Chapter 2
Phytoextraction of Heavy Metals by Fast-Growing Trees: A Review

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2.1 Introduction

The accumulation of heavy metals in excess concentrations in the biosphere leads to environmental contamination. The impact of chemical degradation is a very serious danger and threat for the environment and this process would be irreparable and irreversible with unforeseeable negative consequences if the process of bioremediation does not occur. Bioremediation employs most often microorganisms and plants which degrade, detoxify, or sequester toxic chemicals present in natural waters and soils [1].

Excess concentrations of many metals in the environment could be easily absorbed by plants and animals and therefore affect humans by entering the food chain. The risk assessment for human health, therefore, is assuming an active effort of researchers to develop effective and inexpensive methods for the extraction of contaminants from polluted soils, sediments, and waters. The removal of pollutants from soil by traditional technologies could be more or less successful depending on specific circumstances and costs. During the last few decades, attention has been focused on innovative and cost-effective biological technologies such as phytoremediation, which is based on the use of plants to extract (absorb), destroy, or sequester hazardous contaminants from contaminated growing media [2]. Using plants to clean up the environment is an effective in situ technology which is applicable in the restoration of contaminated soils and waters [1, 3–5].

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Phytoremediation is a complex technology which comprises several techniques with respect to the specificity in physiological (morpho-anatomical, biochemical, and molecular) responses of plants to excessive concentrations of different contaminants. The main criteria for the selection of plants with good remediation potential are their potential to bioaccumulate pollutants and their ability to transform/translocate them in above ground (harvestable) organs as well as high organic production. Many researchers have suggested that the use of trees (rather than smaller plants) in environmental bio-cleaning purposes provides good results because of their long roots which penetrate deep into the ground [6]. According to this, plants used in phytoremediation technologies in order to revitalize contaminated sites have to be hyperaccumulators with deep root systems, and they must possess good potential for using (polluted) groundwaters. All desirable properties are achieved by a specific metabolism defined by enzyme activity, particularly enzymes that are involved in a plant detoxification strategy [7, 8].

According to their ability to accumulate heavy metals, plant species are classified into two groups, i.e., "excluders," characterized by preferential accumulation of heavy metals in roots and low translocation into aerial organs, and "hyperaccumulators," species capable of accumulating and tolerating considerable levels of heavy metals in their shoots [9]. The latter group is able to accumulate above 100 mg kg^{-1} dry weight of Cd, more than 1000 mg kg^{-1} dry weight of Ni, Cu, Co, Pb, and over 10,000 mg kg^{-1} dry weight of Zn and Mn [10] in aerial organs. Besides having a high production of biomass, the accumulation of target metals in harvestable (aboveground) plant organs is pivotal for efficient phytoextraction.

There are several techniques involved in phytoremediation strategies, according to different metabolic processes in mobilization and uptake of metal ions from soil, efficiency of metal translocation to shoots via symplast and apoplast (xylem), sequestration of metals within cells and tissues, transformation of accumulated metals into metabolically less harmful forms. *Phytoextraction* is the most commonly used technique of phytoremediation which involves the utilization of plant-hyperaccumulators for the absorption of pollutants from the soil, their transport, and accumulation (concentration) in the biomass of harvestable organs [11, 12]. High biomass production has been more than welcome in plants used in phytoremediation/phytoextraction projects. Therefore, fresh and/or dry biomass determination has been often included in investigations related to their (bio) concentration potential because it is a significant indicator of specific tolerance to elevated metals in the environment. Plants suitable for successful phytoextraction of heavy metals should be tolerant to high concentrations of metals, and, at the same time, be able to accumulate high amounts of essential and unessential metals in harvestable plant organs [13, 14].

Therefore, the efficiency of phytoextraction is determined by two main factors which should have high values: biomass production and pollutant bioconcentration degree [15]. *Phytostabilization* involves the remediation of polluted soils (and waters) by cultivation of plants whose excluded metabolites in the reaction with metal (metalloids) ions reduce the solubility and mobility of contaminants within the rhizosphere. In this process, plants reduce the bioavailability of contaminants by their absorption, adsorption onto the root surface, or by formation of insoluble compounds, therefore neutralizing their harmful effect on the environment [16]. Accumulation and precipitation of contaminants in the rhizosphere prevent their migration, reduce solubility, and minimize their bioavailability. Growing plants for phytostabilization is useful in preventing soil erosion and reduces the spreading of pollutants from contaminated localities. Phytostabilization can be enhanced by using soil amendments that are effective in the immobilization of metal or metalloid ions [17].

Phytovolatilization involves a process in which plants take up contaminants from the soil and release them in a volatile form into the atmosphere through transpiration. The removal of contaminants, especially organic matters and mercury (Hg), by phytovolatilization could be achieved by the implementation of enzymes which promote plants' capacity to convert metals into volatile chemical forms [18]. *Phytodegradation* is a metabolic strategy of plants in detoxification which involves the uptake and degradation of different xenobiotics within the plant tissues or in soil (water) by enzymes. Rhizodegradation involves the use of plants associated microorganisms in the rhizosphere which carries out the degradation of contaminants in soils [19]. The main prerequisite for successful implementation of phytoremediation is to identify native plants and develop strategies for making hybrids and genetically modified plants which are good candidates for phytoremediation [20]. Significant effort has been made with the aim to identify species suitable for decontamination of heavy metal-polluted environments, primarily soil.

2.2 Phytoremediation/Phytoextraction by Trees

Although herbaceous plants can accumulate higher concentrations of metals and have higher bioconcentration factors compared to woody plants [12, 21], a very useful ecological solution for cleaning contaminated (forest) areas is growing woody plants which are characterized, primarily, by good accumulation capacities for pollutants and high biomass production [22]. Generally, trees have been considered appropriate plants for the utilization of phytoremediation because of their fast and large biomass production with significant economic value, genetic variability, established cultivation practices, high degree of public acceptability, and their contribution to site stability prevention of downward migration of heavy metals by leaching, wind dispersion, or erosion by water [23]. Although the efficiency of phytoremediation could be limited to sites with lower contaminant concentrations and might depend on soil properties and variation from year to year, growing woody plants for phytoremediation/phytoextraction purposes enables the recovery of contaminated sites to their natural conditions and also provides economic returns in obtaining woody biomass which can eventually be used in producing energy [24]. Compared to some other techniques, such as physical excavation and landfill, phytoextraction can significantly reduce the costs of decontaminating and revitalizing chemically degraded lands [25–27].

Phytoextraction by fast-growing, high biomass-producing Salicaceae species, poplar (*Populus* sp.) and willow (*Salix* sp.) have been recognized as a promising approach for the decontamination of polluted soils. However, new and additional data is necessary to improve its large-scale application in the field [23, 28–31]. Poplar and willow forest populations are good vegetation options for phytoextraction and phytostabilization techniques applicable for in situ decontamination of heavy metal-contaminated soils [32, 33]. However, there are several limitations in applying phytoextraction techniques for the remediation of polluted sites, such as a relatively long duration of the process, better absorption of pollutants in shallow layers within the root area, and also the possibility of dissemination of contaminated plant organs (leaves) and the risk of transmission of contaminants to other sites [34].

