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Soil element fractions affect phytotoxicity, microbial biomass and activity in volcanic areas

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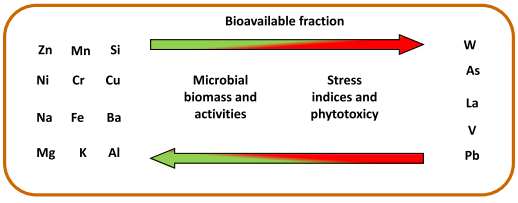
HIGHLIGHTS

- Volcanic soils are peculiar environments for organisms;
- Soil element fractions drove microbial biomass and activity, and phytotoxicity;
- Microbial biomass and activity were affected by reducible and oxidasable fractions;
- Residual fraction drove phytotoxicity more than microbial biomass and activity;
- The investigated volcanic soils showed phytotoxic properties.

GRAPHICAL ABSTRACT



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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, oxidisable 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.

1. Introduction

Soil health and plant productivity are strongly affected by microbial biomass as it is involved in organic matter mineralization and the

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

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

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

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

2. Materials and methods

2.1. Soil sampling

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

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

2.2. Physico-chemical analyses

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

Sequential extraction was applied to measure the concentrations of the elements in the various fractions of the soils as suggested by Ure et al. (1993). These extraction procedures operationally separate the elements into: acid soluble, reducible (associated to Fe-Mn oxides), oxidasable (associated to organic matter content and sulphides) and residual fractions (associated to minerals). According to many authors (He et al., 2006; Ma and Rao, 1997), the first fractions (F1) are considered as bioavailable, the second and third fractions (F2 and F3, respectively) can be potentially bioavailable, whereas the last fraction (F4) is considered not available for organisms. The method for sequential extraction is detailed in Memoli et al. (2018) and briefly reported below.

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

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

Fraction 3 (oxidisable fraction, F3): 10 mL of 8.8 mol L^{-1} hydrogen peroxide solution was carefully added to the residue from the second step. The mixture was digested for 1 h at 22 ± 5 °C and for 1 h at 85 ± 2 °C, and the volume was reduced to <3 mL. A second aliquot of 10 mL of H_2O_2 was added, the mixture was digested for 1 h at 85 ± 2 °C, and the volume was reduced to about 1 mL. The residue was extracted with 50 mL of 1 mol L^{-1} ammonium acetate solution pH 2.0 at 30 ± 10 rpm and 22 ± 5 °C for 16 h. The extract was separated and the residue was washed as in previous steps.

Fraction 4 (residual fraction, F4): the residue from the previous step was digested with aqua regia. In this case, 9.0 mL of HCl (37%) and 3 mL of HNO_3 (69%) were added to 250 mg of soil.

The samples were digested in a microwave oven (CEM MarsX press, USA) according to the procedure described in García-Delgado et al. (2012). The element concentrations in the solutions obtained in each step were determined by ICP-MS (Perkin-Elmer NexION 300). The sum of the concentrations of each element in the four fractions is considered as pseudo-total. One lake sediment sample (BCR-701) certified or with indicative values for extractable metal contents in the three steps of the modified BCR sequential extraction procedure, and indicative values for aqua regia extraction (Rauret et al., 2000), was used to ensure the quality of the results obtained. The accuracy of the obtained values ranged between 80 and 140%.

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

2.3. Biological and phytotoxicological analyses

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

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

Dehydrogenase activity was determined by adding to 1 g of fresh soil 1 mL of 1.5% 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in 0.1 M Tris-HCl buffer at pH 7.5. The reaction mixture was incubated at 30 °C for 24 h in the dark. At the end of incubation, the triphenylformazan (TFF) was extracted with 8 mL of acetone, and the extract was centrifuged at 3500 rpm for 15 min. The absorbance of the supernatant was measured at 546 nm and the results were expressed as mmol of TFF produced in 1 min for 1 g of dry soil.

