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,
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°10′37″ N, 11°22′14″ 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 reforestation 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% HNO₃, 0.2 mL of 60 % HF and 1 mL of 30 % H₂O₂ 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
Five samples were measured per each site. The results were expressed adapted for ten minutes before the measurements. Samples were then filter sterilized and then centrifuged at 10,000 rcf for 5 min. Ergosterol content was measured by HPLC (Perkin Elmer series 200) using a Phenomenex C18 column (250 × 4.6 mm) as a separator and methanol as a mobile phase. Quantification was performed using a Diode Array Detector (DAD, Perkin Elmer series 200) reading the absorbance at 280 nm. Three samples were measured per each site.

2.4.5. Secondary metabolites

Cleaned lichen samples (15–25 mg dw) were extracted in 1 mL cool acetone. Acetone extracts were collected, evaporated and the residues were dissolved in fresh 1.5 mL of acetone. Filtered acetone extracts were also analysed by gradient HPLC under the following conditions: column Tessek SGX C18, flow rate: 0.7 mL min⁻¹, mobile phase: A = H₂O: acetonitrile: H₃PO₄ (80:19:1) and B = 95% acetonitrile. Gradient program: 0 min 25% B, 5 min 50% B, 20 min 100% B, 25 min 25% B. The detection wavelength was 245 nm (detector Ecom LCD 2084). Usnic acid (Aldrich) was used as standard, while the standard of caperatic acid was prepared from crystallized acetone extracts from F. caperata (purity 100%). Three samples were measured per each site.

2.5. Long-term bioindication of environmental quality around the landfill

In addition to elemental analysis, the diversity of epiphytic lichens was used as long-term indicator of the overall effects of landfill emissions in the surrounding environment. As reported in Paoli et al. (2012), Lichen Diversity Values (LDVs) were measured using a sampling grid consisting of four 50 × 10 cm² ladders, each divided into five 10 × 10 cm² units and placed vertically on the N, E, S and W cardinal sides of the bole of each tree, with the base at 1 m above ground. The LDV of the tree corresponds to the sum of frequencies of epiphytic lichens in the grid. The average LDV of sites in group 1 (sites facing the landfill) and group 2 (up to 200 m from the landfill) is the arithmetic mean of the trees sampled within the group. Up to 15 trees were included within each group of sites.

2.6. Data interpretation and statistical analysis

Significance of differences (P < 0.05) was checked by non-parametric statistics: Wilcoxon’s signed rank test was used to compare each trace element and ecophysiological parameter between dependent samples before and after the recovery; Mann-Whitney U test was used to detect differences between sites (group 1 and 2) and the remote area selected for the recovery and between lichen transplants and in situ samples at the landfill. A standardized ratio (on a scale 0–1) was used to indicate the similarity between native and transplanted samples was calculated for each element based on the ratio between the least and the highest concentration. Metal concentrations were interpreted in terms of air pollution (deviation from natural backgrounds) based on a scale desummed from Bargagli and Nimis (2002). LDVs as long-term indicators of environmental quality around the landfill were interpreted in terms of air pollution according to the following scale: 0 = very high (lichen desert), 1–40 = high, 41–80 = moderate, 81–120 = low, > 120 = negligible.

3. Results

3.1. Heavy metals in F. caperata

The content of heavy metals in F. caperata samples harvested around the landfill and exposed in the remote area for 12 months decreased by ca. 25% and showed a level of similarity of 0.52 ± 0.11 with native samples in the remote area (steady state). However, the steady state was not reached. Clean samples taken from the remote area and exposed around the landfill showed a relevant accumulation of heavy metals; transplanted thalli reached about 80% of the concentration of...
Table 1

Standardized ratio (on a scale 0–1) between: A) the level of trace elements in transplants of the lichen *Flavoparmelia caperata* (from the remote area) exposed around the land fill and the corresponding in situ (native) samples at the landfill; B) the level of trace elements in samples taken from the landfill and exposed in the remote area and the corresponding in situ (native) samples in the remote area. Ratios approaching the value 1 indicate a high level of similarity between native and transplanted samples. Average (n = 18) ± SD (95% confidence interval).

