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The Ecoremed protocol for an integrated agronomic approach to characterization and remediation of contaminated soils

Guest editors

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The Ecoremed protocol for an integrated agronomic approach to characterization and remediation of contaminated soils

Guest editors

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Definition of a site as contaminated: Problems related to agricultural soils

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Abstract

For defining a soil as contaminated and to be remediated, it is necessary to make a risk analysis for assessing the risks for human health and environment and the exposure pathways to contaminants. The tools currently available, consider only the direct risks for people who frequent a residential or commercial/industrial site, but the risks related to agricultural soils are not laid down in Italian legislation. In this last case, the risks for human health can be defined as indirect since they are due to the uptake of contaminants by the food crops and to the consequent dietary exposure of population. Therefore, the risks of contamination of food chain are represented only by the bioavailable fraction of potentially toxic elements (PTEs) that can be absorbed by crops, rather than by the total content of PTEs into the soil that often is not mobile. Once environmental characterization and risk analysis allow classification of an agricultural site as contaminated, then its remediation must be undertaken, but it must aim to progressively reduce the bioavailable portion of PTEs. In this regard, the use of environmentally friendly techniques based on the use of natural resources (*e.g.* vegetation and soil microflora) resulted the most suitable both for the reduced costs and for the positive environmental impact. Indeed bio and phytoremediation allow protecting soil and its ecosystem services. The main steps of ecocompatible remediation of contaminated soils carried out in the Ecoremed projects are reported in this volume from the detailed geophysical and geochemical characterization, and the assessment of ecological and dietary risks, to the agriculture-based techniques for remediation and to the evaluation of the impact on ecosystem services of such soils.

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Key words: Soil ecosystem services; bioavailability; direct and indirect risk assessment; ecocompatible remediation.

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Legislative framework

Soil contamination is defined as a process, which is divided into two phases. The first phase involves comparison between the values found in a site and those reported in Table 1 of Annex 5 Part IV of the Italian Environmental Text (Law Decree 152, 2006) which defines the Contamination Threshold Concentration (CSC) in relation to soil use. In the case of public green areas and sites for residential use (column A), the CSCs are lower than for commercial or industrial sites (column B) because it is considered that the sites in question can be frequented for longer hours. No CSC values are provided for agricultural soils. In the event of exceeding the CSC values, the background values (BVs), whether natural or anthropic, must also be considered. Should the values of a particular site be higher than the CSC but lower than the BV, the site is considered NOT contaminated.

In this regard it is worth recalling some of the definitions in Art. 240 of the above-mentioned Law Decree (Table 1). As regards CSCs, from a comparative examination between the values present in the European regulations, albeit with some differences in the classifications of use, it is evident that the Italian legal values are much lower than in other countries (Table 2). The enormous variability of values gives rise to some doubts regarding their toxicological significance. It would appear that the various countries have set the thresholds on a statistical basis, *i.e.* considering the average level of contamination in their respective territories, and increasing the thresholds in the presence of widespread contamination. For example, the fact that a soil with 300 mg/kg of lead is considered potentially contaminated only in Finland and Italy, but not in other countries, has otherwise little sense. Similar examples could be made for all other metals and metalloids, but it is worth stressing the cases of beryllium and tin, because the exceedance of their respective CSCs has led to injudicious land confiscation in the region of Campania. The CSC of beryllium is extremely low in Italy (2 mg/kg), and in accordance with it, all the Italian soils would be classified as potentially contaminated (Figure 1).

The fact that beryllium (together with other minerals) is naturally contained in all volcanic rocks, but also that it is practically insoluble and therefore not absorbed by plants or leached into the groundwater, was not taken into account. Indeed, hardly any European country has set the threshold for beryllium and those which did so (the Czech Republic, the Netherlands and Slovakia) have set much higher thresholds (20-30 mg kg^{-1}).

As regards tin, Italy originally set a much lower threshold than other countries (1 mg kg^{-1} *vs* 900 in the Netherlands). Following a report to the Environment Ministry, within the context of the activities of the Working Group of the Ministerial Directive 23 December 2013 (Art. 1, subsection 1 of Legislative Decree 136, 2013 converted into Law 6, 2014), in which this value could only be a material error, it has been corrected (Art. 13, subsection 3b of Law 116, 2014), replacing the word *tin* with *organic stannic compounds*.

Returning to contaminants that might be hazardous to human health and to the environment, as recalled by Art. 240 of the Single

Environmental Text, in order for a site to be termed *contaminated* an analysis of health and environmental risk has to be performed. However, the procedure as well as the tools available (*e.g.* Software Risknet 2.0, 2017 or ISPRA, 2009) takes into account only the direct risks for those who frequent the sites and is therefore related to hours of exposure to contaminated soil particles that can reach the human target through inhalation, ingestion and skin contact. The risks for the environment linked to the leaching of contaminants into groundwater are also considered.

In the case of agricultural soils, in addition to direct risks, indirect risks for end users who could consume the products obtained on contaminated sites must also be taken into consideration.

Table 1. Art. 240 (definition) of the Legislative Decree 152/06.

Subsect.	Text
b	Contamination Threshold Concentration (CSC): the levels of contamination of environmental matrices that constitute values above which the characterization of the site and the site-specific risk analysis are required, as identified in Annex 5 to the fourth part of this decree. In the case in which the potentially contaminated site is located in an area affected by anthropic or natural phenomena that have led to the exceedance of one or more CSC, the latter are assumed equal to the existing baseline value for all the exceeded parameters
c	Risk threshold concentration (CSR): the levels of contamination of environmental matrices, to be determined case by case with the application of site-specific risk analysis procedure according to the principles illustrated in Annex 1 to the fourth part of this decree and on the basis of the findings of the characterization plan, which if exceeded requires safety measures and remediation. The concentration levels thereby defined constitute acceptable levels for the site
d	Potentially contaminated site: a site in which one or more values of the pollutant concentrations found in environmental matrices are higher than the CSC, prior to carrying out characterization and of site-specific health and environmental risk analysis which allow the contamination status to be determined on the basis of CSR
e	Contaminated site: a site in which the values of CSR are exceeded
f	Non-contaminated site: a site in which the contamination detected in the environmental matrix is lower than the CSC or, if greater, is nonetheless lower than the CSR determined by site-specific health and environmental risk analysis
n	Operative safety measures/securing the site: all the actions performed in a site which is still active aimed at ensuring an adequate level of safety for people and the environment, prior to further permanent safety measures or remediation being carried out on cessation of activity. This also includes contamination containment measures to be carried out until execution of the remediation or until permanent safety measures, in order to prevent the spread of contamination within the same matrix or between different matrixes. In such cases appropriate plans for monitoring and control must be prepared that allow the effectiveness of the adopted solutions to be verified
o	Permanent safety measures/securing the site: all the actions appropriate to permanently isolating the polluting sources with respect to the surrounding environmental matrixes and to ensuring a definitive, high level of safety for people and for the environment. In such cases plans must be provided for monitoring and control and limitations in use with respect to technical forecasts
p	Remediation: all the measures appropriate to eliminating the sources of pollution and the pollutants or to reducing their concentrations in the soil, in the subsoil and in the groundwater to a level equal to or lower than the CSR values
q	Environmental restoration: the actions of environmental and landscaping restoration, also complementary to remediation or permanent safety measures, that allow the site to be restored to the effective and final usability for intended use in compliance with technical specifications

Table 2. Screening values (in Italy, CSC) to define the potentially unacceptable risk for sites for residential use. From Carlon, 2007.

PTEs	European countries										
	AUT	BE(F)	BE(V)	CZE	DNK	FIN	ITA	NLD	POL	SVC	UK
As	50	110	300	70	20	50	20	55	22.5	50	20
Be				20			2	30		30	
Cd	10	6	30	20	5	10	2	12	5.5	20	2
Co				300		100	20	240	45	300	
Cr	250		520	500	1000	200	150	380	170	800	130
Cu	600	400	290	600	1000	150	120	190	100	500	
Hg	10	15	56	10	3	2	1	10	4	10	8
Pb	500	700	700	300	400	200	100	530	150	600	450
Ni	140	470	300	250	30	100	120	210	75	500	
Sb	5			40		10	10	15			
Se							3	100		20	35
Sn				300			1	900	40	300	
Tl	10						1	15			
V				450		150	90	250		500	
Zn		1000	710	2500	1000	250	150	720	325	3000	

AUT, Austria; BEF, Belgium Flanders; BEV, Belgium Walloon; CZE, Czech Republic; DNK, Denmark; FIN, Finland; ITA, Italy; NLD, Netherlands; POL, Poland; SVC, Slovakia; UK, United Kingdom.

However, as mentioned above, the Italian Environmental Text does not provide CSCs for agricultural soils, for which there is no specific procedure. Art. 241 of the Single Environmental Text makes the following provision: a *regulation on remediation, environmental restoration and emergency operative and permanent safety measures, of the areas for agricultural production and husbandry, was adopted by decree of the Minister for the environment and land and sea protection in concert with the Ministers for Industry, Health and Agriculture and Forestry policy*.

The above regulation, albeit drawn up by a technical committee, which included the presence of the working group of the Ministerial Directive of December 23, 2013 (Environmental

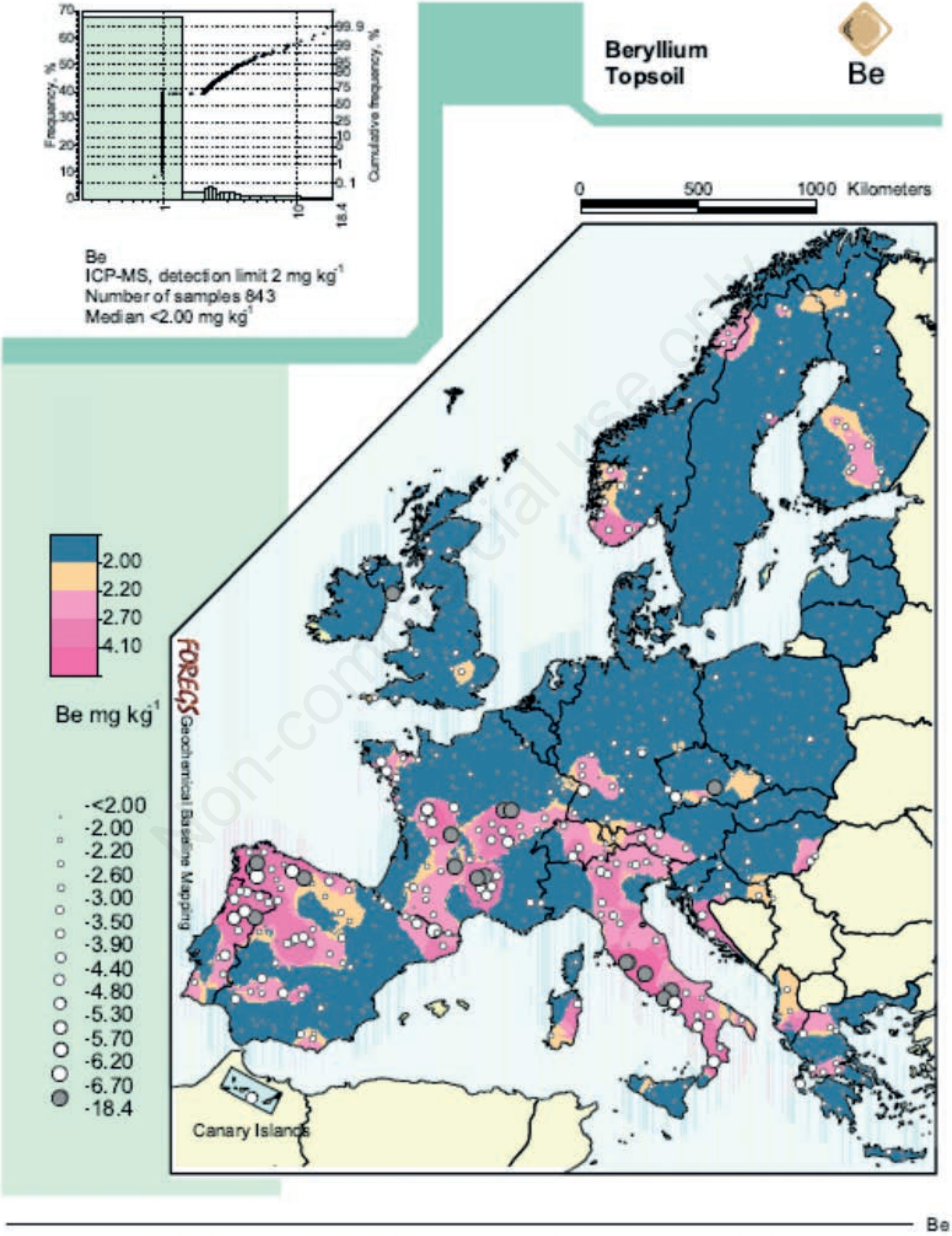

Figure 1. Beryllium background values in Europe (From Foregs, 2005). Geochemical Atlas of Europe, copyright © 2005 EuroGeo Surveys/the Geological Survey of Finland.

Ministry, 2014), has not yet been adopted. In summary, the regulation, which is applied to soil surfaces to be used for agricultural crops, grazing and husbandry, defines: i) the values or range of screening values (Contamination Threshold Concentrations, CSCs) above which site-specific risk assessments must be carried out to ascertain the mobility of contaminants, their transfer to plants, bioavailability and bioaccumulation; ii) the procedure to apply for the execution of site-specific risk assessments associated to pollutant concentrations above the CSC in soils for agricultural production and husbandry - Health Risk Assessment (ISS, 2013); iii) the intervention modes (safety measures, remediation, environmental restoration) for the areas in which unacceptable risk has been detected in order to restore soil functions for agricultural production and husbandry.

The approach of Ecoremed protocol

Environmental characterization

Currently, in the absence of specific regulations, in the LIFE ECOREMED project (Fagnano, 2017) we relied on the provisions in the draft of the regulation mentioned above, and we validated chemical tests on contaminant bioavailability (Rocco *et al.*, 2018) and biological tests on contaminant absorption and accumulation in vulnerable crops (Duri *et al.,* 2018) in order to obtain information about the risks of contaminant accumulation in the edible parts of agricultural products that therefore constitute a threat to the health of consumers.

Furthermore, the analysis of Italian environmental legislation highlighted other weaknesses in relation to setting up the sampling scheme and the depth of the soil layer to be sampled for environmental characterization. In Decree 152 (2006) there are no mandatory rules regarding rational sampling schemes or sampling layers. Normally reference is made to previous laws (Annex 2 to Ministerial Decree 471, 1999), which establish that one sample per hectare be taken and a composite sample of the 0-1 m layer be collected. Clearly, a soil sample 10 cm in diameter cannot be representative of an area of 10,000 m^2 and pollution levels, which are often higher in the top centimetres of soils, could be drastically underestimated if they were diluted in a 1 m layer. For this reason, in the Ecoremed project, several activities were carried out to set up a strategy to attain more representative and more realistic information on the environmental quality of the study areas. In initial research carried out on the Giugliano site (Rocco *et al.,* 2016), discrete and composite samples were compared with two sampling grids (3x3 m and 10x10 m). Our findings demonstrated that making a composite sample causes a dilution effect that leads to underestimating pollution levels, at the risk of overlooking several pollution hotspots: values from composite samples (*i.e.* 276 ppm) were always lower than the discrete samples that make them up (*i.e.* 1707 ppm). In other research conducted at Trentola-Ducenta (Capolupo *et al.,* 2015), we sought to verify whether high-resolution aerial photographs taken by drones could allow analysis of the micromorphology of a lowland field, thus highlighting microbasins in which the accumulation of sediments, and hence of contaminants, could have a higher probability of occurring. Identification of such points is required to steer the subsequent scheme for direct samplings of soil. This method allowed identification of five areas (microbasins) in which copper concentrations were higher.

In a third study made at Giugliano, we requested collaboration from a research group working on another LIFE project (LIFE08 ENV/IT/000408 - SoilConsWeb) which proposed a preliminary series of geophysical measurements to identify anomalies which would then be analysed with direct excavation of pits or soil profiles (Langella *et al.,* 2018).

Opening a soil profile also allows the stratigraphy and possible presence of contaminated materials to be analysed, thus making it possible to choose the layers most representing the form of contamination. For example, from the data gained from *Trench 5* it is evident that the most contaminated layers are only those between 50 and 80 cm: in this case the average sample of the 0-1 m layer would have underestimated the level of contamination.

Furthermore, the reliability of rapid low-cost methods was assessed to design a map of contamination levels so as to guide a subsequent rational sampling scheme of the whole site area (6 ha in this case). With a measurement campaign conducted with X-ray fluorescence (XRF), it was possible to identify, in a few days' work, the areas severely contaminated by chromium, on which then to perform direct sampling for analyses with the official methods.

This approach seemed realistically the most feasible, given costs, times (both extremely low), but mainly the quality and representativeness of the data obtained. The methodological details in question will be presented in Langella *et al.* (2018).

Table 3. Conditions required by current regulations and characteristics of the biomass produced in phytorestoration plants.

Conditions required by Art. 148b, Law Decree 152/06	Characteristics of phytoremediation
The substance or the object originates from a production process of which it constitutes an integral part, and whose primary purpose is not production of such substance or object	The service is soil phytorestoration, and hence it is impossible not to produce the vegetables that are obtained, being an integral and essential part of the process
It is certain that the substance or the object will be used, in the course of the same or in a subsequent production process, by the producer or by a third party	The *plant* material will be used in the same production process (*e.g.* burial of the spontaneous flora, collection of the Brassicaceae and other species intentionally used, cutting and eventual chipping of the wood components) for further agronomic applications in situ according to good agronomic techniques, or *ex situ* in biomass treatment plants
The substance or object can be used directly without any further processing other than normal industrial practice	
Further use is lawful, *i.e.* the substance or object fulfils, for the specific use, all requirements concerning such products and the protection of health and the environment, and will not lead to a negative impact on the environment or human health	The use of biomass for soil fertilization has been a normal agricultural practice for millennia and as such is absolutely legal, as is also legal the use of plant biomass, both for industrial applications, such as the production of electricity or wood, and for those civil purposes (*e.g.* firewood for residential heating)

Bio and phytoremediation

Once environmental characterization and risk analysis allow classification of an agricultural site as contaminated, then its remediation must be undertaken. Such remediation must aim to progressively reduce the bioavailable portion of PTEs (potentially toxic elements, so-called heavy metals).

In this regard, the use of environmentally friendly techniques provided by the Ecoremed protocol (Fiorentino *et al.,* 2018; Ventorino *et al.,* 2018) for the restoration of contaminated farmland in the Agro Aversano, is also mentioned in Law 6, 2014 (Art. 2, subsection 4) which, concerning the Commission which must coordinate the restoration measures of the so-called Land of Fires, reads: *The Commission must also provide, within the measures for remediation and ecosystem rebalancing, for the use of natural regeneration and agroecological systems, through plants with phyto-cleansing properties in accordance with current legislation*.

Therefore, the Ecoremed protocol based on the use of natural resources (vegetation and soil microflora) can be applied, according to the Italian legislation for the operations of remediation, safety measures and environmental restoration, according to the site characteristics and the intervention objectives.

The ecosystem services provided by phytoremediation also extend to reduction of percolate and contaminant leaching, thus also protecting groundwater from contamination (Palladino *et al.,* 2018).

The different fields of use also affect the quality of the biomass of the species used, and in accordance with current legislation, they are to be considered a byproduct of phytoremediation. Indeed, the characteristic of protocols like that of the ECOREMED project is the application of phytorestoration with plant species that have intrinsic qualitative and commercial properties that make the application advantageous, not only in purely scientific terms but also from the economic standpoint: the use of materials produced in the phytoremediation cycle can indeed contribute, albeit in part, to the plant management costs required to implement it. Therefore, agri-food production aside, it is evident that the biomass obtained has economic and commercial properties which, while not constituting the scientific/technical objective of the programme, are commercially exploitable in other production cycles, according to industrial practices. This feature allows that all the produced plant material has all the intrinsic properties of a byproduct, as defined in Decree 152 (2006), Art. 184b, hence completely excluding the nature of *waste*.

For purely explanatory purposes, in Table 3 we report the conditions required by legislation (see Law Decree 152, 2006 and subsequent amendments, Art. 184b c.1) for the classification of a byproduct. What remains excluded is the use of biomass derived from phytoremediation in anaerobic digestion plants. In this case the agricultural use of the digestate is forbidden: it must be subjected to drying processes to reduce the risk of dispersion of hazardous substances possibly contained in it, and then used for energy recovery operations, among which incineration is preferred, as provided for by DIM 5046 of 25/02/2016 (GU no. 90 of 18/04/2016 - S.O. no. 9).

That said, research conducted in the framework of the Ecoremed project (Giudicianni *et al.,* 2017a; 2017b) allowed us to determine that pyrolysis, concentrating potentially toxic elements (PTEs) in the solid product (so-called Biochar), is the best process with the lowest environmental impact for treating biomass produced in phytoremediation plants.

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Geography of soil contamination for characterization and precision remediation of potentially contaminated sites

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Abstract

One of the fundamental problems experienced when evaluations are required (*e.g.* for reclamation, phytoremediation, containment, *etc.*) on agricultural or industrial contaminated sites is the geospatial variability of pollutants. The general lack of ex-ante information on type, quantity, and location of potentially hazardous substances requires the use of proper investigation tools to identify their spatial distribution. In this work we focused on developing and applying an integrated approach based on the following steps: i) preliminary investigations (analysis of aerial photos from different periods of time); ii) indirect investigations (by geophysical and spectrometric methods such as ARP, DUAL-EM, Profiler, Gamma-ray and X-ray fluorescence); iii) direct investigations (pedological and micromorphological); iv) stochastic modelling and critical risk of chemical concentrations being exceeded. The investigated area is located in the region of Campania (southern Italy), close to the main regional volcanic districts (Phlegraean Fields and Somma-Vesuvius). Maps of the apparent electrical resistivity and conductivity, as well as that of the gamma-ray dose, were obtained for the site in question. On analysing the maps, a good correspondence was found among the outputs obtained from measurements at depths between 0 and 2 m, with a spatial resolution that is slightly better for ARP. The procedure of Normalized Sum of Relative Differences on Geophysical Covariates (NSRDGC) was applied to the 15 maps in order to obtain an integrated variability map of all the surveyed anomalies using the proximal sensors. The resulting map identified the most homogeneous areas where five soil profiles and eight trenches were dug. Field morphological observations enabled three different types of anthropogenic materials enriched in Cr and Zn to be identified. According to their morphology in depth, these materials were not emplaced in a single event. Earth movement and landfilling were carried out at several times in the same area, where a mix of contaminated materials and natural soil is found in the first metre, whereas organic materials are visible separately, probably as a consequence of separate excavations and landfill. Soil micromorphology observations evidenced the presence of Cr-enriched organic materials and secondary crystalline gypsum minerals as well as organic matter coatings inside pores. Such observations suggest the possible fate of some contaminants, highlighting soil mineral and organic associations, as well as the presence of pedofeatures indicating downward movement of colloidal materials.

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Introduction

Soil spatial variability is a key issue for land management both in agriculture (*e.g.* precision farming) (Corwin and Lesch, 2003; Mzuku *et al.,* 2005; Aggelopoulou *et al.,* 2010; 2011; Fulton *et al.,* 2011) and in other environmental contexts (*e.g.* contaminated sites) (da Silva *et al.,* 2017; Wang *et al.,* 2017; Shaheen and Iqbal, 2018). When contaminated sites are investigated a twofold spatial variability is experienced due to: i) natural spatial variability of soil properties; and ii) anthropogenic spatial variability due to the sources of the contamination. Moreover, the actual spatial distribution of anthropogenic soil contaminants is almost always unknown. This is the case of most industrial sites where the detailed history of contamination is often lost in time. This is due to two main factors: the loss of historic information of industrial land use for each area of a specific industrial site, and its modification over time (*e.g.* placing/removal of materials). This also holds for most illegal waste dumping, for which type/quantity and especially localization of contaminants are unknown.

Given the above considerations, it is evident that in both analysis and subsequent remediation of contaminated sites, this lack of ex-ante information on the geography of contamination (type/quantity/location) requires that proper investigation tools be adopted to achieve an understanding of the spatial distribution of contamination.

This specific contribution aims to focus on how to acquire such detailed knowledge of the spatial distribution of contamination despite the evidence that this is often neglected and ignored. The regulatory criteria to be adopted in Italy in the case of potential contamination refer to Law Decree 152/06 - Part IV- Title V, according to which a site must be considered potentially contaminated if element concentrations (even only for one element) exceed the established screening values (CSCs). In such cases, environmental characterization of the site has to be performed. Although the law considers the geospatial component of the hypothetical pollution source, its factual application requires a very broad sampling aggregation scheme where *soil surface* refers to a composite sample in the 0-1 m depth range, that could lead to dilute the contaminants, thereby underestimating their concentrations (Rocco *et al*., 2016). According to the law, there are no obligations either for the sampling method to be used (free or systematic) or for the number of sampling points. In many real applications sampling density often refers to the old Decree 471/99 (Annex 2), whereby establishing: i) areas <1 hectare requires at least five sampling points; ii) areas between 1 and 5 ha requires 5 to 15 sampling points; and iii) areas > 5-25 ha requires 15-60 sampling points. These long-established specifications have actually become a standard to which many of the characterization studies of Italian contaminated sites refer. Here we question whether such a small number of observations is really sufficient to define the spatial distribution (in terms of surfaces and depth) of soil contamination. We consider whether the entire site of interest is really contaminated or only parts of it - and also - the extent of this contamination at each detailed location. For the above reasons, here we elaborate a methodological integrated approach, combining many different techniques, aiming to produce a model of contaminant spatial distribution as close as possible to reality.

The approach that we developed is based on the following steps: i) preliminary investigations; ii) indirect investigations; iii) direct investigations; iv) stochastic modelling and critical risk of chemical concentrations exceeded. We considered that almost all geophysical methods are non-destructive, very sensitive and used for describing subsurface properties in engineering, geological, environmental and forensic problems, without digging. There are many methods that differ in the detection of soil physical properties (such as electrical resistivity/conductivity, magnetic susceptibility, velocity of P or S waves). Therefore, the choice of the best method is related to the purpose of prospecting and it is impossible to know in advance which method will perform best. Moreover, such investigations generally enable rapid acquisition of data and can help to transform point-based soil analysis into information on continuous spatial soil properties. Such approaches are already well established in several contexts requiring rapid non-destructive high-resolution soil investigations, such as viticultural zoning at farm scale (Bonfante *et al*., 2015; Tardaguila *et al*., 2018). For all the above reasons, a geospatial survey, using multi-frequency electromagnetic (EM) conductivity meters (such as Profiler EMP-400 and DUAL-EM), automatic resistivity profiling (ARP) and gamma-ray spectrometry as indirect methods, was carried out in order to acquire knowledge on the geospatial distribution of soil physical properties, like electrical resistivity/conductivity, to be used as proxy for understanding the location of buried waste. The maps obtained and related signal anomaly were processed (using geostatistical methods) and represented the basis for a direct survey focusing on the areas where the highest composite anomaly signals occur. A geophysical anomaly (*i.e.* an area where geophysical properties differ from those of the surrounding areas) is due to differences in soil properties and can thus represent a waste burial site in the surveyed area. Profiles and trenches were dug so as to identify: i) possible relationships between anomalies and waste occurrence; and ii) sampling and analyses of allochthonous/contaminated materials. The body of information thereby obtained enables the spatial nature of contamination to be ascertained as a prerequisite for optimal characterization and remediation of a contaminated site.

Materials and methods

Environmental setting

The investigated area is located at Giugliano in Campania (San Giuseppiello site), in the province of Naples, in Campania (southern Italy). The 60,000 m^2 site consists of farmland which, according to preliminary characterization, was potentially contaminated by Cr, Zn and heavy hydrocarbons (C>12) due to illegal burying of tannery sludge and waste. The field, previously used for fruit orchards and arable crops, is subjected to a phytoremediation programme (Fiorentino *et al.,* 2018).

Geologically, the site is located in the Piana Campana graben, to the North of the Phlegrean Fields and NW of the Somma-Vesuvius complex, which are the main active volcanoes of Campania. In this lowland environment a preferential accumulation of pyroclastic deposits is found (Di Vito *et al.*, 2013; Orsi *et al.*, 2004) due to the proximity to these eruptive centres. Therefore, the soils of the site surroundings are the result of a different extent of andosolization, which gave rise to formation of soils classified as Pachi-Vitric Andosols (di Gennaro *et al.,* 2002) and Hypereutric Cambisols (Aric, Humic, Loamic, Tephric) (Vingiani *et al.,* 2018a; 2018b). These soils are known from the literature for their excellent properties (Nanzyo, 2002; Shoji and Takahashi, 2003; Shoji *et al*., 1993) that give high chemical and physical fertility to ecosystems (Maeda *et al.,* 1977; Quantin, 1990), both in volcanic districts and in non-volcanic mountain landscapes (Mileti *et al.,* 2018; Vingiani *et al*., 2013; Vingiani *et al.,* 2014). In spite of these positive properties, Andosols and andic soils have an inner *fragility* to land degradation and pollution (Kabata-Pendias, 2001; Latrille *et al.,* 2003; Tanneberg *et al*., 2001; Vingiani *et al.,* 2015; Zhao *et al.,* 2006), which renders the investigated area highly vulnerable.

Aerial photo analysis and geophysical prospecting

As the first step, all the existing information (topographic and land use maps, aerial photos, *etc*.) on the site was collected in order to identify recent changes that might help identify anomalies or simple modifications of interest. The periods of interest were those preceding and following waste burial. We analysed the aerial photos obtained from the National Geoportal (WMS services) and Google Earth, considering five years, in the period from 1989 to the present. Then we proceeded with the geospatial surveys, applying geophysical methods. Geophysical modelling provides generalized and non-unique solutions to questions concerning the geometry of natural/anthropogenic soil horizons. The methodological approach applied involves the combined use of several indirect surveys to characterize the site, such as automatic resistivity profiling (ARP) and frequency domain electromagnetic induction (Profilers EMP400 and DUAL-EM 642 S). Among the geophysical tools applied in soil science, electrical methods are considered potentially useful to characterize soil. The ARP technique allows to measure the apparent electrical resistivity of soil. This property is related to the spatial and temporal variability of many other soil physical properties (such as structure, water content or fluid composition). Apparent soil electrical resistivity results from the *combination* of all the soil component resistances in the investigated volume. The ARP equipment was developed by Geocarta SA. It consists in a patented mobile multi-electrode system in which several electrodes (eight gear wheels) are automatically inserted into the soil and made to advance (by passive rotation on the soil) during field acquisition (speed <=8 m/s). The distances between the dipoles are 0.5, 1.0 and 1.7 m. In this way it is possible to survey simultaneously a vertical section at three depths. The ARP equipment was towed by a quad and the GPS position for all the measurements was recorded. In addition, electromagnetic induction (EMI) methods in the frequency domain were used to measure the electrical conductivity (the inverse of electrical resistivity) of the subsurface. Two instruments with different characteristics (DUAL-EM 642-S and Profiler EMP-400) were used and acquisition required just one day per technique.

The DUAL-EM 642S induction electromagnetometer (DUAL-EM Inc., Canada) was used with three pairs of sensors arranged in DUAL configuration, separated by 2, 4 and 6 m. Each pair of sensors is able to measure simultaneously at two different depths. Thus DUALEM-642S is able to simultaneously acquire a profile of apparent electrical resistivity (ρa) and apparent magnetic susceptibility (χa) at six different depths and can be configured in two modes: co-planar (HCP) and perpendicular (PRP).

These configurations, which correspond to different measurement depths, make the DUAL-EM-642S very suitable for analysis of both ρa and χa up to 9 m depth. Measurements were made according to parallel profiles with a distance of about 2-4 m.

The Profiler EMP-400 is a portable, digital multi-frequency electromagnetic induction sensor. The user can collect from one (1) to three (3) frequencies simultaneously. The system bandwidth extends from 1 kHz to 16 kHz in 1 kHz steps. The magnitude of the in-phase and quadrature components of the induced secondary field, as well as the apparent conductivity and magnetic susceptibility, are collected and stored for each reading along with a time stamp and GPS data.

Unlike conventional resistivity techniques, with electromagnetic induction sensors no ground contact was required. However, all the systems used allow almost continuous acquisitions to be carried out, with a spacing of about two metres between one row and the next. A single day acquisition was required for each technique. All the available information (maps) was processed in a GIS environment in order to define the areas characterized by anomalies of the signal, greater homogeneity and heterogeneity.

ARP and Profiler EMP-400 data were generally acquired through the parallel profile sampling scheme with 10 cm spacings along the transects, while interfiles between transects ranged between less than 1 m (ARP) to 5-6 m (Profiler).

Another indirect method used, not correlated with those described above, is gamma-ray spectrometry. The gamma-ray spectrometer is designed to measure natural and artificial radioisotopes in the ground. The instrument always measures the complete spectrum, from which it evaluates the cps values in ROIs and calculates the concentrations of elements K [%], eU [ppm] and eTh [ppm]. The natural dose rate (in nGy/h) is calculated from measured concentrations of K, eU and eTh according to IAEA recommendations. The measurements were performed as points and the position of the measured site has been set using external GPS. Using (geo)statistic interpolation it was possible to spatialize the point-based information of K [%], eU [ppm], eTh [ppm] and the natural dose rate (in nGy/h) as was done for resistivity data.

Positioning of soil trenches and profiles

The whole set of observations was crossed using a multivariate statistical approach in order to carry out a rational sampling campaign aimed at complete site characterization. Indeed, the indirect surveys (Profiler, DUAL-EM, ARP, and Gamma-ray) produced a total of 15 information layers as follows: Profiler electrical apparent conductivity at 15 KHz, 10 KHz and 5 KHz; DUAL-EM resistivity at depths 1.0 m, 2.0 m, 3.0 m, 3.2 m, 6.4 m and 9.5 m; ARP resistivity at depths 0.5 m, 1.0 m and 1.7 m; Gamma-ray K [%], eU [ppm] and eTh [ppm].

Each instrument produced a specific density and location of the signal points, for which the data were merged to report all the information on a regular grid at 5 m resolution (high resolution to offset the high density and possible anomalies measured by the instruments). In order to highlight and maximize the global anomaly given by the contribution of all the geophysical sensors, the Normalized Sum of Relative Differences on Geophysical Covariates (NSRDGC) was then calculated as follows:

$$NSRDGC_t = \sum_{i=1}^{15} \sum_{n=1}^{8} \frac{abs(V_t^i - V_n^i)}{V_t^i} \quad (1)$$

for each *t* target pixel, where V_t^i is the value of the i-th geophysical signal at *t* target pixel, V_n^i is the value of the i-th geophysical signal at n-th neighbour pixel, *n* accounts for the 8-adjacent pixels of the target pixel, and *i* accounts for the list of 15 geophysical signals.

