Contribuţii Botanice, *XL*, **2005**
Grădina Botanică "Alexandru Borza"
Cluj-Napoca

IMPROVING THE USE OF LICHENS AS BIOMONITORS OF ENVIRONMENTAL CHANGE UTILIZING STABLE ISOTOPE ABUNDANCES

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Abstract: This study was designed to determine the extent to which the carbon isotope ratio in four species of lichens was influenced by such features of the natural environmental as humidity and rainfall. The range of $\delta^{13}C$ measured for the studied lichens was –26.93‰ to –19.06‰, with a mean value of –23.38‰. The result of this study was found that of natural factors humidity tended to decrease the value of $\delta^{13}C$, and the precipitation to which the lichens were exposed tended to increase $\delta^{13}C$. The effects were minor of the order of 1‰ for all lichens. From their opposite effects, the natural parameters could be assumed to cancel out. We submit that this result is important to the field of the environmental monitoring of pollutants. The outcome of a monitoring study would be only interpretable where the effects of natural environmental factors on a species of lichens were either negligible or systematic.

Introduction

Lichens gain nutrients and minerals almost exclusively from the air and the precipitation of moisture. Carbon dioxide is the most important of lichen nutrients but lichens also utilise nitrogen in one or other of its forms present in the atmosphere. These include gaseous nitrogen, ammonia, various nitrogen gases known collectively as NO_x, and the nitrate ion.

Lichens have evolved highly effective but indiscriminate mechanisms for the absorption and accumulation of low concentration of mineral present in air and moisture. These same physiological properties are responsible for the sensitivity of lichens to atmospheric pollution.

The previously studies [2] have summarized the effect of pollutants on lichens. The stable isotope abundance of carbon in a species of lichens has been investigated in a study relating $\delta^{13}C$ values with know levels of selected pollutants as SO_2, NO_2 and O_3 [1]. To establish a correlation between any pollutant factors and $\delta^{13}C$ in lichens, it is necessary to understanding the effect of the factors in the natural environment that have been shown to affect the growth characteristics of lichens and influence their survival.

Factors in the natural environment that have been shown to affect the growth characteristics of lichens and influence them include rainfall [4] moisture status [7], light [5] and topography [8]. It is well established that the phycobiont in the lichen is sensitive to changes in moisture and light. Exposure of lichens to minimal moisture, under suitable light conditions results in the rapid onset of photosynthesis. Conversely, as an adaptation to high light intensity and temperature, lichens dry rapidly and endure long periods of drought without apparent cell damage.

Carbon is the principal element of the carbohydrates, lipids and proteins that are the predominant metabolites of lichens. Carbon has two stable isotopes ^{12}C, at a natural abundance of 98.89%, and ^{13}C (1.11%) [6]. For two isotopes of any element, the proportion (or ratio) of one isotope relative to the other will very slightly with chemical structure and the environment. Such small changes in the isotope ratio are referred to as fractionation. The extent of this isotope fractionation is expressed in terms of a "δ" value. For carbon, $\delta^{13}C$ is a measure of the change in

the proportion of ^{13}C to ^{12}C in the sample of interest, relative to that in a standard substance. The unit of this measurements is "per mil" (‰).

$$\delta^{13}C = \frac{\left(^{13}C/^{12}C\right)_{lichen} - \left(^{13}C/^{12}C\right)_{s\,tan\,dard}}{\left(^{13}C/^{12}C\right)_{s\,tan\,dard}} x10^{3} \text{ ‰}$$

The international standard for measurements of carbon isotope fractionation is PDB, a carbonate deposit (belemnite) at Peedee in South Carolina, USA.

Materials and Methods

The lichens chosen for this study was species *Cladonia fimbriata* (L1), *Hypogymnia physodes* (L2), *Peltigera cannina* (L3) and *Pseudovernia furfuracea* (L4). The lichen species were collected in 1978, 1989, 1994 and 2003 in three different mountains (Retezat, Hasmasu Mare and Bihor) at altitude of about 1300m. These sampling sites were selected because they are at great distance of any anthropogenic pollution. At time of sampling, field data (temperature, relative humidity, and average monthly precipitation) were recorded.

Carbon isotope composition $\delta^{13}C$ in lichens was determined as described by J.Ehleringer [3].

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 analysed by an isotope ratio mass spectrometer, model 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 international standard PDB. The standard mean deviation was better than 0.3‰.

We have used the following patterns of isotopic variation with stress factors:

I. decrease in irradiance has a physiological effect on photosynthesis and makes $\delta^{13}C$ more negative (we will have a decrease in $\delta^{13}C$)
II. soil moisture decrease produces a decrease in photosynthesis and $\delta^{13}C$ becomes more positive
III. exposed lichens to pollutants exhibited lower carbon isotope discrimination.

