Polycyclic Aromatic Hydrocarbons and Heavy Metal Contaminated Sites: Phytoremediation as a Strategy for Addressing the Complexity of Pollution

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Abstract Since the industrial revolution, soil has been increasingly subjected to continuous negative pressure, largely determined by human activities, which have dispersed heavy metals and many persistent organic compounds causing severe soil contamination. Among pollutants, heavy metals and polycyclic aromatic hydrocarbons (PAHs), which are ubiquitous and generated also from natural resources, are of particular concern. The simultaneous presence of both kinds of pollutants is very common in brownfield sites, and the clean-up of these areas presents technical difficulties and requires appropriate solutions at a reasonable cost. Remediation technologies have often used invasive processes that greatly damage soil characteristics, causing the deterioration of this important resource. In this chapter, the objectives are to briefly examine the processes involved in heavy metal and PAH reactions in soil in order to evaluate the best possible cost-effective remediation strategies for maintaining a high quality of soil and surrounding environment.

Keywords Soil • Remediation technologies • PAHs • Heavy metals • Phytoremediation

1 Introduction

Man is closely dependent on soil functions. Healthy soil ensures clean water, abundant crops and carries out essential functions such as the regulation of the cycle of nutrients and other elements, as well as the flow of water and solutes necessary for the survival of plants and animals. Soil supports the growth of higher plants and biodiversity being an ecological habitat for many organisms. Soil with its high

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buffer capacity works as living filter for waste products. Moreover, soil has the ability to maintain its porous structure to allow the passage of water and air, counteracting the erosive processes [1]. Since the industrial revolution, soil has been increasingly subjected to continuous environmental pressure, largely determined by human activities, which have dispersed heavy metals and many persistent organic compounds causing severe soil contamination. All these activities impair the natural ability of soil to perform the abovementioned functions. Among pollutants, heavy metals and polycyclic aromatic hydrocarbons (PAHs), which are ubiquitous, generated also from natural resources, are of particular concern. The simultaneous presence of both kinds of pollutants is very common in brownfield sites, and the cleanup of these areas presents technical difficulties and requires appropriate solutions at a reasonable cost. Remediation technologies have often used invasive processes that greatly damage soil characteristics, causing the deterioration of this important resource. In this chapter, the objectives are to briefly examine the processes involved in heavy metal and PAH reactions in soil in order to evaluate the best possible cost-effective remediation strategies for maintaining a high quality of soil and surrounding environment.

1.1 Polycyclic Aromatic Hydrocarbons (PAHs)

In the environment, PAH formation can occur as a result of incomplete combustion of organic materials, such as coal, oil, gas, waste, and other organic substances. In addition to these types of contribution, PAHs can also be released into the environment as a result of volcanic activity, forest fires, and burning coal. In soil, high levels of PAHs have been discovered at nearly all industrial sites where fossil fuels have been used in the production processes, including energy generation. The greatest contamination often occurs at former manufactured gas plant sites where generally heavy metals are also present in soils. The molecules of PAHs consist of two or more condensed aromatic rings, fused together via a pair of shared carbon atoms. The placement of the rings can determine linear, angular, or cluster forms. The physical-chemical properties of PAHs mainly depend on the molecular weight and the reactivity of the π electrons. When there is an increase in number of benzene rings and conjugated bonds occur, the delocalization of π electrons increases. The reactivity of the carbon atoms differs depending on the position, as regards both electrophilic substitution and the redox reactions. The reactive positions vary depending on the size of the molecules. PAHs are chemically rather stable; their reactivity is influenced not only by the molecular weight but also by numerous environmental factors such as temperature, light, and oxygen levels which favor the formation of numerous oxidation products and presence of co-pollutants and of materials capable of adsorbing them.

The distribution of PAHs in the environment depends greatly on their chemical–physical characteristics. All PAHs have high melting points and boiling points, low vapor pressure, which is inversely proportional to the number of rings, and low solubility in water, which decreases in the presence of high ions concentration: “salting

out" effect. PAHs are extremely lipophilic, and this feature strongly influences their bioaccumulation.

In soil, the hydrophobic substances tend to reach equilibrium between the solid and the aqueous phases depending on numerous factors including temperature, concentration, amounts of solutes, amount of organic substances, and the characteristics of the contaminant. Their distribution between the liquid and solid soil phases is described by the partition coefficient K_d:

$$K_d = C_S / C_W$$

where C_S is the concentration of the substance in solid phase and C_W concentration of the substance in the aqueous phase. Since the tendency of a hydrophobic substance to distribute in soil between the two phases depends especially on the amount of total organic carbon, K_d can be replaced by K_{OW} the partition coefficient between water and octanol, the organic solvent with similar behavior to that of the organic matter:

$$K_{OW} = C_O / C_W$$

with C_O solubility of the compound in octanol and C_W solubility in water. Generally, $\log K_{OW}$ is inversely related to water solubility and directly proportional to molecular weight of PAHs.

K_{OW} allows evaluating the retention and release of organic compounds in soil and their tendency to bioaccumulate in human and animal tissues through the food chain.

Some properties of the most dangerous PAHs are reported in Table 1.

1.2 Toxicity Effects

It is known that PAHs have negative effects on the environment and human health. The risk to human health is associated with their toxic, mutagenic, genotoxic, teratogenic, and carcinogenic properties [2–4]. Numerous studies have shown that the compounds with 1, 2, and 3 rings are extremely toxic [5], while the higher molecular weight PAHs are considered to be genotoxic [6–8]. Although it has been estimated that 75 % of the total amount of PAHs assimilated enters the body through absorption in the epidermis [9], contamination of the food chain plays an important role in their accumulation since, once ingested, they are rapidly absorbed from the gastrointestinal tract due to their high lipid solubility [10]. Inhalation is another way of ingesting PAHs, following entry into the respiratory system of the particulate matter on which they are adsorbed.

Once in human body, PAHs undergo biotransformation reactions; their elimination depends on the ability to convert them into water-soluble metabolites. However, the formation of reactive metabolic products may determine the mutagenic and

Table 1 Physical-chemical properties of the 16 polycyclic aromatic hydrocarbons selected as priority pollutants by the U.S. Environmental Protection Agency

PAH compounds	PAHs abbreviation	Molecular weight (g mol^{-1})	Solubility at 25 °C (μg L^{-1})	log K_{OW}	log K_{OC}
Naphthalene	Nap	128	12,500	3.30	2.44
Acenaphthylene	AcPy	152	3420	3.94	3.40
Acenaphtene	AcP	154	4000	3.92	3.66
Fluorene	Flu	166	800	4.18	3.86
Phenenatrene	Phe	178	435	4.46	4.15
Anthracene	Ant	178	59	4.54	4.15
Fluoroanthene	FL	202	260	5.20	4.58
Pyrene	Py	202	133	5.18	4.58
Benzo[*a*]anthracene	BaA	228	11	5.76	5.30
Chrysene	Chr	228	1.9	5.81	5.30
Benzo[*b*]fluoroanthene	BbFL	252	2.4	5.80	5.74
Benzo[*k*]fluoroanthene	BkFL	252	0.8	6.00	4.98
Benzo[*a*]pyrene	BaP	252	3.8	6.13	5.74
Benzo[*g*,*h*,*i*]perylene	BghiP	276	0.3	6.63	6.20
Indeno[1.2.3-*c*,*d*] pyrene	InP	276	0.2	6.70	6.20
Dibenzo[*a*,*h*] anthracene	DBA	278	0.4	6.75	6.52

carcinogenic effects in mammals. Following an enzymatic reaction mediated by the cytochrome P450 monooxygenases, the aromatic rings are oxidized giving rise to intermediate epoxide, dihydrodiolepoxide [9]. These intermediates, in particular the diol-epoxides, combine covalently through a nucleophilic attack with the DNA molecules, generating distortions in the structure of the genetic material causing mutations and, therefore, a greater probability of carcinogenesis. Not all PAHs generate damage at the genetic level since not all are the precursors of these reaction intermediates. In particular, most of the PAHs that show carcinogenic properties are formed by more than three benzene rings [11]. The International Agency for Research on Cancer (IARC) has classified many PAHs as "probable or possible human carcinogen" (Group 2A and 2B, respectively), whereas benzo[*a*]pyrene was classified as Group 1 "carcinogenic to humans." In the environment, PAHs are usually found in mixtures and not as single compounds. This makes difficult to define the consequences for human health, due to possible synergistic effects, that make the toxicity of the mixture greater than the sum of the toxicity of individual compounds. Benzo[*a*]pyrene is used as an indicator for assessing levels of contamination and carcinogenic risk, since its carcinogenicity is higher than that of the other PAHs [12].

