

Review

# Phytoremediation—From Environment Cleaning to Energy Generation—Current Status and Future Perspectives

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**Abstract:** Phytoremediation is a technology based on the use of green plants to remove, relocate, deactivate, or destroy harmful environmental pollutants such as heavy metals, radionuclides, hydrocarbons, and pharmaceuticals. Under the general term of *phytoremediation*, several processes with distinctively different mechanisms of action are hidden. In this paper, the most popular modes of phytoremediation are described and discussed. A broad but concise review of available literature research with respect to the dominant process mechanism is provided. Moreover, methods of plant biomass utilization after harvesting, with particular regard to possibilities of “bio-ore” processing for metal recovery, or using energy crops as a valuable source for bio-energy production (bio-gas, bio-ethanol, bio-oil) are analyzed. Additionally, obstacles hindering the commercialization of phytoremediation are presented and discussed together with an indication of future research trends.

**Keywords:** phytoremediation; soil contaminants; plant utilization; metal recovery; bioenergy

## 1. Introduction

The rapid development of the global industry, especially in the field of energy supply, agriculture, mining, metal, and chemical production or transport, contributes to the increase of air, soil, and water pollution [1–4]. On the other hand, the rising environmental awareness of people affects pro-ecological activities, aiming to improve the quality and purity of the environment. It is crucial to maximize the progress in pollution-free production methods and minimize the level of contaminants by applying effective treatment ways. Contaminated soils and residues can be treated by various methods, including: isolation, incineration, stabilization, vitrification, thermal treatment, solvent extraction, chemical oxidation and many more. Above mentioned methods are usually expensive and in most cases result in the generation of secondary waste. Phytoremediation techniques seem to be a sustainable alternative to less environmentally friendly traditional methods of soil purification [5–7]. Phytoremediation is the use of plants and their associated microorganisms with the aim of removal, degradation or isolation of toxic substances from the environment. The word “phytoremediation” derives from the Greek “*phyton*” and the Latin “*remedium*”, which mean “plant” and “to correct”, respectively [8].

Phytoremediation involves using plants as a purifying agent in situ for soil and water systems remediation. The cleanup process may utilize various mechanisms, including phytoextraction, phytovolatilization, phytostabilization, phytodegradation, rhizodegradation, or rhizofiltration [9,10]. Phytoremediation has been widely studied for the removal of heavy metals, radionuclides, hydrocarbons, pesticides, or recently also pharmaceuticals [11–15]. The critical point, ensuring the profitability and

effectiveness of this method, may be achieved by appropriate selection of plant to the type and concentration of contaminants as well as the characteristics of the polluted site. Previous studies indicate that plants used for phytoremediation processes should have high adaptability and significant tolerance to contaminant exposure. Moreover, the high biomass yield, smooth spreading and harvesting along with strong ability to uptake and accumulate pollutants are required [16–18].

Two main parameters determine the plant's ability to remove contaminants from the substrate and to transport them from underground to above-ground parts of plants. The first parameter is the bioconcentration factor (BCF), defined as follows [19]:

$$BCF_{\text{roots}} = C_{\text{roots}}/C_{\text{medium}}, \quad (1)$$

$$BCF_{\text{shoots}} = C_{\text{shoots}}/C_{\text{medium}}, \quad (2)$$

where:  $C_{\text{roots}}$  is concentration of a contaminant in roots,  $C_{\text{shoots}}$  is concentration of a contaminant in shoots and  $C_{\text{medium}}$  is concentration of contaminant in growing medium (i.e., soil or aqueous solution).

The second parameter, called translocation factor (TF), may be calculated using Formula (3) [19]:

$$TF = C_{\text{shoots}}/C_{\text{roots}}, \quad (3)$$

where:  $C_{\text{roots}}$  is concentration of a contaminant in roots,  $C_{\text{shoots}}$  is concentration of a contaminant in shoots.

This paper is a comprehensive review of the recent state-of-the-art in the field of phytoremediation. In this paper, we present a detailed look at the phytoremediation mechanism-plant-contaminant proper selection. This review is organized as follows: the second section focuses on different mechanisms of phytoremediation with an emphasis on the examples of pollutants that may undergo remediation processes. The third section deals with examples of plants and contaminants assigned to the specific phytoremediation mechanism, summarized in the form of a table. In the next part, we describe major factors and the latest promising solution, which influence the efficiency of phytoremediation. Finally, we discuss the challenges and benefits of phytoremediation, and we point out future directions in this field, including the promising processing pathway of post-harvested plants towards bioenergy production. Although, only several reviews in the subject of phytoremediation have been published. Previous works have mainly focused on a single mechanism of phytoremediation, plant, or contaminant. There are also brief reviews describing all of the phytoremediation mechanisms without detailed analysis of plants or pollutants [9,20–22]. While this paper includes an elaborate description of phytoremediation topics taking into account the newest trends to overcome the phytoremediation limitations.

## 2. Mechanisms of Phytoremediation

The main mechanism of phytoremediation with examples of potentially treated contaminants which may undergo corresponding processes are shown in Figure 1.

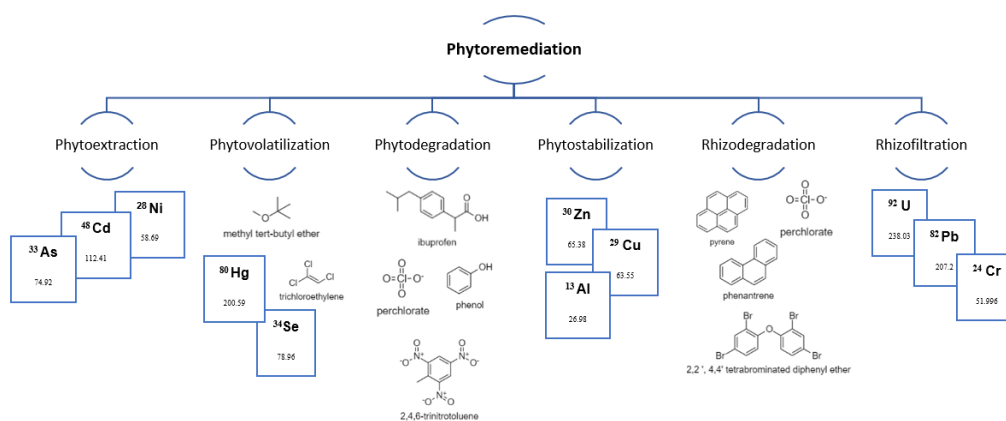


Figure 1. Mechanisms of phytoremediation with examples of removed pollutants.

## 2.1. Phytoextraction

Phytoextraction, also called phytoaccumulation, is a technique applied to remove contaminants from the environment and mainly focuses on the removal of heavy metals and radionuclides. Mechanism of phytoextraction follows several steps, starting from contaminants uptake from soil or water, which are further transported from roots to above-ground plant tissues and finally accumulated therein. Then, plants with collected contaminants are harvested [23,24]. Plants with both TF and BCF >1 have the potential to be used in phytoextraction process [19]. A series of recent studies have investigated the effect of phytoremediation on single-contaminated soil. Yang and Shen [25] characterized the potential of *Typha latifolia* on the remediation of cadmium in wetland soils. Researchers observed that the plant exhibits excellent tolerance for contaminants, however higher concentration of metal in roots than in shoots indicates weak ability to transport accumulated cadmium. Holubík et al. [26] investigated thallium removal by *Sinapis alba* during a hydroponic or semi-hydroponic experiment. In this case, shoots had the highest metal concentration, which indicates that use of *Sinapis alba* in phytoremediation is sufficient to harvest above-ground biomass. Saleem et al. [27] performed research on phytoremediation of copper-contaminated soil by *Linum usitatissimum*. Copper concentration in the soil above 400 mg/kg inhibited plant growth and biomass accumulation. Meanwhile, 140 days after sowing, mainly shoots accumulated copper, and 39–43% of initial Cu concentration was removed from the soil. Zhang and Liu [28] investigated the phytoremediation ability of *Gypsophila paniculata* to accumulate cesium. This plant showed good tolerance for the presence of metal in soil. The highest accumulation was observed in leaves and shoots with translocation factor above 1. After 75 days level of cesium extraction from soil was around 10–12%.

The effects of phytoremediation on multi-contaminated soil have been discussed in many scientific papers. Marathe and Ravichandran [29] investigated *Helianthus annuus* potential to remove heavy metals from soil contaminated by landfill leachate. There were no signs of damage to the plants, and the removal efficiency was about 68% for Pb, 100% for As, and 97% for Hg. Marchiol et al. [30] characterized the ability of *Brassica napus* and *Raphanus sativus* for phytoextraction of Cd, Cr, Cu, Ni, Pb, and Zn from contaminated soil. The *Raphanus sativus* was more tolerant and presented higher metal accumulation in shoots than *Brassica napus*. However, both of these species showed low phytoremediation effect. Gupta and Sintha [31] reported the ability of *Chenopodium album* to accumulate metals from soil amended with tannery sludge. Accumulation of the metals in the plants was as the following: Fe > Mn > Zn > Cr > Cu > Pb > Ni > Cd. The highest concentration of Cr, Pb, Fe, and Cd was observed in leaves, while for Mn and Zn in roots. Keeling et al. [32] compared the phytoextraction efficiency of *Berkheya coddii* for single-contaminated soil with Ni or Co, and soil contaminated with both Ni and Co. Plant accumulation of both metals from single-contaminated soil indicate that the bioconcentration factor increases as total metal concentrations increase. Plants readily accumulated cobalt, irrespectively on the presence of nickel. However, the co-existence of an equal mass concentration of cobalt limited the removal of nickel.

Hyperaccumulator plants are of particular importance when phytoextraction is considered. These plants can accumulate high concentrations of heavy metals. The term hyperaccumulator was first introduced for a species that might accumulate in aerial parts > 1000 mg/kg Ni [33]. Examples of these plants are *Alyssum pintodasilvae* [34] and *Alyssum murale* [35] for nickel, *Pteris vittata* [36] for arsenic, *Solanum nigrum* [37] for cadmium, *Arabidopsis helleri* [38] for zinc. Meanwhile, *Thlaspi caerulescens* is a plant that exhibits hyperaccumulation for both metals—zinc and cadmium [39].

## 2.2. Phytovolatilization

Phytovolatilization concerns mainly the volatile organic compounds [40–42] or volatile inorganic contaminants such as Hg, As, or Se [43–46]. This mechanism of phytoremediation may involve two possible ways of volatilization. These are direct phytovolatilization by steam or leaves and indirect phytovolatilization from the root zone. Direct phytovolatilization includes uptake of contaminants from soil, transforming them into volatile compounds, which are further excreted to the atmosphere

by leaves transpiration or radial diffusion through stem tissues [47]. The mechanism of indirect phytovolatilization involves volatile organic contaminants flux from the subsurface as an effect of plant roots activities e.g., increasing soil permeability, lowering the water table, chemical transport via hydraulic redistribution, or water table fluctuations [47]. Plants applied for phytovolatilization of organic compounds commonly belong to deciduous trees such as *Poplar* and *Salix*, or coniferous trees like *Pinus* [40,48–51]. Sakakibara et al. [45] investigated the phytovolatilization effect of *Pteris vitatta* on soil contaminated with arsenic. Plants converted about 90% of total arsenic uptake into arsenite and arsenate. *Brassica juncea*, *Chara canescens*, and *Salicornia bigelovii* were applied for phytovolatilization of selenium compounds [52,53]. The researchers proved that plants transformed toxic selenium compounds into the relatively non-toxic chemical form of dimethyl selenide. Heaton et al. [54] implemented genetically engineered plants with a modified bacterial mercuric reductase gene—*Arabidopsis thaliana* and *Nicotiana tabacum* for phytoremediation of soil contaminated with mercury and methylmercury. They found that toxic Hg(II) is converted into much less toxic Hg(0) and volatilized from the plants. Moreover, transgenic plants were able to volatilize 3 to 4 times more Hg(0) than control plants without the mercuric reductase gene. Phytovolatilization technique is also widely applied for the treatment of soil irrigated by groundwater with trichloroethylene, tetrachloroethylene, or perchloroethylene [55–59]. It was observed that above 90% of trichloroethylene (TCE) or perchloroethylene (PCE) might be removed from soil. However, a large proportion of contaminants is volatilized right out of the soil. The researchers pointed out that dehalogenation is the primary mechanism responsible for the removal of chlorinated compounds, which was confirmed by the presence of free chloride in the soil after the phytoremediation process. Furthermore, among organic compounds, phytovolatilization may be applied for the removal of methyl tert-butyl ether (MTBE) by *Pinus* or *Salix* [40,50]. The authors observed that signs of *Salix* phytotoxicity related to the reduction of normalized relative transpiration occurred for a high dose of MTBE (400 mg/L). The principal mechanism responsible for MTBE removal was plant transpiration, while plant tissues accumulated the only little amount of organic compound. However, they found that MTBE was not metabolized during transport in the plant and released in the unchanged chemical form.

### 2.3. Phytodegradation

Phytodegradation, also referred to as phytotransformation, is applied for petroleum hydrocarbons, pesticides, insecticides, surfactants, or pharmaceuticals degradation in soil and water. Phytodegradation includes uptake of contaminant from the substrate and its breakdown to low-molecular-weight intermediates via metabolic processes within the plants [60,61]. Enzymes i.e., dehalogenases, oxygenases, and reductases, secreted by plant tissues, are responsible for catalyzing degradation pathways of contaminants [62–64]. Susarla et al. [65] compared the effect of phytodegradation on the sand and aqueous experiment upon contamination with perchlorate by *Myriophyllum aquaticum*. The uptake of perchlorates was 5 times faster for the aqueous environment than for soil, and this probably was due to the influence of chloride ions desorbed from the sand. Degradation pathways of perchlorate were suggested as a stepwise manner to form chloride. Similarly, *Myriophyllum aquaticum* was tested for phytodegradation of trinitrotoluene in aqueous treatment. In this case, the plant removed about 70% of trinitrotoluene (TNT) from the solution [66]. Yoon et al. [67] investigated the mechanism of phytotransformation of 2,4-dinitrotoluene (DNT) in liquid medium by *Arabidopsis thaliana*. The authors observed that plants degrade about 95% of initial 2,4-DNT concentration after 15 days and transformed and incorporated a small part of organic compounds in plant tissue, possibly as a such of lignin and cellulose. Moreover, monoaminonitrotoluene and other unknown metabolites were detected as an intermediate product of 2,4-DNT transformation.

