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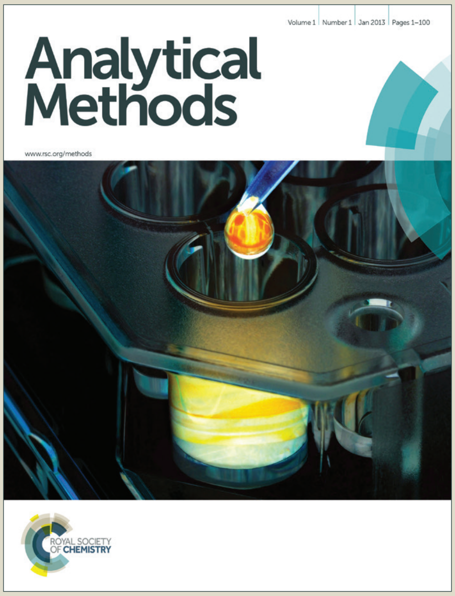

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LICHENS AROUND THE WORLD: A COMPREHENSIVE STUDY OF LICHEN SURVIVAL BIOSTRATEGIES DETECTED BY RAMAN SPECTROSCOPY

I. Miralles[1,2]; H.G.M. Edwards[3]; F. Domingo[1]; S.E. Jorge-Villar[4,5*]

[1] Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas,

[2] Georges Lemaître Centre for Earth and Climate Research, Université Catholique de Louvain-La-Neuve, Belgium.

[3] Department of Chemical and Forensic Sciences, School of Life Sciences, University of Bradford, UK

[4]Area de Geodinámica Interna, Facultad de Humanidades y Educacion, Universidad de Burgos, C/ Villadiego s/n, 09001-Burgos (Spain). E-mails: susanajorgevillar@hotmail.com; seju@ubu.es; phone +34 947 258 772; Fax: +34 947 258 723

[5]National Research Center on Human Evolution (CENIEH), Burgos (Spain).

ABSTRACT

A list of the lichen biomolecules detected by Raman spectroscopy has been compiled and their appearance related with the environmental conditions operating in the lichen habitat over the world. The adaptative climatic strategies of lichens have been analysed as a whole and some interesting and contradictory conclusions arise with regard to other research conclusions reported in the literature, such as the presence of hydrated calcium oxalates and their relationship with desiccated environments or the correlation between climatic conditions and protective pigments or pigment mixtures. The results of this exercise will be useful for our understanding of the biochemical synthetic strategies being employed for the survival of the lichen colonies in hostile terrestrial environments and the prediction of Raman spectral data for extremophiles in a range of novel hot and cold desert conditions. Additionally, a database of all key lichen biomolecules identified by Raman spectroscopy and their characteristic Raman wavenumbers are given for further unambiguos identification.

Keywords: Adaptative strategies, climate, biomolecule, extremophile, calcium oxalate

INTRODUCTION

Lichens are formed by a symbiotic relationship between micro-algae and fungi; the first of these provides chlorophyll and permits a photosynthetic function whereas the fungus provides shelter and humidity. Lichens have colonized most terrestrial environments, even the most hostile cold and hot deserts or high altitude habitats and appear on most substrata, such as rocks, soil, wood, brick, leaves, roofing, paint, walls, etc.[1-8] They are, after microorganisms, pioneer colonizers of sterile areas through physical and chemical mineral substratum degradation and by the addition of organic matter to new soil production.

The capability of lichens to survive under harsh climatic factors and on different substrata has been ascribed to the development of different types of adaptive strategies: Charles Darwin[9] emphasised this in his identification that adaptation of species to a changing environment was the key to its successful survival. Although some endolithic colonisations have been described as lichens[10], most lichens are epilithic and live on the substratum surface and then their survival strategies are necessarily related to the availability of food and water and protection from hazardous external features, such as high or low temperature, desiccation, chemical toxicity or low wavelength, high energy UV-radiation by producing different chemical compounds.[1,3,4,11-15] Dormant and active stages have been described as phases of the life of lichen species, related to the environmental conditions, and can be evaluated by the measurement of their photosynthetic activity. [16-17]

There is a wide range of biomolecules, either organic pigments and oxalates, produced by lichens from different metabolic pathways.[4,8,18-19] Some of these carry out a protective role and, at the same time, act as pigments, giving a characteristic colour to the lichen.[7,8,14,20-22] Oxalates are described as wasted products as a result of methabolic activity; they can play a dual role.[1] By one hand, as water storage under low humidity levels; by the other hand, the calcium oxalate formation process can also help lichens to rock disintegration and to grow into the rock. Although there is a wide range of pigments which often occur in admixture, it has already been reported that there is not a direct relationship between pigments and species in the way that different species use the same protective pigments[4]; also, there is no correlation between the lichen genus and the pigment mixture since different genera can produce the same pigment composition and different species from the same genus could produce different pigment mixtures.[4-5] This means that naturally there will be inherent difficulties in using spectral pigment data for taxonomic purposes. In a similar way, protective biomolecules are not specific for climatic parameters, that is: lichens living under analogous environmental conditions can adopt a different pigment patronage.[1,5] This means that pigment mixtures *per se* cannot be used for lichen species identification or for environmental parameter characterization.

Lichen pigments have been used from ancient times for the manufacture of inks, medicines and perfumes, and for dyeing textiles and garments.[23-27] Lichens play also an essential role in ecosystems by improving biogeochemical soil cycles and increasing the carbon stocks in the soils.[28-31] Nowadays, lichens have a renewed research interest as extremophilic organisms in life and space sciences[32-37] which has itself directed the adoption of new analytical techniques in lichenology. One of these novel analytical techniques is Raman spectroscopy, which has been applied to lichenological studies along with the older infrared and mass spectroscopic techniques. Raman spectroscopy is a non-destructive technique based on the use of laser radiation in the visible or near infrared region of the electromagnetic spectrum for analysing the vibrational spectra in molecules; the basis of this technique relies upon the probing of the chemical bonds in organic molecules and inorganic molecular ions whose Raman spectral signatures are characteristic of each compound or molecular ion. Hence each chemical compound, either organic or inorganic, displays a specific Raman spectral pattern related with its composition and molecular structure and in a particularly advantageous way micro- and macro- analysis can be carried out directly on the sample without the necessity for any physical or chemical pretreatment or manipulation to be carried out on the specimen.[33-34] Because the Raman spectra are obtained using a microscope or lens illuminator then the characteristic molecular information is also derived with a surface spatial resolution of the order of several microns, which gives Raman spectroscopic analysis an added advantage for the examination of heterogeneous lichen encrustations on mineral substrates without chemical extraction or separation being effected from the substrate.