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

Phytotoxicological assays were performed according to EPA (1996) using a monocotyledon (*Sorghum saccharatum* L.) and a dicotyledon (*Lepidium sativum* L.). The phytotoxicity tests were carried out on fresh and sieved (2 mm) samples. Ten seeds for each species were placed in Petri dishes containing an amount of fresh soil equivalent to 10 g of oven-dried soil, subsequently saturated with water. Standard soil (OECD, 1984) and $K_2Cr_2O_7$ were used as negative and positive controls, respectively. After incubation in darkness (72 h, at 25 °C), the number of germinated seeds and the total root length were measured. The results were expressed as effect percentage of germination index (GI), root elongation (E) and seed germination (G) compared to a standard soil (OECD, 1984).

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

2.4. Statistical analyses

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

In order to identify the elements that positively or negatively influenced the biological parameters, four Principal Component Analyses (PCA) were carried out on matrixes containing the mean values, calculated for the sites, of element contents in each fraction (F1: acid soluble, F2: reducible, F3: oxidasable and F4: residual). Furthermore, for each PCA, the first axis scores were correlated (Spearman's test) to the soil biological and phytotoxicological parameters.

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

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

3. Results

3.1. Chemical, biological and phytotoxicological characteristics of the soils

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

The microbial biomasses showed values of C_{mic} ranging from 0.53 and 2.69 mg C g^{-1} d.w. and of C_{fung} ranging between 0.003 and

Table 1
Mean values (±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 (±0.01)	13.0 (±0.54)	27.0 (±1.22)	4.50 (±0.36)	0.18 (±0.02)	0.01 (±0.003)
ELR	6.95 (±0.01)	7.45 (±0.09)	58.4 (±0.40)	8.02 (±0.53)	0.38 (±0.02)	0.03 (±0.003)
ELF	6.98 (±0.01)	10.4 (±0.40)	28.9 (±0.70)	3.57 (±0.23)	0.23 (±0.02)	0.02 (±0.01)
EHR	7.98 (±0.01)	10.6 (±0.13)	14.1 (±0.32)	1.78 (±0.17)	0.11 (±0.01)	0.01 (±0.01)
EHF	6.46 (±0.01)	15.4 (±0.71)	38.7 (±1.31)	5.95 (±1.02)	0.30 (±0.04)	0.05 (±0.01)
MLR	6.57 (±0.01)	46.7 (±0.92)	102 (±1.45)	26.3 (±0.75)	0.65 (±0.04)	0.07 (±0.01)
MLF	6.67 (±0.01)	40.8 (±1.22)	102 (±1.75)	22.3 (±0.18)	0.67 (±0.02)	0.06 (±0.01)
MHR	7.78 (±0.01)	12.0 (±0.75)	26.6 (±0.26)	3.71 (±0.48)	0.24 (±0.03)	0.02 (±0.01)
MHF	7.36 (±0.01)	13.6 (±0.71)	23.0 (±1.28)	5.72 (±1.46)	0.34 (±0.06)	0.05 (±0.05)

