Contribuţii Botanice, *XXXIX*, **2004**
Grădina Botanică “Alexandru Borza”
Cluj-Napoca

CHARACTERIZATION OF LICHENS USING THE NATURAL ISOTOPE ABUNDANCE OF ^{13}C FROM ORGANIC MATERIAL

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Abstract: Lichens are the result of a successful symbiosis between a heterotrophic fungal partner (mycobiont) and a photosynthetic autotroph (photobiont). Lichens possessing different photobiont associations show differences in photosynthetic rates. Lichen groups have also been distinguished by means of carbon isotope ratio. One objective of the present work was to study the carbon isotope ratio among these different lichens groups. Many free-living algae and most cyanobacteria have a CO_2 concentrating mechanism (CCM), which operates under conditions of low CO_2 availability in their environment, such as when the diffusion of CO_2 is low. The CCM functions to accumulate an internal pool of inorganic carbon, which is subsequently used to increase the CO_2 supply to the carboxylating enzyme. Another objective of this work was to study the implications of photosynthetic CO_2 concentrating mechanism on the carbon isotope ratio from organic material of lichens.

Analysis of the $\delta^{13}C$ values of organic material in 42 samples of lichens showed that the photobionts of these lichens were C_3-like. Carbon isotope discrimination, Δ, varies with the type of photobiont: the low Δ for cyanobiont lichens and the higher Δ for phycobiont lichens. The differences in Δ have been attributed to differences in CO_2 transfer resistance and to photosynthetic CO_2 concentrating mechanism (CCM).

The $\delta^{13}C$ values of organic material in lichens fluctuate with altitude and the date collection. These variations are attributable to different pollutants and stress factors, to climatic variables and to changes in $\delta^{13}C$ of the source air CO_2. This is relevant for understanding the impact of global change on natural vegetation.

Introduction

The use of stable isotopes at natural abundance levels has brought a new dimension to our understanding of plant ecology. In the past two decades there have been remarkable advances in the theoretical understanding of discrimination processes, as well as technical developments in mass spectrometry, leading to an exponential growth in applications for natural systems. Stable isotopes provide plant ecologists with a quantitative and integrative framework for chemical, biological and ecological transformations. The range of stable isotopes currently routinely analyzed for ecological applications and their natural abundances are shown in Table 1. Analyses of the relative natural abundances of stable isotopes of carbon ($^{13}C/^{12}C$), oxygen ($^{18}O/^{16}O$), nitrogen ($^{15}N/^{14}N$) and deuterium (D/H) have been used across a wide range of scales, from cellular to community and ecosystem level, contributing much to our understanding of the interactions between biosphere, pedosphere and atmosphere.

Table 1: Average terrestrial abundance of stable isotopes of major elements of interest in ecological studies

Element	Isotope	Natural abundance (‰)
Hydrogen	^{1}H ^{2}H	99.985 0.015
Carbon	^{12}C ^{13}C	99.63 1.110
Oxygen	^{16}O ^{17}O ^{18}O	99.759 0.037 0.204

Theoretical background

Lichens are the result of a successful symbiosis between a heterotrophic fungal partner (mycobiont) and a photosynthetic autotroph (photobiont). They can be separated into three groups on the basis of the type of photobiont partner: phycobiont (green algae), cyanobiont (cyanobacteria), and phycobiont associated with cyanobacteria [6]. Lichens possessing different photobiont associations show different water requirements for the activation of photosynthesis. Cyanobiont lichens surrounded by a gelatinous sheath require liquid water for photosynthetic CO_2 assimilation, while phycobiont associations can fix CO_2 in the presence of water vapor exceeding a critical value. Consequently, lichens possessing different photobiont associations also show differences in photosynthetic rates. These groups have also been distinguished by means of carbon isotope ratio. One objective of the present work was to study the carbon isotope ratio among these different lichens groups.

The initiation and maintenance of the metabolic activity of lichens require that water is taken up and stored. Surplus water may, however, limit the photosynthetic activity of the lichen if this causes swelling of the fungal hypae, which may block the gaseous pores within the hyphal matrix and thus impede the diffusion of CO_2 to the photobiont [3].

