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REVIEW PAPER

Organic matter–microorganism–plant in soil bioremediation: a synergic approach

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Abstract Bioremediation is a natural process, which relies on bacteria, fungi, and plants to degrade, break down, transform, and/or essentially remove contaminants, ensuring the conservation of the ecosystem biophysical properties. Since microorganisms are the former agents for the degradation of organic contaminants in soil, the application of organic matter (such as compost, sewage sludge, etc.), which increases microbial density and also provides nutrients and readily degradable organic matter (bioenhancement–bioaugmentation) can be considered useful to accelerate the contaminant degradation. Moreover, the organic matter addition, by means of the increase of cation exchange capacity, soil porosity and water-holding capacity, enhances the soil health and provides a medium satisfactory for microorganism activity. Plants have been also recently used in soil reclamation strategy both for their ability to uptake, transform, and store the contaminants, and to promote the degradation of organic contaminants by microbes at rhizosphere level. It is widely recognized that plant, through organic materials, nutrients and oxygen supply, produces a rich microenvironment capable of promoting microbial proliferation and activity.

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1 Introduction

Soil pollution can be defined as the introduction of compounds into the soil environment at concentrations that alter its functioning or that are a threat to human health. Soil is, in fact, the basic natural resource for humans which are especially exposed through ingestion of food grown on polluted area and inhalation of contaminated dusts. Pollutants in soil can be originated from several sources, especially in developing countries, which not only experiences a rapid growth of population due to increasing rate of rural urban migration but also industrialization which is accompanied by air, water and soil pollution. The contaminants encountered at these sites include metals (such as lead, cadmium, mercury, chromium and nickel), volatile organic compounds (such as benzene, toluene, and trichloroethylene), and semi-volatile organic compounds (such as total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)). Organic and metal contaminants are found to coexist at many sites (Krishna 2010). The industrial operations which mainly contribute to heavy metals and organic pollutant soil contamination are smelting, mining, metal forging, manufacturing of alkaline storage batteries, combustion of fossil fuel and the spillage of liquids such as oil or solvents (Collins et al. 2002). Moreover, the agricultural activities like application of agrochemicals (fertilizers, pesticides and herbicides), use of sewage sludge in agricultural practices and irrigation with polluted water also could add significant amounts of organic and inorganic contaminants to the soils (Vaca-Paulín et al. 2006; Liu et al. 2006). Among the organic contaminants, PAHs are the most widespread in soils, water and wastewater (Puglisi et al. 2007). PAHs originate mainly from combustion of fossil fuels and direct release of oil and its products (Johnsen et al. 2005).

In order to protect public health and the environment, large resources have been invested to develop efficient remediation technologies. Remediation is a challenge, not only from a technological point of view, but also because of the large costs involved. Various physico-chemical processes (soil washing, electrokinetic, chemical reduction or oxidation of contaminants, incineration) have been developed for treating polluted soil; these approaches are often prohibitively expensive, non-specific and produce secondary contamination. As a result, there has been an increased interest in bio-based treatments commonly known as bioremediation. Bioremediation techniques, which are based on the use of living organisms (microorganisms, plants and earthworms) to degrade and detoxify environmental contaminants, are more cost-effective and ensure the conservation of the site and of its biological potential. As asserted by Adriano et al. (1999), the purpose of soil bioremediation is "not only to enhance the timely degradation, transformation, remediation or detoxification of pollutants by biological means, but also to protect soil quality.

Bioremediation techniques accelerate the naturally occurring degradation of organic compounds by optimizing conditions for biodegradation through, for example, aeration, addition of nutrients and control of pH and temperature. Their primary disadvantages are that biological techniques need a long duration to achieve the required pollutant concentration thresholds and suitable environmental condition to sustain living organisms. However, other than advantages above mentioned, being a natural process it is perceived by the public as an acceptable decontamination treatment for polluted soil.

Bioremediation technologies include natural attenuation, biostimulation, bioventing, bioaugmentation, landfarming, composting, and phytoremediation. The development of these strategies is continuously in progress in order to become effective and reliable for matrices contaminated by a wide range of organic and inorganic pollutants. Many studies about bioremediation have revealed its potential for the detoxification and degradation of the contaminants (Wang and Chen 2007; Weber 2007; Kulkarni et al. 2008). However, the effectiveness of bioremediation technologies depends largely on the contaminant chemistry and concentration and soil.

The main aim of this paper is to review the main agents involved in soil bioremediation: microorganisms, organic matter and plants, while providing emphasis on their synergic action.

2 Biotic and abiotic factors affecting contaminant behavior in soil

Physico-chemical properties of pollutants (e.g. aqueous solubility, polarity, hydrophobicity, lipophilicity and molecular structure) control their fate and behaviour in soil (Reid et al. 2000).

Moreover, several environmental factors, e.g. organic matter (Puglisi et al. 2007), clay minerals (Lair and Sawhney 2002), temperature, water content, pH, salinity, supply of oxygen and nutrients are well known to affect biodegradation of organic contaminants in soil (Kurola and Salkinoja-Salonen 2007). Together with abiotic factors, biotic agents are of great importance in controlling the contaminant degradation in soil environment. The presence of suitable microorganisms for degrading the organic contaminants is critical for the naturally occurring biodegradation. However, some site conditions, such as marginal environmental conditions or high concentrations of contaminants or organic vapors, can limit the microorganism growth and activity (Moreels et al. 2004).

Generally, bacterial metabolic activity and contaminant biodegradation increase with increasing temperature up to an optimum value reported to be around 30–40 °C (Zhang et al. 2005) while a large electrical conductivity and a high or low pH of soil inhibit microbial activity (Luna-Guido and Dendooven 2001; Ramirez-Fuentes et al. 2002). An inhibitory effect of artificial salinity on mineralization of oil has been reported (Rhykerd et al. 1995). Mille et al. (1991) found an inhibitory effect of salinity above 2.4 % NaCl that was greater for the biodegradation of aromatic and polar fractions than for the saturated fraction of petroleum hydrocarbons. However, different results have been obtained when investigating naturally salt-containing soils, since indigenous microorganisms in such environments can be salt-adapted (Geiselbrecht et al. 1998). An interesting phenomenon is that low concentrations of salt (<1 % NaCl) slightly stimulated mineralization in some cases (Ulrich et al. 2009).

Another very important parameter is the moisture level; the optimum moisture level for the biodegradation of petroleum hydrocarbon reported in literature is between 45 and 85 % of the soil's water holding capacity (US EPA 2006). At higher water contents, there is a risk of the onset of anaerobic conditions arising from the slow rate of oxygen diffusion through water. At lower water contents, water availability becomes a limiting factor for microbial activity, movement and bioavailability of contaminants (Treves et al. 2003).