The NSRDGC is calculated on the 1 m resolution grid according to the following steps: i) for each target pixel, a list of 15 values according to the number of sensors and frequencies (*i.e.* auxiliary covariates) is retrieved from the proximal sensing measurements (*e.g.* for signal number two we have V^2_n); ii) for each auxiliary covariate, the list of 8-adjacent pixels to the target pixel is produced and stored in the vector $V^2_{1\ to\ 8}$; iii) the $NSRDGC_t$ at target pixel *t* is calculated by summing the absolute differences of the target pixel with respect to the 8-adjacent pixels and to the values in the neighbourhood given by the 15 auxiliary covariates. By extending the calculation on the whole regular grid, the hotspot map was generated by aggregating all the collected geophysical signals. Relatively high pixel values are found where an important leap occurred between one point and the points around. This procedure was practical since the resulting map aided identification of the areas in which to dig profiles and trenches, according to the spatial distribution of major and/or minor variations in the data fused information. Eight trenches were located at the major geophysical contrasts in order to identify the spatial variability of the site while five profiles were located in the most homogeneous units. Trench size varied between 4 and 7 m in length, with a depth and width of about 2 m, while the profiles had a length of about 2 m, but with the same depth and width. One or two representative profiles were sampled per trench and a photographic report was also produced for a vertical and horizontal reconstruction of the trenches. For each profile morphological description (FAO, 2006) and undisturbed sampling for micromorphological analyses were also carried out, as well as bulk sampling for further chemical and physical analyses.

XRF and micromorphological analyses

A portable handheld XRF analyzer was used for rapid identification of the materials most contaminated by potentially toxic elements (PTEs) directly in the field in order to address the following soil sampling procedure for assessing PTE content and bioavailability (Rocco *et al.,* 2018). Twenty-one elements (As, Ca, Cd, Cr, Cu, Fe, K, Mn, Nb, Ni, Pb, Rb, Sn, Sr, Th, Ti, U, V, Zn, Zr and Y) were measured on smooth uniform surfaces, with complete contact between instrument and sample surface to minimize surface effects. Scanning was carried out with a Delta Professional (Olympus, DPO-4000) using a large window (8 mm^2). The instrument features a

Ta/Au X-ray tube operating at 15–40 kV with integrated large area silicon drift detector (165 eV). Innov-X software was used in Soil mode, which consists of three beams operating sequentially, with acquisition times of 30 seconds per beam. Then, the most significant materials, in terms of soil contamination, were selected and a total of 35 undisturbed samples were collected from soil trenches and profiles by means of Kubiena boxes (5x10x5 cm). Samples were impregnated with polyester crystic resin and large (10x5x0.003 cm) thin sections were produced using the FitzPatrick methodology (1984, 1993). Thin sections were analysed under optical microscope in plain (PPL) and crossed (XPL) polarized light. XRF analyses were also performed on soil thin sections using a small spot collimator (analysis area of 0.07 cm^2), to have a preliminary assessment of micro-pedofeatures contamination.

Results and discussion

Through the study of the aerial photos, we identified changes in land use (from orchard to arable) in different sites as well as the occurrence of traces of temporary roadways that could well have been caused by the transit of heavy vehicles. The photos in question are shown in Figure 1.

Nevertheless, it is self-evident that these signs of heavy vehicle transit are not helpful for understanding the distribution of contaminants in the area. Indeed, the pictures would be useful only if the illegal spillage was associated with the acquisition of simultaneous remotely sensed images (as in the case of drones). Needless to add, such data are very rare when dealing with illegal activities. Indeed,

Figure 1. Aerial photos of the study area from 1989 to the present day.

waste spill activities are generally carried out very quickly and most often overnight.

Mapping of geophysical properties and gamma-ray spectrometry

The geoelectric, electromagnetic and spectrometric investigations carried out in the surveyed site by ARP, DUAL-EM, Profiler and Gamma-ray spectrometry enabled the mapping of the apparent electrical resistivity and conductivity and gamma-ray dose of the investigated site. Analysing the maps obtained from measurement (surface acquisition scheme in Figure 2) at depths between 0 and 2 m (Figures 3-6), it may be stated that there is good correspondence between the outputs of the various instruments, with a spatial resolution that is slightly better for the ARP. In further detail, maps show that part of the variability of the observed geophysical anomalies can be explained by the different land use that most likely results in different soil moisture and hence the differences observed between the investigated soils. In particular: i) the area with low resistivity located at the W site (shown in blue in Figure 3) has a land use of uncultivated grassland amongst prevailing orchard; ii) the N-S variability of geophysical anomalies, which is evident throughout the survey area, may reflect the arrangement of crop rows.

However, land use cannot explain all the other geophysical anomalies. In particular, long-range variability is clearly shown (Figures 3 and 5) with a large central area showing higher resistivity surrounded by two less resistive areas (of these two only the W area has non-arboreal land use). In addition, within each of these areas there is short-range variability with alternating - apparently chaotic - of highly variable resistivity areas. Moreover, the measurements made with different signal frequencies (*e.g.* the case for DUAL-EM is reported in Figures 4 and 5) and hence with different investigated volumes, highlight results with very different mapping areas, especially when observing short-range variability. For example, in Figure 5 (estimated volume depth: 3 m) there are many geophysical anomalies, completely different from the same survey reported in Figure 4 (estimated volume depth: 1.7 m).

It is evident that *a priori* it is impossible to ascertain the nature (natural *vs* anthropogenic) of such variability occurring among the 15 maps obtained from the different sensors used (Figure 7A). On the basis of the 15 maps we then applied the NSRDGC procedure in order to obtain an integrated map of the variability of all surveyed anomalies obtained by proximal sensors. Reported in Figure 7B the map clearly shows higher variability (larger violet circles) in a N-S direction with a few additional large patches. On the basis

Figure 2. Example of an acquisition path for the ARP technique.

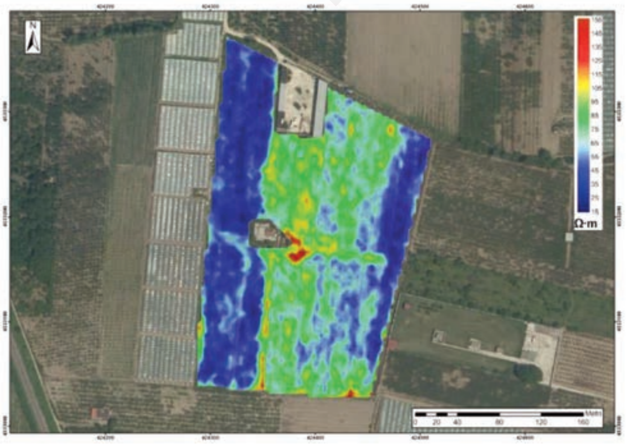

Figure 3. Electrical resistivity map using ARP. Channel 3 - depth: 1.7 m.

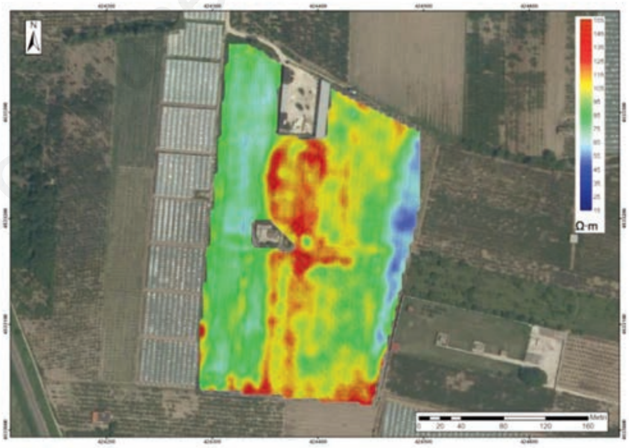

Figure 4. Electrical resistivity map obtained using DUAL-EM (estimated volume depth 1 m).

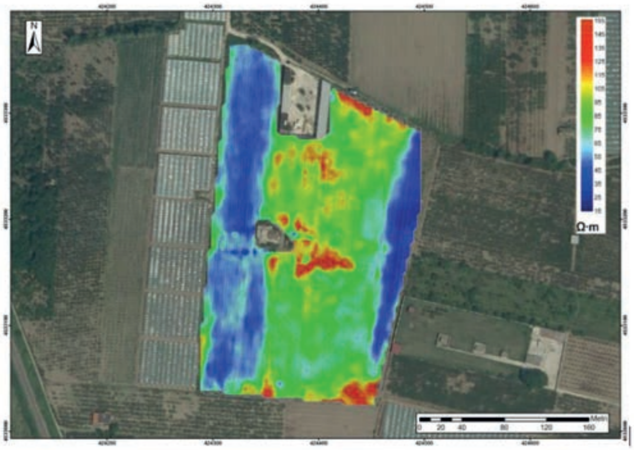

Figure 5. Resistivity map obtained using DUAL-EM (estimated volume depth: 3 m).

of this NSRDGC information, we then located soil profiles in more homogeneous areas and soil trenches in more heterogeneous areas. In so doing, we paid special attention to avoid: i) N-S anomalies due simply to changes in land use; ii) anomalies close to manmade structures (walls, small houses and warehouses, *etc.*); and iii) anomalies already known in the SW portion of the field.

Trenches and profiles

The location of the pedological observations is shown in Figure 8. Soil morphological observations enabled identification of three different types of anthropogenic materials enriched in Cr and Zn: i) coarse material (2-7 cm) and sediments in well-identifiable place of the soil profile, such as in trench 1, where Cr reached a concentration of 2.7%; ii) organic surface material used as *compost* identified in profile 4; material rich in organic component, more or less mineralized, in trenches 2 and 4. Evidence from soil morphology with depth suggests that emplacement of these materials did not take place in a single event, but several earth movements and landfill were carried out in the same area and the effects of these movements were identified from the detailed observation of trenches 2 and 4 (Figure 9). The extreme complexity and variability of the landfill is clearly visible in trench 4, where a mix of pollutants with natural soil is found in the first metre of the central area, whereas organic matter not present in the central part is visible laterally, probably as a consequence of successive excavations. As a general rule, illegal landfills are found in the first two metres, but they also occur at different depths. Indeed, in two of the open trenches (TR2 and TR4) the lower limit of the landfill was not identified and is thus considered to be deeper than 240 cm (bottom of the soil excavation). Further investigation should be carried out to ascertain the depth of the landfill by means of environmental

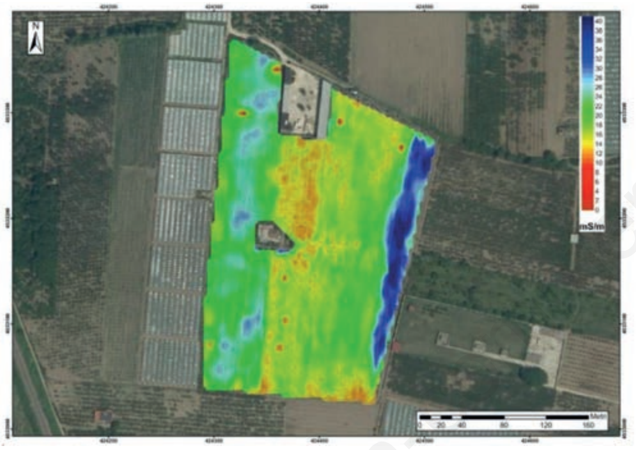

Figure 6. Electrical conductivity obtained using profiler with 15KHz frequency.

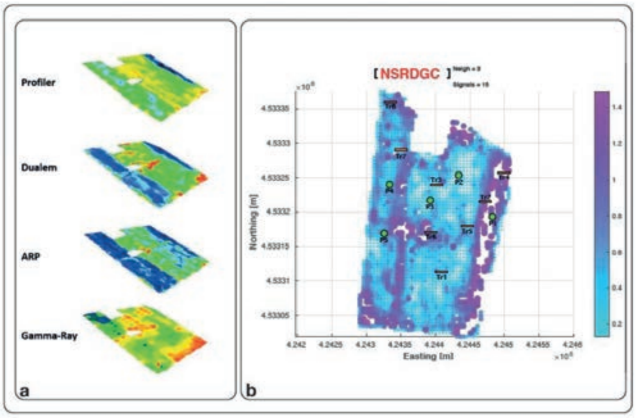

Figure 7. A) Maps obtained from the sensors used; B) map reporting the variability of the normalized sum of relative differences on geophysical covariates (NSRDGC).

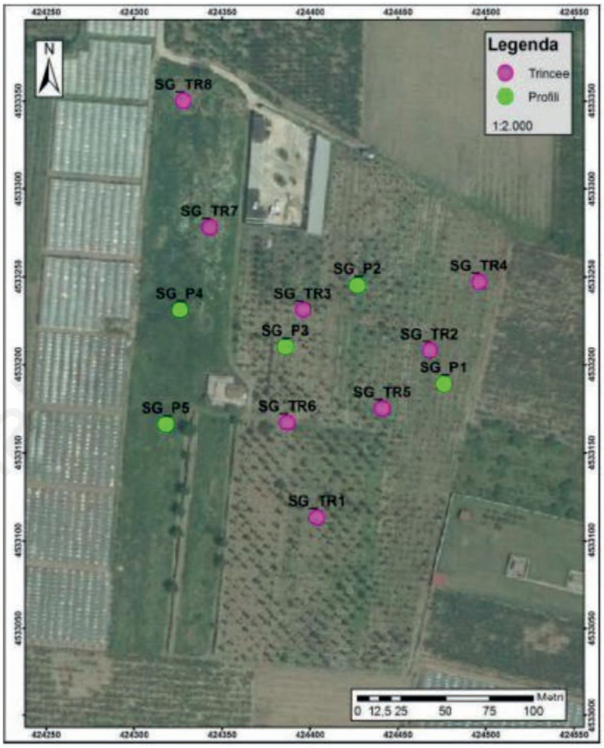

Figure 8. Location of the pedological (trench and profile) observations.

Figure 9. Pictures from trenches 2 and 4.

Figure 10. Picture from trench 3.

core drills. Observations also showed that human impact greatly affected the areas along the field margins whereas within the less disturbed area anthropogenic materials were identified between an abandoned cottage and a shed (trench 3, Figure 10), albeit in smaller amounts and at lower depths compared with the marginal areas. Another less disturbed area was identified in correspondence of profile 2 (Figure 11), which shows signs of anthropic disturbance in the first metre, but starting from 100 cm displays a natural distribution of soil horizons. In this profile two buried soil cycles, typical of volcanic environments, truncated upwards by recent human activities are clearly visible.

Micromorphological analysis

The micromorphological study carried out on thin sections from selected soil horizons enabled the identification of interesting soil pedofeatures. In particular, in one of the most contaminated soils in the TR2: i) blackish aggregates of organic materials standing alone (Figure 12A and B) or mixed with mineral particles (Figure 12C and D); and ii) several carbonate fragments were recognized in deep horizons (175-185 cm). From analysis of the elemental composition (Table 1), the organic materials proved highly enriched in Cr (from 2109 to 3427 mg kg^{-1}), Fe (from 3243 to 4945 mg kg^{-1}), Ca (from 83,907 to 124,982 mg kg^{-1}), S (16,751 to 21,737 mg kg^{-1}) and to a lesser extent Zn (from 92 to 122 mg kg^{-1}), compared with the soil matrix (Table 1 with reference to the light brown soil matrix). Moreover, in many soil pores fine coatings were also identified (Figure 12E and F). As observed, coatings are made by alternate levels of pale yellowish and reddish layers, optically isotropic, indicating downward movements of organic materials with different chemical composition and at different times. Very frequently, acicular small crystals light grey in PPL (Figure 12D and E) and with high interference colours in XPL (Figure 12D and E) were found to be present in association with the organic materials. Due to their small size, general absence in volcanic materials of this environment and their occurrence within organic materials, they were interpreted as minerals of secondary origin. From their composition (Table 1), showing very high content of both S (47,597 mg kg^{-1}) and Ca (80,952 mg kg^{-1}), they were recognized as calcium sulphates and most probably gypsum.

Figure 11. Profile 2, as an example of undisturbed soil.

Table 1. Concentration of selected elements obtained by XRF analyses of soil pedofeatures identified by the micromorphological study.

	Light brown soil matrix 1	Light brown soil matrix 2	Blackish aggregate 1 mg kg^{-1}	Blackish aggregate 2	Carbonate fragments	Acicular crystals
S	1814±474	2686±523	21,737±1288	16,751±1136	3607±717	47,597±1872
K	6902±243	24,187±545	1213±126	2292±154	2229±155	1744±132
Ca	37,231±719	30,028±592	124,982±2180	83,907±1515	123,984±2204	80,952±1441
Ti	414±25	884±37	917±39	967±41	206±21	336±23
V	19±3	29±4	18±4	34±51	5±3	11±3
Cr	37±6	63±7	3427±68	2109±47	22±6	489±17
Mn	113±9	241±12	86±16	73±14	1172±29	78±9
Fe	1340±39	3536±66	3243±64	4945±82	3365±67	1938±47
Zn	ND	32±4	92±6	122±6	54±5	43±4
Sr	55±5	58±5	61±6	51±56	5±6	49±5
Zr	19±4	33±5	35±5	47±62	4±5	15±4

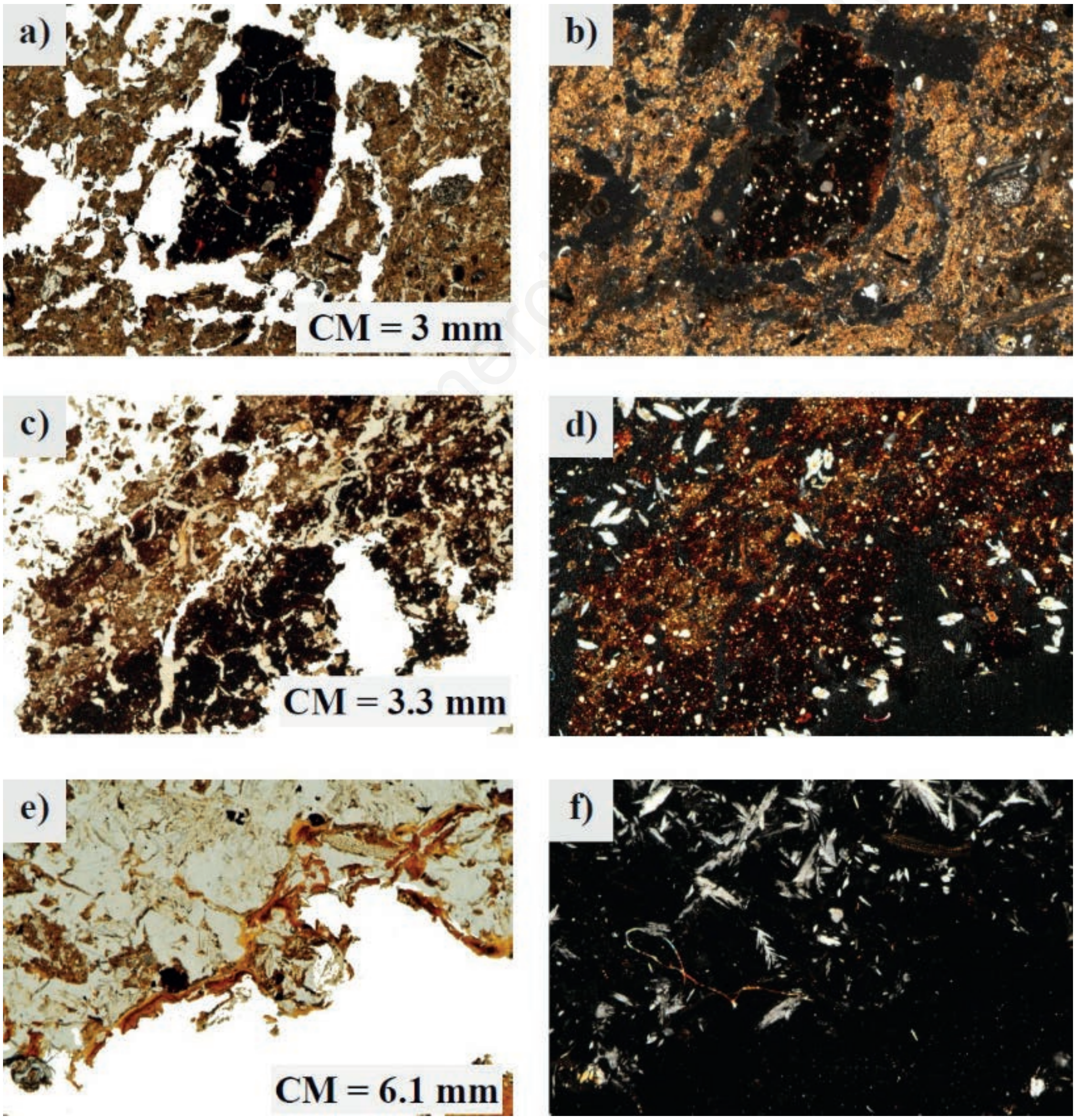

Figure 12. Optical micrographs of selected soil features. a) and b) Blackish aggregate of organic material in carbonate matrix (PPL and XPL, respectively); c) and d) organic material mixed with mineral component (PPL and XPL, respectively); e) and f) organic matter coatings inside a soil pore (PPL and XPL, respectively). PPL, plane polarized light; XPL, crossed polarized light.

Moreover, it is noteworthy that Cr content associated to these sulphate minerals is remarkable (489 mg kg^{-1}).

Conclusions

From the results obtained the geospatial complexity of contaminated sites, along with the complex spatial distribution of contaminants, appears evident. This has to be ascribed to the complex history of the site and type of deposition of pollutant materials.

It is self-evident that, in sites subject to high environmental risk, such site complexity should not be neglected, also given that a large set of proxy technologies (*e.g.* miniaturized sensors) is currently available and can be directly used in the field for precision soil sampling. The correspondence found between hot spots reported in the maps and presence of heavily contaminated materials in field observations (profiles and trenches) showed that it is indeed possible and sustainable to use a powerful approach that integrates various sensors (indirect measurements and direct surveys) to attain detailed geospatial knowledge of contaminated sites and encourage further applications. Moreover, the information thereby obtained can also reduce the number of samples and costs of analysis, and increase the accuracy in delineating site-specific problems (*e.g.* volume of soil contamination). Soil micromorphology application evidenced the presence of: i) organic materials enriched in Cr and surrounded by a carbonate matrix (or mixed with mineral particles) of allochthonous origin; ii) secondary crystalline gypsum minerals showing notable Cr content; and iii) peculiar soil features (such as organic matter coatings) close to the above mentioned organic materials, indicating movement inside pores of colloidal materials. These observations give a picture of the fate of some contaminants, such as the formation of the Cr-associated organic materials and secondary crystalline gypsum, as well as the downward movement of fine particles in depth as PTE carriers.

Finally, we should highlight the urgent need to improve existing legislation, including approaches like that, which we have shown. This would enable both proper characterization of the geography of contamination and hence proper site restoration. Indeed, the proposed approach is economically sustainable and detailed knowledge of contamination geography enables site-specific remediation techniques to be performed, thus avoiding treatment of non-contaminated soils.

It should be also emphasized that indirect techniques enable data to be acquired in a very short time (1 to 3 days' work per technique, for a survey of 6 ha) and can help transform point-based analysis of standard soil observations into information on continuous spatial soil properties. Homogeneous soil property areas can then be identified and studied in detail by direct investigations.

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Assessing the bioavailability of potentially toxic elements in soil: A proposed approach

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Abstract

For risk assessment of soil contamination, there is a growing need for an approach based on the real risk for living organisms, hence considering the bioavailable fraction of contaminants. The maximum allowable limit of a contaminant in soil, as well as the target for restoration, could be established in relation to a level of bioavailable amount of contaminants considered safe and estimated by standardised procedures. In this work we propose a combination of single and sequential chemical extractions to assess the mobility and plant availability of potentially toxic elements (PTEs) in polluted agricultural soils under remediation. The proposed approach was tested in two pilot sites of Campania potentially polluted by Cu and Zn, before and one year after phytoremediation treatment with *Eucalyptus camedulensis* L. and *Populus nigra* L. Specifically, 1 mol L^{-1} NH_4NO_3 (readily soluble and phytoavailable PTEs) and 0.05 mol L^{-1} EDTA at pH 7 (potentially phytoavailable PTEs) single extractions and the EU-BCR sequential scheme (presumed main geochemical PTE forms) were applied. Overall, in the study sites, the estimation of PTE bioavailability by standardised chemical extractions appeared to be effective in monitoring the Zn and Cu plant-available fractions as well as the trend and effectiveness of phytoremediation treatment. After one year from planting, a significant reduction in Cu and Zn bioavailable amounts in soil was observed (NH_4NO_3: from 19 to 60% for Cu, from 43 to 92% for Zn; EDTA: from 4 to 27% for Cu and from 8 to 76% for Zn). Plant uptake was positively related with the bioavailability of PTEs in soil, with a general underestimation of Zn extracted in ammonium nitrate. Both single and sequential extractions suggested a higher mobility of Zn with respect to Cu in soil.

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Key words: Polluted agricultural soil; heavy metals; bioavailability; chemical extractions; phytoremediation.

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Introduction

Despite their evident importance, environmental and human health risks related to soil contamination are still rarely assessed. This difficulty is, in part, due to the high variability in the soil characteristics of the sites of interest, the nature of the contaminants and the target organisms taken into account. Moreover, different evaluation criteria and different threshold levels of soil contaminants are adopted by various countries in Europe and worldwide (Carlon, 2007), most of which are based on the total content of contaminants (Kim *et al.*, 2015). This approach may be useful to assess the extent of the contamination in soil, but is an extremely conservative method and increases the possibility of overestimating the risk, making remediation targets expensive and often unachievable (Kim *et al.*, 2015; Adamo *et al.*, 2018). In recent years, the ever-increasing need to define an approach based on the real risk for living organisms has led researchers to consider the bioavailable fraction of contaminants rather than their total content, *i.e.* the portion of the total content that is available and harmful to target organisms, such as humans, animals or plants, or that is mobile and therefore transferable to water or other sites (Harmsen, 2015; Kim *et al.*, 2015; Adamo *et al.*, 2018). Indeed, several studies have demonstrated that some of the total contaminant content remains immobile in soil or unavailable for living organisms, hence posing no hazard (Harmsen, 2015; Kim *et al.*, 2015). Therefore, the maximum allowable limit of a contaminant in a soil, as well as the restoration target, could be established in relation to a bioavailable amount of the contaminants considered harmless in terms of transfer to the food chain or towards other environmental sectors, rather than in relation to their total content in the soil (Harmsen and Naidu, 2013; Harmsen, 2015).

Potentially toxic elements (PTEs) are inorganic contaminants, which are of great concern because of their persistence in soil and toxicity at certain concentrations (Cristaldi *et al.*, 2017). Some of them are essential elements for living organisms (*e.g.* Cu, Zn, Ni), with toxic effects occurring only at high concentrations, while others (*e.g.* Cd, Pb) are not essential elements and can be toxic even at very low concentrations (Adamo *et al.*, 2018). PTEs in soil may be both anthropogenic and natural in origin, and they are distributed between the soil solution and the various organic and mineral components of the soil. Generally, the PTE fraction in the soil solution is considered to be readily bioavailable for plants, while the fractions associated with soil organic or mineral components prone to be mobilizable in the soil solution are considered potentially bioavailable (Adamo *et al.*, 2018). This PTE repartition is a dynamic equilibrium influenced both by the nature and chemical species of the contaminant and by the chemical and physical characteristics of the soil such as pH, organic carbon, clay and carbonate content, and exchange capacity, that influence PTE retention or release in the soil solution (Adamo *et al.*, 2018). The PTEs in the soil from anthropogenic sources tend to be more mobile and bioavailable than their geogenic counterparts (Mir *et al.*, 2017). All this makes the plant-bioavailable fraction of a given PTE site-specific and difficult to estimate without direct measurement.

Many chemical extractions are available to estimate the bioavailability of PTEs in a soil (single extractions) or their fractionation between the various geochemical forms in which they may exist in the soil (sequential chemical extractions), each of which is more or less suitable for a particular soil or a particular contaminant (Adamo *et al.*, 2018). Some of these extractions have been standardised and taken into consideration in the national legislation of some countries, while others are undergoing standardisation, a process required to make bioavailability a concept that can be included in legislation and in policies for soil protection, as well as a tool for risk assessment (Harmsen, 2015; Kim *et al.*, 2015). That said, it should be borne in mind that bioavailability may also vary according to the target organism considered. Different plant species can absorb and translocate different quantities of the same contaminant from the same soil and can influence the contaminant equilibrium in soil differently, such that the bioavailability measurement of a contaminant in soil is an indication that should be verified with the real absorption by the plant concerned (Kim *et al.*, 2015; Adamo *et al.*, 2018; Duri *et al.,* 2018). In addition, the PTE dynamic equilibrium in soil should be considered in monitoring the remediation target, because the element decrease in the readily bioavailable fraction may be replenished through soil element re-equilibration (Keller and Hammer 2004; Harmsen and Naidu, 2013), and repeated monitoring over time should be done.

Implementation of bioavailability in regulation is still limited, mostly due to the large number of methods claiming to measure bioavailability, but also to the plethora of scientific definitions in use in the literature. The most effective action that scientists can take to support implementation of bioavailability within the monitoring and management of contaminated sites entails: i) choosing and proposing standardised methods for measuring bioavailability such that bioavailability can play a role in regulations; and ii) promoting discussion among stakeholders in order to make bioavailability comprehensible and also measurable. With this conviction in mind, and also with a view to supporting the current Italian debate on managing polluted farmland, in this work a selection of standardised chemical extraction methods were applied in two potentially PTE-contaminated agricultural sites under phytoremediation treatment within the ECOREMED project (LIFE11 ENV/IT/000275). The aim was to assess the mobility, plant availability and the distribution of the PTEs among the main soil geochemical forms before (T0) and after one year (T1) of phytoremediation and to improve predictions of PTE bioavailability from the amount of PTEs taken up by plants. In particular, we applied:

- 1 mol L^{-1} ammonium nitrate extraction (ISO 19730:2008) to determine the readily bioavailable content of PTEs in soil. Element extraction is mainly caused by cation exchange and desorption of cations, favoured by the high ionic strength of the NH_4NO_3 solution. This extraction has yielded a good correlation with the absorption data of plants in agricultural soils (Adamo *et al.*, 2018), and is the official method applied in Germany (DIN 19730, 1997; BBodSchV, 1999), Slovakia (ASP, 2004) and Austria (Standard ON S 2088-2) to assess the risk of PTE transfer from the soil to plants in potentially polluted agricultural soils.
- 0.05 mol L^{-1} EDTA at pH 7.0 (Rauret *et al.*, 2001) to determine the potentially bioavailable content of PTEs in soil. EDTA is a strong chelating agent, which can form stable chelates with many metal ions and can remove organically-bound metals, along with part of the metals occluded in oxides and secondary clay minerals; the Measurement and Testing Programme of the European Commission has standardised this extraction method, and certified reference materials (BCR-700) were produced to assess the quality of the data obtained by using the procedure in question (Adamo *et al.*, 2018).
- the EU-BCR sequential chemical extraction scheme (Rauret *et al.*, 1999) to determine PTE distribution among the main soil geochemical forms. This is a four-step sequential extraction procedure proposed by the Measurements and Testing Programme - MAT (formerly BCR) - of the European Commission; it involves the sequential use of several reagents, fractionating the total amount of PTEs in soil into fractions which become progressively less mobile and bioavailable to plants [*i.e.* i) 0.11 mol L^{-1} acetic acid (easily extractable fraction); ii) 0.5 mol L^{-1} hydroxylamine hydrochloride (reducible fraction); iii) hydrogen peroxide/1 mol L^{-1} ammonium acetate (oxidizable fraction); and iv) hydrochloric acid/nitric acid (residual fraction)]. According to the interaction between each reagent and the polluted soil, the presumed geochemical fractions of PTEs extracted in sequence are: i) soluble, exchangeable and associated to carbonates; ii) associated to amorphous Fe and Mn oxides; iii) bound to organic matter and sulphides; iv) occluded in non-siliceous minerals (Adamo *et al.*, 2018). Certified reference materials for BCR sequential extractions have been produced to assess the quality of the data obtained by using this extraction procedure (CRM 601 and BCR 701).

Materials and methods

The study area and the pilot sites

In Campania (southern Italy), two National Interest Priority Sites (NIPSs) and three Regional Interest Priority Sites (RIPSs) were identified, amounting to a total area of about 200,000 ha with different levels and sources of pollution. One of these sites, namely the *Litorale Domitio-Agro Aversano*, encompassing the plains of the Garigliano and Volturno Rivers and partially the Phlegrean Fields volcanic area, and including much of the farmland in Naples and Caserta provinces, has been in the media limelight since 2008 because of widespread legal and illegal disposal and burning of waste, with consequences for soil and groundwater quality (Rocco *et al.*, 2016; Monaco *et al.*, 2015; Capra *et al.*, 2014).

In this work, in the framework of the LIFE-ECOREMED project, a 1000 m^2 rural area in the municipality of Giugliano (Naples) (GI) and a 3300 m^2 area in Trentola-Ducenta (Caserta) (TD) were used as pilot sites. The two sites were affected by illegal waste disposal. In GI, the waste was mainly from the *manufacture, formulation, supply and use of coating (i.e. paints and varnishes)* and *end-of-life vehicles and their components* (*i.e.*, boat and car batteries) (William, 2005). In a few cases traces of waste burning were visible. In TD, waste predominantly consisted of mixed *construction and demolition* materials, including asphalt and plastic (Rocco *et al.*, 2016). Based on preliminary analytical investigations on the PTE total content in the soils, the two sites were defined as potentially contaminated by Cu and Zn as they exceeded the Italian legal thresholds provided by L.D. 152/06 for public, private and residential green areas of 120 and 150 mg kg^{-1}, for Cu and Zn respectively. Detailed site characterisation, soil sampling and total metal content in soil at T0 are given in Rocco *et al.* (2016).

The approach for bioavailability assessment

Soil characterisation

The soils in question were characterised for their main physical and chemical properties in order to improve the interpretation of the analytical data. All soil samples were air-dried and sieved at 2 mm in accordance with the Italian official procedures (MiPAF, 2000) prior to determining moisture content at 105°C, pH–H_2O (1:2.5 soil:water ratio), electric conductivity (1:5 soil:water ratio), organic carbon (Walkley and Black method), cation exchange capacity (CEC) ($BaCl_2$+TEA at pH 8.1), exchangeable bases and carbonates (Dietrich-Fruehling calcimeter method). Particle size analysis (Andreasen's pipette method) was carried out by wet sieving and by sedimentation in aqueous media after organic matter oxidation by H_2O_2 treatment.

Extraction by 1 mol L^{-1} ammonium nitrate (procedure ISO 19730:2008)

Extraction by 1 mol L^{-1} ammonium nitrate is commonly used to determine the readily soluble and bioavailable content of PTEs in soil (Gryschko *et al.*, 2005). Soil samples (2 mm sieved soil) were extracted for 2 hours on an end-over-end shaker by 1 mol L^{-1} NH_4NO_3 solution in a soil/solution ratio of 1:2.5. The samples were then centrifuged and filtered. PTE content in the extract solution was measured by ICP-AES (inductively coupled plasma-atomic emission spectrometry) (Optima 7300DV, Perkin Elmer).