1.3 PAHs in Soil: Behavior and Effects

In soil, increasing concentrations of PAHs may impair the structure of the microbial community, reducing biomass and inhibiting certain metabolic activities [13]. The negative effects of PAHs differ depending on the compounds; for example, phenanthrene is more toxic than pyrene for the microbial community due to its greater accessibility. Also enzymatic activities are influenced by PAH contamination; dehydrogenase activity appears to be the biological parameter most sensitive to these contaminants in different types of soil [14]. Once in soil, the fate of PAHs is determined [15] by their distribution among the solid, liquid, and gaseous soil phases, which strongly influence the processes of migration and degradation. PAHs of low molecular weight may volatilize into the atmosphere or be leached along the soil profile. Those of high molecular weight may be strongly adsorbed to clay materials and humic substances of the soil. In addition, these compounds can undergo redox reactions of abiotic origin and can be absorbed and biodegraded by microorganisms in the soil.

The role of soil organic matter is of paramount importance in determining the fate of PAHs in soil; due to similarity of PAHs to humic substances, they are strongly adsorbed by soil organic matter. Interactions among PAHs and organic matter have been described according to different models; in particular the distributed reactivity model (DRM) and the dual-mode model (DMM) describe organic matter as a multi-domain material, showing either linear or nonlinear sorption characteristics [16]. PAH sorption can be described by a dual-mode sorption composed of absorption by amorphous humic materials and adsorption to carbonaceous materials such as black carbon [17]. Due to the high affinity, the sorption process is often nonreversible, and it can be considered one of the main factors responsible for the aging process that greatly reduces PAH bioavailability [18]. Therefore, PAHs that are less volatile and less soluble in water accumulate in the soil.

Persistence in the soil depends on the overall result of all the mechanisms of transport and degradation above mentioned [15]. Water solubility should be considered one of the most important physical-chemical properties for PAH biodegradation. Hydrophobicity increases with increased number of fused benzene rings. Thus, low molecular weight PAHs are more quickly released from soil surfaces and therefore are more available for microbial degradation [19]; as a consequence many organisms are able to degrade 2 and 3 ring PAHs, while relatively few have been discovered to degrade 4, 5, and 6 ring PAHs. Because of their chemical–physical characteristics PAHs persist in the environment for long periods and are thus considered persistent organic pollutants (POPs). Nevertheless, these compounds can undergo transformation and degradation processes due to biotic and abiotic reactions. Photodegradation processes have an important relevance in the degradation process; PAHs can be degraded via two mechanisms: direct photolysis by ultraviolet radiation ($\lambda < 290$ nm), and indirect photolysis and photooxidation, due to the action of oxidizing agents (•OH radicals, O_3, NO_x). However, these reactions can result in the production of molecules far more dangerous to the environment, as in

the case of nitro derivatives formed by interaction with NO_x [20]. Other processes of abiotic transformation of these organic contaminants may be derived from the oxidizing action of metal ions such as manganese and iron [21].

Microbial degradation is considered one of the principal mechanisms of PAH removal from soil [10, 22–24]. The metabolic processes involved in the degradation of the PAHs are predominantly aerobic based on oxidation reactions with oxygen or nitrate as electron acceptor. These processes are based on the cleavage by oxidation of the aromatic ring, with consequent formation of metabolites and carbon dioxide. Following exposure to hydrocarbons, the oxidation potential of microbial communities may increase due to adaptation processes [25] that produce an increase or a decrease of specific enzymes. Moreover, new metabolic abilities may develop after selective enrichment of organisms able to transform these pollutants [25–27]. The ability of microorganisms to degrade PAHs may be ascribable to the synthesis and subsequent excretion of enzymes characterized by oxide reductase activities [28, 29]. These enzymes are involved in the degradation process of recalcitrant organic compounds, such as lignin, a complex organic polymer consisting of multiple phenyl propane units. These enzymes (phenoloxidase and peroxidase) are able to oxidize PAHs [30] due both to their low substrate specificity and to the structural similarity of PAHs with lignin, resulting from their aromatic character.

Fungi, especially white-rot fungi (belonging to the group of Basidiomycetes and to a lesser extent of the Ascomycetes), are the organisms mostly involved in the degradation of lignin as oxide reductase producers, mainly peroxidases and laccases. Even brown-rot fungi possess "PAH-degrading" enzymes, which have shown the ability to use PAHs as a sole source of carbon [31]. Also many kinds of saprotrophic bacteria in soil synthesize enzymes with phenol oxidase activity [32, 33]. The degradation has been also promoted by sporogenic bacteria such as *Bacillus* and proteobacteria including *Pseudomonas* [34]. Microbial communities in soil have a very high potential to degrade PAHs. Synergy between various microbial groups promotes complete degradation; for example, by-products generated from the oxidation of PAHs by fungi can be further used by bacteria until complete decomposition. The action of specific bacteria ligninolytic and non-ligninolytic fungi has been reported in detail elsewhere [9]. In contaminated soil, the presence of readily biodegradable substances may produce a reduction of available oxygen and, in these cases, also PAH anaerobic degradation by means of electron acceptors other than oxygen has been described [9].

2 Heavy Metals

Heavy metals are defined as metallic elements that have a relatively high density at least five times greater than that of water. The term is broadly used to also include certain elements such as arsenic, which cannot be formally considered a heavy metal. Heavy metals are naturally present in the soil environment deriving from the pedogenetic processes of parent materials. However, in industrialized countries,

many activities contributed to increase in heavy metals concentration in soil such as industrial activities, mining, waste disposal, etc. Heavy metal soil pollution has been increasing since the beginning of the Industrial Revolution. Since metals are not biodegradable, they tend to persist and accumulate in soils; however, the risks to humans and the environment strictly depend on their bioavailability.

2.1 Toxicity Effects

Heavy metals have been used for thousands of years, and emissions into the environment occur via air, water, and soil. The main hazards to human health from heavy metals are derived from exposure to chromium, lead, cadmium, mercury, and arsenic, the last one being a metalloid associated for its toxicity to heavy metals [35]. The environmental exposure of humans to heavy metals involves a very high degree of complexity, especially near contaminated sites, where the population is frequently exposed to a wide variety of pollutants, whose biological effects may be synergetic. To evaluate the risks in the presence of a complex environmental contamination requires studying of the molecular mechanisms of action of each contaminant and the identification of possible interactions between different biological effects. Regarding heavy metals, the association between environmental exposure and increased incidence of cancer is well known and widely documented for various metals by the International Agency for Research on Cancer. Specifically, chromium (Cr), cadmium (Cd), and nickel (Ni) are considered in Class 1 human carcinogens based on sufficient evidence of a carcinogenic effect on humans [12].