Most recent trends in the studies of phytodegradation processes concern the treatment of aqueous solution contaminated with pharmaceuticals. Regular literature studies involve phytodegradation of antibiotics and nonsteroidal anti-inflammatory drugs [68–70]. One of the examples is the phytodegradation of Ibuprofen by *Phragmites australis* [71]. The researchers concluded that this plant was able to uptake,

translocate, and degrade Ibuprofen (IBP). No symptoms of phytotoxicity were observed, and the plant removed the total amount of initial contaminant concentration within 21 days. Moreover, in the plant tissue, especially in stem and leaves, four types of intermediate products were detected. These were: hydroxy-IBP, 1,2-dihydroxy-IBP, carboxy-IBP, and glucopyranosyloxy-hydroxy-IBP. A similar degradation pathway of Ibuprofen by *Typha Latifolia* observed Li et al. [72]. The authors detected Ibuprofen carboxylic acid, 2-hydroxy-IBP, and 1-hydroxy-IBP as intermediates. Singh et al. [73] investigated the effect of antibiotic—ofloxacin phytodegradation by *Spirodela polyrhiza*. In this case, after 7 days, about 93–98% of ofloxacin was removed. Other studies concern phytoremediation of an antibiotic-tetracycline. Datta et al. [74] reported that *Chrysopogon zizanioides* exhibit high potential for degradation of tetracycline (TC) from aqueous media. After 40 days, TC was completely removed, moreover, some unknown metabolites of TC were detected in the plant root and shoot tissues. Comparable research focused on the degradation of tetracycline by *Lemna gibba* L. performed Topal et al. [75]. In this case, the maximum removal efficiency for TC in the planted reactor was determined as 79.6% at day 10. Li et al. [76] and Ryšlavá et al. [77] evaluated the phytodegradation of carbamazepine by *Zea mays*, *Helianthus annuus*, *Daucus carota* L., and *Apium graveolens* L. Carbamazepine (CBZ) was not readily uptaken by plants, probably due to its hydrophobic properties. However, some intermediates such as amino-CBZ-10, 11-epoxide were detected, thus the epoxidation process was confirmed as one of the possible degradation pathways.

Another group of contaminants, which may be effectively removed from water by phytodegradation, are plant protection products, especially pesticides and insecticides. Xia and Ma [78] investigated the potential of *Eichhornia crassipe* to remove a phosphorus pesticide—ethion. The Authors concluded that the plant removed about 69% of ethion by accumulation. Moreover, after one week of incubation, the concentration of phosphorus pesticide in plant tissue significantly decreased, which indicates the degradation of this compound by the metabolism of the plant. Rani et al. [79] observed phytotransformation of toxic insecticides—phorate by *Brassica juncea*. During this experiment, above 68% of phorate was removed within 5 days. Only phorate sulfoxide was detected as an intermediate product, which confirms that sulfoxidation is an essential transformation pathway for organophosphate insecticides in the plant.

#### 2.4. Phytostabilization

Phytostabilization is widely used for the treatment of soil, wetlands, or mining waste contaminated with metals such as Zn, Pb, Cd, Mn, Cu, Cr, Fe, As, and Ni [80–84]. This method refers to limitation of contaminants bioavailability and its immobilization to avoid bulk erosion, reduce air-borne transport or leaching and thus to prevent the distribution of toxic contaminants to other areas [85]. Phytostabilization involves rhizosphere-induced adsorption and precipitation processes, sorption, or complexation [86–89]. In this process, plants called excluders play a fundamental role, which actively limits metal uptake. These plants can absorb and accumulate metals in their roots and ( $BCF > 1$ ), on the other hand, are characterized by low root-shoot translocation factor ( $TF < 1$ ) [90,91]. One of the solutions to enhance the efficiency of phytostabilization is the so-called aided phytostabilization [92,93]. It is related to the application of inorganic or organic amendments to the substrate. The most common examples are manure compost, biochar, biosolids, activated carbon, diatomite, chalcedonite, dolomite, sand, limestone, bone mill, bottom ash, furnace slag, and red mud [93–96]. Pérez-Esteban et al. [94] observed that the addition of manure had an impact on the reduction of metal concentration in shoots of *B.juncea*, decreasing the value of bioconcentration factor and copper and zinc bioavailability in soil and high accumulation of both metals in roots. This effect, as explained, was related to high pH and the presence of organic matter, which acts as fertilizer. A similar effect was reported by Meeinkuirt et al. [97], who examined the influence of manure on the Cd phytostabilization potential of *Eucalyptus camaldulensis*. The application of amendments improved plant growth and biomass production. Furthermore, plants grown on amended soils had lower Cd accumulation than those grown on the Cd soil alone. The same effects were described by Phusantisampan et al. [98], who demonstrated the potential of *Vetiveri*

*zizanioides* for phytostabilization of cadmium in soil, enhanced by mixing soil with manure. Meanwhile, Lee et al. [92] compared the effect of the bone mill, bottom ash, furnace slag, and red mud application in aided phytostabilization of Pb/Zn mine tailings by *Miscanthus sinensis* and *Pteridium aquilinum*. *M. sinensis* accumulated heavy metals mostly in the roots and had a lower value of translocation factor compared to *P. aquilinum*. Furthermore, Fe-rich amendments such as furnace slag or red mud significantly reduced the amount of soluble and extractable heavy metals.

Another possible direction to improve phytostabilization processes includes roots inoculation with fungus resulting in symbiotic interaction between fungi and plants. Application of mycorrhizal fungus was investigated by Chen et al. [99] and Gu et al. [100] in the phytostabilization of zinc, lead, copper, cadmium, or uranium. In the case of uranium, mycorrhizal decreased concentration of U in shoots and increased U concentration in roots. A comparable effect was observed for Pb, Zn, Cu, and Cd with a higher concentration in roots than in shoots. The Authors concluded that mycorrhizal fungi inoculation reduces metal bioconcentration and translocation factors. Ouari et al. [101] also observed that the inoculation of mycorrhizal fungi onto *Eucalyptus camaldulensis* might improve the plant growth and its tolerance to high copper concentration in soil.

### 2.5. Rhizodegradation

Rhizodegradation also known as a phytostimulation refers to the decomposition of pollutants such as PAHs (polyaromatic hydrocarbons), hydrocarbons or perchlorates, due to the activity of microorganisms in the rhizosphere. This type of phytoremediation may be referred to plant-microorganism cooperation, strongly depending on the interactions between these group of species. Plant exudates being a carbon source, provide beneficial conditions for growth and development of soil microflora, while microorganism such as bacteria and fungus are able to degrade hazardous contaminants to nontoxic products via enzymatic and metabolic processes [102–104]. Rhizodegradation may be an effective way for cleanup of soil contaminated by petroleum, diesel, or oily sludge [105–107]. Maqbool et al. [106] compared the impact of bioaugmentation on rhizodegradation of petroleum hydrocarbons by *Sesbania cannabina*. The plant degraded about 75% of hydrocarbons in the rhizosphere within 120 days due to natural plant-microorganism interaction, whereas bioaugmentation did not improve organic contaminants removal. Comparable research performed Ramos et al. [108], who noticed that after 60 days, reduction of hydrocarbons concentration by *Sebastiania commersoniana* was 60% and after 424 days above 94%. Moreover, vegetated and contaminated soil presented higher microbial density and diversity. Hydrocarbons found in petroleum may be used by microorganism as a carbon source. Lu et al. [109] and Jia et al. [110] investigated rhizodegradation of phenanthrene (Ph) and pyrene (Py), respectively, by *Kandelia candel* and *Avicennia marina*. Researchers stated that the dissipation of phenanthrene and pyrene were significantly higher in the rhizosphere compared to non-rhizosphere zones of sediments. *Kandelia candel* was able to remove 47.7% of Ph and 37.6% of Py after 60 days, while *Avicennia marina* 71–86% Ph and 63–79% Py after 120 days. In both cases, plant root promoted dissipation significantly exceeded uptake and accumulation of hydrocarbons in plant tissue. Some authors have also suggested that perchlorates may be readily removed by rhizodegradation. Yifru et al. [111] examined the potential of *Salix nigra* for biostimulation of perchlorates rhizodegradation. As an electron source, the authors proposed natural and artificial carbon products. Addition of dissolved organic carbon reduced time required for degradation of total perchlorate from 70 days to 9 days. Mwegoha et al. [112] evaluated the effect of biostimulation for perchlorate rhizodegradation by *Salix babylonica* using chicken manure. They observed that the addition of dissolved organic carbon reduces perchlorate uptake and phytoaccumulation in plant tissue.

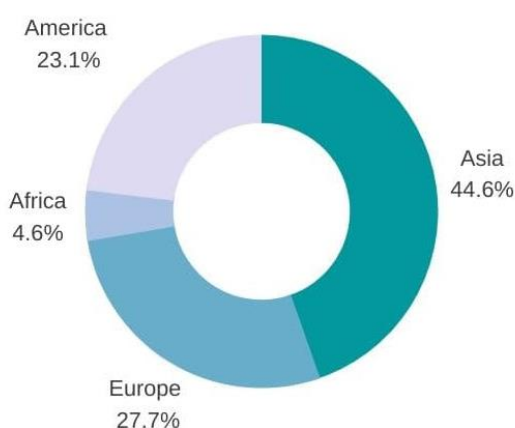
### 2.6. Rhizofiltration

Rhizofiltration is a phytoremediation technique which involves using a plant able to adsorb contaminants occurring in the rhizosphere on the surface of roots or absorb into roots tissue, concentrate and precipitate them [113,114]. Plants with long, fibrous root system covered with root hairs and

having high surface area are particularly desirable to provide effective remediation [115,116]. This method is especially used for the treatment of groundwater or wastewater polluted with heavy metals or radionuclides, such as Ra, U, and Cs [115,117–119]. Uranium rhizofiltration by *Helianthus annuus* and *Phaseolus vulgaris* was investigated by Lee and Yang [113]. Researchers observed that *Helianthus annuus* removed about 80% of initial concentration within 24 hours, while *Phaseolus vulgaris* from 60 to 80%. Moreover, it was concluded that the highest removal efficiency occurred at pH 3–5. Roots analysis revealed that the mechanism of uranium rhizofiltration might be strongly dependent on the adsorption, precipitation, and exchangeable sorption on the root surface. Eapen et al. [116] examined potential of *Brassica juncea* and *Chenopodium amaranticolor* for rhizofiltration of uranium. Plant root systems genetically transformed by *Agrobacterium rhizogenes* have evolved hairy root cultures used as a bioadsorbant. *B. juncea* uptake was 20–23% of uranium from the solution, while *C. amaranticolor* showed only 13% uptake. Tomé et al. [115] applied rhizofiltration for removing uranium and radium by *Helianthus annuus*. In this case, after 2 days plant removed about 50% of uranium and 70% of radium, which accumulated in the roots with deficient translocation factor. In contrast, a specific part of radionuclides was bounded as copious white precipitate. Analogous analysis was performed by Yang et al. [118] for the removal of uranium by *Phaseolus vulgaris*. Obtained result suggested that optimal conditions for uranium removal occurred at moderately acidic pH conditions (pH 3–5) when uranyl cation is the predominant uranyl species, which is readily translocated to plant roots. At pH 5, the *Phaseolus vulgaris* decreased the uranium concentration by 90.2% within 12 h and by 98.9% within 72 h. Root analysis confirmed that rhizofiltration mechanism at pH 7 is based on the adsorption and precipitation on the root surface in the form of insoluble uranium compounds. Veselý et al. [120] proposed treatment of highly polluted solution contaminated by cadmium and lead by rhizofiltration using *Pistia Stratiotes*. The plant exhibited high tolerance to heavy metal stress and excellent capability for metal accumulation. The experiment showed that *Pistia Stratiotes* was able to remove up to 95% of metals after 7 days, and the concentration of Cd and Pb were about 10-fold higher in roots than in leaves.

Information provided in Table 1 summarizes the literature research on phytoremediation. Collected information is grouped according to the dominant mechanism of phytoremediation. Valuable information on the details of experimental work together with main conclusions are presented in concise manner.

Figure 2 presents percentage distribution of research papers included in Table 1 for individual continents.



**Figure 2.** Percentage of research papers from Table 1 for the individual continents.

Taking into consideration percentage of research papers assigned to individual continents it can be concluded that greatest interest in the topic of phytoremediation occurs in Asian countries, followed by Europe and America. However, notable lower share of Africa may be associated with the limitation of the ability to perform phytoremediation process for example due to the tropical weather conditions.

