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Effects of H_2S on CO_2 Gas Exchanges and Growth Rates of the Epiphytic Lichen *Parmelia sulcata* Taylor

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Abstract

The results of a study on the effects of hydrogen sulphide on CO_2 gas exchanges and radial growth of *Parmelia sulcata* are presented. Field work was carried out in a chestnut wood of Acquapassante (Mt. Amiata, Central Italy) near a vent emitting H_2S and CO_2. Gas exchanges were measured at optimal conditions using crossed transplanted thalli collected and re-exposed at 16–30 m from the vent (zone A; maximum H_2S concentration: c. 2 ppm) and at more than 130 m (zone C; maximum H_2S concentration: c. 5 ppb). Corrections for temperature were made using temperature-dependent curves obtained in the laboratory. Radial growth rates were measured every three months in three populations occurring in A, C and in the intermediate zone B, 50–80 m from the vent. The results show that the metabolism of *P. sulcata* is strongly affected by H_2S. At low concentration inhibition of photosynthesis occurs, but the phenomenon is possibly reversible; a prolonged exposition seems to cause permanent damage to the photosynthetic apparatus, with a decrease in photosynthetic capacity, causing a drastic decrease in thallus growth.

Keywords: Growth, hydrogen sulphide, inhibition, lichen, photosynthesis

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1. Introduction

Hydrogen sulphide (H_2S) is the main reduced sulphur compound cycling through the atmosphere (Brown and Bell, 1986; Sawyer et al., 1994), and being highly reactive with metals and salts (Leithe, 1971), it affects the metabolism of higher plants (Koch et al., 1990; Maas et al., 1987; Pezeshki et al., 1991), although it is released in small amounts when these organisms – and lichens as well – are exposed to SO_2 (Rennenberg, 1984, 1989; Gries et al., 1997).

In a study on the effects of H_2S on epiphytic lichens, Tretiach and Ganis (1999) showed that this pollutant affects the species composition of lichen communities, and already at low concentrations (less than 2 ppm) it causes a reduction in lichen biomass and thallus size of foliose and fruticose species. In a following work on the effects of H_2S on the content of selected elements, Tretiach et al. (1999) showed that in *Parmelia sulcata*, a species rather tolerant to this pollutant, the compartimentation of Ca, Mg and Fe is drastically modified when the thalli are exposed at less than 2 ppm H_2S, due to changes in the chemico-physical characteristics of the substratum caused by the pollutant. However, the decreased biomass could be primarily related to changes in CO_2 gain, because it is known that H_2S is a strong inhibitor of photosynthesis in higher plants (Mass et al., 1987) as well as in the diatom *Skeletonema costatum* (Breteler et al., 1991). In this paper we present the results of a study on the effects of hydrogen sulphide on CO_2 gas exchanges and growth rates of a lichen carried out in the field, under natural, low levels of pollution.

2. Material and Methods

Study area

The study was carried out at Acquapassante (Abbadia S. Salvatore, Mt. Amiata, Central Italy), at 1052 m, near a vent whose outgassings (mainly water vapour, H_2S and CO_2) are emitted through an iron chimney that is 5.2 m high. The area hosts a well-developed, mature chestnut (*Castanea sativa*) wood, and the vent is the only source of atmospheric pollution, besides a small road with scarce traffic.

Three zones were selected at increasing distance from the vent in a south-east direction: A at 16–30 m (where the first trees occur), B at 60–90 m, and C at more than 130 m (see Tretiach and Ganis, 1999, Fig. 1). H_2S concentration is around 100 ppm at the chimney, 2 ppm in A, and less than 0.5 ppb in C; SO_2 is absent (Tretiach and Ganis, 1999). Gaseous Hg is 27.4 ± 9.4 ng m^{-3} immediately near the vent (R. Bargagli, pers. comm.).

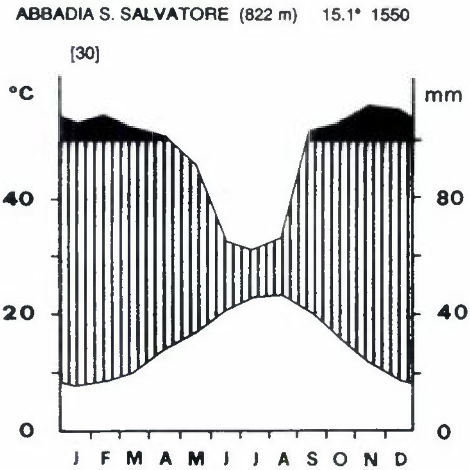


Figure 1. Climatic diagram of Abbadia S. Salvatore, 2 km far from the study area, according to Walter and Lieth (1960); data from Barazzuoli and Salleolini (1992). The x-axis = the months of the year, from January to December, the y-axis = mean monthly temperatures (lower line) and mean monthly precipitations (upper line).

