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Changes of Physiological and Biochemical Parameters in the Lichen *Hypogymnia physodes* (L.) NYL. due to the Action of Air Pollutants - a Field Study

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Summary

EGGER R., SCHLEE D. & TÜRK R. 1994. Changes of physiological and biochemical parameters in the lichen *Hypogymnia physodes* (L.) NYL due to the action of air pollutants. – Phyton (Horn, Austria) 34 (2): 229–242. English with German summary.

The lichen *Hypogymnia physodes* (L.) NYL. was transplanted to three locations with different levels of air pollution near air pollution monitoring stations in the province Salzburg (Austria) for eight months. Samples of the lichen were retrieved at the end of each month to determine net photosynthesis (NPS), dark respiration, the contents of chlorophyll (Chl) and malone dialdehyde (MDA), and the activities of superoxide dismutase (SOD) and acid phosphatase (AP). Single and multiple linear regression analysis between the lichen parameters and the monthly mean concentrations of air pollutants (O_3, SO_2, NO, NO_2) were performed to test for significant relations.

Depending on pollution level of the location, several statistically significant relationships were observed. O_3 showed a positive correlation with the contents of Chl and MDA, as well as SOD activity. SO_2 showed a negative correlation with NPS, a positive one with the MDA content and AP activity, and as well a positive as a negative correlation with the content of Chl. Best single correlations were found between the activity of SOD and O_3 ($r^2 = 0.85$), as well as the content of MDA and O_3 ($r^2 = 0.86$) and SO_2 ($r^2 = 0.88$), respectively. Multiple regression analysis resulted in a significant linear relationship of combinations of two air pollutants with the activities of SOD

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and AP, as well as the contents of MDA and Chl. Highest multiple correlations were estimated between the content of MDA and SO_2/O_3 ($R^2 = 0.91$), the Chl content and SO_2/O_3 ($R^2 = 0.87$), as well as SOD activity and O_3/SO_2 ($R^2 = 0.87$).

Zusammenfassung

EGGER R., SCHLEE D. & TÜRK R. 1994. Veränderungen physiologischer und biochemischer Parameter in der Flechte *Hypogymnia physodes* (L.) NYL. infolge der Einwirkung von Luftschadstoffen. – Phyton (Horn, Austria) 34 (2): 229-242 (Englisch mit deutscher Zusammenfassung.)

Transplantate der Flechte *Hypogymnia physodes* (L.) NYL. wurden acht Monate lang an drei Standorten mit unterschiedlicher Luftbelastung in unmittelbarer Nähe von Luftgütemeßstationen im Bundesland Salzburg (Österreich) exponiert. Am Ende jeden Monats wurden Flechtenproben eingesammelt und die Netto-Photosynthese (NPS), Dunkelatmung, die Gehalte an Chlorophyll (Chl) und Malondialdehyd (MDA) sowie die Aktivitäten der Superoxiddismutase (SOD) und Sauren Phosphatase (AP) bestimmt. Mit Hilfe einfacher und multipler Regressionsanalysen wurde überprüft, ob signifikante Korrelationen zwischen den biologischen Flechtenparametern und den Monatsmittelwerten der registrierten Luftschadstoffe (O_3, SO_2, NO, NO_2) bestehen.

In Abhängigkeit der an den untersuchten Standorten aufgetretenen Schadstoffbelastungen konnten statistisch abgesicherte Zusammenhänge nachgewiesen werden. O_3 korrelierte positiv mit den Gehalten an Chl und MDA sowie der SOD-Aktivität. SO_2 zeigte eine negative Korrelation mit der NPS, eine positive mit dem MDA-Gehalt und der AP-Aktivität sowie eine negative als auch positive mit dem Chl-Gehalt. Die besten einfachen Korrelationen wurden zwischen der SOD-Aktivität und O_3 ($r^2 = 0.85$) sowie zwischen dem MDA-Gehalt und O_3 ($r^2 = 0.86$) bzw. SO_2 ($r^2 = 0.88$) nachgewiesen. Multiple Regressionsanalysen ergaben signifikante Zusammenhänge zwischen der Kombination zweier Luftschadstoffe eines Standortes und den Gehalten an Chl und MDA sowie den Aktivitäten von SOD und AP. Die besten multiplen Korrelationen wurden zwischen dem MDA-Gehalt und SO_2/O_3 ($R^2 = 0.91$), dem Chl-Gehalt und SO_2/O_3 ($R^2 = 0.87$) sowie der SOD-Aktivität und O_3/SO_2 ($R^2 = 0.87$) festgestellt.