**Table 1.** Summary of research on phytoremediation with respect to the main process mechanism.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytoextraction	<i>Typha Latifolia</i>	China	wetland soil	Greenhouse pot experiment (pots—20 × 20 × 20 cm, day/night temperature of 25/18 °C, relative humidity from 70 to 80%) Exposition to contaminants: 90 days	Cd (0.1 and 30 mg/kg soil)	<ul style="list-style-type: none"> <li>Higher bioaccumulation of Cd in roots than in shoots (low TF factor).</li> <li>Accumulation of Cd in <i>T. latifolia</i> tissues was 77.0 and 410.7 mg/kg.</li> </ul>	[25]
	<i>Typha Angustifolia</i>	China	soil (1/2 compost, 1/4 vermiculite and 1/4 sand)	Greenhouse pot experiment (pots—20 cm high, capacity 5 L). Addition of chelator: 2.5, 5, and 10 mM EDTA or citric acid Exposition to contaminants: 20 days + 25 days after chelator application	Cd (50 mg/kg soil) Cu (10 mg/kg soil) Pb (20 mg/kg soil) Cr (10 mg/kg soil)	<ul style="list-style-type: none"> <li>Addition of EDTA, and 5 and 10 mM CA remarkably increased shoots Cd, Pb, and Cr concentration.</li> </ul>	[121]
	<i>Helianthus annuus</i>	Switzerland	loamy topsoil from an agricultural field	Greenhouse pot experiment (16 h (21 °C)/8 h (16 °C) day/night cycle, light intensity at leaf height of 10,900 lux) Addition of chelator: 10 mM EDDS Exposition to contaminants: 4 weeks + 5 days after chelator application	Cu (360 mg/kg soil) Zn (530 mg/kg soil)	<ul style="list-style-type: none"> <li>Addition of EDDS caused signs of toxicity, necrosis symptoms and loss of shoots dry weight.</li> <li>EDDS increased metal solubility in soil and significantly enhanced shoots Cu uptake.</li> </ul>	[122]
	<i>Thlaspi caerulescens</i>	Netherlands	soil contaminated due to atmospheric deposition of metal-bearing dust from a smelter	Greenhouse pot experiment (cone-shaped pots—18 cm in height with a top diameter of 18 cm and a bottom diameter of 14 cm, relative humidity from 30 to 60%, 16 h (20 °C)/8h (18 °C) day/night cycle, artificial light intensity of 400 W/m <sup>2</sup> ) Exposition to contaminants: 113 days	Cd (from 0.51 = 0.03 to 9.6 ± 0.6 mg/kg soil) Zn (from 33.3 ± 1.4 to 776 ± 43 mg/kg soil)	<ul style="list-style-type: none"> <li>Higher concentration of Zn and Cd in shoots than in roots, with TF &gt;1 for both metal.</li> <li>For 100% contaminated soil concentration of Cd in shoots was 749 ± 73mg/kg and of Zn was 4044 ± 400 mg/kg.</li> </ul>	[123]
	<i>Thlaspi goesingense</i>	Austria	soil collected from the vicinity of a former Pb-Zn smelter (sand, silt, clay)	Greenhouse pot experiment Addition of chelator: EDTA and (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> Exposition to contaminants: 143 days + 7 days after chelator application	Cd (6.3 and 19.7 mg/kg soil) Cu (174 and 232 mg/kg soil) Ni (23.9 and 126 mg/kg soil) Pb (12300 mg/kg soil) Zn (714 and 2710 mg/kg soil)	<ul style="list-style-type: none"> <li><i>T. goesingense</i> was a Pb and Zn hyperaccumulator (2840 mg/kg for Pb and 4000 mg/kg for Zn).</li> <li>Addition of EDTA enhanced Cd, Cu, Ni, and Zn accumulation, while ammonium sulfate only Cd and Zn.</li> </ul>	[124]
	<i>Brassica juncea</i>	Italy	soil from farming area contaminated by high metal polluted irrigation water	Greenhouse pot experiment (pots—22 cm diameter plastic, 16 h (25 °C)/8h (20 °C) day/night cycle, relative humidity of 75%, 400 μmol m <sup>-2</sup> s <sup>-1</sup> photon flux density) Addition of chelator: 5 mmol/kg NTA or citric acid Exposition to contaminants: 43 days + 7 days after chelator application	Cd (40 mg/kg soil) Cr (186 mg/kg soil) Cu (313 mg/kg soil) Pb (1331 mg/kg soil) Zn (3326 mg/kg soil)	<ul style="list-style-type: none"> <li><i>B. juncea</i> shoots dry weights suffered notable decreases following NTA application (about 33% loss).</li> <li>Application of NTA increased shoots metal concentrations 2–3 times,</li> </ul>	[125]





Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytoextraction	<i>Brassica napus</i>	Austria	soil from former industrial site	Outdoors pot experiment Addition of chelator: 0.21, 0.41, 0.83, and 1.65 g/kg EDTA Exposition to contaminants: 60 days (application of chelator at 35th day and 50th day) Field-lysimeter experiment (spacing of 15 × 10 cm between individual plants) Addition of chelator: 0.25, 0.50, 1.0 and 2.01 g/kg EDTA Exposition to contaminants: about 6 months	Cu (225 mg/kg soil) Pb (77.1 mg/kg soil) Zn (344 mg/kg soil)	<ul style="list-style-type: none"> <li>Addition of EDTA (1.65 mg/kg soil), increased the labile metal fraction to 34% (Cu), 11% (Pb) and 17% (Zn).</li> <li><i>B.napus</i> accumulated about 20 mg Cu, 2 mg Pb and 120 mg Zn/kg DW in shoots and 130 mg Cu, 30 mg Pb and 180 mg Zn/kg DW in roots of the control plants.</li> </ul>	[126]
	<i>Zea mays</i>	Belgium	dredged sediments from the river (clay, silt, sand)	Greenhouse pot experiment (12 h/12 h day/night cycle, temperature from 18.5 to 22.5 °C, relative humidity from 60 to 70%.) Addition of chelator: 2mmol/kg EDTA, citric acid, and ammonium acetate Exposition to contaminants: 6 weeks (application of chelator 1 day before sowing or at 10th day after sowing)	Cu (145 ± 11 mg/kg soil) Zn (874 ± 61 mg/kg soil) Cd (9 ± 0.2 mg/kg soil) Pb (181 ± 6 mg/kg soil) Ni (58 ± 6 mg/kg soil)	<ul style="list-style-type: none"> <li>Application of EDTA and DTPA increased levels of heavy metals in the aerial biomass and TF value.</li> <li>Addition of EDTA 10 d after germination, compared to 1 day before sowing, better enhanced metal accumulation.</li> </ul>	[127]
		China	soil from disused agricultural field	Greenhouse pot experiment (pots—12 cm diameter and 12 cm height, temperature from 18 to 23 °C) Addition of chelator: EDTA or/and EDDS (variable ratios) Exposition to contaminants: 2 weeks + 2 weeks after chelator application	Pb (2500 mg/kg soil) Cu (500 mg/kg soil) Zn (1000 mg/kg soil) Cd (15 mg/kg soil)	<ul style="list-style-type: none"> <li>Use of 5 mmol/kg of EDTA and EDDS notably reduced the plants growth.</li> <li>Ratio 2:1 of EDTA: EDDS was the most productive to rise the concentrations of Cu, Pb, Zn and Cd in the shoots and TF value.</li> <li>The simultaneous application of EDTA and EDDS (ratio of 2:1) let to obtain the greatest Pb concentration of 647 mg kg DW in the shoots.</li> </ul>	[128]
	<i>Alyssum murale</i>	Albania	soil from ultramafic area	Field experiment (area divided into six 36-m <sup>2</sup> plots) Exposition to contaminants: 3 months	Ni (3500 mg/kg soil)	<ul style="list-style-type: none"> <li>Ni content in the shoots of <i>A. murale</i> reached 9129 mg/kg.</li> <li>For phytoextraction with <i>A. murale</i>, the preferably pH range was between 5.2 and 6.2.</li> </ul>	[129]
		Canada	agricultural soils collected near a historic Ni refinery	Greenhouse pot experiment (pots—18 cm diameter, 16 h (28 °C)/8 h (20 °C) day/night cycle) Exposition to contaminants: 120 days	Ni (1720 and 2570 mg/kg soil) Co (24 and 37 mg/kg soil)	<ul style="list-style-type: none"> <li>Ni accumulation decreased at lower soil pH and increased at higher soil pH.</li> <li>For <i>A. murale</i> shoot Ni concentrations was from 2180–11300 mg/kg</li> </ul>	[130]



Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytoextraction	<i>Populus nigra</i>	Egypt	clay soil	Greenhouse pot experiment (16 h/8h day/night cycle, temperatures of $25 \pm 2$ °C, relative humidity from 40 to 50%, $300 \mu\text{mol m}^{-2}\text{s}^{-1}$ photon flux density) Exposition to contaminants: 30 months	Cd (from 3.9 to 15.6 mg/kg soil) Cu (from 3.6 to 63.6 mg/kg soil) Pb (from 19.11 to 173.3 mg/kg soil)	<ul style="list-style-type: none"> <li>More Cd, Cu, Pb were localized in roots, than in leaves or stem, with low bioconcentration factor and TF factor.</li> <li>The negative impact was observed for higher metal concentration—reduction of plant dry weight.</li> </ul>	[131]
	<i>Populus nigra x Populus maximowiczii</i>	Czech Republic	soil from mining and smelting area	Pot experiment in controlled outdoor vegetation hall Addition of chelator: 3, 6 and 9 mmol/kg EDTA or EDDS Exposition to contaminants: 100 days + 30 days after chelator addition	Pb ( $200 \pm 2$ and $1360 \pm 10$ mg/kg soil)	<ul style="list-style-type: none"> <li>Both EDTA and EDDS were efficient in solubilising Pb in soil.</li> <li>EDTA was more productive than EDDS in desorbing and complexing Pb from soils, and led to mobilization 60% of Pb.</li> <li>The addition of 9 mmol EDTA/kg soil, led to a rise of Pb concentration in <i>Poplar</i> aerial parts (<math>251 \pm 35</math> mg/kg).</li> </ul>	[132]
	<i>Vetiveria zizanioides</i>	USA	soil from firing range	Greenhouse pot experiment (pots—capacity 13.2 L) Addition of chelator: 5 mmol/kg EDTA Exposition to contaminants: 4 months (addition of chelator 1 week before harvesting)	Pb (300–4500 mg/kg soil)	<ul style="list-style-type: none"> <li>Application of EDTA, one week before harvesting notably increased the Pb phytoextraction</li> <li>Pb concentrations was 1390–1450 mg/kg in tissue samples.</li> </ul>	[133]
		Brazil	soil from an area of slag deposition	Field experiment (different spacing between rows (0.80, 0.65 and 0.50 m).) Addition of chelator: 40 mmol/kg citric acid Exposition to contaminants: 69 days (addition of chelator 1 week before harvesting)	Pb (1850 mg/kg soil)	<ul style="list-style-type: none"> <li>The citric acid improved a Pb accumulation in <i>Vetiveria</i> shoots that was about 7 times higher than the control</li> </ul>	[134]
	<i>Rumex crispus Rumex K-1</i>	China	soil form farmland near the Pb/Zn mine	Field experiment (experimental area was split into eight blocks and 32 plots (each plot 3 m × 2 m)) Addition of chelator: 6 mmol/kg EDTA Exposition to contaminants: about 100 days (addition of chelator 1 week before harvesting)	Pb ( $960 \pm 54$ mg/kg) Zn ( $1050 \pm 89$ mg/kg) Cd ( $7.2 \pm 0.92$ mg/kg)	<ul style="list-style-type: none"> <li>High biomass production for both <i>Rumex</i> species—25 t/ha and 18 t/ha.</li> <li><i>Rumex crispus</i> presented higher metal accumulation in tissues</li> <li>Addition of EDTA enhance extraction rate for metals, especially for Pb</li> </ul>	[135]



Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytoextraction	<i>Sedum alfredii</i>	China	paddy soil from Pb/Zn mining site	Field experiment (21 field plots of 1.2 m × 6.8 m) Addition of chelator: MGWL, citric acid and EDTA at a mole ratio of 1:10:2 Exposition to contaminants: about 2 months (addition of chelator 21 days before harvesting)	Pb (268 mg/kg soil) Zn (121 mg/kg soil) Cd (0.99 mg/kg soil)	<ul style="list-style-type: none"> <li>The addition of the chelators mixture (MC) significantly increased biomass yields of <i>S. alfredii</i></li> <li>The uptake of Zn, Cd, and Pb by <i>S. alfredii</i> was enhanced with the addition of MC.</li> <li>Addition of the MC, increased the uptake of Zn, Cd and Pb by <i>S. alfredii</i>, by 65.71%, 47.93% and 78.49%, respectively.</li> </ul>	[136]
		China	soil irrigated with industrial wastewater	Pot experiment (pots—15 cm height and 20 cm diameter) Addition of chelator: 5 and 8 mmol/kg EDTA or citric acid Exposition to contaminants: 30 days	Cd (3.0 ± 0.4 mg/kg) Cu (45.5 ± 1.8 mg/kg) Zn (168.8 ± 16.4 mg/kg) Pb (57.9 ± 1.4 mg/kg)	<ul style="list-style-type: none"> <li>The application of EDTA (5 and 8 mmol/kg) and CA (5 and 8 mmol/kg) had inhibitory effects on the growth of the plants, resulted in reduction in shoot dry biomass</li> <li>The addition of chelators remarkably enhanced the metal concentration in aerial parts of the plants</li> </ul>	[137]
	<i>Viola baoshanensis</i>	China	soil from farmland near the Pb/Zn mine	Field experiment (area of 0.8 ha and consisting of six consecutively rectangle paddy plots, each plant was planted with the space of 20 cm × 20 cm) Addition of chelator: 6 mmol/kg EDTA and 10 mmol/kg (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and NH <sub>4</sub> NO <sub>3</sub> Exposition to contaminants: about 140 days (addition of EDTA 1 week before harvesting and (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and NH <sub>4</sub> NO <sub>3</sub> weeks before)	Pb (975 ± 84 mg/kg) Zn (924 ± 94 mg/kg) Cd (9.8 ± 0.9 mg/kg)	<ul style="list-style-type: none"> <li>The concentrations of Pb, Zn, and Cd in the shoots of <i>V. baoshanensis</i> treated with EDTA were 624, 795, and 25 mg/kg, respectively.</li> <li>The application of ammonium did not have obvious impact on phytoextraction of the metals.</li> </ul>	[138]
	<i>Brassica juncea</i>	Belgium	soil from vicinity of a former radium production site soil naturally contaminated by uraniumiferous shale vein	Greenhouse pot experiment (12 h (16 °C)/12 h (11 °C) day/night cycle) Addition of chelator: 5 mmol/kg EDDS, NTA, citric acid, oxalic acid or ammonium citrate Exposition to contaminants: 4 weeks + 2 weeks after addition of chelator	Cd (1 and 2 mg/kg soil) Zn (704 and 151 mg/kg soil) Cu (372 and 430 mg/kg soil) U (14 and 41 mg/kg soil) Pb (254 and 35 mg/kg soil) Cr (467 and 209 mg/kg soil)	<ul style="list-style-type: none"> <li>Dry biomass of <i>B. juncea</i> shoots on EDDS treated soils was reduced about 50% compared to the controls.</li> <li>EDDS increased shoot concentration for U, Pb, and Cu, 19-, 34-, and 37-times, respectively</li> </ul>	[139]



Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytoextraction	<i>Chenopodium album</i>	India	soil with tannery sludge (10 wt% and 25 wt%)	Greenhouse pot experiment (pots—12 inches in diameter) Exposition to contaminants: 60 days	Fe ( $12.6 \pm 0.3$ and $16.7 \pm 0.2$ mg/kg soil) Mn ( $12.3 \pm 0.2$ and $15.1 \pm 0.3$ mg/kg soil) Zn ( $7.1 \pm 1.2$ and $13.6 \pm 0.4$ mg/kg soil) Cr ( $3.6 \pm 0.1$ and $5.6 \pm 0.1$ mg/kg soil) Cu ( $2.7 \pm 0.1$ and $3.5 \pm 0.0$ mg/kg soil)	<ul style="list-style-type: none"> <li>Uptake of the metals by plants was in the order: Fe &gt; Mn &gt; Zn &gt; Cr &gt; Cu &gt; Pb &gt; Ni &gt; Cd.</li> <li>Shoot length increased for plants treated with sludge.</li> <li>TF indicated that 10 wt% of tannery sludge amendments increased phytoextraction of Cr.</li> </ul>	[31]
	<i>Chenopodium glaucum</i>	China	soil from mercury mining area	Greenhouse pot experiment (temperature from 25 to 30 °C, relative humidity from 40 to 60%) Addition of chelator: 0.05, 0.1 and 0.2 μM ammonium thiosulphate Exposition to contaminants: 55 days + 5 days after addition of chelator	Hg ( $151.13 \pm 5.0$ mg/kg soil)	<ul style="list-style-type: none"> <li>The solubility of mercury remarkably increased with thiosulphate soil treatment.</li> <li>The total Hg concentration increased 1100%, 600% and 200% in roots, stems and leaves of plant for the chelator addition.</li> <li>The average shoot mercury concentration (leaf + stem) obtained in this study was 4.85 mg/kg.</li> </ul>	[140]
Phytovolatilization	<i>Pteris Vittata</i>	Japan	soil from deposit site of neutralized acid mine drainage	Greenhouse pot experiment (temperature varied from 25 (night) to 45°C (day) in summer and 10 (night) and 25 °C (day) in winter) Exposition to contaminants: 18 months	As ( $6540 \pm 380$ mg/kg soil)	<ul style="list-style-type: none"> <li>Plants removed about 90% of the total uptake of As from As contaminated soils.</li> <li>Percentages of arsenic components in sample were 37% for arsenite and 63% for arsenate.</li> </ul>	[45]
	<i>Brassica juncea</i>	USA	upland and wetland soil	Greenhouse pot experiment (temperature of 25 °C, relative humidity of 40%) Exposition to contaminants: 10 days	Selenium compounds (20 and 200 μM)	<ul style="list-style-type: none"> <li><i>Brassica juncea</i> treated with 20 m SeCN removed 30% (w/v) of the Se in 5 days.</li> <li><i>Brassica juncea</i> accumulated mainly organic Se and produced volatile Se in the form of less toxic dimethylselenide.</li> <li>Se volatilization by <i>B.juncea</i> was only 0.7% (w/v) of the total SeCN removed.</li> </ul>	[52]

Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytovolatilization	<i>Salicornia bigelovii</i>	USA	soil mixed with sand	Greenhouse pot experiment (pots—9 cm or 25 cm in diameter, 14 h/10 h day/night cycle) Addition of amendment: 10 g/pot finely-ground pickleweed shoot tissues Exposition to contaminants: 4 months	Se (2.5 and 10 mg/kg soil)	<ul style="list-style-type: none"> <li>Volatilization of Se was remarkably enhanced by incorporation of pickleweed shoot. (2-times during 5-days)</li> <li>The average rate of Se volatilization was <math>1.78 \pm 0.99</math> <math>\mu\text{g/day}</math> per pot (control <math>0.82 \pm 0.12</math> <math>\mu\text{g/day}</math> per pot).</li> </ul>	[53]
	<i>Poplar</i>	USA	soil irrigated by groundwater	Field experiment Exposition to contaminants: 5 months	Trichloroethylene (from 1.4 $\mu\text{g/L}$ to 190 $\mu\text{g/L}$ )	<ul style="list-style-type: none"> <li>The <i>Poplar</i> was able to remove above 99% of the added TCE.</li> <li>Accumulation of chloride was not observed in the plant tissues, while chloride ion concentration increased in the soil</li> </ul>	[55]
	<i>Arabidopsis thaliana</i>	USA	aqueous solution	Hydroponic experiment (ball canning jar—capacity 1000 mL) Plants engineered with a modified bacterial mercuric reductase gene, merA and merB Exposition to contaminants: 7 days	Hg (II) (1 and 5 mg/L)	<ul style="list-style-type: none"> <li>The transgenic plants volatilized 3 to 4-times more Hg(0) than wild-type plants.</li> <li>Plants with Mer A gene removed above 70% of the total Hg(II).</li> </ul>	[54]
	<i>Salix babylonica</i>	China	aqueous solution	Hydroponic experiment in a climate control chamber (temperature of $25.0 \pm 1$ °C under continuous artificial light) Exposition to contaminants: 168 h	Methyl tert-butyl ether (10, 25, 50, 100, 200, and 400 mg/L)	<ul style="list-style-type: none"> <li>Severe signs of toxicity were only found at high doses of MTBE (400 mg/L).</li> <li>Results also showed that all applied MTBE was removed in the presence of <i>Salix</i> for all treatment groups over a 168 h period of exposure.</li> <li>The amount of MTBE from 2.38 mg (10 mg/L)–94.77 mg (400 mg/L) was removed by transpiration.</li> </ul>	[50]

Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytodegradation	<i>Myriophyllum aquaticum</i>	USA	aqueous solution	Outdoor hydroponic experiment (Erlenmeyer flasks—capacity 250 mL, under natural light) Exposition to contaminants: 10 days	Trinitrotoluene (from 80 to 100 mg/L)	<ul style="list-style-type: none"> <li>Roots accumulated the greatest amount of TNT intermediates and were particularly active in sequestering products into ‘bound residues’.</li> <li>About 65% of initial TNT was removed from solution at 10th day.</li> </ul>	[66]
		USA	aqueous solution and sand treatment	Experiment in beaker (beaker—600 mL, temperature of $20 \pm 1$ °C, continuous lights (150 W, 120 V) at 25 cm above the plant) Exposition to contaminants: 10 days	Perchlorate (0.2, 2.0, and 20 mg/L)	<ul style="list-style-type: none"> <li>Perchlorate accumulation was 5 times higher in aqueous solution than in sand treatments.</li> <li>The intermediates detected in the plant tissue indicate that perchlorate was degraded to chloride.</li> <li>Accumulation of perchlorate in the plant tissues was 1.2 g/kg.</li> </ul>	[65]
	<i>Brassica juncea</i>	India	aqueous solution	Greenhouse hydroponic experiment (Erlenmeyer flasks—capacity 250 mL, temperature $25 \pm 2$ °C, 12 h/12 h day/night cycle) Exposition to contaminants: 5 days	Insecticide phorate (5 mg/L)	<ul style="list-style-type: none"> <li>After 5 days, <i>Brassica juncea</i> removed 54% of the phorate with the transformation to phorate sulfoxide</li> <li><i>B. juncea</i> tissue contained only 6.4% (0.32 mg/L) of the total phorate.</li> <li>Phorate sulfoxide (0.32%) was detected as a transformation product.</li> </ul>	[79]
	<i>Azolla pinnata</i>	Iraq	dye-contaminated water	Hydroponic experiment (temperature from 20 to 23 °C, under lamp light at approximation of 10,000–25,000 lux) Exposition to contaminants: 5 days	Methylene blue (5, 15, 25 mg/L)	<ul style="list-style-type: none"> <li>Plant growing in medium with 25 mg/L of methylene blue, removed 38% of dye within 1 h.</li> </ul>	[141]
	<i>Azolla filiculoides</i>	Iran	aqueous solution	Hydroponic experiment (pots - 15 cm height and 10 cm width, natural light and ambient temperature of 30 °C) Exposition to contaminants: 14 days	Phenol (5,10,25,50 mg/L)	<ul style="list-style-type: none"> <li>The growth rate decreased in presence of more than 50 mg/L of phenol.</li> <li>The removal efficiency was more than 97% when phenol concentration was 5 mg/L, for 50 mg/L about 60%.</li> </ul>	[60]



Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytodegradation	<i>Phragmites australis</i>	Netherlands	Perlite	Greenhouse pot experiment (12 h (22 °C)/12 h (17 °C) day/night cycle, relative humidity of 60% (day) and 70% (night)) Exposition to contaminants: 21 days	Ibuprofen (60 µg/L)	<ul style="list-style-type: none"> <li>IBP was completely removed from the solution after 21 days.</li> <li>Four intermediates were detected in the plant tissues: hydroxy-IBP, 1,2-dihydroxy-IBP, carboxy-IBP and glucopyranosyloxy-hydroxy-IBP.</li> </ul>	[71]
	<i>Chromolaena odorata</i>	China	aqueous solution with wastewater	Hydroponic experiment (tanks—48 × 36 × 20 cm, temperature of 20 °C) Addition 0.4 mg/kg <i>Chromolaena odorata</i> L. extract Exposition to contaminants: 15 days	SDS (10 and 20 mg/L)	<ul style="list-style-type: none"> <li>SDS was partially converted into low-molecular weight intermediates.</li> <li>Root was the main part responsible for removing SDS.</li> <li>Addition of plant extract increased SDS phytotransportation.</li> </ul>	[142]
	<i>Eichhornia Crassipes</i>	China	aqueous solution	Greenhouse hydroponic experiment (containers—7 × 12.5 cm, capacity—500 mL or 30 × 25 cm, temperature of 25 ± 1 °C, 14 h/10 h day/night cycle, light intensity 1400 lux) Exposition to contaminants: 240 h or 3 weeks	Phosphorus pesticide ethion (0.01, 0.1 and 1 mg/L)	<ul style="list-style-type: none"> <li>Uptake and phytotransformation of ethion was 69%, while microbial degradation about 12%.</li> <li>The accumulated ethion in <i>Eichhornia Crassipes</i> was reduced by 55–91% in shoots and 74–81% in roots when the plant growing 1 week without ethion addition.</li> </ul>	[78]
	<i>Cyperus Alternifolius</i>	Thailand	synthetic wastewater	Greenhouse hydroponic experiment (temperature of 30 ± 2 °C, 12 h/12 h day/night cycle, relative humidity of 70 ± 5%) Exposition to contaminants: 12 days	Ethanolamines (MEA, DEA, TEA) (1400 mg/L)	<ul style="list-style-type: none"> <li>Ethanolamines was accumulated mainly in plant stems</li> <li>A smaller molecular weight—MEA was taken up the fastest, followed by DEA and TEA.</li> <li>Plants were able to degrade TEA to DEA, then to MEA, then further to acetic acid.</li> </ul>	[143]
	<i>Erythrina crista-galli</i>	Brazil	petroleum-contaminated soil	Greenhouse pot experiment (pots—22- cm height and 24- cm diameter, temperature from 25 °C to 30 °C, relative humidity from 85% to 90%,) Exposition to contaminants: 120 days	Hydrocarbons (25, 50, 75 g/kg soil)	<ul style="list-style-type: none"> <li>Petroleum degradation rate was higher for vegetated pots than for non-vegetated (reduction of hydrocarbons concentration about 49–82% compared to non-vegetated pots at 75 g/kg).</li> </ul>	[144]



Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytostabilization	<i>Lupinus Albus</i>	Spain	soils affected by acid pyrite sludge	Greenhouse pot experiment (pots—capacity 5.5 L, temperature from 25 to 35 °C, relative humidity from 55 to 85%, photosynthetic photon flux density 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) Addition of chelator: 0.5 mM NTA Exposition to contaminants: 3 weeks Field experiment (rows: 35 m long and 50 cm apart, 20 g seeds/m <sup>2</sup> ) Exposition to contaminants: 6 months	As (about 60 nmol/g soil) Cd (about 10 nmol/g soil) Cd (5.5 kg/ha soil) As (92.5 kg/ha soil)	<ul style="list-style-type: none"> <li>Phytoextraction efficiency of Cd and As by plants was very low.</li> <li>The accumulation of heavy metals occurred mostly in root nodules.</li> <li>NTA was efficient in mobilization of Cd and As from polluted soil.</li> </ul>	[145]
	<i>Lupinus uncinatus</i>	Mexico	soil from crop field	Greenhouse pot experiment (pots—15 cm in diameter, day and night temperature from 25 to 29 °C and from 8 to 11 °C) Exposition to contaminants: 18 weeks	Cd (9, 18, and 27 mg/kg soil)	<ul style="list-style-type: none"> <li>The highest shoot Cd concentration of 540 mg Cd/kg DW was found at 27 mg Cd/kg soil.</li> <li>The weak translocation of Cd from roots to shoot was observed with TF value &lt; 1.</li> </ul>	[146]
	<i>Vicia faba</i>	Tunisia	vineyard soil	Greenhouse pot experiment (pots—15 cm diameter) Bacteria inoculation Exposition to contaminants: 65 days	Cu (63.5 mg/kg soil)	<ul style="list-style-type: none"> <li>Bacteria inoculation caused increase the number and the biomass of root nodules of 50%.</li> <li>Reduction of accumulated copper in roots was 35%.</li> </ul>	[147]
	<i>Brassica juncea</i>	Spain	soil with mine tailings	Greenhouse pot experiment (pots—capacity 0.7 L, maximum temperature from 25 to 33 °C, minimum temperature from 6 to 9 °C) Addition of amendments: pine bark compost and manure compost Exposition to contaminants: 110 days	Zn (from 10.6 $\pm$ 0.1 to 18.3 $\pm$ 0.6 mg/kg soil) Cu (from 12 $\pm$ 0.2 to 118 $\pm$ 16 mg/kg soil)	<ul style="list-style-type: none"> <li>Addition of manure reduced metal accumulation in shoots (10–50% reduction of Cu and 40–80% of Zn in comparison with non-amended soils).</li> <li>Manure treatment improved soil fertility and increased plant biomass.</li> </ul>	[94]
	<i>Vetiveria zizanioides</i>	Thailand	mine tailings	Greenhouse pot experiment Addition of amendments: organic fertilizer, Osmocote® and cow manure Exposition to contaminants: 3 months Field experiment (plots 4 $\times$ 4 each) Addition of amendments: organic fertilizer and Osmocote® Exposition to contaminants: above 1 year	Pb (>10000 mg/kg soil)	<ul style="list-style-type: none"> <li><i>V. zizanioides</i> treated with organic fertilizer and cow manure presented the greatest biomass and the highest Pb accumulation (519.5 <math>\mu\text{g}</math> per plant).</li> <li>Low TF (&lt;1) values and BCF for root (&gt;1) were observed.</li> </ul>	[148]
		Thailand	soil contaminated by creek irrigation with creek which passes through drainage from active Zn mines	Greenhouse pot experiment (pots—8-in. diameter and 7 in. height, temperature from 26 to 30 °C, relative humidity from 60 to 80%, 4000–46,000 lux light intensity, 12 h/12 h day/night cycle) Addition of amendments: 10 wt% of cow manure, pig manure, bat manure, or organic pelleted fertilizer Exposition to contaminants: 3 months	Cd (from 33.8 to 35.7 mg/kg soil)	<ul style="list-style-type: none"> <li>Cadmium did not remarkably affected biomass production.</li> <li>Sri Lanka ecotype of <i>Vetiveria zizanioides</i> has accumulated Cd mainly in roots, with TF r values &lt;1 and a BCF for roots &gt;1.</li> </ul>	[98]





Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytostabilization	<i>Vigna radiata</i>	Thailand	soil from an agricultural area	Greenhouse pot experiment (pots—capacity 16 L, 19 cm in top diameter, 15 cm in bottom diameter, 30 cm in height) Addition of amendments: 5, 10, 15 wt% of biochar Exposition to contaminants: 8 weeks	Cd (58 mg/kg soil) Zn (1220 mg/kg soil)	<ul style="list-style-type: none"> <li>10 wt% biochar-amended soil had a positive effect on promoting plant growth and seed yield while 15 wt% biochar had an adverse effect on plant growth.</li> <li>Cadmium and Zn bioavailability in soil decreased with an increasing biochar addition.</li> </ul>	[95]
	<i>Lolium perenne</i>	Chile	mine tailings	Greenhouse pot experiment (pots—12.5 cm in diameter and 12 cm height, temperature of 23 °C) Addition of amendments: 6 and 12 wt.% of biosolid Exposition to contaminants: 6 months	Cd (<0.02 mg/kg soil) Cu (485 mg/kg soil) Zn (41 mg/kg soil) Mo (109 mg/kg soil)	<ul style="list-style-type: none"> <li>Biosolids application increased the dry biomass production of <i>L. perenne</i>.</li> <li>Metals were mostly accumulated in the roots and only a small part was translocated to the shoots.</li> <li>With the addition of biosolids accumulation of copper in the roots was even 166-times higher, than in the shoots of the plants.</li> </ul>	[89]
	<i>Lolium italicum</i>	Italy	soil from mining area	Mesocosm experiment Addition of amendments: 10 and 30 wt.% of compost Exposition to contaminants: 60 days	Zn (437 and 4622 mg/kg soil) Pb (374 and 17,739 mg/kg soil)	<ul style="list-style-type: none"> <li>The accumulation of Pb and Zn in plants tissue of <i>L. italicum</i> decreased more than five times with addition of compost.</li> <li>Addition of compost greatly increased biomass production.</li> <li>Low translocation factor for Pb &lt; 0.11 and for Zn &lt; 0.93.</li> </ul>	[80]
	<i>Miscanthus sinensis</i>	Republic of Korea	mine tailings	Greenhouse pot experiment (pots—20 cm diameter and 25 cm height, temperature from 15 to 25 °C, relative humidity from 60 to 70%) Addition of amendments: 2 wt% of bone mill, furnace slag, bottom ash, or red mud Exposition to contaminants: 90 days	Cu (388.31 mg/kg soil) Zn (2210 mg/kg soil) Pb (3889 mg/kg soil) Cd (32.46 mg/kg soil)	<ul style="list-style-type: none"> <li><i>M. sinensis</i> did not show any visible toxicity symptoms.</li> <li>Application of furnace slag and <i>M. sinensis</i> reduced extractable heavy metals by 56–91%.</li> <li><i>M. sinensis</i> accumulated heavy metals primarily in the roots, with low TF.</li> </ul>	[93]
	<i>Miscanthus sinensis</i> × <i>giganteus</i>	Romania	soil polluted by smelter activity	Field experiment Addition of amendments: 1 wt% of red mud Exposition to contaminants: 1 year	Zn (322 to 780 mg/kg soil) Cd (4.7 to 10.3 mg/kg soil) Pb (154 to 607 mg/kg soil)	<ul style="list-style-type: none"> <li>BCF &lt; 1 indicated that <i>M. sinensis</i> × <i>giganteus</i> was an excluder of heavy metals.</li> <li>Amending soil with red mud decreased the bioavailable fractions of Zn, Cd and Pb.</li> </ul>	[92]

Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Phytostabilization	<i>Rose</i>	India	soil from agricultural field	Pot experiment (pots—capacity 4.5 kg) Exposition to contaminants: 60 days	Cr (25, 50, 100, and 200 mg/kg soil)	<ul style="list-style-type: none"> <li>The rose had good tolerance and presented high Cr accumulation, however presence of Cr reduced of the shoots.</li> <li>Higher concentration of Cr in roots (1985 µg/g DW) followed by shoots (760 µg/g DW).</li> </ul>	[149]
	<i>Jatropha curcas</i>	China	soil from mine site	Greenhouse pot experiment (pots—capacity 2 kg, temperature from 18 to 26 °C, under natural light) Addition of amendments: 0.1, 0.25, 0.5, and 1.0% of limestone Exposition to contaminants: 6 months	Al (10756 to 32,970 mg/kg soil) Zn (287 to 651 mg/kg soil) Cu (324 to 722 mg/kg soil) Pb (266 to 2466 mg/kg soil) Cd (1 to 19.85 mg/kg soil)	<ul style="list-style-type: none"> <li>The highest metal accumulation in <i>J. curcas</i> was in roots.</li> <li>The growth of <i>J. curcas</i> was enhanced due to the increase in soil pH and decrease in bioavailable metals in soil with lime application.</li> </ul>	[150]
	<i>Salix viminalis</i> <i>Salix purpurea</i>	France	soil from settling basin of a former gold mine	Mesocosm experiment (temperature of 20 ± 2 °C, 16 h/8 h day/night cycle, photosynthetic photon flux density 1000 µmol·m <sup>-2</sup> ·s <sup>-1</sup> ) Exposition to contaminants: 45 days	As (24.6 ± 14.5 to 1593 ± 280.3 mg/kg soil) Sb (0.18 ± 0.004 to 83.04 ± 2.53 mg/kg soil) Pb (3.6 ± 0.8 to 221 ± 27.9 mg/kg soil)	<ul style="list-style-type: none"> <li><i>S. purpurea</i> biomass production on garden soil was 2-times higher than that of <i>S. viminalis</i>.</li> <li><i>S. purpurea</i> was more efficient in accumulating As in the plant's upper parts than <i>S. viminalis</i> while <i>S. viminalis</i> showed an ability to transfer Pb and Sb to its shoots whereas <i>S. purpurea</i> did not translocate these elements.</li> </ul>	[151]
Rhizodegradation	<i>Kandelia candel</i>	China	sediment of mangrove wetland	Greenhouse rhizobox experiment (rhizobox—150 mm × 300 mm × 200 mm, temperature from 26 to 32 °C, natural illumination, relative humidity of 85%) Exposition to contaminants: 60 days	Phenanthrene (10 mg/kg soil) Pyrene (10 mg/kg soil)	<ul style="list-style-type: none"> <li>Plants importantly improved the dissipation of Ph (47.7%) and Py (37.6%) from contaminated sediment after 60 days.</li> <li>Plant uptake and accumulation of Ph and Py were very low.</li> </ul>	[109]
	<i>Lolium multiflorum</i>	Japan	soil contaminated with diesel	Greenhouse pot experiment (pots—capacity 0.3 L, temperature of 25 °C, 10 h/14 h day/night cycle relative humidity of 70%, photosynthetic photon flux density of 150 µ mol m <sup>-2</sup> s <sup>-1</sup> ) Exposition to contaminants: 152 days	TPH (7977 ± 146 mg/kg soil)	<ul style="list-style-type: none"> <li>Soil contaminated with 0.8% diesel reduced growth of plants aerial parts.</li> <li>At 63 DAS, the concentration of TPH in the planted soil was much lower than in unplanted soil, with higher number of anaerobic bacteria in soil</li> </ul>	[105]

Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Rhizodegradation	<i>Salix nigra</i>	USA	hydroponic solution and contaminated groundwater	Greenhouse hydroponic experiment (Erlenmeyer flasks- capacity 2 L) Addition: 500 mg/L of DOC derived from acetate, ethanol, organic mushroom compost, or chicken litter extract Exposition to contaminants: up to 70 days	Perchlorate (22 to 25 mg/L and 40 mg/L)	<ul style="list-style-type: none"> <li>The addition of 500 mg/L DOC, caused reduction of initial perchlorate concentrations to below the detection limit of 2 µg/L within 9 d.</li> <li>Improvement of rhizodegradation by organic carbon led to reduction of perchlorate from approximately 430 to 20 mg/kg.</li> </ul>	[152]
	<i>Salix babylonica</i>	USA	hydroponic solution and humic or sandy loam soil	Greenhouse bioreactor experiment (plastic buckets—2 gallons or Erlenmeyer flasks—capacity 2 L) Addition: 150 or 300 mg/L of DOC derived from chicken manure Exposition to contaminants: up to 42 days	Perchlorate (65.85 mg/L to 119.89 mg/L)	<ul style="list-style-type: none"> <li>The plants amended with DOC reduced perchlorate from 65.85 to 2.67 mg/L in 21 days for humic soil and from 68.99 to 0.06 mg/L for sandy loam in 11 days.</li> </ul>	[112]
	<i>Sebastiania Commersoniana</i>	Brazil	petroleum-contaminated soil	Greenhouse pot experiment (pots—22 cm × 24 cm, temperature from 25 to 30°C and relative humidity from 85 to 90%) Exposition to contaminants: 424 days	TPH (25, 50, 75 g/kg soil)	<ul style="list-style-type: none"> <li><i>S. commersoniana</i> did not present any sign of toxicity.</li> <li>Plants after 60-days of growth showed a reduction of petroleum hydrocarbons higher than 60% and the 424-day plants showed a reduction higher than 94%.</li> </ul>	[108]
	<i>Avicennia marina</i>	China	sediments from mangrove wetland	Greenhouse pot experiment (pots—20 cm in diameter for top, 14 cm in diameter for bottom, and 20 cm in height, 12 h/12 h day/night cycle, relative humidity of 85%, temperature from 22 to 26 °C). Exposition to contaminants: 120 days	Phenanthrene and pyrene (5, 10, and 50 mg/kg soil)	<ul style="list-style-type: none"> <li>The dissipation of phenanthrene and pyrene was remarkably improved in the rhizosphere compared with non-rhizosphere sediments.</li> <li>Plant roots promoted dissipation significantly greater than the contribution of direct plant uptake and accumulation of phenanthrene and pyrene.</li> <li>After 120 days removal in rhizosphere zones of Ph was 83.8–86.2% and for Py 68.5–69.1%.</li> </ul>	[110]
	<i>Daucus carota</i>	China	loamy soil	Greenhouse pot experiment (temperature from 25 to 35 °C in the daytime and from 15 to 25 °C in the nighttime, relative humidity of 50 and 75%) Addition: 1, 2, 4 wt% of composted pig manure Exposition to contaminants: 90 days	2, 2', 4, 4'-tetrabrominated diphenyl ether (BDE) (0.4 mg/kg soil)	<ul style="list-style-type: none"> <li>The BCF factors of BDE-47 in <i>Daucus carota</i> was remarkably reduced from <math>229.7 \pm 28.2</math> to <math>43.4 \pm 20.4</math> ng/g and from <math>1.86 \pm 0.5</math> to <math>0.15 \pm 0.03</math>, respectively, with increasing pig manure addition.</li> <li>Rhizodegradation of BDE-47 was improved from 8.6 to 28.5% with increasing addition of pig manure from 0 to 4%.</li> </ul>	[153]



Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Rhizofiltration	<i>Brassica juncea</i>	India	aqueous solution	Hydroponic experiment (Erlenmayer flask—capacity 250 mL, temperature of 25 °C) Exposition to contaminants: 10–12 days	U (25–5000 µM)	<ul style="list-style-type: none"> <li><i>B. juncea</i> could accumulated 20–23% of uranium from the solution.</li> <li>The rate of growth of <i>B. juncea</i> hairy root was retarded, when grown in the presence of 1000–5000 µM uranium solution.</li> </ul>	[116]
	<i>Helianthus annuus</i>	Republic of Korea	Groundwater	Greenhouse hydroponic experiment (glass jars—12 cm × 12 cm × 8 cm, temperature of 25 °C, relative humidity of 80%, 16 h/8 h day/night) Exposition to contaminants: 72 hours	U (30–646 µg/L)	<ul style="list-style-type: none"> <li>More than 80% of the initial uranium concentration in groundwater was removed within 24 h by <i>Helianthus annuus</i>.</li> <li>The maximum uranium removal occurred at pH 3–5.</li> <li>Performed analyses indicated that uranium was mainly accumulated in the roots.</li> </ul>	[113]
	<i>Carex pendula</i>	The Netherlands	synthetic wastewater	Greenhouse hydroponic experiment (pots—capacity 11.4 L, temperature from 18 to 27 °C, 16 h/8 h day/night cycle, light intensity (48–56 mE/m <sup>2</sup> /s), relative humidity of 50%) Exposition to contaminants: 2 weeks	Pb (1, 5, 10 mg/L)	<ul style="list-style-type: none"> <li>The Pb accumulation in root biomass was about 10-folds higher than those in shoot biomass for the 1.0–10 mg/L.</li> <li>For 10 mg/L roots were able to accumulate 1600 µg/g DW, with low TF = 0.1.</li> </ul>	[154]

Table 1. Cont.

