Abid A. Ansari · Sarvajeet Singh Gill
Ritu Gill · Guy R. Lanza
Lee Newman *Editors*

Phytoremediation

Management of Environmental Contaminants, Volume 4

Springer

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Preface

> “*Live as if you were to die tomorrow, learn as if you were to live forever*”
>
> Mahatma Gandhi

Volume 4 of this 5 volume series adds various studies on phytoremediation of organic contaminants from terrestrial and aquatic ecosystems. In this volume, some examples on applications of phytoremediation in wastewater engineering technology have been provided. Various studies on natural and constructed wetlands for phytoremediation have also been included in this volume. The importance of phytoremediation in reclamation and restoration of terrestrial and aquatic ecosystems has been described. Information on uptake, tolerance mechanisms and the role of grasses in phytoremediation of various organic contaminants has also been provided. Plant microbe interactions, bio-retention systems, phenolic compounds and enzymatic applications in phytoremediation of contaminated soil and water have been described in different chapters of this volume. The chapters in volume 4 illustrate how phytoremediation applications using constructed wetlands can also serve in the removal of pathogenic bacteria from contaminated waters. Volume 4 of this book series provides additional accounts of some selected phytoremediation research projects and case histories from specific sites and/or laboratories. The editors and contributing authors hope that one result of publishing this book will be to provide a wide range of useful experimental data derived from global applications of phytoremediation. Hopefully, like the previous three volumes of this book series this volume can also provide new insights into the advantages and disadvantages of phytoremediation to manage the continuing threat of ecosystem degradation resulting from anthropogenic inputs of environmental contaminants.

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Part I
Phytoremediation of Organic Contaminants

Phytoremediation of PCBs and PAHs by Grasses: A Critical Perspective

Esmaeil Shahsavari, Arturo Aburto-Medina, Mohamed Taha, and Andrew S. Ball

Abstract Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are two major environmental contaminants which threaten our health and environment. The removal of these key environmental pollutants from the environment is therefore paramount. Among the cleanup methods currently being used, traditional methods such as chemical and physical treatments tend to be expensive, laborious and may cause secondary contamination. Phytoremediation, the use of plants and associated microorganisms, represents a promising, nondestructive and cost-effective in situ technology for the degradation or removal of contaminants. Grasses belonging to the Poaceae family have drawn significant attention in this regard due to their fast growth, dense, fibrous root systems, and the demonstrated fast removal of PAH and PCB compounds from soils in which these plants have been grown. In this review, we review research on the use of grasses for the degradation of PAHs and PCBs and highlight the benefits of this phytoremediation approach.

Keywords Phytoremediation • Grass • Polycyclic aromatic hydrocarbons • Polychlorinated biphenyls • Plant roots • Endophytes

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1 Introduction

Since the start of the industrial revolution, there has been a steady impact of human activities on the environment. This has resulted in large environmental problems that threaten both environmental and human health. The constant increase in human activities is related to the exponential growth of the world population in the last century, now expected to exceed 8.9 billion by 2050 (UN). Such an increase in the global population also means a faster depletion of natural resources and ever-increasing pressure on the environment, resulting in increasing amounts of chemical and radioactive pollutants into the environment. In addition, inhabitants of major cities are commonly affected by air pollution generated by heavy industries and motor vehicles; the World Health Organization (WHO) estimates that these emissions account for the death of three million people per year worldwide [1].

Water and soil pollution is a huge environmental problem caused by the vast amount of waste generated by human kind; in the European Union (EU) alone, around three million sites of contamination are suspected and 250,000 are known as contaminated sites which need to be cleaned up [2]. In China, it is estimated that 1×10^4 ha of land are contaminated with petroleum hydrocarbons [3]. The situation in aquatic systems is not much better; around 1.7–8.8 million metric tonnes of oil goes into aquatic environments [4] every year. Polycyclic aromatic hydrocarbons (PAHs), a key component of oil have been produced in huge amounts from anthropogenic activities such as oil refining and during incomplete fuel combustion [5], resulting in soil contamination between 1 μg/kg and 300 g/kg [6]. PAHs are of great concern since these compounds have carcinogenic and mutagenic properties and can enter the food chain because of their high persistence in the environment and their ability to bioaccumulate [7, 8].

