# Changes in spectral reflectance of selected Antarctic and South American lichens caused by dehydration and artificially-induced absence of secondary compounds

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## Abstract

Recently, spectral characteristics of lichens are in focus because of increasing number of spectral data applications in remote sensing of treeless polar and alpine regions. Therefore, species-specific spectral reflectance indices are measured in lichen species dominating polar ecosystems. Hydration status of the lichen thalli, as well as the presence of intrathalline secondary metabolites - which are UV-B absorbing compounds - both affects the spectral reflectance curves as well as numeric values of spectral reflectance indices. In the present paper, the reflectance spectra in 380-800 nm was measured in selected lichens to assess the effects of full hydration, and to evaluate the influence of secondary metabolites, they were wash out from lichen thalli with acetone (i.e. acetone rinsing) and then the spectra were also measured. For these experiments, Antarctic (Xanthoria elegans, Leptogium puberulum, Physconia muscigena and Rhizoplaca melanophthalma) and Argentinean lichens from mountain regions (Parmotrema conferendum and Ramalina celastri) were used. Changes in several spectral reflectance indices were evaluated and discussed in relation with hydration status and the absence of secondary metabolites. For the great majority of studied lichens, MCARI (Modified Chlorophyll Absorption in Reflectance Index) was the most effective index to reflect the changes between dry and wet state of thallus.

Key words: reflectance indices, secondary metabolites, hydration

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*Abbreviations:* AR – acetone rinsing, acetone-rinsed, DRY – dry sample, LI – Lichtenthaler Index, MCARI, MCARI1 – Modified Chlorophyll Absorption in Reflectance Index, NDVI – Normalized Difference Vegetation Index, SRPI – Simple Ratio Pigment Index, NPCI – Normalized Pigment Chlorophyll Index, PRI – Photochemical Reflectance Index, WET – wet (fully hydrated) sample

### Introduction

Secondary lichen metabolites serve mainly as sun-screening compounds and have herbivore-deterring functions as well (for review see e.g. Solhaug et Gauslaa 2012). These metabolites screen solar radiation either by absorbance (parietin, melanins) or by reflectance (atranorin) to protect the photobiont against excessive photosynthetically active radiation (McEvoy et al. 2007). Their concentration correlates with light exposure and is positively dependent on UV-B radiation as well (Gautam et al. 2011). Many studies demonstrated that high doses of UV-B induced the synthesis of parietin, usnic acid, melanins, depsidones (lobaric acid, pannarin, etc.), depsides (atranorin, gyrophoric acid, etc.), diphenyl ethers (epiphorellic acids, buellin), bisxanthones (secalonic acids, etc.), mycosporines and mycosporine amino acids (MAAs), scytonemin along with classical pigments (melanin, carotenoids) in lichen thalli (for review see Nguyen et al. 2013). However, too high doses (more than 1.5 W m<sup>-2</sup>) of UV-B may lead to a decrease of UV-B absorbing compounds due to their photodegradation (Estêvão 2015). In addition, lichen secondarymetabolites have been involved in lichen palatability(Gauslaa 2005).

Many secondary compounds can non-

destructively be extracted from air-dry living lichens with acetone without any harmful effects on photosynthetic capacity (Solhaug et Gauslaa 1996) of photobionts and whole lichen thalli (e.g. Solhaug et Gauslaa 2001, Asplund et Wardle 2013, 2016). Since spectral characteristics of lichen thalli are determined by a variety of mycobiont- and photobiont-produced metabolites, the above-mentioned approach of acetone rinsing allows to study the influence of secondary metabolites on lichen spectral reflectance. In our study we hypothesized that the reflectance spectra is associated to both, the content of secondary metabolites as well as the hydration status of lichen thalli. Therefore, lichen thalli rinsed with acetone would reflect the pecularities of the species studied, since the content of secondary metabolites is species-specific. The aim of this study was to compare spectral reflectance curves of chloro- and cyanolichens in wet and dry status and to identify the most sensitive spectral vegetation indices, *i.e.* showing the most apparent change between wet and dry lichen thallus. Another aim was to evaluate the changes in spectral refectance caused by the absence of secondary metabolites

