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The Influence of Sublethal Concentrations of Sulfur Dioxide on Morphology, Growth and Product Yield of the Duckweed *Lemna minor* L.*

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Summary. There was no disturbance in the growth of *Lemna minor* L. with a SO_2 concentration of up to 0.3 ppm in air. A SO_2 concentration of 0.6 ppm caused an initial depression of the growth rate of about 25%, but in the course of adaptation, the rate rose to the values of the control. The average dry weight per frond was not influenced by the SO_2 fumigation. The initial sporadic appearance of chloroses by fumigation with 0.6 ppm SO_2 was considered a sign of the proximate toxicity limit for *Lemna minor* L. With 0.15 ppm SO_2 in air, the size of the fronds was reduced. The average surface of the fronds was diminished by 0.3 ppm SO_2 for about 16% as compared with the control plants.

The protein remained quantitatively uneffected up to a SO_2 concentration of 0.6 ppm. As a qualitative influence of SO_2, the nitrogen content of the proteins remained constant, but the sulfur content of the proteins increased.

Under 0.3 and 0.6 ppm SO_2, the starch content decreased immediately by 20–30%, under 0.15 ppm SO_2 the decrease reached the same level after a longer time than in the case of the higher concentrations.

The SO_2 concentrations up to 0.6 ppm had no effect on chlorophyll concentration.

The contents of C, N, H, P, K, Na, Ca, Mg, Mn, and Fe were not effected by SO_2 fumigation.

Conclusion: SO_2 may have some effects on product yield, even under low concentrations, without provoking acute damage; the plant is able to adapt by regulation of its metabolism, and enters a new steady state.

Introduction

As a consequence of the present technologic situation, the air pollutant sulfur dioxide represents in increasing measure a burden on our atmosphere. In numer-

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ous reports, outward visible and acute damages have been described (Guderian and v. Haut, 1970), which are caused partially by very high SO_2 concentrations as they hardly occur under natural conditions in the air. In more recent investigations, particularly the so-called invisible damage is of more interest, since even very small SO_2 emissions interfere with the metabolism of plants. This microscopically invisible gas damage may also have an economical importance like necrotic damage, since it can manifest itself in loss of product yield and in changes of important plant substances (Guderian, 1966; Jaeger et al., 1972; Boertitz, 1974).

In our investigations, we selected SO_2 concentrations which still permitted growth without outward visible damage to the test plants, and moreover, which were in the scope of the emission limit values of SO_2 which are admissible in Switzerland (Eidgenössische Kommission für Lufthygiene). With this arrangement we had the possibility of examining these limit values for SO_2 fumigation on a wider basis.

Materials and Methods

Lemna minor L., strain number 6580 of the collection of Lemnaceae from Landolt (1957), was cultivated in continuous culture under strictly controlled conditions on a nutrient medium E-NO_3^- modified (Kopp et al., 1974). The cultivation has been described previously (Erismann and Brunold, 1973). Conditions of culture: 4500 lx of permanent light, 25° C, air from outside mixed with SO_2 concentrations: 0, 0.15, 0.3, and 0.6 ppm. The dosage of SO_2 was made by a diffusion method (Schaerer, 1974); the SO_2 content was measured spectrophotometrically with a gas analyzer "Spectrometrics III d^2".

The size of the fronds was determined by measuring the diameters of the fronds (Brunold, 1972) and by calculation of the surfaces of the fronds (Erismann, 1965). The specific growth rate μ was calculated according to Erismann and Brunold (1973). For the determination of the dry weight, the plant material was washed for 1 h in ice-cold distilled water and subsequently dryed at 80° C for 24 h until the weight remained constant. The protein content was measured by the Biuret reaction of Dévény and Gergely (1968) and the content of protein nitrogen as described by Bohley (1967). For the protein sulfur determination the protein extracts of Lemna were first evaporated to dryness and then transformed to ash by heating at 430° C for 24 h. The ashes were put in 0.1 N formic acid and the sulfur content measured by the method of Johnson and Nishita (1952). The evaluation of the starch content was done enzymatically according to a method of Boehringer (1971), modified by Fankhauser (1975). The content of total chlorophyll was measured as reported by Arnon (1949). The analysis of carbon, nitrogen, and hydrogen content of the whole plant was made in the microlaboratory of organic chemistry of the ETH in Zürich. The plant material for the phosphorus and cation determination was resolved after being ashed in HCl (0.1 N). The phosphorus content was then measured colorimetrically by a method published in Kali-Briefe (1971), whereas the cations were determined with the aid of an atomic absorption spectrometer (Feller, 1975).