Soils are also commonly polluted with hydrophobic man-made compounds that tend to be recalcitrant, they are called persistent organic pollutants (POPs) of which PAH are included [9]. These POPs are listed in the Stockholm convention (2004) and include aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, polychlorinated biphenyls (PCB), DDT, dioxins, and furans. The following nine were added to the list in 2010: chlordecone, lindane, hexabromobiphenyl, pentachlorobenzene, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride (PFOS), tetrabromodiphenyl ether and pentabromodiphenylether ('commercial pentabromodiphenyl ether'), hexabromodiphenyl ether and heptabromodiphenyl ether ('commercial octabromdiphenyl ether') [10]. Many of these compounds were used for plant protection and as pest control chemicals and now are some of the most serious contaminants.

As a result of the threats to the environment from these organic contaminants, there is now an urgent need to remove them from the environment. There are a range of traditional removal techniques such as chemical oxidation, thermal treatment, and solvent washing for PAHs and PCBs; these methods are expensive (varying between $50 and $500 per tonne of soil) and further post-labor treatments are required. Therefore, increased attention has been paid to more economic and environmentally friendly remediation approaches such as phytoremediation. The aim of this chapter is to describe and assess the potential of phytoremediation of organic contaminants by grasses.

2 PAH and PCB Compounds

Polycyclic aromatic hydrocarbons (PAHs) are defined as a group of organic compounds, formed of two or more 2–6 aromatic rings (Fig. 1). Fossil fuel refining, timber products processing, iron and steel manufacturing, textile mills, vehicle exhausts, forest fires, and volcanoes are important sources of PAHs [11]; however, the primary source of PAHs is fuel combustion [9]. All PAHs exhibit toxicity properties; the high-molecular-weight PAHs are also potentially carcinogenic.

The 2015 ranking of PAHs in the United States Agency for Toxic Substances and Disease Registry (ATSDR) was 9 [12], while one particular PAH, benzo[*a*]pyrene, ranked 8. The US Environmental Protection Agency (US-EPA) lists 16 PAHs as