### **Material and Methods**

#### **Experimental lichens**

Xanthoria elegans, Leptogium puberulum, Physconia muscigena, and Rhizoplaca melanophthalma were collected at the northern part of James Ross Island (Ulu peninsula), Antarctica. *X. elegans* and *L. puberulum* were collected from small stones at several sites located on the northern slopes (140 m a.s.l., 63°48'51'' S, 57°51'35'' W) of the Berry Hill mesa. *P. muscigena* was collected from a vegetation oasis forming the long-term research plot located in a close neighbourhood of the Mendel station (63°48'03'' S, 57°52'50'' W). The long-term research plot is located at the altitude of 8 m a.s.l. close to the coastal line (for more details *see* Barták et al. 2015). *R. me-lanophthalma* was collected from a coastal terrace (63°48'10'' S, 57°55'09'' W). The altitude of the sampling site was 10 m a.s.l.

Continental lichens were collected from different areas in the province of Cordoba, Argentina. This area has an average temperature in winter of 8.5°C and 16.6°C in summer, precipitations are concentrated in the summer period, with an average of 750 mm per year. *Parmotrema conferendum* Hale, and *Ramalina celastri* (Spreng.) Krog. & Swinsc. were collected from branches and stems of small trees (height below 2 m) from Inti-Yaco, Calamuchita Valley, 90 km south of Córdoba city (57° 30' S, 64°42' W). The collection site is a pristine area with a medium density woodland, located at the altitude of 1084 m a.s.l.

Usnea amblvoclada (Müll. Arg.) Zahlbr. was collected from rocks at Los Gigantes, 80 km west of Córdoba city (31°24' S. 64° 46' W). This is a mountain area with scarce vegetation. The average height above sea level is 1 300 m. This species was chosen because of its wide distribution in the Cordoba province, it can be easily separated from the rock substrate and it had already been used in many biomonitoring studies in the area (Carreras et Pignata 2001, 2002, 2007, Carreras et al. 2005, 2009). The basal parts of lichen thalli with adhering pieces of rock were detached and samples were stored in the laboratory at room temperature until exposure.

#### Sample preparation

In all experiments, the thalli of lichen species were first measured by a spectroreflectometer in dry (DRY) state. Then, the thalli were hydrated in closed Petri dishes with wet paper for 24 h and their spectral reflectance was measured on fullyhydrated thalli (WET). For the third spectral reflectance measurements, WET thalli were left to dry for 48 h at room temperature (25°C) and then rinsed in acetone. After that they were rinsed 4 times with 10 ml of acetone (100%) for 15 min. each time. Then, the rinsed thalli (AR) were left at room temperature for 6 h to evaporate the remaining acetone before performing the spectral reflectance measurements.

#### Spectral reflectance measurements

Reflectance spectra were measured within the range of 380-800 nm using the Poly-Pen RP 400 UV VIS spectroreflectometer (Photon Systems Instruments, Brno, Czech Republic). Lichen thalli (DRY, WET, AR) were placed into a clip that enabled a constant distance from the instrument measuring head. Each sample remained 1 min. in darkness into the clip and then, spectral reflectance and transmittance of the samples were measured. With data from reflected light spectra, several reflectance indices were calculated (*see* Table 1 for the list) using the pre-programmed routines in the instrument. Mean reflectance spectra for individual species were calculated considering five replicates for each treatment (WET, DRY, AR).