Results and Discussion

Previously it was found that the *Lemna minor* L. plants supplied from the 4 unities of the cultivation apparatus were physiologically equivalent (Fankhauser, 1975). Moreover, it has been demonstrated that the effects of SO_2 cannot be attributed to a possible setup of toxic concentrations of sulfite (Fank-

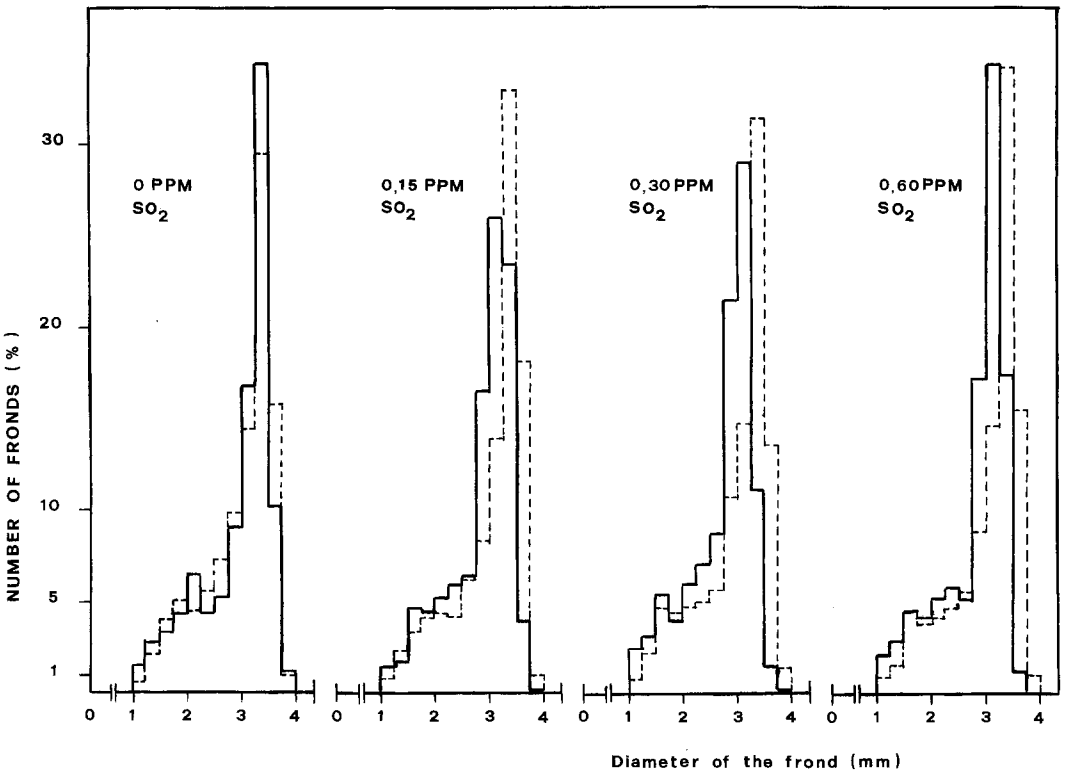

Fig. 1. Dependence of diameters of fronds of *Lemna minor* L. upon SO_2 concentration in air. Duration of SO_2 fumigation 6 weeks. Ordinate: percentage portions from total number of fronds (more than 1000 fronds). Broken lines: before SO_2 fumigation. Unbroken lines: after SO_2 fumigation

hauser, 1975). After these preinvestigations, we began the long-time fumigation with SO_2.

For *Lemna* a concentration of 0.6 ppm SO_2 was found to be the critical concentration because it caused the sporadic appearance of chloroses at the beginning of the SO_2 fumigation time. The proximity of the toxicity limit was also demonstrated by the fact that SO_2 concentrations higher than 0.8 ppm are lethal for *Lemna minor* L.