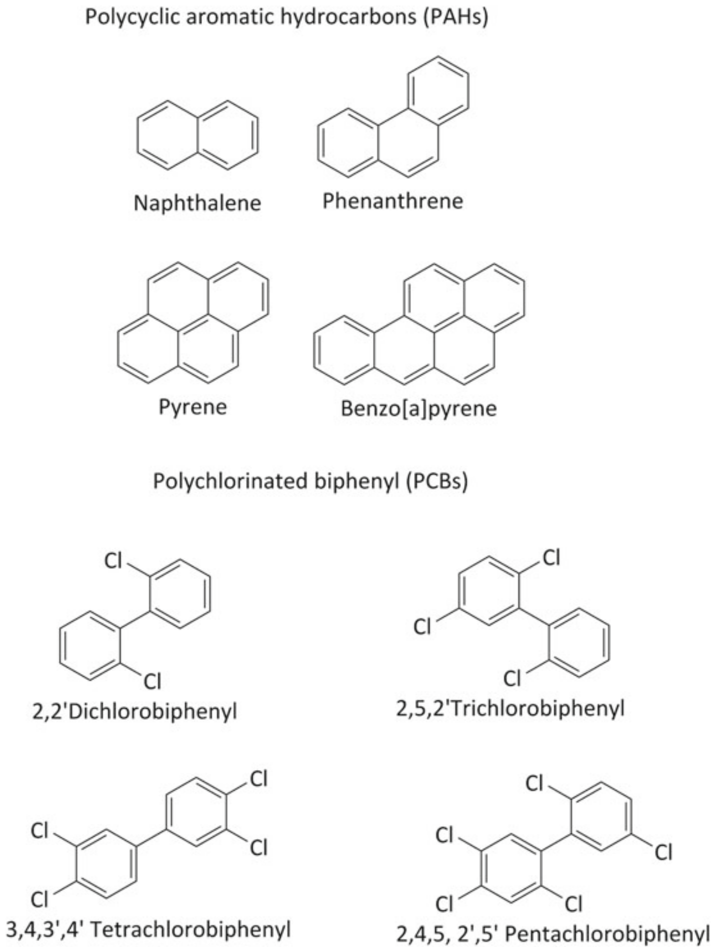

Fig. 1 Representative structure of PAHs and PCBs

priority pollutants. These 16 PAHs include naphthalene, fluorene, acenaphthene, acenaphthylene (2 ring), fluoranthene, phenanthrene, anthracene (3 ring), chrysene, pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene (4 ring), benzo[*a*]pyrene, indeno[1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene (5 ring), and benzo[*g*,*h*,*i*]perylene (6 ring) [13].

Polychlorinated biphenyls (PCBs) represent a major group of persistent organic pollutants (POPs), which contain 1–10 chlorine atoms and comprise 209 different congeners (Fig. 1). PCBs are widely used as coolants and lubricants in transformers, capacitors, heat exchange fluids, paint additives, carbonless copy paper, and plastic. It is estimated that about 1.5 million tons of PCBs were manufactured over 40 years between the1930s and 1970s. The usage of PCBs was banned in the late 1970s, however there is still substantial amounts of PCBs released into the environment from different sources (e.g. old electrical equipment) in conjunction with poor handling and storage, spills, and improper disposal in the past [14–16].

PCBs are classified as carcinogenic to humans. Exposure to PCBs leads to neurological, reproductive, endocrine, and cutaneous disorders. It has also been shown that PCBs are strongly linked to some metabolic diseases, including type 2 diabetes, obesity, metabolic syndrome, and non-alcoholic fatty liver disease [17]. Like PAHs, PCBs are listed as USEPA Priority Pollutants and were ranked 5 by the United States Agency for Toxic Substances and Disease Registry (ATSDR) in the year 2015 [12]. Although PAHs and PCBs are recalcitrant with low aqueous solubility, low bioavailability, and high stability in environments, both are subjected to biological degradation by bacteria, fungi, and plants. For more information regarding PAH degradation by bacteria and fungi, see articles by Bamforth and Singleton [6], Haritash and Kaushik [7], and Mougin [18]; and for a recent review for PCBs, see Passatore et al. [17].

3 Phytoremediation Technique

Phytoremediation is defined as the use of plants or associated microorganisms to remediate contaminated soils, sediments, and water [19, 20]. The term "phytoremediation" contains two words: Greek *phyto* (meaning plant) and Latin *remedium* (meaning to correct or remove an evil) [21]. This method is relatively recent and it has attracted significant attention on the basis of current research. However, like other methods, phytoremediation has both advantages and disadvantages as outlined in Table 1.

Many studies have shown phytoremediation to be very efficient for the removal of a wide range of contaminants such as metals [22–24], POPs [25–29], and hydrocarbons [30–36]. Furthermore, excellent reviews have also previously assessed the potential of phytoremediation [9, 33, 37, 38]. Phytoremediation is a general name and encompasses different techniques. These include phytoextraction, phytofiltration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation, and phytodesalination. The definition and applications of different methods of phytoremediation are presented in Table 2. It is important to note that each of these

Table 1 Advantages and disadvantages of phytoremediation of organic pollutants [90, 91]

Advantages	Disadvantages
Less disruptive to the environment (*in situ*)	Growth limitation due to environmental toxicity
No need for disposal sites	Taking a longer time than other methods
High public acceptance	Results in greater environmental damage and/or pollutant migration due to enhanced solubility of some contaminants
Avoids excavation and heavy traffic	Accumulation of pollutants in firewood
Useful for the treatment of several hazardous materials	Limited by certain climatic and geological conditions
Can be used in combination with other methods	Potential for the rerelease of pollutants to the environment during litter fall
Inexpensive and solar driven	Not successful for all pollutants

Table 2 Different approaches of phytoremediation using grasses [19, 21, 37, 92]