<table>
<thead>
<tr>
<th>Transplants</th>
<th>As</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: to impacted sites</td>
<td>0.75±0.08</td>
<td>0.86±0.11</td>
<td>0.79±0.13</td>
<td>0.86±0.08</td>
<td>0.81±0.09</td>
<td>0.80±0.12</td>
<td>0.86±0.11</td>
</tr>
<tr>
<td>B: to clean sites</td>
<td>0.66±0.25</td>
<td>0.47±0.21</td>
<td>0.35±0.19</td>
<td>0.56±0.21</td>
<td>0.63±0.25</td>
<td>0.45±0.25</td>
<td>0.55±0.10</td>
</tr>
</tbody>
</table>

Table 2

Content of trace elements (average ± SD, µg/g) in the lichen *Flavoparmelia caperata* collected at selected sites around the landfill (May 2013) and after 12 months of recovery (May 2014) in the remote area. Values of native samples in the remote area are also shown. Interpretation of heavy metals is given in terms of air pollution (Bargagli and Nimis, 2002). All concentrations (including those after the recovery) are significantly different respect to those of native samples in the remote area (clean site for recovery). Values followed by different small letters indicate significant variations between samples before vs after the recovery. Values followed by different capital letters indicate significant differences within each period, due to the site (group 1 vs group 2).

<table>
<thead>
<tr>
<th>gr. 1 – sites facing the landfill</th>
<th>gr. 2 – 200 m from the landfill</th>
<th>Remote area for recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After recovery</td>
<td>Before</td>
</tr>
<tr>
<td>As</td>
<td>low</td>
<td>0.37 ± 0.12B</td>
</tr>
<tr>
<td>Cd</td>
<td>low</td>
<td>0.41 ± 0.14B</td>
</tr>
<tr>
<td>Cr</td>
<td>moderate</td>
<td>3.3 ± 0.78</td>
</tr>
<tr>
<td>Cu</td>
<td>high</td>
<td>9.5 ± 1.48</td>
</tr>
<tr>
<td>Fe</td>
<td>low</td>
<td>535 ± 1178</td>
</tr>
<tr>
<td>Pb</td>
<td>moderate</td>
<td>6.8 ± 1.18</td>
</tr>
<tr>
<td>Zn</td>
<td>low</td>
<td>51.3 ± 6.28</td>
</tr>
</tbody>
</table>

Table 3

Content of trace elements (average ± SD, µg/g) in the lichen *Flavoparmelia caperata* exposed around the land fill and in *in situ* samples at the landfill (May 2014). Interpretation of heavy metals is given in terms of air pollution (Bargagli and Nimis, 2002). All concentrations are significantly different respect to those of native samples in the remote area (see Table 2). Within each group, values followed by different small letters indicate significant differences between transplants and *in situ* samples.

<table>
<thead>
<tr>
<th>gr. 1 – sites facing the landfill</th>
<th>gr. 2 – 200 m from the landfill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplants</td>
<td>Transplants</td>
</tr>
<tr>
<td>in situ samples</td>
<td>in situ samples</td>
</tr>
<tr>
<td>As</td>
<td>0.40 ± 0.07</td>
</tr>
<tr>
<td>Cd</td>
<td>0.62 ± 0.11</td>
</tr>
<tr>
<td>Cr</td>
<td>11.2 ± 3.5 moderate</td>
</tr>
<tr>
<td>Cu</td>
<td>20.1 ± 4.2 moderate</td>
</tr>
<tr>
<td>Fe</td>
<td>864 ± 98 moderate</td>
</tr>
<tr>
<td>Pb</td>
<td>21.8 ± 7.3 moderate</td>
</tr>
<tr>
<td>Zn</td>
<td>79.9 ± 12.9 moderate</td>
</tr>
</tbody>
</table>

3.2. Ecophysiological parameters in *F. caperata* before and after the recovery

Ecophysiological parameters have been summarized in Fig. 1. The following signals of alteration were detected in the samples directly facing the landfill (group 1) before the recovery: peroxidation of membrane lipids (TBARS), reduction of the ergosterol content, lower chlorophyll integrity (OD435/415). These symptoms were partially observed up to 200 m from the landfill (group 2). On the whole, caperatic...
Fig. 1. Ecophysiological parameters in the lichen *Flavoparmelia caperata* collected around the landfill (May 2013, Paoli et al., 2015a) and after 12 months of recovery (May 2014) in the remote area. Average ± SD. For a comparison, also values of native samples in the remote area are shown. Total chlorophylls (a+b, µg/mg), chlorophyll degradation (OD_{435/415}), potential quantum yield of PSII (Fv/Fm), TBARS – thiobarbituric acid reactive substances (µmol/g dw), ergosterol content (mg/g dw), caperatic and usnic acid (% dw). * indicates significant differences respect to the native samples in the remote area. Values followed by different small letters indicate significant variations between the samples before and after the recovery. Values followed by different capital letters indicate significant differences within each period, due to the site (group 1 – landfill vs group 2 – 200 m from the landfill).
acid decreased and usnic acid increased approaching the landfill. Lower values of F/Fm were occasionally recorded in the thalli facing the landfill. However, the parameter was not affected on average basis. Observations carried out on F. caperata confirmed signs of discoloration and necrosis especially in those samples collected in front of the cultivated parcels, as previously described (Paoli et al., 2015a).