The analysis of the sizes of the fronds showed that already under 0.15 ppm SO_2, a statistically secure shifting (Kolmogorov test) to smaller diameters of the fronds occurred for one size class of 0.25 mm. Comparing the distribution curves of the diameters of the fronds before and after SO_2 fumigation (Fig. 1), it was clearly evident that especially the newly formed mother fronds were smaller in their measurements under increasing SO_2 influence. We could see this too in the diminution of the average surfaces of the fronds, where there was a loss in surface area with 0.3 and 0.6 ppm SO_2 for 16.5 and 13.8%, respectively. In the determinations of the average dry weight per frond we found no significant differences between control plants and plants under SO_2 treatment (Table 1). This means that the reduction of the average surface areas

Table 1. Average dry weight per frond of *Lemna minor* L. under various SO_2 concentrations. The data (in mg) are the means of 6 parallels. Standard deviations (s)

Fumigation	SO_2 content in air			
	0 ppm	0.15 ppm	0.30 ppm	0.60 ppm
28 days	0.0805 $s=0.0028$	0.0767 $s=0.0039$	0.0770 $s=0.0018$	0.0756 $s=0.0019$
40 days	0.0791 $s=0.0016$	0.0795 $s=0.0038$	0.0786 $s=0.0026$	0.0762 $s=0.0023$
51 days	0.0819 $s=0.0025$	0.0792 $s=0.0020$	0.0805 $s=0.0035$	0.0809 $s=0.0040$

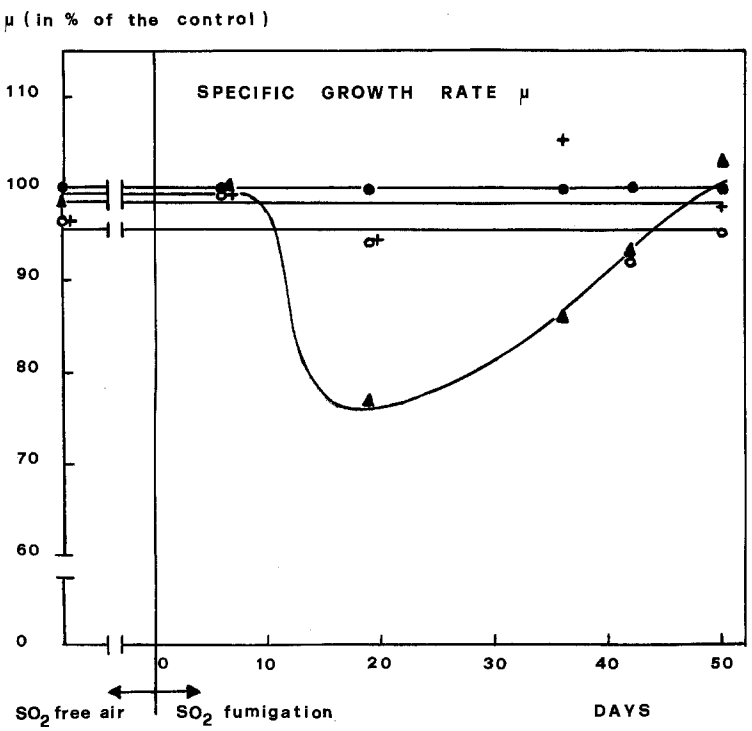

Fig. 2. Specific growth rates μ of *Lemna minor* L. under the following SO_2 concentrations: ●: 0 ppm SO_2 (control); ○: 0,15 ppm SO_2; +: 0,30 ppm SO_2; ▲: 0,60 ppm SO_2. The data are the means of 6 parallels

of the fronds by SO_2 represented only a morphologic alteration but not a diminution of the product yield.

As a further parameter of the product yield measurement we used beside the average dry weight per frond the specific growth rate μ. *Lemna minor* L. grew uninfluenced up to concentrations of 0.3 ppm SO_2 (Fig. 2). The average

Table 2a. Total protein content of *Lemna minor* L. under various SO_2 concentrations. The data (in mg protein per g dry weight) are the means of 6 parallels. Standard deviations (s)

Fumigation	SO_2 content in air			
	0 ppm	0.15 ppm	0.30 ppm	0.60 ppm
22 days	231.8 $s=19.51$	219.8 $s=27.44$	213.9 $s=30.67$	220.1 $s=14.00$
44 days	273.6 $s=7.00$	264.2 $s=4.95$	278.8 $s=9.14$	255.8 $s=14.24$
56 days	264.0 $s=4.98$	263.4 $s=10.15$	259.5 $s=9.98$	255.3 $s=7.27$

