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Corresponding Author: Dr. Francesco Nannoni, Ph.D.

Corresponding Author's Institution: University of Siena

First Author: Francesco Nannoni, Ph.D.

Order of Authors: Francesco Nannoni, Ph.D.; Riccardo Santolini; Giuseppe Protano

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Heavy element accumulation in *Evernia prunastri* lichen transplants around a municipal solid waste landfill in central Italy

Francesco Nannoni[a,*], Riccardo Santolini[b], Giuseppe Protano[a]

[a] Department of Physical, Earth and Environment Sciences, University of Siena, Via del Laterino 8, I-53100, Siena, Italy

[b] Department of Earth, Life and Environmental Sciences, University of Urbino "Carlo Bo", Via Cà le Suore 2/4, I-61029, Urbino, Italy

[*]Corresponding author:
Tel: +39 0577 233950; fax: +39 0577 233945
E-mail address: nannoni@unisi.it (F. Nannoni)
Department of Physical, Earth and Environment Sciences, University of Siena
Via del Laterino 8, I-53100, Siena, Italy

ABSTRACT

This paper presents the results of a biomonitoring study to evaluate the environmental impact of airborne emissions from a municipal solid waste landfill in central Italy. Concentrations of 11 heavy elements, as well as photosynthetic efficiency and cell membrane integrity were measured in *Evernia prunastri* lichens transplanted for 4 months in 17 monitoring sites around the waste landfill. Heavy element contents were also determined in surface soils. Analytical data indicated that emissions from the landfill affected Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn concentrations in lichens transplanted within the landfill and along the fallout direction. In these sites moderate to severe accumulation of these heavy elements in lichens was coupled with an increase in cell membrane damage and decrease in photosynthetic efficiency. Nevertheless, results indicated that landfill emissions had no relevant impact on lichens, as heavy element accumulation and weak stress symptoms were detected only in lichen transplants from sites close to solid waste. The appropriate management of this landfill poses a low risk of environmental contamination by heavy elements.

Keywords: Lichens; Solid waste landfill; Heavy elements; Biomonitoring; Soils.

1. Introduction

Landfilling is currently the main method of solid waste disposal worldwide, as it is the simplest, cheapest and most cost-effective practice to store municipal solid waste (Giusti, 2009). However, the disposal of municipal solid waste in landfills causes concern about possible adverse effects on the environment and human health, such as fires and explosions, unpleasant odours, damage to vegetation, as well as air, soil and groundwater contamination (Chrysikou et al., 2008; El-Fadel et al., 1997; Vrijheid, 2000). These adverse effects are mainly related to the release of inorganic and organic contaminants from the waste landfills via leachate and production of gases and particulate matter (Bogner and Matthews, 2003; Chalvatzaki et al., 2010; Koshy et al., 2009). Much attention has therefore been given to landfill emissions affecting air quality, and the EU has recently prescribed strict regulations on waste disposal in landfills (European Union, Directive 2008/98/EC).

Gas emissions from municipal solid waste landfills are a complex mixture of contaminants such as carbon dioxide, methane, hydrogen sulphide, nitrous compounds and hazardous volatile organic compounds (i.e., polycyclic aromatic hydrocarbons). The characteristics of landfill particulate matter reflect the nature and chemical composition of the disposed waste. Several contaminants such as toxic heavy elements (i.e., Pb, Cr, Cd and Zn) are associated with the airborne particles released from the landfill (Koshy et al., 2009). Municipal solid waste landfill is thus a reservoir and source of several inorganic and organic contaminants in the surface environment.

In the landfill, particulate matter is mainly generated by re-suspension and dispersal by wind of decomposed and altered waste materials. It is also generated by mechanical processes linked to landfill management such as: i) the movement of dustcarts and vehicles over previously deposited waste; ii) the tipping, sorting and compaction of waste by bulldozers; iii) the stockpiling of soil and rubble required for daily waste coverage (Chalvatzaki et al., 2010; Fitz and Bumiller, 2000).

Gaseous and particulate emissions from municipal solid waste landfill may fall down close to the source and accumulate contaminants in soil (Iwegbue et al., 2010; Rizo et al., 2012; Waheed et al., 2010). The contaminants accumulated in soil may be released into watercourses and groundwater (Rajkumar et al., 2010) and be taken up by vegetables and animals, thus constituting a threat to living organisms, including humans (Acosta et al., 2011; Gupta et al., 2010; Krishna and Govil, 2007).

The levels and distribution of contaminants should be monitored in any study assessing the environmental impact of activities related to the management of municipal solid waste in landfill (Biswas et al., 2010; Paoli et al., 2012). In this context, lichens are valuable biomonitoring tools for evaluating air quality and controlling contamination in areas around waste landfills (Nimis et al., 2002; Pirintsos and Loppi, 2008).

Lichens are one of the most sensitive components of the ecosystem. These organisms are able to absorb and accumulate contaminants in their thallus, intercepting airborne materials and solutes of wet and dry precipitations, as well as atmospheric gases (Nash, 2008). Lichens are thus widely used as biomonitors and bioaccumulators of contaminants in air quality and environmental contamination surveys (i.e., Conti and Cecchetti, 2001; Loppi et al., 1997; Nimis et al., 2002; Wolterbeek, 2002).

Lichens are symbiotic, perennial and slow-growing organisms that maintain a fairly uniform morphology over time. They are highly dependent on the atmosphere for their macro and micronutrients. However, due to their large surface area, relatively low growth rate, and lack of waxy cuticle and stomata, lichens can also absorb and accumulate inorganic and organic contaminants such as heavy elements directly from the air. Moreover, several authors have shown that a direct relationship exists between heavy element concentrations in thalli and those in the environment (Bari et al., 2001; Ng et al., 2006; Rodrigo et al., 1999; Sloof, 1995).

Lichens are widely used in biomonitoring studies, which employ either native species, that is species naturally present in the study area (Augusto et al., 2007; Blasco et al., 2008), or transplanted species (Baptista et al., 2008; Bergamaschi et al., 2007; Frati et al., 2005; Sorbo et al., 2008). The latter technique involves the use of thalli removed from areas with little or no contamination and transplanted for a period in selected monitoring sites. Transplant techniques are frequently used when lichens are scarce or absent in the study area.

Changes in the physiology and chemical composition of lichen transplants provide information on the concentration of inorganic and organic contaminants in the air (Demiray et al., 2012; Guttova et al., 2011; Oztetik and Cicek, 2011). In urban and industrial settings, the accumulation of air contaminants such as heavy elements can damage the photosynthetic apparatus (Piccotto et al., 2011; Zambrano and Nash, 2000), decrease the integrity of cell membranes (Paoli et al., 2011) and induce oxidative stress (Carreras et al., 2009; Oztetik and Cicek, 2011) in transplanted lichens.

Lichens accumulate heavy elements through uptake of soluble species in wet depositions and trapping of airborne particles (Williamson et al., 2004). Trapped particles can remain within lichen thalli over long periods of time and may be leached out by acid precipitation or lichen organic compounds (Brown, 1987). As lichens lack a vascular system and roots, there is no interaction with the substratum. This feature eliminates any doubts as to the origin of contaminants, an issue when vascular plants are used to biomonitor air quality.

Besides being useful bioindicators, these characteristics make lichens very sensitive to changes in the chemical composition of air. Lichens can thus serve as "early-warning" indicators of environmental changes and are very helpful in monitoring spatial patterns and temporal trends in heavy element deposition and accumulation (Bennett and Wetmore, 1999, 2000).