2.2.1 Heavy Metals in the Environment

The distribution and mobilization of heavy metals into the biosphere are involved in many forms of environmental contamination. Soil is contaminated when concentrations of nutrients, different chemicals, and trace metals deviate from naturally occurring sources. Widespread accumulation of cadmium, copper, and zinc in soils has been the result of human activities, such as mining, fossil fuel production, irrigation with metal-containing wastewater, agricultural utilization of municipal sewage sludge, the application of herbicides and pesticides, and the application of organic and phosphorus fertilizers. Some heavy metals, especially Pb, enter soil and surface/ground waters directly, by deposition, precipitation, or drainage of atmospheric polluted particles emitted from vehicles which use gasoline with lead as an additive [35].

Pollution is evident when contaminants are present in significantly greater than natural concentrations and when nutrient content, organic matter decomposition, soil microflora, acidic and alkaline buffer capacity, etc. are not capable of enabling normal bioproduction and diversity of plants and animals [36]. Accumulated chemicals and particularly heavy metals in excess concentrations disturb life and self-regulation processes in soil. There are many forest and agricultural localities polluted by heavy metals as the result of atmospheric deposition of industrial and traffic emissions [37]. Regarding the applicability and effectiveness in cleaning the environment, attention must be paid to the research of numerous environmental physicochemical parameters and biological parameters related to plant metabolism.

2.2.2 Physicochemical Properties of Soil and Bioavailability of Metals

Successful phytoextraction can be achieved by the selection of the most suitable genotypes for growing on soils with specific physicochemical properties. The availability of metal ions for root uptake and their toxicity depends on many abiotic factors such as their concentrations (total element content), chemical forms, types of binding, mobility, solubility, etc. According to Free Ion Activity Model (FIAM), the activity of particular metal species in the soil solution is a major determinant in bioavailability [38]. The phytoavailability of heavy metals in soil can be significantly limited by neutral to alkaline pH values, texture, cation exchange capacity, mineral composition, and the concentration of organic matter [39–44]. Cadmium (Cd) is a highly toxic trace element and a soil pollutant which could be easily taken up by roots via metal transporters and translocated to the shoots [45, 46]. Borišev et al. [47] reported that the quantity of essential nutrients present in the soil can have a significant influence on the uptake and accumulation of Cd in plants. They observed increased Cd accumulation in the leaves, stems, and roots of a *Salix viminalis* clone under conditions of Mg and Fe deficiency.

These results indicated that Cd, Mg, and Fe ions use the same transport pathways for their uptake in roots and translocation to aerial plant parts. Thus, competitive interactions can occur between these elements, in both the apoplasm and symplasm regions of the plant. If Cd^{2+} is present in soluble form in the root area, it can reach the root apoplast. In order to reach aboveground plant parts, it has to pass through the root cells' plasma membranes, which requires mobility in both apoplastic and simplastic regions of the plant [20]. Cd ion solubility, and thus mobility, is reduced at pH values above 6–7 and is also dependent on other soil properties [48–50]. An increase in the concentration of other nutrients or heavy metals can also create antagonistic competition between different ions [51]. The capacity for metal ion adsorption in the apparent free space of the plant root can be reduced by competition for membrane transporters. Elevated concentrations of both essential and non-essential metals can result in growth inhibition and toxicity symptoms which could be the result of competition with nutrient ions for the same membrane transporters; however, displacing of essential elements results in deficiency symptoms [46].

Plants phytoextraction capacity (uptake, accumulation, and translocation of metal ions from roots to shoots) could be significantly increased by the addition of chelating substances, which are present in soil. Therefore, different agronomical practices have been developed with the aim to enhance phytoextraction (pH adjustment, addition of fertilizer, or chelating agents). Induced phytoextraction is achieved through the addition of chelating agents (and strong acids) to the soil in order to increase the metals' bioavailability and their translocation from root to shoot [16]. Hammer et al. [32] observed that the use of chelating agents EDTA (Ethylenediamine-tetra-acetic acid) in soil a few weeks before harvesting improves phytoextraction via better translocation of Cu, Pb, and Zn from roots to shoots of *Salix viminalis*. The results of Hernandez-Allica et al. [52] indicated that proper management of EDTA application can reduce metal phytotoxicity and increase the uptake of metals with low phytoavailability. Although the use of soil amendments such as EDTA should increase metal ion activity, the success of its application is not guaranteed. One of the problems is the depletion of ions around roots, so rhizosphere conditions may not reflect bulk soil conditions [53, 54].

The use of EDTA-metal complexes in induced phytoextraction must be carried out with caution because they are highly stable and can easily reach the groundwater together with other heavy metals, consequently inducing environmental damage [52, 55]. Robinson et al. [29] reported that compared to control plants treated with EDTA accumulated higher concentrations of Cd which caused necrosis and abscission in most of the leaves. During this time, leaves were examined and a significantly lower biomass and no significantly higher Cd concentrations were observed. Meers et al. [56] suggested that EDDS (Ethylene-diamine-*N*,*N′*-disuccinic acid) chelating agents might enhance the removal of Cd, Cu, and Zn by *Salix dasyclados*. Although authors concluded that these results are limited to certain soil types, the efficiency of chelating agents has been evident and these preliminary results should be implemented into strategies of field trial organizing. In addition, authors suggested that before applying the method of phytoextraction in field conditions, pot experiments should be performed by screening suitable clones and evaluating the efficiency of chelating agents.

2.2.3 Physiological and Molecular Responses to Excessive Heavy Metals-Plants' Detoxification Strategy

Heavy metals are potentially toxic because they cause many morphological and physiological disorders in excessive concentrations in plant tissue. Phytotoxicity is caused directly or indirectly by the disturbance of cell membranes and the inactivation of many enzymes by the replacement of essential ions in enzymes, such as Fe, Mn, Cu, and Zn. Binding of metals with disulfide groups (–SH) in proteins leads to an inhibition of activity or disruption of their structure [46]. Excessive Pb concentrations in plant cells impair the uptake of essential elements such as N, P, and Mg, which consequently damages metabolism—mineral nutrition, photosynthesis, and transpiration. The inhibition of nutrient cycling and the displacement of essential cations (Ca, Mg, K) by Cu and Ni cations result in a decrease of base cation concentrations in the organic layer [57]. Exposure to excessive Cd concentrations decreases plant growth rate by affecting the water regime and transpiration, photosynthesis, enzyme activity, absorption, and translocation of many macro- and micronutrients [58].

Under stress caused by heavy metals, plants have developed defense mechanisms in order to preserve metabolic rate and stable organic production. They can reduce the uptake of heavy metals into cells, sequestrate them into vacuoles by formation of complexes, bind heavy metal ions by phytochelatins, synthesize osmolytes, such as proline. [59]. Mechanisms of absorption, translocation from roots to shoots and accumulation of metals, the rate of chemical transformation into less toxic compounds in plant cells and tissues, as well as metal redistribution in plant cells are implicated in plant metal tolerance and homeostasis and define the level of tolerance and adaptability [60]. Plants cope with deleterious effects of heavy metal exposure and accumulation, such as oxidative stress and disturbance of cellular ionic homeostasis, by engaging physiological and biochemical detoxification mechanisms such as the activation of enzymes involved in chelation, subcellular compartmentalization and exclusion of pollutants [46]. The activity of enzymes involved in antioxidative protection, catalase, and superoxide dismutase levels increased in leaves and roots of plants grown in heavy metal-polluted soil compared to control plants. Also, the remarkable induction of glutathione *S*-transferase activity in poplar plants grown under Cd excess in soil was recorded [61].