Contaminant degradation has also been shown to be favored in slightly alkaline soils, where hydrocarbon-degrading bacteria become less competitive with increasing acidic conditions (Maeir et al. 2000). Bacteria require nutrient elements, such as nitrogen and phosphorus for incorporation into biomass and the synthesis of cellular components. The presence of these nutrient elements in soil is therefore critical for the biodegradation of organic contaminants (Atlas and Bartha 1992). The optimization of the C:N:P ratio is thought to be one of the most important actions enhancing the rates and extents of petroleum hydrocarbon biodegradation in soil.

Normally, as the time of contact between contaminant and soil increases there is a decrease in chemical and biological availability, a process termed 'ageing' (Hatzinger and Alexander 1995). For example, Uyttebroek et al. (2007) reported a biphasic loss of PAHs in a contaminated soil with phenanthrene and pyrene. In particular, the degradation and volatilization of PAHs was fast during the first 30 days and slow but continuous during the 140 day experimental period.

Sorption to clay minerals and organic soil components (SOM) are considered the dominant processes in the sequestration of organic contaminants in soil. These soil-contaminant contacts influencing their bioavailability, are responsible for the decrease in contaminant degradation (Reid et al. 2000; Semple et al. 2001). Soil particles are bound together by bacterial products and by hyphae of fungi into stable microaggregates (2–20 μm in diameter). These are bound by microbial products into larger microaggregates (20–250 μm in diameter), with bacterial polysaccharides acting as binding agents. Microaggregates are then bound into macroaggregates (>250 μm in diameter), with bacterial polysaccharides acting as binding agents and fungi mycelia increasing the size of macroaggregates. Organic contaminants that are similar to organic matter, i.e. they have phenolic structure, can be entrapped and/or strongly bound within soil aggregate; the resulting bounds lead to stable "almost irreversible" incorporation of pollutants into the soil (Gevao et al. 2000). It has been shown that organisms such as bacteria, earthworms, or plants can access these supposedly unavailable fractions by "facilitated desorption processes" (Park et al. 2001; Stokes et al. 2006) or diffusion back out of the micropore (Johnsen et al. 2005). A laboratory experiment on the biodegradation of phenanthrene in soil proved that fungal mycelia bridged air-filled pores and thereby provided a continuous network of water-paths that mobilized soil bacteria and facilitated the access of the bacteria to the contaminant (Wick et al. 2007).

Humic substances (HSs), both exogenous and endogenous, have been found to greatly strengthen aggregate formation and stability in soil (Piccolo et al. 1997), thus representing an important factor in the control of organic compound incorporation into less or inaccessible compartments. Moreover, chemical, photochemical or enzymatic catalysts can mediate the formation of covalent bonds between pollutants and soil HSs (Gevao et al. 2000). HSs showed an important role on sorption and binding of PAHs or PAH metabolites (Conte et al. 2001). The water-dissolved fraction of humic acids (HAs) can act as carriers of PAH compounds. In an experiment on phenanthrene degradation using *Sphingomonas* sp. and two humic acid concentrations, the increase of HAs increased the rates of phenanthrene degradation (Smith et al. 2009). This can only be interpreted by an HA-mediated transport of phenanthrene to the cells, supplementing diffusive uptake from the freely dissolved phase.

As described for the organic contaminants, heavy metals also can be involved in a series of complex chemical and biological interactions. The most important factors which affect their mobility are pH (Gomes et al. 2001), sorbent nature, presence and concentration of organic and inorganic ligands (Harter and Naidu 1995), including humic and fulvic acids, root exudates and nutrients. Organic matter has a large capacity to adsorb heavy metal nonspecifically because of its high cation exchange capacity and specifically when forming simple covalent bonds and chelates (Stevenson and Fitch 1986). The carboxylic and phenolic groups, present in large number in the structure of humic and fulvic acids, are responsible for the adsorptive capacity of organic matter (Harter and Naidu 1995; Kinniburgh et al. 1996). In a study performed by Kinniburgh et al. (1996) on metal ion binding by humic substances, a prevalence of carboxylic sites was identified at acid pH (median value 2.98), while phenolic type prevailed at basic pH (median value 8.73).

A study aimed to quantify the contribution of mineral and organic soil compounds to the heavy metal sorption capacity, clearly showed that organic compounds are the major source for metal sorption in soil. In this study the organic carbon showed a sorption capability for heavy metals 6–13 times higher than the soil minerals (Lair et al. 2007). Acidification of soil directly influences the types of adsorption to both organic and inorganic soil particles (Sauve et al. 2000). The H^+ ions are exchanging with heavy metals in the cation exchange sites, thus desorbing the non-specifically bound heavy metals (Alloway 1995).

A study on the investigation of the role of organic matter in bounding the zinc in agricultural soils demonstrated that the content of organically bound Zn is related to pH and soil organic matter content (Dabkowska-Naskret 2003). Furthermore, redox reactions, both biotic and abiotic, are of great importance in controlling the oxidation state and thus, the mobility and the toxicity of many elements, such as Cr, Se, Co, Pb, As, Ni and Cu. Reduction in redox potential may cause changes in metal oxidation state, formation of new low-soluble minerals, and reduction of Fe, resulting in release of associated metals (Baumann et al. 2002; Chuan et al. 1996).

3 Monitoring parameters for contamination and decontamination

It is widely recognized that in the monitoring of the effectiveness of the bioremediation approach, the simple determination of the total content of chemical contaminant, even if the most mandatory, do not provide adequate information about the risk faced by living organisms that interact with soil, and downstream to the human health (Middaugh et al. 1993; Andreoni and Gianfreda 2007; Krishnamurti and Naidu 2008). The mobility and bioavailability, and hence potential toxicity of contaminants in soil depend, in fact, on their concentration in soil solution, the nature of their association with other soluble species, and soil ability to release the contaminants from the solid phase (Krishnamurti et al. 2007). Of course for heavy metals, the total metal concentration is of interest, but it is now accepted that understanding the environmental behavior by determining their speciation is of paramount importance. Metals speciation in soils, related to the distribution of an element among chemical forms or specie, is generally carried out with specific extractants which solubilize different phases of metals (Mulligan et al. 2001). Therefore, chemical speciation allows the estimation of the mobile and bioavailable fraction, thus indicating their potential toxicity in the natural compartment of the environment.

On the other hand, for organic contaminants, the monitoring of the reduction of target contaminant concentration is not indicative of decrease in soil toxicity. Incomplete degradation and formation of toxic intermediary metabolites may results in increased soil toxicity during bioremediation (Phillips et al. 2000). In view of this, chemical monitoring is usually insufficient to provide insight into the potential ecological risk of polluted soils; moreover, it does not allow to understand the combined effects of the mixture of all chemicals present at a polluted site, including their bioavailability. Therefore, in order to evaluate the toxic effects of pollution both in laboratory and field studies, a number of biological assays have been developed and standardized (Saterbak et al. 1999, 2000; Dorn and Salanitro 2000). They have been incorporated in the program for ecological assessment of bioremediation at hazardous waste sites and for supporting management decisions for remediation (Maila and Cloete 2005; Płaza et al. 2005).