The study determined some physiological parameters (photosynthetic efficiency and electrical conductivity) and concentrations of 11 heavy elements (As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, V and Zn) in transplants of *Evernia prunastri* (L.) Ach. lichen and in surface soils collected within and around a municipal solid waste landfill in central Italy. Variations in photosynthetic efficiency and cell membrane integrity, as well as the accumulation of heavy elements in transplanted lichens were used to assess the environmental impact of emissions from the municipal solid waste landfill and define the extent of heavy element distribution in air.

This lichen biomonitoring study contributes to understanding of how municipal solid waste landfills affect air quality. To our knowledge, few researches have focused on this topic (Paoli et al., 2012; Protano et al., 2014). The study also provides analytical data on toxic heavy elements in lichens such As, Sb and Tl, which are generally little investigated in such surveys.

2. Material and methods

2.1. Study area

The study area is centred on the Cà Mascio municipal solid waste landfill (MSWL), located about 1 km NNW of the urban centre of Montecalvo in Foglia (Province of Pesaro and Urbino, Marche) in central Italy (Fig. 1). The landfill is located in a hilly zone with reliefs between 200 and 250 m a.s.l. The land is mostly used for agriculture

(cereals), but there are also pastures and woodlands. The prevalent direction of winds is from SSW and SW (Fig. 1).
In the study area, pliocenic marine sediments belonging to the Argille azzurre formation crop out. This lithostratigraphic unit mainly consists of blue-gray clays and marly clays, with interbedded yellowish sandstones and silty clays.
According to the Italian law (Italian Legislative Decree n° 36/2003), the Cà Mascio landfill is classified as "landfill for municipal and not hazardous waste". This landfill consists of seven batches: the old batches, numbered from 1 to 6, were used from 1984 to 2000, and overall they contain about 600,000 m^3 of solid waste. These old batches were covered by a layer of soil and vegetation. The batch 7 was utilized since 2001 and is still in use when this research was carried out (2011).

2.2. Lichen sampling, transplant and laboratory treatment

The lichen transplantation is an effective technique widely used for the determination of heavy element accumulation and for assessing the variation of physiological characteristic (Ayrault et al., 2007; Conti et al., 2004; Godinho et al., 2008; Mikhailova, 2002; Pacheco et al., 2008 Sloof, 1995).
The use of lichen transplants in place of native lichens (lichens grown *in situ*) is mainly due to the following reasons: absence of native lichens in the study area, uniformity of the lichen species utilized in biomonitoring and of the exposure period, possibility to choose the monitoring sites and their number, knowledge of the concentration of chemical elements before exposure, possibility to evaluate the accumulation trend of chemical elements.
For these reasons, and especially because of the absence of native lichens, the transplant technique was employed to assess the influence of Cà Mascio landfill emissions on the air distribution of some heavy elements of environmental concern.
In February 2011 thalli of the fruticose *Evernia prunastri* (L.) Ach. lichen were randomly collected from tree twigs in a woody zone far from contamination sources, located about 25 km S of Siena (central Italy). The *E. prunastri* lichen was chosen because it is easily collected and transplanted. In addition, this species is one of the most used lichens in biomonitoring studies due to its bioaccumulation capacity and widespread distribution (Garty, 2001; Guttova et al., 2011; Loppi and Frati, 2006; Paoli et al., 2011).

Figure 1

The samples of native lichens were immediately transferred to laboratory in polyethylene bags, and left 48 h to acclimate in a climatic-chamber at 15 °C with relative humidity at 60 ± 5% and photoperiod of 12 h at 40 μmol m^{-2} s^{-1} photons of photosynthetically active radiation. In laboratory, the lichen samples were carefully cleaned with plastic tweezers under a binocular microscope to remove extraneous materials such as moss, leaves, bark pieces and soil particles. Finally, the lichen samples were rinsed with ultra-pure water to remove smaller particles from the surface of thalli and were stored in paper bags. The samples used for analytical determinations (n=4) were frozen at -20 °C.

In the study area, the samples of native lichens were transplanted in sites located at different distance from the Cà Mascio MSWL and positioned according to the prevalent directions of winds. Lichen transplants were exposed in 17 sites clustered into the following 4 groups (Fig. 1): landfill sites situated around the Cà Mascio MSWL within a distance of 100 m (n=5); fall-out sites placed along a NNE transect aligned with the prevalent direction of winds, at a distance of between 50 and 500 m from the landfill (n=4); rural sites not affected by the direct influence of the landfill fall-out, at a distance of 250 and 500 m from the plant (n=4); background sites distant from contamination sources including the landfill, at distances of more than 1500 m from the stored waste (n=4).

In each monitoring site, a tree was selected and three lichen thalli were fixed on the branches at a height of about 2.5 m above the ground, using plastic strings. Lichens were exposed for 4 months, from March to June 2011. At the end of the exposure period, lichen samples were detached from the tree and placed in clean plastic bags to avoid contamination.

In the laboratory transplanted lichens were stored in paper bags and frozen at -20 °C. Before the analysis lichen thalli were removed from the freezer and left at room temperature for about 15 minutes and were then carefully cleaned with plastic tweezers under a binocular microscope to remove extraneous material deposited onto the surface. Samples were not washed as the washing procedure may alter their chemical composition (Bettinelli et al., 1996).

Native and transplanted lichens were air-dried and immersed in liquid nitrogen, pulverized and homogenized using a ceramic mortar and pestle. Only peripheral parts of thalli (up to 2 cm from lobe tips) were used for analysis. Each transplanted sample was a mixture of all lichen thalli exposed in the monitoring site.

2.3. Lichen analysis

About 200 mg of powdered lichen material were solubilised with a mixture of 6 mL HNO_3 70%, 1 mL H_2O_2 30% and 0.2 mL HF 60%, in Teflon bombs placed in a microwave digestion system (Milestone Ethos 900) for 30 min. Ultra-pure trace-grade reagents were employed and ultra-high-purity water was used for dilution. Solutions were filtered, diluted to 50 mL and stored in PE bottles before analysis. A certified reference material and a blank of the employed reagents were included in each digestion batch.

The concentrations of As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, V and Zn in native and transplanted lichens were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) using the Perkin Elmer Sciex Elan 6100 spectrometer. Concentrations were expressed as dry weight basis (mg/kg dry weight).

The Standard Reference Material IAEA-336 (Trace and minor elements in lichen) of the International Atomic Energy Agency (Vienna, Austria) was used to check the analytical accuracy. The recoveries were from 90% (Co) to 106% (Cr). Precision was determined by five replicate analyses of each lichen sample and expressed as percent relative standard deviation (% RSD). The analytical precision was within 10% for all the analyzed chemical elements.

2.4. Integrity of cell membranes

To check the integrity of the plasma membrane, the difference in electrical conductivity was carried out by placing a fragment of lichen thalli in deionized water (Munzi et al., 2009; Paoli et al., 2011). This is a simple test to assess the integrity of the plasma membrane: the value of electrical conductivity is related to the degree of damage endured by cell membranes (Marques et al., 2005). In fact, permeability is altered in damaged cell membranes and electrolyte leakage (mainly K^+) occurs (McKersie et al., 1982).

Each lichen sample (about 100 mg of young portion of thalli up to 2 cm from lobe tips) was rinsed several times in deionised water for 3-5 seconds, until stable values of electrical conductivity were obtained. This rinsing was carried out to remove the particles deposited onto the lichen surface that contribute to the electrical conductivity of sample. Afterwards, the lichen sample was immersed into a glass bottle with 50 mL of deionised water and shaken for 60 min. The electrical conductivity of water was measured before and after the lichen was soaked using the conductivity-meter Delta Ohm HD/8706. Thalli were thus boiled for 10 min at 100 °C to determine the total disruption of cell membranes, and electrical conductivity of water was measured again. The values of electrical conductivity were expressed in µS cm^{-1} mL mg^{-1} dry weight at a normalized temperature of 25 °C.

