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Effetti dell'utilizzo del territorio sulla qualità del suolo

Effects of land use on soil quality

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INDEX

Sommario

Il suolo è la principale componente delle aree terrestri e fornisce molti servizi che garantiscono la funzionalità degli ecosistemi; supporta la vita degli organismi, sostiene la produttività delle piante o delle colture, regola gli scambi gassosi tra l'atmosfera e la biosfera, assicura il benessere degli organismi e mantiene la qualità ambientale.

Nell'ultimo secolo, la densità della popolazione umana è notevolmente incrementata causando forti variazioni ambientali dovuti alla necessità, da parte dell'uomo, di soddisfare i propri bisogni e un'eccesiva trasformazione del territorio. I diversi utilizzi del territorio determinano profonde alterazioni della funzionalità dell'ecosistema con conseguenti danni ai processi fisici, chimici e microbiologici. Poiché la formazione del suolo è un processo più lento rispetto al suo consumo, è necessario preservarne la qualità al fine di sostenere la salute dell'ambiente. Poiché le funzioni del suolo sono difficili da misurare, le proprietà del suolo sensibili a determinati cambiamenti possono essere utilizzate come indicatori di qualità.

La presente ricerca fornisce un contributo alle attuali conoscenze sulle caratteristiche dei suoli soggetti a fenomeni di degradazione ambientale dovuti all'uso intensivo ed estensivo del territorio da parte dell'uomo (cave, agricoltura, urbanizzazione) e alla gestione delle riserve naturali. Lo scopo principale della ricerca è stato quello di valutare la qualità di suoli sottoposti a differenti impatti antropici valutando: i) la possibilità di poter recuperare una cava attraverso una singola aggiunta di ammendanti organici; ii) le caratteristiche chimiche e biologiche di suoli agricoli inseriti in un contesto urbano e di suoli forestali all'interno del Parco Nazionale del Vesuvio. Inoltre, sono state investigate le interazioni tra le caratteristiche chimiche dei suoli e il biota ed è stato proposto un minimo numero di indicatori per offrire uno strumento utile ai gestori dei parchi in maniera tale da monitorare, preservare o migliorare la qualità del suolo.

I principali risultati della ricerca mirata al recupero di una cava attraverso una singola applicazione degli ammendanti organici investigati (compost e una mistura di compost e

pollina) ha evidenziato che le caratteristiche dei substrati migliorano a lungo termine in quanto la disponibilità dei metalli decresce notevolmente e si osserva un progressivo incremento della componente microbica. I suoli agricoli inseriti in un contesto urbano e i suoli del Parco Nazionale del Vesuvio sono risultati leggermente contaminati da Cu e Pb, ciò nonostante la componente microbica non sembra essere influenzata dalle loro concentrazioni. Inoltre, solo quattro indicatori, selezionati da venticinque parametri, sembrerebbero sufficienti al fine di monitorare la qualità del suoli nel Parco Nazionale del Vesuvio.

Summary

Soil is a prime component of terrestrial areas that provides many services that guarantee the functionality of the ecosystems, support organism life, sustain plant or crop productivity, regulate gas exchanges between atmosphere and biosphere, allow organism wellness and maintain the environmental quality.

In the last century, human density rapidly increased, causing strong environmental variations to satisfy their needs and leading to an excessive land transformation. Land use changes determine deep alterations of ecosystem functionality that governs physical, chemical and microbiological processes. As soil formation is slower than its consumption, it is necessary to preserve soil quality in order to sustain environment health. Because soil functions are difficult to measure, soil properties that are sensitive to specific changes can be used as indicators of soil quality.

The present research provides a contribution to the actual knowledge on the characteristics of soils affected by environmental degradation due to intensive or extensive use of lands by humans (quarries, agriculture, urbanization) and by management of a natural reserve. The overall aim of the research was to evaluate the quality of soils undergone to different kinds of anthropic impacts. Particularly, the research aimed: i) to exam the feasibility to recover quarries through the single addition of organic amendments; ii) to evaluate the chemical, biological characteristics of agricultural soils inside an urban fabric and of forest soils inside a National Park. Besides, the studies were also focused on the relationships between the chemical characteristics of the soils and the biota and a minimum data set of indicators was proposed in order to provide a useful tool to decision-makers to monitor, conserve or improve soil quality.

The main results of the research focused on quarry recover through a single application of the investigated organic amendments (compost and a mixture of compost and poultry manure) highlighted an improvement of the substrate properties, as, over the time, the metal

availabilities decrease and microbial community increase. The agricultural soils inside the urban fabric and the soils of the Vesuvius National Park appeared slightly contaminated by Cu e Pb, nevertheless, soil microbial biomass were scarcely affected by their concentrations. In addition, only four indicators, selected from twenty-five parameters, appeared enough to monitor the quality of soils inside the Vesuvius National Park.

CHAPTER 1

1. INTRODUCTION

1.1 Land uses and effects on soil characteristics

During the second half of the 20th century, growth rate of human population rapidly increased, reaching more than doubled to 6.5 billion in 2005 and this expansion is expected to continue for several more decades before peaking near 10 billion later in the 21th century (United Nations 1962, 1973, 2007). Contextually, the surface of lands used to satisfy human needs increased as well, causing a strong impact on terrestrial environments, especially on lithosphere. The increase of global population and growing requirement for humans to satisfy their needs have led to an uncontrolled and excessive use of lands.

Different kinds of land use cause complete removal of soils (quarry) to extract rocks for construction industries or strong alterations of soil characteristics (urban, agricultural, industrial, breeding). In natural areas, biodiversity plays important roles in guaranteeing the correct functioning of the ecosystem and providing good conditions for wellness of living organisms. Human activity, modifying soil characteristics, limit soil capability to provide crucial ecosystem services such as primary production, biodiversity, filtering of toxicants and nutrient dynamics (Joimel et al., 2017). Many ecosystems services are related to soil formation and function (Fig. 1). For instance, soil formation addresses important biological processes in terrestrial ecosystems and carbon storage in soils regulates nutrient cycling and gas exchange (Lavelle et al., 2006). At the same time, soils are source of significant amount of CO_2 due to microbial activity (Schlesinger and Andrews, 2000). Human activities, cause wide variability of soil characteristics that can distort or accelerate natural soil processes (Rossiter, 2007).

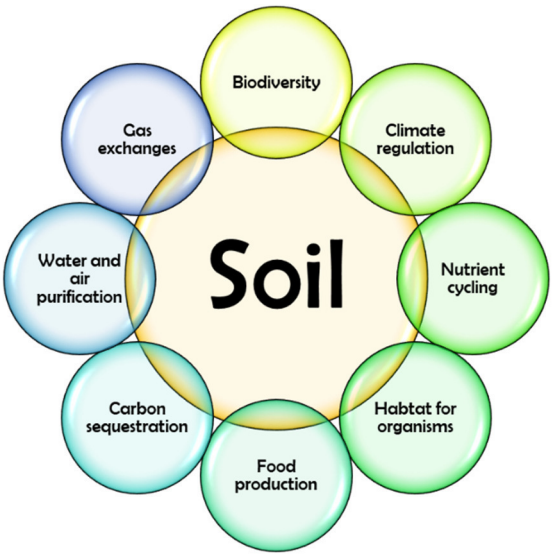

Fig.1 - *Soil ecosystem services*

According to different land uses, difference among soil parameters occur, in fact urban soils can contain much more organic carbon than agricultural soils (Kaye et al., 2005); similarly the accumulation of organic wastes increase carbon storage enhancing mineralization with consequent losses of CO_2 from soils to the atmosphere (Beesley, 2012; 2014). Human activities also cause soil contamination mainly in the form of polycyclic aromatic hydrocarbons (PAHs) and of heavy metals (Maisto et al., 2006, Xiao et al., 2017). Microbial degradation of PAHs is considered the main removal mechanism of these compounds in soil (Semple et al., 2001). Instead, the persistence and fate of metals in soils depend on their solubility and availability (Wuana and Okieimen, 2011).

In more details, urbanization and industrialization cause fragmentation of the landscape that appears as a mosaic of natural and anthropic patches (De Montis et al., 2017) that affect the characteristics of soil surface layer (Liu et al., 2016; Amjadian et al., 2016). Urban and industrial areas depend on far ecosystems, although green areas, such as woodlands, parks, institutional gardens and open spaces, inside them have a key role for organism wellness and ecosystem processes (Scott et al., 2013). In addition, in these environments, conspicuous amounts of contaminants (heavy metals and PAHs) emitted by

domestic heating, municipal and industrial wastes, vehicular traffic, industries occur (Kelly et al., 1996; Norra et al., 2001; Wong et al., 2006; Szolnoki et al., 2013). Instead, agriculture overexploits resources in order to enhance the crop production supplying nutrients and energy into the environments. In fact, the harvesting of crops reduces the accumulation of organic matter on the soil limiting the activity of decomposers that release a few amount of nutrients. For this reason, fertilizers are applied as they have high capability to improve the nutrient status of the soil, especially in terms of N and P. Nevertheless, these substances can represent a potential risk for the environment and organism health, as they are very rich in heavy metals (Nziguheba et al., 2008). For instance, it is reported that the addition of phosphate fertilizers considerably increases the mobility of trace elements, particularly for As (Tokunaga and Hakuta, 2001) and Cd (Bolan et al., 2014) that originate mainly from phosphate rocks used for manufacturing fertilizers. Agricultural practices also require the addition of not degradable compounds or rich in metals to soils to limit the growth of pathogens or undesirable species that can compete with the species of interest for resources. Large quantities of Cu contained in soils where fungicides were applied cause toxicity to plants and microbial communities (Bolan et al., 2014). Instead, not biodegradable substances can bioaccumulate along the food chain, causing damages in organisms belonging to the highest trophic levels (Gąsiorek et al., 2017). Animal breeding also causes alteration of the environment as reduction of the surface layer of soil and porosity, which, in turns, reduce water infiltration and percolation damaging soil structure (Avondo et al., 2013). Moreover, loss of organic matter in soils can lead to reduction of fertility and vegetation regeneration and alteration of nutrient cycle (Pulido-Fernandez et al., 2013; Zhang et al., 2017; Sarker et al., 2018) causing unbalances of different component of the edaphic community. In addition, the deriving anaerobic conditions enhance the production of the reduced compounds that are released into the atmosphere contributing a global warming (Azeem et al., 2014) and changes of pH that facilitates the solubility of cations (Sequi, 1989). Human activities are also

addressed to protect and preserve the naturalness and biodiversity of terrestrial ecosystems through management plans. At this purpose, natural parks are established to: protect ecological integrity of ecosystems for present and future generations, exclude exploitation or occupation inimical to the purposes of designation of the area, provide a foundation for spiritual, scientific, educational, recreational, and visitor opportunities, all of which must be environmentally and culturally compatible (IUCN).

The extensive and intensive use of lands for human needs determines alteration of environmental quality, disfunctioning of ecosystem services and, in turns, soil degradation (Yu et al., 2014), defined as "*the loss of capacity of soil to perform its functions and its ecological services*" (Draft Low 1181, 2013). The main kinds of soil degradation (Fig. 2) are:

- Erosion: natural phenomenon that consists in removal of soil particles by weathering, often as a consequence of human activities;
- Compacting: phenomenon caused by an excessive mechanical pressure due to use of heavy machinery and overgrazing, causing loss of soil porosity and structure and consequently soil fertility;
- Sealing: anthropic action that results in permanent cover of soil due to construction of platforms, building, roads etc.,
- Salinization: accumulation of soluble salts in soil. Normally, it derives by natural phenomena, but sometimes it derives by irrigation of agricultural soils with sea water;
- Desertification: anthropic phenomenon that causes the irreversible loss of soil functions, due to utilization of surface soil layer for agricultural and breeding purposes;

- Contamination: anthropic phenomenon that introduces toxic compounds into the soil.

Fig. 2 – *Different kinds of soil degradation*

1.2 Soil metal contamination

In 400 B.C., Hippocretes considered the “soil health” as an important factor for human health (Krupenicov et al., 2011). Therefore, incorrect and intensive land uses can have adverse effects on soil quality and, then, on human health.

The introduction in soil system of compounds that not have crucial roles in metabolic processes and are not essential for organism life can lead to contamination phenomena. Contamination and pollution are environmental alterations that can be, respectively, defined as "*the consequence of human actions able to alter the condition properties, the availability or the quality of resources within space and time*" and pollution is "*reached when contamination causes adverse effects on organisms, populations, and ecosystems*" (Vighi and Bacci, 1998).

Heavy metals, among the inorganic compounds, play a fundamental role in terrestrial ecosystems as they do not undergo to microbial or chemical degradation and persist in the environment for a long time after their introduction (Radha et al., 1997; Bolan et al., 2014).

They are defined as all elements of the periodic table characterized by a density greater than 5g/dm^3. They are categorized as essential and non-essential metals. Essential metals include elements such as copper (Cu), zinc (Zn), manganese (Mn), nickel (Ni) and iron (Fe) that have important regulatory roles in a great number of biological processes; whereas non-essential metals such as cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As) and chromium (Cr) have not known biological functions and can cause toxicity also at very low intracellular concentrations (Fageria et al., 2009; Chaffai and Koyama, 2011; Sarwar et al., 2017).

The primary source of heavy metals in soils is due to parent material alteration during the pedogenesis. Especially volcanic soils, deriving by pyroclastic materials (Shoji et al., 1978) and rich in neoformed amorphous aluminosilicates and organo-mineral compounds (Eswaran et al., 1993; Tanneberg et al., 2001), show high concentrations of Fe, Cr, Cu, Mn, Ni, Pb and Zn (Vigneri et al, 2017).

Nevertheless, the recent development of industrial, agricultural and mining sectors as well as urbanization have increased soil heavy metal concentrations. For instance, it is known that long-term use of fertilizers in agricultural practise, to enhance crop production, causes release of large amount of metals in soil (Czarnecki and Düring, 2015).

The fate and behaviour of elements in soil depend on their chemical forms (Fernández-Ondoño et al., 2017). Therefore, in studies focusing on environmental risk, it is necessary to investigate not only the total concentrations but also the bioavailable fraction as it indicates the real portion that can be uptaken by soil dwelling organisms (Tack and Verloo, 1995; Peijnenburg et al., 1997; Businelli, 2007; Gomez-Sagasti et al., 2016). The bioavailable fraction of elements can enter cell through the membrane (Wolt, 1994; Semple et al., 2004) and includes (Violante, 2002):

- ✓ the soluble fraction: immediately available and present in the soil liquid phase;

- ✓ the exchangeable fraction: easily available elements and adsorbed on the exchange surfaces;
- ✓ the available reserves: moderately available and combined with mineral or organic forms, or adsorbed in not accessible positions.

The unavailable portion, represented by metals involved in the structural organization of solid inorganic and organic compounds, is greatly resistant to alteration and mineralization.

Unlike pedogenic inputs, heavy metals added in soil by anthropogenic activities are characterized by high bioavailability as they are often added into soil in forms, such as soluble, exchangeable, reducible, oxidasable, with high mobility (Naidu et al., 1996; Lamb et al., 2009).

Metal mobility and bioavailability in soils are strongly regulated by various abiotic factors (*i.e.* soil texture, pH, cationic exchangeable capacity, organic matter content and electric conductivity) that affect processes of sorption/desorption (art. Chimico Degryse et al., 2009; Abreu et al., 2012). In particular, pH and organic matter content are key parameters that control element mobility in soils. At low pH values, the abundance of H^+ increases their competition for anion groups (OH^-, CO_3^{2-}, SO_4^{2-}, Cl^-, S^- and PO_4^{3-}) causing metal release in soil (Tahervand et al., 2017). By contrast, high soil organic matter content allows the complexion of metal ions with insoluble organic compounds, reducing their mobility.

In addition to abiotic factors, also soil dwelling microorganisms and plants, modifying the element oxidation state, affect metal bioavailability. In fact, soil microfauna and plant roots can release protons or oxygen that favour reductions or oxidation reactions. In addition, they can release agents (such as organic acids and siderophores) that bind metals, facilitating their absorption. Nevertheless, it is generally accepted that metals reduce the amount of soil microbial biomass (Brookes and McGrath, 1984; Chander et al., 1995). Besides, metals have negative effects on various enzyme activities, causing decrease in the functional diversity of

the soil ecosystem (Kandeler et al., 1996) and changes in the microbial community structure (Frostegård et al., 1993; Pennanen et al., 1998). However, long-term metal exposure may also lead to the development of metal tolerant microbial populations (Ellis et al., 2003). Due to their sensibility to soil alterations and their relation to soil functionality, soil microbial populations and their activities have been often used as indicators of soil quality assessment (Pankhurst et al., 1995). In particular, soil enzymatic activities are considered as sensitive and early indicators of both natural and anthropogenic disturbances (Giller et al., 1998).

In addition, the adverse effects of heavy metals are also documented on plants as they exert growth inhibition, biomass reduction and photosynthesis rate decrease (Nagajyoti et al., 2010). Besides, heavy metals, due to their similarity with nutrient cations, interfere with their uptake. For instance, As and Cd^{2+} compete with P and Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+} for their absorption, causing plant mineral deficiency (Barceló and Poschenrieder, 1990; DalCorso et al., 2013; Sharma and Archana, 2016).

1.3 Soil quality

Soil is characterized by physical, chemical and biological factors and is the result of continuous conservation and degradation processes. It supports all terrestrial life forms. In order to assure the integrity of terrestrial ecosystems and to recover them from disturbances, such as drought, climate change, pest infestation, pollution, and human exploitation including agriculture is necessary to main high quality of soils (Ellert et al., 1997). In addition, the new environmental constraints that affect forest ecosystem functions and plant or crop productivity can be overcame by soils of high quality.

The widespread definition of soil quality is "*the capacity of soil to function to sustain plant and animal productivities, to maintain or enhance water and air quality and to support human health and habitation*" (Doran and Parkin, 1994; Karlen et al., 1997). The necessity to define the quality of a soil is given by the possibility to provide early warning signs of

adverse trends or to assess sustainable agricultural management (Doran and Zeiss, 2000; Marzaioli et al., 2010; Takoutsing et al., 2016). The physical, chemical and biological properties, that influence soil production and that are sensitive to environmental changes, are typically chosen as soil quality indicators (Nosrati, 2013; Takoutsing et al., 2016). Among soil properties, soil indicators should be simple and easy to measure, should cover the largest possible situations (soil types), including temporal variations, should be highly sensitive to environmental changes and soil management (Saviozzi et al., 2001), should be accessibility and usefulness to producers, scientists, conservationists and policy makers (Rezaei et al., 2006).

Because of the multi-functionality of soil, it is not enough to identify one single property as general indicator of soil quality (Paz-Ferreiro and Fu, 2016). Biological indicators and microbial indicators, quickly responding to environmental changes, have recently attracted attention to define soil quality (Dose et al., 2015; Niemeyer et al., 2012). Besides, they often are correlated with the physico-chemical properties of soil, such as between dehydrogenase activity and chemical indicators, such as soil organic matter, pH and nutrients (Cardoso et al., 2013; Doi and Ranamukhaarachchi, 2009).

To provide a useful tool to decision-makers, quantifying the soil quality is necessary. For this reason, indexes of soil quality are proposed, as they are easy to implement and are quantitatively flexible (Andrews et al., 2002; Swanepoel et al., 2014). Soil quality index normally includes three steps (Fig. 3): (1) choosing appropriate indicators, (2) scoring the indicators, and (3) combining the indicator scores into an index.

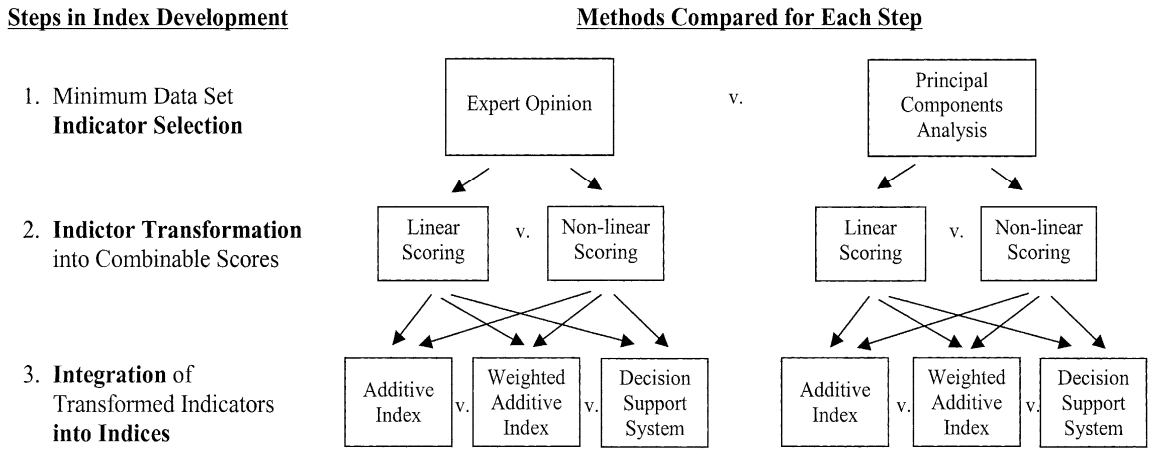

Fig. 3 - *Flow diagram depicting the three steps of index creation and the alternative methods for each step* (Andrews et al., 2002)

As the huge soil properties of soils that contribute to their quality, it is necessary to identify a minimum data set that is a group of soil quality indicators that are chosen, through statistical approaches, mainly on the basis of their importance in soil functionality (Harris et al., 1996). However, soil quality and its assessment is soil- and site-specific, and can vary according to factors, such as climate, land use or inherent soil properties (Karlen et al., 1997).

CHAPTER 2

AIMS OF THE RESEARCH

Land uses, according to management practices and contaminant emissions, differently affect soil chemical and biological characteristics. In order to guarantee high biodiversity, wellness of organisms living in terrestrial ecosystems, environmental quality and correct functionality of the ecosystems it is necessary to maintain high quality of the soils. In this concern, it is essential to monitor the element concentrations in soil system in order to manage it correctly. In the last years, the governments have recognized the importance of soil pollution and particular attention has been focus to study, more in detail, the effects that these compounds have on the ecosystems, in particular on biotic component, to implement new strategies in order to resolve existing problems and manage the best emerging ones.

The overall aim of the research was to investigate soils undergone to different kinds of anthropic impacts. Particularly, the research aimed: i) to exam the feasibility to recover quarries, from which soils were completely removed, through the addition of organic amendments; ii) to evaluate the chemical, biological characteristics of agricultural soils inside an urban fabric and of forest soils inside a National Park. Besides, the studies were also focused on the relationships between the chemical characteristics of the soils and the biota (*i.e.* soil microorganisms or crops). Below, the main aims will be better described taking into account the specific focuses of the investigated researches.

Firstly, in order to restore the ecosystem services lost for elimination of soil, that was removed to use rocks for construction industry, the evaluation of the chemical, biological and ecotoxicological characteristics of two organic amendments added on limestone debris in a mesocosm trial set up in 2004 was performed. In this study, the obtained results in samples collected after one and ten years from the setting up were compared to provide information linked to brief- and long-term effects on the amendment characteristics (the

paper published in *Ecosphere* journal, with DOI: 10.1002/ecs2.2009, is reported in Chapter III, section 3.1).

Recently, crops are grown in small areas inside the urban fabric in order to increase green spaces in complex human-dominated environments. These agricultural soils are affected by integrated inputs of contaminants. In fact, they are already characterized by a peculiar composition of metals deriving by the agricultural practices and besides they receive contaminants coming from the surrounding urban environments. In this research, the concentrations of some metals were investigated and linked to soil microbial parameters in order to highlight the probable effects of metal content on the structure and activity of the microbial component designated to matter cycle and decomposition process. In addition, metal accumulation in different portions on the crops that can be used in the human dietary was evaluated (the paper published in *Applied Soil Ecology* journal, with DOI: https://doi.org/10.1016/j.apsoil.2017.09.035, is reported in Chapter III, section 3.2).

After studying environments directly affected by human activities, the research was focused on a peculiar environment, the Vesuvius National Park. This area is an example of great naturalistic importance in the world but, at the same time, is affected by strong human pressure due to tourism and to high level of urbanization of the metropolitan area of Naples in its surroundings. A contribution to the actual knows out on soil quality assessment was given. In fact, a study was performed in order to select few indicators, according two statistical approaches, among numerous chemical, biological and ecotoxicological parameters useful to evaluate the soil quality by an integrated linear index. The soil quality index was calculated after ranking the investigated parameters by linear scoring technique. The scores were assigned applying *more is better* or *less is better* functions (the paper submitted for publication in *CATENA* journal is reported in Chapter III, section 3.3).

Finally, the Vesuvius National Park was investigated in order to discriminate different (geogenic or anthropogenic) derivations of many metals in the surface soils. Besides, the

availability and mobility of the metals were also investigated. Knowing the metal derivation in soils could be a useful tool to identify the main emission sources and, consequently, regulate them in order to decrease their concentrations in the environment (the paper submitted for publication in *Science of The Total Environment* journal is reported in Chapter III, section 3.4).

CHAPTER 3

3.1 Short- and long- term effects of a single application of two organic amendments

(the paper is published in *Ecosphere*, DOI: 10.1002/ecs2.2009)

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ECOSPHERE

Short- and long-term effects of a single application of two organic amendments

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Abstract. A frequent side-effect of soil treatment with organic amendments is the slow release of harmful metals deriving from the initial matrices, mainly municipal waste and manure from intensive farming. Contamination is amplified by repeated treatments, which is a common practice to maintain soil fertility. The aim of the present research was to compare, in a mesocosm trial, short (one year)- and long-term (ten years) effects of a single application of compost or a mixture of compost and poultry manure to limestone waste. Attention was focused on pH, organic matter content, metal availability, and microbial biomass and activity. Amendment ecotoxicity at ten years after application was also evaluated. A single application reduced the metal availability and metabolic quotient (an index of stress condition). In the long term, an overall improvement of the environmental conditions has been observed, as the microbial biomass increased, respiration decreased (suggesting low energy requirement) and mineralization activity decreased (likely due to high recalcitrance of residual organic matter). In the brief term, poultry manure played a significant role in improving the environmental conditions as it contributed to reduce the metal availability and to enhance the microbial biomass and activity. In the long term, the overall conditions of both the organic amendments appeared favorable for organisms as low ecotoxicity occurred.

Key words: compost; ecotoxicity; heavy metals; microbial activity; microbial biomass; poultry manure.

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Introduction

Organic amendments (i.e., compost, poultry manure, peat, sewage sludge, or others) increase soil organic matter and nutrient concentrations and enhance soil structure, porosity, and water penetration; hence, they are widely employed to improve poor or degraded soils (Gigliotti et al. 1996, Celik et al. 2004, Bastida et al. 2007, Businelli et al. 2009, Hernandez et al. 2015). Amendments rich in humic substances such as quality compost release nutrients gradually (Schnitzer and Khan 1978, Cooperband et al. 2002), and hence, they have a protracted effect on plants and edaphic microorganisms (Caravaca et al. 2002); in contrast, amendments rich in nitrogen (i.e., manure) induce rapid plant growth (Hesse et al. 2004, Delgado et al. 2012, da Silva Oliveira et al. 2017). By releasing organic exudates through the roots, plants can increase the biomass and activity of edaphic microorganisms (Singh et al. 2004, Chaparro et al. 2014).

A possible side-effect of soil treatment with organic amendments is the release of pollutants. Bioaccumulation of inorganic (mainly harmful metals) and persistent organic pollutants (e.g., PCBs and dioxins) is an unavoidable consequence of compost production and is becoming a major

risk due to increasing use of municipal solid waste, sewage sludge, or manure from industrial plants as starting materials (Achiba et al. 2009). The applications of organic amendments are often repeated several times in order to improve soil properties and obtain high yields, but this may increase contamination (Iwegbue et al. 2007, Kidd et al. 2007). Long-term effects of repeated applications of organic amendments have been investigated quite extensively (Ros et al. 2006, Achiba et al. 2009, Hernandez et al. 2015), but the effects of single applications are still unknown.