Biological tests (bioassays), which consist of exposing biological organisms to polluted materials, have been developed by the Organization for Economic Cooperation and Development (OECD), by the US Environmental Protection Agency (US EPA), and by individual researchers for use in assessing soil toxicity related to bacteria, plants and earthworms (Saterbak et al. 1999, 2000; Abbondanzi et al. 2003; Plaza et al. 2009; Hubalek et al. 2007).

Ecotoxicity tests of soil samples can be performed either as direct contact tests with contaminated solid materials or as tests on soil elutriates (Bierkens et al. 1998). Tests on soil water leachates give a quick response but their sensitivity is, however, lower for substances with low water solubility; therefore, contact tests are preferred for soil, even if time and space consuming and therefore more expensive (Wahle and Kördel 1997). The bioassay with the luminescent marine bacterium *Vibrio fischeri* has routinely been used for ecotoxicity evaluation of contaminated soils and water (Loibner et al. 2004; Ros et al. 2008). In the plant tests, the effect of the contaminated soils on the growth and germination of selected monocotyledonous and dicotyledonous plant species and ability of soil to support sustainable growth are assessed (Dorn et al. 1998). Various plant species have been applied to assess species sensitivity in plant bioassays on contaminated soils (Baudgrasset et al. 1993; Gong et al. 2001). Plant test species should be selected based upon the documented exposure pathways and plant receptors appropriate for the present and future use of the site rather than based on the most sensitive species, by default (Saterbak et al. 1999, 2000). The earthworm avoidance, survival, and reproduction protocols are commonly used in terrestrial ecotoxicology to assess the toxicity of compounds in soil (Gibbs et al. 1996; Saterbak et al. 1999, 2000). *Eisenia fetida* is the most commonly used earthworm species in ecotoxicological studies, including waste-site assessments (Dorn et al. 1998; Saterbak et al. 1999, 2000).

However, to obtain useful information on potential ecological risks of polluted or remediated soils, it is recommended to use a battery of tests, including a number of different test species representative of the ecosystem to be protected (Van Straalen and Van Gestel 1993; Keddy et al. 1995). Species selected for such a battery of tests should be taxonomically different, play different roles in (soil) ecosystem(s), and have different routes of exposure (Keddy et al. 1995). Besides this, also the availability of test organisms, their tolerance to variations in physico-chemical soil properties, and the availability of suitable test methods have to be taken into consideration.

Monitoring of contaminated-soil restoration can be also achieved following different unconventional approaches. Microbiological estimates may serve as a good indicator for evaluating the effect of contaminants and/or decontamination on soil. Soil microorganisms, are very sensitive to any ecosystem perturbation and respond rapidly to stressors by adjusting activity rates, biomass, and community structure. The structural diversity of a bacterial community has been found to be very sensitive to environmental changes, reacting by shifts in its composition (Vivas et al. 2008; Moreno et al. 2011). Common methods for the quantification of microorganisms are focused on the measurement of carbon biomass, soil respiration, the number of cultivable bacteria and microbial bioluminescence (Phillips et al. 2000; Maila and Cloete 2005).

Recently, developments in molecular-biology-based techniques have led to rapid and accurate strategies for monitoring, discovery and identification of bacteria and their catabolic genes involved in bioremediation process (Widada et al. 2002). Real-time polymerase chain reaction (RT-PCR) targeting16S rRNA genes has been proposed as a feasible method to estimate bacterial biomass in contaminated environments (Cébron et al. 2008). Polymerase chain reaction (PCR)-based amplification of the 16S and 18S rRNA genes allows the profiling of complex microbial communities on the basis of sequence diversity, independent of cultivation in the laboratory (Muyzer et al. 1993). Among genetic fingerprinting methods, denaturing gradient gel electrophoresis (DGGE) and terminal Restriction Fragment Length Polymorphism (T-RFLP) has been shown to be effective means to determine spatial and temporal changes of soil communities within and between locations under different environmental conditions (Renella et al. 2005; Vivas et al. 2008). Although the above approaches are very valuable for evaluating microbial community structure and activity, most are either fairly sophisticated and/or labor intensive techniques.

Soil enzyme activities have been postulated as useful markers of the impact of pollution on the metabolic activity of soil (Ceccanti et al. 2006; Harris 2003; Labud et al. 2007). Soil enzymes are the catalysts of important metabolic functions, including the decomposition and the detoxification of contaminants; they can rapidly change in response to changes in soil caused by both natural and anthropogenic factors; and they are fairly easy to measure (Nannipieri et al. 2002; Gülser and Erdogan 2008). As a result of these advantages, it has been suggested that soil enzyme activities are useful as early and sensitive indicators of soil alteration in both natural ecosystems and ecosystems altered by anthropogenic activities, and, in addition, they are well suited to measure the impact of pollution on soil quality. Enzymes that have been tested for their potential to monitor bioremediation processes include soil lipases, dehydrogenases, catalases and ureases (Maila and Cloete 2005; Ceccanti et al. 2006; Doni et al. 2012). Among the catalytic proteins tested, soil lipases have shown great potential in monitoring bioremediation of hydrocarbon, since the products released from hydrocarbon biodegradation are the substrate for these enzymes (Margesin et al. 1999). At the same time, dehydrogenase, considered as an indicator of the microbial redox system and of the oxidative activities of the soil (Trevors 1984), has been widely used as a simple method to examine the possible inhibitory effect of the contaminants on the soil microbial activities (Ihra et al. 2003). However, Maila and Cloete (2005) in a recent review concerning the potential, the performance, the variability, and the failure of several bioindicators, concluded that at this stage there is no general guarantee of successful utilization of biological activities as monitoring tools and these latter should be complemented by existing traditional monitoring approaches.

It has been shown that low molecular mass peptides and/or proteins rich in cysteine can be considered as biomarkers of heavy metal pollution (Fojta et al. 2006). Metallothionein like proteins, reduced glutathione and phytochelatins belong to the group of these biomarkers. Due to their affinity to heavy metals, they are involved in detoxifying and maintaining of heavy metal homeostasis in plants (Cobbett and Goldsbrough 2002). The synthesis of these biomolecules can be catalyzed by the presence of metal ions in the intracellular environment (Supalkova et al. 2007).

4 Organic matter addition as a starter of nutrients and microorganisms: effects of different types and stability of organic compounds in soil bioremediation

Contaminated soils are often poor in organic matter and show unfavorable environmental conditions, such as nutrient availability and oxygen concentration, which limit the microbial growth and activity, and thus the contaminant degradation (Jùrgensen et al. 2000). Addition of organic amendments can facilitate the degradation of organic contaminants because they play a role in supplementing nutrients and carbon source in contaminated soil (Namkoong et al. 2002; Oleszczuk 2007; Anastasi et al. 2009). Furthermore, organic amendments, such as sewage sludge and compost, having a high microbial density and diversity can affect the activity and the composition of the autochthonous microbial community and thus the extent of contaminant removal (Ros et al. 2006).