2.5. Photosynthetic efficiency

Photosynthetic efficiency was measured with a plant efficiency analyzer (Handy PEA, Hansatech Instruments Ltd) on well-wet and dark-adapted lichen samples, applying a saturating flash of red light (650 nm) of 2400 µmol s^{-1} m^{-2} for 1 sec. To block the physiological state at the time of the end of the transplant, lichen samples were air-dried and stored at -20 °C in paper bags. Before measurements, a physiological recovery of the samples was carried out. To avoid any osmotic stress by air humidity during de-freezing, lichens were left in dry ambient conditions for 15 min. Thalli were subsequently sprayed with water until wet and the water in excess was removed. Samples were then stored at 4 °C in the dark for 24 h. The outermost 2 cm of thalli was randomly selected for measurements. The selected lichen material was placed within a clip for 10 min to allow full dark adaptation of the photosynthetic pigments. The ratio of variable fluorescence to the maximal fluorescence (F_V/F_M) was used to assess the potential quantum yield of primary photochemistry of PS II (Maxwell and Johnson, 2000).

2.6. Soil sampling, laboratory treatment and analysis

In the 17 sites where lichens were exposed, surface soil samples (5 cm deep) were collected. Each soil sample was a composite sample consisting of 3 sub-samples taken a few metres apart.

In laboratory litter material was manually removed and soil samples were dried at +40 °C and sieved through a 2 mm mesh. Homogenization was carried out by quartering and pulverization procedures. Soil samples were solubilised by acid digestion adding 2 mL HNO_3 70%, 2 mL HF 60% and 1 mL H_2O_2 30% to 250 mg of powdered soil. Ultra-pure trace-grade reagents were used for soil preparation. The mixture was processed in Teflon bombs using a microwave lab station. The solution was filtered and diluted with ultra-pure water to a final volume of 100 mL. A certified reference material and a blank of the employed reagents were included in each digestion batch.

The contents of As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, V and Zn in soil samples expressed as dry weight basis (mg/kg dry weight), were determined by ICP-MS.

The accuracy of analytical determinations was checked using the NIST 2709 (San Joaquin soil) and NIST 2710 (Montana soil) certified reference materials of the National Institute of Standards and Technology. The recoveries were from 91% (Co) to 106% (Sb). The precision was determined by five replicate analyses of each soil sample and expressed as percent relative standard deviation (% RSD). The analytical precision was within 8% for all the analyzed chemical elements.

2.7. Data interpretation and statistical analysis

In order to evaluate the accumulation of heavy elements in transplanted lichens, the Exposed to Control (EC) ratio was utilized. The EC ratio is calculated as the ratio between the element concentration in lichen after its exposure in the study area and in non-exposed control lichen (Frati et al., 2005). To assess the variations in accumulation or loss of heavy elements in transplanted thalli, an interpretative scale consisting of 5-class was used: severe loss EC=0.00-0.25, loss EC=0.25-0.75, normal EC=0.75-1.25, accumulation EC=1.25-1.75 and severe accumulation EC>1.75.

The Shapiro-Wilks W test was applied to verify the normal distribution of analytical data. Statistical differences between datasets were determined through the Student's t test for data normally distributed, and the Mann-Whitney U test for data not normally distributed, at the 5% significance level. Spearman's correlation test was used to identify the significant correlations among the concentrations of heavy elements and the values of physiological parameters in lichens.

3. Results

3.1. Heavy element accumulation in transplant lichens

Table 1 reports the As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, V and Zn concentrations (as mg/kg dry weight) both in native specimens of *Evernia prunastri* collected from an uncontaminated habitat in the Province of Siena (control site) and in transplanted thalli after 4 months of exposure in the background, rural, fallout and landfill sites of the Cà Mascio MSWL area.

Table 1

Heavy element concentrations in transplanted lichens showed the following distribution patterns: i) the mean values of Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn concentrations decreased according to the features of the monitoring sites, as follows: landfill > fallout > rural > background; ii) As, Tl and V concentrations were rather homogeneous in all the selected sites.

Statistical analysis revealed that the Cd, Cr, Sb and Zn concentrations in landfill, fallout and rural transplants differ significantly with respect those in native lichens from control site ($p<0.05$; Tab. 1). Co, Cu and Pb concentrations in lichens from landfill and fallout sites and Ni concentrations in transplants from landfill sites differed significantly from those in control site. There were no significant differences between As, Tl and V concentrations in lichens exposed in landfill, fallout and rural sites and those in native lichens. For all the analyzed heavy elements, no statistically significant differences were found between concentrations in transplanted thalli from background sites and concentrations in native lichens from control site.

The lichen transplants located along a transect in the direction of prevailing winds showed an evident decrease in concentrations of several heavy elements (mainly Cr, Pb and Sb) within about 150 m of the perimeter of the landfill.

Based on the mean EC ratios, Cr, Pb, Sb and Zn showed severe accumulation (EC>1.75) in lichens exposed both in landfill and fallout sites (Fig. 2), and moderate accumulation (1.25 >EC> 1.75) in transplants located in rural areas (except for Sb). For these heavy elements the highest EC ratios (1.96-11.6) pertained to lichens transplanted within a 100 m range of the Cà Mascio MSWL; the accumulation of Cr, Pb, Sb and Zn in the exposed lichens therefore decreased from landfill to rural sites.

Cd, Co, Cu and Ni accumulation in exposed lichens was generally severe in the landfill sites and moderate in fallout sites (Fig. 2). No accumulation of these heavy elements was detected in rural areas (except for Cd).

Analytical data indicated that As, Tl and V were not accumulated in lichens exposed in the monitoring sites of the study area. All heavy element concentrations (except Cu) determined in lichen transplants from background sites were similar to those of native control samples (EC from 0.75 to 1.25).

In summary, EC ratios indicated that Cr, Pb, Sb and Zn were the main airborne contaminants deriving from Cà Mascio MSWL emissions. Taking into account the EC ratios in lichen transplants from landfill sites, the order of accumulation was the following: Sb ≈ Cr > Pb > Zn > Cd ≈ Cu > Co > Ni. The transplants in the landfill and fallout sites showed the highest levels of accumulation, whereas heavy element concentrations in lichens from rural and background sites were generally comparable to those measured in native lichens from an uncontaminated area of central Italy.

Figure 2

3.2. Physiological parameters of transplant lichens

As shown in Figure 3a, the values of electrical conductivity in lichens exposed in the landfill and fallout sites were significantly higher (p<0.05) than those of native lichens in the control site. Conversely, there was no statistically significant difference between the electrical conductivity measured in transplants from rural and background sites and that measured in the control site, although the mean values of this parameter were slightly higher in exposed thalli.

The photosynthetic efficiency (F_V/F_M) of transplanted lichens was lower than that of samples from the control site (Figure 3b). The lowest F_V/F_M ratios pertained to lichens exposed in the landfill and fallout sites, and statistically significant differences were found with respect to the values measured in the native control thalli (p<0.05).

Figure 3

3.3. Correlation among heavy element concentrations and physiological parameters in lichen transplants

Table 2 reports the Spearman correlation coefficients among heavy element concentrations and physiological parameters (electrical conductivity and F_V/F_M) in lichens exposed in all the monitoring sites within the Cà Mascio MSWL area.