In Campania Region (southern Italy), concrete industry produces about 14,026,000 t/yr of limestone debris whose disposal is a major environmental problem (BURC 2006). In parallel, ~2,257,500 t of municipal waste was collected in the last year, with 60% being used for compost production (ORR 2016). With the objective of evaluating the application of organic amendments for reducing the impact of limestone waste while avoiding contaminant accumulation, we investigated short- and long-term effects (one and ten years, respectively) of a single application of two types of organic amendments to limestone material from a local quarry.

Materials and Methods

Mesocosm setting up

The research was carried out in mesocosm trials. In March 2004, 15 pots 1 m in diameter were filled to about 60% of total height (60 cm) with limestone debris with 1–4 cm granulometry, from a quarry in the Caserta area (Campania, Italy). One mesocosm containing compost only was kept as control (C); in seven mesocosms (C_p), compost mixed with expanded clay pellets (70:30 = v:v) was placed above the limestone debris; and in the other seven mesocosms (CP_p), a mixture of compost and poultry manure plus wheat husk and expanded clay pellets (60:10:30 = v:v:v) was placed on the limestone debris (Fig. 1). The amount of compost added to each pot was 150 kg f.w., equivalent to about 1900 t/ha. The compost used for the experiments was produced by a local company (Pomigliano Ambiente S.p.A., Pomigliano, Naples, Italy) from the organic share of municipal wastes and green refuses from tree pruning. Two 1-yr-old specimens of native sclerophyllous shrubs, *Laurus nobilis* L. (bay tree), *Phillyrea angustifolia* L. (jasmine box), and *Quercus ilex* L. (holm oak), were transplanted in C_p and CP_p mesocosms, to a total of six specimens per pot. The mesocosms were put outdoors in the Botanical Garden of Naples and adequately irrigated with distilled water.

From each mesocosm and at each sampling time, that is, March 2004, March 2005, and March 2014, three samples of substrate were collected from the surface layer (0–10 cm) and mixed into a representative composite sample (Fig. 1). The samples were sieved (<2 mm) and analyzed for physico-chemical and biological parameters; in addition, ecotoxicological assays were performed only for the samples collected in 2014.

Physico-chemical and biological analyses

An aliquot of each composite sample was oven-dried (75°C, until constant weight) for physico-chemical analyses. pH in water (1.0:2.5 = w:w) was measured using a pH meter. The organic matter content and total C, N, and metal (Cd, Cr, Cu, Ni, and Pb) concentrations were measured on samples powdered by an agate mortar and pestle (Fritsch Analysette Spartan 3 Pulverisette 0), whereas the metal available fractions were determined in non-pulverized samples. The metals to determine were selected among the contaminants indicated in the National Law (Legislative Decree 75/2010) for organic amendments for agricultural use.

In order to characterize the substrates, the total C, N, and metal concentrations were measured only at the beginning of the experiment. Instead, the organic carbon was measured on samples collected at each sampling time in order to calculate the organic matter content. The organic carbon, in samples previously treated with HCl (10%), and the total concentrations of C and N were evaluated by gas chromatography (CNS Analyzer; Thermo Finnigan, Milan, Italy). The organic matter content was obtained multiplying the organic carbon for 1.724 (Pribyl 2010).

In order to obtain the total concentrations of Cd, Cr, Cu, Ni, and Pb, the substrate samples were previously digested by HF (50%) and HNO_3 (65%) in the 1:2 (v:v) ratio in a microwave oven (Digestion/Drying Module mls 1200; Milestone, Leutkirch, Germany); to obtain the available metal fractions, the substrate samples were extracted with diethylenetriamine pentaacetic acid, $CaCl_2$, and triethanolamine at pH 7.3 $\pm$ 0.05

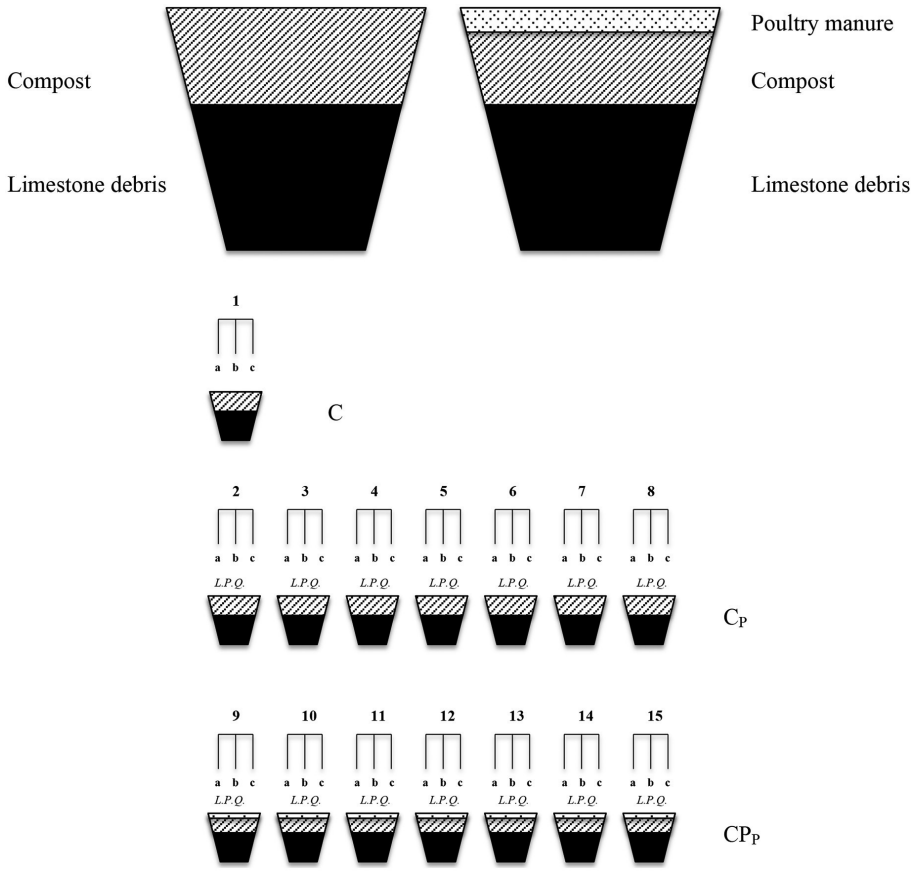

Fig. 1. Scheme of mesocosm setting up (top) and of the sampling procedure (bottom). C, mesocosm with compost without plants; C_P, mesocosms with compost and plants; CP_P, mesocosms with the mixture of compost and poultry manure and plants; *L*, *Laurus nobilis* L. (bay tree); *P*, *Phillyrea angustifolia* L. (jasmine box); *Q*, *Quercus ilex* L. (holm oak); a–c, points of sampling for each mesocosm at each sampling time; 1–15, homogeneous samples from each mesocosm.

(Lindsay and Norvell 1978). The metal concentrations were measured by atomic absorption spectrometry, via graphite furnace (SpectrAA 220 FS; Varian, Sidney, Australia).

Microbial and fungal biomasses and biological activity were measured within three days from collection in samples stored at 4°C. Microbial carbon (C_{mic}) was evaluated by the method of substrate-induced respiration according to Anderson and Domsch (1978), while microbial activity was estimated as potential respiration. Microbial C was measured by CO_2 evolution from soil (3 g of each soil sample) in response to the addition of glucose (2 mL of 75 mmol/L D-glucose), an easily mineralizable substrate, after incubation for 5 d in the dark at 25°C and 55% water holding capacity. CO_2 values were corrected for the CO_2 measured in a blanc and were reported as mg of microbial carbon per g soil. The potential respiration of soil samples was estimated as CO_2 evolution in standard conditions (10 d of incubation at 25°C in the dark, at 55% water holding capacity) and was expressed in mg CO_2 evolved per g soil per time unit.

CO_2 evolution was measured after incubation by NaOH absorption followed by two-phase titration with HCl (Froment 1972). The metabolic quotient, qCO_2 (mg C–CO_2/mg C_{mic}), that is, the degree of activity of the microbial biomass, and the coefficient of endogenous mineralization (CEM, mg C–CO_2/g C_{org}), that is, the rate of organic C mineralization, were calculated using respiration data and microbial C and organic C data, respectively.

Total fungal biomass 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). Soil samples were suspended in a solution (1 g of fresh soil in 100 mL) of phosphate buffer (60 mmol/L, pH 7.5) and homogenized at 4025 g for 2 min. One milliliter of suspension was collected and filtered under vacuum on nitrocellulose filter (pore size: 0.45 μm) and stained with aniline blue. The mass of total mycelia was calculated on the basis of the average values of cross section (9.3×10^{-6} mm^2), density (1.1 g/mL), and dry mass of the hyphae (15% of the wet mass) according to Berg and Söderström (1979). To obtain the fungal fraction of microbial carbon, the values of fungal biomass were converted to 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. All the physico-chemical and biological analyses on the substrates were carried out in triplicate.

Ecotoxicological analyses

The ecotoxicological analyses were carried out only for substrates collected in 2014 and were performed on both raw and sieved (2 mm) samples. Phytotoxicity tests were performed according to EPA (1996) on a monocotyledon (*Sorghum saccharatum* L.) and a dicotyledon plant (*Lepidium sativum* L.). Ten seeds for each species were placed in Petri dishes, containing an amount of fresh organic amendment equivalent to 10 g of oven-dried organic amendment, and subsequently saturated with water. Standard soil (OECD 1984) and $K_2Cr_2O_7$ were used as negative and positive control, respectively. After incubation in darkness (72 h, 25°C), the number of germinated seeds and total root length were measured.

A test with the ostracod *Heterocypris incongruens* was performed according to Chial and Persoone (2003), evaluating the survival and growth as endpoints.

All ecotoxicological analyses were carried out in three replicates, and the results were expressed as percentages relative to the standard soil (OECD 1984).

Statistical analyses

Two-way analysis of variance (ANOVA) was performed considering as fixed factors the mesocosm typologies (C_p and CP_p) and the sampling time (2004, 2005, and 2014) in order to highlight the differences in each parameter attributable to the substrate and/or sampling time. Normality was assessed using the Shapiro–Wilk test and homoscedasticity using equal variance test. The ANOVAs were followed by the post hoc tests of Holm-Sidak.

The relationships between biological and physico-chemical parameters were evaluated by the Spearman test, according to the non-normal distribution of the data, assessed using the Shapiro–Wilk test.

Statistical analyses and graphical displays were performed by Systat_SigmaPlot_12.2 software (Jandel Scientific, San Jose, California, USA).

Results

The total concentrations of C, N, Cd, Cr, Cu, Ni, and Pb as well as C/N ratios in the substrates of the different mesocosm typologies (control, C; compost with plants, C_p; and mixture of compost and poultry manure with plants, CP_p), at the beginning of the experiment (2004), are reported in Table 1. C and N concentrations ranged between 16.9% and 17.5% d.w. and between 1.57% and 1.63% d.w., respectively. Among the investigated metals, Cu and Pb showed higher

Table 1. Mean values of total concentrations of C and N (% d.w.) as well as Cd, Cr, Cu, Ni, and Pb (μg/g d.w.), and mean C/N ratio in the substrates for mesocosms without plants (C), mesocosms with compost and plants (C_p), and mesocosms with a mixture of compost and poultry manure and with plants (CP_p) in 2004.

2004	C	C_p	CP_p	Legislative Decree 75/2010
C	17.0	17.5	16.9	n.r.
N	1.63	1.63	1.57	n.r.
C/N	10.4	10.7	10.8	Max 50.0
Cd	0.41	0.42	0.31	1.5
Cr	11.8	9.60	9.40	n.r.
Cu	149	139	139	230
Ni	21.3	25.4	22.2	100
Pb	118	101	74.0	140

Notes: Also reported are threshold values for organic amendments for agricultural use as established by current Italian legislation. n.r., The Legislative Decree 75/2010 does not report the threshold values for C, N, and Cr. Only the threshold value for the hexavalent Cr (0.5 μg/g d.w.) is reported.

total concentrations relative to Cd, Cr, and Ni, with values of approximately 100 μg/g d.w.

In 2004, the mean pH values were 7.56 in C and 7.70 and 6.80, respectively, in C_p and CP_p (Fig. 2a), and they significantly increased over time, particularly for the CP_p mesocosm (Appendix S1: Table S1).

In 2004, the mean organic matter content was 41.9% d.w. in C, whereas it was 31.1% d.w. and 39.1% d.w., respectively, in C_p and CP_p (Fig. 2b). Later, the organic matter content in C (39.1% d.w. in 2005 and 22.7% d.w. in 2014) was higher than in C_p (28.8% d.w. in 2005 and 17.9% d.w. in 2014) and CP_p (21.5% d.w. in 2005 and 17.4% d.w. in 2014; Fig. 2b). At each sampling time, no statistically significant differences were observed between the organic matter content in C_p and CP_p (Appendix S1: Table S1). A significant decrease in organic matter occurred only in CP_p, from 2004 to 2005 and from 2004 to 2014 (Fig. 2b; Appendix S1: Table S1).

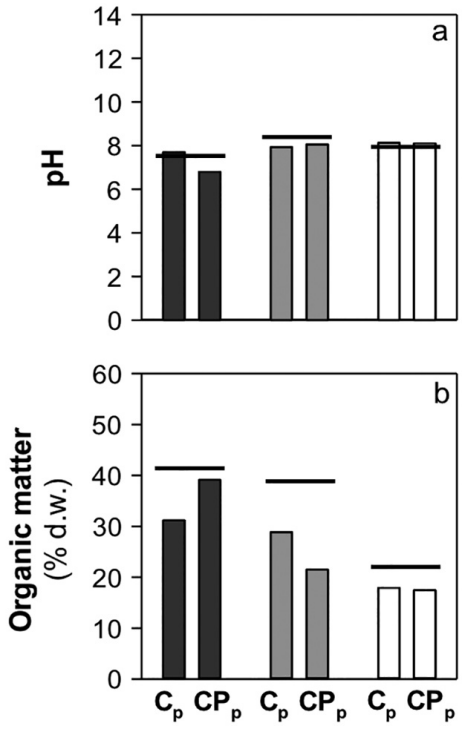

Fig. 2. Mean values (±standard error) of pH (a) and organic matter content (b) in mesocosms with compost (C_p) and mesocosms with a mixture of compost and poultry manure (CP_p) in 2004 (black bars), 2005 (gray bars), and 2014 (white bars). The lines indicate the mean value for the mesocosm without plants (C) at the different sampling times.

At the beginning of the experiment, the available fractions of the metals in C were higher than in C_p (Fig. 3), whereas they were higher than those measured in CP_p only for Cd and Pb (Fig. 3a and 3e). Over time, metal availability decreased in C as well as in the mesocosms with plants (C_p and CP_p) reaching at the end of the experiment comparable values in all three types of mesocosm (Fig. 3). The availability of Cd, both in C_p and in CP_p, did not statistically vary from 2004 to 2005, whereas it decreased significantly in 2014 (Fig. 3a; Appendix S1: Table S1); the availability of the other metals already significantly decreased in 2005 both in C_p and in CP_p with the exception of Ni and Pb in C_p (Fig. 3b, e; Appendix S1: Table S1). The greatest decrease in metal availability was observed for the CP_p mesocosms (Fig. 3). In 2004, the available fractions of Cr, Cu, and Ni were significantly higher in CP_p than in C_p (Fig. 3b–d; Appendix S1: Table S1), whereas in 2005 the available fractions of Cd and Pb were significantly higher in C_p than in CP_p (Fig. 3a, e; Appendix S1: Table S1). In 2014, there were no differences in metal availability between the two mesocosm typologies (Fig. 3; Appendix S1: Table S1).

In 2004, the microbial carbon (C_{mic}) in C (0.25 mg/g d.w.) was comparable to that measured in C_p (0.35 mg/g d.w.) and lower than that measured in CP_p (0.52 mg/g d.w.; Fig. 4a). In all the mesocosm typologies, C_{mic} increased over time reaching in 2014 the value of 3.60, 4.79, and 5.37 mg/g d.w., respectively, in C, C_p, and CP_p (Fig. 4a; Appendix S1: Table S1). Besides, for each sampling time, with the exception of 2005 when C_{mic} in CP_p was significantly higher than in C_p, no significant differences were observed between C_p and CP_p (Fig. 4a; Appendix S1: Table S1). At the beginning of the experiment, fungal carbon (C_{fung}) in C was 0.12 mg/g d.w. and decreased over time to 0.03 and 0.01 mg/g d.w., respectively, in 2005 and 2014 (Fig. 4b). C_{fung} in C_p and CP_p decreased over time (Appendix S1: Table S1) from 0.09 mg/g d.w. (Fig. 4b) to values similar to that measured in C (Fig. 4b); at each sampling time (2004, 2005, and 2014), no statistically significant differences were observed between the two typologies (C_p and CP_p) of mesocosms with plants (Fig. 4b; Appendix S1: Table S1).

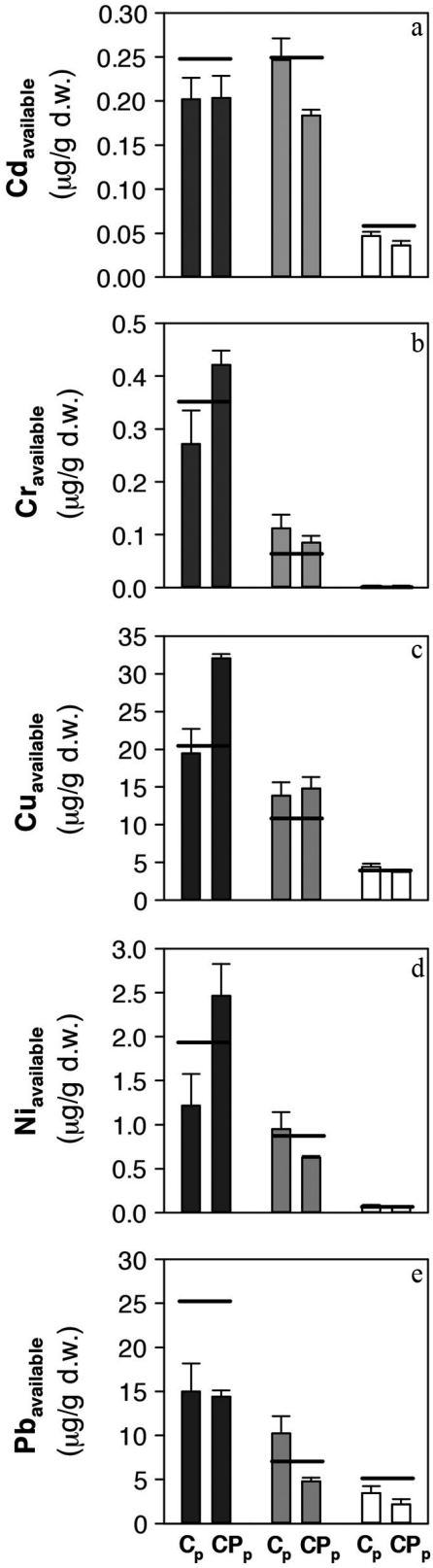

Fig. 3. Mean values (±standard error) of the concentrations of available fractions of Cd (a), Cr (b), Cu (c), Ni (d), and Pb (e) in mesocosms with compost (C_p) and mesocosms with a mixture of compost and poultry manure (CP_p) in 2004 (black bars), 2005 (gray bars), and 2014 (white bars). The lines indicate the mean value for the mesocosm without plants (C) at the different sampling times.

The respiration in C was 14.4 mg CO_2 g^{-1} d.w. 10 d^{-1} at the beginning of the experiment and was slightly higher than in C_p and CP_p where it was, respectively, equal to 10.7 and 13.4 mg CO_2 g^{-1} d.w. 10 d^{-1} (Fig. 4c). Respiration in C drastically decreased reaching in 2014 the mean value of 1.25 mg CO_2 g^{-1} d.w. 10 d^{-1} (Fig. 4c). It also significantly decreased in the mesocosms with plants over time (Appendix S1: Table S1) and reached, at the end of the experiment, values comparable to that measured in C mesocosm (Fig. 4c). With the exception of 2004, when the respiration was significantly higher in CP_p than in C_p, in the other sampling times no differences in respiration between these two mesocosm typologies were detected (Fig. 4c; Appendix S1: Table S1). Initially, the metabolic quotient (qCO_2) in C (14.4 mg C–CO_2/mg C_{mic}) was higher than in C_p (10.4 mg C–CO_2/mg C_{mic}) and CP_p (8.07 mg C–CO_2/mg C_{mic}), but over time it drastically decreased, becoming comparable to those detected in the mesocosm with plants (Fig. 4d). A drastic decrease in qCO_2 was observed in C from 2004 to 2005 and 2014 (Fig. 4d), whereas in C_p and CP_p it decreased just from 2005 to 2014 (Fig. 4d; Appendix S1: Table S1). No differences were observed between qCO_2 values in C_p and CP_p (Fig. 4d; Appendix S1: Table S1). In 2004, the mean value of the CEM in C (14.0 mg C–CO_2/g C_{org}) was lower than those calculated for C_p and CP_p (Fig. 4e) and, over time, it decreased reaching the value of 2.57 mg C–CO_2/g C_{org} in 2014 (Fig. 4e). In the mesocosms with plants, CEM decreased in 2014 reaching values of 6.01 and 7.00 mg C–CO_2/g C_{org} in C_p and CP_p, respectively (Fig. 4e), although different temporal trends were observed in the two mesocosm typologies. In fact, in C_p significant differences were observed only between 2004 and 2014, whereas in CP_p they were detected either between 2004 and 2014 or

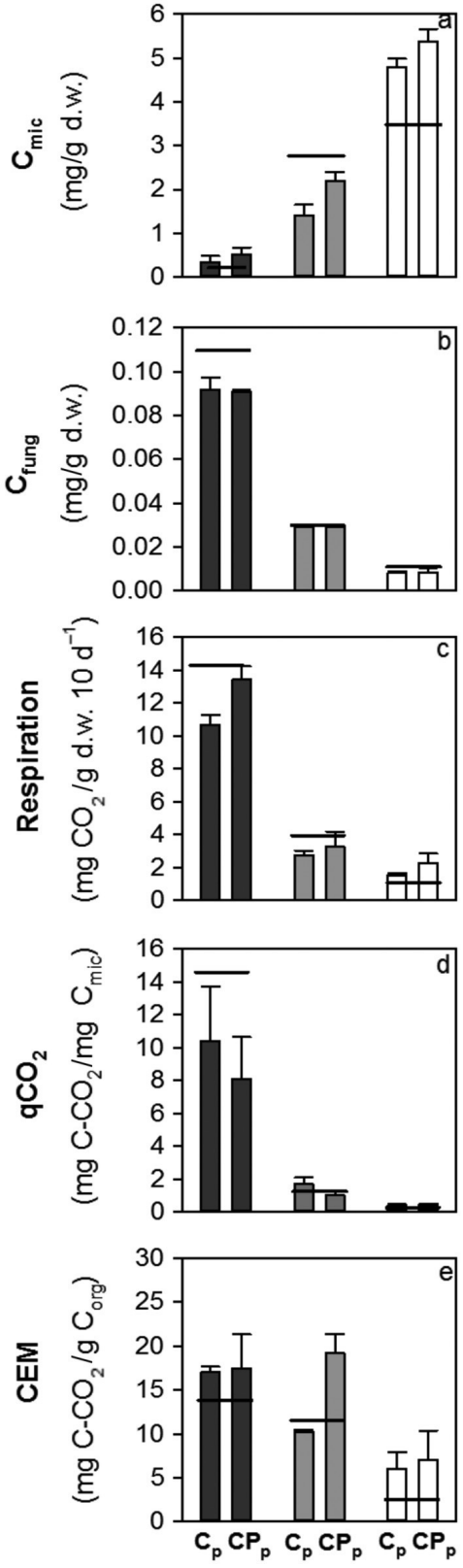

Fig. 4. Mean values (±standard error) of microbial carbon (C_{mic}) (a), fungal carbon (C_{fung}) (b), respiration (Resp) (c), metabolic quotient (qCO_2) (d), and coefficient of endogenous mineralization (e) in mesocosms with compost (C_p) and mesocosms with a mixture of compost and poultry manure (CP_p) in 2004 (black bars), 2005 (gray bars), and 2014 (white bars). The lines indicate the mean value for the mesocosm without plants (C) at the different sampling times.

between 2005 and 2014 (Fig. 4e; Appendix S1: Table S1). Only for the 2005 sampling, significant differences were detected between C_p and CP_p with higher values for CP_p (Fig. 4e).

On the whole, fungal carbon, respiration, and qCO_2 were positively correlated with metal availability and organic matter content, whereas microbial carbon was negatively correlated (Table 2).

The ecotoxicological assays, carried out for the sampling of 2014, showed different responses depending on the tested organisms. The phytotoxicity assays showed percentage effects between 80% and 120% in the substrates of the mesocosm typologies with the exception of root elongation for *Lepidium sativum*, which showed a nearly 150% effect for C_p and CP_p (Fig. 5). The percentage effect of *Heterocypris incongruens* survival was about 86% in C and 60% for C_p and CP_p (Fig. 5); the percentage effect of *H. incongruens* growth was ~65% for C_p and CP_p (Fig. 5). In no ecotoxicological assay, differences were observed between C_p and CP_p.

Discussion

Metal concentrations in the employed amendments were very low, as they did not exceed the threshold values indicated by the National Law (Legislative Decree 75/2010) for organic amendments usable on agricultural soils. Unfortunately, currently the Italian legislation does not consider the acceptable metal thresholds for organic amendments usable to improve degraded or poor soils.

In spite of no differences in total metal concentrations between C_p and CP_p substrates, the poultry manure seems to be the main responsible for the significantly higher Cr, Cu, and Ni availability at the beginning of the experiment (2004), probably because of lower pH. Hernandez et al.

Table 2. Spearman's correlation coefficients assessed between biological and physico-chemical parameters of all the mesocosms.

Parameters	C_{mic}	C_{fung}	Respiration	qCO_2	CEM
pH	0.778***	−0.775***	−0.855***	−0.819***	−0.500*
Organic matter	−0.705***	0.608**	0.604**	0.643**	0.207
$Cd_{available}$	−0.699***	0.595**	0.521*	0.643**	0.243
$Cr_{available}$	−0.874***	0.841***	0.868***	0.878***	0.645**
$Cu_{available}$	−0.856***	0.816***	0.798***	0.839***	0.666**
$Ni_{available}$	−0.874***	0.759***	0.775***	0.827***	0.484*
$Pb_{available}$	−0.903***	0.756***	0.752***	0.847***	0.472*

Note: CEM, coefficient of endogenous mineralization.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

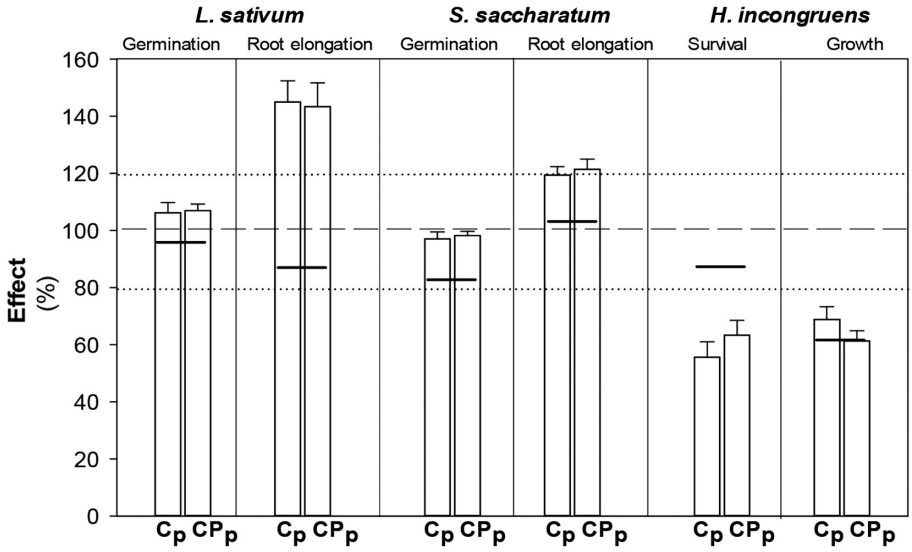

Fig. 5. Mean values of percentage effects of germination and root elongation for *Lepidium sativum* and *Sorghum saccharatum* and percentage effects of survival and growth of *Heterocypris incongruens* for mesocosms with compost and plants (C_p) and mesocosms with a mixture of compost and poultry manure and with plants (CP_p) in 2014. The continuous lines indicate the percentage of effect for mesocosms without plants (C). The dotted line indicates the supposed physiological responses.