Introduction

Cellular injury may be caused by free radicals which are induced upon exposure to air pollutants (Heath 1980). Toxic effects of sulfite, for example, lead to damage of biomolecules and membranes by radical chain reactions (COVELLO & al. 1989). Lipid peroxidation is one of the most important organic expressions of oxidative stress induced by the reactivity of oxygen free radicals. Malone dialdehyde (MDA) is released during the last stages of the breakdown of endoperoxides formed during intramolecular rearrangements in the structure of polyunsaturated fatty acids. Therefore, the determination of MDA can be used as an indicator of the degree of oxidative stress in biological systems (VALENZUELA 1991).

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide radicals ($O_2\cdot^-$) to yield hydrogen peroxide (H_2O_2) and oxygen (O_2). The enzyme is ubiquitous in aerobic organisms where it plays a main role in defense against oxygen radical-mediated toxicity (CADENAS 1989). Thus, the induction of SOD can be an indicator of enhanced $O_2^{\cdot -}$ production (RABINOWITCH & FRIDOVICH 1983). $O_2^{\cdot -}$ production results from numerous reactions, including the decomposition of O_3 (PELEG 1976) and the oxidation of SO_2 (ASADA & TAKAHASHI 1987). SOD was initially induced in spinach (SAKAKI & al. 1983) and Pine (TANDY & al. 1989) by exposure to O_3, and similarly, in poplar leaves by SO_2 (TANAKA & SUGAHARA 1980) and algae by sulfite (KÖCK & al. 1985).

Because of their high sensitivity to air pollutants, lichens are often used as bioindicators. Several reviews have been published summarizing the reported effects of air pollution on the lichen metabolism (e. g. FIELDS 1988, RICHARDSON & NIEBOER 1983). The "transplant technique" has often been used in field experiments where mainly photosynthesis and the chlorophyll content had been determined. In spite of extensive reports on the effects of gaseous immissions (especially of SO_2) on lichens, little is known about the influence of O_3 and NO_2 in environmental concentrations to biological parameters, particularly enzymatic activities.

In the present study we wanted to find out whether O_3, SO_2, NO, and NO_2 affect SOD activity and MDA content in the lichen *Hypogymnia physodes,* and if these lichen parameters could be used as an indicator of ozone levels in the field. In addition, CO_2-exchange and chlorophyll content - known as sensitive indicators of gaseous air pollutants in lichens – were determined. Furthermore, the acitivity of acid phosphatase, an important enzyme of cell metabolism, was investigated.

Material and Methods

Plant material and exposition-sites

Samples of *Hypogymnia physodes* were collected from the stems of 10 willows *(Salix sp.)* in a small unpolluted area in Waldhausen (Oberösterreich) in June 1992. Nearly equal sized, undamaged thalli were picked at a height of 50–200 cm. After cleaning, the thalli were randomly divided into 3 groups, and each group again into eight 2g-samples (dw). The 2g-samples (25–30 thalli) were enclosed into flat plastic screens (cp. CHRIST & TÜRK 1982). Eight lichen containing screens were exposed from July 1992 to February 1993 at each of 3 different air polluted sites (Table 1) near air pollution monitoring stations of the Salzburger government in the province Salzburg (Austria).