Table 2

The most significant positive correlations (p<0.001) were found among the concentrations of heavy elements most accumulated by lichens, i.e. Cd, Cr, Cu, Pb, Sb and Zn. Electrical conductivity was positively correlated with the concentrations of these contaminants (p<0.001). Significant negative correlations (p<0.01) were found among photosynthetic efficiency and Cd, Cr, Cu, Pb, Sb and Zn concentrations. These findings are consistent with the fact that higher electrical conductivity and lower photosynthetic efficiency values correspond to higher heavy element accumulation by lichens.

3.4. Heavy element contents in soils

Table 3 reports the As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, V and Zn contents (as mg/kg dry weight) in soil samples collected from the 17 sites where lichens were exposed. Heavy element contents in soil samples were compared to the respective local natural variability in soil (geochemical background) in order to evaluate the input of emissions from the Cà Mascio MSWL. For this purpose, the enrichment factor (EF) was calculated as $EF=[C]_{element}/[C]_{background}$, where $[C]_{element}$=element concentration in the soil sample and $[C]_{background}$=maximum value of the local geochemical background. Local geochemical background levels of heavy elements were assessed in soil samples collected from the background sites (n=4) located far away from the Cà Mascio MSWL and other possible sources of contamination.

As shown in Table 3, heavy element contents in soils from the study area were rather homogeneous. Using the EF scale proposed by Sutherland (2000), no enrichment was found: EF values were usually less than 1, with mean values from 0.7 for Co, Cu and Pb 1.1 for As. These results suggest that heavy element contents in soils collected close to the landfill (landfill sites) and along a transect in the direction of prevailing winds (fallout sites) were within their respective local natural variability in soil. Therefore, the heavy element contents in soils from the study area must be considered geogenic, due to natural factors and processes such as the nature of the parent rock (clays and marly clays of the Argille azzurre formation), and the features of weathering and pedogenesis.

Heavy element concentrations in soil samples were constantly below contamination thresholds for green public, private and residential areas set by the Italian guidelines (Italian Legislative Decree n° 152/2006; Tab. 4). Vanadium contents slightly above its contamination threshold (90 mg/kg) were found in 6 soil samples randomly distributed in the study area.

Table 3

4. Discussion

It is known that the chemical composition of lichens mainly depends on the availability of chemicals in the environment and that lichens respond to environmental changes (Bačkor and Loppi, 2009; Nimis et al., 2002). Lichens are able to head off and accumulate heavy elements from wet depositions and trapped airborne particles. This ability may be affected by environmental and climatic conditions as well as by the exposure time (Wolterbeek, 2002). Garty et al. (1993; 2001) demonstrated that thalli transplanted from uncontaminated to contaminated areas undergo changes, mainly due to the effects of contaminants. Moreover, several studies have shown that lichens transplanted close to contamination sources can uptake significant amounts of heavy elements within a few weeks or months (Bargagli, 1998; Conti et al., 2004; Paoli et al., 2011).

Chalvatzaki et al. (2010) recently pointed out that waste management techniques such as composting, unloading and sorting of waste and dust re-suspension, as well as meteorological conditions (wind direction and temperature) affect local concentrations of PM_{10} in the surroundings of a municipal solid waste landfill. Furthermore, Koshy et al. (2009) reported that Cr, Ni, Pb and Zn were the main heavy elements in the particulate matter emitted from a MSWL in the UK. Likewise, Paoli et al. (2012) measured high Cd, Cr and Ni concentrations in *F. caperata* thalli collected near a MSWL in Italy.

Analytical data from this work indicated moderate to severe enrichments in heavy elements such as Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn, in lichens transplanted in the landfill and fallout sites. Conversely, lichens exposed in background sites showed no variations in heavy element concentrations with respect to the non-exposed control thalli. As, Tl and V were not accumulated in any of the lichens exposed in the monitoring sites. This finding suggested a geogenic source for the latter elements, in agreement with the results of a study carried out on soils around the largest MSWL in Europe (Malagrotta, Rome; Barbieri et al., 2014).

The highest EC ratios for Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn were measured in lichen transplants within the municipal solid waste landfill and along a transect in the direction of fallout. This evidence suggested that the airborne emissions from the Cà Mascio MSWL affected the heavy element concentrations in air, mainly Cr, Pb, Sb and Zn. This finding agrees with the results of Prechthai et al. (2008), who measured high Cd, Cr, Cu, Pb and Zn concentrations in municipal solid waste from a dumpsite in Thailand.

The main sources of these heavy elements were plastics, rubber, electronic equipment and non-ferrous metallic components (Prudent et al., 1996). In accordance with similar studies (Nannoni et al., 2015; Paoli et al., 2012; Protano et al., 2014), we did not find a specific heavy element acting as tracer of emissions from the Cà Mascio MSWL.

The moderate accumulation of Cu and Zn in lichens from the background sites attested to a wider distribution of these elements in the study area, probably in relation to the spraying of fertilizers and pesticides.

In agreement with Paoli et al., 2012; Protano et al., 2014, we conclude that airborne emissions from the Cà Mascio MSWL caused the accumulation of several heavy elements in lichens, mainly Cr, Pb, Sb and Zn. However, the landfill had a rather modest impact, as the highest concentrations and enrichments in heavy elements were found in lichens within about 150 m of the municipal solid waste landfill. Likewise, Paoli et al. (2012) revealed an increased deposition of some heavy elements limited to sites facing a municipal solid waste landfill in Italy.

Electrical conductivity is a good indicator of air contamination, as this parameter reveals the degree of damage to cell membranes in lichens (Munzi et al., 2009; Paoli et al., 2011; Pearson and Henriksson, 1981). This is because the plasma membrane is the first site of biological interaction with toxic substances, including heavy elements. Electrical conductivity values from this study reveal that the damage to cell membranes of lichens exposed in the monitoring sites differs from that of lichens in control site. The electrical conductivity of transplanted lichens varied as a function of site location: the highest values characterized the thalli exposed in the landfill and fallout sites, suggesting that the highest degree of cell membrane damage concerned the transplants within the Cà Mascio MSWL and the sector most affected by landfill emissions. These differences may be due to the higher heavy element concentrations in the landfill and fallout dispersion areas. Garty et al. (1998a) observed a similar difference in electrical conductivity between lichens exposed in industrial sites and urban centres and those exposed in rural sites.

The significant positive correlations between the concentrations of the main heavy-element contaminants in the study area (Cr, Pb, Sb and Zn) and the values of electrical conductivity in exposed lichens confirmed the hypothesis above. This is consistent with the results of other studies that found higher values of electrical conductivity in lichen transplants affected by heavy element accumulation (Adamo et al., 2003; Garty et al., 2002; Garty et al., 1998b). Paoli and Loppi (2008) also observed that cell membrane damage in *E. prunastri* lichens was correlated with air quality.

Photosynthetic activity, expressed as F_V/F_M, has often been employed in biomonitoring studies as it is considered a general index of the health of lichens and can be used to assess their vitality. The photosynthetic activity of lichens in the field is influenced by several factors, including environmental conditions and anthropogenic disturbances (i.e., high levels of SO_2, NO_x and heavy elements). The accumulation of certain air contaminants in lichen thalli is assumed to coincide with low F_V/F_M ratios. The F_V/F_M ratio of lichens growing in uncontaminated areas (healthy lichens) usually varies from 0.5 to 0.76 (Jensen and Kricke, 2002), and values lower than 0.5 reveal that lichens were exposed to stress. Several studies (i.e., Garty et al., 2000; Karakoti et al., 2014) reported low F_V/F_M ratios for lichens characterized by high heavy element concentrations.