Lead (Pb) and mercury (Hg), frequently associated with environmental contamination, are classified as possible carcinogens (Class 2B) only in some chemical forms. Arsenic, due to its mechanisms of interaction with biological material, is considered a carcinogenic Class 1 contaminant [12]. Heavy metals are able to interact with different stages of the process of carcinogenesis, producing DNA damage directly or indirectly, reducing the efficiency of the defensive systems of the cell. Thus, they act as cancer promoters, in some cases also by modulating the processes of cell adhesion with consequences for the ability to produce metastases. Heavy metals are able to interact with cell components, producing, directly or indirectly, DNA damage; thus, they act as cancer promoters [36, 37].

2.2 Heavy Metals in Soil: Behavior and Effects

Heavy metals from anthropogenic sources are generally more mobile and their fate and transport in soil strictly depend on soil characteristics, which determine the chemical form and speciation of the metal [38]. Once in the soil, heavy metals distribute into different soil phases by precipitation—dissolution and adsorption—desorption reactions. In soils characterized by high contents of humic acids and clay

minerals, metals are strongly retained by complexation and adsorption reactions, which reduce their mobility. pH affects the concentrations of metals in soil solutions by regulating precipitation–dissolution, specific adsorption, and complexation processes thus determining the concentration of most metal ions in the soil pore water. Ion exchange and specific adsorption are the mechanisms by which clay minerals adsorb metal ions from the soil solution. Highly selective sorption occurs at the mineral edges, but differences exist between clay minerals in terms of their ability to retain heavy metals. Also hydrous iron and manganese oxides are particularly effective in influencing metal solubility under relatively oxidizing conditions.

They reduce metal concentrations in soil solutions by both specific adsorption reactions and precipitation. The organic matter in soil has a great influence on metal mobility and bioavailability due to the tendency of metals to form soluble or insoluble complexes with organic matter. The negative charges on soil surfaces, described by cation exchange capacity (CEC), may be pH dependent or permanent. Heavy metals can substitute alkaline cations on these surfaces by exchange reactions; specific adsorption promotes the retention of heavy metals, also by partially covalent bonds. Redox potential (Eh) in soil determines the reduction–oxidation reactions, which control the chemical forms of metals at different oxidation state. Well-aerated soils are characterized by high values of Eh, while soils subject to waterlogging tend to have lower Eh values [38, 39].

Transport and retention are the key processes that determine the fate and behavior of heavy metals in soil. Transport may occur through the soil solution by diffusion or by mass flow or convection. Retention of heavy metals on soil surfaces strongly determines metal release into soil solution and their transport to groundwater. The process of retention comprises chemical and physical adsorption and precipitation. The adsorption processes are essential for the evaluation of the soil as a protective barrier against heavy metals. The distribution of heavy metals between the solid phase and the soil solution is considered to be a fundamental factor in the assessment of the environmental consequences of the accumulation of metals in soil. The soil's ability to hold heavy metals in the solid phase is the fundamental mechanism by which soil protects other environmental matrices. Therefore, it is essential to assess the strength of this holding action and the nature of chemical bonds involved. Different kinds of forces retain metals on surfaces; these forces range from electrostatic to covalent with related bonding energies. Mechanisms that remove metal ions from solution include ion exchange and specific sorption [40]. Adsorbed on solid phases, heavy metals are usually unavailable to environmental processes, including plant uptake.

Thus, adsorption processes influencing the equilibrium between soluble and solid phases determine their fate in the soil environment. Several models have been used to describe the retention/release reactions of metals in soils. The adsorption equations theoretically refer to a state of equilibrium in which the rates of adsorption and desorption are equal. This implies a reversible process; however, some metal species are irreversibly held by the solid phase due to the formation of bonds, which are not exclusively electrostatic. Despite these theoretical limitations, several models are commonly used to describe heavy metals sorption in soil. The most

frequently used equations in soil chemistry are the Langmuir and Freundlich equations. The Langmuir equation (1), although originally derived for gas adsorption on solids, has been used successfully to describe heavy metal adsorption in different kinds of soil [40]:

$$q = \frac{q_{\max} KC}{1 + KC} \tag{1}$$

where q is the amount of metal sorbed per unity of mass of soil, $q_{\max}$ is the maximum amount of metal adsorbed by the soil, C is the equilibrium metal concentration, and K is a constant. The Langmuir equation can be derived from the action mass law, whereas the Freundlich equation (2) derives from the assumption that there is a linear relationship between the surface energy and the sites occupied. The general equation is:

$$q = KC^n \tag{2}$$

where q is the amount of metal sorbed per unity of mass of soil, C is the equilibrium metal concentration, and K and n are Freundlich parameters related to the maximum amount of adsorbable metal and the energy of bonds with which the metal is retained. Many other equations have been used in studying sorption and release of heavy metals in soil and elsewhere reported [41].

Much effort has been spent to quantify heavy metals retained with different kinds of forces by soil surfaces; in particular, in contaminated soils attention has focused on mobile and bioavailable metals [38, 39]. In soil, the chemical forms of heavy metals can be various. Heavy metals can be present as simple or complex ions in the soil solution, adsorbed or precipitated on the solid phases from which they can be released. When not specifically sorbed, they can be replaced by a competing cation by exchange reactions. If the binding mechanisms involve complexation and adsorption, metals are specifically sorbed and they are linked by covalent bonds to soil components. Heavy metals can also be occluded and coprecipitated with oxides, carbonates, and phosphates from which they can be released under specific conditions. In the crystalline lattices of primary minerals, metals are present in chemical forms that are not involved in environmental processes.

Knowledge of the chemical forms of metals is the key to understanding the toxicity, environmental hazards, and possible remedial strategies. Heavy metals are essential for plant and animal life, but can become toxic at high concentrations. Their toxicity for living organisms is closely linked to the bioavailability. In contaminated soils, bioavailability can be assessed by biological and chemical assays. The chemical assays provide information about bioavailability, determining the quantity of metals in soluble form thus in the liquid phase of the soil or easily releasable from the solid phase, for example metals retained with electrostatic bonds. This quantity can be determined either by direct sampling of interstitial water in the soil or through extractions with suitable reagents, such as water or dilute solutions of alkali metals.

Table 2 Some technologies utilized for PAHs and heavy metals

Class type	Technologies	PAHs	Heavy metals
Physical	Soil washing/soil flushing	Yes	Yes
Chemical	Solvent extraction	Yes	Yes
	Supercritical fluid extraction	Yes	Yes
	Precipitation	No	Yes
	Chemical oxidation	Yes	No
	Photocatalytic degradation	Yes	No
	Electrokinetic	Yes	Yes
Thermal	Incineration	Yes	No
	Thermal desorption	Yes	Yes
Biological	Bioremediation	Yes	No
	Phytoremediation	Yes	Yes

Extractions with stronger agents can be used to assess the potential release of metals from the surface of the soil with time [39, 42]. The chemical extraction must be supported by a biological test, for example evaluating the metal content in plants growing in the polluted soil. Chemical and biological tests are unable to produce a direct measure of bioavailability but both provide information about the amount of bioavailable metal [43]. The mobility/bioavailability processes may be used in the choice of remediation technologies. The aim of these technologies may be to remove from the soil the mobile fractions of the metals or to convert them to permanently stable forms. In the first case, chemical additives, which increase the mobility, are used. Alternatively, procedures may be used that reduce the bioavailability and prevent movement of the pollutants from the soil to living organisms [44].

