**SOIL ELEMENT FRACTIONS AFFECT PHYTOTOXICITY, MICROBIAL BIOMASS
AND ACTIVITY IN VOLCANIC AREAS**

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Abstract

Soil quality is strongly affected by microbial biomass that is involved in organic matter mineralization and the supply of nutrients to plants. The effects of trace elements on soil microbial biomass and activity are still controversial, and the contents of the elements in different forms, more than the total amounts, may affect soil microbial community. Volcanic soils are peculiar environments because of their chemical characteristics. Therefore, the aims of this research were to evaluate in volcanic soils: i) the elemental composition; ii) the elemental availability; and iii) the effects of elemental fractions on soil microbial biomass and activity. In order to reach the aims, the BCR sequential extraction method was applied in order to separate 22 elements in different soil fractions: acid soluble, reducible, oxidasable and residual. The studied biological parameters were: microbial and fungal biomasses, soil respiration, metabolic quotient, coefficient of endogenous mineralization, dehydrogenase and hydrolase activities, and phytotoxicity. Among the investigated elements, Al, B, Ba, Ca, Cr, Fe, K, Mg, Mn, Na, Ni, Ti, V and Zn were scarcely available; Cd appeared to be the most ready available element; Zn was mainly present in the acid soluble and in the residual fractions. Microbial biomass and activity appeared to be mainly affected by the reducible and oxidasable fractions of the investigated elements more than the acid soluble or residual ones. With the exception of La and V, the elemental content in the various fractions would seem to stimulate the microbial biomass and activity. Finally, the investigated volcanic soils showed phytotoxic properties.

Key words: acid soluble fraction; reducible fraction; oxidasable fraction; residual fraction; soil enzymatic activity; phytotoxicity.

1. Introduction

Soil health and plant productivity are strongly affected by microbial biomass as it is involved in organic matter mineralization and the supply of nutrients to plants (Bertiller et al., 2009; Nannipieri et al., 2002). Soil factors such as pH, organic matter and water content control microbial growth and activity (Doran and Parkin, 1996; Majer et al., 2002; Mungai et al., 2005; Yadav and Tarafdar, 2001). In addition, the amount of elements in soils can modify the spatial distribution of microorganisms, their enzymatic activity and influence microbial growth (Hansda et al., 2017; Vasquez-Murrieta et al., 2006).

According to kind and amount, elements differently regulate soil biota (Hu et al., 2014). In fact, some elements (*e.g.*, copper, iron, manganese and zinc), having a crucial role in metabolic processes, are essential to ensure plant and animal life, to guarantee the organic matter cycle and to maintain soil functionality; other elements (*e.g.*, cadmium and arsenic) are non-essential, are not involved in biological functions, and can cause damage to organisms also at low concentrations. However, the effects of trace elements on soil biota are still controversial. For instance, Zeng et al. (2007) reported that Pb, a well-known toxic element, can have stimulating effects on soil microbial biomass and activities at low concentrations and inhibitory effects at higher concentrations. In soils affected by exposure of heavy metals for long time, microbial metal tolerance may also develop (Giller et al., 1998).

In addition, soil microorganism activity can regulate element availability. For example, microorganisms involved in the biogeochemical cycling of Fe couple the oxidation of organic matter with the reduction of Fe (III) oxyhydroxides to obtain energy for their metabolism (Roden and Zachara, 1996; Wahid and Kamalam, 1993) and, contextually, the elements associated with Fe (III) mineral phases such as As, Cr, Cd or Pb can be solubilized in the soils (Bonneville et al., 2004). Soil enzymes play vital roles in soils (Angelovičová and Fazekašová, 2014), and, being very sensitive to disturbances (Bhattacharya et al., 2008; Hu et al., 2014), are important components in soil quality assessment. As enzymatic activities in soil provide useful information on extent of

75 environmental impact of toxic metals, some authors (Hinojosa et al., 2004) recommend studying
76 them in addition to soil physico-chemical properties. In particular, dehydrogenase and hydrolase
77 activities are recognized as general indicators of microbial activity in soils (Adam and Duncan,
78 2001; Alef and Nannipieri, 1995; Lundgren, 1981).

79 In the last decades, it is widely reported that soil total element content is not enough to provide
80 information about their effects on biomass or activity of the edaphic community (Krishnamurti and
81 Naidu, 2003; Van Peijnenburg et al., 1997). In fact, elements are present in soils in different forms,
82 such as acid soluble, exchangeable, carbonate-associated, Fe-Mn oxide-associated, organic-
83 associated and residual (Fernández-Ondoño et al., 2017). Element mobility and availability are
84 strongly affected by soil chemical and physical characteristics (Degryse et al., 2009; Meers et al.,
85 2007; Van Peijnenburg et al., 2007), although a contribution due to microbial biomass and activity
86 cannot be excluded (Li et al., 2009).

87 Volcanic soils, derived from pyroclastic materials (Shoji and Ono, 1978), have high baseline
88 contents of some trace elements (De Nicola et al., 2003; Giammanco et al., 1996; Maisto et al.,
89 2006) and are rich in inorganic and organic compounds that bind elements (Eswaran et al., 1993;
90 Tanneberg et al., 2001), regulating their fate in various fractions (Maeda et al., 2003). Moreover,
91 physico-chemical properties of the soil within preferential flow paths may be different than those of
92 matrix flow soil resulting, for instance, in different microbial activity (Bundt et al., 2001a) in both
93 flow domains. In addition, Bundt et al. (2001b) found that the soil of the preferential flow paths can
94 adsorb specifically organically bound metals to a larger extent than in the soil matrix due to its
95 greater content in organic matter. Other authors (Knechtenhofer et al., 2003; McBride et al., 1997;
96 Richards et al., 1998) have also described preferential flow paths in the soil matrix. In order to
97 evaluate whether metal distribution in the soil is mainly controlled by water flow or characteristics
98 of the soil matrix, further investigations are needed, especially in volcanic soils that are particularly
99 rich in some micronutrients and metals.

100 The aims of the present research were to evaluate: i) the elemental composition of the volcanic
101 soils inside Vesuvius National Park; ii) the availability of elements; and iii) the effects of elemental
102 fractions on soil microbial biomass and activity.

103

104 **2. Materials and Methods**

105 2.1 Soil sampling

106 The research was carried out in Vesuvius National Park, established in 1995, which is located 20
107 km SE of Naples and contains Mt. Somma, the original volcano, and Mt. Vesuvius originating from
108 the 79 AD eruption.

109 Inside the park, nine sites were selected to collect surface (0-10 cm) soil samples, as reported in
110 Supplementary material 1. The sampling was carried out on November 2016 and, at each site, five
111 subsamples of soil were collected, after litter removal, and mixed together in order to obtain a
112 representative composite sample to perform the physico-chemical, biological and
113 phytotoxicological analyses.

114

115 2.2 Physico-chemical analyses

116 Fresh soil samples were sieved (< 2 mm). pH was measured on aqueous extracts obtained by
117 adding distilled water to soils (2.5:1 = v:v). The water content was determined by drying fresh soil
118 at 105 °C to reach a constant weight. The organic carbon content (C_{org}) was measured on pulverized
119 and dried (at 105 °C) samples previously treated with HCl (10%) by gas chromatography (Thermo
120 Finnigan, CNS Analyzer). Soil organic matter content was calculated multiplying the C_{org} content
121 by 1.724 as reported by Pribyl (2010). The total carbon, nitrogen and sulfur concentrations were
122 determined by elemental analysis (LECO CHNS-932 analyzer, USA).

123 Sequential extraction was applied to measure the concentrations of the elements in the various
124 fractions of the soils as suggested by Ure et al. (1993). These extraction procedures operationally
125 separate the elements into: acid soluble, reducible (associated to Fe-Mn oxides), oxidizable

126 (associated to organic matter content and sulphides) and residual fractions (associated to minerals).
127 According to many authors (He et al., 2006; Ma and Rao, 1997), the first fractions (F1) are
128 considered as bioavailable, the second and third fractions (F2 and F3, respectively) can be
129 potentially bioavailable, whereas the last fraction (F4) is considered not available for organisms.
130 The method for sequential extraction is detailed in Memoli et al. (2018) and briefly reported below.

131 Fraction 1 (acid soluble fraction, F1): 1 g soil sample was extracted with 40 mL of 0.11 mol L⁻¹
132 acetic acid solution by shaking in a mechanical shaker at 30 ± 10 rpm at 22 ± 5 °C for 16 h. The
133 extract was separated by centrifugation at 5000 rpm g for 20 min, collected in polyethylene bottles
134 and stored at 4 °C until analysis. The residue was washed by shaking for 15 min with 20 mL of
135 doubly deionised water and then centrifuged, discarding the supernatant.

136 Fraction 2 (reducible fraction, F2): 40 ml of 0.5 mol L⁻¹ hydroxylammonium chloride solution at
137 pH 1.5 was added to the residue from the first step, and the mixture was shaken 30 ± 10 rpm at 22 ±
138 5 °C for 16 h. The extract was separated and the residue was washed as in the first step.

139 Fraction 3 (oxidisable fraction, F3): 10 mL of 8.8 mol L⁻¹ hydrogen peroxide solution was
140 carefully added to the residue from the second step. The mixture was digested for 1 h at 22 ± 5 °C
141 and for 1 h at 85 ± 2 °C, and the volume was reduced to less than 3 mL. A second aliquot of 10 mL
142 of H₂O₂ was added, the mixture was digested for 1 h at 85 ± 2 °C, and the volume was reduced to
143 about 1 mL. The residue was extracted with 50 mL of 1 mol L⁻¹ ammonium acetate solution pH 2.0
144 at 30 ± 10 rpm and 22 ± 5 °C for 16 h. The extract was separated and the residue was washed as in
145 previous steps.

146 Fraction 4 (residual fraction, F4): the residue from the previous step was digested with aqua
147 regia. In this case, 9.0 mL of HCl (37%) and 3 mL of HNO₃ (69%) were added to 250 mg of soil.

148 The samples were digested in a microwave oven (CEM MarsX press, USA) according to the
149 procedure described in García-Delgado et al. (2012). The element concentrations in the solutions
150 obtained in each step were determined by ICP-MS (Perkin-Elmer NexION 300). The sum of the
151 concentrations of each element in the four fractions is considered as pseudo-total. One lake

152 sediment sample (BCR-701) certified or with indicative values for extractable metal contents in the
153 three steps of the modified BCR sequential extraction procedure, and indicative values for aqua
154 regia extraction (Rauret et al., 2000), was used to ensure the quality of the results obtained. The
155 accuracy of the obtained values ranged between 80 and 140%.

156 The physico-chemical analyses were carried out on three technical replicates (number of
157 composite samples: 9; number of replicates: 3).