Table 2
Mean and median values (±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 CO_2 g^{-1} d.w. h^{-1}), metabolic quotient (qCO_2, expressed as mg C-CO_2 mg g^{-1} C_{mic}), coefficient of endogenous mineralization (CEM, expressed as mg C-CO_2 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	*L. sativum* (GI)	*L. sativum* (E)	*L. sativum* (G)	*S. saccharatum* (GI)	*S. saccharatum* (E)	*S. saccharatum* (G)
NR	1.54 (±0.48)	0.02 (±0.01)	0.14 (±0.05)	0.02 (±0.008)	0.51 (±0.15)	2.94 (±0.20)	0.19 (±0.01)	−39.1 (±9.57)	−29.0 (±6.47)	−7.41 (±3.70)	42.7 (±3.62)	52.7 (±2.98)	2.70 (±0.00)
ELR	1.43 (±0.06)	0.01 (±0.002)	0.30 (±0.07)	0.06 (±0.01)	1.93 (±0.48)	5.43 (±0.34)	0.36 (±0.01)	−79.0 (±3.60)	−60.4 (±3.22)	−11.1 (±0.00)	48.4 (±3.31)	47.8 (±1.83)	20.7 (±3.60)
ELF	1.72 (±0.48)	0.02 (±0.01)	0.24 (±0.03)	0.04 (±0.004)	1.05 (±0.12)	4.83 (±0.23)	0.15 (±0.01)	−58.7 (±12.9)	−42.2 (±11.6)	−11.1 (±0.00)	53.5 (±4.76)	50.4 (±4.97)	24.3 (±6.24)
EHR	0.53 (±0.23)	0.008 (±0.008)	0.07 (±0.02)	0.04 (±0.009)	0.32 (±0.08)	2.95 (±0.20)	0.00 (±0.00)	−18.0 (±4.50)	−9.81 (±7.24)	−7.41 (±3.70)	53.5 (±7.98)	55.5 (±3.93)	17.1 (±9.53)
EHF	0.81 (±0.06)	0.003 (±0.007)	0.14 (±0.01)	0.05 (±0.004)	0.42 (±0.02)	3.76 (±0.24)	0.15 (±0.01)	−69.1 (±17.3)	−56.2 (±11.9)	−7.41 (±3.70)	51.0 (±4.46)	50.0 (±1.41)	20.7 (±9.53)
MLR	2.50 (±0.29)	0.03 (±0.08)	0.69 (±0.007)	0.07 (±0.001)	0.69 (±0.03)	7.00 (±0.85)	0.44 (±0.004)	−27.3 (±5.39)	−18.1 (±4.38)	−7.41 (±3.70)	62.1 (±3.75)	53.2 (±7.00)	31.5 (±14.4)
MLF	2.70 (±0.08)	0.07 (±0.006)	0.49 (±0.08)	0.05 (±0.01)	0.56 (±0.09)	6.08 (±0.28)	0.50 (±0.02)	−53.8 (±11.8)	−47.6 (±4.11)	−3.70 (±7.41)	38.5 (±8.93)	45.2 (±6.14)	9.91 (±7.21)
MHR	0.99 (±0.39)	0.01 (±0.005)	0.16 (±0.03)	0.04 (±0.01)	0.58 (±0.06)	3.79 (±0.13)	0.06 (±0.01)	−10.4 (±5.84)	−14.9 (±6.54)	−3.70 (±7.41)	36.4 (±10.3)	40.9 (±4.04)	13.5 (±12.5)
MHF	0.82 (±0.03)	0.01 (±0.001)	0.13 (±0.009)	0.04 (±0.003)	0.48 (±0.07)	3.07 (±0.08)	0.07 (±0.01)	−29.2 (±1.51)	−28.6 (±1.50)	0.00 (±0.00)	34.5 (±9.32)	38.5 (±5.75)	13.5 (±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

0.07 mg C g^{-1} d.w. (Table 2). The microbial activities also widely varied (*i.e.*, 0.07–0.69 mg CO_2 g^{-1} d.w. d^{-1} for respiration; 0.32–1.93 mg C-CO_2 g^{-1} C_{org} for CEM; 0.02–0.07 mg C-CO_2 mg g^{-1} C_{mic} for qCO2; 0.00–0.50 mmol TFF min^{-1} g^{-1} d.w. for DHA and 2.94–7.00 mmol FDA min^{-1} g^{-1} d.w. for HA) among the soils (Table 2).

The phytotoxicological assays showed biostimulating effects on seed germination and root elongation for L. *sativum* and inhibiting effects for *S. saccharatum* (Table 2). The assays performed with L. *sativum* showed wider variability as compared to those performed with *S. saccharatum* (Table 2).