Extraction by 0.05 mol L^{-1} EDTA at pH 7

Extraction by 0.05 mol L^{-1} Ethylenediaminetetraacetic acid (EDTA) at pH 7 is commonly used to determine the potentially bioavailable content of PTEs in soil (Rauret *et al*., 1999; Adamo *et al.*, 2018). Soil samples (2 mm sieved soil) were extracted for one hour on an end-over-end shaker by 0.05 mol L^{-1} EDTA at pH 7 solution in a soil/solution ratio of 1:10. The samples were then centrifuged and filtered. PTE content in the extract was measured by ICP-AES (inductively coupled plasma-atomic emission spectrometry) (Optima 7300DV, Perkin Elmer). Quality control of the extraction procedure was carried out using BCR700 certified standard material.

Sequential chemical extraction procedure

The EU-BCR sequential chemical extraction is used to determine PTE distribution among the soil geochemical fractions (Rauret *et al*., 1999; Adamo *et al.*, 2018). Due to the higher values of soil contamination, the sequential extraction was performed only on selected samples of site GI. Soil samples (2 mm sieved soil) were extracted for 16 h on an end-over-end shaker by 0.11 mol L^{-1} acetic acid in a soil/solution ratio of 1:40, in order to extract the acid-soluble fraction (step 1) consisting of exchangeable metals and fraction bound to carbonates. It is the most labile metal fraction in the soil and hence the most dangerous for the environment. The extracts were separated from the solid residue by centrifugation at 3000 rpm for 20 min and the supernatants were filtered into a polyethylene container and then stored at 4°C prior to analysis. Soil residues were washed by adding 20 mL of distilled water, shaken for 15 min on an end-over-end shaker and centrifuged for 20 min at 3000 rpm. The supernatant was then discarded. Forty mL of 0.5 mol L^{-1} hydroxylammonium chloride were then added to soil residues to extract, by shaking for 16 h on an end-over-end shaker, the reducible phase (step 2) consisting of metals bound to Fe and Mn oxides. Separation, filtration and storage of 2nd-step extract, as well as soil residue washing, were performed as described above (Step 1). Thereafter, the soil residues were treated with a suitable addition of 8.8 mol L^{-1} H_2O_2 at 85°C until no foaming occurred, and then evaporated up to 1 mL. This treatment aims to oxidize the organic matter and release bonded metals. Then, to recover these metals and prevent their precipitation or re-adsorption by the soil sample, 40 mL of 1 mol L^{-1} ammonium acetate were added to soil residues. After shaking for 16 h on an end-over-end shaker, the oxidizable phase (step 3) was extracted, representing the amount of metal bound to organic matter and, if present, sulphides. Separation, filtration and storage of 3rd-step extract, as well as soil residue washing, were performed as described above (Step 1). The metal contents in the soil residues (step 4) were then determined by treating the residual soils with *aqua regia* (3:1, v/v, HCl to HNO_3). The metals present in the residual phase are mainly occluded in the crystalline structures of non-siliceous minerals. For checking the quality of data, the sum of the PTE content extracted by the four steps of the sequential procedure (cumulative total) were compared with total PTE contents extracted by a single procedure (unique total) from the same sample. Metal content in the extracts was measured by ICP-AES (inductively coupled plasma-atomic emission spectrometry) (Optima 7300DV, Perkin Elmer). A quality control of the extraction procedure was carried out using BCR701 certified standard material.

Phytoremediation treatments and plant analysis

In the pilot sites of the ECOREMED project, a plant-based remediation protocol was applied, utilizing *Eucalyptus camedulensis* L. and *Populus nigra* L. in GI and TD, respectively (Fiorentino *et al.,* 2018). One year after applying phytoremediation treatment (T1), seven plots with different soil pollution levels were selected in each site. Soil and plant leaves were collected and analysed in order to assess metal uptake by plants and the effect of phytoremediation on metal bioavailability in soil. The data related to plant uptake refer only to site GI due to the absence of leaves on poplar in TD during the sampling period.

Plant leaves were washed with distilled water, oven-dried at 65°C until constant weight and crushed. An aliquot of the samples was then pulverized in a planetary ball mill (PM 200, Retsch). Half a gram of the powdered materials was digested with 6 ml of HNO_3 (65%), 0.5 mL of HF (40%) and 2 mL of H_2O_2 (30%) in a microwave digestion system (Ethos 900, Milestone). Metal content in the digestion solutions was measured by ICP-AES (inductively coupled plasma-atomic emission spectrometry) (Optima 7300DV, Perkin Elmer).

Data analysis

All data are presented as mean values with relative standard deviation of three replicates. A correlation analysis was performed on data from soil single chemical extractions, before and after one year of plant growth, and from total metal content of both leaves and soil.

Results and discussion

Soil physical and chemical properties

Soil physical and chemical properties were analysed in both GI and TD sites. The soil pH-H_2O was neutral in GI (pH 7.3±0.2) and sub-alkaline in TD (pH 8.0±0.2). This is in agreement with the higher carbonate content in TD (174±41 g kg^{-1}) than in GI (7.6±2.5 g kg^{-1}). Electrical conductivity was 0.16±0.03 dS m^{-1} in GI and 0.45±0.3 dS m^{-1} in TD, thus indicating that salinity is not limiting to plant growth and agricultural production. Soil organic carbon content was always below 20 g kg^{-1}, with similar values in the two sites (GI 20±3.1 g kg^{-1}, TD 18±0.7 g kg^{-1}). CEC ranged between 28±3.1 cmol(+) kg^{-1} in TD and 34±3.2 cmol(+) kg^{-1} in GI, with a dominance (~80%) of calcium on the exchange complex. Giugliano and TD soils were sandy loam, with 610±25 g kg^{-1} in GI and 711±29 g kg^{-1} in TD of the soil in the sand fraction.

Single extractions and plant uptake

At T0, in both GI and TD sites, 1 mol L^{-1} NH_4NO_3 extraction almost always showed higher Cu values than the respective *trigger value* of 1 mg kg^{-1}, adopted by several European countries for agricultural areas (ASP, 2004; BBodSchV, 1999; Carlon, 2007); Zn exceeded the *trigger value* of 2 mg kg^{-1} only in concomitance with the highest total concentrations (Table 1, Figure 1).

In both sites the amounts of metals extracted in EDTA (Table 1, Figure 2) were, as expected, always much higher than those extracted in NH_4NO_3. Indeed, the amount of both metals extracted by 1 mol L^{-1} NH_4NO_3 was from 0.1 to 7.9% of respective total contents, while the amount extracted by 0.05 mol L^{-1} EDTA ranged from 13 to 64 % of total content. The potentially bioavailable amount of Zn was in some cases higher than that of Cu (Table 1). The content of both metals in leaves (Table 2) increased with total metal concentration in soil (R^2: 0.534 for Cu and 0.786 for Zn). Zn content in leaves was always higher than Cu (Zn: from 19.4 to 59.2 mg kg^{-1}; Cu: from 5.1 to 19.2 mg kg^{-1}). This is a common trend in plants as observed by Kabata-Pendias (2011) (Zn: 20-46 mg kg^{-1}; Cu: 2-11 mg kg^{-1}) and is related to plant physiology. Moreover, Priya and Jenifer (2014) in their study on Zn and Cu uptake by *Eucalyptus ficifolia* grown on unpolluted soil from Mannarguti (India) found, in leaves, Cu and Zn concentrations around 1.42 and 3.52 mg kg^{-1}, respectively. The larger amount of both metals taken

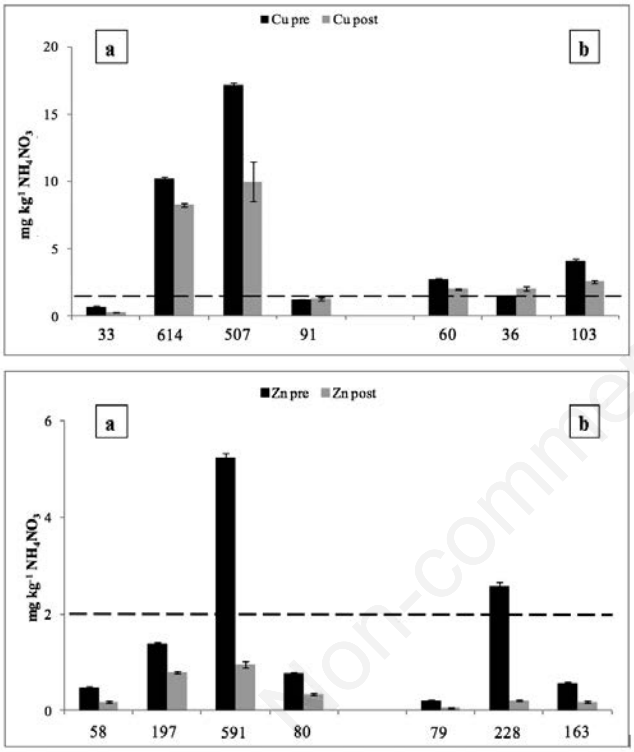

Figure 1. Cu and Zn readily bioavailable amounts (mg kg^{-1}), extracted by 1 mol L^{-1} NH_4NO_3 before and after one year of phytoremediation treatments (pre = T0; post = T1) in Giugliano (A) and Trentola Ducenta (B). The numbers below the bars indicate total metal content in soil samples expressed in mg kg^{-1}. Dotted line = trigger values defined by German DIN 19730 (1997).

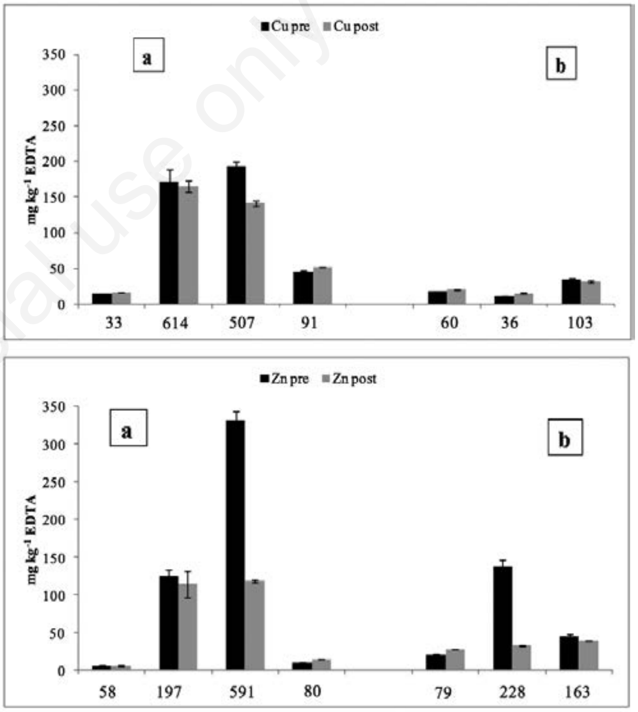

Figure 2. Cu and Zn potentially bioavailable amounts (mg kg^{-1}), extracted by 0.05 mol L^{-1} EDTA pH 7 before and after one year of phytoremediation treatments (pre = T0; post = T1) in Giugliano (A) and Trentola Ducenta (B). The numbers below the bars indicate total metal content in soil samples expressed in mg kg^{-1}.

Table 1. Cu and Zn total and bioavailable concentrations [mean ± SD (min-max), n=10] in Giugliano (GI) and Trentola Ducenta (TD) soils analysed at T0.

Site	Metal	NH_4NO_3 (mg kg^{-1})	EDTA (mg kg^{-1})	Aqua Regia (mg kg^{-1})
GI	Cu	7.66±7.0 (0.69-17.3)	113±88 (15.2-239)	429±530 (33.4-1707)
	Zn	3.00±3.0 (0.49-11.8)	132±148 (6.48-393)	308±329 (58.1-872)
TD	Cu	3.09±0.94 (1.54-4.26)	21.9±8.71 (11.3-35.9)	59.2±20.9 (36.0-103)
	Zn	0.69±0.74 (0.09-2.58)	48.7±35.8 (18.2-139)	136±67.1 (62.2-256)

up by *Eucalyptus* plants grown on polluted GI soil (on average Cu 12.1 mg kg^{-1}, Zn 39.3 mg kg^{-1}) supports the suitability of *Eucalyptus camedulensis* for metal phytoextraction.

A correlation analysis was performed to investigate the relationship between chemically assessed bioavailable Cu and Zn amounts and total content of both metals in soil and in plants. Both amounts of Cu and Zn extracted by 1 mol L^{-1} NH_4NO_3 and 0.05 mol L^{-1} EDTA were positively and significantly ($P<0.05$) correlated with total Cu and Zn content in soil, indicating an increase in bioavailable amounts with total metal content in soil. Also a positive correlation ($P=0.10$) was found between Cu and Zn amounts taken up by plants and total and bioavailable Cu and Zn content in soil.

Sequential extractions

As shown in Figure 3, EU-BCR sequential extractions, carried out on selected samples (GI1-5, Cu 1707 mg kg^{-1}, Zn 972 mg kg^{-1}; GI1-6, Cu 506 mg kg^{-1}, Zn 591 mg kg^{-1}) indicated that Cu was mainly associated to easily reducible mineral phases (54 and 55%, Step 2) and, to a lesser extent, with oxidizable phases (19 and 24%, Step 3). Instead, Zn was equally distributed between HOAc-extractable (35 and 45%, Step 1) and reducible (29 and 43%, Step 2) phases.

The data obtained by sequential extractions indicate a higher mobility of Zn with respect to Cu, confirmed by the larger amounts of Zn accumulated by leaves. Iron and aluminium oxides and organic matter are the soil components most commonly involved in the adsorption of metals in soil. This is indirectly confirmed by the considerable amounts of Cu extracted by EDTA (215±5.3 mg kg^{-1} in GI1-5 and 194±5.5 mg kg^{-1} in GI1-6) and by hydroxylamine (Step 2 EU-BCR) (926±187 mg kg^{-1} in GI1-5 and 279±22.2 mg kg^{-1} in GI1-6).

Effect of phytoremediation treatments on bioavailability

In Figures 1 and 2 the amounts of Cu and Zn extracted by NH_4NO_3 and EDTA solutions before and after one year of phytoremediation treatment in GI and TD sites are compared. A general reduction of both NH_4NO_3 and EDTA bioavailable amounts of Cu and Zn was observed after planting (T1) regardless of the initial

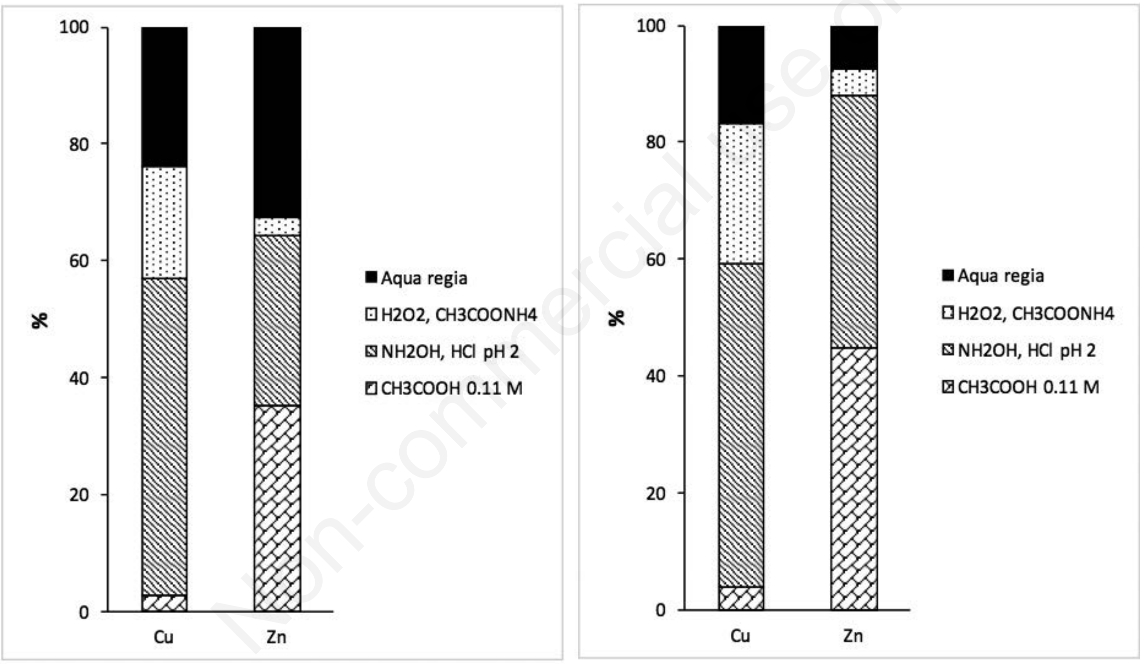

Figure 3. Amounts of sequentially extracted Cu and Zn in Giugliano soil, expressed as a percentage of total contents (A: G1-5, Cu 1707 and Zn 972 mg kg^{-1}; B: G1-6, Cu 506 and Zn 591 mg kg^{-1}).

Table 2. Cu and Zn accumulation in leaves (mg kg^{-1}) of *Eucalyptus camedulensis* and total content in Giugliano soil.

	Total content in leaves (mg kg^{-1})		Total content in soil (mg kg^{-1})	
SUB-PLOT	Cu	Zn	Cu	Zn
GI1-7	8.18±0.6	27.1±2.0	614±7.0	197±1.7
GI6-5	5.10±0.1	19.4±0.6	106±2.3	84.0±1.2
GI6-6	10.9±1.1	20.5±0.9	109±3.7	79.0±1.0
GI8-3	9.15±0.3	20.1±1.0	50.0±1.4	71.0±3.2
GI1-5	19.2±1.3	59.2±1.2	1707±15.3	972±6.7
GI1-6	8.67±0.6	33.9±0.6	507±12.1	591±1.7
GI8-4	6.71±0.9	29.4±0.7	859±6.0	720±6.3

concentrations (NH_4NO_3: from 19 to 60% for Cu, from 43 to 92% for Zn; EDTA: from 4 to 27% for Cu and from 8 to 76% for Zn). When the initial concentration was lower than the legal limit, the amounts of bioavailable fractions before and after phytoremediation did not significantly differ. Despite the reduction observed one year after planting, the bioavailable amounts of Cu were still above the trigger value of 1 mg kg^{-1} defined by German DIN for the NH_4NO_3 procedure. By contrast, the bioavailable amounts of Zn, which were above the German DIN trigger value of 2 mg kg^{-1} prior to planting, dropped below the limit in question after one year of phytoremediation. This result might be related to the greater Zn mobility and bioavailability in soil, as assessed by single and sequential extractions, and to the higher Zn uptake by the plants, as shown by the larger amounts of Zn found in the plant leaves. For both soil samples, the 1 mol L^{-1} NH_4NO_3 solution extracted amounts of Cu similar to the amounts taken up by plants, while an underestimation was observed for Zn.

Conclusions

The criteria to assess the quality of the agricultural soils, safeguarding the healthiness and quality of agro-food products for the protection of human health, cannot take account of only total PTE content in soil, but must also consider their mobility and bioavailability. Indeed, in assessing the contamination level of agricultural areas, attention should mainly be paid to the possible transfer of pollutants from soil to plant, with the consequent entry of contaminants into the food chain.

The approach proposed in the ECOREMED project was based on the chemical monitoring of PTE bioavailability using a combination of single and sequential chemical extractions. Our findings showed that the potential mobility and bioavailability of both metals, though higher for Zn than for Cu, was a reliable indication of the suitability of plant cropping as soil remediation treatment. Indeed, one year after planting *Eucalyptus camedulensis*, a significant reduction in the bioavailable amounts of Cu and Zn in soil (assessed by single chemical extractions) was observed in both sites. A general underestimation of bioavailable Zn assessed by 1 mol L^{-1} NH_4NO_3 was observed from comparison with leaf content. Further detailed information was obtained from the sequential extractions, indicating Zn as being mainly associated to the easily HOAc-extractable and reducible fractions, while Cu was mostly found in reducible and oxidizable phases. In our case study, metal removal from the soil by *Eucalyptus* provides evidence that, albeit in a considerable number of years, the chosen phytoremediation treatment should clean the soil at least of the bioavailable PTE fraction, restoring the area to its original agricultural use for food production.

In light of the above, the study of metal bioavailability in soil of polluted sites would translate into economic benefits from the executive phases of environmental restoration projects, which in the absence of such information should reduce total PTE content in soil below the risk thresholds.

Moreover, knowledge of PTE bioavailable concentrations (hence likely to enter the food chain and affect human health) in Campania's agricultural soils could contribute to the creation of a database from which specific threshold values for the region could be obtained. Such knowledge would also provide a basis for legislative proposals leading to an improvement in the current Law Decree 152/2006.

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Use of the native vascular flora for risk assessment and management of an industrial contaminated soil

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Abstract

This study was carried out in an industrial site contaminated by potentially toxic elements (PTEs) to assess the relationship between spontaneous vegetation and pollution levels, the potential risks for biological communities and ecosystems, and the potential of native plant species for phytoremediation. PTE concentrations had negative effects on plant biodiversity, as determined through changes in the Shannon index, Pielou evenness index and species richness. *Poaceae* and *Asteraceae* were moderately affected by soil contamination, while PTE levels had a negative effect on the other species groups. Cadmium had the greatest effect on plant species diversity, followed by zinc and then lead. The ecological risk index showed a mean value of 4924, corresponding to a very high risk in most plants. Target PTEs for phytoremediation were Cd (3813 on average) followed by Pb (937 on average) contributing to the ecological risk index, respectively from 42 to 81% and from 11 to 24%, in spite of the much higher concentrations of Pb.

The most frequent species were *Holcus lanatus* subsp. *lanatus* and *Silene latifolia* that showed good adaptability to contamination, growing in very high-risk areas. *S. latifolia* reported high concentrations of Tl both in shoot and in roots, at levels typical of hyperaccumulator species. High values of bioaccumulation (BAC_S, BAC_R) and translocation factors (TF) confirmed that this species may be considered a hyperaccumulator of Tl. *Holcus lanatus* and *Silene latifolia* proved the most suitable species respectively for Cd and Pb phytostabilization and can be used in association for soil cover during the summer when soil resuspension is generally more intense and for protecting groundwater from pollutant leaching.

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Key words: Plant biodiversity; bioaccumulation coefficient; ecological risk index; potentially toxic elements; spontaneous flora.

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Introduction

The most hazardous potentially toxic elements (PTEs) in urban and industrial areas are widely acknowledged to be lead and cadmium. Lead (Pb) is the second most hazardous element for human health, causing a decline in mental and cognitive capacities (ATSDR 2015). Pb content in soils can have a geological origin, but vehicle emissions, mining, smelting and battery recycling can release the element into the environment through atmospheric fallout and waste disposal. By contrast, cadmium (Cd) is often used as a doping agent in batteries or in some paints. It is a non-essential element for plants and animals, and exposure even at low concentrations could have carcinogenic effects (Goering *et al.,* 1994).

PTEs can also affect normal plant functions and metabolic processes like photosynthesis, respiration and enzymatic activities (Furini, 2012). They stimulate defence mechanisms to oxidative damages based on antioxidant systems that can differ among plant species (Meharg, 2005; Sytar *et al.,* 2013; Antoniadis *et al.,* 2017). Therefore, composition of native flora in contaminated environments can be affected, causing selection pressure that permits tolerant, bioaccumulator or excluder species to proliferate, eliminating the most sensitive populations (Chowdhury *et al.,* 2016). Moreover, analysis of native vascular flora may also help to select the most suitable species in phytoremediation projects (Barbafieri *et al.*, 2011).

Phytoremediation is a technique for removing contaminants from soils or for interrupting exposure pathways that can be viewed as belonging to the general class of bioremediation systems (Vidali, 2001). It is a promising technique thanks to its cost-effectiveness and the positive effects on local landscapes and soil ecosystem services. Phytoextraction is based on uptake of toxic substances by plant roots and their subsequent accumulation in shoots (Raskin and Ensley, 1999).

An alternative approach, namely phytostabilization, entails the use of vegetation to reduce the mobility of contaminants toward other environmental compartments such as air and groundwater. Plant growth and contaminant uptake, as well as their bioavailability, can be improved by assisting phytoremediation with fertilisers and biostimulants, thereby improving the efficiency of this technique and reducing the restoration time required (Fiorentino *et al.*, 2013, 2017).

The effectiveness of phytoremediation requires selected plants for uptaking or immobilizing PTEs. Furthermore, it is important to find plant species not only tolerant to contaminants, but also adapted to the site-specific conditions, which are often limiting for plant growth, such as soil physical degradation due to compaction and aggregate destruction caused by the weight of waste or by the transit of heavy vehicles (Fiorentino *et al.*, 2018).

The aim of the present study was threefold: i) to assess the risks for biological communities and ecosystems due to PTE pollution; ii) identify target PTEs for phytoremediation; and iii) evaluate the potential for phytoremediation of native species growing *in situ*.

Materials and methods

Site description

The study site is located in the industrial area of the municipality of Marcianise (Campania, southern Italy: 41° 00' 48.9" N - 14° 17' 49.7" E) in the western part of the Campanian Plain at about 24 m a.s.l. It has a typical Mediterranean climate with precipitation mostly occurring in the autumn and winter and a long summer drought.

The area in question is a 3.5-ha plot near an industrial plant for recycling automotive electric batteries classified by the regional authorities as potentially contaminated by Cd and Pb due to past storage of waste from the industrial plant itself. The site was thus classified as contaminated, since risk analysis showed that there was a serious potential risk for workers due to inhalation or dermal contact with contaminated soil particles. A phytoremediation project was approved, using the ECOREMED protocol (2017) based on ecological systems (*i.e.* a poplar stand and permanent meadows) to prevent soil particles becoming air-borne, thereby interrupting exposure pathways to contaminants. The study site presents the typical scenario of a disused industrial area, where the recolonization of natural vegetation has allowed the formation of herbaceous and shrubby secondary plant communities.

Soil analysis

Soil samples both from plots and from the rhizosphere of the most representative species were dried at 50°C until constant weight, homogenized and sieved at 2 mm. The <2 mm fraction was characterized for the following: texture (normalized methods for soil analysis, ISS, 1985), pH-H_2O (1:2.5 soil:water solution ratio), electrical conductivity (1:5 soil:water solution ratio - Conductimeter basic 30, Crison), organic carbon (Walkley and Black method, 1934), nitrogen (Kjeldahl method), carbonate content (Dietrich-Frühling calcimeter method, Loeppert and Suarez, 1996) and PTE concentrations (acid digestion with aqua regia followed by ICP-MS).

The potentially bioavailable fraction of PTEs was estimated by a single extraction with a 0.005 M diethylene triamine pentaacetic acid (DTPA) + 0.01 M triethanolamine (TEA) + 0.01 M calcium chloride ($CaCl_2$) solution adjusted to pH 7.3 (Lindsay and Norwell, 1978). PTE concentration in the solution was determined by inductively coupled plasma-atomic emission spectrometry (Perkin Elmer ICP-AES Optima 7300DV).

Vegetation analysis

On 9 June 2015 the analysis of the spontaneous vegetation was carried out by using nine square plots (3x3 m) selected according to the various vegetation types it presented. In each plot the presence/absence of the plant species, their abundance (expressed as per cent cover) and overall vegetation cover was detected. The plant specimens were directly identified in the field except for dubious cases, which were later identified at the Herbarium Porticense (PORUN) according to Pignatti (1982), Pignatti *et al.* (2018) and Tutin *et al.* (1964-1980, 1993). The nomenclature follows Bartolucci *et al.* (2018) and Galasso *et al.* (2018).

With the data collected, the Shannon-Weiner index, Pielou evenness index and species frequency were calculated for each plot. The Shannon-Weiner diversity index (*H'*) is usually based on the number of species and the abundance of individuals within each species (Magurran, 1988), but in this survey the mean soil cover of the species was used, as suggested by Stefanska-Krzaczek (2012):

$$(H') = -\sum_{i=1}^{S} \frac{ci}{C} \, x \, \log_e \frac{ci}{C} \qquad (1)$$

where *S* is the number of species, *ci* is the mean cover of *i* species on a plot and *C* is the sum of mean covers for all species.

The Pielou evenness index (*J'*) (Grall and Coic, 2006) is used to measure the distribution of species within a site, regardless of species richness. Its value varies from 0 (dominance of one species) to 1 (equitable distribution of species). It is calculated as follows:

$$(J') = \frac{H'}{H'max} \qquad (2)$$

where *H'* = the Shannon-Weiner index and H'max = $\log_e$ of the total number of species S.

Frequency (F), *i.e.* the distribution or dispersion of individual species (Mukhopadhyay *et al.*, 2017), was estimated as percentage of occurrence calculated for the most representative plant species of each plot:

$$(F) = \frac{\text{Number of areas in which a species occurred}}{\text{Total number of areas studied}} \, x \, 100 \qquad (3)$$

Characterization of the most representative species

To analyse the behaviour of the single species detected in the site, plant species of the nine plots showing the highest soil coverage were identified and a variable number of plant samples (>3 when possible) were collected together with their respective rhizosoils. Samples of each species were separated in shoots and roots and then washed with tap water followed by distilled water, oven-dried at 60°C until constant weight and finely ground.

Composite samples of roots and shoots of each selected species were then analysed (acid digestion with *aqua regia* followed by ICP-MS) for PTE content. PTE concentrations were compared to PTE thresholds in forage, considering the native species as a potential pasture (EU Reg. 1275/2013). For metals not considered by this Regulation the mean values found in grasses grown on polluted sites (Kabata-Pendias, 2011) were used as reference.

Potential ecological risk assessment

With the soil contamination data, both in the nine plots and in rhizo-soil of the most representative species, the potential ecological risk index (ERI) was calculated. ERI represents the sensitivity of the biological community to toxic substances and shows the potential ecological risk caused by the overall contamination (Zhao and Li, 2013). The index is also useful to evaluate the adaptability of plant species to soil contamination, given that species growing in high-risk areas might be adapted to contamination. The equation used for calculating ERI (Hakanson, 1980) is:

$$\mathrm{ERI} = \sum_{i=1}^{n} E_r^i = \sum_{i=1}^{n} T_r^i \times C_f^i = \sum_{i=1}^{n} \left(T_r^i \times \frac{C^i}{C_n^i} \right) \qquad (4)$$

where: E_r^i is the monomial potential ecological risk index of the PTE i; T_r^i is the toxic response factor for a specific PTE i (*e.g.* As=10, Cd=30, Cr=2, Cu=5, Pb=5, Tl=10 and Zn=1); C_f^i is the contamination factor of PTE i; C^i is the content of PTE i in the samples (mg kg^{-1}), and C_n^i is the background value of PTE i in the study area (mg kg^{-1}). The toxic response factor for Tl is reported by Liu *et al.* (2018).

In this study, soil background values (BV) of the area from Cicchella *et al.* (2008) were used (As: 10.50; Cd: 0.45; Cr: 10; Cu: 28.50; Pb: 46.50; Zn: 78.0; Tl: 1.00 mg kg^{-1}). The contamination degrees and the potential ecological risk of a single PTE (E_r^i) were classified as low (E_r^i<40), moderate (40 ≤ E_r^i<80), considerable (80 ≤ E_r^i<160), high (160 ≤ E_r^i<320) and very high (E_r^i≥ 320). The overall ecological risk (ERI) was classified as low (ERI <95), moderate (95 ≤ERI <190), high (190 ≤ERI <380) and very high (ERI ≥380) (Rehman *et al.,* 2018).

Calculation of PTE accumulation indices

For each of the most representative species, the following indices were calculated for assessing the ability of plants to accumulate and translocate PTEs: bioaccumulation coefficient in shoots (BACs), bioaccumulation coefficient in roots (BAC_R) and translocation factor (TF). The BACs and BAC_R were calculated as the ratio between the concentration of PTEs in shoots and roots, respectively, and the concentration of PTEs in the rhizospheric soils (Putwattana *et al.,* 2015). The translocation factor was calculated as the ratio between the concentration of PTEs in shoots and that in roots (Baker and Brooks, 1989):

$$BAC_S = \frac{PTE\ concentration\ in\ shoots\ (mg\ kg^{-1})}{PTE\ concentration\ in\ soil\ (mg\ kg^{-1})} \tag{5}$$

$$BAC_R = \frac{PTE\ concentration\ in\ roots\ (mg\ kg^{-1})}{PTE\ concentration\ in\ soil\ (mg\ kg^{-1})} \tag{6}$$

$$TF = \frac{PTE\ concentration\ in\ shoots\ (mg\ kg^{-1})}{PTE\ concentration\ in\ roots\ (mg\ kg^{-1})} \tag{7}$$

To determine the capacity of plants to accumulate the bioavailable fractions of contaminants, a modified bioaccumulation coefficient (mBAC) was calculated for shoots and roots (Hamon and McLaughlin, 1999; Barbafieri *et al.,* 2011; Petruzzelli *et al.,* 2011) as follows:

$$mBAC_S = \frac{PTE\ concentration\ in\ shoots\ (mg\ kg^{-1})}{bioavailable\ PTE\ concentration\ in\ soil\ (mg\ kg^{-1})} \tag{8}$$

$$mBAC_R = \frac{PTE\ concentration\ in\ roots\ (mg\ kg^{-1})}{bioavailable\ PTE\ concentration\ in\ soil\ (mg\ kg^{-1})} \tag{9}$$

To evaluate the presence of hyperaccumulator plants we also compared PTE concentrations in shoots with reference values given by Van der Ent *et al.* (2013).

Statistical analysis

The statistical analyses were all carried out by using Ms Excel 2007 and SPSS 21 (SPSS Inc. Chicago, USA). Pearson correlation analyses were made to investigate the relationships between ERI, soil factors (pH, electric conductivity, organic carbon, total nitrogen, total and bioavailable PTEs) and ecological parameters (H', J', plant species richness and total plant cover) of each plot. Statistical significance in this analysis was defined at P<0.05 and P<0.01.

Results and discussion

Soil features of the nine plots

All the soils showed a good organic carbon content and a subalkaline pH, while EC and $CaCO_3$ were low for all rhizo-soils (Table 1). Soil texture was sandy loam (USDA) without differences among the plots with 15% of clay, 23% of silt and 62% of sand on average (data not shown). The soils of all nine plots showed good fertility, and thus cannot be considered limiting for plant growth.

Soil Pb concentrations ranged from 409 to over 100,000 mg kg^{-1} and exceeded the Italian screening values (SV) for industrial sites (DL 152/06: 1000 mg kg^{-1}) in 89% of cases (Table 2). Soil Cd concentration ranged from 1.6 to 298 mg kg^{-1} and was above SV (15 mg kg^{-1}) in 67% of plots. Soil As concentration ranged from 16 to 861 mg kg^{-1} and exceeded SV (50 mg kg^{-1}) in 56 % of cases.

Soil Cu, Cr, Tl and Zn concentrations were lower than SVs for industrial sites (Cu: 600 mg kg^{-1}; Cr: 800 mg kg^{-1}; Tl: 10 mg kg^{-1}; Zn: 1500 mg kg^{-1}) in all the plots. Due to the very high concentra-

Table 1. Selected soil physico-chemical properties in the plots monitored.

Plots	pH	Electrical conductivity (dS m^{-1})	$CaCO_3$ (g kg^{-1})	Organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})
1	7.2	197.0	15.0	26.9	2.5
2	7.2	174.8	21.4	42.4	6.3
3	7.1	231.0	13.1	42.5	4.4
4	7.0	472.0	87.5	68.3	9.7
5	6.9	524.4	51.9	55.7	4.0
6	7.2	311.5	46.4	48.9	5.5
7	7.4	398.5	41.0	55.9	1.3
8	7.4	238.5	64.1	31.4	0.5
9	7.5	500.9	81.1	16.9	0.6
Average	7.2	338.7	46.8	43.2	3.9
St. err	0.07	45.9	9.1	5.4	1.0

tion of some PTEs (mainly Pb and Cd), the ERI of the nine plots proved very high in 89% of cases, with values up to 26,556 (plot 5), 70 times higher than the maximum threshold reported by Rehman *et al.* (2018). These values were higher than other Pb contaminated sites (Ogunkunle and Fatoba, 2013; Jiang *et al.*, 2014; Kaddour *et al.*, 2017).