The results of many studies suggest the occurrence of different detoxification strategies in poplar species (clones, cultivars) exposed to heavy metals. For instance, the exclusion strategy has been employed by young poplar plants (*Populus × canescens, P. tremula × P. alba*) exposed to different Cd concentrations, according to specific accumulation and distribution of the metal in plant organs. Treatments resulted in significant differences in Cd concentrations in the following order: roots > stem > leaves [62]. Phytochelatins (PCs) are reported to play a significant role in metal sequestration in vacuoles; however, relationships between PC content and metal tolerance in hyperaccumulators are still under consideration [63].

Considering the fact that low molecular mass cysteine-rich proteins, metallothioneins, can neutralize the toxic effect of heavy metals, and also take part in the regulation of gene expression and cell metabolism, Castiglione et al. [64] analyzed the expression of genes belonging to class II metallothioneins (including all those from plants and fungi). The expression profiles of certain genes in stems, leaves, and roots of poplar plants exposed to Zn treatments were differentially affected by Zn in an organ-specific manner, and the relationship between Zn concentration and exposure time was rarely linear. According to the lack of a strict dependency of gene expression and zinc concentration and/or exposure time, authors concluded that tolerance to metals in Villafranca poplar is not based on the "exclusion" mechanism (i.e., restricted uptake and/or limited root-to-shoot translocation). Obtained results indicated the participation of other mechanisms of plants' detoxification strategies (e.g., other low-molecular-weight chelators, vacuolar sequestration).

Results from the in vitro experiment of Macovei et al. [65] with cell suspension cultures have been used as a model system to investigate molecular mechanisms responsible for Cd, Cu, and Zn tolerance of *Populus alba* L. cv. Villafranca. The authors concluded that the *VFMT2* gene, encoding a type 2 metallothionein, was differently regulated in response to the type of metal and its concentration. Phytochelatins, cysteine-rich peptides capable for efficient chelation of heavy metals, are synthesized inductively from reduced glutathione by phytochelatin synthase activity, following plant exposure to various heavy metals, such as Cd, Hg, Cu, Zn, Pb, and Ni [60, 66]. The significance of glutathione in the tolerance of Zn stress in *Populus nigra*, *Populus canescens*, and two transformed *Populus canescens* clones over-expressing a bacterial gene encoding γ-glutamylcysteine synthetase has been investigated by Bittsánszki et al. [67]. Considering an elevated level of glutathione recorded in transgenic poplars along with higher heavy metal uptake than in nontransformed clones, authors concluded that the transgenic poplars were more suitable for the phytoremediation of soils contaminated with Zn than wild-type plants. Complexation of heavy metals with these peptides results in the sequestration of Cd in the vacuole, protecting plant cells from its toxic effects [68].

Besides experiments under controlled/semicontrolled conditions, the performance of the wild-type poplar hybrid *Populus tremula × Populus alba* and a transgenic mutant over-expressing bacterial genes encoding the enzymes of glutathione biosynthesis has been investigated via their cultivation under field conditions for 3 years on a relatively clean (control) site and a site contaminated with heavy metals [69]. Although considerable changes in aboveground biomass accumulation were not recorded, changes in chloroplast structure, as a consequence of over-expression of bacterial gene gsh1 in poplar plants, were evident due to an exchange of the excess glutathione produced between the cytosol and the chloroplasts. The sequestration of heavy metals by phytochelatin complexes in the vacuole may partially prevent changes of chloroplast structure in plants from the contaminated site.

Investigating physiological processes related to the uptake and accumulation of heavy metals by woody plants is of great practical importance for a better understanding of phytoextraction and enlarges the possibilities of the exploitation of trees for the remediation of polluted sites. According to this, the determination of reliable physiological/biochemical indicators for plants successful survival and remediation potential in unfavorable ecological conditions is of crucial importance for distinguishing genotypes with high adaptive potential in contaminated environments. Plant selection criteria for high phytoextraction capacities are: photosynthetic and transpiration potential, produced enzymes involved in detoxification and their activity, biomass production, which is related to growth and survival rate, root system, and other criteria which affect the adaptive ability to tolerate different contaminants [7, 70]. In this sense, the aim of breeding programs is to produce genotypes (cultivars, clones) characterized by superior growth and resistance to high levels of pollutants which have to be extracted from the soil to aboveground plant parts.

2.2.4 Phytoextraction, Photosynthesis, and Water Management

Preserved photosynthetic activity under heavy metal stress conditions benefit plant survival and growth potential in unfavorable (polluted) ecological conditions and enables high biomass production, resulting in successful and efficient phytoextraction. Defining CO_2 photosynthetic assimilation of different woody species is important in order to choose genotypes which are suitable for phytoremediation breeding programs [71, 72]. The high toxicity of divalent heavy metal ions for overall plant metabolism is the most evident in the inhibition of photosynthesis. Multiple inhibitory effects were detected such as leaf chlorosis, decreasing of leaf area, and biomass production. The most depressive effect on the photosynthetic CO_2 assimilation rate according to investigated poplar and willow genotypes was evident in plants grown on diesel fuel and metal mixtures in soil [5]. Küpper et al. [73] observed that during excessive Cd-induced stress, a few mesophyll cells became more inhibited and accumulated more Cd than the majority of cells and this heterogeneity disappeared during acclimation in plants with good bioconcentration potential. Chlorophyll fluorescence parameters related to photochemistry were more strongly affected by Cd stress than nonphotochemical parameters indicating that Cd inhibits photosynthetic light reactions more than the Calvin-Benson cycle.

The substitution of Mg ion in chlorophyll by heavy metals leads to a breakdown in photosynthesis. By growing white poplar clones on excessive Pb concentrations in cell culture media, Katanić et al. [74] found that, depending on Pb levels, plant height was decreased, as was multiplication, and chlorophyll concentration in shoots. Excessive heavy metal presence in plant tissue can affect photosynthesis by reducing photosynthetic CO_2 fixation as a result of the partial closure of stomata in leaves. Heavy metal uptake and translocation from the root zone to the stems and leaves of plants are driven by transpiration, and because of that the water status of the plant tissues and soil moisture are of crucial importance for photosynthesis and organic assimilation [11].

According to Klang-Westin and Perttu [75], water availability is a critical factor for the growth of *Salix* and during some periods of the growing season, water availability will probably be the most limiting growth factor. Transpiration rate is affected by heavy metal stress, and plants which are more adapted to polluted environments need lower water amounts for nutrient absorption. Plant populations, which suffer a strong selective pressure in contaminated conditions, perform lower WUE and higher N, suggesting that plants may be "wasting water" to increase N delivery for photosynthetic apparatuses via the transpiration stream [76]. Becerril et al. [77] found that different metals may have different effects on transpiration and growth in the same plant. Pb caused a drastic reduction of water use efficiency, while Cd inhibited transpiration and carbon assimilation to a similar degree and thus did not change WUE.

Extensive physiological characterizations of the genotypes under a variety of conditions, including heavy metal soil pollution, is likely to reveal more about the specific suitability of each hybrid for site-specific remediation.

2.3 Salicaceae Trees in Phytoremediation/Phytoextraction Technologies

Plants from two genera: *Populus* and *Salix* (family: Salicaceae, order Salicales, class: Magnoliopsida-dicots) are recognized as the most commercially exploited forest trees with great economic importance. Although taxonomic subdivisions have been under continuous revision because of intra/interspecific differentiations [78, 79], these 2 genera, together, consist of 480 species [80, 81] and form a monophyletic group [82]. *Salix* L. (willows) is the largest genus of the family Salicaceae with about 450 species [8, 80]. The genus *Populus* L. (poplars) consists of 30 species with 6 taxonomic sections [80, 83]. It comprises fast-growing deciduous trees commonly named as poplars, cottonwoods, and aspens [84]. The wide distribution of these species over the northern hemisphere and potential to adapt to contrasting environmental conditions rely upon their wide natural variability [85]. Because of their rapid growth rate and a high biomass yield, adaptability to different ecological conditions, genetic intraspecific variability, as a result of a large number of intraspecific hybridizations and differentiations, willows and poplars are valuable resources for bioremediation/phytoextraction uses [27, 86].