Several organic amendments have been used in the bioremediation of contaminated soil, and different biodegradation rates have been obtained. Fresh organic amendments, such as sewage sludge, can be considered a good source of organic matter for soils because of their high content of available nutrients essential for plant and microorganism growth; however, the high proportion of water-soluble organic compounds can increase solubility of soil contaminants and therefore their bioavailability and leaching. Moreover, sewage sludge addition can represent a potential soil contamination source with heavy metals (Gupta and Sinha 2007), organic contaminants (Stevens et al. 2003) and pathogens (Al-Bachir et al. 2003). In view of this, the biological and chemical characterization of sewage sludge is an important requirement prior to sludge disposal to soil. Several studies have shown that sewage sludge composting can significantly reduce organic contaminant and pathogen content in this material (Nielsen 2007; Peruzzi et al. 2011). Furthermore, humification of organic matter may decrease heavy metal and organic contaminant bioavailability by redistributing these elements or compounds from soluble fractions to forms less-readily available to plants and microorganisms, this being related to the formation of pollutant–humic complexes (Garcia et al. 1995; Clemente and Bernal 2006).

Stabilized organic matter is well known to positively affect: (1) the chemical-nutritional status of soil, providing slow-release nutrients, (2) the biochemical status of soil, protecting and preserving the extracellular enzyme activity (humus-enzyme complexes), and (3) the physical status of soil, strengthening the soil aggregate formation and stability (Six et al. 2002). In a laboratory experiment, a compost was added to a soil contaminated by total petroleum hydrocarbon (TPH) in order to evaluate its bioremediation efficiency (Ceccanti et al. 2006). After 3 months of experimentation, a reduction in TPH higher than 50 % was shown. Moreover, compost application stimulated soil microbial metabolism (increase in soil respiration and enzymatic activity). Gallego et al. (2001) noted that the addition of activated sludge from a domestic waste water plant to a natural sandy soil contaminated with diesel (6000 mg kg^{-1}) increased hydrocarbon degradation rate. The effectiveness of the organic wastes from animal source (i.e., cow dung (CD), poultry manure (PM) and pig waste (PW)) as remediation option in stimulating biodegradation of the hydrocarbons in soil was also reported by Adesodum and Mbagwu (2008); these authors showed the following order of efficiency : PM > PW > CD. Soil application of organic residues resulted particularly useful also in the bioremediation of the PAH fraction (Hamdi et al. 2007). Hamdi et al. (2007) reported an increase in the degradation of pyrene and anthracene (but not benzo[a]pyrene) in spiked soil when either aged PAH-contaminated soil, sewage sludge, or decaying rice straw were added in aerobically incubated microcosms for 120 days. In this experiment, the addition of the aged PAH-contaminated soil, containing activated indigenous degraders, has the ability to increase the degradation of the target toxic molecules. Ros et al. (2010) conducted a research on the use of fresh and composted sewage sludge amendments in the landfarming process of a TPH-contaminated soil. In this 8-months bioremediation experiment, fresh organic amendment led to a higher percentage of hydrocarbon degradation (46 %) and an increase in bacterial and fungal population compared to composted organic amendment (36 %), thus indicating a different role of organic matter quality in bioremediation efficiency. However, wide evidences have been provided by a number of authors that application of unstable and/or immature organic amendments may adversely affect soil properties, plant growth, and surrounding water and air compartments (Senesi and Plaza 2007; Ramirez et al. 2008).

The effects of cotton gin crushed compost, poultry manure, sewage sludge and organic municipal solid waste on the bioremediation of a soil polluted with gasoline at two loading rates (5 and 10 %) were studied by Tejada et al. (2008). The results obtained in this study indicated that the addition of organic matter to the soil decreased the extent to which soil microbial biomass, respiration and enzymatic activities were inhibited by gasoline; moreover, this decrease was higher in presence of organic materials with a high humic acid content. Humic acids have greater aromaticity than fulvic acids, and this is also in keeping with the concept of greater numbers of aromatic carboxylic acids in the humic acids. For this reason, the binding of humic acids with gasoline is higher than in fulvic acids. These results are in agreement with those of Kollist-Siigur et al. (2001) who suggested that humic acids had greater binding affinity for PAHs than fulvic acids. Bogan and Sullivan (2003) reported that the addition of fulvic acid to soils that had low humic acid/fulvic acid content greatly enhanced pyrene mineralization by *Mycobacterium austoafricanum*. They also reported a slower progress in PAH sequestration in a soil with high fulvic acid content. Furthermore, Plaza et al. (2009) in an experiment on the interaction of humic acids with PAHs during a composting process showed a decrease of humic acids affinity for phenanthrene and pyrene but an increase in the heterogeneity of binding sites; these changes may be expected to facilitate microbial accessibility to PAHs, thus resulting in a faster and more effective soil cleanup with matured compost, rather than with fresh organic amendments.

Finally, the quantity of organic amendment addition to the contaminated soil should be also determined (Namkoong et al. 2002). Depending on sludge quality, high addition rates or repetitive applications may be accompanied by substantial loads of organic toxic compounds and growth inhibitors that hinder the microbial activity (Barajas-Aceves et al. 2002; Garcia et al. 1994). Moreover, the mineralization of compost derived from sewage sludge in soil has been shown to induce physico-chemical stresses due to a pH decrease and salinity elevation (Li et al. 2001; Hamdi et al. 2006, 2007). In an experiment on the addition of humic acids to a soil contaminated with fresh ^{14}C-labeled pyrene, an increase in the mineralization up to a maximum of more than three times the non-amended rate was shown. At very high HA additions the rate of pyrene mineralization decreased, possibly due to inhibition from pH or salt concentrations (Haderlein et al. 2001). In addition, the application of organic amendments traditionally used in agriculture, both fresh or stabilized (e.g., animal manures and compost), has been also studied in different bioremediation experiments in soils contaminated with heavy metals. Organic matter plays a decisive role in control heavy metal availability through changes in soil chemical properties and by its metal-chelating ability (Plaza et al. 2009). For this reason, organic amendments can enhance bioremediation of heavy metals through various processes that include immobilization, reduction, volatilization and rhizosphere modification.

However, attention should be paid on the heavy metal content in the added amendments.

It is clear that the heavy metal-sequestering effect of humic matter may represent a benefit because it reduces the risk of heavy metal leaching, evaporation, and protects food chain from accumulation. On the other hand, humic matter may also represent a long term storage compartment for heavy metals, thus making the soil a hyperaccumulator environment. In this case, the association of this "hyperaccumulator" system with a "competitive hyperaccumulator", such plant-root system, is necessary to obtain a progressive soil clean up (*phytoremediation*).

5 Microorganism-pollutant interaction

Microorganisms, the core of biological treatments, include bacteria, protozoa, fungi, algae and viruses. The ability of microorganisms to degrade xenobiotic organic compounds derives from their co-evolution with naturally occurring compounds that have analogous molecular structures and by the development of catabolic activity through adaptation on sites under extended periods of pollution (Semple et al. 2003).