Pretreatments of the lichen samples after exposure

Transplanted lichens retrieved at the end of each month were rinsed with distilled water to eliminate dust, placed into distilled water for 5 min to saturate, and then acclimatized in a climate chamber for 48 hours (10°C, 80–90% RH, 12:12 hs dark/light (20 $\mu E\ m^{-2}\ s^{-1}$) exchange). Afterwards, 4–6 thalli were chosen randomly for determination of net photosynthesis and dark respiration. The remaining thalli

Table 1
Description of the exposure sites.

site name	remarks on the location	continuously measured pollutants
Zistelalm	on a mountain (Gaisberg) near the city of Salzburg (1010 m, rural, high concentrations of O_3)	O_3
Winterstall	on a mountain (Dürrnberg) adjacent to the industrial city Hallein (650 m, suburban, higher peak concentrations of SO_2 in winter)	O_3, SO_2
Rudolfsplatz	at a traffic crossing in the city of Salzburg (455 m, urban, heavy automobile pollution)	SO_2, NO, NO_2

were shock frozen with liquid nitrogen and stored at –70°C until analysis. Before analysis, the thalli were ground to a coarse powder with liquid nitrogen, mixed well, and kept on ice. All determinations of an exposure-sample were always performed in one day in the same order.

Measurements were performed at least twice and the results related to the dry weight (dw) of the lichen powder (1 h at 110°C). All extracts were prepared by homogenizing the lichen powder in a blender (Bühler HO4) at 30 krpm and 0°C for 5 min. Absorbances were determined with a Beckman DU-50 spectrophotometer.

Malone dialdehyde (MDA)

Lichen powder (300 mg) was homogenized in 4 ml of 50 mM glycine buffer (pH 3.3) containing 1% insoluble polyvinylpyrrolidone (PVP_i) and 0.2% Tritone X-100. The homogenate was clarified by centrifugation twice at 4°C (20 and 30 min, 30000 g). The determination of malone dialdehyde was based on the method described by ASAKAWA & MATSUSHITA 1979. Aliquots of the supernatant (500 µl) were mixed with 375 µl of 0.5% thiobarbituric acid (TBA, in 25 mM NaOH), 80 µl dist. water, 25 µl 10 mM $FeCl_3$, 20 µl 0.28% butylated hydroxytoluene (in EtOH), and heated in a boiling water bath for 15 min. Blanks contained glycine buffer instead of TBA. After cooling on ice, 250 µl of 35% trichloroacetic acid and 500 µl of chloroform (p.a.) were added. The mixture was shaken, centrifuged (10 min, 2000 g), and the optical density of the supernatant estimated at 532 nm against the blank. The MDA concentration was determined by comparison of the absorption against a standard curve obtained from 1,1,3,3-tetra-methoxypropane (Aldrich), which liberates MDA mole for mole during the acidic assay conditions.

Superoxide dismutase (SOD) activity

Lichen powder (300 mg) was homogenized in 4 ml of 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA, 1% PVP_i, 0.2% Tritone X-100, and 5 mM 2-mercaptoethanol. The homogenate was clarified by centrifugation twice at 4°C (20 and 30 min, 30000 g). The estimation of SOD activity was based on a method described by BEAUCHAMP & FRIDOVICH 1971. The reaction mixture contained 50 mM Tris/maleate buffer (pH 8.6), 0.025 mM nitro-blue-tetrazolium-chloride (Serva), 0.3 mM xanthine

(Merck), as well as appropriate amounts of the crude extract (20-30 µl) and xanthine oxidase (Merck) in a total volume of 3 ml. After the addition of xanthine oxidase the increase in absorbance at 560 nm was recorded automatically at 25°C. SOD activity was calculated according to Mc Cord & Fridovich 1969, who defined one unit of SOD activity as that which inhibits 50% of the control reaction (without crude extract).

Acid phosphatase (AP) activity

Lichen powder (150 mg) was homogenized in 5 ml of 50 mM citrate buffer (pH 4.9) containing 0.2% Tritone X-100. The homogenate was clarified by centrifugation twice at 4°C (20 and 30 min, 30000 g). The estimation of AP activity was based on the method described by Bergmeyer 1970. Aliquots of the supernatant (150 µl) were mixed with 750 µl of 5.5 mM p-nitrophenylphosphate (Serva; in citrate buffer) and incubated at 25°C for 30 min. After terminating the reaction by the addition of 3 ml 0.1 M NaOH, the absorbance at 405 nm was determined against a blank (the crude extract was added after incubation).