The F_V/F_M ratios for lichens exposed in the landfill and fallout sites suggested that environmental conditions within and around the Cà Mascio MSWL caused a decrease in photosynthetic activity. However, the F_V/F_M ratios of transplanted lichens ranged from 0.51 to 0.66, in agreement with non-stressed conditions for lichens (Jensen and Kricke, 2002). As photosynthetic activity is considered a sensitive indicator of contamination stress, this finding suggested that airborne heavy element contamination due to landfill emissions was spatially limited in the study area and not sufficiently severe to determine significant changes in this physiological parameter.

Lastly, analytical data revealed that the selected heavy elements were not accumulated in soils surrounding the Cà Macio MSWL, despite the fact that the landfill site has been operating for 30 years. This finding could be ascribed to the fact that the contribution of heavy elements in soil due to fallout and re-suspension can be masked by run-off and leaching processes affecting the uppermost part of the soil profile (first 5 cm). Our results agree with those of Jain et al. (2005), who report that the concentrations of some heavy elements in soils sampled around a municipal solid waste landfill in Florida were below US regulatory thresholds. Likewise, Nannoni et al. (2015) and Amadi Akobundu and Nwankwoala (2013) did not detect accumulation of heavy elements in soils close to municipal waste dumpsites in Italy and Nigeria, respectively.

5. Conclusions

Analytical data indicated that airborne emissions from the Cà Mascio municipal solid waste landfill affected the Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn concentrations in *Evernia prunastri* lichens transplanted for 4 months within and around the landfill. Moderate to severe accumulation of these heavy elements was detected in lichens exposed within the landfill and along the direction of fallout. In these sites heavy element accumulation coupled with an increase in cell membrane damage and decrease in photosynthetic efficiency in lichens, was mainly due to the airborne particles generated by re-suspension and dispersal of waste materials.

Our results excluded that the Cà Mascio MSWL had a significant impact on heavy element levels in the study area. This statement is supported by the following evidences: (i) heavy elements accumulated only in the lichen transplants exposed within the landfill and along the direction of fallout up to about 150 m from the landfill; (ii) emissions from the solid waste caused weak stress symptoms only in lichens exposed in the landfill and fallout sites; (iii) sites far from the landfill (rural and background) were not affected by emissions from the MSWL. Moreover, in 30-years of waste management at the Cà Mascio landfill, there has been no accumulation of heavy elements in the surrounding soils, as concentrations were within the respective local geochemical background.

In conclusion, conditions in the area around the Cà Mascio MSWL are not remarkably stressful for lichens. Therefore, an appropriate landfilling management poses a relatively low risk of environmental contamination by heavy elements. Our research confirmed that lichens are very sensitive to even small changes in atmospheric concentrations of heavy elements.

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FIGURE CAPTIONS

Figure 1. Map of the study area showing the direction of dominant winds and location of the monitoring sites: ◆ landfill, ● fallout, ○ rural and ■ background sites.

Figure 2. Mean values of EC ratio of lichens exposed in the monitoring sites (background, rural, fallout and landfill) of the study area and relative accumulation/loss scale.

Figure 3. Values (mean ± standard deviation) of electrical conductivity (a) and photosynthetic efficiency (b) in *E. prunastri* lichens collected in the control site and transplanted in the monitoring sites (background, rural, fallout and landfill) of the Cà Mascio MSWL area. * Significant differences of electrical conductivity and photosynthetic efficiency among background, rural, fallout and landfill lichens compared to control ones (p<0.05).

Figure_1
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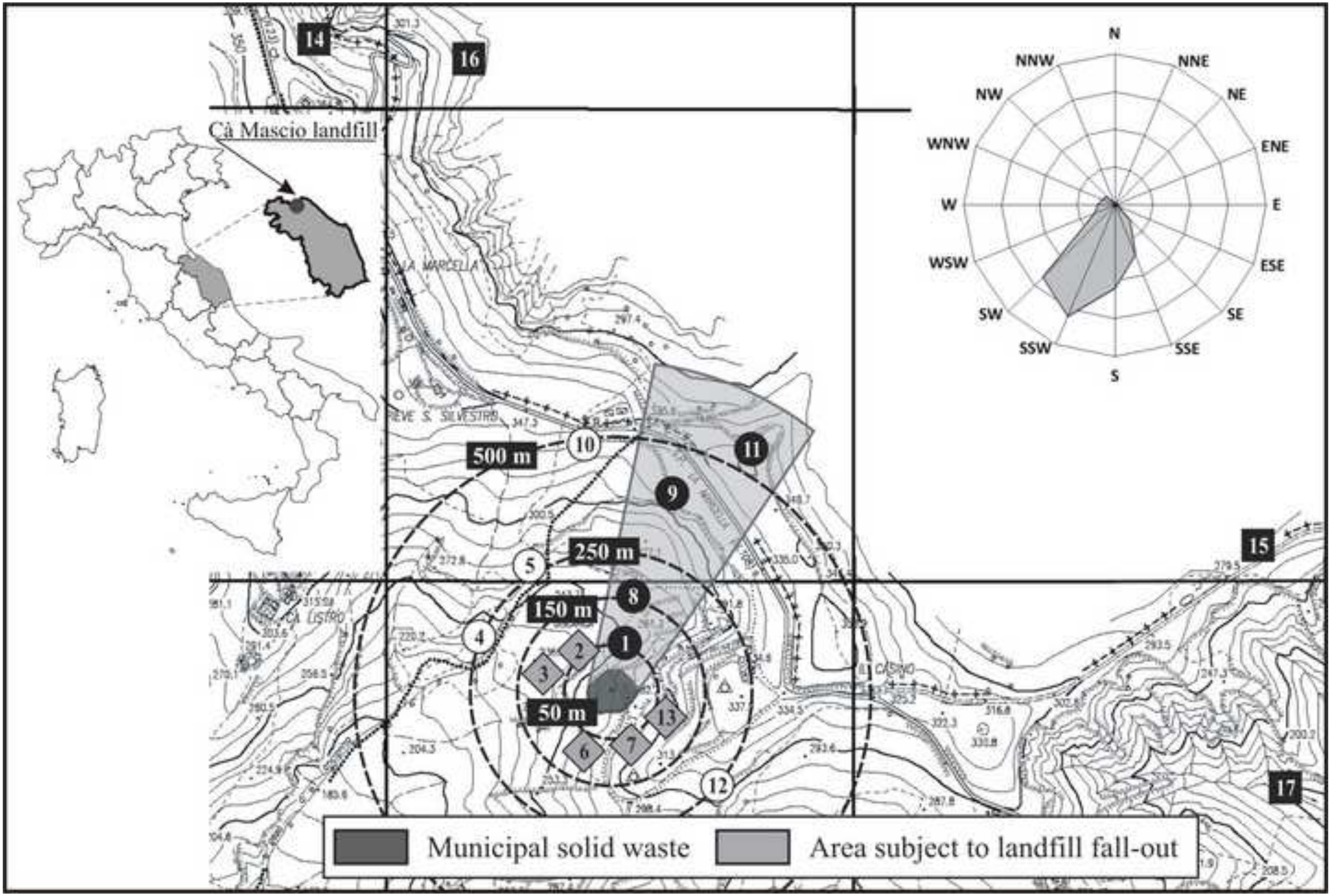