(2015) also observed a slight acidification of degraded soils immediately after the addition of peat and manure as amendments, and Pierzynski et al. (1994) report higher metal availability in acidic soils. However, the initially higher metal bioavailability in CP_p substrates did not cause an inhospitable habitat for microorganisms as lower qCO_2, index of stress conditions (Anderson and Domsch 1993), and higher microbial biomass were observed as compared to C_p. Besides, the highest organic matter content in CP_p seems to stimulate the microbial activity, hiding negative effects from higher metal availability.

In the short time, both types of amendment stimulated the microbial biomass as significant increases were observed in comparison with initial values. This effect involved the bacterial rather than fungal component. As fungi are generally more resistant than bacteria to stress conditions (Dighton 2003), the increase in bacterial biomass appears to reflect a sudden improvement in the environmental conditions since the second year after mesocosm setting up, confirmed by the parallel decrease in the respiration rate and qCO_2. In fact, at the beginning of the study period (2004), when the substrate manipulations during the

mesocosm setting up occurred, the highest respiration might depend on high energy requirement by microorganisms to survive in adverse conditions (Anderson and Domsch 1993). Nevertheless, the stimulation of respiration caused by higher aeration, which likely occurred during the manipulations, could not be ruled out. Poultry manure augmented the microbial biomass (Garcia-Gil et al. 2000); hence, the mineralization activity remained close to the initial level (Usman et al. 2013), whereas the organic matter content halved from ~40% to 20% d.w. The decrease in the organic matter content and the higher CEM suggest a more efficient degradation of the mixture of compost and poultry manure (Doni et al. 2014, Hernandez et al. 2014, Jain et al. 2014) as compared to the compost alone. Besides, both types of amendment caused a sudden overall decrease in metal availabilities that mainly occurred in the mixture where, since the first year after the mesocosm setting, decreases in Cd, Ni, and Pb were highlighted.

A further change occurred in the biological community in both amended substrates, in the form of a decline in fungal carbon and parallel increase in bacterial carbon, already visible after one year but more pronounced in the long term. Over time, the microbial carbon in both amended substrates even exceeded the values reported for soils collected in a mature maquis in the surroundings of Naples during spring (Marzaioli et al. 2010), that is, under climatic conditions similar to those occurring during the investigated observation period. The inhibitory effects on fungi, in the short and long term, could be due to production of antifungal compounds by bacteria or a better use of resources by bacteria vs. fungi in favorable environmental conditions (Meidute et al. 2008). An improvement of the quality of the substrates, inferable from the steady decrease in the qCO_2 index, already started after one year from mesocosm setting and was likely related to the decrease in metal availability. An overall improvement of substrate quality is also likely to account for the observed changes in respiration. In fact, ten years after mesocosm setting, the respiration had decreased to values typical of agricultural soils of the Mediterranean region amended with compost (Ventorino et al. 2012), suggesting a transition from stressed to unstressed environmental conditions. In the long term (2014), the microbial biomass appeared less active, though more abundant, than in the short term, as the CEM values were lower after ten years than after one year in both amended substrates. This is in line with the small variation in organic matter content between 2005 and 2014, probably due to greater recalcitrance to biodegradation of the remaining organic matter (Maisto et al. 2010).

On the whole, our data are consistent with a regulatory effect of metal availability on microbial biomass and activity. In fact, the fungal carbon appeared to be enhanced and, by contrast, the bacterial carbon appeared to be inhibited by increased metal availability. In addition, the parallel increase in qCO_2 suggests a general stress condition (Anderson and Domsch 1993) that favors fungal rather than microbial biomass that is also provided to organic matter mineralization (Dighton 2003).

The ecotoxicological assays, carried out at the end of the experiment, indicated an overall good quality of the amendments. In fact, the tests more effective in revealing toxicity due to direct contact of the organism with the matrix (germination and root elongation) showed effects linked to individual physiological responses, as they were in the range 80–120%. The biostimulation of root elongation in *Lepidium sativum* reflects a greater sensitivity of this species, as already observed in previous researches (Manzo et al. 2008). By contrast, the ecotoxicity tests revealed non-optimal conditions of the water present in the substrates, as inhibition on both survival and growth of *Heterocypris incongruens* was observed for all the investigated substrates.

A comparison of the data from mesocosms with or without plants shows that, after a sudden effect at the moment of mesocosm setting up, the plants had no major effect in either the short or long term on the parameters investigated (i.e., pH, metal availabilities, fungal carbon, respiration, and qCO_2), with the exception of microbial biomass and CEM. In fact, plants seem to slowly enhance the mineralization of organic matter by microorganisms (Bardgett and Wardle 2003 Van Elsas et al. 2006) as after ten years from mesocosm setting, on average, a twofold increase in CEM and a 1.2-fold decrease in organic matter content were observed in C_p and CP_p relative to C. In addition, an improvement of the substrate quality linked to a reduction in metal availabilities was clearly visible (in 2004, Pb available fractions

in C_p or CP_p were ~1.7-fold lower than in C). As the highest decreases in Cd and Pb availabilities were observed in mesocosms with plants (C_p and CP_p), root uptake and accumulation of these metals could not be ruled out (Tangahu et al. 2011, Yoon et al. 2006, Gupta et al. 2014).

In conclusion, the results of the present investigation show that a single application of organic amendments enhanced the microbial biomass, in both the short (one year) and long term (ten years). The data also suggest the bacterial component to be more competitive than the fungi in the long term. The progressive reduction in qCO_2 (a stress index) and respiration (energy requirement) levels observed during the ten-year experiment suggests that initially high stress conditions reduced with time. The applied amendments appeared to be more favorable to microbial growth in the long than short term, despite the long-term reduction in the mineralization rate likely due to the chemical complexity of residual organic matter. Ten years after mesocosm setting, the metal availability drastically decreased. The addition of poultry manure to compost improved chemical and biological parameters especially in the brief time. In the long term, the single application of either of the investigated amendments showed low ecotoxicity, thus confirming their suitability for reducing the impact of limestone waste on the territory.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2009/full

AppendixS1

Table S1. Levels of significance (P values) from the two-way ANOVAs for comparison of pH, organic matter content (O.M.), Cd, Cr, Cu, Ni and Pb available fractions, microbial carbon (C_{mic}), fungal carbon (C_{fung}), respiration (Resp), metabolic quotient (qCO_2), and coefficient of endogenous mineralization (CEM) between substrates (C_p and CP_p) and between different sampling times (2004, 2005 and 2014).

Variable	Substrates	Time	Substrates x Time	*Post-hoc* comparision test										
				Substrates within time			Time within substrates							
							C_p				CP_p			
				2004	**2005**	**2014**		2004	2005	2014		2004	2005	2014
pH	**0.015**	**<0.001**	**0.002**	C_p x CP_p	**<0.001**	0.481	0.904	2004	-	0.314	0.062	2004	-	**<0.001**	**<0.001**
								2005	-	-	0.254	2005	-	-	0.734
O.M.	0.989	**0.009**	0.288	C_p x CP_p	0.245	0.282	0.944	2004	-	0.728	0.181	2004	-	**0.038**	**0.018**
								2005	-	-	0.224	2005	-	-	0.544
C_{mic}	**0.011**	**<0.001**	0.350	C_p x CP_p	0.571	**0.020**	0.944	2004	-	**0.003**	**<0.001**	2004	-	**<0.001**	**<0.001**
								2005	-	-	**<0.001**	2005	-	-	**<0.001**
C_{fung}	0.645	**<0.001**	0.165	C_p x CP_p	0.271	0.107	0.82	2004	-	**<0.001**	**<0.001**	2004	-	**<0.001**	**<0.001**
								2005	-	-	**0.003**	2005	-	-	**<0.001**
Resp	**0.021**	**<0.001**	0.181	C_p x CP_p	**0.008**	0.55	0.416	2004	-	**<0.001**	**<0.001**	2004	-	**<0.001**	**<0.001**
								2005	-	-	0.178	2005	-	-	0.251
qCO_2	0.491	**<0.001**	0.780	C_p x CP_p	0.351	0.792	0.994	2004	-	**0.007**	**0.004**	2004	-	**0.026**	**0.024**
								2005	-	-	0.600	2005	-	-	0.799
CEM	0.107	**0.002**	0.189	C_p x CP_p	0.9	**0.023**	0.779	2004	-	0.141	**0.023**	2004	-	0.617	**0.021**
								2005	-	-	0.245	2005	-	-	**0.012**
$Cd_{available}$	0.122	**<0.001**	0.198	C_p x CP_p	0.956	**0.027**	0.677	2004	-	0.103	**<0.001**	2004	-	0.438	**<0.001**
								2005	-	-	**<0.001**	2005	-	-	**<0.001**
$Cr_{available}$	0.129	**<0.001**	**0.029**	C_p x CP_p	**0.005**	0.547	0.993	2004	-	**0.006**	**<0.001**	2004	-	**<0.001**	**<0.001**
								2005	-	-	**0.028**	2005	-	-	0.084
$Cu_{available}$	**0.008**	**<0.001**	**0.003**	C_p x CP_p	**<0.001**	0.684	0.794	2004	-	**0.034**	**<0.001**	2004	-	**<0.001**	**<0.001**
								2005	-	-	**0.003**	2005	-	-	**<0.001**
$Ni_{available}$	0.121	**<0.001**	**0.01**	C_p x CP_p	**0.002**	0.324	0.974	2004	-	0.415	**0.011**	2004	-	**<0.001**	**<0.001**
								2005	-	-	**0.035**	2005	-	-	0.105
$Pb_{available}$	0.089	**<0.001**	0.297	C_p x CP_p	0.804	**0.034**	0.588	2004	-	0.059	**<0.001**	2004	-	**0.002**	**<0.001**
								2005	-	-	**0.023**	2005	-	-	0.275

Terms of ANOVA models (degrees of freedom in brackets) are: Substrates (1), Time (2), Substrates x Time (2), Residual (12), Total (17). Pairwise multiple comparison by Holm-Sidak method. Significant differences (for $\alpha \leq 0.05$) are highlighted in bold.

CHAPTER 3

3.2 Metal compartmentalization in different biomass portions of *Helianthus annuus* L. and *Sorghum bicolor* L. grown in agricultural field inside an urban fabric

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Metal compartmentalization in different biomass portions of *Helianthus annuus* L. and *Sorghum bicolor* L. grown in an agricultural field inside an urban fabric

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ABSTRACT

Plant-based products comprise the vast majority of human and animal nutrition, and metal concentration and accumulation inside food can affect human health. Recently, agricultural soils are often located in close proximity of urban fabrics and factories, so that risk of contamination is high. A field study, carried out in summer 2014, aimed to: i) evaluate the chemical, biological and ecotoxicological characteristics of an agricultural soil in the neighbourhood of the city of Naples (Southern Italy); ii) detect Cd, Cr, Cu, Ni and Pb concentrations and accumulations in roots of sunflower and sorghum; iii) distinguish the potential main pathway (by roots or leaves) of metal uptake in the two crop species. Metal concentrations were measured in soil, roots, stems, leaves and seeds. Besides, the metal bioaccumulation and translocation factors were also calculated. Soils resulted slightly contaminated by Cu and Pb, deriving by both agricultural practices and urban inputs, but did not differ in biological and ecotoxicological characteristics. Cu was the most abundant metal and roots appeared the main path of metal uptake for both the crop species. In sunflower, roots apparently limit the absorption of Pb as well Cu and Cd uptake by leaves from the atmosphere cannot be excluded. Sunflower, likely for the higher biomass and faster growth rate as compared to sorghum, showed an overall higher metal accumulation, particularly high for Cd and Cu in the seeds.

Capsule: Sorghum and sunflower, grown in an agricultural field inside an urban fabric, showed a different metal compartmentalization and accumulation in biomass portions used in human dietary

1. Introduction

Worldwide, plant-based foods makes an essential component of human and animal diet and their quality is likely to affect to human health. Over the decades, agricultural practices have employed fertilizers, pesticides and other synthesised substances to achieve massive production of crop products. The rapid growth of urbanization and industrialization has often determined the enclosure of agricultural areas in the urban surroundings or in vegetable gardens of the urban fabric (Yan et al., 2007). Sites ranging from former manufacturing/industrial sites down to small individual lots embedded into commercial and residential areas are being considered as potential sites for growing food, mainly vegetables.

As a result potentially toxic elements, such as heavy metals, emitted by a wide range of anthropogenic activities (Kelly et al., 1996; Norra et al., 2001; Thorton, 1991; Wong et al., 2006), might contaminate soils and accumulate in crops grown in urban and peri-urban areas, and become directly or indirectly responsible for a large proportion of the dietary uptake of pollutants by humans and animals (Kabata-Pendias and Pendias, 1989). Metal contaminated crops can represent a serious risk for human health, directly, due to the direct excessive assumption through the diet (Babu et al., 2013) or, indirectly, due to the damaging of the environmental health (Kachenko and Singh, 2006). For instance, Cd and Pb, showing a long biological half-life in humans (Komarnicki, 2005), are associated to a large number of human diseases and are considered carcinogenic (Jarup, 2003); whereas Cu, although an essential nutrient, can cause toxic effects when exceeding certain concentrations (Kabata-Pendias and Mukherjee, 2007).

Soil conditions strongly affect metal transfer to crops, as this process is controlled by various parameters that regulate the metal mobility and

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availability (Kabata-Pendias, 2004). Among the soil properties that affect metal availability, pH plays a fundamental role in determining metal speciation, solubility from mineral surfaces and movement (Mülbachová et al., 2005; Zhao et al., 2010). Apart from soil pH, also high soil organic matter content enhances metal adsorption onto soil constituents (Antoniadis et al., 2008; Hettiarachchi et al., 2003), while soil dissolved organic matter can increase metal mobility and uptake by plant roots (Impellitteri et al., 2002). Furthermore, soil microorganisms, releasing specific compounds that form complexes with metals and modify soil pH (Alkorta et al., 2006; Prasad and Freitas, 2003; Vig et al., 2003), can alter metal bioavailability in soil. At the same time, soil metal availability affects the growth and activity of microorganisms that inhabit it, leading to a distortion of their basic life functions, and especially the processes of decomposition and transformation of organic matter (Gadd, 2004). In general terms, the crop abilities to deal with metals, in terms of tolerance, absorption and accumulation, are linked to soil availability rather than total amount (Willey, 2007).

Metals exhibit different behaviours regarding the plant-soil relationships. Pb that mainly exhibits strong interactions with soil particles (Clemens, 2006) is scarcely soluble and hardly absorbed by roots; on the other hand, Cd is very labile in soil and can be readily taken up by roots and translocated to the upper portions (Gimbert et al., 2008). After root absorption, metals can be differently compartmentalized in the biomass (Gupta and Gupta, 1998; McBride, 2007; Monika and Katarzyna, 2004): Co and Ni are readily transported to the shoots, whereas Al, Cr, Cu and Pb mainly remain in the roots (Vazquez et al., 1994). Besides, different behaviours in metal uptake can be linked to the biomass production and growth rate. In contrast with small crop species with slow rates of biomass production, fast-growing tall crops accumulate moderate levels of metals and feature a significant heavy metal tolerance (Kumar et al., 1995; Wenzel et al., 1999). In addition, biomass production plays a fundamental role in element accumulation. In fact, assessing accumulation on the basis on the sole concentration can provide misleading information. Therefore, the accumulation should be evaluated taking into account both tissue concentration and biomass (Vymazal, 2016). For instance, a greater shoot biomass can compensate for a lower shoot metal concentration (Ebbs and Kochian, 1997, 1998).

Crops can also take up metals directly from the atmosphere through stomata and translocate them to other portions of the plant (Dollard, 1986). Soil or atmosphere contribution to metal transfer and accumulation in crops depends on the type of metals and crop species and it is still strongly controversial (Bi et al., 2009); therefore, the actual environmental quality becomes of particular concern. Starting from the widespread assumption that agricultural soils located in the urban fabrics can represent a potential serious risk for food safety and human health, the aims of the present study are: i) to evaluate the quality (in terms of chemical, physical, biological and ecotoxicological characteristics) of agricultural soils inside the urban fabric; ii) to detect metal (Cd, Cr, Cu, Ni and Pb) concentrations and accumulations in different portions of the plant (roots, stems, leaves, seeds) of two crops, part of the human and animal diet, such as sunflower (*Helianthus annuus* L.), characterised by high biomass and fast growth rate and a short cultivar of grain sorghum (*Sorghum bicolor* L.), featuring a slightly lower plant size and slower growth rate; iii) to distinguish the potential main uptake patterns (by roots or leaves) of uptake depending on the kind of metal and crop species.

2. Materials and methods

2.1. Study area and sampling

The experiment was carried out in Ponticelli (Naples, 40°52′7.57″N, 14°20′22.53″E, 50 m a.s.l.), in a flat agricultural site inside to urban agglomerations in the suburbs of Naples, close to highly frequented urban roads. At 1 km away from the experimental site, a lead pipe factory operated until 1970. The overall size of the studied area, characterized by the Mediterranean climate with warm dry summer and mild wet winters, is 3 ha with a coarse soil texture due to its volcanic origin (Vitale et al., 2017). Soil tillage (0.5 m depth) occurs by mill and the site has been always cultivated with horticultural species (*e.g.* tomato, potato, fennel, aubergine, pepper, kale, ecc.) whose residues are regularly interred after harvest. Fungicides are sometimes used depending on crop type.

The study was carried out from May to September 2014; on May, eight 12 m^2 plots, arranged in fully randomized block design with four replicates, were sown with *Sorghum bicolor* L. (cultivar ROCE) and *Helianthus annuus* L. (cultivar MAS 83.R). During the growth of the crops, the soil was regularly irrigated to fully replenish crop evapotranspiration supplying about 2500 $m^3\ ha^{-1}$ of water by sprinkling and cultivated to control weeds; fungicides were not used in this experiment. Before sowing, the soil was milled and fertilized with 120 kg N ha^{-1} as urea 46%.

On 4th September plants were harvested and partitioned in roots, stems, leaves and seeds. Contextually, at each plot, three samples of soil (diameter: 10 cm; depth: 0–10 cm) in proximity of roots (soil) were collected and mixed to obtain a homogeneous sample to perform chemical, biological and ecotoxicological analyses.

2.2. Soil chemical analyses and indices

In laboratory, the soil samples were sieved (2 mm) and divided in different aliquots to measure: water content, pH, organic carbon (C_{org}) content, total C and N contents as well as total concentrations and available fractions of Cd, Cr, Cu, Ni and Pb.

The water content was determined by drying fresh soil at 105 °C until constant weight, and pH was measured in a soil:distilled water (1:2.5 = v:v) suspension by electrometric method.

The organic carbon content (C_{org}) and total C and N concentrations were evaluated on oven-dried (75 °C, until constant weight) and grounded (Fritsch Analysette Spartan 3 Pulverisette 0) soil samples. The contents of C_{org}, after treatment with HCl (10%), and total C and N were measured by gas-chromatography (Thermo Finnigan, CNS Analyzer). The soil organic matter content was calculated multiplying the C_{org} concentrations by 1.724 as reported by Pribyl (2010).

To measure the total concentrations of Cd, Cr, Cu, Ni and Pb, the oven-dried and grounded soil samples were digested with a mixture of HF (50%) and HNO_3 (65%) at a ratio of 1:2 (v:v) in a micro-wave oven (Milestone mls 1200 – Microwave Laboratory Systems).

The available fractions of Cd, Cr, Cu, Ni and Pb were extracted by oven-dried soil samples with diethylenetriamine pentacetic acid, $CaCl_2$ and triethanolamine at pH 7.3 ± 0.05 (Lindsay and Norwell, 1969).

The total and available concentrations of metals were measured, via graphite furnace, by atomic absorption spectrometry (SpectrAA 20 – Varian). Accuracy was checked by concurrent analysis of standard reference material (BCR CRM 142R – *Commission of the European Communities*, 1994) and recoveries ranged from 86 to 98%.

All the described analyses were performed in triplicates.

The metal contamination degree was evaluated through the contamination (C_f^i) factor as reported below.

$$C_f^i = C_s^i / C_R^i$$

where C_s^i is the total concentration of a single metal in the soil and C_R^i is the concentration reported as limit values by the Italian law (*i.e.* Cd, Cr, Cu, Ni and Pb, respectively, of 2, 150, 120, 120 and 100 μg g^{-1} d.w.) for agricultural soils (D. Lgs.152/06).

2.3. Soil biological analyses and indices

The investigated soils were analysed for microbial and fungal biomass as well as for microbial respiration and functional biodiversity

(catabolic evenness). The analyses were performed within a week after sampling, in quadruplicates, on fresh samples stored at 4 °C.

The microbial biomass was evaluated by SIR, the substrate-induced respiration method (Degens et al., 2000), as CO_2 evolution measured by IRGA determination (Li6262, Licor, USA), after addition of 2 ml D-glucose solution (75 mM) to fresh soil, equivalent to 1 g dry mass, and incubation for 4 h (25 °C in darkness). The total fungal biomass was determined by the membrane filter technique (Sundman and Sivelä, 1978). Fresh soil equivalent to 1 g dry mass was dispersed in 100 ml phosphate buffer (60 mM; pH 7.5) using a blender at 6000 rev min^{-1}. The suspension was transferred to a membrane filter (0.45 μm mesh size), and stained with aniline blue (80% lactic acid). After clearing by immersion oil, 20 microscopic fields were observed at a magnification of 400×. Fungal mycelium was estimated by the intersection method (Olson, 1950) and the mass calculated according to Berg and Söderström (1979). Fungal C was calculated from total-mycelium mass on the basis of mean fungal values of C/N ratio (Killham, 1994) and N content (Swift et al., 1979).

The microbial respiration was evaluated as CO_2 (Li6262, Licor, USA) evolved from fresh soil, after addition of 2 ml of distilled water, incubated in microcosms for 4 h under standard condition (25 °C in darkness).

The catabolic evenness (E) was evaluated by measuring the short-term respiration (incubation for 4 h, at 25 °C, in darkness) after addition of 19 simple organic compounds (Degens et al., 2000). The substrates used in the assay were: 7 amino acids (L-arginine, L-asparagine, D-glucosamine, L-glutamic acid, L-glutamine, L-histidine, L-serine), 2 carbohydrates (D-glucose, D-mannose), and 10 carboxylic acids (L-ascorbic acid, fumaric acid, α-ketobutyric acid, α-ketoglutaric acid, DL-malic acid, malonic acid, pantothenic acid, succinic acid, tartaric acid and uric acid). E was calculated using the Simpson and Yule index (Magurran, 1988), as reported below:

$$E = 1/\Sigma p_i^2$$

where p_i is the percentage of the respiration response due to each substrate respect to the respiration response due to all the substrates.

Some of the performed microbial analyses were used to calculate two indices: the metabolic quotient (qCO_2), i.e. the degree of activity of the microbial biomass, and the coefficient of endogenous mineralization (CEM), i.e. the rate of organic carbon mineralization. The qCO_2 was calculated as ratio between microbial respiration and biomass, whereas the CEM was calculated as ratio between microbial respiration and C_{org}.

2.4. Ecotoxicological assays

To evaluate the ecotoxicity of the soil on which the crops (*H. annuus* and *S. bicolor*) were cultivated, the seed germination and root elongation assays were carried out, in triplicates, on the soils according to the U.S. EPA protocols (EPA, 1996). In more details, 10 seeds (Ecotox Lds) of *Lepidium sativum* L. (dicotyledon) and *Sorghum saccharatum* L. (monocotyledon) were placed in a Petri dish, containing an amount of fresh soil equivalent to 10 g of oven-dried soil, subsequently saturated with water. For each soil sample, standard soil (OECD, 1984) and $K_2Cr_2O_7$ was used as a negative and positive control, respectively. All soil samples were incubated in darkness at 25 °C, and after 72 h the number of germinated seeds and the total root length were evaluated. The results were expressed as percentages with respect to the control.

2.5. Crop analyses

After separation of different portions (roots, stems, leaves and seeds) the vegetable samples were weighted and oven dried at 75 °C, until constant weight, to evaluate the standing biomass that was expressed as dry weight per unit of area.

An aliquot of dry biomass was ground into a fine powder by an agate mortar and pestle (Fritsch pulverisette) to measure, in triplicates, the concentrations of Cd, Cr, Cu, Ni and Pb as above described for the total metal concentrations in the soil. Accuracy was checked by concurrent analysis of standard reference material (BCR CRM 62 – *Commission of the European Communities*, 1994) and recoveries ranged from 86 to 98%.

Successively, in order to evaluate the metal accumulation in each portion of the biomass, the concentrations of each metal was multiplied by dry biomass weight per unit of area (Vymazal, 2016).

2.6. Crop-soil relationships

In order to estimate the root metal uptake from soil and to estimate the metal translocation from roots to the other portions of the plant, the bioaccumulation factor (BF) and the translocation factor (TF) were calculated as reported below:

$$BF = C^i_{\text{roots or leaves or stems or seeds}}/C^i_{\text{available in soil}}$$

$$TF = C^i_{\text{leaves or stems or seeds}}/C^i_{\text{roots}}$$

where C^i is the concentration of a single metal.

2.7. Statistical analyses

The unpaired *t*-test was performed in order to evaluate the differences between soils samples from the two crop species for the chemical, biological and ecotoxicological parameters.

The two-way analysis of variance (ANOVA) was performed in order to evaluate the differences among the biomass portions in each crop and between the two species for metal concentrations, metal accumulations, translocation factors and bioaccumulation factors. The ANOVA tests were followed by the *post hoc* tests of Holm-Sidak.

The relationships among the chemical, biological and ecotoxicological characteristics of the soils as well as those among soil metal availability, metal concentrations or accumulations in the different portions of each crop were evaluated by Spearman test, according to the non-normal distribution of the data, assessed by the Shapiro-Wilk test.

The statistical assays, performed by Systat_SigmaPlot_12.2 software (Jandel Scientific, USA), were considered statistically significant for $P < 0.05$.

3. Results

3.1. Soil physico-chemical characteristics

The investigated physico-chemical characteristics of the soils collected in the plots where sunflower and sorghum were grown are reported in Table 1. Cu and Pb were the most abundant metals with mean values of circa 100 μg g^{-1} d.w., while Cr and Ni concentrations ranged between 5 and 6 μg g^{-1} d.w. and Cd was only negligible (Table 1). In addition, Cu was the most available metal followed by Pb; by contrast, Cd, Cr and Ni availabilities in the soils were scarce (Table 1). On the overall, the investigated physico-chemical characteristics did not statistically differ between the soils of the two crop species (Table 1).

The contamination factors were approximately 1 for Cu and Pb (0.92 and 1.02, respectively, for soils under *H. annuus* and 1.06 and 0.97, respectively, for soils under *S. bicolor*) and lower than 1 for the other investigated metals (*i.e.* 0.03, 0.04 and 0.05, respectively, for Cd, Cr and Ni for soils under *H. annuus* and 0.03, 0.04 and 0.04, respectively, for Cd, Cr and Ni for soils under *S. bicolor*).

3.2. Soil biological and ecotoxicological characteristics

The mean values of microbial and fungal carbon in the soils were, respectively, 517 and 3.05 μg C g^{-1} d.w. and did not statistically differ

Table 1
Mean values (± s.e.) of pysico-chemical characteristics of soils under *H. annuus* (sunflower) and *S. bicolor* (sorghum). Different letters indicate statistically significant differences ($P < 0.05$) for each characteristic between the soils under the two crop species (unpaired *t*-test).