Typical bacterial groups already known for their capacity to degrade hydrocarbons include *Pseudomonas sp.*, *Marinobacter sp.*, *Alcanivorax sp.*, *Microbulbifer sp.*, *Sphingomonas sp.*, *Micrococcus sp.*, *Cellulomonas sp.*, *Dietzia sp.* and *Gordonia sp.* (Brito et al. 2006). Biodegradation of oil by fungi *Rhodotorula*, *Sporobolomyces*, *Aspergillus* and *Penicillium* has been also studied (Head and Swannell 1999).

As suggested by a number of studies (Liste and Felgentreu 2006; Sartoros et al. 2005), pre-exposure of indigenous microorganisms to organic contaminants can influence their degradation capabilities. Liste and Felgentreu (2006) observed similar microbial species richness in a contaminated and pristine soil; however, PAH-degraders (*Alcaligenes piechaudii*, *Pseudomonas putida*, and *Stenotrophomonas maltophilia*) were more abundant in the contaminated soil (1517 mg kg^{-1} total petroleum hydrocarbons and 71.4 mg kg^{-1} PAHs) compared to pristine soil. It is estimated that in 1 g of unpolluted soil, there are only 100 to 1000 cells of hydrocarbon degrading microorganisms, whereas, in 1 g of soil polluted by oil, their number increases to 1×10^6 to 5×10^7 cells, especially if pollution occurred repeatedly and during a long time (Rosenberg and Ron 1996). Generally, organic contaminants are assimilated by microorganisms as a carbon source for growth and energy and an increase in microorganism quantity is regarded as an indicator of contaminant degradation (Shukor et al. 2009).

Surfactants have been frequently used to increase the bioavailability of compounds by incorporating them into micelles, thus increasing their transfer rate into the aqueous phase. The biotransformation and mineralization of a mixture of two PAHs, anthracene and pyrene by an enrichment culture in the presence or absence of Tergitol NP-10, a non-ionic surfactant, and at temperatures of 10 and 25 °C was investigated by Sartoros et al. (2005). The addition of surfactant at 25 °C increased the overall mineralization of anthracene and pyrene to 33.0 and 27.6 %, respectively. However, the addition of surfactant at 10 °C had a negative impact on the overall biotransformation of anthracene and pyrene, reducing them to 20.6 and 14.0 %, respectively. Comparing natural and synthetic surfactants, the former are considered more effective and environmentally friendly in enhancing bioremediation. The addition of sophorolipid, a microbial glycolipid produced from *Candida bombicola*, to a crude oil contaminated soil determined a 80 % biodegradation of saturates and 72 % aromatics in a 8 weeks biodegradation experiment (Seok-Whan et al. 2010). In addition, dissolved humic substances, were found to be promising in enhancing the bioavailability of hydrophobic pollutants in soil. They were found to increase the aerobic biodegradation of PAHs (Bogan and Sullivan 2003; Van Stempvoort et al. 2002) and PCBs (Fava and Piccolo 2002) in soil without exerting any toxicity.

In sites co-contaminated with organic and metal pollutants, the maintenance of a phylogenetically and functionally diverse microbial community can be seriously affected by the synergistic cytotoxic effect of multiple contaminants on soil microorganisms (Lin et al. 2006). A study on methyl tert-butyl ether (MTBE) biodegradation in presence of heavy metals demonstrated that the metal ions Cu^{2+} (at 1 and 10 mg l^{-1}), Cr^{3+} and Zn^{2+} (at 10 mg l^{-1}) determine an inhibitory effect on MTBE degradation by *P. aeruginosa* strain (Chi-Wen et al. 2006). Olaniran et al. (2011) evaluated the inhibitory effect of heavy metals (cadmium, mercury and lead) on the aerobic biodegradation of 1,2-Dichloroethane (1,2-DCA) by autochthonous microorganisms in soil microcosm.

In this study, a dose-dependent relationship between degradation rate of 1,2-DCA and metal ion concentrations was observed for all the heavy metals tested, except for Hg^{2+}.

In soil, heavy metals can have long-term toxic effects within ecosystems and have a negative influence on biologically mediated soil processes. It is generally accepted that accumulation of metal reduces the amount of soil microbial biomass and various enzyme activities, leading to a decrease in the functional diversity in the soil ecosystem and changes in the microbial community structure (Barea et al. 2005). However, metal exposure may also lead to the development of metal tolerant microbial populations (Giller et al. 1998). Changing the valence or charge of a metal through oxido-reduction is necessary for microorganisms resistance. This is accomplished by cell surface electron-transport systems and enzyme-reducing systems that allow bacteria to detoxify and regulate the movement of metal ions. For example, reduction of higher valence species may affect mobilization, e.g., Mn(IV) to Mn(II), or immobilization, e.g., Cr(VI) to Cr(III) (Gadd 2004).

6 Phytoremediation

Phytoremediation, a technology that uses plants to clean up pollutants from the environment, was defined in the '90s as a promising technology for soil remediation (Cunningham and Berti 1993; Raskin et al. 1994). The effectiveness of this technology has been widely demonstrated in soil for many classes of pollutants, like oil hydrocarbons, polycyclic aromatic hydrocarbons, pesticides, dyes, chlorinated solvents, and heavy metals (Kagalkar et al. 2011; Nedunuri et al. 2000; Newman et al. 2001), and has also shown a strong potential for treatment of different contaminated matrices, such as sediments (Bert et al. 2009; Bianchi et al. 2010). According to several authors (Chaney et al. 1997; Salt et al. 1995; Raskin et al. 1997, US EPA 2000), phytoremediation is usually classified on the basis on plant action; the well-known terms of Phytoextraction, Phytostabilization, Phytovolatization and Phytodegradation belong to this classification. At first, the concept of phytoremediation was mainly applied to heavy metal hyperaccumulator species (US 2000) and to hydroponic experiments with singular pollutants, then, in the next years, the research was driven to field studies, in order to obtain significant results for real-field applications, also for other type of pollutants, like toxic organic compounds or herbicides. Also the number of plant species with hyperaccumulator and accumulator character has significantly increased during the time (Jabeen et al. 2009). Examples of plants with these characteristics are following reported: *Brassica spp, Populus spp, Phragmites spp* and *Thlaspi spp,* (EPA, 2000), profit yielding crops, such as *Cannabis sativa* (Linger et al., 2002), *Gossypium spp.*, *Linum spp.* (Angelova et al. 2004), and *Jatropha curcas* (Yadav et al. 2009), plants for biomass production, such as *Salix spp* (Mleczek et al. 2010), horticulture species, such as *Zea mays* and *Lycopersicon esculentum*, (An et al. 2011), and tree species (Pulford 2003). In the recent years, this technology, being considered eco-friendly and cost effective with respect to the traditional technologies, is receiving considerable global attention (Glick 2010).