Figure_2

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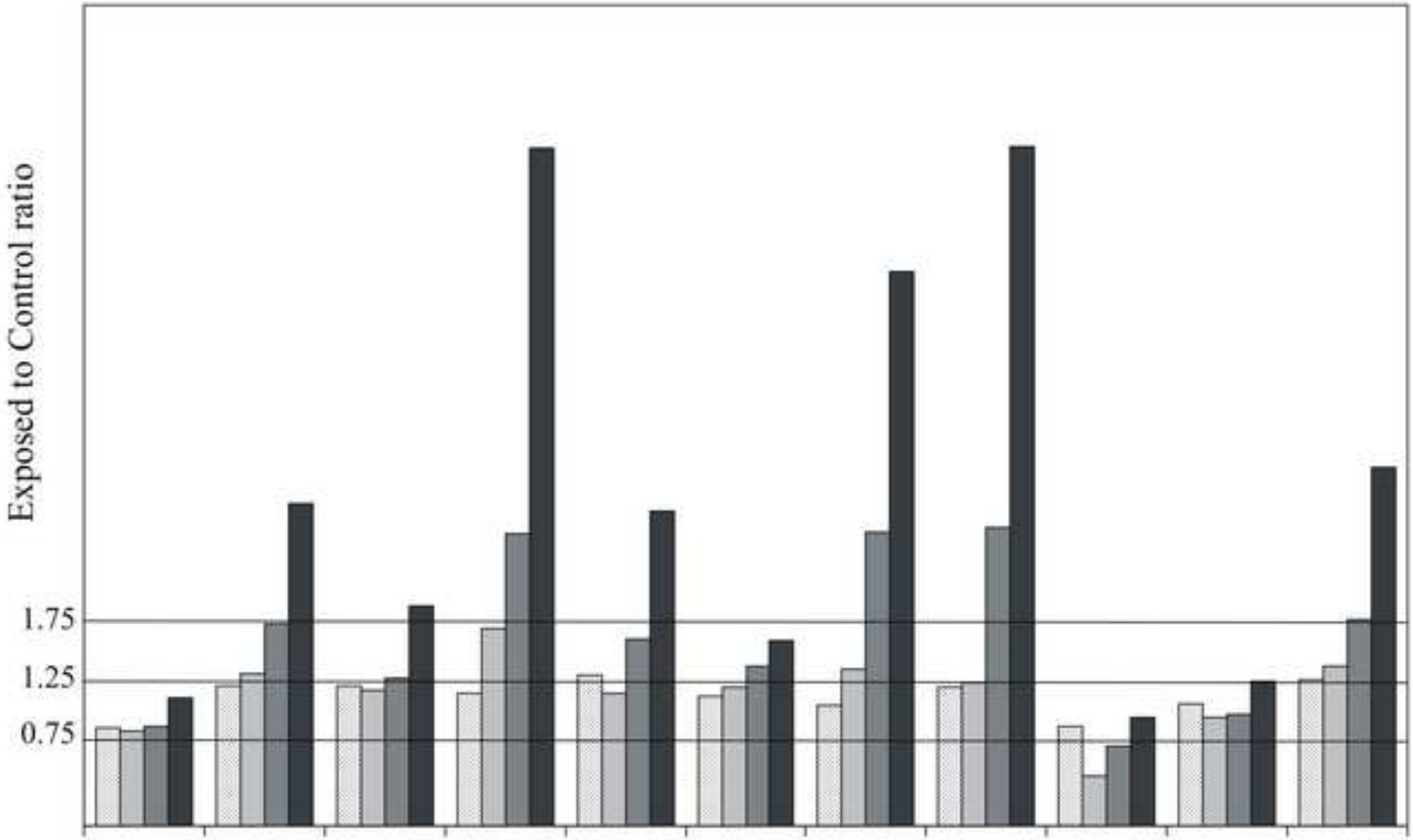

Monitoring sites	As EC ratio		Cd EC ratio		Co EC ratio		Cr EC ratio		Cu EC ratio		Ni EC ratio		Pb EC ratio		Sb EC ratio		Tl EC ratio		V EC ratio		Zn EC ratio	
Background	0.83	N	1.19	N	1.19	N	1.13	N	1.28	A	1.10	N	1.03	N	1.18	N	0.85	N	1.04	N	1.25	N
Rural	0.81	N	1.30	A	1.15	N	1.68	A	1.13	N	1.19	N	1.33	A	1.21	N	0.43	L	0.92	N	1.36	A
Fall-out	0.85	N	1.73	A	1.26	A	2.49	SA	1.59	A	1.36	A	2.50	SA	2.55	SA	0.68	L	0.96	N	1.77	SA
Landfill	1.09	N	2.74	SA	1.88	SA	5.78	SA	2.68	SA	1.58	A	4.72	SA	5.79	SA	0.92	N	1.24	N	3.05	SA

Accumulation/Loss scale: L = loss, N = normal, A = accumulation, SA = severe accumulation

Figure_3
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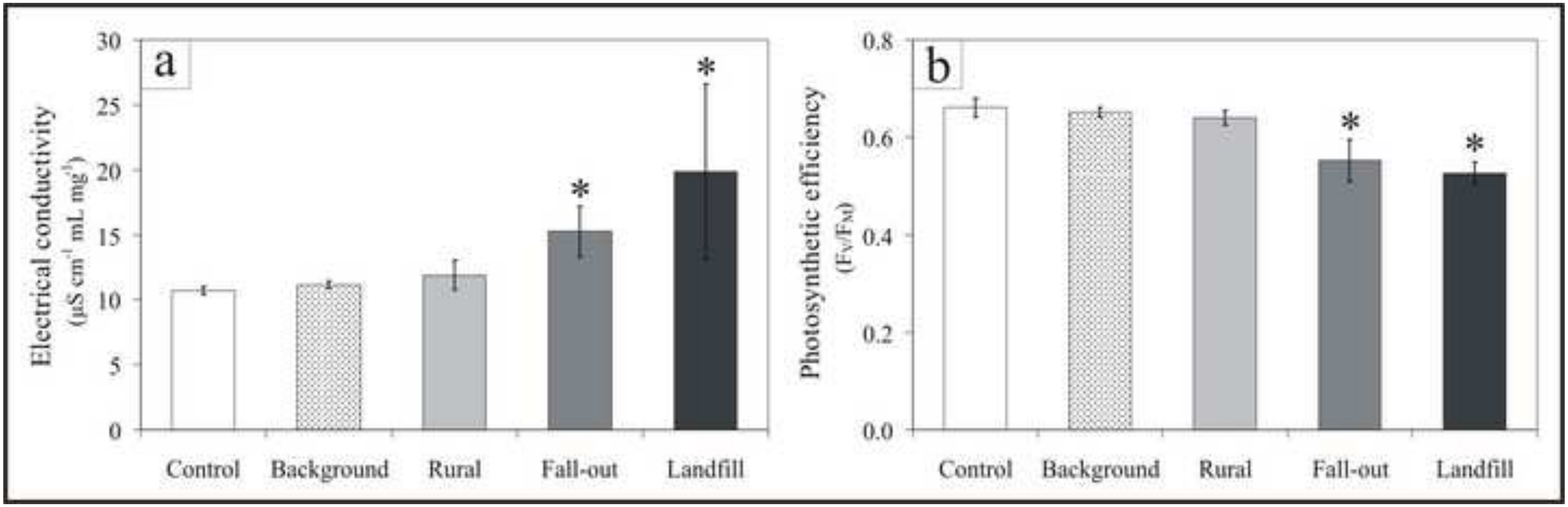

Table 1. Concentrations and mean values of heavy elements (data in mg/kg ± standard deviation) in *E. prunastri* lichens collected in the control site and transplanted in the monitoring sites of the Cà Mascio MSWL area.

