the biological material itself, but on 350 µg of the ammonium sulphate resulting from N determination carried out in tin capsules for ^{15}N isotopic analysis. Five parallel samples were measured in each case.

For ^{13}C isotope analysis, the test material was first finely pulverized using a ball vibration grinding mill, after which 70–100 µg of the powder was transferred to tin capsules of the IRMS.

An on-line system of instruments (mass spectrometer MAT 252 and element analyser CHN 1108 with variable split) was used for isotopic analysis. Flash incineration of the samples took place at 1800 °C with subsequent analysis of the CO_2 and N_2.

Two calibrated ammonia sulphates (+ 10.8 ‰ and 0.4 ‰, measured against atmospheric nitrogen) were used as working standards for ^{15}N analysis. Each sample was prepared twice and measured six times. The consistency of the measurements was better than 0.2 ‰ in 90 % of the cases (MUCCIARONE and DUNBAR 1992; GEHRE et al., 1994). When determining the $\delta^{13}C$ value, the sample material was directly incinerated (JENSEN 1991). ANU sucrose and PEF1 (IAEA standards; $\delta^{13}C$ values – 10,5 ‰ and 31.77 ‰ based on PDB) were used as working standards. The plant samples were measured five times and the consistency of the measurements was better than 0.1 ‰ in 90 % of the cases.

3 Results and discussion

According to the $\delta^{13}C$ values determined (which were between - 22 and - 26 ‰), the photobionts of the lichens *Hypogymnia physodes* and *Pseudevernia furfuracea* belong to the C_3 type of photosynthesis. This corresponds to the findings of TEERI (1981) for *Cladonia* and *Stereocaulon* (- 21.8 to 24.6 ‰). The $\delta^{13}C$ values fluctuate only slightly in the given altitude gradient (Fig. 1). Whereas in *Hypogymnia physodes* the $\delta^{13}C$ value ranges along the altitude gradient between - 24.6 and - 22.9 ‰, a relative minimum emerges for *Pseudevernia furfuracea* at heights between 1,430 and 1,660 m (- 23.5 ‰). The value is significantly less negative at 1,940 m (- 22.3 ‰).

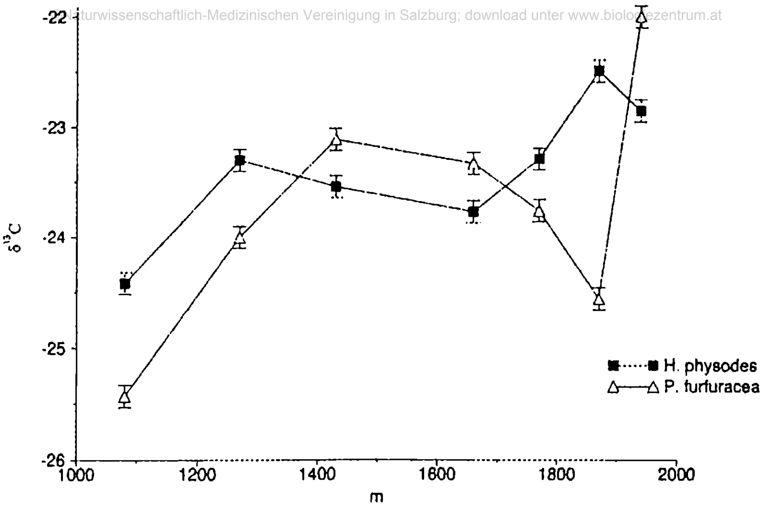


Fig. 1. $\delta^{13}C$ values of *Hypogymnia physodes* (■) and *Pseudevernia furfuracea* (Δ) depending on altitude (eastern central Alps, Lungau, Mt. Katschberg)

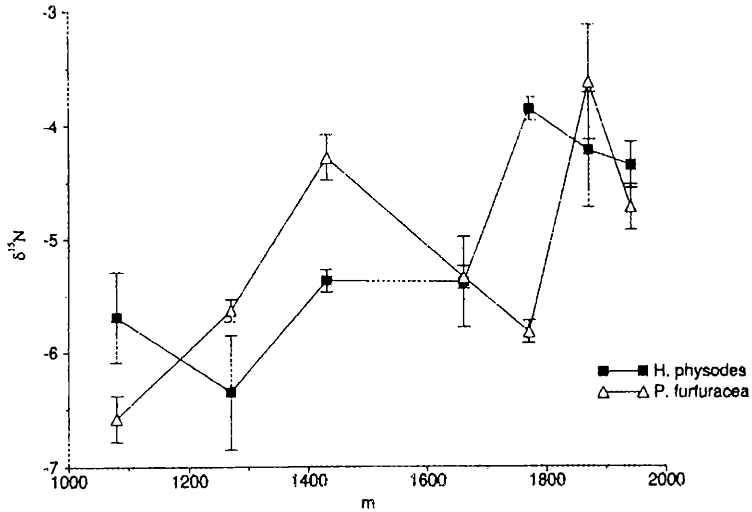


Fig. 2: $\delta^{15}N$ values of *Hypogymnia physodes* (■) and *Pseudevernia furfuracea* (Δ) depending on altitude (eastern central Alps, Lungau, Mt. Katschberg)

