



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

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**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.

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

3

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36 **ABSTRACT**

37 This paper presents the results of a biomonitoring study to evaluate the environmental  
38 impact of airborne emissions from a municipal solid waste landfill in central Italy.  
39 Concentrations of 11 heavy elements, as well as photosynthetic efficiency and cell  
40 membrane integrity were measured in *Evernia prunastri* lichens transplanted for 4  
41 months in 17 monitoring sites around the waste landfill. Heavy element contents were  
42 also determined in surface soils. Analytical data indicated that emissions from the  
43 landfill affected Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn concentrations in lichens  
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47 indicated that landfill emissions had no relevant impact on lichens, as heavy element  
48 accumulation and weak stress symptoms were detected only in lichen transplants from  
49 sites close to solid waste. The appropriate management of this landfill poses a low risk  
50 of environmental contamination by heavy elements.

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

53

54 **1. Introduction**

55 Landfilling is currently the main method of solid waste disposal worldwide, as it is the  
56 simplest, cheapest and most cost-effective practice to store municipal solid waste  
57 (Giusti, 2009). However, the disposal of municipal solid waste in landfills causes  
58 concern about possible adverse effects on the environment and human health, such as  
59 fires and explosions, unpleasant odours, damage to vegetation, as well as air, soil and  
60 groundwater contamination (Chrysikou et al., 2008; El-Fadel et al., 1997; Vrijheid,  
61 2000). These adverse effects are mainly related to the release of inorganic and organic  
62 contaminants from the waste landfills via leachate and production of gases and  
63 particulate matter (Bogner and Matthews, 2003; Chalvatzaki et al., 2010; Koshy et al.,  
64 2009). Much attention has therefore been given to landfill emissions affecting air  
65 quality, and the EU has recently prescribed strict regulations on waste disposal in  
66 landfills (European Union, Directive 2008/98/EC).

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

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

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

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

92 Lichens are one of the most sensitive components of the ecosystem. These organisms  
93 are able to absorb and accumulate contaminants in their thallus, intercepting airborne  
94 materials and solutes of wet and dry precipitations, as well as atmospheric gases (Nash,  
95 2008). Lichens are thus widely used as biomonitors and bioaccumulators of  
96 contaminants in air quality and environmental contamination surveys (i.e., Conti and  
97 Cecchetti, 2001; Loppi et al., 1997; Nimis et al., 2002; Wolterbeek, 2002).

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

106 Lichens are widely used in biomonitoring studies, which employ either native species,  
107 that is species naturally present in the study area (Augusto et al., 2007; Blasco et al.,  
108 2008), or transplanted species (Baptista et al., 2008; Bergamaschi et al., 2007; Frati et  
109 al., 2005; Sorbo et al., 2008). The latter technique involves the use of thalli removed  
110 from areas with little or no contamination and transplanted for a period in selected  
111 monitoring sites. Transplant techniques are frequently used when lichens are scarce or  
112 absent in the study area.

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

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

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

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

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

145

146

## 147 **2. Material and methods**

148

### 149 **2.1. Study area**

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

154 (cereals), but there are also pastures and woodlands. The prevalent direction of winds is  
155 from SSW and SW (Fig. 1).

156 In the study area, pliocenic marine sediments belonging to the Argille azzurre formation  
157 crop out. This lithostratigraphic unit mainly consists of blue-gray clays and marly clays,  
158 with interbedded yellowish sandstones and silty clays.

159 According to the Italian law (Italian Legislative Decree n° 36/2003), the Cà Mascio  
160 landfill is classified as “landfill for municipal and not hazardous waste”. This landfill  
161 consists of seven batches: the old batches, numbered from 1 to 6, were used from 1984  
162 to 2000, and overall they contain about 600,000 m<sup>3</sup> of solid waste. These old batches  
163 were covered by a layer of soil and vegetation. The batch 7 was utilized since 2001 and  
164 is still in use when this research was carried out (2011).

165

## 166 **2.2. Lichen sampling, transplant and laboratory treatment**

167 The lichen transplantation is an effective technique widely used for the determination of  
168 heavy element accumulation and for assessing the variation of physiological  
169 characteristic (Ayrault et al., 2007; Conti et al., 2004; Godinho et al., 2008; Mikhailova,  
170 2002; Pacheco et al., 2008 Sloof, 1995).

