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Original Articles

One year of transplant: Is it enough for lichens to reflect the new atmospheric conditions?

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ABSTRACT

How long does it take a lichen to respond to changes (worsening or improvement) of atmospheric conditions is still discussed. We selected and removed lichen thalli (*Flavoparmelia caperata*) from sites subject to different intensities of pollution around a landfill in Central Italy and exposed them in a remote unpolluted area for 12 months. The content of elements of toxicological concern (As, Cd, Cr, Cu, Pb, Zn) and several physiological parameters in lichen thalli (chlorophyll *a* fluorescence emission, chlorophyll content and integrity, membrane lipid peroxidation, content of secondary metabolites and ergosterol content) were investigated before and after the recovery and hence compared with those of native (and clean) samples of the remote area. In an opposite trial, heavy metals content was investigated in samples taken from the remote area and exposed around the landfill. Values of the transplants were then compared with those of native samples at the landfill.

From chemical point of view, the content of heavy metals decreased (by ca. 25%) in lichen thalli taken from the landfill and exposed in the remote area, however background values were never reached. On the other hand, lichen thalli taken from the remote area and exposed around the landfill accumulated up to ca. 80% of the content of *in situ* samples. The rate of accumulation was higher than the rate of element loss referred to the same temporal interval.

The recovery of physiological parameters, especially those typical of the mycobiont or of the whole lichen symbiosis, was much faster than heavy metal detoxification, and after 12 months transplanted lichens already reflected the new environmental conditions at the remote site.

1. Introduction

It is widely accepted that biomonitoring, i.e. the use of living organisms for monitoring of air pollution, may help for the implementation of environmental policy on air quality and atmospheric pollution control (Pirintsos and Loppi, 2008). Among biomonitors, lichens and mosses are of primary importance as indicators of air quality (Aničić Urošević et al., 2017). Since lichen metabolism depends on the mineral uptake from the atmosphere, these organisms are effective in trapping trace elements from the surrounding environment, well reflecting the environmental levels of heavy metals (Bari et al., 2001; Sloof, 1995). In a recent review, Loppi and Paoli (2017) pointed out the usefulness of lichen biomonitoring as a tool for the implementation of environmental friendly waste management policies. Previous lichen based studies reported on the biological impact of air pollution determined by different waste management strategies, such as waste incineration (Loppi et al., 1995, 2000; Paoli et al., 2015b; Protano et al., 2015; Tretiach et al., 2011), landfilling (Nannoni et al., 2015; Paoli et al., 2012, 2015a), industrial composting (Paoli et al., 2014), and the number of applications around point sources is steadily increasing. Environmental biomonitoring should be regularly included in the process of impact assessment of waste management strategies, evaluating the ecological impacts of specific activities and the effectiveness of environmental recovery, in support of regulatory procedures and providing consistent data for environmental management (Loppi and Paoli, 2017). However, so far the use of bioindicators has been only occasionally introduced into environmental monitoring around landfill sites (Kotovicová et al., 2011; Paoli et al., 2012; Protano et al., 2014).

How long does it take a lichen to respond to changes (worsening or improvement) of atmospheric conditions is still debated. The uptake and release of trace elements are reversible processes influenced by thallus morphology, age, physiological status, pH, duration of exposure,

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microclimatic conditions and obviously, also presence, concentration and type of pollutants in the environment. Uptake mechanisms mainly involve particulate trapping, extracellular ion exchange and intracellular accumulation (see e.g. Bargagli, 1998). It is known that lichens tend to an equilibrium with the surrounding environment and reply faster under a worsening of environmental conditions (e.g., increase of heavy metal depositions) with respect to their improvement (e.g. removal of a pollution emitting source). In fact, they may accumulate heavy metals within weeks or few months following an increase of pollution in the environment (Bargagli, 1998) and show a reduction within a year or two (up to five) after stopping the emissions from an industrial source (Nieboer and Richardson, 1981). Furthermore, chemical and physiological parameters may reflect the change at different rates.

In this study, we simulated the closure of a solid waste landfill in Central Italy by removing lichen thalli (*Flavoparmelia caperata* (L.) Hale) from sampling sites subject to different intensities of pollution and exposing them in a remote unpolluted area for 12 months. Ecophysiological parameters and the variation of the chemical content of the thalli before and after the recovery were analysed. On the contrary, clean samples taken from the remote area were exposed around the landfill and heavy metals were analysed. The study aimed to reply the following questions: 1) to which extent the content of heavy metals in lichen samples decreased after the exclusion of the pollution source and oppositely, to which extent the content of heavy metals in samples from the remote area increased after the exposure around the source? 2) do lichen thalli are able to recover a physiological healthy status? 3) which would be in the long-term the condition of the samples when they remain exposed around the source?

2. Material and methods

2.1. Study area

The investigated landfill (43°52′52″ N, 10°53′21″ E, ca. 60 m a.s.l.) is located in Tuscany (Central Italy). A detailed description of the area is presented in Paoli et al. (2012). The authorized wastes may include scraps of paper, plastics and metals, packing, spent tires, textile products, building materials, ashes from municipal solid waste incinerators, polluted terrain from environment reclamation, etc.