By contrast, larger fluctuations are shown by the $\delta^{15}N$ values (between - 5 and - 7 ‰) in *Pseudevernia furfuracea* and in *Hypogymnia physodes* (Fig. 2). In the foliose lichen *Hypogymnia physodes*, a slight decrease of the $\delta^{15}N$ values was generally found to occur in relation to altitude, whereas in *Pseudevernia furfuracea*, minima appear at 1,430 and 1,870 m.

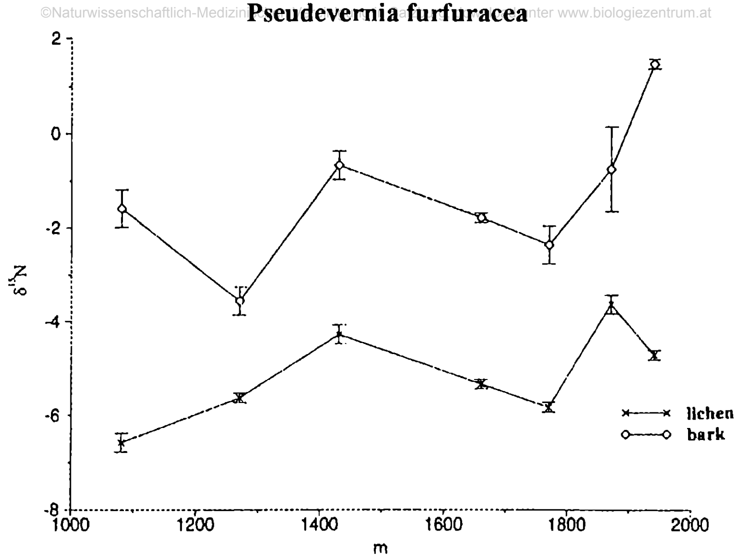
The $\delta^{15}N$ values evidently indicate a pollution influenced plant metabolism (stress metabolism), to which the organism can react to a certain degree with corresponding compensation and repair mechanisms. Within the framework of a biomonitoring network in the central German industrial region of Leipzig-Halle, $\delta^{15}N$ values between -0.2 and -10.1‰ were determined in *Pinus sylvestris* needles depending on the trees' location (JUNG et al., 1993; SCHLEE et al., 1994).

Of special interest is the comparison of the $\delta^{15}N$ values of the lichens *Hypogymnia physodes* and *Pseudevernia furfuracea* with those of the substrate (bark of *Picea abies* or *Larix decidua*; Fig. 3). The marked difference highlights the capacity of the lichens to absorb nitrogen from the air and to convert it in the metabolic process. This ability is evidently developed to a higher degree in the foliose lichen *Hypogymnia physodes* than in the fruticose lichen *Pseudevernia furfuracea.*

A completely different picture emerges for *Cetraria islandica*, which underwent comparative analysis. The substrate (i.e. soil) revealed a very similar $\delta^{15}N$ value as the lichen itself (Fig. 4). Differences from *Hypogymnia physodes* and *Pseudevernia furfuracea* also emerged in a comparison to *Cetraria islandica* gathered at an altitude of 1.940 msm (Fig. 5). Whereas *Hypogymnia physodes* and *Pseudevernia furfuracea* exhibited similar $\delta^{15}N$ values, the $\delta^{15}N$ value of *Cetraria islandica* was around 50 % lower – a finding which also corresponds to the N content in the lichens based on dry mass. By contrast, the $\delta^{13}C$ values and the dry mass correspond to each other in the three lichen species.

The results show that the natural isotopic variation $^{15}N/^{14}N$ and $^{13}C/^{12}C$ appear to be a summary expression of the intensity of metabolism and thus would seem suitable as biomarkers. At the same time, the N metabolism evidently reacts more sensitive to the various environmental factors depending on altitude.

The $\delta^{13}C$ values determined, indicate that - regarding their photosynthetic activity - the photobionts of the lichens may be classified as C_3 plants.