171 The use of lichen transplants in place of native lichens (lichens grown *in situ*) is mainly  
172 due to the following reasons: absence of native lichens in the study area, uniformity of  
173 the lichen species utilized in biomonitoring and of the exposure period, possibility to  
174 choose the monitoring sites and their number, knowledge of the concentration of  
175 chemical elements before exposure, possibility to evaluate the accumulation trend of  
176 chemical elements.

177 For these reasons, and especially because of the absence of native lichens, the transplant  
178 technique was employed to assess the influence of Cà Mascio landfill emissions on the  
179 air distribution of some heavy elements of environmental concern.

180 In February 2011 thalli of the fruticose *Evernia prunastri* (L.) Ach. lichen were  
181 randomly collected from tree twigs in a woody zone far from contamination sources,  
182 located about 25 km S of Siena (central Italy). The *E. prunastri* lichen was chosen  
183 because it is easily collected and transplanted. In addition, this species is one of the  
184 most used lichens in biomonitoring studies due to its bioaccumulation capacity and  
185 widespread distribution (Garty, 2001; Guttova et al., 2011; Loppi and Frati, 2006; Paoli  
186 et al., 2011).

187



## Figure 1

188

189

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

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

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

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

220 Native and transplanted lichens were air-dried and immersed in liquid nitrogen,  
221 pulverized and homogenized using a ceramic mortar and pestle. Only peripheral parts of

222 thalli (up to 2 cm from lobe tips) were used for analysis. Each transplanted sample was  
223 a mixture of all lichen thalli exposed in the monitoring site.

224

### 225 **2.3. Lichen analysis**

226 About 200 mg of powdered lichen material were solubilised with a mixture of 6 mL  
227 HNO<sub>3</sub> 70%, 1 mL H<sub>2</sub>O<sub>2</sub> 30% and 0.2 mL HF 60%, in Teflon bombs placed in a  
228 microwave digestion system (Milestone Ethos 900) for 30 min. Ultra-pure trace-grade  
229 reagents were employed and ultra-high-purity water was used for dilution. Solutions  
230 were filtered, diluted to 50 mL and stored in PE bottles before analysis. A certified  
231 reference material and a blank of the employed reagents were included in each digestion  
232 batch.

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

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

243

### 244 **2.4. Integrity of cell membranes**

245 To check the integrity of the plasma membrane, the difference in electrical conductivity  
246 was carried out by placing a fragment of lichen thalli in deionized water (Munzi et al.,  
247 2009; Paoli et al., 2011). This is a simple test to assess the integrity of the plasma  
248 membrane: the value of electrical conductivity is related to the degree of damage  
249 endured by cell membranes (Marques et al., 2005). In fact, permeability is altered in  
250 damaged cell membranes and electrolyte leakage (mainly K<sup>+</sup>) occurs (McKersie et al.,  
251 1982).

252 Each lichen sample (about 100 mg of young portion of thalli up to 2 cm from lobe tips)  
253 was rinsed several times in deionised water for 3-5 seconds, until stable values of  
254 electrical conductivity were obtained. This rinsing was carried out to remove the  
255 particles deposited onto the lichen surface that contribute to the electrical conductivity

256 of sample. Afterwards, the lichen sample was immersed into a glass bottle with 50 mL  
257 of deionised water and shaken for 60 min. The electrical conductivity of water was  
258 measured before and after the lichen was soaked using the conductivity-meter Delta  
259 Ohm HD/8706. Thalli were thus boiled for 10 min at 100 °C to determine the total  
260 disruption of cell membranes, and electrical conductivity of water was measured again.  
261 The values of electrical conductivity were expressed in  $\mu\text{S cm}^{-1} \text{ mL mg}^{-1}$  dry weight at a  
262 normalized temperature of 25 °C.