Padmavathiamma and Li (2007) have extensively reviewed the metal hyper-accumulation in plants, while the role of rhizosphere has been published in specific reviews (McGrath et al. 2001; Fitz and Wenzel 2002; Wenzel et al. 2004). Moreover, Meagher (2000) gave a description of mechanisms of plants phytoremediation for heavy metals and toxic organic compounds. For phytoremediation of soil polluted by heavy metals, the main actions are of two kinds: phytostabilization and phytoextraction. The former strategy involves the immobilization of these contaminants at root level, avoiding their dispersion by the wind and their transfer to the aquifer. In addition, plants with their root apparatus contribute to physically stabilize the soil, thus avoiding the run-off of soil particles (White et al. 2006). Alternatively, the latter strategy takes advantage from the ability of plants to hyperaccumulate metals (Turnau et al. 2005). Some examples about phytoremediation of organic and inorganic contaminants are summarized in Table 1. A field study was performed to assess the role of Indian mustard in phytoremediation of chromium-contaminated substrata. A significant increase in Cr accumulation (0.64–4.19 mg/g dw, stem; and 0.77–1.1 mg Cr/g dw, root) was observed in response to Cr stress, thus showing that Indian mustard is a potential hyperaccumulator specie (Diwan et al. 2010). In a field experiment, the absorption characteristics of different plant species (tomato, maize, greengrocery, cabbage, and

Table 1 Examples about phytoremediation of organic and inorganic contaminants

Plants	Contaminants	Results	References
Indian mustard (*Brassica juncea*)	Cr	Significant increase in Cr accumulation in response to Cr stress	Diwan et al. (2010)
Tomato (*Lycopersicon esculentum*), maize (*Zea mays*), greengrocery (*Brassica chinensis*), cabbage (*Brassica oleracea*), japan clover herb (*Kummerowia striata*)	Cd, Pb, Cr, Cu, Fe	Greater amounts of heavy metals absorption in tomato plant species; the accumulation increased when tomato was intercropped with other plant species	An et al. (2011)
Willows (*Salix viminalis*)	TPH	Higher THP reduction (57 % of original concentrations) in sediments planted with willows compared to unplanted sediments	Vervaeke et al. (2003)
Mangrove (*Rizophora mangle*)	TPH	Higher THP reduction in planted sediments compared to unplanted sediments	Moreira et al. (2011)
Alfalfa (*Medicago sativa*)	PCB	Significant decrease in soil PCB concentration after one and 2 years of alfalfa planting (31.4 and 78.4 %, respectively)	Tu et al. (2011)
Rye grass (*Lolium perenne*), white clover (*Trifolium repens*), celery (*Apium graveolens*)	PAH	Mixed culture of rye grass, white clover, and celery resulted more effective in removing PAH (52 %) with respect to monocultures (45 %) and to the control soil (30 %)	Meng et al. (2010)

Japan clover herb) and planting patterns (monoculture and intercropping) for heavy metals (Cd, Pb, Cr, Cu, and Fe) were studied. The authors found that tomato absorbed greater amounts of heavy metals and that the accumulation increased when tomato was intercropped with other plant species; on the other hand, the levels of most of the heavy metals were reduced in maize intercropped with other plant species, making intercropping maize a feasible method for obtaining safe harvest (An et al. 2011).

Several studies about phytoremediation were focused on decontamination and reclamation of ex mine-tailings sites (Clemente et al., 2003; Archer and Caldwell 2004; Chehregani et al. 2009) and on phytomanagement of these contaminated areas (Dominguez et al. 2008).

Several studies have also involved the use of chelating agents, in order to improve the bioavailability of heavy metals and their uptake by plants. In 2004, Alkorta et al. (2004) have extensively reviewed the role of chelating agents, such as ethylene diamine tetraacetic acid (EDTA), questioning about their use in phytoremediation: the authors, in fact focused on the risk of adverse environmental effects due to metal mobilization during extended periods of time, and proposed the use of chelating agent less harmful to the environment such as citric acid and ethylene diamine disuccinic acid (EDDS). The suitability of EDDS in phytoremediation systems was also proven by Meers et al. (2008), especially in terms of its biodegradation. Furthermore, the mathematical approach, now, has reache d a greater importance, in the management of phytoremediation studies. In a recent study, the authors developed a mathematical model to select plant in order to optimize the potential of metal phytoextraction, thus characterizing the nonlinear behaviour of the soil–plant interaction with heavy metal pollution (Guala et al. 2011).

It is well known that plants promote the degradation of organic compounds by immobilization, removal, and promotion of microbial degradation. Some organic compounds are transported across plant membranes, released through leaves via evapotranspiration (phytovolatilization) or extracted, transported and accumulated in plant tissues (phytoextraction) or degraded via enzymatic processes (phytodegradation). Some of the non-volatile compounds are sequestered in plants and are less bioavailable (phytostabilization) (Megharaj et al. 2011).

Kuiper et al. (2004) and Newman and Reynolds (2004) published reviews on phytodegradation of organic pollutants, at root level (rhizodegradation), and Dzantor (2007) addressed the state of rhizosphere "engineering" for rhizodegradation of xenobiotic contaminants. Soil microflora plays, in fact, vitally important role during rhizoremediation of xenobiotics (Johnsen et al. 2005; Semple et al. 2007). The interaction among microbial degrader, plant and PAHs in soil might be regulated through rhizospheric processes (de Carcer et al. 2007).

Plants have many important functions in the stimulation of the microbial metabolism, providing a carbon source for microorganism activity, transferring the oxygen from air to the soil (Olson et al. 2003; Yu et al. 2006), and releasing root exudates which can serve as substrates for the total microbial community activity, thus increasing the number of microorganisms (Salt et al. 1998). Several studies regarding the microbial community involved in phytoremediation, indicated that the composition and size of the microbial community in the rhizosphere depends on plant species, plant age, and soil type (Campbell 1985; Atlas and Bartha 1998). These results agree with other studies that have found a strong species dependence on the ability of phytoremediation systems to promote hydrocarbon degradation (Liste and Alexander 2000, Wiltse et al. 1998). This may be due to alterations in root exudate patterns (type and amount), which are depending on plant species and stage of plant development (Fletcher and Hegde 1995), but may also be due to differences in root architecture (Aprill and Sims 1990); the soil exploration by roots helps in making tightly in contact plants, microorganisms, nutrients and contaminants between each other (Cunningham et al. 1996), thus enhancing the biodegradation of organic pollutants. The ability of plants to enhance TPH degradation in contaminated sediments has been found also by Vervaeke et al. (2003) in a study reporting a higher THP reduction in sediments planted with willows (57 % of the original concentrations) compared to unplanted sediments. The role of plants is probably due to their release of radical excretions such as carbon, energy, nutrients, enzymes and oxygen for the microbial population of the rhizosphere (Anderson et al. 1993). Typically, higher and more diverse microbial populations have been seen in rhizospheric soil compared to unvegetated soil thus contributing to increase the hydrocarbon degradation (Siciliano et al. 2003).