The climate of the study area is suboceanic and mesothermic. The data of Fig. 1 refer to Abbadia S. Salvatore, located at 822 m, only 2 km far from Acquapassante. Rainfall is high, c. 1550 mm/year. There are 110 rainy days per year, concentrated in October–November and in late winter. In summer, from June to August, there is a pronounced drought period, with a minimum of c. 60 mm of rain in July, and mean temperatures of c. 24°C; winter temperatures are relatively high, with a minimum of 2°C in January, due to the mitigating influence of the Thyrrenian sea (Barazzuoli and Salleolini, 1992). Fogs are a further important feature of the Mt. Amiata climate (Arrigoni and Nardi, 1975).

The species

The selected species was *Parmelia sulcata* Taylor, an epiphytic foliose lichen which is present throughout the transect studied by Tretiach and Ganis (1999) with high cover and biomass, although the thalli occurring near the vent are generally smaller and some are partially discoloured, with necrotic and bleached portions.

Gas exchange measurements

To verify whether the CO_2 gas exchange rates of *Parmelia sulcata* are affected by H_2S, four healthy C-thalli of *P. sulcata* c. 7 cm in diameter were collected, thoroughly cleaned of mosses, bark fragments and debris, hung up on a bole in Petri dishes covered by a thin mesh of nylon, and kept fully hydrated by spraying distilled water. After 24 h their CO_2 gas exchanges were measured in the field at optimum thallus water content and saturating light (400 µmol photons $m^{-2}s^{-1}$), as determined in previous experiments. The day after the thalli were exposed immediately near the vent at c. 40 ppm H_2S, and their CO_2 gas exchanges monitored at increasing time intervals at the same conditions of the previous day.

The effects on photosynthesis of the maximum H_2S concentrations at which the lichens were exposed in A and C were investigated by monitoring the gas exchanges of crossed transplanted thalli before and after exposure (in the absence of wind) in the two zones.

In a first experiment six healthy C-thalli of *P. sulcata* were cleaned and put in Petri dishes; three thalli were then maintained hydrated for three days in C, and their gas exchanges monitored daily as control, whereas the other three thalli were kept one day in C, then moved to A, and again to C; their gas exchanges were also monitored daily, after 24 h of exposure in each zone.

In a second experiment the gas exchanges of six thalli of similar size, three from A and three from C, were compared before and after crossed transplants. Thalli were kept humid for 24 h in their original zone, their gas exchanges measured, and then transferred to the other zone (A-thalli to C, and vice versa); their gas exchanges were monitored after 24 h of exposure.

In these two experiments net photosynthesis and dark respiration were calculated as the average of the three highest series of measurements of three CO_2 gas exchange curves obtained at decreasing thallus water content. Minor corrections were made taking into account temperature-dependence curves of net photosynthesis and respiration obtained in the laboratory using the same thalli, because in the field this factor could not be controlled.

The CO_2 gas exchange rates were measured in a closed system with an infrared gas analyzer, model LICOR-6250 (Licor Inc., Lincoln, Neb., USA; estimated precision ±0.2–0.3 ppm CO_2; cuvette volume 4,000 cm^3; internal volume 130 cm^3; flow rate 1,100–1,200 cm^3 min^{-1}) connected to a data-logger. Fresh weight was determined immediately after three measurements, each of them lasting 40 s. Because c. 40–60 s were necessary to reach a stable gradient of CO_2 depletion within the cuvette, each series of measurements lasted c. 2–3 min. In the field natural spectrum light was provided by a fiber illuminator Fl-400 (Walz, Effeltrich, Germany); PAR flux in the 400–700 nm waveband was measured using a Licor Quantum meter model LI 185A inserted within the cuvette at the same position as the lichen thalli.