Net photosynthesis (NPS) and dark respiration (DR)

Immediately after acclimatization, 4–6 thalli were wetted (approx. 120% rel. water content) and NPS as well as DR were measured by infrared absorption analysis in an open system with a BINOS (Leybold) at 10°C and 400 µE m^{-2} s^{-1}. After reaching the maximum NPS, DR was determined. For calculating the rate of NPS the maximal recorded peaks were used, and the results were related to the dry weight of the thalli ($1^1/_2$ h at 110°C).

Chlorophyll (Chl)

Lichen powder (100 mg) was extracted with 20 ml dimethylsulfoxide (p.a.) for 40 min at 65°C in the dark. After centrifugation for 15 min (2000 g), absorbances were measured and the concentration of Chl was determined according to Arnon 1949, however by exchanging A_{663} to A_{665} (cp. Ronen & Galun 1984).

Statistical analysis

Linear regression analysis (Statistical Programm SPSS for Windows) were used to determine the degree of relationship between the biological parameters and the monthly mean concentrations of air pollutants. To express the relationship coefficients of single (r^2) and multiple (R^2) determination (the squares of the single (r) and multiple (R) correlation coefficient), and the probability at the 5% level ($P \leq 5\%$) were quoted. The coefficient of determination expresses the proportion (or percentage if multiplied with 100) of variation of the dependent variable (biological lichen parameter) which can be explained by variation of the independent variable(s) (concentration of air pollutants).

Results

On the location Zistelalm, O_3 was the main air pollutant. The monthly mean concentrations in the study period 1992/93 ranged from 68 to 114 µg/m^3, monthly half and eight hour maximum values ranged from 98 to 198 and from 96 to 174 µg/m^3, respectively (Table 2). No visible injuries were observed on the transplanted lichens during the exposition period.

Table 2

Monthly mean concentrations (M), as well as maximal half hour (mh)-, eight hour (m8)-, and day (md) mean concentrations [µg/m³] of the registered air pollutants at the study sites during the exposition period 1992/93.

	Zistelalm			Winterstall						Rudolfsplatz								
	O_3			O_3			SO_2			SO_2			NO_2			NO		
	M	mh	m8	M	mh	m8	M	mh	md	M	mh	md	M	mh	md	M	mh	md
Jul	102	198	174	82	184	166	6	54	9	7	22	12	75	146	104	89	341	161
Aug	114	178	164	84	174	162	5	32	7	6	21	10	61	190	84	96	418	153
Sep	86	144	122	46	94	80	5	23	8	13	63	23	46	113	69	131	468	180
Oct	68	108	104	26	74	68	6	42	14	10	36	16	61	142	92	129	468	203
Nov	70	102	96	34	72	66	6	76	13	10	51	20	71	161	88	185	889	339
Dec	68	98	96	20	66	62	10	197	29	16	92	29	73	221	108	160	979	343
Jan	74	108	100	34	70	66	8	152	33	22	92	41	73	148	106	163	671	328
Feb	76	114	102	30	88	70	14	172	40	21	85	44	83	198	136	128	815	370

On the site Zistelalm (Table 3), O_3 showed a significant positve correlation with the MDA content ($r^2 = 0.86$, $P \leq 1\%$) and the activity of SOD ($r^2 = 0.77$, $P \leq 1\%$) in the transplanted lichens. According to the statistical analysis, the variation of the MDA content was caused by 86% and that one of SOD activity by 77% due to the action of O_3. The rest of the measured lichen parameters did not correlate significantly with O_3 (Table 3). Statistical analysis of the biological parameters (Table 5) also showed a significant positive relationship between the MDA content and SOD activity ($r^2 = 0.66$, $P \leq 5\%$).