Phytoremediation method	Definition	Suitable contaminants	Usage of grasses
Phytoextraction	Contaminants accumulate in harvestable biomass (e.g. shoot)	Metals	
Phytofiltration	Contaminants from aquatic system are removed by plants	Metals	
Phytostabilization	Contaminants mobility and bioavailability are eliminated or reduced	Metals	Recommended
Phytovolatilization	Contaminants are taken up by the plants and released into the atmosphere	Volatile organic compounds such as MTBE, methyl-tert-butyl ether	
Phytodegradation	Organic contaminants are degraded by plant enzymes inside plant tissues	Herbicides and TNT	Recommended
Rhizodegradation	Contaminants are degraded by microbes associated with plant roots	PAHs	Recommended
Phytodesalination	Surplus salt is removed from saline soils by halophytes	Salt	Recommended

methods is suitable for different types of contaminants. There is a growing number of studies showing successful phytoremediation of POPs, PAHs and other contaminants using different types of grasses [22, 39–47], and some of the studies are very recent [48–50] suggesting the use of grasses is popular and effective.

4 Advantages of Grasses Used for Phytoremediation of Organic Compounds

Plants are the main agents of phytoremediation, and selection of appropriate plants for specific contaminants is a crucial step. This is because all plants do not show the same potential for phytoremediation as a result of different morphology, physiology, genetic background and root exudates. Irrespective of the fate of organic compounds in plants, the first step in dealing with most organic contaminants by phytoremediation is through the use of plants with a fibrous root system. Grass root systems show the highest root surface area per m^3 of soil relative to other plants and can be developed up to 3 m in the soils, providing a very large surface area for microbial colonization by soil microorganisms and ample space for the interaction of contaminants and microorganisms. In addition, the genetic diversity of grasses helps them survive in unfavorable soil conditions such as contaminated soils [51]. Merkl et al. [52] performed a pre-selection of 57 native species of plants containing 18 legumes, 19 grasses, 3 sedges, and 17 other herbaceous species. The authors found that the most extensive root system belonged to some grasses and sedges.

Moreover, grasses are fast growing and cover the contaminated area quickly, preventing the leaching of contaminants from the soil. In addition, the lower maintenance cost for grasses (e.g. lower fertilizer requirements) make them good candidates for the phytoremediation of organic compounds. Whilst it can be argued that the decontamination of sites by phytoremediation requires more time compared to other methods, the interaction of plants, especially grasses with endophytic microorganisms may lead to enhanced remediation beyond that of other technologies [39, 53]. Many grasses benefit from endophytic partnerships with both bacteria and fungi. Endophytes are defined as microorganisms (mostly bacteria, fungi, and actinobacteria) which live inside the plants without showing any disease symptoms [54]. Some cool season grasses such as tall fescue, perennial ryegrass, and meadow fescue infected with a fungal endophyte (*Neotyphodium* sp.) exhibit enhanced tolerance to abiotic and biotic stress [55].

It has been shown that *Neotyphodium* endophytes may enhance the phytoremediation of metals [56–58], salt [59], and petroleum hydrocarbons [45]. Aged petroleum-contaminated soil has been shown to be effectively remediated using *Festuca arundinacea* and *Festuca pratensis* containing the endophytic fungi *Neotyphodium coenophialum* and *Neotyphodium uncinatum*. Grasses infected with endophytic fungi showed a larger percentage of degradation of the total petroleum hydrocarbons (TPH), suggesting they may be more efficient for TPH removal [45]. However, our knowledge about the effects of *Neotyphodium* fungi on the degradation of other organic contaminants (e.g. PCBs) is limited. Endophytic bacteria from grasses have also alone showed significant potential for the bioremediation of contaminants [60–62]. Extensive reviews about the benefits of endophytic bacteria in the phytoremediation of contaminants have been recently published [53, 63–65].