	H. annuus	*S. bicolor*
pH	7.66[a] (± 0.03)	7.67[a] (± 0.03)
Water content (% d.w.)	12.4[a] (± 1.54)	15.0[b] (± 0.75)
C (% d.w.)	2.46[a] (± 0.23)	2.48[a] (± 0.31)
N (% d.w.)	0.23[a] (± 0.02)	0.25[a] (± 0.03)
Organic matter (% d.w.)	2.68[a] (± 0.36)	2.70[a] (± 0.50)
Total Cd (μg g^{-1} d.w.)	0.08[a] (± 0.02)	0.08[a] (± 0.003)
Total Cr (μg g^{-1} d.w.)	5.99[a] (± 0.32)	5.83[a] (± 0.43)
Total Cu (μg g^{-1} d.w.)	105[a] (± 5.92)	114[a] (± 7.54)
Total Ni (μg g^{-1} d.w.)	5.58[a] (± 1.24)	4.92[a] (± 0.44)
Total Pb (μg g^{-1} d.w.)	102[a] (± 5.07)	96.9[a] (± 3.49)
Available Cd (μg g^{-1} d.w.)	0.07[a] (± 0.01)	0.07[a] (± 0.01)
Available Cr (μg g^{-1} d.w.)	0.001[a] –	0.001[a] –
Available Cu (μg g^{-1} d.w.)	23.8[a] (± 7.24)	20.6[a] (± 3.69)
Available Ni (μg g^{-1} d.w.)	0.01[a] (± 0.003)	0[a] (± 0.004)
Available Pb (μg g^{-1} d.w.)	9.71[a] (± 3.69)	9.71[a] (± 4.37)

Table 2
Mean values (± s.e.) of microbial carbon (C_{mic}), fungal carbon (C_{fung}), respiration (Resp). catabolic evenness (Cat. Ev.), coefficient of endogenous mineralization (CEM), metabolic quotient (qCO_2) and ecotoxicological assays (%E percentages of root elongation or seed germination with respect to the control) of soils under *H. annuus* (sunflower) and *S. bicolor* (sorghum). Different letters indicate statistically significant differences ($P < 0.05$) for each characteristic between the soils under the two crop species (unpaired *t*-test).

	H. annuus	*S. bicolor*
C_{mic} (μg C g^{-1} d.w.)	526[a] (± 17.9)	508[a] (± 41.6)
C_{fung} (μg C g^{-1} d.w.)	3.18[a] (± 0.60)	2.92[a] (± 0,37)
Resp (μg CO_2 g^{-1} d.w. h^{-1})	1.51[a] (± 0.45)	2.39[a] (± 0.27)
Cat. Ev. ($1/\Sigma pi^2$)	14.9[a] (± 0.13)	14.0[b] (± 0.31)
CEM (μg C-CO_2 $g^{-1}C_{org}$)	11[a] (± 4)	19[a] (± 1)
qCO_2 (μg C-CO_2 $mg^{-1}C_{mic}$)	0.81[a] (± 0.25)	1.35[a] (± 0.27)
Lepidium sativum L.		
Root elongation (%E)	108[a] (± 10.1)	92[a] (± 6.9)
Seed germination (%E)	89[a] (± 3.9)	90[a] (± 1.7)
Sorghum saccharatum L.		
Root elongation (%E)	115[a] (± 18.0)	125[a] (± 22.2)
Seed germination (%E)	89[a] (± 1.6)	88[a] (± 3.2)

between sunflower and sorghum plots (Table 2). The catabolic evenness was statistically higher under sunflower than under sorghum (Table 2). Despite of not statistically significant differences between the soils of the two crop species, the microbial activity (respiration, CEM and qCO_2) was slightly higher in soil under sorghum as compared to soil under sunflower (Table 2).

The percentages of the root elongation and seed germination performed with both *L. sativum* and *S. saccharatum* on soils under sunflower and sorghum were on average 110 and 89, compared to the control, respectively; no statistically significant differences between the soils of the two crop species were detected (Table 2).

3.3. Relationships among the chemical, biological and ecotoxicological characteristics of the soils

Catabolic evenness was negatively correlated to total Cu concentration (r_s: −0,881, $P < 0.001$). In addition, root elongation of *L. sativum* was negatively correlated to water content (r_s: −0.905, $P < 0.001$) and total Cu (r_s: −0.690, $P < 0.05$) concentration; whereas, root elongation of *S. saccharatum* was positively correlated to organic matter (r_s: 0.976, $P < 0.001$) content and negatively correlated to total Cd concentration (r_s: −0.690, $P < 0.05$). Seed germination of *L. sativum* was negatively correlated to organic matter (r_s: −0.703, $P < 0.05$) content and available fraction of Cd (r_s: −0.679, $P < 0.05$); whereas, seed germination of *S. saccharatum* was positively correlated to pH (r_s: 0.801, $P < 0.01$).

3.4. Metal concentrations and accumulation in different portions of sunflower and sorghum, and translocation factors

After crop harvesting, crop biomass was partitioned in the different portions. In sunflower, the biomass decreased in the order seeds (1036 g m^{-2} ± 101) > stems (722 g m^{-2} ± 24) > roots (280 g m^{-2} ± 72) > leaves (252 g m^{-2} ± 24); whereas in sorghum the order was stems (440 g m^{-2} ± 39) > leaves (175 g m^{-2} ± 14) > roots (110 g m^{-2} ± 27) > seeds (75 g m^{-2} ± 21). Statistically significant differences between sunflower and sorghum were evidenced in stem ($P < 0.05$) and seeds ($P < 0.01$).

Metal concentrations statistically differed among the various portions of the crop biomass (Fig. 1), and for both sunflower and sorghum, the highest concentrations were, on average, measured in the roots whereas the lowest in the seeds (Fig. 1). Among the metals, Cu showed the highest concentrations in each biomass portion of either crop species, with values ranging from 7.11 μg g^{-1} d.w. in the stems to 57.9 μg g^{-1} d.w. in the roots of sunflower, and ranging from 6.25 μg g^{-1} d.w. in the seeds to 28.6 μg g^{-1} d.w. in the roots of sorghum (Fig. 1). Cr, Ni and Pb were negligible in some portions, especially in seeds (Fig. 1). The comparison between the crop species highlighted that root Cd, Cr and Pb concentrations were statistically higher in sorghum; conversely, root Cu concentrations were statistically higher in sunflower (Fig. 1). Besides, the metal concentrations in the other biomass portions were mainly higher (sometimes statistically significant) in sunflower (Fig. 1). Taking into account both crop species, leaf Cu concentrations were positively correlated to Cu seed(r_s: 0.857, $P < 0.01$) or root (r_s: 0.690, $P < 0.05$) concentrations as well as leaf Ni concentrations were positively correlated to Ni stem concentrations (r_s: 0.973, $P < 0.001$).

As observed for metal concentrations, also metal accumulation varied among the various portions of the crop biomass (Fig. 2). The highest metal accumulation was, on average, detected in the roots, although Cd and Cu accumulations were particularly high in the seeds of sunflower (Fig. 2). The highest metal accumulations were observed for Cu, especially in sunflower, in all the biomass portions and for Pb in roots of both crop species (Fig. 2). Metal accumulation in the different biomass portions did not statistically differ between the crop species with the exception of Cu in roots, Cd and Ni in stems, Cd and Cu in seeds that were statistically higher in sunflower (Fig. 2). Taking into account both crop species, leaf Cd accumulations were positively correlated to seed Cd accumulations (r_s: 0.786, $P < 0.05$). The accumulations of Cu in leaves were positively correlated to those in seeds or roots (r_s: 0.738, $P < 0.05$ for both) as well as seed Cu accumulations were positively correlated to root Cu accumulations (r_s: 0.762, $P < 0.05$). In addition, leaf Ni or Pb accumulations in leaves were positively correlated to the respective metal accumulations in stems (r_s: 0.919, $P < 0.001$ for Ni and r_s: 0.736, $P < 0.05$ for Pb).

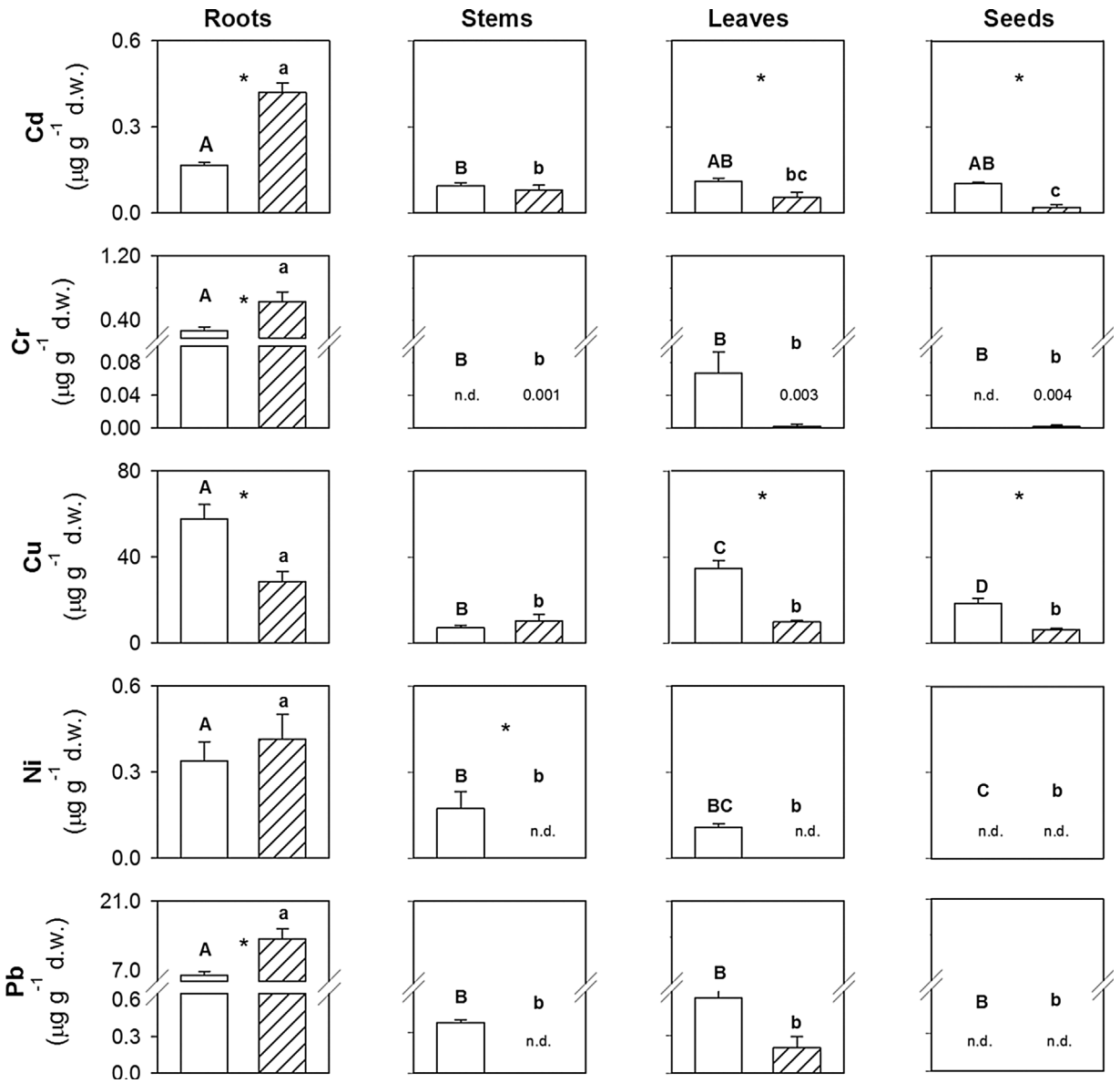

Fig. 1. Mean concentrations (± s.e.) of metals in roots, stems, leaves and seeds of sunflower (white bars) and sorghum (coarse pattern bars). Asterisks indicate statistically significant differences ($P < 0.05$) in metal concentrations in each biomass portion between sunflower and sorghum; capital and lower case letters indicate statistically significant differences ($P < 0.05$), for each metal concentration, among the different portions in sunflower and sorghum, respectively (two-way analysis of variance with Holm-Sidak *post hoc* test).

The TFs, estimation of the potential metal translocation from roots to the other portions of the biomass, were lower than 1 for both crop species (Table 3). The TFs for Cr, Cu, Ni and Pb statistically differed among the various biomass portions in sunflower, whereas they did not statistically differ for any metal in sorghum (Table 3; Supplementary material 1). Besides, they were statistically higher in sunflower than in sorghum, with the exception of the TF for Cu that was similar in both crop species (Table 3; Supplementary material 1).

3.5. Crop-soil relationships

In order to assess the likely metal root absorption and subsequent allocation in the different biomass portions of each crop species, the BFs were calculated and reported in Table 4. The BFs for roots were higher than 1 for all the investigated metals and for both crop species with the exception of Pb for sunflower (Table 4). For this last species, also the BFs of Cd, Cr, Cu and Ni for leaves and that of Cd for seeds were higher than 1 (Table 4); for sorghum, the BFs of Cr for leaves and seeds were higher than 1 (Table 4). Particularly high were the BFs for Cr and Ni in roots of both the crop species, and for Cr in leaves of sunflower (Table 4). In sunflower, the BFs of Cd for roots were statistically higher than those for stems, the BFs of Cu for roots were statistically higher than those for stems and seeds, the BFs of Cu for leaves were higher than those for stems as well as the BFs of Pb for roots were higher than those for seeds (Table 4; Supplementary material 2); in sorghum, the BFs of all the investigated metals for roots were statistically higher than those for stems, leaves and seeds (Table 4; Supplementary material 2). The comparison between the crop species highlighted that the BFs of all the investigated metals for the roots were statistically higher in sorghum than in sunflower with the exception of Cu that instead was statistically higher in sunflower (Table 4; Supplementary material 2). Besides, the BFs of Cd for the seeds were statistically higher in sunflower than sorghum; the BFs of Cu calculated for leaves were statistically higher in sunflower (Table 4; Supplementary material 2).

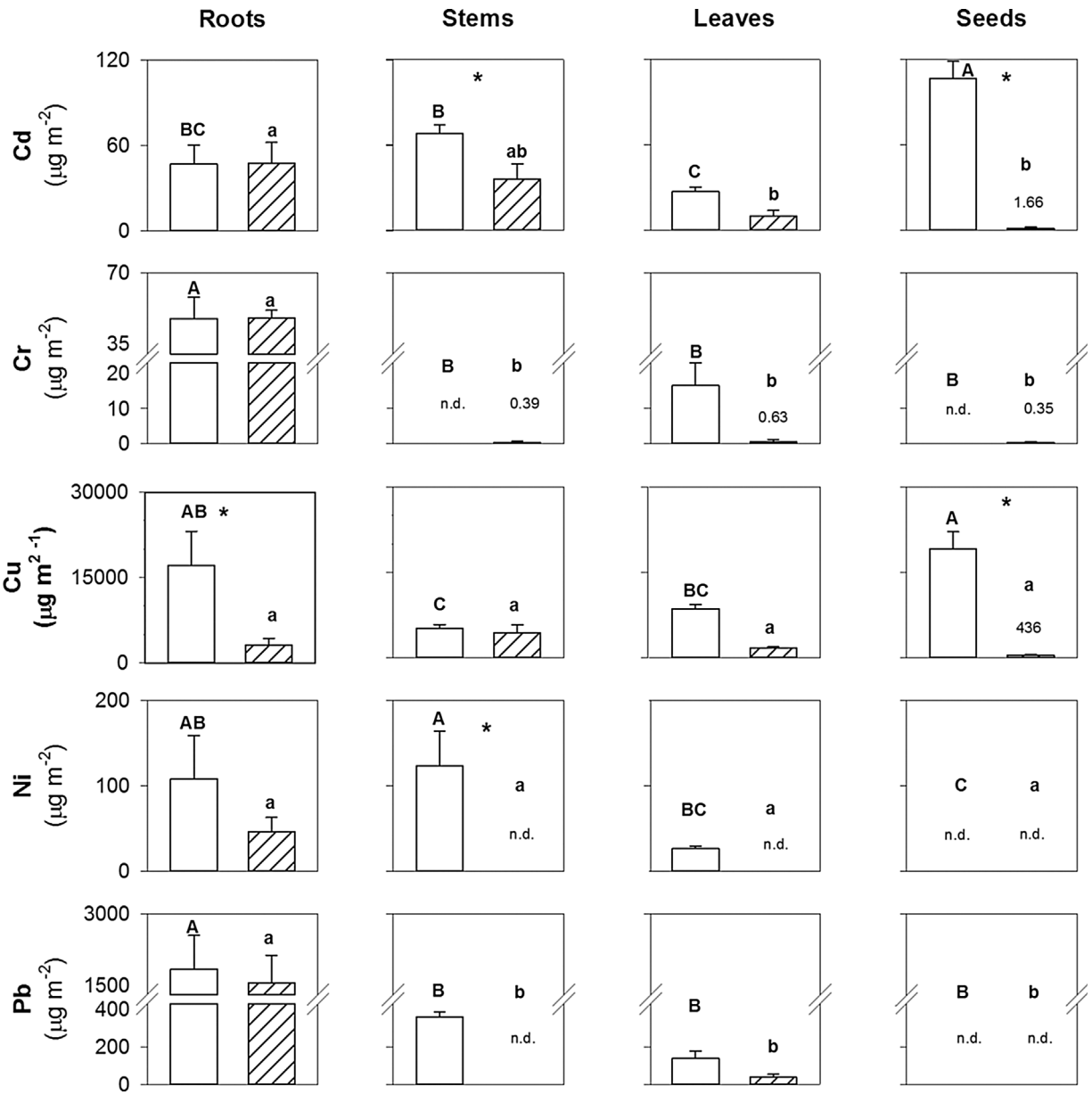

Fig. 2. Mean accumulations (± s.e.) of metals in roots, stems, leaves and seeds of sunflower (white bars) and sorghum (coarse pattern bars). Asterisks indicate statistically significant differences ($P < 0.05$) in metal accumulations in each biomass portion between sunflower and sorghum; capital and lower case letters indicate statistically significant differences ($P < 0.05$), for each metal accumulation, among the different portions in sunflower and sorghum, respectively (two-way analysis of variance with Holm-Sidak *post hoc* test).

Table 3
Mean values of translocation factor calculated on Cd, Cr, Cu, Ni and Pb concentration in different portions of biomass in two crop species.

	H. annuus			*S. bicolor*		
	stems	leaves	seeds	stems	leaves	seeds
Cd	0.57	0.67	0.63	0.20	0.13	0.05
Cr	0.00	0.28	0.00	0.00	0.00	0.01
Cu	0.13	0.65	0.34	0.34	0.39	0.25
Ni	0.46	0.36	0.00	0.00	0.00	0.00
Pb	0.09	0.11	0.00	0.00	0.02	0.00

Table 4
Mean values of bioaccumulation factor calculated on Cd, Cr, Cu, Ni and Pb concentration in different portions of biomass in two crop species.

	H. annuus				*S. bicolor*			
	roots	stem	leaves	seeds	roots	stem	leaves	seeds
Cd	2.27	0.57	1.56	1.44	6.15	0.20	0.77	0.29
Cr	183	0.00	42.0	0.00	818	0.00	1.61	4.14
Cu	2.53	0.13	1.62	0.87	1.70	0.34	0.57	0.37
Ni	68.7	0.65	24.9	0.00	239	0.00	0.00	0.00
Pb	0.69	0.09	0.08	0.00	1.60	0.00	0.02	0.00

In order to evaluate the possible relationships between soil and crop species, the correlations between soil metal content and metal concentrations or accumulations in the various crop portions were tested. The findings showed that soil Cr availability was positively correlated to leaf Cr concentration or accumulation (r_s: 0.756, $P < 0.05$ for both) as well as soil Cd and Ni availabilities were positively correlated to the respective root metal accumulation (r_s: 0.786, $P < 0.05$ for Cd and r_s: 0.762, $P < 0.05$ for Ni).

4. Discussion

4.1. Abiotic characteristics of the soils

In spite of pH, water content, total C and N concentrations that showed values comparable to those typical of agricultural areas of the Mediterranean Region (Grignani et al., 2012), the studied soil was richer of organic C as compared to the 75% of arable soils in this Region that exhibit values < 2% d.w. (Jones et al., 2004; Zdruli et al., 2004), but showed values similar to those obtained in the same site under different crop species (Vitale et al., 2017), likely due to the interring of vegetable residues of previous crops after harvesting. In arable soils of southern Europe, organic C content is usually very small, due to synergy between natural and human factors that influence soil organic matter degradation (Zdruli et al., 2004).

The investigated agricultural soil showed a low degree of contamination (Caeiro et al., 2005). Only Cu and Pb, with contamination factors approximately equal to 1, can be considered dangerous in the investigated agricultural area, and their derivations likely could be linked to both agricultural practices and urban inputs. In fact, it is widely reported that the main input of Cu in agricultural soils is due to the massive uses of fungicides (He et al., 2005) as well as that of Pb in urban soil is due to vehicular traffic (Davis and Burns 1999; Maisto et al., 2011; Sutton et al., 1995).

The mean percentage of the availability of each metal with respect to the correspondent total content decreased in the order Cd (93.8%) > Cu (20.4%) > Pb (9.76%) > Ni (0.90%) > Cr (0.02%). The same trend was found by Baldantoni et al. (2010) and St. Luce et al. (2017) for agricultural soils, respectively, in the South of Italy and Quebec. The availability of Cd depends on soil pH and organic matter content, although plant roots can absorb only a little portion, as free hydrated ions are present in nanomolar range in most soils (Wagner, 1993).

4.2. Effects of the crop species on the biotic characteristics of the soils

The microbial and fungal biomasses in the investigated soils showed values comparable to those reported for other Italian agricultural areas, whereas the microbial activity appeared lower (Ventorino et al., 2012). However, the comparison with arable soils of Central Europe highlighted that in the investigated soils the microbial biomass were more abundant and active (Kautza et al., 2004). These findings together with the good level of diversity (the values of the catabolic evenness were relative high) suggest the microbial community in the investigated soil adopted, over the time, mechanisms facing the environmental stressors such as land disturbance, amount and kind of pollutants as well as exposure time (Iram et al., 2009; Zafar et al., 2007). These responses would seem more evident for the bacterial than fungal component of the microbial community. In the studied soil, C_{fun}/C_{mic} ratio was < 1%, showing a fungal component particularly reduced. The low ratio between fungal and bacterial biomass highlighted that bacteria were favoured and more competitive than fungi in utilization of readily accessible organic matter (De Boer et al., 2005; Grayston et al., 1996; Lynd et al., 2002). Agricultural land management causes catastrophic effects on fungi (Kuske et al., 2002; Peixoto et al., 2006), as it physically breaks the hyphae and severely damages the mycelium, consequently hampering the stability of soil aggregates whose particles are transiently bound together by fungal hyphae (Ibekwe et al., 2002; Six et al., 2006).

Despite of not statistically significant differences in microbial or fungal carbon contents between the soils under the two crop species, they were slightly higher under sunflower, where a significant higher functional diversity (*i.e.* catabolic evenness) was observed. The size of microbial community can be often related to the diversity of specific functional groups (Chang et al., 2001; Helgason et al., 1998). The faster root growth of sunflower, with a root biomass twice greater than that of sorghum, could produce a presumably higher amount of root exudates, providing an important source of energy and carbon for microorganisms (Roder et al., 1988) and, in turns, enhancing the microbial biomass and functional diversity. In addition, allelopathic effects contrasting the microbial development and diversity in soils under sorghum cannot be excluded. This supposition can be corroborated by the higher microbial activity (*i.e.* respiration, CEM and qCO_2) that can indicate stress conditions (Anderson and Domsch, 1993), in the soils under sorghum than in those under sunflower, although the differences were not statistically significant. Sorghum allelopathy has been reported in a series of experiments (Cheema and Khaliq, 2000) as well as, recently, allelochemicals and secondary products have been isolated and identified from sorghum shoots, roots, and root exudates (Alsaadawi and Dayan, 2009; Weston et al., 2013).

4.3. Relationships between soil metal availability and crop metal concentration or accumulation

Sorghum and sunflower did not seem to affect the soil metal availability, as it did not statistically vary in the soils under the two crop species. Besides, the differences in metal concentration and accumulation in the different biomass portions of sorghum and sunflower would seem to be linked to different behaviour in metal absorption and allocation of the species.

Sunflower and sorghum showed different growth rates as, at the end of the same period, the former produced a total biomass approximately three-fold higher than the latter. The comparison of the percentage contributions of the single portion of the biomass, as compared to the total, highlighted that they were higher in sunflower. Particularly, sunflower allocated high energy in the production of seeds that represented approximately 45% of the total biomass; whereas sorghum apportioned higher energy in production of stems that represented approximately 55% of the total biomass.

Roots would seem the main via of metal uptake for both the crop species as they showed higher concentrations of all the investigated metals as compared to stems, leaves or seeds. This supposition is also corroborated by the higher BF_{roots} than BF_{leaves}, BF_{stems} or BF_{seeds}. Anyway, roots of sunflower would seem to limit the Pb absorption as the BF_{roots} was lower than 1. For many plants (*i.e. Arabidopsis thaliana* L., *Funaria hygrometrica* L. and *Lemna minor* L.), it has been recognized a defence strategy to stop Pb entering the root tissues by excluding it (Krzesłowska, 2011; Mishra et al., 2006; Samardakiewicz et al., 2012), through the synthesis and deposition of callose, between the plasma membrane and the cell wall, that forms a mechanical barrier. However, the synthesis of callose is not a general pattern in plants in response to Pb; in fact, for instance in *Zea mays*, the synthesis of callose is in response to cadmium or arsenic (Pirselova et al., 2012).

Among the investigated metals, Cu appeared the most abundant in both crop species, likely for its importance as nutrient for plants. The higher Cu and Cd concentrations in leaves and seeds of sunflower could be due to a more efficient translocation from roots as compared to sorghum, even if a direct uptake from the atmosphere by leaves cannot be excluded. In fact, sunflower with a wider leaf extension could intercept a higher amount of air particulate than sorghum (Shahid et al., 2017). As the TFs were lower than 1, metal translocations from roots to the aboveground biomass would seem to be negligible for both the crop species although different behaviours in metal translocation between sunflower and sorghum were observed. In fact, the translocation in the different biomass portions statistically varied in sunflower but not in sorghum and for the former it was globally higher than for the latter, suggesting a higher efficiency of translocation and a more pronounced differentiation in metal accumulation in the various biomass portions. For instance, in sunflower Cd showed almost the same concentrations in roots, stems, leaves and seeds, suggesting a homogenous redistribution of this metal among the different biomass portions; by contrast, in sorghum a Cd root accumulation can be supposed, as the concentrations

were at least four-fold higher than in the other aboveground biomass portions. Besides, Pb would seem to be uptaken from soils to roots and there stocked as in sunflower Pb concentration in roots was at least ten-fold higher than in stems and leaves (Pb seed concentration was undetectable) whereas in sorghum it was at least twenty-fold higher than in leaves (Pb stem and seed concentrations were undetectable). In both the crop species, Cr and Ni were more abundant, although with low values, in the roots than in the other biomass portions where the concentrations were negligible.

The lack of metal translocation from roots to the aboveground biomass portions together with the higher accumulation of some metals in stems or seeds would suggest their uptake from air through leaves and consequent translocation to other portions of the aboveground biomass. Anyway, in sunflower Cd and Cu translocation appeared more conspicuous than in sorghum, suggesting that in the former the activity of sequestering pathways of these metals in roots, the efficiency of radial symplastic passage through the root and across the endodermis and, finally the xylem loading activity, the efflux activity from xylem parenchyma cells into the xylem (Clemens, 2006), were more important than in the latter. Anyway, it cannot be excluded that Cd and Cu accumulated in the seeds of sunflower can derive by transport from the leaves via floema. In fact, various authors report for various crop species a metal translocation from leaves towards the active growing regions (Bi et al., 2009; Patrick and Offler, 2001).

Although root metal concentrations were, on average, lower in sunflower than in sorghum, except for Cu, it can be stated that sunflower was a better root metal accumulator. Thus could be due to the higher biomass and growth rate of sunflower as compared to sorghum. This concept could explain the differences in metal accumulation in the different portions of the biomass between the crop species, especially for seeds that were approximately 14 times higher in sunflower than in sorghum. Cd and Cu showed high tendency to accumulate in seeds, other than roots, representing the principal route to entry in the human food chain (McLaughlin et al., 1999), as more than 45% of seed biomass is constituted by oil, an essential component of the Mediterranean dietary.

