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Monitoring with lichens – Conductivity methods assess salt and heavy metal damage more efficiently than chlorophyll fluorescence

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ABSTRACT

In the lab, we exposed three foliose lichen species, *Lobaria pulmonaria*, *Parmelia sulcata* and *Xanthoria aureola*, to 0, 0.01, 0.2, and 0.6 M NaCl in combinations with copper and zinc (0, 10, 100, 500 μM). High salt concentrations adversely affected the lichen membrane integrity as measured by conductivity methods, whereas the potential photosystem II efficiency (Fv/Fm) was tolerant. High light was necessary to reduce Fv/Fm in thalli exposed to salt, whereas high light did not aggravate the conductivity. The seashore species *X. aureola* was much more resistant to salt than the old forest species *L. pulmonaria*. With respect to Cu and Zn, used concentrations had no *(P. sulcata*, *X. aureola)* or small *(L. pulmonaria)* effects on Fv/Fm. However, both heavy metals substantially increased conductivity in all species, consistent with membrane damage. Thus, the conductivity method detected high salt, high copper and high zinc stress much more efficiently than did the chlorophyll fluorescence method. This suggests that membrane integrity of the mycobiont is more sensitive to salt and heavy metal stress than potential photosystem II efficiency of its autotrophic partners.

1. Introduction

Lichens, being symbiotic associations between a mycobiont and its photobionts(s), a green alga and/or a cyanobacterium, are often used for monitoring various pollution stresses e.g. (Conti and Cecchetti, 2001; Nimis et al., 2002). When lichens are used for biomonitoring, measured responses can be accumulation of various pollutants (Yemets et al., 2014), or adverse effects of pollutants on the lichens' physiological status (Conti and Cecchetti, 2001). Because lichens are long-lived organisms, and because they are poikilohydric organisms with little control of water and gas exchange, they are useful indicators not only of air pollution (Bačkor and Loppi, 2009; Garty, 2001), but also of other man made disturbances like sudden increases in light exposure during logging of forests (Gauslaa et al., 2001).

In nature, lichens face various stressors such as SO_2, heavy metals, salt, and high light. Some of these agents may interact, meaning that a stressor may aggravate the damage caused by another co-occurring one. Many heavy metals reduce the viability of lichens, and the potential photosystem II (PSII) efficiency (Fv/Fm) has widely been used for assessing adverse effects on photosynthesis (e.g. Garty, 2001). Heavy metals may damage biological membranes, causing leakage of ions. Such damage can be quantified by measuring conductivity after immersing lichens into de-ionized water (e.g. Bačkor and Loppi, 2009). In addition to adverse effects of SO_2, other harmful gases and heavy metals, lichens can experience osmotic stress due to high salt exposures. Such stress may become aggravated by excess light that strongly impacts viability measured as reduced Fv/Fm. Lichens species often respond differently to various pollutants. For example, species taking advantage of NH_3 often suffer from exposures of NO_2 and SO_2 (van Dobben and ter Braak, 1999).

This study was motivated by a recent lichen transplantation experiment along a highway quantifying accumulation of salt and heavy metals in winter, with concurring assessment of lichen viability by chlorophyll fluorescence and relative growth rates (Yemets et al., 2014). These lichens were exposed to a complex mixture of heavy metals from abrasion of tires, lubricating oils, brake pads or fuel additives (Pacyna and Pacyna, 2001), de-icing salt, and episodes of high light stress. To reduce the effects of confounding factors present in a natural roadside environment, this study aims to assess effects of various stressors on lichens one by one, and in fixed combinations under controlled laboratory conditions. Our main hypotheses are: (i) High light aggravates the salt stress effects on lichens. (ii) Adverse effects of heavy metals and salt are additive. In testing these

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hypotheses, we compare the photobiont susceptibility to treatments using chlorophyll fluorescence tools for detecting PSII damage, and the mycobiont susceptibility using conductivity methods for indicating membrane damage. The conductivity method is mainly a mycobiont stress indicator because the mycobiont comprises approx. 90% of total lichen biomass. Finally, we compare the susceptibility of applied treatments for the following lichens: *Lobaria pulmonaria* – an air pollution-susceptible species (Brodo et al., 2001; Sigal and Johnston, 1986), *Parmelia sulcata* – a pollution-resistant species (Von Arb et al., 1990), and *Xanthoria aureola*, a salt-tolerant species belonging to an air pollutant-tolerant genus (Silberstein et al., 1996), particular with respect to nitrogen pollution (Gaio-Oliveira et al., 2005; Johansson et al., 2011).

2. Materials and methods

2.1. Lichen materials

All lichens were collected in autumn 2011 in southeastern Norway. *Lobaria pulmonaria* (L.) Hoffm., a cephalolichen with the green alga *Dictyochloropsis reticulata* as a primary and the nitrogen fixing cyanobacterium *Nostoc sp*. as a secondary photobiont was collected on *Fagus sylvatica* L. in an old, open-shaded forest near Larvik (59°05′31″N, 9°56′52″E; 200 m above sea level). *Parmelia sulcata* Taylor, a chlorolichen with the green algae *Trebouxia* as photobionts was collected on stems of *Tilia cordata* Mill. in an alley along a local farm road in Ås (59°40′N and 10°45′E; 100–150 m.a.s.l). *Xanthoria aureola* (Ach.) Erichsen, a chlorolichen with *Trebouxia arboricola* as photobionts was collected on sun-exposed rocks at the seashore in Hvaler (59°02′46″N and 10°55′45″E; 2 m.a.s.l). We carefully removed residual tree bark, debris, associated bryophytes and other lichens from collected thalli. They were brought to the lab on the collection date, air dried at room temperatures, and then stored at −20 °C until experiments, the recommended way of storing lichens for later physiological experiments (Honegger, 2003).