From physiological point of view, the recovery appeared much faster respect to the reduction of heavy metals in the thalli, so that the values of the investigated ecophysiological parameters after the recovery approximated those of native samples in the remote area. In fact, at the end of the recovery there were no significant differences between samples from group 1 and 2.

In addition, those parameters related to the mycobiont were much more responsive than those of the lichen photobiont. In details, TBARS, a decomposition product of polyunsaturated fatty acids produced during the peroxidation of membrane lipids (Mittler, 2002), were significantly higher before the recovery reflecting a condition of stress. After the recovery, TBARS decreased down to the level of native samples. Ergosterol content, an indicator of mycobiont viability, clearly increased comparing samples before and after the recovery (both in group 1 and 2). Final concentrations were equal or even higher than those of native samples. The overall content of secondary metabolites roughly doubled after the recovery, reaching that of native samples.

Concerning the parameters related to the photobiont, chlorophyll integrity as reflected by OD_{435/415} and chlorophyll a fluorescence emission did not point significant variations after the recovery. On the other hand, the level of total chlorophylls in thalli from the remote area was lower than the samples before the recovery. Even after the recovery, despite total chlorophylls clearly tended to decrease, they barely reached the level of native samples.

3.3. Long-term indicators of environmental quality at the experimental sites

This section replies to the question “which would be the state of the samples if they remain exposed around the landfill?”. As general overview, lower lichen diversity values (LDVs) correspond to those sites characterized by the highest heavy metal depositions. At the end of the experiment of environmental recovery (2014), LDVs indicated moderate air pollution in front of the landfill (52 ± 10) and moderate with tendency to low air pollution at 200 m (76 ± 24). A further evolution (2016) pointed out a stable condition in sites in front of the landfill (LDV = 53 ± 11) and at 200 m (LDV = 70 ± 15), the tendency for a partial alteration of LDVs in one site of group 2.

Between 2014 and 2016, the closure of a cultivated parcel in the SE side of the landfill was completed (coverage with a grassy mantel) and a significant reduction of heavy metal depositions (in particular Cr, Cu, Pb, Zn) in native lichens was observed in the closest sampling site. However, the cultivated parcels were shifting to another sector of the landfill (SW direction). Therefore, in sites of group 1 a condition of moderate (Cd, Cu, Fe, Pb, Zn), up to high (Cr) air pollution recorded in 2014 (Table 3), evolved in 2016 into low pollution for Cd (0.83 µg/g), moderate pollution for Fe (1082 µg/g) and Pb (34.6 µg/g), high for Cu (27.3 µg/g) and very high for Zn (167 µg/g). In spite of their average decreases, Cr levels in native lichens remained high (11.8 µg/g).

4. Discussion

4.1. Ecophysiological parameters

Dumping activities are the cause of worse environmental conditions in close proximity to the landfill (Paoli et al., 2015a). The results of the present study confirmed the following signals of stress detected approaching the landfill: concerning the lichen mycobiont, membrane lipid peroxidation, lower ergosterol content, altered production of secondary metabolites, while concerning the lichen photobiont, partial chlorophyll degradation and partial bleaching and necrosis of the thalli.

Also a long term indicator of environmental conditions, such as the diversity of epiphytic lichens (LDVs), confirms a partial alteration approaching the facility. Noteworthy, we estimated that about 25% of the samples in group 1 and 12% of samples in group 2, died and disappeared during the reference period (due to heavy necrosis or total bleaching of the thalli). Hence, all measurements refer to the status of the remaining material.

