

UNIVERSITÀ DEGLI STUDI DI CASSINO E
DEL LAZIO MERIDIONALE

Ph.D. course in
Models, Methods, and Technologies for Engineering

Curriculum: Civil and Environmental Engineering

XXXV cycle

Application of phytoremediation technique for removal of polycyclic aromatic hydrocarbons and potential toxic elements from contaminated soils

SSD: ICAR/03

Course Coordinator
Prof. Fabrizio Marignetti

Ph.D. Student
Ilaria Gabriele

Supervisor
Prof. Marco Race

ACKNOWLEDGEMENTS

This doctoral scholarship was funded by the Italian Ministry of University and Research (MUR).

ABSTRACT

The issue of polycyclic aromatic hydrocarbons (PAHs) and potential toxic elements (PTEs) is widespread in soils involving environmental systems and human health. At the same time, research on eco–friendly alternatives for the treatment of PAH and PTE-contaminated soils and their reuse after remediation must be stepped up. Among effective treatments employed to remediate contaminated soils with PAHs and PTEs, phytoremediation gained increasing attention due to its eco-friendly interaction and cost-effectiveness features. For this purpose, this doctoral thesis firstly examined the phytoremediation influencing factors involved in the removal of PAHs and PTEs from contaminated soils. In a realistic approach, soil organic matter (SOM), plant species, environmental parameters (pH, soil oxygenation, and temperature), bioavailability, autochthonous microorganisms, and vegetation competition and resulted among the main parameters affecting PAHs and PTEs removal through phytoremediation.

Phytoremediation was confirmed to be a good alternative to conventional physical-chemical and thermal methods, as the latter may cause serious chemical modification of the site and interference with ongoing activities. Among different plant species, the application of *Cannabis sativa* L. in phytoremediation of contaminated soils represent a promising remediation strategy, also to implement encouraging feedbacks in terms of land use and creation of circular schemes. In this context, the present thesis investigated 60 days phytoremediation by *C. sativa* L. at laboratory scale for pyrene (PYR)-contaminated soil (i.e., 50, 100 and 150 mg kg^{-1} dry soil), resulting in PYR removal up to 95% in planted soil, 35% higher than in the unplanted control. The interaction between the plant roots and

microorganisms in rhizosphere was likely associated with PYR removal in the study. As a proof, the highest dehydrogenase (DHO) activity, of 66.26 μg INTF g^{-1} dry soil, which is a possible indirect indicator related to PYR degradation was measured in the soil with the highest PYR removal. Negative effects of PYR on *C. sativa* L. roots and shoots development were observed, reaching a decrease up to 56 and 42 % respectively.

Afterwards, the present thesis work focused on the phytoremediation of PAH and Cu-contaminated soil (i.e., 50–150 and 350 $mg \cdot kg^{-1}$, respectively) by *C. sativa* L. for 50 days, a very frequent condition of real polluted sites. PYR and phenanthrene (PHE) were used as model compounds for low (LMW) and high molecular weight (HMW) PAHs, respectively, in the study. PHE and PYR removal reached 93 and 61%, 98 and 48%, and 97 and 36% in the three testes conditions, respectively, while the highest Cu extraction amounted to 58 $mg \cdot kg^{-1}$. The plants biomass decreased with the increase of the initial concentration of PYR and PHE in soil. Hence, the height of the plants grown under the 300 mg ΣPAHs kg^{-1} condition, was found to be the lowest, reaching a final value of about 10 cm, approximately three times lower than the plants grown in the uncontaminated controls. Finally, this thesis evaluated a rough cost assessment in dollar currency for the entire phytoremediation process including seed purchase, general remediation management, required working expanse for pre-planting, planting, harvesting and post-harvesting operations. A total cost of USD 47 per ton was obtained for the 100 mg ΣPAHs kg^{-1}, which achieved the highest PAH and Cu removal, and biomass production (i.e., 78 and 11%, and 0.32 g, respectively) compared to the other two conditions after 50 d.

TABLE OF CONTENTS

ABSTRACT I
LIST OF FIGURES VI
LIST OF TABLES VIII
INTRODUCTION 10
I.1 Phytoremediation of PAHs and HMs contaminated soils 15
I.2 Heavy Metals bioavailability 20
I.2.1 Impact of plants on HMs bioavailability 20
I.3 Effect of additives on HMs phytoremediation 20
I.4 Biomass post-process treatment 21
CHAPTER 1. Phytoremediation of a pyrene- contaminated soil: a critical review of the key factors affecting the fate of pyrene 23
1.1 Introduction 23
1.2 Pyrene: properties, distribution, and removal 24
1.3 Environmental presence and fate of pyrene 25
1.4 Physico-chemical mechanisms of pyrene removal from contaminated soils 27
1.5 Phytoremediation of pyrene contaminated soils 28
1.6 Mechanisms of phytoremediation aimed at removing pyrene from contaminated soils 29
1.6.1 Treatment time and mechanisms 29
1.6.2. Accumulation of pyrene in plant tissue and fate of contaminated plant biomass 30
1.7 Markers for pyrene biodegradation in the rhizosphere 31
1.7.1 Enzymatic evaluation 31
1.7.2 Pyrene degraders in rhizosphere 32
1.7.3 Effect of pyrene on biomass production 34
1.8 Main factors influencing the fate of pyrene during phytoremediation 34
1.8.1 Pyrene bioavailability 34
1.8.2 Aging of contamination 35
1.8.3 Presence of organic matter in soil 35

1.8.4 Environmental factors 36
1.8.5 Plant species and/or mixed crops 37
1.8.6 Co-presence of potential toxic elements..... 37
1.9 Additives used to strengthen phytoremediation of pyrene-contaminated soils 38
1.9.1. Soil amendments 39
1.9.2 Effect of surfactants..... 40
1.9.3 Effect of bioaugmentation 41
1.10 Conclusions and potential future investigations 48
CHAPTER 2. Phytoremediation of a pyrene-contaminated soil by *Cannabis sativa* L. At different initial pyrene concentration 50
2.1 Introduction 50
2.2 Materials and methods.....52
2.2.1 Experimental soil 52
2.2.2 Spiking procedure for soil.....52
2.2.3 Experimental design 53
2.2.4 Analytical procedures 54
2.2.5 Statistical analysis.....55
2.2.6 Calculations 56
2.3 Results and discussion.....56
2.3.1 PYR removal from soil after 60 days phytoremediation with *C. sativa* L. 56
2.3.2 Bioavailable PYR fraction in soil 58
2.3.3 PYR content, accumulation and translocation in roots and shoots of *C. sativa* L. 59
2.3.4 Production of *C. sativa* L. biomass in PYR-contaminated and uncontaminated soil.....61
2.3.5 Assessment of DHO activity in planted and unplanted soil 63
2.3.6 Identification of PYR-degrading bacteria.....65
2.4 Conclusions.....66
CHAPTER 3. Phytoremediation of Pah- and Cu-Contaminated soil by *Cannabis sativa* L.: Preliminary Experiments on a Laboratory Scale 68
3.1 Introduction 68
3.2 Materials and Methods 71
3.2.1 Chemicals 71
3.2.2 Soil 71

3.2.3 Spiking procedure for soil.....71
3.2.4 Experimental setup 72
3.2.5 Analytical methods and sampling.....72
3.2.6 Statistical analysis.....73
3.2.7 Economic evaluation 74
3.3 Results 74
3.3.1 Residual PAH concentrations in soil after the growth of *C. sativa* L.....74
3.3.2. Copper removal from soil after 50 d of phytoremediation with *C. sativa* L. 78
3.3.3 *C. sativa* L. biomass response to PYR-PHE and Cu contaminated soil over 50-d phytoremediation.....79
3.3.4 Preliminary economic evaluation 81
3.4 Conclusions 83
Conclusions 85
REFERENCES 88

LIST OF FIGURES

Fig. 1.1 Predominant mechanisms involved during phytoremediation aimed at removing pyrene from contaminated soils..... 31

Fig. 1.2. Main enzymes monitored in the rhizosphere and their roles in pyrene degradation during phytoremediation of pyrene-contaminated soils. 32

Figure 1.3 Main effects produced by the supplementation of additives in phytoremediation of pyrene-contaminated soils..... 38

Figure 2.1 Pyrene (PYR) removal (%) after 60 days of phytoremediation in an artificially spiked soil having an initial PYR concentration of 50 (P1), 100 (P2) and 150 (P3) mg·kg TS-1. The same letters (Tukey test) do not represent statistical differences ($p>0.05$) between the experimental conditions. Values are the averages of triplicate analyses and error bars indicate the standard deviation. 57

Figure 2.2 Evolution of the height of the *C. sativa* L. plants used in the PYR-contaminated soil (P1, P2 and P3 conditions) and in the unspiked soil. The same letters (Tukey test) do not represent statistical differences ($p>0.05$) between the experimental conditions. Values are the averages of triplicate analyses and error bars indicate the standard deviation. 62

Figure 2.3 DHO activity in 50 (P1), 100 (P2) and 150 (P3) mg PYR·kg-1 dry contaminated soil and uncontaminated control (P0) after 60-days phytoremediation with *C. sativa* L. The same letters (Tukey test) do not represent statistical differences ($p>0.05$) between the experimental conditions. Values are the averages of triplicate analyses and error bars indicate the standard deviation..... 64

Figure 2.4 Abundance (%) of bacterial diversity (at phylum level) living in the rhizosphere around C. sativa L. roots in P3 (150 mg PYR kg TS-1) after 60 days of growth (A). Panel B is related to the unplanted control. 66

Figure 3.1. Residual pyrene (PYR) and phenanthrene (PHE) concentrations (mg each PAH kg−1) in the unplanted and planted soil, the latter being treated with 50-d phytoremediation by *Cannabis sativa* L. PYR t0 and PHE t0 represent the initial concentrations. The reported values are the means of three replicate analyses. The error bars display the RSD (residual standard deviations). The same letter represents no

significant differences (p > 0.05) among total PAH removal after 50 days of phytoremediation. 75

Figure 3.2. Total PAH removal (%) in the unplanted and planted conditions after 50 d of *Cannabis sativa* L.-induced phytoremediation. The reported values are the means of three replicate analyses. The error bars display the RSD (residual standard deviations). The same letter represents no significant differences ($p > 0.05$) among total PAH removal after 50 days of phytoremediation. 77

Figure 3.3. Measurements of the height (cm) of *Cannabis sativa* L. during the phytoremediation of PAH- and Cu-contaminated soil (PC1, PC2, and PC3 conditions) and the unspiked control. Values are the means of three replicate analyses. The error bars display the RSD (residual standard deviations). The means are interpolated with linear regressions, which are described by equations and determination coefficients (R^2) on the graph. The same letter represents no significant differences (p > 0.05) between the reached height at the end of the experiment. 81

LIST OF TABLES

Table 0.1 Conventional techniques commonly used to remediate contaminated soil by PAHs and PTEs 12

Table 0.1.1 Phytoremediation investigation in which PAHs and HMs were used concurrently. 16

Table 1.1 Chemical and physical properties of pyrene 24

Table 1.2 Monitoring studies reporting the country/region, the concentration, and the major causes of pyrene presence in sites highly impacted by polycyclic aromatic hydrocarbons (PAHs) worldwide. 25

Table 1.3 - Different bacterial species and strains capable for pyrene degradation. 33

Table 1.4 Effect of different additives, surfactants and plant growth promoting bacteria on the phytoremediation of pyrene-contaminated soils 43

Table 2.1. Physical-chemical properties of the soil used in the experimental campaign. 52

Table 2.2 Initial and final pyrene (PYR) concentration under the different experimental conditions 58

Table 2.3 . Initial and residual bioavailable pyrene (PYR) concentrations after 60 days of phytoremediation with *C. sativa* L. under P1, P2 and P3 operating conditions. The concentrations of bioavailable PYR in the unplanted control tests are also reported. 58

Table 2.4 Shoot/root concentration factors (SCFs/RCFs) and the root-to-shoot transfer factors (TFs) obtained under the P1, P2 and P3 conditions after 60 days of *C. Sativa* L. growth. 59

Table 2.5 Fresh and dry biomass (g) of roots and aboveground parts of *C. sativa* L. after 60 days of phytoremediation at three different initial pyrene (PYR) concentrations and in an unspiked soil. The same letters (a, b, c and d) obtained with the Tukey test do not represent statistical differences ($p > 0.05$) between the experimental conditions. Values are the averages of triplicate analyses and error bars indicate the standard deviation. 63

Table 3.1 - Cu removed per g of dry biomass (mg g^{-1}) and Cu removal efficiency after 50 days of *Cannabis sativa* L. growth under the PC1, PC2 and PC3 operating conditions. The same letter represents no significant differences ($p > 0.05$) among total Cu removal efficiency after 50 days of phytoremediation. 78

Table 3.2 Fresh and dry biomass (g) of aerial parts and roots of *Cannabis sativa* L. after 50 d of phytoremediation in PC1, PC2, and PC3 (i.e., 100, 200, and 300 mg ΣPAHs kg^{-1}). The obtained values are the means of three replicate analyses and the RSD (residual standard deviations). 80

Table 3.3 Obtained costs for working hours (i.e., pre-plant cultivation, planting, harvesting and post-harvesting operations), management (i.e., field and laboratory work, and general expenses) and seed purchase. 82

INTRODUCTION

Over the last decades, significant exertions around the world have been made to elucidate the impact of soil quality on human health and environmental safety [1,2]. The outcomes of these studies have shown that soil is the sink of numerous chemical and biological stressors, which could cause health problems (e.g., heart disease, chronical illnesses and cancer) and ecosystem degradation [3,4]. Occurrence of contaminants in soil, directly affects the loss of biodiversity along with the physical structure functions such as nutrients recycling and water holding capacity [5]. In this context, sustainable technologies with the aim to clean up the contaminated soil are urgently required [6].

Among ubiquitous contaminants in soil, polycyclic aromatic hydrocarbons (PAHs) are organic compounds which have received concern due to their toxic, teratogenic, mutagenic and carcinogenic properties [7]. PAHs are composed by two to six benzene rings, sharing no less than two carbon atoms, assembled in different structures (i.e., linear, angular and cluster) [8]. PAHs are divided into two main classes, low molecular weight (LMW) and high molecular weight (HMW) PAHs [9]. The LMW PAHs (i.e., 2–3 fused rings) such as naphthalene, phenanthrene (PHE) and fluorene demonstrate to be significantly less toxic and readily degradable compared to the HMW PAHs (i.e., 4–7 fused rings), as chrysene and benzo(a)pyrene which are recalcitrant and more persistent in the ecosystem [10]. Despite the existence of hundreds different PAHs, the United Stated Environmental Agency (USEPA), since the 1970s, classified 16 PAHs of priority concern which represented a crucial role as a standardized set of compounds to be analyzed in the environmental matrices [11]. Although the limitations in accounting for the harmfulness of contaminated sites, the 16 PAHs listed by the USEPA were significant to cover the wide range of PAHs occurring in contaminated samples with a practical number of representative compounds to screen [12]. This list of compounds still represents a benchmark for environmental policy and sciences [13].

The extensive incidence of PAHs in the environment is due to their production from saturated hydrocarbon combustion under oxygen-deficiency condition [14]. Thus, forest fires and volcanic eruptions can contribute to the natural rate of the PAHs emissions while

the most relevant anthropogenic sources include stationary fossil fuel combustion in plants and mobile combustion in diesel and petrol engines [15].

Ones released, subjected to atmospheric agents in the atmosphere, PAHs final destinations, are sediment and soil due to their low solubility in water and lipophilic features [16]. PAHs are easily sorbed onto soil particles [17]. In this context, Zhao et al. [18] demonstrated the adsorption PAHs onto soil organic matter (i.e., fulvic and humic acids, humins and lignin). Also, Kuśmierz et al. [19] obtained an increased PAH adsorption by increasing the organic matter in soil.

Likewise, soils are the major target for potential toxic elements (PTEs), which over the certain maximum acceptable levels represent a type of environmental issue [20]. PTEs in soils may have geogenic origin (i.e., originate from soil) or, in most of the cases, are the outcomes of anthropogenic practices [21]. The uncontrolled mining activities, industry expansion, increase of waste, and improper use of fertilizers and chemical pesticides resulted in PTEs soil contamination [22].

The release of PTEs may be accidental, as a consequence of agricultural practices, dry and wet depositions from the atmosphere, nevertheless it can also be deliberate, when PTEs are spread or suppressed in the soil [23].

Soil characteristics (i.e., organic matter content, pH, cation exchange capacity, and redox potential) govern the mobility and bioavailability of PTEs in the soil [24]. The potential toxicity, persistence, and bioaccumulation properties of PTEs in the environment pose a serious risk to human health and environmental security [25]. Indeed, agricultural plants grown on PTEs contaminated soil accumulate contaminant which are subsequently transferred into the human food chain [26]. The most common PTEs for consideration are heavy metals (HMs) and metalloid [27]. Due to its non-biodegradable properties, HMs cannot be degraded, and can be retained in different oxidation state in soil [28]. HMs are non-degradable by any biological or physical process and persist in the soil for long time, which pose a long-term risk for the environment [29].

Several elements, classified as HMs, such as Cu, Mn, Zn, and Fe, are micronutrients for plants, yet excessive quantity of those can reduce productivity by altering and hindering the naturally present microbial communities in the soil [30]. Additionally, As, Cu, Cd, Cr, Ni, Pb, Zn, and Hg, has been designated as priority contaminants by the USEPA [31].

Due to their mutual sources, PAHs and HMs co-exist in numerous contaminated soils [32,33]. It has been estimated globally, over 10 million contaminated soils exist, including

100.000 and 80.000 sites in the United States and in Europe, respectively [34]. As reported by United States Environmental Protection Agency (USEPA) [35], about 30% of contaminated sites in the US contain a single contaminant (e.g., HMs, PAHs, or polychlorobiphenyls), while about 70% are characterized by the co-presence of more types of contaminants. Total area of contaminated lands is about $3 \cdot 10^6$ ha and raises at a rate of about 46,700 ha annually in China [36]. Among the contaminated sites, about 12 and 4 % are contaminated by mixed HMs and PAHs in the US and Canada, respectively [37]. In this context coal consumption has been identified as the main source of PAHs and HMs pollution [33,38].

Traditional physical-chemical and thermal techniques to treat the co-contaminated soil by PAHs and PTEs, such as excavation, ion exchange, chemical extraction, surfactants washing, and incineration are onerous, energy-consuming, and could cause secondary pollution, which strongly limit their large-scale utilization [39]. Indeed, the estimated cost for the application of soil flushing, excavation and incineration resulted between \$150 – \$250, \$270–\$460 and \$914–\$1540 per ton of treated soil, respectively [40]. Principle and drawbacks of conventional treatment methods are reported in the Table 0.1.

Table 0.1 Conventional techniques commonly used to remediate contaminated soil by PAHs and PTEs

Method	Technique	Principles	Drawbacks
Physical	Excavation Fixation Soil replacement Capping	Reversible transformations which do not involve the transformation and removal of PAHs and PTEs	Temporary solution, applicable to small, serious-contamination sites
Physical/chemical	Precipitation Stabilization Oxido-reduction Ion exchange Immobilization Electro-kinetic	Sequence of operations which involve the chemical transformation of PAHs and PTEs	Output current to be treated; extraction agents cost
Thermal	Incineration Vitrification	Modification of the mechanical and/or	Energy consumption, high

	physical/chemical properties of PAHs and HMs through temperature	cost, modification of soil structure

In contrast, biological treatment which involve the use of hyperaccumulator plants and microorganisms in the soil, through volatilization, degradation, stabilization and translocation, are promising and gained the global attention [41]. Among biological treatments, phytoremediation refers to the remediation process mediated by the plants and associated living-organisms in the portion of soil closed to the roots system [42].

Phytoremediation technology can be applied to remediate co-contaminated soil with PTEs and PAHs [43]. For instance, Zhang et al. [44], demonstrated that co-planting with *Lolium perenne*, *Agrostis stolonifera*, and *Elymus dahuricus*, could promote the simultaneously phenanthrene (PHE) degradation and copper uptake, from contaminated soil. Also, Tao et al. [45], obtained a benzo(a) pyrene (B[a]P) removal of 82% and Cd phytoextraction of about 20% after the application of *Sedum alfredii* and saponin for 90 days. Song et al. [46], found that about 65% of ∑PAHs and 41% of Cd were removed after 150 days treatment with Bermuda grass (*Cynodon dactylon*), compared to 38% and 15% removed in the unplanted controls. The outcome of phytoremediation treatment depends on several factors (i.e., plant species, root conformation, environmental condition), along with biological and physical-chemical properties of the soil matrix [47]. For instance, soil organic matter (SOM) can significantly influence the bioavailability and speciation of PAHs and HMs respectively and thus the phytoremediation effectiveness [48].

Moreover, over the last decades, the interest on studying the impact of soil organic and inorganic amendments (e.g., biochar, compost, organic acid, surfactants, red mud and vermiculite) on PTEs mobility and PAHs bio-accessibility in contaminated soils, has greatly increased [49–51].

Plants used in the phytoremediation process are able to bioaccumulate PTEs in their tissues depending on the ability of the plant to uptake the contaminants, and other influencing factors [52]. This result in a drawback of phytoextraction associated with the production of waste biomass which is identified as a secondary pollutant due to its PTEs content [53]. Consequently, integrating phytoremediation with plant biomass valorization and elements recovery are promising alternatives to the direct biomass disposal [40,54]. The new alternatives include thermochemical (e.g., incineration, gasification, and pyrolysis) and biochemical (e.g., fermentation and anaerobic digestion) processes [55].

This Ph.D. thesis is composed of three chapters including a preliminary bibliographic study. Various aspects of PAHs have been addressed in a published review, which is also a later chapter in this thesis. An in-depth look at PTEs and their interaction with PAHs besides the post-biomass valorization in phytoremediation processes have also been reported as further subsections of the introduction.

The structure of the present thesis is reported as follows:

- Subsections of the introduction examine the phytoremediation of PAH and PTE contaminated soils focusing on the critical issues involved in the application of the technique;
- chapter 1 is an overview of the literature, focusing on the of phytoremediation of PYR–contaminated soils and the knowledge of the key factors influencing the process to shed light on what scientific literature lacks;
- chapter 2 investigate the performance of *C. sativa* L., for remediating a PYR contaminated soil, here used as the model PAH compound, at different initial PYR concentrations by assessing the extractable concentration, the bioavailable fraction and enzymatic activity. Microbiological analysis has been performed at this stage;
- chapter 3 investigates the phytoremediation by *C. sativa* L. of PAHs (i.e., PYR and PHE) and copper contaminated soil. A preliminary economic evaluation has been performed with the aim to provide information about the phytoremediation feasibility.

  A conclusion section highlights the main findings of this doctoral thesis and discusses their relevance to existing literature. Recommendations for future studies are expressed in this chapter.

I.1 Phytoremediation of PAHs and HMs contaminated soils

The usage of plants to remediate the PAHs and HMs contaminated soil has been widely employed as a sustainable, cost-effective and eco-friendly technology [56]. The phytoremediation treatment involves several mechanisms by which plants can affect HMs and PAHs in soil such as phytoextraction, rhizofiltration, phytovolatilization, phytostabilization, phytodegradation and rhizodegradation [57].

Since HMs are non-degradable by physical and biological processes, numerous studies reported hyperaccumulating and non-hyperaccumulating plants have the ability to uptake and accumulate HMs, in their tissues [58].

Hyperaccumulator refers to plant species which have the capability to remove and translocate HMs from soil to the plant tissues [59]. According to the criteria to screen out hyperaccumulators species: bioconcentration factor (BF), that is the shoot-to-soil ratio of element concentration, is > 1 and the translocation factor (TF), the shoot-to-root ratio of element concentration is > 1 [60]. Hyperaccumulators have the capability to uptake 100 times more metal concentration than a common non-accumulating specie via plant membrane cellular proteins, up to about 1–2% of the total dry weight [61–63].

For instance, in At et al. [64], *Pteris vittata* L. accumulated Zn into leaves, with a maximum of 737 mg kg^{-1} under field conditions for 105 d. Cu concentration up to about 1800 mg kg^{-1} DW in *Brassica napus* L. with citric acid addition was reported by Zaheer et al. [65].

Also, PAHs can accumulate in plant tissues, though, the degradation by microorganisms in the rhizosphere has been reported as the main mechanism for PAHs removal in phytoremediation applications [66,67]. According to Liao et al. [68], the accumulation of PHE and PYR of about 21 and 1 mg kg^{-1} respectively, was considerably lower compared to the total PAHs removal, in *Zea mays* L grown in 50 mg each PAHs kg^{-1} of contaminated soil. In the PAH-phytoremediation, the BF is generally between 0.001 and 0.4, resulting higher (i.e., up to 2) only in the cases of high initial soil contamination levels [69].

In this context, the co-presence of HMs and PAHs influence the outcome of the phytoremediation process [70]. Indeed, the presence of 71 mg·kg^{-1} of pyrene (PYR) enhanced Cd removal by ryegrass due to promotion of rhizospheric bacteria and plant growth acceleration according to Li et al. [71]. Table 0.1.1 shows the literature in which the HMs and PAHs contaminated soil was treated by phytoremediation process.

Table 0.1.1 Phytoremediation investigation in which PAHs and HMs were used concurrently.

Chemical	Artificially spiked soil	Plant specie	Concentration (mg/kg)	Additives	Reference
Copper Pyrene	✓	*Zea mays*	50 Cu +100 Pyr	EDTA and citric acid,	[72]
Copper Pyrene	✓	*Brassica juncea*	50 Cu + 250 Pyr 100 Cu + 250 Pyr 50 Cu + 500 Pyr 100 Cu + 500 Pyr	-	[73]
Copper Pyrene	✓	*Cynodon dactylon L* (Bermudagrass)	200 Cu + 50 Pyr 400 Cu + 50 Pyr 200 Cu + 100 Pyr 400 Cu + 100 Pyr 200 Cu + 500 Pyr 400 Cu + 500 Pyr	-	[74]
Cadmium Pyrene Phenanthrene	✓	*Acorus calamus*	10 Cd + 50 Phe + 100 Pyr 10 Cd + 25 Phe + 50 Pyr 20 Cd + 50 Phe + 100 Pyr 20 Cd + 25 Phe + 50 Pyr	-	[75]
Cadmium Σ4PAHs (Pyrene Chrysene Benzo(b)fluoranthene Benzo(k)fluoranthene)	X	*Fire phoenix*	150 Σ4PAHs + 15 Cd 200 Σ4PAHs + 15 Cd	*Mycobacterium*	[76]
Cadmium Phenanthrene Pyrene	✓	*Juncus subsecundus*	5 Cd + 50 Phe + 50 Pyr 10 Cd + 50 Phe + 50 Pyr 20 Cd + 50 Phe + 50 Pyr	-	[77]

Copper Cadmium Lead Benzo(a)pyrene	✓	*Tagetes patula*	5 B[a]P + 20 Cd 5 B[a]P + 50 Cd 5 B[a]P + 1000 Pb 5 B[a]P + 3000 Pb 5 B[a]P + 100 Cu 5 B[a]P + 500 Cu	-	[78]
Cadmium Σ3PAHs (Phenanthrene, pyrene, anthracene)	✓ PAHs X Cd	*Sedum alfredii*	5,53 Cd + 250 Σ3PAHs	-	[79]
Cadmium Phenanthrene Anthracene Pyrene	✓ PAHs X Cd	*Sedum alfredii*	0.59 Cd + 174 Phe + 184 Pyr +180 Ant	Pig manure vermicompost	[79]
Copper Cadmium Lead Pyrene	✓	*Festuca arundinacea*	100 Pyr + 100 Cu 100 Pyr + 500 Cu 100 Pyr + 100 Cd 100 Pyr + 500 Cd 100 Pyr + 500 Pb 100 Pyr + 2000 Pb	-	[80]
Pyrene Nickel	✓	*Scirpus triqueter*	100 Pyr + 300 Ni	Plant-growth-promoting-bacteria	[81]
Pyrene Nickel	✓	*Sorghum sudanense (Piper) Stapf.*	200 Ni + 50 Pyr	Nitrilotriacetic acid and tea saponin	[82]
Pyrene Lead	✓	*Perennial ryegrass*	107 Pyr + 238 Pb	Alkyl polyglucoside, citric acid, and nitrilotriacetic acid	[83]
Pyrene Cadmium	✓	Willow	5 Cd + 15 Pyr	Urea	[84]

Pyrene Nickel	✓	*Bidens pilosa L.*	350 Pyr + 450 Ni	Polyaspartic acid (PASP), aminotriacetic acid (NTA)	[85]
Benzo[a]pyrene Chromium	✓	*Zea mays L.*	50 Cr + 1 B[a]P 50 Cr + 5 B[a]P 50 Cr + 10 B[a]P 100 Cr + 1 B[a]P 100 Cr + 5 B[a]P 100 Cr + 10 B[a]P	-	[86]
Pyrene Nickel	✓	*Scirpus triqueter*	357 Pyr + 296 Ni	*Bacillus subtilis*, nitrilotriacetic acid and alkyl polyglucoside	[87]
Pyrene Lead	✓	*Scirpus triqueter*	184 Pyr + 454 Pb	*Alkyl polyglucoside and nitrilotriacetic acid*	[88]
Cadmium Pyrene	✓	*Oudemansiella radicata*	5 Cd + 200/400 Pyr 15 Cd + 200/400 Pyr 30 Cd + 200/400 Pyr	-	[89]
Phenanthrene Cadmium	X	Willow	8.9 Phe + 7.1 Cd	*EDTA-ethyl lactate*	[90]
Naphthalene Phenanthrene Fluoranthene Chrysene Lead Zinc	✓	*Helianthus annuus*	20 Nap 20 Phe 20 Flt 7.5 Chr 500 Pb 1000 Zn	-	[91]

Pyrene Zinc		*Brassica Juncea* *Festuca Arundinacea*		-	[92]
Cadmium Lead Zinc ∑16PAHs	✓	*Medicago sativa L.* *Solanum nigrum L.*	Cd 8.6 and 6.5 Pb 441.4 and 201.6 Zn 326.5 and 153.1 ∑16PAHs 22.0 and 18.4	*Amendments and microbial agents*	[93]
Cadmium PAHs		*Xanthium sibiricum*		-	[94]
Cadmium PAHs		*Solanum Nigrum L.*		EDTA, cysteine, salicylic acid and Tween 80	[30]
Copper Lead Zinc PAHs (creosote)	X Copper X Lead X Zinc ✓ PAHs	*Festuca arundinacea* *Schreb* *Brassica juncea L.* *Salix viminalis L.*	Cu 1760 Pb 6840 Zn 3560 PAHs 154	-	[95]

2

I.2 Heavy Metals bioavailability

Since only a little amount of HMs content in the soil exist as soluble compounds available for bioaccumulation, increasing HMs bioavailability could be a strategy to improve the efficiency of phytoextraction [96]. Bioavailability refers to the portion of HM available for incorporation into biota [97]. Heavy metals/metalloids, according to their bioavailability can be classified as follows, as reported by Prasad et al. [98]:

(i) Readily bioavailable (i.e., Cd, Ni, Zn, As, Se, Cu);
(ii) Moderately bioavailable (i.e., Co, Mn, Fe),
(iii) At least bioavailable (i.e., Pb, Cr)

HMs bioavailability in soil is governed by intrinsic compound solubility and soil physical-chemical properties, which directly affect the binding of HMs to soil particles [99]. Different factors, including the presence of chelating agents, pH, redox potential and microbial activity, have shown large impact on solubility and bioavailability of HMs in soil [100].

I.2.1 Impact of plants on HMs bioavailability

Plants, during the phytoremediation process, could significantly alter the solubility and bioavailability of HMs as root exudates, thus acidifying the rhizosphere zone by lowering soil pH, could promote the desorption of HMs from insoluble complexes to free ion form [101]. Plant roots can also secrete metal-mobilizing compounds in the rhizosphere, for instance phytosiderophores, organic acids, and carboxylates which can affect the HMs solubility, mobility and bioavailability [102,103].

Additionally, living organisms into the rhizosphere can release enzymes and chelating agents, which lead to the formation of HM–chelate complexes, thus improving heavy metal uptake and translocation significantly increase heavy metal availability and uptake by plants [104]. These microorganisms (e.g., plant growth-promoting rhizobacteria) could influence soil physical properties such as soil structure and biochemical properties such as pH, soil redox state, soil enzymes that influence the solubility and bioavailability of heavy metals [105].

I.3 Effect of additives on HMs phytoremediation

Endophytic bacteria can secrete siderophores, organic acid and mobilizing HMs phosphate, which can increase the HMs bioavailability to rhizobacteria and plants [106]. Consequently, inoculating the host plant with plant growth promoting bacteria can improve the efficiency of phytoremediation of soil contaminated by HMs [107]. An increased *Brassica juncea* biomass production of 12.0%–17.4% and a higher vanadium accumulation up to 32.0% more, were achieved inoculating with endophytes *Serratia* PRE01 and *Arthrobacter* PRE05 by Wang et al. [108]. *Micrococcus sp*. TISTR2221 increased the root length and the toal dry biomass of *Z. mays* L. in cadmium-contaminated soils [109]. On the contrary, according to Mendoza-Hernández et al. [110], inoculating with *Enterobacter MC156* resulted in the reduction of the bioaccumulation of metals (e.g., Cu, Al, Ni and Pb) in *Brassica juncea* probably due to metals phytostabilization phenomena. Chelate-assisted phytoremediation involve the use of chemical or organic agents which can form a complex with the metal ion and consequently promote HMs bioavailability and the bioaccumulation by plants [111]. These chelating agents, such as ethylene diamine tetraeacetic acid (EDTA) and ethylenediaminedisuccinic acid (EDDS), although effective in enhancing the phytoremediation treatment, are non-biodegradable, persistent, potentially phytotoxic and pose risk for metal leaching dispersion into the environment [112]. On the contrary, organic chelating agents, such as organic acid (e.g., citrate, tartaric and oxalic acid), are readily biodegradable in soil, which will be promising for chelate-assisted phytoextraction[113]. The microbe-assisted phytoremediation is an emerging technology for phytoremediation, which pose a low to the environment compared to chemical-assisted phytoremediation, and it consist in the augmentation of the biological system of plants capable of removing HM [114].

I.4 Biomass post-process treatment

Regarding the developed biomass for phytoremediation applications in contaminated soils, which main contain high amount of contaminants, different biomass post-treatments have been proposed [115]. Since, the phytoremediation involves the accumulation of contaminants in the plant tissues, the contaminated bio-waste, if unproperly managed, could easily return to the environments generating a secondary pollution source [116]. The management of the developed biomass plays a key role in the whole sustainability of phytoremediation projects [117]. The treatments of contaminated biomass can be classified as reported by Liu et al. [118] in:

- Thermal (e.g., incineration, pyrolysis, gasification, hydrothermal modification);
- Biological (e.g., microbial stabilization, fermentation and humification);
- Chemical/physical (e.g., compression landfill and synthesis of nanomaterials).

Organic substances such as lignin, hemicellulose and cellulose confer to the biomass produced during phytoremediation a high calorific value, which, when thermochemically treated, are transformed into solids (i.e., ash, biochar), liquids (i.e., oil) or gases (i.e., syngas, biogas) [119]. The HMs distribution after the thermal treatment in the obtained products will decide their future use as compost, catalyst material or the additional operation to separate the contaminant fraction [55]. If the HMs content result under the acceptable limits, composting could be implied for the biochemical conversion of the biomass produced and eventually become an amendment for the phytoremediation site itself [120]. On the other hand, anaerobic digestion is promising due to the possibility of biogas production in anaerobic fermentation [121]. In this contest, according to Tian & Zhang [122], up to 1 $mg \cdot L^{-1}$ of Cd in the feedstock does not show an inhibitory effect on the cumulative biogas yields. Pretreatments to increase production efficiency have been proposed in this area [123].

CHAPTER 1. PHYTOREMEDIATION OF A PYRENE-CONTAMINATED SOIL: A CRITICAL REVIEW OF THE KEY FACTORS AFFECTING THE FATE OF PYRENE

1.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread as the result of human-related and natural activities, having both pyrogenic (combustion) or petrogenic (originating from petroleum) origins [124]. Gas expelled from volcanoes and wildfires represent the main natural sources of PAHs, while fossil fuel combustion in vehicles, coking plants and industrial productive activities are the anthropogenic sources that mostly increase the quantity of PAHs in the environmental matrices. PAHs can be additionally formed during the slow transformation of complex organic molecules in soil [125], or during industrial thermal processes and, as a consequence, petroleum, natural gas, and bio-gas are rich in these compounds [126]. PAHs belong to the group of persistent organics pollutants (POPs) and consist of two or more fused aromatic rings organized in a linear, angular or a cluster structure. In general, low-molecular-weight PAHs with less than 4 rings are easier to degrade than high-molecular-weight PAHs, which are particularly recalcitrant to chemical and biological degradation [127–129]. Humans can be exposed to PAHs contamination through different ways such as ingestion, inhalation, or skin adsorption, with the ingestion of contaminated food being the predominant path of exposure [130]. Although more than a hundred PAHs exists, the United States Environmental Protection Agency (USEPA) listed sixteen compounds of priority concern, based upon their toxicity, easier exposure, and persistency in the contaminated sites [131]. In the last decades, this list of the 16 PAHs has played a significant role mainly for environmental science and policy issues, but questions are recently opened on the update of the environmental regulatory system based on recent toxicology studies, possibly extending the classification to additional PAHs in the future [132,133]. Among PAHs, pyrene (4 fused rings) has gained attention as a result of its high toxicity, mutability, ubiquitous presence in all environmental matrices, high persistence and recalcitrance to biodegradation [134]. Particularly related to the subject of this article, recent research has shown a negative influence of pyrene on plant life cycle in function of the global increased temperature, posing the problem of a possible future aggravation of the risk caused by pyrene in the environment due to climate change [135]. Pyrene is repeatedly found in lands intended for agriculture as the predominant PAH due to the industrial practices described above,

urgently demanding for the implementation of remediation technologies [136,137]. The success of these technologies is intimately correlated to some factors (e.g. pH, site temperature, bioavailability), thus knowing the environmental behavior of pyrene is crucial to assess the efficiency and select the most suited remediation technique. Apart from the pyrene concentration in soil and the additional variables cited above, the environmental and ecotoxicological properties of pyrene strongly depend on the content of natural organic matter in soil [125]. Indeed, hydrophobicity and bioaccumulating characteristics make pyrene highly prone to be adsorbed onto suspended particulates and biota, and accumulated in soil and sediments [138,139].

Despite the existence of different thermal and chemical methods to remediate pyrene-polluted soils, such as incineration, solvent extraction and oxidation by chemical agents, green biological technologies offer several advantages [140]. For instance, phytoremediation has been successfully applied due to its cost-effectiveness, the possibility to remediate pollutants from different environmental compartments [141] and the eco-friendly interaction with the surrounding environment [142]. Although phytoremediation is a consolidate technology, current knowledge needs a review that includes a critical approach about the emerging phytoremediation results for the removal of pyrene from contaminated sites [143,144] and the main mechanisms occurring between the contaminant and the plant. Also, the use of soil amendments requires an in-depth analysis as a strategy to enhance the phytoremediation of pyrene-contaminated sites in terms of both removal percentage and rate. Hence, this study aims to provide a guidance for future research on pyrene removal from soils through phytoremediation and to boost the development of new methods capable to improve the efficiency of the process.

1.2 Pyrene: properties, distribution, and removal

Pyrene, $C_{16}H_{10}$, is composed of 4 fused rings assembled in a flat aromatic structure (IUPAC 1998). Pyrene belongs to group 4 in the classification for carcinogenicity provided by International Agency Research on Cancer (IARC), meaning that the agent (mixture or circumstances) does not show evidence for carcinogenic induced effects to humans. In contrast, studies report that pyrene can meet transformation processes and be transformed into more hazardous compounds such as benzo(a)pyrene, which belongs to group 1, i.e. those substances certainly carcinogenic to humans [145]. Pyrene shows a strong photo-mutagenic behavior, meaning that pyrene can adsorb sunlight radiation and be transformed or cause toxic reactions [146]. The main properties of pyrene are summarized in Table 1.1.