At the study site Winterstall, the O_3 concentration was lower than on the site Zistelalm. The monthly average values ranged from 20 to 84 µg/m³, monthly half and eight hour peak values ranged from 66 to 184

Table 3

Coefficients of determination (r^2) between the biological parameters in *Hypogymnia physodes* and the monthly mean concentrations of the registered air pollutants at the three study sites (n = 8, * = $P \leq 5\%$, ** = $P \leq 1\%$, "ns" = non significant (P = > 5%); in brackets is quoted whether a positive (+) or negative (-) correlation exists).

	Zistelalm	Winterstall		Rudolfsplatz		
	O_3	O_3	SO_2	SO_2	NO_2	NO
NPS	ns	ns	0.65* (–)	ns	ns	ns
Chl	ns	0.63*(+)	0.67* (–)	0.73** (+)	ns	ns
MDA	0.86** (+)	ns	0.88** (+)	ns	ns	ns
SOD	0.77** (+)	0.85**(+)	ns	ns	ns	ns
AP	ns	ns	ns	0.62* (+)	ns	ns

Table 4

Coefficients of single (r^2) and multiple (R^2) determination between biologicial parameters in *Hypogymnia physodes* and monthly mean concentrations of the registered air pollutants at the study sites Wintertall and Rudolfsplatz (n = 8, * = $P \leq 5\%$, ** = $P \leq 1\%$).

	Winterstall						Rudolfsplatz		
	Chl		MDA		SOD		Chl		AP
O_3	0.63*	SO_2	0.88**	O_3	0.85**	SO_2	0.73**	SO_2	0.62*
SO_2	0.67*	SO_2+O_3	0.91**	O_3+SO_2	0.87**	SO_2+NO	0.77*	SO_2+NO_2	0.70*
O_3+SO_2	0.87**					SO_2+NO_2	0.77*		

and from 62 to 166 μg/m^3, respectively. Monthly averages for SO_2 were relatively low, ranging from 5 to 14 μg/m^3. As shown by the half hour maximum values (Table 2), which reached 197 μg/m^3, relatively high short term levels of SO_2 were registered. Maximum day concentrations ranged from 7 to 40 μg/m^3. Only few lichens collected at the end of January and February showed visible injuries on some parts of the thalli. These parts, however, were removed after sample collection and thus, not used for analysis.

A significant positve relationship between O_3 and SOD activity (r^2 = 0.85, $P \leq 1\%$) was also estimated at the site Winterstall. In contrast to the site Zistelalm, the Chl content showed a weak positive, but signifcant relationship with O_3 ($r^2 = 0.63$, $P \leq 5\%$). The MDA content showed a significant positive correlation with SO_2 ($r^2 = 0.88$, $P \leq 1\%$), but not with O_3 as on the location Zistelalm. Furthermore, SO_2 concentrations were inversely correlated with NPS ($r^2 = 0.65$, $P \leq 5\%$) and Chl content ($r^2 = 0.67$, $P \leq 5\%$).

Linear multiple regression analysis (Table 4) gave a significant correlation of both gases, SO_2 and O_3, with the content of Chl ($R^2 = 0.87$, $P \leq 1\%$), the MDA content ($R^2 = 0.91$, $P \leq 1\%$), and the activity of SOD ($R^2 = 0.87$, $P \leq 1\%$). But as well the multiple correlation of the MDA content as that one of SOD activity with both immissions are only slightly higher than the corresponding single correlation with SO_2 or O_3 alone (Table 4). In contrast, the variation of the Chl content in the exposed lichens is best explained by the action of SO_2 and O_3 together. Compared with the single correlation – Chl with SO_2 ($r^2 = 67\%$) and O_3 ($r^2 = 63\%$) respectively – the multiple relationship with both air pollutants increased to 87%.

Weak, but still significant correlations were also determined between the biological lichen parameters (Table 5). NPS correlated negatively with the content of MDA and the activity of AP, and positively with the Chl content. The Chl content showed a negative relationship with the MDA content, and a positive one with SOD activity.