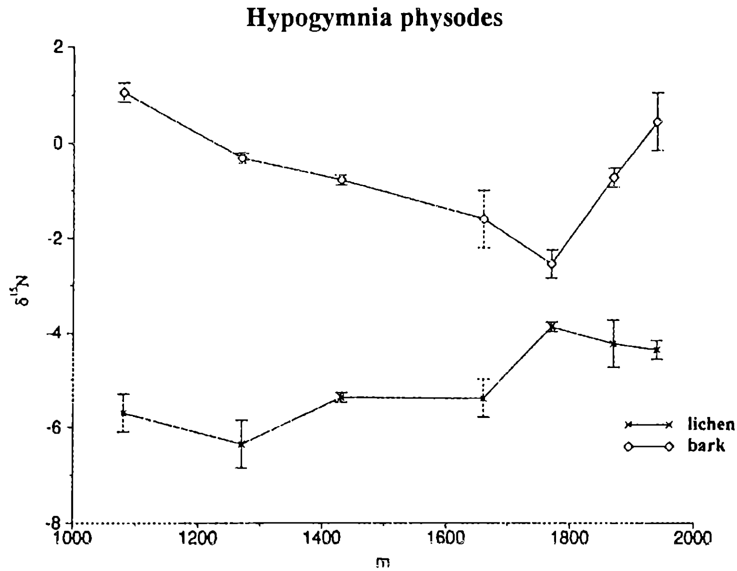


Fig. 3: Comparison of $\delta^{15}N$ values in the lichens (x) *Hypogymnia physodes* (below) and *Pseudevernia furfuracea* (above) and the corresponding bark (◊) depending on altitude (eastern central Alps, Lungau, Mt. Katschberg)

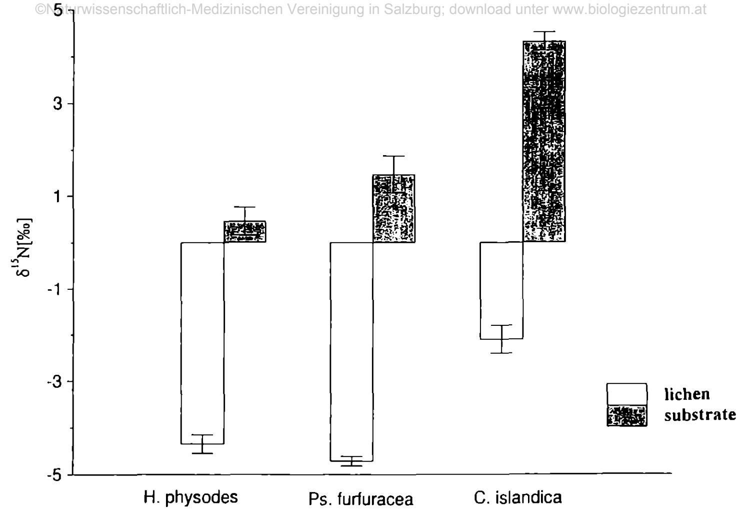


Fig. 4. Comparison of $\delta^{15}N$ values of *Hypogymnia physodes*, *Pseudevernia furfuracea* und *Cetraria islandica* and the corresponding substrate (altitude 1,940 m NN, eastern central Alps, Lungau, Mt. Katschberg)

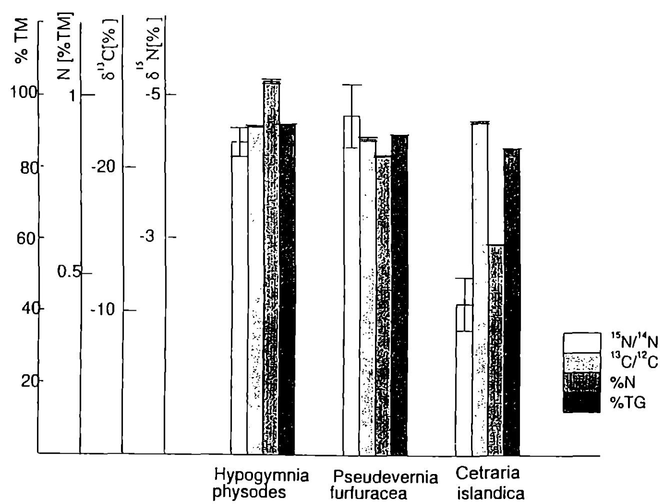


Fig. 5: $\delta^{15}N$ values, $\delta^{13}C$ values, N content and dry mass (TG) of *Hypogymnia physodes*, *Pseudevernia furfuracea* and *Cetraria islandica* (altitude 1,940 m NN, eastern central Alps, Lungau, Mt. Katschberg)

However, if bioindication is to be performed with the lichens being used as an "early warning system" (cf. BARTHOLMESS et al., 1994), further physiological and biochemical parameters must be investigated. In particular there is currently a lack of direct data concerning the composition of the atmosphere at the specific location. Correlation between the natural isotopic variation and the degree of pollution is difficult to determine. More efforts have to be undertaken to quantify the natural isotopic variation.

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