Table 1.1 Chemical and physical properties of pyrene

Property	Description/value	References
Color/form	Colorless solid	[147]

Average molecular mass Monoisotopic mass	202.26 g/mol 202.078 g/mol	[148]
Melting point Boiling point	150.62 °C 394 °C	[149]
Solubility in water at 25 °C	0.135 mg/L	[150]
Octanol-water partition coefficient Log (K_{ow})	5.18	[148]
Octanol-air partition coefficient Log (K_{oa})	8.61	[151]
Gas-hexadecane Ostwald solubility coefficients (log L_h)	8.72	[152]
Henry's law constant at 298.15 K	92 mol/kg·bar	[153]

1.3 Environmental presence and fate of pyrene

Pyrene is often monitored as an indicator of the amount of PAHs in contaminated soils and sediments [154]. Monitoring studies demonstrate the recurrence of pyrene in a wide variety of soils from different regions in all continents worldwide ([155–157]. The origin of PAHs pollution is often estimated through the PAH isomer ratio [158]. For instance, an anthracene to phenanthrene (Ant/Phe) ratio over 0.10 indicates a pyrogenic origin. A fluoranthene to pyrene (Flu/Pyr) ratio indicates a petroleum origin when below 0.4. PAHs derive from the burning of liquid fossil fuels or vegetable waste and coal when the Flu/Pyr ratio is between 0.4-0.5 or > 0.5, respectively [159,160].

A correlation was found between the high presence of pyrene in soils and the low air temperature [161]. A direct proportionality was also observed between increased pyrene concentrations in soil and the total organic carbon (TOC) content, in favor of the hypothesis that high natural organic matter concentrations in soil can influence the retention and mobility of pyrene [162]. Further monitoring studies are reported in Table 1.2, from which extensive burning of biomasses and non-renewable resources are the primary causes of pyrene distribution in soil.

Table 1.2 Monitoring studies reporting the country/region, the concentration, and the major causes of pyrene presence in sites highly impacted by polycyclic aromatic hydrocarbons (PAHs) worldwide.

Site	Region	Total PAH concentration range ($mg \cdot kg^{-1}$)	Pyrene to total PAH ratio	Main source	References

Gyeonggi province (18 sites)	South Korea	19.53–672.93	17,7%	Biomass burning	[143]
Ulsan (20 sites) and Pohang (19 sites)		125.01–3106.27 51.94–8106.21	24.0%-44.6%		
Aura river, an urbanized Amazon river	Northern Brazil	2.4982-2.8658	20%	Nonrenewable resources combustion	[163]
Urbanized and countryside areas of Hong Kong	Japan	0.007–0.410	10.6% in rural areas-16% in urban soils	Biomass burning	[164]
Paddy soil from different areas	Japan	0.529-2.180	14%	Burning of agricultural waste (before mid-1970s) and fossil fuel (after amid-1970s)	[161]
Background locations	UK	0.042-11.200	90%	Long-range atmospheric transport and deposition	[165]
	Norway	0.008.6-1.050	50%		
Agricultural soil from Pearl River mouth	South China	0.0033-4.0790	Predominant	Incomplete combustion of nonrenewable resources	[166]
Agricultural soil in Shanghai	China	0.0026-0.0054	33.9%	Vegetable waste, and coal combustion	[167]
City and surrounding areas in Shanghai	China	0.442-19.7	Predominant	Combustion	[168]
Urban Soils of Xi'an	China	0.1499-5.770	42.4-72.2%	Burning combustibles in engine and coal for energy	[144]
Soil Surrounding a Coal-Fired Thermal Power Plant	India	0.00159-0.227	10.7%	Fly ash, gas from vehicles, and biomass burning	[169]
Cropping Soils	China	0.12599-0.79665	One of the predominant	Biomass, coal, and	[170]

				petroleum burning	
Agricultural Soils	China	0.25049-9.38726	Prevalent in the 60% of the total	Fossil fuel burning in plants and vehicles	[171]
Agricultural Soils of Shandong	China	0.1115-2.744.1	One of the predominant by point sources	Pyrogenic	[172]
Urban soils	East coast of Florida	1.869 0.478–1.079	From about 42 to 296 $\mu g \cdot kg^{-1}$	Pyrogenic	[173]
Background soils Urban soils in Tyumen	Russia	0.1135 0.341	Predominant together with high molecular weight-PAHs	Intensive anthropogenic activities	[174]
Polish arable soils	Poland	0.080-7.264	4–6 rings hydrocarbons were 74% of total PAHs	Pyrogenic	[175]
Residential area in Hong Kong	China	n.d.-19.5	Predominant	Traffic; improper exhausted oils disposal	[176]
Chemical Petrol-chemical Residential areas (Tarragona)	Spain	0.1495 ± 0.1072 0.2117 ± 0.2891 0.0524 ± 0.0077	20-35% 22-26% 16-23%	Vehicular traffic emissions	[177]

n.d. not detected

1.4 Physico-chemical mechanisms of pyrene removal from contaminated soils

Different techniques have been involved for pyrene removal from soil [178]. Based on reported studies, physico-chemical along with thermal processes have been effectively applied to remove pyrene from contaminated soils, although resulting in interferences with the ecosystem, high energy consumption and elevated operating costs. In particular, chemical extraction can be pursued by soil washing, which consists in the use of a washing agent capable to solubilize pyrene [179]. A nearly complete removal of pyrene (92%) has been achieved using soil washing driven by the anionic surfactant sodium dodecylbenzenesulfonate at a dosage of 10 $g \cdot l^{-1}$, but toxic by-products can be generated causing a serious damage to the environment [180]. Oxidizing agents (e.g. persulfate) have been successfully used to achieve chemical oxidation of pyrene, while a strong oxidizing compound can severely hinder the soil bacterial abundance [181,182]. Finally, thermal processes employ heat to volatilize and/or destroy the contaminant but involve elevated operating costs and can be responsible for an irreversible change in the soil

structure [183,184]. Double dielectric barrier discharge plasma was proposed to reduce soil physico-chemical changing during thermal treatment yet high energy requirement is needed [185].

Bearing in mind the need to develop non-invasive methods, innovative processes, such as nano-remediation and electrokinetic remediation, are gaining attention due to the opportunity of reducing potential damaging ecological consequences [186]. For instance, in nano-remediation applications, pyrene removal can be achieved through the use of zerovalent iron (ZVI) as a function of treatment time and nanoparticles dose [187]. Using ZVI, no specific ecotoxicity of the by-products (i.e. iron oxide) has been found [188] and the operative costs are kept low. When the aim is also to reduce the damage to the soil microflora, an alternative approach is represented by electrokinetic remediation. Through this technique, a constant voltage in an anode-cathode system is applied, allowing the removal of pyrene via charged species mobilization to electrodes [189]. Periodic polarity-reversal in electrokinetics application for pyrene removal has also been found to be beneficial for the microbial growth and, thus, pyrene biodegradation [178,190]. Hence, the first main issue related to the application of conventional techniques for pyrene removal from soils is related to the interference with the ecosystem and application site, while the second main issue is associated with the high costs required. For this reason, the application of mixed bacterial cultures, the use of transgenic systems and multi-process phytoremediation have strongly been encouraged [191–193].

1.5 Phytoremediation of pyrene contaminated soils

Phytoremediation offers different advantages against the conventional physico-chemical methods, as the latter may cause serious chemical modification of the site and interference with ongoing activities [194]. Overall, the most considerable advantages of phytoremediation deal with the possibility to concomitantly remove different contaminants, the non-interference with the ecosystem and the site, and the low operating costs. Indeed, the estimated costs for phytoremediation are about 4-5, 10-20, 10-20, 10-15 times lower than costs required for electrokinetics, soil washing with water/solubilizing substances, landfilling and vitrification, respectively, without taking into consideration the extra costs related with long term monitoring, soil excavation, recycling and transport [195].

To highlight the economic convenience of phytoremediation, it has been reported that the total costs of a two years project, including both direct and indirect costs, were equal to approximately 40 dollars per cubic meter of soil treated [196]. The cost of an integrated phytoremediation approach to restore a contaminated land has been reported to amount to 100´000 euros ha^{-1}, compared to the 1-2 million required for physical/chemical treatment in the same area [197]. In a real case study, it has been estimated that the entire cost of a thermal treatment is about 2-3 thousand US $ per cubic meter of soil [198],

which represents a considerably limiting factor in several applications. Contrariwise, the most considerable disadvantages that can limit the efficacy of phytoremediation are related to contaminant bioavailability, the concentration of co-contaminants, which can be toxic for the plant over a certain threshold, plant characteristics and potential bioaccumulation of the plants involved. Especially, this is of a great concern for agricultural soils where it is easier for the contaminants to enter the food chain and directly affect human health [199].

1.6 Mechanisms of phytoremediation aimed at removing pyrene from contaminated soils

1.6.1 Treatment time and mechanisms

Phytoremediation is a natural approach consisting in the utilization of plants to remediate contamination from different environmental compartments. This technology employs single plants or mixed crops to phytoextract or degrade contaminants from contaminated environmental compartments [200]. The time needed for a successful implementation of phytoremediation is strictly related to the plant species involved [201]. For instance, an almost complete removal of pyrene, from about 15 $mg \cdot kg^{-1}$, can be obtained after more than 100 days of treatment [202];. In other cases, longer treatment times only resulted in 60% pyrene removal from aged contaminated soils [203].

Phytoremediation mechanisms, which include extraction, degradation, volatilization, and stabilization by plants can work individually or co-occur in the environmental recovery of a soil (Fig. 1.1) [195]. Phytostabilization involves the immobilization of the contaminant in soil by mechanisms such as sorption and accumulation by roots or immobilization into the root area, preventing the migration of pyrene through different ways [204]. It has been proved that, in spite of a not complete pyrene degradation, the treatment can be beneficial to impede the translocation and spread of the contaminant, making it harmless [205]. In phytovolatilization, the contaminant is released in the air through transpiration by plant leaves. Phytovolatilization is not a significative mechanism to remove pyrene from contaminated sites, since pyrene is difficult to mobilize being tightly bound to the plant tissues due to its hydrophobic behavior [206].

Rhizodegradation is considered as the most significant mechanism for pyrene removal from soil by phytoremediation [207–210]. Rhizodegradation involves the conversion of the complex structure of pyrene molecule to simpler products by the action of living organisms in the portion of soil near the root system, the so-called rhizosphere. Pyrene is degraded by subsequent steps. At the start, the ring breaks into a main metabolite that is 4-dihidroxy-phenantrene. This is subsequently transformed into naphthol and 1,2-dihydroxynaphtalene, which are further degraded through salicylic and phthalic acids pathway [211]. Phthalic acid can be further metabolized by microorganisms during

pyrene degradation indicating that phthalic acid is not an end product during degradation of pyrene [212].

Investigating the existence and vitals of microorganisms during phytoremediation, the USEPA documented a 100-fold higher prevalence of microorganisms in rhizospheric than in non-rhizospheric soils (USEPA, 2000). Nonetheless, rhizodegradation of pyrene mainly involves the bioaccessible part of the contaminant, thus being strongly influenced by the pyrene bioavailability in soil [213], which is an important limiting factor in numerous cases.

1.6.2. Accumulation of pyrene in plant tissue and fate of contaminated plant biomass

Phytoextraction, which is referred to the extraction of contaminants through accumulation in plant tissues [214], is of interest for phytoremediation of contaminated soils by pyrene. During the growth phase, plants can accumulate the contaminant in roots, stem and leaves, and pyrene is eventually removed by harvesting the plant when the treatment is terminated [215]. The uptake of pyrene by plants from soil is often negligible since pyrene is strongly bound to soil organic particles and is not considerably dissolved in soil pore water [209]. The overall accumulation in roots is considered as the main domain for pyrene accumulation, considerably predominant comparing to the accumulation in shoots [216]. Detailed information about accumulation of pyrene in plant biomass are reported in the supporting information accompanying the manuscript (S1. Accumulation of pyrene in plant tissues).

Eventually, the contaminated plant biomass may be subjected to different side-stream processes that are generally evaluated case by case depending on the contaminant concentration in plant tissues [217]. Thermal treatment is an option, with pyrolysis being more sustainable and environmentally friendly than conventional incineration [218]. However, considering that pyrene is not at all or present at little concentrations in the plant biomass, alternatives can be considered. Kumar et al. [219] evaluated the possibility to produce bioenergy from plant biomass after phytoremediation of PAH-contaminated soils.

In addition to biological degradation processes, pyrene can possibly be stabilized through humification and polymerization reactions that lead to the incorporation of pyrene into soil organic substances or its transformation into non-solvent extractable fraction. With this aim, the treatment with plants plays a crucial role, enhancing the microbiological processes through the excretion of enzymes in soil and the phyto-availability of pyrene [220].

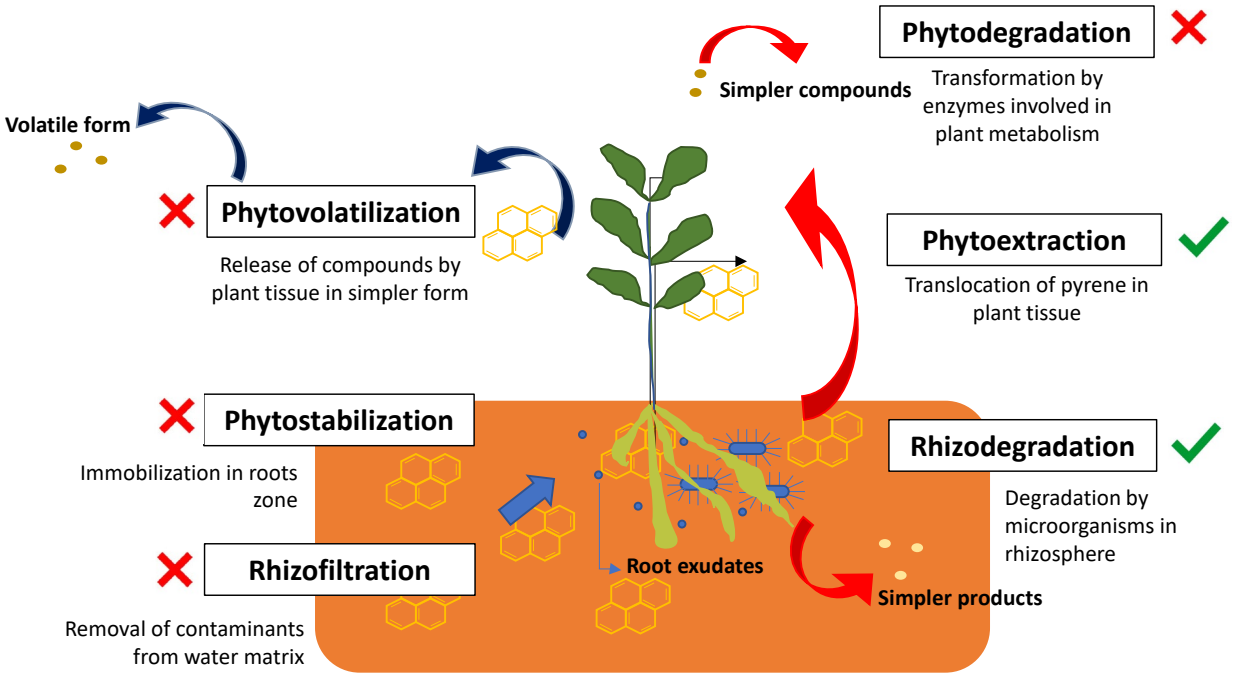


Fig. 1.1 Predominant mechanisms involved during phytoremediation aimed at removing pyrene from contaminated soils.

1.7 Markers for pyrene biodegradation in the rhizosphere

Considering the relevance assumed by synergic biodegradation through plant-microorganism symbiotic effect [221], an examination of the microbial community in soil or/and the estimation of the enzymatic activity are important markers to assess the degradation of pyrene during phytoremediation [222]. It is clear that the significance of the plant species, or plant combinations, is among the primary factors involved in pyrene removal, since these can directly affect the phytoextraction and rhizodegradation mechanisms through expanding the microbial population in soil [223,224].

1.7.1 Enzymatic evaluation

Extracellular enzymes secreted by plant besides rhizosphere microorganisms could play an important role for the degradation of pyrene [225]. Catalase (CAT), dioxygenase (DOX), peroxidase (POD), polyphenol oxidase (PPO), dehydrogenase (DHO), urease (URE), and phosphatase (PHO) are typical enzymes, which have been diffusively monitored in phytoremediation of pyrene-contaminated soils since they are directly-indirectly involved in metabolic processes including pyrene decomposition into simpler products [80,226]. The existence of these enzymes is mostly associated with the removal of pyrene in soil [227]. The function of different enzymes monitored during phytoremediation of pyrene-polluted soils is summarized in Fig. 1.2.

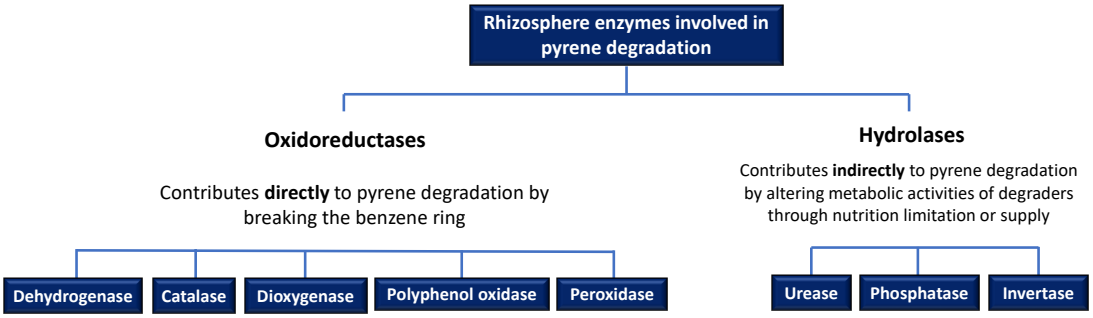


Fig. 1.2. Main enzymes monitored in the rhizosphere and their roles in pyrene degradation during phytoremediation of pyrene-contaminated soils.

The presence of enzymes in soil depends on the pyrene concentration as the suppression of living microorganisms is more evident at increasing pyrene concentrations. Due to its high stability and toxicity, pyrene can alter the life cycle of plants and soil microorganisms, often leading to a higher death rate and a consequent lower enzymatic activity [228]. Also, the proximity to the root area is another aspect affecting the enzymatic presence in a soil. Several plant species are capable to extend the action of degradative microorganisms to a large volume of soil, beyond the proximity to the roots. URE and PHO enzymes have been found in a pyrene-contaminated soil in the treatment with *Kandelia candel (L.) Druce*, also farther than the region encircling the roots. Nevertheless, URE and PHO concentrations diminished at increasing distances from the rhizosphere [208].

CAT and PPO production were promoted by the *B. maximowicziana* plant in soil with the initial pyrene concentration of approximately 10 $mg \cdot kg^{-1}$. At these concentration levels, pyrene serves as carbon source for microbial growth [229]. When pyrene concentration was increased to 121 $mg \cdot kg^{-1}$, the PPO concentration significantly decreased. The enhanced enzymatic activity involved in the degradation of pyrene in cultivated soil was responsible for the higher removal that was reached in an uncultivated soil [229]. Different from the report of Lu et al. [229], no significant changes in DHO activities was observed by Li et al. [76]. This could be ascribed to a different secretion by plant roots, as also reported by Ye et al. [211].

According to Dai et al. [230], the treatment with *Fire phoenix* species promoted the production of DHO, POD and CAT enzymes, but explicated a negative influence on PPO. The changing in the enzymatic activity produced by the plant species positively contributed to pyrene removal, being DHO, POD and CAT all oxidoreductase enzymes. Liu et al. [231] also observed a PPO decrease in the presence of pyrene and showed a negative correlation between the concentration of PPO and pyrene removal ($R^2 = 0.947$).

Targeting microorganisms, enhanced by plant interaction with soil, might be useful as a way of predicting phytoremediation efficiencies and pyrene dissipation [232,233].

1.7.2 Pyrene degraders in rhizosphere

Recently, different genera encouraged by the presence of the *Fire phoenix* plant, have been targeted as those mainly accountable for promoting pyrene degradation in the roots area ([230]. These genera are capable to use pyrene as energy and sole carbon source [234,235]. Bacteria accountable for pyrene degradation have the possibility to populate the root system of the plant, also forming a biofilm. The biofilm colonized on plant roots can promote new biomass production, reduce pyrene stress, and enhance biodegradation [236]. Most microorganisms depicted to be able to mineralize pyrene have dioxygenase genes [237]. For instance, genome investigations on *Mycobacterium* strains demonstrated two morphotypes (PO1 and PO2), which possess a complete gene for pyrene degradation [238]. Further bacterial strains and their taxonomy affiliation are reported in Table 1.3. Most bacteria reported are Gram-negative and the strains are related to different genera, belonging to the phyla of *Proteobacteria, Actinobacteria*, and *Firmicutes*.

Table 1.3 - Different bacterial species and strains capable for pyrene degradation.

Taxonomy affiliation	Bacterial strain	Reference
Actinobacteria	*Mycobacterium PO1* *Mycobacterium PO2*	[238]
	Rhodococcus sp. T1	[239]
	Mycolicibacterium sp. Pyr9	[236]
	Arthrobacter pokkalii P3B162	[234]
	Rhodococcus sp. ASDP3	[212]
Proteobacteria	*Pseudomonas sp. ASDP1* *Burkholderia sp. ASDP2*	
	Sphingobium barthaii KK22	[234]
	K. pneumoniae AWD5	[238]
	Bosea NS2 *Acinetobacter sp. NFL* *Enterobacter sp. BT*	[240]
	Achromobacter xylosoxidans PY4	[241]
	Pseudomonas aeruginosa PDB1	
	Pseudomonas fragi DBC	[221]
	Klebsiella pneumoniae AWD5 *Alcaligenes faecalis BDB4* *Hydrogenophaga PYR1*	[242]
	Roseobacter	[243]
Firmicutes	*Bacillus subtilis*	[87]
	Bacillus frigoritolerans Z2B-19	[244]
	Bacillus pumilus Bp-11	

Basidiomycota	*Basidioascus persicus EBL-C16*	[245]

1.7.3 Effect of pyrene on biomass production

Due to its toxicity, pyrene alters the primary carbon metabolism. Pyrene exercises a deleterious impact on signal transduction and primary processes implicated in plant development. These mechanisms exerted by pyrene on plants produce macroscopical and observable effects, such as reduced shoots number, height, stem diameter, and root extension [100]. Plants are capable to grow up in pyrene-polluted soils without showing apparent signs of stress. However, a significant lower biomass production is evident in comparison to the type grown up in uncontaminated soils [246]. Pyrene causes oxidative stress on plants, which are capable to implement defense mechanisms such as anatomical adaption [247]. As reaction to pyrene stress, maize raises antioxidant system but not enough when pyrene concentration exceeds 100 ppm, resulting in reduced growth variables [248]. The damaging effect of pyrene on plant development is more evident as the concentration of the contaminant increases. For instance, a significant decrease of alfalfa biomass productivity was observed when pyrene was dosed at concentrations higher than 49 $mg \cdot kg^{-1}$ [249]. Lu et al. [80] observed tall fescue (*Festuca arundinacea*) biomass similar to that grown in non-contaminated samples below a pyrene concentration of 100 $mg \cdot kg^{-1}$, while 200-500 mg of pyrene per kg of soil drastically inhibited the plant growth, with shoot and root biomass productivity being reduced by 66.9 and 46.7%, respectively. Probably, pyrene concentrations up to 150 $mg \cdot kg^{-1}$ can be tolerated by alfalfa and ryegrass and used by rhizosphere microorganisms as a source of organic carbon [250]. A more severe impact of pyrene is frequently observed on the root part rather than on the leaves [251]. Cheema et al. [252] observed that tall fescue shoots biomass (dry weight) was about 50%, while roots biomass only stood for 30% of the entire plant weight when pyrene concentration in soil exceeded 336 $mg \cdot kg^{-1}$ compared to the plant grown in uncontaminated controls. Since the accumulation in plant tissue could be a possible mechanism involved in pyrene reduction from soil, the correlation between a reduced biomass productivity and a decrease in pyrene removal potential is of interest [83].

1.8 Main factors influencing the fate of pyrene during phytoremediation

1.8.1 Pyrene bioavailability

During phytoremediation of pyrene-contaminated soils, bioavailability of pyrene is an essential key factor to consider as it represents the relevant exposure dose available for degradation by living organisms [253], but also a possible toxicity exposure for plants and associated microorganisms in soil [254]. The accessible quantity of pyrene for

biodegradation is the portion available to cross the cellular membrane of organisms [255] and is strictly related to the risk assessment of contaminated sites [256]. The interaction between the plant or the rhizosphere microrganisms and pyrene can be hindered or enhanced as a function of pyrene bioavailability in soils [257], which in turn depends on the aging time of contamination. Generally, aged planted soils result in a lower pyrene bioavailability [258]. Pyrene bioavailability can be studied from two different viewpoints: chemical and biological. Chemical extraction provides information about the quantity of pyrene available for biodegradation while biological analysis offers the possibility to deeply understand the toxicity effects exerted on living species [259].

1.8.2 Aging of contamination

Fresh spiked soils and soils with aged pyrene contamination may show differences in terms of phytoremediation efficiency. Pyrene loss through mechanisms not connected to plants can more easily occur in freshly spiked soils [260]. Chigbo & Batty [261] observed inhibition of plant biomass growth in freshly spiked soils in comparison to the aged soils, confirming the higher toxicity effect of the contaminant towards *Brassica Juncea*. As the aging process proceeds, pyrene is less prone to be degraded by living species due to the movement and incorporation in the soil matrix via physico-chemical and biological interactions, including sorption/desorption onto the soil organic matter (SOM) [262]. Experimental tests show how the extractable pyrene fraction quickly decreases over the first 15 days [263]. On the contrary, the aging process lasts for several years in real scenarios resulting in a stronger interaction between pyrene and soil media [264].

Differences may emerge due to the variety of the pyrene-degrading community in aged soils, which has adapted for longer to different environmental conditions than in freshly spiked soils [265]. Indeed, microorganisms developed in long-term pyrene contamination show a better acclimatization, lowering their sensitivity to pyrene concentrations [266]. The use of *Potamogeton crispus L.* improved pyrene degradation in aged sediments more than in freshly spiked soil. Planting resulted in more enhanced bioavailability (+73.9%) and biodegradation activity (+277%) of pyrene as compared to unaged sediments (13.1% and 150%), respectively, during 36 days-experiments [267]. Such variances highlight the issue associated with real field conditions, which can considerably differ from greenhouse experiments, and the application of real environmental samples to validate the effectiveness of phytoremediation treatment.

1.8.3 Presence of organic matter in soil

Pyrene can be present in soil as a free substance, or weakly or strongly adsorbed and/or combined to SOM. Pyrene tends to interact with non-aqueous phases and SOM becoming potentially inaccessible for microbial degradation, since microorganisms are known to degrade compounds in a soluble form [268]. Furthermore, pyrene sorption under high SOM conditions is strong and nearly instantaneous [269]. Conversely, dissolved organic

matter (DOM) is the part of SOM that may positively contribute to increase pyrene bioavailability [270]. Also, SOM is an essential source of energy to promote the growth of the microbial community in soil [271]. In addition to SOM amount, sorption, degradability via phytoremediation and mobility of pyrene critically vary depending on the type of carbonaceous materials within the soil matrix, as described elsewhere [272].Chefetz et al. [273] reported the significance of both aliphatic and aromatic moieties of organic matter in sorption of pyrene. Also, the age of SOM influences the type of organics present and, thus, pyrene distribution according to C-normalized distribution coefficients (K_{oc}) [274]. Therefore, no significant alfalfa phytoremediation enhancement was observed in aged soil contamination with high SOM content (8.51%), being the latter the predominant factor limiting the process [275].

A strong dependence between the SOM content and the phytoremediation mechanisms implicated in pyrene removal has been observed. Degradation of pyrene by *P. crispus* decreases with increasing SOM content in pyrene contaminated sediments [276]. In particular, under high SOM (6.3%) conditions, adsorption or covalent binding of pyrene to SOM are the dominant forces for pyrene retention in soil, also limiting pyrene availability [277]. In contrast, Sun et al. [129] evaluated the degradation of pyrene under low SOM (3.75 $g \cdot kg^{-1}$) conditions by the incidence of ryegrass *(Lolium perenne),* which contributed to pyrene degradation by 21.3% of the total degradation. A great amount of abiotic loss occurred, contributing for the 57.2% to the total pyrene degradation, probably due to aerobic conditions and sunlight exposure that increased photodegradation and volatilization processes.

1.8.4 Environmental factors

Pyrene-degrading microorganisms have shown a higher efficiency in terms of pyrene degradation when temperature ranges between 25 and 42 °C. At increasing temperatures, pyrene is more bioavailable and, thus, more biodegradable, however posing risks for a higher pyrene release into the environment [278]. With regard to pH, values out of the interval 5.0-8.0 can result in the inhibition of the biological activity of pyrene degraders [236]. Indeed, in a recent investigation, Yang et al. [279] observed that the optimal pH range for the vital functions of *Mycolicibacterium* sp. Pyr9 was 7-8, with a high pyrene removal efficiency also at a pH up to 9. When pH was risen above pH 10 or decreased below pH 5, the activity of *Mycolicibacterium* sp. Pyr9 was clearly inhibited.

The presence of pyrene in soil throughout conditions of oxygen deficit during biotransformation of organic mixture may suggest that aeration promoted by plants roots can definitely make a significant contribution to the improvement of pyrene degradation by rhizosphere-living microorganisms [280]. Hence, a positive association was found between the redox potential and pyrene degradation in pyrene contaminated sediment [267,281]. Soil oxygenation is directly related to the incidence of pore water, which is responsible to redirect the microbial population but even the accumulation of pyrene in

plant tissues via roots uptake and, consequently, the translocation in the aerial part [282]. Furthermore, the presence of organic and inorganic nutrients in soil can enhance microbial growth and plant expansion, thus promoting pyrene removal [145].

1.8.5 Plant species and/or mixed crops

The potential utilization of certain plant species for phytoremediation of soils contaminated by pyrene are thoroughly related to plant morphology, the possibility to accumulate the contaminant in its different portions and biochemical interactions in rhizosphere, which have the capability to expand or limit the degrading microbial community in that region [210,283]. Besides the utilization of a single species cultivation, employing mixed crops is promising as an improvement of the population of pyrene degraders can be obtained [224,284–286]. Indeed, the remediation efficiency of a pyrene-contaminated soil in the presence of both alfalfa and rape was up to 11.03 and 16.29% higher than only using single alfalfa and rape, respectively, after 98 days of treatment [285]. Grasses *(Poaceae)* and legumes *(Fabaceae)* showed evidence for pyrene removal due to the composition of root exudates, being effective on stimulating the activity of the rhizospheric microbial population [287].

According to the carbon fixation pathway employed, plants are classifiable in different groups, such as C3 and C4 [288]. Comparing the effectiveness of various plant species, higher performance in terms of pyrene removal was observed in C4 species (e.g. sudan grass, vetiver, maize) compared to C3 species (e.g. cowpea, wallaby grass), whilst C3 and C4 plant species have been shown similar accumulation potentials in shoots and roots [246]. However, the overall phytoremediation efficiency is greater with C4 than C3 plants, indicating that a different content of organic acids and sugars in roots of C4 plants could promote pyrene degradation [289].

1.8.6 Co-presence of potential toxic elements

The co-existence of potential toxic elements (PTEs) with pyrene is widespread as the result of a broad range of anthropic practices [290]. Co-contamination with PTEs is considered a more serious environmental issue since the incidence of two or more components may induce harmful effects on plants and related microorganisms in rhizosphere, thus affecting the remediation process [291]. The competition with PTEs can even limit the adsorption processes of pyrene to the lipophilic substances in soil [292]. Likewise, different investigations showed that the co-existence of PTEs may hinder the activity of microorganisms accountable for the degradation of pyrene resulting in a reduced treatment efficiency [78].

The stress due to the presence of copper (Cu) up to a concentration of 20 $mg \cdot kg^{-1}$ was not considered responsible for affecting pyrene removal by *Juncus subsecundus* after 70 days of treatment [77], whereas Cu negatively impacted pyrene removal in a soil planted with *Brassica juncea* when Cu was present at a concentration of 100 $mg \cdot kg^{-1}$ over 60

days of plant trial [73]. In such cases, Chigbo et al.[72] suggested that a combined treatment with chelators could decrease the inhibiting effects of PTEs and concomitantly improve the phytoextraction and degradation of pyrene. Co-existence of Cd could even influence the translocation factor of pyrene in *Zea mays* [293]. Increasing levels of Cd up to 150 $mg \cdot kg^{-1}$ led to a lower pyrene removal rate by *Sedum alfredii* [145]. The removal rate of pyrene was negatively impacted by co-existence of Cd due to a high Cd toxicity to soil enzymes [227]. Zhang et al. [294] observed that pyrene was removed at a lower extent in a nickel (Ni)/pyrene co-contaminated soil than in a soil polluted with the sole Ni. The translocation factor was affected by a lead (Pb)/pyrene synergistic action in the co-occurrence of 300 and 30 $mg \cdot kg^{-1}$ of Pb and pyrene, respectively. The presence of Pb doubled the accumulation of pyrene in ryegrass roots [295]. Also, the pyrene removal efficiency increased from $59.1 \pm 2.1\%$ to $68.7 \pm 2.3\%$ in pyrene- and Pb/pyrene-amended soils due to a shift in the rhizospheric community and a higher abundance of microorganisms including genes capable to mineralize pyrene ([296]. Other studies also suggest that the use of chelators can increase the efficiency of phytoremediation of soils co-contaminated with pyrene and PAHs [297].

1.9 Additives used to strengthen phytoremediation of pyrene-contaminated soils

The performance of phytoremediation of pyrene-contaminated soils can be improved by adding compounds (e.g. nutrients, chelating agents, bacterial growth stimulators) or seeding rhizospheric and endophytic bacteria to soil [298,299]. As illustrated in Fig. 1.3, the aim is to improve the quality of the soil treated by increasing the bioaccessibility of the contaminant, the biomass growth and the biological degradation, thus the success of the treatment [300,301].

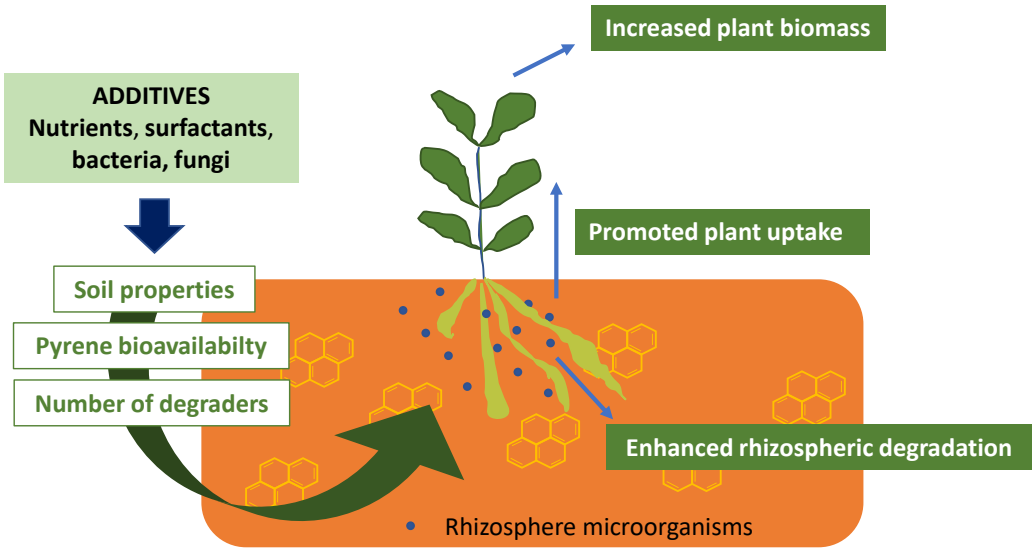


Figure 1.3 Main effects produced by the supplementation of additives in phytoremediation of pyrene-contaminated soils.

1.9.1. Soil amendments

A list of the major amendments used for stimulating phytoremediation of pyrene-containing soils is reported in Table 1.4. Pyrene degradation has been reported to improve in the presence of most amendments. The results suggest that different amendments stimulate plant growth, phytoextraction and enhance soil microbial degradation. In particular, biodegradation of pyrene can be highly stimulated by adding organic compounds, which can redirect the microbial community towards pyrene degradation [302], mitigate the damaging effects on plants produced by pyrene [303], and limit the harmful effects of pyrene contamination through adsorption onto the aromatic part of the molecule, consequently improving plant biomass [304]. Also, the discharge of pyrene from amendment particles or molecules may improve pyrene bioavailability [305,306]. For instance, stress and damage induced on *Scirpus triqueter L.* by the co-existence of Pb and pyrene was reduced dosing 50 $mg \cdot L^{-1}$ of organic acids (glutaric acid, citric acid (CA) and succinic acid) in planted soil [307]. Increased bioavailability of pyrene and interaction with roots and soil bacteria due to compost supplementation promoted pyrene removal by alfalfa and ryegrass [145]. Besides promoting rhizospheric biodegradation, dosing caffeine solution could enhance phytoextraction in Cucurbita pepo *(sp. pepo cv. Gold Rush)* due to an increased pyrene solubility. This is particularly effective in aged soils where pyrene molecules are more tightly bound to soil and less available for biodegradation [301]. Precise attention must be given to the dosage of these amendments. Indeed, dosing 1 % of biochar in ryegrass planted soil was not effective for phytoremediation of pyrene-containing soils ([308], while a 3% biochar/soil mixture in ryegrass planted soil increased pyrene removal. However, a 5% biochar/soil mix inhibited biomass production [306].

Different can be the effect of the supplementation of nutrients, such as phosphorus (P) and nitrogen (N), which are essential for plant development. The addition of P has been reported to slow down pyrene removal in the treatment with ryegrass *(Lolium multiflorum Lam.)* [309]. *Fire Phoenix* was able to release substances in soil, particularly associated to available phosphorus, which led to approximately 80% of pyrene removal later than 150 days in a pot cultivation experiment [310]. The supplement of a high dose of inorganic N through N-based fertilizers with urea at a pyrene-urea ratio of 4.5:1 (in pots cultivated with bermudagrass *Cynodon dactylon L.*) improved the performance of the treatment compared to unplanted controls, due to an increased population of pyrene degraders and a higher production of biosurfactants and root exudates from the microbe/plant consortium [311]. Urea addition, aimed to achieve a final N concentration in soil of 1.29 $g \cdot kg^{-1}$, did not improve the pyrene removal rate but accumulation in willow tissues decreased at the end of the treatment [84]. An improved phytoremediation efficiency with *Koelreuteria paniculata* has been attributed otherwise to the total N increase, naturally released by the plant [312]. Mineral nitrogen in *Medicago sativa L.*

roots could promote soil microorganisms including pyrene degrading bacteria activity [313].

1.9.2 Effect of surfactants

Surfactants have both a hydrophobic tail and a hydrophilic head and are classifiable in anionic, cationic, non-ionic, and zwitterionic as a function of the charge of the polar head group [314]. The provision of surfactants in phytoremediation of pyrene-contaminated soils is an encouraging method due to the effectiveness of surfactants in enhancing biodegradation and mass transfer along with promoting the transformation of pyrene from bound to bioavailable fractions [136]. Indeed, results show that adding surfactants can improve pyrene phytoremediation treatment. Most of the surfactants proposed are natural non-ionic surfactants, which do not exert high toxicity to plants at certain dosage. It is well known that microbial degradation principally occurs in the dissolved phase and is limited by mass-transfer processes. Thus, the supplementation of surfactants enhances pyrene desorption from the solid to the liquid phase besides what achieved through phytoextraction and the activity of pyrene degrading bacteria [136].