2.3.1 *Willows (***Salix** *spp.)*

The efficiency of phytoextraction using *Salix* spp. depends on soil type, contamination level, the accumulation of metals in harvestable parts, as well as on the yield of aboveground biomass [87–89]. Next to the many favorable characteristics for phytoextraction such as rapid growth, high biomass production, deep root systems, as well as the ability to uptake large amounts of heavy metals, willow species have great potential for vegetative propagation having the ability to form roots from stem cuttings [90]. Easy vegetative propagation by cuttings and the production of a large number of new shoots leads to high biomass production in a short period of time. Therefore, willows are commonly used as an energy source [91, 92]. Vegetative propagation, i.e., clonal plantations, reduces variability between plants in comparison with plants produced from seeds [93]. Different species of this genus show significant differences in the accumulation of heavy metals [28, 94]. Heavy metal accumulation and biomass production in *Salix* species showed a complex relationship as a result of different developments of individual taxa [23, 95]. In general, *Salix* species are not hyperaccumulators, but some clones could be grown in heavily contaminated soils, accumulating large amounts of heavy metals due to their fast growing and high biomass production [96, 97].

In recent years, a large number of studies have been conducted in order to identify the most efficient willow clones which could be used in cleaning ecosystems which have been contaminated by heavy metals. Their tremendous genetic variability could be used in creating genotypes with high biomass production in contaminated environments [28, 93]. Various literature data have been obtained from investigations conducted in different experimental conditions and the results are often difficult to compare. The in vitro studies performed with cell cultures and shoots (cuttings) in many controlled experimental conditions could reveal possible uses of selected plants in phytoextraction methods [98]. This technique is often used in short-term experiments with the aim of testing the effect of excessive heavy metals in growth media on their uptake, accumulation, and translocation in plants [99, 100]. Several studies indicated that growing plants as tissue cultures enables the selection of genetic materials for use in genetic engineering [101]. In addition, numerous works highlighted this approach as suitable in the determination of relevant *Salix* genotypes for phytoextraction [64, 102].

Willows have a long life cycle, therefore short screening technologies such as growing plants in water culture—hydropones, are suitable for the selection of genotypes for phytoremediation/phytoextraction utilization [100, 103, 104]. There are numerous advantages of applying this growing method, such as precise definitions of the substance concentrations taken up by roots, the possibility for controlling the temperature and aeration, visual monitoring of root growth and aboveground parts, etc. In addition, this technique reduces the period of growth, duration of treatments, and also variability due to environmental factors. The analyses of morphological parameters of *Salix* species exposed to excessive heavy metals in nutrient solution showed significant variability. In general, disturbed plant growth as a result of elevated concentrations of heavy metals in nutrient solution was observed, regardless of the applied metals and their concentrations [14, 97].

Generally, cadmium (Cd) in plants suppresses root growth—length and biomass production [105]. The root length reduction and decrease in shoot biomass, as a primary toxic effect of heavy metals, are confirmed on several *Salix* species and genotypes [54, 87, 94, 106]. Furthermore, Luković et al. [107] reported a higher reduction in biomass of roots, leaves, and stems in willows than in poplars, with the same applied Cd concentrations. Zacchini et al. [14] have found that total root length was significantly reduced by 50 % in analyzed *Salix* clones treated with 50 μM of Cd. Contrary to this, Cocozza et al. [108] found no reduction in root length of *Populus nigra* and *Salix alba* in the same experimental conditions regarding applied Cd treatment and duration of the experiment. Root length and active absorption area play a predominant role in the absorption of water and nutrients; therefore, metal uptake is more strongly related to root length than root weight [28]. In addition to that, root elongation is an important parameter in screening tests for highlighting different plant sensitivity to Cd [14, 109].

Analyses of growth parameters of six fast-growing trees (four willows and two poplars) showed that roots are more sensitive to the presence of Cd than shoots [110]. According to the tolerance index (Ti), a significant negative correlation was estimated between plant biomass and Cd concentration in plants [72]. In this context, Dickinson and Riddell-Black [93] reported that productivity represented the most important trait in the uptake of heavy metals. Beside the reduction of biomass, visual symptoms such as chlorosis and necrosis are common occurrences of heavy metal toxic effects in leaves. Cosio et al. [111] observed the occurrence of chlorosis in all plants with applied Cd treatments (5, 10, 50, and 200 μM Cd) while necrosis was obtained at concentrations of 10 and 50 μM Cd. At the same time, they recorded severe root and shoot biomass reductions which were more than 90 % in plants exposed to the highest applied Cd concentration. This indicated that the root growth, elongation, and absorption zones were restricted by exposure to Cd causing a decrease in root capacity for nutrient and metal uptake, resulting in the inhibition of plant growth.

Contrary to this, Borišev et al. [54] did not detect chlorosis and a decrease in shoot biomass production with Pb-EDTA-treated willow plants. Since the accumulated Pb was retained mostly in roots, the photosynthetic plant parts were protected from the toxic effects of metals. The results also indicated an increase in root length of plants grown on media with excessive Cd. Literature data showed variation in biomass production as a response to different Cu and Zn concentrations. In a comparison between treated and untreated plants, excess concentrations of heavy metals may cause a decrease, an increase, or may have no significant effect on plant growth and biomass production [105, 111]. The results of numerous studies illustrated high genetic variation associated with the tolerance of the *Salix* genus toward heavy metals [14, 112–114].

The variation in accumulation of heavy metals between *Salix* clones is confirmed in several studies, particularly pointing to the efficiency of the use of willows in Cd phytoextraction [5, 115, 116]. Yang et al. [104] compared 39 willow clones in order to establish the best clone for the application of phytoextraction. The authors observed that the shoot Cd contents varied up to 91-fold among the clones, ranging from 29.8 (a hybrid *S. babylonica* × *S. alba*) to 2726.52 kg plant^{-1} DW (*S. babylonica*), respectively. The accumulation and allocation of heavy metals in plant tissues determine various remediation goals and relevance. Therefore, three principal patterns of Cd distribution can be distinguished [104, 117, 118]. Stem Cd accumulators are plant genotypes with a capacity of cadmium accumulation and retention mainly in the stem, while the leaves and roots have smaller Cd concentrations.

According to the same researchers, leaf Cd accumulators are characterized by high Cd content in leaves and clones with this type of Cd tissue (organ) allocation are suitable for phytoextraction. Root Cd accumulators are clones with high Cd content in roots with low transport to aboveground plant parts. Species/clones with such characteristics are good candidates for phytostabilization. Cadmium allocation is clearly species specific, but its visualization is important for understanding patterns of Cd accumulation and translocation [119]. Different patterns were observed by mapping Cd distribution in roots between species and clones within the *Salicaceae* family in the accumulation of Cd [108]. Observations suggested that the allocation of Cd within the root profile could not be used as a single parameter for the translocation of metals; other parameters must also be taken into count. Furthermore, the localization of Cd is dependent on the age of the leaves. It is smaller in young in comparison to old leaves, while in old leaves it is also dependent on the treatment duration and its concentration in nutrient solution. The superior ability of willows to translocate and concentrate Cd in leaves with respect to poplar clones is confirmed in several studies [14, 107, 118].