5. Conclusion

In conclusions, the investigated agricultural soil located in the urban fabric appeared slightly contaminated by Cu and Pb that would seem to derive by both agricultural practices and urban inputs. Besides, the soils under the two crop species did not differ in biological and ecotoxicological characteristics. For both sorghum and sunflower, roots would seem the main via of metal uptake as they showed higher concentrations of all the investigated metals as compared to stems, leaves or seeds and the BF_{roots} were higher than BF_{leaves}, BF_{stems} or BF_{seeds}. In addition, the metal translocation from roots to the aboveground biomass portions would seem to be negligible for both sunflower and sorghum. Cu appeared the most abundant metal in both the crop species; differently from roots of sorghum, those of sunflower would seem to limit the Pb absorption. In addition, for sunflower, Cd and Cu uptake by leaves from the atmosphere cannot be excluded. As the cultivar of sunflower chosen for the study is characterised by higher biomass and faster growth rate as compared to the sorghum cultivar, it showed an overall higher metal accumulation in the various biomass portions. In particular, the accumulation of Cd and Cu in the seeds of both the crop species could represent damage for human health, as they are included in the Mediterranean dietary.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.apsoil.2017.09.035.

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Supplementary material 1. Levels of significance (P values) from the two-way ANOVAs for comparison of translocation factors calculated on Cd, Cr, Cu, Ni and Pb concentrations in different portions of biomass (stems, leaves and seeds) and in two crop species.

				Post-hoc comparision test									
					Species within portion				Portion within specie				
Variable	Species	Portions	Species x portions		**stems**	**leaves**	**seeds**		***H. annuus***			***S. bicolor***	
									leaves	seeds		leaves	seeds
Cd	**<0.001**	0.371	0.087	Sunflower vs sorghum	**<0.001**	**<0.001**	**<0.001**	stems	0.376	0.671	stems	0.298	0.094
								leaves	-	0.488	leaves	-	0.405
								seeds	-	-	seeds	-	-
Cr	**0.008**	**0.001**	**0.002**	Sunflower vs sorghum	1.000	**<0.001**	0.912	stems	**<0.001**	1.000	stems	0.995	0.999
								leaves	-	**<0.001**	leaves	-	0.981
								seeds	-	-	seeds	-	-
Cu	0.554	**0.012**	**0.050**	Sunflower vs sorghum	0.109	0057	0.495	stems	**0.002**	0.115	stems	0.688	0.726
								leaves	-	**0.047**	leaves	-	0.613
								seeds	-	-	seeds	-	-
Ni	**<0.001**	**0.020**	**0.020**	Sunflower vs sorghum	**<0.001**	**0.003**	1.000	stems	0.391	**0.003**	stems	1.000	1.000
								leaves	-	**0.007**	leaves	-	1.000
								seeds	-	-	seeds	-	-
Pb	**0.002**	**0.013**	0.052	Sunflower vs sorghum	**0.006**	**0.004**	1.000	stems	0.392	**0.012**	stems	0.773	1.000
								leaves	-	**0.003**	leaves	-	0.892
								seeds	-	**-**	seeds	-	-

Terms of ANOVA models (degrees of freedom in brackets) are: Species (1), Portions (2), Species x Portions (2), Residual (18), Total (23). Pairwise multiple comparison by Holm-Sidak method. Significant differences (for $\alpha = 0.05$) are highlighted in bold.

Supplementary material 2. Levels of significance (P values) from the two-way ANOVAs for bioaccumulation factors calculated on Cd, Cr, Cu, Ni and Pb concentrations in different portions of biomass (roots, stems, leaves and seeds) and in two crop species.

					Post-hoc comparision test									
					Species within portion					Portion within specie				
Variable	Species	Portions	Species x Portions		**Roots**	**Stems**	**Leaves**	**Seeds**		***H. annuus***			***S. bicolor***	
										Leaves	Seeds		Leaves	Seeds
Cd	0.063	**<0.001**	**<0.001**	Sunflower vs Sorghum	**<0.001**	0.354	0.060	**0.009**	Roots	0.170	0.139	Roots	**<0.001**	**<0.001**
									Stems	0.105	0.159	Stems	0.425	0.809
									Leaves	-	0.763	Leaves	-	0.439
									Seeds	-	-	Seeds	-	-
Cr	0.080	**<0.001**	**0.021**	Sunflower vs Sorghum	**<0.001**	1.000	0.807	0.980	Roots	0.867	0.853	Roots	**<0.001**	**<0.001**
									Stems	0.960	1.000	Stems	0.992	1.000
									Leaves	-	0.992	Leaves	-	1.000
									Seeds	-	-	Seeds	-	-
Cu	**0.006**	**<0.001**	0.101	Sunflower vs Sorghum	**0.031**	0.564	**0.008**	0.182	Roots	0.056	**<0.001**	Roots	**0.018**	**0.006**
									Stems	**0.002**	0.052	Stems	0.895	0.934
									Leaves	-	0.094	Leaves	-	0.826
									Seeds	-	-	Seeds	-	-
Ni	0.198	**<0.001**	0.066	Sunflower vs Sorghum	**0.005**	0.991	0.652	1.000	Roots	0.718	0.773	Roots	**0.001**	**0.001**
									Stems	0.884	0.991	Stems	1.000	1.000
									Leaves	-	0.958	Leaves	-	1.000
									Seeds	-	-	Seeds	-	-
Pb	0.121	**<0.001**	**0.017**	Sunflower vs Sorghum	**<0.001**	0.716	0.794	1.000	Roots	0.084	**0.047**	Roots	**<0.001**	**<0.001**
									Stems	0.972	0.977	Stems	0.997	1.000
									Leaves	-	0.933	Leaves	-	1.000
									Seeds	-	-	Seeds	-	-

Terms of ANOVA models (degrees of freedom in brackets) are: Species (1), Portions (3), Species x Portions (3), Residual (24), Total (31). Pairwise multiple comparison by Holm-Sidak method. Significant differences (for $\alpha = 0.05$) are highlighted in bold.

CHAPTER 3

3.3 Set of indicators to assess the quality of andosols: a study case

(the paper is submitted for publication in *CATENA*)

Set of indicators to assess the quality of andosols: a study case

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Abstract

Maintaining of high quality of soils is necessary to let healthy living ecosystems and to protect them from disturbances. Among different kinds of soils, andosols, widespread on the Earth, are peculiar for their chemical composition that, in turns, affects the microbial community. The present research aimed to test if: the soil quality index calculated for the andosols of the Vesuvius National Park had high scores; site specific factors (such as altitude, seasonality, traffic flux) influenced the SQIs; a few selected parameters provided SQIs similar to those obtained using the twenty-five investigated parameters. The integrated soil quality index (SQI), calculated for all the parameters, showed intermediate values. Among the investigated site-specific factors, altitude and seasonality influenced the SQIs. At low altitude and in spring, most favourable conditions occurred for soil microbial biomass growth and activities. In order to individuate the Minimum Data Set (MDS), two statistical approaches were performed. In the first approach, not redundant parameters and showing the highest loading values were considered (MDS_1), whereas in the second approach, those with the highest sum of correlation coefficients were considered (MDS_2). In the investigated andosols, (total carbon content, total Ni concentration, qCO_2 and percentage effect of root elongation for *S. saccharatum* L., indicators selected in the MDS_1, appeared enough to define the soil quality. Besides, total carbon content appeared the main driver of soil quality even if a particular role is given by ecotoxicological parameters that likely are linked to soil inorganic components deriving by specific volcanic substrates.

Keywords: Minimum data set; chemical parameters; biological parameters; ecotoxicological parameters.

47 **1. Introduction**

48 Soils support all the terrestrial life forms and represent the substrate where fundamental activities
49 occur, guarantying important ecosystem services. Therefore, maintaining the quality of soil is
50 necessary to let healthy living ecosystem, to allow integrity of terrestrial ecosystems and to protect
51 them from disturbances (Ellert et al., 1997).

52 The original definition of soil quality was related to its capacity to function within ecosystem
53 and land use boundaries, to sustain productivity, maintain environmental quality, and promote plant
54 growth as well as animal health (Doran and Parkin, 1994). Therefore, soil quality depends on
55 numerous physical, chemical and biological properties that individually or jointly regulate soil
56 functioning. The physical and chemical properties provide information about soil production and
57 are sensitive to environmental changes (Takoutsing et al., 2016). Instead, dynamic soil properties
58 such as soil organic matter and most microbial attributes are more responsive to management
59 practices and disturbance (Li et al., 2013) and provide information about short-term effects of
60 environmental changes on soil functioning (Dose et al., 2015). Soil microbial parameters may
61 provide an 'early warning' of system collapse and allow to react before irreversible damages occur
62 (Renella et al., 2007). Some biological and microbial parameters are often correlated to soil
63 physico-chemical properties (Doi and Ranamukhaarachchi, 2009), but they depend on other
64 characteristics of the investigated area. Also ecotoxicological tests, providing information about the
65 effects of pollutants on organisms, are usually used in combination with chemical analyses
66 (Manzano et al., 2017) to offer tools for monitoring and to establish target criteria of soil quality
67 (Leitgib et al., 2007). In particular, phytotoxicity tests are often applied in soil monitoring as plants
68 strongly depend on its properties. Alterations in plant growth and physiological processes may
69 reflect the presence of toxic compounds and water and nutrient deficiency (Gyuricza et al., 2010).

70 Soil quality is soil- and site- specific, and can vary according to controlling factors, such as
71 inherent soil properties or climate (Karlen et al 1997). Peculiar environments are volcanic areas as
72 they are rich in non-essential elements, have excellent water-holding and nutrient capacity (unless

leached extensively), and their worldwide extent is estimated at less than 1% of the total soil area on Earth. Andosols display unique morphological, physical and chemical properties as they develop from ash, tuff and pumice, known to present "amorphous" fractions such as allophane, imogolite, ferrihydrite and Al/Fe–humus complexes (Shoji et al., 1993). It is reported that, in volcanic soils of temperate forests, the microbial activity can be limited due to high soil C/N ratios and acidity that can contribute to a decrease of the microbial biomass contextually to an increase of the metabolic quotient, a stress indicator. Thus suggested a low efficient use of organic C by soil microbial biomass (Xu et al., 2006).

As soil is multi-functional, therefore a single property cannot be used as general indicator of soil quality (Rezaei et al., 2006). Soil indicators, surrogates of a soil attribute that determines how well a soil functions, should be simple and easy to measure, sensitive to environmental changes and useful in a wide range of soil types. In addition, they should be selected on the basis of their capability to assess small variations in soil ecosystem functioning that are linked to soil management or climatic changes (Rezaei et al., 2006).

In order to reduce the number of indicators necessary to define the soil quality, enhancing work efficiency and condensing labour time and expense, the identification of a minimum data set (MDS), based on statistical procedures, of indicators from a total data set could be convenient. Various indicators of soil quality have been proposed and many statistical techniques have been used to select the MDS (Li et al., 2013). The Principal Component Analysis (PCA) is a widely used method to identify the main factors that better drive specific soil functions (Brejda et al., 2000). In addition, the selection of a reduced number of indicators to evaluate the soil quality could be a useful tool for management practices.

In order to implement the current knowledge about the characteristics of andosols and assessment their quality, the soils of the Vesuvius National Park, a peculiar example of a volcanic system of great naturalistic interest affected by high touristic pressure and human impact, were investigated. In this concern, the hypothesis of the research were: the soil quality index calculated

for the soils of the Vesuvius National Park had high scores (H_1); site specific factors (such as altitude, seasonality, traffic flux) influenced the SQIs (H_2); few selected parameters provided SQIs similar to those obtained using all the investigated parameters (H_3). The findings of the research can provide information both at local and global scales, as they can be as useful tool in management plans and can increase the knowledge about the relationships among the chemical, biological and ecotoxicological characteristics of andosols.

2. Materials and Methods

2.1 Study area and sampling

Since decades, only one road (Ercolano, E) conduced to the crater; recently, in 2012 another road (Matrone, M) was opened but accessible only to old military buses that transport tourists to the crater from April to October. Eight sites (four at 600 m a.s.l., L and four at 900 m a.s.l., H) in proximity of each road (Fig. 1) were selected in order to collect surface soils (0-10 cm). At each site, soils were collected at six different points and mixed together in order to obtain a homogeneous sample to perform the analyses. A total of sixteen sites were investigated twice, on November 2015 (Autumn, A) and April 2016 (Spring, S).

2.2 Soil physico-chemical analyses

In laboratory, the soil samples were sieved (2 mm) and divided in different aliquots to measure: water content (WC), pH, organic matter (OM), total C (C_{tot}) and N (N_{tot}) contents as well as total concentrations and available fractions of Cr, Cu, Ni and Pb.

The WC was determined by drying fresh soil at 105 °C until to reach constant weight, and pH was measured in a soil: distilled water (1:2.5=v:v) suspension by electrometric method.

Instead, the organic carbon (C_{org}) was measured on soil samples in order to calculate the organic matter content. The OM content obtained by samples previously treated with HCl (10%) was

124 evaluated by gas-chromatography (Thermo Finnigan, CNS Analyzer) and the OM content was
125 obtained multiplying the C_{org} for 1.724 (Pribyl, 2010).
126 C_{tot} and N_{tot} concentrations were evaluated on oven-dried (105 °C, until constant weight) and
127 grounded (Fritsch Analysette Spartan 3 Pulverisette 0) soil samples by gas-chromatography
128 (Thermo Finnigan, CNS Analyzer). Successively, C/N ratios were calculated.
129 In addition, the total concentrations of Cr, Cu, Ni and Pb were measured, via graphite furnace,
130 by atomic absorption spectrometry (SpectrAA 20 - Varian) afterdigestion with a mixture of HF
131 (50%) and HNO_3 (65%) at a ratio of 1:2 (v:v) in a micro-wave oven (Milestone mls 1200 -
132 Microwave Laboratory Systems) of oven-dried (105 °C, until constant weight) and grounded soil
133 samples. The available fractions of Cr, Cu, Ni and Pb were extracted by oven-dried (105 °C, until
134 constant weight) soil samples with diethylenetriaminepentacetic acid, $CaCl_2$ and triethanolamine at
135 pH 7.3 ± 0.05 (Lindsay and Norvell, 1969). Accuracy was checked by concurrent analysis of
136 standard reference material (BCR CRM 142R - *Commission of the European Communities*, 1994)
137 and recoveries ranged from 86 to 98%.
138 All the described analyses were performed in triplicates.
139
140 2.3 Soil biological analyses and indices
141 The investigated soils were analysed for microbial and fungal biomass as well as for microbial
142 respiration. The analyses were performed within a week after sampling, in quadruplicates, on fresh
143 samples stored at 4 °C. Microbial carbon (C_{mic}) was evaluated by the method of substrate-induced
144 respiration (SIR) according to Anderson and Domsch (1978), while microbial activity was
145 estimated as potential respiration (Resp). The CO_2 evolution from the samples at 55% of water
146 holding capacity was measured by NaOH absorption followed by two-phase titration with HCl
147 (Froment, 1972), after incubation at 25 °C in tight containers for 5 and 10 days, respectively, to
148 evaluate C_{mic} and Resp. Total fungal biomass (TFB) was assayed by membrane filter technique
149 (Sundman and Sivela, 1978), after staining with Aniline Blue, determining hypha length by

150 intersection method (Olson, 1950) with an optical microscope (Optika, B-252). The results obtained
151 by the biological analyses were used to calculate two indices: the metabolic quotient (qCO_2), i.e. the
152 degree of activity of the microbial biomass, and the coefficient of endogenous mineralization
153 (CEM), i.e. the rate of organic carbon mineralization. The qCO_2 was calculated as ratio between
154 microbial respiration ($C\text{-}CO_2$) and biomass (C_{mic}), whereas the CEM was calculated as ratio
155 between microbial respiration ($C\text{-}CO_2$) and C_{org}.
156
157 2.4 Ecotoxicological assays
158 The seed germination and root elongation assays were carried out, in triplicates, on the soils
159 according to the U.S. EPA protocols (US EPA, 1996). In more details, 10 seeds (EcotoxLds) of
160 *Lepidium sativum* L. (dicotyledon) and *Sorghum saccharatum* L. (monocotyledon) were placed in a
161 Petri dish, containing an amount of fresh soil equivalent to 10 g of oven-dried soil, subsequently
162 saturated with water. For each soil sample, standard soil (OECD, 1984) and $K_2Cr_2O_7$ was used as a
163 negative and positive control, respectively. All soil samples were incubated in darkness at 25 °C,
164 and after 72 h the number of germinated seeds and the total root length were evaluated. The results
165 of germination (Germ) and root elongation (Elong) for both species were expressed as effect
166 percentages with respect to the control. The germination index (GI) was calculated multiplying
167 mean seed germination by mean root elongation, and then expressed as percentage with respect to
168 the germination index for the control, as reported below:
169 $$GI(\%) = ((OECD_{Elong\ x\ Germ} - Sample_{Elong\ x\ Germ}) / OECD_{Elong\ x\ Germ}) \times 100$$
170
171 2.5 Soil Quality Index (SQI)
172 An integrated soil quality index was calculated taken into account the physico-chemical,
173 biological and ecotoxicological indicators that were ranked by linear scoring technique according to
174 Liebig et al. (2001). The scores, ranging from 0 to 1, were assigned applying *more is better* or *less*
175 *is better* functions. The *more is better* function was applied to organic matter, water and total C and

176 N contents, C/N ratio, Cmic, TFB, Resp and CEM; whereas the *less is better* function was applied
177 to qCO_2, total concentrations and available fractions of metals (Marzaioli et al., 2010). The
178 maximum score for pH was attributed to 7 (Liebig et al., 2001), whereas the maximum scores for
179 the ecotoxicological indicators were attributed to 100 % of the percentage effects.

180 The SQI was calculated, for each site and each sampling time, summing the indicator scores and
181 dividing for the number of indicators (Andrews et al., 2003):

$$SQI = \sum_{i=1}^{n} \frac{\mathrm{S}i}{\mathrm{n}}$$

182 Where SQI is soil quality index, *S* is the score assigned to each indicator and *n* is the number of the
183 selected indicators.

184

185 2.6 Selection of the Minimum Data Set (MDS)

186 Three-way analysis of variance (ANOVA), followed by the *post hoc* test of Holm-Sidak, was
187 performed in order to select the soil indicators that showed significant differences ($P < 0.05$)
188 according the seasons, the roads, the altitudes or their interactions (Bhardwaj et al., 2011). The
189 selected indicators were used in the standardized principal component analysis, PCA (Liu et al.,
190 2014). As the principal components (PCs) with eigenvalues < 1 have less variation than the
191 individual variable, only the PCs with eigenvalues > 1 were considered for the identification of the
192 MDS (Askari and Holden, 2015). Within each PC, indicators with absolute values within 10% of
193 the highest weighted loading were selected for the MDS. When, for each PC, more than one
194 indicator showed the values to be selected for the MDS, two approaches were performed for the
195 selection. The first approach retained, for each PC, in the MDS the indicators that were not
196 correlated, eliminating the redundancy. The second approach retained, for each PC, in the MDS the
197 indicators having the highest correlation sum (Rezaei et al., 2006; Raiesi and Kabiri, 2016).

198

199 2.7 Statistical analyses

200 The normality of the data distribution was assessed by the Shapiro-Wilk test.

201 The three-way analysis of variance (ANOVA) was performed in order to evaluate the differences
202 among the seasons, roads or altitudes and also to select the indicators for principal component
203 analysis (PCA). The ANOVA tests were followed by the *post hoc* test of Holm-Sidak.

204 The relationships among the chemical, biological and ecotoxicological characteristics of the soils
205 as well as those among the soil total and available metal concentrations were evaluated by
206 Spearman test as the data showed a non-normal distribution.

207 The unpaired t-test was performed in order to evaluate the differences between the soils sampled
208 along Matrone (M) and Ercolano (E) roads, at low (L) and high (H) altitudes and in autumn (A) and
209 spring (S) seasons for the SQIs.

210 The statistical assays, performed by Systat_SigmaPlot_12.2 software (Jandel Scientific, USA),
211 were considered statistically significant for $P < 0.05$. The PCA analysis carried out to select the
212 MDS, as above described, was performed by Past v. 3.15 (Øyvind Hammer, Oslo).

213

214 **3. Results**

215 3.1 Physico-chemical, biological and ecotoxicological parameters of the soils and soil quality
216 (SQI)

217 The results of the physico-chemical, biological and ecotoxicological indicators of the soils
218 collected in autumn and spring (Tables 1 and 2), highlighted wide variability among the sites.
219 Almost all the parameters showed statistically significant differences between soils collected at
220 different altitudes (Table 3) with values higher at low than high altitudes with the exception of total
221 Cr and Ni concentration. Besides, the chemical, biological and ecotoxicological parameters
222 appeared statistically higher in spring than in autumn; although soil respiration, organic matter
223 content, elongation and germination of *S. saccharatum* L. were higher in autumn (Table 3). Only
224 some parameters showed statistically significant differences between the two roads (Table 3). In
225 particular, C/N ratio, organic matter content, root elongation of *S. saccharatum* L. and fungal

biomass were higher in the soils collected along Matrone road, whereas total Pb concentration and Cu available fraction were higher in the soils collected along Ercolano road (Table 3). The SQIs, calculated taking into account the 25 parameters at the 32 sites, of the soils collected in the Vesuvius National Park showed values ranging from 0.44 and 0.67. Differences of the SQIs were statistically significant ($P < 0.001$) only for the soils collected in different seasons (Fig. 2) with values higher (0.55) in autumn than in spring (0.47); whereas they were not statistically different for altitudes and roads. In fact, the mean values of SQIs were 0.52 and 0.56, respectively, for soils collected at high and low altitudes, and 0.52 and 0.55, respectively, for the soils collected along Ercolano and Matrone roads (Fig. 2).

3.2 Selection of the MDSs and deriving soil quality indices

To individuate the MDS, 20 parameters were selected (Table 3) as they significantly differ for season, road, altitude or their interactions (Bhardwaj et al., 2011) and used in the PCA.

The PCA identified 5 PCs with eigenvalues > 1, explaining the 81.4 % of the variance of the soil parameters (Table 4). The results of the PCA highlighted that the parameters with absolute values within 10% of the highest weighted loading were three (organic matter and water contents, and total carbon concentrations) for the PC1, two (total Ni concentration and percentage effect of germination index for *L. sativum* L.) for the PC2, three (total Cu and Pb concentrations and Cu available fraction) for the PC3, two (microbial carbon and qCO_2) for the PC4, and two (qCO_2 and percentage effect of root elongation for *S. saccharatum* L.) for the PC5 (Table 4).

The outcomes of the PCA were used in order to select the MDSs deriving by the two statistical approaches. The MDS_1 was obtained selecting the not redundant parameters and those with the highest loading values. Under PC1, organic matter, water and total C contents showed similar eigenvectors (Table 4) and the total carbon content was selected as it showed the highest loading value (Table 4). Under PC2, total Ni concentration was selected as showed the highest loading value (Table 4) and was not correlated with total C content, previously selected (Table 5).Under

252 PC3 no parameters were selected as total Cu and Pb concentrations, showing the highest loading
253 values, were correlated to the parameters previously selected (Table 5). By contrast, qCO_2 and root
254 elongation of *S. saccharatum* L. were selected under PC4 and PC5, respectively, as they showed the
255 highest loading values (Table 4) and were not correlated with the other parameters (Table 5).
256 Finally, the MDS_1 included: total carbon content, total Ni concentration, qCO_2 and percentage
257 effect of root elongation for *S. saccharatum* L. The SQI_1, calculated taking into account only the
258 indicators included in MDS_1 ranged between 0.31 and 0.75 (Fig. 3). The SQI_1 were significantly
259 higher in soils collected at low than high altitude ($P < 0.05$) and in autumn than in spring ($P < 0.01$);
260 whereas they were not statistically different between the roads (Fig. 3).
261 The MDS_2, obtained considering the parameters with the highest correlation sums, included:
262 water content, microbial carbon, qCO_2, total Ni concentration, Cu available fraction, percentage
263 effect of germination index for *L. sativum* L. and of root elongation for *S. saccharatum* L. (Table 6).
264 The SQI_2 calculated according the MDS_2 ranged between 0.40 and 0.68 and did not highlight any
265 significant differences between roads, altitudes or seasons.
266

267 **4. Discussions**

268 The wide ranges of values observed for the investigated parameters suggest high heterogeneity
269 of the soils inside the Vesuvius National Park according to both microhabitat and microclimatic
270 conditions. In particular, altitude and seasonality, more than traffic flow, appeared to affect the soil
271 characteristics. In fact, plant cover and density influenced the quality and the amount of organic
272 matter that accumulates that, in turns, regulated the soil abiotic and biotic parameters. In fact, along
273 the altitudinal transect the plant cover drastically changed passing from dense forests rich in pine
274 and holm oak specimens, at the bottom, to a sparse shrub, at the top. The densest plant cover
275 reduces the incidence of solar radiation on soil that, in turns, causes a temperature decrease and a
276 moister content increase in the underwood (Gaudio et al., 2017). Therefore, at low altitude and in
277 spring, most favourable conditions occurred for soil microbial and fungal biomass and activities

278 (De Marco et al., 2013). This supposition is also corroborated by a reduction of stress conditions for
279 the soil microbial community as proved by the lowest respiration rates and qCO_2 values (Fließbach
280 and Mäder, 1997) particularly in soils collected in spring. The highest microbial activity occurring
281 during the spring caused an accumulation of organic matter rich in recalcitrant compounds, less
282 biodegradable also supported by higher C/N ratios, slightly lower CEM values and by the most
283 abundance of fungal biomass, that generally attach more complex compounds (Maisto et al., 2010).
284 On the whole, the traffic flow did not appear to affect soil microbial biomass and activity as, in
285 contrast with that is widely reported in the scientific literature (Chodaka et al., 2013), no negative
286 effects were observed in the investigated area. In fact, in spring when the highest amount of metals
287 was measured, also the highest microbial biomass was detected. Thus can be explained by the likely
288 metal insoluble forms present in the soils or by the tolerance mechanisms developed by the
289 microbial community (Giller et al., 1998). The presence of metal insoluble forms in the investigated
290 soils can be corroborated by the lack of correspondence between the trends of total concentration
291 and available fraction of each metal with the exception of Cu. The unnoticeable effects of soil metal
292 content on microbial biomass and activities were also confirmed by the absence of statistically
293 significant differences between soils collected along the two roads, although the highest contents of
294 Pb and Cu, recognized as markers of vehicular traffic (De Silva et al., 2016), were measured in the
295 soils collected along Ercolano characterized by intensive and continuous traffic flux. Instead, Ni
296 could be a representative indicator of various metals (*i.e.* Cr, Cu, K and V) that in the volcanic areas
297 are basal mineralogical components deriving by the alteration of the parent material (De Nicola et
298 al., 2003), although its derivation from vehicular traffic cannot be excluded (De Silva 2016). The
299 phytotoxicity assays would seem to highlight unremarkable damages on soil biota as the toxicity
300 effects were lower than 20 %, assumed as physiological variation of the population (APAT, 2002).
301 Anyway, *Lepidium sativum* L., showing slight toxicity in soils collected in spring, appeared more
302 sensitive than *S. saccharatum* L.

On the whole, the SQI, calculated taking into account all the investigated parameters and based on the function *more is better* and *less is better*, confirmed that the soil quality was strongly affected by high organic matter content and other deriving parameters (*i.e*. water content, C/N ratio, microbial biomass and activity) as well as low metal content and phytotoxicity.

The MDS_1 and MDS_2, and the deriving SQI_1 and SQI_2, selected the main drivers of soil quality in the investigated area. Both the MDSs selected qCO_2, root elongation of *S*. *saccharatum* L. and total Ni content. The three common indicators belonged to chemical, biological and ecotoxicological parameters. The qCO_2 and percentage effect of root elongation for *S*. *saccharatum* L. are direct expression of soil biota; Ni is a mineralogical component of volcanic areas and marker of vehicular traffic. In particular, as they notoriously suggest stress conditions of the soil, low values of these indicators provide high soil quality. Finally, the key role of organic matter content in defining the quality of the investigated volcanic soils is confirmed by the presence of total carbon in the MDS_1. Besides, the SQI_{2+Ctot} calculated after the addition of total carbon content to the MDS_2 showed the same level of significance of the differences observed for the SQI_1 according the altitudes.