Adding Tween®80 at concentration lower than 13.2 $mg \cdot kg^{-1}$ enhanced pyrene uptake by ryegrass and red clover [315]. Rhamnolipid addition (25.8 $mg \cdot L^{-1}$) led to an increase of the root cells permeability, which enhanced the uptake of pyrene by ryegrass [316]. 1000 $mg \cdot kg^{-1}$ of dodecanol (Brij35) inhibited ryegrass and alfalfa growth, but at lower concentration (up to 250 $mg \cdot kg^{-1}$) significantly promoted the phytoremediation of pyrene up to 59% more than in free-surfactants control [317]. Triton X-100 did not result in an higher accumulation of pyrene in maize (*Zea mayze L.*) but enhanced the microbial community [318]. In a pyrene-Cd contaminated soil, the degradation of pyrene was promoted (up to 135% more than with no plant) by the addition of tea saponin, a green biosurfactant produced by plants [319].

The concomitant application of more than one surfactant has also been proposed. Combining sodium dodecyl benzene sulfonate and Tween®80 at concentration of 100 $mg \cdot kg^{-1}$ to ryegrass was beneficial for pyrene removal, reaching 91.4% by promoting the transition of pyrene from bound to bioavailable fraction as well as increasing the degrading bacterial population [136]. Combining surfactants sodium dodecyl benzene sulfonate (SDBS) and Tween 80 at concentration <150 $mg \cdot kg^{-1}$ was more effective than applying the single Tween 80 with the same amount [320]. Yang et al. [321] observed a higher pyrene solubilization by applying a mixture of Tween®80 and saponin rather than only Tween®80. Alkyl polyglucoside (APG) mixed with nitritotriacetic acid (NTA) had the highest performance in improving the bioaccessibility of pyrene. The activity of DHO was higher when combining the application of NTA and APG, indirectly affected by changing in the solubility of pyrene [322]. NTA and APG at 1 and 2 $g \cdot kg^{-1}$ of soil, respectively, had no significant negative impact on *Scirpus triqueter* yield and their combined application had a positive effect on pyrene degradation [88]. An increase in

phytoremediation efficiency, applying APG combined to CA and NTA, was also explained through a higher chlorophyll content in plant biomass [83].

Up to now, a little attention has been given to the leaching of pyrene after surfactants addition aimed at extracting and analyzing the supernatant to investigate the presence of pyrene, as an indicator of a potential pollution of deeper soil layers or groundwater.

1.9.3 Effect of bioaugmentation

The utilization of plant-growth regulators and simultaneous bioaugmentation of fungi and microbes capable of processing pyrene can be an alternative way to strengthen the phytoremediation of pyrene-contaminated soils [322–324]. The addition of a target microbe known for degrading the contaminant involves augmented bioremediation [279]. The bacterial strains used come from both contaminated sites and synthetic cultivation media. All the bacteria and fungi considered are endophytes and can colonize the roots without causing damage to the plant.

In this context, Rostami et al. [325] remarked that the inoculation of *Pseudomonas aeruginosa* allowed to achieve an 83% pyrene degradation in a soil planted with *Sorghum bicolor* as plant species. 10 $mg \cdot kg^{-1}$ of indole acetic acid added to the *Sorghum bicolor* cultivated soil increased roots biomass and the microbial activity in the rhizosphere [326]. The addition of the endophytic *Staphylococcus* sp. BJ06 bacterium led to a pyrene removal of 56% from an initial pyrene concentration of 50 $mg \cdot kg^{-1}$ and to a decrease of pyrene uptake of about 30 and 44% in ryegrass roots and leaves, respectively, in comparison to the non-inoculated soil [327]. Also, the supplementation of the endophytic *Serratia* sp. PW7 bacterium led to a decreased pyrene concentration in wheat tissues [151].

The same aim has the placement of fungal species in pyrene-contaminated soils during phytoremediation. The presence of fungi results in an optimized symbiotic association with the host plant and in an accelerated pyrene dissipation besides the promotion of a healthy plant growth [328]. In a 7 month experiment, two varieties of *Festuca* plant inoculated with endophytic fungi *Neotyphodium coenophialum* and *Neotyphodium uncinatum* showed a 20% of higher pyrene removal compared to non-inoculated controls [329]. The mycorrhizal- arbuscular fungus, *Glomus mosseae,* added to ryegrass enhanced the degradation of pyrene and biomass growth [330]. *Glomus mosseae* increased concentration of pyrene in maize roots, with pyrene then translocating in maize stems due to action carried out by extracellular mycelium [331]. An increased pyrene uptake by roots with a low translocation in shoots was observed with alfalfa treatment after the addition of *Glomus mosseae* and *G. etunicatum,* which also led to a higher pyrene degradation (23.4 and 47.0%, respectively) [328]. Glomalin-associated soil protein, promoted in mycorrhizal-arbuscular fungi association, contributed to high pyrene removal from soil, either promoting the pyrene transfer from soil into solution and degradation by microorganisms [332]. Finally, a higher accumulation in roots was

stimulated in *Festuca arundinacea* through the addition of endophytic fungus, *Lewi*a asp., compared to the free-inoculum treatment [333].

Table 1.4 Effect of different additives, surfactants and plant growth promoting bacteria on the phytoremediation of pyrene-contaminated soils

Plant species	Common name	Added compound	Soil basic properties	Application scale	Fate of pyrene	Duration of the experiment (days)	References
1. Additives							
Sorghum bicolor	Sorghum	Landfill leachate	OC, 0.2%; pH, 7.2	G	Removal increased by 29%	90	[298]
Lolium prenne L.	Ryegrass	Biochar	TOM, 30.5 ± 0.18 $g \cdot kg^{-1}$; pH, 7.43 ± 0.10;	G	Removal increased reaching 79% dosing biochar (3% w/t biochar/soil); 5% of biochar increased free radicals thus biomass decrease to 65%	60	[306]
Cucurbita pepo	Ssp. pepo cv. Gold Rush	Caffeine	Soil 1: carbon fraction, 0.056 ± 0.03% Standard soil: carbon fraction, 5.2 ± 1%[s]	G	Uptake increased	28	[301]
Kandelia Candel	Kandelia rheedii	Humic acid (6.7%)	OM 6%; pH 7.19 [s]	G	Reduced biomass and removal by plant	180	[334]
Medicago sativa *Lolium perenne*	Alfalfa Ryegrass	Compost (5% in mass ratio with soil)	Alluvional soil: pH, 6.71, TOC, 4.6 $mg \cdot kg^{-1}$; red soil pH, 6.06, TOC, 1.6 $mg \cdot kg^{-1}$	G	Increased removal up to 40%	56	[145]
Brassica napus *Medicago Sativa* *Lolium perenne*	Alfalfa Ryegrass	Compost (1% in mass ratio with soil)	OC, 400 $g \cdot kg^{-1}$; pH, 9.4 [s]	G	Any significant impact in improving phytoremediation	90	[305]
Lolium multiflorum Lam.	Ryegrass	P	OM, 30 $mg \cdot kg^{-1}$; pH, 6.0	FC	Slower removal by plant	175	[309]
Cynodon dactylon L.	Bermuda grass	Urea	TC, 0.97±0.02%; pH, 6.1±0.1	G	Increased degradation by plant	100	[311]

Salix	Willow	Urea	pH, 7.78, OM, 26.61 mg·kg^{-1}	G	No significant effect on removal although uptake in root and bark decreased	60	[84]
Zea. mays	Maize	EDTA and/or citric acid	Standard commercial soil [s]	G	All chelates used increased the dissipation up to 90.4 % for citric acid	60	[72]
2. *Surfactants*							
Lolium multiflorum	Ryegrass	Tea saponin	OM, 19.6 g·kg^{-1}; pH, 8.3	FC	Increased accumulation in plant tissue (mostly in roots)	40	[319]
Agropyron elongatum	Tall weathgrass	Tween®80	pH, 8.35±0.04; DOC, 44 ± 3 mg·L^{-1}; TOM, 3.2 ± 0.1%;	G	Enhanced degradation by 130%. Not influenced uptake by plant	60	[335]
Zea mays L.	Maize grain	Triton X-100; rhamnolipid; saponin	OM, 1.3%, pH, 6.54	G	Not influenced pyrene accumulation by plant. Enhanced reduction rate	90	[318]
Bidens Pilosa L.	Blackjack	Tea saponin	OM, 19,73 g·kg^{-1}	G	Not improved promotion on pyrene removal by plant	120	[85]
Lolium perenne	Perennial ryegrass	Alkyl polyglucoside, nitric acid and nitrilotriacetic acid	pH, 8.3, OM, 19.6 g·kg^{-1}	FC	Dissipation increased up to 3 times more than control with plant alone	60	[83]
Schoenoplectus triqueter *L.*	Scirpus triqueter	Alkyl polyglucoside and nitrilotriacetic acid	pH, 8.3; OM, 19.6 g·kg^{-1}	G	Increased dissipation of pyrene from about 35 to 60%	60	[336]
Sorghum sudanense (Pipe)r Stapf.	Sudan grass	Tea saponin and nitrilotriacetic acid	pH, 8.3; OM, 19.6 g·kg^{-1}	G	Accumulation in shoot increased by 20,14% (1 g·kg^{-1} addition)	42	[82]
Sorghum bicolor	Sorghum	Alkyl polyglucoside and	pH, 8.3; OM; 19.6 g·kg^{-1}	G	Increased pyrene bioaccessible fraction	60	[322]

		nitrilotriacetic acid					
Festuca arundinacea	Tall fescue	Tween®80; triton X-100	OM, 2.1 %; pH, 5.95	G	Increased plant biomass compared to unamended soil. Triton displayed the highest removal rate (from 64.4 to 79.1%)	60	[337]
Lolium multiflorum	Tagetes patula	Tea saponin	n. a.	G	Transformation of pyrene from associated to bioaccessible fraction and increased degradation ratio	40	[338]
3. Plant growth promoting bacteria (PGPB) and bioaugmentation							
Sorghum bicolor	Sorghum	*Pseudomonas aeruginosa* + indole acetic acid (IAA)	pH, 7.5; OM, 2%	G	Increased plant biomass. Increased removal rate showing the best performance with IAA	90	[325]
Jatropha curcas L.	Jatropha	*Pseudomonas aeruginosa PDB1, Pseudomonas fragi DBC, Klebsiella pneumoniae AWD5, Alcaligenes faecalis BDB4 and Acinetobacter sp. PDB4*	OC, 17.5 $g \cdot kg^{-1}$; pH, 5.58	G	Removal reached 97% in 60 d	60	[221]
Medicago sativa L.	Alfalfa	Rhizobium meliloti	TOC, 11.1; pH 6.4	FC	Increased phytoextraction and degradation	90	[339]
Triticum aestivum L.	Wheat	*Eendophytic bacterium*	n. a.	G	Accumulation decreased by 35.7-86.3% in the shoots and by 26.8–	14	[340]

		Serratia sp. PW7			60.1% in the roots after 8-day cultivation		
Salix Viminalis L.	Salix	*Crucibulum laeve (white rot-fungus)*	OM, 20.1 g·kg^{-1}; pH, 8.7	G	45% of removal than natural attenuation	60	[341]
Trifolium repens L.	*White clover*	Mycolicibacterium sp. Pyr9	pH, 6.02; OC, 14.3 g·kg^{-1}	G	Pyrene content in shoots and roots decreased by 25%–30% and 33%–42%	30	[236]
Sorghum; onobrychis sativa	Sorghum; sainfoin	Bacterial mixture cultivated on nutrients agar	n. a.	FC	Increased phytoremediation efficiency	120	[342]
Medicago sativa L.	Alfalfa	*Glomus mosseae; G. etunicatum*	pH, 6.02; OM, 2.41%	G	Promoted degradation. Increased accumulation in root but reduction in shoot	70	[328]
Medicago sativa L.	Alfalfa	*Arthrobacter oxydans; staphylococcus auricularis; stenotrophomonas maltophilia,*	pH, 5.86; OM, 11.7 g·kg^{-1}	G	Significantly enhanced degradation and uptake	45	[211]
Schoenoplectus triqueter *L.*	*Scirpus triqueter*	Klebsiella pneumoniae	pH, 8.31 ± 0.06; OM, 19.6 ± 0.3 g·kg^{-1}	FC	13% and 27% higher removal efficiency in Pyr and Pyr-Ni soils	60	[294]
Zea mays L.	Maize	*Glomus mosseae*	OM, 1.26%; pH, 6.20	G	Increased accumulation in roots and translocation to stem	60	[331]
Triticum aestivum; vigna radiata; solanum melongena	Wheat; mungbean; eggplant	*Glomus mosseae*	pH, 7.9; TOC 3.6 g·kg^{-1}	G	Improved tolerance to plant contamination and microbial degradation	65	[343]
Festuca arundinacea	Tall fescue	Lewia sp.	OM, 4.2 %; pH, 7.53	G	Pyrene removal in plant-symbiotic association was 37 times higher in perlite than only plant treatment; in soil, removal of pyrene by inoculated plants increased by 6.5%.	45	[333]

Schoenoplectus triqueter *L.*	*Scirpus triqueter*	NTA, alkyl poluglucoside, Bacillus subtilis named HD-1	pH, 8.3; OM, 19.6 $g \cdot kg^{-1}$	G	The removal of pyrene goes from 19% (with the plant alone) to 59.78% with the application on both chemical and bioaugmentation	60	[336]
Triticum aestivus; zea mays L.	Wheat and maize	Enterobacter *sp. 12J1*	pH, 6.74; OM, 34.3 $g \cdot kg^{-1}$	G	Pyrene removal increased by 43–65% in the live bacterium-inoculated planted soils	40	[344]

S: spiked soil; G: greenhouse; FC: field condition; OC: organic carbon; TOM: total organic matter; OM: organic matter; TOC: total organic carbon; TC: total carbon; SOM: soil organic matter; DOC: dissolved organic carbon; n.a.: not available

2

1.10 CONCLUSIONS AND POTENTIAL FUTURE INVESTIGATIONS

Phytoremediation is an effective, sustainable, and reliable technique for the remediation of pyrene-contaminated soils, involving only simple equipment, resulting in low operating costs, and having no-interference with the ecosystem. The present review pointed out that pyrene is mostly removed through rhizodegradation (i.e. the action of microorganisms in the rhizosphere region), significantly promoted by the specific plant species involved. The phytoremediation efficiency strongly depends on remediation conditions (i.e. remediation time, water content, temperature), plant species (physiology, lipophilicity) and soil/sediment (i.e. initial pyrene concentration, origin, amount of SOM) characteristics. In any case, none of these factors, observed in the current paper, can limit plant growth and applicability of the treatment.

At higher pyrene concentrations than those that plants can resist, a preliminary treatment to reduce pyrene concentration in soil can be used. Different additives have been proposed to further enhance the activity of pyrene degraders, including both biostimulation and bioaugmentation approaches. To promote pyrene removal, surfactants are capable to enhance pyrene transformation from an inaccessible to a bioavailable fraction, yet the optimum dose of these substances requires more in-depth studies. Bioaugmentation is promising due to an increased pyrene removal potential, possibility to improve soil quality and influence plant uptake. Despite giving a little contribution compared to rhizosphere degradation, the uptake of pyrene through phytoextraction into plant tissues can simultaneously occur, with roots being the major location of pyrene accumulation. Both mechanisms strongly depend on pyrene availability and concentration in soil, plant species and environmental conditions. A key aspect is the plant biomass fate, trying to minimize landfilling and supporting virtuous resource recovery strategies by, for instance, promoting bioenergy production with the aim to minimize costs and environmental impact.

Results from studies shown in literature reveal critical issues that should be progressed in the future. Greenhouse experiments are conducted under controlled conditions with homogenous parameters and soil matrix. In contrast, field conditions

can considerably affect the plant growth along with the microbial community developed in the rhizosphere, indicating that the effect of the treatment scale (real vs. lab-scale) needs to be more deeply studied. Due to a high K_{ow}, pyrene is strongly bound to SOM, thus the application of substances capable to improve pyrene mobilization (i.e. chelating agents and surfactants) seems to be necessary for the future progress of the technology. Drawbacks connected to compounds addition, particularly at high dosage (e.g. 5% in mass ratio with soil), involve a possible exerted phytotoxicity to plants and the production of reactive molecules (i.e. free radicals). As this can lead to a biomass reduction, a further investigation is needed.

CHAPTER 2. PHYTOREMEDIATION OF A PYRENE-CONTAMINATED SOIL BY *CANNABIS SATIVA* L. AT DIFFERENT INITIAL PYRENE CONCENTRATION

2.1 Introduction

Soil contamination by hazardous organic compounds has increased over the years due to productive activities, fossil fuel combustion and vehicular traffic, posing relevant risks for natural ecosystems, biodiversity and human safety [298]. Polycyclic aromatic hydrocarbons (PAHs) are among the main organic compounds released by anthropogenic sources [345]. PAHs contain two or more benzene rings fused in different structures [346], and this results in high toxicity, mutability and carcinogenic effects induced by PAHs [347,348]. In particular, pyrene (PYR) has four fused rings and is included in the 16 PAHs of priority concern listed by the United States Environmental Protection Agency (US EPA). PYR is ubiquitous in the environment and has been found in soil in a great amount from different regions worldwide [57,168,171]. Due to its hydrophobic and lipophilic characteristics, PYR tends to be sorbed onto solid particles and accumulate in soils and sediments by atmospheric transport, deposition and rains [16]. Consequently, a constant need for effective techniques capable to remediate PYR-contaminated sites exists, with the aim to ensure human and environmental protection [349].

Traditional remediation technologies, e.g. thermal treatment, soil washing and chemical oxidation, are frequently invasive and involve elevated energy supply entailing high operating costs [350,351]. On the contrary, green technologies offer cost benefits in addition to a more sustainable interaction with the environment [352]. In this view, phytoremediation has successfully been applied over the years to treat different contaminated soils and groundwater, with research still ongoing to find new approaches to improve the process efficiencies [284,324]. Phytoremediation consists in the use of plants and associated microorganisms in soil to remove different compounds from environmental compartments [353,354]. With regard to PAHs, phytoremediation is capable for PAH removal from contaminated soils by different

mechanisms [355,356], which generally involve accumulation, degradation, stabilization and volatilization [195,357]. Enzymes can actively participate during PAH degradation catalyzing many important biochemical reactions in soil, such as the synthesis and decomposition of humus, the hydrolysis of organic compounds and their conversion into more bioavailable forms [358–362]. Finally, plants interact with PAHs in the rhizosphere via the transpiration stream [363].

Among the plant species used to remediate PAH-contaminated soils, *Cannabis sativa* L. has wide margins for investigation. Hemp, a fibrous plant with a high biomass productivity, has been reported to be suitable to survive under potential stress conditions associated with the presence of toxic elements [364]. It was suggested that hemp could have the ability to avoid cell damage induced by heavy contamination by activating molecular protection mechanisms [365]. *C. sativa* L. showed a high tolerance to chrysene and benzo(a)pyrene at concentrations of 25, 50, and 75 $\mu g \cdot g^{-1}$, being suitable for the remediation of the contaminated fortified soil [366]. Moreover, *C. sativa* L. is capable of extracting high concentrations of potential toxic metals in its roots and aerial parts [367]. Thus, the application of industrial hemp in phytoremediation of PYR-contaminated soils would represent a promising remediation strategy, also to generate positive feedbacks in terms of land use emissions and creation of circular schemes [217]. Indeed, besides the phytoremediation potential, industrial hemp is a precious resource to implement novel second-generation biorefineries aimed at bioenergy and biomaterials recovery [217,219,368,369]. The energy supplied from renewable resources will play a significant role in the global energy demand in the next decades to fulfill the transformations required by the ecological transition and mitigate the impact of climate change [370–372].

In the present work, we propose for the first time the phytoremediation of a PYR-contaminated soil by *C. sativa* L. Three increasing PYR concentrations were tested (i.e., 50, 100 and 150 $mg \cdot kg^{-1}$ dry soil). Plants were grown in greenhouse conditions for 60 days in 300 mL volume pots. The initial and final concentration of total and butanol extractable PYR in soil along with the total PYR concentration in plant tissues on day 60 were evaluated. The *C. sativa L.* height was monitored every week, while hemp dry biomass of roots and aerial parts was determined after 60 days when harvested. Soil dehydrogenase (DHO) activity was measured at the end of the experimental campaign. The specific objectives of this study were to: 1) investigate the PYR removal potential of hemp; 2) explore the mechanisms of phytoremediation focusing on a) PYR bioavailability and b) enzymatic activity in soil; 3) evaluate the effect of PYR on plant growth, biomass production and phytoextraction.

2.2 Materials and methods

2.2.1 Experimental soil

20 kg of soil, with no history of contamination, was manually collected at a depth of approximately 20 cm from the rural level in Sora, Lazio region, Italy (41°43′05″N, 13°36′48″ E). The soil was stored in a hermetic glass vessel and transported to the laboratory. The collected soil was air dried in a laboratory oven (Argolab TCF-50, China) at 45 °C until constant weight, passed through a 2 mm mesh to remove the coarse fraction and ensure homogeneity and kept at room temperature prior to being used [230]. The soil characterization is reported in Table 2.1. Based on the Textural Classification System of the United States Department for Agriculture, the soil texture was typical of that of a silt loam.

Table 2.1. Physical-chemical properties of the soil used in the experimental campaign.

Parameter	Soil
	Average
Sand (%) [a]	32.4 ± 0.9
Silt (%) [a]	57.3 ± 1.6
Clay (%) [a]	10.3 ± 1.5
pH	7.8 ± 0.02
Total solids (%)	97.92 ± 0.03
Volatile solids (%)	1.62 ± 0.01
Total organic carbon (g·kg TS^{-1})	4.07 ± 0.25
Total nitrogen (g N·kg TS^{-1})	1.71 ± 0.3
Electric conductivity ($mS \cdot cm^{-1}$)	1.79 ± 0.01

[a] Tested samples were sieved through different mesh diameters (i.e., sand 0.05-2.00 mm, silt 0.002-0.050 mm, clay <0.002 mm).

2.2.2 Spiking procedure for soil

The selected PAH was PYR (analytical grade ≥98%), which was purchased from Sigma-Aldrich (Germany). PYR was dissolved in acetone (VWR, Italy grade 100%) and added to the uncontaminated soil to obtain three initial theoretical concentrations of 50, 100 and 150 mg PYR·kg^{-1} dry soil (P1, P2 and P3 conditions, respectively). The

same amount of acetone but no PYR was added to another portion of uncontaminated soil to obtain the PYR-free control (P0 condition). The above mentioned PYR concentrations were selected to simulate the contamination values of representative real contaminated soils [145,163,373]. Also, the concentrations used reflect the concentration values tolerated by different plants. For instance, PYR concentrations of 50, 100, and 150 mg kg^{-1} were reported to be lower than the upper tolerable concentration of alfalfa and ryegrass [145]. Afterwards, the contaminated soil was placed under a fume hood and manually mixed daily to completely evaporate the solvent [80]. Finally, to reproduce the effect of aging and better incorporate PYR in the soil matrix, the contaminated soil was stored in the darkness at room temperature based on the aging procedure described by Lukić et al. [374]. The aging period was 2 months according to Wang et al. [145]. After the 2 months aging, the measured initial PYR concentrations were slightly different from the theoretical values, as reported in Table 2.

2.2.3 Experimental design

The experimental soil was placed in plastic pots of 300 mL volume and shut with filter paper at the bottom to avoid soil and chemical loss when watering [75]. Three replicates of each operating condition (i.e., 50, 100, 150 mg PYR·kg^{-1} dry soil with plant) were prepared. Three further pots per each PYR concentration were used as controls in the sole presence of soil without the plant. Three controls were run with the plant in the absence of PYR. The same operating conditions (i.e., humidity, temperature, light/dark cycles) and experimental duration of the main tests were used for the controls.

The feminized seeds of *C. sativa* L. (i.e., certified Carmagnola variety) were preliminarily soaked in warm water for 12 h before planting them in each single pot at a depth of about 1 cm [375]. The pots with the PYR-contaminated soil and the controls were then placed in a growth chamber in the dark for one week or until the first leaves appeared [376]. From that moment, the phytoremediation experiments were officially started. For the vegetative growth (i.e., 40 days) and flourishing (i.e., 20 days) periods, 18/6 h and 12/12 h light/dark cycles were used, respectively [377,378]. The temperature ranged between 18 and 25°C, while the humidity varied from 45 to 55% for the whole duration of the experiment. Each pot was irrigated three times per week using deionized water at 50% of soil water holding capacity to maintain a constant soil moisture and avoid excessive drainage [318]. The soil moisture content was monitored

by weighing the pots [379]. The position of the pots was changed periodically at random [380,381]. The plants were observed and measured every week to take note of changes in appearance and biomass growth. Finally, the plants were harvested after 60 days of phytoremediation.

2.2.4 Analytical procedures

Total (TS) and volatile solids (VS) were measured in accordance with standard methods reported in APHA (1988) and US EPA (2002). A Sentix/940 pH-electrode (WTW, USA) was used in a suspension 1:2 (soil:water) to determine the pH. The electrical conductivity (EC) was determined by measuring the electrical resistance of a 1:5 (soil:water) suspension with a TetraCon/925 (WTW, USA) conductivity cell. Total organic carbon (TOC) in soil was measured by using 1 g of soil and a 0.1 $mol \cdot L^{-1}$ pyrophosphate and sodium hydroxide solution, prior to being analyzed with a TOC-L Series (Shimadzu, Japan) equipment. Total Kjeldahl nitrogen (TKN) analysis was performed to measure the concentration of ammonia-N and organic-N using a K-425 speed digester (Büchi, Switzerland). $N\text{-}NH_4^+$ was measured with an A6/25 ammonium cell test (WTW, Germany). Organic-N was measured as a difference between TKN and $N\text{-}NH_4^+$. Sulfate concentrations were analyzed by ion chromatography (IC) using an 883 Basic IC Plus (Metrohm, Switzerland).

PYR was extracted from contaminated soil samples using the procedure described by Sun et al. [384]. 3-4 sub-samples were taken from each pot and then mixed. The collection point of each single sub-sample was chosen avoiding any anomalous areas and the edges of the pot, mainly focusing on the middle-central part of the pot. Briefly, a contaminated soil sample of 0.5 g, dried under ventilated and dark conditions, was placed in a 25 mL flask with 10 mL acetone (VWR, Italy grade 100%) and 1 mL of 10 $mg \cdot L^{-1}$ chrysene (VWR, GC/MS grade 100%) solution as internal standard (recovery efficiency of 99±1%). Subsequently, the flask was ultrasonicated for 30 minutes keeping the temperature between 39 and 41 °C before filtering the sample on a 0.45 μm glass microfiber filter (Whatman, USA) prior to analysis. PYR was analyzed using a LC-20AD HPLC (Shimadzu, Japan) equipped with a 159 Kinetex® 3.5 μm PAH (150x4.6 mm) column (Phenomenex, USA) heated at 35 °C and an SPD-20A UV detector (Shimadzu, Japan) set at 254 nm. A water/acetonitrile (VWR, HPLC grade) solution (50:50) was used as eluent phase at a flowrate of 1.2 $mL \cdot min^{-1}$ (Volk and Gratzfeld Huesgen, 2011).

All harvested hemp plants were cut into roots and above parts, washed with tap water, and then rinsed with deionized water. The roots and aboveground parts were weighed and then subjected to oven-dried at 50 °C overnight and their oven-dried weights were measured until a constant value was reached [145]. The PYR in the aboveground parts and roots was extracted according to the procedure reported by [77]. According to Kelsey et al. [385], the amount of PYR extracted with butanol is correlated with the bioavailable PYR fraction. For the determination of PYR bioavailability, a solution with 20 mL butanol (VWR, Italy grade ≥96%) and 1 mL of 50 $mg \cdot L^{-1}$ chrysene was placed in a 25 mL flask. Then, 2 g of dried soil were added and the flask was shaken on rotary tables at 160 rpm for 16 hours in the dark [386]. The extracted sample was filtered on a Whatman glass microfiber filter (0.45 μm) before analysis.

Soil DHO activity was determined as modified from Trevors et al. [387], using iodonitrotetrazolium chloride (INT) (Alfa Aesar, Italy grade 95%) as substrate and iodonitrotetrazolium formazan (INTF) (Sigma-Aldrich, Germany grade ≥98%) as product of the enzymatic activity, according to the methods reported by Madrid et al. [388]. Briefly, 0.2 mL of INT (0.4%) solution and 0.4 mL of deionized water were added to 1 g of dried contaminated sample. As a control, the same procedure was performed for 1 g of soil with 0.6 mL of deionized water. The samples were incubated in the dark at room temperature and shaken on a rotary table for 20 h. Afterwards, 10 mL of methanol was added, and the solution was further shaken. The extracted sample was filtered on a Whatman glass microfiber filter (0.45 μm). The INTF formed was determined by using an 8453 UV-visible spectrophotometer (Agilent, USA). INTF was quantified spectrophotometrically at 490 nm.

The composition of the microbial community populating the 150 $mg \cdot kg^{-1}$ PYR-contaminated soil (P3) after 60 days of treatment in both planted and unplanted conditions was analyzed through a preliminary DNA extraction with a ZR Fecal/Soil Microbe DNA MiniPrep™ Kit (Zymo Research, USA). The income of DNA extracted from the sample was assessed as described by Claassen et al. [389]. Polymerase chain reaction (PCR) amplification, Illumina sequencing, sequence filtering and taxonomic classification were performed as according to Haleyur et al. [390]. Library quantification was determined through a 2100 Bioanalyzer (Agilent Technologies, USA).

2.2.5 Statistical analysis

The obtained data were processed by one-way analysis of variance (ANOVA) followed by a Tukey test to evaluate statistically significant differences between the tested working parameters. The correlation between different conditions (i.e., PYR removal, DHO activities and hemp biomass production) was estimated by the standard Pearson sample correlation coefficient ($r_{variable,1\text{-}variable,2}$) with both-sided alternative. All statistical analyses were performed with the XLStat statistical software for Microsoft Excel (2021.1. version, Addinsoft, USA). The result, by the standard of the study, was assumed statistically significant with $p < 0.05$.

2.2.6 Calculations

The shoot concentration factor (SCF) and the root concentration factor (RCF) were calculated as follows [77]:

SCF = PYR concentration in the aerial parts/PYR concentration in soils

RCF = PYR concentration in the aerial parts/PYR concentration in soils

The translocation factor (TF) was calculated as:

TF (%) = (PYR concentration in aerial parts/PYR concentration in root)·100

The components used for calculating each mass balance were the accumulation of PYR in the roots and the aerial parts, the PYR loss in the unplanted control, the accumulation of PYR in the aerial parts of *C. sativa* L. grown in the PYR-free control.

2.3 Results and discussion

2.3.1 PYR removal from soil after 60 days phytoremediation with *C. sativa* L.

In the present study, *C. sativa* L. was used for PYR removal from an artificially spiked soil over a 60-days treatment. The PYR removal percentage after 60 days in both planted and unplanted soil is depicted in Figure 2.1. The final PYR removal efficiency exceeded 68% under all operating conditions. The P1 condition (i.e., 50 mg PYR·kg^{-1} dry soil) resulted in the highest PYR removal efficiency (i.e., 94.52%), while P2 and P3 conditions allowed to achieve a PYR removal of 73.50 and 68.22%, respectively. All planted conditions showed a significant statistical difference with the corresponding unplanted control tests (Figure 2.1), indicating that the presence of *C. sativa* L. was crucial in PYR depletion from soil. PYR removal was not significantly different (p>0.05) in the unplanted controls of P2 and P3 conditions. The residual PYR

concentrations after 60 days of treatment under each operating condition are reported in Table 2.2.

Findings of previous studies confirm that PYR removal is higher in the presence of plants, with different plant species investigated for this purpose [313,326]. Zhang et al. [391] used the species *Scirpus triqueter* and reported that the degradation of 80 mg PYR·kg TS^{-1} reached a PYR removal percentage of approximately 65 and 54% in the rhizospheric and non-rhizospheric soil, respectively, compared to a 43% removal efficiency observed in an unplanted soil after 80 days of treatment. High PYR degradation efficiencies were achieved when using *Vetiveria zizanioides*, *Rohdea japonica* and *Typhafr orientalis* species (86, 84, and 77%, respectively) after 14 weeks, and were 66% higher than those observed in the unplanted control experiments (Wang et al., 2008). Our results are also consistent with Rostami et al. [326], where the removal percentage of PYR in unplanted controls was 47% at the PYR concentration of 150 mg*kg^{-1} in a soil with 2% organic matter.

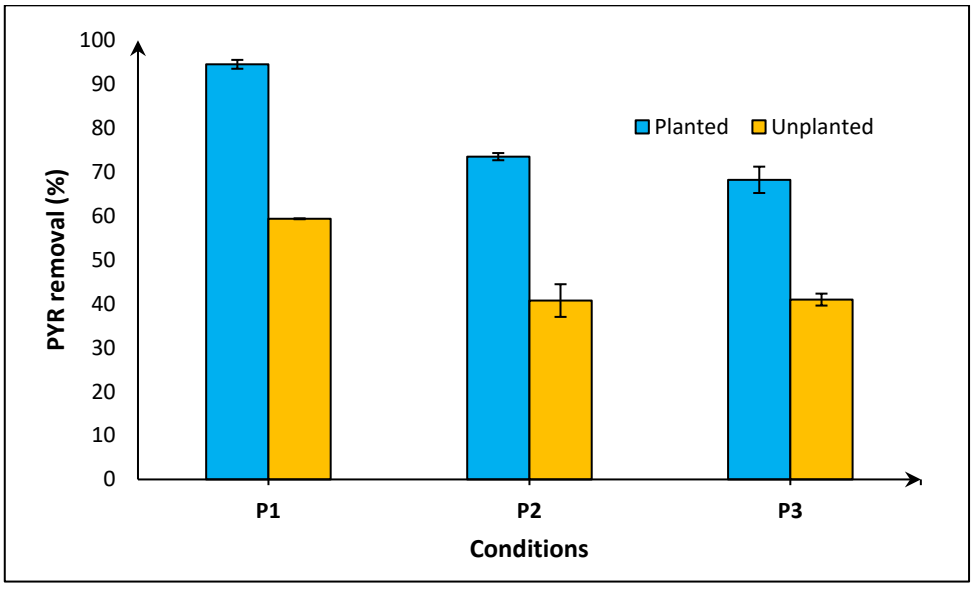


Figure 2.1 Pyrene (PYR) removal (%) after 60 days of phytoremediation in an artificially spiked soil having an initial PYR concentration of 50 (P1), 100 (P2) and 150 (P3) mg·kg TS-1. The same letters (Tukey test) do not represent statistical differences ($p>0.05$) between the experimental conditions. Values are the averages of triplicate analyses and error bars indicate the standard deviation.

Table 2.2 Initial and final pyrene (PYR) concentration under the different experimental conditions

Conditions	*PYR (mg·kg TS^{-1})*			
	t_0		t_{60}	
P1 + plant	38.52	± 0.53	2.11	± 1.01
P2 + plant	89.04	± 2.72	23.60	± 0.82
P3 + plant	155.23	± 3.59	49.32	± 3.01
P1 no plant	38.52	± 0.53	15.65	± 0.10
P2 no plant	89.04	± 2.72	52.15	± 3.72
P3 no plant	155.23	± 3.59	91.01	± 1.36

EU national legislations impose a maximum acceptable PYR concentration in soil in order to define that soil as safe. For instance, in Dutch legislation, the tolerable threshold is 40 mg PYR kg^{-1}. In Italian legislation, the Decree 152/2006 reports a PYR threshold contamination concentration of 50 mg kg^{-1} for industrial soil [393]. The "Interim Canadian Environmental Quality Criteria for Contaminated Sites", issued in 1991, indicate a PYR concentration of 100 mg·kg^{-1} for the industrial and commercial sites as the threshold to protect environmental and human health. However, a real environmental quality standars (EQSs) assessment to improve the understanding of how to implement the PAHs soil quality guidelines for PYR is lacking in the guidelines revised in 2010. In any case, these considerations imply that the process investigated in this study would allow to efficiently decrease PYR concentration below the above mentioned threshold values.

2.3.2 Bioavailable PYR fraction in soil

PYR bioavailability in soils is a fundamental parameter to determine the effectiveness of any biological remediation technique, including phytoremediation [127,394]. The bioavailable PYR fractions, for instance the water-soluble and acid-soluble fractions, can be desorbed by root exudates and be directly accessible to plants [395]. The concentration of the bioavailable PYR fraction measured at the beginning and after 60 days of phytoremediation is reported in Table 2.3.

Table 2.3 . Initial and residual bioavailable pyrene (PYR) concentrations after 60 days of phytoremediation with C. sativa L. under P1, P2 and P3 operating conditions. The concentrations of bioavailable PYR in the unplanted control tests are also reported.

Conditions	*Bioavailable PYR (mg·kg TS^{-1})*

	t_0		t_{60}	
P1 + plant	33.49	± 0.41	2.55	± 0.65
P2 + plant	75.06	± 2.67	23.93	± 0.93
P3 + plant	136.86	± 1.21	46.01	± 1.22
P1 no plant	33.49	± 0.41	13.88	± 0.33
P2 no plant	75.06	± 2.67	45.10	± 0.04
P3 no plant	136.86	± 1.21	82.66	± 9.92

The high bioavailability along with the high total PYR removal efficiency suggests that PYR removal was most likely the result of biotic processes, such as microbial degradation, opposed to sorption onto soil constituents. This is also corroborated by the low presence of soil organic matter (1.6%, expressed as VS, over the total dry matter of soil), which is normally associated with a low PYR bioavailability but rather with the abiotic sequestration of PYR in soil [270]. Moreover, a higher reduction of the bioavailable PYR fraction after 60 days of treatment was observed in planted soils compared to unplanted controls. These results are consistent with what observed by Parrish et al. [396], who found a reduction of PYR bioavailability in the presence of plant roots as long as phytoremediation was performed.

2.3.3 PYR content, accumulation and translocation in roots and shoots of *C. sativa* L.

After 60 days of growth and flourishing of hemp in contaminated soils, the amount of PYR adsorbed onto the root surface was 15.79, 32.54 and 177.03 mg PYR·kg TS^{-1} under the P1, P2 and P3 conditions, respectively. In the aerial parts, the detected PYR amounts were 5.65, 16.27, 40.37 mg PYR·kg TS^{-1} of biomass.

It has been reported that PYR is not prone to be susceptible to translocation mechanisms in plants due to its lipophilic characteristics [397]. Hence, the SCF, RCF and TF were used to evaluate the translocation potential of PYR in *C. sativa* L (Table 2.4).

Table 2.4 Shoot/root concentration factors (SCFs/RCFs) and the root-to-shoot transfer factors (TFs) obtained under the P1, P2 and P3 conditions after 60 days of *C. Sativa* L. growth.

Conditions	SCF	RCF	TF (%)
P1	0.14	0.40	0.35

P2	0.18	0.36	0.50
P3	0.26	1.14	0.22

As shown in Table 2.4, the calculated TFs values were not higher than 0.50 in this study. Nonetheless, the TFs calculated did not show a regular trend with the initial PYR concentration used in soil. Low TF values indicate that *C. sativa* L. took up PYR from soil and transferred it to the aerial parts with a low efficiency [398].

PYR was not detected in the roots of the plant grown in the unspiked control, while a PYR amount of 1.87 ± 0.81 mg·kg^{-1} of biomass was found in the aerial parts. The accumulation of PYR in the aerial parts of the plants was also reported to occur from ambient air, possibly due to the entrapment of fine contaminated soil particles [399]. Yet, the uptake of soil-bound PYR directly via the stomata or the PYR deposition on the leaves surface are pathways for PYR accumulation in plant shoots [129]. In this study, the extraction of PYR by the plant grown in the unspiked control served as a control for aerial adsorption.

