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Combining spectroscopic techniques and chemometrics for the interpretation of lichen biomonitoring of air pollution

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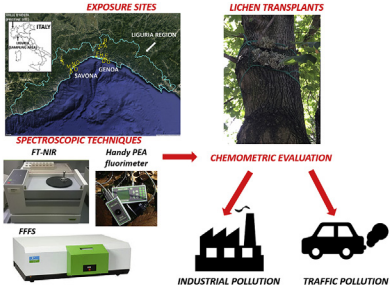
HIGHLIGHTS

- Lichens, used as biomonitors of air pollution, were analyzed by spectroscopic techniques.
- Analytical data were elaborated by means of chemometrics tools.
- Results obtained with spectroscopic techniques were compared with the values of atmospheric pollutants.
- NIRS was able to differentiate between samples exposed in a polluted or not polluted area.
- FFFS was able to highlight different type of pollution, industrial vs. traffic.

GRAPHICAL ABSTRACT



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ABSTRACT

A screening evaluation of lichen thalli, based on spectroscopic techniques coupled with chemometrics, is proposed as fast, simple and "green" method for the biomonitoring of air pollution. For two consecutive years, lichen thalli of *Pseudevernia furfuracea* were exposed for three months in selected sites of Liguria (NW-Italy) according to different levels and types of air pollution. At the end of the exposure period, transplanted thalli were analyzed by a set of monitoring techniques, including Front-Face Fluorescence Spectroscopy (FFFS), Near Infrared Spectroscopy (NIRS) and Plant Efficiency Analyser (PEA). Data were compared with values of air pollutants recorded during the exposure period by the Regional Agency for Environmental Protection, in order to relate lichen physiological indicators with the effects of atmospheric concentrations.

A chemometric evaluation of the analytical signals, including principal component analysis (PCA) and quadratic discriminant analysis (QDA), was performed; the mean prediction rate of the discriminant models calculated on the FFFS emission spectra ranged from 70 to 75% on the external test sets. Front-face fluorescence spectroscopy proved to be a promising technique for the determination of level and type of pollutants in lichen thalli.

1. Introduction

Lichens are symbiotic associations between a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont, which can be either a green alga or a cyanobacterium (Nash

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III, 2006). Lacking organs for active water uptake, structures for regulating gas exchanges and permeability barrier for water, lichens are susceptible to absorb water, nutritive substances and gases directly from the atmosphere. Thus, they are extremely sensitive to the presence of substances that alter the atmospheric composition (e.g. SO_2 and NO_x) and are among the most widely used biomonitors of air pollution (Bargagli and Mikhailova, 2002). For biomonitoring studies, lichens may be used as bioaccumulators, to estimate the accumulation of trace elements within the lichen thalli over space and time (Bargagli and Mikhailova, 2002), or as bioindicators, to assess any alteration of the community diversity and composition (Giordani, 2007) and to estimate changes of physiological biomarkers in response to atmospheric pollutants (Jensen and Kricke, 2002; Mikhailova, 2002). From a physiological perspective, it has been widely demonstrated that the exposure of lichens to many gaseous pollutants (i.e. SO_2 and NO_2) may causes membrane injury, ultrastructural alterations, pigment degradation and/or impairment of photosynthetic function (Deltoro et al., 1999; Malaspina et al., 2015, 2018). Conventionally, these biomarkers may be evaluated by means of spectrophotometric or fluorimetric techniques. Recently, the assessment of the efficiency of the photosynthetic process in the algal population is one of the most common biomarkers used (Calatayud et al., 1996; Maxwell and Johnson, 2000; Malaspina et al., 2018). The use of direct light fluorimeter (Plant Efficiency Analyser, PEA) allows obtaining information on the efficiency of the photosynthetic processes on the tylacoid membranes of the algal chloroplasts, from the connectivity between PSII reaction centres to the electron flow to PSI. Particularly, PEA records the maximum quantum yield of primary photochemistry of the photobiont (measured by the ratio F_V/F_M) and other fluorescence parameters, which can be consider as highly sensitive and reliable tools for studying changes in photosynthetic apparatus and in its working efficiency caused by the negative effects of atmospheric pollution. Differently, when consider lichen as a bioaccumulator, we can obtain information on their trace elements content, thus on the atmospheric contaminants.