Plant community characteristics of the nine plots

In all, 34 species and 17 families were recorded in the nine plots (Table 2). The most abundant families were *Poaceae* and *Asteraceae*. The highest species diversity was reported in the plots with lower ecological risk and PTE content (plots 1, 3 and 4), while the lowest values were found in the plots with highest risk (plots 5 and 9), as also reported by Vidic *et al.* (2006) who reported that the highest biodiversity and evenness of spontaneous plant species were found in the plots with the lowest concentration of Cd, Pb and Zn furthest away from a lead smelter chimney. In particular, plot 5 had a very high concentration of PTEs, with invasive alien species such as *Artemisia annua* L. and *Erigeron sumatrensis* Retz. *Poaceae* were present in all nine plots, also with only one species in the most contaminated plots (5 and 9), *Asteraceae* were present in only 56% of plots, while *Fabaceae* were present with only one species in the least contaminated plot, thus suggesting a decreasing tolerance to soil contamination from *Poaceae* to *Asteraceae* to *Fabaceae,* confirming the results obtained by many authors in other countries (Wang *et al.*, 2004; Shu *et al.*, 2005; Weiersbye *et al.,* 2006; Gawronski and Gawronska, 2007; Mansfield *et al.,* 2014; Salas-Luevano *et al.,* 2017; Nguemte *et al.,* 2018). The overall soil cover of nine plots was always close to 100%, even in the most contaminated plots, thus confirming the findings of Bes *et al.* (2010) who found that elimination of the most PTE-sensitive species allows the more tolerant species to proliferate, which thus occupy the space left by the former.

Cd and Pb reported the highest contribution to the ecological risk index (Table 3). Cd contributed from 54 to 81 % of the ERI, while Pb contributed from 12 to 21% of the ERI, confirming that these elements must be considered the target PTEs of this site. Furthermore, these PTEs are considered the most hazardous also for human health. Indeed, the reference doses of oral soil ingestion

Table 2. PTE levels, ERI, biodiversity and plant cover for the plant communities of the plots.

Plots descriptors	1	2	3	4	5	6	7	8	9
As (mg kg^{-1})	14	34	19	16	861	87	92	94	97
Cd (mg kg^{-1})	1.6	41.9	13.9	5.3	298	91.7	97.6	63.1	153.2
Cr (mg kg^{-1})	44	48	47	48	69	61	62	44	55
Cu (mg kg^{-1})	145	218	339	133	609	606	252	181	340
Pb (mg kg^{-1})	409	10,505	2998	1567	100,000	26,538	26,600	17,508	39,263
Tl (mg kg^{-1})	1.8	1.7	2.2	1.6	1.6	8.6	5.7	2.1	3.8
Zn (mg kg^{-1})	217	251	209	178	450	401	250	172	429
ERI	198	3491	1209	512	26,556	7931	8226	5356	12651
Soil cover (%)	100	95	90	95	100	95	90	100	100
Shannon-W index (H’)	1.76	1.10	1.64	2.14	0.06	0.97	1.44	0.90	0.08
Pielou index (J’)	0.76	0.69	0.70	0.79	0.08	0.54	0.58	0.43	0.10
Species number	11	5	11	15	2	6	12	8	2
Poaceae	2	2	3	3	1	3	4	5	1
Fabaceae	1	0	0	0	0	0	0	0	0
Asteraceae	2	1	2	6	0	0	2	0	0
Miscellaneous species	6	2	6	6	1	3	6	3	1

Table 3. Mean values of ecological risk factor (E_r^i) and potential ecological risk index (ERI) in the different plots.

				E_r^i				ERI
Plots	As	Cd	Cr	Cu	Pb	Tl	Zn	
1	13	107	8.9	26	23	18.3	2.8	199
2	32	*2793*	9.6	38	*597*	17.6	3.2	*3491*
3	18	*927*	9.4	60	170	22.7	2.7	*1209*
4	15	*357*	9.7	23	89	16.1	2.3	*512*
5	*820*	*19907*	13.8	112	*5682*	16.6	5.8	*26,557*
6	83	*6113*	12.2	124	*1508*	86.4	5.1	*7932*
7	88	*6510*	12.5	44	*1511*	57.3	3.2	*8227*
8	90	*4207*	8.8	32	*995*	21.8	2.2	*5356*
9	92	*10,213*	11.0	60	*2231*	38.3	5.5	*12,651*
Average	139	*5681*	10.7	58	*1423*	32.8	3.6	*7348*
St. Err.	86	2096	0.6	12	589	8.1	0.5	2770

Very high risk is reported in italics.

(RfD in mg d^{-1} kg^{-1} body weight) given by USEPA (1989) are very low: 0.0010 for Cd and 0.0035 mg for Pb. It should be noted that Cd had the highest ecological risk, even though the Pb concentrations in soil were much higher. This was due to the higher toxicity of Cd for the biosphere even at low concentrations (Duri *et al.,* 2018; Zhao *et al.,* 2018). The degree of risk due to the other PTEs was lower, excepted for As in plot 5 (very high risk).

Correlation analysis (Table 4) revealed that the ecological risk index has the greatest deleterious effect on plant diversity and evenness ($P<0.01$), but also on the total number of species, as also reported by Andreucci *et al.* (2006) This effect was more evident in the miscellaneous group ($P<0.05$), confirming the findings of Koptsik *et al.* (2003). As regards the single elements, the total soil content of Cd, followed by Pb and Zn, affected the plant community in question, showing a significant negative correlation with diversity, evenness and species richness. The reduction in plant species diversity by these three elements has also been reported by others in industrial areas elsewhere (Vangronsveld *et al.,* 1996; Vidic *et al.,* 2006). This effect was more evident in the miscellaneous group that seemed to be the best indicators of soil PTE pollution while the other groups were unaffected by soil pollution, according to the results of Hernandez and Pastor (2008). The better adaption to soil contamination of *Poaceae* and *Asteraceae* has already been reported extensively elsewhere (Wang *et al.*, 2004; Shu *et al.*, 2005; Gawronski and Gawronska, 2007; Nguemte *et al.,* 2018) and confirmed that such families appear more resistant than other families to soil contamination.

Behaviour of the most representative species

An inventory of the 12 most representative species identified in the nine plots is shown in Table 5. *Artemisia annua* (native to East Europe and Asia) and *Sorghum halepense* (native to tropical areas of Africa and Asia) are considered invasive aliens in Italy. The most frequent families were *Poaceae* and *Asteraceae*. The most frequent species were *Holcus lanatus* subsp. *lanatus* (78% of plots) and *Silene latifolia* (56% of plots). Most of the inventoried species of *Asteraceae* family are common for areas contaminated by industrial waste like *Artemisia vulgaris* (Wojcik *et al.,* 2014), *Cirsium arvense* (Desjardins *et al.,* 2014) and *Poaceae* such as *Elymus repens* subsp. *repens* and *Dactylis glomerata* subsp. *glomerata* (Dygus, 2013). *Rubus ulmifolius* was also reported by Massa *et al.* (2010) in an industrial area in northern Italy.

Characterization of rhizo-soils of the more representative species

Soil PTE concentrations of the 12 rhizo-soils are shown in Table 6 along with PTE content in plants. Also the rhizo-soils showed very high PTE contents, reflecting average values found in the nine plots (see Table 2).

The ecological risk index calculated in the rhizo-soils of the 12 most representative species showed very high risk in all habitats except for *Artemisia vulgaris*, *Dittrichia viscosa* subsp. *viscosa* and *Epilobium tetragonum* subsp. *tetragonum*. The rhizosphere of *Rubus ulmifolius* and *Silene latifolia* showed the highest ERI values, thus suggesting a particular adaptability of the latter two species to PTE contamination, in agreement with Moreira *et al.* (2011) who reported the same tolerance in a site contaminated by Pb, As and Ni.

The Cu concentration in shoots ranged from 9 to 56 mg kg^{-1} with the highest value in *S. latifolia*, that showed concentrations above values recorded by Kabata-Pendias (2011) (21 mg kg^{-1}) along with *D. glomerata*, *C. arvense*, *E. repens* and *B. nigra*. Total

Table 4. Correlations between biodiversity markers for plant communities, ERI, pseudototal, bioavailability and other soil properties.

	Shannon index	Evenness index	Species number	Poaceae	Fabaceae	Asteraeae	Miscellaneous species	Plant cover
ERI	–0.84**	–0.88**	–0.71*	–0.47	–0.33	–0.54	–0.68*	0.37
Cu (mg kg^{-1})	–0.58	–0.53	–0.58	–0.33	–0.33	–0.55	–0.47	0.04
Cu DTPA (mg kg^{-1})	+0.20	+0.21	+0.14	–0.01	–0.22	0.07	0.27	–0.54
Pb (mg kg^{-1})	–0.80**	–0.84**	–0.68*	–0.46	–0.30	–0.51	–0.65	0.37
Pb DTPA (mg kg^{-1})	–0.41	–0.46	–0.18	+0.48	–0.48	–0.49	–0.16	–0.05
Zn (mg kg^{-1})	–0.82**	–0.79*	–0.81**	–0.66	–0.23	–0.59	–0.73*	0.32
Zn DTPA (mg kg^{-1})	+0.47	+0.39	0.46	–0.03	–0.35	0.73	0.39	–0.47
As (mg kg^{-1})	–0.63	–0.68	–0.54	–0.43	–0.18	–0.36	–0.53	0.37
As DTPA (mg kg^{-1})	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cd (mg kg^{-1})	–0.85**	–0.89**	–0.72*	–0.47	–0.33	–0.55	–0.69*	0.37
Cd DTPA (mg kg^{-1})	–0.22	–0.23	–0.07	0.29	–0.33	–0.32	0.01	–0.32
Cr (mg kg^{-1})	–0.55	–0.59	–0.42	–0.32	–0.37	–0.33	–0.35	–0.06
Cr DTPA (mg kg^{-1})	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tl (mg kg^{-1})	–0.13	–0.07	–0.09	0.21	–0.55	–0.31	0.00	–0.31
Tl DTPA (mg kg^{-1})	–0.59	–0.60	–0.49	–0.24	–0.05	–0.53	–0.39	0.16
Ph-H_2O	–0.50	–0.48	–0.43	–0.06	–0.04	–0.55	–0.36	0.22
EC (μS cm^{-1})	–0.42	–0.62	–0.20	–0.36	–0.39	0.12	–0.24	0.17
Carbonates (g kg^{-1})	–0.50	–0.61	–0.35	–0.29	–0.31	–0.08	–0.43	0.41
OC (%)	+0.38	+0.30	+0.46	0.20	–0.38	0.56	0.39	–0.50
Total N (%)	+0.48	+0.50	+0.35	–0.12	–0.17	0.65	0.25	–0.29

**Significant at the 0.01 level; *significant at the 0.05 level; n.d., not detectable.

Cu concentrations in roots ranged from 15 to 70 mg kg^{-1} with the highest accumulation in *S. latifolia*.

Zn shoot concentrations ranged from 23 to 69 mg kg^{-1}, with the maximum in *D. glomerata*. Zn shoot concentrations above the value of 31.5 mg kg^{-1}, recorded by Kabata-Pendias (2011) in plants grown in polluted soils, were reported for all the species except for *A. vulgaris*, *E. tetragonum*, *R. ulmifolius* and *B. nigra.* Zn concentration in the roots ranged from 13 to 97 mg kg^{-1}, with the maximum concentration observed for *E. tetragonum*.

Shoot Cd concentrations ranged from 0.2 to 9.4 mg kg^{-1}, with the maximum concentration in *A. annua*. All plant species except *A. vulgaris, D. viscosa and E. tetragonum* accumulated Cd above the threshold of 1.0 mg kg^{-1} for forage (EU Reg. 1275/2013), suggesting a potential pollutant transfer to food chains in nearby areas by soil resuspension. Cd concentrations in roots ranged from 0.3 to 41.2 mg kg^{-1}, the maximum value occurring in *S. latifolia*.

Pb shoot concentrations ranged from 32 to 326 mg kg^{-1}, reaching a maximum in *D. glomerata*. All plant species accumulated Pb above legal PTE thresholds in plants (EU REG 1275/2013 30 mg

Table 5. Botanical characteristics of the species collected from the site, analysis of frequency and abundance.

Species	Family	Frequency (%)
Holcus lanatus L. subsp. *lanatus*	*Poaceae*	77.8
Silene latifolia Poir.	*Caryophyllaceae*	55.6
Elymus repens (L.) Gould subsp. *repens*	*Poaceae*	44.4
Epilobium tetragonum L. subsp. *tetragonum*	*Onagraceae*	44.4
Dactylis glomerata L. subsp. *glomerata*	*Poaceae*	33.3
Dittrichia viscosa (L.) Greuter subsp. *viscosa*	*Asteraceae*	33.3
Artemisia annua L.	*Asteraceae*	22.2
Artemisia vulgaris L.	*Asteraceae*	22.2
Cirsium arvense (L.) Scop.	*Asteraceae*	22.2
Rubus ulmifolius Schott	*Rosaceae*	22.2
Ballota nigra L. subsp. *meridionalis* (Bég.) Bég.	*Lamiaceae*	11.1
Sorghum halepense (L.) Pers.	*Poaceae*	11.1

Table 6. PTE concentrations (mg kg^{-1} d.w.) in plants and rhizo-soils of the native species.

Species		As	Cd	Cr	Cu	Pb	Tl	Zn	ERI
Artemisia annua	Soils	17.0	4.9	51.0	107	1428	1.70	148	472
	Shoots	0.4	9.4	2.1	14	106	0.10	40	
	Roots	2.1	8.5	3.9	33	321	0.60	38	
Artemisia vulgaris	Soils	15.0	0.7	37.0	67	208	1.70	121	111
	Shoots	0.3	0.4	1.6	9	33	0.04	23	
	Roots	0.4	0.4	1.8	15	17	0.30	18	
Ballota nigra subsp. *meridionalis*	Soils	86.0	55	65	187	21135	5.90	184	5057
	Shoots	0.6	5.9	2.5	35	176	1.70	31	
	Roots	0.1	20.0	1.7	28	671	4.10	34	
Cirsium arvense	Soils	22.0	21.9	47.0	572	4663	2.10	281	1880
	Shoots	0.1	3.8	3.8	24	213	0.20	54	
	Roots	0.3	7.6	1.8	39	197	0.60	47	
Dactylis glomerata subsp. *glomerata*	Soils	54.5	50.7	47.0	155	11795	2.30	164	4167
	Shoots	0.3	3.8	7.0	44	323	0.30	69	
	Roots	2.6	17.9	3.1	47	590	0.90	53	
Dittrichia viscosa subsp. *viscosa*	Soils	13.0	2.5	52.0	224	609	1.90	313	286
	Shoots	0.1	0.8	2.0	15	47	0.10	33	
	Roots	0.9	0.3	10.2	20	16	0.40	13	
Elymus repens subsp. *repens*	Soils	70.0	59.7	52.0	477	16085	5.20	278	5102
	Shoots	0.2	5.2	2.7	28	283	1.00	44	
	Roots	1.8	26.2	2.3	38	1407	1.80	67	
Epilobium tetragonum tetragonum	Soils	14.0	2.1	49.0	160	561	1.70	235	224
	Shoots	0.1	0.2	1.6	12	32	0.03	28	
	Roots	0.1	1.8	2.5	50	101	0.20	97	
Holcus lanatus subsp. *lanatus*	Soils	15.0	5.8	46.0	159	1707	1.50	208	553
	Shoots	0.3	1.3	2.5	11	70	0.10	33	
	Roots	1.0	4.9	3.3	46	358	0.40	71	
Rubus ulmifolius	Soils	479.0	225	62.0	490	69631	2.70	439	19,604
	Shoots	0.1	2.0	2.5	14	106	0.20	29	
	Roots	0.2	3.6	2.6	32	174	0.60	48	
Silene latifolia	Soils	159.0	175	67.0	460	49647	9.40	437	14,873
	Shoots	0.9	7.7	2.9	56	217	102.50	41	
	Roots	20.7	41.2	3.1	70	3404	44.00	81	
Sorghum halepense	Soils	54.0	81.7	47.0	276	20449	1.80	267	6739
	Shoots	0.4	1.0	2.4	16	74	0.20	50	
	Roots	0.1	2.2	2.8	36	76	0.30	72	

kg^{-1}). This behaviour suggests, as for Cd, a potential Pb transfer to the food chain by dust lift. Root Pb concentration ranged from 16 to as high as 3404 mg kg^{-1}. The maximum root PTE content was reported in *S. latifolia*.

As concentrations in shoots ranged from 0.1 to 0.9 mg kg^{-1}. Despite the high concentrations of As in soils, no plant species accumulated As above the threshold for forage of 2.0 mg kg^{-1} (EU Reg 1275/2013). Concentrations of As in plant roots ranged from 0.05 to 20.70 mg kg^{-1}. The highest concentration in shoots and roots was reported in *S. latifolia.*

Cr shoot concentrations ranged from 1.6 to 7.0 mg kg^{-1}, with a maximum concentration in *D. glomerata* while Cr root concentrations ranged from 1.65 to as high as 10.20 mg kg^{-1}, the maximum being found in the roots of *D. viscosa*.

Tl concentrations ranged from 0.03 to 102.54 mg kg^{-1}, with maximum concentrations observed in *S. latifolia*. Only *S. latifolia*, *E. repens* and *B. nigra* accumulated Tl above the concentrations of terrestrial plants according to Kabata-Pendias (2011) (0.51 mg kg^{-1}). Tl concentrations in the roots ranged from 0.21 to 43.99 mg kg^{-1}, the maximum occurring in *S. latifolia*.

Almost all collected species showed higher than normal PTE concentrations. These results indicate that the species were tolerant to such metals in varying degrees. In particular, *S. latifolia* accumulated the highest concentration of As, Cd, Cu, Tl and Pb in its roots. This behaviour had already been observed in the *Silene* genus (Chaabani *et al.,* 2017; Wojcic *et al.,* 2017; Yildirim *et al.,* 2017) and some species ((*Silene vulgaris* (Moench) Garcke)) are perennial facultative metallophytes with high tolerance to multi-element polluted soils (Schat *et al.*, 1996). Such characteristics, along with a wide range of adaptation (Sloan *et al.,* 2012), make this plant genus of great interest for phytoremediation purposes (Garcia-Gonzalo *et al.*, 2017). *D. glomerata* accumulated the highest concentration of Zn, Pb and Cr in its shoots, confirming the results of Swiercz *et al.* (2015) who reported a high concentration of the three elements in a two-cycle pot experiment.

None of the species showed metal concentrations that allow them to be defined as hyperaccumulators according to the concentration criteria (100 mg kg^{-1} for Cd, Se and Tl; 300 mg kg^{-1} for Co, Cu and Cr; 1000 mg kg^{-1} for Ni, Pb and As; 3000 mg kg^{-1} for Zn; and 10,000 mg kg^{-1} for Mn) of Van der Ent *et al.* (2013) except for *Silene latifolia* which accumulated in its shoots Tl concentrations above 100 mg kg^{-1}. This result finds agreement with Escarrè *et al.* (2011). BAC$_S$, BAC$_R$ and TF values are shown in Table 7. Data pertaining to the element As are not shown because none of the

Table 7. BAC$_S$, BAC$_R$ and TF of native plant species.

Plant species		Cd	Cr	Cu	Pb	Tl	Zn
Artemisia annua	TF	*1.10*	0.54	0.42	0.33	0.23	*1.04*
	BAC$_S$	*1.92*	0.04	0.13	0.07	0.08	0.27
	BAC$_R$	*1.74*	0.08	0.30	0.22	0.36	0.26
Artemisia vulgaris	TF	0.89	0.89	0.59	*1.95*	0.12	*1.28*
	BAC$_S$	0.57	0.04	0.14	0.16	0.02	0.19
	BAC$_R$	0.64	0.05	0.23	0.08	0.19	0.15
Ballota nigra subsp. *meridionalis*	TF	0.30	*1.47*	*1.24*	0.26	0.41	0.91
	BAC$_S$	0.11	0.04	0.19	0.01	0.28	0.17
	BAC$_R$	0.36	0.03	0.15	0.03	0.69	0.19
Cirsium arvense	TF	0.50	*2.11*	0.62	*1.08*	0.33	*1.16*
	BAC$_S$	0.17	0.08	0.04	0.05	0.09	0.19
	BAC$_R$	0.35	0.04	0.07	0.04	0.28	0.17
Dactylis glomerata subsp. *glomerata*	TF	0.21	*2.26*	0.95	0.55	0.29	*1.30*
	BAC$_S$	0.07	0.15	0.29	0.03	0.12	0.42
	BAC$_R$	0.35	0.07	0.30	0.05	0.42	0.32
Dittrichia viscosa subsp. *viscosa*	TF	*2.52*	0.20	0.72	*2.84*	0.15	2.52
	BAC$_S$	0.33	0.04	0.07	0.08	0.03	0.11
	BAC$_R$	0.13	0.20	0.09	0.03	0.22	0.04
Elymus repens subsp. *repens*	TF	0.20	*1.14*	0.73	0.20	0.54	0.65
	BAC$_S$	0.09	0.05	0.06	0.02	0.19	0.16
	BAC$_R$	0.44	0.05	0.08	0.09	0.35	0.24
Epilobium tetragonum tetragonum	TF	0.10	0.64	0.24	0.31	0.14	0.29
	BAC$_S$	0.08	0.03	0.07	0.06	0.02	0.12
	BAC$_R$	0.84	0.05	0.31	0.18	0.12	0.41
Holcus lanatus subsp. *lanatus*	TF	0.26	0.76	0.23	0.20	0.24	0.47
	BAC$_S$	0.22	0.05	0.07	0.04	0.07	0.16
	BAC$_R$	0.85	0.07	0.29	0.21	0.29	0.34
Rubus ulmifolius	TF	0.56	0.98	0.42	0.61	0.40	0.61
	BAC$_S$	0.01	0.04	0.03	0.00	0.08	0.07
	BAC$_R$	0.02	0.04	0.07	0.00	0.21	0.11
Silene latifolia	TF	0.19	0.94	0.80	0.06	*2.33*	0.50
	BAC$_S$	0.04	0.04	0.12	0.00	*10.90*	0.09
	BAC$_R$	0.23	0.05	0.15	0.07	*4.68*	0.19
Sorghum halepense	TF	0.47	0.86	0.44	0.97	0.65	0.70
	BAC$_S$	0.01	0.05	0.06	0.00	0.12	0.19
	BAC$_R$	0.03	0.06	0.13	0.00	0.19	0.27

bioaccumulation coefficients reported values higher than one. As regards Cd, *A. annua* showed BAC$_S$, BAC$_R$ and TF higher than 1, with a high concentration in the shoots. For Tl *S. latifolia* reported BAC$_S$, BAC$_R$ and TF higher than 1, confirming the hyperaccumulator hypothesis of this plant species. According to our results, *S. latifolia* and *A. annua* have the potential to be used for phytoextraction of Cd and Tl, respectively. The modified bioaccumulation coefficient for the shoots (mBAC$_S$) and the modified bioaccumulation coefficient for the roots (mBAC$_R$) for each species were also calculated for Cd and Pb, the most hazardous elements (Table 8).

According to the values listed in Table 8, *D. viscosa* and *A. annua* for Cd, *A. vulgaris*, *D. viscosa* and *E. tetragonum* for Pb, reported the highest ability to accumulate the potential bioavailable fraction to their shoots (mBAC$_S$>1). Nevertheless, their use in phytoextraction is not recommended because of their low biomass produced. Indeed, for maximising the amount of PTE uptake per unit area, high biomass-yielding crops (such as *Arundo donax* L.) are preferred even if they show lower PTE concentrations (Fiorentino *et al.,* 2013; 2017).

Table 8. Soil DTPA, extracted Pb and Cd, mBACS and mBACR of native plant species.

Plant species		Cd	Pb
Artemisia annua	DTPA	3.6 (mg kg^{-1})	304.60 (mg kg^{-1})
	mBAC$_S$	*2.61*	0.35
	mBAC$_R$	*2.36*	*1.05*
Artemisia vulgaris	DTPA	0.6 (mg kg^{-1})	23.60 (mg kg^{-1})
	mBAC$_S$	0.63	*1.42*
	mBAC$_R$	0.71	0.73
Ballota nigra subsp. *meridionalis*	DTPA	60.8 (mg kg^{-1})	1660.00 (mg kg^{-1})
	mBAC$_S$	0.10	0.11
	mBAC$_R$	0.33	0.40
Cirsium arvense	DTPA	13.0 (mg kg^{-1})	852.00 (mg kg^{-1})
	mBAC$_S$	0.29	0.25
	mBAC$_R$	0.59	0.23
Dactylis glomerata subsp. *glomerata*	DTPA	25.5 (mg kg^{-1})	3103.40 (mg kg^{-1})
	mBAC$_S$	0.15	0.10
	mBAC$_R$	0.70	0.19
Dittrichia viscosa subsp. *viscosa*	DTPA	0.3 (mg kg^{-1})	31.46 (mg kg^{-1})
	mBAC$_S$	*3.03*	*1.50*
	mBAC$_R$	*1.20*	0.53
Elymus repens subsp. *repens*	DTPA	143.2 (mg kg^{-1})	6598.00 (mg kg^{-1})
	mBAC$_S$	0.04	0.04
	mBAC$_R$	0.18	0.21
Epilobium tetragonum tetragonum	DTPA	1.2 (mg kg^{-1})	26.60 (mg kg^{-1})
	mBAC$_S$	0.14	*1.19*
	mBAC$_R$	*1.41*	*3.79*
Holcus lanatus subsp. *lanatus*	DTPA	3.1 (mg kg^{-1})	382.20 (mg kg^{-1})
	mBAC$_S$	0.40	0.18
	mBAC$_R$	*1.56*	0.94
Rubus ulmifolius	DTPA	42.6 (mg kg^{-1})	2576.00 (mg kg^{-1})
	mBAC$_S$	0.05	0.04
	mBAC$_R$	*0.08*	*0.07*
Silene latifolia	DTPA	46.8 (mg kg^{-1})	1754.00 (mg kg^{-1})
	mBAC$_S$	0.16	0.12
	mBAC$_R$	0.88	*1.94*
Sorghum halepense	DTPA	1.2 (mg kg^{-1})	117.60 (mg kg^{-1})
	mBAC$_S$	0.88	0.63
	mBAC$_R$	*1.88*	0.65

D. viscosa, E. tetragonum, S. halepense, A. annua and H. lanatus were efficient at accumulating bioavailable Cd in roots (mBAC$_R$>1), while *E. tetragonum*, *A. annua* and *S. latifolia* accumulated Pb efficiently in roots (mBAC$_R$>1). The high accumulation efficiency of Cd on the part of *D. viscosa* was also reported by Barbafieri *et al.* (2011) in a mining area. Instead, *E. tetragonum* was also native of an industrial area (Moreira *et al.,* 2011) where showed a high Zn accumulation. *H. lanatus* displayed the potential for phytoremediation of a mining area (Favas and Pratas, 2015) while *S. halepense* was studied previously in a battery recycling site (Salazar *et al.* 2014) where it showed good Pb phytostabilization capacity. *S. latifolia* was only found spontaneously growing in a mining area by Escarrè *et al.* (2011) with a high accumulation of Tl in its shoots. Thus the above species may be considered suitable for Cd and Pb phytostabilization protocols aimed at revegetating the area, stabilizing the soil with their root systems. In addition, they can act as a barrier limiting wind erosion and the consequent dispersion of contaminated soil particles in the environment.

Conclusions

The composition of native vegetation can give interesting information about the distribution of contamination and of the consequent ecological risks in contaminated industrial sites. Our findings indicate that plant species diversity is negatively affected by PTE contents. *Poaceae* and *Asteraceae* species were more tolerant to contamination, while *Fabaceae* and other families were strongly affected by the very high levels of PTEs. Calculation of the ecological risk index afforded insights into the impact of contaminants on ecosystems, highlighting the fact that cadmium is more hazardous than lead despite its lower concentrations due to its higher toxicity for the biosphere.

Comparing PTE contents in plant biomass with thresholds fixed for forage crops can provide useful indications on potential PTE transfer in the food chain. Analyses of Cd and Pb accumulation in plants, above the threshold for forage, confirmed their hazardousness, suggesting that there might be a potential transfer of pollutants to the food chain through the dispersion and fallout of contaminated soil particles in surrounding farmland, thus reinforcing the need of a barrier to reduce contaminant mobility.

In addition, both analysis of PTE concentrations in plants and calculation of bioaccumulation and translocation factors allowed species suitability for PTE phytoextraction or phytostabilization to be determined. *Silene latifolia* was identified as a hyperaccumulator of Tl. *Holcus lanatus* subsp. *lanatus* and *S. latifolia* were the most frequent species on the site and also proved well adapted to the site-specific conditions, growing in areas with the highest ERI. The above two species also proved the most suitable for phytostabilization respectively of Cd and Pb, accumulating the two elements in their roots. They could therefore be used in association to increase soil cover during the summer in order to avoid generally more intense wind erosion during the dry season.

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Assisted phytoremediation for restoring soil fertility in contaminated and degraded land

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Abstract

The results of experiments carried out in the National Interest Priority Site (NIPS) Agro Aversano-Litorale Domizio in Campania (southern Italy) within the framework of the project ECOREMED (LIFE11 ENV/IT/275) are used as a basis for reporting the main threats to soil fertility. In this paper we focus on soil degradation and contamination due to illegal waste disposal and burning, widespread phenomena in the NIPS in question, evaluating different options for soil remediation with agriculture-based techniques in light of their impact on the ecosystem services of soils. Bioremediation and assisted phytoremediation are recognized as the best options for protecting human health and enhancing environmental quality, maintaining agricultural soil functions with the application of relatively low-cost protocols.

The purpose of agriculture-based remediation techniques is threefold: i) make the area in question safe, interrupting exposure to contaminant pathways through ecological structures such as dense reed plantations or dense turfgrass combined with high-density tree rows for reducing ground wind speed; ii) remediation, aiming to reduce the bioavailable fraction of potentially toxic elements (PTEs); iii) environmental restoration, to improve environment and landscape quality of degraded, but not contaminated land. The technical steps for carrying out phytoremediation projects and the results of their application are described for the following case studies monitored in Campania: i) agricultural soil contaminated by bioavailable Cd (*San Giuseppiello* site in Giugliano); ii) industrial soil heavily contaminated by Pb and Cd (*Ecobat* site in Marcianise); iii) agricultural soils potentially contaminated by non-bioavailable PTEs and organic pollutants (*Giugliano* and *Trentola-Ducenta* sites); iv) physically degraded soil (*Teverola* site). In all the case studies phytoremediation proved a low-cost tool to reduce risks for human health and enhance environmental quality, whilst maintaining soil fertility and improving ecosystem services.

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Introduction

Soil fertility

Soil plays a pivotal role in regulating natural and socio-economic processes for sustaining human survival. It is a critical component of food and water safety that represents the main current and future challenge of balancing human activities against protection of the environment (Hatfield *et al.*, 2017). Soils are a biodiverse pool of habitats, flora and fauna species and genes, as well as an archive of geological and archaeological heritage (European Commission, 2006). The ability to perform such functions is referred to as *soil fertility*, recognized as the interrelation between: i) chemical properties such as the availability of micro- and macro-nutrients and organic matter (SOM) content, pH, salinity, cation exchange capacity (CEC); ii) physical properties, or the stability and dimension of soil aggregates originating from the interaction between mineral and organic soil components, that are strongly related to soil porosity, air content and water retention; iii) biological properties, or the balanced interaction between micro-macro biota living in the soil. These three aspects are closely interrelated. Therefore their balance is responsible for soil fertility in its entirety. By way of example, in a highly unstructured soil (poor physical fertility) with low oxygen availability due to the soil's high micro-porosity, the activity of aerobic N cycling bacteria is severely limited as well as the availability of this macronutrient for crops. Therefore, low oxygen availability can limit root growth and reduce soil colonization by vegetation.

Soil organic matter is the main pillar of soil fertility (Doran and Parkin, 1994), as its content is linked with numerous soil functions (Franzluebbers, 2002), namely nutrient retention, organic C storage, soil aggregation and microbial diversity and growth (Murphy, 2015). In degraded soils (*i.e.* poor fertility, low organic matter content and compacted), the microbial equilibrium is broken with a severe reduction in biodiversity (Singh, 2003) and a significant increase in pathogenic or parasitic rather than beneficial organisms (Abawi and Widmer, 2000). Therefore soil management with a view to increasing its organic C content is pivotal to enhancing crop productivity and all the above aspects of soil fertility.

Among agronomic practices, tillage has the greatest impact on SOC decline, since it increases residues and OM oxidative degradation and disrupts soil structure (Holland, 2004). The loss of SOC reduces air/water movements and physical fertility (high bulk density, penetration resistance and low porosity) as well as chemical fertility (high denitrification, low cation and anion exchange capacity and availability of N, P, and S). Consequently, low quantity and quality of SOM induce a progressive depletion in microbial biodiversity (Fierer *et al.*, 2009). At global scale, soil is a source or sink of OC, contributing to CO_2 emissions and sequestration balance (Jones *et al.*, 2005; Schils *et al.*, 2008). The most effective strategies to optimize SOC sequestration are to reduce soil disturbance (*i.e.* conservative tillage) and the increase in C inputs through cover crops and organic fertilization (*i.e.* compost) (Alluvione *et al.*, 2013). Furthermore, SOC makes soil more resilient and resistant to soil against compaction, erosion and biodiversity loss and limits pollution diffusion through chelation and adsorption of potentially toxic compounds.

Soil degradation

Soil degradation involves the partial or total impairment of soil ecosystem functions. Complex interactions between natural factors (*e.g.* soil, climate, vegetation cover, topography) and anthropogenic processes (*e.g.* urbanization, land management, overgrazing) are involved in soil degradation. The European Union has acknowledged ten threats to soil functions: i) water and wind erosion; ii) decline in organic matter; iii) contamination; iv) sealing; v) compaction; vi) soil biodiversity loss; vii) salinization; viii) floods and landslides; ix) desertification; x) acidification (JRC, 2011; European Commission, 2006a; 2006b; 2012). In Italy, the most vulnerable soils are located in Southern coastal and upland areas, in the main islands and in some plain areas of Northern Italy with high population density (Costantini *et al.*, 2009; Salvati *et al.*, 2011).

After soil sealing, *soil erosion* and mass movements are the most widespread causes of land degradation in Europe: about 13% of arable land is subjected to unsustainable soil losses (>5 t $ha^{-1}yr^{-1}$) (Panagos *et al.*, 2015). What has most impact on land degradation is the action of water rather than wind (JRC, 2011). Human activities involving mismanagement and intensive cultivation of soils, deforestation, overgrazing and urbanization exacerbate soil erosion. Low or absent vegetation cover and crop residues expose soil to the direct impact of rainfall and consequent water run-off (García-Ruiz, 2010). Tillage strongly affects the detachment processes of soil, having both direct and indirect effects on its properties (Holland, 2004), such that soil losses in the Mediterranean are mainly concentrated from August to October, just after the soil has been tilled (Diodato *et al.,* 2009; Fagnano *et al.,* 2012).