In literature, PYR accumulation in plants has been attributed to the lipid content present in the roots [397,400,401]. The PYR accumulation in roots and aerial parts here observed can, thus, be due to the high content of lipids and sugars normally observed in the tissues of *C. sativa* L. cultivars [402]. A previous study showed a PYR concentration of 462 ± 18 mg PYR·kg^{-1} in the roots of *S. triqueter* grown on 100 mg PYR·kg TS^{-1} of contaminated soil [81]. This indicates that different physiological characteristics of plants and especially the root system developed likely play a pivotal role in governing the PYR removal from soil [403]. However, once accumulated in the roots, it is possible that PYR degradation is not complete, negatively affecting both plant growth and further PYR accumulation [81].

The amount of PYR taken up by roots and aerial parts is highly correlated with the bioavailable PYR fraction, as well as the total PYR concentration, soil water content and the microbial community in soil [395]. For instance, an increased PYR accumulation in plant tissues as a function of initial PYR concentration in soil was observed by Gao and Zhu (2004), where PYR in roots increased from 21.5 ±3.20 to 112 ±6.49 mg kg^{-1} of biomass with an initial PYR concentration in soil of 48.7 ±3.76 and 172 ±9.21 mg PYR kg^{-1} of contaminated soil, respectively.

Besides plant accumulation, the fate of PYR in spiked soils during phytoremediation can be ruled by percolating, photo-degradation, volatilization and biodegradation mechanisms as well as supplementary abiotic losses [229]. Carrying

out some simple mass balances, we observed that 40.62, 58.56 and 58.64% of PYR removal was attributed to abiotic mechanisms under the P1, P2 and P3 operating conditions, respectively. The amount of PYR found in roots made up only the 0.004, 0.005 and 0.010% of the PYR removed for P1, P2 and P3, respectively. Similarly, the contribution of the aerial parts of *C. sativa* L. in PYR removal was irrelevant. The remaining percentages of 59.38, 41.44 and 41,35% for P1, P2 and P3, respectively, can be ascribed to microbial degradation, which resulted in the highest PYR depletion contribution, confirming what reported in section 3.2. According to Zhang et al. [404], plant root exudates might enhance PYR biodegradation promoting the activity of rhizosphere microorganisms and enzymes. In contrast, in the unplanted soil, PYR removal may be due to the metabolic activity of the microorganisms naturally present in the soil and/or partially to photochemical oxidation and leaching [15].

2.3.4 Production of *C. sativa* L. biomass in PYR-contaminated and uncontaminated soil

According to previous studies, increasing PYR concentrations in soil are associated with the decrease of the plant biomass growth, as for instance observed for alfalfa by Alves et al. [405]. Therefore, the height of the plants here used was measured every week to investigate the ability of hemp to grow in the PYR-contaminated soil. The height of *C. sativa* L. during the 60-days experiment is shown in Figure 2.3. Under P1, P2, and P3 conditions, the final height of the plant was averagely 36, 29 and 42% lower than that observed in the PYR-free control ($p < 0.05$), respectively. The height of the plant in the uncontaminated soil reached the highest value of about 70 cm after 60 days. No significant difference ($p > 0.05$) was observed among the plants grown at the three different initial PYR concentrations. Plants developed an average height of 45 cm after 60 days (Figure 2.3) in the PYR-contaminated soil.

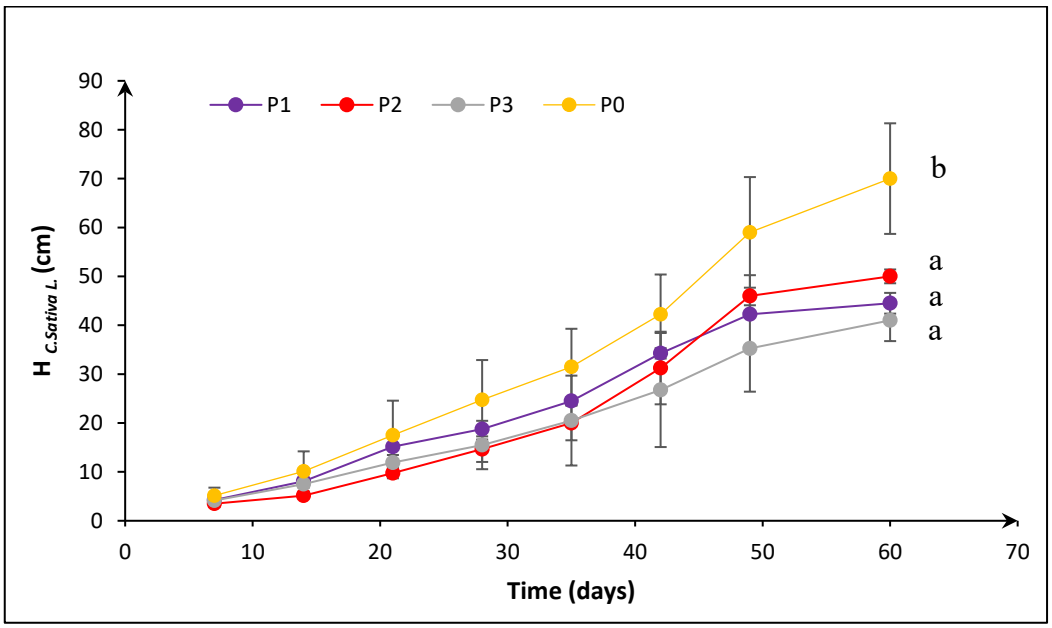


Figure 2.2 Evolution of the height of the *C. sativa* L. plants used in the PYR-contaminated soil (P1, P2 and P3 conditions) and in the unspiked soil. The same letters (Tukey test) do not represent statistical differences (p>0.05) between the experimental conditions. Values are the averages of triplicate analyses and error bars indicate the standard deviation.

Yet, PYR concentration showed adverse effects on roots and shoot dry biomass, as reported in Table 2.4. Considering the dry biomass weight, the above parts decreased by 28, 36 and 42% in P1, P2 and P3 respectively, compared to the uncontaminated control (P0). A more severe biomass reduction was observed in roots. Increasing the PYR concentration, the root biomass decreased by 41, 50 and 56% compared to the roots grown in the unspiked soil (P0). Statistical analysis showed no influence on the biomass weight up to P1 concentration (Table 2.4). At higher PYR concentrations (i.e., P2 and P3 conditions), a significant difference ($p < 0.05$) was observed in comparison to P1 and P0. This can be attributed to a more severe effect of PYR concentrations higher than 50 mg kg^{-1} on the biomass growth [248]. The presence of such PYR concentrations could alter root morphology, hindering the nutrients and water uptake and resulting in a decreased biomass response [81]. Negative effects of PYR on biomass growth were also reported by Wang et al. [406], who observed that the total biomass of a mangrove species (i.e., *Kandelia obovata*) decreased by approximately 28% when grown in a contaminated soil with a PYR concentration of 10 mg·kg^{-1}. Similarly, Zhang et al. [135] reported that PYR contamination reduced the height and

biomass of spruce. Despite reduced biomass, *C. sativa* L. did not exhibit visible sign of stress and toxicity (e.g., seeds germination, appearance of spots, yellowing tissues and damage in leaves) proving that phytoremediation in PYR-contaminated soils is feasible with this plant.

Table 2.5 Fresh and dry biomass (g) of roots and aboveground parts of *C. sativa* L. after 60 days of phytoremediation at three different initial pyrene (PYR) concentrations and in an unspiked soil. The same letters (a, b, c and d) obtained with the Tukey test do not represent statistical differences ($p > 0.05$) between the experimental conditions. Values are the averages of triplicate analyses and error bars indicate the standard deviation.

	Spiked PYR concentration (mg·kg TS^{-1})			
Conditions	0	50	100	150
		Fresh weight		
Roots (g)	0.52 ± 0.05 ^{a}	0.30 ± 0.04 ^{a}	0.21 ± 0.08 ^{b}	0.19 ± 0.08 ^{b}
Above parts (g)	3.49 ± 0.11 ^{c}	2.84 ± 0.07 ^{c}	1.11 ± 0.08 ^{c}	0.96 ± 0.08 ^{d}
		Dry weight		
Roots (g)	0.12 ± 0.03 ^{a}	0.07 ± 0.02 ^{a}	0.06 ± 0.02 ^{b}	0.05 ± 0.02 ^{b}
Above parts (g)	0.70 ± 0.08 ^{c}	0.56 ± 0.03 ^{c}	0.23 ± 0.02 ^{d}	0.21 ± 0.05 ^{d}

2.3.5 Assessment of DHO activity in planted and unplanted soil

The DHO activity in soil measured after 60 days of treatment is shown in Figure 2.3. DHO was monitored as an indicator of microbial activity in soil and a possible indirect indicator related to PYR degradation [407], as DHO functions are associated with microbial oxidoreduction processes in soil [408].

The role of PYR, either stimulating or inhibitory, can increase or decrease the levels of DHO activity in soils [75,409]. Different studies reported an increase in DHO activity possibly related to PYR and its intermediates, which could serve as carbon source for microbial metabolism and growth.

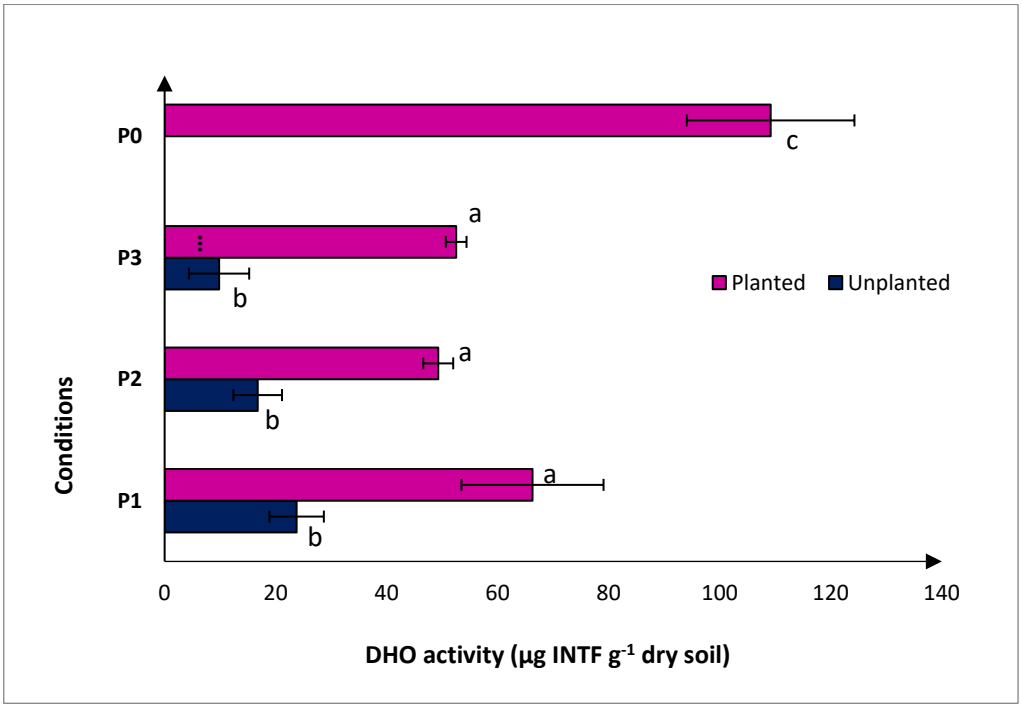


Figure 2.3 DHO activity in 50 (P1), 100 (P2) and 150 (P3) mg PYR·kg-1 dry contaminated soil and uncontaminated control (P0) after 60-days phytoremediation with *C. sativa* L. The same letters (Tukey test) do not represent statistical differences ($p>0.05$) between the experimental conditions. Values are the averages of triplicate analyses and error bars indicate the standard deviation.

In the present study, after 60 days of hemp growth, the DHO activity ranged between 50 and 110 µg INTF g^{-1} dry soil in planted soils. In unplanted controls, DHO was between 10 and 24 µg INTF g^{-1} dry soil, indicating that the DHO activity was up to 10 times higher in planted soils. Hence, the presence of hemp roots significantly ($p<0.05$) enhanced the activity of the native microbial community in soil. The highest DHO activity was found under the P1 operating condition, which was also associated with the highest PYR removal efficiency. This is also indicated by the Pearson coefficient (i.e., rDHO-PYR = 0.761) that demonstrates a good correlation between the DHO activity and PYR removal, being consistent with previous studies, which observed a direct correlation between PYR degradation and the dehydrogenation process [286,329]. Indeed, a higher DHO activity is frequently indicated as the main reason of PYR degradation in planted soils by rhizospheric microflora [404].

Increasing initial PYR concentrations had a negative impact on DHO activity (Figure 2.3), as also reported in previous studies. According to Sivaram et al. [246], a

significant reduction in DHO activity was observed in PYR-spiked soil compared to an uncontaminated control. In contrast, Baran et al. [410] observed a positive correlation between the presence of PYR and DHO activity. This was ascribed to an adaptation of the soil microflora to PYR, which was used as a carbon and energy source.

2.3.6 Identification of PYR-degrading bacteria

The phylogenetic analysis showed that *Proteobacteria* and *Firmicutes* were the most abundant bacterial phyla in soil accounting for 16 and 25%, respectively, after the 60 days of growth and flourishing of *C. sativa* L. under the P3 condition (Figure 2.4). Species belonging to archaea were completely absent. The genus *Pseudomonas*, 2.13% abundant in the analyzed samples, was associated with PYR degradation according to previous studies [411] as well as the *Bacteroidota* phylum [412], which showed an abundance of 5%. The *Actinobacteria* phylum was relatively abundant in the soil (i.e., 5%) and the bacteria belonging to it can be considered active PYR-degraders according to Ho et al. [413], including the *Mycobacterium* genus that was the most abundant among *Actinobacteria* [414,415]

In the unplanted controls, the microbial community considerably shifted and was mostly enriched in *Cyanobacteria* accounting for more than 42% of the total reads in the sample (Figure 2.4). A genus of *Cyanobacteria* termed Leptolyngbya_EcFYyyy-00 was found in the unplanted control, while it was not detected in the planted soil. A genus of *Bacillus* was also found at a higher extent in the control rather than in the planted soil sample (i.e., 20 and 10%, respectively). Thus, the presence of *C. sativa* L. could promote the growth of different species associated with PYR degradation.

In our study, the bacterial diversity is limited due to the PYR concentrations used. We only report the bacterial abundance relative to the P3 condition because those referred to the other two conditions (i.e. P1 and P2) were not significantly different. Based on reported studies, loss of bacterial diversity was detected even in the low (i.e., 20 $mg \cdot kg^{-1}$) PYR-contaminated soil ([416]. Since in the present study the PYR concentration is higher (i.e. from 50 to 150 $mg \cdot kg^{-1}$), we assume that a substantial change in the bacterial community in soil is more likely to occur at lower PYR concentrations than those used.

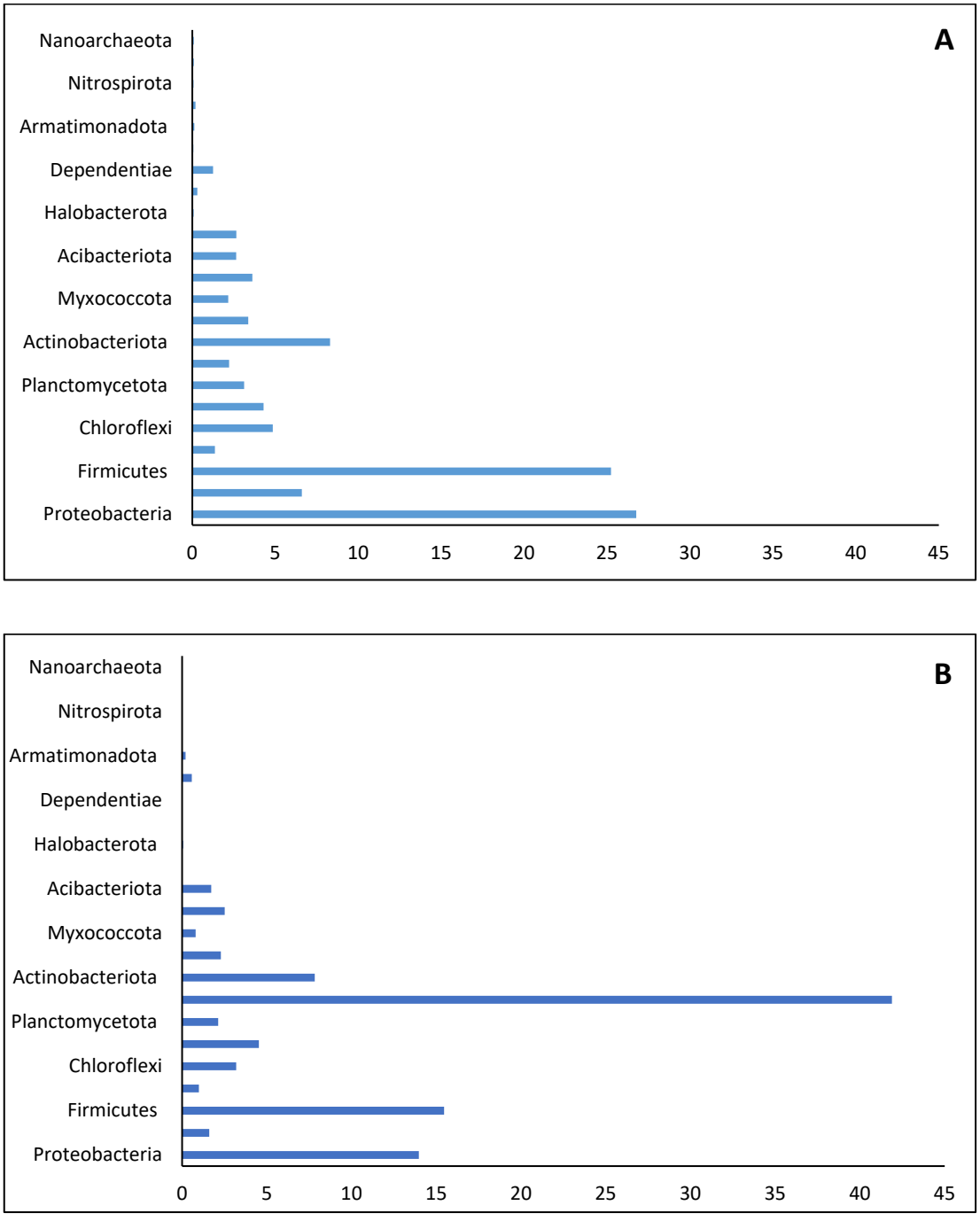


Figure 2.4 Abundance (%) of bacterial diversity (at phylum level) living in the rhizosphere around *C. sativa* L. roots in P3 (150 mg PYR kg TS-1) after 60 days of growth (A). Panel B is related to the unplanted control.

2.4 Conclusions

This study shows that phytoremediation with *C. sativa* L. for PYR-contaminated soil is capable for removing PYR up to approximately 95%, when an initial PYR concentration of 50 mg·kg TS^{-1} is used. After 60 days of treatment, concentrations of 15.79, 32.54 and 177.03 mg PYR·kg TS^{-1} under the P1, P2 and P3 conditions, respectively, were found in the roots of *C. sativa* L. Hence, higher initial PYR concentrations in soil resulted in a higher PYR uptake in the plant, and this led to a decrease of the weight of roots and above parts by 41-58 and 28-52%, respectively, when compared to the PYR-free controls. Positive correlations were found between PYR degradation and the elevated DHO presence in soil, likely indicating that the presence of *C. sativa* L. enhanced the activity of the rhizospheric PYR-degrading flora. The high level of PYR bioavailability suggests that PYR removal was mostly the result of biotic processes rather than abiotic loss.

CHAPTER 3. PHYTOREMEDIATION OF PAH- AND CU-CONTAMINATED SOIL BY *CANNABIS SATIVA* L.: PRELIMINARY EXPERIMENTS ON A LABORATORY SCALE

3.1 Introduction

Soil co-contamination by polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs) due to productive activities, intensive farming practices and waste incineration, has caused growing concern in the last decade [417]. PAHs are widespread organic pollutants in the soil compartment and consist of two or more aromatic rings fused in different structures, thus resulting in high toxicity, mutability and possible carcinogenic effects on humans [130,418]. HMs naturally occur throughout the rigid upper part of the lithosphere, whereas several HMs, such as copper (Cu), can be used by factories (e.g., the petrochemical industry) and agriculture practices, and subsequently released into the environment [419,420]. Therefore, HMs can be present in the soil in different forms such as free metal ions, and soluble metal complexes, incorporated into silicate structures and/or soil organic matter, oxides, hydroxides and carbonates [421,422].

As a result of the adsorption of PAHs and HMs onto soil particles, urban soils generally reflect centuries of practices or the legacy of industrial activities. Indeed, soil is the main sink for PAHs due to their affinity to naturally occurring organic matter [423]. Adsorption of contaminants onto soil particles represents a key mechanism determining the fate of PAHs and HMs in the environment due to various interactions (e.g., hydrophobic, electrostatic) [424,425]. In addition, particle dimension and pore volume can improve PAH adsorption in the soil matrix [425] and affect the migration patterns of HMs at different scales [426]. For instance, Zhao et al. [427] showed PAH

adsorption of up to 160 mg·kg^{-1} onto organic substances in soil after 24 h. Additionally, Lim et al. [428] obtained adsorption (i.e., up to about 4 mg Pb·kg^{-1}) of HMs ions onto soil particles after only 90 min.

An increase in the urban population has also led to new sources of the mentioned pollutants accumulating in soils [429]. Indeed, various types of combined pollution (e.g., multiple HMs, multiple PAHs and HMs + PAHs) have been recently found in some global survey areas (e.g., China, Poland, Australia) and even in far-off Antarctic continents [234]. It has been estimated that approximately half a billion hectares of soil were contaminated worldwide by HMs and/or PAHs, which exceeded the regulatory levels [430]. To define a soil as harmless, the EU national legislations impose a maximum acceptable PAH and HM concentrations in soil (e.g., 50 mg each PAH kg^{-1} for industrial use and 50 mg·Cu kg^{-1}) [431,432].

The co-existence of both PAHs and HMs in the environment is a more complex condition compared to the presence of a single class of those pollutants [80]. Thus, the remediation of such polluted soils represents a systemic challenge for environmental science and management [93], and the use of sustainable, effective and eco-friendly remediation practices is increasingly crucial to tackle the costs and preserve the environment [433]. In this context, several conventional strategies are currently available for the remediation of PAH- and HM-contaminated soils, and can only deal with a single type of pollutant rather than a complex mixture, thus requiring a sequence of remediation processes to be performed [434]. Overviewing the literature, remediation techniques that can contemporarily remove both PAHs- and HMs in contaminated soils mainly include physical-chemical processes (i.e., soil washing, electrokinetic technology and supercritical fluid extraction), which are generally invasive and can cause deterioration and damage to natural resources [435]. Therefore, bioremediation can be alternatively employed [436].

Among the biological processes, phytoremediation has been effectively applied for the remediation of co-contaminated soils by PAHs and HMs [93]. Phytoremediation refers to the employment of plants and living species associated with them to remove contaminants from the soil through several mechanisms, such as phytoextraction and rhizodegradation [437]. Phytoremediation has largely been used in recent years due to

its improved costs and benefits compared to other conventional techniques, in addition to the less impacting interaction with the ecosystem [438]. On the contrary, the thickness of the contaminated soil layer to be treated can be a problem as it can limit the application of the technique at the field-scale [439]. However, only a small amount of information is available in the literature on the phytoremediation of co-contaminated soils by PAHs and HMs, indicating the need for further research [440]. For instance, PAHs could alter the HM uptake mechanism by plants, whereas HMs could influence the PAH biodegradation process by rhizobacteria [441–443].

Cannabis sativa L. has recently been reported as an affordable option for phytoremediation and bioenergy production purposes [217,444]. For instance, *C. sativa* L. was shown to grow under potential stress conditions induced by the presence of PAHs such as pyrene (PYR) in contaminated soil, being suitable for the phytoremediation of soils contaminated by up of 150 mg PYR kg^{-1} [445]. *C. sativa* L. has also been observed capable of phytoextracting HMs, the distribution of which was found throughout the roots and aerial parts of the plant at different HM concentrations [217]. To the best of our knowledge, however, the phytoremediation potential of *C. sativa* L. under PAH and HM co-contamination conditions has never been tested.

In the present study, we investigate for the first time the phytoremediation of a PAH- and HM-contaminated soil (i.e., 50-150 and 350 $mg \cdot kg^{-1}$, respectively) by *C. sativa* L. The type and concentration of pollutants were selected to represent the contamination of several real polluted sites in which the co-existence of PAHs and HMs is a common environmental issue [45,446] Phytoremediation is a complex phenomenon involving several influencing factors to the used soil, the contaminant, the plant species and external parameters (e.g., precipitation, temperature distribution, terrain slope) [200,447]. Since most of the abovementioned variables are not directly manageable in the field condition due to the spatial and temporal variability of soil, which affect the processes occurring in the root layer, the experiments were conducted at a laboratory scale. The ultimate goal of this lab-scale experiment is to provide the preliminary results necessary for the future implementation of a field-scale study.

Therefore, the initial and final concentrations of extractable PYR, PHE and Cu in soil were evaluated. The height of *C. sativa* L. was monitored every week, while the

dry hemp biomass of roots and aerial parts was determined after 50 d when harvested. Finally, a cost evaluation of phytoremediation to assess its upscaling perspective was conducted.

3.2 Materials and Methods

3.2.1 Chemicals

PYR (purity ≥98%) and PHE (purity ≥98%) were purchased from Sigma–Aldrich (Steinheim, Germany). Cu as copper (II) chloride anhydrous (purity ≥99%) was provided by Acros Organics–Thermo Fisher Scientific (New Jersey, USA). Acetone (purity 100%) and acetonitrile (HPLC purity) were purchased by VWR (Fontenay-sous-Bois, France). MilliQ water with an electrolytic conductivity equal to 0.05 μS cm^{-1} was used as the background for preparing all solutions.

3.2.2 Soil

A portion of uncontaminated soil was collected at a depth of 0-20 cm from topsoil in Sora, Lazio region, Frosinone province, Italy (41°43′05″N, 13°36′48″ E). The soil was placed in a hermetically sealed glass vessel, then transferred to the laboratory, dried in a TCF-50 oven (Argolab, Carpi, Italy) at 45 °C until reaching a constant weight, and finally sieved through a 2-mm mesh. The soil was classified as silt loam, by containing sand (0.05–2.00 mm) at 32.4%, silt (0.002–0.050 mm) at 57.3%, and clay (< 0.002 mm) at 10.3%. The soil was characterized by a total solid (TS) and volatile solid (VS) contents of 97.92 and 1.62%, respectively, a pH value of 7.80, an electric conductivity (EC) of 1.79 $mS \cdot cm^{-1}$, a total organic carbon (TOC) of 3.20 g kg^{-1} and a total nitrogen (TN) of 0.22 g kg^{-1}.

3.2.3 Spiking procedure for soil

The same amount of PHE and PYR was firstly dissolved in acetone, which was subsequently added to the uncontaminated soil in order to obtain an initial theoretical concentrations of 100 (PC1), 200 (PC2) and 300 (PC3) mg ΣPAHs kg^{-1} in the contaminated soil. An identical amount of acetone (1 $L \cdot kg^{-1}$) was also added to the uncontaminated control soil (PC0) to guarantee the same initial conditions [445]. After the complete evaporation of the solvent in the fume hood, copper (II) chloride anhydrous was dissolved in a water solution, which was subsequently added to the

PAH-polluted soil by achieving a constant concentration of 350 mg Cu·kg^{-1} under all conditions tested, as reported by Bonilla-Bird et al. [448].

3.2.4 Experimental setup

The artificially contaminated soil was placed in 300 mL pots covered at the bottom with 5-13 μm filter paper (VWR, Germany) to avoid soil and chemical losses. Three different concentrations of phenanthrene (PHE) and PYR, here used as model compounds for low (LMW) and high molecular weight (HMW) PAHs, respectively, were tested (i.e., 50, 100 and 150 mg each PAH·kg^{-1} dry weight of soil). Additionally, a constant copper concentration of 350 mg·kg^{-1} was considered. Plants were placed in a grow box under controlled conditions for 50 d in 300 mL volume pots. Three replicates were prepared for each operating condition (i.e., PC1, PC2 and PC3 planted) in the presence of the plant by adding one seed of *C. sativa* L. (Carmagnola variety) in a single pot, and three replicates were prepared for the controls without the plant (i.e., PC1, PC2 and PC3 unplanted) [449]. Prior to sowing, the seeds were soaked in a water bath for 12 h. Afterwards, the pots with the planted PAH- and Cu- contaminated soil were placed in a growth chamber in the dark until the first leaves appeared [376]. The growth chamber was utilized to provide an 18/6 h light/dark cycle required for plant vegetative growth [378]. Additionally, unplanted pots (i.e., without the plant) were operated as control groups under the same operating conditions (i.e., duration of the test, water content, temperature, light/dark cycles) of the planted tests. The temperature varied from 15 to 22°C, while the humidity was between 45 and 50% during the 50-day growth period. Each pot was watered with distilled water at 50% of the soil water holding capacity to maintain a constant soil moisture, which was monitored by weighing the pots and avoiding unwanted drainage [318][44]. The position of the pots was sporadically and randomly changed to avoid edge effects [450]. Fresh and dry biomass of the plant was measured at the end of the 50-d treatment when harvested. The plant was divided into roots and aerial parts, which were weighed separately.

3.2.5 Analytical methods and sampling

TS and VS contents were determined according to standard methods [451]. TOC was extracted from soil by a 0.1 $mol \cdot L^{-1}$ pyrophosphate and sodium hydroxide solution and subsequently evaluated by using a TOC-L Series (Shimadzu, Japan) apparatus [452]. pH was quantified with a Sentix/940 pH-electrode (WTW, USA) in a 1:2 suspension (soil:water). A TetraCon/925 (WTW, USA) conductivity cell was employed to determine the electrical conductivity (EC) in a 1:5 soil-to-water suspension. TN was spectrophotometrically measured using a Spectroquant® nitrogen cell test (Merk, Germany). In order to constitute the representative samples for analysis, after the 50-d treatment, 3-4 sub-samples were taken from the selected pots and finally mixed. Possible anomalous areas such as topsoil and the edges of the pot were avoided, mainly sampling from the middle-central part of the pot. Afterwards, 0.5 g of the soil sample was dried under ventilated and dark conditions, and finally extracted for PYR and PHE quantification according to Sun et al. [384]. The sample was filtered on a 0.45 μm glass microfiber filter (Whatman, USA) prior to the analysis. A LC-20AD HPLC (Shimadzu, Japan) equipped with a Kinetex® 3.5 μm PAH (150x4.6 mm) column (Phenomenex, USA) heated at 35 °C and an SPD-20A UV detector (Shimadzu, Japan) set at 254 nm was employed to analyze the amount of each PAH [445]. The total concentration of Cu in the soil samples, after 50 d phytoremediation, was obtained by an acidic mineralization process. Atomic adsorption spectrometry (AAS) using a Varian Model 55B SpectrAA (Agilent Technologies, USA) F-AAS equipped with a flame (acetylene/air) and a deuterium lamp for background correction was employed to evaluate the concentrations of Cu ions in the solution [453]. The plant height was measured every week to monitor the evolution of the biomass growth. At the end of the experiments (i.e., 50 d), the *C. sativa* L. plants were harvested in soil by cutting the stem on the bottom of the topsoil. The plants were divided into roots and above parts, washed with tap water, and then rinsed with deionized water. The fresh biomass weight was evaluated when harvested. The biomass was then oven-dried at 50 °C until a constant weight [145], and then weighed.

3.2.6 Statistical analysis

Statistically significant differences between the obtained parameters were processed by a one-way analysis of variance (ANOVA) followed by a Tukey‘s honestly significant difference (HSD) test. According to the standard of the study, the results were assumed to be statistically significant when $p < 0.05$.

3.2.7 Economic evaluation

A rough cost assessment in dollar currency was conducted on the entire phytoremediation process. The cost considered for the purchase of the *C. sativa* L. seeds was 0.22 $\$\cdot seed^{-1}$. The input costs for planting, management and monitoring procedures were estimated according to da Silva Benevenute et al. [454]. Taking into account a biomass production of 1.23 $kg\cdot plant^{-1}$, the maximum hemp yield was 3,600 $plants\cdot ha^{-1}$. The required working hours for *C. sativa* L. pre-plant cultivation, planting, harvesting and post-harvesting operations, and the general remediation management was 0.033, 0.033, 0.19 and 0.017 h $plant^{-1}$, respectively. The used cost for hourly field and laboratory work, and general expenses were USD 12 and 15, respectively. Among PC1, PC2 and PC3, the condition with the highest biomass yield was selected for the economic evaluation [366]. A soil density of 1.2 ton m^{-3} was used for the calculation.

3.3 Results

3.3.1 Residual PAH concentrations in soil after the growth of *C. sativa* L.

In the present study, plants of *C. sativa* L. were used for the phytoremediation of a PAH- and HM-contaminated soil. The residual PYR and PHE concentrations in both planted and unplanted soil after 50 d of treatment in greenhouse conditions are illustrated in Fig. 3.1. Final PHE concentrations of 2.93, 1.77 and 4.28 mg $PHE\cdot kg^{-1}$ (Fig. 3.1) were obtained in the planted conditions (i.e., PC1, PC2 and PC3, respectively). Under the same conditions, a significantly lower ($p < 0.05$) removal was obtained for PYR than that achieved for PHE. Indeed, the residual concentrations of PYR in PC1, PC2 and PC3 were 18.19, 31.14, and 66.33 $mg\cdot kg^{-1}$ (Fig. 3.1), respectively, which approximately halved under all planted conditions compared to the initial concentration (i.e., 100, 200, and 300 mg $\Sigma PAHs\ kg^{-1}$).

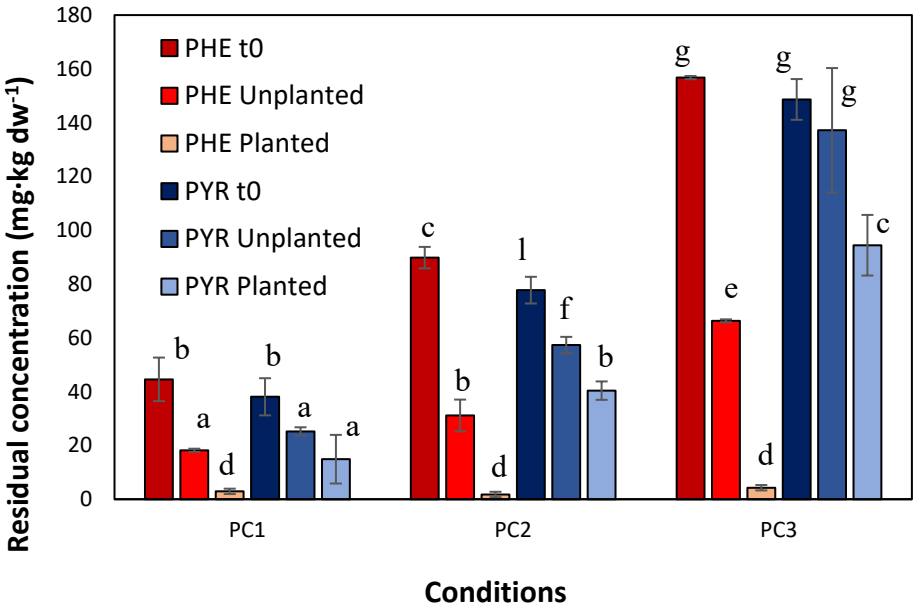


Figure 3.1. Residual pyrene (PYR) and phenanthrene (PHE) concentrations (mg each PAH kg−1) in the unplanted and planted soil, the latter being treated with 50-d phytoremediation by *Cannabis sativa* L. PYR t0 and PHE t0 represent the initial concentrations. The reported values are the means of three replicate analyses. The error bars display the RSD (residual standard deviations). The same letter represents no significant differences ($p > 0.05$) among total PAH removal after 50 days of phytoremediation.

Being characterized by 3 and 4 rings, respectively, PHE and PYR have different structural features, which induce distinct ecological effects and consequently different responses of the bacterial communities towards their degradation [455]. Additionally, compared to HMW PAHs, an LMW compound can be more volatile, show higher solubility in water and be less prone to be adsorbed onto suspended particles and biota, thus being more susceptible to biodegradation [456]. The results shown in Fig. 3.1 are supported by previous studies reporting bioremediation techniques, in which the biodegradation of LMW PAHs was faster and simpler than that of HMW compounds [457]. Similarly, Sun et al. [129] showed that PHE and PYR concentrations were reduced from 100 to 1.6 mg kg^{-1} and 25 mg kg^{-1}, respectively, after planting soil with ryegrass (*Lolium perenne*) for 60 d. Additionally, Campbell et al. [366] showed a lower removal of benzo[a]pyrene (i.e., 14-21%) compared to that obtained for chrysene (i.e.,

20-30%) in soil remediated by *C. sativa* L. after 45 d of growth. The removal of PHE and PYR can also be deeply influenced by the initial PAH concentration in soil [405]. The PYR removal efficiency decreased as the initial mg ΣPAHs kg^{-1} in soil increased, while similar values of PHE removal efficiency were observed under the PC1, PC2 and PC3 conditions. The total PAH removals in the planted PC1, PC2 and PC3 (i.e., 100, 200 and 300 ΣPAHs kg^{-1}, respectively) were 78, 75 and 68%, respectively, which were significantly higher ($p < 0.05$) than those obtained in the corresponding unplanted controls (Fig. 3.2) after 50 d. These results suggest that an important fraction of PHE and PYR was removed through rhizodegradation mechanisms in the presence of *C. sativa* L., as recently reported by Gabriele et al. [445]. In the unplanted controls, both abiotic (i.e., volatilization, percolation, photo-degradation) and biotic (i.e., biodegradation by autochthonous microorganisms) contributions could occur (Fig. 3.2) [129]. Plant-promoted PAH biodegradation was previously reported by Lu et al. [208] as the major mechanism involved in soil during PHE and PYR removal. The growth of Kandelia candel supported the removal of 48 and 38% of PHE and PYR, respectively, after 60 days with an initial concentration of 20 ΣPAHs kg^{-1} (i.e., 10 mg each PAH kg^{-1}). Approximately 98 and 85% of PHE and PYR removal, respectively, was observed by Cheema et al. [252] in the planted soils with tall fescue (*Festuca arundinacea*) contaminated by about 400 ΣPAHs kg^{-1}, due to increased biological activity in the rhizosphere. The lower PHE and PYR removals obtained here can be attributed to the presence of copper in the soil affecting rhizobacterial activity.

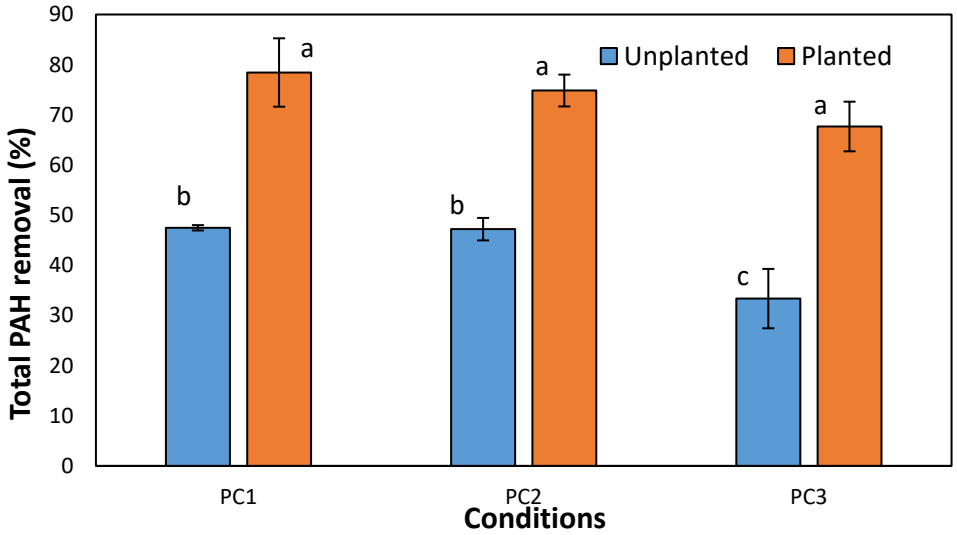


Figure 3.2. Total PAH removal (%) in the unplanted and planted conditions after 50 d of *Cannabis sativa* L.-induced phytoremediation. The reported values are the means of three replicate analyses. The error bars display the RSD (residual standard deviations). The same letter represents no significant differences ($p > 0.05$) among total PAH removal after 50 days of phytoremediation.