The main conventional analytical techniques used to determine element concentration consist of atomic absorption spectrophotometry techniques such as ICP-AES and ICP-MS (Bargagli and Nimis, 2002). Although these techniques are accurate and reliable in giving a quantitative result, they require long laboratory procedures and they are not able to establish unambiguously a relation between any change in the lichen physical and chemical properties and the individuals pollutants in the atmosphere (Nimis et al., 2002).

In this paper, we tested an alternative approach, which combining information from different analytical sources, could potentially provide a comprehensive evaluation of the complex chemical phenomena that occur in complex matrices. For this reason, spectroscopic techniques (e.g. visible (VIS), near infrared (NIR) and mid infrared (MIR) spectroscopy) were considered in order to integrate the assessment of atmospheric pollution by means of lichens. Spectroscopic analysis exploits the interaction of electromagnetic radiation with atoms and molecules to provide qualitative and quantitative chemical and physical (structural) information that is contained within the wavelength or frequency spectrum of energy that is either absorbed or emitted. Spectroscopy in the visible, near and mid-infrared ranges is an increasingly growing technique due to its cost, rapidity, simplicity, and safety, as well as its ability to measure multiple attributes simultaneously without monotonous sample preparation, making it suitable to be implemented on a routine basis. Near infrared spectroscopy (NIRS), Front-Face Fluorescence Spectroscopy (FFFS) and Plant Efficiency Analyser (PEA) are not expensive and 'green' because no reagents are required and thus no waste is produced.

By using the application of mathematical and statistical techniques, chemometrics allows to extract chemical and physical information from complex multidimensional data (Wold and Sjöström, 1972), which are currently observed in spectroscopy techniques. Chemometrics often relies on visualization to help the chemist to obtain the required information, and the most used method in this respect is principal component analysis (PCA). PCA extracts information from data tables by transforming them into plots (Massart and Vander Heyden, 2004).

In our previous work (Casale et al., 2015), we showed that NIR spectroscopy coupled with chemometrics was able to generate a lichen 'fingerprint' capable of discriminating between samples exposed in a polluted or non-polluted area. Differently, FFFS is usually applied on food samples for classification purposes (Ruoff et al., 2006; Sádecká et al., 2009), whereas, according to our knowledge, this technique was not investigated for lichen biomonitoring.

The present study aimed at testing the use of different analytical spectroscopic approaches, coupled with chemometrics, as rapid and simple tools for assessing effects of air pollutants on lichen thalli. For achieving this goal, thalli of the fruticose lichen *Pseudevernia furfuracea* (L.) Zopf v. *furfuracea*, collected from a pristine area, have been transplanted for three months to 15 sites in the Liguria region (NW-Italy), characterized by contrasting levels and type of atmospheric pollution, as measured by the regional Environmental Protection Agency (ARPAL). Lichen samples have been analyzed by FFFS, NIRS and PEA and data elaborated by multivariate data analysis (chemometrics), in order to compare the performances of these spectroscopic techniques and to highlight possible synergic or complementary information.

2. Materials and methods

2.1. Lichen sampling and sample preparation

The fruticose epiphytic lichen *Pseudevernia furfuracea* (L.) Zopf v. *furfuracea* was selected because it is widely used in biomonitoring studies with transplants (Blasco et al., 2011; Kodnik et al., 2015; Nascimbene et al., 2014; Sorbo et al., 2008; Tretiach et al., 2007a, 2011).

Lichen thalli 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 (Malaspina et al., 2014). Collecting lichens from the north side of tree allows work with material adapted to homogeneous regime of diffuse light (Tretiach et al., 2007b). Samples were picked up, at 1.5–2.0 m above the ground, together with a piece of the supporting branch, using garden shears. The material was taken to the laboratory in paper bags and left to dry out at room temperature and low light overnight ($\approx 5\ \mu mol\ m^{-2}\ sec^{-1}$), to minimize a rise in the F_V/F_M caused by recovery from natural photoinhibition (Gauslaa and Solhaug, 2004). Samples were divided into two groups: one, including samples that were never exposed in the experimental sites, were kept in freezer until the end of the experiments (control), whereas the second group included one hundred and fifty thalli which were randomly selected and prepared to be exposed in the 15 exposure sites. In the laboratory, lichen thalli were fixed by means of plastic bands on plastic nets (of ca. 25 × 15 cm) and put into paper bags.

2.2. Study area and sampling sites

Fifteen sites (A - Q) distributed in an area of ca. 200 Km^2 in Liguria region (NW Italy) (Fig. S1) were selected for exposure.