Assisted phytoremediation for restoring soil fertility in contaminated and degraded land

Nunzio Fiorentino, Mauro Mori, Vincenzo Cenvinzo, Luigi Giuseppe Duri, Laura Gioia, Donato Visconti, Massimo Fagnano

Department of Agricultural Sciences, University of Naples Federico II, Portici (NA), Italy

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.

Correspondence: Nunzio Fiorentino, Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055 Portici (NA), Italy.
E-mail: nunzio.fiorentino@unina.it

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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

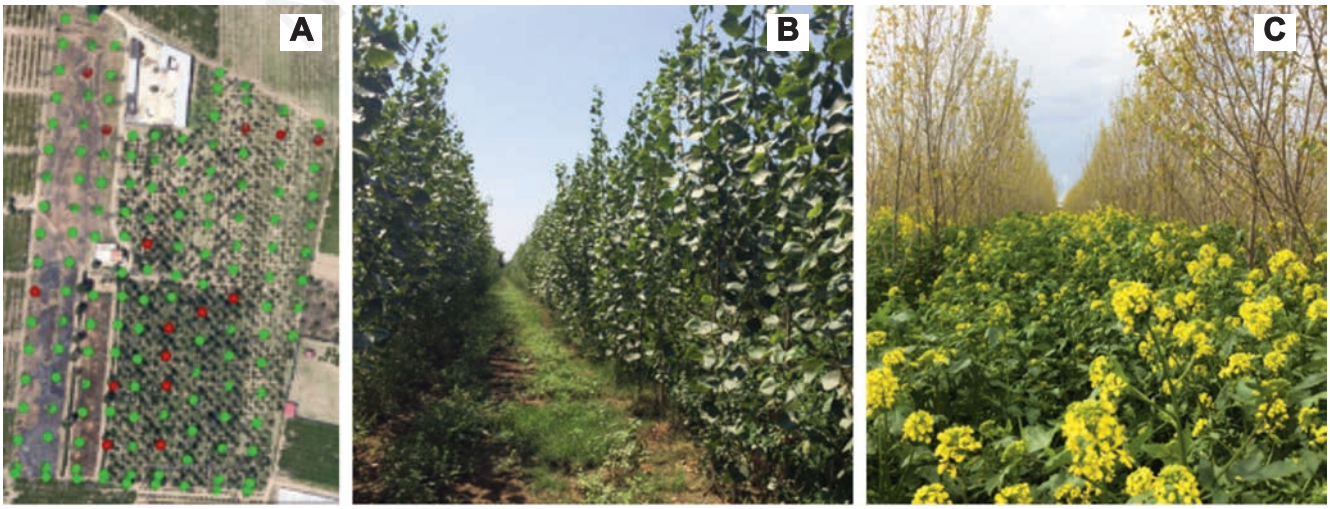

Figure 1. A) Aerial photo of the San Giuseppiello site; B) poplar grassed rows; C) Indian mustard in the hot spot contaminated by Cd.

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

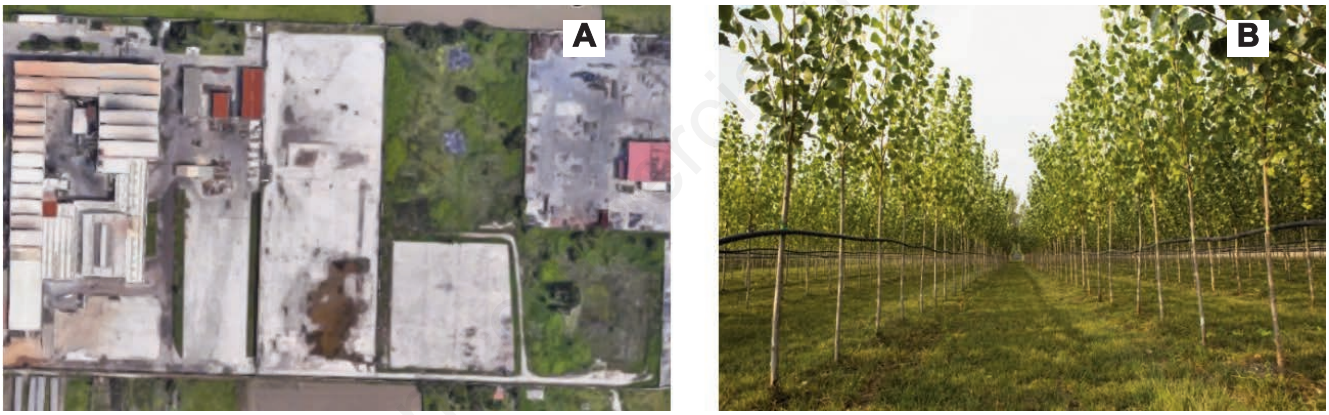

Figure 2. A) Aerial photo of the Ecobat site; B) poplar grassed rows for making safe the site.

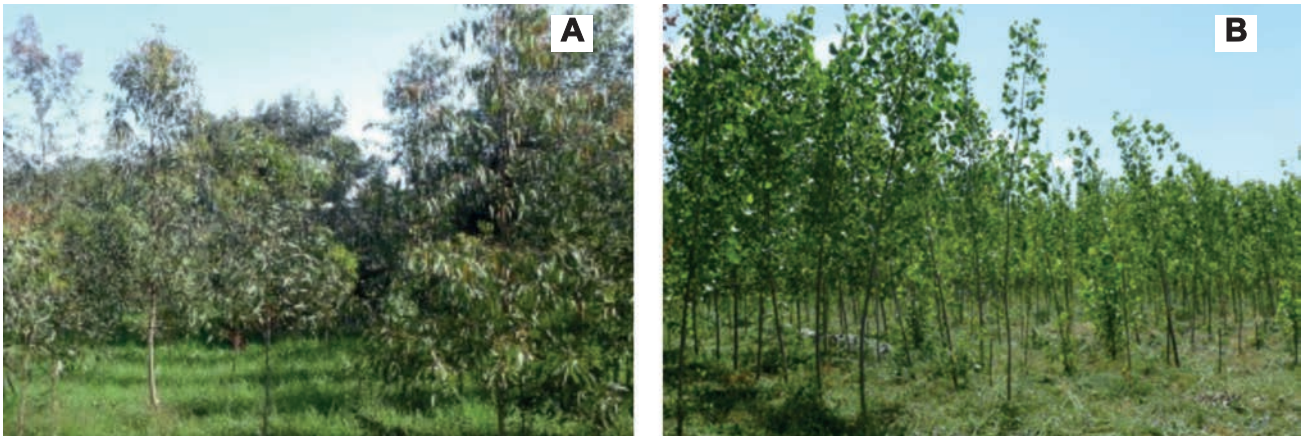

Figure 3. A) Eucalyptus stand at Giugliano; B) poplar stand at Trentola.

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.

Figure 4. Giant reed stand at Teverola 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.

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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