Table 5

Coefficients of determination (r^2) of the biological parameters in *Hypogymnia physodes* at the three study sites ($n = 8$, * = $P \leq 5\%$, ** = $P \leq 1\%$, "ns" = non significant ($P > 5\%$); in brackets is quoted whether a positive (+) or negative (–) correlation exists.)

	Zistelalm				Winterstall				Rudolfsplatz			
	NPS	Chl	MDA	SOD	NPS	Chl	MDA	SOD	NPS	Chl	MDA	SOD
hl	ns				0.50* (+)				ns			
MDA	ns	ns			0.56* (–)	0.62* (–)			ns	0.61* (+)		
SOD	ns	ns	0.66* (+)		ns	0.62* (+)	ns		ns	ns	ns	
AP	ns	ns	ns	ns	0.62* (–)	ns	ns	ns	ns	0.52* (+)	ns	ns

At the study site Rudolfsplatz, SO_2, NO, and NO_2 were monitored continuously. Compared with the location Winterstall, monthly mean concentrations of SO_2 (6–22 µg/m^3) were higher and monthly day peak values (10–44 µg/m^3) higher in sommer and lower in winter. In contrast, short term levels of SO_2 were lower than at the site Winterstall, particularly in winter (Table 2), as indicated by the monthly half hour peak values (21–92 µg/m^3). NO_2 concentrations were always lower than those of NO. Monthly mean values of NO_2 ranged from 46 to 83, and those of NO from 89 to 185 µg/m^3. Monthly half hour peak concentrations of NO_2 reached 221, and those of NO 979 µg/m^3. Similar to the study site Winterstall, few lichens of the January- and February-samples showed some visible thalli injuries, which, however, were removed after sample collection.

Data from the study site Rudolfsplatz gave a significant direct correlation between the Chl content and SO_2 ($r^2 = 0.73$, $P \leq 1\%$), and a weak, but still significant one between the activity of AP and SO_2 ($r^2 = 0.62$, $P \leq 5\%$). In contrast to the site Winterstall, SO_2 did not correlate significantly with the MDA content (Table 3). As shown by the multiple linear regression analysis (Table 4), the relationship between Chl content and SO_2 increased slightly if NO or NO_2 is included ($R^2 = 0.77$, $P \leq 5\%$). Thus, nitrogen oxides enhanced the positive action of SO_2 on the Chl content in the transplanted lichens at this study site. The influence of NO_2 also increased the correlation between AP activity and SO_2. The multiple relationship between the activity of AP and the combination SO_2/NO_2 ($R^2 = 0.70$, $P \leq 5\%$) was 8% higher than with SO_2 alone ($r^2 = 0.62$). Between the Chl content and AP activity there was also a weak positive, but still significant correlation (Table 5).

Dark respiration did not correlate significantly with pollutant levels at any of the study sites.

Discussion

The presented results show a significant correlation between the gaseous immissions O_3, SO_2, NO, and NO_2 – single or in combination – and various biological parameters of *Hypogymnia physodes* transplanted to three locations with different levels of air pollution in the province Salzburg (Austria).

At the location Zistelalm, O_3 was the main air pollutant. Concentrations of SO_2 or NO_2 were very low and not monitored continuously. This site was therefore especially appropriate to investigate the influence of O_3 on the lichens. From the biological parameteres only SOD activity and particularly the content of MDA correlated significantly with O_3. Also between the two lichen parameters – SOD activity and MDA content – a significant positive relationship exists. This indicates that there was a close relation between the increased formation of superoxide radicals and the formation of MDA – a product of lipidperoxidation. It also points out that O_3 may primarily act on cell membranes. Because of its high reactivity, an accumulation of O_3 in one cell compartment, similar to SO_2 in the stroma, is not possible. The greatest part of O_3 taken up as well as the derived radicals may react already with the plasma membrane.

Studies with higher plants are consistent with these findings. SAKAKI & al. 1983 observed a linear increase of MDA during fumigation of spinach with O_3. PAULS & THOMPSON 1980 reported on the alteration of the physical properties of isolated microsomal membranes and a significant formation of MDA after exposure to O_3. Conifers exposed to O_3 showed an increase in SOD activity (TANDY & al. 1989).