263

### 264 ***2.5. Photosynthetic efficiency***

265 Photosynthetic efficiency was measured with a plant efficiency analyzer (Handy PEA,  
266 Hansatech Instruments Ltd) on well-wet and dark-adapted lichen samples, applying a  
267 saturating flash of red light (650 nm) of  $2400 \mu\text{mol s}^{-1} \text{ m}^{-2}$  for 1 sec. To block the  
268 physiological state at the time of the end of the transplant, lichen samples were air-dried  
269 and stored at -20 °C in paper bags. Before measurements, a physiological recovery of  
270 the samples was carried out. To avoid any osmotic stress by air humidity during de-  
271 freezing, lichens were left in dry ambient conditions for 15 min. Thalli were  
272 subsequently sprayed with water until wet and the water in excess was removed.  
273 Samples were then stored at 4 °C in the dark for 24 h. The outermost 2 cm of thalli was  
274 randomly selected for measurements. The selected lichen material was placed within a  
275 clip for 10 min to allow full dark adaptation of the photosynthetic pigments. The ratio of  
276 variable fluorescence to the maximal fluorescence ( $F_v/F_M$ ) was used to assess the  
277 potential quantum yield of primary photochemistry of PS II (Maxwell and Johnson,  
278 2000).

279

### 280 ***2.6. Soil sampling, laboratory treatment and analysis***

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

284 In laboratory litter material was manually removed and soil samples were dried at +40  
285 °C and sieved through a 2 mm mesh. Homogenization was carried out by quartering and  
286 pulverization procedures. Soil samples were solubilised by acid digestion adding 2 mL  
287  $\text{HNO}_3$  70%, 2 mL HF 60% and 1 mL  $\text{H}_2\text{O}_2$  30% to 250 mg of powdered soil. Ultra-pure  
288 trace-grade reagents were used for soil preparation. The mixture was processed in  
289 Teflon bombs using a microwave lab station. The solution was filtered and diluted with

290 ultra-pure water to a final volume of 100 mL. A certified reference material and a blank  
291 of the employed reagents were included in each digestion batch.

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

294 The accuracy of analytical determinations was checked using the NIST 2709 (San  
295 Joaquin soil) and NIST 2710 (Montana soil) certified reference materials of the  
296 National Institute of Standards and Technology. The recoveries were from 91% (Co) to  
297 106% (Sb). The precision was determined by five replicate analyses of each soil sample  
298 and expressed as percent relative standard deviation (% RSD). The analytical precision  
299 was within 8% for all the analyzed chemical elements.

300

### 301 ***2.7. Data interpretation and statistical analysis***

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

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

315

## 316 **3. Results**

317

### 318 ***3.1. Heavy element accumulation in transplant lichens***

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

### Table 1

324

325

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

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

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

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

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

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

356 In summary, EC ratios indicated that Cr, Pb, Sb and Zn were the main airborne  
357 contaminants deriving from Cà Mascio MSWL emissions. Taking into account the EC

358 ratios in lichen transplants from landfill sites, the order of accumulation was the  
359 following:  $Sb \approx Cr > Pb > Zn > Cd \approx Cu > Co > Ni$ . The transplants in the landfill and  
360 fallout sites showed the highest levels of accumulation, whereas heavy element  
361 concentrations in lichens from rural and background sites were generally comparable to  
362 those measured in native lichens from an uncontaminated area of central Italy.

363

364

365

## Figure 2

### 366 *3.2. Physiological parameters of transplant lichens*

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

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

377

378

## Figure 3

379

### 380 *3.3. Correlation among heavy element concentrations and physiological parameters* 381 *in lichen transplants*

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

385

386

## Table 2

387

388 The most significant positive correlations ( $p < 0.001$ ) were found among the  
389 concentrations of heavy elements most accumulated by lichens, i.e. Cd, Cr, Cu, Pb, Sb  
390 and Zn. Electrical conductivity was positively correlated with the concentrations of  
391 these contaminants ( $p < 0.001$ ). Significant negative correlations ( $p < 0.01$ ) were found  
392 among photosynthetic efficiency and Cd, Cr, Cu, Pb, Sb and Zn concentrations. These

393 findings are consistent with the fact that higher electrical conductivity and lower  
394 photosynthetic efficiency values correspond to higher heavy element accumulation by  
395 lichens.