Mechanism	Plant	Research Location	Medium	Experimental Details	Contaminant and Initial Concentration	Conclusions	Reference
Rhizofiltration	<i>Pistia Stratiotes</i>	Nigeria	aqueous solution	Hydroponic experiment (aquarium—one meter by half a meter, kept in semi-shaded area) Exposition to contaminants: 21 days	Cr (1800–2400 mg/L) Pb (1800–2400 mg/L) Ni (1800–2400 mg/L)	<ul style="list-style-type: none"> <li>No visible toxicity symptoms were observed.</li> <li>The accumulation of heavy metals in the plants ranging from 121.96 µg/g to 304.52 µg/g for Cr, 182.12 µg/g to 419.43 µg/g for Pb and 261.81 µg/g to 446.33 µg/g for Ni, in the 1800 mg/L initial concentration</li> </ul>	[155]
		Czech Republic	soil contaminated due to the atmospheric deposition of potentially risk elements from the Pb smelter	Greenhouse pot experiment (pots—capacity 2 L) Addition: 10 mM acetic, tartaric, citric, and oxalic acid Exposition to contaminants: 21 days	Cd (5.68 ± 0.1 mg/kg soil) Pb (822 ± 14 mg/kg soil) Zn (267 ± 36 mg/kg soil)	<ul style="list-style-type: none"> <li>Citric acid led to mobilization of 71%, 181%, and 112% of Cd, Pb, and Zn, respectively while tartaric acid mobilized 70%, 155%, and 135% of Cd, Pb, and Zn</li> <li>The BCF was 2–5 times higher for juvenile plants than mature plants.</li> <li>Juvenile and mature plants after 3 weeks removed more than 80% of Cd, Pb, and Zn.</li> <li>Higher concentration of metal was observed for roots than leaves (low TF values).</li> </ul>	[156]
		Czech Republic	aqueous solution	Greenhouse hydroponic experiment (glass pots—capacity 250 mL, temperature of 25°C (day) and 18°C (night), relative humidity of 75% in the day and 55% at the night, 18 h/6 h day/night cycle) Addition of 5% nitric acid Exposition to contaminants: 14 days	Pb (25 mg/L and 125 mg/L) Cd (3.5 mg/L and 10.5 mg/L)	<ul style="list-style-type: none"> <li>The accumulation of Pb in plants was the highest during the first 4 days</li> <li>10 times higher in roots than in leaves.</li> <li>Maximal Cd accumulation in roots was 3923 mg/kg (at 14th day) while for Pb was 42,862 mg/kg (at 4th day).</li> </ul>	[120]

DAS—day after sowing, DOC—dissolved organic carbon, DTPA—diethylenetriaminepentaacetic acid, DW—dry weight, EDDS—ethylene diamine disuccinic acid, EDTA—ethylenediaminetetraacetic, HEDTA—hydroxyethylethylenediaminetriacetic acid, MGWL—monosodium glutamate waste liquid, NTA—nitriloacetic acid, PAH—polyaromatic hydrocarbons, TPH—total petroleum hydrocarbo.



### 3. Parameters Affecting Phytoremediation Process

The cleanup process of contaminated soils, which involves the application of plants, depends on variety of factors. The most important of these include soil parameters, properties, concentration and phytoavailability of pollutants, and also plant species [157,158].

#### 3.1. Soil pH

pH is one of the factors affecting the efficiency of soil phytoremediation. Soil pH affects the adsorption and desorption of contaminants in soil. It is the parameter which controls metal solubility. Generally, metals present higher mobility under acidic and reducing conditions than under alkaline and oxidizing conditions [159]. The capacity of soil to adsorb cationic metals increases with pH increasing [160]. At high pH values, the metal ions are virtually non available for plants [161]. Moreover, it was observed that variable pH conditions are crucial for plant growth. Willscher et al. [162] investigated the growth and ability of *Helianthus tuberosus* for the phytoextraction of heavy metals (Cd, Cu, Fe, Mn, Ni, Zn, Pb) under different pH conditions (4.0, 4.5, 5.0, 5.5, and 6.0). The best growth of roots achieved control plants at pH 5, and for leaves and stems it was obtained at pH 5.0 and 5.5 for controls, whereas at slight (highest metal concentration equal 62.5 mg/kg) and medium (highest metal concentration equal 125 mg/kg) heavy metal concentrations, best growth of roots were obtained at pH 5.5 and 6. For slightly and medium contaminated soil, the highest accumulation of all metals in shoots occurred at pH 4.0 and was considerably higher than at pH 5.0 or 6.0. Bagga and Peterson [163] compared the accumulation of arsenic by *Asparagus Fern* from contaminated soil (300 pm) growing on the soil at pH 4.5, 5, 6, and 7. The highest concentration of arsenic in plant tissue occurred at pH 5 ( $480 \pm 17.37$  mg/kg), followed by pH 4 > pH 6 > pH 7. A similar effect evaluated Brown et al. [164] for zinc and cadmium uptake by plants concerning soil pH (5.06–7.04). *Thlaspi caerulescens* exhibits metal stress only in low pH treatments. For Zn and Cd contaminated yard soil, the highest concentration of Zn and Cd in shoots was observed at pH 5.07 and steadily declined with the increase in pH. Research carried out by Chen et al. [165] concerning the addition of citric acid to lowering soil pH and decreased the adsorption of Pb and Cd. The effect of decreased in soil adsorption was more evident for cadmium than for lead. Moreover, addition of citric acid stimulated metals transportation from root to shoot of radish. Hattori et al. [166] compared the effect of soil pH (3.5 and 5.0) on cadmium uptake by *Helianthus annuus*, *Hibiscus cannabinus*, and *Sorghum vulgare*. It was observed that when the soil pH decreased, the amount of Cd dissolved in soil water increased. In case of *Helianthus annuus* and *Hibiscus cannabinus* at low pH (3.5) Cd accumulation increased above twofold compared to the control soil (pH 5), while for *Sorghum vulgare* decreased due to roots sensitivity for low pH. In a study, Saleh [167] controlled the uptake of radionuclides  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  by *Eichhornia crassipes* at variable pH conditions (2.9, 4.9, 8.9 and 10.9). Researcher concluded that *Eichhornia crassipes* might tolerate pH values from 4 to 10, while the highest uptake rate for both radionuclides was observed at pH 4.9. Meanwhile, at low pH value (2.9), protons might compete for the plant adsorption sites with the radioisotope cations, respectively  $\text{Cs}^+$  and  $\text{Co}^{2+}$ . Singh et al. [168] investigated potential of *Lemna minor* for removal of Pb at pH 5.0–9.0. The lowest toxic effect of Pb was found at pH 5, but in contrast the highest percent of removal occurred at pH 9. However, the maximum bioconcentration factor (0.9) was observed at pH 6 for lead content 10 mg/L.

#### 3.2. Inorganic Fertilizers

Fertilization may promote plant growth and biomass production. Moreover, the addition of fertilizers may enhance the phytoremediation process. The first group of amendments is inorganic fertilizers which supply three main nutrients: nitrogen (N), phosphorus (P), and potassium (K). Fertilization of N is essential for promoting leaves growth and forms protein and chlorophyll. Phosphorus plays important role in roots and flower formation, while potassium is responsible for stem and root development [169,170]. However, for optimal plant growth, important is not only the amount of added fertilizer but also the ratio between N, P, and K, which depends on nutrient deficiency



in soil and plant requirements. Moreover, inappropriate ratios of N, P, and K fertilizer may have negative effect on the absorption and utilization of nutrients. [171]. Wu et al. [172] investigated the effect of nutrient addition on the phytoremediation efficiency of Cu contaminated soil by *Brassica juncea*. The addition of fertilizer N (urea) and P (superphosphate) significantly increased plant shoot yield. Nitrogen and phosphorus increased the amount of chlorophyll in the leaves. Moreover, N and P applied at 100 and 200 mg/kg, respectively resulted in the highest Cu uptake. Schwarz et al. [173] characterized phytoextraction of cadmium with *Thlaspi caerulescens*, enhanced by N-fertilization. Soils were amended with increasing rates of N-nitrate ( $\text{NaNO}_3$ ) or N-ammonium ( $(\text{NH}_4)_2\text{SO}_4$ ) in the amount of 0, 20, 80, 200 mg N/kg. *T. caerulescens* responded positively to the increasing nitrogen fertilization on both soils and the nitrogen supply significantly improved metal extraction. However, the ammonium fertilization led to a lower biomass production than nitrate. Similar studies by Liao et al. [170] investigated the effect of a different form of nitrogen fertilizers for arsenic accumulation by *Pteris vittata*. Research evaluated the potential of several nitrogen suppliers i.e.,  $\text{NH}_4\text{HCO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{KNO}_3$ , and urea. As accumulation was greater due to higher biomass, when N fertilizer was added, especially with the addition of  $\text{NH}_4^+$ -N source. Furthermore, the total arsenium uptake and/or accumulation within the plants grown under different forms of N fertilizer, decreased as  $\text{NH}_4\text{HCO}_3 > (\text{NH}_4)_2\text{SO}_4 > \text{urea} > \text{Ca}(\text{NO}_3)_2 > \text{KNO}_3 > \text{control}$ . Jacobs et al. [174] evaluated the efficiency of  $\text{KNO}_3$  and  $\text{NH}_4\text{NO}_3$  fertilizers addition on phytoremediation of Cd and Zn contaminated soil by *Noccaea caerulescens*. A slight favorable effect of nitrogen fertilization on biomass production occurred only in soils with low initial nitrogen content (under  $25 \mu\text{g/g NO}_3^-$ ). Above this concentration, fertilization caused decrease in shoot Cd and Zn concentration. Moreover, there was no difference with biomass increase with application of the two N fertilizers ( $\text{KNO}_3$  or  $\text{NH}_4\text{NO}_3$ ). Research performed by Di Luca et al. [175] focused on the improvement of chromium phytoremediation by *Pistia stratiotes* applying a different concentrations of P and N nutrients. P and N concentrations were 5 mg/L or 10 mg/L. It was observed that nutrients addition significantly increased Cr removal and enhanced Cr translocation to leaves. The decrease in the relative growth rate due to Cr exposure was reduced by nutrient addition at 5 mg/L of P or N, suggesting an improving effect of nutrient enrichment on the Cr tolerance of *P. stratiotes*, whereas the addition of 10 mg/L of P or N increased Cr toxicity. Fertilization may be also effective way to improve phytoremediation process of soil contaminated with crude oil. Merkl et al. [176] studied the influence of fertilizer level on plant growth and oil dissipation. Fertilizer was applied twice in a concentration of 200, 300, and 400 mg/kg soil, each of N, P, and K (commercial fertilizer NPK 20-20-20). The medium fertilizer concentration (300 mg/kg) resulted in the highest root growth and maximal oil dissipation (18.4%) after 22 weeks. While, the highest fertilizer level produced best shoot growth and highest oil dissipation after 14 weeks, but it reduced root biomass production. Application of controlled-release fertilizers (CRF) is another approach presented in the study Cartmill et al. [177] to enhance phytoremediation of petroleum-contaminated soil (3000, 6000 and 15,000 mg/kg). It was found that plant adaptation to contaminants, growth, photosynthesis, and chlorophyll content of *Lolium multiflorum* were improved by the addition of CRF (4, 6, or 8 kg/m<sup>3</sup>). Moreover, soil contaminated with 6000 or 15,000 mg/kg had enhanced petroleum hydrocarbons degradation with fertilization. In contrast, the application of fertilizers not always give the desired effect. Jayaweera et al. [178] investigated removal of Fe (9.27 mg/L) from synthetic wastewaters by *Eichhornia crassipes*. Plants were growing under variable nutrient conditions with 28 mg/L of total nitrogen (TN) and 7.7 mg/L of total phosphorous (TP). Another experiments were performed with 2-fold, 1/2-fold, 1/4-fold and 1/8-fold of these nutrient concentrations. Plants grown without nutrition showed the highest phytoremediation efficiency of 47% after the 6th week of growth, with the highest accumulation of 6707 Fe mg/kg DW. These studies proved that *Eichhornia crassipes* grown under nutrient-poor conditions is very good Fe accumulator. Similarly, Ji et al. [179] observed that the addition of fertilizers ( $\text{NH}_4\text{NO}_3$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ) for phytoremediation of Cd-contaminated soil by *Solanum nigrum* had no significant effect on plant biomass.