5 Disadvantages of Grasses Used for the Phytoremediation of Organic Compounds

Apart from the benefits of grasses in phytoremediation applications, there are some disadvantages of using grasses in phytoremediation. Unlike legumes, grasses cannot fix nitrogen and this would be a disadvantage relative to legumes in regard to phytoremediation of contaminated soil with PAHs and PCBs as many of these soils already exhibit poor nutrition status (low nitrogen).

In addition, seed dormancy, quality and lifespan of seeds, low emergence, and germinations rate represent additional disadvantages when grasses are used for phytoremediation. Merkl et al. [52] reported that native grasses showed the poorest germination rate relative to legumes in pre-screen tests for selection for use in eastern Venezuela for phytoremediation of petroleum hydrocarbons. Also, the grasses could not propagate effectively when compared to legumes. Gaskin et al. [66] screened nine perennial Australian native grasses in a soil contaminated with 60:40 diesel/oil mixture at concentrations of 1 % (w/w) and 0.5 % (w/w). Their results showed that while at least three of the grasses showed the potential for phytoremediation of hydrocarbons, seedling emergence of all grasses was low.

6 Phytoremediation of PAHs and PCBs by Grasses

Plants can absorb the organic compounds, take up, translocate or metabolize them. The fate of the organic chemicals in plants depends on some factors such as lipophilicity, expressed as octanol–water partition coefficient (log K_{ow}), acidity constant (pKa), aqueous solubility (Sw), octanol solubility (So), and concentration of the contaminants. However, overall the log K_{ow} plays the most significant role [63]. It is generally believed that compounds with log K_{ow} values between 0.5 and 3 can be taken up by plants while compounds with values higher than log K_{ow} 4 are not easily taken up by the plant root system [19]. Rhizodegradation represents the main mechanism for the phytoremediation of PAHs and PCBs as many of them generally have log $K_{ow} > 4$, suggesting that plants are incapable of uptake in significant quantities. Like other plant roots, the presence of grass roots enhances microbial activity through the release of nutrients, root exudates, and oxygen into the contaminated soil [35]. In brief, the main effects of the plant rhizosphere are:

- Enhancing bioavailability of contaminants.
- Improving soil aeration and soil quality.
- Enhancing co-metabolism and genetic induction of some functional genes involved in the degradation of contaminants.
- Increasing the population of biosurfactant-producing microorganisms.

Many review papers have shown how the plant roots can enhance microbial ability as well as increase the degradation rate of organic contaminants [15, 33, 67]. Several studies have also shown the successful degradation of PAHs in soils by grasses (Table 3). The primary work by Aprill and Sims [68] showed that when eight

Table 3 Grasses used in some phytoremediation studies of PAHs and PCBs

Grass type	Contaminants	Conditions	References
PAHs			
Eight prairie grasses	Chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a*, *h*)] anthracene	Greenhouse/controlled environments	Aprill and Sims [68]
Tall fescue (*Festuca arundinacea*)	Benzo[*a*]pyrene	Greenhouse/controlled environments	Epuri and Sorensen [69]
Prairie buffalograss (*Buchloe dactyloides*)	PAHs	Field	Qiu et al. [78]
Kleingrass (*Panicum coloratum*)			
Mixture of 12 other warm- and cool-season grasses			
Switchgrass (*Panicum virgatum*)	PAHs (from manufactured gas plant)	Greenhouse/controlled environments	Pradhan et al. [93]
Little Bluestem grass (*Schizachyrium scoparium*)			
Tall fescue (*Festuca arundinacea*)	Benzo[*a*]pyrene	Greenhouse/controlled environments	Banks et al. [70]
Slender oat grass (*Avena barbata*)	Phenanthrene	Greenhouse/controlled environments	Miya and Firestone [94]
Tall fescue (*Festuca arundinacea*)	PAHs	Greenhouse/controlled environments	Ho and Banks [95]
Tall fescue (*Festuca arundinacea*)	Phenanthrene and pyrene	Greenhouse/controlled environments	Cheema et al. [75]
Perennial rye grass (*Lolium perenne*)	Pyrene	Greenhouse/controlled environments	D'Orazio et al.[74)
Tall fescue (*Festuca arundinacea*)	Pyrene and metals	Greenhouse/controlled environments	Lu et al. [96]
Commercial pasture seed mixture, composed of annual ryegrass, legumes and vetches and *Avena strigos*	PAHs	Field	Pizarro-Tobías et al. [76]
PCBs			
Tall fescue (*Festuca arundinacea*)	Aroclor 1260	Greenhouse/controlled environments	Epuri and Sorensen [69]
Tall fescue (*Festuca arundinacea*)	Aroclor 1248	Greenhouse/controlled environments	Chekol et al. [83]
Rye grass (*Lolium multiflorum*)			
Rye grass (*Lolium multiflorum*)	Aroclor 1242	Greenhouse/controlled environments	Ding et al. [85]
Ryegrass (*Lolium perenne*)	PCBs	Greenhouse/controlled environments	Lu et al. [97]
Tall fescue (*Festuca arundinacea*)	PCBs	Greenhouse/controlled environments	Li et al. [86]
Switchgrass (*Panicum virgatum*)	PCB congeners (PCB 52, PCB 77 and PCB 153)	Greenhouse/controlled environments	Liang et al. [89]