Many free-living algae and most cyanobacteria have a CO_2 concentrating mechanism (CCM), which operates under conditions of low CO_2 availability in their environment, such as when the diffusion of CO_2 is low. The CCM functions to accumulate an internal pool of inorganic carbon, which is subsequently used to increase the CO_2 supply to the carboxylating enzyme. It may well be assumed that this mechanism participates in the photosynthetic process of lichenised algae and cyanobacteria too [9]. The CCM may in fact confer a particular advantage to lichens, since the resistance to CO_2 diffusion in the hydrated fungal matrix may be high. Uptake of CO_2 by lichens may be relatively transient, a function of thallus water content, and regulated by variations in atmospheric humidity, dewfall, and precipitation. Without the possibility of regulating water content by stomata, lichens must steer between sufficient water to reactivate photobiont photosynthesis and CO_2 uptake limited by diffusion in a water-saturated thallus.

Another objective of thus work was to study the implications of photosynthetic CO_2 concentrating mechanism on the carbon isotope ratio from organic material of lichens.

The fractionation of stable carbon isotopes observed in all photosynthetic organisms is determined by factors as:

(i) carboxylation enzyme activity (Rubisco/PEP carboxylase)

There are substantial isotopic fractionations associated with enzymatic reactions catalyzing carboxylation and decarboxylation. The isotopic fractionation associated with carboxylation depends on the enzyme involved and thus on photosynthetic pathway: Rubisco for C_3 plants and PEP carboxylase for C_4 plants [2].

The relative variations of carbon isotopic composition in organic material from plants ($\delta^{13}C$ ‰) are presented in Table 2.

Table 2: Range of $\delta^{13}C$ ‰

Plant	$\delta^{13}C$ [‰]	Δ [‰]
C_3	-35 to -22	28 - 14
C_4	-20 to -10	12 - 4
CAM	-34 to -10	27 - 2

(ii) the activity of the biophysical CO_2 concentrating mechanism (CCM) that affects photosynthetic efficiency and carbon isotope discrimination.

CCM increases the CO_2 concentration at the carboxylation site. CO_2 is accumulated within a partially closed comportment, rising the CO_2 concentration at the carboxylation sites.

One of the implications of CCM is a reduced discrimination against $^{13}CO_2$, which has been reported for both C_4 plants, plants with CAM-metabolism, and for algae possessing the CCM. C_3 plants and algae without such mechanisms show higher discrimination of $^{13}CO_2$ present in the atmosphere [7].

(iii) CO_2 transfer resistance between the atmosphere and the sites of carboxylation

Lichens with green algae (phycobiont) can assimilate CO_2 in the presence of high air humidity, while those with cyanobacteria require liquid water, and then will have a greater diffusion limitation and lower discrimination.

Thallus structure, morphology and water content have been shown to play a significant role in determining differences in CO_2 diffusion resistance and consequent changes in isotopic discrimination in lichens [8].

(iv) isotopic composition of the source air (atmospheric CO_2).

Materials and Methods

Lichen material

The lichen species used in this study are presented in Table 3, along with their photobiont type, algae genus, and collection sites.

Table 3: Lichen species studied

Species	**Photobiont association**	**Photobiont genus**	**Collection site**
Peltigera canina	Cyanobiont	Nostoc	Soil
Hypogymnia physodes	Phycobiont	Trebouxia	Tree
Pseudevernia furfuraceea	Phycobiont	Trebouxia	Tree

We studied 42 lichens collected from different mountains (Retezat Mountains, Hasmasu Mare Mountains and Bihor Mountains) at different altitudes (from 1130m to 1500m) and in different years (1978, 1987, 1989, 1994 and 2003).

Carbon isotope analysis

Carbon isotope composition ($\delta^{13}C$) and carbon isotope discrimination (Δ) in organic material were determined as described by J. Ehleringer [4].