### 3.3. Organic Amendments

Organic matter content is one of the most important soil component, and it has the ability to retain heavy metals in soil, due to metal-organic matter interactions and thus limits metal phytoavailability [180]. It has been proved that increase of organic matter in the soil might reduce the metal ions [181]. The poor organic matter content in contaminated soil may limit the plant growth, slow colonization, and microbial activity [182,183], which inhibit natural succession and remediation effects [184]. Organic amendments may include i.e., chicken, cow, horse and pig manure, compost, sludge, biochar, humic acids. Pillai et al. [185] investigated the effect of organic manure addition (1 g powdered cow dung/kg soil) on the phytoremediation potential of *Vetiveria zizanioides*, growing on soil contaminated with chromium. High biomass production was observed in soil with organic manure. Moreover, the addition of cow dung prevents chromium toxicity, which was visible as a yellowing of the plant leaves growing on soil without organic additives. Chromium uptake by *Vetiveria zizanioides* was improved with cow manure. Wai Mun et al. [184] applied chicken manure to improve cleanup of sand tailings contaminated with Pb by *Hibiscus cannabinus*. It was stated that the application of organic fertilizer promoted biomass production as well as higher accumulation capacity of Pb in plant tissues. Application of pig manure compost for phytoremediation of PAH-contaminated soil was studied in the research performed by Cheng et al. [186] and Wang et al. [187]. It has been found that addition of organic compost increased shoot biomass yield. Wang et al. proved that the dissipation of PAHs: phenanthrene, pyrene, and anthracene were greatly improved with using soil-manure composition, while Cheng et al. observed enhancement in dissipation only for pyrene. Several studies performed by Wei et al. [188–190] compared the effect of addition of urea (0.5, 1, and 2 g/kg) and chicken manure (50, 100, and 200 g/kg) on cadmium accumulation different plants. First research applied *Solanum nigrum* for phytoremediation of soil contaminated with cadmium (10, 25, and 50 mg/kg). It was concluded that application of fertilizers led to increase of the Cd phytoextraction efficiency of *S. nigrum* by enhancing its shoot biomass production. Moreover, urea did not affect Cd concentration in plant tissue, while chicken manure decreased the Cd phytoavailability and tissues cadmium concentration. It suggests that the application of urea may be suitable as fertilizer for the phytoextraction process and chicken manure for metal phytostabilization in soil. These results were compared with another study concerning the potential of *Taraxacum mongolicum* for phytoremediation of Cd-contaminated soil (2.5, 5, 10 and 25 mg/kg). The same effect was observed, with increasing plant biomass with fertilization. It was also confirmed that chicken manure significantly decreased Cd concentration in plant tissue by decreasing extractable Cd in soil. Similar research performed by Nwaichi et al. [191] focused on cadmium accumulation by *Mucunapruriens* and *Sphenostylis stenocarpa*, growing under urea and chicken manure fertilization (0.8 g/pot). In contrast, the researcher observed that the addition of chicken manure significantly improved the translocation factor by >1, while urea did not affect metal transport from roots to shoots. Moreover, the chicken manure treatment remarkably increased the shoot Cd concentration, while its application decreased Cd solubilization in comparison to urea addition.

Recent research showed that using biochar may strongly influence the cleanup efficiency of contaminated soil by plants. Houben et al. [192] concluded that the addition of biochar (1, 5, and 10 wt%) to Cd, Pb, Zn-contaminated soil might increase metals bioavailability and biomass yield of *Brassica napus*. The reduction of metal content in soil reached 71%, 87% and 92% for Cd, Zn, and Pb respectively in the presence of 10 wt% biochar. However, addition of organic amendment caused reduction in metal concentrations in shoots, but the biomass production was remarkably improved as a result of the soil fertility improvement. Meanwhile, Han et al. [193] performed a greenhouse pot experiment for the phytoremediation of soil contaminated with total petroleum hydrocarbons (TPHs) by ryegrass. In contrast, negative impact on the growth of ryegrass and the degradation of TPHs by ryegrass was observed. Moreover, Saum et al. [194] used biochar or its mixture with compost to enhance phytoremediation of oil-contaminated soil by petroleum hydrocarbons. It was observed that addition of biochar to contaminated soil suppressed the population of oil-degrading bacteria. Lower level of petroleum degradation in the biochar treatment probably might be related to the smaller populations of



PAH-metabolizing microbes. Several studies showed that the addition of humic acid to contaminated soil might also be a possible solution to enhance phytoremediation efficiency. Humic acids, which contain acidic groups such as carboxyl and phenolic OH functional groups, play an essential role in the transport, phytoavailability, and solubility of heavy metals [195]. Angin et al. [196] studied the effect of humic acid (100, 200, and 400 kg/ha) in enhancing boron and lead accumulation by *Vetiveria zizanioides*. Humic acid application increased Pb phytoavailability in soil and improved Pb removal. The highest boron uptake was for plant growing under the addition of 400 kg/h. However, incorporation of HA to Pb or B contaminated soil did not influence the translocation from roots to shoots. HA addition might increase permeability of root cell membranes and thus allowing for more effortless transfer of metals [197]. Also, it was confirmed for Cu accumulation in *Chrysopogon zizanioides* tissues by Vargas et al. [198]. Researcher concluded that addition of humic acids (10 and 20 g/kg) promoted root growth and increased Cu concentrations in plant roots due to formation of soluble metal-organic complexes, while translocation factor was reduced. In contrast, Evangelou et al. [195] observed that using of humic acids (2 g/kg soil) enhanced *Nicotiana tabacum* uptake of cadmium from contaminated soil. Moreover, upon organic amendment cadmium concentration in shoots increased. It was suggested that this effect was related to pH reduction and higher cadmium availability. A study of Wong et al. [199] showed adverse effects of humic acid addition on phytoremediation of pyrene-contaminated sediments by *Kandelia candel*. The pyrene removal was tremendously higher for sediments without organic amendment (decreased from 89% to 29%). Moreover, total plant biomass was reduced by 50% for humic acid addition. Only for roots pyrene accumulation was slightly higher growing on soil with HA.

#### 3.4. Contaminant Concentration

Uptake of contaminants from soil depends on its concentration. Some of the contaminants, especially at higher concentration may compete with micro- and macronutrients such as P, Ca, Mg or Fe and thus present toxicity effect on plant growth or vital processes [200,201]. Generally higher pollutant concentration makes it more difficult for a plant to accumulate or degrade them. Gomes et al. [202] investigated the effect of variable Cd concentration (0, 15, 25, 45, 90  $\mu\text{mol}/\text{m}^3$ ) on plant growth and phytoremediation capacity of *Eucalyptus camaldulenses*. Shoots and roots biomass production was negatively affected by an increase in Cd concentration. Moreover, the highest cadmium concentration caused visible symptoms of phytotoxicity, such as yellowing of leaves or blackening and thickening of roots. These symptoms might be related to deficiencies of several nutrients essential for the formation, expansion, and operation of chloroplasts. Decreases in total K, Ca, and Mg contents might be related to competition for bivalent ion binding sites by Cd. For Cd concentration in soil equal 45  $\mu\text{mol}/\text{m}^3$ , metal concentration in shoots and roots was the lowest. Studies performed by Dheeba and Sampathkumar [203] concerning influence of variable Cr concentration (10, 20, 30, 40, 50 mg/kg) on growth and accumulation of metal by five species *Helianthus annuus*, *Zea mays*, *Sorghum bicolor*, *Vigna radiate* and *Arachis hypogaea*. It was shown that plants had different tolerance to chromium pollution. *Vigna radiate* and *Arachis hypogaea* shoot length were reduced by more than 50% when compared to control with the increase in contaminants concentration, and also fresh weight was reduced by about 50% at 50 mg/kg Cr concentration. Meanwhile, for all plants, pigment levels significantly decreased at 10–50 mg/kg of chromium in comparison to control. The greatest Cr concentration in roots was in the order of *Zea mays* > *Sorghum bicolor* > *Helianthus annuus* > *Arachis hypogaea* > *Vigna radiate*. Impact of cadmium concentration (0.1 and 30 mg/kg) on growth and removal efficiency of *Typha latifolia* was presented by Yang and Shen [25]. The highest plant shoot and root lengths were for 1 mg/kg Cd treatment with 89.4 and 18.3 cm, while growth at 30 mg Cd/kg treatment reduced biomass production, but plants did not show any toxicity symptoms. The Cd concentration in plant roots and shoots of Cd were 51.6 and 26.0 mg/kg (for 1 mg/kg) and 279 and 131 mg/kg (for 30 mg/kg), respectively. Phytoextraction potential of *Linum usitatissimum* growing on soil differentially spiked with copper (200, 400, and 600 mg/kg soil) was evaluated by Saleem et al. [27]. Results suggested that plant was able to grow up to 400 mg Cu/kg level without any inhibition in growth. Further increase of Cu



concentration caused a reduction in plant growth, total chlorophyll and carotenoids content, and biomass production. However, even at high concentration plant was able to accumulate a significant amount of Cu in roots and shoots.

### 3.5. Mobility, Bioavailability and Chelating Agents

The problem of low mobility and bioavailability of metals is an essential factor affecting phytoextraction efficiency. A new approach demonstrates the possibility of performing chelate assisted phytoextraction. Chelators may enhance the solubility of metals in soil and thus improve its phytoavailability as well as boost metal translocation from roots to above-ground plant parts [127,137,204]. There is a wide choice of chelating agents for enhancing phytoextraction described in the literature. The classification consists of natural and synthetic substances [205]. The group of natural chelators includes mainly low-molecular-weight organic acids such as citric acid, vanillic acid, gallic acids, oxalic acid, or tartaric acid, whereas the synthetic group contains among other things EDTA, EDDS, DTPA, HEDTA, and NTA [127,205–211]. Meers et al. [127] compared the effectiveness of synthetic aminopolycarboxylic acids with low molecular weight, biodegradable organic acids on the phytoextraction ability of *Zea mays* planted on the soil contaminated by Cu, Zn, Cd, Pb, and Ni. Only the addition of EDTA and DTPA increased metal accumulation in above-ground plant tissue and higher the value of the translocation factor. Moreover, the application of chelating agents 10 days after germination more efficient than before sowing of plant for higher accumulation of metal in shoots. The addition of NTA or acids did not have the expected results, mainly due to rapid mineralization and too low dosage. A similar effect observed Kos and Lestan [212] with an application of citric acid on vineyard soil contaminated with copper. While, Quartacci et al. [213] observed that the overall accumulation of Cd, Zn, and Cu in *Brassica juncea* has been improved upon NTA treatment. Several studies also compared the performance of EDTA and EDDS. The authors concluded that EDDS might be more active on multi-contaminated soil and assist in metal translocation from roots to shoots. Furthermore, Luo et al. [214] suggested the combined application of EDTA and EDDS for phytoextraction of Cu, Pb, Zn, and Cd by *Zea mays*. The most efficient ratio was 2:1 of EDTA: EDDS, which led to significantly increased the concentration of heavy metals in shoots and total metal uptake.

Although synthetic chelators can enhance phytoextraction efficiency by increasing metal solubility and bioavailability, their application may adversely affect plant growth, and biomass as well as promote necrosis and chlorosis symptoms. These might be the consequence of excessive metal concentration in soil and its toxicity to plants [132]. Moreover, the application of poor biodegradable synthetic chelators such as EDTA or DTPA that may be persistent in the environment, will be a highly risky action for the due to the possibility of secondary pollution of groundwater [214–216].

### 3.6. Plant Growth, Biomass Production and Accumulation Capacity

Plants used for phytoremediation of the pollutant from contaminated soil should exhibit a fast growth rate and high biomass production. Moreover, an extended root system for exploring large soil areas is favorable. Excellent tolerance and resistance to stress induced by the high contaminant concentration in the soil are necessary. Phytoextraction also requires the capability to accumulate a high concentration of contaminants (hyperaccumulator), simultaneously with high translocation factor from roots to above-ground plant tissues. Keller et al. [217] compared various high biomass plants (*Brassica juncea*, *Nicotiana tabacum*, *Zea mays*, and *Salix viminalis*) with hyperaccumulator plant (*Thlaspi caerulescens*) growing on soil contaminated with Zn, Cd, and Cu. *T. caerulescens*, characterized by small biomass was the most efficient plant for Cd and Zn removal with very high concentrations in the shoots. Among plants with high biomass production, *Salix viminalis* was able to accumulate a high concentration of Cd and Zn, while *Nicotiana tabacum* effectively removed Cd and Cu. Moreover, the difference between root system distribution was observed. *T. caerulescens* formed a shallow root system, which was able to remove contaminants from shallow soil zones (0.2 m), whereas *Zea mays* and *Salix viminalis* colonized the soil at depth and thus were more suitable for deep contamination (0.7 m).

These results indicate the importance of proper choosing plant species to type and concentration of contaminants. Similar research performed Zhuang et al. [135] investigated the potential of high biomass plants (*Vertiveria zizanioides*, *Dianthus chinensis*, *Rumex K-1* (*Rumex upatientia* × *R. timschmicus*), *Rumex crispus*, and *Rumex acetosa*) in comparison to metal hyperaccumulators (*Viola baoshanensis* and *Sedum alfredii*). Among the high biomass plants, *R. crispus* extracted Zn and Cd of 26.8 and 0.16 kg/ha, respectively, which was comparable to Zn accumulation by *Sedum alfredii* and Cd by *Viola baoshanensis*. However, *Vertiveria zizanioides*, which presented the highest biomass, accumulated only a small amount of each metal, with a much higher concentration in root than in shoot. Tolra'et al. [218] compared phytoremediation properties of hyperaccumulator *Thlaspi praecox* and non-hyperaccumulator species of *Thlaspi arvense*. *Thlaspi arvense* exposed to Cd exhibited toxicity symptoms in leaves in the form of chlorosis, while any symptoms were observed in *T. praecox*. Moreover *T. praecox* presented considerably higher root elongation rates than *T. arvense* under control conditions. *T. arvense* accumulated higher root Cd concentrations than *T. praecox*, while shoot Cd accumulation was significantly higher in *T. praecox*, which was above 2500 µg Cd/g DW. Similarly, Shen et al. [219] compared uptake and transport of Zn in the hyperaccumulator *Thlaspi caerulescense* and non-hyperaccumulator *Thlaspi ochroleucum*. *T. caerulescense* was able to tolerate 500 mmol/m<sup>3</sup> Zn in solution without affecting growth and up to 1000 mmol/m<sup>3</sup> with a 25% decrease in dry weight, while in case of *T.ochroleucum* severe toxicity was observed for Zn concentration 500 mmol/m<sup>3</sup>. Moreover, *T. caerulescense* accumulated a higher concentration of Zn in shoots, while *T.ochroleucum* in roots. Presented results indicated that *T. caerulescense* exhibit a strongly expressed constitutive sequestration mechanism, which detoxifies a large amount of Zn in plant tissue.