In a study about phytoremediation with the species *Rizophora mangle* of mangrove sediments contaminated by total petroleum hydrocarbon, after 90 days, a high decontamination efficiency (87 %) was observed. This large efficiency in the remediation was favored by the large growth of bacteria at rhizosphere level (Moreira et al. 2011).

Therefore, the combined use of plants and hydrocarbon degrading bacteria inoculants could have a great potential for improving remediation processes. However, the competition with resident microorganisms in soil can limit the persistence and colonization behavior of inoculated microorganisms. Afzal et al. (2012) in a study about the influence of the bacteria inoculation method (seed imbibement and soil inoculation) on microbial growth, observed that the colonization efficiency was higher when the microorganisms were inoculated in soil. In addition to these factors, the physicochemical properties of soil have been considered the main variables influencing the survival and activity of an inoculated strain and, subsequently, the efficiency of contaminant degradation (Afzal et al. 2011). A reduction in seed germination and biomass (shoot and root) production of Italian ryegrass was shown by Afzal et al. (2011) when the plant was grown in a sandy soil compared to a loamy soil. The better root development and higher production of root exudates in the loamy soil, probably contributed to the better colonization of the inoculants strains and more efficient contaminant degradation. An in situ phytoremediation trial was developed in order to investigate the function of alfalfa during a 2-year bioremediation of an agricultural soil contaminated with polychlorinated biphenyls (PCBs). After the first and second years of remediation, planting alfalfa significantly decreased the initial soil PCB concentrations by 31.4 and 78.4 %, respectively. Moreover, the presence of alfalfa significantly increased soil dehydrogenase and fluorescein diacetateesterase activities during the remediation. Changes in soil bacterial community structure and diversity were shown by PCR–DGGE fingerprinting. Some well-known PCB-degrading bacteria, such as *Chloroflexis p*., may have contributed to the rhizoremediation of PCBs. (Tu et al. 2011). In addition, the effects of monocultures or mixed cultures of different plant species on PAH phytoremediation has been investigated by Meng et al. (2010). Mixed culture of rye grass, white clover, and celery resulted more effective in removing PAH (52 %) with respect to monocultures (45 %) and to the control soil (30 %), after 75 days of treatment.

7 Organic matter-microorganisms-plants: the synergic action

Microbial–plant interactions were largely investigated during the last 50 years; however, these studies aimed mainly to plant–pathogen interactions. Only 10 years ago, the ecology of microbes in the rhizosphere was focused on decontamination processes. The existence of a higher percentage of microbial population and activity in planted soils comparatively to the unplanted soils, indicates that plant growth coupled with active microbial activities at root level and represents the main mechanism of the TPHs biodegradation (Miya and Firestone 2001). Annual and perennial plants transfer 30–70 % of new fixed photosynthetic carbon to the roots of which 30–90 % is transferred directly into the rhizosphere (Olson et al. 2003). These contributions of carbon from plants to the soil stimulate microbial communities and thus the degradation of organic contaminants (Liste and Alexander 2000; Binet et al. 2000; Chen et al. 2003; Robinson et al. 2003; Yu et al. 2006; Kamath et al. 2004). However, several studies underlined that the poor chemical-physical condition of many polluted soils may fail to support vegetation or restrict the depth and proliferation of plant roots (Scullion and Malik 2000). Moreover, pollutants may cause a significant reduction in the plant growth (e.g. Chaney et al. 1997; Siddiqui et al. 2001). For example, in crude oil contaminated sites the reduced growth of plants has been explained by the effects of small aliphatic, aromatic, naphthalic and phenolic like compounds that may reduce respiration, transpiration, photosynthesis and hormonal stress response (Trapp et al., 2005). These effects, however, varied with individual plant species and their physiological responses to contaminants (Vega-Jarquin et al., 2001). In addition, stress caused by contaminants may result in a loss of structural and functional diversity of microorganisms, thus altering nutrient cycles (e.g. Belyaeva et al. 2005; Khan and Scullion 1999).

The addition of organic amendments to a contaminated matrix have been known to improve soil physical, chemical, and biological properties and, consequently, plant growth. Some examples about phytoremediation coupled with the application of organic amendments of contaminated soils and sediments are summarized in Table 2. Farfel et al. (2005) showed that grass coverage was significantly improved by biosolid addition in an urban soil contaminated by Pb, thereby reducing exposure to contaminated soil. Similarly Helmisaari et al. (2007) observed the recolonization of natural vegetation and increased dwarf shrub survival, by addition of organic material (household compost and woodchips), in a heavy metal–polluted forest. Similarly, in a soil contaminated by pyritic mine waste the addition of cow manure was capable, in a short-term, of facilitating the initial re-vegetation (*Chenopodium album* L.) and of preventing soil acidification, thus decreasing heavy metal bioavailability (Walker et al. 2004). The reduction in heavy metal mobility due to pH increase as a consequence of organic matter addition (municipal waste compost, biosolid compost) was reported also by Pérez de Mora et al. (2006). In this study, a generally enhancement in soil microbial functions (increase microbial biomass C (MBC), MBC/TOC ratio and dehydrogenase and aryl-sulphatase enzyme activities) were found in the amended and planted soil. Moreover, the amendments employed and the development of a root system induced shifts in the microbial community structure. This is particularly interesting in soil remediation of contaminated soils, since changes in soil microbial populations can also affect soil functionality, thereby influencing nutrient turnover and the restoration processes of the affected soil.

The stimulation of soil metabolic processes and plant development was also showed in presence of manure in a mesoscale phytoremediation experiment (*Paulownia tomentosa* and *Cytisus scoparius*) of a contaminated soil (Macci et al. 2012). The synergic effect of roots and organic matter was effective in the reclamation of the hydrocarbon and metal polluted soil, with *Paulownia tomentosa* more efficient than *Cytisus scoparius* in extracting metals. The effectiveness of soil amendments in the phytoremediation of polluted soils has been reported in several studies, both at laboratory and field scale. In 1 year field experiment, organic matter and *Populus nigra* were effective in the reclamation of polluted soil; a reduction in both inorganic (60 %) and organic (80 % TPH and 60 % PCBs) contaminants was showed (Doni et al. 2012). In this study, the treatment with only organic matter, showed a lower reduction in organic contaminants (30 % TPH), and as expected, not significant reduction in the total metal concentration. However, in each treatment, the increase of biological parameters (dehydrogenase, β-glucosidase and phosphatase enzyme

Table 2 Examples about phytoremediation coupled with the application of organic amendments of contaminated soils and sediments