A hydroponic survey of metal resistance and accumulation of Cd and Zn in 20 clones of willows and poplar species revealed that *S. dasyclados* (315 mg Cd kg^{-1}) and *S. smithiana* (3180 mg Zn kg^{-1} dry weight) had the largest metal content in leaves, while *S. matsudana*, *S. fragilis*, and *S. purpurea* have been shown as the best metal-tolerant species [113]. A very important characteristic for phytoextraction is metal tolerance which is manifested as a combination of high metal accumulation with a reduction of its damaging effects. Moreover, metal tolerance seems to be associated with low metal transport, thus protecting aboveground plant parts which are involved in photosynthesis [97]. Cosio et al. [111] indicated that *S. viminalis* grown in hydroponics with 20 μM Cd performed as a highly tolerant plant species, with no reduction in biomass. The concentration of heavy metals which trigger the injury of leaves is still not established for tree species, due to the lack of consistent methods which are needed to characterize metal toxicity. According to Kabata-Pendias and Pendias [120], heavy metal toxicity in crop plants occurs when the heavy metal concentration in foliage exceeds 5–10 ppm for Cd, 150–500 ppm for Zn, or 15–20 ppm for Cu. The adaptation of willows to toxic metals could be achieved by gradually enlarging concentrations of heavy metals in the nutrient solution [121].

The genes for metal resistance may become expressed if the clones were gradually adapted to high metal concentrations, therefore this could improve metal resistance properties to elevated metal concentrations in media [113]. Furthermore, the allocation of heavy metals in aerial plant parts is the most important feature in the effective utilization of plants for phytoextraction. Zn distribution in *Salix* demonstrated that high amounts of Zn are concentrated in the leaves and the stem due to high mobility and easy transport to aerial parts [106, 122]. *S. matsudana* showed high Zn content in shoots (4497.7 μg plant^{-1} dry weight) and in combination with high biomass production, showed the highest phytoextraction potential in 12 analyzed clones [112]. On the other hand, Cu noticeably accumulated in the roots with low transport to aerial parts. Short-term exposure in hydroponics may not be sufficient for metal accumulation to occur in aerial parts of willows, causing higher metal concentrations in the roots [123]. Therefore, Zacchini et al. [14] pointed out fundamental aspects such as uptake, tolerance, and translocation to the aerial parts as the selecting criteria in screening tests for phytoremediation.

The phytoextraction capacity of plants could be changed if they were exposed to metal mixtures with respect to one metal which is present in excessive concentrations [124]. Dos Santos et al. [113] observed the accumulation of metals with a cocktail treatment of Cd and Zn. They found a significant reduction of Zn and Cd accumulation in mixed treatments in most cases. The possible reason for that is ion competition between metals. Consequently, the clones with a high uptake of combination heavy metals in their mixture are still not defined due to the effect of antagonism between metals [125].

In order to investigate antagonism between Cd and Ni ions in plant absorption, two willow genotypes (*Salix alba*—clone 68/53/1 and *Salix nigra*—clone 0408) were analyzed in the presence of elevated concentrations of Cd, and Ni. They were subjected to a combined treatment of both Cd and Ni, in two concentrations (10^{-4} and 10^{-5} M L^{-1}) in water culture solutions. Some symptoms of toxicity were evident at 10^{-4} M L^{-1} of applied heavy metals.

Both heavy metals accumulated mostly in roots, but translocation to aboveground plant parts was sufficient enough to confirm a good phytoextraction potential of analyzed genotypes, especially regarding Cd. The combined treatment of both Cd and Ni significantly reduced the metals' accumulation indicating a strong antagonistic relationship between these two elements. The determined antagonism between Cd and Ni probably occurs due to competition for the same metal transporters and carriers which enable the transport of metal ions to the stems and leaves of investigated willow genotypes [126]. Pajevic et al. [127] found that the content of Pb in plant tissue was higher in the treatment where only Pb was applied, compared to the plants grown on soil contaminated with metal mixtures, which indicated competition between other ions in Pb uptake [127].

The results obtained by investigations of phytoextraction capacity of willow genotypes grown under hydroponic experimental conditions should be confirmed by experiments performed in soil/field-growing conditions. Willows as pioneer trees are a common species grown on different soils, often severely contaminated, and therefore are widely used in screening strategies for identifying trees suitable in phytoextraction techniques [128]. Greenhouse experiments are conducted with the aim to separate different clones for their possible use in field environmental conditions. Willows showed considerable differences in metal uptake, translocation, and accumulation. Their adaptability and resistance to excess metals in tissues depend on clone characteristics [129]. Although the capacity of willows for heavy metal accumulation varies due to experimental conditions, Watson et al. [130] have found a significant correlation between Cu and Ni accumulation in the *Salix* clones grown in hydroponics and the accumulation of the same metals in *Salix* clones grown in the field.

A large number of studies highlighted that among the fast-growing trees, *Salix* species are leading candidates in the removal of Cd [131, 132]. In addition, genotypic variation in metal transport and allocation among the organs plays an important role in remediation. Cd and Zn are mainly translocated to aboveground plant parts, whereas As, Cr, Ni, Cu, and Pb are dominantly retained in roots [30, 88, 94]. Several studies confirmed that Cd and Zn are mostly concentrated in young leaves [23, 131, 132]. In addition, Vysloužilová et al. [87] showed that the amount of Cd and Zn removed by willow leaves were up to 83 % for Cd and 71 % for Zn with respect to total absorbed metal concentrations. The high Zn-transfer factor to aboveground plant parts might be the mechanism which enables the elimination of excess metals from plant via defoliation [33]. In many plants grown in chemically contaminated soils, the lead (Pb) mainly accumulates in roots, rather than in leaves. Jensen et al. [133] reported that in aboveground plant parts, Pb was more concentrated in leaves than in twigs, which is in disagreement with results obtained by Evangelou et al. [132] who found higher Pb content in the stem than in the leaves. In general, willows showed a low uptake of arsenic (As).

The removal of As from soil to aboveground parts was less than 1 %, which indicated that willows are not suitable for cleaning sites contaminated with arsenic [88]. Many obtained results indicated that the highest removal of heavy metals could be found in moderately contaminated soils, while phytoextraction potential is significantly decreased in extremely contaminated soils [88]. Heavy metal uptake and translocation are reduced in extremely polluted soils as a consequence of biomass reduction—leaves undergo chlorosis, necrosis, and partial defoliation [87]. Pot experiments have limitations that are reflected in relatively short growing periods which alter heavy metal uptake by plants; therefore, long-term field experiments are needed for the evaluation of results from hydroponic and pot experiments.

Although methods of growing plants under field conditions are still not widespread, these types of experiments provide more realistic data which indicate the ability of plants to carry out phytoextraction [134]. The influence of soil properties on metal uptake and biomass production on moderately contaminated soils was specified in different studies. It has been proven that willows are not suitable for the remediation of heavily contaminated soils, but they could be very effective in the remediation of moderately contaminated soils [133]. Their ability to remove heavy metals under field conditions is particularly limited in calcareous soils because of strong metal-ions bonding at alkaline pH levels [55, 135]. The comparison of Cd and Zn uptake by *Salix viminalis* grown in acidic and alkaline (calcified) soils showed that *Salix* produced more biomass and showed higher metal uptake and translocation in shoots in the acidic soils, with lower pH values [56]. Hammer et al. [32] found that during the 5 year-long experiments conducted on polluted soil, the biomass of willows increased every year, whereas metal concentrations decreased linearly causing an increase in annual metal uptake with time. The total extraction of heavy metals was at a maximum of about 60 g Cd ha^{-1} per year and about 5 kg Zn ha^{-1}. In addition, they confirmed the existence of Cd storage in leaves as an important physiological trait for remediation and pointed out the necessity of collecting leaves as well as shoots in order to clean up contaminated soils.