The presence of nutrients and organic acids can stimulate the metabolic activity of bacteria, which in turn can produce enzymes (e.g., dehydrogenase) and other substances improving plant growth [57,458]. Microorganisms in the rhizosphere could degrade PHE and PYR by releasing extracellular enzymes in the soil that mediate metabolic pathways, such as redox reactions, using these compounds as growth substrates and carbon sources [459]. For instance, Ghosal et al. [460] observed the assimilation of PHE by the Ochrobactrum sp. strain PWTJD, which was isolated from contaminated soil using PHE as a sole source of carbon and energy supply. Among different bacterial genera and species, the presence of bacterial varieties identified as being capable of biodegrading organic compounds such as *Bacteroidetes, Sphingomonas,* and *Rhodococcus* can be positively correlated with the degradation of PYR and PHE in soil [136,461,462]. In the co-presence of PHE and PYR, biodegradation likely occurred by cometabolism, which consists of the microorganism's ability to degrade an HMW compound while using an LMW compound as a growth substrate [463].

3.3.2. Copper removal from soil after 50 d of phytoremediation with *C. sativa* L.

The amount of Cu per gram of dry *C. sativa* L. biomass and the Cu removal efficiency after 50 d of phytoremediation under the PC1, PC2 and PC3 conditions are presented in Table 3.1.

Table 3.1 - Cu removed per g of dry biomass (mg g^{-1}) and Cu removal efficiency after 50 days of *Cannabis sativa* L. growth under the PC1, PC2 and PC3 operating conditions. The same letter represents no significant differences ($p > 0.05$) among total Cu removal efficiency after 50 days of phytoremediation.

Conditions	Cu removed per gram of dry biomass (mg·g^{-1})	Cu removal efficiency	
		Values (%)	Tukey's HSD test
PC1	46.04	11.06 ± 6.01	a
PC2	57.66	6.91 ± 12.95	a
PC3	n.d.	n.d.	n.a.

n.d. = not detectable; n.a. = not applicable

In the present study, a removal of about 57 and 46 mg Cu g^{-1} of dry biomass (Table 3.1) was achieved in PC1 and PC2, respectively. The obtained result was higher compared to the Cu removal of about 1.5 mg·g^{-1} recently reported in the literature by Placido and Lee [464]. In this context, the presence of PAHs can alter the mechanisms of metal uptake, thus affecting the phytoextraction kinetics and extent [465]. The improvement of bacterial activity in the presence of hemp at a specific PAH concentration in the soil (i.e., up to 200 ΣPAHs kg^{-1}) can alleviate the stress on the plant induced by toxic metals and enhance metal extraction [466]. However, this effect vanished as the amount of PAHs increased above a certain threshold (i.e., 300 ΣPAHs kg^{-1}), at which point the Cu concentration did not vary in the soil after the 50-d phytoremediation (Table 3.1). Therefore, the phytoextraction potential of *C. sativa* L. was also dependent on the biomass development and growth [467], which was significantly lower ($p < 0.05$) in PC3 than the other examined conditions (Fig. 3.3). Most of the existing literature reported the inhibition of biomass growth as a function

of the initial PAH concentration in the presence of toxic metals above a certain threshold. For instance, Lin et al. [74] showed that the copper phytoextraction potential of corn (*Zea mays* L.) was reduced by 50% above 100 mg·kg^{-1} PYR, thus leading to a decrease in the biomass growth rate [468].

With regard to the removal mechanisms, it has been reported that *C. sativa* L. could accumulate Cu in the plant tissue [469] likely due to glutathione-sulfide reductase and phospholipase D-α as the antioxidant enzymes, which can protect the plant from oxidative stress induced by reactive species in the presence of Cu [470]. Also, other studies reported an increase in aldo-keto reductase, an NAD(P)H-dependent enzyme, in *C. sativa* L. grown under Cu stress. Consequently, the relative abundance of these enzymes could influence the accumulation of Cu in plant tissues [471]. Therefore, Cu can be highly accumulated in the root system architecture compared to the upper parts of the plant. This is further supported by the achievements of other studies that reported a maximum copper accumulation of 0.01, 1.5 and 0.03 mg·g^{-1} of *C. sativa* L. in flowers, roots and shoots, respectively [364,472,473].

3.3.3 *C. sativa* L. biomass response to PYR-PHE and Cu contaminated soil over 50-d phytoremediation

The occurrence of biomass growth in roots and aerial components is one of the key factors for PYR, and PHE removal and Cu extraction from contaminated soil through phytoremediation [80,81]. The presence of a large root biomass promotes degradation through the action of rhizosphere microorganisms while providing a larger surface area for metal accumulation [82,83].

In this study, the development of *C. sativa* L. biomass was monitored during the 50 d of phytoremediation, measuring the plant height under the PC1, PC2, and PC3 conditions tested (Figure 3.3). The results are summarized in Table 3.2. The height of the plants decreased with the increase of the initial concentration of PYR and PHE (Figure 3.3). The height of the plants grown under the PC3 condition was found to be the lowest, reaching a final value of 10.5 cm (Figure 3.3), about three times lower than the plants grown in the uncontaminated condition. The final heights achieved under the PC1 and PC2 conditions were 26.5 and 20 cm, respectively, which were 35 and

43% lower than those obtained in the uncontaminated controls (Figure 3.3). The most elevated plant (i.e., 35 cm) was detected in the PC0, which was the condition without PHE, PYR, and Cu contamination (Figure 3.3). These results are supported by Cameselle and Gouveia [84], who showed that a high PAH concentration of 300 mg kg^{-1} of soil led to a reduction of turnip (*Brassica rapa*) height from 11 to 9.9 cm after 26 days. Likewise, Reddy et al. [85] obtained a decreased height of tall fescue by 55% in PAH- and HM-contaminated soil (i.e., 150 and 750 mg kg^{-1}, respectively) compared to the uncontaminated control after 61 days of phytoremediation.

In general, by considering the dry biomass of *C. sativa* L., the root growth in the contaminated soil decreased by approximately 25, 65, and 71% (Table 3.2) in PC1, PC2, and PC3, respectively, compared to the uncontaminated soil. The condition PC2 (i.e., 200 mg ΣPAHs kg^{-1}) was found to be the threshold above which a severe biomass reduction was observed (i.e., 65% less, Table 3.2). This result is in agreement with those obtained by Lu et al. [19], who observed a drastic inhibition (i.e., approximately 50%) of tall fescue growth in PYR-contaminated soil (i.e., 200–500 mg·kg^{-1}). This is likely due to contamination with both PAHs and Cu, which hindered plant development [86].

Table 3.2 Fresh and dry biomass (g) of aerial parts and roots of *Cannabis sativa* L. after 50 d of phytoremediation in PC1, PC2, and PC3 (i.e., 100, 200, and 300 mg ΣPAHs kg^{-1}). The obtained values are the means of three replicate analyses and the RSD (residual standard deviations).

Conditions	Aerial parts	Roots	Total
		Fresh weight (g)	
PC0	3.59 ± 0.13	0.59 ± 0.02	2.10 ± 0.01
PC1	3.33 ± 0.14	0.29 ± 0.04	1.81± 0.01
PC2	1.20 ± 0.03	0.22 ± 0.01	0.71± 0.01
PC3	1.05 ± 0.07	0.12 ± 0.02	0.59± 0.05
		Dry weight (g)	
PC0	0.37 ± 0.01	0.06 ± 0.01	0.43± 0.01
PC1	0.29 ± 0.01	0.03 ± 0.01	0.32± 0.01
PC2	0.12 ± 0.02	0.02 ± 0.01	0.15± 0.02
PC3	0.11 ± 0.02	0.016 ± 0.02	0.12± 0.02

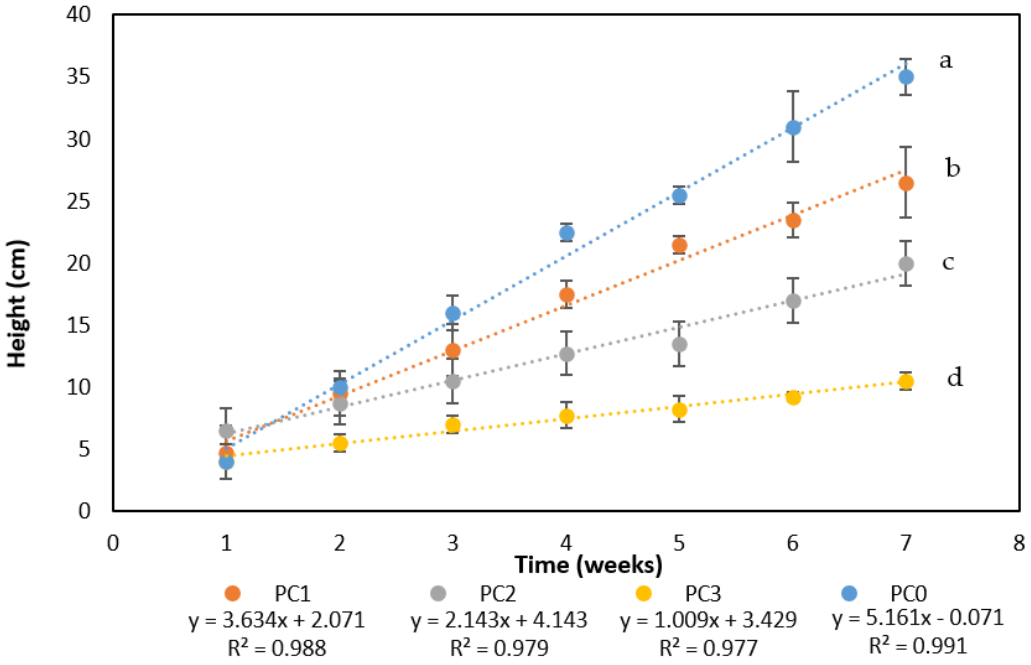


Figure 3.3. Measurements of the height (cm) of *Cannabis sativa* L. during the phytoremediation of PAH- and Cu-contaminated soil (PC1, PC2, and PC3 conditions) and the unspiked control. Values are the means of three replicate analyses. The error bars display the RSD (residual standard deviations). The means are interpolated with linear regressions, which are described by equations and determination coefficients (R^2) on the graph. The same letter represents no significant differences ($p > 0.05$) between the reached height at the end of the experiment.

3.3.4 Preliminary economic evaluation

This study shows that PAH- and Cu-contaminated soil can be remediated through phytoremediation by *C. sativa* L. (Figure 3.2 and Table 3.1). Therefore, a rough economic assessment considering the seed purchase, the required working hours for *C. sativa* L. pre-planting, planting, harvesting, and post-harvesting operations, and the general remediation management was performed.

A total cost of USD 47 per ton was obtained for the PC1 condition (i.e., 100 mg ΣPAHs kg^{-1}, Table 3.3), which achieved a higher PAH and Cu removal, and biomass production (i.e., 78 and 11%, and 0.32 g, respectively) compared to the PC2 and PC3 conditions (Figure 3.2, Table 3.1 and 3.2) after 50 d. In a realistic approach, additional long-term monitoring and in-field studies are needed to verify time and cost data of in

situ phytoremediation since several factors (e.g., autochthonous microorganisms, genetic assortment, and vegetation competition) could severely affect the remediation technique [87,88]. Additionally, the treatment cost of the obtained biomass after each phytoremediation cycle should be considered. In this context, the employment of plant biomass as an energy source is attractive due to further profits [89,90] deriving from bioenergy production (e.g., biogas). For instance, Huence et al. [91] demonstrated that the presence of trace contaminants in plant biomass (e.g., HMs) did not limit their biomethane potential during anaerobic digestion. Moreover, an integrated approach combining soil remediation with post-process valuable elements recovery has been proven to enhance the economic feasibility of the phytoremediation treatment [92].

Table 3.3 Obtained costs for working hours (i.e., pre-plant cultivation, planting, harvesting and post-harvesting operations), management (i.e., field and laboratory work, and general expenses) and seed purchase.

Operations	**Cost (USD per ton)**
Purchase of the *C. sativa* L. seeds	1.35
Pre-plant cultivation Planting Harvesting Post-harvesting	25.30
Management	20.24

The mentioned cost value (i.e., USD 47 per ton, Table 3.3) was slightly higher than that indicated by Cristaldi et al. [93] (i.e., USD 5–40 per ton), who did not take the monitoring costs into account. Indeed, the achieved cost here evaluated can be halved to approximately USD 27 per ton by neglecting the cost of monitoring. In each case, the phytoremediation cost was lower than that necessary for performing other conventional remediation techniques [94,95]. For instance, the employment of physical–chemical techniques (i.e., USD 100–500 per ton), such as soil washing, requires a high cost for extracting agents (e.g., surfactants, chelating agents), which should be further increased by the treatment of spent soil washing solution (e.g., about USD 50 per ton) [24]. Additionally, thermal remediation (e.g., vitrification) can show

a cost of up to USD 425 per ton due to the involvement of a high energy demand to reach the desired temperatures and the need for long-term monitoring [16]. Other solutions such as landfilling are costly (i.e., up to USD 500 per ton) and should be integrated with soil excavation and transportation procedures, as well as biogas production monitoring costs [93].

Considering the removal efficiencies, the application of the conventional Fenton technique resulted in a PAH removal comprised between 43 and 69% after 24 h [96]. Sequential application of chelating agents and innovative surfactants for enhanced electro-remediation exhibited a ∑PAH removal of 43 and 48%, respectively [97]. Up to 40 and 90% of PAHs (i.e., fluorene acenaphthene and fluoranthene) and HMs (i.e., Pb, Ni, and Zn), respectively, were removed after the application of soil flushing with Triton X-100 and EDTA at a flow rate of 0.534 mL min^{-1} through column experiments [98]. Although the higher HM removal efficiencies and shorter application times compared to the phytoremediation, the abovementioned techniques could lead to severe issues towards the ecosystem.

Moreover, it is largely accepted that a conventional remediation process of a site contaminated by hazardous compounds (e.g., PAHs, toxic metals) can also lead to greenhouse gas emissions (e.g., 58,000 ton of CO_2 per year for thermal desorption) [99]. Thus, it is increasingly important to adopt soil remediation approaches entailing the fulfilment of sustainable development principles, promoting energy-saving and low carbon-emitting or even carbon-neutral technologies as much as possible [100].

3.4 Conclusions

This study demonstrates that *C. sativa* L. can grow under stress conditions induced by the co-occurrence of PAHs and Cu in the soil. PHE and PYR removal up to 152 and 54 $mg \cdot kg^{-1}$, respectively, was obtained after a 50 d treatment. Additionally, approximately 57 and 46 mg $Cu \cdot g^{-1}$ of dry *C. sativa* L. biomass were removed under the PC2 and PC1 conditions, respectively. Under the PC2 and PC3 conditions, a severe biomass reduction was achieved (i.e., >65%). Considering the plant height, the biomass decreased with the increase of the initial PYR and PHE concentrations. The

economic assessment revealed a total cost of USD 47 per ton of soil, which was lower than that of conventional remediation techniques.

Although this study provides encouraging preliminary results, the influencing factors to calibrate a future field-scale experiment need further investigation. In order to understand the phytoremediation mechanisms primarily involved in this study, further analyses (e.g., microbiological analyses, plant tissue extractions) are also required. Finally, other studies should be aimed, for instance, at improving phytoremediation with soil conditioners and non-toxic chelating agents (e.g., ethylenediamine-N,N'-disuccinic acid) for enhancing PAH rhizodegradation and metal mobilization, respectively.

CONCLUSIONS

PAHs and PTEs are widespread in the soil, and drawbacks of conventional remediation techniques in addition to the expenditures for alternative disposal of polluted soils lead to search for more effective, environmentally–friendly and sustainable approaches. In this context, the present doctoral thesis showed that it is possible to achieve high efficiencies in terms of PAH (e.g., PYR and PHE) and PTE (i.e., Cu) removal and cost saving by employing phytoremediation.

The thesis demonstrated the removal potential of *Cannabis sativa* L. by reaching 94.52%, 73.50 and 68.22% in the 50, 100 and 150 $mg \cdot kg^{-1}$ PYR contaminated soils, respectively, after 60 days of phytoremediation. The DHO activity, which contributes directly to PYR degradation, ranged between 50 and 110 μg INTF g^{-1} dry soil in planted soils at the end of the experiment, about 10 times higher compared to unplanted controls. Hence, the presence of hemp roots significantly enhanced the activity of the native microbial community in soil responsible for the degradation of PYR. As a proof, the phylogenetic analysis showed that the genus *Pseudomonas* and *Bacteroidota* phylum, associated with PYR degradation, were 2.13% and 5% abundant, respectively, in the analyzed samples. A severe biomass reduction between 41–58 and 28–52% was observed in roots and aerial parts of *C. sativa* L. grown in contaminated conditions compared to uncontaminated control, respectively, demonstrating that PYR could exert toxicity and inhibit the regular functions of living species.

Subsequently, assuming the co-existence of both PAHs and PTEs in the environment as a more complex and frequent condition compared to the presence of a single class of those pollutants, the present thesis evaluated the phytoremediation of PHE, PYR- and Cu-contaminated soil by *C. sativa* L. The

PHE and PYR removal resulted between 93 - 61%, 98 - 48%, and 97 - 36%, respectively, in 100, 200, 300 mg ΣPAHs kg^{-1} and 350 mg Cu kg^{-1} contaminated soils. Besides, the highest Cu extraction amounted to 58 $mg \cdot kg^{-1}$ of *C. sativa* L. dry biomass. The biomass growth of this study was affected more severely by initial co-contamination than in the previous existence of the single PYR in the soil. Indeed, the height reached by the *C. sativa* L. plants after 50 days of growth in soil contaminated with PYR (i.e., 50 mg kg), amounted to 40 cm, while the plant grown in co-contaminated soil (i.e., 50 mg PYR kg^{-1}, 50 mg PHE kg^{-1}, 350 mg Cu kg^{-1}) barely reached 8 cm. The economic assessment revealed a total cost of USD 47 per ton of treated soil, which resulted lower than that of conventional remediation techniques.

Also, regarding the phytoremediation removal efficiency, by increasing the initial contaminant co-concentration, PYR removal efficiency decreased by about 25%. This was probably due to the inhibition of biomass growth as a function of the initial PAH and Cu concentration above a certain threshold and exerted toxicity on microorganisms in the rhizosphere. In this regard, different additives could be employed to further enhance the activity of degraders, including both biostimulation and bioaugmentation. The addition of selected nutrients and additives could be optimal for plant growth, promoting PAH rhizodegradation and metal mobilization, and therefore, a further opportunity for favoring the in−situ restoration. In this context, the use of non-toxic chelating agents capable to enhance PAHs and HMs transformation from an inaccessible to a bioavailable fraction is promising, yet the optimum dose of these substances requires more in-depth studies. In a real-scale field approach, additional long-term monitoring is required to assess time and cost data of in situ phytoremediation since several factors (e.g., autochthonous microorganisms, genetic assortment, and vegetation competition) could severely affect the efficiency of this remediation technique. Finally, further studies should be addressed to evaluate the future fate of the hemp biomass produced during the phytoremediation treatment by maximizing the recovery of energy and resources. The employment of plant biomass, after the

phytoremediation treatment, as an energy resource (e.g., feedstock to anaerobic co-digestion) is attractive due to further profits and contributes to the sustainability of phytoremediation projects.

REFERENCES

[1] J.J. Steffan, E.C. Brevik, L.C. Burgess, A. Cerdà, The effect of soil on human health: an overview, Eur J Soil Sci. 69 (2018) 159–171. https://doi.org/10.1111/ejss.12451.

[2] Y.-N. Kim, K.-A. Lee, M. Lee, K.-R. Kim, Synergetic effect of complex soil amendments to improve soil quality and alleviate toxicity of heavy metal(loid)s in contaminated arable soil: toward securing crop food safety and productivity, Environmental Science and Pollution Research. 29 (2022) 87555–87567. https://doi.org/10.1007/s11356-022-21752-3.

[3] M.D. Kirk, S.M. Pires, R.E. Black, M. Caipo, J.A. Crump, B. Devleesschauwer, D. Döpfer, A. Fazil, C.L. Fischer-Walker, T. Hald, A.J. Hall, K.H. Keddy, R.J. Lake, C.F. Lanata, P.R. Torgerson, A.H. Havelaar, F.J. Angulo, World Health Organization Estimates of the Global and Regional Disease Burden of 22 Foodborne Bacterial, Protozoal, and Viral Diseases, 2010: A Data Synthesis, PLoS Med. 12 (2015) e1001921. https://doi.org/10.1371/journal.pmed.1001921.

[4] G. Genchi, M. Sinicropi, A. Carocci, G. Lauria, A. Catalano, Mercury Exposure and Heart Diseases, Int J Environ Res Public Health. 14 (2017) 74. https://doi.org/10.3390/ijerph14010074.

[5] Y. Yang, S.E. Hobbie, R.R. Hernandez, J. Fargione, S.M. Grodsky, D. Tilman, Y.-G. Zhu, Y. Luo, T.M. Smith, J.M. Jungers, M. Yang, W.-Q. Chen, Restoring Abandoned Farmland to Mitigate Climate Change on a Full Earth, One Earth. 3 (2020) 176–186. https://doi.org/10.1016/j.oneear.2020.07.019.

[6] Q. Wu, W. Mo, J. Liu, S. Peng, Q. Li, R. Wan, Remediation of high-concentration Cr(VI)-contaminated soils with FeSO4 combined with biostimulation: Cr(VI) transformation and stabilization, Journal of Hazardous Materials Advances. 8 (2022) 100161. https://doi.org/10.1016/j.hazadv.2022.100161.

[7] K.-H. Kim, S.A. Jahan, E. Kabir, R.J.C. Brown, A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects, Environ Int. 60 (2013) 71–80. https://doi.org/10.1016/j.envint.2013.07.019.

[8] U. v Okere, https://doi.org/10.1016/j.scitotenv.2019.05.403, J Bioremediat Biodegrad. s1 (2011). https://doi.org/10.4172/2155-6199.S1-006.

[9] R.O. Bua, A. Contino, A. Giuffrida, Polycyclic aromatic hydrocarbons in Mullus surmuletus from the Catania Gulf (Sicily, Italy): distribution and potential health risks, Environmental Science and Pollution Research. 28 (2021) 7756–7765. https://doi.org/10.1007/s11356-020-11052-z.

[10] L. Duan, R. Naidu, P. Thavamani, J. Meaklim, M. Megharaj, Managing long-term polycyclic aromatic hydrocarbon contaminated soils: a risk-based approach, Environmental Science and Pollution Research. 22 (2015) 8927–8941. https://doi.org/10.1007/s11356-013-2270-0.

[11] P. Gao, M. Xu, Y. Liu, E.B. da Silva, P. Xiang, L.Q. Ma, Emerging and legacy PAHs in urban soils of four small cities: Concentrations, distribution, and sources, Science of The Total Environment. 685 (2019) 463–470. https://doi.org/10.1016/j.scitotenv.2019.05.403.

[12] J.T. Andersson, C. Achten, Time to Say Goodbye to the 16 EPA PAHs? Toward an Up-to-Date Use of PACs for Environmental Purposes, Polycycl Aromat Compd. 35 (2015) 330–354. https://doi.org/10.1080/10406638.2014.991042.

[13] Y. Liu, P. Gao, J. Su, E.B. da Silva, L.M. de Oliveira, T. Townsend, P. Xiang, L.Q. Ma, PAHs in urban soils of two Florida cities: Background concentrations, distribution, and sources, Chemosphere. 214 (2019) 220–227. https://doi.org/10.1016/j.chemosphere.2018.09.119.

[14] N.-D. Dat, M.B. Chang, Review on characteristics of PAHs in atmosphere, anthropogenic sources and control technologies, Science of The Total Environment. 609 (2017) 682–693. https://doi.org/10.1016/j.scitotenv.2017.07.204.

[15] H.I. Abdel-Shafy, M.S.M. Mansour, A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation, Egyptian Journal of Petroleum. 25 (2016) 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011.

[16] Y. Jiang, X. Hu, U.J. Yves, H. Zhan, Y. Wu, Status, source and health risk assessment of polycyclic aromatic hydrocarbons in street dust of an industrial city, NW China, Ecotoxicol Environ Saf. (2014). https://doi.org/10.1016/j.ecoenv.2014.04.031.

[17] A. Ukalska-Jaruga, G. Debaene, B. Smreczak, Dissipation and sorption processes of polycyclic aromatic hydrocarbons (PAHs) to organic matter in

soils amended by exogenous rich-carbon material, J Soils Sediments. 20 (2020) 836–849. https://doi.org/10.1007/s11368-019-02455-8.

[18] N. Zhao, F. Ju, Q. Song, Z. Qi, H. Ling, Quantitative assessment of the contribution of soil organic matter functional groups and heteroatoms to PAHs adsorption based on the COSMO-RS model, Science of The Total Environment. 846 (2022) 157415. https://doi.org/10.1016/j.scitotenv.2022.157415.

[19] M. Kuśmierz, P. Oleszczuk, P. Kraska, E. Pałys, S. Andruszczak, Persistence of polycyclic aromatic hydrocarbons (PAHs) in biochar-amended soil, Chemosphere. 146 (2016) 272–279. https://doi.org/10.1016/j.chemosphere.2015.12.010.

[20] L. Borůvka, O. Vacek, J. Jehlička, Principal component analysis as a tool to indicate the origin of potentially toxic elements in soils, Geoderma. 128 (2005) 289–300. https://doi.org/10.1016/j.geoderma.2005.04.010.

[21] J. Zhan, X. Li, P. Christie, L. Wu, A review of soil potentially toxic element contamination in typical karst regions in southwest China, Curr Opin Environ Sci Health. 23 (2021) 100284. https://doi.org/10.1016/j.coesh.2021.100284.

[22] P.O. Oladoye, O.M. Olowe, M.D. Asemoloye, Phytoremediation technology and food security impacts of heavy metal contaminated soils: A review of literature, Chemosphere. 288 (2022) 132555. https://doi.org/10.1016/j.chemosphere.2021.132555.

[23] R. Terzano, I. Rascio, I. Allegretta, C. Porfido, M. Spagnuolo, M.Y. Khanghahi, C. Crecchio, F. Sakellariadou, C.E. Gattullo, Fire effects on the distribution and bioavailability of potentially toxic elements (PTEs) in agricultural soils, Chemosphere. 281 (2021) 130752. https://doi.org/10.1016/j.chemosphere.2021.130752.

[24] A. El-Naggar, S.S. Lee, J. Rinklebe, M. Farooq, H. Song, A.K. Sarmah, A.R. Zimmerman, M. Ahmad, S.M. Shaheen, Y.S. Ok, Biochar application to low fertility soils: A review of current status, and future prospects, Geoderma. 337 (2019) 536–554. https://doi.org/10.1016/j.geoderma.2018.09.034.

[25] X. Xiao, J. Zhang, H. Wang, X. Han, J. Ma, Y. Ma, H. Luan, Distribution and health risk assessment of potentially toxic elements in soils around coal industrial areas: A global meta-analysis, Science of The Total Environment. 713 (2020) 135292. https://doi.org/10.1016/j.scitotenv.2019.135292.

[26] J. Rinklebe, V. Antoniadis, S.M. Shaheen, O. Rosche, M. Altermann, Health risk assessment of potentially toxic elements in soils along the Central Elbe

River, Germany, Environ Int. 126 (2019) 76–88. https://doi.org/10.1016/j.envint.2019.02.011.

[27] A. Freddo, C. Cai, B.J. Reid, Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar, Environmental Pollution. 171 (2012) 18–24. https://doi.org/10.1016/j.envpol.2012.07.009.

[28] P. Mahajan, J. Kaushal, Role of Phytoremediation in Reducing Cadmium Toxicity in Soil and Water, J Toxicol. 2018 (2018) 1–16. https://doi.org/10.1155/2018/4864365.

[29] S. Khalid, M. Shahid, N.K. Niazi, B. Murtaza, I. Bibi, C. Dumat, A comparison of technologies for remediation of heavy metal contaminated soils, J Geochem Explor. 182 (2017) 247–268. https://doi.org/10.1016/j.gexplo.2016.11.021.

[30] C. Yang, Q. Zhou, S. Wei, Y. Hu, Y. Bao, Chemical-Assisted Phytoremediation of Cd-PAHs Contaminated Soils Using Solanum Nigrum L., Int J Phytoremediation. 13 (2011) 818–833. https://doi.org/10.1080/15226514.2010.532179.

[31] Z. Li, Z. Ma, T.J. van der Kuijp, Z. Yuan, L. Huang, A review of soil heavy metal pollution from mines in China: Pollution and health risk assessment, Science of The Total Environment. 468–469 (2014) 843–853. https://doi.org/10.1016/j.scitotenv.2013.08.090.

[32] G. Fazeli, A. Karbassi, S. Khoramnejadian, T. Nasrabadi, Evaluation of Urban Soil Pollution: A Combined Approach of Toxic Metals and Polycyclic Aromatic Hydrocarbons (PAHs), Int J Environ Res. 13 (2019) 801–811. https://doi.org/10.1007/s41742-019-00206-8.

[33] H. Sarma, N.F. Islam, P. Borgohain, A. Sarma, M.N.V. Prasad, Localization of polycyclic aromatic hydrocarbons and heavy metals in surface soil of Asia's oldest oil and gas drilling site in Assam, north-east India: Implications for the bio-economy, Emerg Contam. 2 (2016) 119–127. https://doi.org/10.1016/j.emcon.2016.05.004.

[34] H. Lee, H.-K. Kim, H.-J. Noh, Y.J. Byun, H.-M. Chung, J.-I. Kim, Source identification and assessment of heavy metal contamination in urban soils based on cluster analysis and multiple pollution indices, J Soils Sediments. 21 (2021) 1947–1961. https://doi.org/10.1007/s11368-020-02716-x.

[35] USEPA 2018, Recent Developments for In Situ Treatment of Metal Contaminated Soils, (n.d.).

[36] Z. Li, Z. Ma, T.J. van der Kuijp, Z. Yuan, L. Huang, A review of soil heavy metal pollution from mines in China: Pollution and health risk assessment,

Science of The Total Environment. 468–469 (2014) 843–853. https://doi.org/10.1016/j.scitotenv.2013.08.090.

[37] P. Duan, L. Jiao, J. He, Y. Yang, Effect of dissolved organic matter and heavy metals ions on sorption of phenanthrene at sedimentary particle scale, J Hazard Mater. 436 (2022) 129175. https://doi.org/10.1016/j.jhazmat.2022.129175.

[38] S. Wu, S. Zhou, H. Bao, D. Chen, C. Wang, B. Li, G. Tong, Y. Yuan, B. Xu, Improving risk management by using the spatial interaction relationship of heavy metals and PAHs in urban soil, J Hazard Mater. 364 (2019) 108–116. https://doi.org/10.1016/j.jhazmat.2018.09.094.

[39] Z. Wang, P. Luo, X. Zha, C. Xu, S. Kang, M. Zhou, D. Nover, Y. Wang, Overview assessment of risk evaluation and treatment technologies for heavy metal pollution of water and soil, J Clean Prod. 379 (2022) 134043. https://doi.org/10.1016/j.jclepro.2022.134043.

[40] A. Grzegórska, P. Rybarczyk, A. Rogala, D. Zabrocki, Phytoremediation—From Environment Cleaning to Energy Generation—Current Status and Future Perspectives, Energies (Basel). 13 (2020) 2905. https://doi.org/10.3390/en13112905.

[41] P. Pathak, D. Bhattacharya, Phytoextraction of heavy metals by weeds: Physiological and molecular intervention, in: Handbook of Bioremediation, Elsevier, 2021: pp. 49–59. https://doi.org/10.1016/B978-0-12-819382-2.00004-1.

[42] V. Shah, A. Daverey, Phytoremediation: A multidisciplinary approach to clean up heavy metal contaminated soil, Environ Technol Innov. 18 (2020) 100774. https://doi.org/10.1016/j.eti.2020.100774.

[43] Y. Li, J. Ma, Y. Li, C. Xiao, X. Shen, J. Chen, X. Xia, Nitrogen addition facilitates phytoremediation of PAH-Cd cocontaminated dumpsite soil by altering alfalfa growth and rhizosphere communities, Science of The Total Environment. 806 (2022) 150610. https://doi.org/10.1016/j.scitotenv.2021.150610.

[44] Y. Zhang, M. Li, A. Su, X. Lv, Y. Qiu, Y. Xu, Co-planting improves the phytoremediation efficiency of combined phenanthrene and copper co-contaminated soils, J Clean Prod. 382 (2023) 135380. https://doi.org/10.1016/j.jclepro.2022.135380.

[45] Q. Tao, J. Li, Y. Liu, J. Luo, Q. Xu, B. Li, Q. Li, T. Li, C. Wang, Ochrobactrum intermedium and saponin assisted phytoremediation of Cd and B[a]P co-

contaminated soil by Cd-hyperaccumulator Sedum alfredii, Chemosphere. 245 (2020) 125547. https://doi.org/10.1016/j.chemosphere.2019.125547.

[46] X. Song, C. Li, W. Chen, Phytoremediation potential of Bermuda grass (Cynodon dactylon (L.) pers.) in soils co-contaminated with polycyclic aromatic hydrocarbons and cadmium, Ecotoxicol Environ Saf. 234 (2022) 113389. https://doi.org/10.1016/j.ecoenv.2022.113389.

[47] K. Kumar Yadav, N. Gupta, A. Kumar, L.M. Reece, N. Singh, S. Rezania, S. Ahmad Khan, Mechanistic understanding and holistic approach of phytoremediation: A review on application and future prospects, Ecol Eng. 120 (2018) 274–298. https://doi.org/10.1016/j.ecoleng.2018.05.039.

[48] R. Li, W. Tan, G. Wang, X. Zhao, Q. Dang, H. Yu, B. Xi, Nitrogen addition promotes the transformation of heavy metal speciation from bioavailable to organic bound by increasing the turnover time of organic matter: An analysis on soil aggregate level, Environmental Pollution. 255 (2019) 113170. https://doi.org/10.1016/j.envpol.2019.113170.

[49] K.N. Palansooriya, S.M. Shaheen, S.S. Chen, D.C.W. Tsang, Y. Hashimoto, D. Hou, N.S. Bolan, J. Rinklebe, Y.S. Ok, Soil amendments for immobilization of potentially toxic elements in contaminated soils: A critical review, Environ Int. 134 (2020) 105046. https://doi.org/10.1016/j.envint.2019.105046.

[50] S. Khan, M. Waqas, F. Ding, I. Shamshad, H.P.H. Arp, G. Li, The influence of various biochars on the bioaccessibility and bioaccumulation of PAHs and potentially toxic elements to turnips (Brassica rapa L.), J Hazard Mater. 300 (2015) 243–253. https://doi.org/10.1016/j.jhazmat.2015.06.050.

[51] I. Gul, M. Manzoor, J. Kallerhoff, M. Arshad, Enhanced phytoremediation of lead by soil applied organic and inorganic amendments: Pb phytoavailability, accumulation and metal recovery, Chemosphere. 258 (2020) 127405. https://doi.org/10.1016/j.chemosphere.2020.127405.

[52] A. Khan, S. Khan, M.A. Khan, Z. Qamar, M. Waqas, The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review, Environmental Science and Pollution Research. 22 (2015) 13772–13799. https://doi.org/10.1007/s11356-015-4881-0.

[53] Z. Han, Z. Guo, Y. Zhang, X. Xiao, Z. Xu, Y. Sun, Adsorption-pyrolysis technology for recovering heavy metals in solution using contaminated biomass phytoremediation, Resour Conserv Recycl. 129 (2018) 20–26. https://doi.org/10.1016/j.resconrec.2017.10.003.

[54] P. Cozma, R.-M. Hlihor, M. Rosca, M. Minut, M. Diaconu, M. Gavrilescu, Coupling Phytoremediation with Plant Biomass Valorisation and Metal Recovery: an Overview, in: 2021 International Conference on E-Health and Bioengineering (EHB), IEEE, 2021: pp. 1–4. https://doi.org/10.1109/EHB52898.2021.9657551.

[55] V.-N. Edgar, F.-L. Fabián, P.-C.J. Mario, V.-R. Ileana, Coupling Plant Biomass Derived from Phytoremediation of Potential Toxic-Metal-Polluted Soils to Bioenergy Production and High-Value by-Products—A Review, Applied Sciences. 11 (2021) 2982. https://doi.org/10.3390/app11072982.

[56] Y. Li, J. Ma, Y. Li, C. Xiao, X. Shen, J. Chen, X. Xia, Nitrogen addition facilitates phytoremediation of PAH-Cd cocontaminated dumpsite soil by altering alfalfa growth and rhizosphere communities, Science of The Total Environment. 806 (2022) 150610. https://doi.org/10.1016/j.scitotenv.2021.150610.

[57] I. Gabriele, M. Race, S. Papirio, G. Esposito, Phytoremediation of pyrene-contaminated soils: A critical review of the key factors affecting the fate of pyrene, J Environ Manage. (2021). https://doi.org/10.1016/j.jenvman.2021.112805.

[58] N. Hattab-Hambli, M. Motelica-Heino, M. Mench, Aided phytoextraction of Cu, Pb, Zn, and As in copper-contaminated soils with tobacco and sunflower in crop rotation: Mobility and phytoavailability assessment, Chemosphere. 145 (2016) 543–550. https://doi.org/10.1016/j.chemosphere.2015.11.051.

[59] O. Sytar, S. Ghosh, H. Malinska, M. Zivcak, M. Brestic, Physiological and molecular mechanisms of metal accumulation in hyperaccumulator plants, Physiol Plant. (2020) ppl.13285. https://doi.org/10.1111/ppl.13285.

[60] J. Li, H.K. Gurajala, L. Wu, A. van der Ent, R. Qiu, A.J.M. Baker, Y. Tang, X. Yang, W. Shu, Hyperaccumulator Plants from China: A Synthesis of the Current State of Knowledge, Environ Sci Technol. 52 (2018) 11980–11994. https://doi.org/10.1021/acs.est.8b01060.

[61] X.E. Yang, X.X. Long, H.B. Ye, Z.L. He, D.V. Calvert, P.J. Stoffella, Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (Sedum alfredii Hance), Plant Soil. 259 (2004) 181–189. https://doi.org/10.1023/B:PLSO.0000020956.24027.f2.

[62] M. Shrivastava, A. Khandelwal, S. Srivastava, Heavy Metal Hyperaccumulator Plants: The Resource to Understand the Extreme Adaptations of Plants Towards

Heavy Metals, in: Plant-Metal Interactions, Springer International Publishing, Cham, 2019: pp. 79–97. https://doi.org/10.1007/978-3-030-20732-8_5.

[63] G. DalCorso, E. Fasani, A. Manara, G. Visioli, A. Furini, Heavy Metal Pollutions: State of the Art and Innovation in Phytoremediation, Int J Mol Sci. 20 (2019) 3412. https://doi.org/10.3390/ijms20143412.

[64] Z.-Z. An, Z.-C. Huang, M. Lei, X.-Y. Liao, Y.-M. Zheng, T.-B. Chen, Zinc tolerance and accumulation in Pteris vittata L. and its potential for phytoremediation of Zn- and As-contaminated soil, Chemosphere. 62 (2006) 796–802. https://doi.org/10.1016/j.chemosphere.2005.04.084.

[65] I.E. Zaheer, S. Ali, M. Rizwan, M. Farid, M.B. Shakoor, R.A. Gill, U. Najeeb, N. Iqbal, R. Ahmad, Citric acid assisted phytoremediation of copper by Brassica napus L., Ecotoxicol Environ Saf. 120 (2015) 310–317. https://doi.org/10.1016/j.ecoenv.2015.06.020.