5 Conclusions

The investigated volcanic soils, although located inside a National Park, would seem to be affected by human activities as the SQIs, calculated taking into account twenty-five parameters, varied from 0.44 to 0.67 showing intermediate values in a score ranging from 0 to 1.

The soil quality appeared to be linked also to site-specific factors. In particular, altitude and seasonality that conditioned the type and density of plant cover as well as the microclimatic conditions influenced the SQIs. At low altitude and in spring, most favourable conditions occurred for soil microbial biomass growth and activities. In addition, also the overall characteristics of the investigated volcanic soils could affect the microbial community and the phytotoxicity. Thus was

328 also confirmed by low values of respiration rates and qCO_2, indicators of stress conditions,
329 particularly in soils collected in spring.

330 A limited number of the investigated parameters, such as total carbon content, total Ni
331 concentration, qCO_2 and percentage effect of root elongation for *S. saccharatum* L., selected in the
332 MDS_1, provided the same kind of information about the quality of the investigated soils. In fact, the
333 deriving SQI_1 showed the same levels of significance of the differences of soil quality according the
334 altitude and seasonality obtained by the SQI, calculated by considering all the twenty-five
335 parameters. However, total carbon content appeared the main driver of soil quality. The novel result
336 of the research was the presence of an ecotoxicological parameter beyond chemical and biological
337 parameters as indicator of soil quality. The important role of the ecotoxicological parameter in
338 defining the quality of andosols could be related to the integration of numerous abiotic factors;
339 among them the inorganic components deriving by specific volcanic substrates cannot be excluded.

340 At local scale, the present research suggested that, in management practices, the parameters
341 selected in the MDS_1 should be considered in order to monitor the soil quality of the Vesuvius
342 National Park.

343

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350

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446 **Figure captions**

447 Fig. 1 Map of the sampling sites.

448 Fig. 2 Mean values (± s.e.) of the soil quality index (SQI) calculated taking into account all the 32
449 soil indicators for soils collected along the two roads (Matrone: M, Ercolano: E), at two different
450 altitudes (H: 900 m a.s.l.; L: 600 m a.s.l.), and at different seasons (A: autumn; S: spring). The
451 asterisks indicate the significant differences between the seasons ($P < 0.001$).

452 Fig. 3 Mean values (± s.e.) of the soil quality index (SQI_1) calculated taking into account the 4
453 indicators selected for the Minimum Data Set, MDS_1 for soils collected along the two roads
454 (Matrone: M, Ercolano: E), at two different altitudes (H: 900 m a.s.l.; L: 600 m a.s.l.), and at
455 different seasons (A: autumn; S: spring). The asterisks indicate the significant differences
456 between the altitudes ($P < 0.05$) and the seasons ($P < 0.01$).

457 Fig. 4 Mean values (± s.e.) of the soil quality index calculated taking into account the 7 indicators
458 selected for the Minimum Data Set, MDS_2 (SQI_2), and calculated taking into account the
459 indicators of the MDS_2 plus total carbon content (SQI_{2+Ctot}) for soils collected along the two
460 roads (Matrone: M, Ercolano: E), at two different altitudes (H: 900 m a.s.l.; L: 600 m a.s.l.), and
461 at different seasons (A: autumn; S: spring). The asterisks indicate the significant differences
462 between the altitudes ($P < 0.01$) for the SQI_{2+Ctot}.

Table 1

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Table 1- Mean values (± s.e.) of pH, water content (WC, expressed as % d.w.), organic matter content (OM, expressed as % d.w.), total C and N concentrations (expressed as % d.w.), C/N ratios, total concentrations and available fractions of metals (expressed as μg g^{-1} d.w.) in soils collected in autumn and in spring at the investigated area. In bold are reported the maximum and minimum values.

	pH	WC	OM	C_{tot}	N_{tot}	C/N	Cr_{tot}	Cu_{tot}	Ni_{tot}	Pb_{tot}	Cr_{avail}	Cu_{avail}	Ni_{avail}	Pb_{avail}
MHA	6.89	**13.9**	4.14	**2.04**	0.22	9.21	12.4	53.2	4.70	**35.5**	0.00	**1.33**	**0.03**	1.02
	±0.09	±1.18	±0.82	±0.34	±0.03	±0.29	±0.58	±0.64	±1.07	±1.06	±0.00	±0.18	±0.01	±0.16
MLA	6.85	**70.7**	**29.5**	**17.3**	**0.60**	28.4	11.8	**41.4**	6.38	70.6	0.01	5.44	0.30	9.36
	±0.08	±24.6	±10.4	±5.58	±0.18	±0.90	±1.45	±2.85	±1.91	±6.93	±0.00	±1.77	±0.08	±2.34
EHA	**6.93**	21.6	7.85	4.18	0.62	**7.53**	15.6	45.1	7.06	43.6	0.00	4.22	0.06	2.83
	±0.39	±6.29	±2.56	±1.47	±0.12	±2.26	±0.56	±7.49	±0.49	±2.56	±0.00	±0.48	±0.03	±0.93
ELA	6.75	28.6	7.75	5.96	0.42	14.58	**7.0**	84.3	**2.96**	68.2	0.00	6.05	0.09	6.97
	±0.18	±5.45	±2.20	±1.61	±0.11	±2.65	±1.77	±21.1	±0.28	±9.99	±0.00	±2.48	±0.03	±3.17
MHS	6.65	20.9	6.13	4.36	0.27	15.1	20.1	97.4	17.70	38.9	0.00	3.86	0.07	**0.87**
	±0.12	±5.91	±2.71	±1.72	±0.08	±1.57	±1.56	±7.80	±0.51	±1.80	±0.00	±0.43	±0.01	±0.51
MLS	**6.03**	42.8	24.0	13.6	0.45	**34.4**	18.5	89.9	14.5	72.9	0.04	8.86	**0.52**	**57.7**
	±0.17	±10.6	±2.23	±2.25	±0.12	±3.88	±2.33	±10.6	±1.50	±2.27	±0.04	±3.40	±0.37	±51.7
EHS	6.67	17.2	**4.12**	2.82	**0.21**	16.0	**24.1**	88.8	**18.0**	49.7	0.00	6.11	0.02	1.53
	±0.24	±2.04	±0.62	±0.22	±0.04	±4.39	±2.06	±6.84	±0.79	±4.25	±0.00	±0.99	±0.00	±0.68
ELS	6.79	32.3	10.3	10.4	0.59	16.4	11.1	**153**	9.50	**106**	0.00	**20.9**	0.28	12.0
	±0.44	±11.0	±4.03	±3.37	±0.13	±2.52	±1.77	±46.2	±0.87	±13.4	±0.00	±5.92	±0.14	±0.82

Table 2

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Table 2- Mean values (± s.e.) of microbial biomass carbon (C_{mic}, expressed as mg C g^{-1} d.w.), fungal biomass ($Biom_{fung}$, expressed as mg g^{-1} d.w.), soil respiration (Resp, expressed as mg CO_2 g^{-1} d.w. d^{-1}), metabolic quotient (qCO_2, expressed as mg C-CO_2 mg^{-1} C_{mic}), coefficient of endogenous mineralization (CEM, expressed as mg C-CO_2 g^{-1} C_{org}) and effect percentages of germination index, germinated seeds and root elongation of *Lepidium sativum* L. and *Sorghum saccharatum* L. (Lep_{GI}, Lep_{Germ}, Lep_{Elong}, Sor_{GI}, Sor_{Germ} and Sor_{Elong}, respectively) in soils collected in autumn and in spring at the investigated area. In bold are reported the maximum and minimum values.

	C_{mic}	$Biom_{fung}$	Resp	qCO^2	CEM	Lep_{GI}	Lep_{Germ}	Lep_{Elong}	Sor_{GI}	Sor_{Germ}	Sor_{Elong}
MHA	**0.49**	**0.16**	0.49	0.12	**2.17**	-1.67	**102.0**	**101**	-8.88	91.0	**118**
	±0.12	±0.03	±0.07	±0.03	±0.64	±4.21	±2.00	±5.63	±9.99	±6.10	±6.57
MLA	0.93	0.34	**1.24**	**0.30**	1.04	-16.5	98.5	119	**-9.07**	**94.6**	113
	±0.34	±0.05	±0.18	±0.14	±0.11	±2.15	±5.41	±5.68	±2.80	±3.43	±6.04
EHA	1.34	0.19	1.13	0.05	**1.01**	**-0.78**	94.6	106	6.12	92.8	100
	±0.22	±0.03	±0.18	±0.01	±0.33	±17.3	±1.80	±18.7	±14.0	±5.07	±11.1
ELA	1.07	0.23	0.91	0.07	1.62	-5.28	**92.8**	113	2.51	87.5	115
	±0.26	±0.04	±0.22	±0.02	±0.76	±8.63	±5.07	±8.82	±5.10	±4.51	±8.17
MHS	1.30	0.26	0.24	**0.04**	1.63	-44.0	99.2	145	9.42	81.6	108
	±0.40	±0.04	±0.11	±0.01	±0.26	±17.2	±0.85	±17.0	±8.00	±6.45	±9.30
MLS	**2.97**	**0.63**	0.68	0.06	1.18	-15.1	98.3	145	14.2	80.8	106.3
	±0.51	±0.06	±0.15	±0.00	±0.21	±23.8	±1.68	±6.30	±6.97	±4.39	±9.54
EHS	1.05	0.18	**0.19**	0.07	2.13	**-45.9**	98.4	**162**	19.1	**80.0**	101
	±0.18	±0.03	±0.05	±0.01	±0.44	±5.00	±0.95	±15.2	±4.63	±4.07	±4.95
ELS	1.81	0.20	0.46	0.07	2.07	-41.6	98.4	144	**33.4**	82.5	**79.6**
	±0.34	±0.04	±0.17	±0.01	±0.35	±1.67	±0.95	±2.35	±8.91	±4.79	±6.69

Table 3

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Table 3 – Level of significance (F values) from the three-way ANOVA for comparison of soil quality parameters between road, altitude and season.

Indicator	Road	Season	Altitude	Season x Road	Season x Altitude	Road x Altitude	Season x Road x Altitude
pH	1.03	3.39	1.04	1.49	0.16	0.73	1.60
WC	2.48	0.50	10.64**	0.43	0.75	3.38	1.94
C_{tot}	3.50	0.06	28.70***	0.37	0.00	4.12	2.53
N_{tot}	0.90	1.15	5.42*	0.23	1.49	1.48	6.19*
C/N	18.93***	8.93**	37.64***	0.05	0.76	17.13***	0.80
OM	7.57*	0.15	16.22***	0.04	0.01	9.17**	1.28
Cr_{tot}	1.20	34.84***	26.98***	0.15	1.27	17.75***	0.52
Cu_{tot}	2.74	14.64***	2.44	0.13	0.29	5.23*	0.15
Ni_{tot}	3.75	165.43***	21.85***	1.49	9.46**	13.83**	0.016
Pb_{tot}	6.82*	6.75*	61.77***	4.05	2.56	0.38	2.98
Cr_{avail}	1.15	0.83	1.84	0.83	0.84	1.17	0.74
Cu_{avail}	5.55*	9.00**	11.59**	2.04	3.36	0.99	2.56
Ni_{avail}	1.41	1.01	5.92*	0.08	0.98	1.06	0.013
Pb_{avail}	0.77	1.01	2.36	0.73	1.12	0.95	0.66
$Biom_{fung}$	25.49***	9.37**	27.70***	13.20**	2.01	17.44***	2.61
C_{mic}	0.21	13.38**	8.34**	7.01*	6.22*	3.21	0.05
Resp	0.008	26.21***	8.64**	1.79	0.16	6.89*	3.46
qCO_2	3.04	3.41	1.99	4.69*	1.43	1.53	0.90
CEM	0.45	0.90	0.69	2.49	0.00	2.96	1.21
LEP_{GI}	0.33	26.93***	0.19	0.27	0.00	1.63	0.98
$LEPE_{long}$	0.24	22.72***	0.043	0.22	1.82	0.85	0.05
LEP_{Germ}	2.70	0.67	0.58	2.06	3.1	0.10	0.01
SOR_{GI}	2.58	1.39	0.031	1.55	2.76	0.37	0.28
$SORE_{long}$	4.57*	5.25*	0.44	0.65	2.12	0.00	2.88
SOR_{Germ}	0.15	8.65**	0.00	0.14	0.06	0.16	0.77

* P<0.05; ** P<0.01; *** P<0.001.

Table 4
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Table 4 - Results of principal component analysis of statistically significant soil quality indicators. The data in bold indicated the highly weighted variables.

PCs parameters	PC1	PC2	PC3	PC4	PC5
Eigenvalues	6.14	4.21	2.51	1.40	1.19
Variance (%)	32.32	22.17	13.23	7.41	6.28
Cumulative percent	32.32	54.49	67.72	75.13	81.41
Eigenvectors/factor loading					
WC	**0.363**	-0.097	0.081	0.126	-0.165
$Biom_{fung}$	0.303	0.129	0.190	-0.019	0.301
C_{mic}	0.235	0.259	-0.008	**-0.404**	0.303
Resp	0.237	-0.259	0.027	-0.334	0.028
qCO_2	0.195	-0.211	0.035	**0.421**	**-0.414**
C_{tot}	**0.388**	-0.035	0.023	0.118	-0.099
N_{tot}	0.304	-0.095	0.107	-0.335	-0.031
C/N	0.263	0.127	0.190	0.320	0.280
OM	**0.369**	-0.069	0.120	0.177	-0.030
LEP_{GI}	0.092	**0.400**	0.023	-0.072	-0.239
LEP_{elong}	0.094	0.368	0.050	0.153	-0.126
SOR_{germ}	-0.020	-0.274	-0.043	0.088	0.094
SOR_{elong}	-0.073	-0.163	0.323	0.298	**0.435**
Cu_{aivail}	0.134	0.145	**-0.482**	0.141	0.115
Ni_{aivail}	0.295	0.041	-0.021	-0.156	0.258
Pb_{tot}	0.184	0.080	**-0.437**	0.127	0.051
Cu_{tot}	-0.068	0.227	**-0.446**	0.265	0.123
Ni_{tot}	-0.065	**0.420**	0.198	0.099	-0.067
Cr_{tot}	-0.058	0.326	0.344	-0.047	-0.235

Table 5

Table 5 - Correlation matrix for the highly weighted soil quality indicators under the first five PCs. The correlation coefficients in bold were significant at $P < 0.05$.

	C_{tot}	WC	OM	Ni_{tot}	Pb_{tot}	Cu_{tot}	Cu_{avail}	qCO_2	C_{mic}	LEP_{GI}
WC	**0.92**									
OM	**0.87**	**0.82**								
Ni_{tot}	-0.04	-0.15	-0.08							
Pb_{tot}	**0.65**	**0.51**	**0.48**	-0.09						
Cu_{tot}	-0.09	-0.23	-0.26	**0.53**	0.24					
Cu_{avail}	**0.50**	**0.40**	**0.36**	0.09	**0.65**	0.30				
qCO_2	0.05	0.07	0.09	-0.32	-0.02	-0.24	-0.06			
Cmic	**0.59**	**0.48**	**0.48**	**0.38**	**0.48**	0.26	**0.39**	**-0.38**		
LEP_{GI}	0.32	0.24	**0.21**	**0.65**	0.23	**0.36**	**0.45**	0.04	**0.58**	
SOR_{Elong}	-0.05	0.01	0.05	-0.25	**-0.35**	**-0.39**	-0.29	-0.04	-0.30	**-0.41**

Table 6 - Correlation coefficients and correlation sums for highly weighted soil quality indicators under PC with multiple high factor loadings.

PC_1 variables	C_{tot}	WC	OM
Correlation coefficient			
C_{tot}	1	0.927	0.871
WC	0.927	1	0.882
OM	0.871	0.882	1
Sum of correlation	2.798	2.809	2.753

PC_3 variables	Cu_{aivail}	Pb_{tot}	Cu_{tot}
Correlation coefficient			
Cu_{aivail}	1	0.650	0.306
Pb_{tot}	0.650	1	0.246
Cu_{tot}	0.306	0.246	1
Sum of correlation	1.956	1.896	1.552

The correlation sum is the sum of the absolute value of correlation coefficient for each variable.

Figure 1

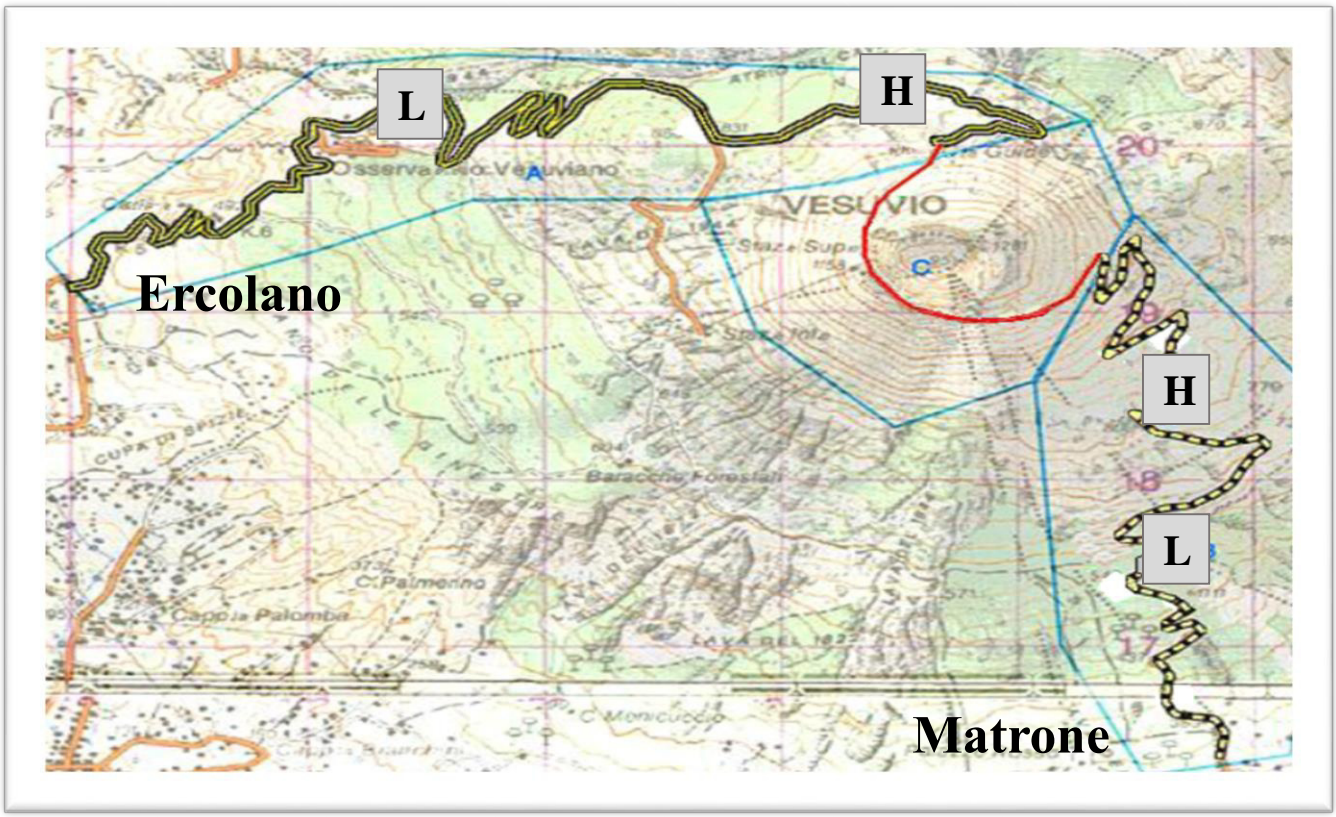

Fig. 1

Figure 2

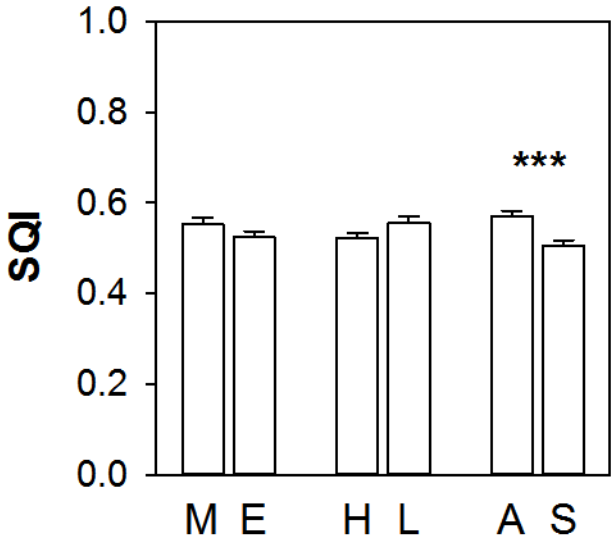

Fig. 2

Figure 3

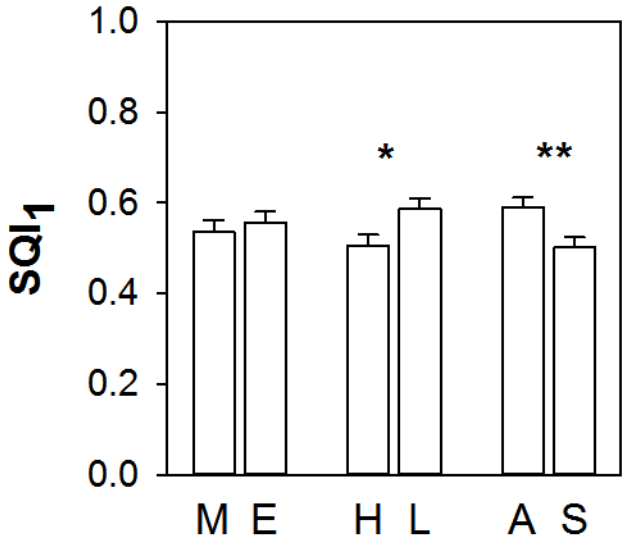

Fig. 3

Figure 4

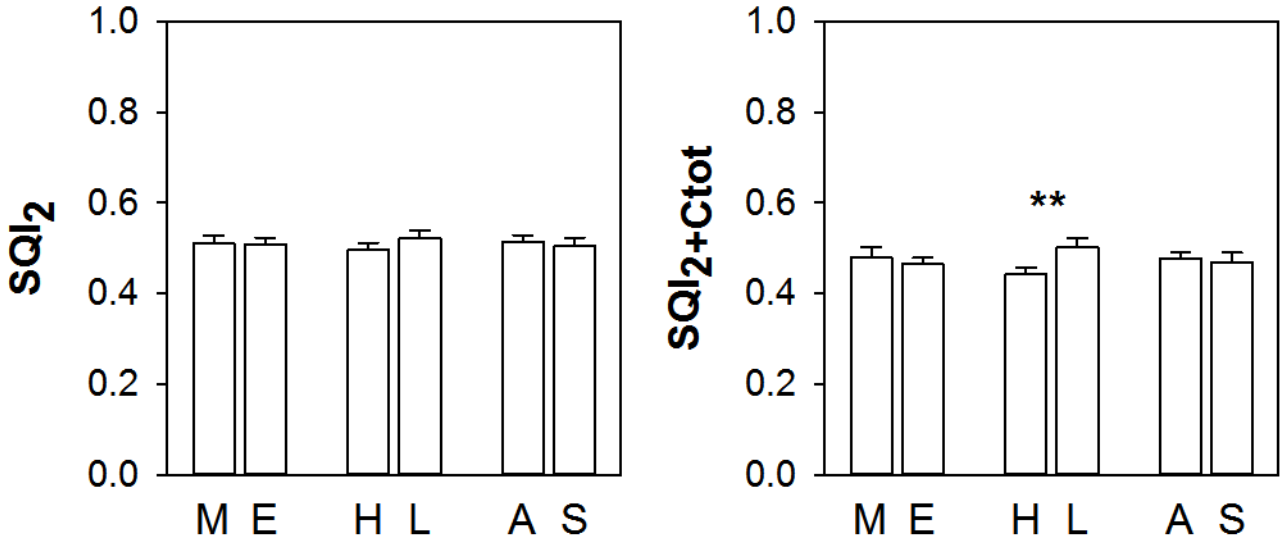

Fig. 4

CHAPTER 3

3.4 Total and fraction content of elements in volcanic soil: geochemical or anthropogenic derivation

(the paper is submitted for publication in *Science of the Total Environment*)

TOTAL AND FRACTION CONTENT OF ELEMENTS IN VOLCANIC SOIL: GEOCHEMICAL OR ANTHROPOGENIC DERIVATION

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Abstract

Soil element composition derives by parent material disaggregation during pedogenesis and weathering processes but also by anthropogenic inputs. Elements are present in soils in different chemical forms that affect their availability and mobility. The aim of the study was to evaluate the main derivation, geochemical or anthropogenic, of elements in the soils of the Vesuvius National Park (a natural environment strongly affected by human impacts). Besides, the effects of age of the lava from which soils derive, different vegetation covers, traffic fluxes along the two roads connecting the Vesuvius crater and altitudes of the sites on the pseudo-total element concentrations and on their contents in different fractions were investigated. To reach the aims, BCR sequential extraction was performed in order to determine the distribution of elements into: exchangeable/water-soluble, reducible, oxidasable and residual fractions. The relationship between the main environmental media and distribution of elements was discussed using non-metric multidimensional scaling (NMDS). The findings showed that, with the exception of Cd, Cu, Pb and Zn that would seem to derive also by human activities, the other investigated elements (Al, As, B, Ba, Ca, Cd, Cr, Cu, Fe, K, La, Mg, Mn, Na, Ni, P, Pb, Si, Ti, V, W and Zn) mainly had a geochemical derivation. The highest element accumulations in the soils at low altitude could be attributable to an integrated effect of plant cover, vicinity of downtowns and traffic flux. The exchangeable/water-soluble fraction of elements appeared more linked to lava age; the reducible and oxidasable ones to plant cover; the residual one to the chemical composition of the parent material that gave origin to the soils.

Keywords: pseudo-total content; element fractionation; contamination factor; pollution load index, risk assessment code

1. Introduction

Soil element composition derives by the integration of local conditions such as geology, climate and hydrology, and it strongly depends on parent material disaggregation during pedogenesis and weathering processes (De Nicola et al., 2003; Martínez Cortizas et al., 2003). Nevertheless, also external factors, such as anthropogenic activities, directly affect the element composition of soil surface layer (Buccianti et al., 2015). In fact, during the last years, human activities (*i.e*. tourism, agriculture, urbanization and industrialization) have caused an increase of major and trace elements in soils (Wiseman et al., 2013). In addition, human activities are confirmed as primary sources of elements in the air gaseous phase or particulates that can reach the surface soils through dry or wet depositions (De Nicola et al., 2003; Werkenthin et al., 2014). For instance, As, Se, Sb and Hg, showing high affinity for the volatile phase, can be aerial transported and are often associated to long distance contamination (Buccianti et al., 2015). As a result, the human activities can determine a significant modification of the elemental status of the soils. Therefore, the identification of the main derivation of a single soil element by geogenic or anthropogenic sources could be difficult especially for those that have both the origins (Cicchella et al., 2005; Buccianti et al., 2015).

Elements are mainly present in soils as water-soluble, exchangeable, carbonate-associated, Fe-Mn oxide-associated and organic-associated forms (Fernández-Ondoño et al., 2017). Besides, some elements can be strongly bound to silicates, representing the residue form, and cannot be available from organisms (Denaix et al., 1999; Tanneberg et al., 2001). Recently, in order to evaluate element fate in soil system, element mobility along the soil profile and the potential element bioavailability or toxicity, the identification of the element amount in different soil geochemical phases and not only the total content is required (Adamo et al., 2007). Soil element fractionation, distribution and mobility depend not only on chemical composition of the parental material (Maeda et al., 2003), but also on various chemical and physical characteristics of soil, such as pH, cationic exchangeable capacity, water and organic matter contents (Peijnenburg et al., 2007; Degryse et al., 2009).

