

Influence of Dust from a Limestone Quarry on Chlorophyll Degradation of the Lichen *Physcia adscendens* (Fr.) Oliv.

A. Zaharopoulou,¹ T. Lanaras,² and M. Arianoutsou¹

¹Department of Biology, Division of Ecology and Systematics, University of Athens, GR-15784, Greece and ²Department of Botany, University of Thessaloniki, P.O. Box 109, GR-54006, Greece

Dust is among the substances usually referred to as pollutants affecting lichen growth and occurrence. Dust splashed or blown on to roadside trees and shrubs usually leads to a reduction of bark acidity, thus encouraging lichen assemblages characteristic of plants with alkaline bark (i.e., *Xanthorion*) (Hawksworth and Rose 1976). Dust from limestone quarries and cement and lime works is also alkaline and has the same neutralizing effect on bark, promoting the development of lichen species normally confined to limestone rocks (e.g., *Physcia* spp., *Xanthoria* spp.). A number of methods are used to estimate the effect of airborne pollutants on lichens. Changes in the rate of photosynthesis, chlorophyll content and chlorophyll degradation are normally more informative than parameters such as species composition and cover (De Wit 1983).

The present work was designed to examine the relative sensitivity of the epiphytic lichen *Physcia adscendens* (Fr.) Oliv. to dust emitted from a limestone quarry in the metropolitan area of Thessaloniki, North Greece.

MATERIALS AND METHODS

The study was carried out at Asvestochori ("Limestone Village"), 15 km NE of Thessaloniki, Greece. The source of air pollution was a limestone quarry located in this village and operating there for over 50 years.

The foliose lichen *Physcia adscendens* (Fr.) Oliv. growing on branches and twigs of *Quercus coccifera* L. evergreen shrubs was collected in late March 1989 from five sites, all located in the broader area of Asvestochori (Fig. 1). Distance from the source of dust emission is 5 km for site A facing southwest, 3 km for site B facing south, 1 km for site C facing north and less than 1 km for sites D and E facing west and south, respectively.

Voucher specimens of the lichen were deposited at the herbarium of the University of Thessaloniki (TAU). The twigs were transported to the laboratory where the thalli were removed for pigment determination. Chlorophyll was extracted as described by Hiscox and Israelstam (1979) with minor modifications. Thalli (10-

Send reprint requests to Dr. Margarita Arianoutsou at the above address.

20 mg) were placed in vials containing 4 ml DMSO (Merck, Germany) and incubated in the dark at 65 °C for 1 hour. Cell debris was removed by centrifugation at 15,000 g for 1 min. The turbidity of the cell-free extracts (A_{750}) was < 0.003. The cell-free extract was measured with a LKB Ultrospec II spectrophotometer at the appropriate wavelengths. Total chlorophyll content was determined according to Jeffrey and Humphrey (1975) using DMSO as an extraction solvent (Hiscox and Israelstam 1979). The chlorophyll to phaeophytin ratio was determined by taking the ratio of the absorbance at 435 and 415 nm, according to Ronen and Galun (1984) and Boonpragob (1987). Data were expressed as percentages due to variability in numbers of lichen algae within a thallus (Plummer and Gray 1972).

RESULTS AND DISCUSSION

The disruption of photosynthetic processes has been so far correlated to the concentration of sulphur dioxide in the air (Hawksworth and Rose 1970; Nash 1973; Moser et al. 1980; Kauppi and Mikkonen 1980; De Wit 1983; Kardish et al. 1987; Boonpragob 1987; McCune 1988) and it is attributed to either irreversible chlorophyll oxidation at pH 3-4.5 or chlorophyll conversion to phaeophytin at even lower pH values. The breakdown of chlorophyll to phaeophytin in plant tissues as a response to air pollution has been demonstrated by Dorries (1932) and Nash (1973).

Chlorophyll and phaeophytin contents were used as the criteria for injury caused by air pollution to *Physcia adscendens* (Table 1). The chlorophyll concentration decreased, while the percentage of phaeophytin increased with decreasing distance from the pollution source and consequently increasing load of dust (Table 1). This indicates that airborne pollution results in the destruction of chlorophyll. The evidence presented here shows that the destruction of chlorophyll in the thalli of the lichen can also occur as a result of pollution by limestone dust.

Table 1. Chlorophyll concentration and percentage of phaeophytin in thalli of *Physcia adscendens* along the load gradient of dust.