[66] Š. Petrová, J. Rezek, P. Soudek, T. Vaněk, Preliminary study of phytoremediation of brownfield soil contaminated by PAHs, Science of The Total Environment. 599–600 (2017) 572–580. https://doi.org/10.1016/j.scitotenv.2017.04.163.

[67] J.J.A. Dominguez, C. Inoue, M.-F. Chien, Hydroponic approach to assess rhizodegradation by sudangrass (Sorghum x drummondii) reveals pH- and plant age-dependent variability in bacterial degradation of polycyclic aromatic hydrocarbons (PAHs), J Hazard Mater. 387 (2020) 121695. https://doi.org/10.1016/j.jhazmat.2019.121695.

[68] C. Liao, X. Liang, G. Lu, T. Thai, W. Xu, Z. Dang, Effect of surfactant amendment to PAHs-contaminated soil for phytoremediation by maize (Zea mays L.), Ecotoxicol Environ Saf. 112 (2015) 1–6. https://doi.org/10.1016/j.ecoenv.2014.10.025.

[69] S.Č. Alagić, V.P.S. Jovanović, V.D. Mitić, J.S. Cvetković, G.M. Petrović, G.S. Stojanović, Bioaccumulation of HMW PAHs in the roots of wild blackberry from the Bor region (Serbia): Phytoremediation and biomonitoring aspects, Science of The Total Environment. 562 (2016) 561–570. https://doi.org/10.1016/j.scitotenv.2016.04.063.

[70] Y. Sun, Q. Zhou, Y. Xu, L. Wang, X. Liang, Phytoremediation for co-contaminated soils of benzo[a]pyrene (B[a]P) and heavy metals using ornamental plant Tagetes patula, J Hazard Mater. 186 (2011) 2075–2082. https://doi.org/10.1016/j.jhazmat.2010.12.116.

[71] G. Li, Z. Wang, Y. Lv, S. Jia, F. Chen, Y. Liu, L. Huang, Effect of culturing ryegrass (Lolium perenne L.) on Cd and pyrene removal and bacteria variations in co-contaminated soil, Environ Technol Innov. 24 (2021) 101963. https://doi.org/10.1016/j.eti.2021.101963.

[72] C. Chigbo, L. Batty, Chelate-assisted phytoremediation of Cu-pyrene-contaminated soil using Z. mays, Water Air Soil Pollut. (2015). https://doi.org/10.1007/s11270-014-2277-2.

[73] C. Chigbo, L. Batty, R. Bartlett, Interactions of copper and pyrene on phytoremediation potential of Brassica juncea in copper-pyrene co-contaminated soil, Chemosphere. (2013). https://doi.org/10.1016/j.chemosphere.2012.11.007.

[74] Q. Lin, K.L. Shen, H.M. Zhao, W.H. Li, Growth response of Zea mays L. in pyrene-copper co-contaminated soil and the fate of pollutants, J Hazard Mater. (2008). https://doi.org/10.1016/j.jhazmat.2007.04.132.

[75] N. Jeelani, W. Yang, L. Xu, Y. Qiao, S. An, X. Leng, Phytoremediation potential of Acorus calamus in soils co-contaminated with cadmium and polycyclic aromatic hydrocarbons, Sci Rep. (2017). https://doi.org/10.1038/s41598-017-07831-3.

[76] N. Li, R. Liu, J. Chen, J. Wang, L. Hou, Y. Zhou, Enhanced phytoremediation of PAHs and cadmium contaminated soils by a Mycobacterium, Science of the Total Environment. (2021). https://doi.org/10.1016/j.scitotenv.2020.141198.

[77] Z. Zhang, Z. Rengel, H. Chang, K. Meney, L. Pantelic, R. Tomanovic, Phytoremediation potential of Juncus subsecundus in soils contaminated with cadmium and polynuclear aromatic hydrocarbons (PAHs), Geoderma. (2012). https://doi.org/10.1016/j.geoderma.2012.01.020.

[78] Y. Sun, Q. Zhou, Y. Xu, L. Wang, X. Liang, Phytoremediation for co-contaminated soils of benzo[a]pyrene (B[a]P) and heavy metals using ornamental plant Tagetes patula, J Hazard Mater. (2011). https://doi.org/10.1016/j.jhazmat.2010.12.116.

[79] K. Wang, J. Zhang, Z. Zhu, H. Huang, T. Li, Z. He, X. Yang, A. Alva, Pig manure vermicompost (PMVC) can improve phytoremediation of Cd and PAHs co-contaminated soil by Sedum alfredii, J Soils Sediments. 12 (2012) 1089–1099. https://doi.org/10.1007/s11368-012-0539-4.

[80] M. Lu, Z.Z. Zhang, J.X. Wang, M. Zhang, Y.X. Xu, X.J. Wu, Interaction of heavy metals and pyrene on their fates in soil and tall fescue (Festuca arundinacea), Environ Sci Technol. (2014). https://doi.org/10.1021/es403337t.

[81] X. Chen, X. Liu, X. Zhang, L. Cao, X. Hu, Phytoremediation effect of Scirpus triqueter inoculated plant-growth-promoting bacteria (PGPB) on different fractions of pyrene and Ni in co-contaminated soils, J Hazard Mater. (2017). https://doi.org/10.1016/j.jhazmat.2016.12.009.

[82] X. Liu, S. Shen, X. Zhang, X. Chen, R. Jin, X. Li, Effect of enhancers on the phytoremediation of soils polluted by pyrene and Ni using Sudan grass (Sorghum sudanense (Piper) Stapf.), Environmental Science and Pollution Research. (2020). https://doi.org/10.1007/s11356-020-09934-3.

[83] X. Liu, L. Cao, X. Zhang, J. Chen, Z. Huo, Y. Mao, Influence of alkyl polyglucoside, citric acid, and nitrilotriacetic acid on phytoremediation in pyrene-Pb co-contaminated soils, Int J Phytoremediation. (2018). https://doi.org/10.1080/15226514.2018.1460305.

[84] Y. Li, T. Xie, Y. Zha, W. Du, Y. Yin, H. Guo, Urea-enhanced phytoremediation of cadmium with willow in pyrene and cadmium contaminated soil, J Hazard Mater. (2020). https://doi.org/10.1016/j.jhazmat.2020.124257.

[85] X. Liu, Y. Mao, X. Zhang, P. Gu, Y. Niu, X. Chen, Effects of PASP/NTA and TS on the phytoremediation of pyrene-nickel contaminated soil by Bidens pilosa L., Chemosphere. (2019). https://doi.org/10.1016/j.chemosphere.2019.124502.

[86] C. Chigbo, L. Batty, Phytoremediation for co-contaminated soils of chromium and benzo[a]pyrene using Zea mays L., Environmental Science and Pollution Research. 21 (2014) 3051–3059. https://doi.org/10.1007/s11356-013-2254-0.

[87] X. Liu, X. Hu, X. Zhang, X. Chen, J. Chen, X. Yuan, Effect of Bacillus subtilis and NTA-APG on pyrene dissipation in phytoremediation of nickel co-contaminated wetlands by Scirpus triqueter, Ecotoxicol Environ Saf. (2018). https://doi.org/10.1016/j.ecoenv.2018.02.028.

[88] T. Chen, X. Liu, X. Zhang, Y. Hou, X. Chen, K. Tao, Enhanced Scirpus triqueter phytoremediation of pyrene and lead co-contaminated soil with alkyl polyglucoside and nitrilotriacetic acid combined application, J Soils Sediments. (2016). https://doi.org/10.1007/s11368-016-1394-5.

[89] R. Chen, Z. Zhou, Y. Liu, J. Jiang, Q. Li, H. Song, D. Pei, H. Xu, Mycoremediation potential and tolerance responses of Oudemansiella radicata in cadmium-pyrene co-contaminated soil, J Soils Sediments. 15 (2015) 1083–1093. https://doi.org/10.1007/s11368-015-1093-7.

[90] Y.Y. Sun, H.X. Xu, J.H. Li, X.Q. Shi, J.C. Wu, R. Ji, H.Y. Guo, Phytoremediation of soils contaminated with phenanthrene and cadmium by

growing willow (Salix × Aureo - Pendula CL 'j1011'), Int J Phytoremediation. 18 (2016) 150–156. https://doi.org/10.1080/15226514.2015.1073668.

[91] D. Kluk, T. Steliga, Potential of Helianthus annuus for phytoremediation of lead, zinc, total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) contaminated soil, Nafta-Gaz. 75 (2019) 379–387. https://doi.org/10.18668/NG.2019.07.01.

[92] L.C. Batty, M. Anslow, Effect of a Polycyclic Aromatic Hydrocarbon on the Phytoremediation of Zinc by Two Plant Species (Brassica Juncea and Festuca Arundinacea), Int J Phytoremediation. 10 (2008) 236–251. https://doi.org/10.1080/15226510801997549.

[93] X. Cao, X. Cui, M. Xie, R. Zhao, L. Xu, S. Ni, Z. Cui, Amendments and bioaugmentation enhanced phytoremediation and micro-ecology for PAHs and heavy metals co-contaminated soils, J Hazard Mater. 426 (2022) 128096. https://doi.org/10.1016/j.jhazmat.2021.128096.

[94] N. Jeelani, W. Yang, Y. Qiao, J. Li, S. An, X. Leng, Individual and combined effects of cadmium and polycyclic aromatic hydrocarbons on the phytoremediation potential of Xanthium sibiricum in co-contaminated soil, Int J Phytoremediation. 20 (2018) 773–779. https://doi.org/10.1080/15226514.2018.1425666.

[95] S. Roy, S. Labelle, P. Mehta, A. Mihoc, N. Fortin, C. Masson, R. Leblanc, G. Châteauneuf, C. Sura, C. Gallipeau, C. Olsen, S. Delisle, M. Labrecque, C.W. Greer, Phytoremediation of heavy metal and PAH-contaminated brownfield sites, Plant Soil. 272 (2005) 277–290. https://doi.org/10.1007/s11104-004-5295-9.

[96] V. SHEORAN, A.S. SHEORAN, P. POONIA, Factors Affecting Phytoextraction: A Review, Pedosphere. 26 (2016) 148–166. https://doi.org/10.1016/S1002-0160(15)60032-7.

[97] W. Adams, R. Blust, R. Dwyer, D. Mount, E. Nordheim, P.H. Rodriguez, D. Spry, Bioavailability Assessment of Metals in Freshwater Environments: A Historical Review, Environ Toxicol Chem. 39 (2020) 48–59. https://doi.org/10.1002/etc.4558.

[98] M.N. v. Prasad, Phytoremediation of Metal-Polluted Ecosystems: Hype for Commercialization, Russian Journal of Plant Physiology. 50 (2003) 686–701. https://doi.org/10.1023/A:1025604627496.

[99] R.-Y. Kim, J.-K. Yoon, T.-S. Kim, J.E. Yang, G. Owens, K.-R. Kim, Bioavailability of heavy metals in soils: definitions and practical

implementation—a critical review, Environ Geochem Health. 37 (2015) 1041–1061. https://doi.org/10.1007/s10653-015-9695-y.

[100] C. Zhang, Z. Yu, G. Zeng, M. Jiang, Z. Yang, F. Cui, M. Zhu, L. Shen, L. Hu, Effects of sediment geochemical properties on heavy metal bioavailability, Environ Int. 73 (2014) 270–281. https://doi.org/10.1016/j.envint.2014.08.010.

[101] E. Kurek, M. Majewska, Microbially Mediated Transformations of Heavy Metals in Rhizosphere, in: Toxicity of Heavy Metals to Legumes and Bioremediation, Springer Vienna, Vienna, 2012: pp. 129–146. https://doi.org/10.1007/978-3-7091-0730-0_8.

[102] M. Shenker, T.W. -M. Fan, D.E. Crowley, Phytosiderophores Influence on Cadmium Mobilization and Uptake by Wheat and Barley Plants, J Environ Qual. 30 (2001) 2091–2098. https://doi.org/10.2134/jeq2001.2091.

[103] A. Ullah, S. Heng, M.F.H. Munis, S. Fahad, X. Yang, Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: A review, Environ Exp Bot. 117 (2015) 28–40. https://doi.org/10.1016/j.envexpbot.2015.05.001.

[104] M. Gavrilescu, Enhancing phytoremediation of soils polluted with heavy metals, Curr Opin Biotechnol. 74 (2022) 21–31. https://doi.org/10.1016/j.copbio.2021.10.024.

[105] N. Abdu, A.A. Abdullahi, A. Abdulkadir, Heavy metals and soil microbes, Environ Chem Lett. 15 (2017) 65–84. https://doi.org/10.1007/s10311-016-0587-x.

[106] A.G. Babu, J.-D. Kim, B.-T. Oh, Enhancement of heavy metal phytoremediation by Alnus firma with endophytic Bacillus thuringiensis GDB-1, J Hazard Mater. 250–251 (2013) 477–483. https://doi.org/10.1016/j.jhazmat.2013.02.014.

[107] S. Zaidi, S. Usmani, B.R. Singh, J. Musarrat, Significance of Bacillus subtilis strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in Brassica juncea, Chemosphere. 64 (2006) 991–997. https://doi.org/10.1016/j.chemosphere.2005.12.057.

[108] L. Wang, H. Lin, Y. Dong, B. Li, Y. He, Effects of endophytes inoculation on rhizosphere and endosphere microecology of Indian mustard (Brassica juncea) grown in vanadium-contaminated soil and its enhancement on phytoremediation, Chemosphere. 240 (2020) 124891. https://doi.org/10.1016/j.chemosphere.2019.124891.

[109] C. Sangthong, K. Setkit, B. Prapagdee, Improvement of cadmium phytoremediation after soil inoculation with a cadmium-resistant Micrococcus sp., Environmental Science and Pollution Research. 23 (2016) 756–764. https://doi.org/10.1007/s11356-015-5318-5.

[110] J.C. Mendoza-Hernández, O.R. Vázquez-Delgado, M. Castillo-Morales, J.L. Varela-Caselis, J.D. Santamaría-Juárez, O. Olivares-Xometl, J. Arriola Morales, G. Pérez-Osorio, Phytoremediation of mine tailings by Brassica juncea inoculated with plant growth-promoting bacteria, Microbiol Res. 228 (2019) 126308. https://doi.org/10.1016/j.micres.2019.126308.

[111] Y. You, J. Dou, Y. Xue, N. Jin, K. Yang, Chelating Agents in Assisting Phytoremediation of Uranium-Contaminated Soils: A Review, Sustainability. 14 (2022) 6379. https://doi.org/10.3390/su14106379.

[112] S. Dipu, A.A. Kumar, S.G. Thanga, Effect of chelating agents in phytoremediation of heavy metals, Remediation Journal. 22 (2012) 133–146. https://doi.org/10.1002/rem.21304.

[113] Firdaus-e-Bareen, Chelate Assisted Phytoextraction Using Oilseed Brassicas, in: 2012: pp. 289–311. https://doi.org/10.1007/978-94-007-3913-0_11.

[114] C. Kaur, B. Bhandari, A. Srivastava, V.P. Singh, Rhizobacteria Versus Chelating Agents: Tool for Phytoremediation, in: 2020: pp. 249–266. https://doi.org/10.1007/978-981-15-2679-4_9.

[115] S. Muthusaravanan, N. Sivarajasekar, J.S. Vivek, S. Vasudha Priyadharshini, T. Paramasivan, N. Dhakal, Mu. Naushad, Research Updates on Heavy Metal Phytoremediation: Enhancements, Efficient Post-harvesting Strategies and Economic Opportunities, in: 2020: pp. 191–222. https://doi.org/10.1007/978-3-030-17724-9_9.

[116] U. Song, H. Park, Importance of biomass management acts and policies after phytoremediation, J Ecol Environ. 41 (2017) 13. https://doi.org/10.1186/s41610-017-0033-4.

[117] M. Vigil, M.F. Marey-Pérez, G. Martinez Huerta, V. Álvarez Cabal, Is phytoremediation without biomass valorization sustainable? — Comparative LCA of landfilling vs. anaerobic co-digestion, Science of The Total Environment. 505 (2015) 844–850. https://doi.org/10.1016/j.scitotenv.2014.10.047.

[118] Z. Liu, K.-Q. Tran, A review on disposal and utilization of phytoremediation plants containing heavy metals, Ecotoxicol Environ Saf. 226 (2021) 112821. https://doi.org/10.1016/j.ecoenv.2021.112821.

[119] Y. Jiang, M. Lei, L. Duan, P. Longhurst, Integrating phytoremediation with biomass valorisation and critical element recovery: A UK contaminated land perspective, Biomass Bioenergy. 83 (2015) 328–339. https://doi.org/10.1016/j.biombioe.2015.10.013.

[120] W. Fang, Y. Wei, J. Liu, Comparative characterization of sewage sludge compost and soil: Heavy metal leaching characteristics, J Hazard Mater. 310 (2016) 1–10. https://doi.org/10.1016/j.jhazmat.2016.02.025.

[121] M. Hejna, E. Onelli, A. Moscatelli, M. Bellotto, C. Cristiani, N. Stroppa, L. Rossi, Heavy-Metal Phytoremediation from Livestock Wastewater and Exploitation of Exhausted Biomass, Int J Environ Res Public Health. 18 (2021) 2239. https://doi.org/10.3390/ijerph18052239.

[122] Y. Tian, H. Zhang, Producing biogas from agricultural residues generated during phytoremediation process: Possibility, threshold, and challenges, Int J Green Energy. 13 (2016) 1556–1563. https://doi.org/10.1080/15435075.2016.1206017.

[123] F.R. Amin, H. Khalid, H. Zhang, S. u Rahman, R. Zhang, G. Liu, C. Chen, Pretreatment methods of lignocellulosic biomass for anaerobic digestion, AMB Express. 7 (2017) 72. https://doi.org/10.1186/s13568-017-0375-4.

[124] N. Hamid, J.H. Syed, M. Junaid, A. Mahmood, J. Li, G. Zhang, R.N. Malik, Elucidating the urban levels, sources and health risks of polycyclic aromatic hydrocarbons (PAHs) in Pakistan: Implications for changing energy demand, Science of the Total Environment. (2018). https://doi.org/10.1016/j.scitotenv.2017.11.080.

[125] A. Cachada, E. Ferreira da Silva, A.C. Duarte, R. Pereira, Risk assessment of urban soils contamination: The particular case of polycyclic aromatic hydrocarbons, Science of the Total Environment. (2016). https://doi.org/10.1016/j.scitotenv.2016.02.012.

[126] Y. Hu, Y. Xia, F. Di Maio, F. Yu, W. Yu, Investigation of polycyclic aromatic hydrocarbons (PAHs) formed in three-phase products from the pyrolysis of various wastewater sewage sludge, J Hazard Mater. (2020). https://doi.org/10.1016/j.jhazmat.2020.122045.

[127] F. Bianco, M. Race, S. Papirio, G. Esposito, Removal of polycyclic aromatic hydrocarbons during anaerobic biostimulation of marine sediments, Science of the Total Environment. (2020). https://doi.org/10.1016/j.scitotenv.2019.136141.

[128] B. Gupta, S. Puri, I.S. Thakur, J. Kaur, Enhanced pyrene degradation by a biosurfactant producing Acinetobacter baumannii BJ5: Growth kinetics, toxicity and substrate inhibition studies, Environ Technol Innov. (2020). https://doi.org/10.1016/j.eti.2020.100804.

[129] T.R. Sun, L. Cang, Q.Y. Wang, D.M. Zhou, J.M. Cheng, H. Xu, Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil, J Hazard Mater. (2010). https://doi.org/10.1016/j.jhazmat.2009.11.124.

[130] A. Polachova, T. Gramblicka, O. Parizek, R.J. Sram, M. Stupak, J. Hajslova, J. Pulkrabova, Estimation of human exposure to polycyclic aromatic hydrocarbons (PAHs) based on the dietary and outdoor atmospheric monitoring in the Czech Republic, Environ Res. (2020). https://doi.org/10.1016/j.envres.2019.108977.

[131] USEPA, Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons, Office of Research and Development. (1993).

[132] J.T. Andersson, C. Achten, Time to Say Goodbye to the 16 EPA PAHs? Toward an Up-to-Date Use of PACs for Environmental Purposes, Polycycl Aromat Compd. (2015). https://doi.org/10.1080/10406638.2014.991042.

[133] L.H. Keith, The Source of U.S. EPA's Sixteen PAH Priority Pollutants, Polycycl Aromat Compd. (2015). https://doi.org/10.1080/10406638.2014.892886.

[134] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures., IARC Monographs on the Evaluation of Carcinogenic Risks to Humans / World Health Organization, International Agency for Research on Cancer. (2010).

[135] Y. Zhang, V. Virjamo, W. Du, Y. Yin, K. Nissinen, L. Nybakken, H. Guo, R. Julkunen-Tiitto, Effects of soil pyrene contamination on growth and phenolics in Norway spruce (Picea abies) are modified by elevated temperature and CO2, Environmental Science and Pollution Research. (2018). https://doi.org/10.1007/s11356-018-1564-7.

[136] H. Lu, W. Wang, F. Li, L. Zhu, Mixed-surfactant-enhanced phytoremediation of PAHs in soil: Bioavailability of PAHs and responses of microbial community structure, Science of the Total Environment. (2019). https://doi.org/10.1016/j.scitotenv.2018.10.385.

[137] E. V. Yakovleva, D.N. Gabov, B.M. Kondratenok, Y.A. Dubrovskiy, Two-Year Monitoring of PAH in the Soils and Pleurozium schreberi under the Impact of Coal Mining, Polycycl Aromat Compd. (2020). https://doi.org/10.1080/10406638.2019.1709213.

[138] D. Dong, P. Li, X. Li, Q. Zhao, Y. Zhang, C. Jia, P. Li, Investigation on the photocatalytic degradation of pyrene on soil surfaces using nanometer anatase TiO2 under UV irradiation, J Hazard Mater. (2010). https://doi.org/10.1016/j.jhazmat.2009.09.132.

[139] A.C. Umeh, R. Naidu, O.J. Owojori, K.T. Semple, Bioavailability and Bioaccessibility of Hydrophobic Organic Contaminants in Soil and Associated Desorption-Based Measurements, in: 2020. https://doi.org/10.1007/698_2020_521.

[140] Sakshi, S.K. Singh, A.K. Haritash, Polycyclic aromatic hydrocarbons: soil pollution and remediation, International Journal of Environmental Science and Technology. (2019). https://doi.org/10.1007/s13762-019-02414-3.

[141] J. Truu, M. Truu, M. Espenberg, H. Nõlvak, J. Juhanson, Phytoremediation and Plant-Assisted Bioremediation in Soil and Treatment Wetlands: A Review, Open Biotechnol J. (2015). https://doi.org/10.2174/1874070701509010085.

[142] G.F.C. Lama, A. Errico, S. Francalanci, L. Solari, F. Preti, G.B. Chirico, Evaluation of flow resistance models based on field experiments in a partly vegetated reclamation channel, Geosciences (Switzerland). (2020). https://doi.org/10.3390/geosciences10020047.

[143] L. Kim, H.J. Jeon, Y.C. Kim, S.H. Yang, H. Choi, T.O. Kim, S.E. Lee, Monitoring polycyclic aromatic hydrocarbon concentrations and distributions in rice paddy soils from Gyeonggi-do, Ulsan, and Pohang, Appl Biol Chem. (2019). https://doi.org/10.1186/s13765-019-0423-7.

[144] H. Bao, S. Hou, H. Niu, K. Tian, X. Liu, F. Wu, Status, sources, and risk assessment of polycyclic aromatic hydrocarbons in urban soils of Xi'an, China, Environmental Science and Pollution Research. (2018). https://doi.org/10.1007/s11356-018-1928-z.

[145] M.C. Wang, Y.T. Chen, S.H. Chen, S.W. Chang Chien, S. V. Sunkara, Phytoremediation of pyrene contaminated soils amended with compost and planted with ryegrass and alfalfa, Chemosphere. (2012). https://doi.org/10.1016/j.chemosphere.2011.12.063.

[146] J. Yan, L. Wang, P.P. Fu, H. Yu, Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list, Mutat Res Genet Toxicol Environ Mutagen. (2004). https://doi.org/10.1016/j.mrgentox.2003.10.004.

[147] M.D. Larrañaga, R.J. Lewis, R.A. Lewis, Hawley's Condensed Chemical Dictionary, Sixteenth Edition, 2016. https://doi.org/10.1002/9781119312468.

[148] Y. Gao, C.D. Collins, Uptake pathways of polycyclic aromatic hydrocarbons in white clover, Environ Sci Technol. (2009). https://doi.org/10.1021/es900662d.

[149] W.M. Haynes, CRC Handbook of Chemistry and Physics 95th Edition (Internet Version 2015), 2015.

[150] S.H. Yalkowsky, Y. He, P. Jain, Handbook of Aqueous Solubility Data - Second edition, 2010.

[151] X. Zhu, W. Wang, D.E. Crowley, K. Sun, S. Hao, M.G. Waigi, Y. Gao, The endophytic bacterium Serratia sp. PW7 degrades pyrene in wheat, Environmental Science and Pollution Research. (2017). https://doi.org/10.1007/s11356-016-8345-y.

[152] S. Yousefinejad, F. Honarasa, M. Chaabi, New relationship models for solvent-pyrene solubility based on molecular structure and empirical properties, New Journal of Chemistry. (2016). https://doi.org/10.1039/c6nj02319c.

[153] J. Reza, A. Trejo, Temperature dependence of the infinite dilution activity coefficient and Henry's law constant of polycyclic aromatic hydrocarbons in water, Chemosphere. (2004). https://doi.org/10.1016/j.chemosphere.2004.04.020.

[154] J. Liu, H. Su, X. Wei, J. Xue, Progress in Bioremediation of Pyrene, Recent Innovations in Chemical Engineering (Formerly Recent Patents on Chemical Engineering). (2020). https://doi.org/10.2174/2405520412666191009104644.

[155] J.J. Nam, A.J. Sweetman, K.C. Jones, Polynuclear aromatic hydrocarbons (PAHs) in global background soils, Journal of Environmental Monitoring. (2009). https://doi.org/10.1039/b813841a.

[156] S. Sushkova, T. Minkina, I. Deryabkina, V. Rajput, E. Antonenko, O. Nazarenko, B.K. Yadav, E. Hakki, D. Mohan, Environmental pollution of soil with PAHs in energy producing plants zone, Science of the Total Environment. (2019). https://doi.org/10.1016/j.scitotenv.2018.11.080.

[157] N.A.O. Offiong, E.J. Inam, H.S. Etuk, J.P. Essien, Current status and challenges of remediating petroleum-derived PAHs in soils: Nigeria as a case study for developing countries, Remediation. (2019). https://doi.org/10.1002/rem.21630.

[158] N. Ozaki, T. Kindaichi, A. Ohashi, PAHs emission source analysis for air and water environments by isomer ratios — Comparison by modified Cohen's d, Science of the Total Environment. (2020). https://doi.org/10.1016/j.scitotenv.2020.136831.

[159] M.B. Yunker, R.W. Macdonald, R. Vingarzan, R.H. Mitchell, D. Goyette, S. Sylvestre, PAHs in the Fraser River basin: A critical appraisal of PAH ratios as indicators of PAH source and composition, Org Geochem. (2002). https://doi.org/10.1016/S0146-6380(02)00002-5.

[160] M. Liu, J. Hu, Y. Lin, H. Ke, J. Lian, Y. Xu, K. Chen, H. Zheng, M. Chen, M. Cai, Full-depth profiles of PAHs in the Western South China Sea: Influence of Upwelling and Mesoscale Eddy, Chemosphere. (2021). https://doi.org/10.1016/j.chemosphere.2020.127933.

[161] K. Honda, M. Mizukami, Y. Ueda, N. Hamada, N. Seike, Residue level of polycyclic aromatic hydrocarbons in Japanese paddy soils from 1959 to 2002, Chemosphere. (2007). https://doi.org/10.1016/j.chemosphere.2007.03.040.

[162] H. Jia, D. Hou, M. Dai, H. Lu, C. Yan, Effects of root exudates on the mobility of pyrene in mangrove sediment-water system, Catena (Amst). (2018). https://doi.org/10.1016/j.catena.2017.10.022.

[163] L.C. Sousa, C.C.S. Rodrigues, R.A. Mendes, J.A.M. Corrêa, PAH Profiles in Suspended Particulate Matter from an Urbanized River Within the Brazilian Amazon, Bull Environ Contam Toxicol. (2020). https://doi.org/10.1007/s00128-020-02912-y.

[164] H.B. Zhang, Y.M. Luo, M.H. Wong, Q.G. Zhao, G.L. Zhang, Distributions and Concentrations of PAHs in Hong Kong Soils, Environmental Pollution. (2006). https://doi.org/10.1016/j.envpol.2005.08.031.

[165] J.J. Nam, G.O. Thomas, F.M. Jaward, E. Steinnes, O. Gustafsson, K.C. Jones, PAHs in background soils from Western Europe: Influence of atmospheric deposition and soil organic matter, Chemosphere. (2008). https://doi.org/10.1016/j.chemosphere.2007.08.010.

[166] G.Y. Yang, T. Bin Zhang, S.T. Gao, Z.X. Guo, H.F. Wan, Y.X. Gao, Source and distribution characteristics of polycyclic aromatic hydrocarbons in agricultural soils in the Pearl River Delta, Huanjing Kexue/Environmental Science. (2007).

[167] J. Jia, C. Bi, X. Guo, X. Wang, X. Zhou, Z. Chen, Characteristics, identification, and potential risk of polycyclic aromatic hydrocarbons in road dusts and agricultural soils from industrial sites in Shanghai, China, Environmental

Science and Pollution Research. (2017). https://doi.org/10.1007/s11356-016-7818-3.

[168] Y.F. Jiang, X.T. Wang, F. Wang, Y. Jia, M.H. Wu, G.Y. Sheng, J.M. Fu, Levels, composition profiles and sources of polycyclic aromatic hydrocarbons in urban soil of Shanghai, China, Chemosphere. (2009). https://doi.org/10.1016/j.chemosphere.2009.01.027.

[169] M.M. Gune, W.L. Ma, S. Sampath, W. Li, Y.F. Li, H.N. Udayashankar, K. Balakrishna, Z. Zhang, Occurrence of polycyclic aromatic hydrocarbons (PAHs) in air and soil surrounding a coal-fired thermal power plant in the south-west coast of India, Environmental Science and Pollution Research. (2019). https://doi.org/10.1007/s11356-019-05380-y.

[170] Y. Li, L. Long, J. Ge, L. xuan Yang, J. jin Cheng, L. xiang Sun, C. Lu, X. yang Yu, Presence, distribution and risk assessment of polycyclic aromatic hydrocarbons in rice-wheat continuous cropping soils close to five industrial parks of Suzhou, China, Chemosphere. (2017). https://doi.org/10.1016/j.chemosphere.2017.06.055.

[171] G. Liu, J. Niu, W. Guo, X. An, L. Zhao, Ecological and health risk-based characterization of agricultural soils contaminated with polycyclic aromatic hydrocarbons in the vicinity of a chemical plant in China, Chemosphere. (2016). https://doi.org/10.1016/j.chemosphere.2016.08.056.

[172] W. Ge, Q.Q. Cheng, C. Chai, L.S. Zeng, J. Wu, Q.H. Chen, X.W. Zhu, D. Ma, Pollution Characteristics and Source Analysis of Polycyclic Aromatic Hydrocarbons in Agricultural Soils from Shandong, Huanjing Kexue/Environmental Science. (2017). https://doi.org/10.13227/j.hjkx.201608199.

[173] K. Banger, G.S. Toor, T. Chirenje, L. Ma, Polycyclic aromatic hydrocarbons in urban soils of different land uses in Miami, Florida, Soil Sediment Contam. (2010). https://doi.org/10.1080/15320380903548516.

[174] T. Minkina, S. Sushkova, E. Konstantinova, B. Kumar Yadav, S. Mandzhieva, A. Konstantinov, V. Khoroshavin, O. Nazarenko, E. Antonenko, Polycyclic Aromatic Hydrocarbons in Urban Soils Within the Different Land Use: A Case Study of Tyumen, Russia, Polycycl Aromat Compd. (2019). https://doi.org/10.1080/10406638.2018.1540997.

[175] B. Maliszewska-Kordybach, B. Smreczak, A. Klimkowicz-Pawlas, H. Terelak, Monitoring of the total content of polycyclic aromatic hydrocarbons (PAHs) in

arable soils in Poland, Chemosphere. (2008). https://doi.org/10.1016/j.chemosphere.2008.07.009.

[176] M.K. Chung, R. Hu, K.C. Cheung, M.H. Wong, Pollutants in Hong Kong soils: Polycyclic aromatic hydrocarbons, Chemosphere. (2007). https://doi.org/10.1016/j.chemosphere.2006.09.062.

[177] M. Nadal, M. Schuhmacher, J.L. Domingo, Long-term environmental monitoring of persistent organic pollutants and metals in a chemical/petrochemical area: Human health risks, Environmental Pollution. (2011). https://doi.org/10.1016/j.envpol.2011.04.007.

[178] S. Gitipour, G.A. Sorial, S. Ghasemi, M. Bazyari, Treatment technologies for PAH-contaminated sites: a critical review, Environ Monit Assess. (2018). https://doi.org/10.1007/s10661-018-6936-4.

[179] E. Madadian, S. Gitipour, L. Amiri, M. Alimohammadi, J. Saatloo, The application of soil washing for treatment of polycyclic aromatic hydrocarbons contaminated soil: A case study in a petrochemical complex, Environ Prog Sustain Energy. (2014). https://doi.org/10.1002/ep.11759.

[180] Q. Zhao, L. Weise, P. Li, K. Yang, Y. Zhang, D. Dong, P. Li, X. Li, Ageing behavior of phenanthrene and pyrene in soils: A study using sodium dodecylbenzenesulfonate extraction, J Hazard Mater. (2010). https://doi.org/10.1016/j.jhazmat.2010.07.111.

[181] Y. Gou, Q. Zhao, S. Yang, H. Wang, P. Qiao, Y. Song, Y. Cheng, P. Li, Removal of polycyclic aromatic hydrocarbons (PAHs) and the response of indigenous bacteria in highly contaminated aged soil after persulfate oxidation, Ecotoxicol Environ Saf. (2020). https://doi.org/10.1016/j.ecoenv.2019.110092.

[182] R. Medina, P.M. David Gara, A.J. Fernández-González, J.A. Rosso, M.T. Del Panno, Remediation of a soil chronically contaminated with hydrocarbons through persulfate oxidation and bioremediation, Science of the Total Environment. (2018). https://doi.org/10.1016/j.scitotenv.2017.10.326.

[183] F. Renoldi, L. Lietti, S. Saponaro, L. Bonomo, P. Forzatti, Thermal desorption of a PAH-contaminated soil: A case study, in: Advances in Ecological Sciences, 2003.

[184] F. Bianco, G. Monteverde, M. Race, S. Papirio, G. Esposito, Comparing performances, costs and energy balance of ex situ remediation processes for PAH-contaminated marine sediments, Environmental Science and Pollution Research. (2020) 1–12.

[185] Y. Abbas, W. Lu, Q. Wang, H. Dai, Y. Liu, X. Fu, C. Pan, H. Ghaedi, F. Cheng, H. Wang, Remediation of pyrene contaminated soil by double dielectric barrier discharge plasma technology: Performance optimization and evaluation, Environmental Pollution. (2020). https://doi.org/10.1016/j.envpol.2020.113944.

[186] S. Kuppusamy, P. Thavamani, K. Venkateswarlu, Y.B. Lee, R. Naidu, M. Megharaj, Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions, Chemosphere. (2017). https://doi.org/10.1016/j.chemosphere.2016.10.115.

[187] M.C. Chang, H.Y. Kang, Remediation of pyrene-contaminated soil by synthesized nanoscale zero-valent iron particles, J Environ Sci Health A Tox Hazard Subst Environ Eng. (2009). https://doi.org/10.1080/10934520902784609.

[188] P. Bardos, C. Merly, P. Kvapil, H.P. Koschitzky, Status of nanoremediation and its potential for future deployment: Risk-benefit and benchmarking appraisals, Remediation. (2018). https://doi.org/10.1002/rem.21559.

[189] S. Xu, S. Guo, B. Wu, F. Li, T. Li, An assessment of the effectiveness and impact of electrokinetic remediation for pyrene-contaminated soil, J Environ Sci (China). (2014). https://doi.org/10.1016/j.jes.2014.09.014.

[190] T. Li, Y. Wang, S. Guo, X. Li, Y. Xu, Y. Wang, X. Li, Effect of polarity-reversal on electrokinetic enhanced bioremediation of Pyrene contaminated soil, Electrochim Acta. (2016). https://doi.org/10.1016/j.electacta.2015.11.097.

[191] I. Hussain, G. Aleti, R. Naidu, M. Puschenreiter, Q. Mahmood, M.M. Rahman, F. Wang, S. Shaheen, J.H. Syed, T.G. Reichenauer, Microbe and plant assisted-remediation of organic xenobiotics and its enhancement by genetically modified organisms and recombinant technology: A review, Science of the Total Environment. (2018). https://doi.org/10.1016/j.scitotenv.2018.02.037.

[192] J. Li, W. Chen, W. Zhou, Y. Wang, M. Deng, S. Zhou, Synergistic degradation of pyrene by Pseudomonas aeruginosa PA06 and Achromobacter sp. AC15 with sodium citrate as the co-metabolic carbon source, Ecotoxicology. (2020). https://doi.org/10.1007/s10646-020-02268-3.

[193] X.D. Huang, Y. El-Alawi, D.M. Penrose, B.R. Glick, B.M. Greenberg, A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils, Environmental Pollution. (2004). https://doi.org/10.1016/j.envpol.2003.09.031.

[194] A. Vasavi, R. Usha, P.M. Swamy, Phytoremediation- An overview review, Journal of Industrial Pollution Control. (2010).

[195] A. Cristaldi, G.O. Conti, E.H. Jho, P. Zuccarello, A. Grasso, C. Copat, M. Ferrante, Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review, Environ Technol Innov. (2017). https://doi.org/10.1016/j.eti.2017.08.002.

[196] X. Wan, M. Lei, T. Chen, Cost–benefit calculation of phytoremediation technology for heavy-metal-contaminated soil, Science of the Total Environment. (2016). https://doi.org/10.1016/j.scitotenv.2015.12.080.

[197] N. Fiorentino, M. Mori, V. Cenvinzo, L.G. Duri, L. Gioia, D. Visconti, M. Fagnano, Assisted phytoremediation for restoring soil fertility in contaminated and degraded land, Italian Journal of Agronomy. (2018).

[198] L.R.P. de Andrade Lima, L.A. Bernardez, M.G. dos Santos, R.C. Souza, Remediation of Clay Soils Contaminated with Potentially Toxic Elements: The Santo Amaro Lead Smelter, Brazil, Case, Soil Sediment Contam. (2018). https://doi.org/10.1080/15320383.2018.1493717.

[199] H. Farraji, N.Q. Zaman, R.M. Tajuddin, H. Faraji, Advantages and disadvantages of phytoremediation: A concise review, Int J Env Tech Sci. (2016).

[200] K. Kumar Yadav, N. Gupta, A. Kumar, L.M. Reece, N. Singh, S. Rezania, S. Ahmad Khan, Mechanistic understanding and holistic approach of phytoremediation: A review on application and future prospects, Ecol Eng. (2018). https://doi.org/10.1016/j.ecoleng.2018.05.039.

[201] H. Hamdi, S. Benzarti, I. Aoyama, N. Jedidi, Rehabilitation of degraded soils containing aged PAHs based on phytoremediation with alfalfa (Medicago sativa L.), Int Biodeterior Biodegradation. (2012). https://doi.org/10.1016/j.ibiod.2011.10.009.

[202] Y. He, J. Chi, Pilot-scale demonstration of phytoremediation of PAH-contaminated sediments by Hydrilla verticillata and Vallisneria spiralis, Environmental Technology (United Kingdom). (2019). https://doi.org/10.1080/09593330.2017.1398783.