396

### 397 **3.4. Heavy element contents in soils**

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

408 As shown in Table 3, heavy element contents in soils from the study area were rather  
409 homogeneous. Using the EF scale proposed by Sutherland (2000), no enrichment was  
410 found: EF values were usually less than 1, with mean values from 0.7 for Co, Cu and Pb  
411 1.1 for As. These results suggest that heavy element contents in soils collected close to  
412 the landfill (landfill sites) and along a transect in the direction of prevailing winds  
413 (fallout sites) were within their respective local natural variability in soil. Therefore, the  
414 heavy element contents in soils from the study area must be considered geogenic, due to  
415 natural factors and processes such as the nature of the parent rock (clays and marly  
416 clays of the Argille azzurre formation), and the features of weathering and pedogenesis.  
417 Heavy element concentrations in soil samples were constantly below contamination  
418 thresholds for green public, private and residential areas set by the Italian guidelines  
419 (Italian Legislative Decree n° 152/2006; Tab. 4). Vanadium contents slightly above its  
420 contamination threshold (90 mg/kg) were found in 6 soil samples randomly distributed  
421 in the study area.

422

423

### **Table 3**

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#### 427 **4. Discussion**

428 It is known that the chemical composition of lichens mainly depends on the availability  
429 of chemicals in the environment and that lichens respond to environmental changes  
430 (Bačkor and Loppi, 2009; Nimis et al., 2002). Lichens are able to head off and  
431 accumulate heavy elements from wet depositions and trapped airborne particles. This  
432 ability may be affected by environmental and climatic conditions as well as by the  
433 exposure time (Wolterbeek, 2002). Garty et al. (1993; 2001) demonstrated that thalli  
434 transplanted from uncontaminated to contaminated areas undergo changes, mainly due  
435 to the effects of contaminants. Moreover, several studies have shown that lichens  
436 transplanted close to contamination sources can uptake significant amounts of heavy  
437 elements within a few weeks or months (Bargagli, 1998; Conti et al., 2004; Paoli et al.,  
438 2011).

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

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

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



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

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

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

475 Electrical conductivity is a good indicator of air contamination, as this parameter  
476 reveals the degree of damage to cell membranes in lichens (Munzi et al., 2009; Paoli et  
477 al., 2011; Pearson and Henriksson, 1981). This is because the plasma membrane is the  
478 first site of biological interaction with toxic substances, including heavy elements.

479 Electrical conductivity values from this study reveal that the damage to cell membranes  
480 of lichens exposed in the monitoring sites differs from that of lichens in control site.

481 The electrical conductivity of transplanted lichens varied as a function of site location:  
482 the highest values characterized the thalli exposed in the landfill and fallout sites,  
483 suggesting that the highest degree of cell membrane damage concerned the transplants  
484 within the Cà Mascio MSWL and the sector most affected by landfill emissions. These  
485 differences may be due to the higher heavy element concentrations in the landfill and  
486 fallout dispersion areas. Garty et al. (1998a) observed a similar difference in electrical  
487 conductivity between lichens exposed in industrial sites and urban centres and those  
488 exposed in rural sites.

489 The significant positive correlations between the concentrations of the main heavy-  
490 element contaminants in the study area (Cr, Pb, Sb and Zn) and the values of electrical  
491 conductivity in exposed lichens confirmed the hypothesis above. This is consistent with  
492 the results of other studies that found higher values of electrical conductivity in lichen  
493 transplants affected by heavy element accumulation (Adamo et al., 2003; Garty et al.,

494 2002; Garty et al., 1998b). Paoli and Loppi (2008) also observed that cell membrane  
495 damage in *E. prunastri* lichens was correlated with air quality.

496 Photosynthetic activity, expressed as  $F_V/F_M$ , has often been employed in biomonitoring  
497 studies as it is considered a general index of the health of lichens and can be used to  
498 assess their vitality. The photosynthetic activity of lichens in the field is influenced by  
499 several factors, including environmental conditions and anthropogenic disturbances  
500 (i.e., high levels of  $SO_2$ ,  $NO_x$  and heavy elements). The accumulation of certain air  
501 contaminants in lichen thalli is assumed to coincide with low  $F_V/F_M$  ratios. The  $F_V/F_M$   
502 ratio of lichens growing in uncontaminated areas (healthy lichens) usually varies from  
503 0.5 to 0.76 (Jensen and Kricke, 2002), and values lower than 0.5 reveal that lichens  
504 were exposed to stress. Several studies (i.e., Garty et al., 2000; Karakoti et al., 2014)  
505 reported low  $F_V/F_M$  ratios for lichens characterized by high heavy element  
506 concentrations.

