SHORT COMMUNICATION

Variations in the Mineral Composition of Herbarium Plant Species Collected During the Last Three Centuries

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ABSTRACT
Mineral content (dry weight basis) was determined for herbarium specimens of 12 C₃ plants (trees, shrubs and herbs) collected during the last 250 years in N.E. Spain. Present values of Al, Ca, Cu, Sr, Fe, P, Mg, Mn, K, Na, S, and Zn were always lower than in any other period of the last three centuries. Only one C₄ plant was analysed. It presented a similar pattern to the C₃ plants. These results are in accordance with experimental results that have shown that the mineral content of plants grown in elevated CO₂ is generally lowered. Increased atmospheric CO₂ and other anthropogenic environmental changes are suggested as possible causes of the changes in mineral content.

Key words: Leaf mineral content, Al, Ca, Cu, Sr, Fe, P, Mg, Mn, K, Na, S, Zn, herbaria, last three centuries.

INTRODUCTION
Changes in the atmospheric and terrestrial environments produced by man’s activities have been found to be accompanied by changes in the herbarium specimens collected during the last centuries. Woodward (1987) found a decrease in stomatal number; Peñuelas and Matamala (1990) found decreases in stomatal density, nitrogen content and specific leaf area; and in the same material, Peñuelas and Azcón-Bieto (1992) found a decrease in Δ¹³C that suggested an increase in carbon assimilation or a decrease in stomatal conductance and, therefore, an increase in water use efficiency (in parallel with the increase in atmospheric CO₂).

Increased atmospheric CO₂ concentrations may have resulted in decreased mineral concentrations of plant tissue if mineral uptake has not increased at the same rate as carbon uptake (Eamus and Jarvis, 1989). The aim of this study was to complete the previous studies of herbarium specimens collected during the last three centuries with the analysis of their mineral composition, and to explore the possible causes of the changes.

MATERIALS AND METHODS
The following species were studied: Pinus uncinata Ramond Ex De. in Lam. et De., Pinus pinea L., Abies glutinosa Gaertn., Betula pendula L., Juniperus communis L., Ceratonia siliqua L., Buxus sempervirens L., Pistacia lentiscus L., Helleborus foetidus L., Rhododendron ferrugineum L., Amaranthus caudatus L., Papaver alpinum L., and Gentiana alpina Vill. Leaves had been stored in the herbarium of the ‘Institut Botànic de Barcelona’ and in the herbarium of the ‘Departament de Botànica de la Universitat de Barcelona’. They had been fumigated with DDT. In most cases, specimens of the same species had been collected in similar habitats and in the same seasons during the periods studied (1750–60, 1850–90, 1940–50, and 1982–8). The drying procedure had been very similar for all specimens (herbarium press). Wherever possible (most times), only leaves on the same terminal position of the herbarium shoot were sampled. Two to four leaves from different herbarium shoots were analysed for each period and species. Leaves were dried for 6 d at 60 °C. Samples of more than 0.1 g were digested in a microwave Whirlpool AVM 635 using open fluorinated ethylene propylene flasks (Nalgé Company) with an acid mixture of nitric (60%)- perchloric (60%) acids. The digestion programme was set at 100% (650 W) for 10 min. Once

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samples were efficiently oxidized (1 cm³ remaining), they were diluted with deionized water (10 cm³). Flasks were stoppered and shaken by hand for thorough mixing of the contents. The sample solution was poured into a polystyrene sample tub, placed in an autosampler carousel and analysed for 12 elements by ICAP–AES (inductively coupled argon plasma emission spectrometer) Polyscan Thermo Jarrel ASH Model 61E.

To avoid the variability due to species genetic differences and to focus on the environmental variability, values relative to present content were used in calculations. We tried to avoid variability due to leaf age and position and to light environment of the collected plants by sampling the herbarium material as uniformly as possible. We always tried to choose the same leaf position and the same, or the closest possible, site and season of collection, but it was not always feasible.