158

159 2. 3 Biological and phytotoxicological analyses

160 The biological analyses were performed within three days from the collection on soil samples
161 stored at 4 °C. Microbial carbon (C_{mic}) was evaluated by the method of substrate-induced
162 respiration (SIR) according to Anderson and Domsch (1978). Total fungal biomass (TFB) was
163 assayed by membrane filter technique (Sundman and Sivela, 1978), after staining with Aniline Blue,
164 determining hypha length by the intersection method (Olson, 1950) with an optical microscope
165 (Optika, B-252). To obtain the fungal fraction of microbial carbon, the values of fungal biomass
166 were converted in fungal carbon (C_{fung}) on the basis of mean values reported for C/N ratio (Killham
167 1994) and N content (Swift et al., 1979) in fungi.

168 The microbial activity was estimated as potential respiration. The CO_2 evolution from the
169 samples at 55% of water holding capacity was measured after incubation in tight containers for 10
170 days at 25 °C by NaOH absorption followed by two-phase titration with HCl (Froment 1972). The
171 metabolic quotient, qCO_2 ($mg\ C-CO_2\ mg^{-1}\ C_{mic}$), *i.e.*, the degree of activity of the microbial
172 biomass, and the coefficient of endogenous mineralization, CEM ($mg\ C-CO_2\ g^{-1}\ C_{org}$), *i.e.*, the rate
173 of organic C mineralization, were calculated using respiration data and microbial C or organic C
174 data, respectively (Anderson and Domsch, 1993).

175 Dehydrogenase activity was determined by adding to 1 g of fresh soil 1 ml of 1.5 % 2,3,5-
176 triphenyltetrazolium chloride (TTC) dissolved in 0.1 M Tris-HCl buffer at pH 7.5. The reaction
177 mixture was incubated at 30 °C for 24 h in the dark. At the end of incubation, the triphenylformazan

178 (TFF) was extracted with 8 ml of acetone, and the extract was centrifuged at 3500 rpm for 15 min.
179 The absorbance of the supernatant was measured at 546 nm and the results were expressed as mmol
180 of TFF produced in one minute for 1 g of dry soil.

181 Hydrolase activity was determined by adding to 1 g of fresh soil 7.5 ml of 60 mM potassium
182 phosphate at pH 7.6 and 0.100 ml of fluorescein diacetate (FDA). The reaction mixture was
183 incubated at 30 °C for 20 min. At the end of incubation, the fluorescein was extracted with 7.5 ml of
184 acetone and the extract was centrifuged at 5000 rpm for 5 min. The absorbance of the supernatant
185 was measure at 490 nm and the the results were expressed as mmol of fluorescein produced in one
186 minute for 1 g of dry soil (Adam and Duncan, 2001).

187 Phytotoxicological assays were performed according to EPA (1996) using a monocotyledon
188 (*Sorghum saccharatum* L.) and a dicotyledon (*Lepidium sativum* L.). The phytotoxicity tests were
189 carried out on fresh and sieved (2 mm) samples. Ten seeds for each species were placed in Petri
190 dishes containing an amount of fresh soil equivalent to 10 g of oven-dried soil, subsequently
191 saturated with water. Standard soil (OECD, 1984) and K₂Cr₂O₇ were used as negative and positive
192 controls, respectively. After incubation in darkness (72 h, at 25 °C), the number of germinated
193 seeds and the total root length were measured. The results were expressed as effect percentage of
194 germination index (GI), root elongation (E) and seed germination (G) compared to a standard soil
195 (OECD, 1984).

196 All the biological and phytotoxicological analyses were carried out on triplicate samples
197 (number of composite samples: 9; number of replicates: 3).

198

199 2.4 Statistical analyses

200 To assess the normality of the distribution of the data sets, the Shapiro-Wilk test was performed.

201 In order to identify the elements that positively or negatively influenced the biological
202 parameters, four Principal Component Analyses (PCA) were carried out on matrixes containing the
203 mean values, calculated for the sites, of element contents in each fraction (F1: acid soluble, F2:

204 reducible, F3: oxidasable and F4: residual). Furthermore, for each PCA, the first axis scores were
205 correlated (Spearman's test) to the soil biological and phytotoxicological parameters.

206 The relationships between the biological or phytotoxicological characteristics and the element
207 contents in acid soluble (F1), reducible (F2), oxidasable (F3) and residual (F4) fractions of the
208 investigated soils were evaluated by Spearman's test, according to the non-normal distribution of
209 the data.

210 All the statistical tests were considered statistically significant when $P < 0.05$. The multivariate
211 statistical tests were performed by Past v. 3.15 (Øyvind Hammer, Oslo), the univariate statistical
212 tests were performed by the Systat_SigmaPlot_12.2 software (Jandel Scientific, San Rafael, CA,
213 USA). The graphs were created using the SigmaPlot12 software (Jandel Scientific, San Rafael, CA,
214 USA).

215

216 3. Results

217 3.1 Chemical, biological and phytotoxicological characteristics of the soils

218 The values of pH, organic matter and water contents as well as C, N and S concentrations in the
219 investigated volcanic soils are reported in Table 1. The pH values ranged from 6.46 to 7.98,
220 whereas the other parameters showed wide variability (*i.e.*, 7.45 – 46.7 % d.w. for organic matter
221 content; 14.1 – 102 % d.w. for water content; 1.78 – 26.3 % d.w. for C concentration; 0.11 – 0.67 %
222 d.w. for N concentration; 0.01 – 0.07 % d.w. for S concentration) among the soils (Table 1).

223 The microbial biomasses showed values of C_{mic} ranging from 0.53 and 2.69 mg C g⁻¹ d.w. and of
224 C_{fung} ranging between 0.003 and 0.07 mg C g⁻¹ d.w. (Table 2). The microbial activities also widely
225 varied (*i.e.*, 0.07 – 0.69 mg CO₂ g⁻¹ d.w. d⁻¹ for respiration; 0.32 – 1.93 mg C-CO₂ g⁻¹ C_{org} for CEM;
226 0.02 – 0.07 mg C-CO₂ mg g⁻¹ C_{mic} for qCO₂; 0.00 - 0.50 mmol TFF min⁻¹ g⁻¹ d.w. for DHA and
227 2.94 – 7.00 mmol FDA min⁻¹ g⁻¹ d.w. for HA) among the soils (Table 2).

228 The phytotoxicological assays showed biostimulating effects on seed germination and root
229 elongation for *L. sativum* and inhibiting effects for *S. saccharatum* (Table 2). The assays performed

230 with *L. sativum* showed wider variability as compared to those performed with *S. saccharatum*
231 (Table 2).

232 In the soils, the mean percentage contribution of each fraction to the pseudo-total concentration
233 showed similar trends for K, Cr, V, Al, Ti, Fe, Ni and Ba following gradient $F1 < F2 < F3 < F4$ (Fig.
234 1; Supplementary material 2). For these elements, the sum of the first three fractions represented
235 approximately the 20% of the pseudo-total concentrations (Fig. 1, Supplementary material 2). For B,
236 Ca, Na, Mg, Mn and Zn, the highest fraction was F4, but the gradient was $F2 < F1 < F3 < F4$, with
237 the exception of Zn that showed similar percentage contributes in F1 and F4, and in F2 and F3 (Fig.
238 1). P, Cu, Si, As, La, W and Pb showed the lowest percentage contribution in F1 and F2 and the
239 highest in F3 (Fig. 1). Differently from the other elements, Cd showed the gradient $F4 < F3 < F2 <$
240 $F1$ (Fig. 1).

241

242 3.2 Relationships among the element contents in different fractions and biological or
243 phytotoxicological characteristics of the soils

244 The performed PCAs highlighted that the same element in the various fractions (F1-F4)
245 differently affected the distribution of the investigated sites along the first axis (Fig. 2). The
246 correlations between the site distribution according the soil chemical composition and the biological
247 responses showed that CEM and HA were positively correlated to the first axis of the PCA
248 performed with the element contents in F1; qCO_2 , CEM, respiration and HA were positively
249 correlated to the first axis of the PCA performed with the element contents in F2; qCO_2 , respiration
250 and HA were positively correlated to the first axis of the PCA performed with the element contents
251 in F3 (Table 3).

252 In order to specifically evaluate the relationships between the element contents in each fraction
253 and the biological and phytotoxicological responses, single correlations were performed.

254 Microbial and fungal carbon contents and some biological activities (*i.e.*, respiration, CEM,
255 DHA and HA) were positively correlated to the contents of Mn, Ni and W in F1 and negatively

256 correlated to La in F1 (Table 4). The microbial carbon content was also positively correlated to the
257 contents of B and K in F1 (Table 4). The statistically significant correlations between the microbial
258 activities with the elements in the F1 fraction were mainly positive with the exception of those with
259 Al, Fe, La and P (Table 4).

260 The number of statistically significant correlations found between microbial and fungal
261 biomasses or biological activities with the element contents in the F2 or F3 fractions were higher
262 than that found for the F1 fraction. They were mainly positive with the exception of La, P and V in
263 F2 (Table 4). Few positive correlations were found between the microbial biomasses or activities
264 and the element contents in the F4 fraction (Table 4).

265 Seed germination and root elongation for *L. sativum* and *S. saccharatum* were unaffected by soil
266 elemental components of the F1, F2, or F3 extracts when considered together using PCA (Table 3).
267 Germination index and germination of *L. sativum* were negatively correlated with F4 components.
268 However, when viewed individually, seed behaviour of these species was positively linked to a
269 variety of different elements in F1 extracts, including Fe and La for *L. sativum* and heavy metals
270 such as Cd and Pb in *S. saccharatum* (Table 4). Root elongation was also stimulated by Pb in this
271 species. Extracts from F2 had few phytotoxicological impacts, excepting As in *L. sativum* (Table 4).
272 There was a suite of predominantly negative correlations among elements and *L. sativum* and a
273 stimulation by Cu in *S. saccharatum* (Table 4). Elements from F4 similarly negatively affected *L.*
274 *sativum* but had no effect on *S. saccharatum* (Table 4).

275

276 4. Discussion

277 The investigated volcanic soils presented high pseudo-total concentrations of Al, Ca, Fe and K
278 and low concentrations of Cd and Cr. These results are in agreement with Ramos et al. (2017) who
279 report, for volcanic rock powder deriving by Nova Prata region (Brazil), scarce retention of Cd and,
280 by contrast, high retention of Al, attributable to the presence of an aluminosilicate glassy matrix. In
281 addition, elemental inputs into the soil surface layer due to atmospheric deposition cannot be

282 excluded (De Nicola et al., 2003; Martínez-Cortizas et al., 2003). The comparison of the element
283 concentrations with those reported for soils of the Picos Fissural Volcanic System (Parelho et al.,
284 2014) highlighted that K, Cu, As, Ba and Pb were higher, whereas P, V, Cr, Mn, Ni and Zn were
285 lower in the investigated soils.