Results and Discussions

The range of $\delta^{13}C$ measured for the studied lichens (56 samples) was –26.93‰ to –19.06‰. The mean value was –23.38‰ (Fig.1).

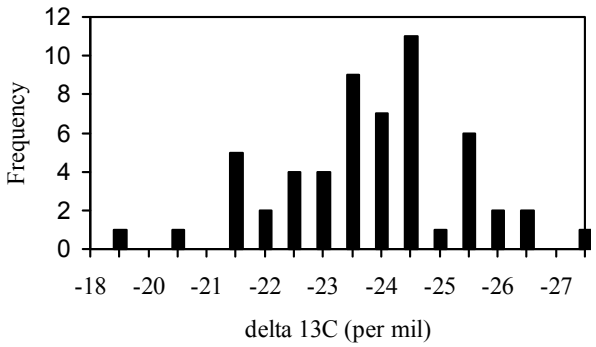

Fig. 1: The distribution frequency of δ ^{13}C (‰) values for the 56 samples

The ^{13}C content of the lichens in natural setting is dictated by effects associated with assimilation of carbon, metabolism and biosynthesis and the ultimate cellular carbon budget.

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 lichens if this causes swelling of the fungal hyphae, which may block the gaseous pores within the hyphal matrix and thus impede the diffusion to the photobiont.

Also, the most lichens 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 lower or when HCO_3^- becomes the dominating inorganic carbon source. One of the implication of photosynthetic CO_2 concentrating mechanism, where CO_2 is accumulated within a partially closed compartment, is a reduced discrimination against $^{13}CO_2$.

We have found a weak but negative correlation between $\delta^{13}C$ and relative humidity (Fig.2, 3, 4) and a positive correlation between $\delta^{13}C$ and average monthly precipitation at the studied sites (Fig.5, 6, 7). This correlation was found for all the studied lichens L1, L2, L3 and L4.

Effect of rising humidity on $\delta^{13}C$ is the opposite of that of increasing rainfall.

Each point in Fig.2-7 represents the average of measurements for six samples of L1-L4 lichens collected in the same site and the same date.

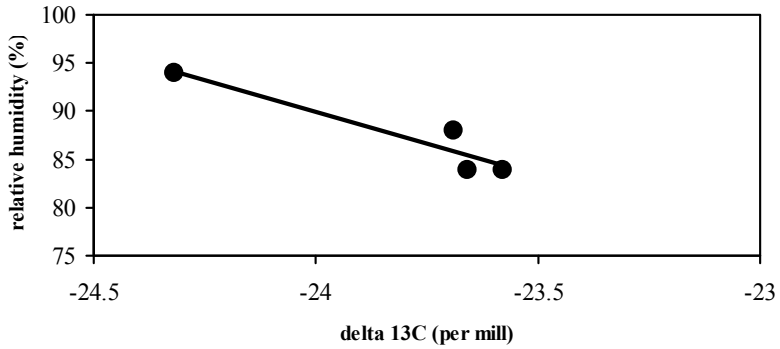

Fig. 2: The relationship between $\delta^{13}C$ (‰) and relative humidity (%) for *Cladonia fimbriata*

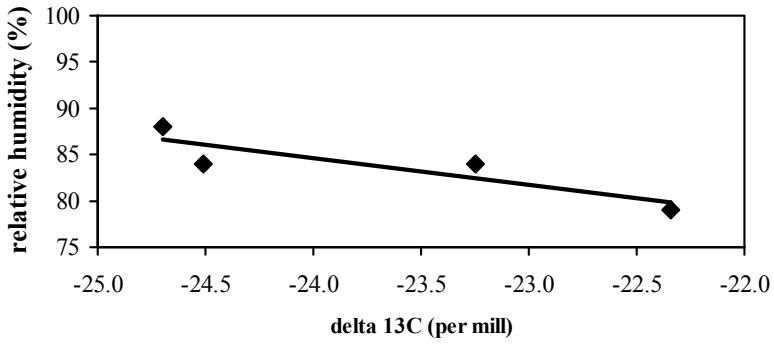

Fig. 3: Relationship between $\delta^{13}C$ (‰) and relative humidity (%) - *Hypogymnia physodes*

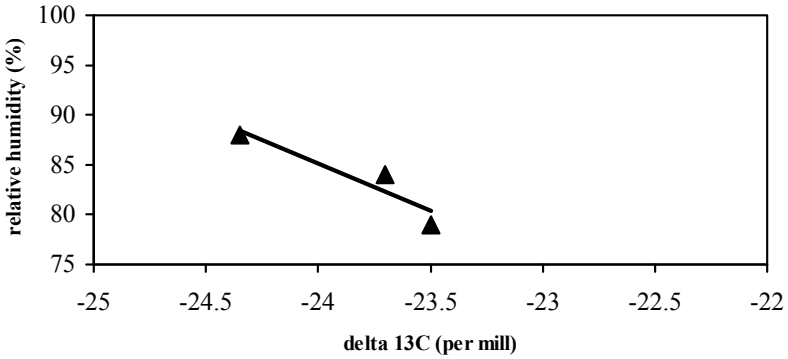

Fig. 4: Relationship between $\delta^{13}C$ (‰) and relative humidity (%) - *Pseudevernia furfuraceea*