Although obtaining a Raman spectrum is intrinsically not difficult, the characterization of each compound in a mixture from its characteristic Raman bands is complex because of several problems, such as the weakness of the Raman signal, fluorescence background emission, band overlapping, wavenumber shifts, etc.[33,38] Furthermore, several technical parameters can affect the observed spectrum, such as laser wavelength, which can influence the onset of fluorescence or resonance effects; spectral resolution, related to changes in band width caused by molecular or ionic environmental effects and so result in band overlapping or asymmetry; laser power, since high laser irradiance at the specimen in Watts per square cm can burn the sample or result in chemical degradation or the induction of molecular or structural changes.[38-40]

Organic compounds give a multi-band Raman spectrum, from which three or four bands, usually the strongest, are used as characteristic Raman signatures; however, the relative intensity of the observed peaks can change with regard to the wavelength of the laser used for exciting the Raman effect.[33,39] This means that sometimes, what seems to be a major feature important could exhibit a significantly reduced intensity using a different laser wavelength other than that reported in the literature database; the actual interpretation of the spectral data and consequent identification of the biomolecules present becomes difficult especially in a complex biological system such as that of a lichen colony and its attendant mineral encrustation.

In this work, we make a summary of the main chemicals produced by lichens over the world detected by Raman spectroscopy with the goal of connecting lichen species, climate and lichen protective biomolecules. From our compendium, the relationship between oxalates and lichens in dry and wet environments will be examined and the production of protective pigment admixtures in different climates discussed.

There are many articles in which studies of lichen pigments have been carried out using different analytical techniques but the focus of this paper is where Raman spectroscopy has been used for the molecular characterization. The focus on Raman spectroscopy is owing to the increasing interest in using a nondestructive technique. Despite the increased adoption of this technique for lichenological systems analysis the resulting spectrum is often complex to interpret for nonspecialists. This will be the first time that a data compendium of lichen pigments and calcium oxalates related with climate parameters has been published and hence will provide a good Raman spectral database for the interpretation of lichen behaviour, especially for lichenologists using Raman spectroscopy for the first time for chemical identification in lichen systems.

MATERIAL AND METHODS

Because of the change in relative intensity of the spectral signatures observed under different operational experimental conditions, we have selected for our database (Table 1) only the most significant spectral features observed using either 1064 or 785 nm laser excitation as fluorescence or resonance Raman effects generally become more significant in the visible region of the electromagnetic spectrum, such as that found using 532 or 514 nm laser radiation. Although the wavenumber region between 1700-1000 cm^{-1} is, normally, the most representative area in a Raman spectrum for biomolecular identification, in our experience, we have seen that, for some compounds, characteristic bands are detected in the 700-200 cm^{-1} region and these too can be quite definitive for molecular or molecular ion identification.

Lichens have been grouped under six different climates (Table 1 and Table 2a, 2b, 2c, 2d; see references in table 1): Polar (cold and dry), Oceanic (temperate and wet), Mediterranean (temperate but with wet and dry seasons), Sub-desert (hot and dry), High mountain+desert (high diurnal temperature variation but very dry in the case of the analyzed specimens) and, finally, Tropical (warm and wet).

From all literature references analysed for this study, we have included in our table eighty-eight lichen specimens, comprising sixty-six different species belonging to thirty-seven different genera. Each species cited in the revised papers has been given a different number; additionally, for clarity when one particular species appears in different papers with clearly identified different pigment mixtures, we have assigned to it the same number but we have added a suffix letter (one species, several specimens). For a genus sp, we have assigned different identifying numbers each time it appears, in the absence of any further literature taxonomic clarification.

In some of the studies analyzed for this work, authors have characterized only the most common compounds, such as carotene, chlorophyll, cellulose or oxalates; this could be explained because a) occasionally, no other molecule gives Raman bands in a lichen analysis owing to particular lichen environmental protective strategies; b) because of the analytical parameters chosen for the study or c) because of the analytical spectrometer limitations. Sometimes, weak Raman spectral signatures remain unassigned, as can be seen in some figures in the original articles. When wavenumbers do not appear in the text or tables and no spectral assignments have been forthcoming it is impossible to assess what compounds have then been detected by Raman spectroscopy - in this case, sometimes they are shown as an “unknown compound”; here we have not attempted to reassign the work of others in our analysis and "unknown compounds" remain as such, although they are discussed further later in an appropriate section in this paper.

For this work, we do not distinguish spatially where a compound was found, i.e in the lichen thallus, apothecia, encrustation etc. All pigments were detected using Raman spectroscopy, although from other studies, using different and destructive analytical techniques, more pigments may have been reported, the goal of this review is to compare those bio-molecules identified non-destructively using Raman spectroscopy.

RESULTS AND DISCUSSION

Results of the lichen specimens, climates and biomolecules found by Raman spectroscopy are shown in tables 2a, 2b, 2c and 2d.