Sites	As	Cd	Co	Cr	Cu	Ni	Pb	Sb	Tl	V	Zn
Control											
Native 1	0.21 ± 0.02	0.02 ± 0.004	0.2 ± 0.01	1.4 ± 0.06	3.2 ± 0.16	2.2 ± 0.07	1.4 ± 0.04	0.07 ± 0.02	0.003 ± 0.001	0.9 ± 0.04	14 ± 0.17
Native 2	0.21 ± 0.02	0.03 ± 0.003	0.18 ± 0.03	1.3 ± 0.02	2.9 ± 0.31	1.7 ± 0.11	1.2 ± 0.01	0.08 ± 0.01	0.008 ± 0.002	2 ± 0.09	19.3 ± 0.11
Native 3	0.29 ± 0.04	0.09 ± 0.008	0.17 ± 0.03	2.2 ± 0.09	4.4 ± 0.61	2.3 ± 0.05	3.1 ± 0.06	0.12 ± 0.03	0.007 ± 0.001	1.6 ± 0.06	20.6 ± 0.18
Native 4	0.24 ± 0.02	0.07 ± 0.009	0.31 ± 0.06	1.6 ± 0.08	4.3 ± 0.92	2.1 ± 0.09	3.3 ± 0.05	0.10 ± 0.01	0.003 ± 0.001	1.2 ± 0.03	14.8 ± 0.2
Mean	0.24 ± 0.04	0.05 ± 0.03	0.22 ± 0.06	1.6 ± 0.37	3.7 ± 0.77	2.1 ± 0.28	2.3 ± 1.1	0.09 ± 0.02	0.005 ± 0.003	1.4 ± 0.48	17.2 ± 3.3
Background											
MV-14	0.2 ± 0.01	0.06 ± 0.002	0.25 ± 0.01	1.8 ± 0.03	4.7 ± 0.05	2.1 ± 0.02	2.5 ± 0.04	0.09 ± 0.01	0.004 ± 0.001	1.5 ± 0.03	21 ± 0.38
MV-15	0.21 ± 0.01	0.05 ± 0.008	0.28 ± 0.02	1.9 ± 0.02	4.8 ± 0.06	2.4 ± 0.06	2.1 ± 0.05	0.11 ± 0.02	0.004 ± 0.001	1.4 ± 0.02	22 ± 0.29
MV-16	0.16 ± 0.02	0.03 ± 0.001	0.20 ± 0.01	1.5 ± 0.02	5.3 ± 0.07	1.8 ± 0.05	2.8 ± 0.03	0.13 ± 0.03	0.006 ± 0.002	1.4 ± 0.04	18.8 ± 0.21
MV-17	0.22 ± 0.02	0.08 ± 0.006	0.31 ± 0.02	2.2 ± 0.03	4.2 ± 0.03	2.7 ± 0.03	2 ± 0.04	0.09 ± 0.02	0.003 ± 0.001	1.5 ± 0.03	24.3 ± 0.28
Mean	0.2 ± 0.03	0.05 ± 0.02	0.26 ± 0.05	1.8 ± 0.31	4.7 ± 0.47	2.3 ± 0.39	2.3 ± 0.38	0.1 ± 0.02	0.004 ± 0.001	1.4 ± 0.08	21.5 ± 2.3
Rural											
MV-4	0.15 ± 0.01	0.05 ± 0.006	0.22 ± 0.03	2.2 ± 0.04	3.6 ± 0.04	3.0 ± 0.04	2.7 ± 0.03	0.09 ± 0.01	0.002 ± 0.001	1 ± 0.02	23 ± 0.24
MV-5	0.18 ± 0.02	0.05 ± 0.009	0.25 ± 0.01	3.2 ± 0.05	4.1 ± 0.07	2.1 ± 0.02	3 ± 0.04	0.1 ± 0.02	0.002 ± 0.001	1.3 ± 0.02	26.3 ± 0.31
MV-10	0.19 ± 0.01	0.07 ± 0.01	0.23 ± 0.02	2.3 ± 0.01	4.7 ± 0.02	2.5 ± 0.02	2.7 ± 0.03	0.12 ± 0.02	0.001 ± 0.001	1.1 ± 0.01	23.4 ± 0.1
MV-12	0.25 ± 0.03	0.07 ± 0.006	0.38 ± 0.07	3.3 ± 0.07	4.2 ± 0.05	2.2 ± 0.03	3.5 ± 0.06	0.11 ± 0.01	0.004 ± 0.002	1.7 ± 0.03	21.1 ± 0.25
Mean	0.19 ± 0.04	0.06 * ± 0.008	0.27 ± 0.07	2.8 * ± 0.57	4.2 ± 0.43	2.4 ± 0.41	3 ± 0.39	0.11 * ± 0.01	0.002 ± 0.001	1.3 ± 0.31	23.4 * ± 2.2
Fall-out											
MV-1	0.21 ± 0.03	0.09 ± 0.003	0.31 ± 0.01	8.5 ± 0.12	7.9 ± 0.1	2.4 ± 0.04	10.8 ± 0.08	0.58 ± 0.07	0.002 ± 0.001	1.3 ± 0.02	33.8 ± 0.5
MV-8	0.16 ± 0.01	0.06 ± 0.004	0.23 ± 0.04	2.9 ± 0.04	4.9 ± 0.54	2.1 ± 0.03	5.7 ± 0.12	0.2 ± 0.02	0.001 ± 0.001	0.95 ± 0.01	23.6 ± 0.12
MV-9	0.22 ± 0.01	0.09 ± 0.007	0.3 ± 0.02	3 ± 0.05	5.2 ± 0.52	3 ± 0.04	4.2 ± 0.07	0.15 ± 0.05	0.008 ± 0.002	1.7 ± 0.02	24.9 ± 0.17
MV-11	0.24 ± 0.04	0.1 ± 0.002	0.31 ± 0.05	5.5 ± 0.09	8.2 ± 0.68	2.5 ± 0.02	6.4 ± 0.07	0.32 ± 0.03	0.002 ± 0.001	1.5 ± 0.04	38.1 ± 0.22
Mean	0.2 ± 0.03	0.08 * ± 0.016	0.28 * ± 0.04	5 * ± 2.6	6.5 * ± 1.7	2.5 ± 0.37	6.8 * ± 2.8	0.31 * ± 0.19	0.003 ± 0.003	1.4 ± 0.32	30.1 * ± 7

Landfill											
MV-2	0.19 ± 0.01	0.07 ± 0.003	0.26 ± 0.01	4.9 ± 0.09	5.3 ± 0.05	3.5 ± 0.05	6.2 ± 0.09	0.24 ± 0.03	0.003 ± 0.001	1.2 ± 0.02	35.1 ± 0.43
MV-3	0.2 ± 0.01	0.07 ± 0.006	0.27 ± 0.04	3.4 ± 0.03	5.1 ± 0.05	2.2 ± 0.02	4.7 ± 0.05	0.15 ± 0.01	0.002 ± 0.001	1.3 ± 0.01	35.2 ± 0.44
MV-6	0.22 ± 0.02	0.24 ± 0.003	0.35 ± 0.01	9.3 ± 0.19	14.6 ± 0.14	3.8 ± 0.07	15.4 ± 0.11	0.59 ± 0.09	0.001 ± 0.001	1.1 ± 0.03	69.9 ± 0.67
MV-7	0.31 ± 0.01	0.14 ± 0.007	0.53 ± 0.07	18.9 ± 0.16	15.1 ± 0.13	4.7 ± 0.06	16 ± 0.23	0.97 ± 0.12	0.005 ± 0.001	1.9 ± 0.03	78.7 ± 0.94
MV-13	0.36 ± 0.02	0.09 ± 0.004	0.59 ± 0.02	7.1 ± 0.15	7.1 ± 0.06	3.3 ± 0.05	6.3 ± 0.05	0.28 ± 0.01	0.013 ± 0.003	3 ± 0.08	45.5 ± 0.57
Mean	0.25 ± 0.08	0.12 * ± 0.07	0.4 * ± 0.15	8.7 * ± 6.1	9.4 * ± 5	3.5 * ± 0.9	9.7 * ± 5.5	0.44 * ± 0.34	0.005 ± 0.005	1.7 ± 0.79	52.9 * ± 20.3

* Significant differences among the mean contents of heavy elements in background, rural, fall-out and landfill lichens compared to control ones (*p*<0.05).