507 The  $F_V/F_M$  ratios for lichens exposed in the landfill and fallout sites suggested that  
508 environmental conditions within and around the Cà Mascio MSWL caused a decrease in  
509 photosynthetic activity. However, the  $F_V/F_M$  ratios of transplanted lichens ranged from  
510 0.51 to 0.66, in agreement with non-stressed conditions for lichens (Jensen and Kricke,  
511 2002). As photosynthetic activity is considered a sensitive indicator of contamination  
512 stress, this finding suggested that airborne heavy element contamination due to landfill  
513 emissions was spatially limited in the study area and not sufficiently severe to determine  
514 significant changes in this physiological parameter.

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

525

526

527

528 **5. Conclusions**

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

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

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

552

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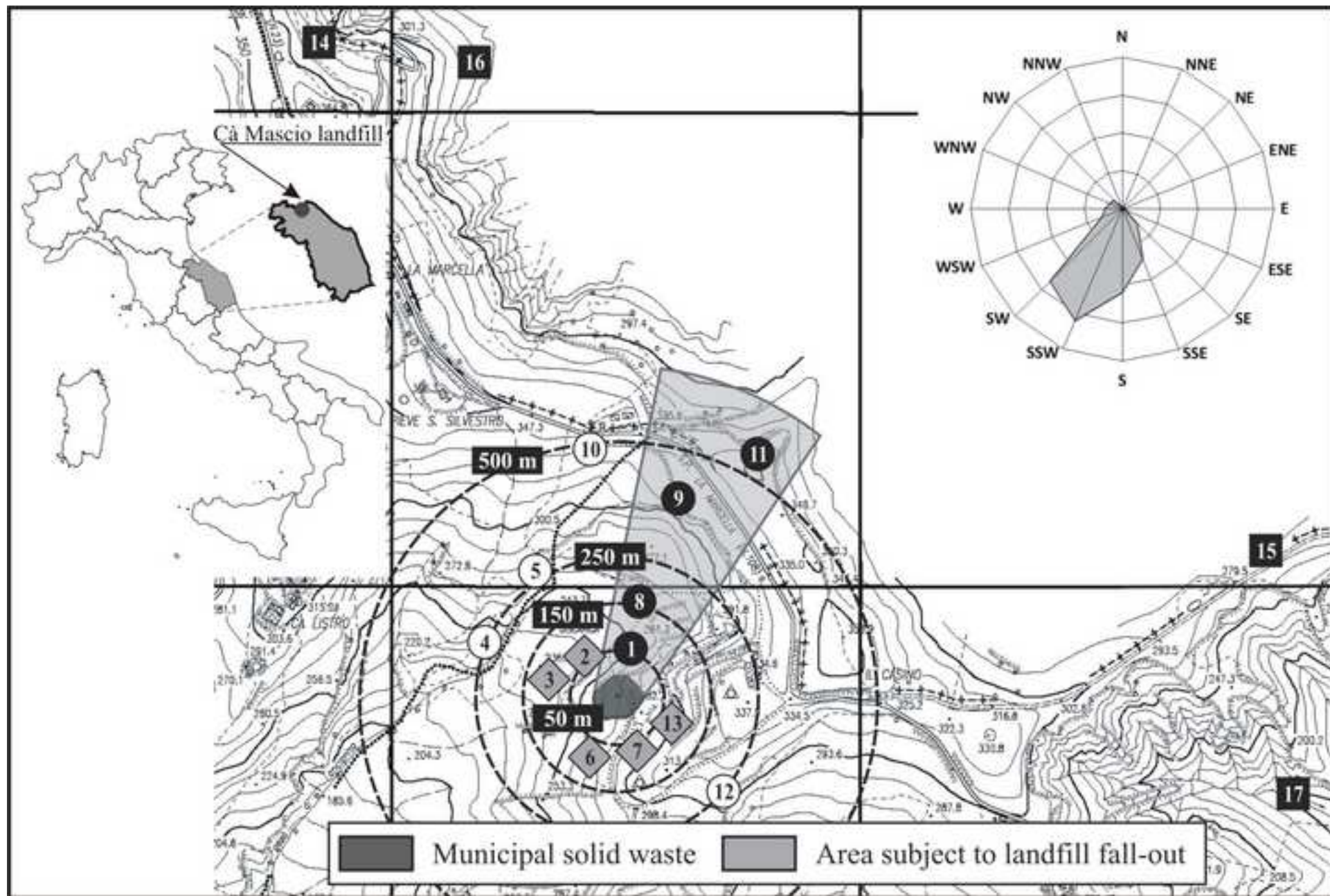
## FIGURE CAPTIONS