CAROTENOIDS AND CHLOROPHYLL

Although chlorophyll is ubiquitously found in lichens, it has been only detected using Raman spectroscopy, in forty two specimens (47,7%) of those compiled for this review (Tables 2a, 2b, 2c, 2d; Table 3). It has been appreciated that chlorophyll is difficult to detect in natural samples when a green or blue laser was used for Raman analyses.[33]

Contrary to the case of chlorophyll, carotenes were generally found (92%) and yet not described in only seven specimens (8%); this is perhaps surprising since the carotenoids are a group of pigments which give a strong Raman spectroscopic response, particularly when a green or blue laser was used because of the resonance Raman enhancement. An electronic absorption band for carotenoids occurs near 500 nm and when green laser excitation is used the characteristic Raman bands (centred near 1510, 1150 and 1000 cm^{-1}) are resonantly enhanced.[33,41] Carotenes fulfil a multiple role: they act as UV-radiation screen, are antioxidants and DNA repairers. Despite the presence of this multifunctional pigment, lichens also produce other protective biomolecules (Tables 3 and 4).

Although it is tempting to suggest that the assignment of specific carotenoids in lichens and other organisms is possible from their Raman spectrum, Oliveira et al. (2010)[41] have shown that the precise characterization of those compounds in natural samples is ambiguous because of the interaction of these molecules with other compounds in the cell, producing significant wavenumber shifts of the characteristic Raman bands, so the interpretation of the Raman spectrum through comparison with pure carotenoid standards must be undertaken with caution. Hence in this study, we have replaced specified "carotenoids" with the generic term in our table since in some cases authors have apparently not considered the consequence of such potential wavenumber shifts in their assignments.

CALCIUM OXALATES

Calcium oxalates are compounds which provide a strong Raman spectrum whichever laser wavelength is used for exciting the sample. However, for 35,2% of specimens no oxalate was detected (Figure 1).

In Figure 1, the percentage of oxalates related to the environmental conditions are shown. The Polar climate is characterized by cold temperatures and low relative humidity in air; what we have called High-mountain/desert shows some similarities with this Polar climate since those lichens were collected in the Atacama desert[4,33,39] with some of the lowest terrestrial humidities. It is interesting to note that, despite the extreme desiccation experienced by lichens in both areas, there are large numbers of specimens without oxalates detected.

Subdesert climates cannot be used for establishing conclusions here because only four specimens were analysed from these regions.

Tropical regions show similar proportions of specimens with and without oxalates to the Polar climate, despite the high rainfall. The temperate Oceanic climate shows a high ratio of specimens with oxalates in comparison with those showing an absence of the calcium oxalates. In Mediterranean areas, with a wet and cold season and another one hot and drier, all specimens showed that oxalate was present (Figure 1).

The role played by calcium oxalates in the lichen survival strategy is controversial: although they are clearly generated as secondary products by lichen metabolic processes, some authors have attributed to them a water storage function.[1,4,5,12,20,42-46] Furthermore, weddellite, calcium oxalate dihydrate, is metastable at temperatures in excess of 5 degrees C and thermodynamically reverts in time to the more stable whewellite, calcium oxalate monohydrate. It has been proposed by some authors that the excess of water could be used by the lichen in drought periods. However, this suggestion does not appear to be supported by the observation that lichens with weddellite detected in the Oceanic and Mediterranean climates involve 47,8% and 80% respectively (Figure 1), whereas in dry climates such as High mountains/desert (14,4%) and in Polar regions (27,8%) this is lower (Figure1). In Subdesert climates 50% of lichens show the presence of weddellite but, as we have previously pointed out, the low number of lichen specimens studied makes this conclusion non representative.

In summary, taking into account the total number of specimens; 71,7% of lichens (38/53 spcms) from Oceanic, Mediterranean and Tropical climates show the presence of either calcium oxalate monohydrate and dihydrate or both whereas only 45,7% of specimens from the more extreme desert climates (subdesert, high mountains/desert and polar, 16/35 specimens) produce the same compounds; i.e. almost three quarters of lichens living under wet climates produce oxalates, whereas less than half of the lichens living under dry climates produce those molecules; therefore, the hypothesis that oxalates could work as a water storage must be revised.

OTHER BIOMOLECULES

From the biomolecules identified by Raman spectroscopy (Table 4), the characterization of parietin as a lichen pigment is outstanding since it appears in no fewer than twenty-one specimens (18,42%). The production of this pigment is apparently unrelated with any specific climate. The next most common lichen pigments identified by Raman spectroscopy are rhizocarpic acid, which appears in 9,65% of specimens and lecanoric acid (7,89%) followed by calycin, gyrophoric acid and usnic acid (7,02% each).

Most commonly, it seems that only one pigment is identifiable if at all in any climate (Figure 2), but it is significant that the production of four or more pigments is commonly found in the oceanic climate. Again, the hypothesis that the most inhospitable environmental conditions are associated with the most complex pigment mixture synthesised by lichen systems is questionable.[47]

CONCLUSIONS

Several conclusions may be made as a result of the current study.

Biomolecules produced by lichens under different climatic conditions and in a range of environments detected using Raman spectroscopy and reported in the literature have been reviewed. As a result, from this assimilation of spectral data and a comparative analysis, it is clear that some hypotheses made on the basis of more localised studies are not supportable.

Raman spectroscopy is a suitable technique for lichen biomolecule identification without sample manipulation or pretreatment. In particular, the ability to detect carotenoids alone and in admixture without extraction is valuable. The detection of chlorophyll is rather more problematic and this can be ascribed to the laser excitation wavelengths used.

Calcium oxalate, either as a mono- or dihydrate, appears more often in specimens from wet climates, such as oceanic, tropical or Mediterranean than in specimens from drier areas, such as polar, desert and subdesert regions. From this work, we conclude that the hypothesis made in the literature that whewellite and weddellite act as water reservoirs for lichens under drought conditions is not sustainable and that more studies should be carried out to understand properly their function. it is also very interesting that only calcium oxalates are detected in the spectral data and there is no evidence for the presence any other metal oxalate or oxalic acid, even under conditions of calcium deficiency, such as on granite rock substrates: it is believed this reflects the insolubility of the calcium oxalate compared with its magnesium or alkali metal congeners by which means the oxalic acid waste product is removed more effectively from the growth area.