Lichens samples were combusted by dry combustion in excess of oxygen with a view to converting organic samples to CO_2 for isotopic analysis. CO_2 was purified on cryogenic traps and then analyzed by a dual-inlet isotope ratio mass spectrometer (Modele ATLAS 86 designed by Varian). The carbon isotope ratio of the sample CO_2 was compared with that of a reference CO_2 calibrated against PDB (international standard). The standard mean deviation was better than 0.3‰. The carbon isotope composition of air was considered -8.0‰ [4]. Carbon isotope composition of plant material (δ_p) and source air CO_2 (δ_a) as:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \tag{1}$$

Results and Discussions

The mean values of the relative carbon isotope variation ($\delta^{13}C_{mean}$) are presented in Table 4.

Table 4: The average of $\delta^{13}C$ for *Peltigera canina*, *Hypogymnia physodes* and *Pseudevernia furfuraceea* lichens

Lichen species	**$(\delta^{13}C)_{mean}$ [‰]**	**Photosynthetic pathway**
Peltigera canina	-22.66	C_3
Hypogymnia physodes	-23.32	C_3
Pseudevernia furfuraceea	-23.71	C_3

The mean values were obtained from 15 samples of *Peltigera canina*, 14 samples of *Hypogymnia physodes* and 13 samples of *Pseudevernia furfuraceea*. The $\delta^{13}C$ values determined indicate that regarding their photosynthetic activity-the photobionts of these lichens may be classified as C_3 plants. However these values are closed to C_4 plants, especially for *Peltigera canina*. This is because of the implications of photosynthetic CO_2 concentrating mechanisms. To investigate the effect of CCM on isotopic discrimination in these lichens, we have studied the variation of $\delta^{13}C$ and Δ in three lichens species collected at the same place and in the same year.

Values of carbon isotope composition ($\delta^{13}C$) and carbon isotope discrimination (Δ) for *Peltigera canina, Hypogymnia physodes* and *Pseudevernia furfuraceea* are presented in Table 5.

Table 5: Variation of carbon isotope discrimination of different lichens

No	Lichens species	Photobiont	Mountain	Year	$\delta^{13}C$ [‰]	Δ [‰]
1.	Peltigera canina	Cyanobiont	Hăşmaşu Mare	1989	-21.57	13.86
2.	Peltigera canina	Cyanobiont	Hăşmaşu Mare	2003	-22.39	14.71
3.	Hypogymnia physodes	Phycobiont	Hăşmaşu Mare	1989	-24.51	16.92
4.	Hypogymnia physodes	Phycobiont	Hăşmaşu Mare	2003	-24.70	17.12
5.	Pseudevernia furfuraceea	Phycobiont	Hăşmaşu Mare	1989	-23.42	15.78
6.	Pseudevernia furfuraceea	Phycobiont	Hăşmaşu Mare	2003	-24.25	16.65

Carbon isotope discrimination varies among different lichen groups from 13.86‰ to 17.12‰. The results for the cyanobiont lichen group were quite different from those for the phycobiont lichens. These differences were observed between species collected in the same habitat and the same year. The observed differences in Δ have been attributed to differences in CO_2 transfer resistance related to the type of photobiont present in the symbiotic association. In particular, *Peltigera canina*, which require liquid water for net CO_2 fixation, would show high diffusion resistance and low Δ (13.86‰ and 14.71‰). The CCM would explain the higher carboxylation efficiency at high water content and the low Δ observed in these lichens.

Because *Hypogymnia physodes*, a phycobiont lichen, have lower CCM activity, the discrimination against $^{13}CO_2$ is increased (16.92‰ and 17.12‰). This is shown also by the isotopic composition of these lichens, which is depleted by about 3‰. *Hypogymnia physodes* can fix CO_2 in the presence of water vapor, so the CO_2 diffusion resistance from air to chloroplast is less than in *Peltigera canina*.

$\delta^{13}C$ of the lichens were shown that could to be related to such factors as temperature, moisture and humidity, slope and altitude.

The $\delta^{13}C$ values fluctuate slightly with altitude gradient for both lichens *Hypogymnia physodes* and *Pseudevernia furfuraceea* (Fig. 1).

A cause for this variation is that along an altitude gradient lichens are exposed to numerous natural and man-made pollutants and stress factors (SO_2, O_3, NO_x). It was shown that lichens react sensitively to any pollution [5]. At higher elevation the ozone is of particular importance as pollutant.