3 Remediation of Heavy Metal and PAH Contaminated Soils

Due to concerns over health risks, many remediation technologies of soils contaminated with heavy metals or PAHs have been proposed and used. When the soil is simultaneously polluted by both these contaminants, the process of remediation presents considerable technical and economic difficulties. In Table 2, some remediation technologies for PAHs and heavy metals are schematically reported. Remediation technologies can be formally classified in Physical, Chemical, Thermal, and Biological according to the different processes adopted. They can be applied "in situ" or "ex situ" after excavation of soil.

Only few technologies are applicable when both contaminants are simultaneously present in soil, since we have to consider that heavy metals can severely reduce the biodegradation of PAHs. Of course, strategies of train technology can be applied; however, with a view to saving of time and costs the use of the same technology represents the best choice wherever possible. Some remediation technologies

able to address both heavy metals and PAH contaminated soils are synthetically reported.

3.1 Soil Washing

Soil washing (SW) is a physical/chemical "ex situ" treatment which exploits the tendency of contaminants to adhere to soil finest particles. The technology is based on the intimate vigorous mixing of excavated soil with a solution, typically water, followed by a classification step, which separates soil into different size fractions. Contaminants, which are attached to coarse fractions through forces of adhesion and compaction, are removed by abrasive scouring and scrubbing action and concentrate into a smaller volume of soil through particle size separation [45]. This technology has been successfully applied to concentrate the heavy metals into a reduced soil mass (typically 5–30 % of the original soil volume) for subsequent treatment, whereas clean or slightly polluted fractions can be returned to the site as fill, or otherwise used. The technology is highly practicable when metals are mostly present as water insoluble forms and in such case the technology should be strictly considered a physical process. In addition, it is essential that a relevant fraction of soil particles be of coarse size. Soil washing has been used also for PAHs [46], but although it is possible to address both kinds of contaminants the real application is extremely difficult due to the need to separate heavy metals in the solid phases and PAHs in the liquid one.

Treatment is often difficult due to the low solubility of the hydrophobic organic contaminants, such as PAHs, which, being strongly lipophilic, tend to be adsorbed to the organic substance present on the fine fractions of the soil (silt and clay). The efficiency of treatment is limited or reduced especially when these contaminants are present in high concentrations. However, if their concentration is high even on the larger particles, the washing of soil with surfactant agents can be a viable solution [47]. In this case, surfactants added to the wash solution, to a concentration of a few g L^{-1}, can increase the rate of desorption of PAHs and their transfer from the soil. Surfactants are capable of lowering the interfacial tension and to collect the PAHs by forming micelles [48, 49]. Surfactants are particularly attractive for remediation because they are characterized by low toxicity and high biodegradability and, thus, are more environmentally compatible than other systems based on organic solvents. However, the efficiency of physical soil washing has not been tested when these contaminant classes coexist.

Soil washing as a chemical technology has been recently investigated for mixed contamination by PAHs and heavy metals, also "in situ" (soil flushing). Sequential flushing using two chemical agents: a surfactant (5 % Igepal) and a chelant 0.2 M ethylenediaminetetraacetic acid (EDTA) have been evaluated for the remediation of soils contaminated by heavy metals and PAHs. Chelant released heavy metals, while PAHs were removed by surfactant flushing, but the process is highly dependent on site and contaminants' characteristics and requires further improvements

for full scale application [50]. The same conclusions were also reported by the authors for different chemical agents [51]. Very positive results of this cleanup strategy have been reported following the use of carboxymethyl-β-cyclodextrin (50 g L^{-1}) and carboxymethyl chitosan (5 g L^{-1}). Repeated washing cycles using these solutions have been reported to efficiently remove about 90 % of total PAHs and heavy metals [52].

3.2 Electrokinetic Technology

Electrokinetic remediation technology (EKRT) is a treatment that was originally developed for soils with a high clay content, contaminated by heavy metals. The technology is based on the insertion of electrodes in the contaminated soil. A direct current with low electric potential is applied to the electrodes; as a result different contaminant transport mechanisms are generated:

- Electromigration, which involves the transport of ions and other polar complexes dissolved in the pore soil solution, caused by the electrical potential applied
- Electroosmosis, the transport of ions and dissolved contaminants due to the movement of the interstitial soil solution, generated by the presence of the electrical double layer on the charged surfaces of the solid phase. Nonionic species are also transported along with the induced water flow
- Electrophoresis, the movement inside pore solution of colloidal particles with surface charge caused by the applied electrical potential

Among the processes of treatment available, electrokinetic remediation is interesting because of the advantages, linked to the possibility of being employed in situ and in clay soils. Many studies have investigated the electrokinetic removal of heavy metals from contaminated soil [53]. To improve the removal of metals and reduce the time for cleaning, systems may be employed that aim to adequately control pH by increasing the movement of the acid front that promotes the release of metals from the solid phase of the soil and their migration. The use of additives such as EDTA produced conflicting results depending on the cases studied [54–57]. Metal ions are transported to the electrode with the opposite charge (electromigration). The electroosmotic flow that is generated provides a driving force for the movement of solubilized contaminants [58]. Moreover, the treatment is able to simultaneously remove heavy metals and organic compounds. Electrokinetics has been used to remove PAHs from contaminated soils. Often the technology has been applied in conjunction with other treatments such as ultrasounds, to enhance electroosmotic or Fenton processes to promote oxidative/reductive processes [59]. In other cases, to improve the efficiency of removing PAHs, surfactants are employed. Positive results have been obtained at laboratory scale on Manufactured Gas Plant (MGP) soils. The PAHs are solubilized by surfactants in the presence of cyclodextrins and migrate significantly to the cathode [60]. Cyclodextrins are cyclic oligosaccharides derived from the degradation of starch by bacteria that have the ability to solubilize both

heavy metals and PAHs. In particular, it has been reported that a modified cyclodextrin, hydroxypropyl β-cyclodextrin (HPCD), is capable of solubilizing both some PAHs and heavy metals simultaneously [61].

Cyclodextrins are particularly effective for the removal of phenanthrene in clay soils [62]. Also in this case the compound is collected at the cathode, due to the electroosmotic flow [63, 64]. This study is particularly interesting because the technology is applied to both classes of contaminants present in the soil at the same time. These authors underline both the potential and the drawbacks of this technology. In particular, there is a need to produce higher electroosmotic flow with higher concentration HPCD to obtain significant phenanthrene removal efficiency. Moreover, it is necessary to adjust soil pH towards acidic values to increase nickel removal efficiency. The effectiveness of EKRT is closely dependent on soil properties such as its buffering capacity, organic matter content, heterogeneity, and presence of coarse material. These conclusions were confirmed by an accurate study of EKRT application to marine sediments simultaneously polluted by heavy metals and PAHs. The technology is not viable without the appropriate additives. Heavy metal removal was enhanced only if EDTA was applied at both sides of the electrokinetic cell, while even after surfactant Tween 80 addition the efficiency of the technology was not satisfactory for PAH removal [57].

3.3 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is based on using a gas as solvent in conditions of pressure and temperature higher than the critical values; in the supercritical state, the fluid exhibits the high density and low compressibility of a classic liquid solvent and the high diffusivity and low viscosity typical of a gas. In terms of power solvation, as this characteristic is directly dependent on density, for solutes of similar molecular polarity, the supercritical fluid may be considered a good solvent, capable of dissolving amount of substance comparable to those obtained with equal amounts of organic solvents. At the same time, its excellent transport capacity facilitates better penetration in the soil matrix allowing a near complete extraction of solutes with advantages in terms of extractive high yields and reduced extraction times [65].