286 The low abundance of K, Cr, V, Al, Ti, Fe, Ni and Ba in F1, F2 and F3, with respect to the
287 pseudo-total concentrations, identified these elements as the most scarcely available. The same
288 behaviour was detected for B, Ca, Na, Mg, Mn and Zn although their relative abundances in F4, the
289 residual fraction, were lower (50-80%) than those observed for the previously cited elements (80-
290 90%). Therefore, sorption mechanisms of B, Ca, Na, Mg, Mn and Zn to inorganic and organic
291 components can be supposed in agreement with the findings reported by Adamo et al. (2003) for
292 volcanic soils of the Solofrana river valley. In addition, it has to be noted that B, Ca, Na, Mg and
293 Mn appeared to be mainly associated to the acid soluble (F1) or reducible fractions (F2), whereas
294 Cu, Si, As, La, W and Pb were associated with the oxidasable (F3) or residual fractions (F4).

295 Cd and Zn showed particular behaviours as compared to the other elements, as they showed high
296 availability in the acid soluble fraction that represented approximately the 60 and 40 % of the
297 pseudo-total concentration, respectively. In addition, the 30% of Cd was in the reducible fraction
298 and the 40% of Zn in the residual fraction. As the availabilities of Cd and Zn in the acid soluble
299 fraction of the investigated volcanic soils were higher than in other volcanic soils of Campania
300 Region (Adamo et al., 2003), it can be supposed that these elements were likely derived by human
301 activities according to Chlopecka et al. (1996). That was also the case of As, Cu, La, P, Pb, Si and
302 W that were associated to the oxidasable or residual fractions or Cr, V and Al that showed high
303 ratios between the sum of the first three fractions and the pseudo-total concentrations, reflecting a
304 great tendency to bioavailability (Xiao et al., 2015). In particular, Cr, which is considered acutely
305 toxic, would seem to form strong complexes with soil minerals and likely to be present in the less
306 available form, Cr (III), more than as Cr (VI); in fact Papassiopi et al. (2009) reported that, in soil

307 highly contaminated by Cr (VI), the percentage contribution of Cr acid soluble fraction decreases
308 whereas the oxidasable and reducible fractions increase.

309 Metal acid soluble, reducible and oxidasable fractions, showing high potential availability to
310 plants or soil-dwelling organisms, are considered to be reactive, whereas the residual fractions are
311 considered to be chemically and biologically inactive (Tanneberg et al., 2001; Ure et al., 1993). The
312 acid soluble fractions of B, K, Mn, Ni and W likely stimulated the microbial and fungal biomasses
313 as well as the microbial activities, suggesting that the metals in the acid soluble fractions were
314 present in contents lower than the threshold values to negatively affect them. Also Cd did not
315 negatively affect the biological activities in the investigated volcanic soils likely due to its presence
316 in fine particles and not in ready available ionic forms.

317 The acid soluble fractions of P and K (the main investigated macro-nutrients) would seem to
318 have opposite effects on the coefficient of endogenous mineralization and microbial respiration.
319 Overall, K would seem to be limiting organic matter decomposition whereas P, although poorly
320 represented with respect to the pseudo-total content, would not seem to be a limiting factor as also
321 confirmed by the mean value of C/P lower than 186 (Sinsabaugh et al., 2009). In fact, the
322 coefficient of endogenous mineralization and microbial respiration was enhanced with the increase
323 of K acid soluble fraction and with the decrease of P acid soluble fraction.

324 The performed PCAs highlighted that the sites exhibited different elemental composition among
325 each fraction. In addition, the contribution of the single element in the characterizing the soil of
326 each site depended on the considered fraction. For instance, As and Pb contributed in opposite
327 trends to characterize F1 but not F2-F4 of the investigated soils. Therefore, as universal trends for
328 single elements in all the fractions were not found, in order to evaluate the effects of soil chemical
329 composition on the biological parameters, the single correlations were taken into account. The
330 positive correlations found between biological parameters and metal reducible or oxidasable
331 fractions suggest that metals likely were strongly bound to various components of the volcanic soils,
332 such as Fe-Mn oxides, organic matter and sulphides that limited their bioavailabilities. This finding

333 agreed with the well-known association of Cr, Cu and As to soil organic matter in forming non-
334 soluble high molecular weight organic complexes (Balasoiu et al., 2001). By contrast, the negative
335 effects caused by the reducible fractions of La and V on many biological activities could be due to
336 the amount of Fe-Mn oxides insufficient to link them or to the weak linkages with Fe-Mn oxides.
337 These oxides precipitate in soils as a result of Mn^{2+} oxidation, promoted by bacteria and fungi, and
338 form highly reactive biofilms (Villalobos et al., 2006) that may explain the numerous positive
339 correlations between microbial activities and element contents in the reducible fraction. The adverse
340 effect of the V reducible fraction on dehydrogenase activity agrees with the results found by Xiao et
341 al. (2015) for stone coal smelting soils.

342 Overall, the scarce potential bioavailability (F1-F3) of the metals in the investigated volcanic
343 soils would seem to stimulate more than inhibit the microbial enzymatic activity likely due to un-
344 limiting availability of organic matter content that, precipitating potentially toxic metals as
345 insoluble complexes, can act as a protective cover for enzymes and can also form enzyme-humus
346 complexes that protect the activities of soil enzymes from metal inhibition (Coppolecchia et al.,
347 2011; D'Ascoli et al., 2006; Tripathy et al., 2014). The investigated soils would seem to be suitable
348 environments for microbial communities as higher values of microbial biomass, hydrolase and
349 dehydrogenase activities and lower respiration and qCO_2 values were observed as compared to
350 volcanic soils of non human impacted areas (D'Ascoli et al., 2006; Dube et al., 2009; Parelho et al.,
351 2016). The high biological activities in the investigated volcanic soils would seem to be due to the
352 relative high organic matter that supplies energy-rich substrates to soil microorganisms, fostering
353 the positive effects of lower potential metal bioavailability. Also soil pH could have affect on
354 enzymatic activities, as it controls both metal lability and enzyme production by microorganisms
355 (Coppolecchia et al., 2011; Xu et al., 2017).

356 Contrary to what was observed for microbial biomass and activity, soil phytotoxicity would
357 seem to be mainly linked to elements in F4 for *L. sativum*, which as an aggregate negatively
358 influenced seed performance through the PCA (Table 3). However, there were numerous

359 statistically significant correlations between soil fraction elemental concentrations and seed
360 germination and growth and these differed for *L. sativum* and *S. saccharatum*. *L. sativum* responded
361 positively to the presence of different metals in the F1 fraction, but negatively to those in the F2-F4
362 fractions. In contrast, *S. saccharatum* was stimulated by trace metals, including Cd and Pb in the F1
363 and Cu in the F3. These different responses for these test organisms agreed with those reported by
364 other authors (Baderna et al., 2014; Czerniawska-Kusza et al., 2006). Biostimulation is often
365 considered an initial trigger of hormesis, an adaptive response to low doses of toxicant resulting in a
366 relevant preliminary stimulation of an effect which turns into strong toxicity when toxic doses rise
367 (Calabrese and Baldwin, 2003). The high phytotoxicity of the investigated soils is also confirmed
368 by the higher percentage effects compared to those observed for volcanic urban or agricultural soils
369 in the downtown or surroundings of Naples, Southern Italy (Manzo et al., 2008, 2010, Memoli et al.,
370 2017). Besides, microorganisms and plants differently responded to various metals in the same
371 fraction according to the levels of sensitivity typical of various organism types (Isidori et al., 2003),
372 as it was the case of Pb in F1 and F4 or As in F2 and F3 that were involved in phytotoxic effects but
373 not in microbial responses.

374

375 **5. Conclusions**

376 Among the investigated elements, K, Cr, V, Al, Ti, Fe, Ni and Ba were the most scarcely
377 available. B, Ca, Na, Mg, Mn and Zn showed similar partitioning although their relative abundances
378 in the residual fraction were lower than those observed for the first group of elements. Cd and Zn
379 showed unique behaviours as compared to the other elements: Cd appeared the most ready available
380 element (60% of the pseudo-total concentration in the acid soluble fraction and 30% in the
381 reducible fraction) and Zn was mainly present in the acid soluble (40% of the pseudo-total
382 concentration) and in the residual (40% of the pseudo-total concentration) fractions.

383 The microbial biomass and activity appeared mainly affected by the reducible and oxidisable
384 fractions of the investigated elements more than the acid soluble or residual ones. With the

385 exception of La and V, the element content in the various fractions stimulated the microbial
386 biomass and activity.

387 Although the relationships between elements and microbial biomass or activity suggest that the
388 level of contamination of the investigated volcanic soils was not such to negatively affected the soil
389 microbial community, the soil matrix showed phytotoxic properties to *L. sativum* perhaps as acid
390 soluble or reducible As and Pb in these soils.

391

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401

402 **7. References**

- 403 Adam, G., Duncan, H., 2001. Development of a sensitive and rapid method for the measurement of
404 total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.*
405 33, 943–951.
- 406 Adamo, P., Denaix L., Terribile F., Zampella M., 2003. Characterization of heavy metals in
407 contaminated volcanic soils of the Solofrana river valley (southern Italy). *Geoderma* 117, 347–
408 366.
- 409 Alef, K., Nannipieri, P., 1995. *Methods in Applied Soil Microbiology and Biochemistry*, third ed.
410 Academic Press, San Diego.

411 Anderson, T. H., Domsch, K. H., 1978. A physiological method for the quantitative measurements
412 of microbial biomass in soil. *Soil Biol. Biochem.* 10, 215–221.

413 Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO₂ (qCO₂) as a specific activity
414 parameter to assess the effects of environmental conditions, such as pH, on the microbial
415 biomass of the soil. *Soil Biol. Biochem.* 25, 393–395.

416 Angelovičová, L., Fazekašová, D., 2014. Contamination of the soil and water environment by
417 heavy metals in the former mining area of Rudňany (Slovakia). *Soil & Water Res.* 9, 18–24.

418 Baderna, D., Colombo, A., Romeo, M., Cambria, F., Teoldi, F., Lodi, M., Diomede, L., Benfenati,
419 E., 2014. Soil quality in the Lomellina area using *in vitro* models and ecotoxicological assays.
420 *Environ. Res.* 133, 220–231.

421 Balasoiu, C.F., Zagury, G.J., Deschenes, L., 2001. Partitioning and speciation of chromium, copper,
422 and arsenic in CCA contaminated soils: influence of soil composition. *Sci. Total Environ.* 280,
423 239–255.

424 Bertiller, M.B., Marone, L., Baldi, R., Ares, J.O., 2009. Biological interactions at different spatial
425 scales in the Monte desert of Argentina. *J. Arid Environ.* 73, 212–221.