prairie grasses were planted in soil, increased degradation of chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*)]anthracene was observed compared with the control. Epuri and Sorensen [69] reported that the planting of tall fescue in contaminated soil led to a decrease in benzo[*a*]pyrene volatilization, enhanced mineralization, and increased solvent extractability after 180 days of plant incubation.

Banks et al. [70] investigated the effects of tall fescue plants on highly adsorbed, recalcitrant benzo[*a*]pyrene degradation. The result from that study showed that the level of residual benzo[*a*]pyrene in vegetated soil was lower (44 %) than in control soils (53 %). However, the authors did not observe any difference in the bacterial community in planted and unplanted soils. Chen and Banks [71] also showed that tall fescue plants enhanced the degradation of pyrene relative to the control in a greenhouse study. The pyrene level decreased from 758 mg/kg to below detection limit after 91 d of plant incubation compared to 82 mg/kg for the unplanted control after 147 days. Phenanthrene and pyrene have been used widely as model PAHs in many studies based on grass phytoremediation [72–75]. Cheema et al. [75] performed a greenhouse experiment to investigate the impact of tall fescue in soil spiked with different concentrations of phenanthrene (11–344 mg/kg) and pyrene (15–335 mg/kg). The results showed that the presence of phenanthrene and pyrene did not affect plant biomass at lower concentrations; however, biomass reduction was observed when the concentration of PAHs was increased in the soil. The authors also observed higher microbial viable counts, water-soluble phenolic compounds, and dehydrogenase activity in planted soil compared with unvegetated soil. In terms of PAHs removal, PAHs degradation rates were higher for phenanthrene (1.88–3.19 %) and pyrene (8.85–20.69 %) compared to degradation rates in unplanted soil.

Only limited studies on the phytoremediation of PAH-contaminated soils using grasses have been conducted in the field [76–78]. Pizarro-Tobías et al. [76] applied bioremediation (*Pseudomonas putida* strains) and rhizoremediation (annual grasses) methodologies for soil restoration in a field-scale trial in a protected Mediterranean ecosystem after a controlled fire. Their results showed that the site had returned to pre-fire status after 8 months of monitoring, with PAH concentrations falling from 398 mg/kg down to 36.8 mg/kg in planted soil treatments. Like PAHs, several studies have shown the degradation of PCBs in soils planted with grasses (Table 3) relative to unplanted soils [69, 79–82]. In regard to the phytoremediation of PCBs by grasses, Epuri and Sorensen [69] evaluated the effect of tall fescue plants on Aroclor 1260 (hexachlorobiphenyl)-contaminated loamy sand during 180 day experiments. The authors found that while the tall fescue plants had no effect on hexachlorobiphenyl volatilization and soil binding, the plants increased the mineralization as well as decreased extractability of Aroclor 1260.