In supercritical fluid extraction, the extracted contaminants are solubilized into the supercritical solvent from which can be separated by changing pressure and temperature conditions. Excavated soil extracted with a stream of SFE is not negatively affected and can be returned to the site, while the solvent can be recycled for further extraction. Although there are many substances that can be in supercritical conditions, in practice the fluid most commonly employed is carbon dioxide [66] because it has a critical point ($T_c = 31.08$ °C; $P_c = 73.8$ bar) which allows to work under relatively mild conditions of temperatures and pressures, such as those commonly used in industrial systems. This characteristic is particularly useful both in terms of energy and because of the possibility of reducing degradation in the case of extraction of thermolabile substances.

The technology can be used for heavy metals [67, 68] by adding as modifier a complexing agent which is able to react with the charged ions to form neutral complexes that can be dissolved in the supercritical CO_2 [69]. Soil pH, moisture, temperature, and chemical forms of the metal species in the soil [68, 70] largely influence the efficiency of the remediation of metal ions from various solid and liquid matrices [71]. PAHs also can be successfully treated [46, 72]. Efficiency of PAH remediation has been obtained by the use (in addition to CO_2) of modifiers such as pentane, acetone, and methanol [73]. The increased efficiency can be attributed to the modifier's ability to break strong hydrophobic interactions between the soil matrix and the PAHs [74, 75]. The SFE can be used as a first step in train technology with the aim of extracting contaminants; the contaminants and any used solvent can be further treated more cost effectively. Furthermore, in the separation step, it is possible to bring back carbon dioxide under gaseous conditions, allowing the total release of this nontoxic, chemically inert gas. In comparison with conventional solvent extraction, SFE requires shorter extraction times and reduced solvent usage without leaving toxic residues in the soil [71]; however, further improvements are necessary for the extraction of both PAHs and heavy metals from multi-contaminated soils.

3.4 Phytoremediation

Phytoremediation is a technology that uses the natural biological processes of plants and rhizosphere microorganisms for removal or transformation of contaminants in soil. The technology is applied "in situ" and is characterized by its positive impacts on the environment and the low cost. Phytoremediation can be employed for the treatment of organic contaminants including PAHs and inorganics such as heavy metals. Depending on the interaction between plants and the soil to be treated and the physiological action that the plant exerts on the pollutants, the technology has been formally divided into different subcategories according to the remediation mechanisms: degradation, extraction, and stabilization. Although phytoremediation has greater economic and ecological benefits in comparison with conventional methods, it also has limitations. The main advantages of this methodology are its low cost, its non-invasiveness, landscape restoration, increased activity and diversity of soil microorganisms, and decreased human exposure to polluted substrates.

The main disadvantages include the long time required for completion of the reclamation due to slow growth of the plants, the poor efficiency in contaminants removal when present at low bioavailability, and the inability of the roots to reach the contaminant at considerable depths. Although increasing attention is been focused on this decontamination technology, its full scale application is still in a consolidation phase [38]. This also depends on the fact that every remediation is site-specific, and for each case involves numerous interdependent variables (soil and its characteristics, type, concentration and depth of the contaminant, plant species, etc.). However, this remediation approach is especially promising for

addressing both PAHs and heavy metals, since plants are able to accumulate heavy metals and positively promote PAH-degrading microorganisms' proliferation in the rhizosphere.

Degradation rate of PAHs by phytoremediation mainly depends on the specific characteristics of the plants. In general, the rate of degradation of PAHs increased in presence of plants compared with non-vegetated soil. Several grasses and legumes have been found to promote the removal of PAHs from contaminated soil [76, 77]. *Medicago sativa* plants have led to an improvement of the physical–chemical properties of polluted soils restoring the initial values of pH and reducing salinity. These improvements have reduced the toxicity of the soil resulting in elongation of plant roots and positive impact on microbial activity in the rhizosphere. The presence of *Medicago sativa* has improved the degradation of benzo[*a*]pyrene, starting from a very high concentration [78]. In situ phytoremediation of PAH contaminated soil by intercropping alfalfa (*Medicago sativa* L.) with tall fescue (*Festuca arundinacea* Schreb.) has been positively tested. With the combination of *M. sativa*/*F. arundinacea*, high concentrations of PAHs were found in plants (270/284 μg kg^{-1} respectively). Intercropping of the two species led to percentages of removal of PAHs in soil up to 30 %, with an effect particularly relevant for hydrocarbons of high molecular weight, 30.9 % for 4 rings PAHs and 33.4 % for 5/6 rings PAHs. Intercropping also increased the number of PAH-degrading bacteria and microbial activity in soil [79].

The characteristics of the contamination play a very important role in the efficiency of phytoremediation; the action of plants is quite different in aged compared to freshly polluted soil. In a comparison of soils spiked with PAHs and soils with aged contamination, the efficiency of phytoremediation was quite different. In spiked soils after the growth of the plants, the PAHs concentration was reduced up to 80 %. In particular, the compounds with 2 or 3 rings underwent a greater reduction. In soils containing aged PAHs, the concentration of all hydrocarbons decreased up to 25 % [80]. Often in the presence of high amounts of contaminants, even the most resistant plants fail to grow. In many plants, the presence of high levels of metals induces the synthesis of ethylene from stress, which inhibits the elongation of the roots and causes a severe deprivation of iron, which in turn inhibits the synthesis of chlorophyll and chloroplasts [81].

A strategy that overcomes this limit involves the use of bacteria, which promote plant growth (PGPB). These bacteria are able to increase both the number of seeds that germinate and the amount of biomass produced from plants. With the addition of PGPB, a phytoremediation process is faster and more efficient. Plant growth-promoting bacteria can positively influence plant growth increasing the uptake of nutrients from the environment, reducing in the meantime the negative effects of phytopathogenic organism [82]. Until the early 1990s, field and laboratory studies have suggested that inoculation with non-pathogenic bacteria can have positive effects on the health of plants and their growth, and thus an increase in yield and their usage was widespread for applications in the agricultural field. Bacteria may supply atmospheric nitrogen to plants and produce siderophores, which can increase the available iron in soil and synthesize auxins and cytokinins, which promote

various stages of plant growth. Bacteria employ any one, or more, of these mechanisms under different conditions; moreover, they produce enzymes that can promote plant development. The use of PGPR has been shown to positively influence the efficiency of phytoremediation both for PAHs and heavy metals [83].

In the case of inorganic contaminants, two main strategies can be used: phytostabilization and phytoextraction. Phytostabilization involves the ability of roots to immobilize the contaminants in the root zone while stabilizing the soil, thus reducing metal leaching and aerial dispersion of contaminated soil particles. Phytostabilization is particularly suitable in those cases when the concentration is so high, such as in the mining sites, that the processes of phytoextraction would require too long to achieve remediation goals; thus, it is essential to maintain the metals in nonmobile forms in soil. Moreover, the growing plants can control hydraulic fluxes and are able to improve the structural stability of soil decreasing the erosive processes and the consequent migration of contaminants providing an adequate immobilization. During phytostabilization, plants must not be removed. Phytostabilization can also be used as a transitory solution for those sites where the removal of metals seems to be unsustainable due to the long time required as well as high cost required. Of course, this technique does not imply the definitive removal of pollutants, which remain immobilized in the site [84].

The fundamental principle of phytoextraction is to use the plant as an extractant capable of absorbing metals from soil by means of the root system and transfer them through the transpiration flow in the aerial part. At end of growth plants can be harvested, removing the adsorbed metals from the soil. The efficiency of the absorption processes depends on the properties of the soil, on the physical–chemical properties of the contaminants, on their chemical form, and on the characteristics of the plant species. Phytoextraction was originally thought for the use of hyperaccumulator plants capable of absorbing metals from the soil and to concentrate them in the aerial part. Almost all hyperaccumulator species have been identified in soils with very high concentrations of heavy metals, in which selected varieties are able to grow in a particularly hostile environment [85].