426 Bhattacharya, P., Tripathy, S., Chakrabarti, K., Chakraborty, A., Banik, P., 2008. Fraction- ation
427 and bioavailability of metals and their impact on microbial properties in sewage irrigated soil.
428 *Chemosphere* 72, 543–550.

429 Bonneville, S., Cappellen, P.V., Behrends, T., 2004. Microbial reduction of iron (III)
430 oxyhydroxides: effects of mineral solubility and availability. *Chem. Geol.* 212, 255–268.

431 Bundt, M., Widmer, F., Pesaro, M., Zeyer, J., Blaser, P., 2001a. Preferential flow paths: biological
432 ‘hot spots’ in soils. *Soil Biol. Biochem.* 33, 729–738.

433 Bundt, M., Zimmermann, S., Blaser, P., Hagedorn, F., 2001b. Sorption and transport of metals in
434 preferential flow paths and soil matrix after the addition of wood ash. *Eur. J. Soil Sci.* 52, 423–
435 431.

436 Calabrese, E.J., Baldwin, L.A., 2003. The hormetic dose–response model is more common than the
437 threshold model in toxicology. *Toxicol. Sci.* 71, 246–250.

438 Chlopecka, A., Bacon, J.R., Wilson, M.J., 1996. Forms of cadmium, lead and zinc in contaminated
439 soils from South West Poland. *J. Environ. Qual.* 25, 69–79.

440 Coppolecchia, D., Puglisi, E., Vasileiadis, S., Suciú, N., Hamon, R., Beone, G.M., Trevisan, M.,
441 2011. Relative sensitivity of different soil biological properties to zinc. *Soil Biol. Biochem.* 43,
442 1798–1807.

443 Czerniawska-Kusza, I., Ciesielczuk, T., Kusza, G., Cichoń, A., 2006. Comparison of the
444 Phytotoxkit Microbiotest and chemical variables for toxicity evaluation of sediments. *Environ.*
445 *Toxicol.* 21, 367–372.

446 D’Ascoli, R., Rao, M.A., Adamo, P., Renella, G., Landi, L., Rutigliano, F.A., Terribile, F.,
447 Gianfreda, L., 2006. Impact of river overflowing on trace element contamination of volcanic
448 soils in south Italy: Part II. Soil biological and biochemical properties in relation to trace element
449 speciation. *Environ. Pollut.* 144, 317–326.

450 De Nicola, F., Maisto, G., Alfani, A., 2003. Assessment of nutritional status and trace element
451 contamination of holm oak woodlands through analyses of leaves and surrounding soils. *Sci.*
452 *Total Environ.* 311, 191–203.

453 Degryse, F., Smolders, E., Parker, D.R., 2009. Partitioning of metals (Cd, Co, Cu, Ni, Pb, Zn) in
454 soils: concepts, methodologies, prediction and applications - a review. *Eur. J. Soil Sci.* 60, 590–
455 612.

456 Doran, J.W., Parkin, T.B., 1996. Quantitative indicators of soil quality: a minimum data set. In:
457 Doran, J.W., Jones, A.J. (Eds.), *Methods for Assessing Soil Quality*. SSSA Special Publication
458 no. 49, Madison, WI, pp. 25–37.

459 Dube, F., Zagal, E., Stolpe, N., Espinosa, M., 2009. The influence of land-use change on the
460 organic carbon distribution and microbial respiration in a volcanic soil of the Chilean Patagonia.
461 *Forest Ecol. Manag.* 257, 1695–1704.

462 EPA, 1996. Ecological effects test guidelines. OPPTS 850.4200. Seed Germination/Root elongation
 463 Toxicity Test. EPA-712-C-96-154.

464 Eswaran, H.E., Van Den Berg, E.V., Reich, P., 1993. Organic carbon in soils of the world. *Soil Sci.*
 465 *Soc. Am. J.* 57, 192–194.

466 Fernández-Ondoño, E., Bacchetta, G., Lallena, A.M., Navarro, F.B., Ortiz, I., Noelia Jiménez, M.,
 467 2017. Use of BCR sequential extraction procedures for soils and plant metal transfer predictions
 468 in contaminated mine tailings in Sardinia. *J. Geochem. Explor.* 172, 133–141.

469 Froment, A., 1972. Soil respiration in a mixed oak forest. *Oikos* 23, 273–277.

470 García-Delgado, C., Cala, V., Eymar, E., 2012. Influence of chemical and mineralogical properties
 471 of organic amendments on the selection of an adequate analytical procedure for trace elements
 472 determination. *Talanta* 88, 375–384.

473 Giammanco, S., Valenza, M., Pignato, S., Giammanco, G., 1996. Mg, Mn, Fe and V con-
 474 centrations in the ground waters of Mount Etna (Sicily). *Water Res.* 30, 378–386.

475 Giller, K.E., Witter, E., Mcgrath, S.P., 1998. Toxicity of heavy metals to microorganisms and
 476 microbial processes in agricultural soils: A review. *Soil Biol. Biochem.* 30, 1389–1414.

477 Hansda, A., Kumar, V., Anshumali, A., 2017. Influence of Cu fractions on soil microbial activities
 478 and risk assessment along Cu contamination gradient. *Catena* 151, 26–33.

479 He, Z.L., Zhang, M., Yang, X.E., Stoffella, P.J., 2006. Release behaviour of copper and zinc from
 480 sandy soils. *Soil Sci. Soc. Am. J.* 70 (5), 1699–1707.

481 Hinojosa, M.B., García-Ruíz, R., Viñegla, B., Carreira, J.A., 2004. Microbiological rates and
 482 enzyme activities as indicators of functionality in soils affected by the Aznalcóllar toxic spill.
 483 *Soil Biol. Biochem.* 36, 1637–1644.

484 Hu, X.F., Jiang, Y., Shu, Y., Hu, X., Liu, L., Lua, F., 2014. Effects of mining wastewater discharges
 485 on heavy metal pollution and soil enzyme activities on the paddy fields. *J. Geochem. Explor.* 147,
 486 139–150.

487 Isidori, M., Lavorgna, M., Nardelli, A., Parrella, A., 2003. Toxicity identification evaluation of
488 leachates from municipal solid waste landfills: a multispecies approach. *Chemosphere* 52, 85–94.

489 Killham, K., 1994. *Soil Ecology*. Cambridge University Press, Cambridge.

490 Knechtenhofer, L.A., Xifra, I.O., Scheinost, A.C., Flühler, H., Kretzschmar, R., 2003. Fate of heavy
491 metals in a strongly acidic shooting-range soil: small-scale metal distribution and its relation to
492 preferential water flow. *J. Plant Nutr. Soil Sci.* 166, 84–92.

493 Krishnamurti, G.S.R., Naidu, R., 2003. Solid-solution equilibria of cadmium in soils. *Geoderma*
494 113, 17–30.

495 Li, Y.T., Rouland, C., Benedetti, M., Li, Li, F., Pando, A., Lavelle, P., Dai, J., 2009. Microbial
496 biomass, enzyme and mineralization activity in relation to soil organic C, N and P turnover
497 influenced by acid metal stress. *Soil Biol. Biochem.* 41, 969–977.

498 Lundgren, B., 1981. Fluorescein diacetate as a stain of metabolically active bacteria in soil. *Oikos*
499 36, 17e22.

500 Ma, L., Rao, G., 1997. Chemical fractionation of cadmium, copper, nickel, and zinc in
501 contaminated soils. *J. Environ. Qual.* 26, 259–264.

502 Maeda, M., Zhao, B., Ozaki, Y., Yoneyama, T. 2003. Nitrate leaching in an Andisol treated with
503 different types of fertilizers. *Environ. Pollut.* 121, 477–487.

504 Maisto, G., De Nicola, F., Iovieno, P., Prati, M.V., Alfani, A. 2006. PAHs and trace elements in
505 volcanic urban and natural soils. *Geoderma* 136, 20–27.

506 Maisto, G., Manzo, S., De Nicola, F., Carotenuto, R., Rocco, A., Alfani, A., 2011. Assessment of
507 the effects of Cr, Cu, Ni and Pb soil contamination by ecotoxicological tests. *J. Environ. Monit.*
508 13, 3049–3056.

509 Majer, B.J., Tscherko, D., Paschke, A., 2002. Effects of heavy metal contamination of soils on
510 micronucleus induction in *Tradescantia* and on microbial enzyme activities: a comparative
511 investigation. *Mutat. Res.* 515, 111–124.

512 Manzo, S., De Luca Picione, F., Rocco, A., Carotenuto, R., Maisto, G., 2010. Urban and
513 agricultural soil ecotoxicity and heavy metal contamination. *Fresen. Environ. Bull.* 19, 1749–
514 1755.

515 Manzo, S., De Nicola, F., De Luca Picione, F., Maisto, G., Alfani, A., 2008. Assessment of the
516 effects of soil PAH accumulation by a battery of ecotoxicological tests. *Chemosphere* 71, 1937–
517 1944.

518 Martínez-Cortizas, A., García-Rodeja Gayoso, E., Nóvoa Muñoz, J.C., Pontevedra Pombal, X.,
519 Buurman, P., Terribile, F., 2003. Distribution of some selected major and trace elements in four
520 Italian soils developed from the deposits of the Gauro and Vico volcanoes. *Geoderma* 117, 215–
521 224.

522 McBride, M.B., Richards, B.K., Steenhuis, T., Russo, J.J., Sauve, S., 1997. Mobility and solubility
523 of toxic metals and nutrients in soil fifteen years after sludge application. *Soil Sci.* 162 (7), 487–
524 500.

525 Meers, E., Du Laing, G., Unamuno, V., Ruttens, A., Vangronsveld, J., Tack, F.M.G., Verloo, M.G.,
526 2007. Comparison of cadmium extractability from soils by commonly used single extraction
527 protocols. *Geoderma* 141, 247–259.

528 Memoli, V., Esposito, F., De Marco, A., Arena, C., Vitale, L., Tedeschi, A., Magliulo V., Maisto,
529 G., 2017. Metal compartmentalization in different biomass portions of *Helianthus annuus* L. and
530 *Sorghum bicolor* L. grown in an agricultural field inside an urban fabric. *Appl. Soil Ecol.* 121,
531 118–126.

532 Memoli, V., Eymar, E., García-Delgado, C., Esposito, F., Santorufo, L., De Marco, A., Barile, R.,
533 Maisto, G., 2018. Total and fraction content of elements in volcanic soil: natural or
534 anthropogenic derivation. *Sci. Total Environ.* 625, 16–26.

535 Mungai, W.N., Motavalli, P.P., Kremer, R.J., Nelson, K.A., 2005. Spatial variation in soil enzyme
536 activities and microbial functional diversity in temperate alley cropping systems. *Biol. Fertil.*
537 *Soils* 42, 129–136.