Another cause for the variation of $\delta^{13}C$ with altitude may be a result of a complex combination of interrelated climatic variables, such as precipitation, atmospheric pressure, and temperature. For both species, an increase in $\delta^{13}C$ values may be associated with a higher annual rainfall at the same site.

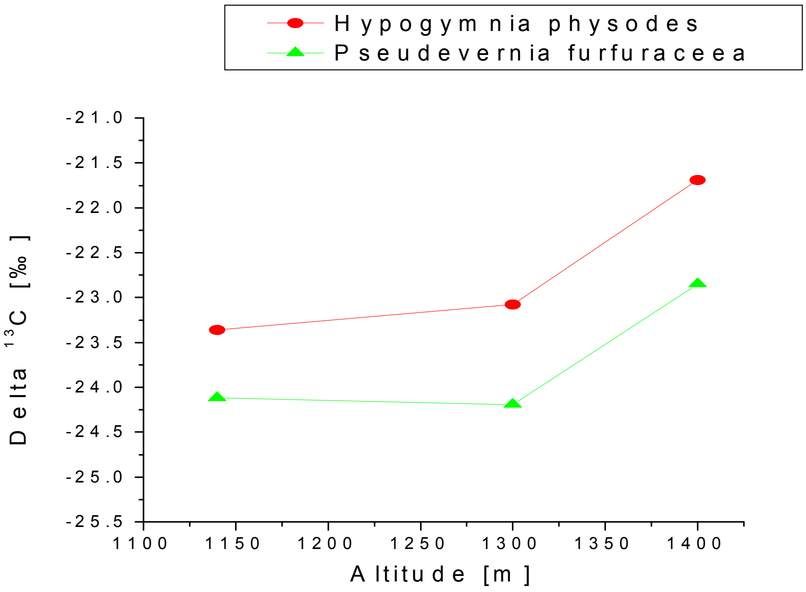

Fig. 1: δ^{13}C values of *Hypogymnia physodes* and *Pseudevernia furfuraceea* depending on altitude (Bihor Mountain, year 1978)

The stress factors have a direct or indirect effect on photosynthetic apparatus of the lichens, and therefore on ^{13}C composition in the organic material from lichens. In Fig. 2 there is a variation of δ^{13}C with altitude gradient, but *Pseudevernia furfuraceea* shows a significantly more negative value at 1500m.