Reed canary grass, switch grass, tall fescue, and deer tongue were all tested, among other plants (alfalfa, flat pea, *Sericea lespedeza*) in terms of the phytoremediation of PCB-contaminated soil. Approximately 62 % removal of PCB was observed in treated soil while only around 18 % was removed from the unplanted control soils. Greatest contaminant removal for grasses was observed in alfalfa and canary grass for legumes and grasses respectively after 4 months of growth in PCB-amended soil. The biodegradation of Aroclor 1248 was influenced by the presence of plants and plant–bacterial interactions [83]. PCB-contaminated soil, specifically contaminated with Aroclor 1260 at concentrations of 90–4200 μg/g, was treated with several grasses such as *Festuca arundinaceae*, *Glycine max*, *Medicago sativa*, *Phalaris arundinacea*, *Lolium multiflorum*, *Carex normalis*, and three varieties of *Cucurbita pepo* under controlled greenhouse conditions in Canada [84]. The authors reported that varieties of *C. pepo* extracted more PCBs from the soil compared to the other plants. All plants only showed signs of stress when the concentration of PCBs was 4200 μg/g (highest tested); however, at two lower concentrations of PCBs (250 and 90 μg/g) no effect on plant heath was observed. Overall, the results indicated that the planted soil did not enhance the degradation of PCBs.

Another study evaluated the microbial communities in planted or unplanted soil with ryegrass (*Lolium multiflorum* L.) in a PCB, Aroclor 1242-contaminated soil (8 mg /kg). At the end of 90 days, the presence of plants significantly enhanced Aroclor 1242 degradation compared with soils without ryegrass [85]). Phospholipid fatty acids (PLFAs) profiling showed that the distance from the rhizosphere impacted the PLFA profiles, confirming a distance-dependent selective enrichment of competent microorganisms involved in the degradation of this PCB. Li et al. [86] used tall fescue and alfalfa alone or in combination to evaluate the phytoremediation of PCB-contaminated soil in a greenhouse experiment. The results showed that the highest removal of PCBs was found in tall fescue single plant treatment, followed by a tall fescue and alfalfa combination. The authors concluded that tall fescue on its own produced greatest biomass and could extract more PCBs from soil relative to mixed plants. However, the highest gene copies of *bphA*, *bphD.1.B*, *bphD.2.A*, and *bphD.2.A*/*B* genes (i.e. genes involved in degradation of PCBs) as well as total bacteria counts and dehydrogenase enzyme activity was observed in the tall fescue/alfalfa treatment.

7 Future Aspects

Many studies have been carried out on the phytoremediation of PAHs and PCBs using grasses, but major gaps in our knowledge remains due to the complexity of contaminants, microbes, grasses, as well as environmental factors. Therefore, further work needs to be performed and a suggestion of the future research requirements is shown in Fig. 2. The literature shows that most of the phytoremediation of PAHs and PCBs studies have been carried out in greenhouse or controlled environments (Table 3). To our knowledge, there are very few reports in the literature involving field experiments of PCBs degradation by grasses. It is obvious that many of PAH- and PCB-contaminated sites are also co-contaminated with other pollutants (e.g. metals); in addition, the climatic conditions and other soil factors are more complex in the field rather than greenhouse. To further assess the potential of the grass phytoremediation strategy, more studies need to be performed in the field. In addition, many of the studies on grass phytoremediation are based on spiking the soils with PAHs and