538 Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and
 539 biochemical processes in soil. In: Burns, R.G., Dick, R.P. (Eds.), *Enzymes in the Environment:
 540 Activity, Ecology, and Applications*. Marcel Dekker, New York, USA, pp. 1–33.

541 OECD, 1984. Terrestrial plants: growth test. *OECD Guidelines for Testing of Chemicals*, Paris n.
 542 208.

543 Olson, F.C.W., 1950. Quantitative estimates of filamentous algae. *Trans. Am. Microsc. Soc.* 69 (3),
 544 272–279.

545 Papassiopi, N., Kontoyianni, A., Vaxevanidou, K., Xenidis, A., 2009. Assessment of chromium
 546 biostabilization in contaminated soils using standard leaching and sequential extraction
 547 techniques. *Sci. Total Environ.* 407, 925–936.

548 Parelho, C., Rodrigues, A.S., Cruz, J.V., Garcia, P., 2014. Linking trace metals and agricultural land
 549 use in volcanic soils — A multivariate approach. *Sci. Total Environ.* 496, 241–247.

550 Parelho, C., Rodrigues, A.S., Barreto, M.C., Ferreira, N.G.C., Garcia, P., 2016. Assessing microbial
 551 activities in metal contaminated agricultural volcanic soils – An integrative approach. *Ecotoxicol.
 552 Environ. Saf.* 129, 242–249.

553 Pribyl, D.W., 2010. A critical review of the conventional SOC to SOM conversion factor.
 554 *Geoderma* 156 (3-4), 75–83.

555 Ramos, C.G., Querol, X., Dalmora, A.C., de Jesus Pires, K.C., Schneider, I.A.H., Oliveira, L.F.S.,
 556 Kautzmann, R.M., 2017. Evaluation of the potential of volcanic rock waste from southern Brazil
 557 as a natural soil fertilizer. *J. Clean. Prod.* 142, 2700–2706.

558 Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Barahona, E., Lachica, M., Ure, A.M., Davidson,
 559 C.M., Gomez, A., Lück, D., Bacon, J., Yli-Halla, M., Muntau, H., Quevauviller, P., 2000.
 560 Application of a modified BCR sequential extraction (three step) procedure for the
 561 determination of extractable trace metal contents in a sewage sludge amended soil reference
 562 material (CRM 483), complemented by a three-year stability study of acetic acid and EDTA
 563 extractable metal content. *J. Environ. Monit.* 2, 228–233.

564 Richards, B.K., Steenhuis, T.S., Peverly, J.H., McBride, M.B., 1998. Metal mobility at an old,
 565 heavily loaded sludge application site. *Environ. Pollut.* 99, 365–377.

566 Roden, E.E., Zachara, J.M., 1996. Microbial reduction of crystalline iron (III) oxides: influence of
 567 oxide surface area and potential for cell growth. *Environ. Sci. Technol. Lett.* 30, 1618–1628.

568 Shoji, S., Ono, T., 1978. Physical and chemical properties and clay mineralogy of Andosols from
 569 Kitakami, Japan. *Soil Sci.* 126, 297–312.

570 Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial
 571 organic nutrient acquisition in soil and sediment. *Nature* 462, 795–798.

572 Sundman, V., Sivelä, S., 1978. A comment on the membrane filter technique for estimation of
 573 length of fungal hyphae in soil. *Soil Biol. Biochem.* 10, 399–401.

574 Swift, M.J., Heal, O.W., Anderson, J.M., 1979. *Decomposition in terrestrial ecosystem*. Blackwell
 575 Scientific Publications, Oxford.

576 Tanneberg, H., Jahn, R., Meijer, E.L., Kleber, M., 2001. Sorption (kinetic and capacity) and
 577 desorption of trace elements in volcanic soils of Italy and the Azores. COST Action 622, *Soil*
 578 *Resources of European Volcanic Systems*. Volcanic Soils: Properties, Processes and Land Use,
 579 International Workshop, Abstracts. 3– 7 October, Ponta Delgada (S. Miguel), Azores, Portugal,
 580 pp. 58– 59.

581 Tripathy, S., Bhattacharyya, P., Mohapatra, R., Som, A., Chowdhury, D., 2014. Influence of
 582 different fractions of heavy metals on microbial ecophysiological indicators and enzyme
 583 activities in century old municipal solid waste amended soil. *Ecol. Engin.* 70, 25–34.

584 Ure, A.M., Quevauviller, PH., Muntau, H., Griepink, B., 1993. Speciation of heavy metals in soils
 585 and sediments. An account of the improvement and harmonization of extraction techniques
 586 undertaken under the auspices of the BCR of the Commission of European Communities. *Int. J.*
 587 *Environ. Anal. Chem.* 51, 135–151.

588 Van Peijnenburg, W.J.G.M., Posthuma, L., Eijsacker, H.J.P., Allen, H.E., 1997. Conceptual
 589 framework for implementation of bioavailability of metals for environmental management

590 purposes. *Ecotoxicol. Environ. Saf.* 37, 163–172.

591 Van Peijnenburg, W.J.G.M., Zablotskaja, M., Vijver, M.G., 2007. Monitoring metals in terrestrial
592 environments within a bioavailability framework and a focus on soil extraction. *Ecotoxicol.*
593 *Environ. Saf.* 67, 163–179.

594 Vasquez-Murrieta, M.S., Migueles-Gardunno, I., Franco-Hernandez, O., Govaerts, B., Dendooven,
595 L., 2006. C and N mineralization and microbial biomass in heavy-metal contaminated soil. *Eur. J.*
596 *Soil Biol.* 42, 89–98.

597 Villalobos, M., Lanson, B., Manceau, A., Toner, B., Sposito, G., 2006. Structural model for the
598 biogenic Mn oxide produced by *Pseudomonas putida*. *Am. Mineral.* 91, 489–502.

599 Wahid, P.A., Kamalam, N.V., 1993. Reductive dissolution of crystalline and amorphous Fe (III)
600 oxides by microorganisms in submerged soil. *Biol. Fertil. Soils* 15, 144–148.

601 Yadav, R.S., Tarafdar, J.C., 2001. Influence of organic and inorganic phosphorus supply on the
602 maximum secretion of acid phosphatase by plants. *Biol. Fertil. Soils* 34, 140–143.

603 Xiao, X., Yang, M., Guo, Z., Jiang, Z., Liu, Y., Cao, X., 2015. Soil vanadium pollution and
604 microbial response characteristics from stone coal smelting district. *Trans. Nonferrous Met. Soc.*
605 *China* 25, 1271–1278.

606 Xu, Z., Yu, G., Zhang, X., He, N., Wang, Q., Wang, S., Wang, R., Zhao, N., Jia, Y., Wang, C.,
607 2017. Soil enzyme activity and stoichiometry in forest ecosystems along the North-South
608 Transect in eastern China (NSTEC). *Soil Biology & Biochemistry* 104, 152–163.

609 Zeng, L.S., Liao, M., Chen, C.L., Huang, C.Y., 2007. Effects of lead contamination on soil
610 enzymatic activities, microbial biomass, and rice physiological indices in soil-lead-rice (*Oryza*
611 *sativa* L.) system. *Ecotoxicol. Environ. Saf.* 67, 67–74.

617 **Figure captions**

618 Fig. 1 – Mean values ($\pm$ s.e.) of percentage contributes of elements in acid soluble (F1), reducible
619 (F2), oxidasable (F3) and residual (F4) fractions to pseudo-total concentration.

620

621 Fig. 2 – Projection in the plane of the results of four Principal Component Analyses (PCA)
622 performed using the mean values, calculated for the sites, of element contents in each fraction (F1:
623 acid soluble, F2: reducible, F3: oxidasable and F4: residual). The loading values of each parameter
624 to the PC1 are reported in the table adjacent the PCA.

Table 1 - Mean values ($\pm$ s.e.) of pH, organic matter content (OM, expressed as % d.w.), water content (W.C., expressed as % d.w.), total C, N and S content (expressed as % d.w.) in soils collected at Natural Reserve (NR), Ercolano (E) and Matrone (M) roads, at low (L) and high (H) altitudes and in proximity of the roads (R) or distant from the roads (F).

	pH	OM	W.C.	C	N	S
NR	7.04 ($\pm$ 0.01)	13.0 ($\pm$ 0.54)	27.0 ($\pm$ 1.22)	4.50 ($\pm$ 0.36)	0.18 ($\pm$ 0.02)	0.01 ($\pm$ 0.003)
ELR	6.95 ($\pm$ 0.01)	7.45 ($\pm$ 0.09)	58.4 ($\pm$ 0.40)	8.02 ($\pm$ 0.53)	0.38 ($\pm$ 0.02)	0.03 ($\pm$ 0.003)
ELF	6.98 ($\pm$ 0.01)	10.4 ($\pm$ 0.40)	28.9 ($\pm$ 0.70)	3.57 ($\pm$ 0.23)	0.23 ($\pm$ 0.02)	0.02 ($\pm$ 0.01)
EHR	7.98 ($\pm$ 0.01)	10.6 ($\pm$ 0.13)	14.1 ($\pm$ 0.32)	1.78 ($\pm$ 0.17)	0.11 ($\pm$ 0.01)	0.01 ($\pm$ 0.01)
EHF	6.46 ($\pm$ 0.01)	15.4 ($\pm$ 0.71)	38.7 ($\pm$ 1.31)	5.95 ($\pm$ 1.02)	0.30 ($\pm$ 0.04)	0.05 ($\pm$ 0.01)
MLR	6.57 ($\pm$ 0.01)	46.7 ($\pm$ 0.92)	102 ($\pm$ 1.45)	26.3 ($\pm$ 0.75)	0.65 ($\pm$ 0.04)	0.07 ($\pm$ 0.01)
MLF	6.67 ($\pm$ 0.01)	40.8 ($\pm$ 1.22)	102 ($\pm$ 1.75)	22.3 ($\pm$ 0.18)	0.67 ($\pm$ 0.02)	0.06 ($\pm$ 0.01)
MHR	7.78 ($\pm$ 0.01)	12.0 ($\pm$ 0.75)	26.6 ($\pm$ 0.26)	3.71 ($\pm$ 0.48)	0.24 ($\pm$ 0.03)	0.02 ($\pm$ 0.01)
MHF	7.36 ($\pm$ 0.01)	13.6 ($\pm$ 0.71)	23.0 ($\pm$ 1.28)	5.72 ($\pm$ 1.46)	0.34 ($\pm$ 0.06)	0.05 ($\pm$ 0.05)

Table 2 - Mean and median values ($\pm$ s.e.) of microbial and fungal carbon (C_{mic} and C_{fung} , expressed as mg C g⁻¹ d.w.), respiration (Resp, expressed as mg CO₂ g⁻¹ d.w. h⁻¹), metabolic quotient (qCO₂, expressed as mg C-CO₂ mg g⁻¹ C_{mic}), coefficient of endogenous mineralization (CEM, expressed as mg C-CO₂ g⁻¹ C_{org}), hydrolase activity (HA, expressed as mmol FDA min⁻¹ g⁻¹ d.w.), dehydrogenase activity (DHA, expressed as mmol TFF min⁻¹ g⁻¹ d.w.), effect percentage of germination index (GI), root elongation (E) and seeds germination (G) of *L. sativum* L. and *S. saccharatum* L. in soils collected at Natural Reserve (NR), Ercolano (E) and Matrone (M) roads, at low (L) and high (H) altitudes and in proximity of the roads (R) or distant from the roads (F).