[203] M. García-Sánchez, Z. Košnář, F. Mercl, E. Aranda, P. Tlustoš, A comparative study to evaluate natural attenuation, mycoaugmentation, phytoremediation, and microbial-assisted phytoremediation strategies for the bioremediation of an aged PAH-polluted soil, Ecotoxicol Environ Saf. (2018). https://doi.org/10.1016/j.ecoenv.2017.08.012.

[204] N.S. Bolan, J.H. Park, B. Robinson, R. Naidu, K.Y. Huh, Phytostabilization. A green approach to contaminant containment, 2011. https://doi.org/10.1016/B978-0-12-385538-1.00004-4.

[205] T. Chekol, L.R. Vough, Assessing the potential of using phytoremediation for pyrene-contaminated soils, Remediation. (2004). https://doi.org/10.1002/rem.20014.

[206] A. Balasubramaniyam, The Influence of Plants in the Remediation of Petroleum Hydrocarbon- Contaminated Sites, Pharmaceutical Analytical Chemistry: Open Access. (2015). https://doi.org/10.4172/2471-2698.1000105.

[207] Y.Z. Gao, L.Z. Zhu, Phytoremediation for phenanthrene and pyrene contaminated soils, Journal of Environmental Sciences. (2005).

[208] H. Lu, Y. Zhang, B. Liu, J. Liu, J. Ye, C. Yan, Rhizodegradation gradients of phenanthrene and pyrene in sediment of mangrove (Kandelia candel (L.) Druce), J Hazard Mater. (2011). https://doi.org/10.1016/j.jhazmat.2011.09.031.

[209] H. Jia, H. Wang, H. Lu, S. Jiang, M. Dai, J. Liu, C. Yan, Rhizodegradation potential and tolerance of Avicennia marina (Forsk.) Vierh in phenanthrene and pyrene contaminated sediments, Mar Pollut Bull. (2016). https://doi.org/10.1016/j.marpolbul.2016.06.075.

[210] A.K. Sivaram, P. Logeshwaran, S.R. Subashchandrabose, R. Lockington, R. Naidu, M. Megharaj, Comparison of plants with C3 and C4 carbon fixation pathways for remediation of polycyclic aromatic hydrocarbon contaminated soils, Sci Rep. (2018). https://doi.org/10.1038/s41598-018-20317-0.

[211] J. Ye, H. Yin, H. Peng, J. Bai, Y. Li, Pyrene removal and transformation by joint application of alfalfa and exogenous microorganisms and their influence on soil microbial community, Ecotoxicol Environ Saf. (2014). https://doi.org/10.1016/j.ecoenv.2014.08.031.

[212] S. Vaidya, K. Jain, D. Madamwar, Metabolism of pyrene through phthalic acid pathway by enriched bacterial consortium composed of Pseudomonas, Burkholderia, and Rhodococcus (PBR), 3 Biotech. (2017). https://doi.org/10.1007/s13205-017-0598-8.

[213] J. Wei, X. Liu, Q. Wang, C. Wang, X. Chen, H. Li, Effect of rhizodeposition on pyrene bioaccessibility and microbial structure in pyrene and pyrene-lead polluted soil, Chemosphere. (2014). https://doi.org/10.1016/j.chemosphere.2013.09.105.

[214] J. Suman, O. Uhlik, J. Viktorova, T. Macek, Phytoextraction of heavy metals: A promising tool for clean-up of polluted environment?, Front Plant Sci. (2018). https://doi.org/10.3389/fpls.2018.01476.

[215] A. Mahar, P. Wang, A. Ali, M.K. Awasthi, A.H. Lahori, Q. Wang, R. Li, Z. Zhang, Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review, Ecotoxicol Environ Saf. (2016). https://doi.org/10.1016/j.ecoenv.2015.12.023.

[216] A. Sobhani, S.Y. Salehi-Lisar, R. Motafakkerazad, A. Movafeghi, Uptake and Distribution of Phenanthrene and Pyrene in Roots and Shoots of Wheat (Triticum aestivum L.), Polycycl Aromat Compd. (2020). https://doi.org/10.1080/10406638.2020.1744166.

[217] H.T. Rheay, E.C. Omondi, C.E. Brewer, Potential of hemp (Cannabis sativa L.) for paired phytoremediation and bioenergy production, GCB Bioenergy. (2020). https://doi.org/10.1111/gcbb.12782.

[218] J. He, V. Strezov, R. Kumar, H. Weldekidan, S. Jahan, B.H. Dastjerdi, X. Zhou, T. Kan, Pyrolysis of heavy metal contaminated Avicennia marina biomass from phytoremediation: Characterisation of biomass and pyrolysis products, J Clean Prod. (2019). https://doi.org/10.1016/j.jclepro.2019.06.285.

[219] S. Kumar, R. Singh, V. Kumar, A. Rani, R. Jain, Cannabis sativa: A plant suitable for phytoremediation and bioenergy production, in: Phytoremediation Potential of Bioenergy Plants, 2017. https://doi.org/10.1007/978-981-10-3084-0_10.

[220] Y. Gao, X. Hu, Z. Zhou, W. Zhang, Y. Wang, B. Sun, Phytoavailability and mechanism of bound PAH residues in filed contaminated soils, Environmental Pollution. (2017). https://doi.org/10.1016/j.envpol.2016.11.076.

[221] L.P. Singha, P. Pandey, Rhizobacterial community of Jatropha curcas associated with pyrene biodegradation by consortium of PAH-degrading bacteria, Applied Soil Ecology. (2020). https://doi.org/10.1016/j.apsoil.2020.103685.

[222] Y. Zhang, C. Peng, Z. Guo, X. Xiao, R. Xiao, Polycyclic aromatic hydrocarbons in urban soils of China: Distribution, influencing factors, health risk and regression prediction, Environmental Pollution. (2019). https://doi.org/10.1016/j.envpol.2019.07.098.

[223] M. Houshani, S.Y. Salehi-Lisar, R. Motafakkerazad, A. Movafeghi, Uptake and distribution of phenanthrene and pyrene in roots and shoots of maize (Zea mays

L.), Environmental Science and Pollution Research. (2019). https://doi.org/10.1007/s11356-019-04371-3.

[224] S. Wei, S. Pan, Phytoremediation for soils contaminated by phenanthrene and pyrene with multiple plant species, J Soils Sediments. (2010). https://doi.org/10.1007/s11368-010-0216-4.

[225] P. Agarwal, B.S. Giri, R. Rani, Unravelling the Role of Rhizospheric Plant-Microbe Synergy in Phytoremediation: A Genomic Perspective, Curr Genomics. (2020). https://doi.org/10.2174/1389202921999200623133240.

[226] Y. Wu, Y. Xu, N. Zhou, A newly defined dioxygenase system from Mycobacterium vanbaalenii PYR-1 endowed with an enhanced activity of dihydroxylation of high-molecular-weight polyaromatic hydrocarbons, Front Environ Sci Eng. (2020). https://doi.org/10.1007/s11783-019-1193-5.

[227] N. Jeelani, W. Yang, H.L. Zhu, S. An, Phytoremediation for co-contaminated soils of cadmium and pyrene using Phragmites australis (common reed), Int J Phytoremediation. (2020). https://doi.org/10.1080/15226514.2020.1780411.

[228] A. Lipińska, J. Wyszkowska, J. Kucharski, Diversity of organotrophic bacteria, activity of dehydrogenases and urease as well as seed germination and root growth Lepidium sativum, Sorghum saccharatum and Sinapis alba under the influence of polycyclic aromatic hydrocarbons, Environmental Science and Pollution Research. (2015). https://doi.org/10.1007/s11356-015-5329-2.

[229] S. Lu, Y. Teng, J. Wang, Z. Sun, Enhancement of pyrene removed from contaminated soils by Bidens maximowicziana, Chemosphere. (2010). https://doi.org/10.1016/j.chemosphere.2010.08.022.

[230] Y. Dai, R. Liu, Y. Zhou, N. Li, L. Hou, Q. Ma, B. Gao, Fire Phoenix facilitates phytoremediation of PAH-Cd co-contaminated soil through promotion of beneficial rhizosphere bacterial communities, Environ Int. (2020). https://doi.org/10.1016/j.envint.2019.105421.

[231] R. Liu, N. Xiao, S. Wei, L. Zhao, J. An, Rhizosphere effects of PAH-contaminated soil phytoremediation using a special plant named Fire Phoenix, Science of the Total Environment. (2014). https://doi.org/10.1016/j.scitotenv.2013.12.027.

[232] R. Child, C.D. Miller, Y. Liang, R.C. Sims, A.J. Anderson, Pyrene Mineralization by Mycobacterium sp. Strain KMS in a Barley Rhizosphere , J Environ Qual. (2007). https://doi.org/10.2134/jeq2007.0008.

[233] H. Lu, J. Sun, L. Zhu, The role of artificial root exudate components in facilitating the degradation of pyrene in soil, Sci Rep. (2017). https://doi.org/10.1038/s41598-017-07413-3.

[234] J. Zeng, Q. Zhu, Y. Li, Y. Dai, Y. Wu, Y. Sun, L. Miu, H. Chen, X. Lin, Isolation of diverse pyrene-degrading bacteria via introducing readily utilized phenanthrene, Chemosphere. (2019). https://doi.org/10.1016/j.chemosphere.2019.01.189.

[235] S. Liu, Y. Hou, G. Sun, Synergistic degradation of pyrene and volatilization of arsenic by cocultures of bacteria and a fungus, Front Environ Sci Eng. (2013). https://doi.org/10.1007/s11783-012-0470-3.

[236] J. Yang, Y. Gu, Z. Chen, Y. Song, F. Sun, J. Liu, M.G. Waigi, Colonization and performance of a pyrene-degrading bacterium Mycolicibacterium sp. Pyr9 on root surfaces of white clover, Chemosphere. (2021). https://doi.org/10.1016/j.chemosphere.2020.127918.

[237] G. Gupta, V. Kumar, A.K. Pal, Microbial Degradation of High Molecular Weight Polycyclic Aromatic Hydrocarbons with Emphasis on Pyrene, Polycycl Aromat Compd. (2019). https://doi.org/10.1080/10406638.2017.1293696.

[238] P. Wanapaisan, N. Laothamteep, F. Vejarano, J. Chakraborty, M. Shintani, C. Muangchinda, T. Morita, C. Suzuki-Minakuchi, K. Inoue, H. Nojiri, O. Pinyakong, Synergistic degradation of pyrene by five culturable bacteria in a mangrove sediment-derived bacterial consortium, J Hazard Mater. (2018). https://doi.org/10.1016/j.jhazmat.2017.08.062.

[239] X. Jia, Y. He, L. Huang, D. Jiang, W. Lu, n-Hexadecane and pyrene biodegradation and metabolization by Rhodococcus sp. T1 isolated from oil contaminated soil, Chin J Chem Eng. (2019). https://doi.org/10.1016/j.cjche.2018.03.034.

[240] B. Gupta, S. Puri, I.S. Thakur, J. Kaur, Comparative evaluation of growth kinetics for pyrene degradation by Acinetobacter pittii NFL and Enterobacter cloacae BT in the presence of biosurfactant, Bioresour Technol Rep. (2020). https://doi.org/10.1016/j.biteb.2019.100369.

[241] A. Nzila, C.O. Ramirez, M.M. Musa, S. Sankara, C. Basheer, Q.X. Li, Pyrene biodegradation and proteomic analysis in Achromobacter xylosoxidans, PY4 strain, Int Biodeterior Biodegradation. (2018). https://doi.org/10.1016/j.ibiod.2018.03.014.

[242] Z. Yan, Y. Zhang, H. Wu, M. Yang, H. Zhang, Z. Hao, H. Jiang, Isolation and characterization of a bacterial strain: Hydrogenophaga sp. PYR1 for anaerobic

pyrene and benzo [a] pyrene biodegradation, RSC Adv. (2017). https://doi.org/10.1039/c7ra09274a.

[243] H. Zhou, S. Zhang, J. Xie, H. Wei, Z. Hu, H. Wang, Pyrene biodegradation and its potential pathway involving Roseobacter clade bacteria, Int Biodeterior Biodegradation. (2020). https://doi.org/10.1016/j.ibiod.2020.104961.

[244] A. Lipińska, J. Wyszkowska, J. Kucharski, Microbiological and Biochemical Activity in Soil Contaminated with Pyrene Subjected to Bioaugmentation, Water Air Soil Pollut. (2021). https://doi.org/10.1007/s11270-020-04950-y.

[245] A. Kamyabi, H. Nouri, H. Moghimi, Characterization of pyrene degradation and metabolite identification by Basidioascus persicus and mineralization enhancement with bacterial-yeast co-culture, Ecotoxicol Environ Saf. (2018). https://doi.org/10.1016/j.ecoenv.2018.07.098.

[246] A.K. Sivaram, P. Logeshwaran, R. Lockington, R. Naidu, M. Megharaj, Impact of plant photosystems in the remediation of benzo[a]pyrene and pyrene spiked soils, Chemosphere. (2018). https://doi.org/10.1016/j.chemosphere.2017.11.081.

[247] H. Song, Y.S. Wang, C.C. Sun, Y.T. Wang, Y.L. Peng, H. Cheng, Effects of pyrene on antioxidant systems and lipid peroxidation level in mangrove plants, Bruguiera gymnorrhiza, Ecotoxicology. (2012). https://doi.org/10.1007/s10646-012-0945-9.

[248] M. Houshani, S.Y. Salehi-Lisar, A. Movafeghi, R. Motafakkerazad, Growth and antioxidant system responses of maize (Zea mays L.) seedling to different concentration of pyrene in a controlled environment, Acta Agric Slov. (2019). https://doi.org/10.14720/aas.2019.113.1.03.

[249] S. Fan, P. Li, Z. Gong, W. Ren, N. He, Promotion of pyrene degradation in rhizosphere of alfalfa (Medicago sativa L.), Chemosphere. (2008). https://doi.org/10.1016/j.chemosphere.2007.10.068.

[250] J.L. Kirk, J.N. Klironomos, H. Lee, J.T. Trevors, The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil, Environmental Pollution. (2005). https://doi.org/10.1016/j.envpol.2004.06.002.

[251] A. Esmaeili, O. Knox, A. Juhasz, S.C. Wilson, Advancing prediction of polycyclic aromatic hydrocarbon bioaccumulation in plants for historically contaminated soils using Lolium multiflorum and simple chemical in-vitro methodologies, Science of The Total Environment. (2021). https://doi.org/10.1016/j.scitotenv.2020.144783.

[252] S.A. Cheema, M.I. Khan, X. Tang, C. Zhang, C. Shen, Z. Malik, S. Ali, J. Yang, K. Shen, X. Chen, Y. Chen, Enhancement of phenanthrene and pyrene degradation in rhizosphere of tall fescue (Festuca arundinacea), J Hazard Mater. (2009). https://doi.org/10.1016/j.jhazmat.2008.12.027.

[253] C. Fernández-López, R. Posada-Baquero, J.L. García, J.C. Castilla-Alcantara, M. Cantos, J.J. Ortega-Calvo, Root-mediated bacterial accessibility and cometabolism of pyrene in soil, Science of The Total Environment. (2020). https://doi.org/10.1016/j.scitotenv.2020.143408.

[254] J.J. Ortega-Calvo, J. Harmsen, J.R. Parsons, K.T. Semple, M.D. Aitken, C. Ajao, C. Eadsforth, M. Galay-Burgos, R. Naidu, R. Oliver, W.J.G.M. Peijnenburg, J. Römbke, G. Streck, B. Versonnen, From Bioavailability Science to Regulation of Organic Chemicals, Environ Sci Technol. (2015). https://doi.org/10.1021/acs.est.5b02412.

[255] J. Wei, X. Zhang, X. Liu, X. Liang, X. Chen, Influence of root components of celery on pyrene bioaccessibility, soil enzymes and microbial communities in pyrene and pyrene-diesel spiked soils, Science of the Total Environment. (2017). https://doi.org/10.1016/j.scitotenv.2017.04.083.

[256] X. Chen, X. Cheng, H. Meng, K. Kumar, H. Li, H. He, W. Du, S. Yang, S. Li, L. Zhang, Past, Present, and Future Perspectives on the Assessment of Bioavailability/Bioaccessibility of Polycyclic Aromatic Hydrocarbons: A 20-year systemic review based on scientific econometrics, Science of The Total Environment. (2021). https://doi.org/10.1016/j.scitotenv.2021.145585.

[257] K.E. Gerhardt, X.D. Huang, B.R. Glick, B.M. Greenberg, Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges, Plant Science. (2009). https://doi.org/10.1016/j.plantsci.2008.09.014.

[258] X. Ye, J. Ma, J. Wei, K. Sun, Q. Xiong, Comparison of the bioavailability of benzo[a]pyrene (B[a]P) in a B[a]P-contaminated soil using the different addition approaches, Sci Rep. (2019). https://doi.org/10.1038/s41598-019-40813-1.

[259] X.Y. Ma, X.C. Wang, H.H. Ngo, W. Guo, M.N. Wu, N. Wang, Bioassay based luminescent bacteria: Interferences, improvements, and applications, Science of the Total Environment. (2014). https://doi.org/10.1016/j.scitotenv.2013.08.028.

[260] M.J. Smith, T.H. Flowers, H.J. Duncan, H. Saito, Study of PAH dissipation and phytoremediation in soils: Comparing freshly spiked with weathered soil from a former coking works, J Hazard Mater. (2011). https://doi.org/10.1016/j.jhazmat.2011.06.033.

[261] C. Chigbo, L. Batty, Phytoremediation potential of Brassica juncea in Cu-pyrene co-contaminated soil: Comparing freshly spiked soil with aged soil, J Environ Manage. (2013). https://doi.org/10.1016/j.jenvman.2013.05.041.

[262] M. Alexander, Aging, bioavailability, and overestimation of risk from environmental pollutants, Environ Sci Technol. (2000). https://doi.org/10.1021/es001069+.

[263] R. Wei, J. Ni, L. Guo, L. Yang, Y. Yang, The effect of aging time on the distribution of pyrene in soil particle-size fractions, Geoderma. (2014). https://doi.org/10.1016/j.geoderma.2014.04.027.

[264] X. An, B. Xiao, X. Di, H. Dong, H. Tang, Research progress on aging of organic pollutants in geosorbents: a review, Acta Geochimica. (2017). https://doi.org/10.1007/s11631-016-0129-z.

[265] M.D. Jones, D.R. Singleton, D.P. Carstensen, S.N. Powell, J.S. Swanson, F.K. Pfaender, M.D. Aitken, Effect of incubation conditions on the enrichment of pyrene-degrading bacteria identified by stable-isotope probing in an aged, PAH-contaminated soil, Microb Ecol. (2008). https://doi.org/10.1007/s00248-007-9352-9.

[266] A. Klimkowicz-Pawlas, B. Maliszewska-Kordybach, Effect of anthracene and pyrene on dehydrogenases activity in soils exposed and unexposed to PAHS, Water Air Soil Pollut. (2003). https://doi.org/10.1023/A:1023619023030.

[267] F. Meng, J. Chi, Effect of Potamogeton crispus L. on bioavailability and biodegradation activity of pyrene in aged and unaged sediments, J Hazard Mater. (2017). https://doi.org/10.1016/j.jhazmat.2016.11.001.

[268] C. Wang, Z. Wang, Z. Li, R. Ahmad, Two-liquid-phase system: A promising technique for predicting bioavailability of polycyclic aromatic hydrocarbons in long-term contaminated soils, Chemosphere. (2017). https://doi.org/10.1016/j.chemosphere.2016.11.111.

[269] P.N. Diagboya, F.M. Mtunzi, K.O. Adebowale, B.I. Olu-Owolabi, Assessment of the effects of soil organic matter and iron oxides on the individual sorption of two polycyclic aromatic hydrocarbons, Environ Earth Sci. 80 (2021) 227. https://doi.org/10.1007/s12665-021-09530-9.

[270] H. Lin, X. Xia, S. Bi, X. Jiang, H. Wang, Y. Zhai, W. Wen, Quantifying Bioavailability of Pyrene Associated with Dissolved Organic Matter of Various Molecular Weights to Daphnia magna, Environ Sci Technol. (2018). https://doi.org/10.1021/acs.est.7b05520.

[271] L.B. Martínez-García, G. Korthals, L. Brussaard, H.B. Jørgensen, G.B. De Deyn, Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties, Agric Ecosyst Environ. (2018). https://doi.org/10.1016/j.agee.2018.04.018.

[272] S. Ahn, D. Werner, R.G. Luthy, Physicochemical characterization of coke-plant soil for the assessment of polycyclic aromatic hydrocarbon availability and the feasibility of phytoremediation, Environ Toxicol Chem. (2005). https://doi.org/10.1897/04-564R.1.

[273] B. Chefetz, A.P. Deshmukh, P.G. Hatcher, E.A. Guthrie, Pyrene sorption by natural organic matter, Environ Sci Technol. (2000). https://doi.org/10.1021/es9912877.

[274] R.J. Smernik, R.S. Kookana, The effects of organic matter-mineral interactions and organic matter chemistry on diuron sorption across a diverse range of soils, Chemosphere. (2015). https://doi.org/10.1016/j.chemosphere.2014.05.066.

[275] R. Wei, J. Ni, X. Li, W. Chen, Y. Yang, Dissipation and phytoremediation of polycyclic aromatic hydrocarbons in freshly spiked and long-term field-contaminated soils, Environmental Science and Pollution Research. (2017). https://doi.org/10.1007/s11356-017-8459-x.

[276] S. Liu, F. Meng, Z. Ding, J. Chi, Phytoremediation of PAH-contaminated sediments with different organic matter contents by Potamogeton crispus L., Int J Phytoremediation. (2018). https://doi.org/10.1080/15226514.2018.1488811.

[277] T. Chekol, L.R. Vough, R.L. Chaney, Plant-soil-contaminant specificity affects phytoremediation of organic contaminants, Int J Phytoremediation. (2002). https://doi.org/10.1080/15226510208500070.

[278] Q. Zhang, H. Wang, X. Xia, S. Bi, H. Lin, J. Chen, Elevated temperature enhances the bioavailability of pyrene to Daphnia magna in the presence of dissolved organic matter: Implications for the effect of climate warming, Environmental Pollution. (2020). https://doi.org/10.1016/j.envpol.2020.115349.

[279] Y. Yang, Y. Liu, Z. Li, Z. Wang, C. Li, H. Wei, Significance of soil microbe in microbial-assisted phytoremediation: an effective way to enhance phytoremediation of contaminated soil, International Journal of Environmental Science and Technology. (2020). https://doi.org/10.1007/s13762-020-02668-2.

[280] S. Thiele, Bioformation of polycyclic aromatic hydrocarbons in soil under oxygen deficient conditions, Soil Biol Biochem. (2002). https://doi.org/10.1016/S0038-0717(01)00204-8.

[281] Y. He, J. Chi, Phytoremediation of sediments polluted with phenanthrene and pyrene by four submerged aquatic plants, J Soils Sediments. (2016). https://doi.org/10.1007/s11368-015-1221-4.

[282] F. Wu, K. Tian, J. Wang, H. Bao, W. Luo, H. Zhang, H. Hong, Accumulation and translocation of phenanthrene, anthracene and pyrene in winter wheat affected by soil water content, Ecotoxicol Environ Saf. (2019). https://doi.org/10.1016/j.ecoenv.2019.109567.

[283] H.H. Liste, M. Alexander, Plant-promoted pyrene degradation in soil, Chemosphere. (2000). https://doi.org/10.1016/S0045-6535(99)00216-7.

[284] N.H. AL Sbani, S.R.S. Abdullah, M. Idris, H.A. Hasan, M.I.E. Halmi, O.H. Jehawi, N.I. Ismail, PAH-degrading rhizobacteria of Lepironia articulata for phytoremediation enhancement, Journal of Water Process Engineering. (2020). https://doi.org/10.1016/j.jwpe.2020.101688.

[285] P. Sheng-wang, W. Shi-qiang, Y. Xin, C. Sheng-xian, The Removal and Remediation of Phenanthrene and Pyrene in Soil by Mixed Cropping of Alfalfa and Rape, Agric Sci China. (2008). https://doi.org/10.1016/S1671-2927(08)60185-6.

[286] S.A. Cheema, M. Imran Khan, C. Shen, X. Tang, M. Farooq, L. Chen, C. Zhang, Y. Chen, Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation, J Hazard Mater. (2010). https://doi.org/10.1016/j.jhazmat.2009.12.044.

[287] S. Alagić, B.S. Maluckov, V.B. Radojičić, How can plants manage polycyclic aromatic hydrocarbons? May these effects represent a useful tool for an effective soil remediation? A review, Clean Technol Environ Policy. (2015). https://doi.org/10.1007/s10098-014-0840-6.

[288] J. Srivastava, H. Chandra, A. R., S.J. S. Kalr, Response of C3 and C4 Plant Systems Exposed to Heavy Metals for Phytoextraction at Elevated Atmospheric CO2 and at Elevated Temperature, in: Environmental Contamination, 2012. https://doi.org/10.5772/31333.

[289] C. Nabais, G. Labuto, S. Gonçalves, E. Buscardo, D. Semensatto, A.R.A. Nogueira, H. Freitas, Effect of root age on the allocation of metals, amino acids and sugars in different cell fractions of the perennial grass Paspalum notatum (bahiagrass), Plant Physiology and Biochemistry. (2011). https://doi.org/10.1016/j.plaphy.2011.09.010.

[290] E. Konstantinova, T. Minkina, A. Konstantinov, S. Sushkova, E. Antonenko, A. Kurasova, S. Loiko, Pollution status and human health risk assessment of

potentially toxic elements and polycyclic aromatic hydrocarbons in urban street dust of Tyumen city, Russia, Environ Geochem Health. (2020). https://doi.org/10.1007/s10653-020-00692-2.

[291] P. Dhanwal, A. Kumar, S. Dudeja, V. Chhokar, V. Beniwal, Recent advances in phytoremediation technology, in: Advances in Environmental Biotechnology, 2017. https://doi.org/10.1007/978-981-10-4041-2_14.

[292] H. Sun, X. Ren, L. Zhao, Sorption of pyrene on different constituents of rice straw in the presence of phenanthrene, benzo[a]pyrene, and phenols, Water Air Soil Pollut. (2014). https://doi.org/10.1007/s11270-014-2048-0.

[293] N. Hechmi, N. Ben Aissa, H. Abdennaceur, N. Jedidi, PHYTOREMEDIATION POTENTIAL OF MAIZE (ZEA MAYS L.) IN CO-CONTAMINATED SOILS WITH PENTACHLOROPHENOL AND CADMIUM, Int J Phytoremediation. (2013). https://doi.org/10.1080/15226514.2012.723067.

[294] X. Zhang, C. Su, X. Liu, Z. Liu, X. Liang, Y. Zhang, Y. Feng, Effect of plant-growth-promoting rhizobacteria on phytoremediation efficiency of Scirpus triqueter in pyrene-Ni co-contaminated soils, Chemosphere. (2020). https://doi.org/10.1016/j.chemosphere.2019.125027.

[295] S. Khan, S. Rehman, Q. Cao, N. Jehan, M.T. Shah, Uptake and translocation of lead and pyrene by ryegrass cultivated in aged spiked soil, Int J Environ Pollut. (2011). https://doi.org/10.1504/IJEP.2011.039089.

[296] S. Khan, A.E.L. Hesham, G. Qing, L. Shuang, J. He, Biodegradation of pyrene and catabolic genes in contaminated soils cultivated with Lolium multiflorum L, J Soils Sediments. (2009). https://doi.org/10.1007/s11368-009-0061-5.

[297] A.C. Agnello, D. Huguenot, E.D. van Hullebusch, G. Esposito, Citric acid- and Tween® 80-assisted phytoremediation of a co-contaminated soil: alfalfa (Medicago sativa L.) performance and remediation potential, Environmental Science and Pollution Research. (2016). https://doi.org/10.1007/s11356-015-5972-7.

[298] N. Salehi, A. Azhdarpoor, M. Shirdarreh, The effect of different levels of leachate on phytoremediation of pyrene-contaminated soil and simultaneous extraction of lead and cadmium, Chemosphere. (2020). https://doi.org/10.1016/j.chemosphere.2020.125845.

[299] H. Zhang, Y. Liu, X. Shen, M. Zhang, Y. Yang, S. Tao, X. Wang, Influence of multiwalled carbon nanotubes and sodium dodecyl benzene sulfonate on bioaccumulation and translocation of pyrene and 1-methylpyrene in maize (Zea

mays) seedlings, Environmental Pollution. (2017). https://doi.org/10.1016/j.envpol.2016.10.093.

[300] V. Kumar, A. Kaushal, M.P. Shah, K. Singh, Phytoaugmentation technology for phytoremediation of environmental pollutants: current scenario and future prospects, in: Bioremediation for Environmental Sustainability, 2021. https://doi.org/10.1016/b978-0-12-820318-7.00016-2.

[301] R.R. Navarro, H. Ichikawa, K. Morimoto, K. Tatsumi, Enhancing the release and plant uptake of PAHs with a water-soluble purine alkaloid, Chemosphere. (2009). https://doi.org/10.1016/j.chemosphere.2009.04.021.

[302] I.K.U. Adam, M. Duarte, J. Pathmanathan, A. Miltner, T. Brüls, M. Kästner, Microbial communities in pyrene amended soil–compost mixture and fertilized soil, AMB Express. (2017). https://doi.org/10.1186/s13568-016-0306-9.

[303] Y. Hou, X. Liu, X. Zhang, X. Chen, K. Tao, Effects of key components of S. triqueter root exudates on fractions and bioavailability of pyrene–lead co-contaminated soils, International Journal of Environmental Science and Technology. (2016). https://doi.org/10.1007/s13762-015-0927-6.

[304] F. Bianco, M. Race, S. Papirio, P. Oleszczuk, G. Esposito, The addition of biochar as a sustainable strategy for the remediation of PAH–contaminated sediments, Chemosphere. (2021). https://doi.org/10.1016/j.chemosphere.2020.128274.

[305] A. Ghanem, V. D'Orazio, N. Senesi, Effects of compost addition on pyrene removal from soil cultivated with three selected plant species, Clean (Weinh). (2013). https://doi.org/10.1002/clen.201200601.

[306] G. Li, F. Chen, S. Jia, Z. Wang, Q. Zuo, H. He, Effect of biochar on Cd and pyrene removal and bacteria communities variations in soils with culturing ryegrass (Lolium perenne L.), Environmental Pollution. (2020). https://doi.org/10.1016/j.envpol.2020.114887.

[307] X. Zhang, J. Chen, X. Liu, X. Chen, L. Liu, Y. Niu, R. Wang, The relief effects of organic acids on Scirpus triqueter L. under pyrene–lead stress, Environmental Science and Pollution Research. (2019). https://doi.org/10.1007/s11356-019-04976-8.

[308] T. Han, Z. Zhao, M. Bartlam, Y. Wang, Combination of biochar amendment and phytoremediation for hydrocarbon removal in petroleum-contaminated soil, Environmental Science and Pollution Research. (2016). https://doi.org/10.1007/s11356-016-7236-6.

[309] T.L. Lalande, H.D. Skipper, D.C. Wolf, C.M. Reynolds, D.L. Freedman, B.W. Pinkerton, P.G. Hartel, L.W. Grimes, Phytoremediation of pyrene in a Cecil soil under field conditions, Int J Phytoremediation. (2003). https://doi.org/10.1080/16226510390856439.

[310] N. Xiao, R. Liu, C. Jin, Y. Dai, Efficiency of five ornamental plant species in the phytoremediation of polycyclic aromatic hydrocarbon (PAH)-contaminated soil, Ecol Eng. (2015). https://doi.org/10.1016/j.ecoleng.2014.12.008.

[311] O.A. Thompson, D.C. Wolf, J.D. Mattice, G.J. Thoma, Influence of nitrogen addition and plant root parameters on phytoremediation of pyrene-contaminated soil, Water Air Soil Pollut. (2008). https://doi.org/10.1007/s11270-007-9552-4.

[312] T. Liu, F. Zhu, W. Yan, X. Chen, X. Huang, R. Wang, X. Wang, H. Kang, X. Yi, Assessment of Koelreuteria paniculata Seedling for Phytroremediation of Pyrene-Contaminated Soils, Water Air Soil Pollut. (2018). https://doi.org/10.1007/s11270-018-4044-2.

[313] V. D'Orazio, A. Ghanem, N. Senesi, Phytoremediation of pyrene contaminated soils by different plant species, Clean (Weinh). (2013). https://doi.org/10.1002/clen.201100653.

[314] S. Paria, Surfactant-enhanced remediation of organic contaminated soil and water, Adv Colloid Interface Sci. (2008). https://doi.org/10.1016/j.cis.2007.11.001.

[315] Y. Gao, Q. Shen, W. Ling, L. Ren, Uptake of polycyclic aromatic hydrocarbons by Trifolium pretense L. from water in the presence of a nonionic surfactant, Chemosphere. (2008). https://doi.org/10.1016/j.chemosphere.2008.02.032.

[316] L. Zhu, M. Zhang, Effect of rhamnolipids on the uptake of PAHs by ryegrass, Environmental Pollution. (2008). https://doi.org/10.1016/j.envpol.2008.01.004.

[317] Y.-Z. GAO, W.-T. LING, L.-Z. ZHU, B.-W. ZHAO, Q.-S. ZHENG, Surfactant-Enhanced Phytoremediation of Soils Contaminated with Hydrophobic Organic Contaminants: Potential and Assessment, Pedosphere. (2007). https://doi.org/10.1016/s1002-0160(07)60050-2.

[318] C. Liao, X. Liang, G. Lu, T. Thai, W. Xu, Z. Dang, Effect of surfactant amendment to PAHs-contaminated soil for phytoremediation by maize (Zea mays L.), Ecotoxicol Environ Saf. (2015). https://doi.org/10.1016/j.ecoenv.2014.10.025.

[319] X. Liu, L. Cao, Q. Wang, X. Zhang, X. Hu, Effect of tea saponin on phytoremediation of Cd and pyrene in contaminated soils by Lolium

multiflorum, Environmental Science and Pollution Research. (2017). https://doi.org/10.1007/s11356-017-9515-2.

[320] H. Ni, W. Zhou, L. Zhu, Enhancing plant-microbe associated bioremediation of phenanthrene and pyrene contaminated soil by SDBS-Tween 80 mixed surfactants, J Environ Sci (China). (2014). https://doi.org/10.1016/S1001-0742(13)60535-5.

[321] M. Yang, J. Ke, Q. Zhang, X. He, Effects of Mixed Surfactant on Enhancing High Concentration Anthracene and Pyrene Removal from Contaminated Soil, Water Air Soil Pollut. (2019). https://doi.org/10.1007/s11270-019-4172-3.

[322] T. Chen, X. Liu, X. Zhang, X. Chen, K. Tao, X. Hu, Effect of alkyl polyglucoside and nitrilotriacetic acid combined application on lead/pyrene bioavailability and dehydrogenase activity in co-contaminated soils, Chemosphere. (2016). https://doi.org/10.1016/j.chemosphere.2016.03.127.

[323] L.P. Singha, N. Sinha, P. Pandey, Rhizoremediation prospects of Polyaromatic hydrocarbon degrading rhizobacteria, that facilitate glutathione and glutathione-S-transferase mediated stress response, and enhance growth of rice plants in pyrene contaminated soil, Ecotoxicol Environ Saf. (2018). https://doi.org/10.1016/j.ecoenv.2018.08.069.

[324] S. Rostami, A. Azhdarpoor, The application of plant growth regulators to improve phytoremediation of contaminated soils: A review, Chemosphere. (2019). https://doi.org/10.1016/j.chemosphere.2018.12.203.

[325] S. Rostami, A. Azhdarpoor, M.R. Samaei, Removal of Pyrene from Soil Using Phytobioremediation (Sorghum Bicolor-Pseudomonas), Health Scope. (2017). https://doi.org/10.5812/jhealthscope.62153.

[326] S. Rostami, A. Azhdarpoor, M. Rostami, M.R. Samaei, The effects of simultaneous application of plant growth regulators and bioaugmentation on improvement of phytoremediation of pyrene contaminated soils, Chemosphere. (2016). https://doi.org/10.1016/j.chemosphere.2016.07.026.

[327] K. Sun, J. Liu, L. Jin, Y. Gao, Utilizing pyrene-degrading endophytic bacteria to reduce the risk of plant pyrene contamination, Plant Soil. (2014). https://doi.org/10.1007/s11104-013-1875-x.

[328] Y. Gao, Q. Li, W. Ling, X. Zhu, Arbuscular mycorrhizal phytoremediation of soils contaminated with phenanthrene and pyrene, J Hazard Mater. (2011). https://doi.org/10.1016/j.jhazmat.2010.09.076.

[329] M. Soleimani, M. Afyuni, M.A. Hajabbasi, F. Nourbakhsh, M.R. Sabzalian, J.H. Christensen, Phytoremediation of an aged petroleum contaminated soil

using endophyte infected and non-infected grasses, Chemosphere. (2010). https://doi.org/10.1016/j.chemosphere.2010.09.034.

[330] X.Z. Yu, S.C. Wu, F.Y. Wu, M.H. Wong, Enhanced dissipation of PAHs from soil using mycorrhizal ryegrass and PAH-degrading bacteria, J Hazard Mater. (2011). https://doi.org/10.1016/j.jhazmat.2010.11.116.

[331] F.Y. Wu, X.Z. Yu, S.C. Wu, X.G. Lin, M.H. Wong, Phenanthrene and pyrene uptake by arbuscular mycorrhizal maize and their dissipation in soil, J Hazard Mater. (2011). https://doi.org/10.1016/j.jhazmat.2011.01.024.

[332] Y. Gao, J. Zong, H. Que, Z. Zhou, M. Xiao, S. Chen, Inoculation with arbuscular mycorrhizal fungi increases glomalin-related soil protein content and PAH removal in soils planted with Medicago sativa L., Soil Biol Biochem. (2017). https://doi.org/10.1016/j.soilbio.2017.08.023.

[333] A. Cruz-Hernández, A. Tomasini-Campocosio, L.J. Pérez-Flores, F.J. Fernández-Perrino, M. Gutiérrez-Rojas, Inoculation of seed-borne fungus in the rhizosphere of Festuca arundinacea promotes hydrocarbon removal and pyrene accumulation in roots, Plant Soil. (2013). https://doi.org/10.1007/s11104-012-1292-6.

[334] L. Ke, W.Q. Wang, T.W.Y. Wong, Y.S. Wong, N.F.Y. Tam, Removal of pyrene from contaminated sediments by mangrove microcosms, Chemosphere. (2003). https://doi.org/10.1016/S0045-6535(02)00811-1.

[335] K.Y. Cheng, K.M. Lai, J.W.C. Wong, Effects of pig manure compost and nonionic-surfactant Tween 80 on phenanthrene and pyrene removal from soil vegetated with Agropyron elongatum, Chemosphere. (2008). https://doi.org/10.1016/j.chemosphere.2008.06.005.

[336] X. Hu, X. Zhang, X. Liu, L. Cao, J. Chen, Z. Huo, The Contribution of Pyrene Degrading Bacteria and Chemical Reagents to Scirpus triqueter Phytoremediation of Pyrene and Ni Co-contaminated Soil, Water Air Soil Pollut. (2017). https://doi.org/10.1007/s11270-017-3484-4.

[337] S.A. Cheema, M.I. Khan, X. Tang, C. Shen, M. Farooq, Y. Chen, Surfactant enhanced pyrene degradation in the rhizosphere of tall fescue (Festuca arundinacea), Environmental Science and Pollution Research. (2016). https://doi.org/10.1007/s11356-016-6987-4.

[338] Q. Wang, X. Liu, X. Zhang, Y. Hou, X. Hu, X. Liang, X. Chen, Influence of tea saponin on enhancing accessibility of pyrene and cadmium phytoremediated with Lolium multiflorum in co-contaminated soils, Environmental Science and Pollution Research. (2016). https://doi.org/10.1007/s11356-015-5784-9.

[339] Y. Teng, Y. Shen, Y. Luo, X. Sun, M. Sun, D. Fu, Z. Li, P. Christie, Influence of Rhizobium meliloti on phytoremediation of polycyclic aromatic hydrocarbons by alfalfa in an aged contaminated soil, J Hazard Mater. (2011). https://doi.org/10.1016/j.jhazmat.2010.11.126.