The recovery of physiological parameters in samples exposed to the remote area appeared much faster respect to the decrease of heavy metals, indicating that from this point of view, transplanted samples well reflected the improvement of environmental quality induced by the transplant. Those parameters related to the mycobiont were much more responsive. The content of TBARS generally increases following and exposure of lichens to high concentrations of toxic elements (Bačkor et al., 2009, 2010). Ergosterol content correlates with the amount of metabolically active fungal cells (Ekblad et al., 1998) and is sensitive to the exposure to heavy metals, which can reduce the integrity of cell membranes of the mycobiont. Therefore, when environmental conditions improve, a reduction of TBARS production and an increase of ergosterol content should be expected in previously stressed samples, as in our case. However, the removal of a polluting source does not automatically prevent physiological damages in previously exposed samples, since elements accumulated at intracellular level may still produce toxic effects and residual intracellular uptake can still occur from the extracellular fractions. Chlorophyll degradation by OD_{435/415} is generally well correlated with the accumulation of heavy metals in lichen thalli (e.g., Garty et al., 2000). The parameter did not pinpoint the improvement of environmental conditions gained by the transplant in the remote area. Samples from the landfill (both group 1 and 2) underwent a remarkable decrease of total chlorophylls when exposed in the remote area, however, after 12 months, concentrations were still higher than those of native samples. Nutrient availability and light regime (lichens in the remote area were likely subject to higher irradiance) could partially explain the decrease of chlorophyll content.

4.2. Heavy metals

For a complete recovery (steady state), the concentrations of trace elements in transplanted thalli should be equal to those of the samples in the remote (clean) area. By means of the transplant to the remote area we induced a significant reduction of Cd, Cr, Cu, Pb and Zn concentrations in lichen thalli; nevertheless, background values of native samples were never reached.

Lichens would be expected to show a reduction in elemental content within a year or two after a decrease in emissions from an industrial source (Nieboer and Richardson, 1981). However, the effective decrease depends on the location of the elements inside the thalli. Besides particulate entrapment of atmospheric depositions on their thallus surface, lichens uptake elements by means of ionic intracellular and extracellular processes. Beside passive accumulation, there is also a biological regulation of internal concentrations: elements can be mobilized in the thallus suggesting an easy interchange with the environment (Godinho et al., 2009). It is thus clear, that any real detoxification (reaching equilibrium with the surrounding environment, not just the mere loss of simply trapped particles) can be detected using younger, metabolically active and less particle-contaminated, material. Comparing central and peripheral parts of F. caperata, Loppi et al. (1997) measured similar concentrations for seven elements. Elements of limited metabolic significance (Al, Cd, Pb) had higher concentrations in the central parts, suggesting that they are trapped in the medulla. Elements essential for lichen metabolism (Co, Cu, Mo, Zn) had higher concentrations in the metabolically active peripheral parts, suggesting that they are displaced from one part of the thallus to another (Loppi et al., 1997). It has been shown that elements with intracellular location have longer residence time (Nieboer et al., 1979), while elements linked to extracellular binding sites can be more easily displaced during
wetting and drying cycles and hence have a shorter turnover time (Richardson and Nieboer, 1980). We do not have enough data to forecast the time necessary for the polluted lichens to reach a full equilibrium with their new unpolluted environment, however, we may suppose a period of 24–30 months, that is consistent with the biological mean residence times of heavy metals of 1–2.5 years for the new growing parts of lichen thalli reported by Nieboer and Richardson (1981), and also matches the residence times of 200–600 days indicated by Reis et al. (1999) and of 1.2–2.4 years reported by Walther et al. (1990). The role of time in elements uptake and release has been modelled by Reis et al. (1999), who introduced the concept of “re-membrance time”, that is the time a lichen transplant has “memory” of its element concentration. In other words, element concentrations in lichens tend to reach the equilibrium with atmospheric concentrations, but the rate at which this equilibrium is reached is far from being constant and probably depends on the concentrations of elements in the atmosphere and the physiological status of the lichens (Godinho et al., 2008, 2011). Kularatne and de Freitas (2013) assessed the ability of the lichen Parmotrema reticulatum transplanted to sites with different inten-sities of pollution to reflect Cu, Cr, Pb and Zn concentrations of native samples in the same sites, intended as the point of equilibrium. Transplanted lichens achieved the levels of all four heavy metals of native samples within four to five seasons (12–15 months) in industrial contexts, within three to four seasons (9–12 months) in commercial areas and within two to three seasons (6–9 months) in residential areas (Kularatne and de Freitas, 2013).

Godinho et al. (2011) transplanted lichens from clean to polluted sites for 6 months and moved part of them again to clean sites for 3 months. By investigating elemental content, they highlighted the rele-vance of fast and reversible processes during uptake and/or release mechanisms of lichens, rather than non-reversible ones (Godinho et al., 2011). Furthermore, weather conditions and the hydration of the thallus may influence uptake and/or release mechanisms (Godinho et al., 2008; Kularatne and de Freitas, 2013).