In this framework, the Vesuvius National Park is a good environmental model to provide a

72 contribution to the present knowledge about the evaluation of geochemical or anthropogenic
73 derivation of some elements in the soils. In fact, the soils of the Vesuvius are andosols and, deriving
74 by pyroclastic materials (Shoji et al., 1978), are rich in neoformed amorphous aluminosilicates and
75 organo-mineral compounds that have high capacity to bind elements (Eswaran et al., 1993;
76 Tanneberg et al., 2001). It is been reported that the chemical species composition of the Vesuvius
77 substrates is a function of the age of the lava and pyroclastic materials and of the time and degree of
78 fractionation. In fact, Belkin et al. (1998) have reported that silicate-melt inclusions showed a
79 decrease of some components such as total alkalis, SO_3, Cl, Li, B and Sr and a decrease of Zr and Y
80 passing from samples of lava of 25000 yr B.P. to 1631-1944 A.D. The Vesuvius is located at few
81 kilometres from Naples, one of the most populated cities where various and intensive human
82 activities occur. In addition, itself is a touristic destination of thousands of people per year who
83 reach the crater by any kinds of vehicles.
84 The aims of the study were to evaluate the main derivation (geochemical or anthropogenic) of
85 elements in the soils of the Vesuvius National Park and the element fate in the soils. The
86 identification of the main source of contaminants can be useful to contain their emissions in order to
87 preserve and/or restore the soils quality inside the park. To reach the aims, the element fractions
88 were detected according the BCR sequential extraction (recommended by the European Community
89 Bureau of Reference, 1987, and modified by Ure et al., 1993) in order to separate the elements into:
90 exchangeable/water-soluble, reducible (associated to Fe-Mn oxides), oxidasable (associated to
91 organic matter content and sulphides) and residual fractions (associated to minerals). The
92 exchangeable/water-soluble fractions are considered as bioavailable, the reducible and oxidasable
93 fractions can be potentially bioavailable, whereas the residual fraction is considered not available
94 for organisms (Ma and Rao, 1997; He et al., 2006; Rodriguez et al. 2009). Besides, other aim of the
95 research was to investigate the relationships between the element pseudo-total or fraction contents
96 to different: i) age of the lava from which soils derive, ii) different vegetation covers, iii) traffic
97 fluxes along the two roads (one accessible over the year long and the other one accessible only for

six months a year) connecting the Vesuvius crater, iv) altitudes of the sites (approximately 600 and 900 m a.s.l.).

2. Materials and methods

2.1 Study area

The Vesuvius National Park was established in 1995 and is located 12 km SE of Naples. It covers an area of 8482 ha and contains Mt. Somma (maximum height: 1132 m a.s.l.), the original volcano, and Mt. Vesuvius (maximum height: 1281 m a.s.l.), originated from 79 A.D. eruption. The vegetation of Mt. Vesuvius is constituted by native Mediterranean vegetation based in trees (such as holm oak, maple, alder) and shrubs (such as myrtle, laurel, viburnum, brambles, brooms), but are present some species such as black pine and black locust (De Nicola et al., 2003; De Marco et al., 2013).

Vesuvius is one of the most studied volcanoes because it has been active for about 25000 years and for the alternation of explosive and effusive activities. At the present, Vesuvius is in a quiescent phase and the last eruption started in 1913 and finished with the paroxystic phase in 1944 (Rolandi, 2010). Because of the various eruptions, the slopes of the Vesuvius present diversified landscapes as result of different lava flows.

In this concern, the study focused on the soils in proximity of the two roads that lead to the Vesuvius cone: Matrone (M) and Ercolano (E). At high altitude (H), the soils derive by the 1937 and 1891-1893 eruptions, respectively at Matrone and Ercolano roads, whereas at low altitude (L), the soils in proximity of Matrone road derive by the 1906 eruption whereas those in proximity of Ercolano by the 1944 one (Table 1).

2.2 Soil sampling

In the last decades, Vesuvius is subject to intensive touristic flux. Ercolano road was, for a long time, the unique road to reach the crater, but in 2012 also Matrone road was opened to reach the

124 crater only by old military vehicles and only from April to October. On November 2016, a total of
125 eight sites were selected along each road: four sites were selected at high altitude (approximately,
126 900 m a.s.l.) and four at low altitude (approximately, 600 m a.s.l.). At each altitude, two sites were
127 selected at each edge of the road and two at approximately 30 m from the previous towards the
128 vegetation (Table 1). At each site, five subsamples of surface soil (0-10 cm) were collected and
129 mixed to obtain a homogeneous sample, in order to perform the physico-chemical analyses.

130

131 2.3 Physico-chemical analyses

132 All the physico-chemical analyses were carried out per triplicate on sieved (< 2mm) soil
133 samples. pH was measured with pH-meter on aqueous extract obtained adding distilled water to soil
134 (2.5:1; v:v). The water content was determined by drying fresh soil at 105 °C until to reach constant
135 weight. The total carbon, nitrogen and sulfur concentrations were determined by elemental analysis
136 (LECO CHNS-932 analyzer, USA). The organic carbon content was measured by gas-
137 chromatography (Thermo Finnigan, CNS Analyzer) on dried samples previously treated with HCl
138 (10%). The soil organic matter content was calculated multiplying the C_{org} concentrations by 1.724
139 as reported by Pribyl (2010).

140 The sequential extraction was applied to study the fractionation of metals in the soils and
141 determine their mobility and potential bioavailability as suggested by Bureau of Community
142 Research (BCR) (Rauret et al., 2000). Therefore, in order to determine the exchangeable/water-
143 soluble fraction (F1), to 1 g of dry soil was added 40 mL of acetic acid 0.11M. The samples were
144 shaken for 16 h at 30 ± 10 rpm at 22 ± 5 °C in a mechanical shaker. The extract was separated by
145 centrifugation at 5000 rpm for 20 min, the supernatant passed through 0.45 mm filter, collected in
146 polyethylene bottles and stored at 4°C until analyses. The residue was washed by shaking for 15
147 min with 20 mL of doubly deionised water and then centrifuged, discarding the supernatant. To the
148 residue, to determine the fraction associated to Fe – Mn oxides (F2), 40 mL of hydroxylamine

149 hydrochloride 0.5M at pH 1.5 was added. The samples were shaken for 16 h at 30 ± 10 rpm at 22 ±
150 5 °C in a mechanical shaker. The extract was separated by centrifugation at 5000 rpm for 20 min,
151 the supernatant passed through 0.45 mm filter, collected in polyethylene bottles and stored at 4°C
152 until analyses. The residue was washed as described as in the previous step. To the residue, to
153 determine the oxidasable fraction (associated to organic matter content and sulphides, F3), 10 mL of
154 8.8 M hydrogen peroxide was added. The mixture was digested for 1 h at 22 ± 5 °C and for another
155 1 h at 85 ± 2°C, and the volume was reduced to less than 3 mL. A second aliquot of 10 mL of H_2O_2
156 was added, the mixture was digested for 1 h at 85 ± 2°C, and the volume was reduced to about 1
157 mL. The residue was extracted with 50 mL of 1M of ammonium acetate, adjusted to pH 2.0, at 30 ±
158 10 rpm and 22 ± 5°C for 16 h. The extract was separated and the residue was washed as in previous
159 steps. The residual fraction (associated to minerals, F4) was determined by treating the residue from
160 step 3 with aqua regia. In this phase, to 250 mg of soil were added 9 mL of HCl (37%) and 3 mL of
161 HNO_3 (69%) and the samples were digested in microwave oven (CEM MarsX press, USA)
162 according to the procedure described in García-Delgado et al. (2012). The element concentrations in
163 the solutions obtained in each step were determined by ICP-MS (Perkin-Elmer NexION 300). The
164 sum of the concentrations of each element in the four fractions is considered as pseudo-total.

165

166 2.4 Quantification of soil pollution

167 In order to assess the soil contamination level and the risk index, the contamination factor (CF),
168 pollution load index (PLI) and risk assessment code were calculated. The contamination factor (CF)
169 is the ratio between the pseudo-total concentration of each element in the soil at each edge of the
170 road and its background value (*i.e.* the element concentration in soil collected at the natural reserve
171 inside the Vesuvius National Park):

$$CF = \frac{C_{element}}{C_{\text{background}}}$$

172 The pollution load index (PLI) is the geometric mean of the CF values for the *n* elements
173 (Madrid et al., 2002):

174 $$PLI = \sqrt[n]{CF1\ x\ CF2\ x\ CF3\ \ldots\ldots x\ CFn}$$

175 The risk assessment code (RAC), used as a risk index for heavy metals Cd, Cu, Pb, Zn was
176 calculated as follows (Liang et al., 2017):

$$RAC = \frac{amount\ elements\ of\ F1\ in\ HMs}{total\ amount\ of\ HMs}\ x\ 100$$

177

178 2.5 Statistical analyses

179 The unpaired t-test was performed in order to evaluate the differences between soils samples
180 from the two roads (Ercolano and Matrone) or altitudes (high and low) for each element fractions of
181 the BCR sequential extraction. The similarity of the sites according the mean value of the contents
182 of each element in the fractions (F1 - F4) was investigated through the multivariate analysis of the
183 non-metric multidimensional scaling (NMDS), based on the Euclidean distance. In addition, the
184 confidence ellipses (for α = 0.05) for lava ages, traffic flows and vegetation covers were
185 superimposed on the NMDS in order to evaluate their effects on element distribution. The NMDS
186 analyses were performed using the R 3.1.1 programming environment (R Core Team 2016) with
187 functions from Vegan^ package, whereas t-test was performed using Sigmaplot 12.0.

188

189 **3. Results**

190 3.1 Relationships between soil element contents and categorical variables

191 The results of the physico-chemical characteristics of the soils collected at the investigated sites
192 are reported in Table 2. The soil pH values ranged from 6.46 to 7.98 (Table 2). The organic matter

193 and water contents showed wide variability among the sites as well as total C, N and S contents
194 with ranges of 1.78 - 26.3% d.w., 0.11 - 0.67% d.w. and 0.01 - 0.07% d.w., respectively (Table 2).
195 The pseudo-total element concentrations determined as summation of F1, F2, F3 and F4, showed
196 that, at all the sites, Cr and Cd were traceable, whereas Al, K, Ca and Fe were the most abundant
197 elements (Table 3).
198 Cd, Cr and W were among the less abundant elements, at the sites, in all the fractions (Table 3,
199 Supplementary material). Instead, the elements with the highest concentrations varied according to
200 the fractions. In fact, Ca was the most abundant (3218 μg g^{-1} d.w.) in the F1 and F2 (the
201 exchangeable/water-soluble and reducible fractions, respectively); Al was abundant in the F3
202 (oxidasable fraction), whereas Al and K in the F4 (residual fraction; Supplementary material).
203 The NMDS performed using the results of the F1 showed that the soils distributed according
204 both the altitude (axis 1) and the kind of road (axis 2); that performed using the results of the F2 and
205 F3 (Fig. 1a, 1b and 1c) showed that the soils mainly separated according to the altitude (axis 1);
206 whereas that performed using the results of the F4 showed a soil separation mainly due to the kind
207 of road (Fig. 1a, 1b and 1c). The variability among the soils was wider in the first three NMDS, also
208 showing similar values, but it was narrower in the NMDS performed with the F4 results (Fig. 1a, 1b
209 and 1c).
210 The soils originated by the four lava ages clearly separated for the F1 and F2, whereas the soils
211 originated from the lava flow of 1906, with similar concentrations of Mg, Mn and K in the F1, and
212 1944, with similar concentrations of Pb and B in the F2, separated from those originated from the
213 lava flow of 1891-1893 and 1937 for the F3 (Fig. 1a). By contrast, no separation among the soils
214 coming from lava with different ages was observed for the F4 (Fig. 1a).
215 The soils covered by different plant (shrub or tree) mainly separated according to the element in
216 the F2 and F3 (Fig. 1b). The soils covered by shrubs were characterized by similar concentrations of
217 K, As, Si and La in F2 and by similar concentrations of La, W, V, Si, Cd, Ca and Na in F3. Instead,

218 the soils covered by trees showed similar concentrations of Mn, Ca, Fe, Al, W and Ba in F2 and
219 similar concentrations of Al in F3 (Fig. 1b).
220 According to the kind of traffic flow (intense or less intense), the soils clearly separated for the
221 element contents in the F1, a narrow separation was observed for the F2 and F3 and no separations
222 were observed for F4 (Fig. 1c). In the F1, the soils affected by low traffic flow showed similar
223 concentrations of As, Ba, V and Mg, whereas those affected by high traffic flow showed similar
224 concentrations of Cd, Na, Si, Zn, Ca and Al (Fig. 1c).
225
226 3.2 Comparison of pollution level and element fractionations in the soils collected along the
227 roads or at different altitudes
228 As the performed NMDS analyses highlighted that the element concentrations in the soils mainly
229 separated according the altitude and the proximity to the two roads with different kind of traffic, a
230 deeper analyses of the results was performed for these site typologies.
231 The CFs showed differences according the site typologies, in fact they were higher than 1 for 12
232 (*i.e*. As, B, Ca, Cd, Cu, K, Na, P, Pb, Si, W and Zn) out of the 22 investigated elements with values
233 particularly high for Cd, Cu and Zn for the soils collected along Ercolano road (Table 4); whereas
234 they were higher than 1 for 11 elements (*i.e*. Ba, Ca, Cd, Cr, Cu, Mg, Mn, Ni, Pb, Si and Zn) with
235 values of Cd particularly high for the soils collected along Matrone road (Table 4). The CFs showed
236 values higher than 1 for 4 (*i.e*. Cd, Cu, Si and Zn) out of the 22 investigated elements for the soils
237 collected at high altitude (Table 4); they were higher than 1 for all the elements (particularly higher
238 were the values for Cd, Cu, Pb and Zn) with the exception of La, P and Ti for the soils collected at
239 low altitude (Table 4).
240
241 The PLIs, calculated only for the elements with CFs higher than 1, were 1.55, 1.37, 1.29 and
242 1.52, respectively, for the soils collected along Ercolano road, Matrone road, at high altitude and
243 low altitude.

As Cd, Cu, Pb and Zn appeared the main contaminants in the investigated soils, the RAC were calculated. The results showed high risk for Cd in MH_1 and MH_2 (67%) and EL_2 (63%), medium risk for Zn (25% in EL_1 50% in EH_1), and low risk for Cu and Pb with RAC < 10%.

The soils collected along Ercolano road, with high traffic flow, showed statistically higher concentrations of Na in F1, Na and Si in F2, P and La in F3 as compared to the soils collected along Matrone road, with low traffic flow (Table 5). Instead, the concentrations of Mn in F1, Cd, Ni, Ti and Zn in F2, Mg and Ti in F3 were statistically higher in the soils collected along Matrone road (Table 5). No statistically significant differences for the element contents in F4 between the soils collected along the two roads were observed (Table 5).

The percentage ratios between element fraction and pseudo-total content (Fig. 2) of the following elements showed the same gradient for the soils collected along both the roads:

- Al, Ba, Fe and V: F4 > F3 > F2 > F1;
- As, La, P and W: F3 > F4 > F2 > F1;
- Cr, K, Mg and Na: F4 > F3 > F1 > F2;
- Cu: F3 > F4 > F1 > F2;
- Mn: F4 > F1 > F2 > F3;
- Ca: F4 > F1 > F3 > F2;
- Cd: F1 > F2 > F3 > F4.

Conversely, the percentage contribute of the fractions to the pseudo-total concentrations for B, Ni, Pb, Si, Ti and Zn strongly varied between the soils collected along the two roads (Fig. 2).

The soils collected at high altitude showed statistically higher contents of Ti in F3; whereas the soils collected at low altitude showed statistically higher contents of Mn, Na and Ni in F1, Cd, Na, Ni, Si and Zn in F2, and Cr, La, Mg, Pb and Zn in the F3 fractions (Table 5). No statistically significant differences for the element contents in the F4 fractions between the soils collected at the two altitudes were observed (Table 5).

269 The percentage ratios between element fraction and pseudo-total content (Fig. 3) of the following
270 elements showed the same gradient for the soils collected along both the altitudes:
271 - Al, Fe, Ni and V: F4 > F3 > F2 > F1;
272 - La, P and W: F3 > F2 > F4 > F1;
273 - Cr, K, Mg, Na and Ti: F4 > F3 > F1 > F2;
274 - Cu: F3 > F4 > F1 > F2;
275 - Ca: F4 > F1 > F3 > F2;
276 - Si: F3 > F1 > F4 > F2.
277 Conversely, the percentage contribute of the fractions to the pseudo-total concentrations for As,
278 B, Ba, Cd, Mn, Pb and Zn contents differed for the soils collected at the two altitudes with higher
279 values in F3 than in F4 (Fig. 3).
280
281 **4. Discussion**
282 The wide variability of pH values, organic matter, C, N and S contents observed in the sampled
283 soils likely was linked to the parent material disaggregation, weathering processes and topography
284 (Lozano-Garcia et al., 2016, Li et al., 2017). Besides, also plant cover gave an important role; in
285 fact, the different plant species differently contribute to litter amount, chemical composition and
286 decay, influencing the soil organic matter quality (De Marco et al., 2012).
287 The traceable (*i.e*. Cr and Cd) and dominant (*i.e*. Al, K, Ca and Fe) probably derive from leukite,
288 $K[AlSi_2O_6]$ and augite $(Ca,Mg,Fe)_2(Si,Al)_2O_6$, two of the most abundant minerals in Vesuvian
289 rocks (Vingiani et al., 2013). In addition, pseudo-total concentrations of some elements in the
290 investigated soils agreed with those reported for volcanic rock powder by Ramos et al. (2017). The
291 low concentrations of toxic elements (such as Cr, Cd and Pb) could be attributable to the scarce
292 potentiality of volcanic rocks to bind them, whereas the abundance of Al, a widely recognized toxic
293 element, could derive by the alteration of aluminosilicate glassy matrix (Ramos et al., 2017), that
294 are peculiar components of andosols. The less abundant elements were traceable also in each

fraction (F1-F4), whereas Ca was the most abundant element in F1 and F2, Al in F3 and K in F4, suggesting that these elements outnumber in different chemical forms.

The main drivers of element fractionations in the soils would seem to be linked to specific site characteristics such as altitude and proximity to the two roads. These site characteristics integrate the effects due to different lava ages, plant covers, traffic flows and types, and microclimatic conditions. The outcomes of the NMDS suggested that the chemical composition of the soils mainly depended on lava age. In fact, the similar element contents of the residual fraction (F4), which represents the portion of elements bound to the primary and secondary minerals, suggested a comparable chemical composition of the lava, whereas the weathering time of the lava would seem to affect the availability and mobility (F1, F2 and F3) of different elements. In particular, the soils deriving by the lava of 1906 showed similar concentrations of Mg, Mn and K in F1, whereas those deriving by the lava of 1944 showed similar concentrations of Pb and B in F2.

Also plant cover appeared to have an important role in element fractionations, especially for F2 and F3, as a clear separation was observed in the NMDS. Plants have a direct effect on soil elemental composition as root exudates, changing the rhizosphere pH, modify the oxidation status of the elements and, in turns, their mobility and fate in the soil (Houben and Sonnet, 2015). In addition, also the amount and quality of litter deriving from different plant species, affecting the soil organic matter content, are important drivers the soil element mobility (Degryse et al., 2009; Abreu et al., 2012). In the Vesuvius National Park, it is evident a clear role of soil element fractionations due to different types of plant cover. In fact, the element mobility and availability of the soils collected at low altitude of Ercolano road, deriving by the lava flow of 1944 and covered by lichens and herbaceous species clearly separated by those of the other soils.

However, in addition to the geochemical derivation of the elements of the soils inside the Vesuvius National Park the anthropogenic one can not be excluded, especially for Cd, Cu, Pb and Zn that are widely recognized as markers of vehicular traffic (De Silva et al., 2016; Wang et al., 2017) and that are the main responsible of the higher CFs and PLIs for the soils collected at low

altitude. These soils, more than those at high altitude, were more exposed to the direct effect of the traffic flow along the two roads connecting the crater of the Vesuvius (at the highest altitude a lower traffic flow was observed) and they also endured the effects of air particulates coming from the nearby cities. The deposition of air particulate deriving by direct and indirect inputs decreases with the increase of distance from the source of emission (Zhang et al., 2017).

In conclusion, the investigated elements in the soils of the Vesuvius National Park would seem mainly to have geochemical derivation. Exceptions were observed for Cd, Cu, Pb and Zn that would seem to derive also by human activities. These elements, especially Cd, can represent a potential high risk for the investigated soils according with the criteria used in sediments (Sundaray et al., 2011). The highest element accumulations in the soils at low altitude could be attributable to an integrated effect of the site characteristics (*i.e*. plant cover, vicinity of downtowns, traffic flux and microclimatic conditions). Lava age and plant cover strongly affected the soil element fractionation. In particular, the exchangeable/water-soluble fraction appeared more linked to lava age, whereas the reducible and oxidasable fractions to plant cover. The residual fraction of elements, that was comparable among the investigated soils, suggested a similar chemical composition of the parent material that originated, over the time, the present soils.

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347

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467 **Figure captions**

468 Fig. 1 – NMDS biplot for the element concentrations in each fraction (F1, F2, F3 and F4) in the
469 investigated soils with the superimposition of the confidence ellipses (for $\alpha = 0.05$) relative to (a)
470 lava ages (1891-1893, 1906, 1937, 1944), (b) plant covers (shrub or tree) and (c) traffic flows (less
471 intense or intense).

472 Fig. 2 – Percentage contributes of elements in exchangeable/water-soluble (F1, oblique lines),
473 reducible (F2, dots), oxidasable (F3, grey) and residual (F4, grid) fractions of the soils collected
474 along Ercolano and Matrone roads.

475 Fig. 3 – Percentage contributes of elements in exchangeable/water-soluble (F1, oblique lines),
476 reducible (F2, dots), oxidasable (F3, grey) and residual (F4, grid) fractions of the soils collected at
477 high and low altitudes.

Table 1
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Table 1. Description and characteristics of the investigated sites inside the Vesuvius National Park.

Site	Geographical coordinates	Age of the pedogenetic substrate	Altitude (m a.s.l.)	Distance from the road (m)	Vegetation cover	Litter layer (cm)
EL_1	40°49'49.156''N 14°24'0.273''E	1944	596	0	Holm oak, broom, lichens, euphorbia, black locust, ivy	< 1 cm
EL_2	40°49'49.156''N 14°24'0.273''E	1944	596	30	Broom, euphorbia, lichens	0 cm
EH_1	40°49'51.935''N 14°25'28.606''E	1891-1893	900	0	Broom, euphorbia	< 1 cm
EH_2	40°49'51.935''N 14°25'28.606''E	1891-1893	900	30	Pine, broom	5-7 cm
ML_1	40°48'19.04''N 14°26'13.361''E	1906	570	0	Pine, broom, holm oak, mosses	5-7 cm
ML_2	40°48'19.04''N 14°26'13.361''E	1906	570	30	Pine, broom, holm oak, mosses	5-7 cm
MH_1	40°48'55.246''N 14°26'18.679''E	1937	820	0	Pine, broom, mosses, bramble	1-2 cm
MH_2	40°48'55.246''N 14°26'18.679''E	1937	820	30	Pine, broom, bramble, holm oak	4-5 cm

Table 2. Mean values (± s.e.) of pH, organic matter content and water content (OM and WC, expressed as % d.w.), total C, N and S concentrations (expressed as % d.w.) in soils collected inside the Vesuvius National Park along Ercolano (E) and Matrone (M) roads, at low (L) and high (H) altitudes, in proximity (1) and far (2) from the road.

	pH	OM	WC	C	N	S
EL_1	6.95	7.45 (± 0.09)	58.4 (± 0.40)	8.02 (± 0.53)	0.38 (± 0.02)	0.03 (± 0.003)
EL_2	6.98	10.4 (± 0.40)	28.9 (± 0.70)	3.57 (± 0.23)	0.23 (± 0.02)	0.02 (± 0.01)
EH_1	7.98	10.6 (± 0.13)	14.1 (± 0.32)	1.78 (± 0.17)	0.11 (± 0.01)	0.01 (± 0.01)
EH_2	6.46	15.4 (± 0.71)	38.7 (± 1.31)	5.95 (± 1.02)	0.30 (± 0.04)	0.05 (± 0.01)
ML_1	6.57	46.7 (± 0.92)	102 (± 1.45)	26.29 (± 0.75)	0.65 (± 0.04)	0.07 (± 0.01)
ML_2	6.67	40.8 (± 1.22)	102 (± 1.75)	22.13 (± 0.18)	0.67 (± 0.02)	0.06 (± 0.01)
MH_1	7.78	12.0 (± 0.75)	26.6 (± 0.26)	3.71 (± 0.48)	0.24 (± 0.03)	0.02 (± 0.01)
MH_2	7.36	13.6 (± 0.71)	23.0 (± 1.28)	5.72 (± 1.46)	0.34 (± 0.06)	0.05 (± 0.05)

Table 3
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Table 3. Mean values of pseudo-total concentrations of elements in soil collected inside the Vesuvius National Park along Ercolano (E) and Matrone (M) roads, at low (L) and high (H) altitudes, in proximity (1) and far (2) from the road.

	Al	K	Ca	Fe	Na	Mg	Si	P	Ti	Ba	Mn	Cu	V	Zn	Pb	La	As	Ni	B	W	Cr	Cd
											($\mu g\ g^{-1}$ d.w.)											
EL_1	24005	20052	15584	12586	5325	3792	2458	793	633	364	342	43.6	46.0	51.9	39.8	23.9	5.68	9.01	10.5	4.14	2.15	0.16
EL_2	25497	24006	13718	11785	4755	2931	2532	953	614	219	289	262	49.0	49.7	50.6	25.4	20.2	7.31	8.98	4.83	1.69	0.27
EH_1	12829	8591	14701	7529	2901	2814	1767	590	442	234	186	35.8	34.7	44.3	16.2	14.0	2.47	6.48	3.37	2.13	1.26	0.07
EH_2	17143	11113	10245	8100	2065	2677	1376	690	450	232	199	40.2	33.2	15.1	15.6	17.1	4.49	6.05	4.28	3.14	1.33	0.14
ML_1	20410	12985	13987	9486	2921	4117	1960	421	426	338	427	34.1	40.3	55.3	50.8	15.4	3.51	10.3	5.74	2.99	3.35	0.31
ML_2	18321	11268	13539	10968	2483	4817	1657	484	427	297	457	28.2	35.3	54.0	49.5	15.1	3.49	12.1	4.84	3.24	3.23	0.26
MH_1	15288	11478	10486	8449	3050	3179	2094	467	407	271	213	26.0	32.4	13.6	9.40	14.5	2.16	7.20	2.24	2.33	1.04	0.03
MH_2	11603	12324	11128	9987	2976	3303	1496	622	573	241	242	28.1	32.8	16.6	12.7	17.1	3.10	7.02	3.16	2.62	0.78	0.06

Table 4
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Table 4. Contamination factors (CFs) for the soils collected inside the Vesuvius National Park along Ercolano (E) and Matrone (M) roads, at low (L) and high (H) altitudes. The values higher than 1 are reported in bold.

	Roads		Altitudes	
	E	**M**	**H**	**L**
Al	0.97	0.80	0.70	**1.08**
As	**1.58**	0.59	0.59	**1.58**
B	**1.01**	0.59	0.48	**1.11**
Ba	1.00	**1.10**	0.93	**1.16**
Ca	**1.14**	**1.03**	0.98	**1.20**
Cd	**3.15**	**3.30**	**1.53**	**4.92**
Cr	0.89	**1.15**	0.61	**1.43**
Cu	**3.47**	**1.06**	**1.18**	**3.35**
Fe	0.94	0.91	0.80	**1.05**
K	**1.07**	0.81	0.73	**1.15**
La	1.00	0.78	0.78	1.00
Mg	0.84	**1.06**	0.82	**1.07**
Mn	0.92	**1.22**	0.77	**1.38**
Na	**1.28**	0.96	0.94	**1.29**
Ni	0.95	**1.20**	0.88	**1.27**
P	**1.10**	0.72	0.86	0.96
Pb	**1.77**	**1.77**	0.78	**2.76**
Si	**1.64**	**1.46**	**1.36**	**1.74**
Ti	0.95	0.81	0.83	0.93
V	0.98	0.85	0.80	**1.03**
W	**1.12**	0.88	0.80	**1.19**
Zn	**2.02**	**1.75**	**1.13**	**2.65**

Table 5. P values of the t-test performed on the concentrations of the elements in F1, F2 and F3 in soils collected inside the Vesuvius National Park to evaluate differences between roads (Ercolano *vs*. Matrone) or altitudes (high *vs*. low).