[340] X. Zhu, W. Wang, K. Sun, X. Lin, S. Li, M.G. Waigi, W. Ling, Inoculating wheat (Triticum aestivum L.) with the endophytic bacterium Serratia sp. PW7 to reduce pyrene contamination, Int J Phytoremediation. (2017). https://doi.org/10.1080/15226514.2017.1284747.

[341] X. Ma, X. Li, J. Liu, Y. Cheng, F. Zhai, Z. Sun, L. Han, Enhancing Salix viminalis L.–mediated phytoremediation of polycyclic aromatic hydrocarbon–contaminated soil by inoculation with Crucibulum laeve (white-rot fungus), Environmental Science and Pollution Research. (2020). https://doi.org/10.1007/s11356-020-10125-3.

[342] M.M. Baneshi, R.R. Kalantary, A.J. Jafari, S. Nasseri, N. Jaafarzadeh, A. Esrafili, Effect of bioaugmentation to enhance phytoremediation for removal of phenanthrene and pyrene from soil with Sorghum and Onobrychis sativa, J Environ Health Sci Eng. (2014). https://doi.org/10.1186/2052-336X-12-24.

[343] G.H. Rabie, Role of Arbuscular Mycorrhizal Fungi in Phytoremediation of Soil Rhizosphere Spiked with Poly Aromatic Hydrocarbons, Mycobiology. (2005). https://doi.org/10.4489/myco.2005.33.1.041.

[344] X. Sheng, X. Chen, L. He, Characteristics of an endophytic pyrene-degrading bacterium of Enterobacter sp. 12J1 from Allium macrostemon Bunge, Int Biodeterior Biodegradation. (2008). https://doi.org/10.1016/j.ibiod.2007.12.003.

[345] J. Sánchez-Avila, J. Bonet, G. Velasco, S. Lacorte, Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant, Science of The Total Environment. 407 (2009) 4157–4167. https://doi.org/10.1016/j.scitotenv.2009.03.016.

[346] A.T. Lawal, Polycyclic aromatic hydrocarbons. A review, Cogent Environ Sci. (2017). https://doi.org/10.1080/23311843.2017.1339841.

[347] L. Sørensen, E. Rogers, D. Altin, I. Salaberria, A.M. Booth, Sorption of PAHs to microplastic and their bioavailability and toxicity to marine copepods under co-exposure conditions, Environmental Pollution. (2020). https://doi.org/10.1016/j.envpol.2019.113844.

[348] B.-K. Lee, Sources, Distribution and Toxicity of Polyaromatic Hydrocarbons (PAHs) in Particulate Matter, in: Air Pollution, 2010. https://doi.org/10.5772/10045.

[349] J. de Boer, M. Wagelmans, Polycyclic Aromatic Hydrocarbons in Soil – Practical Options for Remediation, Clean (Weinh). (2016). https://doi.org/10.1002/clen.201500199.

[350] C. Li, K. Zhou, W. Qin, C. Tian, M. Qi, X. Yan, W. Han, A Review on Heavy Metals Contamination in Soil: Effects, Sources, and Remediation Techniques, Soil Sediment Contam. (2019). https://doi.org/10.1080/15320383.2019.1592108.

[351] F. Bianco, G. Monteverde, M. Race, S. Papirio, G. Esposito, Comparing performances, costs and energy balance of ex situ remediation processes for PAH-contaminated marine sediments, Environmental Science and Pollution Research. 27 (2020) 19363–19374. https://doi.org/10.1007/s11356-020-08379-y.

[352] H. Nouri, S. Chavoshi Borujeni, R. Nirola, A. Hassanli, S. Beecham, S. Alaghmand, C. Saint, D. Mulcahy, Application of green remediation on soil salinity treatment: A review on halophytoremediation, Process Safety and Environmental Protection. (2017). https://doi.org/10.1016/j.psep.2017.01.021.

[353] B. Panneerselvam, S. Priya K, Phytoremediation potential of water hyacinth in heavy metal removal in chromium and lead contaminated water, Int J Environ Anal Chem. (2021) 1–16. https://doi.org/10.1080/03067319.2021.1901896.

[354] M.H. Saier, J.T. Trevors, Phytoremediation, Water Air Soil Pollut. 205 (2010) 61–63. https://doi.org/10.1007/s11270-008-9673-4.

[355] S.H. Awa, T. Hadibarata, Removal of Heavy Metals in Contaminated Soil by Phytoremediation Mechanism: a Review, Water Air Soil Pollut. 231 (2020) 47. https://doi.org/10.1007/s11270-020-4426-0.

[356] L.C. Batty, C. Dolan, The potential use of phytoremediation for sites with mixed organic and inorganic contamination, Crit Rev Environ Sci Technol. (2013). https://doi.org/10.1080/10643389.2011.604254.

[357] S. Muthusaravanan, N. Sivarajasekar, J.S. Vivek, T. Paramasivan, Mu. Naushad, J. Prakashmaran, V. Gayathri, O.K. Al-Duaij, Phytoremediation of heavy metals: mechanisms, methods and enhancements, Environ Chem Lett. 16 (2018) 1339–1359. https://doi.org/10.1007/s10311-018-0762-3.

[358] J. Pandey, S. Chand, S. Chaurasiya, R. Kumari, D.D. Patra, R.K. Verma, S. Singh, Effect of tannery sludge amendments on the activity of soil enzymes and

phytoremediation potential of two economically important cultivars of geranium (Pelargonium graveolens), Soil and Sediment Contamination: An International Journal. 28 (2019) 395–410. https://doi.org/10.1080/15320383.2019.1594688.

[359] S. Abbas, M.T. Javed, Q. Ali, H.J. Chaudhary, M. Rizwan, Alteration of plant physiology by the application of biochar for remediation of organic pollutants, in: Handbook of Bioremediation, Elsevier, 2021: pp. 475–492. https://doi.org/10.1016/B978-0-12-819382-2.00030-2.

[360] J.C. Sanchez-Hernandez, J. Notario del Pino, Y. Capowiez, C. Mazzia, M. Rault, Soil enzyme dynamics in chlorpyrifos-treated soils under the influence of earthworms, Science of The Total Environment. 612 (2018) 1407–1416. https://doi.org/10.1016/j.scitotenv.2017.09.043.

[361] M.A. Rao, R. Scelza, R. Scotti, L. Gianfreda, ROLE OF ENZYMES IN THE REMEDIATION OF POLLUTED ENVIRONMENTS, J Soil Sci Plant Nutr. 10 (2010). https://doi.org/10.4067/S0718-95162010000100008.

[362] S. Gangola, S. Joshi, S. Kumar, S.C. Pandey, Comparative analysis of fungal and bacterial enzymes in biodegradation of xenobiotic compounds, in: Smart Bioremediation Technologies, Elsevier, 2019: pp. 169–189. https://doi.org/10.1016/B978-0-12-818307-6.00010-X.

[363] P.J. Harvey, B.F. Campanella, P.M.L. Castro, H. Harms, E. Lichtfouse, A.R. Schäffner, S. Smrcek, D. Werck-Reichhart, Phytoremediation of polyaromatic hydrocarbons, anilines and phenols, Environmental Science and Pollution Research. 9 (2002) 29–47. https://doi.org/10.1007/BF02987315.

[364] R. Ahmad, Z. Tehsin, S.T. Malik, S.A. Asad, M. Shahzad, M. Bilal, M.M. Shah, S.A. Khan, Phytoremediation Potential of Hemp (Cannabis sativa L.): Identification and Characterization of Heavy Metals Responsive Genes, Clean (Weinh). 44 (2016) 195–201. https://doi.org/10.1002/clen.201500117.

[365] S. Citterio, A. Santagostino, P. Fumagalli, N. Prato, P. Ranalli, S. Sgorbati, Heavy metal tolerance and accumulation of Cd, Cr and Ni by Cannabis sativa L., Plant Soil. (2003). https://doi.org/10.1023/A:1026113905129.

[366] S. Campbell, D. Paquin, J.D. Awaya, Q.X. Li, Remediation of benzo[a]pyrene and chrysene-contaminated soil with industrial hemp (Cannabis sativa), Int J Phytoremediation. (2002). https://doi.org/10.1080/15226510208500080.

[367] G. Deng, M. Yang, M.H. Saleem, M. Rehman, S. Fahad, Y. Yang, M.S. Elshikh, J. Alkahtani, S. Ali, S.M. Khan, Nitrogen fertilizer ameliorate the remedial

capacity of industrial hemp (Cannabis sativa L.) grown in lead contaminated soil, J Plant Nutr. (2021). https://doi.org/10.1080/01904167.2021.1881553.

[368] C. Moscariello, S. Matassa, G. Esposito, S. Papirio, From residue to resource: The multifaceted environmental and bioeconomy potential of industrial hemp (Cannabis sativa L.), Resour Conserv Recycl. 175 (2021) 105864. https://doi.org/10.1016/j.resconrec.2021.105864.

[369] S. Papirio, S. Matassa, F. Pirozzi, G. Esposito, Anaerobic Co-Digestion of Cheese Whey and Industrial Hemp Residues Opens New Perspectives for the Valorization of Agri-Food Waste, Energies (Basel). 13 (2020) 2820. https://doi.org/10.3390/en13112820.

[370] V. Daioglou, J.C. Doelman, B. Wicke, A. Faaij, D.P. van Vuuren, Integrated assessment of biomass supply and demand in climate change mitigation scenarios, Global Environmental Change. 54 (2019) 88–101. https://doi.org/10.1016/j.gloenvcha.2018.11.012.

[371] T. Beringer, W. Lucht, S. Schaphoff, Bioenergy production potential of global biomass plantations under environmental and agricultural constraints, GCB Bioenergy. (2011). https://doi.org/10.1111/j.1757-1707.2010.01088.x.

[372] Sri Shalini S., Palanivelu K., Ramachandran A., V. Raghavan, Biochar from biomass waste as a renewable carbon material for climate change mitigation in reducing greenhouse gas emissions—a review, Biomass Convers Biorefin. 11 (2021) 2247–2267. https://doi.org/10.1007/s13399-020-00604-5.

[373] A. Anastasi, T. Coppola, V. Prigione, G.C. Varese, Pyrene degradation and detoxification in soil by a consortium of basidiomycetes isolated from compost: Role of laccases and peroxidases, J Hazard Mater. 165 (2009) 1229–1233. https://doi.org/10.1016/j.jhazmat.2008.10.032.

[374] B. Lukić, D. Huguenot, A. Panico, M. Fabbricino, E.D. van Hullebusch, G. Esposito, Importance of organic amendment characteristics on bioremediation of PAH-contaminated soil, Environmental Science and Pollution Research. 23 (2016) 15041–15052. https://doi.org/10.1007/s11356-016-6635-z.

[375] C.Y. Wang, F. Wang, T. Wang, X.L. Yang, Y.R. Bian, F.O. Kengara, Z.B. Li, X. Jiang, Effects of Autoclaving and Mercuric Chloride Sterilization on PAHs Dissipation in a Two-Liquid-Phase Soil Slurry, Pedosphere. (2011). https://doi.org/10.1016/S1002-0160(10)60079-3.

[376] L.L. Cook, R.S. Inouye, T.P. McGonigle, Evaluation of four grasses for use in phytoremediation of Cs-contaminated arid land soil, Plant Soil. 324 (2009) 169–184. https://doi.org/10.1007/s11104-009-9942-z.

[377] A.F. Vogelmann, J.C. Turner, P.G. Mahlberg, Cannabinoid Composition in Seedlings Compared to Adult Plants of Cannabis sativa, J Nat Prod. 51 (1988) 1075–1079. https://doi.org/10.1021/np50060a004.

[378] B.T. Borille, M.C.A. Marcelo, R.S. Ortiz, K. de C. Mariotti, M.F. Ferrão, R.P. Limberger, Near infrared spectroscopy combined with chemometrics for growth stage classification of cannabis cultivated in a greenhouse from seized seeds, Spectrochim Acta A Mol Biomol Spectrosc. 173 (2017) 318–323. https://doi.org/10.1016/j.saa.2016.09.040.

[379] C. Kaya, L. Tuna, D. Higgs, Effect of Silicon on Plant Growth and Mineral Nutrition of Maize Grown Under Water-Stress Conditions, J Plant Nutr. 29 (2006) 1469–1480. https://doi.org/10.1080/01904160600837238.

[380] R.M.N. Kucey, Increased Phosphorus Uptake by Wheat and Field Beans Inoculated with a Phosphorus-Solubilizing Penicillium bilaji Strain and with Vesicular-Arbuscular Mycorrhizal Fungi, Appl Environ Microbiol. 53 (1987) 2699–2703. https://doi.org/10.1128/aem.53.12.2699-2703.1987.

[381] S.Y. XU, Y.X. CHEN, K.F. LIN, X.C. CHEN, Q. LIN, F. LI, Z.W. WANG, Removal of Pyrene from Contaminated Soils by White Clover, Pedosphere. (2009). https://doi.org/10.1016/S1002-0160(09)60117-X.

[382] W.G. Walter, Standard methods for the examination of water and wastewater, (1961).

[383] B.A. Schumacher, Methods for the determination of total organic carbon (TOC) in soils and sediments, (2002).

[384] F. Sun, D. Littlejohn, M. David Gibson, Ultrasonication extraction and solid phase extraction clean-up for determination of US EPA 16 priority pollutant polycyclic aromatic hydrocarbons in soils by reversed-phase liquid chromatography with ultraviolet absorption detection, Anal Chim Acta. (1998). https://doi.org/10.1016/S0003-2670(98)00186-X.

[385] J.W. Kelsey, B.D. Kottler, M. Alexander, Selective Chemical Extractants To Predict Bioavailability of Soil-Aged Organic Chemicals, Environ Sci Technol. 31 (1997) 214–217. https://doi.org/10.1021/es960354j.

[386] W. Zhou, Y. Lu, S. Jiang, Y. Xiao, G. Zheng, L. Zhou, Impact of sludge conditioning treatment on the bioavailability of pyrene in sewage sludge, Ecotoxicol Environ Saf. (2018). https://doi.org/10.1016/j.ecoenv.2018.07.088.

[387] J.T. Trevors, C.I. Mayfield, W.E. Inniss, Measurement of Electron Transport System (ETS) activity in soil, Microb Ecol. 8 (1982) 163–168. https://doi.org/10.1007/BF02010449.

[388] F. Madrid, M. Rubio-Bellido, J. Villaverde, M. Tejada, E. Morillo, Natural attenuation of fluorene and pyrene in contaminated soils and assisted with hydroxypropyl-β-cyclodextrin. Effect of co-contamination, Science of The Total Environment. 571 (2016) 42–49. https://doi.org/10.1016/j.scitotenv.2016.07.110.

[389] S. Claassen, E. du Toit, M. Kaba, C. Moodley, H.J. Zar, M.P. Nicol, A comparison of the efficiency of five different commercial DNA extraction kits for extraction of DNA from faecal samples, J Microbiol Methods. 94 (2013) 103–110. https://doi.org/10.1016/j.mimet.2013.05.008.

[390] N. Haleyur, E. Shahsavari, S.S. Jain, E. Koshlaf, V.B. Ravindran, P.D. Morrison, A.M. Osborn, A.S. Ball, Influence of bioaugmentation and biostimulation on PAH degradation in aged contaminated soils: Response and dynamics of the bacterial community, J Environ Manage. 238 (2019) 49–58. https://doi.org/10.1016/j.jenvman.2019.02.115.

[391] X. Zhang, X. Liu, S. Liu, F. Liu, L. Chen, G. Xu, C. Zhong, P. Su, Z. Cao, Responses of Scirpus triqueter, soil enzymes and microbial community during phytoremediation of pyrene contaminated soil in simulated wetland, J Hazard Mater. 193 (2011) 45–51. https://doi.org/10.1016/j.jhazmat.2011.07.094.

[392] J.Y. Wang, L. Yang, C.-C. Tseng, H.-L. Hsu, Application of Phytoremediation on Soil Contaminated by Pyrene, Environ Eng Sci. 25 (2008) 829–838. https://doi.org/10.1089/ees.2006.0194.

[393] M. Grifoni, I. Rosellini, P. Angelini, G. Petruzzelli, B. Pezzarossa, The effect of residual hydrocarbons in soil following oil spillages on the growth of Zea mays plants, Environmental Pollution. 265 (2020) 114950. https://doi.org/10.1016/j.envpol.2020.114950.

[394] M.A.A. Wijayawardena, R. Naidu, M. Megharaj, D. Lamb, P. Thavamani, T. Kuchel, Using soil properties to predict in vivo bioavailability of lead in soils, Chemosphere. 138 (2015) 422–428. https://doi.org/10.1016/j.chemosphere.2015.06.073.

[395] X. Wu, L. Zhu, Evaluating bioavailability of organic pollutants in soils by sequential ultrasonic extraction procedure, Chemosphere. 156 (2016) 21–29. https://doi.org/10.1016/j.chemosphere.2016.04.113.

[396] Z.D. Parrish, M.K. Banks, A.P. Schwab, Assessment of contaminant lability during phytoremediation of polycyclic aromatic hydrocarbon impacted soil, Environmental Pollution. (2005). https://doi.org/10.1016/j.envpol.2005.02.012.

[397] Y. Gao, L. Zhu, Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils, Chemosphere. (2004). https://doi.org/10.1016/j.chemosphere.2004.01.037.

[398] Y. Sun, Q. Zhou, Uptake and translocation of benzo[a]pyrene (B[a]P) in two ornamental plants and dissipation in soil, Ecotoxicol Environ Saf. 124 (2016) 74–81. https://doi.org/10.1016/j.ecoenv.2015.09.037.

[399] R. Schroll, B. Bierling, G. Cao, U. Dörfler, M. Lahaniati, T. Langenbach, I. Scheunert, R. Winkler, Uptake pathways of organic chemicals from soil by agricultural plants, Chemosphere. 28 (1994) 297–303.

[400] C.T. Chiou, G. Sheng, M. Manes, A partition-limited model for the plant uptake of organic contaminants from soil and water, Environ Sci Technol. 35 (2001) 1437–1444.

[401] H.R. Arvanaghi, Y. Aminfar, A.A. Sadeghan, N. Dolatabadi, Phytoremediation of polycyclic aromatic hydrocarbons (PAHs): air-plant-soil interactions, J Biodivers Enviromental Sci. 10 (2017) 178–184.

[402] Y. HUANG, Y. DENG, D. LI, L. ZHAO, H. TANG, S. HUANG, Effect of growth and physiology-chemistry of hemp (Cannabis sativa) seedlings under cadmium stress, Plant Fiber Sciences in China. (2017) 5.

[403] J. Wang, X. Chen, W. Yan, C. Ning, T. Gsell, Both artificial root exudates and natural Koelreuteria paniculata exudates modify bacterial community structure and enhance phenanthrene biodegradation in contaminated soils, Chemosphere. 263 (2021) 128041. https://doi.org/10.1016/j.chemosphere.2020.128041.

[404] H. Zhang, Z. Dang, X.Y. Yi, C. Yang, L.C. Zheng, G.N. Lu, Evaluation of dissipation mechanisms for pyrene by maize (ZEA MAYS L.) in cadmium co-contaminated soil, Global Nest Journal. (2009). https://doi.org/10.30955/gnj.000599.

[405] W.S. Alves, E.A. Manoel, N.S. Santos, R.O. Nunes, G.C. Domiciano, M.R. Soares, Phytoremediation of polycyclic aromatic hydrocarbons (PAH) by cv. Crioula: A Brazilian alfalfa cultivar, Int J Phytoremediation. (2018). https://doi.org/10.1080/15226514.2018.1425663.

[406] W. Wang, X. Zhang, J. Huang, C. Yan, Q. Zhang, H. Lu, J. Liu, Interactive effects of cadmium and pyrene on contaminant removal from co-contaminated sediment planted with mangrove Kandelia obovata (S., L.) Yong seedlings, Mar Pollut Bull. 84 (2014) 306–313. https://doi.org/10.1016/j.marpolbul.2014.04.046.

[407] R. Liu, Y. Dai, L. Sun, Effect of Rhizosphere Enzymes on Phytoremediation in PAH-Contaminated Soil Using Five Plant Species, PLoS One. 10 (2015) e0120369. https://doi.org/10.1371/journal.pone.0120369.

[408] A. Wolinska, Z. Stepniewsk, Dehydrogenase Activity in the Soil Environment, in: Dehydrogenases, InTech, 2012. https://doi.org/10.5772/48294.

[409] Y. Hong, D. Liao, J. Chen, S. Khan, J. Su, H. Li, A comprehensive study of the impact of polycyclic aromatic hydrocarbons (PAHs) contamination on salt marsh plants Spartina alterniflora: implication for plant-microbe interactions in phytoremediation, Environmental Science and Pollution Research. 22 (2015) 7071–7081. https://doi.org/10.1007/s11356-014-3912-6.

[410] S. Baran, J.E. Bielińska, P. Oleszczuk, Enzymatic activity in an airfield soil polluted with polycyclic aromatic hydrocarbons, Geoderma. 118 (2004) 221–232. https://doi.org/10.1016/S0016-7061(03)00205-2.

[411] J. Ma, L. Xu, L. Jia, Characterization of pyrene degradation by Pseudomonas sp. strain Jpyr-1 isolated from active sewage sludge, Bioresour Technol. 140 (2013) 15–21. https://doi.org/10.1016/j.biortech.2013.03.184.

[412] G. Charalampous, E. Fragkou, K.A. Kormas, A.B. De Menezes, P.N. Polymenakou, N. Pasadakis, N. Kalogerakis, E. Antoniou, E. Gontikaki, Comparison of Hydrocarbon-Degrading Consortia from Surface and Deep Waters of the Eastern Mediterranean Sea: Characterization and Degradation Potential, Energies (Basel). 14 (2021) 2246. https://doi.org/10.3390/en14082246.

[413] Y. Ho, M. Jackson, Y. Yang, J.G. Mueller, P.H. Pritchard, Characterization of fluoranthene- and pyrene-degrading bacteria isolated from PAH-contaminated soils and sediments, J Ind Microbiol Biotechnol. 24 (2000) 100–112. https://doi.org/10.1038/sj.jim.2900774.

[414] D.-W. Kim, K. Lee, D.-H. Lee, C.-J. Cha, Comparative genomic analysis of pyrene-degrading Mycobacterium species: Genomic islands and ring-hydroxylating dioxygenases involved in pyrene degradation, Journal of Microbiology. 56 (2018) 798–804. https://doi.org/10.1007/s12275-018-8372-0.

[415] J.M. DeBruyn, T.J. Mead, G.S. Sayler, Horizontal Transfer of PAH Catabolism Genes in Mycobacterium : Evidence from Comparative Genomics and Isolated Pyrene-Degrading Bacteria, Environ Sci Technol. 46 (2012) 99–106. https://doi.org/10.1021/es201607y.

[416] H.W. Zhou, A.H.Y. Wong, R.M.K. Yu, Y.D. Park, Y.S. Wong, N.F.Y. Tam, Polycyclic Aromatic Hydrocarbon-Induced Structural Shift of Bacterial

Communities in Mangrove Sediment, Microb Ecol. 58 (2009) 153–160. https://doi.org/10.1007/s00248-008-9456-x.

[417] S.-H. Liu, G.-M. Zeng, Q.-Y. Niu, Y. Liu, L. Zhou, L.-H. Jiang, X. Tan, P. Xu, C. Zhang, M. Cheng, Bioremediation mechanisms of combined pollution of PAHs and heavy metals by bacteria and fungi: A mini review, Bioresour Technol. 224 (2017) 25–33. https://doi.org/10.1016/j.biortech.2016.11.095.

[418] F. Bianco, M. Marcińczyk, M. Race, S. Papirio, G. Esposito, P. Oleszczuk, Low temperature–produced and VFA–coated biochar enhances phenanthrene adsorption and mitigates toxicity in marine sediments, Sep Purif Technol. 296 (2022) 121414. https://doi.org/10.1016/j.seppur.2022.121414.

[419] N. Nawrot, E. Wojciechowska, S. Rezania, J. Walkusz-Miotk, K. Pazdro, The effects of urban vehicle traffic on heavy metal contamination in road sweeping waste and bottom sediments of retention tanks, Science of The Total Environment. 749 (2020) 141511. https://doi.org/10.1016/j.scitotenv.2020.141511.

[420] P.B. Tchounwou, C.G. Yedjou, A.K. Patlolla, D.J. Sutton, Heavy Metal Toxicity and the Environment, in: 2012: pp. 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6.

[421] H.B. Bradl, Adsorption of heavy metal ions on soils and soils constituents, J Colloid Interface Sci. 277 (2004) 1–18. https://doi.org/10.1016/j.jcis.2004.04.005.

[422] S.D. Young, Chemistry of Heavy Metals and Metalloids in Soils, in: 2013: pp. 51–95. https://doi.org/10.1007/978-94-007-4470-7_3.

[423] Y. Yang, N. Zhang, M. Xue, S. Tao, Impact of soil organic matter on the distribution of polycyclic aromatic hydrocarbons (PAHs) in soils, Environmental Pollution. 158 (2010) 2170–2174. https://doi.org/10.1016/j.envpol.2010.02.019.

[424] X. Guo, L. Luo, Y. Ma, S. Zhang, Sorption of polycyclic aromatic hydrocarbons on particulate organic matters, J Hazard Mater. 173 (2010) 130–136. https://doi.org/10.1016/j.jhazmat.2009.08.065.

[425] L. Tang, F.O. Gudda, C. Wu, W. Ling, H. El-Ramady, A. Mosa, J. Wang, Contributions of partition and adsorption to polycyclic aromatic hydrocarbons sorption by fractionated soil at different particle sizes, Chemosphere. 301 (2022) 134715. https://doi.org/10.1016/j.chemosphere.2022.134715.

[426] B. Huang, Z. Yuan, D. Li, M. Zheng, X. Nie, Y. Liao, Effects of soil particle size on the adsorption, distribution, and migration behaviors of heavy

metal(loid)s in soil: a review, Environ Sci Process Impacts. 22 (2020) 1596–1615. https://doi.org/10.1039/D0EM00189A.

[427] N. Zhao, F. Ju, H. Pan, Z. Tang, H. Ling, Molecular dynamics simulation of the interaction of water and humic acid in the adsorption of polycyclic aromatic hydrocarbons, Environmental Science and Pollution Research. 27 (2020) 25754–25765. https://doi.org/10.1007/s11356-020-09018-2.

[428] S.-F. Lim, A.Y.W. Lee, Kinetic study on removal of heavy metal ions from aqueous solution by using soil, Environmental Science and Pollution Research. 22 (2015) 10144–10158. https://doi.org/10.1007/s11356-015-4203-6.

[429] K. Ciarkowska, F. Gambus, J. Antonkiewicz, T. Koliopoulos, Polycyclic aromatic hydrocarbon and heavy metal contents in the urban soils in southern Poland, Chemosphere. 229 (2019) 214–226. https://doi.org/10.1016/j.chemosphere.2019.04.209.

[430] L. Liu, W. Li, W. Song, M. Guo, Remediation techniques for heavy metal-contaminated soils: Principles and applicability, Science of The Total Environment. 633 (2018) 206–219. https://doi.org/10.1016/j.scitotenv.2018.03.161.

[431] M. Thiombane, S. Albanese, M. di Bonito, A. Lima, D. Zuzolo, R. Rolandi, S. Qi, B. de Vivo, Source patterns and contamination level of polycyclic aromatic hydrocarbons (PAHs) in urban and rural areas of Southern Italian soils, Environ Geochem Health. 41 (2019) 507–528. https://doi.org/10.1007/s10653-018-0147-3.

[432] M. Grifoni, I. Rosellini, P. Angelini, G. Petruzzelli, B. Pezzarossa, The effect of residual hydrocarbons in soil following oil spillages on the growth of Zea mays plants, Environmental Pollution. 265 (2020) 114950. https://doi.org/10.1016/j.envpol.2020.114950.

[433] C. Wu, F. Li, S. Yi, F. Ge, Genetically engineered microbial remediation of soils co-contaminated by heavy metals and polycyclic aromatic hydrocarbons: Advances and ecological risk assessment, J Environ Manage. 296 (2021) 113185. https://doi.org/10.1016/j.jenvman.2021.113185.

[434] M. Saeedi, L.Y. Li, J.R. Grace, Simultaneous removal of polycyclic aromatic hydrocarbons and heavy metals from natural soil by combined non-ionic surfactants and EDTA as extracting reagents: Laboratory column tests, J Environ Manage. 248 (2019) 109258. https://doi.org/10.1016/j.jenvman.2019.07.029.

[435] K.R. Reddy, A.Z. Al-Hamdan, P. Ala, Enhanced Soil Flushing for Simultaneous Removal of PAHs and Heavy Metals from Industrial Contaminated Soil, J Hazard Toxic Radioact Waste. 15 (2011) 166–174. https://doi.org/10.1061/(ASCE)HZ.1944-8376.0000076.

[436] F. Bianco, M. Race, S. Papirio, P. Oleszczuk, G. Esposito, Coupling of desorption of phenanthrene from marine sediments and biodegradation of the sediment washing solution in a novel biochar immobilized–cell reactor, Environmental Pollution. 308 (2022) 119621. https://doi.org/10.1016/j.envpol.2022.119621.

[437] M.D. Fernández Rodríguez, M.C. García Gómez, N. Alonso Blazquez, J.V. Tarazona, Soil Pollution Remediation, in: Encyclopedia of Toxicology, Elsevier, 2014: pp. 344–355. https://doi.org/10.1016/B978-0-12-386454-3.00579-0.

[438] I. Sharma, Bioremediation Techniques for Polluted Environment: Concept, Advantages, Limitations, and Prospects, in: Trace Metals in the Environment - New Approaches and Recent Advances, IntechOpen, 2021. https://doi.org/10.5772/intechopen.90453.

[439] M. Laghlimi, B. Baghdad, H. el Hadi, A. Bouabdli, Phytoremediation Mechanisms of Heavy Metal Contaminated Soils: A Review, Open J Ecol. 05 (2015) 375–388. https://doi.org/10.4236/oje.2015.58031.

[440] L. Li, P. Zhu, X. Wang, Z. Zhang, Phytoremediation effect of Medicago sativa colonized by Piriformospora indica in the phenanthrene and cadmium co-contaminated soil, BMC Biotechnol. 20 (2020) 20. https://doi.org/10.1186/s12896-020-00613-2.

[441] S. Deng, T. Ke, Y. Wu, C. Zhang, Z. Hu, H. Yin, L. Guo, L. Chen, D. Zhang, Heavy Metal Exposure Alters the Uptake Behavior of 16 Priority Polycyclic Aromatic Hydrocarbons (PAHs) by Pak Choi (Brassica chinensis L.), Environ Sci Technol. 52 (2018) 13457–13468. https://doi.org/10.1021/acs.est.8b01405.

[442] S. Zhang, H. Yao, Y. Lu, X. Yu, J. Wang, S. Sun, M. Liu, D. Li, Y.-F. Li, D. Zhang, Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater, Sci Rep. 7 (2017) 12165. https://doi.org/10.1038/s41598-017-12437-w.

[443] E. Meers, A. Ruttens, M. Hopgood, E. Lesage, F.M.G. Tack, Potential of Brassic rapa, Cannabis sativa, Helianthus annuus and Zea mays for phytoextraction of heavy metals from calcareous dredged sediment derived

soils, Chemosphere. 61 (2005) 561–572. https://doi.org/10.1016/j.chemosphere.2005.02.026.

[444] C. Moscariello, S. Matassa, G. Esposito, S. Papirio, From residue to resource: The multifaceted environmental and bioeconomy potential of industrial hemp (Cannabis sativa L.), Resour Conserv Recycl. 175 (2021) 105864. https://doi.org/10.1016/j.resconrec.2021.105864.

[445] I. Gabriele, M. Race, S. Papirio, P. Papetti, G. Esposito, Phytoremediation of a pyrene-contaminated soil by Cannabis sativa L. at different initial pyrene concentrations, Chemosphere. 300 (2022) 134578. https://doi.org/10.1016/j.chemosphere.2022.134578.

[446] X. Song, C. Li, W. Chen, Phytoremediation potential of Bermuda grass (Cynodon dactylon (L.) pers.) in soils co-contaminated with polycyclic aromatic hydrocarbons and cadmium, Ecotoxicol Environ Saf. 234 (2022) 113389. https://doi.org/10.1016/j.ecoenv.2022.113389.

[447] M. Laghlimi, B. Baghdad, H. el Hadi, A. Bouabdli, Phytoremediation Mechanisms of Heavy Metal Contaminated Soils: A Review, Open J Ecol. 05 (2015) 375–388. https://doi.org/10.4236/oje.2015.58031.

[448] N.J. Bonilla-Bird, Y. Ye, T. Akter, C. Valdes-Bracamontes, A.J. Darrouzet-Nardi, G.B. Saupe, J.P. Flores-Marges, L. Ma, J.A. Hernandez-Viezcas, J.R. Peralta-Videa, J.L. Gardea-Torresdey, Effect of copper oxide nanoparticles on two varieties of sweetpotato plants, Plant Physiology and Biochemistry. 154 (2020) 277–286. https://doi.org/10.1016/j.plaphy.2020.06.009.

[449] V. Jayanthy, R. Geetha, R. Rajendran, P. Prabhavathi, S. Karthik Sundaram, S. Dinesh Kumar, P. Santhanam, Phytoremediation of dye contaminated soil by Leucaena leucocephala (subabul) seed and growth assessment of Vigna radiata in the remediated soil, Saudi J Biol Sci. 21 (2014) 324–333. https://doi.org/10.1016/j.sjbs.2013.12.001.

[450] C. Shen, X. Tang, S.A. Cheema, C. Zhang, M.I. Khan, F. Liang, X. Chen, Y. Zhu, Q. Lin, Y. Chen, Enhanced phytoremediation potential of polychlorinated biphenyl contaminated soil from e-waste recycling area in the presence of randomly methylated-β-cyclodextrins, J Hazard Mater. 172 (2009) 1671–1676. https://doi.org/10.1016/j.jhazmat.2009.08.064.

[451] APHA, AWWA, WEF (2005) Standard methods for the examination of water and wastewater, (2007).

[452] P. Mattei, A. Cincinelli, T. Martellini, R. Natalini, E. Pascale, G. Renella, Reclamation of river dredged sediments polluted by PAHs by co-composting

with green waste, Science of The Total Environment. 566–567 (2016) 567–574. https://doi.org/10.1016/j.scitotenv.2016.05.140.

[453] G. Trancone, D. Spasiano, M. Race, V. Luongo, A. Petrella, F. Pirozzi, U. Fratino, A.F. Piccinni, A combined system for asbestos-cement waste degradation by dark fermentation and resulting supernatant valorization in anaerobic digestion, Chemosphere. 300 (2022) 134500. https://doi.org/10.1016/j.chemosphere.2022.134500.

[454] S. da Silva Benevenute, J.H. Freeman, R. Yang, How do pinching and plant density affect industrial hemp produced for cannabinoids in open field conditions?, Agron J. 114 (2022) 618–626. https://doi.org/10.1002/agj2.20882.

[455] M. Ahmad, J. Ling, Y. Zhang, W. Sajjad, Q. Yang, W. Zhou, J. Dong, Effect of pyrene and phenanthrene in shaping bacterial communities in seagrass meadows sediments, Arch Microbiol. 203 (2021) 4259–4272. https://doi.org/10.1007/s00203-021-02410-7.

[456] J.K. Pannu, A. Singh, O.P. Ward, Influence of peanut oil on microbial degradation of polycyclic aromatic hydrocarbons, Can J Microbiol. 49 (2003) 508–513. https://doi.org/10.1139/w03-068.

[457] P.T. de S. e Silva, V.L. da Silva, B. de B. Neto, M.-O. Simonnot, Phenanthrene and pyrene oxidation in contaminated soils using Fenton's reagent, J Hazard Mater. 161 (2009) 967–973. https://doi.org/10.1016/j.jhazmat.2008.04.042.

[458] B.S.M. Singh, D. Singh, N.K. Dhal, Enhanced phytoremediation strategy for sustainable management of heavy metals and radionuclides, Case Studies in Chemical and Environmental Engineering. 5 (2022) 100176. https://doi.org/10.1016/j.cscee.2021.100176.

[459] H. Sarma, A.R. Nava, M.N.V. Prasad, Mechanistic understanding and future prospect of microbe-enhanced phytoremediation of polycyclic aromatic hydrocarbons in soil, Environ Technol Innov. (2019). https://doi.org/10.1016/j.eti.2018.12.004.

[460] D. Ghosal, A. Dutta, J. Chakraborty, S. Basu, T.K. Dutta, Characterization of the metabolic pathway involved in assimilation of acenaphthene in Acinetobacter sp. strain AGAT-W, Res Microbiol. 164 (2013) 155–163. https://doi.org/10.1016/j.resmic.2012.11.003.

[461] X. Li, Y. Song, Y. Bian, C. Gu, X. Yang, F. Wang, X. Jiang, Insights into the mechanisms underlying efficient Rhizodegradation of PAHs in biochar-amended soil: From microbial communities to soil metabolomics, Environ Int. 144 (2020) 105995. https://doi.org/10.1016/j.envint.2020.105995.

[462] M. Roy, Catabolic Versatility of Sphingobium sp. Strain PNB Capable of Degrading Structurally Diverse Aromatic Compounds, J Bioremediat Biodegrad. 04 (2013). https://doi.org/10.4172/2155-6199.1000173.

[463] A. Nzila, Update on the cometabolism of organic pollutants by bacteria, Environmental Pollution. 178 (2013) 474–482. https://doi.org/10.1016/j.envpol.2013.03.042.

[464] D.F. Placido, C.C. Lee, Potential of Industrial Hemp for Phytoremediation of Heavy Metals, Plants. 11 (2022) 595. https://doi.org/10.3390/plants11050595.

[465] C.M.R. Almeida, A.P. Mucha, M.F.C. Delgado, M. Isabel Caçador, A.A. Bordalo, M.T.S.D. Vasconcelos, Can PAHs influence Cu accumulation by salt marsh plants?, Mar Environ Res. 66 (2008) 311–318. https://doi.org/10.1016/j.marenvres.2008.04.005.

[466] A.C. Agnello, M. Bagard, E.D. van Hullebusch, G. Esposito, D. Huguenot, Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation, Science of The Total Environment. 563–564 (2016) 693–703. https://doi.org/10.1016/j.scitotenv.2015.10.061.

[467] H. Ali, E. Khan, M.A. Sajad, Phytoremediation of heavy metals—Concepts and applications, Chemosphere. 91 (2013) 869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075.

[468] N. Kashyap, K. Roy, V.S. Moholkar, Mechanistic investigation in Co-biodegradation of phenanthrene and pyrene by Candida tropicalis MTCC 184, Chemical Engineering Journal. 399 (2020) 125659. https://doi.org/10.1016/j.cej.2020.125659.

[469] L. Arru, S. Rognoni, M. Baroncini, P.M. Bonatti, P. Perata, Copper localization in Cannabis sativa L. grown in a copper-rich solution, Euphytica. 140 (2004) 33–38. https://doi.org/10.1007/s10681-004-4752-0.

[470] E. Bona, F. Marsano, M. Cavaletto, G. Berta, Proteomic characterization of copper stress response inCannabis sativa roots, Proteomics. 7 (2007) 1121–1130. https://doi.org/10.1002/pmic.200600712.

[471] B. Elisa, F. Marsano, M. Cavaletto, G. Berta, Copper stress in Cannabis sativa roots: morphological and pro-teomic analysis, Caryologia. 60 (2007) 96–101. https://doi.org/10.1080/00087114.2007.10589553.

[472] V. Angelova, R. Ivanova, V. Delibaltova, K. Ivanov, Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp), Ind Crops Prod. 19 (2004) 197–205. https://doi.org/10.1016/j.indcrop.2003.10.001.

[473] R.N. Malik, S.Z. Husain, I. Nazir, Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan, Pak J Bot. 42 (2010) 291–301.