From our results, there is no apparent relationship between pigments or pigment mixtures produced and climate; More specific studies should be carried out to clarify what mechanism dictates the adoption of a particular survival strategy related to stressed or temperate environmental conditions.

It has also not been observed previously that the presence of a single pigment is the most common strategy regardless of mild climate. Also, whereas it could have reasonably been thought that lichens would have produced four or more pigments in the most hostile and extreme environmental conditions, in reality this mixture is produced only in rather more gentle oceanic climates; of the literature surveyed, only one subdesert specimen produced four pigments in admixture.

The most common lichen pigment is parietin, followed by rhizocarpic acid and then lecanoric acid, calycin, gyrophoric acid and usnic acid. It doesn't seem to have any relationship between any climate and a pigment type. The most common pigments appear in different climatic conditions.

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TABLES

Table 1. Climate, lichen genus and species, site locations and specimen numbers.

Table 2a: Number of lichen specimens and biochemical compounds found by using Raman spectroscopy on Polar climate.

Table 2b: Number of lichen specimens and biochemical compounds found by using Raman spectroscopy on Oceanic climate.

Table 2c: Number of lichen specimens and biochemical compounds found by using Raman spectroscopy on Mediterranean and Subdesert climates.

Table 2d: Number of lichen specimens and biochemical compounds found by using Raman spectroscopy on High Montain+Desert and Tropical climates.

Table 3: Lichen chemicals by specimens and climates. SPCMS: specimens; SPCS: species; Ca OX.: calcium oxalate (unspecified hydration); WEDDE. OX. DIHYDRATE: Weddellite (oxalate dihydrate); WHEWE. OX. MONOHYDRATE: Wewellite (oxalate monohydrate); PIGMENTS: other pigments than carotene and chlorophyll.Table 4: Number of times each pigment was found under different climates, by using Raman spectroscopy and total percentage of each pigment found in the total specimen number.

FIGURES

Figure 1. Representation by pie-chart of: a) Percentage of lichen specimens with and/or without oxalates; b) Type of oxalates found in different world climate regions. The world climate regions considered in this study are: Polar (18 specimens [spcms]), Oceanic (23 spcms), Mediterranean (10 spcms), Subdesert (4 spcms), High-mount/desert (13 spcms) and Tropical (20 spcms). Climate Map zones of the world: Original uploader was Waitak at en.wikipedia Later version(s) were uploaded by Splette at en.wikipedia. - Transferred from en.wikipedia; transfered to Commons by User:Legoktm using CommonsHelper.

https://en.wikipedia.org/wiki/Climate#/media/File:ClimateMap_World.png

Figure 2. Representation by pie-chart of pigments occurrence: number of pigments in each region (without carotenoids and chlorophyll). The world climate regions considered in this study are: Polar (18 speciments [spcms]), Oceanic (23 spcms), Mediterranean (10 spcms), Subdesert (4 spcms), High-mount/desert (13 spcms) and Tropical (20 spcms).

Climate Map zones of the world: Original uploader was Waitak at en.wikipedia Later version(s) were uploaded by Splette at en.wikipedia. - Transferred from en.wikipedia; transfered to Commons by User:Legoktm using CommonsHelper.

https://en.wikipedia.org/wiki/Climate#/media/File:ClimateMap_World.png

Table 1: Climate, lichen genus and species, site locations and specimen numbers. (SPEC: Specimen)

CLIMATE	GENUS	SPECIES	SITE	SPEC
Polar	*Acarospora*	*Acarospora chlorophana*[14,20,43,48,]	1-Football Saddle, northern Victoria Land, Antarctica; 2- Harrow Peaks, Victoria Land.	1a
Polar	*Acarospora*	*Acarospora chlorophana*[32]	Football Saddle, northern Victoria Land.	1b
Polar	*Acarospora*	*Acarospora chlorophana*[3]	Victoria Land (Antarctica)	1c
Polar	*Acarospora*	*Acarospora sp.*[49]	Signy Island in the maritime Antarctic.	2a
Polar	*Buellia*	*Buellia sp.*[50]	Mars Oasis on Alexander Island.	3
Polar	*Caloplaca*	*Caloplaca sublobulata* [13,14]	1-Signy I.; 2- Leonie Island in Marguerite Bay, off the Antarctic Peninsula.	4
Polar	*Caloplaca*	*Caloplaca saxicola* [14,48,51]	1-Crater Cirque, Victoria Land.	5a
Polar	*Candelaria*	*Candelaria sp.*[14]	Victoria Land.	6
Polar	*Candelariella*	*Candelariella sp.*[14]	Victoria Land.	7
Polar	*Lecidea*	*Lecidea sciatrapha*[14]	Signy I.	8
Polar	*Lepraria*	*Lepraria sp.* [14]	Signy I.	9
Polar	*Rhizocarpon*	*Rhizocarpon geographicum*[14]	Victoria Land	10
Polar	*Umbilicaria*	*Umbilicaria antarctica*[14]	Signy I.	11
Polar	*Usnea*	*Usnea antarctica*[14]	Signy I.	12
Polar	*Xanthoria*	*Xanthoria elegans*[52]	Harrow Peaks, Victoria Land, Antarctica.	13a
Polar	*Xanthoria*	*Xanthoria elegans* [13, 14,32]	1-Harrow Peaks, Victoria Land; 2-Leonie Island in Marguerite Bay, off the Antarctic Peninsula; 3-Mc-Murdo Valley (Victoria Land).	13b
Polar	*Xanthoria*	*Xanthoria mawsonii*[52]	Harrow Peaks, Victoria Land, Antarctica.	14a
Polar	*Xanthoria*	*Xanthoria mawsonii*[14]	Rothera, Graham Land.	14b
Oceanic	*Aspicilia*	*Aspicilia calcarea* [12]	Vizcainos (Spain).	15
Oceanic	*Caloplaca*	*Caloplaca holocarpa*[12]	Vizcainos (Spain).	16
Oceanic	*Caloplaca*	*Caloplaca decipiens*[12]	Tañabueyes (Spain).	17
Oceanic	*Caloplaca*	*Caloplaca teicholyta*[12]	Jaramillo de la Fuente (Spain).	18
Oceanic	*Caloplaca*	*Caloplaca saxicola*[12]	Villamoron (Spain).	5b
Oceanic	*Caloplaca*	*Caloplaca saxicola*[12]	Olmos de Picaza (Spain).	5c
Oceanic	*Candelariella*	*Candelariella medians*[12]	Tanabueyes (Spain).	19
Oceanic	*Chroodiscus*	*Chroodiscus megalophthalmus*[53]	New Zealand.	20
Oceanic	*Diploicia*	*Diploicia canescens*[54]	Frocester, Gloucester (UK)	21
Oceanic	*Diploschistes*	*Diploschistes scruposus*[55]	Sahagún (Spain)	22
Oceanic	*Dirina*	*Dirina massiliensis forma sorediata*[44]	Lincolnshire (UK)	23a
Oceanic	*Dirina*	*Dirina massiliensis forma sorediata*[42]	Laulivery, Cornwall and Norfolk (UK)	23b
Oceanic	*Dirina*	*Dirina massiliensis forma*	Cape Clear Island, Co.	23c