The moisture is an important factor influencing the $\delta^{13}C$ in the lichen. Photosynthesis in lichens takes place only in light after the lichen thallus has been wetted by air-born moisture or through direct exposure to liquid water. The conclusion is that under humid condition, ^{12}C enriched components accumulated, reducing the $\delta^{13}C$ value. This result is shown for L1, L2, L3 species (Fig.2, 3, 4) where with increasing humidity, $\delta^{13}C$ values decreasing.

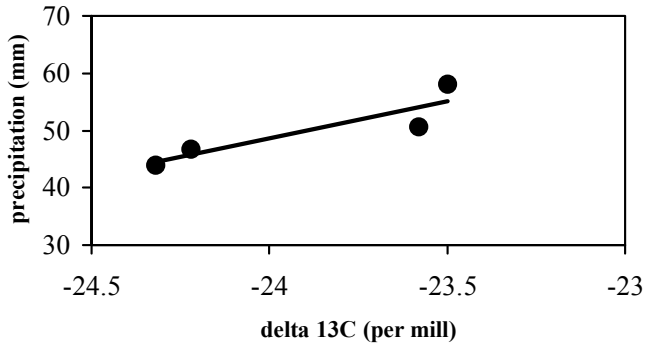

Fig. 5: The relationship between $\delta^{13}C$ (‰) and average monthly precipitation (mm) for *Cladonia fimbriata*

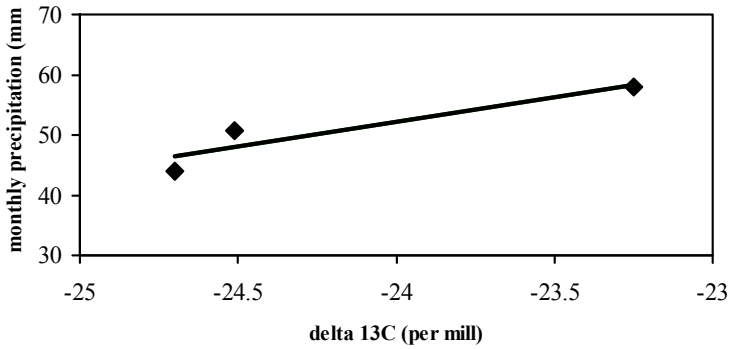

Fig. 6: The relationship between $\delta^{13}C$ (‰) and monthly average precipitation (mm) *Hypogymnia physodes*

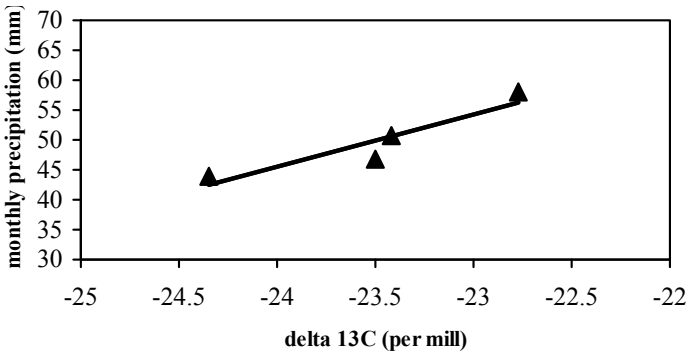

Fig. 7: The relationship between $\delta^{13}C$ (‰) and monthly precipitation (mm) - *Pseudevernia furfuraceea*

Lichens have no specialized mechanism for water uptake and no way of maintaining a water balance. In lichens, saturation levels of water are thought to lead to increased resistance to CO_2 transport into the lichen, with a subsequent of reduction in discrimination, that is an increase of $\delta\ ^{13}C$ ‰ in lichen. With increasing water content of the lichen thallus, $\delta\ ^{13}C$ values rise until the rate of diffusion of CO_2 into the thallus becomes rate limiting. This result is shown for L1, L2, L3 species in Fig.5, 6, 7: with increasing of the monthly precipitation, $\delta\ ^{13}C$ values increasing, too. Simply stated the more liquid water there is in the lichen environment, the higher (less negative) the $\delta\ ^{13}C$ value.

Conclusions

The measurements showed a negative correlation between $\delta^{13}C$ and relative humidity. Under humid condition, ^{12}C enriched components accumulated, reducing the $\delta^{13}C$ value, so with increasing humidity, $\delta^{13}C$ values are decreasing.