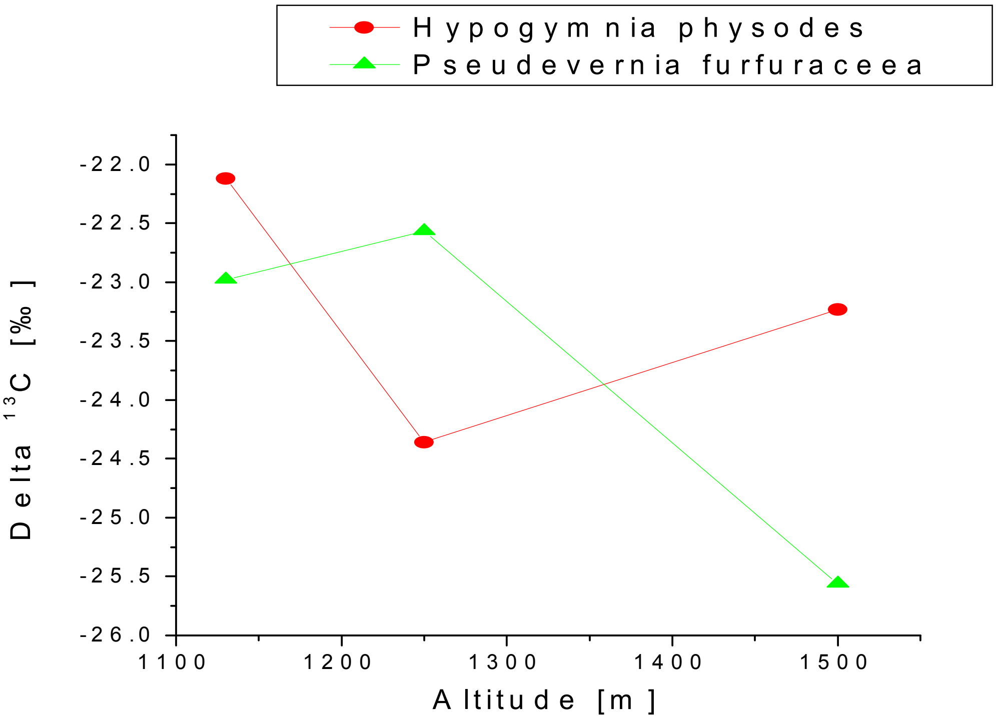

Fig. 2: δ^{13}C values of *Hypogymnia physodes* and *Pseudevernia furfuraceea* depending on altitude (Retezat Mountain, year 1994)

We have supposed that this trend of *Pseudevernia furfuraceea* is because these lichens are particularly sensitive to air pollutants. It is possible to be more stress factors at that altitude (1500m) and at that collection date (1994).

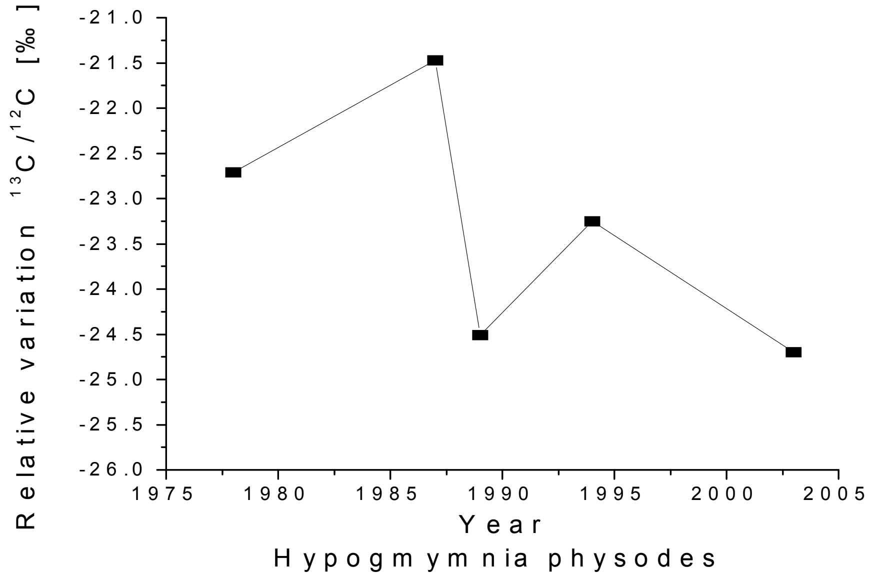

Fig. 3: $\delta^{13}C$ variation of *Hypogymnia physodes* depending on collection dates

In figure 3 is shown the variation in $\delta^{13}C$ among different collection dates.

The variations in $\delta^{13}C$ are attributable to changes in $\delta^{13}C$ of the source air CO_2. It is known that the CO_2 atmospheric is increasing each year and this increase is accompanied by a year to year decrease in $\delta^{13}C$ of the CO_2 [1].

However, there are two $\delta^{13}C$ values in Fig. 3 that show a higher value than we would expect. The $\delta^{13}C$= - 21.47‰ for the lichens collected in 1987 may be the result of great contamination after Chernobyl. This contamination has had a significant impact on photosynthetic capacity of lichens, and in response on $\delta^{13}C$ values. The $\delta^{13}C$= - 23.25‰ for the lichens collected in 1994 is possible to be the result of a higher annual rainfall because the phycobiont is sensitive to change in moisture.

Conclusions

Analysis of the $\delta^{13}C$ values of organic material in 42 samples of lichens showed that the photobionts of these lichens were C_3-like. Carbon isotope discrimination, Δ, varies with the type of photobiont: the low Δ for cyanobiont lichens and the higher Δ for phycobiont lichens. The differences in Δ have been attributed to differences in CO_2 transfer resistance and to photosynthetic CO_2 concentrating mechanism (CCM).

The $\delta^{13}C$ values of organic material in lichens fluctuate with altitude and the date collection. These variations are attributable to different pollutants and stress factors, to climatic variables and to changes in $\delta^{13}C$ of the source air CO_2. This is relevant for understanding the impact of global change on natural vegetation.