Several species have been recognized as hyperaccumulators of different metals. Often, however, hyperaccumulators have a reduced biomass production that does not allow relevant removal of heavy metals. To overcome this limitation, it is possible to use plants commonly employed in agriculture that have a higher biomass production. In this case, the efficiency of metal removal may be increased modifying the bioavailability of contaminants by the use of suitable chemical additives in the soil: "assisted phytoextraction" [86, 87]. The efficiency of technology strictly depends on the pollutants' bioavailability, which in turn is determined by the chemical and physical characteristics of the soil [38]. Therefore, it is very important to evaluate the bioavailability of heavy metals in soil since only the "bioavailable" amount can be absorbed by plants. When bioavailability is low, it may be increased by the use of metal mobilizing treatments, for example, by addition of chelating agents (EDTA, etc.) [88–91]. In the opposite case, however, if the quantity of heavy metals in the soil solution is too high, it is possible to reduce the soluble amounts,

for example by changing the pH of the soil or by adding absorbent materials, which possess specific adsorption sites for the metals [44].

In the soil, a contaminant can interact with the surfaces of the solid phase, with reactions of adsorption—desorption and precipitation—dissolution. A fraction of the contaminant remains in the liquid phase, where it is transported with the soil solution. Contaminant's bioavailability for plants depends on all these reactions, and it is influenced by the chemical characteristics of the contaminants and by soil characteristics such as pH, organic matter, clay content, cation exchange capacity, and redox potential [39]. Heavy metal phytoextraction is a very attractive remediation strategy since it enables the use of a biological technique to remove nonbiodegradable contaminants from a contaminated site. Before phytoextraction could be effectively applied, the specific conditions of the contaminated site must be considered. In general, several preliminary aspects linked to the distribution of contamination must be verified:

- Whether the site is large enough to grow plants with an adequate opportunity to carry out normal agricultural practices
- Whether the treatment can be employed for a sufficiently long period of time
- Whether the concentration of pollutants is not too high to create problems of phytotoxicity to the used plants
- Whether the contaminants are in the soil depth explored by plant roots

In addition to the concentration of metals in plants, it is also essential to determine the total accumulation [92], resulting from the product of the concentration of the metals in the tissues of plants for the biomass produced. This quantity provides the amount of metal actually removed and thus of the efficiency of the technology. In this way, estimates can be made on the time needed to complete the remediation. Of course, to optimize the absorption of metals, it is essential that plants are able to grow and develop properly. It is shown that the appropriate use of fertilizers and phytohormones helps to overcome difficulties in plant growth due to phytotoxicity and to increase plant biomass production [93–98].

At the end of this brief review of technologies, it can be said that although several technologies are able to decontaminate soils polluted by metals and PAHs, it is not easy and often not possible to use the same technology at the same time for the two classes of contaminants. As an example, chemical soil washing of metals requires reagents that are quite different from those needed to solubilize PAHs. Similarly, electrokinetic is not easy to manage so that it can act simultaneously on metals and PAHs. Further studies must be carried out to apply a single technology in the presence of simultaneous contamination by metals and PAHs. A very promising technology choice seems to be the use of phytoremediation. As previously described, many plants species have the ability to take up high levels of metals and translocate them from roots to shoots. In the meantime, in the presence of growing plants, the processes of degradation of organic compounds are increased due to synergistic effects between plants and microbial communities in soil and the induced chemical changes in the rhizosphere. Some, among the others, plant species used in phytoremediation of heavy metals and PAHs are reported in Table 3.

Table 3 Some plant species used in phytoremediation of heavy metals and PAHs

Plants species	Metals	PAHs
Brassica juncea	Cu, Ni, Pb, As, Cd, Cr	Pyr, BkFL, AcPy, Ant
Pteris vittata	Cu, Ni, Zn, As	Phe
Thlaspi caerulescens	Zn, Cd, Ni, Pb, Hg	
Heliantus annuus	Pb, Cu, Ni, Zn, As	
Arobiodopsis thaliana	Zn, Cd, As, Hg	
Zea mays	Zn, Cu, Pb	Ant, Phe, BkFL
Medicago sativa	As, Cd, Cu	Ant, Pyr, Nap
Panicum virgatum		Ant, Pyr, BaP, BaA
Sorghastrum nutans	Zn, Cd, Ni, Pb	Chr, BaP, BaA, DBA
Festuca rubra	Pb, Zn, Cd	Nap
Festuca arundinacea	Pb	Nap, Chr, BbFL,BkFL, DBA
Echinacea purpurea	Cu, Cr, Pb, Zn	Flu, Pyr, BaA, Chr
Fire Phoenix		Chr, BbFL, BkFL, DBA
Trifolium pretense		Nap
Glycine max		Ant, BaA

Numerous researches have demonstrated the efficiency of the use of plants for the remediation of soils contaminated by organic compounds mainly by the process of rhizodegradation [16]. Many studies have highlighted the ability of the plants to facilitate the degradation of organic pollutants in soil. Plants and microorganisms have many symbiotic relationships making the rhizosphere a field of intense microbial activity with an increase in the number of microbial communities, able to improve the physical and chemical properties of the soil [99]. The efficiency of heavy metals phytoextraction can be greatly increased by modulating contaminants bioavailability in soil. If phytoextraction is planned to remove the bioavailable fractions of metals [39], it offers a sustainable approach to remediation since at the end of the treatment the quality of soil is increased. Even with the limitations related to the specific characteristics of the technology, phytoremediation appears as a versatile solution, cost-effective, and of high environmental quality for the remediation of soils simultaneously contaminated by heavy metals and PAHs.

4 Case Study

A phytoremediation feasibility test, at a microcosm scale, was carried out with the aim of evaluating the efficiency of two plant species, *Brassica juncea* and *Zea mays*, in the simultaneous remediation of a soil polluted by Pb and PAHs. These two plants were selected due to their ability to grow in the Mediterranean climate and in polluted soils. Moreover, the species seem particularly appropriate because their deep-rooted system can explore a large portion of soil.

4.1 Experimental Procedure

The soil used in this study was collected from a former industrial site in northern Italy where various industrial activities had been carried out since the beginning of the last century. The soil resulted simultaneously contaminated by PAHs (10000 mg kg^{-1}) and Pb (120 mg kg^{-1}).

Soil samples were air-dried and ground to pass through a 2 mm sieve before characterization analysis. Soil pH, cation-exchange capacity (CEC), and soil texture were determined according to Soil Science Society of America (SSSA) methods of soil analysis [100]. The contaminated soil was characterized by a pH value of 7.58, a CEC of 19.6 $c_{(+)}$mol kg^{-1}, and the following texture: sand 68.0 %, silt 19.8 %, and clay 12.2 %.