		sorediata[42]	Cork, S.W. (Ireland).	
Oceanic	*Haematomma*	*Haematomma ochroleucum var. Porphyrium*[56]	Goxhill Hall, Lincolnshire (UK).	24
Oceanic	*Lecanora*	*Lecanora muralis*[57]	Menithwood, Worcestershire (UK).	25
Oceanic	*Lecidea*	*Lecidea fuscoatra*[58]	Bastavales, La Coruña (Spain).	26
Oceanic	*Ochrolechia*	*Ochrolechia parella*[59]	San Lorenzo church (Santiago de Compostela, Spain).	27a
Oceanic	*Ochrolechia*	*Ochrolechia parella*[59]	Bridge of Merza (Pontevedra, Spain), Monastery of Toxosoutos (La Coruña, Spain), Os Anxeles church (La Coruña, Spain).	27b
Oceanic	*Porpidia*	*Porpidia cinereoatra*[58]	Bastavales, La Coruña (Spain).	28
Oceanic	*Porpidia*	*Porpidia macrocarpa*[58]	Bastavales, La Coruña (Spain).	29
Oceanic	*Xanthoria*	*Xanthoria parietina*[52]	Portpatrick, SW Scotland (UK).	30a
Oceanic	*Xanthoria*	*Xanthoria parietina*[12]	Burgos (Spain).	30b
Oceanic	*Xanthoria*	*Xanthoria parietina*[60]	S.W. Scotland and west Yorkshire (UK).	31
Mediterranean	*Acarospora*	*Acarospora oxytona*[57]	Sierra Nevada (Spain).	32a
Mediterranean	*Acarospora*	*Acarospora oxytona*[20]	Picon (Spain).	32b
Mediterranean	*Aspicilia*	*Aspicilia calcarea*[43]	Sicily and Égadi Islands (Italy).	33
Mediterranean	*Caloplaca*	*Caloplaca aurantia*[57]	Southern Spain.	34
Mediterranean	*Dirina*	*Dirina massiliensis forma sorediata*[61]	Palazzo Farnese, Caprarola (Italy).	23d
Mediterranean	*Dirina*	*Dirina massiliensis forma sorediata*[42]	Palazzo Farnese, Caprarola (Central Italy); SS. Niccolo e Cataldo near Lecce (S.E. Italy);	23e
Mediterranean	*Dirina*	*Dirina massiliensis forma sorediata*[43]	Sicily and Égadi Islands (Italy).	23f
Mediterranean	*Dirina*	*Dirina massiliensis f. massiliensis*[43]	Tremiti Islands, San Nicola (Italy).	23g
Mediterranean	*Lecanora*	*Lecanora sulfurea*[43]	Tremiti Islands, San Nicola (Italy).	35
Mediterranean	*Tephromela*	*Tephromela atra*[43]	Monte Sicily, Égadi Islands (Italy).	36
Sub-desert	*Diploschistes*	*Diploschistes diacapsis*[1,34]	Tabernas Desert (SE, Spain).	37
Desert	*Dirina*	*Dirina massiliensis forma sorediata*[42]	E. of Alexandria (Egypt).	23h
Sub-desert	*Lepraria*	*Lepraria crassissima*[1,34]	Tabernas Desert (SE, Spain).	38
Sub-desert	*Squamarina*	*Squamarina lentigera* [1,34]	Tabernas Desert (SE, Spain).	39
High mountain + desert	*Acarospora*	*Acarospora sp.*[33]	Atacama Desert (Chile)	2b