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STUDIUL LICHENILOR FOLOSIND VARIAȚIA NATURALĂ A ^{13}C DIN MATERIA ORGANICĂ A ACESTORA

(Rezumat)

Studiul lichenilor sub cât mai multe aspecte este justificat datorită rolului lor în formarea solului, în prevenirea degradării acestuia, dar mai ales pentru că distribuția lor este corelată cu nivelele de poluare din mediul înconjurător.

Una din cele mai noi metode de studiu a lichenilor este determinarea compoziției izotopice a ^{13}C din materia organică din licheni prin metoda spectrometriei de masă [6].

În studiul de față a fost determinat conținutul de ^{13}C din 42 eșantioane de licheni aparținând la trei specii diferite (*Peltigera canina, Hypogymnia physodes* şi *Pseudovernia furfuraceea*), colectate în diferiți ani, de la diferite altitudini din Munții Retezat, Hăşmaşu Mare şi Munții Bihor.

Variația conținutului de ^{13}C s-a exprimat ca variația relativă a conținutului de ^{13}C din probă față de un standard, şi este notată cu $\delta^{13}C$ ‰ (per mil). Precizia măsurătorilor exprimată prin eroarea medie pătratică a fost de ± 0.3 ‰.

Rezultatele obținute au dus la următoarele concluzii:

(i) Valorile $\delta^{13}C$ determinate pentru toți lichenii studiați variază între –21.21‰ şi –26.93‰, ceea ce îi încadrează în categoria plantelor C_3 la care în timpul fotosintezei se consumă cu preferință $^{12}CO_2$. Pentru plantele C_3, $\delta^{13}C$ variază între –21‰ şi –35‰ [3].

(ii) S-a găsit un gradient $\delta^{13}C$ cu altitudinea care pentru specia *Hypogymnia physodes* este între –21.21‰ (1130 m) şi –26.09‰ (1520 m). Se poate presupune că la diferite înălțimi, lichenii sunt expuşi la factori de stres diferiți, cum ar fi concentrația de ozon şi care afectează direct sau indirect procesul de fotosinteză din fotobiont.

(iii) Între diferitele grupuri de licheni apar diferențe în compoziția izotopică. Astfel, pentru lichenii *Peltigera canina* colectați din Munții Bihor în anul 1978 şi care aparțin grupului de licheni *Cianobiont*, s-a găsit o valoare medie $\delta^{13}C$ = -23.67‰, iar pentru lichenii *Hypogymnia physodes* colectați din aceeaşi munți, în acelaşi an şi care aparțin grupului de licheni *Ficobiont*, s-a găsit valoarea medie $\delta^{13}C$ = -22.71‰, cu aproximativ 1‰ mai mare în conținutul relativ de ^{13}C. Explicația acestei diferențe se găseşte în viteza diferită de fotosinteză a celor două grupuri de licheni [9]. Lichenii avand asociați fotobionți diferiți au preferințe diferite pentru apa necesară fotosintezei. Lichenii din grupul *Cianobiont* au nevoie de apă în stare lichidă pentru asimilarea fotosintetică a CO_2, în timp ce lichenii din grupul *Ficobiont* pot să fixeze CO_2 în prezența vaporilor de apă. Altfel spus, diferența observată între valorile $\delta^{13}C$ ale celor două grupuri de licheni este datorată diferenței în rezistența de transfer a CO_2 spre cloroplaste, care este diferită pentru diferiți fotobionți (în cazul de față alga verde şi cianobacteria).

(iv) Analiza variației $\delta^{13}C$ pentru lichenii colectați la date diferite arată de asemenea un gradient de variație. În toate cazurile au fost aleşi licheni colectați la aceeaşi înălțime. Gradientul de variație a $\delta^{13}C$ cu data colectării arată o sărăcire a conținutului de ^{13}C. Această sărăcire este explicată prin schimbările în $\delta^{13}C$ şi concentrația de CO_2 a sursei de CO_2 (care este CO_2 atmosferic) şi care în 14 ani s-a modificat. Acest lucru este relevant pentru înțelegerea impactului schimbărilor globale asupra vegetației.