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

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

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

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

Microbial and fungal carbon contents and some biological activities (*i.e.*, respiration, CEM, DHA and HA) were positively correlated to the contents of Mn, Ni and W in F1 and negatively correlated to La in F1 (Table 4). The microbial carbon content was also positively correlated to the contents of B and K in F1 (Table 4). The statistically significant correlations between the microbial activities with the elements in the F1 fraction were mainly positive with the exception of those with Al, Fe, La and P (Table 4).

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

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

4. Discussion

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

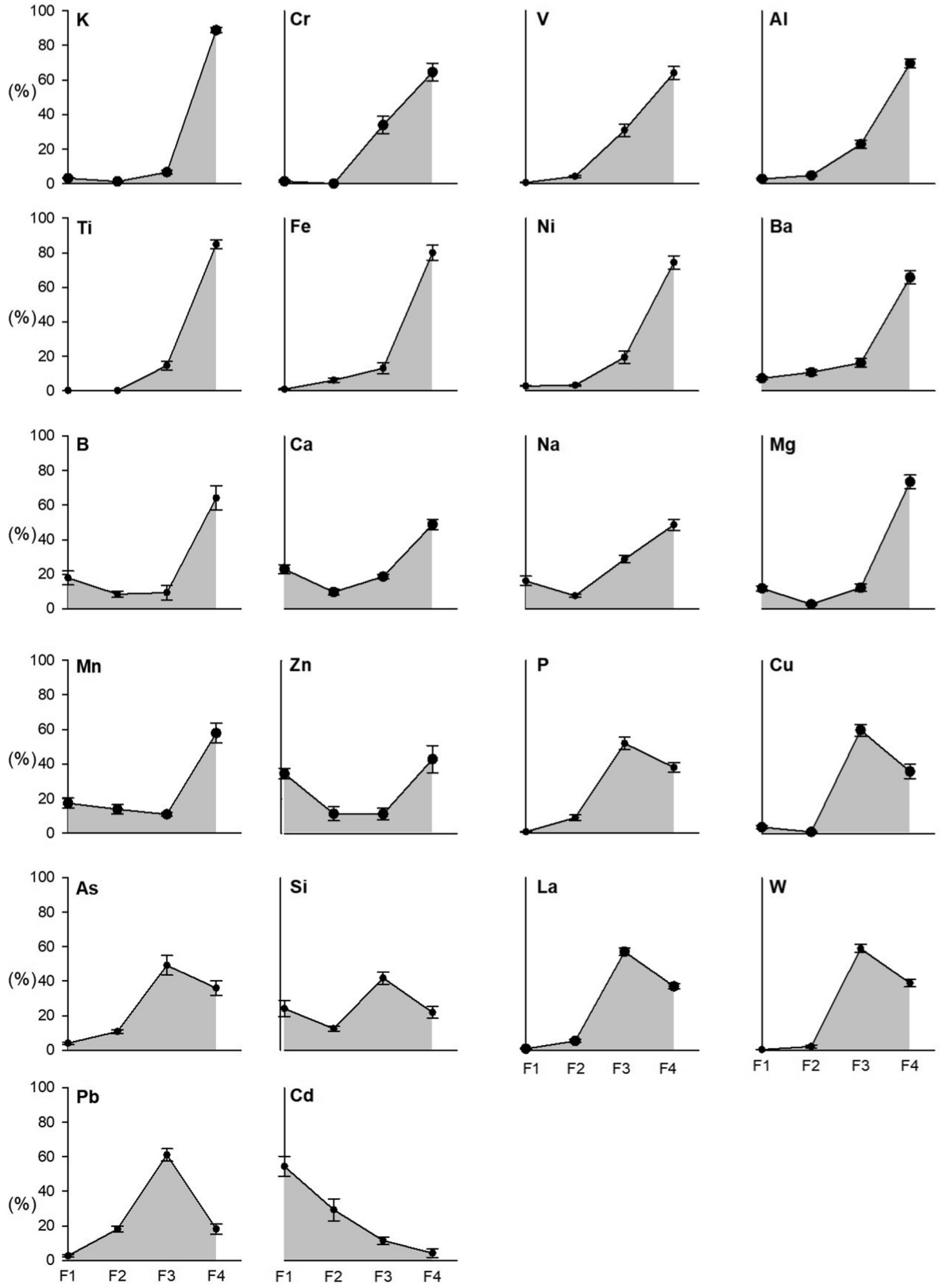


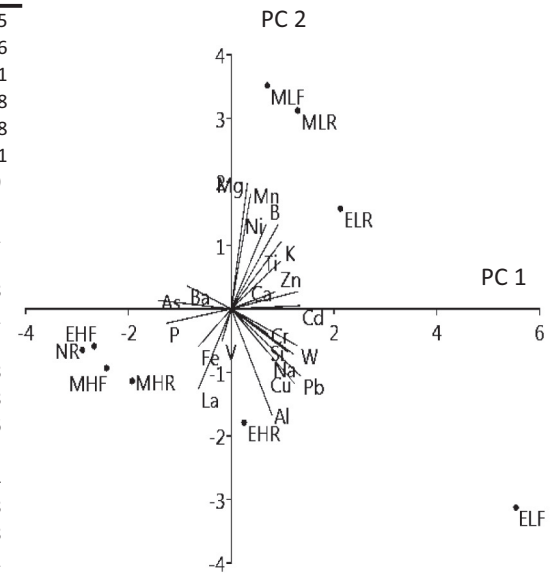
Fig. 1. – Mean values (± s.e.) of percentage contributes of elements in acid soluble (F1), reducible (F2), oxidasable (F3) and residual (F4) fractions to pseudo-total concentration.

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

The low abundance of K, Cr, V, Al, Ti, Fe, Ni and Ba in F1, F2 and F3, with respect to the pseudo-total concentrations, identified these elements as the most scarcely available. The same behaviour was detected for B, Ca, Na, Mg, Mn and Zn although their relative abundances in F4, the residual fraction, were lower (50–80%) than those observed for

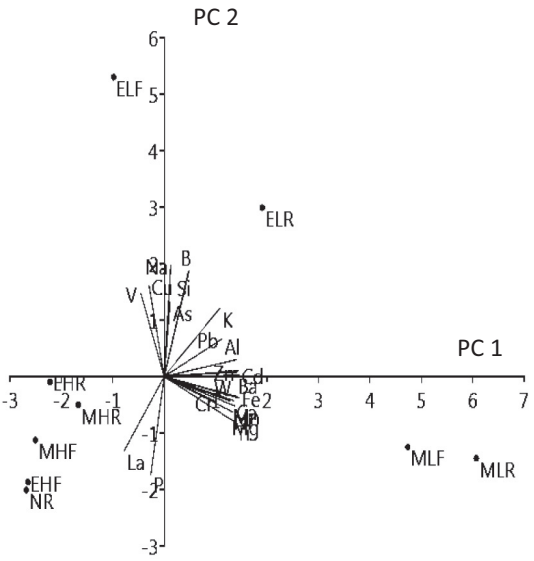
F1

	PC 1
As	-0.85
P	-0.76
Ba	-0.51
Fe	-0.38
La	-0.38
V	-0.11
Mg	0.19
Mn	0.23
Ni	0.41
Cr	0.43
Al	0.48
Ca	0.52
B	0.55
Ti	0.58
K	0.58
Si	0.66
Na	0.73
Cu	0.74
W	0.78
Zn	0.78
Cd	0.81
Pb	0.81