Though negative effects of O_3 on CO_2-exchange and Chl content are known in many higher plants, these parameters did not correlate significantly with O_3 in *Hypogymnia physodes* at the study site Zistelalm. As shown by laboratory experiments, CO_2-exchange and Chl content in lichens seem to be relatively insensitive against the action of O_3. High concentrations of O_3 – 2 ppm (4 mg/m^3) for 0.5 h and 6 ppm (12 mg/m^3) for 2.5 h – did not alter photosynthetic $^{14}CO_2$-fixation in *Cladonia rangiformis* (BROWN & SMIRNOFF 1978). ROSENTRETER & AHMADJIAN 1977 found no essential decrease in Chl concentration of *Cladonia arbuscula* nor of the phycobiont of *Cladonia stellaris* when exposed to a range of concentrations from 0.1 to 0.8 ppm (0.2 to 1.6 mg/m^3) O_3 over a period of one week.

At the study site Winterstall, O_3 and SO_2 were measured continuously. O_3 concentration was lower than on the site Zistelalm. At this site, O_3 showed a high correlation with SOD activity. In contrast to the site Zistelalm, O_3 did not correlate significantly with the content of MDA, but showed a positive relationship with the Chl content. A direct correlation between the content of Chl and O_3 was also observed by ROSENTRETER & AHMADJIAN 1977. The chlorophyll content of *Cladonia arbuscula* and of the *Trebouxia* phycobiont isolated from *Cladonia stellaris* increased after exposure for one week to an O_3 concentration of 0.1 ppm (0.2 mg/m^3). O_3 concentrations above 0.1 ppm, however, did not signifcantly affect the chlorophyll content.

SO_2 showed a weak inverse, but significant correlation with NPS and Chl content. The negative effects of SO_2 to NPS and Chl in lichens are confirmed by many other investigators (e. g. FIELDS 1988, HENRIKSSON & PEARSON 1981). SO_2 is highly soluble in aqueous phases. Hydration of SO_2 forms sulfurous acid that produces protons, bisulfite, and sulfite. In these forms, SO_2 acts as an acidic toxin at specific metabolic sites (HEATH 1980). When lichen species are exposed to SO_2, NPS is reduced more at pH 5.0–7.0 than above pH 7.0 (TÜRK & WIRTH 1975). An inhibition of the Calvin Cycle may be caused by cytoplasmatic and especially stromal acidification (PFANZ & al. 1987a,b) and a competitive inhibition of ribulose biphosphate carboxylase by sulfite (ZIEGLER 1977).

PUCKETT & al. 1973 showed that chlorophyll destruction by SO_2 is a function of the pH, with a marked decrease of chlorophyll as the pH is lowered. They also suggested that SO_2 functioned directly as an oxidizing agent, hence the chlorophyll molecules were irreversibly oxidized. Thus, the degradation of chlorophyll may be caused by oxidation or by conversion of chlorophyll into phaeophytin exchanging Mg-ions by protons.

Analysis with both pollutants, SO_2 and O_3, resulted in a multiple correlation which was much better than the relationship between Chl content and SO_2 or O_3 alone. That means the variation of the Chl content in the lichens transplanted at the site Winterstall was mainly due to the action of both gases together, SO_2 acting negatively and O_3 positively.

SO_2 induced lipidperoxidation in biomembranes as indicated by its positive correlation with the MDA content. This is in agreement with laboratory (PEARSON & HENRIKSSON 1981) and field experiments (PEARSON & RODGERS 1982), where an increase of electrolyte leakage in lichens were measured after exposure to SO_2. In addition, also O_3 played a role in the destruction of lichen lipids as shown by the multiple regression.

There was a high positive correlation between SOD activity and O_3. Although this correlation is increased slightly if SO_2 was included for statistical analysis, O_3 was surely the main factor causing changes in SOD activity at the site Winterstall. This is consistent with other observations, where O_3 and SO_2, either single or in combination, caused an induction of antioxidant activity in forest trees (CASTILLO & al. 1987, MELHORN & al. 1986).

