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A biological method to monitor early effects of the air pollution caused by the industrial exploitation of geothermal energy

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Biomonitoring early effects of geothermal air pollution.

Abstract

The suitability of a set of ecophysiological parameters, to be used as early warning indicator to detect signs of a worsening environment around geothermal power plants, was tested by comparison with the diversity of epiphytic lichens, a well-established indicator of geothermal air pollution. Samples of the lichen *Evernia prunastri* were transplanted around a geothermal power plant at Larderello (Tuscany, Italy) and at a control site, and integrity of cell membranes, concentration of chlorophyll *a*, *b* and carotenoids, chlorophyll integrity and variations in pH of thalli were measured. The results showed that cell membrane damage, expressed by changes in electrical conductivity, could be used to detect early (exposure periods as short as 1 month) deleterious effects of geothermal air pollution.

Keywords: Biomonitoring; Ecophysiology; Italy; Lichens; Non-fossil resources

1. Introduction

Italy was the first country in the world to exploit geothermal energy for the production of electricity and the industrial town of Larderello (Tuscany) has been powered by geothermal energy since 1908. The region of Tuscany hosts all the existing Italian geothermal power plants, which produce altogether 800 MW, accounting for 26% of the regional electricity requirement (Cappetti and Ceppatelli, 2005).

Geothermal energy is regarded as an environmentally friendly source when compared with fossil fuels (Rybach, 2005); nevertheless it is well-known that its industrial exploitation for power generation may affect the surrounding environment. Geothermal airborne emissions from power plants consist mainly of H_2O (95%), non-condensable gases e.g. CO_2 (4.5%), H_2S, CH_4, NH_3, H_2 (Barbier, 1997) and trace elements such as Hg, As, B and Rn, which are potentially toxic for human health (Layton et al., 1981) and plants (Bussotti et al., 2003).

Lichen biomonitoring proved to be a reliable tool for assessing the biological effects of geothermal air pollution (Loppi, 2000) and lichens have been used often as bioindicators of air quality in geothermal areas (Loppi, 1996; Loppi and Nascimbene, 1998; Loppi et al., 2002a,b, 2006). However, although the diversity of epiphytic lichens responds fairly well to air pollutants emitted by geothermal power plants, this indicator requires a time lag for tracking changes in air quality, which must be sufficient to allow for modification in species number and composition. In other words, changes can be measured only after damage has occurred at community or at least at species level. On the other hand, monitoring changes at the ecophysiological level can allow detecting early stress symptoms.

The aim of this study was to check the suitability of a set of ecophysiological parameters measured in transplanted samples of the lichen *Evernia prunastri* to be used as early warning indicator to detect signs of a changing environment around geothermal power plants.

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2. Experimental

The effects of geothermal power plant emissions have been investigated by the diversity of epiphytic lichens, a well-established indicator, and ecophysiological parameters of transplanted *E. prunastri* thalli, namely integrity of cell membranes, concentration of chlorophyll *a*, *b* and carotenoids, chlorophyll integrity and variations in pH of the thalli. *E. prunastri* was selected because of its known sensitivity to air pollution, the availability of thalli in unpolluted areas of Tuscany and the ease of preparation for analysis.

2.1. Study area

The study was carried out at two monitoring sites in the geothermal area of Larderello: around the Valle Secolo power plant and at a control site, located close to the Cornate-Fosini Nature Reserve, far from any local source of air pollution. The Valle Secolo power plant consists of two 60 MW units and is the largest geothermal plant in Italy. Fluxes of the main airborne pollutants emitted are 255 kg/h H_2S, 11.3 g/h Hg and 2.6 g/h As. Non-condensable gases are discharged at high temperature (around 200 °C), however, as the power plant chiefly uses direct-contact condensers, part of the H_2S is naturally emitted from 16 cooling towers as a result of cooling water stripping with air, while only part is dispersed with most of the non-condensable gases from the stacks (50 m high). In 2007, two AMIS plants (a patented H_2S abatement technology for geothermal application) went in operation at Valle Secolo, with a considerable reduction, up to 80%, of H_2S emissions (Sabatelli, in litt.). Geothermal power plants are the only important source of impact on natural ecosystems in the area (Bussotti et al., 2003).

2.2. Sampling design

For each monitoring site, a sampling area of 500 × 500 m was selected. This size was chosen since 500 m is the distance generally accepted where effects of geothermal emission from power plants can be detected (Loppi, 1996; Loppi and Bargagli, 1996). The sampling area was centered around the emission point in correspondence of the power plant (43°14′12″N, 10°52′38″E) and randomly selected at the control site (43°11′23″N, 10°55′00″E). To allow for geographical variation, each sampling area was divided into four sampling quadrants of 250 × 250 m.