Experiments were carried out at microcosm scale using 300 g of the contaminated soil. A total of 1.0 g of *B. juncea* seeds or six seeds of *Z. mays* were used in five replicates for each species per microcosm with five unplanted controls run simultaneously. Experiments were carried out in a growth chamber in controlled conditions: 14 h of light with a temperature of 24 °C and 10 h in the dark at 19 °C. Relative humidity was maintained at 70 %. The growing period lasted 3 months, after which plants started to decay. The additive, Ethylenediamine-N,N'-disuccinic acid (EDDS) 10 mM, was added 7 days before harvesting. PAHs decrease in soil was tested by analyzing the concentrations in soil at the beginning and end of the experiments in vegetated and non-vegetated microcosms. At the end of the growth period, plants were collected and shoots were separated from roots and washed with deionized water. Pb uptake by plants was measured determining Pb concentration in roots and shoots of the two selected species. Aggregate stability, used as an index of soil quality, was determined by the single sieve method [101–104], using soil samples of 10 g of the 1–2 mm size air-dried samples. The soil material retained on the sieve was oven dried, weighed, and then corrected for sand content. The wet aggregate stability (WAS) was calculated as:

$$\mathrm{WAS\%} = \left[\text{Retained Soil Material} - \text{Sand}\right] / \left[\text{Soil Sample} - \text{Sand}\right] \times 100$$

4.2 Analytical Methods

The soil samples in this study were extracted using EPA method 3550, with a mixture of acetone/hexane (1:1 v/v). Soil extracts were analyzed by GC/MS, according to US EPA method 8270C, using a Thermofinnigan "TRACE DSQ" GC-MS with a quadrupolar analyzer and PTV injector (DB 5 ms capillary column, 30 m × 0.25 mm ID, 0.25 μm stationary phase film thickness). All reagents were pesticide quality. The compounds determined by the analysis were the 16 PAHs in the US EPA list of priority pollutants: naphthalene, acenaphtylene, acenaphthene, fluorene, phenantrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, crysene, benzo[*b*]

	%
Nap	38.1
AcPy	49.8
AcP	33.7
Flu	41.5
Phe	48.8
Ant	50.9
FL	38.3
Pyr	40.3
BaA	28.2
Chr	40.8
BbFL	23.7
BkFL	69.0
BaP	38.5
InP	35.9
DBA	46.4
BghiP	22.7

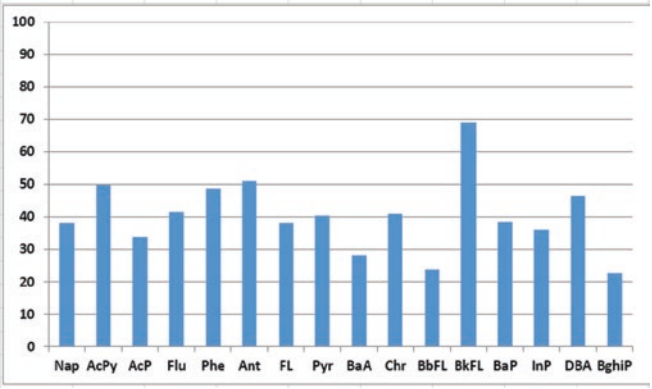

Fig. 1 Percentage degradation of each single PAH in vegetated soil with *B. juncea*. Percentages were calculated using mean concentration values

fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene, and benzo[*g*,*h*,*i*]perilene.

Pb concentrations in soils and plants were determined by EPA method 3051 via atomic absorption spectrophotometry using flame AAS (Varian AA 240FS).

4.3 Results

4.3.1 Effect on PAH Remediation

At the end of the experiment, in the non-vegetated microcosms, PAH concentrations were the same as at the beginning of the experiment, 10150 ± 189 mg kg^{-1}. After the growing period in microcosms vegetated by *B. juncea* and *Z. mays*, the PAH concentration in soil decreased to 6146 ± 396 and 6293 ± 402 mg kg^{-1}, respectively, with a similar reduction of about 40 and 38 %. The degradation rate of PAHs with a large number of aromatic rings and high molecular weight increased more than those with a small number of aromatic rings, and the best results have generally been obtained for those PAHs with high molecular weight (5–6 rings). The addition of EDDS had no effects on PAH degradation, and no significant differences were found for each single PAH between soil treated or untreated with EDDS. This might be attributed to the fact that EDDS had no toxic effects on microbial communities [105]. Concentrations of each single compound tend to decrease in vegetated pots, both with *B. juncea* and *Z. mays*. To observe the efficiency of the two plant species in the degradation of the PAHs, percentage degradation values were calculated by considering the decrease in the concentration of a single PAH in the vegetated soil, with respect to that in non-vegetated soil (Figs. 1 and 2).

	%
Nap	46.1
AcPy	46.6
AcP	34.6
Flu	40.8
Phe	88.0
Ant	51.9
FL	29.0
Pyr	28.2
BaA	40.3
Chr	22.0
BbFL	13.6
BkFL	72.7
BaP	33.7
InP	21.7
DBA	24.2
BghiP	17.1

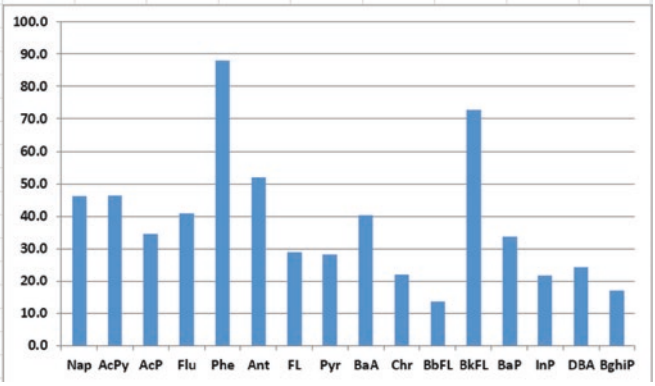

Fig. 2 Percentage degradation of each PAH in vegetated soil with *Z. mays*. Percentages were calculated using mean concentration values

In the case of soil vegetated with *B. juncea*, all the PAHs were degraded by at least more than 20 %, (benzo[*b*]fluoranthene, benzo[*g*,*h*,*i*]perylene) compared to degradation in non-vegetated soil. The highest percentage degradation was obtained for benzo[*k*]fluoranthene (69 %), while plant growing resulted in about 50 % of degradation for acenaphthylene, phenanthrene, anthracene, and dibenzo[*a*,*h*]anthracene. The remaining were degraded with an abatement percentage of about 30–40 %. *B. juncea* promoted degradation up to about 40 % of total PAH content. Similar results (about 38 %) of total PAH content were also obtained in microcosms planted with *Z. mays*, but the percentages of degradation of the single PAH were different (Fig. 2).

Most of the PAH degradation percentages increased by 20–40 % compared to those in non-vegetated soil. The lowest value of degradation was obtained for benzo[*b*]fluoranthene (10 %), while anthracene was degraded to 50 %. Benzo[*k*]fluoranthene and phenanthrene showed the highest degradations of 72 % and 88 % respectively. Thus, plants promoted the degradation of PAHs in the contaminated soil with different trends for the two investigated species. As is well known, plant growing stimulates the microbial biomass involved in PAH degradation [106, 107]. Microbial investigation (data not reported) showed that most (97 %) of the isolated bacterial strains belong to the phylum Proteobacteria in accordance with previous findings [108] and showed that growing plants were able to increase biodegradation of organics [109]. The process is highly complex, and the success of remediation depends on the specific site conditions. In this soil, the PAHs are derived from a long-time contamination, but although aged PAHs are considered to be of difficult degradation [110], the results can be considered highly positive. However, we must take into account that this high efficiency of phytoremediation is strictly linked to the specificity of microcosm experiments, where intimate contact between soil and roots exists, largely different from that in the field.

Table 4 Effect of addition of EDDS 10 mM increased Pb concentrations in shoots compared with the control

	B. juncea		*Z. mays*	
	Control	EDDS	Control	EDDS
Roots	7.5 ± 0.89	93.8 ± 2.1	6.8 ± 0.75	130 ± 4.3
Shoots	4.3 ± 0.11	51.2 ± 1.6	3.1 ± 0.10	40.3 ± 1.8

4.3.2 Plant Uptake of Lead

At the end of the growing cycle, the Pb concentrations in the tissues of *B. juncea* and *Z. mays* were determined. In the control microcosms (untreated soil), the concentration of Pb was very low in both the shoots and roots of *B. juncea* and *Z. mays*. The results highlighted the need to use assisted phytoextraction to remove Pb from the soil. Of the numerous additives reported in the literature for "assisted phytoextraction", EDTA is most commonly used, due to its high complexing capacity for most metals, such as Pb, Cu, Cd, and Zn, which generally leads to an increase in metal translocation from soil to plants [91, 111]. However, given the persistence in the soil of chelating agents such as EDTA, there is a greater risk that mobilized metals will leach into the ground or surface water. Hence, research is now aimed at new mobilizing agents that have no adverse effects on the environment while they promote the bioavailability of contaminants.