High mountain + desert	*Acarospora*	*Acarospora sp. orange-yellow*[4]	Atacama Desert (Chile)	40
High mountain + desert	*Acarospora*	*Acarospora sp. Brown*[4]	Atacama Desert (Chile)	41
High mountain + desert	*Acarospora*	*Acarospora cf. Schleichera*[39]	Atacama Desert (Chile)	42a
High mountain + desert	*Acarospora*	*Acarospora Schleichera greenish-yellow*[4]	Atacama Desert (Chile)	42b
High mountain + desert	*Aspicilia*	*Aspicilia caesiocinerea agg*[15]	High Atlas Mountains near Oukaimeden, about 48 km south of Marrakech (Morrocco).	43
High mountain + desert	*Candelariella*	*Candelariella genus orange-yellow*[4]	Atacama Desert (Chile).	44
High mountain + desert	*Lecidea*	*Lecidea Tessellata Florke*[62]	Utah, Colorado Plateau (USA).	45
High mountain + desert	*Rhizocarpa*	*Rhizocarpa sp. White*[4]	Atacama Desert (Chile)	46
High mountain + desert	*Rhizoplaca*	*Rhizoplaca sp. White*[4]	Atacama Desert (Chile)	47
High mountain + desert	*Xanthopamundia*	*Xanthopamundia sp. White*[4]	Atacama Desert (Chile)	48
High mountain + desert	*Xanthomendoza*	*Xanthomendoza mendozae* [32]	Atacama Desert (Chile)	49a
High mountain + desert	*Xanthomendoza*	*Xanthomendoza mendozae red*[4]	Atacama Desert (Chile)	49b
Tropical	*Buslicia*	*Buslicia sp.*[5]	Kilauea, Hawaii (USA).	50
Tropical	*Calenia*	*Calenia triseptata* [63]	Tortuguero National Park (Costa Rica).	51
Tropical	*Candelariela*	*Candelariela concolor*[5]	Kilauea, Hawaii (USA).	52
Tropical	*Caloplaca*	*Caloplaca crosbyae*[5]	Kilauea, Hawaii (USA).	53
Tropical	*Cladonia*	*Cladonia coniocraea*[5]	Kilauea, Hawaii (USA).	54a
Tropical	*Cladonia*	*Cladonia coniocraea*[5]	Kilauea, Hawaii (USA).	54b
Tropical	*Cryptothecia*	*Cryptothecia rubrocincta*[7]	Cantareira, Sao Paulo (Brazil); Pinheiros, Santuario de Caraja, Minas Gerais (Brazil).	55
Tropical	*Dirinaria*	*Dirinaria aegialita*[5]	Kilauea, Hawaii (USA).	56a
Tropical	*Dirinaria*	*Dirinaria aegialita*[5]	Kilauea, Hawaii (USA).	56b
Tropical	*Dirinaria*	*Dirinaria applanata*[5]	Kilauea, Hawaii (USA).	57a
Tropical	*Dirinaria*	*Dirinaria applanata*[5]	Kilauea, Hawaii (USA).	57b
Tropical	*Dirinaria*	*Dirinaria sp*[5]	Kilauea, Hawaii (USA).	58
Tropical	*Echinoplaca*	*Echinoplaca strigulacea*[63]	Monteverde Biological Reserve, (Costa Rica).	59
Tropical	*Hyperphysia*	*Hyperphysia adglutinata*[5]	Kilauea, Hawaii (USA).	60
Tropical	*Parmotrema*	*Parmotrema sp.*[5]	Kilauea, Hawaii (USA).	61

Tropical	*Parmotrema*	*Parmotrema tinctorum Del. Ex Nyl.*[64]	Campus of the Federal University of Juiz de Fora, in Juiz de Fora city, Minas Gerais State (Brazil).	62
Tropical	*Ramalina*	*Ramalina umbilicata*[5]	Kilauea, Hawaii.	63
Tropical	*Tricharia*	*Tricharia carnea*[63]	Near La Selva Biological Station (Costa Rica).	64
Tropical	*Xanthoparmelia*	*Xanthoparmelia scabrosa*[65]	Hong Kong (China).	65
Tropical	*Xanthoparmendia*	*Xanthoparmendia sp*[5].	Kilauea, Hawaii (USA).	66

Table 2a Number of lichen specimens and biochemical compounds found by using Raman spectroscopy on Polar climate.

	POLAR																	
MIXTURE	1a	1b	1c	2a	3	4	5a	6	7	8	9	10	11	12	13a	13b	14a	14b
Weddellite	X			X	X					X		X						
Whewellite	X		X	X	X			X			X	X						
Calcium oxalate															X		X	
Carotene	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chlorophyll																		
Cellulose															X		X	
Atranorin										X	X							
Calycin								X	X	X								
Fumarprotocetraric acid											X			X				
Gyrophoric acid										X			X					
Parietin						X	X	X							X	X	X	X
Pulvinic acid			X															
Pulvinic dilactone								X	X									
Rhizocarpic acid	X	X	X	X								X						
Usnic acid														X				
C2 (Glycosidic linkage)															X		X	
C5 (Metabolic product)				X														

Table 2b Number of lichen specimens and biochemical compounds found by using Raman spectroscopy on Oceanic climate.

	OCEANIC																						
MIXTURE	15	16	17	18	5b	5c	19	20	21	22	23a	23b	23c	24	25	26	27a	27b	28	29	30a	30b	31
Weddellite	X		X		X		X	X	X		X	X					X		X			X	
Whewellite				X		X		X		X	X			X	X			X	X				X
Calcium oxalate																					X		
Carotene	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chlorophyll				X	X	X				X						X	X	X	X	X			
Cellulose									X			X	X	X			X	X	X		X		X
Anthraquinone																							**X**
Aspicilin	X																						
Atranorin				X						X													
Calycin							X																
Diploschistesic acid										X													
Emodin			X	X	X	X																	
Erythrin	X											X	X										
Fragilin	X																						
Gyrophoric acid				X												X							
Lecanoric acid										X		X	X										
Parietin		X	X		X	X				X											X	X	X
Parietinic acid		X	X		X	X																	
Pulvinic acid anhydride							X																
Rhizocarpic acid							X																
Stictaurin							X																
Stictic acid								X															
Teloschistin			X		X	X																	
C1 (Polyphenolic acid)									X														
C2 (Glycosidic linkage)																					X		
C3 (Unknown compound)																							**X**
C5 (Metabolic product)								X						X									X

Table 2c: Number of lichen specimens and biochemical compounds found by using Raman spectroscopy on Mediterranean and Subdesert climates.

	MEDITERRANEAN										**SUB-DESERT**			
MIXTURE	32a	32b	33	34	23d	23e	23f	23g	35	36	37	23h	38	39
Weddellite	X	X		X	X	X	X	X		X		X	X	
Whewellite	X	X	X	X	X		X	X	X	X	X		X	X
Calcium oxalate														
Carotene	X	X	X		X	X					X	X	X	X
Chlorophyll											X		X	X
Cellulose					X	X						X		
Anthraquinone				**X**										
Calycin													X	
Emodin											X			
Erythrin					X	X						X		
Fumarprotocetraric acid													X	
Lecanoric acid					X	X					X	X		X
Parietin				X										X
Rhizocarpic acid	X	X									X			
Usnic acid											X			X
C3 (Unknown compound)	**X**													
C5 (Metabolic product)			X	X			X	X	X	X				

Table 2d: Number of lichen specimens and biochemical compounds found by using Raman spectroscopy on High Montain+Desert and Tropical climates.