Growth measurements

In December 1997, three groups of chestnuts were selected in the three zones of the study area. Trees were c. 18 m high, c. 60 years old, and with a trunk circumference of 100–150 cm, inclination not exceeding 5°, and with no signs of obvious damage. On each tree three or four healthy thalli of *P. sulcata* larger than 4 cm in diameter were selected [Armstrong (1974) showed that in *Parmelia* species the linear growth rate is constant for thalli larger than 1.5 cm in diameter]. For each thallus, exposition on the bole, height above ground, maximum and minimum diameter were recorded. Every three months the thallus radial growth rate was measured with the aid of a callipers (precision: 0.05 mm), from a plastic-covered needle infixed in the centre of the thallus to the top margin of 5–9 lobes without soralia. To recognize the lobes, they were marked on a 1:1 b/w photograph.

3. Results

CO_2 gas exchanges

An evident depression of photosynthetic activity occurred when C-thalli were exposed for more than 20 minutes to 40 ppm H_2S (Fig. 2). Dark respiration was not affected at this concentration (data not shown).

The variation in photosynthetic activity resulting from the exposure of healthy C-thalli to the H_2S concentration of zone A is shown in Fig. 3. A significant, pronounced depression of net photosynthesis, ranging from 25–40%, occurred in all C-thalli moved from C after 24 h in A. The depression was still partly reversible, because their photosynthetic activity recovered more or less completely when the thalli were transferred again to C. On the contrary, the gas exchange rates measured at the end of each day at optimal light and thallus water content conditions did not change significantly in the three thalli, maintained fully hydrated for three days in C as control.

The photosynthetic capacity of C-thalli was approx. double that of A-thalli of similar size (but not of the same age, see later). This difference (7.15 against 3.5 mg CO_2 $g^{-1}h^{-1}$, Fig. 4) could not be attributed to a different chlorophyll content, which was similar (data not shown). Interestingly, photosynthesis of C-thalli decreased when they were exposed for 24 h in A, whereas that of A-thalli remained practically constant after their exposure in C, in virtual absence of H_2S in the air.

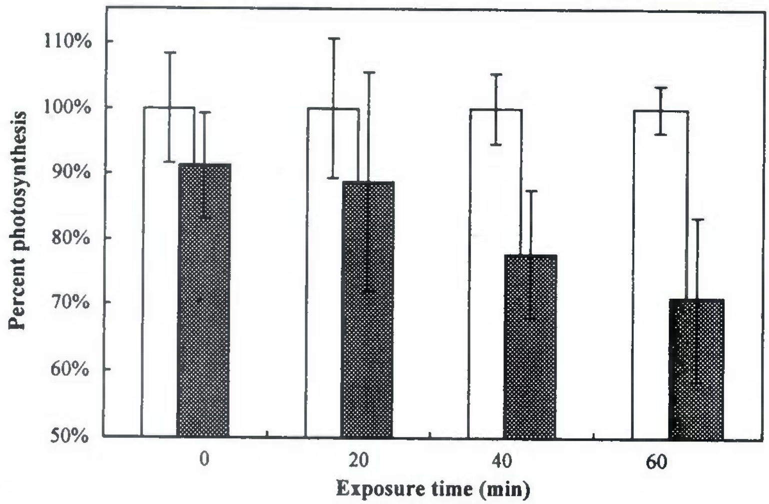


Figure 2. Per cent variation in net photosynthesis of four C-thalli of *Parmelia sulcata* before (white columns) and after (shaded columns) exposure to 40 ppm H_2S at increasing time intervals (min, abscissa).

Growth

A positive, highly significant correlation (r^2=0.792, p<1%) was found between radial growth rate of single thalli and distance of the lichens from the H_2S emitting vent (Fig. 5D), whereas no significant correlation was found with other variables (thallus height above ground, bole circumference, exposure; see Fig. 5A–C). The mean growth rate of all C-thalli was about three times that of A-thalli (c. 0.4 against 0.15 cm per year, Table 1), B-thalli having an intermediate value of 0.22 cm per year. The difference among zones remained practically constant in winter, spring and autumn (Fig. 6); only in summer, when the thallus growth was low in all three zones, the difference between the mean values recorded in A and B was not statistically significant. The growth reduction in summer is evidently related to the decrease in precipitation and air humidity occurring in that season, a typical feature of the Mt. Amiata climate (Fig. 1).