Many field experiments confirmed a dissimilarity incapacity for biomass production and metal accumulation within the *Salix* genus [31, 136]. Mleczek et al. [92] revealed the significant differences among the eight *S. viminalis* clones and one *S. alba* clone. The maximum rate of difference between the highest and lowest heavy metal content in the shoots of investigated *Salix* clones were 84 % for Cd, 90 % for Cu, 167 % for Hg, 190 % for Pb, and 36 % for Zn. Phytoextraction potential for Cd, Zn, Pb, Cu, Cr, and Ni using willows (*Salix* sp.) and poplars (*Populus* sp.) has been tested by Algreen et al. [137]. The results obtained after 10 years of field experiments, indicated very low phytoextraction efficiency for investigated heavy metals: the highest was registered in willows for Cd, but still not very high, below 0.5 %. Despite this, benefits from using willows in the decontamination of soils by the process of phytoextraction techniques are significant.

Rosselli et al. [33] found that the results of phytoextraction potential of fast-growing trees conducted in field conditions are in correlation with those grown in pots, under controlled conditions. However, bio-concentration factors for Cu, Zn, and Cd were higher in pot experiments. Authors explained the obtained results by the restricted volume of soil prospected by the roots and thus better ion uptake. In contrast to this study, Jensen et al. [133] revealed a two to tenfold higher metal uptake (Cd, Zn, Pb, Cu) in field trials in comparison to metal extraction by willow plants in growth-chamber experiments. Regarding Cd and Zn absorption by plants, authors obtained high percentages of their removal, up to 0.13 % of total soil Cd and 0.29 % of total Zn. These percentages are small, but represent the most soluble fraction. The risk, therefore, of groundwater and subsurface water leaching, is reduced.

*2.3.2 Poplars (*Populus *spp.)*

In order to investigate the efficiency of poplar species, hybrids, and cultivars in the removal of heavy metals from contaminated sites, numerous experiments have been set differing in the type of nutrient medium, heavy metal concentration and application (single metal or combined contamination using several metals), and the level of control of cultivation conditions. The results obtained from these experiments which were performed under controlled or semi-controlled conditions provide reliable guidelines for the selection of highly efficient genotypes. The accomplishment of these experiments under laboratory instead of field conditions enables precise control of growth conditions, as well as of heavy metal concentration and their bioavailability. The phytoremediation/phytoextraction potential of poplars has been tested in vitro (cell culture experiments), in hydroponic systems, using pot experiments (sand or soil), as well as in field experiments.

In vitro experiments have been successfully used to test the specificity of poplar species' (hybrids, cultivars) potential to withstand excesses of heavy metals in cultivation media. Many authors marked in vitro screening as a useful tool in studies aimed to test the ability of poplar clones to take up, tolerate, and survive heavy metal stress [64, 102, 138]. However, considering the lower availability of heavy metals in soil, the higher juvenility of the in vitro material, and the complexity of interactions between plants and their habitat, reliable evaluation of the particular genotype performance under conditions of a contaminated environment becomes necessary [139].

Recently, Di Lonardo at al. [102] investigated As, Cd, Cu, and Zn phytoremediation potential using in vitro multiplied microshoots of a commercial and two autochthonous *Populus alba* clones. Obtained data showed that plants might be able to accumulate high levels of heavy metals with no unfavorable effects on their biomass production. Higher concentrations of applied metals in roots than in shoots of poplar plants suggested a metal exclusion strategy of tested clones. Metal content was generally higher in the shoots than in the roots in all the clones. The highest content of all metals in shoots was recorded in the fast-growing commercial clone, suggesting biomass production as the key factor in evaluating the phytoextraction capacity of *P. alba* clones.

With the aim to evaluate the potential of four white poplar (*Populus alba* L.) clones (Villafranca, L-12, L-80, and LBM) for nickel (Ni) phytoextraction, Katanić et al. [138] carried out experiments using the shoot tips for cultivation on a solid growth medium with the addition of different nickel concentrations. Higher concentrations of nickel in the growth medium had significant inhibitory effects on plant fresh mass and especially on the photosynthetic pigments content, while the presence of Ni in the concentration of 10^{-3} M caused a serious disturbance of growth and decay in investigated clones. Authors singled out genotypes L-80 and L-12 as convenient candidates for phytoextraction and phytostabilization, as well as for the reforestation of areas moderately contaminated with Ni.

White poplar (*Populus alba* L.) genotypes have also been tested in vitro for Pb tolerance and accumulation. These tests were aimed to evaluate genotype performance in phytoremediation projects and landscaping in areas endangered by Pb contamination [139]. Some white poplar genotypes considered to be interesting for biomass production, landscaping, and horticulture were cultivated on media supplemented with different concentrations of Pb. The obtained results distinguish investigated genotypes according to the ability of Pb accumulation and two of them achieved a significantly higher lead shoot content compared to the widespread control genotype (almost 200 % and 125 % higher, respectively). The investigation of several poplar genotypes by Pajević et al. [127] showed a very high capacity for metal accumulation, especially for Pb (average content in plant tissue was 300 $\mu g \cdot g^{-1}$ dry mass) with the highest translocation factor. Compared to the control group, bioaccumulation factors for Cd were also high, but the translocation factor was lower, depending on the genotype investigated.

A commercial clone Villafranca of *Populus alba* L. has been used in the experiment conducted by Castiglione et al. [64] which was aimed to investigate tolerance to high concentrations of zinc (Zn) using an in vitro model system with shoot cultures. Applied Zn concentrations (0.5–4 mM) negatively affected chlorophyll content and the rate of adventitious root formation although to different extents. With the aim to explore the role of aluminum (Al) in the tolerance of poplars to heavy metals, Bojarczuk [140] carried out in vitro experiments using adventitious bud cultures of *P. tremula* L. × *P. alba* L. Plants originating from cultures grown in the presence of Al showed greater tolerance to the presence of Al and Cu in the medium than plants derived from cultures grown on media without Al. Although high concentrations of Cu and Pb inhibited shoot and root development, the author recommended in vitro selection of tolerant plants in order to obtain valuable material for research on mechanisms of plant sensitivity to metal toxicity.

Nikolić et al. [141] calculated the tolerance index (TI) on the basis of shoot weight in both treated and control plants (shoot fresh weight in plants from polluted soils × 100/shoot fresh weight in control plants) in order to define the tolerance of *P. deltoides* to soil contamination. The pot culture experiment was established using soil contaminated with Cd, Pb, and Ni. The metals were applied separately, or in combination in lower and higher concentrations. The lowest tolerance of *P. deltoides* was found after Ni treatment, probably due to the highest ability of translocation of this metal from roots to shoots (with respect to cadmium and lead). The obtained results of a very high bioconcentration factor for Cd and moderate tolerance, indicated that some poplar genotypes might be considered for evaluation of phytoextraction potential in outdoor/field experiments. In hydroponic experiments with plants concurrently exposed to several metals, Migeon and coworkers [142] identified clones of *P. nigra* and *P. maximowiczii* × *P. nigra* as highly tolerant to the heavy metals applied, with the TI value above 100.