Sites	Total chlorophyll concentration mg/g dw, mean \pm SE, n = 15	Percent pheophytin mean \pm SE, n = 15
A	2.52 \pm 0.20	19.64 \pm 2.17
B	2.54 \pm 0.25	21.57 \pm 2.64
C	1.95 \pm 0.18	34.00 \pm 2.85
D	1.93 \pm 0.16	27.74 \pm 1.29
E	1.69 \pm 0.18	30.43 \pm 2.30

The bark of *Quercus coccifera* has a pH value of about 5 in areas free of air pollution (personal data). It is expected that in the sites neighboring a limestone quarry, the pH will be higher because of the neutralizing effect of windspread dust particles which are rich in ammoniates, phosphates and nitrates. At pH values

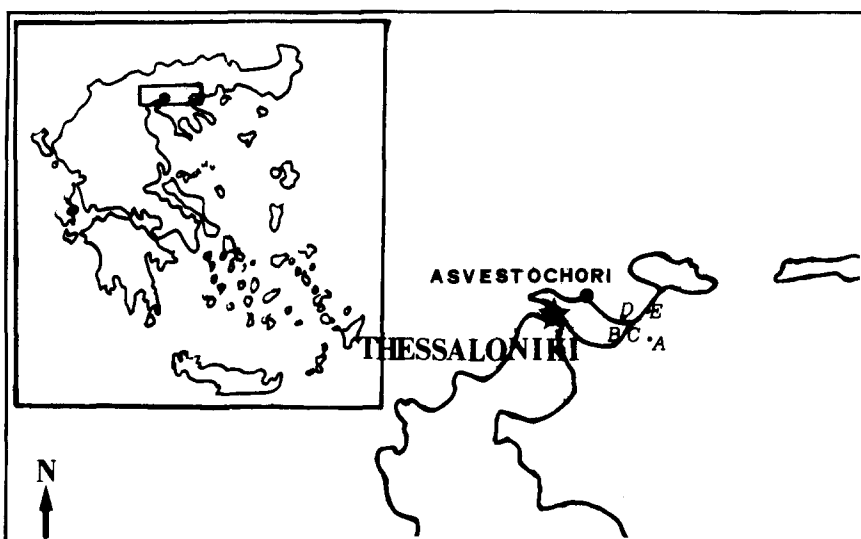


Figure 1 Sketch of the broader area of Asvestochori.

higher than 5, chlorophyll destruction occurs as there is interference with the photosynthetic electron transport system (Hawksworth and Rose 1976).

The process of bark nitrification due to nitrophytic effects of such dust particles has been reported to be optimal at pH 6.8-7.3 and non-existent below 3.7 (James 1973). This eutrophication is generally reflected in the occurrence of lichen association *Xanthorion* (James 1973), which actually occurs in all of the study sites and is represented mainly by *Physcia adscendens* and *Xanthoria parietina* (L.) Th. Fr..

In conclusion, it appears that air pollution by limestone quarry dust particulates led to the formation of the *Xanthorion* community, a community which is not normally found in such inland sites, although the representatives of this community are negatively affected by dust.

REFERENCES

- Boonpragob K (1987) Seasonal effects of air pollution on physiology of the lichen *Ramalina menziesii*. A dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Arizona State University
- De Wit T (1983) Lichens as indicators of air quality. *Environ Monit Assess* 3: 273-282
- Dorries W (1932) Über die Brauhbarkeit der spectroscopic Phaophytinprobe in der Rauchshaden-Diagnostik. *Zeitschr Pflanzenkr Gallenk* 42: 257-273
- Garty J, Kardish N, Hagemeyer J, Ronen R (1988) Correlations between the concentration of adenosine triphosphate, chlorophyll degradation and the amounts of airborne heavy metals and sulfur in a transplanted lichen. *Arch Environ Contam Toxicol* 17: 601-611
- Hawksworth DL, Rose F (1970) Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. *Nature* 227: 145-148
- Hawksworth DL, Rose F (1976) Lichens as pollution monitors. *Studies in Biology*

- no 66, Edward Arnold Publishers, England
- Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* 57: 1332-1334
- James PW (1973) Introduction. In: Ferry BW, Baddeley MS, Hawksworth DL (eds) *Air pollution and lichens*, Athlone Press, London, pp 1-5
- Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanzen (BBP)* 167: 191-194
- Kardish N, Ronen R, Bubrick P, Garty J (1987) The influence of air pollution on the level of ATP and on chlorophyll degradation in the lichen *Ramalina duriaei* (De Not.) Bagl. *New Phytol* 106: 697-706
- Kauppi M, Mikkonen A (1980) Floristic versus single species analysis in the use of epiphytic lichens as indicators of air pollution in a boreal forest region, Northern Finland. *Flora* 169: 255-281
- McCune B (1988) Lichen communities along O_3 and SO_2 gradients in Indianapolis. *The Bryologist* 91: 223-228
- Moser TJ, Nash TH III, Clark WD (1980) Effects of a long-term field sulphur dioxide fumigation on Arctic caribou forage lichens. *Can J Bot* 58: 2235-2240
- Nash TH III (1973) Sensitivity of lichens to sulphur dioxide. *The Bryologist* 76: 333-339
- Plummer GL, Gray BD (1972) Numerical densities of algal cells and growth in the lichen genus *Cladonia*. *Amer Mid Nat* 87: 355-365
- Ronen R, Galun M (1984) Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. *J Exp Bot* 24: 239-245

Received June 1, 1992; accepted December 30, 1992.