4. Discussion

As far as we know, there has been only one previous study on the effects of H_2S on lichen growth, carried out by the Cornwall Trust for Nature Conservation (1986). According to their results, geothermal emissions

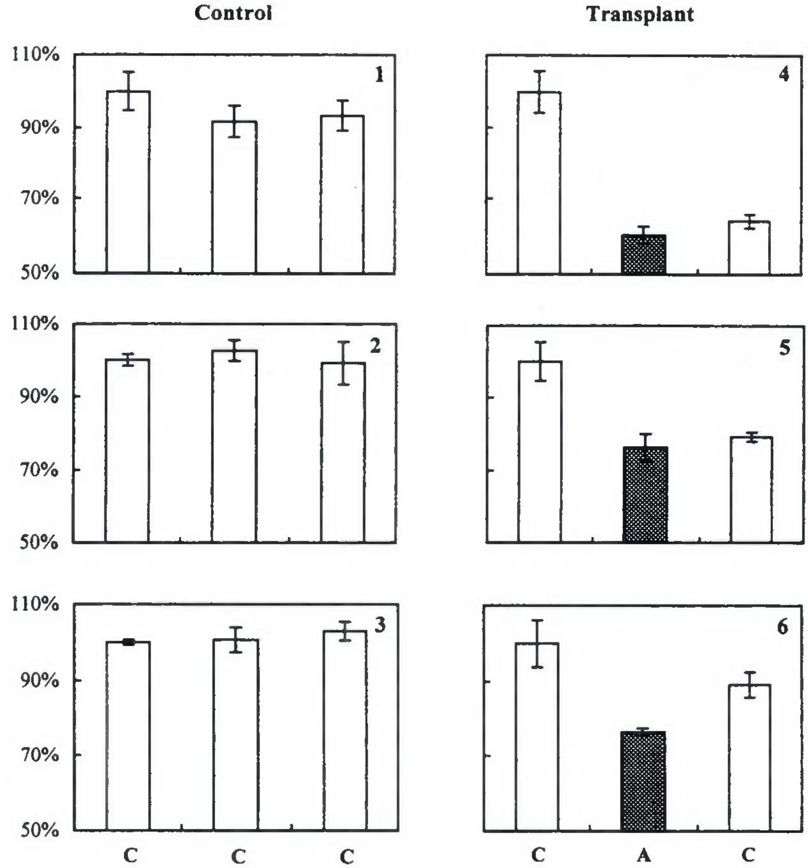


Figure 3. Per cent variation in net photosynthesis of six C-thalli of *Parmelia sulcata*, three (1–3) maintained fully hydrated for three days in C (control), and three (4–6) transferred to A during the second day and then again to C (transplant).

Table 1. Mean yearly radial growth (cm) of *Parmelia sulcata* in the three zones of the transect

	Growth (cm per year)	Number of lobes measured	Coefficient of variation
A	0.148±0.075	48	51%
B	0.216±0.084	32	39%
C	0.398±0.110	34	28%
$F_{(A-B)}$	14.2**		
$F_{(A-C)}$	149.1**		
$F_{(B-C)}$	56.3**		

F = ANOVA's values (* = 1%<p<5%; ** = p<1%).