The reliable evaluation of tolerance and performance in poplar plants exposed to excessive concentrations of heavy metals in the growth medium can be evaluated by other parameters, besides plant biomass, such as morpho-anatomical parameters, photosynthetic parameters, and water regime parameters [107]. The importance of different parameters in an indication and evaluation of tolerance to soil contamination was confirmed by the results of Pilipović et al. [143]. They investigated the influence of excessive Cd, Ni, and Zn concentrations in soil on pigment concentrations, photosynthesis, and activity of the nitrate reductase enzyme in *P. deltoides* clones and *Populus* × *euramericana*.

The obtained results for heavy metal tolerance of poplars indicated a significant correlation between investigated parameters: variations in aboveground and root biomass production, depending on heavy metal treatment, were in correlation with variations in obtained results for physiological parameters. Pietrini et al. [118] investigated the sensitivity of photosynthesis in *Populus* × *canadensis* and *P. nigra* L. plants to cadmium (50 μM $CdSO_4$) under hydroponic conditions. Both net photosynthesis and transpiration were considerably lower in treated plants, but high concentrations of phytochelatins were recorded in the leaves of both species. Also, the same authors showed that the confinement of Cd accumulation accompanied with the absence of phytochelatins in necrotic tissues of *Salix alba* L. leaves represents an efficient strategy for maintaining high photosynthetic activity in the willow genotypes.

Bioindication and phytoextraction potentials of poplars (*Populus nigra* × *maximovitzii* × *P. nigra var. Italica*; *Populus* × *euramericana*; *P. deltoides*) for Cd and Ni were investigated using hydroponic cultures under glasshouse conditions by Nikolić et al. [144]. Although the highest accumulation of both heavy metals was found in roots, their distribution in shoots in the same experimental treatment was metal specific: poplars preferentially accumulated Ni in leaves while Cd in stems. The obtained results also elucidated the potential of tested poplars as bioindicators of environmental pollution, even in the absence of other toxicity symptoms, such as growth reduction or chlorosis. There are implications in literature data which suggest that poplars might engage not only one, but several mechanisms related to accumulation patterns and tolerance to increased levels of heavy metals in the growth substrate. Sebastiani et al. [145] studied the effects of organic waste enriched with nonhazardous levels of Zn, Cu, Cr, and Cd on biomass partitioning and heavy metal accumulation in plant organs in two poplar clones.

Authors observed the presence of both phytoextraction and phytostabilization physiological strategies in studied plants, considering active ion transport and accumulation of Zn in leaves of both clones, Cu retention in the roots, and nonspecific intermediate transport of Cr. Similar conclusions have been drawn for a poplar clone cultivated in a sand-vermiculite substrate under glasshouse conditions treated with different Zn concentrations [146]. The higher bioaccumulation coefficient of the control group than of Zn-treated plants, the continuous Zn uptake during the growing season, and accumulation of Zn in old leaves, suggest that investigated clones employ both excluding and compartmentation mechanisms, confirming the potential of poplars to be used for plantations in Zn-contaminated soils.

Results related to heavy metal resistance and accumulation in various plant species obtained by using in vitro experimental systems always need to be confirmed by field performance trials [102, 130]. The phytoextraction potential of many plant species has been tested in hydroponic systems. These methods of cultivation are useful means for the selection of appropriate plants for the removal of heavy metals from contaminated substrates, due to short periods of plant growth and the treatment duration, as well as reduced variability of environmental factors [14]. Migeon et al. [142] employed the nutrient film technique to screen poplar clones for the tolerance and accumulation of trace elements. After 4 weeks of exposure to multipollution solutions containing 10 μM Cd, Cu, Ni, and Pb, and 200 μM Zn, the highest Cd, Zn, and Ni concentrations in leaves were measured in *Populus trichocarpa* and its hybrids. Among studied clones, the highest concentration of Cu was measured in *Populus deltoides* hybrids.

In recent decades, researchers were faced with the necessity to find alternatives for fossil fuel consumption in heat and electric power systems. With this aim, the short-rotation coppice (SRC) systems of growing plants were studied and developed with the aim to select appropriate bioenergy crops. Short rotation coppice cultures (SRC) are intensively managed, high-density plantations of multi-shoot trees, and its cultivation regime allows higher biomass yields per unit of land area [147]. The establishment of SRC on soils contaminated with heavy metals might fulfill several objectives. First of all, considerable amounts of heavy metals might be removed through repeated coppicing of the aerial biomass of plants [6]. Also, this renewable energy source is both economically and ecologically very attractive [148]. In this sense, extensive studies have been related to poplar cultivation in SRC systems [149].

These investigations were based on the ability of poplars to accumulate relatively high concentrations of certain metals, along with high biomass production exploitable for energy production. Laureysens et al. [24] studied the variation in heavy metal accumulation and biomass production among 13 poplar clones cultivated under SRC systems, which were established on a site which was moderately polluted by heavy metals. Clones with very high concentrations of all metals measured were not found, but significant clonal differences in accumulation were evident for most metals. Among the heavy metals measured, Cd, Zn, and Al were the most efficiently taken up by plants. Results presented by Laureysens and coworkers [147] related to an SRC field trial with 17 different poplar clones established on a former waste disposal site suggested that the selection and improvement of poplar clones for phytoextraction should be focused on biomass production, shoot survival, and metal concentration in the biomass.

2.4 Other Commonly Used Fast-Growing Trees in Phytoremediation/Phytoextraction Technologies

In the last 20 years, there have been numerous studies on heavy metal tolerance and uptake by plants, mostly examining willows and poplars from the Salicaceae family. Though they have great potential in the phytoextraction of pollutants and high biomass production, species like eucalypts, black locust, birch, and paulownia are better adapted to nutrient-poor, acidic soils, and harsh environments. In temperate regions, poplars, willows, and black locusts (*Robinia pseudoacacia* L.) predominate as short rotation woody crops [150]. They are characterized as excellent coppicing species with very intensive and fast growth [151]. They are able to survive droughts and severe winters, tolerate infertile and acidic soils in contrast to other tree species, and are widely used for erosion control and reforestation [150]. Concerning abovementioned characteristics and adaptations, they represent suitable candidates for phytoextraction purposes, which were confirmed in numerous researches [152–154]. Besides the aforementioned, it is noteworthy that the black locust is able to form a symbiotic relationship with nitrogen-fixing bacteria, which represents a well-adapted trait for survival in soils rich with heavy metals.

Potentive biomass species include over 700 Eucalyptus species (*Eucaliptus* spp.), commonly known as eucalypts, which are native plants to the Australian region. Eucalypts are the most promising energy crops in semitropical and tropical areas [150]. They have been successfully planted as exotics due to their fast growth and tolerance of harsh, disturbed environments involving many effective adaptations: indefinite growth, coppicing, lignotubers, drought, fire, insect resistance, and tolerance of soil acidity and low fertility [155]. The potential of eucalypts in the phytoextraction of different trace elements has been reported in several researches conducted in hydroponic trials [156–159], pot experiments [160–162], and field trials [163, 164] for the reclamation of sewage effluents, municipal wastewater, and heavy metal-contaminated substrates.