Dumping activities are generally a source of particulate matter and landfill gases containing a mixture of contaminants. Particulate material can be easily and reversibly trapped and released. Therefore, airborne particulate matter from landfilling operations may be highly enriched in heavy metals depending on the nature of the materials, the waste/terrain initially used for coverage and hence the resuspension from the surface of the site (e.g., Vega et al., 2001; Koshy et al., 2009; Chalvatzaki et al., 2010). Hence, for lichens growing around landfill sites, a relevant amount of deposition can be represented by particulate matter. Protano et al. (2014) exposed the fruticose lichen Pseudevernia furfuracea around the largest municipal solid waste landfill in Europe (Malagrotta, Rome, Italy) and detected a significant bioaccumulation of As, Cd, Cr, Cu, Ni, Pb and Zn after 4 months, with values of Cu and Pb which further increased after 8 months and a large contribution due to traffic. In fact, in case of multiple air pollution sources, such as industrial sites, agricultural areas, residential districts and road traffic, lichens uptake of particulate matter around landfills and waste incinerators may integrate the accumulation from each source (Protano et al., 2014, 2015).

In our study, samples transplanted to the remote area have probably lost a relevant percentage of particulate matter deposited on the thallus surface and/or dispersed between intercellular spaces or associated to extracellular binding sites in the cell wall (especially cations). It is noteworthy in this sense that the main loss occurred to samples in group 1 (sites facing the landfill), likely exposed to heavier particulate depo-sitions. The remaining fraction likely consisted in cations entered and accumulated within the mycobiont and the photobiont cells through energy-dependent and plasma membrane controlled systems (Bargagli, 1998). The intracellular fraction is likely difficult to be removed and may represent the residual content of the thalli after 12 months in the remote area. However, these statements should be verified, e.g. by sequential elutions experiments. Furthermore, during lichen growth in an unpolluted environment, trace elements at intracellular level could have been distributed between the new cells and hence their concentra-tions reduced.

Despite the recovery was simulated in an unpolluted environment, residual emissions and/or the persistence of contaminants around the closed source should be also accounted. For example, Rusu et al. (2006) investigated the patterns of heavy metals in the lichen Hypogymnia physodes exposed around a large mine waste dump after the closure of a mineral processing plant and a copper smelter. They found out increased concentrations of Cu, Fe, Pb and Zn as likely residual cont-aamination from soils, waste dump and tree banks on which the lichens were exposed, in spite of the closure of the ore-processing plant and smelter prior to the transplant.

Noteworthy, when we exposed F. caperata from the remote area to the landfill, the data pointed out a relevant accumulation of heavy metals, so that transplanted thalli gained about 80% the level of heavy metals respect to native lichens. Consequently, it is reasonable to hypothesize that a longer period (24 months) should be safe to reach, by means of transplanted thalli, the concentrations of native lichens. This would be particularly helpful in long-term monitoring programmes (i.e., repeated surveys carried out in the same sampling points): we would avoid exploiting native populations (which can be scarce in polluted sites) and would extend the advantages of the transplants to biomonitoring studies with native samples (e.g., selected material with known pre-exposure concentrations, known duration of the exposure, application of specific sampling designs, higher amount of material available). In addition, data interpretation could benefit of natural/alteration scales eventually available for native lichens.

5. Conclusions

When transferred to a remote area, lichen thalli collected around a landfill showed improved chemical and ecophysiological parameters. From chemical point of view, 12 months of recovery of the contami-nated samples were not enough to reflect the chemical condition of native samples in the remote area selected for recovery, despite some elemental concentrations significantly decreased. Native samples in the remote area (which is considered the status of a lichen in equilibrium with its surrounding environment) pointed out a condition of very low air pollution. Transplanted samples at the end of the reference period still pointed out a condition of low up to moderate pollution (Cr being an exception – high pollution). Clean samples taken from the remote area and exposed around the landfill accumulated up to 80% of the content of trace elements in native samples at the landfill.

Twelve months in the remote area allowed lichen thalli recovering a physiological healthy status. The levels of secondary metabolites and ergosterol, as well as peroxidation of membrane lipids and chlorophyll a fluorescence emission, approximated those of native samples in the remote area. In the long-term, native samples used as bioaccumulators around the landfill pointed out an overall condition of moderate air pollution for some elements (high for Cr, according to the parcel cul-tivated); the biodiversity of epiphytic lichens (LDVs) reflected moderate air pollution, limited to those sites directly facing the landfill.

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