	C_{mic}	C_{fung}	Resp	qCO ₂	CEM	HA	DHA	<i>L. sativum</i> (GI)	<i>L. sativum</i> (E)	<i>L. sativum</i> (G)	<i>S.</i> <i>saccharatum</i> (GI)	<i>S.</i> <i>saccharatum</i> (E)	<i>S.</i> <i>saccharatum</i> (G)
NR	1.54 ($\pm$ 0.48)	0.02 ($\pm$ 0.01)	0.14 ($\pm$ 0.05)	0.02 ($\pm$ 0.008)	0.51 ($\pm$ 0.15)	2.94 ($\pm$ 0.20)	0.19 ($\pm$ 0.01)	-39.1 ($\pm$ 9.57)	-29.0 ($\pm$ 6.47)	-7.41 ($\pm$ 3.70)	42.7 ($\pm$ 3.62)	52.7 ($\pm$ 2.98)	2.70 ($\pm$ 0.00)
ELR	1.43 ($\pm$ 0.06)	0.01 ($\pm$ 0.002)	0.30 ($\pm$ 0.07)	0.06 ($\pm$ 0.01)	1.93 ($\pm$ 0.48)	5.43 ($\pm$ 0.34)	0.36 ($\pm$ 0.01)	-79.0 ($\pm$ 3.60)	-60.4 ($\pm$ 3.22)	-11.1 ($\pm$ 0.00)	48.4 ($\pm$ 3.31)	47.8 ($\pm$ 1.83)	20.7 ($\pm$ 3.60)
ELF	1.72 ($\pm$ 0.48)	0.02 ($\pm$ 0.01)	0.24 ($\pm$ 0.03)	0.04 ($\pm$ 0.004)	1.05 ($\pm$ 0.12)	4.83 ($\pm$ 0.23)	0.15 ($\pm$ 0.01)	-58.7 ($\pm$ 12.9)	-42.2 ($\pm$ 11.6)	-11.1 ($\pm$ 0.00)	53.5 ($\pm$ 4.76)	50.4 ($\pm$ 4.97)	24.3 ($\pm$ 6.24)
EHR	0.53 ($\pm$ 0.23)	0.008 ($\pm$ 0.008)	0.07 ($\pm$ 0.02)	0.04 ($\pm$ 0.009)	0.32 ($\pm$ 0.08)	2.95 ($\pm$ 0.20)	0.00 ($\pm$ 0.00)	-18.0 ($\pm$ 4.50)	-9.81 ($\pm$ 7.24)	-7.41 ($\pm$ 3.70)	53.5 ($\pm$ 7.98)	55.5 ($\pm$ 3.93)	17.1 ($\pm$ 9.53)
EHF	0.81 ($\pm$ 0.06)	0.003 ($\pm$ 0.007)	0.14 ($\pm$ 0.01)	0.05 ($\pm$ 0.004)	0.42 ($\pm$ 0.02)	3.76 ($\pm$ 0.24)	0.15 ($\pm$ 0.01)	-69.1 ($\pm$ 17.3)	-56.2 ($\pm$ 11.9)	-7.41 ($\pm$ 3.70)	51.0 ($\pm$ 4.46)	50.0 ($\pm$ 1.41)	20.7 ($\pm$ 9.53)
MLR	2.50 ($\pm$ 0.29)	0.03 ($\pm$ 0.08)	0.69 ($\pm$ 0.007)	0.07 ($\pm$ 0.001)	0.69 ($\pm$ 0.03)	7.00 ($\pm$ 0.85)	0.44 ($\pm$ 0.004)	-27.3 ($\pm$ 5.39)	-18.1 ($\pm$ 4.38)	-7.41 ($\pm$ 3.70)	62.1 ($\pm$ 3.75)	53.2 ($\pm$ 7.00)	31.5 ($\pm$ 14.4)
MLF	2.70 ($\pm$ 0.08)	0.07 ($\pm$ 0.006)	0.49 ($\pm$ 0.08)	0.05 ($\pm$ 0.01)	0.56 ($\pm$ 0.09)	6.08 ($\pm$ 0.28)	0.50 ($\pm$ 0.02)	-53.8 ($\pm$ 11.8)	-47.6 ($\pm$ 4.11)	-3.70 ($\pm$ 7.41)	38.5 ($\pm$ 8.93)	45.2 ($\pm$ 6.14)	9.91 ($\pm$ 7.21)
MHR	0.99 ($\pm$ 0.39)	0.01 ($\pm$ 0.005)	0.16 ($\pm$ 0.03)	0.04 ($\pm$ 0.01)	0.58 ($\pm$ 0.06)	3.79 ($\pm$ 0.13)	0.06 ($\pm$ 0.01)	-10.4 ($\pm$ 5.84)	-14.9 ($\pm$ 6.54)	-3.70 ($\pm$ 7.41)	36.4 ($\pm$ 10.3)	40.9 ($\pm$ 4.04)	13.5 ($\pm$ 12.5)
MHF	0.82 ($\pm$ 0.03)	0.01 ($\pm$ 0.001)	0.13 ($\pm$ 0.009)	0.04 ($\pm$ 0.003)	0.48 ($\pm$ 0.07)	3.07 ($\pm$ 0.08)	0.07 ($\pm$ 0.01)	-29.2 ($\pm$ 1.51)	-28.6 ($\pm$ 1.50)	0.00 ($\pm$ 0.00)	34.5 ($\pm$ 9.32)	38.5 ($\pm$ 5.75)	13.5 ($\pm$ 12.5)
Median	1.43	0.02	0.16	0.04	0.56	3.79	0.15	-39.1	-28.9	-7.41	48.4	50.0	17.1

Table 3 – Spearman coefficients of the correlations between the scores of the first axis of each PCA performed using the element contents in acid soluble (F1), reducible (F2), oxidasable (F3), residual (F4) fractions and the biological or phytotoxicological responses of the investigated soils. The correlation coefficients in bold with the asterisk indicate the levels of significance (* P < 0.05; ** P < 0.01; *** P < 0.001).

	C _{mic}	C _{fung}	Resp	qCO ₂	CEM	HA	DHA	<i>L.</i> <i>sativum</i> (GI)	<i>L.</i> <i>sativum</i> (E)	<i>L.</i> <i>sativum</i> (G)	<i>S.</i> <i>saccharatum</i> (GI)	<i>S.</i> <i>saccharatum</i> (E)	<i>S.</i> <i>saccharatum</i> (G)
PC1 F1	0.467	0.550	0.617	0.417	0.733*	0.733*	0.367	-0.233	-0.167	-0.525	0.469	0.133	0.63
PC1 F2	0.650	0.650	0.833**	0.700*	0.667*	0.933***	0.583	-0.017	-0.067	-0.149	0.318	0.017	0.462
PC1 F3	0.533	0.617	0.767*	0.667*	0.583	0.833**	0.633	-0.283	-0.267	-0.525	0.644	0.367	0.622
PC1 F4	-0.017	0.067	-0.150	-0.267	0.233	-0.250	0.083	-0.700*	-0.550	-0.708*	0.092	0.067	0.118

Table 4 – Spearman correlations among the element contents in acid soluble (F1), reducible (F2), oxidasable (F3), residual (F4) fractions and biological or ecotoxicological characteristics of the investigated soils. The correlation coefficients in bold with the asterisk indicate the levels of significance (* P < 0.05; ** P < 0.01; *** P < 0.001).

F1	C _{mic}	C _{fung}	Resp	qCO ₂	CEM	HA	DHA	<i>L. sativum</i> (GI)	<i>L. sativum</i> (E)	<i>L. sativum</i> (G)	<i>S.</i> <i>saccharatum</i> (GI)	<i>S.</i> <i>saccharatum</i> (E)	<i>S.</i> <i>saccharatum</i> (G)
Al	-0.417	-0.383	-0.433	-0.583	0.100	-0.367	-0.717*	0.250	0.367	-0.087	-0.050	-0.066	0.118
As	-0.233	-0.400	-0.300	-0.016	-0.550	-0.383	-0.066	0.117	0.033	0.446	-0.268	-0.100	-0.311
B	0.667*	0.533	0.817**	0.783**	0.783**	0.867***	0.733*	-0.450	-0.533	-0.210	-0.025	-0.367	0.331
Ba	-0.383	-0.500	-0.100	0.350	0.033	0.083	-0.350	0.433	0.33	0.516	-0.420	-0.483	0.016
Ca	0.066	0.350	0.283	0.350	0.267	0.467	0.100	0.100	0.167	-0.315	0.469	0.333	0.429
Cd	0.567	0.633	0.583	0.400	0.383	0.633	0.600	-0.483	-0.400	-0.621	0.711*	0.417	0.639
Cr	0.041	0.226	0.167	0.033	0.041	0.335	-0.159	0.427	0.510	-0.136	0.681*	0.552	0.527
Cu	-0.33	-0.250	-0.333	-0.583	-0.117	-0.317	-0.467	-0.083	0.050	-0.411	0.410	0.417	0.202
Fe	-0.383	-0.383	-0.450	-0.400	-0.350	-0.400	-0.683*	0.900***	0.900***	0.630	-0.226	-0.066	0.160
K	0.750*	0.600	0.900***	0.583	0.967***	0.800**	0.717*	-0.333	-0.383	-0.393	0.184	-0.083	0.365
La	-0.767*	-0.667*	-0.833***	-0.700*	-0.617	-0.783**	-0.933***	0.667*	0.733*	0.393	-0.167	0.083	-0.252
Mg	0.383	0.333	0.583	0.767**	0.267	0.683*	0.483	0.150	-0.016	0.358	-0.176	-0.300	-0.025
Mn	0.917***	0.800**	0.933***	0.567	0.717*	0.733*	0.917***	-0.200	-0.300	-0.175	0.117	0.016	0.101
Na	-0.317	-0.433	-0.183	-0.167	0.400	-0.083	-0.417	-0.267	-0.183	-0.324	-0.075	-0.300	0.336
Ni	0.900***	0.700*	0.950***	0.517	0.733*	0.850***	0.733*	-0.066	-0.167	-0.078	0.218	-0.016	0.286
P	-0.400	-0.350	-0.600	-0.317	-0.867***	-0.633	-0.233	0.083	0.083	0.332	-0.167	0.100	-0.420
Pb	0.283	0.600	0.267	0.000	0.283	0.283	0.250	-0.200	-0.016	-0.761**	0.812**	0.717*	0.555
Si	-0.333	-0.383	-0.117	-0.016	0.450	0.033	-0.400	-0.167	-0.083	-0.324	-0.008	-0.250	0.420
Ti	0.167	0.417	0.367	0.250	0.233	0.417	0.167	0.267	0.300	-0.254	0.544	0.533	0.311
V	0.317	0.016	0.200	-0.117	0.350	0.133	-0.083	0.350	0.283	0.393	-0.310	0.417	-0.008
W	0.778**	0.695*	0.703*	0.402	0.695*	0.703*	0.561	-0.117	-0.126	-0.140	0.113	-0.167	0.350
Zn	0.117	0.400	0.217	0.150	0.133	0.417	0.050	0.016	0.150	-0.455	0.745**	0.550	0.605