EDDS can be considered a valid alternative to EDTA for lead phytoextraction, and its efficiency is often greater than that of EDTA. These results could be due to the calcium present in the soil. In fact, the interaction of lead and EDTA can decrease due to the competition between lead and calcium for this complexing agent. Although the complex Ca-EDTA has a much lower stability constant ($\log K = 10.6$) than the Pb-EDTA complex ($\log K = 17.9$), the high solubility of calcium along with its high concentration in the soil makes this cation a powerful competitor of Pb. Regarding EDDS, the complexation constant ($\log K = 12.7$) with Pb is lower than that of the Pb-EDTA complex [112]. However, the low stability of the complex Ca-EDDS ($\log K = 4.3$) did not lead to a significant reduction in the concentration of Pb mobilized; in fact EDDS often has been reported to show an higher extractive efficiency than EDTA in the presence of significant amounts of Ca [88, 91]. In this experiment, the addition of the mobilizing agent (EDDS) led to a significant increase in Pb uptake by the plants (Table 4).

In both plant species, the amount of Pb was higher in the roots than in the shoots. The plants are able to uptake the metal but only partially translocate it to the aerial parts; in fact, as is well known, roots act with a defense mechanism against toxic elements. However, the addition of EDDS to the soil also promoted the translocation of Pb in the aerial parts of the plants. Pb concentration in the aerial parts reached 51.2 mg kg^{-1} for *B. juncea* and 40.3 mg kg^{-1} for *Z. mays*. Without addition of EDDS addition, the values in the controls were 4.3 and 3.1 mg kg^{-1} for *B. juncea* and *Z. mays*, respectively. The amount of contaminant extracted by the plants is a result of two dynamic processes, metal uptake, and biomass production and can be expressed

	B. juncea		Z. mais	
	Control	EDDS	Control	EDDS
shoots	3.0	35.8	2.1	27.4

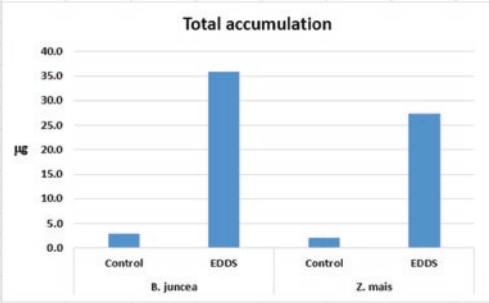

Fig. 3 Effect of EDDS 10 mM addition on Pb total accumulation in the aerial parts of plants

as "total accumulation" [92]. This is calculated as the product of the concentration of the metal in plant tissues and the respective dry biomass. The data are reported in Fig. 3. Total accumulation provides an estimation of the amount of Pb removed from the polluted soil and thus the phytoextraction efficiency. Results showed that the increase in metal bioavailability using EDDS promoted higher Pb accumulation in plants. In this experiment, *B. juncea* showed a greater efficiency than *Z. mays* and should be considered the best candidate for further phytoextraction tests at the field scale.

Effect of chelators on shoot biomass production was not significant, since EDDS was added only a few days before harvesting. This was necessary because the chelating agent could promote leaching of Pb in lower soil horizons. Plants absorbed and translocated a fraction of the metal mobilized by the treatments, and a certain amount could remain in the soil solution with an increased risk of percolation. Thus, the persistence of a high mobility of Pb after harvest should be avoided. Leaching can be countered by the degradation of the chelating agent with the consequent release of Pb, which tends to form stable precipitates due to the alkaline conditions of this soil. Therefore, the effects induced by the addition of chelating agents should be considered not only in relation to the increased Pb uptake by plants but also to the residual effects in the soil, including the metal's release from decaying roots.

4.3.3 The Effect on Soil Quality

If one of the primary aims of the remediation process is to leave a good quality environment, evaluation of the physical properties of the soil is a very important issue [113]. Among the physical soil parameters, an important index to define the functionality of the soil is the stability of the structure. Aggregate stability influences a wide range of physical and biogeochemical processes in soil including the movement and storage of water and air in the pore system. The pore system provides zones rich of biological activity where plant roots can grow. At the end of the growth cycle, wet aggregate stability was determined using a wet-sieving methodology, in vegetated and non-vegetated microcosms. The results show how the growth

of plants has improved soil structure stability. This positive effect can be ascribed to the high development of roots, which can release polysaccharide material through exudates; this may act as a binding agent promoting the increase of larger aggregates, reducing soil bulk density [114–116]. The wet aggregate stability increased from 27 to 61 % in the case of vegetation microcosms with *B. juncea* and 32–60 % in microcosm with *Z. mays*. The increased stability of the structure derived from the presence of plants highlights the improved soil quality following the green remediation approach. The aggregate stability of the soil is an important indicator of the quality of the soil. In fact, a good structural stability is able to counteract the process of compaction of the soil, typical of contaminated sites, and to increase water retention capacity, properties which in turn promote the growth of roots and improve physiological functions of the plant [117]. Good structural properties also provide better support for microbial communities; this effect can increase the efficiency of PAH degradation in subsequent cycles of growth.

4.3.4 Concluding Remarks

In the reported case study, a feasibility test was carried out to evaluate phytoremediation as a candidate technology for cleaning up a site contaminated by PAHs and Pb. Phytoremediation was shown to be a viable green remediation strategy for both Pb and PAHs. In particular, phytoremediation of PAHs appears to be a very promising technology for removing these contaminants from contaminated soils. Promoting an adequate substrate for microbial growth, plants greatly enhanced the degradation of PAHs, while in the meantime they were able to uptake, after addition of EDDS, a certain amount of the bioavailable fraction of Pb.

5 Conclusions

Understanding the mechanisms involved in the process of degradation of PAHs is important for promoting the use of green remediation strategies at contaminated sites. Soft technologies are certainly an advantageous alternative, being noninvasive and less expensive compared to traditional methods. Moreover, the application of bioremediation and phytoremediation improves the physical and biological properties of soils. In particular, phytoremediation promotes the activity of microbial species able to metabolize recalcitrant organic compounds and can speed up the process of natural attenuation very efficiently. Some contaminated sites can play a very important economic role after remediation. Sites located in areas of strategic importance from the point of view of production and trade can be transformed into high income activities, but also sites that can be devoted to service facilities and utilities can play an important role in protecting and promoting the local economy.

If remediation should ensure adequate levels of economic and financial sustainability, or generate economic benefits for the communities concerned, remediation

technologies should also be directed toward recovering and preserving the local environment, starting from the function that soils can perform in the protection of the entire ecosystem. The opportunity to achieve a double purpose (social/environmental or economic/environmental) makes it essential to set up an integrated strategy of intervention based on the recovery of soil quality as an integral part of environmental restoration. This approach not only offers the advantage of stimulating economic development and employment in the areas subject to this work but also promotes the culture of "reuse", instead of that of excavation and landfilling. The positive effects associated with recovery strategies based on the principle of sustainability are very important: for the reduction of environmental risks and reclamation of degraded areas. In such contexts and conditions, when the aim of clean-up is the recovery of soil and environment quality, rather than the achievement of numerical values of pollutant concentrations, remediation of contaminated sites can become an important opportunity for local sustainable development and increased well-being.

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