#### Spectral indices

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Normalized Difference Vegetation Index (NDVI)
Reference: Rouse et al. (1974)
    Equation: NDVI = (RNIR - RRED) / (RNIR + RRED)
Modified Chlorophyll Absorption in Reflectance Index (MCARI1)
Reference: Haboudane et al. (2004)
    Equation: MCARI1 = 1.2 * [2.5 * (R790- R670) - 1.3 * (R790- R550)]
Modified Chlorophyll Absorption in Reflectance Index (MCARI)
Reference: Daughtry et al. (2000)
    Equation: MCARI = [(R700- R670) - 0.2 * (R700- R550)] * (R700/ R670)
Simple Ratio Pigment Index (SRPI)
Reference: Peñuelas et al. (1995)
   Equation: SRPI = R430 / R680
Photochemical Reflectance Index (PRI)
Reference: Gamon et al. (1992)
   Equation: PRI = (R531 - R570) / (R531 + R570)
Normalized Pigment Chlorophyll Index (NPCI)
Reference: Peñuelas et al. (1994)
    Equation: NPCI = (R680 - R430) / (R680 + R430)
Lichtenthaler Index
Reference: Lichtenthaler (1996)
    Equation: LI = (R790 - R680) / (R790 + R680)
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 Table 1. List of spectral reflectance indices used in this study with sources of the equations for particular indices.

## **Results and Discussion**

Spectral reflectance curves were speciesspecific, as expected (see Fig. 1). Spectral reflectance curves curves in N. commune and L. puberulum exhibited more or less linear part within the range of 400 to 680 nm followed by a red-edge increase which was more pronounced in wet compared to dry samples. In hydrated Antarctic chlorolichens (X. elegans, P. muscigena, and R. melanophthalma), local peaks were apparent in 530 and 640 nm, followed by the red-edge increase in the wavelengths above 680 nm. In the three species, local minimum was found in 675 nm similarly to the data presented by Barták et al. (2017). The increase in the red-edge region was biphasic showing a higher rate within the range of 680 to 710 nm and the other in the range of 710 to 800 nm. The presence of red-edge increase in spectral reflectance is a characteristic of chlorolichens that has been reported and documented for many species exhibiting a great variety of colors of thallus (see Bechtel et al. 2002 - Umbilicaria torrefacta, Van der Veen et Csantho 2005 for Parmelia isidiata, Cladina arbuscula, Cetraria cucullata, Stereocaulon rivulorum, and other species). In general, hydrated chlorolichens samples from Antartica showed lower reflectance values than in the dry state. On the other hand, lichens from Córdoba province (R. celastri, U. amblyoclada and P. conferendum) had similarly-shaped spectral reflectance curves with the peaks and local minima in the same wavelengths in the DRY and WET state. The peak at 550 nm was higher in WET than in DRY state.

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**Fig. 1.** Reflectance spectra measured in lichen thalli and *N. commune* colony in dry state  $(DRY - \bullet)$  and fully hydrated state  $(WET - \circ)$ . Missing spectrum for DRY *R. melanophthalma* is due to insufficient amount of lichen thalli.

In wet state, however, the peak in 540-550 nm was more pronounced than in dry state. This peak has already been reported in green chlorolichens (see e.g. Rees et al. 2004). In general, spectral reflectance reached higher values in wet than drv state in the wavelengths of 540-550 nm in the three Argentinean lichens. A local minimum found at 670-680 nm corresponds to the maximum absorption of chlorophyll. These lower values were more pronounced in wet than in dry state in both, Antarctic and Argentinean chlorolichens. In cyanolichens L. puberulum and N. commune, the minimum was found only in wet state. Xie (2008) reports that in cyanolichens (Lobaria hallii and Peltigera praetextata) green light was less reflected than chlorolichen (Lobaria pulmonaria) which may partly explain the slightly higher cyanobacterial photosynthetic CO<sub>2</sub> uptake in green light.

Among the spectral reflectance indices, MCARI, was the most sensitive index (*i.e.* showing the highest difference between DRY and WET samples) to reflect the changes between DRY and WET state (Table 2) with differences over 100% in all species. MCARI indices showed the largest change in *X. elegans due* to differences in 670 and 700 nm (*see* Fig. 1).