Plants and organic matter	Contaminats	Results	References
Princess (*Paulownia tomentosa*), Scotch broom (*Cytisus scoparius*) Horse manure	TPH, Cu, Cd, Ni, Zn, Pb, Cr	The synergic effect of roots and organic matter was effective in the reclamation of the hydrocarbon and metal polluted soil, with *Paulownia tomentosa* (reduction >50 %) more efficient than *Cytisus scoparius* (reduction <40 %) in extracting metals	Macci et al. (2012)
Poplar (*Populus nigra*) Horse manure	TPH, PCB, Cu, Cd, Ni, Zn, Pb, Cr	A reduction in both inorganic (60 %) and organic (80 % TPH and 60 % PCBs) contaminants was showed in planted soil. The treatment with only organic matter, showed only a reduction (30 % TPH) in total petroleum hydrocarbon	Doni et al. (2012)
Crop (*Brassica juncea*) Cow manure, compost	Zn, Cu, Pb, Cd, As	The addition of cow manure and compost had a greater influence on soil remediation	Clemente et al. (2005)
Tomato (*Lycopersicon esculentum*) Tannery sludge	Cr, Fe	Significantly higher accumulation of metals in the different parts of the plants grown on soil amended with tannery sludge	Singh et al. (2004a)
Sunflower (*Helianthus annuus*) Tannery sludge	Cr, Fe, Zn and Mn	Significantly higher accumulation of metals in the different parts of the plants grown on soil amended with tannery sludge	Singh et al. (2004b)
Paspalum (*Paspalum vaginatum*), Tamarix (*Tamarix gallica*) Green compost	TPH, Cu, Cd, Ni, Zn, Pb, Cr	Higher THP and heavy metals reduction in planted sediments compared to unplanted sediments	Bianchi et al. (2010)

activities) over the time indicated the activation of microbial metabolism favored by the organic matter application and the plant roots-microorganisms interaction. In a 4-year phytoremediation (*Brassica juncea* L.) of a site affected by the toxic spill of pyrite residue contaminated by heavy metals (Zn, Cu, Pb, Cd) and arsenic, the addition of cow manure and compost had a greater influence on soil remediation (Clemente et al. 2005). Soil amendments together with the *B. juncea* contributed to the occurrence of natural attenuation processes, increasing soil microbial biomass and improving soil fertility, as indicated by the appearance of spontaneous vegetation. Gupta and Sinha (2006) studied the extractability of metals in different tannery sludge amendment and the potential of *Sesamum indicum* L. var. T55 (sesame) for the removal of metals from tannery waste contaminated site. They stated that the level of extractable Zn, Ni and Cd in both diethylene triamine pentaacetic acid (DTPA) and EDTA extractants increased with the increase in sludge amendments. In agreement with the results reported by Singh et al. (2004b), the tannery sludge favored the growth of the plants at low amendment rates (25 %). Furthermore, Singh et al. (2004a; 2004b) observed also a significantly higher accumulation of metals in the different parts of the plants *Lycopersicon esculentum* (Singh et al. 2004a) and *Helianthus annuus* (Singh et al. 2004b) grown on soil amended with tannery sludge. The synergic action of plant and organic matter in soil phytoremediation has also been investigated in association with a nonionic-surfactant (such as Tween 80) with the aim to improve bioavailability of PAHs (Cheng and Wong 2008). In this study, the effects of pig manure compost (PMC) and Tween 80 on the removal of ^{14}C–Pyrene (Pyr) from soil cultivated with *Agropyron elongatum* has been evaluated. The results showed that the addition of PMC increased the dissipation of Pyr in vegetated soil from 12.1 to 58.7 %, while the co-addition of Tween 80 and PMC further enhanced the dissipation of about 90.3 %, suggesting that the co-application of PMC and Tween 80 could improve phytoremediation of Pyrene-contaminated soil. A study aimed to examine the effects of soil humic acids (HA, natural surfactants) on phytoremediation of PAH-contaminated soil showed that HA application as amendments between 20 and 200 mg kg^{-1} consistently increased pyrene mineralization by indigenous microorganisms (Liang et al. 2007). Atiyeh et al. (2002) found that plant growth increased progressively with increasing concentrations of HA in the range of 50–500 mg kg^{-1}, but growth decreased at HA concentrations exceeded 500–1,000 mg kg^{-1}. In addition, a study aimed to assess the effects of exogenous dissolved HA on pyrene removal from vegetated and non vegetated sediments showed that a high humic acid concentration (6.7 %) led to a significant reduction in pyrene degradation (Ke et al. 2003). In particular, it has been found that in the absence of HA, planted sediments had a significantly higher pyrene removal when compared to the non-vegetated ones. However, when HA was added, no significant difference was found between vegetated and non-vegetated sediments in pyrene removal. This trend has been explained by the reduction of plant growth in terms of total biomass, thus confirming the plant growth inhibition when high concentration of HA were added.

Recently, phytoremediation assisted by HSs has been explored as a sustainable reclamation technology for turning slightly-polluted dredged marine sediments into a matrix feasible for productive use (Bianchi et al. 2010). The properties of sediments can differ significantly from those of soils, and therefore, technologies that work well for soils may be not effective for sediments. The high water content, the compactness, the often high salinity, the poor aeration and the nutrient content of clay sediments that can hinder the root growth, are the most common problems in treating this kind of matrix. The study on marine sediment phytoremediation (using a combination of the grass specie, *Paspalum vaginatum* and the shrub specie, *Tamarix gallica*) showed the necessity of a bio-physical pre-conditioning of sediments by mixing them with a calcareous material from excavating activities and applying green compost in order to create an environment suitable for plant growth and heavy metal uptake (Bianchi et al. 2010). Nine months after the beginning of the experiment, the healthy state of the plants and the decrease in sediment organic and inorganic contaminants indicated the efficiency and success of this technology for sediments reclamation. In this experiment, a correlation among humic substances, hydrocarbon concentration and dehydrogenase activity has been reported (Bianchi and Ceccanti 2010), thus confirming that humic substances are capable of binding with hydrocarbons, becoming a substrate for specialized microorganisms which enhance their degradation through oxydoreductase enzymes (Vacca et al. 2005).

8 Conclusions

Background knowledge on the chemistry and potential risks of toxic compounds in terms of concentration and solubility, together with biological, physical and chemical properties of contaminated soils must drive the selection of the most appropriate remediation option.

The different bioremediation strategies, mainly based on microorganism rich organic waste and plant action, have been found effective in the reclamation of polluted soil. In particular, several studies have demonstrated how microorganisms and organic matter can facilitate the degradation of organic contaminants, while plants enhanced heavy metal decontamination. However, the simultaneous adoption of these technologies can better fulfill the objectives of pollutant immobilization and degradation in sites contaminated by both inorganic and organic compounds, respectively, thus actually controlling threats to human beings and environmental quality. Moreover, the strength of the rhizosphere and its associated microbial communities thorough the external management based on the addition of organic amendments and the use of particular plants, greatly promoted soil bioremediation; similar approach applied to very problematic matrices, such as mine soil and polluted sediments, have been found very effective, not only for bioremediation process, but also for the improvement of the physical, chemical and biological properties.

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