2.2. Experimental design

Before experiments, we hydrated the lichens and kept them at 18–20 °C under low light (10 μmol photons m^{-2} s^{-1}) for 24 h. In all experiments, $n = 5$ replicate thalli.

In the first experiment, we immersed lichens into 0.01, 0.20 and 0.60 M NaCl in de-icing salt solutions and in deionized water as a control. The de-icing salt used on roads in Norway during winter consists of 96.5% NaCl, 0.4% $CaSO_4$, 0.2% $MgSO_4$, 0.06% $MgCl_2$, 0.05% MgO, and 2.8% water (Analytical data from Norwegian Public Roads Administration). All thalli were immersed in the solutions for 20 h under low light (10 μmol photons m^{-2} s^{-1}) from fluorescent tubes. Afterwards, half of the thalli were exposed to high light (650 μmol photons m^{-2} s^{-1} with equal levels of red, green and blue light) from a LED panel (SL3500 RGB, Photon Systems Instruments, Brno, Czech Republic) for 4 h while the other half remained under low light for the same time.

In the second experiment, we immersed lichens into copper and zinc solutions of 10, 100 and 500 μM in addition to a deionized water control under low light (10 μmol photons m^{-2} s^{-1}) for 24 h at 20 °C. The copper and zinc solutions were prepared from $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$.

In the third experiment, we immersed lichens into solutions containing 0.01, 0.20 or 0.60 M salt combined with 10, 100 or 500 μM copper or zinc under low light (10 μmol photons m^{-2} s^{-1}) for 24 h at 20 °C.

2.3. Chlorophyll fluorescence

Potential quantum yield of PSII (Fv/Fm) was measured with a portable chlorophyll fluorometer PEA (Plant Efficiency Analyser, Hansatech Instruments Ltd, King's Lynn, Norfolk, UK) before and after the treatments. Thalli were dark adapted for 15 min before measurements. Chlorophyll fluorescence parameters were recorded in three different positions on each thallus; the mean value was used as one observation.

2.4. Conductivity measurement

Electrolyte leakage in lichens was determined by a portable conductivity meter (Mettler-Toledo International Ltd, Singapore). The initial electrical conductivity in μS cm^{-1} (Ci) of the deionized water was measured in advance as a blank. After the salt treatment, we rinsed the lichens 3 times 5 min in deionized water in order to remove unbounded sodium and potassium ions. After rinsing, thalli were shaken in deionized water for 12 h before measurement of the electrical conductivity of the solution (Cv). Finally, the thalli were boiled at 100 °C for 15 min in water bath to cause total rupture of cell membranes and release all electrolytes; cooled to 25 °C and the final electrical conductivity (Cf) was measured. A conductivity index indicating loss of membrane integrity was calculated as: $((Cv-Ci)/Cf) \times 100$.

2.5. Statistical analyses

We analyzed the data with two-way ANOVA after checking for normal distribution and equal variance. All statistical analyses were performed using the statistical package Minitab ver. 16.2.2.

3. Results

3.1. Effects of salt and/or high light on viability parameters

Salt did not affect potential quantum yield of PSII (Fv/Fm) in any of the lichens exposed to low light (Fig. 1). However, lichens receiving high light were photoinhibited at the highest salt concentrations, evidenced by the significant interaction between salt and light (Fig. 1; Table 1). The increase in photoinhibition at the higher salt concentrations was substantially larger for *L. pulmonaria* than for *P. sulcata*, and for *X. aureola* in particular (Fig. 1; Table 1).

By contrast, increasing salt concentration increased the electrolyte leakage at high as well as at low light. The increase in electrolyte leakage was much greater for *L. pulmonaria* than for *P. sulcata*, whereas *X. aureola* experienced just slight increases

Table 1
Two-way ANOVA for combined effects of salt and high light on potential photosystem II efficiency (Fv/Fm) and loss of membrane integrity (ion leakage measured as percent increase in conductivity) of *Lobaria pulmonaria*, *Parmelia sulcata* and *Xanthoria aureola*.

Source	df	*F*	*P*	*F*	*P*	*F*	*P*
		L. pulmonaria		*P. sulcata*		*X. aureola*	
Potential photosystem II efficiency (Fv/Fm)							
Salt	3	25.58	0.000	1.86	0.157	7.85	0.000
Light	1	523.06	0.000	144.21	0.000	48.06	0.000
Salt × light	3	20.27	0.000	3.79	0.020	4.15	0.014
Error	32						
Total	39						
Loss of membrane integrity (conductivity index)							
Salt	3	133.24	0.000	87.37	0.000	51.94	0.000
Light	1	3.17	0.084	2.46	0.127	0.15	0.698
Salt × light	3	0.73	0.541	1.40	0.262	0.07	0.973
Error	32						
Total	39						