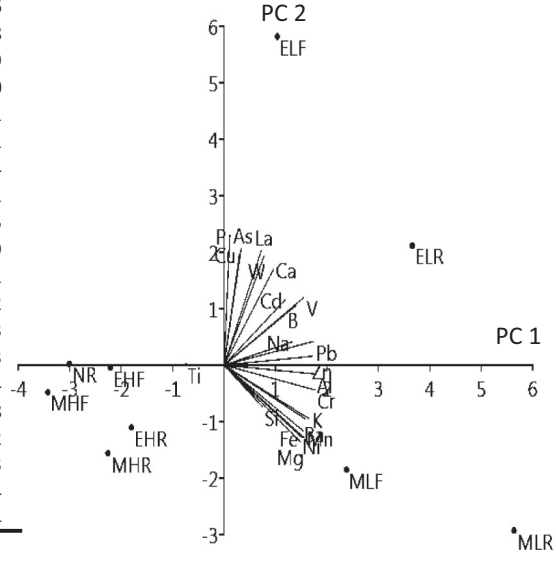
F2

	PC 1
La	-0.52
V	-0.30
Cu	-0.19
P	-0.17
As	0.08
Na	0.08
Si	0.31
B	0.32
Cr	0.67
K	0.71
Pb	0.75
W	0.85
Mg	0.89
Ti	0.89
Mn	0.90
Ni	0.91
Al	0.93
Zn	0.94
Ca	0.94
Ba	0.96
Fe	0.96
Cd	0.97



F3

	PC 1
Ti	-0.41
P	0.06
Cu	0.16
As	0.18
La	0.39
Si	0.40
W	0.41
Ca	0.51
Cd	0.64
Na	0.71
B	0.75
Mg	0.79
Fe	0.81
Ni	0.82
V	0.83
Mn	0.83
Ba	0.84
K	0.88
Zn	0.92
Pb	0.93
Al	0.94
Cr	0.94



F4

	PC 1
Si	-0.60
Cr	-0.06
Cd	-0.02
Ni	0.28
P	0.36
Mg	0.50
V	0.61
W	0.70
Ba	0.71
Na	0.82
B	0.83
Cu	0.85
La	0.85
Ca	0.86
Al	0.87
Pb	0.87
K	0.90
Fe	0.93
As	0.95
Mn	0.95
Zn	0.97
Ti	0.98

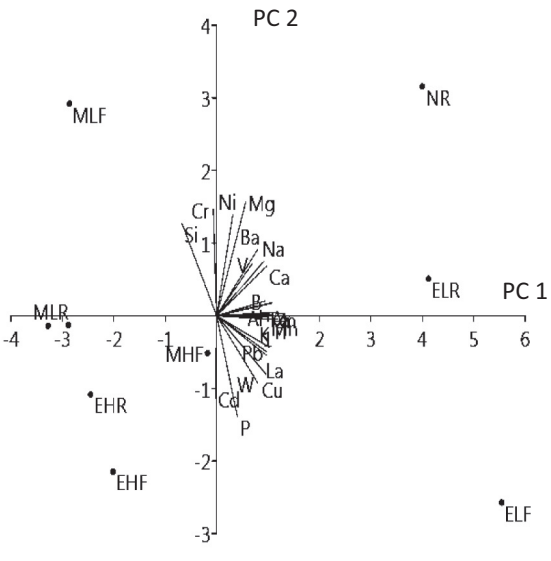


Fig. 2. – Projection in the plane of the results of four Principal Component Analyses (PCA) performed using the mean values, calculated for the sites, of element contents in each fraction (F1: acid soluble, F2: reducible, F3: oxidasable and F4: residual). The loading values of each parameter to the PC1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

the previously cited elements (80–90%). Therefore, sorption mechanisms of B, Ca, Na, Mg, Mn and Zn to inorganic and organic components can be supposed in agreement with the findings reported by Adamo et al. (2003) for volcanic soils of the Solofrana river valley. In addition, it has to be noted that B, Ca, Na, Mg and Mn appeared to be mainly associated to the acid soluble (F1) or reducible fractions (F2), whereas Cu, Si, As, La, W and Pb were associated with the oxidasable (F3) or residual fractions (F4).