PRI and LI were the other indices reflecting the change between DRY and WET, especially in P. muscigena. It is well established that PRI is associated with xanthophyll pigments pool activity, de-epoxidation state of the pigments in particular. The relation is reported for higher plants (e.g. Ripullone et al. 2011), and mosses (Lovelock et Robinson 2002). Therefore, PRI has been used as a measure of xanthophyll cycle activity on a diurnal scale in chlorophyll-possessing autotrophs. Recently, PRI was used as a measure of photosynthetic light-useefficiencv(LUE) and as an indicator of stress in higher plants and lichens, too (Weber et Hill 2016). Our study shows, that PRI is a sensitive indicator of hydrationrelated changes in physiological activity of

chlorolichens (see Table 3). The differences found in PRI were consistent with the data previously presented by Barták et al. (2016). For, N. commune, Trnková et Barták (2016) reported dehydration-induced changes in PRI in Antarctic Nostoc sp. colonies as well. In the latter study, PRI changed to dehydration within a narrow range (-0.05 to 0.02) which is well comparable to our data (see DRY and WET data in Table 2). In WET N. commune and L. puberulum cyanolichens, PRI was related to xanthophylls pigments (see Zakar et al. 2016 for review). However, the influence of several carotenoids, such as *e.g.* β-cryptoxanthin, caloxanthin, nostoxanthin and orange carotenoid protein can not be excluded from the reflectance in the carotenoid wavelength range. PRI showed only slight differences between the DRY and WET state in the studied species (see Table 2), with the exception of P. muscigena.

Regarding NDVI, the largest relative change was found for *P. muscigena*, and attributed to prevailing color of thalli which is greyish in dry state and dark to fresh green in fully hydrated state (Barták, unpublished data). SRPI, showed the largest difference in *X. elegans* followed by *R. melanophthalma*.

Acetone rinsing brought some minor changes to the values of spectral reflectance indices (see Table 2, Table 3). In some lichen species (P. muscigena, L. puberulum and U. amblyoclada), the changes in numeric values were relatively small (below 25%). This was probably due to the fact that acetone rinsing affects mainly UV-B screening compounds (190-350 nm) from dry lichen thallus while photosynthetic pigments remain unaffected. This is evident in the lichen U. amblyoclada whose secondary metabolites are composed almost exclusively of usnic acid, absorbing within the range of 200-400 nm (for Usnea genus review, see Fernández-Moriano et al. 2016), thus this species was strongly affected by the acetone washed out.

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Species		NDVI	MCARI1	MCARI	SRPI	PRI	NPCI	LI
R. melanophthalma	DRY	0.290	0.219	0.035	0.298	-0.065	0.543	0.283
	WET	0.308	0.309	0.070	0.465	-0.046	0.366	0.332
	AR-WET	0.445	0.102	0.049	0.815	-0.095	0.129	0.521
N. commune	DRY	0.349	0.029	0.004	0.909	-0.030	0.056	0.377
	WET	0.322	0.082	0.018	0.856	-0.049	0.079	0.341
	AR-WET	0.392	0.092	0.022	0.879	-0.070	0.065	0.428
P. muscigena	DRY	0.293	0.083	0.014	0.688	-0.032	0.192	0.258
	WET	0.511	0.189	0.110	0.638	-0.084	0.221	0.566
	AR-WET	0.566	0.205	0.107	0.623	-0.084	0.233	0.603
L. puberulum	DRY	0.320	0.035	0.003	0.573	-0.079	0.275	0.277
	WET	0.334	0.052	0.011	0.723	-0.049	0.169	0.316
	AR-WET	0.360	0.049	0.009	0.737	-0.026	0.153	0.324
X. elegans	DRY	0.231	-0.150	0.007	0.082	-0.223	0.848	0.187
	WET	0.309	0.225	0.108	0.143	-0.197	0.750	0.355
	AR-WET	0.318	0.307	0.141	0.307	-0.141	0.537	0.374
R. celastri	DRY	0.290	0.219	0.035	0.298	-0.065	0.543	0.283
	WET	0.329	0.397	0.185	0.700	-0.030	0.179	0.382
	AR-WET	0.393	0.410	0.136	0.525	-0.074	0.311	0.459
P. conferendum	DRY	0.334	0.280	0.070	0.751	-0.036	0.163	0.366
	WET	0.435	0.628	0.281	0.821	-0.007	0.099	0.506
	AR-WET	0.489	0.755	0.465	0.827	-0.017	0.096	0.593
U. amblyoclada	DRY	0.266	0.223	0.053	0.495	-0.045	0.356	0.287
	WET	0.325	0.379	0.162	0.404	-0.055	0.428	0.383
	AR-WET	0.376	0.432	0.198	0.368	-0.067	0.464	0.442