F2	C _{mic}	C _{fung}	Resp	qCO ₂	CEM	HA	DHA	<i>L. sativum</i> (GI)	<i>L. sativum</i> (E)	<i>L. sativum</i> (G)	<i>S.</i> <i>saccharatum</i> (GI)	<i>S.</i> <i>saccharatum</i> (E)	<i>S.</i> <i>saccharatum</i> (G)
Al	0.750**	0.617	0.917***	0.750**	0.733*	0.850***	0.833***	-0.517	-0.517	-0.463	0.393	0.100	0.487
As	0.370	0.333	0.350	0.133	0.500	0.183	0.567	-0.900***	-0.833***	-0.804**	0.251	0.133	0.227
B	0.400	0.517	0.517	0.217	0.600	0.583	0.333	-0.350	-0.283	-0.603	0.477	0.233	0.450
Ba	0.583	0.533	0.833***	0.733*	0.617	0.933***	0.550	0.016	-0.066	-0.078	0.310	0.016	0.437
Ca	0.467	0.533	0.550	0.667*	0.100	0.517	0.800**	-0.400	-0.467	-0.210	0.251	0.200	0.075
Cd	0.717*	0.717*	0.767**	0.767**	0.533	0.800**	0.850***	-0.450	-0.467	-0.341	0.301	0.016	0.429
Cr	0.604	0.604	0.822**	0.842***	0.574	0.822**	0.782**	-0.208	-0.277	-0.239	0.298	0.099	0.364
Cu	-0.317	-0.233	-0.183	-0.233	0.133	0.033	-0.483	-0.033	0.066	-0.271	0.226	0.033	0.345
Fe	0.800**	0.617	0.933***	0.733*	0.767**	0.900***	0.733*	-0.050	-0.183	0.043	0.000	-0.250	0.227
K	0.533	0.517	0.800**	0.583	0.817**	0.833***	0.533	-0.267	-0.267	-0.516	0.494	0.183	0.605
La	-0.433	-0.417	-0.550	-0.483	-0.767**	-0.683*	-0.333	0.267	0.217	0.385	-0.218	0.167	-0.605
Mg	0.333	0.300	0.533	0.650	0.183	0.633	0.367	0.317	0.150	0.428	-0.117	-0.200	-0.050
Mn	0.783**	0.867***	0.783**	0.433	0.467	0.617	0.883***	-0.317	-0.350	-0.411	0.393	0.367	0.109
Na	-0.100	-0.066	0.117	0.100	0.567	0.283	-0.233	-0.033	0.033	-0.297	0.083	-0.167	0.429
Ni	0.583	0.583	0.717*	0.453	0.450	0.650	0.483	0.367	0.250	0.157	0.134	0.117	0.033
P	-0.133	-0.117	-0.350	-0.200	-0.717*	-0.483	0.066	0.000	-0.066	0.358	-0.285	0.033	-0.613
Pb	0.717*	0.883***	0.657	0.317	0.433	0.617	0.717*	-0.383	-0.333	-0.533	0.510	0.367	0.303
Si	0.267	0.283	0.533	0.400	0.783**	0.650	0.167	-0.100	-0.050	-0.455	0.393	0.066	0.655
Ti	0.500	0.383	0.633	0.633	0.333	0.650	0.500	0.333	0.150	0.446	-0.218	-0.250	-0.118
V	-0.300	0.167	-0.417	-0.667*	-0.016	-0.400	-0.667*	0.483	0.633	-0.061	-0.167	0.217	0.134
W	0.667*	0.533	0.767**	0.767**	0.400	0.750**	0.867***	-0.517	-0.583	-0.280	0.335	0.066	0.387
Zn	0.509	0.678*	0.644	0.627	0.322	0.746**	0.644	-0.254	-0.254	-0.356	0.502	0.305	0.385

F3	C _{mic}	C _{fung}	Resp	qCO ₂	CEM	HA	DHA	<i>L. sativum</i> (GI)	<i>L. sativum</i> (E)	<i>L. sativum</i> (G)	<i>S.</i> <i>saccharatum</i> (GI)	<i>S.</i> <i>saccharatum</i> (E)	<i>S.</i> <i>saccharatum</i> (G)
Al	0.633	0.533	0.850***	0.750**	0.600	0.800**	0833***	-0.500	-0.550	-0.472	0.477	0.217	0.487
As	0.333	0.283	0.316	0.133	0.450	0.217	0.500	-0.917***	-0.833***	0.804**	0.326	0.133	0.345
B	0.540	0.469	0.731*	0.627	0.766**	0.775**	0.661*	-0.679*	-0.644	-0.703*	0.476	0.095	0.658
Ba	0.283	0.283	0.600	0.800**	0.400	0.750**	0.383	0.150	0.050	0.150	0.126	-0.083	0.331
Ca	0.100	0.200	0.250	-0.016	0.633	0.233	-0.033	-0.033	0.117	-0.665*	0.577	0.417	0.655
Cd	0.267	0.300	0.517	0.617	0.583	0.683*	0.433	-0.633	-0.550	-0.726*	0.586	0.167	0.807**
Cr	0.800**	0.817**	0.867***	0.567	0.583	0.750**	0.900***	-0.400	-0.417	-0.507	0.527	0.376	0.370
Cu	0.033	0.050	0.267	0.350	0.350	0.467	0.133	-0.550	-0.433	-0.699	0.686*	0.283	0.840***
Fe	0.367	0.233	0.650	0.883***	0.300	0.750**	0.533	0.033	-0.133	0.254	-0.016	-0.217	0.168
K	0.200	0.267	0.517	0.750**	0.233	0.633	0.400	0.100	0.016	0.017	0.243	0.117	0.269
La	0.433	0.317	0.417	0.117	0.600	0.233	0.533	-0.800**	-0.733*	-0.769**	0.301	0.133	0.345
Mg	0.317	0.300	0.600	0.767**	0.250	0.700*	0.450	0.167	0.033	0.192	0.117	-0.017	0.168
Mn	0.317	0.300	0.600	0.767**	0.250	0.700*	0.450	0.167	0.033	0.192	0.117	-0.016	0.168
Na	0.267	0.283	0.467	0.450	0.750**	0.550	0.200	-0.033	0.016	-0.341	0.266	-0.050	0.580
Ni	0.400	0.267	0.617	0.917***	0.233	0.683*	0.667*	-0.183	-0.333	0.157	-0.025	-0.217	0.151
P	-0.100	-0.066	-0.200	-0.333	0.200	-0.300	0.000	-0.733*	-0.600	-0.691*	0.058	0.033	0.075
Pb	0.850***	0.850***	0.817***	0.550	0.550	0.717*	0.917***	-0.450	-0.450	-0.463	0.444	0.250	0.370
Si	0.200	0.333	0.267	0.000	0.066	0.333	-0.033	0.467	0.533	-0.114	0.711*	0.633	0.479
Ti	-0.671	-0.700*	-0.467	0.066	-0.200	-0.283	-0.450	-0.066	-0.133	0.358	-0.678*	-0.700*	-0.286
V	0.683*	0.500	0.833***	0.500	0.917***	0.800**	0.617	-0.483	-0.500	-0.507	0.318	-0.050	0.546
W	0.550	0.433	0.533	0.283	0.600	0.450	0.650	-0.883***	-0.867***	-0.683	0.167	-0.083	0.261
Zn	0.627	0.780**	0.712*	0.475	0.610	0.661*	0.729*	-0.390	-0.373	-0.560	0.400	0.271	0.282

F4	C _{mic}	C _{fung}	Resp	qCO ₂	CEM	HA	DHA	<i>L. sativum</i> (GI)	<i>L. sativum</i> (E)	<i>L. sativum</i> (G)	<i>S.</i> <i>saccharatum</i> (GI)	<i>S.</i> <i>saccharatum</i> (E)	<i>S.</i> <i>saccharatum</i> (G)
Al	0.283	0.117	0.283	-0.200	0.533	0.033	0.250	-0.533	-0.483	-0.647	0.276	0.217	0.210
As	0.050	0.083	-0.066	-0.183	0.233	-0.283	0.217	-0.600	-0.483	-0.647	0.100	0.167	0.067
B	0.000	0.117	-0.100	-0.350	0.133	-0.317	0.133	-0.567	-0.400	-0.831***	0.444	0.533	0.193
Ba	-0.066	-0.250	-0.117	-0.117	0.200	-0.330	0.100	-0.500	-0.500	-0.245	-0.335	-0.267	-0.176
Ca	0.050	0.133	-0.183	-0.350	0.100	-0.400	0.150	-0.483	-0.433	-0.324	-0.351	-0.150	-0.437
Cd	-0.075	0.000	-0.277	-0.176	-0.445	-0.143	-0.042	-0.370	-0.286	-0.132	0.173	0.084	0.072
Cr	0.700*	0.700*	0.633	0.300	0.250	0.383	0.867***	-0.400	-0.467	-0.306	0.243	0.317	-0.118
Cu	-0.117	-0.067	-0.183	-0.283	0.283	-0.283	-0.050	-0.567	-0.400	-0.717*	0.176	0.133	0.261
Fe	0.217	0.150	0.050	-0.217	0.417	-0.100	0.217	-0.683*	-0.633	-0.463	-0.243	-0.283	-0.126
K	0.317	0.183	0.267	-0.033	0.683*	0.083	0.250	-0.433	-0.367	-0.516	0.016	-0.100	0.244
La	-0.133	-0.217	-0.200	-0.333	0.367	-0.267	-0.200	-0.433	-0.350	-0.376	-0.251	-0.317	0.016
Mg	0.333	0.317	0.066	-0.267	0.167	-0.200	0.333	-0.317	-0.383	0.008	-0.586	-0.350	-0.714*
Mn	0.367	0.233	0.217	-0.133	0.583	0.033	0.300	-0.583	-0.567	-0.385	-0.285	-0.350	-0.109
Na	-0.016	0.133	-0.150	-0.517	0.283	-0.400	-0.083	-0.100	0.016	-0.472	0.008	0.233	-0.151
Ni	0.533	0.533	0.350	-0.033	0.433	0.150	0.500	-0.367	-0.433	0.114	-0.427	-0.333	-0.487
P	-0.617	-0.450	-0.633	-0.450	-0.200	-0.433	-0.683*	-0.100	0.100	-0.341	0.192	0.100	0.336
Pb	0.383	0.350	0.350	0.083	0.517	0.183	0.517	-0.817***	-0.717*	-0.848***	0.393	0.250	0.655
Si	0.417	0.233	0.300	0.133	0.133	0.200	0.250	0.517	0.317	0.778**	-0.393	-0.267	-0.487
Ti	0.167	0.333	0.167	-0.117	0.433	-0.033	0.317	-0.500	-0.350	-0.761**	0.385	0.350	0.345
V	-0.367	-0.150	-0.450	-0.367	-0.267	-0.600	-0.066	-0.450	-0.333	-0.507	0.058	0.317	-0.218
W	0.300	0.350	0.300	0.217	0.517	0.267	0.333	-0.417	-0.267	-0.699*	0.502	0.283	0.665
Zn	0.500	0.483	0.367	-0.033	0.700*	0.167	0.400	-0.383	-0.317	-0.507	0.008	-0.050	0.118