Compaction affects around 36% of European soils (JRC, 2011), with a similar percentage of Italian soils being affected, especially in hills and lowland areas with their fine soil texture and low organic carbon content (APAT, 2017). In agriculture, most soil compaction is caused by farm machinery, soil tillage and livestock (Hamza and Anderson, 2005). Severe soil compaction by heavy machinery can also occur in temporary disposal sites or in construction sites, leading to a substantial reduction in infiltration rates from 70 to 99% (Gregory *et al.*, 2006). Compacted soils show high bulk density and low porosity that decrease air permeability and hydraulic conductivity, thus altering biogeochemical patterns (Pagliai *et al*., 2000). Plant growth and yield are affected by limited root development, elongation and accessibility of nutrients (Pagliai *et al.*, 2000).

The accumulation of water-soluble salts in soil, known as *salinization*, affects around 3 million ha of European soils, mainly situated in arid areas of the Mediterranean (JRC, 2011). One of the main causes of salinization is the unsustainable use of groundwater resources leading to salt water intrusion in coastal lowlands (Salvati *et al*., 2011). Further, inappropriate irrigation practices, such as use of brackish water or insufficient drainage, can contribute to the accumulation of salts in soil. Such phenomena are likely to be aggravated in the future as a result of increased evapotranspiration and water demand due to climate change (Costantini and Lorenzetti, 2013). The increase in salt content of soil circulating solution has adverse effects on soil structural stability, bulk density and permeability, facilitating surface crust formation (Tejada and Gonzalez, 2005). In addition, salinization can inhibit plant growth, influencing water availability, gas exchange, photosynthesis and protein synthesis (Maggio *et al.,* 2011).

Contamination consists in the external input of xenobiotics such as inorganic/organic potentially toxic compounds that are not present in natural soils of a specific area and that could represent a risk for human and ecosystem health (Fagnano, 2018). In Europe, there are estimated to be about 2.5 million potentially contaminated sites (*i.e.* sites where there is evidence of polluting activities but where detailed information and assessment is lacking), about 14% of which (340,000 sites) are contaminated and need remediation measures. Disposal and treatment of waste, whether municipal or industrial, as well as industrial and commercial activities, are responsible for two thirds of local contamination mainly due to potentially toxic elements (35%), mineral oils (24%) and polycyclic aromatic hydrocarbons (11%) (Van Liedekerke *et al.*, 2014). Effects of these contaminants on animal and human health and on the environment depend on many factors such as chemical properties and form, bioavailability and toxicity, potential for dispersion, and solubility in water or fat. As potentially toxic elements (PTEs) are neither biologically nor chemically degraded, they are long-lasting and accumulate in soil (Bolan *et al.*, 2014). However, soils are the last repository for most hydrophobic organic contaminants from environmental matrices, such as hydrocarbons and polycyclic aromatic hydrocarbons (PAHs), which are rapidly absorbed on particles and organic matter (Latimer and Zheng, 2003). In agricultural areas, PTEs may be taken up by plants and consequently transferred to human beings through the food chain (Kidd *et al*., 2015). In residential and industrial areas, the risks are mostly ascribed to erosion and air-borne dispersion of polluted soil particles that can be transferred to humans through ingestion, inhalation and dermal contact (Heal *et al.,* 2012), as well as to pollutant leaching into the groundwater (Prasanna *et al.,* 2011).

Agronomic approaches to mitigating soil degradation

The cultivation of perennial crops on degraded Mediterranean farmland is a promising solution to restore soil fertility and improve soil ecosystem services. Such a practice would reduce soil tillage, increase soil cover and result in the consequent litter effect. Among perennial crops, biomass species, such as feedstock for bioenergy or bio-based products, could also increase farm income, thus helping to reduce abandonment and depopulation of inland hill areas (Fagnano *et al.*, 2015; Bonfante *et al.*, 2017; Impagliazzo *et al.*, 2017). The environmentally safe use of such biomasses even in the event of their contamination by PTEs has been proved by using pyrolysis (Giudicianni *et al.,* 2017a, b). Bioenergy crop cultivation on good quality agricultural soil, that could be used for food production, is generating land-use conflict. Such negative effects could be limited by growing non-food bioenergy perennial crops on marginal or degraded land, unsuitable and often economically unattractive for agricultural food production. If appropriately managed, perennial biomass crops minimise greenhouse gas (GHG) emissions (Smith and Olesen, 2010) without depleting soil nutrients, water supplies, or negatively impacting biological and landscape diversity (Forte *et al.*, 2015; Zucaro *et al.*, 2015). Deep dense root systems and permanent vegetative cover allow a reduction in runoff and soil erosion (Fagnano *et al.*, 2015; Fernando *et al*., 2015). Low soil disturbance due to the reduced need for tillage and agricultural practices decreases soil compaction, thus providing benefits to soil structure, porosity and biodiversity, that is considered as pillars of soil ecosystem functions (Brussaard, 2007). Consequently, the cultivation of perennial crops enhances organic matter content, thus extending nutrient storage and availability, as well as the capacity to chelate and filter contaminants, thereby reducing their diffusion (Pardo *et al.,* 2014; Barbosa *et al.*, 2015; Arco-Lazaro *et al.,* 2017). However, compared with degraded land, where the vegetation cover is often low and sparse, perennial crop cover may have a strong positive impact on aesthetic landscape values, also reducing the risk of inappropriate use. In addition, by producing bioenergy from degraded and marginal land such crops can contribute to supporting social and economic development of the local farming community through the use of land with little or no previous productivity (Bonfante *et al.*, 2017).

Phytoremediation

Protocols for reducing contaminant levels in soils include chemical and physical treatments with a very high efficiency, always associated with high soil disturbance. This means that conventionally remediated soils present a significant reduction in contaminants, balanced by severe drawbacks regarding physical, chemical and biological fertility (Gil-Díaz *et al.,* 2016). In addition, physical and chemical treatments can prove costly and hence may not be applicable at a large scale (Gil-Díaz *et al.,* 2016). This is why the most frequently adopted strategy for remediating soil consists in soil excavation and consequent disposal.

In a substantial proportion of cases, landscape degradation and reduction in chemical and physical fertility are the main constraints affecting agroecosystem productivity. In such cases, rather than a reduction in total contaminant levels, the pathways of contaminant exposure have to be blocked off, thereby reducing risks for human health and the environment potentially resulting from their dispersion towards other environmental compartments (*i.e.* air and groundwater) (Kuppusamy *et al.*, 2017). Phytoremediation consists of a pool of agricultural techniques aimed at reducing the concentration or the risk related to the presence of organic and inorganic contaminants by using plants and soil fertilization (or amendment) (Greipsson, 2011; Ali *et al.*, 2013). In a broader perspective phytoremediation may have the following aims (Vangronsveld *et al.*, 2009): i) metal accumulation in plant tissues (phytoextraction); ii) risk management: gentle soil remediation, phytostabilization and/or making the site safe; iii) increase in soil fertility and improvement in its ecosystem services.

Phytoextraction serves to increase accumulation of PTEs in easily harvestable crops in order to concentrate metals in biomass to be disposed of or used in other chemical conversion processes. The main PTE sinks are trunks, culms and leaves (Halim *et al.,* 2003) and in some cases also belowground organs such as rhizomes (Fiorentino *et al.*, 2017).

PTE uptake can be significantly increased by selecting appropriate species and enhancing PTE transfer to plant tissues with specific cropping techniques. This process can significantly reduce the bioavailable fraction of PTEs to values below the risk thresholds. The most suitable species for phytoextraction may have several of the following characteristics (Alkorta *et al.,* 2004): i) tolerance to high PTE content; ii) PTE accumulation in easily harvestable organs; iii) fast-growing biomass; iv) high biomass yield; v) high root growth; vi) easy cropping management; vii) genetically stable attributes; viii) biomass useful for energy production or *green* chemistry; ix) not appreciated by grazing animals.

Short rotation forestry (SRF) crops (*e.g.* poplar, eucalyptus, willow) fit perfectly with the above-mentioned attributes, proving to be appropriate for both reclaiming soils and producing biomass (Abhilash *et al.,* 2012). SFR-based phytoremediation allows high productivity of (potentially) polluted areas to be maintained, lowering soil PTE content below the thresholds of risk without any detrimental effect on soil fertility. Indeed, perennial crop cultivation minimises soil disturbance (no tillage), favouring C storage in the soil with the conversion of crop residues (*e.g.* litter) and root exudates into soil organic matter (SOM).

Another tool consists in using *Brassicaceae* species (*e.g. Brassica juncea* L., known as Indian mustard). These herbaceous crops are known to be hyperaccumulators of several PTEs, meaning that PTE content in their tissues is usually several orders of magnitude higher than in other plants. Finally, there are some lignocellulosic herbaceous crops for biomass production, such as *Arundo donax* L (giant reed) with a preferential allocation of PTEs in rhizomes suitable for harvest at the end of the phytoremediation programme with a significant removal of PTEs from the soil-root layer. A short list of some crops which may be used for phytoextraction is provided in Table 1.

Phytostabilization is the use of plants to reduce PTE bioavailability and mobility in the secondary source of pollution (contaminated soil) (Sylvain *et al.,* 2016) and limit exposure for residents or workers who frequent a site. Another well-recognized mechanism of phytostabilization is the reduction of bioavailable PTEs in the vadose zone through root compartmentalization and metal precipitation (Bolan *et al.,* 2011). This process has the key role of protecting groundwater from contamination (Barbosa *et al.,* 2015; Palladino *et al.*, 2018). In addition, there are reduction/precipitation mechanisms that inactivate the toxicity of metals in the root layer, thus limiting their availability (Mahar *et al.,* 2016). Phytostabilization may also be used to limit dust lifting which may

Table 1. Tree and herbaceous crops suitable for phytoextraction.

Species	PTE extracted	Sink tissue	Reference
Populus nigra L.	Cd	Trunk, leaf	Vangronsveld *et al*., 2009
Eucalyptus camaldulensis Dehnh.	Cd	Trunk, leaf	Luo *et al*., 2016
Salix viminalis L.	Cd, Zn	Trunk, leaf	Vangronsveld *et al*., 2009
Arundo donax L.	Cd, Cr	Shoot, leaf, rhizome	Barbosa *et al*., 2015 Fiorentino *et al*., 2013; 2017
Brassica carinata A.	As, Cd, Cr, Cu, Ni, Pb, Zn	Shoot, leaf	Marchiol *et al*., 2004 Soriano and Fereres, 2003
Brassica juncea L.	As, Cd, Cr, Cu, Pb, Zn	Shoot, leaf	Clemente *et al*., 2005 Marchiol *et al*., 2004
Brassica nigra L.	As, Cd, Cr, Cu, Ni, Pb, Zn	Shoot, leaf	Marchiol *et al*., 2004

be a predominant source of exposure in Mediterranean areas during the summer drought (June-August). In such cases fast-growing species should be chosen, able to rapidly colonise the soil and form a compact turf to hinder wind erosion (Pardo *et al.,* 2014; Boisson *et al.,* 2016). To achieve this goal, the most effective tool consists in perennial grasses including both microthermal and macrothermal species. Intercropping such species is designed to rapidly cover the soil during the wet-cold season with microthermal species (*e.g.* ryegrass or fescue), and ensure high soil cover during the dry season with drought-resistant grasses such as Bermuda grass (*Cynodon dactylon L.*) or dallisgrass (*Paspalum spp.*) (Table 2). In some cases limiting the access to polluted/degraded sites is mandatory to avoid improper grazing and food production. The most effective way to do so is to transplant fast-growing species such as giant *(Arundo donax* L.*) or* common reed (*Phragmites australis* (Cav.) Trin.), that are tolerant to high pollution levels and to anoxic conditions of unstructured soils (Barbosa *et al.,* 2015). The above species create a green barrier, limiting the illegal use of contaminated sites during the phytoremediation period. Many tree species suited for phytoextraction can also make a contribution in terms of phytostabilization (Table 1), especially by reducing ground wind speeds and consequent wind erosion.

Rhizodegradation is based on the ability of plants to modify and enhance the soil microflora through the release of root exudates usable as a growth substrate by microorganisms (Passatore *et al.*, 2014). Another effect of this technique is to increase aerobic conditions in the soil due to the effect of root growth on soil porosity (Leigh *et al.*, 2002; Smith *et al.*, 2007). This enhances the activity of aerobic soil microflora able to oxidize organic pollutants. The effects described above are important in the *rhizosphere* which is a specific volume of soil surrounding roots (within a maximum distance of 2-5 mm) and represents the keystone in restoring polluted soils through phytoremediation (Ventorino *et al.*, 2018). Rhizodegradation is the main technique when the pollution in question predominantly consists in organic compounds (Terzaghi *et al.*, 2018). Root activity enhances the activity of microorganisms naturally present in the soil and capable of degrading organic compounds. All agronomic practices aimed at increasing root growth and efficiency can positively affect rhizodegradation. In this context, organic fertilization and inoculation with mycorrhizal fungi and/or *Trichoderma* (Audet and Charest, 2007; Miransari, 2011; Fiorentino *et al.,* 2013) also play a major role, as discussed in the next section. In addition, rhizodegradation can also use massive inoculum of native bacteria (Ventorino *et al.*, 2018) in order to increase the degradation of organic pollutants with the application of microbial formulations designed using bacteria adapted to site-specific conditions.

Phytoremediation efficiency can be enhanced through two agronomic techniques commonly employed in food crop management: fertilization with composted organic matter and the enforcement of the activity of plant-root associated bacteria and or fungi (Fiorentino *et al*., 2013, Sessitsch *et al.*, 2013). Compost is a fertilizer obtained through a process of aerobic stabilization of crop residues and also from organic municipal solid waste. Compost use in phytoremediation positively affects soil fertility according to the mechanisms described below: i) improvement in soil structure due to the formation of stable mineral-organic aggregates; ii) enhancement of microbial driven degradation of organic pollutants (see rhizodegradation); iii) activation of N-cycling bacteria.

These effects allow suitable conditions for crop growth even in hostile environments. For phytoremediation purposes we must highlight the compost effect on PTE mobility and bioavailability (Fagnano *et al.,* 2011; Huang *et al.,* 2016) due to direct immobilization from compost (Vanegas *et al.*, 2015) and the formation of

Table 2. Grasses and herbaceous crops for phytostabilization.

Species	Main effects	Reference
Lignocellulosic crops		
Miscanthus sinensis	PTE reduction in soil solution Limitation of site access	Barbosa *et al.*, 2015
Arundo donax L.	Reduction of bioavailable PTE fraction Limitation of site access Limitation of wind erosion	Fiorentino *et al.*, 2013
Phragmites australis	PTE allocation to rhizomes Limitation of site access	Bacchetta *et al.*, 2015
Microthermal grasses		
Lolium perenne	Reduction in dust lift Reduction in bioavailable fraction of Cu, Pb, Mn	Padmavathiamma and Li, 2010 Karami *et al.*, 2011 Golda *et al.*, 2016
Poa pratensis	Reduction in dust lift Reduction in bioavailable fraction of Mn, Pb	Padmavathiamma and Li, 2010
Festuca spp. *Agrostis spp.* *Phleum pratense* *Bromus inermis* *Elymus spp.*	Reduction in dust lift Reduction in bioavailable fraction of Cu, Zn, Pb	Mahar *et al.*, 2016
Macrothermal grasses		
Paspalum spp.	Reduction in wind erosion	Mekonnen *et al.*, 2015
Cynodon dactylon	Reduction in wind erosion	Kort *et al.*, 1998
Piptatherum miliaceum	Reduction in water and wind erosion	Arco-Lazaro *et al.*, 2017

insoluble complexes (Achiba *et al.*, 2009). With these mechanisms, humic substances reduce the passive PTE mobility (diffusion, mass transport), limiting vertical or lateral flows towards adjacent water bodies. In addition, organic fertilization is associated to an increase in active PTE mobility (root uptake), thanks to the formation of humus-metal complexes, prone to be broken up by organic acids of plant exudates, increasing the PTE bioavailable fraction (Fiorentino *et al.,* 2013). Mycorrhizal fungi are fairly common in the rhizosphere and are able to establish a mutualistic symbiosis with plant roots. Arbuscular mycorrhizal fungi can foster plant colonisation of contaminated/degraded soils (Audet and Charest, 2007; Miransari, 2011), enhancing root uptake of nutrients and PTEs according to the following mechanisms: i) dilution of PTE contents in plant tissues due to increased biomass yield (Kaldorf *et al.,* 1999); ii) exclusion of metals through precipitation or chelation in the rhizosphere (Christie *et al.,* 2004); iii) direct uptake in fungi tissues that limit transport in plant tissues (Christie *et al.,* 2004).

Another viable option for assisting phytoremediation is represented by root inoculation with *Trichoderma spp*. (Fiorentino *et al.,* 2013), endophytic plant opportunistic symbionts, used as biofertilizers (Fiorentino *et al.*, 2018) and biocontrol agents for plant diseases (Brotman *et al.*, 2010; Lorito *et al.*, 2010), such as *F. oxysporum, R. solani, Phytophthora spp.* and *Verticillium spp*. In addition, different *Trichoderma* species are recognized for their production of a large number of secondary metabolites with antibiotic activity (Harman *et al.*, 2004). Thanks to the production of a large variety of depolymerising enzymes, *Trichoderma spp.* are able to use a large group of compounds as a source of carbon and nitrogen. Together with the abundant production of conidia, the adaptability to different environmental conditions makes *Trichoderma* highly competitive with respect to the common soil microflora. Some strains establish strong and lasting colonisation of the roots, penetrating surfaces even below the epidermis (Woo and Lorito, 2007). This fungi-root interaction promotes above-ground and below-ground plant growth (Harman *et al.,* 2004).

In recent years, the ability of some *Trichoderma* strains to biodegrade or tolerate a wide range of contaminants has also been demonstrated, allowing their use in phytoremediation of soils contaminated by hydrocarbons (Harman *et al.,* 2004). A widely used strain for this purpose is *T. harzianum* T22 which greatly increases the effectiveness of plants used for phytoremediation, as proved by experiments showing a significant decrease in soil metal content due to inoculated fern and giant reed and a significant increase in root biomass, compared to control plants (Harman *et al.,* 2004; Fiorentino *et al.,* 2013).

Materials and methods for implementing phytoremediation plants

The following agronomic practices are necessary for implementing phytoremediation plants. They have to be locally calibrated after geophysical (Langella *et al.,* 2018) and geochemical (Rocco *et al.,* 2018) characterization and phytoscreening (Visconti *et al.,* 2018) in a perspective of precision remediation.

Removal of waste, stones and pre-existing flora

This activity is obviously required especially in waste disposal areas or on sites subjected to illegal spills. To proceed with removal an inventory of the different categories of waste present has to be drawn up by referring to the European Waste Catalogue (EWC) code, estimating the quantities to be managed and the costs of disposal in authorised landfills. The same approach should be adopted in agricultural sites on which perennial crops not suited to phytoremediation are already present. The biomass of these crops must be disposed of following the previously described procedure. If there are any species that can be used for phytoremediation (*e.g.* poplar), they should be integrated within phytoremediation plantations.

Compost fertilization

Compost fertilization is recommended in physically and chemically degraded soils according to the reference dose of 20-90 Mg ha^{-1} (f.w.), in order to recover fertility (*e.g.* an SOM increase of 1.5% in the 0-20 cm depth layer requires 90 Mg ha^{-1} of compost with a dry matter content of 80% and a humification coefficient of 0.5). The same fertilization rate is recommended to enhance microbial biodegradation of organic pollutants (Ventorino *et al.,* 2018). On soils whose fertility has not been compromised, the compost fertilization rate may be 10-20 Mg ha^{-1} (f.w.) (Fiorentino *et al.,* 2013) in order to promote plant growth and increase PTE bioavailability. It is recommended to use high quality compost that complies with the limits established by the legislation on fertilizers (Law Decree 75/2010). Spreading of the compost should be performed one month before planting/sowing of the crop (winter) so as to facilitate its stabilization (if necessary), thus avoiding any phytotoxicity.

Soil tillage

Deep tillage (40-60 cm) should be made with a ripper during the late summer so as to operate in optimal working conditions in terms of soil moisture. Indeed, soil structure can be seriously compromised by performing tillage on wet soils (*e.g.* winter or early spring). Soil ripping is recommended on compacted soils, previously subjected to the transit of heavy vehicles and/or waste disposal. Soil preparation prior to crop transplant (*e.g.* trees) and/or seeding (*e.g.* brassica species, grass) must carried out with a rotary-hoe in order to mix compost and crop residues in the 0-20 cm soil layer.

Transplant of trees and herbaceous lignocellulosic species

Trees as well as rhizomatous species (*e.g.* giant reed) should be transplanted in late winter and during the dormancy stage. Dense planting layouts (3x1 m) should be employed for tree species in order to create planting espaliers with high phytoextraction efficiency and to reduce ground wind speed and the consequent lifting and dispersion of contaminated soil particles.

For phytoremediation plants aimed at increasing the landscape value of a site (when phytoextraction is not required) a less dense planting layout can be employed (5x5 m; 7x7 m; 10x10 m) in order to allow the growth of tall forest species (*e.g.* poplar and willow). The planting layout of rhizomatous species will be 1x1 m in loose soils, where rhizome growth is not limited, while a higher density (0.60x0.60 m) is recommended in heavy soils.

Tree species transplanted at a high density will be managed as short rotation forestry, harvesting biomass every 3-5 years. An annual pattern will be adopted for giant reed harvest. Temporary storage and PTE analysis of biomasses will be required after each harvest in order to plan their disposal to conform with the code of the European Waste Catalogue (EWC), or their reuse (energy, timber, ...), as they are considered the byproduct of phytoremediation (Fagnano, 2018).

Grassing

The seeding of microthermal grasses (*e.g.* ryegrass, fescue) should be carried out when the soil temperature is above 10°C and during the rainy season (*i.e.* the period from September to December). When irrigation water is available *in situ* the suitable time for sowing can be extended until April. For macrothermal species (*e.g.* Bermuda grass) seeding must be carried out in late spring (April-May) in order to reach a soil temperature close to 18-20°C, whilst ensuring adequate soil moisture in order to promote seed germination. Grass mowing and baling must be planned every month from June to November. In the case of phytoextraction plants, temporary storage, PTE analysis and subsequent disposal must be carried out according to the approach described in the previous point of this section.

Irrigation

When irrigation water is available, it is recommended to set up an irrigation system to ensure that the water requirements of tree crops and macrothermal grasses are met in the very early stages of growth (emergence irrigation). Irrigation should be performed during the whole summer season in order to limit dust lift from the soil, ensuring a higher soil moisture content and grass cover and increasing the phytoextraction capacity of plants (Palladino *et al.,* 2018). When water available on site is not suitable for crops, irrigation can be performed by using safe water from other sites. Alternatively, drought-resistant species can be transplanted (*e.g.* eucalyptus, giant reed, common reed).

Monitoring phytoremediation efficiency

As with all the other restoration techniques, the effects of phytoremediation have to be monitored when the plants are *in situ*. PTE phytoextraction can be monitored by sampling plant biomass before each harvest in order to determine the PTE content and moisture (after drying at 60°C until sample weight is constant) of the various plant organs (trunk, branches, leaves). The proportion between stems, branches and leaves on a minimum number of 20 plants also needs to be estimated so as to be able to quantify their biomass yield per unit area according to the amount of total biomass (whole shoot) harvested on the site. The PTE content of the dry biomass of the various organs multiplied by the biomass produced per hectare represents the PTE quantity (*e.g.* kg/ha or g/ha) removed at each harvest. A similar approach should be followed for grass after each mowing. Soil samples must be collected in the root layer (0-30 cm and 30-60 cm depth) for monitoring changes in soil bioavailable PTE (Rocco *et al.,* 2018). Annual bioavailable soil PTE dynamics are strictly correlated with crop uptake (*e.g.* SRF trees, grasses and intercropped *Brassicaceae*) and the comparison between concomitant measurements of PTEs in soils and crops allows estimation of the PTE fraction liable to leached into the groundwater.

To restore the contaminated sites to food production after application of phytoremediation protocols, indirect risk of PTE transfer to the food chain must be excluded. A quite efficient monitoring protocol consists in growing heavy metal hyperaccumulator food crops (*e.g. brassicaceae* such as Indian mustard and rocket; *compositae* such as chicory) on plots established in the hot spots with higher PTE content (Duri *et al.,* 2018). Finally, monitoring the effects of grasses on dust lift as a consequence of turf formation can be carried out by setting specific samplers of particulate in the inter-row area. Amongst the most commonly used samplers suitable for this purpose is the Big Spring Number Eight or the Modified Wilson and Cook (Gao *et al.*, 2016; Mendez *et al.*, 2016). PTE content of the air particulate is linked to wind erosion from the polluted soil and hence to the turf effect on particulate lift.

Results of assisted phytoextraction in Campania case studies

Agricultural soil contaminated by bioavailable PTEs

The features of the San Giuseppiello site (Figure 1A) are described by Duri *et al.* (2018). In this case the aims were: i) to remediate the soil by gradually reducing the bioavailable fraction of PTEs (*i.e.* Cd, Pb) which could accumulate in foodstuffs; ii) interrupting the exposure pathways of pollutants by making the site safe; iii) improving landscape quality of a degraded land.

High-density (3x1 m) poplars were used, intercropped with

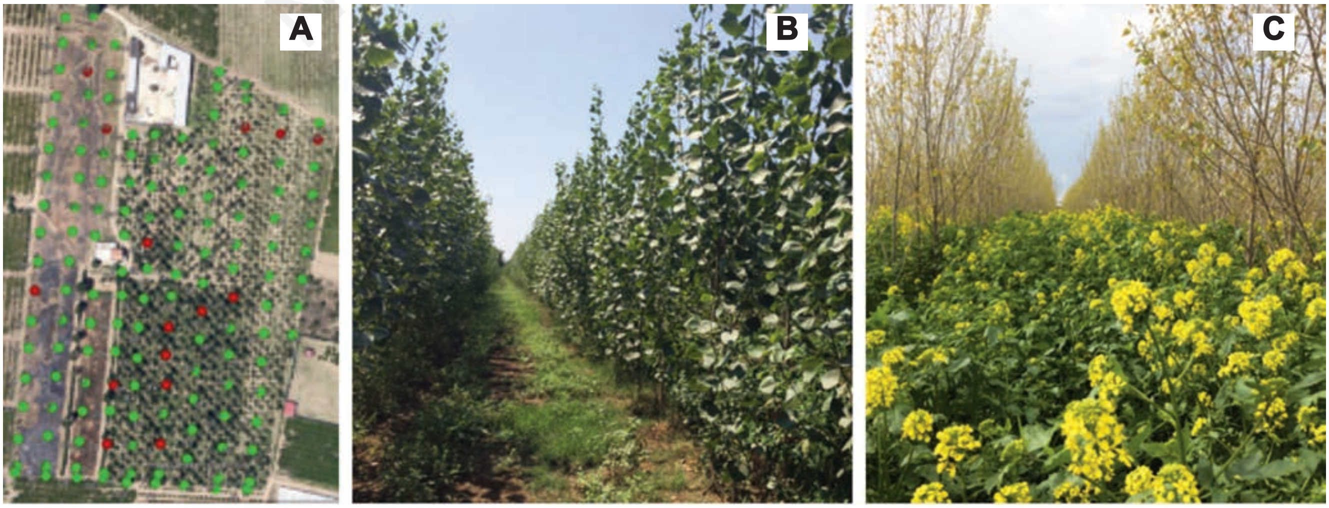

Figure 1. A) Aerial photo of the San Giuseppiello site; B) poplar grassed rows; C) Indian mustard in the hot spot contaminated by Cd.

natural meadows selected by frequent lawn mowing in order to combine the phytoextractive ability of trees with the effect of turf in preventing lifting and dispersion of soil particles (Figure 1B).

In the hot-spot areas with high bioavailable PTE concentrations, remediation was enhanced by intercropping Indian mustard (Figure 1C).

Industrial soil highly contaminated by PTEs (Ecobat site)

In the Ecobat site (Figure 2), an auto vehicle battery recycling plant, our analysis revealed an intolerable health risk due to air dispersion of Pb-polluted soil particles that can be transferred to humans through ingestion, inhalation and dermal contact. The features of the site were described elsewhere by Visconti *et al*. (2018). When phytoextraction is not viable for removing PTEs due to their excessive concentration, as in industrial areas, sites can be secured by interrupting the exposure pathways to contaminants.

In the Ecobat site dense permanent grassing was made to protect workers' health and allowed safe fruition of the site by interrupting dust lift. Deep soil ripping was performed to reduce soil compaction. Compost fertilization at a high dose (40 t ha^{-1}) was then carried out to improve both plant growth and soil structure for reducing soil dust. Microthermal species (*Lolium perenne, Festuca rubra, Poa pratensis*) were sown in October to ensure rapid soil cover, as also reported by Golda *et al*. (2016). Macrothermal species (*Cynodon dactylon, Paspalum vaginatum*) were sown in April to obtain a dense stand of turfgrass during the dry season when wind erosion could be higher. A poplar stand was added to reduce ground wind speed and the consequent risk of soil particle lift-off and dispersion. In this case, the Pb-contaminated poplar wood will be used in the smelter for producing lead bullion, replacing petroleum coke as a reducing agent. Another option for sites with similar characteristics may be to crop common reed or giant reed characterized by a dense and continue soil covering, reducing the downward flow of pollutants towards the groundwater, limiting site accessibility and immobilizing PTEs in the rhizomes and in the upper soil layers (Barbosa *et al.,* 2015; Fiorentino *et al.,* 2017). In this case, the quality of biomass produced must be verified, analysing the content of PTEs and comparing it with the anticipated regulatory limits for their use as by-products.

Soil contaminated by non-bioavailable PTEs and/or organic pollutants (Giugliano, Trentola-Ducenta sites)

In the Giugliano site the soil was potentially contaminated by Cu, Zn and heavy hydrocarbons ($C>12$), while the Trentola site showed the presence of several tons of abandoned waste and the soil was potentially contaminated by heavy hydrocarbons (Monaco *et al*., 2015; Rocco *et al.,* 2016). In these cases (Figure 3) the focus

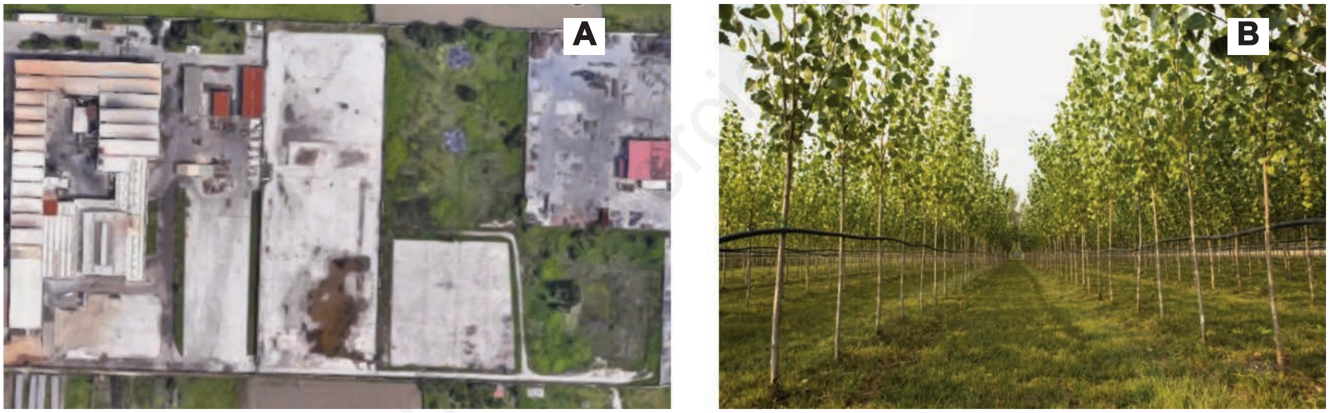

Figure 2. A) Aerial photo of the Ecobat site; B) poplar grassed rows for making safe the site.

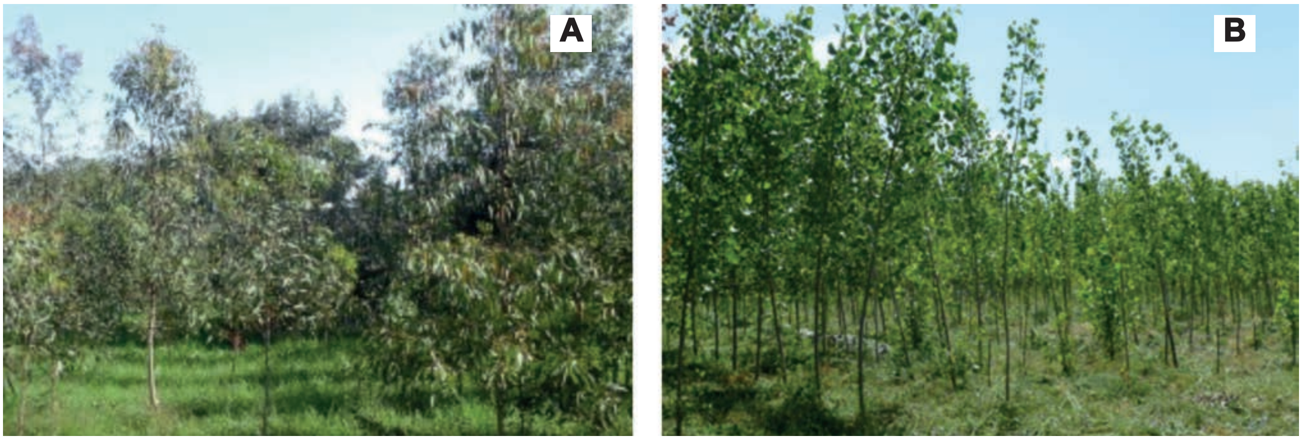

Figure 3. A) Eucalyptus stand at Giugliano; B) poplar stand at Trentola.

of phytoremediation was: i) to remove abandoned waste, thereby improving landscape quality; ii) limit the lift and dispersion of contaminated soil particles; iii) enhance biodegradation of organic compounds by soil microflora. The control of dust lift was achieved with permanent grasses, combined with biomass trees (*i.e.* eucalyptus and poplar). The above crops are able to explore large amounts of soil with their root system and hence to reduce the content of organic compounds through rhizodegradation. This activity was enhanced with the use of specific microbial consortia (bioremediation), as described by Ventorino *et al.* (2018). It must be pointed out that in this specific case there are no risks of pollutant transfer to crops, whether non-food or food (*e.g.* orchards, vegetables). Thus the biomass produced in such sites can be used without limitation (*e.g.* as a structuring agent in composting plant, for energy recovery).