	Ercolano *vs*. Matrone			**high *vs*. low**		
	F1	**F2**	**F3**	**F1**	**F2**	**F3**
Cd	n.s.	0.05	n.s.	n.s.	0.02	n.s.
Cr	n.s.	n.s.	n.s.	n.s.	n.s.	0.05
La	n.s.	n.s.	0.009	n.s.	n.s.	0.007
Mg	n.s.	n.s.	0.04	n.s.	n.s.	0.04
Mn	0.05	n.s.	n.s.	0.04	n.s.	n.s.
Na	0.009	0.02	n.s.	0.01	0.02	n.s.
Ni	n.s.	n.s.	n.s.	0.05	n.s.	n.s.
Ni	n.s.	0.04	n.s.	n.s.	0.05	n.s.
P	n.s.	n.s.	0.05	n.s.	n.s.	n.s.
Pb	n.s.	n.s.	n.s.	n.s.	n.s.	0.03
Si	n.s.	0.005	n.s.	n.s.	0.003	n.s.
Ti	n.s.	0.05	0.05	n.s.	n.s.	0.02
Zn	n.s.	0.006	n.s.	n.s.	0.002	0.03

n.s. = not significant ($p > 0.05$)

Figure 1a

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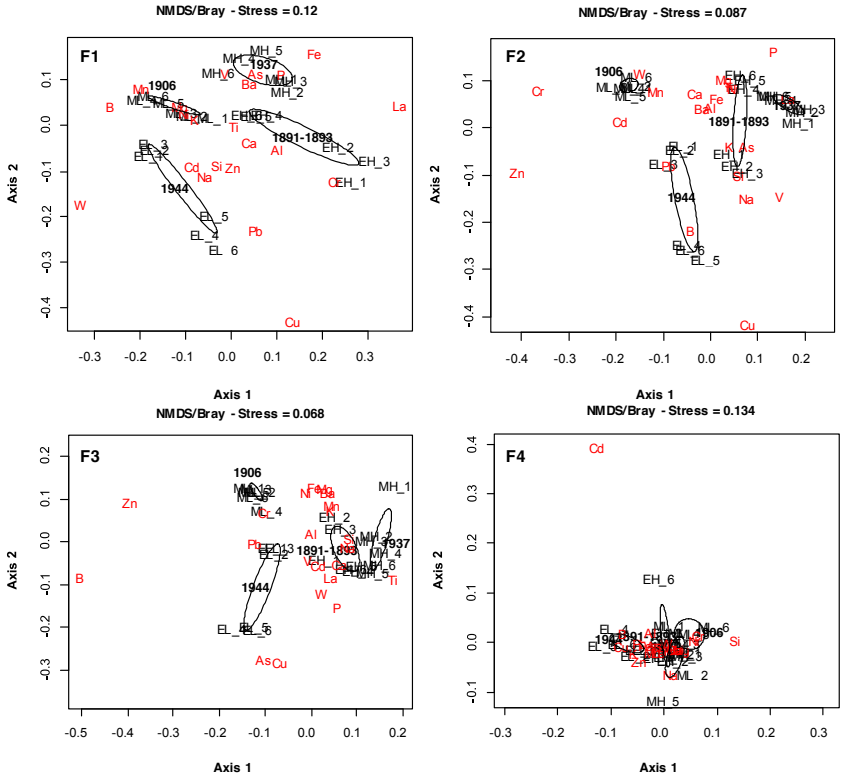

Fig. 1a

Figure 1b

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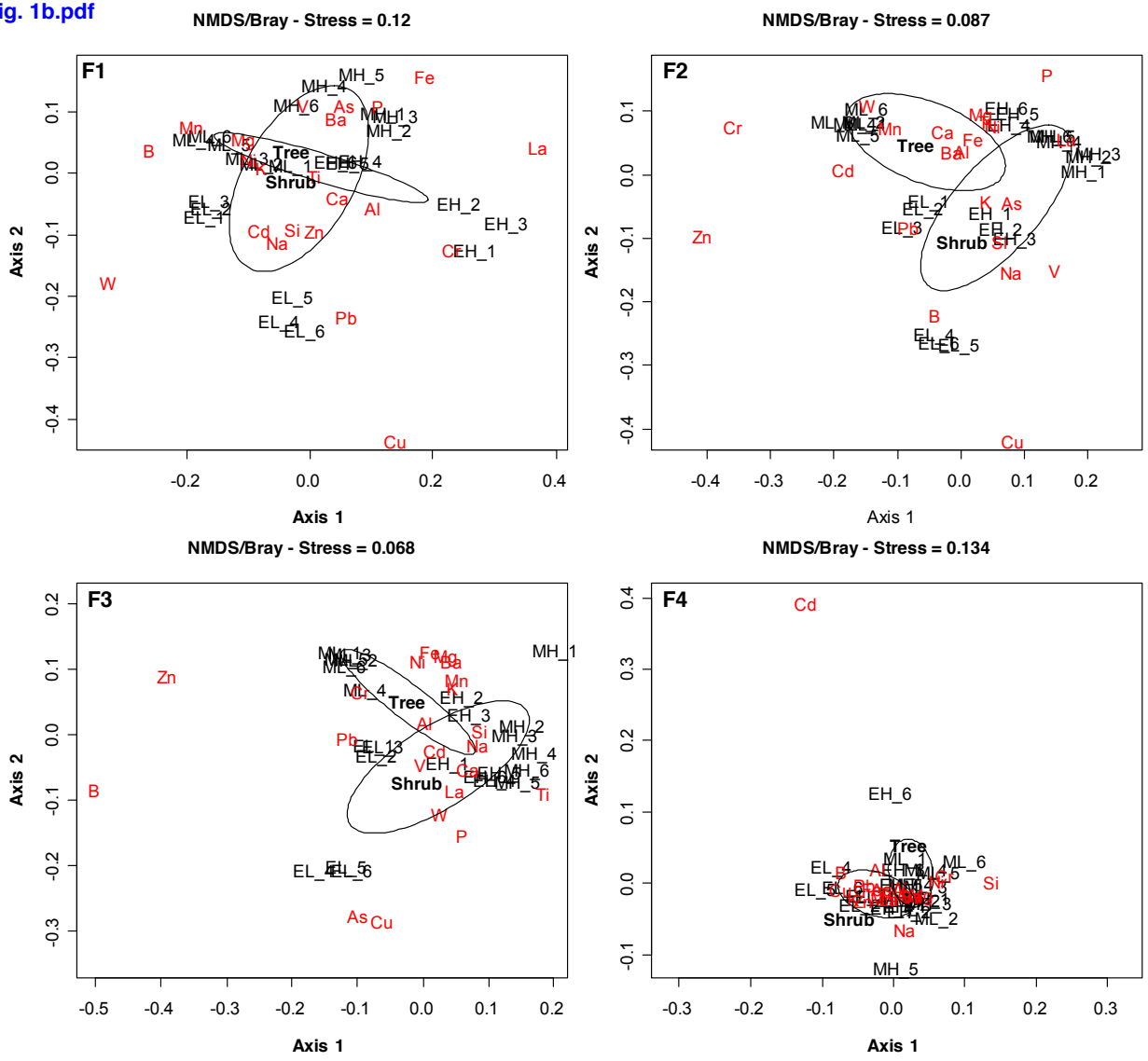

Fig. 1b

Figure 1c

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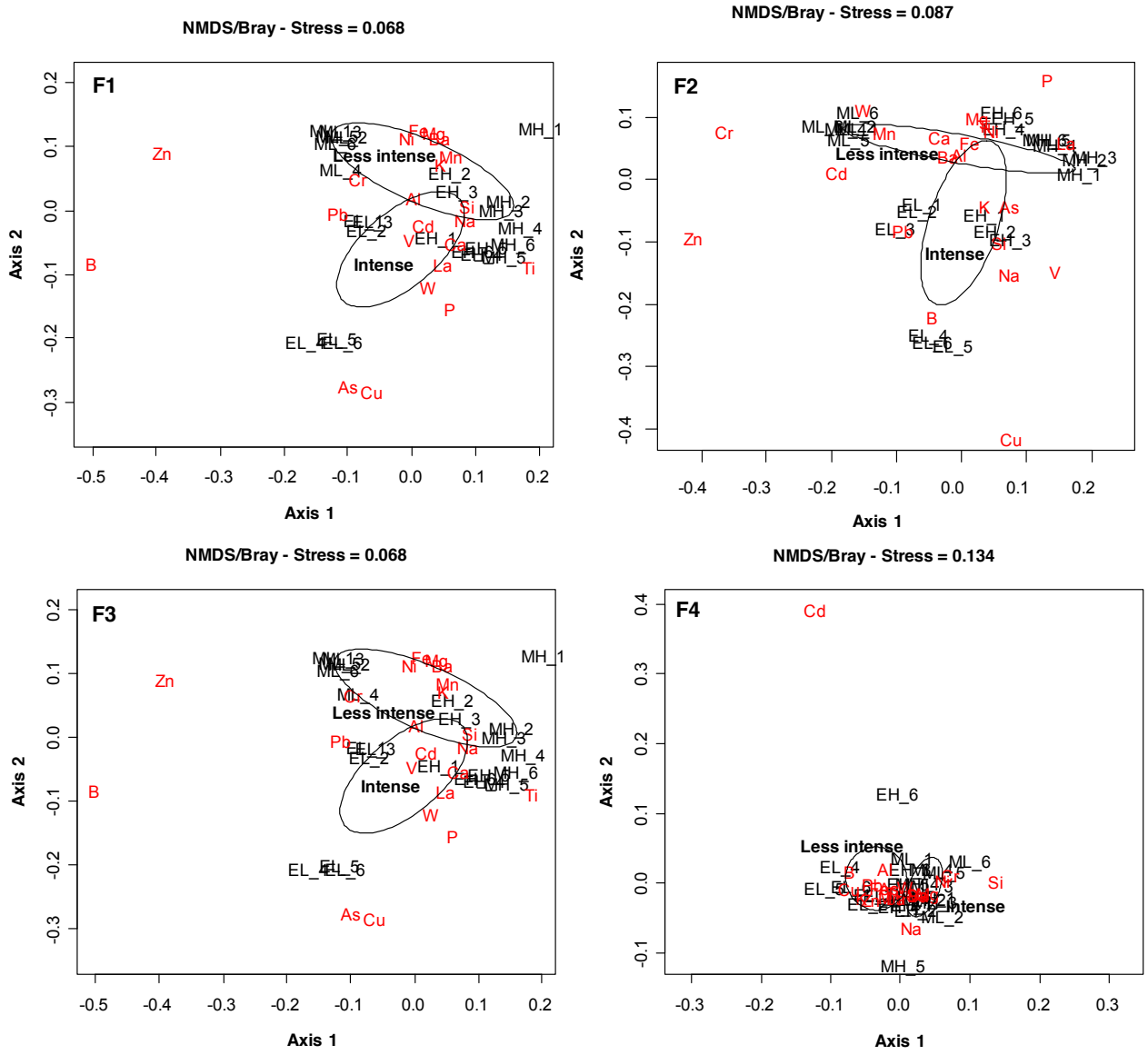

Fig. 1c

Figure 2
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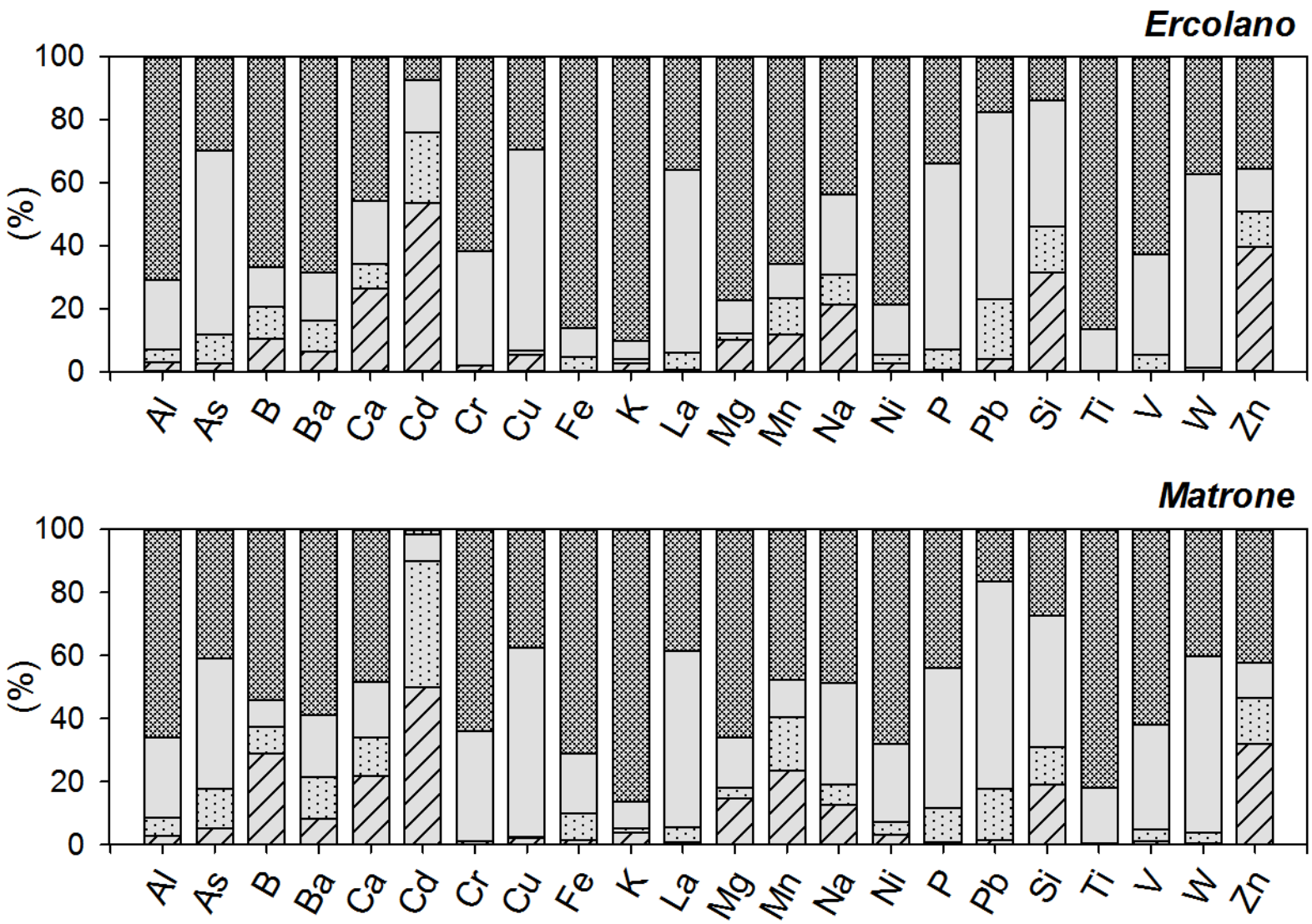

Figure 3
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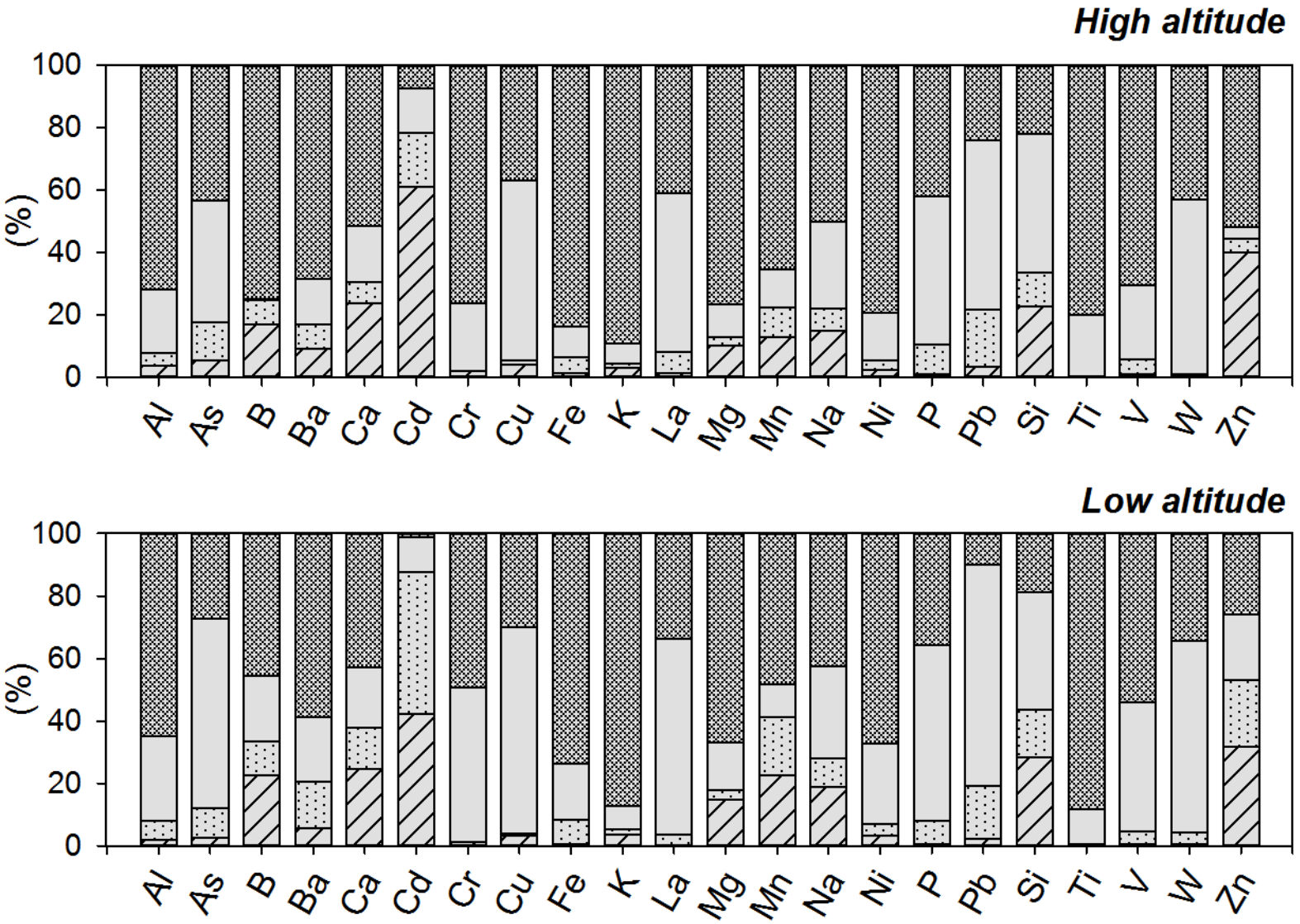

Supplementary material - Element concentrations (μg g^{-1} d.w.) in single fractions (F1, F2, F3 and F4) of BRC sequential extraction for investigated soils.

Fraction 1

	EL_1	EL_2	EH_1	EH_2	ML_1	ML_2	MH_1	MH_2
Al	390	1114	504	361	245	169	713	461
As	0.07	0.07	0.1	0.22	0.16	0.13	0.16	0.17
B	1.91	1.13	0.07	0.39	1.21	1.83	0.63	0.91
Ba	21.8	9.89	18.7	18.9	20.5	17.5	29.4	25.6
Ca	4133	3025	5854	1762	3540	3364	2093	1972
Cd	0.06	0.17	0.04	0.07	0.12	0.08	0.02	0.04
Cr	0	0.04	0.07	0.01	0.05	0.01	0.02	0
Cu	0.57	23.6	3.2	1.12	0.47	0.51	0.93	0.37
Fe	24.5	44.4	67.9	38.4	63.7	28.7	289	139
K	713	499	252	306	558	484	451	339
La	0.03	0.08	0.33	0.11	0.07	0.01	0.23	0.2
Mg	567	187	276	265	786	866	366	323
Mn	47.8	34.9	15.1	27.6	146	137	33.4	33.3
Na	1349	1534	333	333	240	236	567	407
Ni	0.22	0.28	0.09	0.2	0.42	0.33	0.23	0.14
P	1.31	1.94	4.16	10.6	4.08	4.14	3.97	5.26
Pb	0.52	2.66	1.27	0.34	0.56	0.45	0.17	0.23
Si	1171	1126	330	222	202	168	625	394
Ti	1.46	1.17	1.71	0.66	1.65	1.38	1.23	0.6
V	0.07	0.38	0.04	0.15	0.32	0.19	0.59	0.36
W	0	0.06	0	0	0.02	0.01	0	0.01
Zn	12.9	19.5	22.4	6.75	18.2	16.4	5.09	4.62

	Fraction 2							
	EL_1	**EL_2**	**EH_1**	**EH_2**	**ML_1**	**ML_2**	**MH_1**	**MH_2**
Al	1439	778	494	705	1450	1350	576	572
As	0.62	0.60	0.33	0.44	0.38	0.44	0.30	0.38
B	0.90	1.36	0.47	0.13	0.47	0.58	0.30	0.03
Ba	53.5	20.3	18.3	17.7	57.9	56.1	24.6	16.1
Ca	1782	666	784	965	2310	2614	644	729
Cd	0.07	0.06	0.01	0.02	0.17	0.15	0.00	0.02
Cr	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
Cu	0.18	3.07	1.21	0.17	0.13	0.16	0.19	0.14
Fe	644	405	356	358	1278	1043	593	403
K	310	214	155	110	236	213	170	101
La	0.54	0.42	1.33	1.33	0.70	0.72	0.87	0.71
Mg	87.4	35.0	80.4	50.5	198	206	111	62.7
Mn	44.8	24.7	24.0	21.5	96.5	141	18.1	15.3
Na	626	590	218	134	173	161	229	199
Ni	0.26	0.16	0.22	0.12	0.60	0.49	0.32	0.15
P	23.8	9.98	50.1	90.8	48.6	70.9	34.4	56.4
Pb	4.80	9.15	4.45	2.88	8.98	9.87	1.19	1.84
Si	495	426	211	130	244	203	226	169
Ti	0.97	0.51	0.66	0.58	2.01	1.79	0.98	0.92
V	1.00	3.98	2.42	0.98	1.11	0.92	2.05	1.20
W	0.05	0.04	0.00	0.06	0.27	0.11	0.02	0.02
Zn	9.53	3.94	6.89	0.25	15.5	16.5	0.00	0.00

Fraction 3

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

Fraction 4

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

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

Fraction 1

	EL_1	EL_2	EH_1	EH_2	ML_1	ML_2	MH_1	MH_2
Al	390	1114	504	361	245	169	713	461
As	0.07	0.07	0.1	0.22	0.16	0.13	0.16	0.17
B	1.91	1.13	0.07	0.39	1.21	1.83	0.63	0.91
Ba	21.8	9.89	18.7	18.9	20.5	17.5	29.4	25.6
Ca	4133	3025	5854	1762	3540	3364	2093	1972
Cd	0.06	0.17	0.04	0.07	0.12	0.08	0.02	0.04
Cr	0	0.04	0.07	0.01	0.05	0.01	0.02	0
Cu	0.57	23.6	3.2	1.12	0.47	0.51	0.93	0.37
Fe	24.5	44.4	67.9	38.4	63.7	28.7	289	139
K	713	499	252	306	558	484	451	339
La	0.03	0.08	0.33	0.11	0.07	0.01	0.23	0.2
Mg	567	187	276	265	786	866	366	323
Mn	47.8	34.9	15.1	27.6	146	137	33.4	33.3
Na	1349	1534	333	333	240	236	567	407
Ni	0.22	0.28	0.09	0.2	0.42	0.33	0.23	0.14
P	1.31	1.94	4.16	10.6	4.08	4.14	3.97	5.26
Pb	0.52	2.66	1.27	0.34	0.56	0.45	0.17	0.23
Si	1171	1126	330	222	202	168	625	394
Ti	1.46	1.17	1.71	0.66	1.65	1.38	1.23	0.6
V	0.07	0.38	0.04	0.15	0.32	0.19	0.59	0.36
W	0	0.06	0	0	0.02	0.01	0	0.01
Zn	12.9	19.5	22.4	6.75	18.2	16.4	5.09	4.62

	Fraction 2							
	EL_1	**EL_2**	**EH_1**	**EH_2**	**ML_1**	**ML_2**	**MH_1**	**MH_2**
Al	1439	778	494	705	1450	1350	576	572
As	0.62	0.60	0.33	0.44	0.38	0.44	0.30	0.38
B	0.90	1.36	0.47	0.13	0.47	0.58	0.30	0.03
Ba	53.5	20.3	18.3	17.7	57.9	56.1	24.6	16.1
Ca	1782	666	784	965	2310	2614	644	729
Cd	0.07	0.06	0.01	0.02	0.17	0.15	0.00	0.02
Cr	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
Cu	0.18	3.07	1.21	0.17	0.13	0.16	0.19	0.14
Fe	644	405	356	358	1278	1043	593	403
K	310	214	155	110	236	213	170	101
La	0.54	0.42	1.33	1.33	0.70	0.72	0.87	0.71
Mg	87.4	35.0	80.4	50.5	198	206	111	62.7
Mn	44.8	24.7	24.0	21.5	96.5	141	18.1	15.3
Na	626	590	218	134	173	161	229	199
Ni	0.26	0.16	0.22	0.12	0.60	0.49	0.32	0.15
P	23.8	9.98	50.1	90.8	48.6	70.9	34.4	56.4
Pb	4.80	9.15	4.45	2.88	8.98	9.87	1.19	1.84
Si	495	426	211	130	244	203	226	169
Ti	0.97	0.51	0.66	0.58	2.01	1.79	0.98	0.92
V	1.00	3.98	2.42	0.98	1.11	0.92	2.05	1.20
W	0.05	0.04	0.00	0.06	0.27	0.11	0.02	0.02
Zn	9.53	3.94	6.89	0.25	15.5	16.5	0.00	0.00

Fraction 3

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

Fraction 4

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

CHAPTER 4

GENERAL CONCLUSIONS

Different land uses alter soil characteristics that, in turns, cause dysfunctions of ecosystem services. Therefore, soil quality evaluation and monitoring need in order to provide useful tools to decision-makers in management plans.

The main results of the research focused on quarry recover through a single application of the investigated organic amendments (compost and a mixture of compost and poultry manure) highlighted a progressive improvement of the substrate properties. In fact, the microbial biomass increased, metal availabilities and microbial stress conditions decreased. Besides, after ten years from the application, the ecotoxicity of the substrates was low. The mixture of compost and poultry manure rather than only compost appeared the most suitable amendment.

In the agricultural soils inside the urban fabric and in the soils of the Vesuvius National Park, Cd availability was particularly high, and Cu and Pb can be considered contaminants as their total concentrations were similar to the threshold values of the Italian law. Nevertheless, soil microbial biomass appeared tolerant as they were scarcely affected by their concentrations, the overall conditions were unstressed for microorganisms and the ecotoxicity was negligible.

The study of the soils inside the Vesuvius National Park showed that only four indicators, selected from twenty-five parameters, appeared enough for decision-makers to monitor soil quality in management plans. The four selected indicators were total carbon content, total Ni concentration, metabolic quotient and root elongation of *S. saccharatum* L. that belong at soil chemical, biological and ecotoxicological parameters. The innovative aspect of this minimum data set is the presence of an ecotoxicological parameter that commonly are not considered in soil quality evaluation.

Future perspectives could be focused on the effects of soil element fractionation on the biological parameters in terms of microbial biomass, diversity and activity. In addition, as soil quality is soil- and site- specific, further investigations in different land uses, such as urban, agricultural and industrial environments, could be performed to select minimum data sets to monitor soil quality.

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