Fig. 1

Fig. 2

Supplementary material 1 - Description and characteristics of the soils collected at Natural Reserve (NR), Ercolano (E) and Matrone (M) roads, at low (L) and high (H) altitudes and in proximity of the roads (R) or distant from the roads (F) inside the Vesuvius National Park

Site	Geographical coordinates	Altitude (m a.s.l.)	Distance from the road (m)	Vegetation cover	Litter layer (cm)
NR	40°49'22.900''N 14°25'45.819''E	530	0	Pine, holm oak, broom, lichens, mosses	4-5
ELR	40°49'49.156''N 14°24'0.273''E	596	0	Holm oak, broom, lichens, euphorbia, black locust, ivy	< 1
ELF	40°49'49.156''N 14°24'0.273''E	596	30	Broom, euphorbia, lichens	0
EHR	40°49'51.935''N 14°25'28.606''E	900	0	Broom, euphorbia	< 1
EHF	40°49'51.935''N 14°25'28.606''E	900	30	Pine, broom	5-7
MLR	40°48'19.04''N 14°26'13.361''E	570	0	Pine, broom, holm oak, mosses	5-7
MLF	40°48'19.04''N 14°26'13.361''E	570	30	Pine, broom, holm oak, mosses	5-7
MHR	40°48'55.246''N 14°26'18.679''E	820	0	Pine, broom, mosses, bramble	1-2
MHF	40°48'55.246''N 14°26'18.679''E	820	30	Pine, broom, bramble, holm oak	4-5

Supplementary material 2 - Element concentrations ($\mu\text{g g}^{-1}$ d.w.) in single fractions (F1, F2, F3 and F4) of BRC sequential extraction of investigated soils.

Element concentrations	F1								
	EL_1	EL_2	EH_1	EH_2	ML_1	ML_2	MH_1	MH_2	NR
Al	390	1114	504	361	245	169	713	461	378
As	0.07	0.07	0.10	0.22	0.16	0.13	0.16	0.17	0.21
B	1.91	1.13	0.07	0.39	1.21	1.83	0.63	0.91	0.21
Ba	21.8	9.89	18.743	18.743	20.5	17.5	29.4	25.6	15.0
Ca	4133	3025	5854	1762	3540	3364	2093	1972	1539
Cd	0.06	0.17	0.04	0.07	0.12	0.08	0.02	0.04	0.04
Cr	0.00	0.04	0.07	0.01	0.05	0.01	0.02	0.00	0.00
Cu	0.57	23.6	3.2	1.12	0.47	0.51	0.93	0.37	0.61
Fe	24.5	44.4	67.9	38.4	63.7	28.7	289	139	51.0
K	713	499	252	306	558	484	451	339	353
La	0.03	0.08	0.33	0.11	0.07	0.01	0.23	0.20	0.12
Mg	567	187	276	265	786	866	366	323	238
Mn	47.8	34.9	15.1	27.6	146	137	33.4	33.3	41.0
Na	1349	1534	333	333	240	236	567	407	290
Ni	0.22	0.28	0.09	0.20	0.42	0.33	0.23	0.14	0.21
P	1.31	1.94	4.16	10.6	4.08	4.14	3.97	5.26	10.3
Pb	0.52	2.66	1.27	0.34	0.56	0.45	0.17	0.23	0.37
Si	1171	1126	330	222	202	168	625	394	177
Ti	1.46	1.17	1.71	0.66	1.65	1.38	1.23	0.6	0.69
V	0.07	0.38	0.04	0.15	0.32	0.19	0.59	0.36	0.28
W	0.00	0.06	0.00	0.00	0.02	0.01	0.00	0.01	0.002
Zn	12.9	19.5	22.4	6.75	18.2	16.4	5.09	4.62	4.52

Element concentrations	F2								
	EL_1	EL_2	EH_1	EH_2	ML_1	ML_2	MH_1	MH_2	NR
Al	1439	778	494	705	1450	1350	576	572	584
As	0.62	0.60	0.33	0.44	0.38	0.44	0.30	0.38	0.52
B	0.90	1.36	0.47	0.13	0.47	0.58	0.30	0.03	0.21
Ba	53.5	20.3	18.3	17.7	57.9	56.1	24.6	16.1	14.9
Ca	1782	666	784	965	2310	2614	644	729	934
Cd	0.07	0.06	0.01	0.02	0.17	0.15	0.00	0.02	0.01
Cr	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Cu	0.18	3.07	1.21	0.17	0.13	0.16	0.19	0.14	0.11
Fe	644	405	356	358	1278	1043	593	403	359
K	310	214	155	110	236	213	170	101	104
La	0.54	0.42	1.33	1.33	0.70	0.72	0.87	0.71	1.37
Mg	87.4	35.0	80.4	50.5	198	206	111	62.7	36.0
Mn	44.8	24.7	24.0	21.5	96.5	141	18.1	15.3	30.2
Na	626	590	218	134	173	161	229	199	95.4
Ni	0.26	0.16	0.22	0.12	0.60	0.49	0.32	0.15	0.20
P	23.8	9.98	50.1	90.8	48.6	70.9	34.4	56.4	92.3
Pb	4.80	9.15	4.45	2.88	8.98	9.87	1.19	1.84	3.83
Si	495	426	211	130	244	203	226	169	91.1
Ti	0.97	0.51	0.66	0.58	2.01	1.79	0.98	0.92	0.90
V	1.00	3.98	2.42	0.98	1.11	0.92	2.05	1.20	1.14
W	0.05	0.04	0.00	0.06	0.27	0.11	0.02	0.02	0.02
Zn	9.53	3.94	6.89	0.25	15.5	16.5	0.00	0.00	0.00

Element concentrations	F3								
	EL_1	EL_2	EH_1	EH_2	ML_1	ML_2	MH_1	MH_2	NR
Al	6083	4253	2568	4449	7354	5746	2596	2074	3115
As	3.17	17.8	0.85	2.47	1.67	1.81	0.65	1.13	1.98
B	3.45	1.52	0.00	0.05	1.46	0.41	0.00	0.00	0.00
Ba	84.5	19.3	39.1	25.9	97.00	62.8	50.2	26.0	18.5
Ca	3074	3510	2390	1874	2661	1793	2157	1904	2100
Cd	0.03	0.03	0.02	0.02	0.02	0.02	0.00	0.01	0.002
Cr	1.09	0.74	0.28	0.38	1.86	1.54	0.24	0.10	0.40
Cu	24.0	204	20.3	26.0	23.4	17.8	14.9	14.4	11.8
Fe	1414	533	720	828	3245	2422	980	747	688
K	1313	507	759	640	1597	1085	745	552	542
La	15.2	16.7	6.44	9.69	9.57	9.14	7.18	8.72	11.5
Mg	464	201	315	299	1078	755	412	263	202
Mn	38.6	18.3	28.2	21.8	62.4	44.4	31.3	20.3	19.3
Na	1490	931	841	532	1161	770	853	871	586
Ni	1.92	0.79	0.81	1.23	4.39	3.39	1.00	1.01	0.87
P	542	669	268	356	169	223	208	297	379
Pb	29.5	32.1	7.54	8.20	37.4	36.1	5.43	7.57	8.30
Si	602	776	966	683	1045	690	748	553	669
Ti	108	41.0	63.3	66.6	25.7	62.0	73.9	176	56.2
V	18.7	21.3	6.70	7.99	18.1	13.0	9.64	7.25	7.36
W	2.87	3.06	1.08	1.96	1.44	2.11	1.35	1.39	1.73
Zn	12.5	7.99	6.58	0.00	11.9	12.4	0.00	0.00	0.34

Element concentrations	F4								
	EL_1	EL_2	EH_1	EH_2	ML_1	ML_2	MH_1	MH_2	NR
Al	16093	19351	9262	11628	11361	11055	11403	8496	16270
As	1.82	1.74	1.19	1.36	1.29	1.11	1.06	1.42	1.90
B	4.21	4.97	2.83	3.72	2.59	2.02	1.31	2.22	6.33
Ba	204	169	158	170	163	161	167	173	214
Ca	6594	6517	5674	5644	5476	5768	5592	6524	7304
Cd	0.00	0.01	0.00	0.03	0.00	0.00	0.00	0.00	0.002
Cr	1.06	0.92	0.91	0.95	1.42	1.67	0.78	0.68	1.42
Cu	18.8	31.3	11.1	12.9	10.1	9.70	9.98	13.2	15.3
Fe	10503	10802	6386	6876	4899	7475	6587	8698	9845
K	17715	22787	7425	10057	10595	9486	10112	11331	13870
La	8.14	8.21	5.92	5.96	5.11	5.26	6.19	7.44	6.73
Mg	2673	2509	2142	2062	2055	2990	2291	2654	3191
Mn	210	212	119	128	122	135	131	174	184
Na	1860	1700	1509	1066	1347	1316	1401	1498	2019
Ni	6.62	6.08	5.37	4.50	4.92	7.85	5.66	5.72	6.32
P	226	272	268	233	199	186	221	263	219
Pb	4.99	6.67	2.89	4.20	3.79	3.13	2.62	3.08	4.75
Si	189	203	260	341	469	595	495	379	448
Ti	522	571	377	382	397	362	331	395	532
V	26.2	23.3	25.6	24.1	20.8	21.3	20.1	24.0	32.6
W	1.21	1.67	1.05	1.12	1.26	1.02	0.96	1.20	1.16
Zn	17.0	18.3	8.45	8.07	9.76	8.72	8.55	12.0	14.6