The landfill site is located over an impermeable natural clay layer, surrounded to the N, W and S by a vegetation belt dominated by *Quercus cerris* and *Q. pubescens*. The neighbouring area is hilly, characterized by vineyards, olive plantations and woodlands, while the eastern side (lowland), is characterized by inhabited areas and plant nurseries.

Cultivated parcels, once closed, are covered by a waste layer (terrain) to stabilize the surface, drainage systems, compact clay, soil bentonite and a vegetative soil layer (up to 100 cm, according to the slope). A grassy mantle and/or reafforestation with local vegetation complete the recovery.

2.2. Experimental design

The closure of the facility was simulated removing lichens from impacted sites and exposing them to clean sites. Doing this, it was assumed that no residual emissions affected the samples. However, residual contamination may still occur in the surrounding environment after the closure of a polluting source (e.g., Rusu et al., 2006) and toxicological effects may still occur due to the previously accumulated contaminants. In order to allow the recovery of the samples, based on previous studies (Paoli et al., 2012, 2015a), we selected the sites with the highest depositions: three of them directly facing the landfill (highly impacted – group 1) and three others located at about 200 m from the landfill (moderately impacted – group 2). Sites within group 2 correspond to the outer margin of the vegetation belt surrounding the landfill, which roughly ranges up to 200 m. The sampling sites are represented by circular plots (60 m diameter).

In each sampling site, 15 thalli of the foliose lichen *Flavoparmelia caperata* were collected from the bark of 3–5 holm oak trees (above 1 m from the ground), so that about 45 thalli were available within each group (May 2013). The thalli were selected randomly irrespectively of their morphological condition, therefore also visually altered thalli (with signs of discoloration and necrosis) have been included. Element bioaccumulation and the physiological status of the samples were assessed in a fraction of this material, randomly selected before the recovery.

The recovery site (43°10′37″ N, 11°22′14″ E) was selected in a remote area far from pollution sources. The high quality of this environment is witnessed by the presence of a nearby oak forest widely colonized by a large population of *Lobaria pulmonaria*, a sensitive macrolichen, considered as an indicator of humid environments with high air quality. In fact, this remote area has been employed as background site for several monitoring studies (e.g., Paoli et al., 2016) and *F. caperata* is widely diffused there.

During 12 months of the transplant, mean maximum and minimum temperature were respectively 20–8 °C in the remote area and 21–11 °C at the landfill, total rainfall was about 1100 mm in the remote area and 1500 mm at the landfill. The average number of 'rainy days' ($>$ 1 mm) was 101 in the remote area and 119 at the landfill. Data are obtained from the closest operating meteorological stations (Hydrological Meteorological Monitoring Centre of the Region Tuscany, http://www.sir.toscana.it).

Samples have been exposed in the remote area for a whole year (May 2013–May 2014), distributed into three homogeneous sub-groups and bound with strings to the branches of three holm oaks (the recovery substrates, at about 2 m from the ground and ensuring the same conditions of exposure). Each thallus was marked and numbered. The selected trees are characterized by the presence of roughly horizontal branches, to which our thalli have been easily bound. In a parallel trial, unpolluted ('clean') samples of *F. caperata* were collected from the remote area and exposed around the landfill, allowing a comparison of the rate of accumulation in 'clean' samples with that of disaccumulation in 'polluted' samples. Field measurements of solar radiation, occasionally carried out at the experimental sites with a LI-1400 datalogger (LI-COR) – between 12:00 and 2 pm during sunny days – showed that samples in the remote area received more light than *in situ* samples at the landfill (950–1500 and 600–1300 μmol s^{-1} m^{-2}, respectively). The following procedures have been applied to all samples.

2.3. Trace elements content

In the laboratory, samples were carefully cleaned under a stereoscopic microscope to remove extraneous material deposited on the surface, such as mosses, bark pieces and soil particles. The peripheral part of the thalli (roughly up to 5 mm from lobe tips) was selected for the analysis; this choice is foreseen by the protocols generally applied in the field of passive biomonitoring with foliose lichens. In the case of *F. caperata,* this part can be easily separated from the bark, being distinguishable by a paler colour and absence of rhizinae. Samples were pulverized and homogenized with a ceramic mortar and pestle. About 200 mg of powdered lichen material were mineralized with a mixture of 6 mL of 70% HNO_3, 0.2 mL of 60 % HF and 1 mL of 30 % H_2O_2 in a microwave digestion system (Milestone Ethos 900) at 280 °C and 55 bar. The concentrations of selected elements of toxicological concern (As, Cd, Cr, Pb, V, Zn) and Fe (being associated to soil contamination of the samples) were determined by ICP-MS (Perkin Elmer – Sciex, Elan 6100) and expressed on a dry weight basis (μg/g dw). Analytical quality was checked by the Standard Reference Material IAEA-336 'lichen'. Precision of analysis was estimated by the coefficient of variation of 4 replicates and was within 10% for all elements. Three replicates were measured at each site. The concentrations of trace elements in lichen