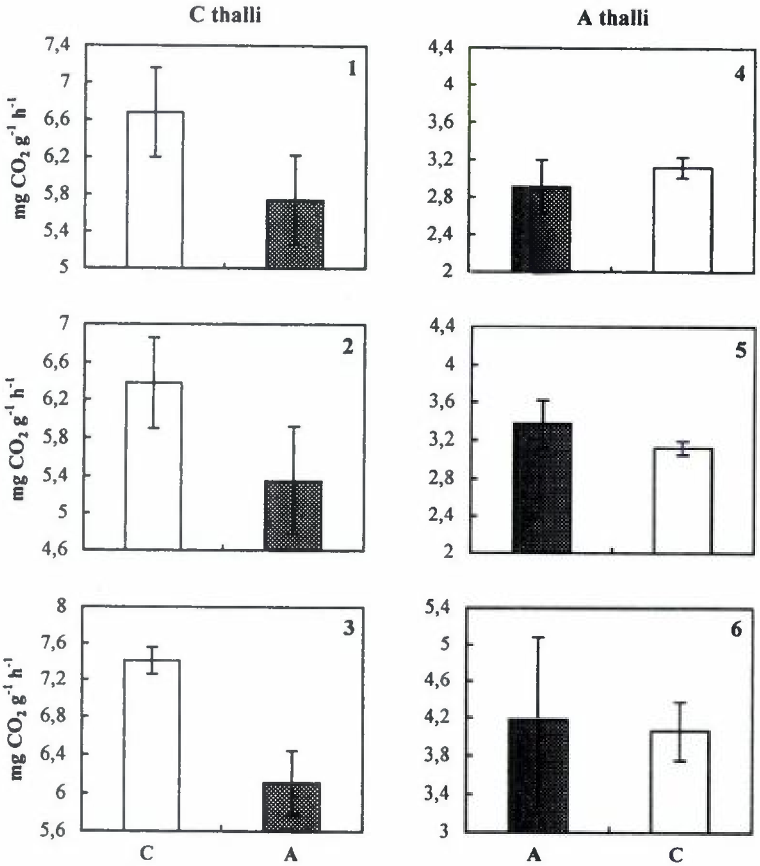


Figure 4. Net photosynthesis of six thalli of *Parmelia sulcata*, three collected in C (C-thalli, 1–3) and three in A (A-thalli, 4–6), measured first after 24 h of optimal hydration in their original zone (C and A, respectively), and then after 24 h of optimal hydration in the other zone.

containing H_2S do not influence lichen growth. On the contrary, we detected a significant, constant difference between the radial growth rate of thalli exposed to 2 ppm H_2S, and those selected as control; furthermore, a positive correlation was found between the distance of single thalli from the H_2S emitting vent, and their yearly growth rate (Fig. 5D).

The high data spread of our growth measurements (Table 1) should not be considered as excessive. It is well known that lichen growth is not constant during the year (Fisher and Proctor, 1978), and is generally highly variable, because some lobes remain quiescent for a certain time, having an intermittent

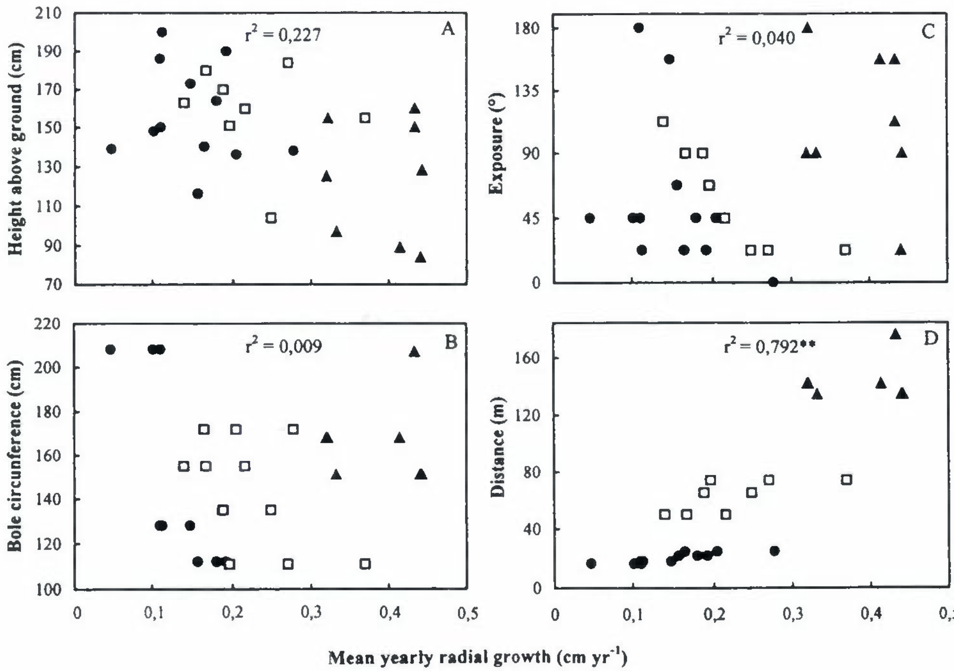
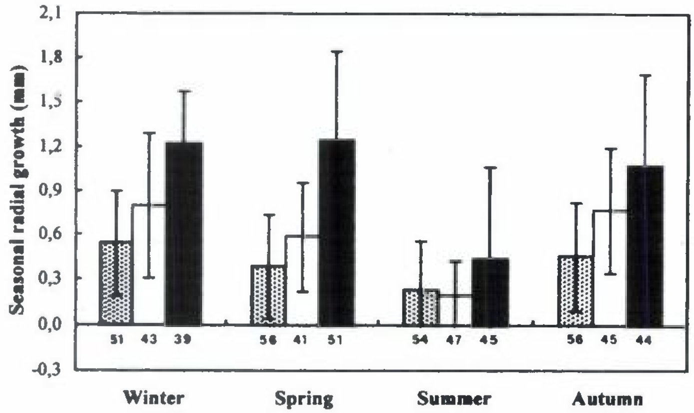