	HIGH MOUNTAIN + DESERT													TROPICAL																				
MIXTURE	2b	40	41	42a	42b	43	44	45	46	47	48	49a	49b	50	51	52	53	54a	54b	55	56a	56b	57a	57b	58	59	60	61	62	63	64	65	66
Weddellite			X					X												X				X	X								
Whewellite				X		X		X						X	X					X		X				X				X	X		X
Calcium oxalate																																	
Carotene	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chlorophyll	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Cellulose																																X	
Atranorin																			X				X		X								
Calycin	X	X					X																										
Chiodectonic acid																				X													
Confluentic acid																				X													
Gyrophoric acid																					X	X	X	X									
Lecanoric acid																													X				
Parietin				X								X	X				X																
Pulvinic dilactone	X	X					X									X																	
Rhizocarpic acid				X	X																												
Usnic acid										X	X							X										X					X
C1 (Polyphenolic acid)						X		X																								X	
C3(Unknown compound)					X																												
C4 (Unknown compound)												X																					

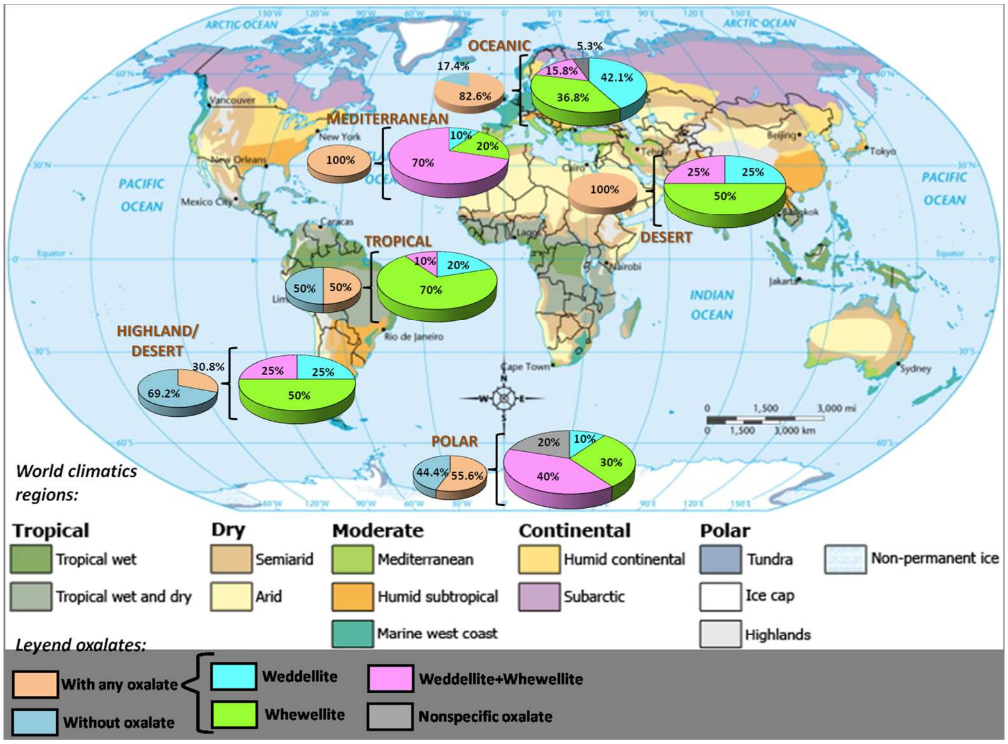
TABLE 3: Lichen chemicals by specimens and climates. SPCMS: specimens; SPCS: species; Ca OX.: calcium oxalate (unspecified hydration); WEDDE. OX. DIHYDRATE: Weddellite (oxalate dihydrate); WHEWE. OX. MONOHYDRATE: Wewellite (oxalate monohydrate); PIGMENTS: other pigments than carotene and chlorophyll.

Climate	Speci men	Cellulose	Wedde. Ox. dihydrate	Whewe. Ox. monohydrate	Ca Ox.	Carotene and/or chlorophyll	Pigments
Polar 18 spcms 14 spcs	1a		X	X		1	1
	1b					1	1
	1c			X		1	2
	2a		X	X		1	1
	3		X	X		1	0
	4					1	1
	5a					1	1
	6			X		1	3
	7					1	2
	8		X			1	3
	9			X		1	2
	10		X	X		1	1
	11					1	1
	12					1	2
	13a	X			X	1	1
	13b					1	1
	14a	X			X	1	1
	14b					1	1
Oceanic 23 spcms 18 spcs	15		X			1	3
	16					1	2
	17		X			1	4
	18			X		2	3
	5b		X			2	4
	5c			X		2	4
	19		X			1	4
	20		X	X		0	1
	21	X	X			1	0
	22			X		2	4
	23a		X	X		1	0
	23b	X	X			1	2
	23c	X				1	2
	24	X		X		1	0
	25			X		1	0
	26					2	1
	27a	X	X			2	0
	27b	X		X		2	0
	28	X	X	X		2	0
	29					2	0
	30a	X			X	1	1