There is a positive correlation between $\delta^{13}C$ and average monthly precipitation at the studies sites: with increasing of the monthly precipitation, $\delta^{13}C$ values increasing, too. The effects were minor of the order of 1‰, excepting L3 species where the effect was ±3‰.

From their opposite effects, the natural parameters could be assumed to cancel out. We submit that this result is important to the field of environmental monitoring of pollutants. The outcome of a monitoring study would be only interpretable where the effects of natural environmental factors on a species of lichen were either negligible or systematic.

BIBLIOGRAPHY

1. Batts, J.E., Calder, L.J., Batts, B.D., 2004, Utilizing stable isotope abundances of lichens to monitor environmental change, *Chemical Geology*: 204, 345-368.
2. Conti, M.E., Cecchetti, G., 2001, Biological monitoring: lichens as bioindicators of air pollution assessment- a review, *Environ. Pollut*: 114, 471-492.
3. Ehleringer, J.R., 1991, *$^{13}C/^{12}C$ Fractionation and Utility in Terrestrial Plant Studies.* In: Carbon Isotope Techiques, D.C.Coleman, B.Fry (eds.), *Academic Press*., Inc.San Diego: 174-176, 187-200.
4. Eldridge, D.J., Tozer, M.E., 1997, Environmental factors relating to the distribution of terricolous bryophytes and lichens in semi-arid Eastern Australia, *Briologist*, **100**: 28-39.
5. Gremmen, N.J.M., Huiskes, A.H.L., Francke, J.W., 1994, Epilithic macrolichen vegetation of the Argentine islands, Antarctic Peninsula, *Antarct. Sci.*, **6**: 463-471.
6. Hoefs, J., 1997, *Stable Isotope Geochemistry*, Springer-Verlag, Berlin.
7. Lange, O.L, Belnap, J., Reichenberger, H., Meyer, A., 1997, Photosynthesis of green algal soil crust lichens from arid lands in Southern Utah, USA-role of water content on light temperature responses of CO_2 exchange, *Flora*, **192**: 1-15.

8. Pharo, E.J., Beattie, A.J., 1997. Bryophyte and lichen diversity-acomparative study, *Aust. J.Ecol.*, **22**: 151-162.

UTILIZAREA LICHENILOR CA BIOMONITORI PENTRU SCHIMBĂRILE DE MEDIU FOLOSIND COMPOZIȚIA IZOTOPICĂ A ^{13}C

(Rezumat)

Scopul acestui studiu a fost de a determina efectul pe care factori din mediul înconjurător, cum ar fi umiditatea şi cantitatea de precipitații, influențează compoziția izotopică a carbonului în materia organică din câteva specii de licheni. Metoda de determinare a variațiilor relative a ^{13}C în probele de licheni a fost spectrometria de masă pentru rapoarte izotopice. Pentru acest studiu au fost selectate patru specii de licheni (*Cladonia fimbriata*, *Hypogymnia physodes*, *Peltigera cannina* şi *Pseudevernia furfuracea*) care au fost colecate din trei masive muntoase diferite (Munții Retezat, Munții Haşmaşu Mare şi Munții Bihor) de la altitudinea de aproximativ 1300 m. Alegerea acestor zone de colectare a lichenilor a fost determinată de condiția de a fi cât mai departe de orice sursă de poluare. Valorile $\delta^{13}C$ determinate pentru lichenii studiați au variat de la –26.93‰ la -19.06‰, cu o valoare medie de –23.38‰. Măsurătorile au arătat o corelație negativă intre $\delta^{13}C$ şi umiditatea relativă a mediului înconjurator. În condiții de umiditate mai mare, ^{12}C se acumulează în materia organică din licheni reducând valoarea $\delta^{13}C$. Altfel spus, cu creşterea umidității valorile $\delta^{13}C$ descresc. Între $\delta^{13}C$ şi media lunară a precipitațiilor la locul colectării lichenilor s-a găsit o corelație pozitivă. Cu creşterea mediei lunare a precipitațiilor, valorile $\delta^{13}C$ cresc de asemenea. Efectele sunt destul de mici, de ordinul 1‰. Aşa cum rezultă din datele experimentale, aceşti parametrii naturali par să-şi reducă reciproc efectele asupra valorilor $\delta^{13}C$. Acest rezultat este important atunci când se doreşte să se utilizeze lichenii ca biomonitori de poluare a mediului inconjurător. Un studiu de monitorizare a poluării atmosferice cu ajutorul lichenilor poate fi interpretabil numai atunci când efectele factorilor naturali ca umiditatea şi precipitațiile sunt fie neglijabile, fie sistematice. Studiul de față a demonstrat că efectele celor doi factori naturali (umiditatea relativă şi cantitatea de precipitații) se anulează reciproc.