Figure 5. Correlation between mean yearly radial thallus growth rate (abscissa) and thallus height above ground (A), bole circumference (B), exposure (C), and distance of each thallus from the H_2S emitting vent (D). A-thalli (circle); B-thalli (square); C-thalli (triangle). * = 1%<p< 5%; ** = p<1%.

growth (Linkola, 1918; Phillips, 1969; Hale, 1970). Apparently, however, this phenomenon occurred more frequently in A, because the data spread is generally higher in A than in B or C (Table 1); this might be due to the natural fluctuation of H_2S pollution, which is highly variable due to changes in direction and intensity of air currents, and emission of outgassings from the vent. Interestingly, the mean yearly growth rate measured in the control zone C is similar to the value reported by Fisher and Proctor (1978) for thalli of *P. sulcata* from Devonshire (England), an area climatically rather similar to Mt. Amiata. Linkola (1918) and Degelius (1964), who worked in more continental areas of Scandinavia, found lower values (1.6 and 2.2 mm per year, respectively).

The negative correlation between H_2S and lichen growth is congruent with the effects of this pollutant on CO_2 gas exchanges, because, although our experimental design was somewhat empirical, there are no doubts that H_2S



	Winter	Spring	Summer	Autumn
$F_{(A-B)}$	9,62**	7,53**	0,36	15,7**
$F_{(A-C)}$	86,6**	84,2**	4,33*	39,5**
$F_{(B-C)}$	19,9**	37,9**	5,94*	7,58**

Figure 6. Seasonal radial growth (mm) in 30 thalli of *Parmelia sulcata*, 12 from A, 9 from B, and 9 from C. Number below each bar = lobes measured; F = ANOVA's values (* = 1%<p<5%; ** = p<1%).

reduces lichen photosynthesis, whereas respiration is not affected. At low concentration, inhibition of photosynthesis is possibly reversible if exposure to the pollutant is short (Fig. 3). After a long time, however, it seems to cause permanent damage, with a decrease in photosynthetic capacity: A-thalli had indeed considerably lower rates than C-thalli (Fig. 4). The inhibitory effect of H_2S on photosynthesis was already known in higher plants. Maas et al. (1988) showed that after exposure of 14 days at 2.5 ppm H_2S, the gross photosynthesis of *Spinacia oleracea* decreases to 30% of the control. The same authors suggested that this phenomenon might be due to a decreased electron transport efficiency, as already pointed out by de Kok et al. (1983b). Sivaraja et al. (1988) demonstrated that H_2S interacts with the Mn-protein of the multienzymatic complex involved in the photolysis of water, at the PSII level. The inhibition of enzymatic activity would be reversible, but permanent damage could occur at high H_2S concentration due to the leakage of the Mn bounded metal ions from the enzyme, which becomes inactive. If this hypothesis is correct, then fluorescence techniques would be the best way to verify in the field the H_2S effects on lichen species with different tolerance to hydrogen sulphide.

The situation faced in our study, however, is complicated by further factors. First, in our field work measurements were carried out at 360 ppm CO_2. The A-thalli, however, were exposed not only to higher concentration of H_2S, but also of CO_2. This implies that the actual photosynthesis of A-thalli might be somewhat higher too, because at 360 ppm CO_2 photosynthesis is only 80% of the maximum value recorded at the saturating concentration of 600 ppm CO_2. Second, the thalli were exposed also to the indirect effects of the pollutant, for instance the chemico-physical modifications of tree bark (Tretiach et al., 1999). This means that the decrease in growth, and the degenerative processes of the central parts of thalli, are not only due to a decrease in CO_2 gain, but also to the additional, negative effects of different other factors, such as the decreased intracellular calcium content, the increased iron content, and the possible acidification of the cytoplasm (Tretiach et al., 1999).

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