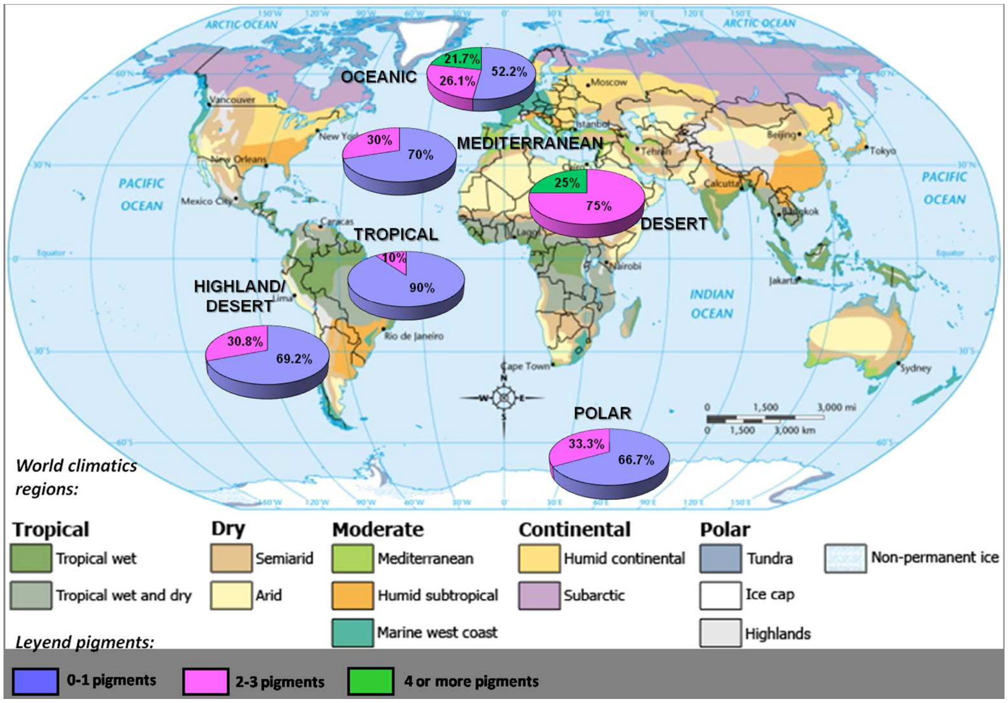
	30b		X			1	1
	31	X		X		1	2
Mediterranean 10 spcms 6 spcs	32a		X	X		1	1
	32b		X	X		1	1
	33			X		1	0
	34		X	X		0	2
	23d	X	X	X		1	2
	23e	X	X			1	2
	23f		X	X		0	0
	23g		X	X		0	0
	35			X		0	0
	36		X	X		0	0
Subdesert 4 spcms 4 spcs	37			X		2	4
	23h	X	X			1	2
	38		X	X		2	2
	39			X		2	3
High mount+desert 13 spcms 11 spcs	2b					2	2
	40					2	2
	41		X			2	0
	42a			X		1	2
	42b					2	1
	43			X		2	0
	44					2	2
	45		X	X		2	0
	46					2	0
	47					2	1
	48					2	1
	49a					1	1
	49b					2	1
Tropical 20 spcms 17 spcs	50			X		2	0
	51			X		2	0
	52					2	1
	53					2	1
	54a					2	1
	54b					2	1
	55		X	X		2	2
	56a					2	1
	56b			X		2	1
	57a					2	2
	57b		X			2	1
	58		X			2	1
	59			X		2	0
	60					2	0
	61					2	1
	62					1	1
	63			X		2	0
	64			X		2	0
	65					2	0
	66			X		2	1

TABLE 4: : Number of times each pigment was found under different climates, by using Raman spectroscopy and total percentage of each pigment found in the total specimen number.

Compound	Polar	Oceanic	Mediter.	Subdesert	High mount +desert	Tropical	Total	% total pigments
Anthraquinone		1	1				**2**	1.75
Aspicilin		1					**1**	0.88
Atranorin	2	2				3	**7**	6.14
Calycin	3	1		1	3		**8**	7.02
Chiodectonic acid						1	**1**	0.88
Confluentic acid						1	**1**	0.88
Diploschistesic acid		1					**1**	0.88
Emodin		5		1			**6**	5.26
Erythrin		3	2	1			**6**	5.26
Fragilin		2					**2**	1.75
Fumarprotocetraric acid	2	1		1			**4**	3.51
Gyrophoric acid	2	2				4	**8**	7.02
Lecanoric acid		3	2	3		1	**9**	7.89
Parietin	**7**	8	1	1	3	1	**21**	18.42
Parietinic acid		4					**4**	3.51
Pulvinic acid	1						**1**	0.88
Pulvinic acid anhydride		1					**1**	0.88
Pulvinic dilactone	2				3	1	**6**	5.26
Rhizocarpic acid	5	1	2	1	2		**11**	9.65
Stictaurin		1					**1**	0.88
Stictic acid		1					**1**	0.88
Teloschistin		4					**4**	3.51
Usnic acid	1			2	2	3	**8**	7.02
TOTAL PIGMENTS	**25**	**42**	**8**	**11**	**13**	**15**	**114**	**100.01**



Representation by pie-chart of: a) Percentage of lichen specimens with and/or without oxalates; b) Type of oxalates found in different world climate regions. The world climate regions considered in this study are: Polar (18 specimens [spcms]), Oceanic (23 spcms), Mediterranean (10 spcms), Subdesert (4 spcms), High-mount/desert (13 spcms) and Tropical (20 spcms). Climate Map zones of the world: Original uploader was Waitak at en.wikipedia Later version(s) were uploaded by Splette at en.wikipedia. - Transferred from en.wikipedia; transfered to Commons by User:Legoktm using CommonsHelper.



Representation by pie-chart of pigments occurrence: number of pigments in each region (without carotenoids and chlorophyll). The world climate regions considered in this study are: Polar (18 speciments [spcms]), Oceanic (23 spcms), Mediterranean (10 spcms), Subdesert (4 spcms), High-mount/desert (13 spcms) and Tropical (20 spcms).

Climate Map zones of the world: Original uploader was Waitak at en.wikipedia Later version(s) were uploaded by Splette at en.wikipedia. - Transferred from en.wikipedia; transfered to Commons by User:Legoktm using CommonsHelper.