Degraded soil and disfigurement of the landscape (Teverola)

The most frequently recognized problem in many areas of Campania is the abandonment of waste in fallow farmland and soil compaction due to the transit of motor vehicles (Fagnano, 2018). The Teverola site was used by the local municipality for temporary waste storage, leading to soil physical degradation and the disfigurement of the landscape, which in turn placed major constraints on the provision of ecosystem services. In this case, in addition to the removal of the waste concerned, phytoremediation was used for environmental and landscape restoration by applying all the techniques that improve the physical fertility of the soil, such as fertilization with compost and minimum tillage. In this way the best species proved to be giant reed (*Arundo donax* L.) due to its high adaptability to low fertility soils, its reduced maintenance needs and the high shoot density, which can also limit access to the site.

Conclusions

The integrated approach including agronomic, geochemical, geophysical, microbiological, hydraulic engineering protocols proposed by the ECOREMED project is a cost-effective and environmentally friendly strategy to remediate degraded land or render contaminated soils safe. The budget required for the proposed approach amounts to 100,000 euros ha^{-1}, proving more affordable than chemical and physical techniques whose costs range from 1-2 M euros ha^{-1} (*e.g.* capping with concrete platforms) to 8-10 M euro ha^{-1} (*e.g.* digging and dumping).

In addition, phytoremediation preserves soil resources, and improves ecosystem services, due to the combination of low input soil management techniques (*i.e.* soil ripping for reducing soil compaction, compost fertilization) and permanent soil covering by vegetation. This is in accordance with Italian law 6/2014 (Art. 2, par. 4) which clearly defines soil protection as being mandatory in remediating natural or agricultural sites.

The fields of application of phytoremediation can be classified (according to Italian environmental law) as ensuring public safety or remediation. In the first case, the aim is to interrupt exposure to contaminant pathways by using an ecological structure such as a dense turfgrass combined with dense tree rows for reducing ground wind speed and limit water percolation and contaminants leaching toward groundwater. A preliminary analysis of native vegetation of the contaminated sites could help to choose the most suitable species under the site-specific conditions in question. In the case of contamination of a deep soil layer (*e.g.* >2.0 m) phytoremediation is not sufficient and must be combined with other engineering approaches (*e.g.* hydraulic barriers, syringing) for isolating groundwater from contaminants. Phytoremediation is not suitable for securing sites contaminated by organic pollutants that can move in a gaseous phase such as light hydrocarbons and PAHs.

Figure 4. Giant reed stand at Teverola site.

In the case of remediation, understood as the elimination of contaminants and restoration of the previous environmental conditions of the site, phytoremediation can act only on the bioavailable fractions of PTEs, while it is not suitable for uptaking and eliminating the non-bioavailable forms. In such cases, typical of mine waste contamination or industrial plants, if the levels of contamination represent an intolerable risk for human health, the Authorities may opt for safety measures described in the previous point or for other engineering approaches such as soil washing. If contamination is due to organic compounds, such as in petrochemical plants, or results from the fallout of toxic fumes due to waste combustion, grasses and tree species may help bioremediation performed with bacteria or fungi thanks to rhizosphere effects. In all cases the use of vegetation could help achieve environmental restoration of contaminated and remediated sites by improving soil fertility and ecosystem services.

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Bioassays for evaluation of sanitary risks from food crops cultivated in potentially contaminated sites

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Abstract

Environmental pollution in agricultural contexts cannot be neglected since food crops can be an important exposure pathway for soil pollutants. In Italy the Law on the Environment (Law Decree 152/2006) is unclear with regard to agricultural areas, since the specific regulation provided for in Art. 241 has not been enacted. Current risk analysis is based only on direct risk (*i.e.* via ingestion and inhalation of soil particles), while indirect risk via food consumption is not considered. For defining potential risks for consumers, assessment of exposure to potentially toxic elements (PTEs) can be made through the analysis of their content in food crops and the estimation of the related hazard quotient (HQ) based on the ratio between PTE intake through food and the health risks due to PTE doses during a lifetime. In order to obtain a precautionary estimate, the worst case approach was followed by selecting species well known for their PTE accumulation capacity, such as chicory (*Cichorium intybus* L.), lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), radish (*Raphanus sativus* L.) and rocket salad (*Eruca vesicaria* L.), and by cultivating these plants in hotspots with the highest PTE concentrations. The PTE contents in such crops show wide variability, with values from 0.005 to 0.054 mg kg^{-1} for arsenic (As), from 0.004 to 3.9 mg kg^{-1} for cadmium (Cd), from 0.17 to 0.79 mg kg^{-1} for chromium (Cr), from 0.22 to 1.18 mg kg^{-1} for copper (Cu), from 0.03 to 0.43 mg kg^{-1} for lead (Pb) and from 1.43 to 25.06 mg kg^{-1} for zinc (Zn). Lead and Cd contents in vegetables exceeded thresholds established by EC Reg. 1881/2006 in 14% and 25% of samples, respectively. By contrast, from HQ analysis, any potential risk of dietary exposure due to ingestion of As, Cr, Cu, Pb or Zn was excluded. Only Cd content in lettuce, spinach and chicory resulted in a potential health risk due to intake of such foodstuffs. The proposed method could resolve the lacuna in current Italian legislation regarding the evaluation of risks for human health due to the intake of contaminants through the food chain.

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Introduction

The content of potentially toxic elements (PTEs) in agricultural soils, their bioaccumulation in food crops and their dispersion in the environment are major exposure pathways for humans (Chen *et al*., 2018). However, in Italy current environmental legislation (Law Decree 152/2006) does not provide for assessing the suitability of a soil for agricultural use. Currently, the main risk analysis software programs, such as ReasOnable Maximum Exposure - R.O.M.E. (APAT), GIUDITTA (Milan province) and RBCA ToolKit (GSI), only allow calculation of direct health risks due to dermal contact, ingestion and inhalation of contaminants by people who frequent a site, while indirect risks, associated to PTE uptake by food crops and their consumption, are not taken into account.

In the rest of Europe, only Germany and Austria have guidelines for assessing the suitability of soil for crop production, reporting trigger values based on PTE bioavailable contents in soil (Carlon, 2007), thus requiring analysis of PTEs in crops whenever the trigger level is exceeded. The bioavailability of PTEs is an important aspect, because it can show the potential mobility of elements and the consequent potential uptake by plants as described by Adamo *et al.* (2014) and Rocco *et al.* (2018). Nevertheless, its assessment by chemical methods (*i.e.* by using extractants with different strength) allows indirect evaluation, while direct assessment can be made by analysing PTE accumulation in food crops.

Lead and Cd contents in foodstuffs can be evaluated by comparing them with the thresholds reported by EC Reg. 1881/2006 for some vegetables, while for As and mercury (Hg) the values could be compared with the thresholds reported in EC Dir. 32/2002 relative to forage. For other potentially toxic contaminants, health risks could be assessed by estimating the intake of contaminants through the most common food in normal diets, as described by national or international agencies (ISS, 2013; US EPA, 1989).

These methods have already been used and validated in other surveys (Zheng *et al*., 2007; Huang *et al*., 2008; Beccaloni *et al*., 2013; Osaili *et al*., 2016; Salvo *et al*., 2018), in which estimation of contaminant intake allowed identification of the effective risks for human health that were often unrelated to major soil contaminants. Indeed, in an agricultural area near a Zn smelting plant, Zheng *et al.* (2007) excluded risks derived from accumulation in foodstuffs of the elements with higher concentrations (Zn, Cu and Hg), but highlighted potential risks due to Cd and Pb. On the other hand, in industrial areas, Huang *et al.* (2008) did not find health risks for each single element (Hg, As, Cr, Cu, Ni, Pb and Zn), but focused on calculating aggregate risk due to their cumulative effect. Beccaloni *et al.* (2013) found no health risks associated to consumption of local vegetable products in potentially contaminated croplands in Sardinia, while research conducted in Sicily (Salvo *et al*., 2018) revealed health risks due to Cd and As accumulation in vegetables cultivated in fields close to a petrochemical plant, correlating the contamination of soil and food crops with the use of contaminated groundwater for irrigation.

To evaluate the suitability of a field for agricultural use, a precautionary assessment can be made by using the worst case approach in which food crops well known for their PTE accumulation capacity, such as lettuce (Antoniadis *et al*., 2017; França *et al.*, 2017), spinach (Agrawal *et al.*, 2007*)*, chicory (Aksoy, 2008), rocket salad (Mourato *et al.*, 2015) and radish (Liu *et al.*, 2006) are cultivated in potentially contaminated soils to evaluate the risk of PTE transfer to the food chain.

In light of the above, the aim of this work was to evaluate the suitability for agricultural use (*i.e*. the risks for human health due to contaminant accumulation in foodstuffs) of a site defined as potentially contaminated by Cr and Zn due to illegal tannery sludge disposal, in which a more detailed environmental characterization highlighted some hot spots potentially contaminated also by As, Cd, Cu and Pb (Adamo *et al.,* 2017).

Materials and methods

Description of the study area

The study area (60,000 m^2) is situated in the Campania plain in which fruit (*e.g*. strawberry, melon and peach) and vegetables (*e.g.* tomato, lettuce, broccoli) are mainly cultivated. In 2008 the site was sequestered due to illegal disposal of tannery sludge, and from 2015 onwards it was characterized and remediated by using the ECOREMED protocol (2017).

Experimental model

To evaluate the potential uptake of PTEs without uncertainties due to field conditions (*e.g*. leaching and weather adversity), some soil hot spots with higher PTE concentrations were collected from the study area and used in a pot experiment in a greenhouse. The *worst case* approach was used, which means growing selected crop species already known for their ability to accumulate PTEs in their edible portions in soils from such hot spots.

Soil and vegetable selection

Starting from a previous detailed characterization (Figure 1) four areas were selected with different contaminant levels (F4 with the highest concentration of Cd, F2 with the highest concentration of Cr and Zn, C13 with the highest concentration of Pb, and A7 with the lowest concentration of PTEs as control) (Table 1). Massive samples (1 m^2 x 0.2 m) of soils were collected and transported to an experimental greenhouse at the University of Naples.

The following five species commonly grown in the field area and known for their high PTE accumulation capacity were selected: i) Lettuce (*Lactuca sativa* L.): known to accumulate Pb, As, Hg (Antoniadis *et al.*, 2017) and Zn (França *et al.*, 2017); ii) Spinach (*Spinacia oleracea* L.): reported by Agrawal *et al.* (2007) as accumulating various PTEs (Pb, Cd, Cr and As); iii) Chicory (*Cichorium*

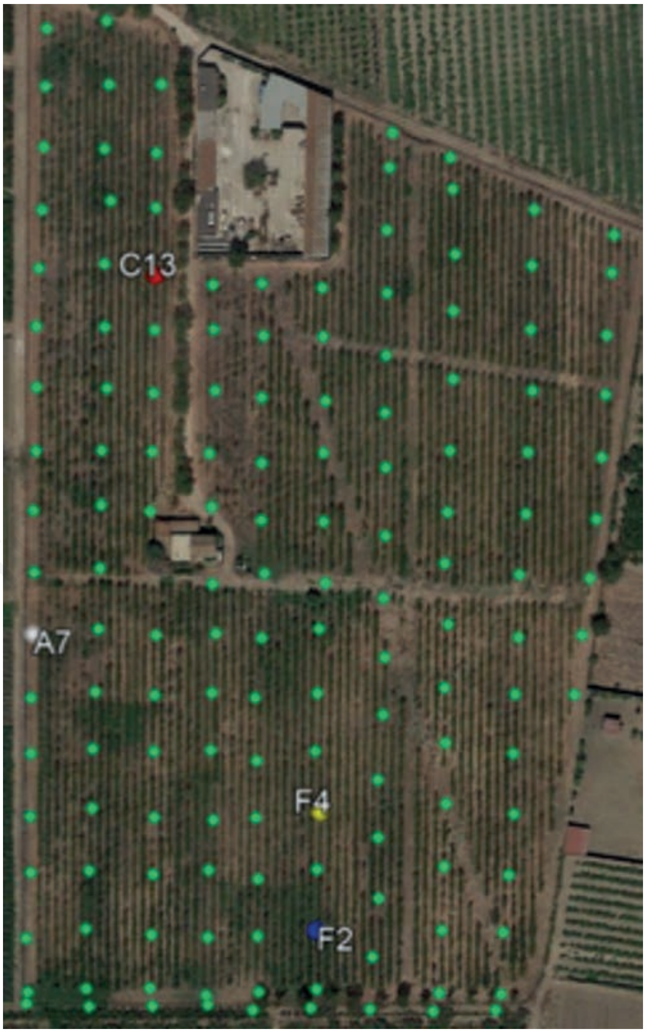

Figure 1. Hot spots in the experimental site (A7 = control; F2 = highest Cr and Zn concentration; F4 = highest Cd concentration; C13 = highest Pb concentration) (from Adamo *et al.*, 2017).

Table 1. Total and bioavailable content (mg kg^{-1}) of PTEs in the selected hot spots.

Hot spots	Copper		Lead		Zinc		Arsenic		Cadmium		Chromium	
	T	B	T	B	T	B	T	B	T	B	T	B
A7	29	0.11	45	0.06	122	0.14	13	---	0	0.05	112	0.30
C13	88	0.45	*147*	0.02	*684*	0.82	20	---	1	0.07	*650*	0.54
F2	49	0.17	63	0.01	*846*	1.78	16	---	1	0.01	*2399*	0.23
F4	64	0.19	70	0.01	*342*	0.49	20	---	*13*	0.18	*716*	0.50

T, total content; B, bioavailable content. Values in italics exceed the Italian screening values (DL 152/2006).

intybus L.): accumulating Pb, Cd and Zn (Bandiera *et al.*, 2016); iv) Rocket salad (*Eruca vesicaria* L.): accumulating Pb and Zn (Nikaido *et al.*, 2010); v) Radish (*Raphanus sativus*): known for its ability to accumulate Pb, Cd, As and Cr (Liu *et al.*, 2006).

Experimental activities: preparation, sampling and analysis

The selected soil was homogenized, sieved and used to fill 1.5-L pots in which the five plant species were grown, with five replicates per crop. During the first ten days of December 2016, all plants were seeded except the lettuce, which was transplanted, while baby leaf lettuce was seeded at the beginning of February 2017. For all plants the optimal growth conditions (temperature, water, *etc.*) were guaranteed.

The plants were cultivated until commercial maturity, harvested, weighed and washed with tap water and then with deionized water to remove any residual soil particles from the samples. Determination of PTE concentrations in fresh plant samples was made by using ICP-MS after acid digestion (HNO_3) in a microwave oven.

Total PTE contents in soil samples were determined by *aqua regia* extraction, while the readily soluble and bioavailable content of PTEs was extracted by 1 mol L^{-1} ammonium nitrate (DIN 19730, 1997). The metal contents in the extracts were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Health risk assessment

To evaluate the potential non-cancer risk of PTEs, the hazard quotient (HQ) equation was used for each PTE (US EPA, 1986; 2000), while the hazard index (HI) equation (US EPA, 1989; Huang *et al.*, 2008) was used to assess the cumulative potential effects of PTEs. The calculation formulas are shown below:

$$HQ = ADD/RfD \qquad (1)$$

where:
HQ is the hazard quotient for a single PTE (adimensional);
ADD is the average daily dose of the element (mg kg^{-1} day^{-1});
RfD is the reference dose of the element (mg kg^{-1} day^{-1}) that represents an estimate of daily oral exposure of the population which is likely to be without an appreciable risk of detrimental effects during a lifetime. If the ratio exceeds unity (HQ>1) there could be a potential risk to human health.

Exposure to contaminants is estimated by calculating the average daily dose (ADD) as follows:

$$ADD = [(C \times IR) \times EF \times ED]/(BW \times AT_{ADD}) \qquad (2)$$

where:
ADD is the average daily dose of the element (mg kg^{-1} day^{-1});
C is the concentration of an element in a vegetable (mg g^{-1});
IR is the per capita food consumption rate differentiated by age group (g day^{-1});
EF is the exposure frequency (day $year^{-1}$), indicating the number of days in one year in which an individual comes into contact with the contaminant (it is a variable parameter which may depend on seasonality and the availability of the food in question). The highest degree of caution is to consider 365;
ED is the exposure duration (years), indicating the number of years during which the population is exposed to intake of contaminated food. If the real exposure time is unknown, a precautionary value can be considered 70 years. If the risk assessment is for children, the maximum value of the age group is considered (*e.g.* for the 0-10 years age group, ED is equal to 10);
BW is the body weight (kg). Internationally a value of 60 (for adults) is generally considered;
AT_{ADD} is the time over which exposure is averaged (days), AT_{ADD} = ED x 365.

As mentioned above, it is important to calculate also the potential cumulative risk due to the addition of single risks:

$$HI = \Sigma HQ = ADD_1/RfD_1 + ADD_2/RfD_2 + ADD_n/RfD_n \qquad (3)$$

where:
HI is the hazard index as the sum of single hazard quotients (adimensional);
$ADD_{[1,2\ldots n]}$ are the average daily doses of the single PTEs (mg kg^{-1} day^{-1});
$RfD_{[1,2\ldots n]}$ are the reference doses of the single PTEs (mg kg^{-1} day^{-1}).

When HI exceeds unity, there could be a potential health risk. The single parameters used for calculating HQ values from national and international agencies (INAIL, 2015; EFSA, 2006, 2015) are reported in Table 2.

Statistical analysis

One-way ANOVA was performed by using SPSS (version 21). Mean separation was carried out by using the LSD test.

Results and discussion

As shown in Table 3, there were no phytotoxic effects depending on soil PTE contents, since plant growth was not significantly affected by the extent of soil contamination (Table 4). From Table 5, it may be noted that 14% and 25% of samples exceeded the European thresholds (EC Reg. 1881/06) for Pb and Cd, respectively. With regard to As, only in two plots were soil concentrations (Table 4) close to Italian screening values for potential contamination in residential/urban soils (20 mg kg^{-1}), but the bioavailability

Table 2. Parameters used for calculating the hazard quotient.

Intake rate (grams day^{-1})	Adult	Child
Radish	32.1	16.7
Spinach	135.0	77.8
Lettuce*	100.8	43.3
Rocket	18.5	3.4
Chicory	150.0	75.9
BW (kg)	60	26
EF (day $year^{-1}$)	350	350
ED (year)	70	10
ATADD (days)	25550	3650
Reference dose (mg kg^{-1} day^{-1})		
As	0.0003	
Cd	0.0005	
Cr	1.5	
Pb	0.0035	
Cu	0.04	
Zn	0.3	

*Lettuce and baby leaf lettuce.

of all samples was almost zero. Therefore As content in all foodstuffs (Table 5) was always very low (maximum value = 0.05 mg kg^{-1} f.w. in the 4th harvest of rocket), as also shown by Corradini *et al.* (2017) in their survey. As a consequence, HQ for adults (Table 6) and children (Table 7) were low, with a maximum value of 0.2 in chicory. It should be pointed out that As concentrations in vegetables that could represent health risks (*i.e.* HQ=1) are very low both for adults (0.14 for spinach) and children (0.10 for spinach), due to the high toxicity of this element (Table 7).

Copper content in soils was low (Table 4) and normal for this kind of volcanic soil (De Vivo *et al.*, 2012). Levels of bioavailable Cu (Table 5), as assessed by NH_4NO_3, were below the trigger value of 1 mg kg^{-1} defined by German DIN (1997) on agricultural areas, in agreement with the moderate accumulation in vegetables (maximum value was 1.2 mg kg^{-1} f.w. in the 1st harvest of rocket), thus confirming the tolerance to Cu of rocket salad (Zhi *et al.*, 2015) and its low storage capacity as demonstrated by Ali *et al.* (2012). No significant relation was found between soil content and vegetable accumulation (Table 8). HQ values were very low both for adults (0.06 for chicory and spinach, Table 6) and children (0.08 for spinach), thanks to the low toxicity of this element (HQ=1 for children can be reached with 14 mg kg^{-1} of Cu in spinach and chicory, Table 7). The levels of Cr in the soil were very high (up to 2399 mg kg^{-1}) due to tannery sludge disposal. For this reason the site was classified as potentially contaminated (Table 4). However, the bioavailable values were low (up to 0.54 mg kg^{-1}), as well as accumulation in vegetables (maximum value was 0.79 mg kg^{-1} f.w. in lettuce and rocket, Table 5). Similar values were also found by

Table 3. Vegetable yields (g pot^{-1} f.w.).

Hot spots	Chicory	Lettuce	B.L. Lettuce	Radish	Rocket 1st	Rocket 4th	Spinach
A7	37.32^{b}	95.07^{a}	32.99	16.67^{c}	16.25^{b}	20.05	20.79^{c}
C13	47.72^{ab}	51.07^{b}	24.01	60.21^{a}	26.28^{a}	34.89	33.28^{a}
F2	53.50^{a}	93.88^{a}	39.17	32.77^{b}	15.39^{b}	40.99	28.59^{ab}
F4	42.07^{ab}	112.96^{a}	31.46	39.35^{b}	19.02^{b}	32.08	25.60^{bc}

Table 4. Soil physical and chemical properties (0-30 cm).

Hot spots	NO_3-N ppm	NH_4-N ppm	C %	N %	C/N	Clay %	Loam %	Sand %	USDA Class.
A7	13	2	1.77	0.17	10.5	16.0	29.5	54.5	Sandy loam
C13	42	4	2.84	0.45	6.4	14.5	25.5	60.0	Sandy loam
F2	19	4	2.04	0.25	8.3	16.0	25.5	58.5	Sandy loam
F4	19	4	1.66	0.21	7.9	15.0	25.5	59.5	Sandy loam

Table 5. PTE concentrations in soils (mg kg^{-1} dw) and in vegetable edible parts (mg kg^{-1} f.w.).

PTEs	Hot spots	Soil T	Soil B	Chicory	Lettuce	B.L. Lettuce	Radish	Rocket 1st	Rocket 4th	Spinach
Arsenic	A7	13	0.01	0.01^{Acd}	0.01^{Ad}	0.04^{Aab}	0.02^{ABbc}	0.02^{bd}	0.04^{ABa}	0.00^{Ad}
	C13	20	0.01	0.03^{Aa}	0.01^{Aa}	0.02^{Aa}	0.01^{Ba}	0.02^{a}	0.02^{Aa}	0.02^{Aa}
	F2	16	0.01	0.03^{Aab}	0.01^{Abc}	0.02^{Abc}	0.03^{Aab}	0.02^{bc}	0.04^{ABa}	0.01^{Ac}
	F4	20	0.01	0.02^{Abd}	0.01^{Acd}	0.03^{Ab}	0.03^{ABbc}	0.01^{d}	0.05^{Ba}	0.02^{Abd}
Copper	A7	29	0.11	1.05^{Aa}	0.45^{Ab}	0.48^{Ab}	0.38^{Ab}	0.58^{Cb}	0.60^{Bb}	0.89^{Ba}
	C13	88	0.45	0.87^{Ab}	0.40^{Acd}	0.52^{Ac}	0.22^{Ad}	1.18^{Aa}	0.83^{Ab}	1.02^{ABab}
	F2	49	0.17	1.02^{Aa}	0.49^{Ab}	0.48^{Ab}	0.31^{Ab}	0.96^{ABa}	0.91^{Aa}	0.80^{Ba}
	F4	64	0.19	0.90^{Ab}	0.54^{Ac}	0.56^{Ac}	0.30^{Ad}	0.90^{Bb}	0.80^{ABb}	1.13^{Aa}
Chromium	A7	112	0.30	0.26^{Aab}	0.22^{Ab}	0.46^{Ba}	0.17^{Bb}	0.29^{ab}	0.36^{Bab}	0.26^{Aab}
	C13	650	0.54	0.34^{Abc}	0.39^{Ab}	0.79^{Aa}	0.18^{Bc}	0.39^{b}	0.53^{ABb}	0.46^{Ab}
	F2	2399	0.23	0.40^{Abd}	0.27^{Ad}	0.53^{Bbc}	0.41^{Abd}	0.58^{d}	0.79^{Aa}	0.33^{Acd}
	F4	716	0.50	0.36^{Aab}	0.22^{Ab}	0.47^{Ba}	0.18^{Bb}	0.37^{ab}	0.53^{ABa}	0.37^{Aab}
Zinc	A7	122	0.14	5.34^{Cbc}	3.55^{Bc}	5.53^{Abc}	1.43^{Ac}	6.03^{Bbc}	9.07^{Bb}	18.42^{Ba}
	C13	684	0.82	13.61^{Bb}	6.30^{Bc}	7.28^{Ac}	2.71^{Ac}	16.04^{Aab}	20.51^{Aa}	20.63^{ABa}
	F2	846	1.78	21.14^{Aab}	11.67^{Ac}	7.78^{Acd}	3.63^{Ad}	17.37^{Ab}	24.13^{Aa}	25.06^{Aa}
	F4	342	0.49	9.14^{BCbc}	7.51^{ABc}	6.69^{Acd}	2.11^{Ad}	10.37^{Bbc}	13.11^{Bb}	21.94^{ABa}
Cadmium	A7	0	0.05	0.04^{Ba}	0.03^{Ba}	0.03^{Ba}	0.01^{Aa}	0.01^{a}	0.05^{Ba}	0.03^{Ba}
	C13	1	0.07	0.07^{Ba}	0.02^{Ba}	0.02^{Ba}	0.00^{Aa}	0.01^{a}	0.03^{Ba}	0.01^{Ba}
	F2	1	0.01	0.12^{Ba}	0.10^{Ba}	0.02^{Ba}	0.01^{Aa}	0.01^{a}	0.04^{Ba}	0.03^{Ba}
	F4	13	0.18	*3.98*^{Aa}	*2.23*^{Ab}	*1.76*^{Ac}	*0.14*^{Ad}	*0.47*^{d}	*1.36*^{Ac}	*2.61*^{Ab}
Lead	A7	45	0.06	0.04^{Ab}	0.03^{Ab}	0.08^{Ab}	0.08^{Cb}	0.06^{b}	*0.34*^{Aa}	0.05^{Ab}
	C13	147	0.02	0.07^{Ac}	0.06^{Ac}	0.12^{Abc}	*0.17*^{Bb}	0.09^{bc}	0.27^{Aa}	0.10^{Abc}
	F2	63	0.01	0.04^{Ac}	0.04^{Ac}	0.08^{Abc}	*0.43*^{Aa}	0.09^{bc}	0.14^{Ab}	0.05^{Ac}
	F4	70	0.01	0.06^{Ab}	0.03^{Ab}	0.09^{Ab}	0.05^{Cb}	0.06^{b}	*0.40*^{Aa}	0.08^{Ab}

Concentrations exceeding the thresholds of EC Reg. 1881/06 are marked in italics; interactions of the same plant on different soils are expressed in capital letters, while interactions of different plants on the same soils are expressed in lower case.

Table 6. Estimation of the hazard quotient for adults (to 70 years).

PTEs	Hot spots	Chicory	S.E	Lettuce	S.E	B.L. Lettuce	S.E	Radish	S.E	Rocket 1st	S.E	Rocket 4th	S.E	Spinach	S.E.
As	A7	0.11	0.03	0.03	0.00	0.20	0.05	0.04	0.00	0.02	0.00	0.04	0.01	0.04	0.00
	C13	0.23	0.07	0.08	0.02	0.13	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.12	0.04
	F2	0.22	0.07	0.07	0.03	0.11	0.05	0.05	0.01	0.02	0.01	0.04	0.01	0.06	0.02
	F4	0.19	0.05	0.05	0.03	0.18	0.03	0.05	0.00	0.01	0.00	0.05	0.00	0.17	0.08
Cu	A7	0.06	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.05	0.00
	C13	0.05	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.05	0.00
	F2	0.06	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.05	0.00
	F4	0.05	0.01	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.06	0.00
Cr	A7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zn	A7	0.04	0.00	0.02	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.13	0.00
	C13	0.11	0.01	0.03	0.00	0.04	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.15	0.01
	F2	0.17	0.01	0.06	0.01	0.04	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.20	0.01
	F4	0.07	0.01	0.04	0.01	0.04	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.16	0.01
Cd	A7	0.21	0.04	0.08	0.02	0.10	0.02	0.01	0.00	0.01	0.00	0.03	0.00	0.14	0.01
	C13	0.36	0.03	0.06	0.01	0.06	0.01	0.00	0.00	0.01	0.00	0.02	0.00	0.06	0.00
	F2	0.58	0.06	0.32	0.02	0.08	0.01	0.01	0.00	0.01	0.00	0.03	0.00	0.12	0.01
	F4	*19.07*	2.24	*7.06*	0.99	*5.67*	0.46	0.14	0.00	0.28	0.01	0.83	0.15	*11.27*	0.39
Pb	A7	0.03	0.00	0.02	0.00	0.04	0.01	0.01	0.00	0.00	0.00	0.03	0.00	0.03	0.00
	C13	0.04	0.01	0.03	0.00	0.06	0.00	0.02	0.00	0.01	0.00	0.02	0.00	0.06	0.01
	F2	0.03	0.00	0.02	0.00	0.04	0.00	0.06	0.01	0.01	0.00	0.01	0.00	0.03	0.00
	F4	0.04	0.01	0.01	0.00	0.04	0.00	0.01	0.00	0.01	0.00	0.03	0.01	0.05	0.01

Values exceeding unity are marked in italics.

Table 7. Estimation of the hazard quotient for children (from 3 to 10 years).

PTEs	Hot spots	Chicory	S.E	Lettuce	S.E	B.L. Lettuce	S.E	Radish	S.E	Rocket 1st	S.E	Rocket 4th	S.E	Spinach	S.E.
As	A7	0.13	0.04	0.03	0.00	0.20	0.05	0.05	0.00	0.01	0.00	0.02	0.01	0.05	0.00
	C13	0.26	0.08	0.08	0.02	0.13	0.02	0.02	0.01	0.01	0.00	0.01	0.00	0.16	0.05
	F2	0.25	0.08	0.07	0.03	0.10	0.05	0.06	0.01	0.01	0.00	0.02	0.01	0.08	0.02
	F4	0.22	0.06	0.05	0.03	0.18	0.03	0.05	0.01	0.00	0.00	0.02	0.00	0.22	0.11
Cu	A7	0.07	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.06	0.00
	C13	0.06	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
	F2	0.07	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00
	F4	0.06	0.01	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00
Cr	A7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zn	A7	0.05	0.00	0.02	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00
	C13	0.13	0.01	0.03	0.00	0.04	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.20	0.01
	F2	0.20	0.01	0.06	0.01	0.04	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.26	0.01
	F4	0.09	0.01	0.04	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.01
Cd	A7	0.25	0.05	0.08	0.02	0.10	0.02	0.01	0.00	0.00	0.00	0.01	0.00	0.19	0.01
	C13	0.42	0.03	0.06	0.01	0.06	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.08	0.00
	F2	0.68	0.07	0.32	0.02	0.08	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.15	0.01
	F4	*22.27*	2.62	*7.00*	0.98	*5.62*	0.45	0.17	0.00	0.12	0.00	0.35	0.06	*14.99*	0.52
Pb	A7	0.03	0.00	0.01	0.00	0.04	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.04	0.00
	C13	0.05	0.01	0.03	0.00	0.05	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.08	0.01
	F2	0.04	0.00	0.02	0.00	0.04	0.00	0.08	0.01	0.00	0.00	0.01	0.00	0.05	0.01
	F4	0.05	0.01	0.01	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.07	0.01

Values exceeding unity are marked in italics.

Table 8. Correlation between PTE contents in soils and plants.

PTEs	Soil	Tot *vs* Bioav.	Chicory	Lettuce	B.L. Lettuce	Radish	Rocket 1st	Rocket 4th	Spinach
As	Tot	-	0.7371	0.6872	–0.2728	–0.4712	–0.2276	–0.2346	0.9263
	Bioav.		-	-	-	-	-	-	-
Cu	Tot	0.9143	–0.9369	–0.2967	0.5711	*–0.9819*	0.9289	0.5678	0.5750
	Bioav.		–0.7769	–0.6576	0.2563	–0.9385	0.8545	0.3809	0.3444
Cr	Tot	–0.5040	0.8385	0.0517	–0.0045	*0.9705*	*0.9911*	*0.9828*	0.0246
	Bioav.		0.0337	0.5036	0.5229	–0.6937	–0.4106	–0.3359	0.8056
Zn	Tot	0.9263	*0.9644*	0.8178	0.9726	*0.9774*	*0.9931*	*0.9993*	0.7951
	Bioav.		*0.9929*	0.9345	0.8996	*0.9819*	0.8806	0.9320	0.9145
Cd	Tot	0.9438	*0.9999*	*0.9995*	*0.9996*	*0.9991*	*0.9996*	*0.9995*	*0.9996*
	Bioav.		0.9413	0.9344	0.9449	0.9393	0.9440	0.9430	0.9445
Pb	Tot	–0.3237	0.7993	*0.9504*	0.9795	–0.0171	0.6467	–0.0944	0.8709
	Bioav.		–0.4647	–0.1180	–0.1782	–0.4351	–0.5472	0.3103	–0.4101

Values significant per P≤0.05 are reported in italics.

Cao *et al.* (2010) (lettuce with 0.35 mg kg^{-1} f.w.) and by Guerra *et al.* (2012) (rocket salad 0.25 mg kg^{-1} f.w.) in other contaminated soils. Chromium concentrations in vegetables were significantly correlated with soil values only for radish and rocket (Table 8). HQ values were close to zero in all vegetables thanks to the very low toxicity of chromium. Indeed, HQ may be equal to 1 with unrealistic concentrations in vegetables: for children from 500 mg kg^{-1} f.w. with spinach to 12,000 mg kg^{-1} with rocket. Zn values were also very high in the soil at the site (846 mg kg^{-1}, Table 4) but the NH_4NO_3 bioavailable amounts were low and below the German DIN trigger value of 2 mg kg^{-1} (Table 5). In this case vegetables accumulated high Zn concentrations with values often higher than 10 mg kg^{-1} f.w., up to 25 mg kg^{-1} in spinach (Table 5). Similar values were also found by Ferri *et al.* (2015) (up to 18 mg kg^{-1} f.w.), thus confirming results obtained by Rocco *et al.* (2018) in other potentially contaminated sites of the same area. Zinc concentrations in plants were positively correlated with total Zn content in soils, for all vegetables, resulting not significant only for spinach (Table 8). In spite of these high concentrations, HQ values were always low, with a maximum value of 0.26 with spinach for children (Tables 6 and 7), thanks to the low toxicity of this element. Also in this case, HQ may be equal to 1 with unrealistic concentrations in vegetables: for children from 105 mg kg^{-1} f.w. with spinach to 2381 mg kg^{-1} with rocket. It must be pointed out that Zn is a beneficial microelement for human health, such that Zn deficiency is known to clinically affect several organ systems such as the epidermal, gastrointestinal, central nervous, immune, skeletal and reproductive systems (Hambidge, 2000). Therefore extensive research has been carried out to produce Zn-fortified foods by cultivating crops in Zn-rich soils (Brown *et al.*, 2010). As regards Cd, a hot spot was selected with very high concentration (13 mg kg^{-1}, Table 5), in which the bioavailable values (Table 5) were higher than the German DIN trigger value of 0.1 mg kg^{-1}. In this plot, the accumulation in vegetables was very high and exceeded the thresholds of EC Reg. 1881/06 (Root and tuber vegetables, stem vegetables = 0.1 mg kg^{-1} f.w.; leaf vegetables, fresh herbs, leafy brassica = 0.2 mg kg^{-1} f.w.), with the maximum value of 3.9 mg kg^{-1} f.w. in chicory, higher than values reported by Bandiera *et al.* (2016) (2.5 mg kg^{-1} f.w.) in other contaminated sites. Cadmium concentrations in vegetables and soil were highly correlated for all crops (Table 8). HQ values showed a potential risk for adult and children only for four crops, with values of 5.67-5.62 for baby leaf lettuce, 7.06-7.00 in lettuce; 11.27-14.99 for spinach and 19.07-22.27 for chicory, while radish and rocket salad did not represent a risk for human health, as they have a low intake rate in standard diets. Considering the high toxicity of this element, the potentially dangerous concentrations are very low (HQ=1, Table 9) and values measured in chicory, spinach, baby leaf lettuce and lettuce cultivated in this case study exceeded 19, 11, 7, and 5 times, respectively, these values for adults, and 22, 15, 7, and 5 times, respectively, for children.