**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.

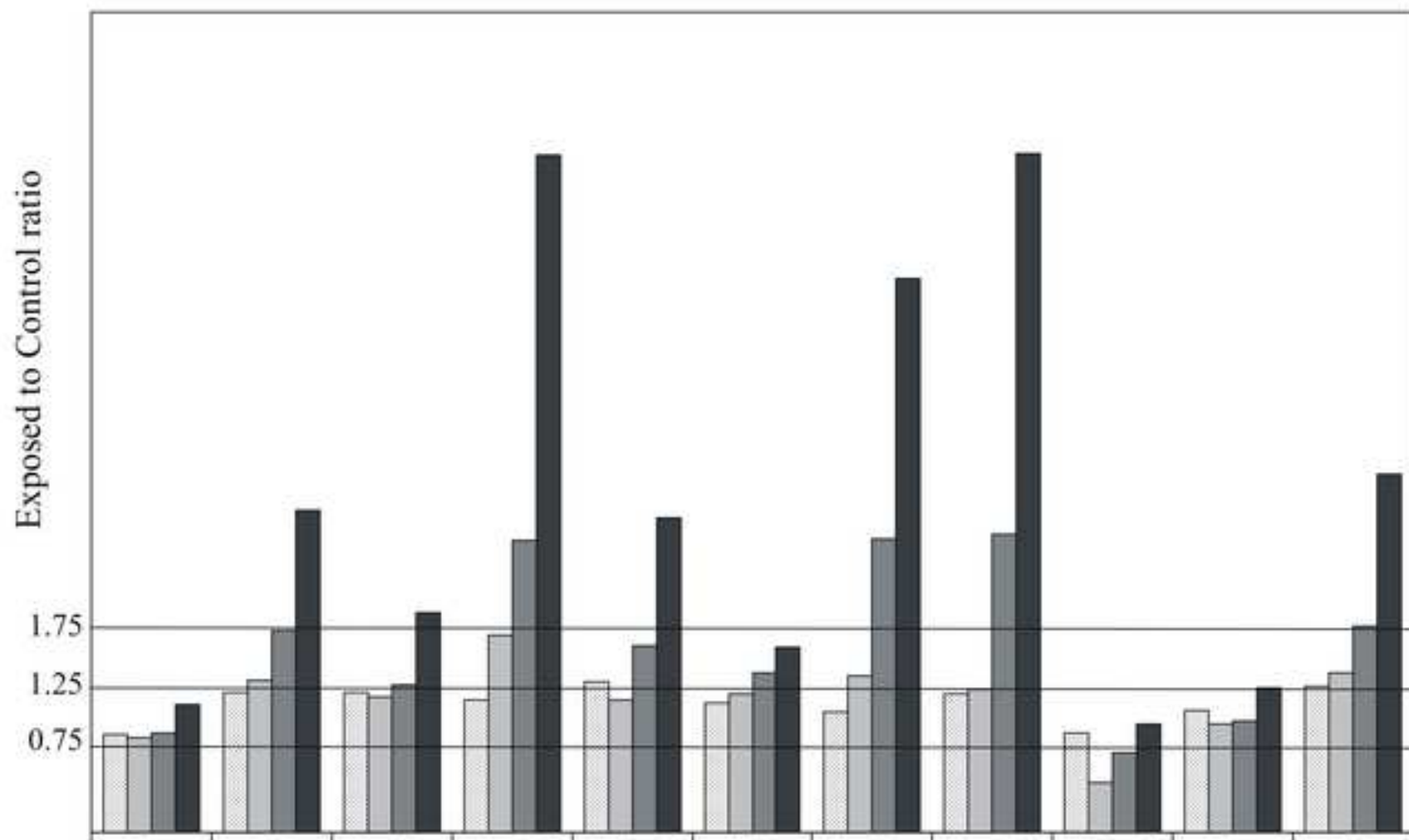
**Figure 3.** Values (mean  $\pm$  standard deviation) of electrical conductivity (a) and photosynthetic efficiency (b) in *E. prunastri* lichens collected in the control site and transplanted in the monitoring sites (background, rural, fallout and landfill) of the Cà Mascio MSWL area. \* Significant 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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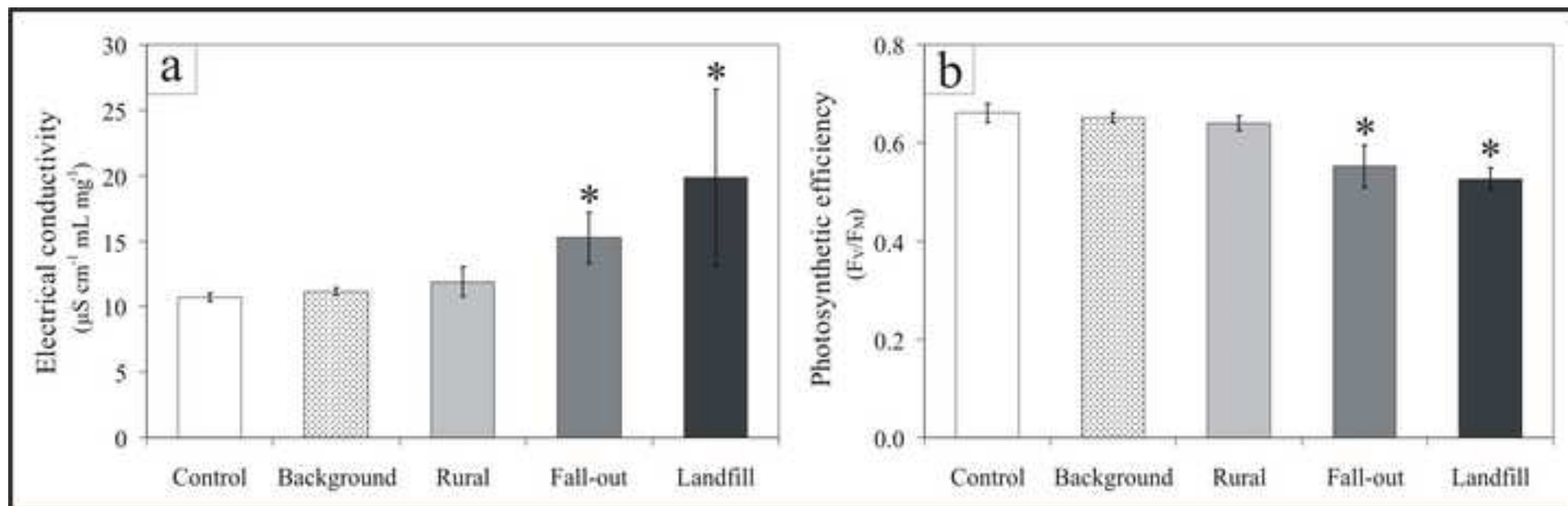


	As	Cd	Co	Cr	Cu	Ni	Pb	Sb	Tl	V	Zn
Monitoring sites	EC ratio	EC ratio	EC ratio	EC ratio	EC ratio	EC ratio	EC ratio	EC ratio	EC ratio	EC ratio	EC ratio
Background	0.83 N	1.19 N	1.19 N	1.13 N	1.28 A	1.10 N	1.03 N	1.18 N	0.85 N	1.04 N	1.25 N
Rural	0.81 N	1.30 A	1.15 N	1.68 A	1.13 N	1.19 N	1.33 A	1.21 N	0.43 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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1 **Table 1.** Concentrations and mean values of heavy elements (data in mg/kg  $\pm$  standard deviation) in *E. prunastri* lichens collected in the control site  
 2 and transplanted in the monitoring sites of the Cà Mascio MSWL area.  
 3

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

5 **Table 2.** Spearman correlation coefficients among the heavy element concentrations and physiological parameters [electrical conductivity (EC) and  
6 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; \*  $p < 0.01$ ; \*\*  $p < 0.001$

8

9 **Table 3.** Content of heavy elements (data in mg/kg  $\pm$  standard deviation) in soil samples collected in the monitoring sites of the Cà Mascio  
 10 MSWL area.  
 11

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