The location Rudolfsplatz is situated in the city of Salzburg, at a very busy traffic crossing. This site is therefore characterized by the presence of high concentrations of automobile exhausts, with the major pollutants NO and NO_2. While SO_2 immissions have decreased in the city Salzburg during the last years, nitrogen oxides have become the primary air pollutants.

Many authors reported a decrease in chlorophyll content when lichens were exposed to air pollutants, especially SO_2. The same result was also observed at the location Winterstall. Therefore it is interesting to note that SO_2, alone and in combination with NO or NO_2, showed a positive correlation with the Chl content. HOLOPAINEN 1983 attributed the increase in chlorophyll content in the studied lichens to a fertilizing effect of a nitrogen-rich dust deposition from a nearby factory. Our results indicate that the increase of Chl in the lichen *Hypogymnia physodes* at the study site Rudolfsplatz seems to be a more general effect of air pollution. Previous results from investigations in Switzerland support this opinion. VON ARB & BRUNOLD 1990 showed that the lichen *Parmelia sulcata* contained high amounts of Chl in the central and most polluted part of the city Biel. Also VON ARB & al. 1990, who studied several locations in the northern part of Switzerland and its bordering area, found the same effects with *Parmelia sulcata*. They observed signifcant positive correlations between the Chl content and the concentrations of SO_2, NO, and NO_2 – single or in combination.

The direct correlation of SO_2 with the content of Chl as well as the insignificant relationship of SO_2 to both the MDA content and NPS are in contrast to the results received at the site Winterstall. This in spite of the fact that the monthly mean concentrations of SO_2 were always lower at Winterstall than at the site Rudolfsplatz. However, short term levels of SO_2 were higher at Winterstall (particularly in winter) than at the site Rudolfsplatz. Therefore it is probable, that these higher short time concentrations of SO_2 might have been responsible for the negative effects to Chl and NPS, and the increase of MDA in the lichens at the study site Winterstall.

Transplanted lichens were not negatively affected by NO or NO_2. Similar to SO_2, NO_2 can act as an acidifying agent, because it produces the strong acids HNO_2 and HNO_3. When dissolving in the liquid phase of the plant tissue, NO_2 forms nitrite and nitrate, which can be reduced enzymatically to ammonium (NH_4^+) and thus be used for N-metabolism (ROWLAND & al. 1987). By the reduction of NO_2^- to NH_4^+, H^+ is consumed and OH^- is produced thereby preventing acidification. Lichens seem to be relatively insensitive to NO_2 as shown by NASH 1976. He fumigated several lichen species with 1 (1.92), 2 (3.84), 4 (7.68) and 8 ppm (15.36 mg/m^3) NO_2. He found only a signifcant reduction of chlorophyll after fumigation with the unrealistic concentrations of 4 and 8 ppm.

Rudolfsplatz was the only site where AP activity correlated significantly with air pollutants. It showed a slight positive correlation with SO_2 and a higher one with the combination SO_2/NO_2. This is not in agreement with results of BAUER & KREEB 1974, who found in most cases a negative correlation between the concentration of air pollutants and the activity of AP in lichens from differently polluted sites in the town of Esslingen. On a few sites, however, they also observed an increase of AP activity with air pollution. The positive effects on AP activity might be explained by the fact that plants are able to metabolize SO_2 and nitrogen oxides (RENNENBERG 1984, ROWLAND & al. 1987) resulting in an increase of metabolism, which is indicated by higher activities of AP.

In conclusion, the presented results show that the action of ambient concentrations of air pollutants on the lichen *Hypogymnia physodes* depends on the location and thus, on the number, kind, and concentration of the present air pollutants. Therefore, the results may not be generalized. In regions where O_3 is the main air pollutant (in the country) SOD activity and MDA content in the lichen *Hypogymnia physodes* could perhaps be used to indicate O_3 levels. But this needs to be investigated further in the field.

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