Lead is ubiquitous throughout the Campania plain both due to geogenic and anthropogenic contamination (De Vivo *et al.*, 2012). In this study area a selected hot spot (147 mg kg^{-1}, Table 5) showed bioavailable values below the German DIN trigger value of 0.1 mg kg^{-1} (Table 5). Nevertheless, Pb content in rocket salad and radish exceeded the thresholds of EC Reg. 1881/06 (vegetables excluding

Table 9. Potential health risk concentrations (HQ=1) mg kg^{-1} f.w.

			Adult (to 70 years)		
PTEs	Chicory	Salad*	Radishes	Rocket	Spinach
As	0.13	0.19	0.58	1.01	0.14
Cu	16.69	24.82	77.87	135.29	18.54
Cr	625.71	930.81	2920.00	5073.36	695.24
Zn	125.14	186.16	584.00	1014.67	139.05
Cd	0.21	0.31	0.97	1.69	0.23
Pb	1.46	2.17	6.81	11.84	1.62
			Children (3-10 years)		
EPTs	Chicory	Salad*	Radishes	Rocket	Spinach
As	0.11	0.19	0.49	2.38	0.10
Cu	14.28	25.03	65.07	317.44	13.93
Cr	535.59	938.57	2440.29	11903.83	522.55
Zn	107.12	187.71	488.06	2380.77	104.51
Cd	0.18	0.31	0.81	3.97	0.17
Pb	1.25	2.19	5.69	27.78	1.22

*Lettuce and baby leaf lettuce.

Table 10. Hazard Index value for adults (A; to 70 years) and children (C; from 3 to 10 years).

Hot spots	Chicory		Lettuce		B.L. Lettuce		Radishes		Rocket 1st		Rocket 4th		Spinach	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C
A7	0.46	0.53	0.16	0.16	0.39	0.39	0.07	0.09	0.04	0.02	0.11	0.05	0.39	0.52
C13	0.79	0.92	0.22	0.21	0.31	0.31	0.05	0.06	0.06	0.03	0.09	0.04	0.44	0.59
F2	*1.06*	*1.24*	0.49	0.49	0.28	0.28	0.13	0.16	0.06	0.02	0.11	0.05	0.45	0.61
F4	*19.42*	*22.69*	*7.19*	*7.13*	*5.95*	*5.90*	0.21	0.25	0.31	0.13	0.93	0.40	*11.71*	*15.57*

Values exceeding hazard thresholds are marked in italics.

leafy brassica = 0.10 mg kg^{-1} f.w.; leafy brassica, excluding fresh herbs = 0.30 mg kg^{-1} f.w.) not only in the hot spot but also in the other plots with lower soil Pb concentrations. Indeed, only for lettuce and baby leaf lettuce was there a significant correlation between concentrations in vegetables and soils (Table 8). However, HQ values were low for all crops, with maximum values of 0.063 in radish for adults and 0.078 in spinach for children (Tables 6 and 7), well below unity. Pb concentrations in vegetables representing a potential risk for human health (*e.g.* 2.15 mg kg^{-1} in lettuce) were considered attainable in contaminated agricultural areas (Kabata-Pendias, 2011), but the values measured in this study area were much lower, thus excluding any risk for consumer health and confirming the findings of Agrelli *et al.* (2017) in a broad-based survey made on vegetables produced in the same area.

A key aspect was the cumulative effect of single non-carcinogenic effects of PTEs: even though an element does not give an HQ>1 it could contribute to an overall potential disease if added to the effect of other PTEs.

As shown in Table 10, many vegetables grown in the high Cd plot (F4) showed an HI>1, but also chicory grown on F2 showed an overall HI>1. In both cases the highest contribution to the overall hazard index was due to Cd followed by As. In all other cases, the cumulative risk was well below unity.

Conclusions

Environmental characterization of an agricultural area affected by illegal disposal of tannery sludge highlighted very high concentrations of Cr and Zn in the soil. However, both our analysis of the direct risk for workers and people who could frequent this site and of the indirect risk for consumers of vegetables cultivated in this soil excluded any risk for these two elements thanks to their low to moderate toxicity.

Instead, our analysis made by using highly precautionary parameters showed that the potential risks for consumers were due to Cd, even in plots in which its soil total content was low. The legal methodology for carrying out environmental characterization, based on a few samples per hectare and the comparison between the total content of PTEs in soils and the screening values reported in specific tables, does not allow the suitability of a potentially contaminated site to be evaluated for agricultural purposes. This estimation could be improved by comparing the readily bioavailable PTE contents with trigger values reported in German guidelines, as proposed by Rocco *et al.* (2018), and then by biological assays with specific metal-accumulating crops with which a realistic risk assessment of food chain contamination can be achieved, even in soils showing low total contents of PTEs.

In conclusion, the proposed methodology aims to fill a gap in the Italian environmental legislation that fails to give due consideration to the agricultural use of soils and the consequent risk for consumers due to contaminant intake from food crops.

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Responses of bacterial community structure and diversity to soil eco-friendly bioremediation treatments of two multi-contaminated fields

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Abstract

Biodiversity of soil microbial populations could be altered and affected by anthropogenic pressures due to the release of organic and inorganic xenobiotic compounds and/or the application of remediation practices. Therefore, the assessment of the response of microbiota to environmental pollution and to bioremediation techniques is a critical issue in soil ecology. In this study a culture-independent approach was used to investigate the indigenous bacterial community structure in two contaminated soils of a National Interest Priority Site in Campania (southern Italy) and to monitor the impact of different remediation technologies. Our results show that bacterial populations shifted in the polluted soils over time after the application of compost and microbial inoculum. Statistical analyses based on the similarity of DGGE profiles show that the bacterial community structure and diversity was not affected by contamination. Hence the main change in similarity levels was induced by sampling time and by the interaction between soil eco-friendly bioremediation treatments.

Introduction

The harmful effects of environmental pollution do not impact only on human health, but also on ecosystems, landscape and soil biodiversity. Several studies have shown that in a polluted environment the number of animal, plant and microbial species could be greatly reduced (Øvreås *et al.*, 1998; Singh, 2003). Therefore, a contaminated site loses both ecological and economic value, and could become more vulnerable to other anthropogenic and natural pressures (Beier *et al.*, 2005). However, although organic pollutants reduce microbial biodiversity (Sutton *et al.,* 2013), they could stimulate the growth of some microbial species able to use them as a carbon source (Ventorino *et al.*, 2014). Among the cheapest technologies available for soil remediation, the use of compost (Chen *et al.*, 2015) as well as inoculation of selected microorganisms able to use organic xenobiotic compounds as a carbon source are considered eco-friendly and effective (Fiorentino *et al.,* 2013; 2017). Indeed, not only can compost improve soil quality but it is also a source of bacteria with putative suppressive effect and of nutrients for stimulating growth and activity of soil microbial populations able to degrade organic contaminants and promote plant fitness (Pepe *et al.*, 2013; Ventorino *et al.*, 2016; Taiwo *et al.*, 2016; Parillo *et al.*, 2017). Therefore, the presence of specific pollutants, as well as the use of bioremediation techniques, could affect the microbial community structure of a soil (Ventorino *et al.*, 2018a). However, the high microbial biodiversity and the complex relationships among bacterial populations and biotic and abiotic processes influencing their activities in soil make it difficult to evaluate soil microbial response to contamination and remediation practices (Bastida *et al.*, 2016). In this context, it is necessary to use biomonitoring techniques for assessing soil microbial structure and diversity in order to establish and apply the best method for cleaning up contaminated soils. Since it is generally accepted that by using culture-dependent methods it is possible to recover less than 1% of the microbial populations living in environmental samples (Amann *et al.*, 1995), the use of culture-independent methods, such as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), allows changes in the microbial community structure to be evaluated. In fact, molecular methods based on a metagenomic approach allow direct analysis of microbial populations in their natural habitat, thus avoiding the isolation and cultivation of the different microbial species according to their growth requirements. PCR-DGGE based on 16S ribosomal DNA and denaturing gradient gel electrophoresis fingerprinting technology is being increasingly used to assess changes in soil bacterial communities in a wide range of environments (Li *et al.*, 2006; Gupta *et al.*, 2016; Ventorino *et al.*, 2016, 2018a).

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In this context, this study was carried out to assess the impact of contamination as well as the use of environmentally compatible techniques for soil remediation on diversity of bacterial communities in soil samples collected from two multi-contaminated fields of the area of the Litorale Domitio Agro Aversano (Giugliano and Trentola Ducenta), used as pilot fields in the LIFE-Ecoremed project.

Materials and methods

Study sites and soil sampling

The study sites were two fallow rural fields, Trentola Ducenta

(TD) and Giugliano (G), contaminated both organically and with potentially toxic elements (PTEs) due to unauthorised waste disposal. These sites were used as pilot fields in the LIFE-Ecoremed project to validate environmentally compatible techniques for soil remediation (Ecoremed, 2017). In April 2014, some plots were amended with 20 t ha^{-1} of compost from the organic fraction of municipal soil waste. In addition, all plots were inoculated twice (October 2014 and April 2015) with a microbial consortium selected for its ability to use hydrocarbons as a carbon source (Ecoremed, 2017). Sixteen soil samples (10 from TD and 6 from G) were collected from the top soil (0-20 cm depth) at four sampling times: December 2013 (T0), after waste removal and before any bioremediation practices; May 2014 (TC), after compost addition; October 2014 (TIa), after the first inoculation of microbial consortium; April 2015 (TIb), after the second inoculation of microbial consortium (Tables 1 and 2 for sites TD and G, respectively). From each plot three 1-kg sub-samples were collected, homogenized and analysed to determine the diversity of bacterial communities.

Genomic DNA extraction and PCR-DGGE analysis

Total microbial DNA was extracted by using the FastDNA Spin Kit for Soil (MP Biomedicals, Illkirch Cedex, France) according to the manufacturer's specifications. DGGE analysis of bacterial communities was performed using the primers V3f (5'-CCTACGGGAGGCAGCAG-3') and V3r (5'- ATTACC GCGGCTGCTGG -3'), spanning the 200-bp region of the 16SrDNA of *Escherichia coli* (Muyzer *et al.*, 1993). A GC-clamp was added to the forward primer according to Muyzer *et al.* (1993). The PCR mixture and conditions were performed according to Ventorino *et al.* (2017). DGGE analysis was performed in a polyacrylamide gel [8% (wt/vol) acrylamide-bisacrylamide (37:5:1)] with a denaturing gradient of 30-60% using a Bio-Rad DCode Universal Mutation System (Bio-Rad Laboratories, Milan, Italy) as previously described (Pepe *et al.*, 2011).

Statistical analysis

Phoretix 1 advanced version 3.01 software (Phoretix International Limited, Newcastle upon Tyne, England) was used to detect the DGGE bands automatically, to determine matching bands and to perform a cluster analysis as previously indicated by Ventorino *et al.* (2013). The correlation matrix of the band patterns was performed using the method described by Saitou and Nei (1987). Finally, the percentage of similarity (S) of the bacterial community was estimated by analysing the resulting matrix using the average linkage method in the cluster procedure of Systat 5.2.1.

Table 1. Characteristics of plots and soil samples collected over time from each plot before and after the different bioremediation treatments applied in Trentola Ducenta.

Plot	Contamination*		Time°			
	C>12 (mg kg^{-1})	Zn (mg kg^{-1})	T0 Sample	TC Sample	TIa Sample	TIb Sample
6-3	93	-	1	11	21	31
6-7	106	-	2	12	22	32
21-1	132	163	3	13	23	33
21-5	150	-	4	14	24	34
21-9	206	-	5	15	25	35
32-3	109	-	6	16	26	36
32-4	329	-	7	17	27	37
32-5	176	-	8	18	28	38
32-7	541	228	9	19	29	39
32-8	250	-	10	20	30	40

*Organic and inorganic pollutant concentration (mg kg^{-1}) in soil samples measured after waste removal and before any bioremediation practices (T0), for more details please see Monaco *et al.*, 2015; Rocco *et al.*, 2016; Ventorino *et al.* (2018b). °Sampling time: T0, after waste removal and before any bioremediation practices in December 2013; TC, after compost addition in May 2014; TIa, after the first inoculation of microbial consortium in October 2014; TIb, after the second inoculation of microbial consortium in April 2015.

Table 2. Characteristics of plots and soil samples collected over time from each plot before and after the different bioremediation treatments applied in Giugliano.

Plot	Contamination*		Time°			
	C>12 (mg kg^{-1})	Cu (mg kg^{-1})	T0 Sample	TC Sample	TIa Sample	TIb Sample
1-3	533	219	1	7	13	19
1-8	75.7	110	2	8	14	20
6-7	79.5	33	3	9	15	21
8-2	705	91	4	10	16	22
8-5	401	96	5	11	17	23
8-8	590	53	6	12	18	24

*Organic and inorganic pollutant concentration (mg kg^{-1}) in soil samples measured after waste removal and before any bioremediation practices (T0), for more details please see Monaco *et al.*, 2015; Rocco *et al.*, 2016; Ventorino *et al.* (2018b). °Sampling time: T0, after waste removal and before any bioremediation practices in December 2013; TC, after compost addition in May 2014; TIa, after the first inoculation of microbial consortium in October 2014; TIb, after the second inoculation of microbial consortium in April 2015.

Results and discussion

The PCR-DGGE culture-independent approach was employed to obtain a qualitative fingerprint of the bacterial communities due to the effect over time of the environmentally compatible restoration treatments on the resident soil bacterial community of multi-contaminated soil sites TD and G (Figures 1 and 2).

In TD, the comparison of DGGE patterns showed important changes in bacterial community structure over time, revealing complex profiles and hence a high diversity of bacteria in all soils. In fact, the number of distinct DNA bands ranged from 21 at the beginning of the bioremediation treatment (T0, Figure 1A; TC, Figure 1B) whereas a considerable increase in the number of bands (up to 34) was observed after inoculation treatments (Figure 1C and D).

In site G, high bacterial diversity was also observed in all soil samples showing a number of bands of 28-29 (Figure 2). Although the number of bands remained constant during the experiment, their position and intensity strongly varied over time (Figure 2A-D) as also observed in TD. Improvement in the biodiversity of the bacterial populations after remediation treatments could be correlated with a disappearance of a stress factor, such as depletion of pollutants, to an increment in the abundance of taxonomic units and a redistribution of the bacterial specimen in the soil interpreted as the recovery of the resilience of the matrix (Ruffini Castiglione *et al.*, 2016).

As shown in Figure 3, statistical analysis of the DGGE profiles revealed that approximately 25-30% of the bacterial populations of the two soils remained stable during the experimental period without microbial perturbations (Figure 3A and B, cluster 1). These persistent bacteria could represent autochthonous populations whose growth and activity are not affected by anthropogenic activity (xenobiotic compounds and bioremediation techniques) and environmental pressures. Autochthonous naturally occurring bacteria possess some traits that may be used to survive and grow in specific habitats, enhancing their environmental survival (Søborg *et al.*, 2013). Information related to responses of autochthonous microbiota to pollution and to remediation treatment could help to

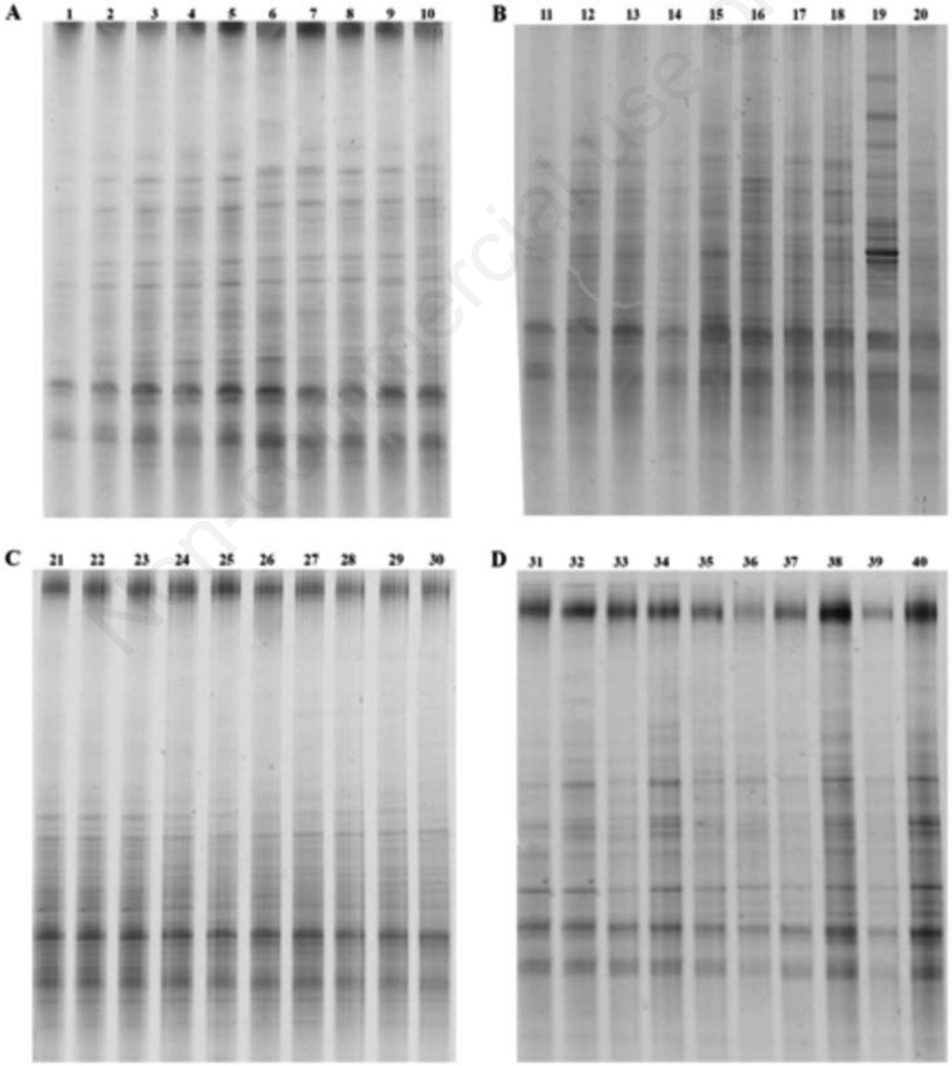

Figure 1. DGGE profiles of bacterial populations from soil samples of the Trentola Ducenta site collected before any bioremediation practices (A), after compost addition (B), after the first inoculum addition (C) and the second inoculum addition (D). See the text and Table 1 for the details of each sample.

assess the impact of environmental perturbation (Islam *et al.*, 2011) and to detect putative biomarkers (Ventorino *et al.*, 2018b). DGGE analysis revealed important microbial shifts that accounted for about 70% of bacteria in both TD and G (Figure 3A and B, cluster 1). This behaviour is a typical fluctuation observed in the zymogenous population selected by environmental conditions. Interestingly, cluster analysis of TD (Figure 3A) and G (Figure 3B) soils detected two main groups in both sites: cluster 2 (Figure 3A and B) grouped the soil samples collected before any bioremediation practices (T0) and after compost addition (TC); cluster 3 (Figure 3A and B) included soils sampled after the first (TIa) and second (TIb) inoculum addition. In both sites a dramatic shift in bacterial community structure was detected after inoculation treatments, since cluster 2 shared only about 30% of similarity with cluster 3 (Figure 3). Interestingly, within each of the major clusters delineated, the subgroupings of the prokaryotes (Figure 3A; cluster 4, 5, 6 and 7) were always similar and clearly associated to sampling times (T0, TC, TIa and TIb). Indeed, in TD soils, clusters 4 and 6 shared a 60% similarity with clusters 5 and 7, respectively. Within each subgroup low alterations in the bacterial community structure were observed, showing a similarity level ranging from 80 to 100%. Similarly, in site G four subclusters were identified on the basis of sampling time in which slight changes within the bacterial populations were observed with similarity level from 90 to 100% (Figure 3B; clusters 4, 5, 6 and 7). It is well known that the resident microbiota is able to adapt and acclimate to soil pollutants and/or to bioremediation treatments (Haritash and Kaushik, 2009) even if allochthonous organisms could affect fluctuations of the autochthonous microbial groups in capturing an important part of the overall energy influx during bioaugmentation of soils (Dejonghe *et al.*, 2001).

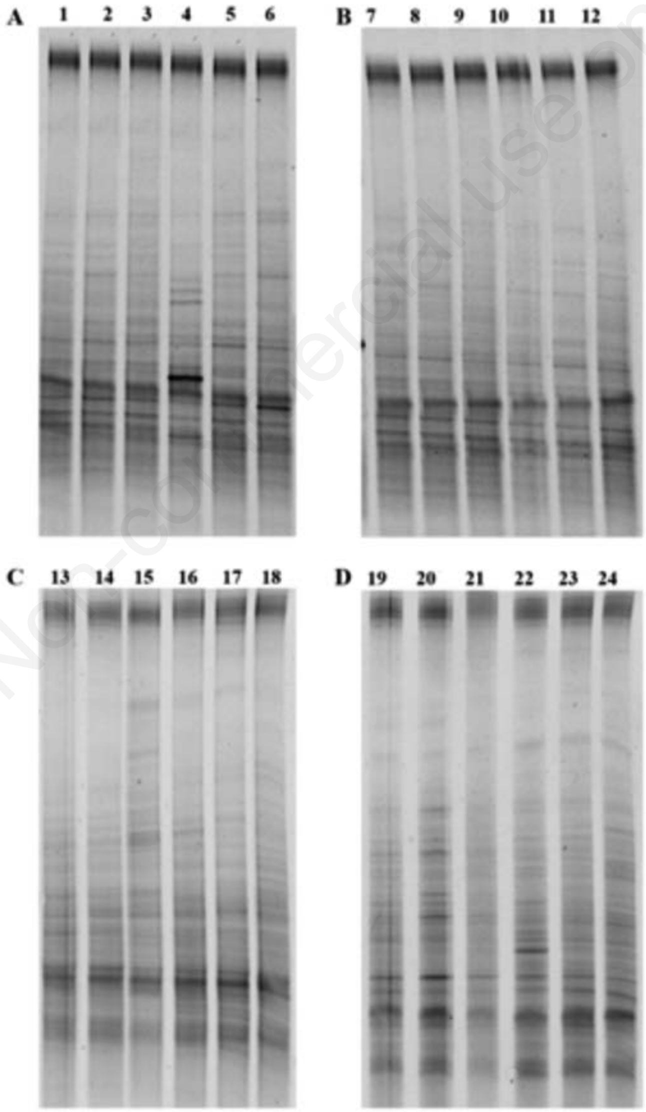

Figure 2. DGGE profiles of bacterial populations from soil samples of the Giugliano site collected before any bioremediation practices (A), after compost addition (B), after the first inoculum addition (C) and the second inoculum addition (D). See the text and Table 2 for the details of each sample.

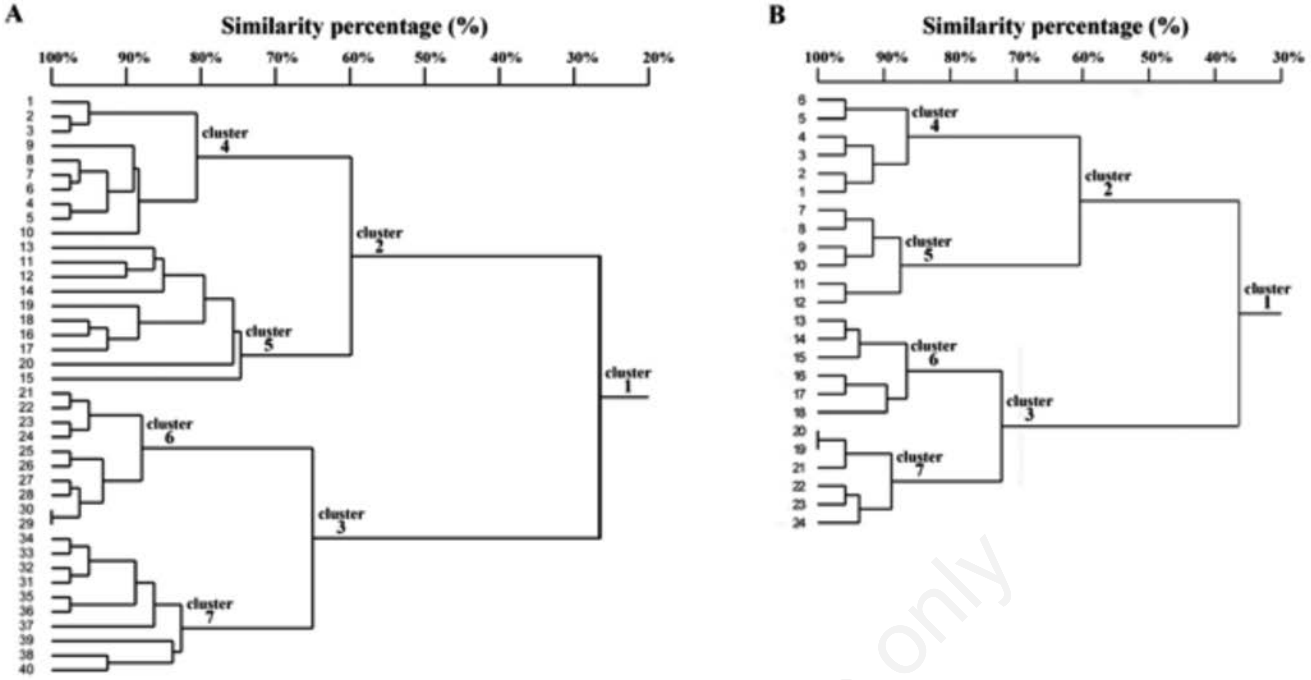

Figure 3. Dendrogram showing the degree of similarity (%) of PCR-DGGE profiles of the bacterial populations from soil samples of Trentola Ducenta (A) and Giugliano (B) site. See the text, and Tables 1 and 2 for the description of each sample.

Conclusions

In conclusion, the responses of bacterial community structure and diversity were not affected by contamination and hence the main change in similarity levels was induced by sampling time and by interaction between soil environmentally friendly bioremediation treatments. This behaviour suggests that a processes of acclimatization (Lladó *et al.*, 2015) to the polluted environment occurred especially with regard to allochthonous and/or zymogenous bacterial populations.

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Monitoring and modelling the role of phytoremediation to mitigate non-point source cadmium pollution and groundwater contamination at field scale

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Abstract

The study presented in this paper was carried out within the framework of the EU-Life+ EcoRemed project with the main aim of evaluating the effectiveness of a phytoremediation technique (the EcoRemed protocol) developed for field-scale reclamation of contaminated agricultural sites. The methods discussed here should be viewed as a proof-of-concept and their potential is shown at the Trentola test site subjected to low cadmium contamination, a potentially toxic element (PTE), presenting hazards for human health and the environment. Advanced monitoring equipment and modelling tools are employed to estimate water flow and solute transport in the soil-vegetation-atmosphere system, with specific emphasis to assess potential contaminant movement towards the groundwater. The strength of the phytoextraction process is evaluated by comparing two basic scenarios: i) a bare soil only condition (BS scenario); and ii) a distributed phytoextraction activity in the field obtained by planting poplar trees (*Populus nigra spp*) (PP scenario). Water and contaminant balance are modelled at plant scale by using the HYDRUS-1D code based on the Richards equation and the advective-dispersive equation, respectively. Sensitivity analyses enabled specific features of the phytoextraction process to be identified and showed the importance of setting up cost-effective monitoring devices to capture the main features of the problem under study. Moreover, especially with the aim of transferring the knowledge gained to public bodies and stakeholders, we suggest that the plant-scale monitoring and modelling activities be conveniently complemented with a modelling exercise applied to the entire field area, using the three-dimensional HydroGeoSphere (HGS) modelling tool.

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Introduction

The remediation of contaminated agricultural soils aims primarily to minimise the transfer of potentially toxic substances into the food chain and towards the groundwater (Kutílek and Nielsen, 1998). Most of the techniques devoted to the remediation of soils polluted with potentially toxic elements (PTEs) act directly on the mobile and bioavailable fraction of contaminants. As can be found extensively in national legislation and guidelines, a concentration threshold is fixed and given by the sum of the fraction adsorbed in the solid phase and the fraction dissolved in the liquid phase (Ferguson, 1999; Carlon, 2007). An overall reduction in the concentrations of hazardous substances and compounds can be achieved through processes that promote desorption and subsequent removal during the liquid phase. In general, this is the basic mechanism by which most modern techniques (*e.g.* soil washing techniques, electrokinetic methods, heat treatment) work to restore a contaminated site.

Phytoremediation is a relatively new technique for removing contaminants from soil profiles that can be viewed as belonging to the general class of bioremediation systems (Vidali, 2001). In particular, phytoextraction is based on the action of sequestration or chelation of toxic substances by plant roots and their subsequent accumulation (natural hyper-accumulation) in the plant tissues (Raskin and Ensley, 1999; Mir *et al.,* 2017). It is a promising technique not only because of its simplicity of practical implementation and cost effectiveness, but also because of the positive effects on the local landscape where it is applied. These reasons make phytoextraction a widely used technique, especially to remediate agricultural land. In spite of requiring relatively long remediation periods, it enables soil fertility to be preserved by maintaining use of the land for agriculture (Moosavi and Seghatoleslami, 2013).

The above reasoning became the basis and guided the setting-up of a project whose acronym is *EcoRemed*, funded in 2012 by the European Commission, Directorate-General Environment, within the call *ENV.E-4 - Life Environment & Eco-innovation*. The trigger for the research and demonstration activities was the large media attention drawn to the alleged contamination of agricultural soils in the provinces of Naples and Caserta (Campania, southern Italy) and the related negative effects on farm products (Agrelli *et al.*, 2017; Komínková *et al.*, 2018). This situation has had a devastating impact on the agri-food sector of the entire region, which is not only unjustified (as clearly demonstrated below), but also damaging to the rights of all the people involved in related economic activities. With specific reference to the coastal strip northwest of Naples known as the Litorale Domitio/Agro-aversano, the overall aims of this project were to demonstrate that soil can be remediated using an environmentally effective, low-impact method (EcoRemed protocol) that primarily points to bringing soil back to its agricultural use (Monaco *et al.*, 2015). This benefit sustains landowners economically, providing temporary non-food trees as biomass for energy production, and setting up a fruitful link between universities and research institutes on the one hand, and public bodies and stakeholders on the other, with a view to promoting laws and technical guidelines on the subject of remediation of agricultural soils. The EcoRemed project is coordinated and managed by the Interdepartmental Center for Environmental Research (C.I.R.AM.) of the University of Naples Federico II, one of the oldest research centres (it was established in 1992) of this university and which has brought together scholars of environmental issues from ten different university departments.

The fate of a substance in the environment depends on biological, chemical, physical and hydrological processes that are highly interconnected and act almost simultaneously. In this paper we specifically deal with the physical and hydrological processes in an attempt to highlight certain aspects of the phytoextraction technique that are sometimes overlooked or even ignored. The success of outcomes obtained when employing this technique depends on the degree of contamination of the site and the complexity of interactions among the water flow and solute transport processes taking place in the soil-plant-atmosphere continuum. Firstly, phytoextraction is not always applicable because remediation may take too long, a period that might not be compatible with certain needs of stakeholders and the community. Moreover, phytoremediation can yield some unpredictable results when compared with other traditional remediation systems for contaminated sites. Several authors have shown poor effects of phytoextraction mainly due to some drawbacks including phytotoxicity, a relatively low production of biomass of hyperaccumulator plants, or even the propensity of the roots to avoid uptake from the areas with higher pollutant concentrations. An alternative approach may be the use of vegetation stands to increase the partitioning factors in such a way as to decrease the mobile fraction. This technique, known as phytostabilization, but also immobilization or natural reclamation, operates both physically (through the transpiration of plants) and chemically by increasing the buffer capacity of the soil thanks to the increase in biomass represented by root exudates (Vidali, 2001). Chemical mechanisms can be promoted by adding specific soil amendments such as fertiliser agents that can increase the buffer capacity of the soil. With these measures, phytostabilization is considered an alternative system, which yields better success rates.

Moreover, and more importantly for certain aspects of the problem at hand, uncertainties are usually related to the actual solute concentration reductions gained with the remediation process, but are also certainly due to the difficulty of predicting the rate of contaminant extraction by trees. Schematically, the presence of vegetation can affect the following processes of the hydrological cycle more or less significantly: rain interception, soil hydraulic response, water and solute uptake and biomass adsorption of metal solutes. The synergistic effect of these possible changes leads to a reduction in the redistribution of rainfed infiltrated water, with substantial reductions in the risk of transferring contaminants from the soil surface to the deepest soil layers and to the groundwater.

Tackling point or non-point pollution phenomena in soil and groundwater is quite a complex task partly because of the inherent heterogeneity of the porous materials, but mainly because of the fact that soil and the underlying aquifer are intimately interconnected via the hydrological processes occurring in the soil-vegetation-atmosphere system, which in turn can be altered by human settlements, intensive agricultural practices, waste storage and disposal, as well as accidental or even illegal spills. These complexities make it difficult to predict *a priori* the actual impact that contamination will have on the environment, in terms of both spatial extent and persistence. Therefore, risk assessment of contamination should describe and interpret such complexities adequately in order to evaluate correctly the probability of occurrence of specific damage to human health and the ecosystem.

To encourage a wider use of phytoremediation with a clear understanding of its limitations, we suggest the use of analytical-numerical approaches. In other words, in-depth knowledge of the physical, chemical and biological processes together with the soil-vegetation interactions enables phytoremediation protocols to be suitably implemented by exploiting the outcomes of computer model simulations. The study presented in this paper was undertaken to shed light on the effectiveness of a poplar plantation to remove cadmium from the soil in a small agricultural field (the Trentola site) in the province of Caserta (Pietrini *et al.*, 2010; Dai *et al.*, 2013; Redovniković *et al.*, 2017; Michels *et al.*, 2018). Two different scenarios were designed to evaluate the fate of contaminant plumes and the effect of vegetation on the migration velocity of cadmium to the deepest soil layers and towards the underlying aquifer. The HYRUS-1D model (Šimůnek *et al.*, 2015) was used to evaluate different scenarios for phytoremediation along the soil profile at the scale of individual poplar plants. Moreover, the three-dimensional water flow and solute transport processes occurring in the entire study area assuming the absence or presence of plants were simulated through the HydroGeoSphere (HGS) 3-D model (Brunner and Simmons, 2012). Information about local weather conditions as well as soil and vegetation characteristics were retrieved directly through field measurements.