2.3. Sampling tactic

The four suitable deciduous *Quercus* trees closest to the center of the four sampling quadrants were examined. Trees were deemed suitable if well lit, with girth >70 cm, and with trunk near straight, not damaged, and without parts with >25% cover of bryophytes.

2.4. Lichen sampling

During February 2004, for each tree an index of lichen diversity (ILD) was calculated as the sum of frequencies of epiphytic lichens in a sampling grid consisting of four 50 × 10 cm ladders, each divided into five 10 × 10 cm quadrates. This grid was positioned systematically on the N, E, S and W cardinal sides of the bole of each tree, at a height of 1 m above ground. The ILD of the tree was the sum of frequencies of epiphytic lichens of each ladder; the ILD of the monitoring site was the arithmetic mean of the ILD measured for each sampled tree. In case of identification problems during field sampling, specimens were collected and identified later in the laboratory. The ILD values were interpreted in terms of air pollution effects according to the following scale (Loppi et al., 2006): 0 = very high (lichen desert), 1–40 = high, 41–80 = moderate, 81–120 = low, >120 = negligible.

2.5. Lichen transplants

Prunus spinosa twigs (ca. 15–30 cm in length) carrying *E. prunastri* thalli were pruned at the beginning of February 2004 from an unpolluted area of Tuscany. About 100 twigs were transplanted to the monitoring sites after a 4-day acclimation period in a climatic chamber at a temperature of 15 ± 2 °C, relative humidity of 55 ± 5% and photoperiod of 12 h with a light flux of ca. 40 μmol $m^{-2} s^{-1}$ PAR photons. Twigs were suspended using nylon strings at 2–2.5 m above ground on the same trees investigated for ILD measurements and retrieved after 1, 4 and 10 months of exposure, i.e. in March, June and November 2004.

2.5.1. Pigment content and chlorophyll degradation

Samples of 20–25 mg each were used for the analysis of pigment content and chlorophyll integrity, selecting only the outermost 2 cm of the lobes. Samples were first rinsed six times for 5 min each in $CaCO_3$-buffered acetone to remove lichen substances and subsequently pigments were extracted in PVPi-buffered DMSO (Barnes et al., 1992). The absorbances at 750, 665, 649, 480, 435 and 415 nm were determined spectrophotometrically (Agilent 8453E UV–visible). Concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) were calculated according to Wellburn (1994) and expressed on a thallus dry mass basis. Chlorophyll degradation was expressed by the $OD_{435/415}$ ratio (Ronen and Galun, 1984).

2.5.2. Assessment of damage to cell membranes and pH of the thalli

A simple test to check the integrity of the plasma membrane enclosing lichen cells is to place a piece of lichen thallus in deionized water and measure the variation in electric conductivity since this value is proportional to the degree of damage endured by cell membranes and hence to solute concentration (Marques et al., 2005). Thalli were first placed in a humid chamber (RH > 90%) for 24 h to stabilize electrolyte leakage (Buck and Brown, 1979), then about 100 mg of lichen material was rinsed three times for 5 s in deionized water to remove particles deposited onto the lichen surface (Garty et al., 1993). Afterward, thalli were soaked for 1 h in 50 mL of deionized water. The integrity of cell membranes was estimated by the change in the electric conductivity of the solution, measured with a conductivity-meter (Crison Basic 30), and expressed in μS cm^{-1} mL mg^{-1}.

To measure the pH of the thalli, 500 mg of dried *E. prunastri* material were powdered in liquid nitrogen, shaken for 1 h in 50 mL of deionized water and centrifuged for 5 min at 4000 rpm. Measurements were performed on the clear fluid fraction using a pH-meter (HI 8424, Hanna Instruments).

2.6. Statistical analysis

The significance of differences was tested by the Kolmogorov–Smirnov two-sample non-parametric test.

3. Results

3.1. Diversity of epiphytic lichens

Thirty-nine epiphytic lichen species were recorded during the study (Table 1). *Physcia adscendens*, *Physcia biziana*, *Xanthoria parietina*, *Candelariella xanthostigma*, *Lecanora chlarotera* and *Lecidella elaeochroma* were the only species common to both monitoring sites. Mean as well as total species richness was higher at the control site; the lowest ILD values were found in the surroundings of the Valle Secolo power plant.

3.2. Lichen transplants

Values of electrical conductivity, a measure of cell membrane damage, and pH of thalli differentiated Valle Secolo from the control site (Table 2). At Valle Secolo electrical conductivity reached a peak in June, probably because of the cumulative effects of pollution and drought, recurring to March