RESULTS AND DISCUSSION

Present contents of Al, Ca, Cu, Sr, Fe, P, Mg, Mn, K, Na, S, and Zn in herbarium leaves were always lower than values from any other period in the last three centuries (Fig. 1). However, a great variability in the relative changes was found (Fig. 1). The maximum variability was seen for Al, Sr, Fe, Mn, and Na, probably because of their lower concentrations and the associated difficulty of precise measurement.

Only one C₄ plant, *Amaranthus caudatus* L., was analysed. Present values were also lower than in the past decades and centuries but the sparse data (only one species) make it difficult to draw any significant conclusion on changes in C₄ plants (data not shown).

There are several factors such as genetics, age and position of leaf, light environment, source of nutrients, or soil characteristics, that affect plant elemental composition (Jones et al., 1991). We tried to avoid most of these factors of variability as explained in Materials and Methods. However, there are still some

![Relative changes in leaf content](image-url)

**Fig. 1.** Relative (to the 100% present average for each species) changes in the content of several elements (measured on a dry weight basis) of herbarium-stored leaves of 12 C₃ species of trees, shrubs, and herbs from Catalonia (N.E. Spain), collected over the last 250 years. Averages of overall means (±SEM) for 1750–60, 1850–90, 1925–50, and 1982–90 samples are represented. Atmospheric CO₂ concentrations for each date are based on ice-core studies and the Mauna Loa data (Neftel et al., 1985; Friedli et al., 1986).
remaining factors that might explain the decrease in leaf element content.

Because some of the measured elements, Ca, K, and Na, move readily in the xylem, they can be expected to increase in the upper portions of the plant as the transpiration stream evaporates, leaving these elements behind (Jones et al., 1991). The decrease of their content in present leaves might be explained by a decrease in stomatal conductance, in agreement with our previous results of decreased 13C discrimination (Peñuelas and Azcón-Bieto, 1992) and decreased stomatal density (Peñuelas and Matamala, 1990) in the same herbarium material, and in accordance with meteorological data of the zone where plants were collected, that show that the 1980s were drier than the 1940s (Burgueno, 1989). The same could be true for Zn, Cu, and Fe leaf contents that have been found to be positively correlated with transpiration rate (Sándor and Zoltánne, 1981).

The fact that the same elemental patterns are seen in Ca (phloem-immobile so that its concentration is proportional to transpiration), P (phloem-mobile and indicative of general nutrient status), and Na and Al (actively excluded by roots and other tissues) is in accordance with the difficulty that additional optimum amounts of other mineral nutrients can be obtained by plants, and with the nutrient dilution produced by the increase in carbohydrates and by the decrease in transpiration of plants growing under higher CO2 availabilities (Lemon, 1983). These results are also in accordance with experimental results showing that the mineral content of plants grown in elevated CO2 is generally lower compared to controls (Mousseau and Saugier, 1992; Overdieck, 1993). There could also be an indirect effect of increased CO2 on mineral composition through changes in temperature and humidity due to the greenhouse effect (Houghton et al., 1990).

This decrease of mineral content might be a short-term phenotypic response within the period of rapid CO2 rise of the past 200 years. Over longer terms of CO2 increase, a genotypic adaptation could also be likely, as has been found for the stomatal density of fossil leaves of Salix herbacea L. extending back over 140 ka (Beering et al., 1993).

However, we could not clearly conclude that increased CO2 is the major cause of the decline in mineral composition of herbarium specimens through time because the decline is not uniform, even though CO2 has increased steadily, and because the C4 plant (not CO2-limited) and the C3 plants studied changed in parallel through time. There are several other factors that might also explain the changes in concentration: soil weathering or acid rain could reduce mineral nutrient content through the stimulation of nutrient leaching from soils. However, this does not seem to be the case in soils of Catalonia (Vallejo, personal communication). Another source of variation may be the long-term patterns of litter and forest product utilization by man that represents a continuous drain of nutrients from ecosystems over the last centuries. The landscapes of Europe have changed so much that the growing conditions are hardly comparable. Other less intensively managed landscapes could be more appropriate to sample for unbiased signals of this kind. Nevertheless, in the short-term, the results reported here indicate further that human activities are influencing vegetation.

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LITERATURE CITED