Table 2b. Nitrogen and sulfur content of proteins of *Lemna minor* L. after 56 days and SO_2 fumigation. The data (in mg per g dry weight) are the means of 6 parallels. Standard deviations (s). Ratio nitrogen/sulfur

	SO_2 content in air			
	0 ppm	0.15 ppm	0.30 ppm	0.60 ppm
N content	44.27 $s=3.48$	44.67 $s=4.70$	42.29 $s=2.59$	42.19 $s=2.27$
S content	4.83 $s=0.279$	5.25 $s=0.432$	5.46 $s=0.255$	5.61 $s=0.206$
N/S	9.17	8.51	7.75	7.52

doubling times of the number of fronds were 41.4 h for the control plants, 42.8 h for the plants fumigated with 0.15 ppm SO_2, and 42 h for the plants fumigated with 0.3 ppm SO_2. Under 0.6 ppm SO_2, the specific growth rate reached the value of the control after an initial depression of about 25% (doubling time to 54 h) in the course of an adaptation time of nearly 4 weeks. We found a similar adaptation in the rate of DNA synthesis, which first decreases under 0.6 ppm SO_2 to two-thirds of the normal level; after an adaptation time, the reduced rate of synthesis seems to be compensated by a prolongated synthesis phase (Stoeckli et al., 1975). The ability for adaptation of our test plant was also observed with a kinetic experiment at the rate of the sulfate uptake but here the adaptation to the SO_2 treatment was faster (Schaerer, 1975).

Fischer (1967) and Jaeger et al. (1972) found a stimulation of the protein synthesis by short-time application and a distinct inhibition by long-time application of SO_2. Godzik and Linskens (1974) reported an enhancement of the free amino acids and the ammonia under SO_2 fumigation and they postulated that the lowered protein content under SO_2 is due to a higher degradation. In contrast to these results, we did not find a quantitative change in the total protein content of *Lemna minor* L. up to 0.6 ppm SO_2 (Table 2a). The nitrogen content of the proteins remained unaffected by SO_2, but there was an increase

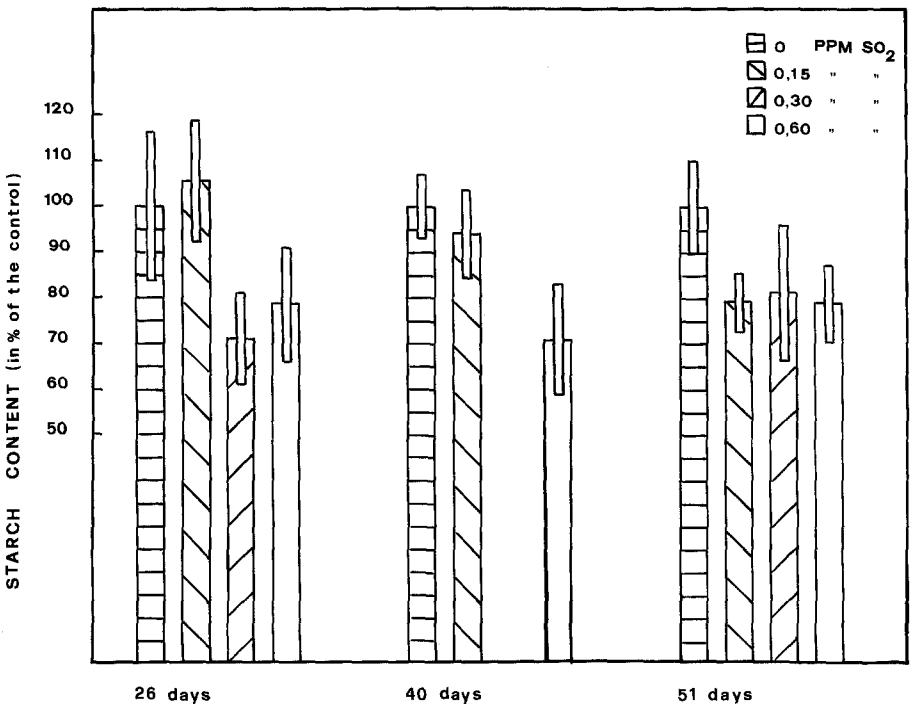

Fig. 3. Starch content of *Lemna minor* L. after 26, 40, and 51 days of SO_2 fumigation (0, 0,15, 0,3, and 0,6 ppm SO_2). The data are the means of 6 parallels. Standard deviations