**Table 2.** The effects of dry (DRY), wet (WET), and wet thallus that has been acetone-rinsed (AR on spectral reflectance indices (NDVI - Normalized Difference Vegetation Index, MCARI1, MCARI - Modified Chlorophyll Absorption in Reflectance Index, SRPI - Simple Ratio Pigment Index, PRI - Photochemical Reflectance Index, NPCI - Normalized Pigment Chlorophyll Index, LI - Lichtenthaler Index – for equations *see* Table 1.) of the experimental lichen species and *N. commune*. Species *R. melanophthalma*, *N. commune*, *P. muscigena*, *L. puberulum*, *X. elegans* were from James Ross Island (Antarctica), species *R. celastri*, *P. conferendum* and *U. amblyoclada* from Cordóba Province (Argentina).

In *X. elegans*, the cortical orange parietins is UV-B absorbing screen which could be easily washed out from dry thallus by acetone (*e.g.* Nybakken et al. 2004). However, since the main absorbance is in UV-B range (*see* Araújo et al. 2015 for review), removal of parietin by acetone rinsing did not lead to a change in spectral reflectance of acetone-rinsed *X. elegans* at the wavelengths of 400-500 nm. Contrastingly to the above-mentioned three species, other species showed much bigger changes in acetone-rinsed thalli. This was specifically seen in *R. melanophthalma* (all spectral indices – *see* Table 3), *R. celastri* (PRI), and *X. elegans* (SRPI). For *R. melanophthalma*, such a huge difference can be attributed to specific mosaic pattern of pale yellow and brownish-black structures. The black parts are apothecia that in some cases may form a majority of the upper surface of the lichen. Therefore, the optical properties of *R. melanophthalma* may differ to a large extent due to relative proportion of black apothecia in the upper surface of *R. melanophthalma* thallus.

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	P melanonhthalma	Ncommune	P musciaena	l nuberulum	Y elegans	R celastri	P conferendum	II. amhluoclada
1010	n.meiunopintiiuimu	7.0	r. muscigenu	L. puberululli	A. Eleguns	N. CEIU3LII	7. conjerendum	21.0
NDVI	5.9	-7.9	74.6	4.2	33.8	11.7	30.4	21.9
MCARI1	40.9	180.1	127.1	49.8	-249.9	44.8	124.1	69.9
MCARI	101.5	351.4	709.4	230.3	1393.3	81.2	298.4	203.7
SRPI	56.2	-5.9	-7.2	26.1	73.3	57.5	9.4	-18.3
PRI	-29.6	61.2	159.7	-37.4	-11.8	-116.3	-79.5	22.2
LI	17.3	-9.6	118.9	13.8	89.4	25.9	38.3	33.3
	R.melanophthalma	N.commune	P. musciaena	L. puberulum	X. eleaans	R. celastri	P. conferendum	U. amblvoclada
NDVI	-44.5	-21.7	-10.8	-7.8	-2.9	-19.5	-12.4	-15.7
MCARI1	67.0	-12.2	-8.5	5.8	-36.4	-3.3	-20.2	-14.0
MCARI	30.0	-22.2	2.7	18.2	-30.6	26.5	-65.5	-22.2
SRPI	-75.3	-2.7	2.4	-1.9	-114.7	25.0	-0.7	8.9
PRI	-106.5	-42.9	0.0	46.9	28.4	-146.7	-142.9	-21.8
11	56.0	25.5	65	25	5 /	20.2	17.2	15.4

Relative change in spectral reflectance indices (WET - DRY)\*100/DRY (%)

**Table 3.** Relative changes in spectral reflectance indices (NDVI, MCARI, SRPI, PRI, and LI) caused by hydration of a thallus (changes related to WET and DRY thallus are reported), and acetone rinsing (AR) – changes are related to WET thalli compared to acetone-rinsed (AR) thalli.

Thus, follow-up studies should be focused on spectral reflectance of *R. melanophthalma* in response to dehydration to point out the likely reason of the changes in spectral reflectance indices.

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