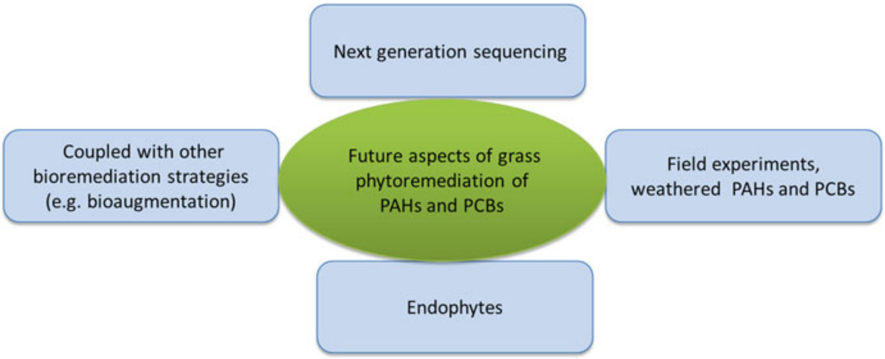

Fig. 2 Future aspects of grass phytoremediation of PAHs and PCBs

PCBs; in this case, bioavailability is not a limitation to phytoremediation. In reality, bioavailability of weathered PAHs and PCBs is one of the main limitations, since PAHs and PCBs tend to strongly attach to the soil particles. Therefore, more studies on weathered PAH- and PCB-contaminated soils are required.

Microbial communities play an important role in the degradation of both PAHs and PCBs and should be monitored during the phytoremediation of PAHs and PCBs to elucidate their microbial dynamics and to identify the microorganisms responsible for the degradation. Next-generation sequencing (NGS), metagenomics opens a new horizon to investigate different aspects of the microbial communities such as species richness and distribution as well as information on the functional genes present in the microbial communities. Furthermore, no prior knowledge of the organisms or specific genes is required in order to evaluate whole microbial communities [87]. Further studies using metagenomics can lead to a better understanding of active microorganisms involved in PAHs and PCBs degradation in the rhizosphere of grasses. As mentioned earlier, plant endophytes can represent a practical solution in the degradation of PAHs and PCBs. Some of the advantages of using endophytic bacteria include [53]:

- Endophytic bacteria are less affected by biotic and abiotic stresses than rhizosphere bacteria.
- The population of endophyte degraders is higher than rhizosphere bacteria.
- Genetic manipulation (genetic engineering) of endophytic bacteria is much easier than plants (in this case, grasses) where genetic engineering of PAHs and PCBs degradation pathways is needed.
- Symbiosis of endophytic PAH and PCB degraders with grasses leads to the degradation of contaminants inside the plants, resulting in reduced toxicity to other organisms and any subsequent biomagnification.
- Many endophytes contribute to enhanced plant growth, resulting in increased stress resistance of plants to contaminants such as PAHs and PCBs.

Our current understanding of the role of endophytic bacteria in grasses used for the phytoremediation of PAHs and PCBs is still incomplete. Only a few endophytic bacteria involved in degradation of PAHs and PCBs have been isolated and investigated. Recently Khan et al. [88] isolated an endophytic bacteria (*Pseudomonas putida*, PD1) from poplar which showed phenanthrene degrading ability when inoculated in willow and perennial ryegrass. The results of this study showed that the presence of PD1 not only increased (by 25–40 %) the removal rate of phenanthrene by willow and grasses but also the PD1 strain promoted root and shoot growth.

Toxicity associated with PAHs and PCBs is the main constraint to grass phytoremediation. Therefore, phytoremediation is not always successful; consequently, a combination of other bioremediation methods can represent long-term solutions in PAH- and PCB-contaminated soils. In one study, polychlorinated biphenyl (PCB) congeners (PCB 52, 77, and 153) were subjected to switch grass phytoremediation and bioaugmentation with *Burkholderia xenovorans* LB400Y [89]. The results showed that total PCB removal was greatest, with an average of 47.3 % in switch grass/LB400Y-treated soil. In addition, the presence of switch grass supported LB400Y survival in the soil. The authors concluded that the use of phytoremediation in conjunction with bioaugmentation might represent a sustainable approach to eliminate or degrade recalcitrant PCB congeners in soils. Our understanding about the interaction of grass with other microorganisms is not clear and remains to be elucidated in future studies.

In conclusion, grass phytoremediation is a promising, cost-effective, and environmental-friendly strategy to degrade or remediate PAH- and PCB-contaminated soils. However, most of the results reported to date have been obtained from the phytoremediation of PAHs and PCBs by grasses in greenhouses or spiked soil. There is now an urgent need to move to field studies. Is grass phytoremediation going to be a successful strategy in the real PAH- and PCB-contaminated environments? The answer to this question will be addressed through the application of field studies and the development of new molecular microbial and environmental analytical techniques.

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