of the sulfur content of the proteins which is evident from the nitrogen/sulfur ratio (Table 2b). The enhanced sulfur content of the proteins by SO_2 is in agreement with the results obtained by Weigl and Ziegler (1962), Faller and Hoefner (1968), and Jaeger et al. (1972). They have also reported that sulfur dioxide can be included in normal sulfur metabolism by incorporation in S-amino acids.

In starch content, we found an immediate decrease of 20–30% with 0.3 and 0.6 ppm SO_2 in the air (Fig. 3). In contrast to the growth rate, this decrease proved to be definitive, which indicates a change in the starch metabolism. With 0.15 ppm SO_2, the starch content decreased after a longer SO_2 incubation at the same level as with higher SO_2 concentrations. The question of whether the reduced starch content is the result of a degradation or a decelerate synthesis remains unanswered by our data. Boertitz (1968) reported a gradual hydrolysis of the starch under the effect of SO_2 at which the ability for a de novo synthesis of starch remains intact until lethal damage occurs. In opposition to findings of Fischer (1973) and Daessler and Schenk (1973), the concentration of chlorophyll as a critical quantity in the correct function of the photosynthetic apparatus is not influenced by SO_2 concentrations up to 0.6 ppm. Accordingly, an inhibition of the photosynthesis by SO_2 could be conditioned by attacks on the enzyme level (Ziegler, 1972, 1973).

Table 3. Percentage portions of C, N, H, P, K, Na, Ca, Mg, Mn, and Fe from the dry mass of *Lemna minor* L. after SO_2 fumigation of 6 or 8 weeks. The data are the means of 6 parallels. Standard deviations (s)

	SO_2 content in air			
	0 ppm	0.15 ppm	0.3 ppm	0.6 ppm
C content	38.85 $s=0.86$	38.42 $s=0.68$	39.44 $s=0.48$	38.72 $s=0.44$
N content	6.26 $s=0.33$	6.01 $s=0.24$	6.07 $s=0.17$	5.98 $s=0.14$
H content	5.39 $s=0.11$	5.32 $s=0.08$	5.53 $s=0.04$	5.45 $s=0.26$
P content	1.35 $s=0.093$	1.47 $s=0.192$	1.36 $s=0.102$	1.34 $s=0.092$
K content	4.58 $s=0.027$	4.45 $s=0.150$	4.23 $s=0.160$	4.34 $s=0.120$
Na content	1.26 $s=0.097$	1.23 $s=0.061$	1.30 $s=0.071$	1.21 $s=0.097$
Ca content	0.741 $s=0.037$	0.721 $s=0.026$	0.692 $s=0.046$	0.756 $s=0.028$
Mg content	0.093 $s=0.005$	0.089 $s=0.007$	0.092 $s=0.010$	0.093 $s=0.005$
Mn content	0.098 $s=0.006$	0.105 $s=0.004$	0.117 $s=0.010$	0.113 $s=0.006$
Fe content	0.050 $s=0.013$	0.048 $s=0.006$	– –	0.046 $s=0.005$

Schaerer (1975) found an increase in content of the sulfate in *Lemna minor* L. with advancing duration of the SO_2 fumigation. This fact corresponds to various investigations where an enhanced sulfur content after SO_2 incubation is repeatedly found. On the other hand, a SO_2 fumigation of 6 weeks caused no essential changes in the percentage portions of phosphorus, potassium, sodium, calcium, magnesium, manganese, and iron, and the fumigation of 8 weeks no changes in the percentage portions of carbon, nitrogen, and hydrogen (Table 3).

Summarizing the results, we can say that there are no irreversible damages for *Lemna minor* L. up to 0.6 ppm SO_2. Although we can demonstrate morphologic and physiologic effects, even under 0.15 ppm SO_2, the plant is able to adapt itself to a SO_2 concentration of 0.6 ppm by regulation of its metabolism, and to enter into a new steady state. These investigations are based on model experiments, comparable only with reservation to ecologic freeland experiments.

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