Experimental works and methods

The study site of Trentola-Ducenta

The case study reported in this paper was carried out in one of the experimental fields of the EcoRemed project near the town of Trentola-Ducenta (province of Caserta; 40°58’32.2” N, 14°08’59.0” E, and 68 m a.s.l.). It is one of the 77 municipalities of the National Interest Priority Site of Campania (southern Italy) *Litorale Domitio/Agro-Aversano* and identified as an appropriate site for analysis because it had been subjected to illegal storage of waste consisting mainly of mixed construction and demolition materials, including asphalt and plastic (Rocco *et al.*, 2016).

Figure 1 shows the field boundary (red line), marking an area of approximately 4500 m^2, where soil samples were collected to determine soil physical, chemical and hydraulic properties. Frequent measurements of near-surface (soil depth 0-15 cm) soil-water content were carried out in different positions of the test site by using the Time Domain Reflectometry (TDR) technique through mobile equipment specifically designed at the Laboratory of Soil Hydrology of the University of Naples Federico II. The study area was equipped with the following monitoring systems:

In two soil pits (Figure 2A) the following sensors were placed: three Frequency Domain Reflectometer (FDR) ruggedized sensors

Figure 1. Experimental site at Trentola-Ducenta (Caserta Province, Italy).

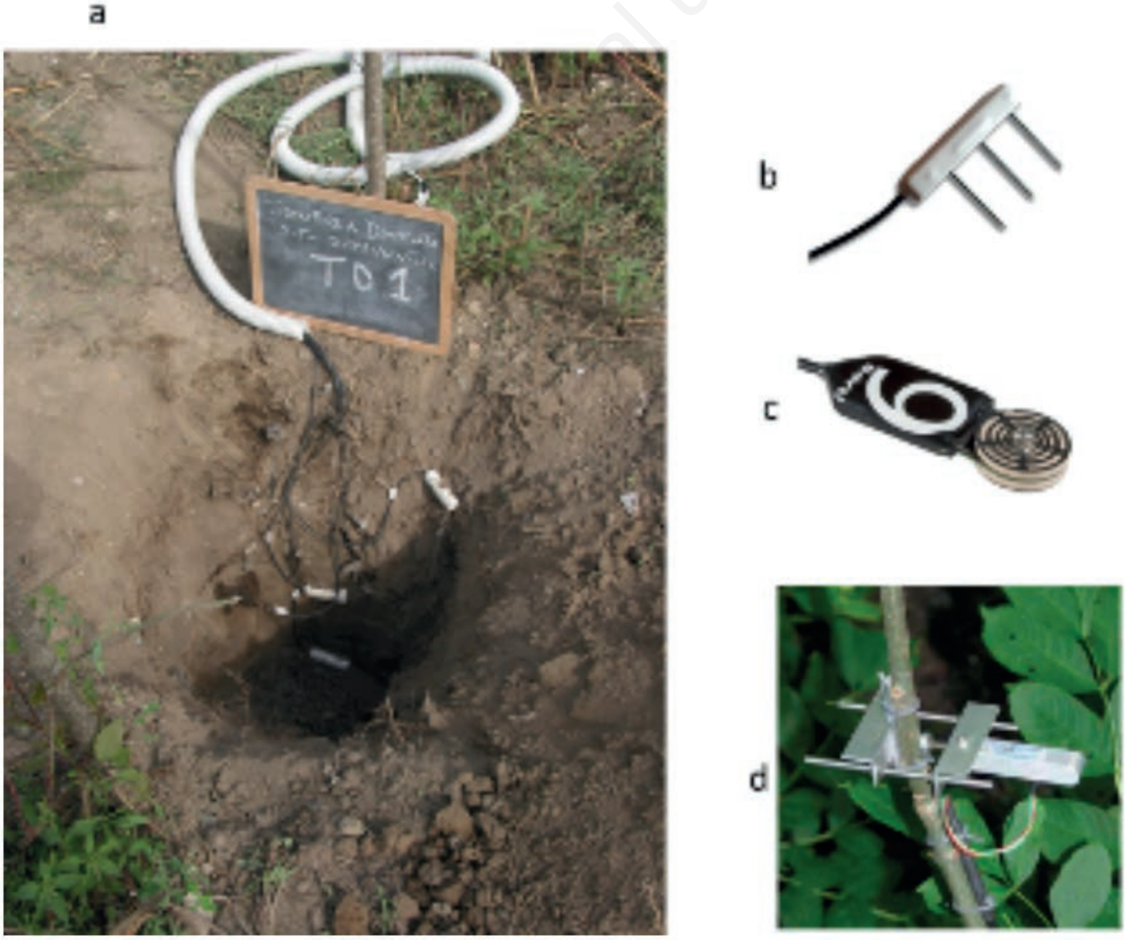

Figure 2. A) Picture of one the two soil pits; B and C) sensors used for calibrating the parameters of Hydrus-1D model; D) the dendrometer used for the calibration of evapotranspiration fluxes of the poplars.

(GS3 sensor by Decagon Devices Inc., USA) to measure simultaneously the volumetric soil water content, θ (cm^3 cm^{-3}), temperature, T (°C), and apparent electrical conductivity, *ECa* (dS m^{-1}) at the soil depths of 20 cm, 40 cm and 60 cm (Figure 2B); two soil-water potential sensors (MPS-6 by Decagon Devices Inc., USA) located at the soil depths of 20 cm and 40 cm for measuring soil matric pressure head (ψ) (Figure 2C); an electronic dendrometer DD-S by Ekomatic mounted on the stem of each of the two poplar trees (Figure 2D) for monitoring micro-variations in plant diameter to obtain proxy information on actual plant transpiration.

Note that the GS3 sensors located at the two soil depths of 20 cm and 40 cm were inserted horizontally and, together with the MPS-6 sensors located at the same depths, provided pairs of soil-water retention $\theta(\psi)$ data points throughout the monitoring period. Moreover, the MPS-6 sensors enabled the soil-water fluxes to be computed from knowledge of the gradient of total hydraulic head ($H=z+y$) and information about the soil hydraulic conductivity characteristic, $K(\theta)$. It should be noted, however, that the MPS-6 sensor has sensitivity from -9 kPa (*i.e.* a suction head of about 0.92 m of water) all the way to air dry (a value of about -100,000 kPa, equal to a suction head of about 10,200 m of water). The installation of sensors along the soil profile for monitoring water balance is shown in Figure 2A. All sensors were connected to a datalogger for recording the measurements. The dendrometric data were used for direct calibration of the crop parameters that were then employed for running the computer simulations.

Weather data taken from a meteorological station close to the test site were collected to determine the reference evapotranspiration, with the equation of Penman-Monteith, and used in the crop module of the HYDRUS-1D model. Figure 3A shows daily temperature and rainfall during the observation period. Instead, Figure 3B shows, as an example, the observed soil water contents at the three soil depths in pit #1. The two plots in this figure highlight the contrast between wet (from October to April) and dry seasons (from April to October), which is typical of a Mediterranean climate.

Distribution of cadmium (Cd) on near-surface soil was determined by carrying out a sampling campaign on a regular grid 25x25 m. Twenty-three samples were collected and analysed at the Mass Spectrometry Laboratory of the University of Naples Federico II using the EPA 6010C 2007 method. Table 4 in Capolupo *et al.* (2018) provides the descriptive statistics of Cd concentration with average and standard deviation values of 0.546 mg kg^{-1} and 0.179 mg kg^{-1}, respectively.

Modelling transport processes in the soil-vegetation-atmosphere system

At the pedon scale, theoretical studies (Dagan, 1986; Sposito, 1986) and many experimental investigations (Santini, 1975; Vauclin *et al.,* 1979) have demonstrated that water flow and solute transport processes in soil and in the rhizosphere are basically one-dimensional along the vertical direction and governed by the well-known Richards equation (Davison *et al.,* 2018; Šimůnek *et al.,* 2018):

$$S_w S_s \frac{\partial\psi}{\partial t} + \theta_s \frac{\partial S_w}{\partial t} = \nabla\cdot[\mathbf{K}\cdot k_r\,\nabla(z+\psi)] + \sum\Gamma_{ex} + Q \qquad (1)$$

which is obtained by combining the mass balance equation and Darcy's law. In Eq. (1), t is time, z is elevation, ψ is matric pressure head, S_w is water saturation, θ is volumetric soil water content and θ_s is soil water content at full saturation of the porous medium, S_s is the specific storage, **K** is the tensor of soil hydraulic conductivity, whereas k_r is the relative hydraulic conductivity. Soil water content, θ, and relative hydraulic conductivity, k_r, depend on matric pressure head, ψ, through the soil water retention, $\theta(\psi)$, and hydraulic conductivity, $k_r(\psi)$ or $K(\psi)$, functions. Moreover, the term $S(G_{ex})$ represents the internal fluid exchanges between domains (*e.g.* surface, subsurface, macropores, pumping wells, *etc.*) and Q is the external fluid exchanges (*e.g.* evapotranspiration, snow melt, *etc.*).

In the remainder of the paper we refer to the units of m, cm or mm for the length dimension, and to the units of hours, days or years for the dimension of time. After selecting the appropriate initial and boundary conditions for the flow field under study, while knowing the relevant soil hydraulic functions $\theta(\psi)$ and $K(\psi)$, solving even simple problems of practical interest requires Eq.(1) to be solved numerically by using specific, well-validated algorithms (Romano *et al.,* 1998; Brunone *et al.,* 2003).

The problem of solute movement in the subsurface is considerably more complex. This complexity is often exacerbated by the nature of the solute. However, the movement and fate of a chemical species (the solute) in an agricultural soil are mainly governed by two basic processes: convection and dispersion. In

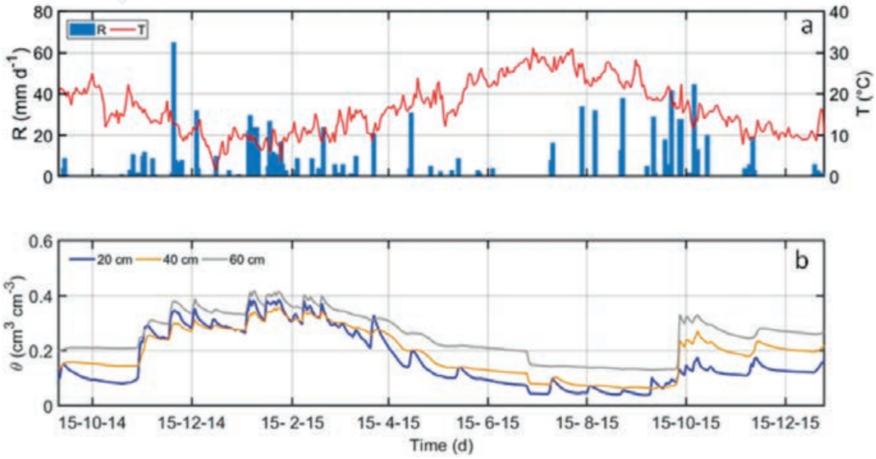

Figure 3. A) Daily temperature and rainfall values during the observation period; B) soil water content at three depths in soil profile.

general, the space-time variations of solute concentrations are described by the following convection-dispersion equation (CDE):

$$\frac{\partial}{\partial t}(\theta c + \rho s) = \frac{\partial}{\partial z}\left(\theta D_e \frac{\partial c}{\partial z} - qc\right) - \zeta(\psi) \qquad (2)$$

where, together with soil depth z and time t, c is the concentration of the substance in the liquid phase, ρ is oven-dry soil bulk density, D_e is effective dispersion (that describes the global mixing caused by both convection and dispersion processes), s is the concentration of the substance in the solid phase, and q is Darcy's flux of water as computed by Eq.(1).

The relationships between the variables θ, ψ, and K are rather complex and depend on many factors, but in most practical cases they are expressed by the soil water retention, $\theta(\psi)$, and hydraulic conductivity functions $K(\psi)$, which are commonly referred to as soil hydraulic properties.

Determining soil physical and hydraulic properties

Soil hydraulic properties were determined by extracting undisturbed soil cores from different depths of three distinct soil horizons. All soil cores were obtained by driving steel cylinders (0.08 m in diameter and 0.15 m in height) vertically into the soil using a hand-operated device while excavating the soil around the cylinder by hand to reduce disturbance during sampling. Before performing the hydraulic tests, the top of each undisturbed soil core (approximately 0.03-0.04 m) is removed and set aside for particle-size analysis, determination of soil organic carbon (SOC) content, and for measuring soil water retention data points in the dry range through the pressure plate apparatus. Particle-size distribution was determined by using standard laboratory techniques based on a set of sieves and the soil hydrometer (Gee and Or, 2002). Particle-size data are primarily grouped into sand (<2000-0.05 μm), silt (50-2 μm) and clay (<2 μm) fractions according to the USDA classification. The grouped data (% sand, % silt, and % clay) are used to derive the textural class of each soil sample according to the USDA textural triangle. Soil organic carbon content was determined with the dichromate method (Mebius, 1960), whereas by convention the soil organic matter value was obtained by multiplying the SOC value by the constant factor of 1.724. The height of soil that remains in the core was slowly wetted from below (see the procedure suggested by Romano *et al.,* 2002), until saturated, using a de-aerated 0.005 M CaSO4 solution. Saturated water content, θ_{sat}, was measured by the gravimetric method (Topp and Ferré, 2002), whereas saturated hydraulic conductivity, K_{sat}, was measured in the laboratory by the falling-head method (Reynolds *et al.,* 2002).

Soil hydraulic properties, namely the soil water retention and hydraulic conductivity functions, were determined by subjecting each soil core in the laboratory to an evaporation experiment, analysing the measured variables through the modified Wind method (Peters and Durner, 2008) and using the optimization approach proposed by Romano and Santini (1999). The evaporation experiment starts from an initially saturated soil core and involves the measurements with time of suction head profiles and soil core weights. Starting from a condition of hydrostatic equilibrium, with a nearly zero suction head at the bottom of the soil core, the evaporation experiment is performed by draining the core with a small fan placed near the top and sealing the lower end of the core completely (Nasta *et al.,* 2013). During the transient flow event, the total weight of the soil sample (with a load cell) and the matric head (by horizontally inserted micro-tensiometers) at different positions along the soil core are measured at frequent but irregular times. All data are recorded automatically and processed using a data-logger and a personal computer. On completion of all the hydraulic tests, we measured the oven-dry bulk density by putting the cores in a ventilated oven at the constant temperature of 105°C. In this study, the soil hydraulic functions are described by the bimodal hydraulic model proposed by Durner (1994). For further information on this model the reader is directed to the HYDRUS-1D manual (Šimůnek *et al.,* 2015). Romano *et al.* (2011) and Romano and Nasta (2016) also demonstrated the effectiveness of employing bimodal soil hydraulic properties to obtain better predictions from Richards-based models of unsaturated flow.

Simulated scenarios

The HYDRUS 1-D model (Šimůnek *et al.,* 2018) uses the Richards equation to describe water flow in soil and the advection-dispersion equation to represent solute transport. The main assumptions in this study were as follows: i) one-dimensional flow domain; ii) preferential flow paths are considered; iii) time-invariant contaminant root uptake; iv) phytoremediation based on root extraction of solutes.

The root uptake model was defined by a macroscopic approach for determining the sink term in the Richards equation. This term describes the capacity of the roots to draw water from the soil. The root uptake process is limited when water availability is limited, according to the formulation of the Feddes model. The sink term is delimited by specific threshold values, typical of poplar trees. Contaminant root uptake was computed through two different methods, one passive and one active. The former assumes that solute uptake is proportional to the water sink term and to the concentration of the solute dissolved in water. Active uptake was calculated using Michaelis-Menten kinetics. The maximum theoretical uptake was termed uptake potential $A_p(t)$, which is characteristic of plant-solute coupling. The Michaelis-Menten constant K_m [M L^{-3}] is assumed as K_m = 0.09537 μg cm^{-3} and A_p=0.0271 μg cm^{-2} d^{-1} for Cd^{2+}.

The upper boundary conditions were imposed by using the daily values of rainfall and potential evaporation, whereas potential transpiration represents the potential root water uptake exerted along the soil profile. Reference evapotranspiration was calculated using the equation of Penman-Monteith (Allen *et al.,* 1998). The initial conditions were imposed at hydrostatic equilibrium, while the lower boundary was free drainage.

As for the ion-matrix interactions, the adsorption of ions on the solid matrix is considered to be well described by a linear model applicable to all soil horizons. The distribution coefficient K_d was inferred from studies carried out on similar soils, using the value of K_d =140 mL/g (Anderson and Christensen, 1983), while the diffusion coefficient in free water was set according to the standard reference values (Shackelford and Daniel, 1991).

Numerical simulations were organised into three steps: pre-contamination, contamination and remediation. The first step (lasting one year) was a spin-up time step required to establish suitable initial conditions in the soil profiles. Spontaneous shrubby vegetation was present at the soil surface. During the second step, *true* contamination was simulated by considering the mean value of Cd concentration, 0.546 mg kg^{-1} (see previous Section). In the third step, phytoremediation of soil was evaluated by comparing the bare soil (BS scenario) with *Populus nigra spp* (PP scenario) scenarios. The simulated period of the third step spanned from September 24th, 2014 to January 15th, 2016. According to an experimental study, this tree species is not affected by toxicity at experimental concentrations. The parameters of Feddes' model for root water uptake were estimated by analogy with similar trees (Guittonny-Larchevêque *et al.,* 2016).

Results and discussion

One of the main issues that arouse when one simulates environmental problems is *how well the simulated transport process resemble the actual one.* Soil water content and its temporal variation are of paramount importance to assess the success of a phytoremediation technique. Therefore, in this study we consider that the good description of soil water flow in a field site is a good proxy of the goodness of the transport simulation.

For this work we consider soil water content as a *witness* variable to evaluate the robustness of model implementation. To do so, we compared observed and simulated soil water content data at the three installation depths in the scatterplot in Figure 4.

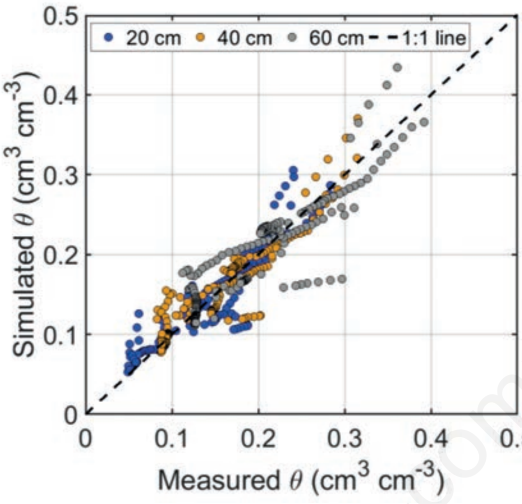

Figure 4. Comparison between measured and simulated soil water content values at the three installation depths.

A robust prediction of soil water dynamics can be diagnosed by the alignment of data pairs around the identity line, depicted by the 1:1 line. The root mean squared error, RMSE, values ranged from 0.023 cm^3 cm^{-3} for soil water content at 40 cm depths to 0.033 cm^3 cm^{-3} for soil water content at the depth of 60 cm, while the RMSE value for the depth of 20 cm shows an intermediate value (0.023 cm^3 cm^{-3}). These results may be considered acceptable by following the guidelines provided by Moriasi *et al.* (2007). Indeed the RMSE values have the same order of magnitude as the measurement errors obtained by the monitoring devices (see the GS3 Decagon manual, available at: http://manuals.decagon.com/Manuals/13822_GS3_Web.pdf).

Besides the model performance on the soil water status, the present study focused on the phytoextraction capacity of poplar. In doing so, in Figure 5 we show how complex processes can be monitored with different variables.

The timespan of the graphs is from April 5th to May 7th, 2015 and the time unit is set in hours. This was a period of rapid poplar growth, with trunk diameter increasing from 2.1 cm to 4.0 cm. Initially, soil water storage induced by the antecedent wet season sustained transpiration and cadmium accumulation. As the water content decreased, available water also decreased (see the red line of soil water potential) by inducing root water stress, up to the minimum limit of soil matric potential (y= −527 kPa) on the 480th hour of simulation. Water stress prompted a decrease in actual *simulated* transpiration rate, associated with a decreased *measured* daily difference in trunk diameter (0.02 mm). As soon as rainfall events (33 mm in all) replenished the soil profile, soil matric potential increased and stabilized at around y= −33 kPa, alleviating water stress. Consequently, poplars could again transpire at maximum velocity by supporting potential transpiration.

Very few experiments show attempts to correlate stem variation with other physiological or meteorological variables pertaining to poplar, such as pre-dawn leaf water potential (Giovannelli *et al.,* 2007), vapour pressure deficit (McLaughlin *et al.,* 2003) or groundwater table depth (Xiao *et al.,* 2014). We present here an effective correlation, albeit in a qualitative manner, of stem diameter daily variations with simulated actual transpiration. Further research is desirable, as it should be noted that the dendrometer is a low-cost sensor and relatively easy to maintain. Once an effective relationship could be developed between dendrometric measurements and poplar water status, the use of the dendrometers

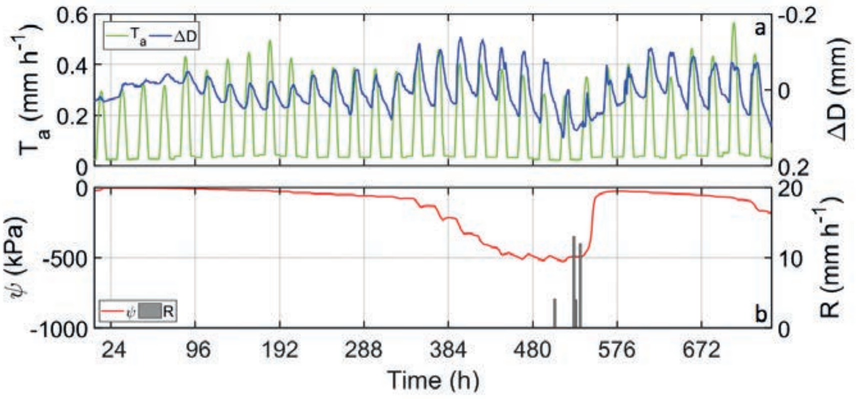

Figure 5. Hourly values of A) simulated actual transpiration (green line) and detrended dendrometer measurements (blue line) in the period between April 5, 2015 and May 7, 2015; B) soil matric potential (red line) and rainfall amount (grey bar).

would allow direct control on the effectiveness of irrigation practices or phytoremediation protocols.

The simulated water balance components pertaining to BS and PP scenarios gave us the chance to prove the effectiveness of the phytoremediation protocol (Figure 6). Total rainfall for both simulations was 1124 mm. Cumulative deep drainage, D_{PP} (solid green line) in the PP scenario (total amount of 205 mm) decreased by 36.5% when compared to D_{BS} (dashed green line) simulated in the BS scenario (total amount of 323 mm). This difference was mainly due to higher cumulative actual evapotranspiration (ET_{PP}, solid red line) losses (886 mm) than the sole evaporative (E_{BS}, red dashed line) loss in the BS scenario (812 mm).

In our study the deep drainage in the PP scenario was 18.6% of total rainfall, while it represented 29.3% of total rainfall in the BS scenario. Fan *et al.* (2015) came to similar results, concluding that the presence of a *Pinus elliotii* forest reduces deep drainage with interception and root uptake processes.

Below we evaluate the results in terms of Cd concentration at the soil bottom boundary (1 m depth), and in Figure 7 we show the typical breakthrough curves as defined by Van Ommen (1985) for scenarios BS (dashed line) and PP (solid line).

The relatively rapid release of cadmium through the lower boundary of the modelled soil profile is due to the preferential flow processes, induced by macroporosity. Indeed, for this reason we used dual porosity water retention curves (Durner, 1994). The peak in solute release for scenario BS occurs on April 1st and is equal to 1.17×10^{-9} mg mm^{-3} d^{-1}, while the peak pertaining to scenario PP occurs four days later, on April 5th, and amounts to 0.23×10^{-9} mg mm^{-3} d^{-1}, representing only 19.7% of the former. The large difference in Cd elution between the two scenarios and the consistent reduction in the release of cadmium towards the water table can be explained by two mechanisms. The former is the retardation of Cd migration along the downward direction due to root water extraction. Higher transpiration induced by the poplar tree reduce drainage, and hence cadmium has less chance to migrate towards the bottom boundary. The latter is attributable to the absorption of the solute by the roots and the relative accumulation in the poplar's plant organs. These two mechanisms act together, and their effects reinforce each other in reducing the concentration of cadmium in the soil water moving towards the groundwater. When the cadmium is removed by poplar roots from the soil water and accumulated in the plant organs, its concentration in the soil solution decreases very rapidly. Nonetheless, these two combined mechanisms take effect only if the poplar plant is able to sustain potential transpiration. This eventuality can only take place when two conditions coexist: i) presence of available water (above field capacity) in the root zone during the growing season (Nasta and Gates, 2013); and ii) high evapotranspiration fluxes. This effect is evident from the perusal of Figure 8.

In the first 100 days of simulation, transpiration decreased due to drying conditions of soil water (subplot *a* in Figure 8). Along with this effect, we observed a concomitant decrease of cadmium phytoextraction in the vadose zone (subplot *b* in Figure 8). Therefore an optimal irrigation routine should be ensured with the aim of inhibiting root water stress (Gibson *et al.,* 2017; Gibson *et al.,* 2018).

With a view to considering this study as a proof-of-concept, monitoring and modelling activities are basically discussed to show their potential for assessing the effectiveness of techniques to remediate a PTE-contaminated soil. We would emphasise that one-dimensional simulations performed at the scale of individual trees should be conveniently integrated with results obtained by running a three-dimensional model at the scale of the entire field. This latter modelling task, albeit even more demanding than the former, definitely gives a more detailed picture of the problem at hand. Also, we envisage it as a key step to properly and effectively transfer the scientific results to the realm of the public officers and stakeholders who should take decisions on the management of contaminated soils and possibly give remediated fields back to their owners to restore farming practices. Capolupo *et al.* (2018) recently showed that coupling photogrammetry by unmanned aerial systems (UAS) and

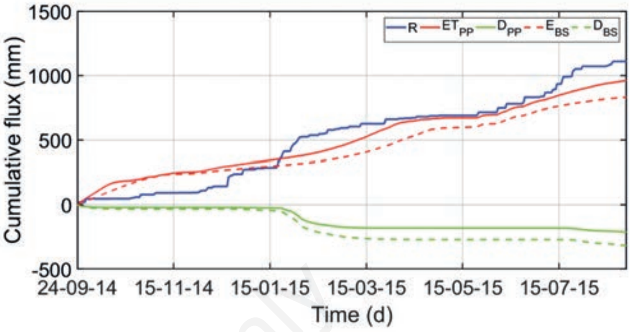

Figure 6. Daily cumulative values of rainfall, actual evapotranspiration and drainage for poplar (PP), actual evaporation and drainage for bare soil (BS).

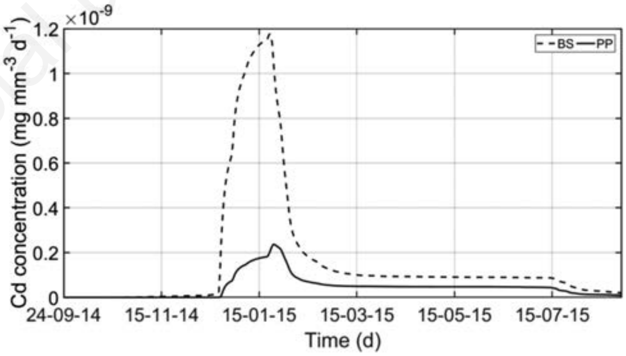

Figure 7. Daily values of Cd concentration at the bottom boundary of the soil profile simulated for BS and PP.

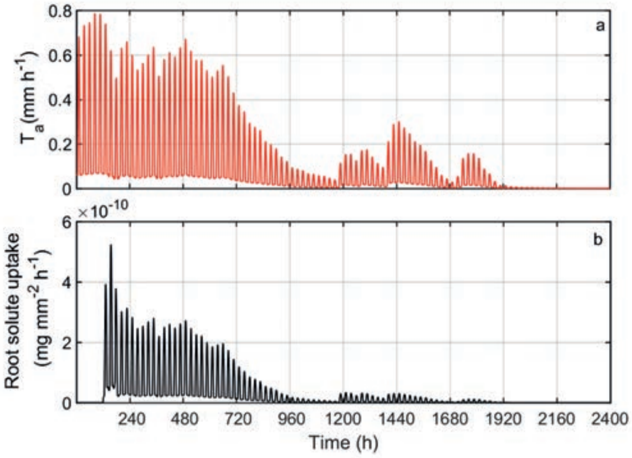

Figure 8. Hourly values of actual root water (A) and solute (B) uptake during the first 100 days of simulation.

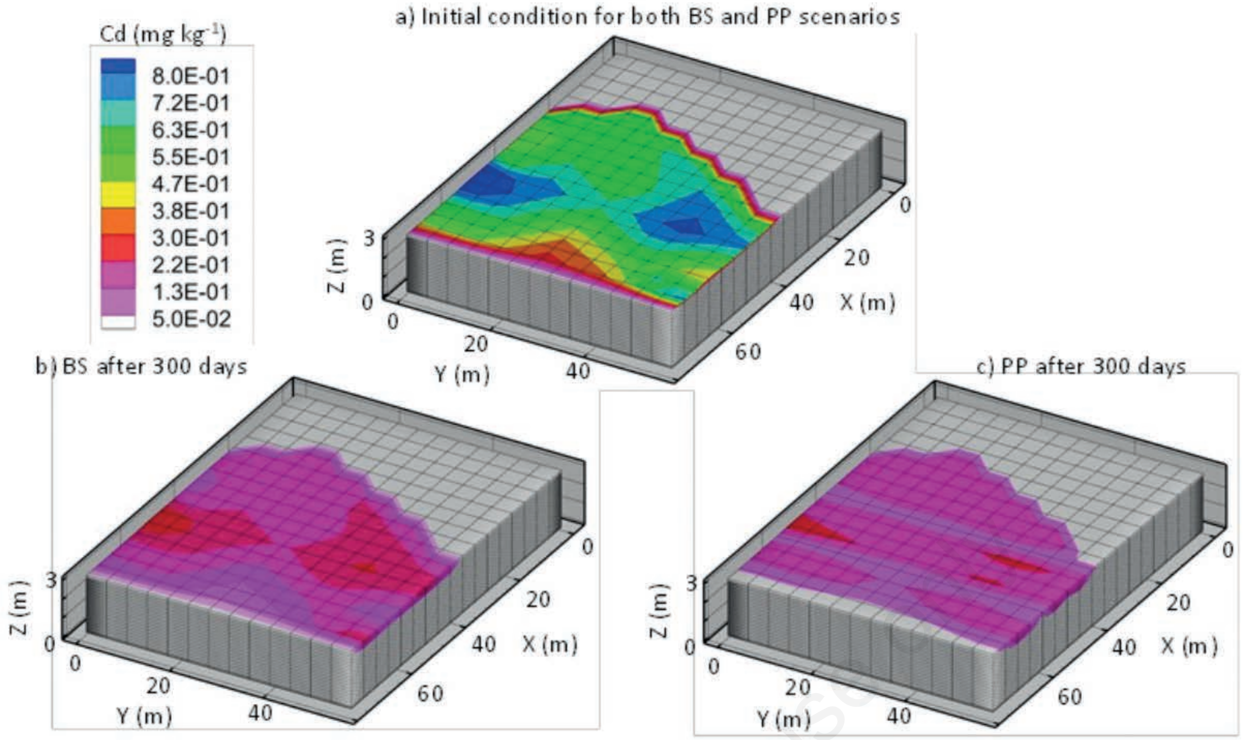

Figure 9. Maps of Cd concentrations in both scenarios at the beginning (A) and after 300 days of model simulations for scenario BS (B) and scenario PP (C).

the HGS model is a powerful tool for field-scale modelling activities.

In the PP scenario, poplars were virtually allocated in a block-designed rectangular grid with an inter-row distance of 10 m and a spacing of 5 m. Figure 9 displays the snapshots of initial soil surface Cd concentrations (Figure 9A). The spatial-average initial Cd concentration on the soil surface is 0.37 mg kg^{-1} in both scenarios. After 300 days of simulation the differences in terms of Cd concentrations are considerable when comparing the map deriving from scenario BS (Figure 9B) and the map from PP (Figure 9C).

The total Cd concentration absorbed by the poplar roots is governed by the time-varying solute uptake exerted by the hybrid poplar roots. The beneficial effect of a poplar plantation not only reduces Cd concentration on the soil surface, but also removes Cd along the soil profile by reducing the risk of contaminant transport towards the groundwater. Indeed, the numerical simulations featuring in the BS scenarios show high levels of Cd (even >0.3 mg kg^{-1}) concentration along the soil profile (Lu *et al.,* 2017). The simulations in the 3D environment evidenced that the phytoextraction efficiency assumes its maximum value during the autumn due to the availability of enough water to ensure solute uptake and to reduce the water stress conditions. Indeed, Cd concentrations diminished by about 64% during the winter, and just 36% during the summer period (Capolupo *et al.,* 2018). As demonstrated in previous studies (Robinson *et al.,* 2000; Wu *et al.,* 2010), poplar trees strongly contribute to the reduction in Cd concentrations by lowering its values to about one third (0.04 mg kg^{-1} and 0.12 mg kg^{-1} respectively for scenarios BS and PP). In another study Lugli and Mahler (2015) demonstrated that other plant species (such as vetiver grass) are able to remove 11% of initial cadmium concentrations after 48 months.

Conclusions

In this study we provided basic guidelines to integrate soil samplings, field campaigns, monitoring activities and 1D and 3D flow and contaminant transport simulators in the soil-vegetation-atmosphere system in order to evaluate the performance of phytoremediation of cadmium in a small, potentially contaminated site. Among all the remediation strategies, phytoremediation can be considered the most cost-effective, environmentally friendly and practical approach. The scenario-based simulations proved the effectiveness of phytoremediation for reducing cadmium concentrations and potential risk in food quality (Ding *et al.,* 2018). However, water stress partially inhibited the contaminant uptake and might compromise the phytoextraction of cadmium. In general, transpiration greatly decreased during the dry season in rainfed fields, and hence the removal of cadmium diminished. Under such circumstances, the supply of irrigation water might alleviate water stress and buffer phytoextraction by preventing the potential migration of cadmium to the aquifer.

This scenario-based approach can be considered as a proof-of-concept and might be extended to compare the effectiveness of other fast-growing trees with the performance of poplar plantations for the phytoremediation of cadmium (Wu *et al.,* 2010; Mayerová *et* *al.,* 2017). Moreover, the integration of field measurements and 3D flow simulators can be used in future investigations to predict the fate of other potentially hazardous PTEs (such as copper, chromium, mercury and zinc) in the study area. By combining intense data acquisition and robust numerical modelling, the modelling scenarios will provide the chance to gain insights into the complex interplays between soil, water and vegetation governing the water balance and solute transport. We envision the integration of measurement techniques and modelling approaches as learning tools, with a view to transferring precious and basic knowledge to stakeholders and decision-makers. Indeed, the model projections will have tremendous practical relevance to society by promoting sustainable strategies to combat soil contamination.

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