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	*L. sativum* (GI)	*L. sativum* (E)	*L. sativum* (G)	*S. saccharatum* (GI)	*S. saccharatum* (E)	*S. saccharatum* (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

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

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

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

The performed PCAs highlighted that the sites exhibited different elemental composition among each fraction. In addition, the contribution of the single element in the characterizing the soil of each site depended on the considered fraction. For instance, As and Pb contributed in opposite trends to characterize F1 but not F2–F4 of the investigated soils. Therefore, as universal trends for single elements in all the fractions were not found, in order to evaluate the effects of soil chemical composition on the biological parameters, the single correlations were taken into account. The positive correlations found between biological parameters and metal reducible or oxidasable fractions suggest that metals likely were strongly bound to various components of the volcanic soils, such as Fe-Mn oxides, organic matter and sulphides that limited their bioavailabilities. This finding agreed with the well-known association of Cr, Cu and As to soil organic matter in forming non-soluble high molecular weight organic complexes (Balasoiu et al., 2001). By contrast, the negative effects caused by the reducible fractions of La and V on many biological activities could be due to the amount of Fe-Mn oxides insufficient to link them or to the weak linkages with Fe-Mn oxides. These oxides precipitate in soils as a result of Mn^{2+} oxidation, promoted by bacteria and fungi, and form highly reactive biofilms (Villalobos et al., 2006) that may explain the numerous positive correlations between microbial activities and element contents in the reducible fraction. The adverse effect of the V reducible fraction on dehydrogenase activity agrees with the results found by Xiao et al. (2015) for stone coal smelting soils.

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

Contrary to what was observed for microbial biomass and activity, soil phytotoxicity would seem to be mainly linked to elements in F4 for L. *sativum*, which as an aggregate negatively influenced seed performance through the PCA (Table 3). However, there were numerous statistically significant correlations between soil fraction elemental concentrations and seed germination and growth and these differed for L. *sativum* and *S. saccharatum*. *L. sativum* responded positively to the presence of different metals in the F1 fraction, but negatively to those in the F2–F4 fractions. In contrast, *S. saccharatum* was stimulated by trace metals, including Cd and Pb in the F1 and Cu in the F3. These different responses for these test organisms agreed with those reported by other authors (Baderna et al., 2014; Czerniawska-Kusza et al., 2006). Biostimulation is often considered an initial trigger of hormesis, an adaptive response to low doses of toxicant resulting in a relevant preliminary stimulation of an effect which turns into strong toxicity when toxic doses rise (Calabrese and Baldwin, 2003). The high phytotoxicity of the investigated soils is also confirmed by the higher percentage effects compared to those observed for volcanic urban or agricultural soils in the downtown or surroundings of Naples, Southern Italy

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	*L. sativum* (GI)	*L. sativum* (E)	*L. sativum* (G)	*S. saccharatum* (GI)	*S. saccharatum* (E)	*S. saccharatum* (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	*L. sativum* (GI)	*L. sativum* (E)	*L. sativum* (G)	*S. saccharatum* (GI)	*S. saccharatum* (E)	*S. saccharatum* (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*****	**−0804****	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	*L. sativum* (GI)	*L. sativum* (E)	*L. sativum* (G)	*S. saccharatum* (GI)	*S. saccharatum* (E)	*S. saccharatum* (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	*L. sativum* (GI)	*L. sativum* (E)	*L. sativum* (G)	*S. saccharatum* (GI)	*S. saccharatum* (E)	*S. saccharatum* (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	0283	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

(Manzo et al., 2008, 2010; Maisto et al., 2011; Memoli et al., 2017). Besides, microorganisms and plants differently responded to various metals in the same fraction according to the levels of sensitivity typical of various organism types (Isidori et al., 2003), as it was the case of Pb in F1 and F4 or As in F2 and F3 that were involved in phytotoxic effects but not in microbial responses.

5. Conclusions

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

The microbial biomass and activity appeared 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 element content in the various fractions stimulated the microbial biomass and activity.

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

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