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NIR spectroscopy as a tool for discriminating between lichens exposed to air pollution

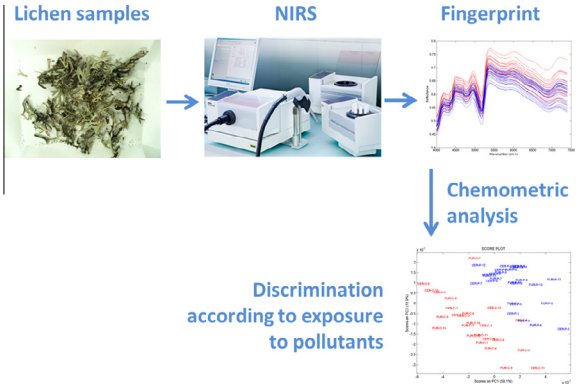
Monica Casale [a], Lucia Bagnasco [a], Paolo Giordani [a,*], Mauro Giorgio Mariotti [b], Paola Malaspina [b]

[a] University of Genoa, Department of Pharmacy, Via Brigata Salerno, 13, I-16147 Genoa, Italy
[b] University of Genoa, Department of Earth Sciences, Environment and Life (DISTAV), Corso Europa, 26, I-16126 Genoa, Italy

HIGHLIGHTS

- Lichens as biomonitors of air pollution.
- NIR spectroscopy for analysing lichen samples.
- PCA as a multivariate display method to visualise the NIR data.
- LDA to discriminate between lichens according to their exposure to pollutants.

GRAPHICAL ABSTRACT



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ABSTRACT

Lichens are used as biomonitors of air pollution because they are extremely sensitive to the presence of substances that alter atmospheric composition. Fifty-one thalli of two different varieties of *Pseudevernia furfuracea* (var. *furfuracea* and var. *ceratea*) were collected far from local sources of air pollution. Twenty-six of these thalli were then exposed to the air for one month in the industrial port of Genoa, which has high levels of environmental pollution.

The possibility of using Near-infrared spectroscopy (NIRS) for generating a 'fingerprint' of lichens was investigated. Chemometric methods were successfully applied to discriminate between samples from polluted and non-polluted areas. In particular, Principal Component Analysis (PCA) was applied as a multivariate display method on the NIR spectra to visualise the data structure. This showed that the difference between samples of different varieties was not significant in comparison to the difference between samples exposed to different levels of environmental pollution.

Then Linear Discriminant Analysis (LDA) was carried out to discriminate between lichens based on their exposure to pollutants. The distinction between control samples (not exposed) and samples exposed to the air in the industrial port of Genoa was evaluated. On average, 95.2% of samples were correctly classified, 93.0% of total internal prediction (5 cross-validation groups) and 100.0% of external prediction (on the test set) was achieved.

* Corresponding author.
E-mail address: giordani@difar.unige.it (P. Giordani).

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1. Introduction

Lichens are symbiotic associations between a fungus (usually an ascomycete, the mycobiont) and a population of unicellular algae (the autotrophic photobiont) (Honegger, 1991). Unlike vascular plants that mainly take up nutrients from the soil through their roots, lichens take up water, solutes and gases over the entire thallus (Nash, 2006), and thus depend entirely on the atmosphere for nutrition. For this reason, lichens are extremely sensitive to the presence of substances that alter the composition of the atmosphere, such as phytotoxic gases (e.g. SO_2 or NO_x), heavy metals or organic compounds. These features make them excellent biomonitors of air pollution (Nimis et al., 2002). Atmospheric pollutants may affect lichens at different levels of biological organization, by determining e.g., alterations in community diversity and composition (Giordani, 2007), accumulation of trace elements within the lichen thalli (Bargagli and Mikhailova, 2002) or causing other eco-physiological effects, such as changes to their metabolism (Jensen and Kricke, 2002; Mikhailova, 2002), e.g. decreasing their photosynthetic efficiency (Malaspina et al., 2014a) or causing the synthesis of biomarkers of oxidative stress (Cuny et al., 2002). From an applied perspective, in many urban areas where in situ lichens do not occur, eco-physiological effects are typically evaluated on transplanted lichen material collected from pristine sites and transferred to polluted target areas (Godinho et al., 2008, 2004; Malaspina et al., 2014b,c).

Other techniques are often used for assessing the physiological effects of pollution on lichens. The analysis of chlorophyll a (Chl a) fluorescence emitted by the autotrophic photobiont allows changes in photosynthetic apparatus and in its working efficiency under different environmental conditions to be investigated (Calatayud et al., 1996; Maxwell and Johnson, 2000; Piccotto et al., 2011). Chlorophyll degradation can also be assessed with a spectrophotometric test by measuring a decreasing amount of chlorophyll a and b, estimated by means of OD435/415 ratio (Boonpragob, 2002).