Meanwhile, plants applied for phytostabilization treatment should avoid excessive uptake and transport of contaminants thus present low accumulation in steams (excluders) with a low value of the translocation factor [80]. Moreover, in this process, the crucial requirements are related to morphology, density, and penetration depth of root [97,220,221]. Plants with high root biomass, or fibrous rooting system are excellent candidates for metal stabilization in soil [222,223].

Favorable candidates for phytoremediation might be engineered plants. Transgenic plants with unique genes promotes fast growth rate, development of deep rooting system, abilities to detoxify hazardous pollutants, or tolerance to various, very often harsh climatic conditions. However, this technique despite the many benefits might bring potential environmental risk due to the possibility for invasion into natural plant communities [224]. The transgenic *Beta vulgaris* L. with gene that synthesizes glutathione have been reported by Liu et al. [225] as an efficient agent for removal of Cd, Zn, and Cu from aqueous solution. The modified plants presented higher tolerance to heavy metals and stronger accumulation than wild-type plant. Similar, He et al. [226] overexpressed bacterial γ-glutamylcysteine synthetase in the cells of *Populus tremula* × *P.alba*. Transgenic plants were characterized by higher Cd uptake, accumulation in aerial parts, and tolerance to the presence of metal in nutrient solution. Meanwhile, Sharma and Yeh [227] proved that genetic engineered *Arabidopsis* and *Nicotiana tabacum* showed 4–7 times higher accumulation of Fe than wild-type plants. As well phytodegradation of acetochlor by *Arabidopsis thaliana* with oxygenase component of the bacterial N-dealkylase system was evaluated by Chu et al. [228]. The Authors concluded that transformed plants were able to eliminate about 80% of acetochlor (5 mg/kg soil) within 30 days, and above 94% (20 µM) in aqueous solution within 48 hours. In the study, Zhang et al. [229] reported that *Pascopyrum smithii* with bacterial genes flavodoxin-cytochrome P450 Xp1A, favodoxin reductase Xp1B, and nitroreductase nfsI was able to remove and detoxify more hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX), and 2, 4, 6-trinitrotoluene (TNT) than wild-type plants.

### 3.7. Microbial Activity

Microbial activity in the rhizosphere has been considered as important parameter that has strong functions in plant growth and metal uptake. Microbes are involved in many significant processes associated with nutrient acquisition, cell elongation, metal detoxification and alleviation of stress in

plants [230]. A group of microbes in the soil, participating in phytoremediation, includes such as plant growth promoting bacteria (PGPB), phosphate-solubilizing bacteria (PSB), mycorrhizal-helping bacteria (MHB), and arbuscular mycorrhizal fungi (AMF) [231]. Jeong et al. [232] tested the ability of phosphate-solubilizing bacteria for enhancing Cd bioavailability and phytoextraction potential of *Brassica juncea* and *Abutilon theophrasti*. Phosphate-solubilizing bacteria solubilize insoluble phosphates of soil into soluble plant available forms by secreting various organic acids, and therefore are able to stimulate plant nutrition and growth [233]. However, performed analysis revealed that inoculation with *Bacillus megaterium* increased Cd accumulation by two folds compared to uninoculated plants, while did not remarkably affect plants biomass. Meanwhile, it has been suggested that the incorporation of plant growth promoting bacteria seems to be useful approach to improve plant growth and shoot and root biomass production [234,235]. These bacteria could make the plants more tolerant to harmful contaminants, lower stress ethylene levels and decreased concentrations of proline and malondialdehyde [236]. Marques et al. [237] found that inoculation of *Helianthus annuus* with PGPB reduced biomass losses growing on Zn and Cd contaminated soil. However, bacterial community decreased Zn and Cd accumulation in plant tissue. This strategy may be a reliable approach in phytostabilization. Meanwhile, Rajkumar and Freitas [238] observed that inoculation *Brassica juncea* with PGPB facilitate above-ground biomass production and at higher Ni concentration (300 mg/kg) in soil increased metal uptake by shoot and root compared to the uninoculated plant. Moreover, it was concluded that bacteria strains protect the plants against the toxic effects of nickel, probably due to the production of phytohormone—indole acetic acid (IAA), siderophore, and solubilization of phosphate. Several researches have been demonstrated the effect of arbuscular mycorrhizal fungi (AMF) on phytoremediation potential of plants. In the study Bhaduri and Fulekar [239] investigated the potential of *Ipomea aquatica* supported by AMF for Cd contaminated soil phytoremediation. Results showed that AMF enhanced accumulation of cadmium in plant tissues. Furthermore, inoculated plants exhibited improved Cd tolerance and resistance under stress conditions and thus lower reduction of biomass growing on contaminated soil than non-AMF plants. Similar research were performed by Gunathilakae et al. [240] with AMF inoculated *Eichhornia crassipes*. AMF colonization enhanced plant growth, biomass production, relative growth rate and Cd concentration in roots and shoots.

#### 4. Phytoremediation—Benefits and Limitations

Phytoremediation involves a group of cost-effective and eco-sustainable green processes based on several mechanisms, which finally led to pollutants removal from aqueous or soil ecosystems and is a promising alternative for traditional remediation technologies. The estimated cost of phytoremediation amounts to \$5–\$40/ton of contaminated soil [241]. Calculation prepared by Wan et al. [242] proved that total cost of phytoremediation of soil contaminated with arsenic, cadmium, and lead was \$37.7/m<sup>3</sup>, which is significantly lower than costs for other remediation techniques such as solidification (\$87–\$190/m<sup>3</sup>) [243], extraction (\$240–\$290/m<sup>3</sup>) [243], or vitrification (\$75–\$425/ton) [241]. The significant advantage of this method is a vast variety of plants demonstrating the potential for accumulation, degradation, or stabilization of a satisfactory amount of contaminants. This group of plants includes diverse species i.e., grasses, legumes, aquatic and marsh plants, deciduous, and coniferous trees. Moreover, the broad spectrum of pollutants such as heavy metals, radionuclides, polyaromatic hydrocarbons, surfactants, pesticides, or pharmaceuticals may be subject to phytoremediation. The advantage of in-situ performed remediation is a limitation of contaminants spread with air or water, and prevention of secondary pollution. Despite many advantages phytoremediation technique has not still become Worldwide used technology. However, information provided for example by the U.S. Environmental Protection Agency (EPA) indicate that phytoremediation has been successfully used at many sites across the country. Moreover EPA pointed out that this techniques is used because requires less equipment and work than other remediation methods as well as helps control soil erosion and improves air quality. Also in the U.S exist dedicated companies offering commercial phytoremediation services targeting particular contaminants. One of the main disadvantage of phytoremediation is related to



the duration of treatment processes. It might be a slow and time-consuming process, which last from months to several years [244]. It should be mentioned that another obstacle is related to seasonality of the phytoremediation, which loses efficiency during the winter season. Moreover, phytoremediation may be limited by agronomic challenge—quality of the soil. Poor soil structure and low nutrition level might be the factors which have significant impact on remediation efficiency. Thereby proper preparation of field including irrigation and fertilization is required and in turn, phytoremediation costs might increase [245]. The majority of previous research in the field of phytoremediation has only focused on a greenhouse experiment, maintained at special conditions (temperature, humidity, photoperiod), so recreating of this condition in the field experiment may be problematic. Furthermore, the phytoremediation process may be affected by several factors, for example, soil texture, soil pH, fertilization, coexistent pollutants, and climatic conditions, thus fields have to be appropriately adapted to provide high removal effectiveness. Moreover, harvested plants with accumulated contaminants may be recognized as a hazardous waste. A challenging area in the field of phytoremediation is plant disposal and thus suitable utilization methods are required. Some researchers proposed composting and compaction as a post-harvested plant management [246]. Moreover, application of crops as a “bio-ore”—high grade and useful material for metal recovery was investigated. Thermal, thermo-chemical and chemical methods were used for extraction of nickel from biomass with obtaining respectively, ferronickel,  $\text{Ni}^{2+}$  salts, and  $\text{Ni}^0$  [247]. Vaughan et al. [248] proposed combustion and leaching of nickel from tropical hyperaccumulator plant. This process led to producing unique impurity nickel hydroxide. A step forward in plant utilization is also novel adsorption—pyrolysis technology for recovering copper and cadmium from contaminated biomass after the phytoremediation process [249]. The possibility of using a contaminated biomass as an adsorbent with functional groups, able to react with metals and retain them within biomass was proved.

## 5. Energy Generation from Harvested Plants

Recently, utilization of plant biomass as a non-fossil material for renewable, clean energy production progressively increases and currently is the 4th largest energy source in the world [250,251]. Biomass may be easily converted into bio-solid (chips, pellets, briquettes), bio-liquid (methanol, ethanol, diesel), and bio-gaseous (hydrogen, biogas, syngas) fuels using thermochemical or biological methods. The conversion methods include such as combustion, pyrolysis, gasification, fermentation, and anaerobic decomposition [252]. Biomass might be called “CO<sub>2</sub>-neutral” or “zero CO<sub>2</sub>-emission” energy source, since equal or even higher amount of carbon dioxide is used during plant photosynthesis processes than released when it is burned [253]. In this case, there is no net increase in the atmospheric CO<sub>2</sub> correlated with plant biomass use as fuel, in contrast to fossil fuels. Energy crops for bioenergy production should be characterized by high yield, fast growth, low fertilizer input, low energy input to its production, and low costs [254]. Similar requirements are imposed for plant applied for phytoremediation process. Thus, recent studies suggested that also contaminated plant biomass from phytoremediation might be a promising source for bioenergy production [255,256]. Some authors hypothesized that energy crops with high biomass such as *Populus*, *Salix*, *Pinus*, *Helianthus annuus* might be not only promising candidates as phytoremediation species, but also their biomass can be economically valorized for renewable energy production (bio-ethanol, bio-diesel, bio-gas, or bio-energy) [257]. Table 2 shows examples of plant efficient in phytoremediation processes as well as in bio-energy production.

Witters et al. [258] predicted that Silage maize might be used for phytoremediation of soil contaminated with Cd with simultaneous application of post-harvest biomass as a source for renewable energy production. Silage maize biomass production was 20 Mg DW per hectare per year with Cd accumulation 0.022 kg per hectare per year. Performed life cycle analysis (LCA) revealed that Silage maize biomass might be converted by anaerobic digestion to biogas, with a production of 12,459 MJ energy per hectare per year, pointed out the positive effect of metals on energy production. Hunce et al. [259] compared the potential of *Helianthus annuus* and *Silybum marianum* growing on contaminated and non-contaminated soil, for biogas production. It was concluded that the presence of



trace elements in plant biomass did not limit the potential of energy recovery. The biogas production potential of *S. Marianum* biomass (194–223 mL/g) was higher than that from *H. annuus* (134–154 mL/g). Meanwhile, Meers et al. [260] performed the field experiment using *Zea mays* for removal of Cd, Zn and Pb from contaminated soil. Application of energy *Zea mays* will valorized potential of phytoremediation techniques due to its biomass conversion to biogas via anaerobic digestion as a sustainable waste management. It was estimated that *Zea mays* biomass from field experiment might be converted in 33,000–46,000 kWh of renewable energy per hectare per year, which as a substituent of fossil energy, that will help reduce up to 21,000 kg per hectare per year CO<sub>2</sub>. In the study, Balsamo et al. [261] investigated effectiveness of grasses for remediation of lead contaminated soil, and biofuel production from their contaminated biomass. The Authors stated that the presence of lead in the grass material feedstock did not adversely affect the outcomes of the conversion processes. Furthermore, it was concluded that grasses might be a promising candidate for bioethanol or bio-crude oil production.

**Table 2.** Examples of plants applied in phytoremediation and bioenergy production.

Plant	Phytoremediation Process	References	Bio-Energy Production	References
<i>Helianthus annuus</i>	Phytoextraction of Zn and Cu Rhizofiltration of U	[122]	Bio-diesel Bio-gas	[262,263]
<i>Zea mays</i>	Phytoextraction of Pb, Cu, Zn, Cd, Ni	[127,128]	Bio-ethanol Bio-gas	[264,265]
<i>Brassica juncea</i>	Phytoextraction of Cd, Cr, Cu, Zn, Pb Rhizofiltration of U	[125]	Bio-diesel	[266]
<i>Miscanthus</i>	Phytostabilization of Cu, Zn, Pb, Cd	[92,93]	Bio-ethanol Bio-gas	[267,268]
<i>Salix</i>	Phytovolatilization of MTBE Rhizodegradation of perchlorate	[50,112,152]	Bio-ethanol Bio-gas	[269,270]

## 6. Summary

A large number of papers published in recent years indicate that phytoremediation is gaining interest both for scientists as well as for practical purposes. Searching for new plant species, contaminants that can be removed via phytoremediation techniques, or novel methods to enhance biomass yield and efficiency of the cleanup process are still in the developing stage. However, despite successes of phytoremediation confirmed by laboratory-scale greenhouse experiments, there is a gap in the field research, where the phytoremediation process is depending on real conditions and may be affected by numerous factors. Thus, there is a need to investigate phytoremediation at the field scale. Furthermore, an essential aspect of phytoremediation, which supposed to be envisaged, is the economic and ecological valorisation of contaminated biomass of plants after harvesting. There is still a need for further experiments to develop a productive and profitable method for plant biomass processing, when “bio-ore” generation with metal recovery is considered. An approach of combining phytoremediation aiming at biomass generation and its utilization as energy source should be more intensively investigated. This two-track approach for interconnection of phytoremediation processes with renewable bioenergy production from contaminated crops might bring tangible benefits, especially related to simultaneous clean up-process of large areas and thus significant amount of alternative energy production from waste, also taking into account reduction of CO<sub>2</sub> production in comparison to using fossil fuel. This will allow to call phytoremediation “zero waste” sustainable environmental technology for soil remediation. Moreover, an interesting approach of research may be related to investigations on mutual symbiotic interactions between various plant or microbial activity in terms of enhancing plant growth, and thus phytoremediation efficiency. Key aspect is also development in the field of plant engineering, which provide plant unique features. Furthermore, a focus may be on investigations of factors affecting plant growth and plant selection for obtaining valuable products possible to be extracted (not only e.g., metals, but even biologically active compounds).

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