Table 2. Spearman correlation coefficients among the heavy element concentrations and physiological parameters [electrical conductivity (EC) and photosynthetic efficiency (F_V/F_M)] in lichens exposed in the Cà Mascio MSWL area. Only significant values are reported.

	As	Cd	Co	Cr	Cu	Ni	Pb	Sb	Tl	V	Zn	EC	F_V/F_M
As	1												
Cd	0.777 **	1											
Co	0.946 **	0.804 **	1										
Cr	n.s.	0.836 **	0.689 *	1									
Cu	n.s.	0.695 *	n.s.	0.667 *	1								
Ni	n.s.	0.618 *	n.s.	n.s.	n.s.	1							
Pb	n.s.	0.746 **	n.s.	0.917 **	0.821 **	n.s.	1						
Sb	n.s.	0.735 **	n.s.	0.840 **	0.921 **	n.s.	0.947 **	1					
Tl	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1				
V	0.779 **	n.s.	0.667 *	n.s.	n.s.	n.s.	n.s.	n.s.	0.852 **	1			
Zn	n.s.	0.825 **	0.610 *	0.890 **	0.650 *	0.650 *	0.801 **	0.759 **	n.s.	n.s.	1		
EC	n.s.	0.817 **	0.641 *	0.847 **	0.684 *	n.s.	0.846 **	0.839 **	n.s.	n.s.	0.787 **	1	
F_V/F_M	n.s.	-0.653 *	n.s.	-0.719 *	-0.632 *	n.s.	-0.735 **	-0.741 **	n.s.	n.s.	-0.834 **	-0.669 *	1

n.s. = not significant; * p < 0.01; ** p < 0.001

Table 3. Content of heavy elements (data in mg/kg ± standard deviation) in soil samples collected in the monitoring sites of the Cà Mascio MSWL area.

Sites	As	Cd	Co	Cr	Cu	Ni	Pb	Sb	Tl	V	Zn
Background											
MV-14	6.7 ± 0.17	0.23 ± 0.02	14.2 ± 0.23	102 ± 1.3	28.7 ± 0.41	63.7 ± 0.72	16 ± 0.18	0.41 ± 0.02	0.41 ± 0.009	95.1 ± 1.2	90.5 ± 1.1
MV-15	6.2 ± 0.28	0.26 ± 0.02	13.1 ± 0.29	107 ± 1.7	35.6 ± 0.64	60.2 ± 0.75	19.6 ± 0.34	0.52 ± 0.04	0.44 ± 0.007	101 ± 2.3	98.1 ± 1.8
MV-16	6.9 ± 0.19	0.21 ± 0.04	13.9 ± 0.31	99.4 ± 2.1	38.7 ± 0.55	58.1 ± 0.65	14.3 ± 0.22	0.44 ± 0.03	0.39 ± 0.007	92.9 ± 1.8	92.2 ± 1.8
MV-17	6.3 ± 0.21	0.25 ± 0.03	12.7 ± 0.2	105 ± 3	30.1 ± 0.54	62.4 ± 0.97	18.5 ± 0.41	0.5 ± 0.05	0.42 ± 0.008	108 ± 2.7	97.5 ± 2
Rural											
MV-4	5.9 ± 0.1	0.19 ± 0.02	13.7 ± 0.4	96.3 ± 1.9	29.9 ± 0.46	60.9 ± 0.91	13.1 ± 0.06	0.49 ± 0.02	0.4 ± 0.006	93.5 ± 1.8	104 ± 1.2
MV-5	7.3 ± 0.25	0.2 ± 0.03	13 ± 0.16	87.8 ± 0.8	25.2 ± 0.4	55.9 ± 0.62	14.5 ± 0.22	0.44 ± 0.01	0.38 ± 0.012	80.8 ± 1.3	85.3 ± 0.69
MV-10	8.9 ± 0.1	0.24 ± 0.03	14.3 ± 0.37	106 ± 1.7	32.2 ± 0.29	62.6 ± 1.1	15.5 ± 0.12	0.49 ± 0.02	0.4 ± 0.011	85.4 ± 1.5	93.4 ± 2
MV-12	7.3 ± 0.11	0.15 ± 0.04	10.7 ± 0.3	69.4 ± 0.9	19.2 ± 0.46	43.1 ± 0.94	12.5 ± 0.16	0.32 ± 0.02	0.39 ± 0.008	49.3 ± 0.7	59 ± 0.63
Fall-out											
MV-1	7.4 ± 0.15	0.18 ± 0.02	13.1 ± 0.15	97.8 ± 1.1	29.4 ± 0.42	60 ± 0.47	13.5 ± 0.11	0.53 ± 0.01	0.36 ± 0.009	93.9 ± 1.2	94.3 ± 1.8
MV-8	8.8 ± 0.2	0.13 ± 0.02	12.9 ± 0.23	88.7 ± 0.9	25.5 ± 0.21	52.7 ± 0.69	11.6 ± 0.12	0.42 ± 0.02	0.4 ± 0.012	82.2 ± 0.8	83.6 ± 0.61
MV-9	7.5 ± 0.23	0.22 ± 0.01	13.2 ± 0.26	81.3 ± 1.1	23.8 ± 0.21	54.6 ± 0.91	15.8 ± 0.13	0.42 ± 0.01	0.37 ± 0.008	71.5 ± 1.2	76.9 ± 1.5
MV-11	7.2 ± 0.14	0.22 ± 0.01	13.6 ± 0.21	103 ± 0.7	28.7 ± 0.33	61.9 ± 0.75	16.7 ± 0.25	0.51 ± 0.02	0.4 ± 0.003	98.6 ± 1.2	95.2 ± 1.9
Landfill											
MV-2	7.1 ± 0.2	0.2 ± 0.02	13.9 ± 0.2	93.5 ± 1	25.7 ± 0.4	56.7 ± 0.62	15 ± 0.2	0.52 ± 0.01	0.38 ± 0.008	95.3 ± 1.1	95.8 ± 1.2
MV-3	7.1 ± 0.33	0.17 ± 0.05	11.3 ± 0.32	85.6 ± 0.9	22.5 ± 0.24	47 ± 0.84	11.1 ± 0.1	0.4 ± 0.02	0.32 ± 0.005	62.5 ± 1	73.8 ± 0.65
MV-6	7.9 ± 0.23	0.16 ± 0.01	13.7 ± 0.21	84 ± 1.1	25.6 ± 0.55	56.7 ± 0.74	14.2 ± 0.1	0.43 ± 0.02	0.41 ± 0.007	82.7 ± 1.2	83.6 ± 1.5
MV-7	7.5 ± 0.15	0.2 ± 0.03	12.9 ± 0.32	85.2 ± 1	27.4 ± 0.48	59.2 ± 1.2	13 ± 0.11	0.45 ± 0.02	0.36 ± 0.003	82.9 ± 1.4	87.6 ± 1.7
MV-13	7.9 ± 0.12	0.17 ± 0.03	12.6 ± 0.26	79.9 ± 1.4	23.3 ± 0.49	53.7 ± 1	12.6 ± 0.12	0.43 ± 0.02	0.37 ± 0.006	75.1 ± 1.1	79.9 ± 0.43
Contamination threshold in soils for public, private and residential green areas (Italian Legislative Decree n° 152/2006)											
	20	2	20	150	120	120	100	10	1	90	150