This paper represents a preliminary study into the use of Near-infrared spectroscopy (NIRS) for generating a 'fingerprint' of lichens capable of discriminating between samples from polluted and non-polluted areas.

Near-infrared spectroscopy offers a number of important advantages over traditional chemical methods, because it is a rapid and non-destructive method and it requires minimal or no sample preparation. Moreover, NIRS is less expensive because no reagents are required and thus no waste is produced. Finally, the versatility of NIRS instruments makes them useful tools for control analyses. However, in order to take advantage of the positive features of NIRS, the analyst must overcome limitations in sensitivity and selectivity that arise from the relatively weak and highly overlapping spectral bands found in the NIR region. Thus, a key step in the implementation of successful NIRS analysis is the use of chemometrics (Casale et al., 2012; Oliveri et al., 2013; Bagnasco et al., 2014).

Thanks to recent technical developments and advances in chemometrics, the NIR technique has been widely adopted as a powerful means of routine control analyses in many industrial sectors (Blanco and Villarroya, 2002).

The development of portable NIR equipment has enabled on-site measurements to determine, for example, the degree to which soil is contaminated with heavy metals, oils or fuels (Kooistra et al., 2001; Stallard et al., 1996; Malley et al., 1999; Chakraborty et al., 2010). NIR spectroscopy was tested also as a fast method for characterizing the toxic effects of air pollution on trees (Gäb et al., 2006). Also, as a further application in the field of plant science, NIR spectroscopy has been used to monitor the quality of fruit post-harvest (Cavaco et al., 2009). In biomonitoring studies, spectral reflectance response in Vis–NIR regions has been used to examine potential alterations of physiological parameters in lichen thalli exposed to air pollutants (Garty et al., 1997). However, spectral changes were not processed using a chemometrical approach.

In recent years, a lot of progress has been made in the spectroscopy field. In particular, remote sensing and hyperspectral imaging showed themselves to be the perfect candidate for an automated procedure in biomonitoring natural vegetation and air pollution (Agresti et al., 2013; Serranti et al., 2013a,b). This study evaluates for the first time the speed and reliability of NIR spectroscopy and chemometrics in supporting traditional methods used in the discrimination between lichen samples according to their exposure to pollutants. Transplanted thalli of two varieties (var. *furfuracea* and var. *ceratea*) of the fruticose lichen *Pseudevernia furfuracea*, which is frequently selected in biomonitoring surveys were used (Malaspina et al., 2014a) and the distinction between control samples (collected far from local sources of air pollution) and samples exposed to the air in the industrial port of Genoa was evaluated.

2. Materials and methods

2.1. Lichen species

P. furfuracea (L.) Zopf v. *furfuracea* is a fruticose, meso-xerophytic, epiphytic lichen widespread throughout upland areas of Europe, in the mountain and subalpine belts, including the Mediterranean mountains (Nimis, 1993). The photosynthetic partner is represented by *Trebouxia* algae. The species grows on bark at sites with diffuse light, but is scarce at sites with very high direct solar irradiation (Nimis and Martellos, 2008). *P. furfuracea* had been frequently employed in biomonitoring studies also in coastal cities (Tretiach et al., 2007; Sorbo et al., 2008; Malaspina et al., 2014a,b). *P. furfuracea* has two chemical varieties, which are distinguished on the basis of a set of secondary metabolites, with special reference to some polyphenolic compounds (depsides and depsidones): var. *ceratea* contains olivetoric acid in the medulla, whereas var. *furfuracea* has physodic acid (Culberson, 1965). Moreover, both varieties produce other secondary metabolites, such as chloroatranorin, atranorin and oxiphysodic acid, this latter being a major compound in var. *furfuracea*.

2.2. Samples exposure

Fifty-one thalli of both varieties of *P. furfuracea* were collected from northerly exposed barks of *Picea abies* (L.) H. Karst in a forest area of Valtournenche (Valle d'Aosta, Italy) at 1900 m a.s.l., far from local sources of air pollution. Samples were taken to the laboratory and subjected to a pre-conditioning period of two days in wet chambers with low light (5 $\mu mol\ m^{-2}\ s^{-1}$), to stabilise a rise in the vitality caused by recovery from natural photoinhibition (Gauslaa and Solhaug, 2004). Afterwards, 25 thalli (13 of *furfuracea* and 12 of *ceratea* variety) were kept in the freezer until the end of the experiments and used as control samples, while 26 thalli were exposed to the air for one month (from October, 1st to October, 29, 2013) in a highly polluted area in the industrial port in the city of Genoa (NW Italy) (Malaspina et al., 2014c).

The exposed thalli were fixed to a plastic screen and attached to a support covered with a shade cloth (UV stabilized HDPE agro shade net, shade factors 40%), in order to minimize the possible photoinhibition of the thalli due to high solar radiation (Malaspina et al., 2014a). The transplanted thalli were retrieved at the end of the exposure period and transported back to the laboratory in paper bags for the analysis.