Another promising species, paulownia (*Paulownia* spp.), which was introduced into North America and Europe, has been recently used for phytoremediation due to its ability to tolerate high concentrations of metals, strong transpiration rates, rapid growth, and high biomass production [165–167]. The effects of heavy metals on plant biomass production may vary, from stimulating to deleterious effects, mostly dependent on the applied concentration and the duration of metal exposure. The effect of cadmium on plant biomass, metal accumulation, and distribution within plants tissue was the main object of research in several studies. Pietrini et al. [159] examined morpho-physiological and biochemical responses of two eucalypt genotypes (hybrid clones of *Eucalyptus camaldulensis* × *Eucalyptus globulus* spp.), by exposing 1-year-old rooted cuttings to Cd in solution during 1 month. The presence of Cd in nutrient solution reduced the root growth of selected clones up to 30 and 50 %. The reduction of biomass was less pronounced in the aboveground parts, and this was followed with a similar decrease in root/shoot biomass between eucalypt clones. Noteworthy is the fact that the accumulation of Cd in roots of tested clones has been very high (up to 14778 mg kg^{-1}), while the accumulation in stems reached the values of approximately 600 mg kg^{-1} of dry weight. Accumulation in the leaves of selected clones was 20-fold smaller compared to the values in the stem. These findings unlock notable perspectives for the future utilization of these species in phytoremediation purposes. Fine et al. [158] reported that the biomass of *Eucalyptus camaldulensis* saplings was not adversely affected during a 1-month exposure to Cd and organic ligands (EDTA and EDDS) in hydroponic solution. Older and bigger plants were used for the second experiment where plants were treated with significantly higher concentrations of Cd (89 mM Cd).

The obtained results indicated significantly reduced growth of saplings after Cd treatments (with the absence of ligands), up to 40 % in comparison to the control group. Treatments with ligands added showed concentration-dependant influence on sapling growth, which had stimulating or deleterious effects. Also, EDTA has been proven as a more effective ligand than EDDS, possibly due to the higher stability constant of its complex with Cd. In the author's opinion, *E. camaldulensis* may successfully be used for the phytoextraction of cadmium from soils that are contaminated at environmental concentrations, with chelating agents' assistance. Gomes et al. [168] had reported deleterious effects on *E. camaldulensis* plants grown in the presence of 90 μM of Cd in solution, with obvious symptoms of Cd toxicity, like wilted growth and leaf chlorosis, as well as blackened and thickened roots. Increased root/shoot translocation rate led to high Cd concentrations in shoots. Such adverse effects of heavy metals may be reduced by associating plants with arbuscular mycorrhizal fungi. The roots of *E. globulus* were inoculated with arbuscular mycorrhizal and saprobe fungi in order to prevent harmful effects of excessive concentration of Cd in nutrient solution [169]. According to the authors, inoculation with fungi led to the redistribution of absorbed Cd mostly in the stem of plants where the harmful effects during the development of the plant were minor, explaining why arbuscular mycorrhizal fungi conferred resistance of eucalyptus to the toxic impact of Cd in spite of high accumulation of this metal in the plant. Contrary to the various responses in the presence of Cd, certain eucalypt species may have remarkable tolerance to aluminum (Al).

Silva et al. [156] have subjected six eucalypt species and clones to different concentrations of Al^{3+} in solution. Root growth and elongation was either stimulated or unaffected by low to intermediate Al concentrations and Al mostly accumulated in the roots, and differed among investigated species. The restriction of Al translocation from roots to shoots may provide a mechanism of protecting the shoots from the harmful effects of the metal. Such results indicated the potential of selected eucalypts in the phytostabilization of aluminum-contaminated soils. It is suggested that aluminum phytoextraction potential of these species should be investigated further, with prolonged exposure to the contaminant. Furthermore, such trials would be very useful if we keep in mind that the exploitation of short-rotation eucalypts may lead to a notable reduction in exchangeable Ca and Mg and enhance exchangeable Al in the soil over time [170]. Assareh et al. [157] have evaluated the bioconcentration (enrichment) coefficient, metal uptake, and translocation among three eucalypt species (*E. camaldulensis*, *E. microtheca,* and *E. occidentalis*).

Enrichment coefficients varied between species and depended on metals applied: *E. occidentalis* had a greater ability to accumulate Zn, *E. camaldulensis* had a higher ability for Cu uptake, in the stem. Another promising species with a high affinity to accumulate Zn in aboveground biomass are paulownia species. Their very high annual increment in biomass, up to 150 t ha^{-1} [171], with extensive deep-digging root systems and high transpiration rates makes these plants an effective natural pump capable of absorbing large quantities of water and pollutants from the soil [172]. The significant removal of trace elements from substrates and a rather low rate of metal absorption [165] are useful parameters in specifying the future strategy for the utilization of these species in phytoremediation/phytoextraction. At rather high Zn concentrations (above 2000 μM in the nutrient solution), Azzarello et al. [172] have determined typical plant stress symptoms in exposed *Paulownia tomentosa* plants, such as growth inhibition and the loss of leaf area. However, when plants were exposed to lower Zn concentrations in the nutrient solution, the accumulation of zinc in aboveground biomass had exceeded 1500 μg g^{-1} with insignificant effects on plant growth parameters, confirming the fact that paulownia species may be suitable candidates for phytoextraction processes.

Authors of this study have proposed a tolerance mechanism to high Zn levels in *P. tomentosa* plants, throughout the use of advanced mechanisms that are able to sequester the heavy metals in specific cell structures, such as the petiole cell walls and the vacuoles in the root hairs, or they may be capable of extruding a percentage of the Zn in exudates located on the surface of the petiole. To our knowledge, there is a lack of data when it comes to hydroponic screening of black locusts (*Robinia pseudoacacia* L.) and their phytoextraction ability. Župunski et al. [154] have determined the importance of genotype (half-sib) selection in accumulation and tolerance of Cd, Ni, and Pb. Specific half-sib families of black locusts have showed promising perspectives for phytoextraction of Cd and Ni.

The effects of different heavy metals on growth, uptake, and tolerance of fast-growing species like eucalypts, birch, and paulownia have been reported in several soil phytoextraction studies with the assistance of synthetic chelators [165, 167], and without chelator assistance [132, 160–162, 173, 174]. Doumett et al. [165] have grown paulownia plantlets in soil rich with Cd, Cu, Pb, and Zn in order to determine whether complexing agents (EDTA, tartarate, and glutamate) have influenced metal uptake by plants and mobilization in soil. The visual assessment did not show any signs of phytotoxicity, and neither did it affect the whole plant dry weight. Tartarate and glutamate have shown good potential in complexing heavy metals, very similar to those of EDTA, followed by the absence of a significant increment of metal leaching probability.

A comparison between plant metal accumulation and the bioavailable metal fraction in soil has shown that uptake and translocation were not mainly dependent on the bioavailable fraction and that the predominant mechanism for metal accumulation was not the concentration gradient between the soil and plant tissues. The phytoremediation potential of paulownia species is mostly assigned to its high biomass production rather than its uptake and accumulation potential. The same authors [167] investigated the influence of repeated applications of tartarate and glutamate (50 mmol kg^{-1} of soil) on Cd, Cu, Pb, and Zn distribution between a contaminated soil sample and *Paulownia tomentosa*. Cu, Pb, and Zn uptake was stimulated by repeated glutamate applications. Cd and Pb were mostly isolated in the roots of paulownia plants and were excluded from aboveground biomass. Such an exclusion mechanism can explain the observed tolerance of *Paulownia tomentosa* to Cd and Pb, but with evident limits in phytoextraction potential for these elements. Furthermore, no significant effect was found in soil on heavy metal bioavailability and concentration, thus decreasing the potential risk of leaching into groundwater [165].

The potential of 13 eucalypt clones for both heavy metal uptake and biomass production from natural and polluted soils was assessed by Mughini et al. [161]. All tested clones have survived and grown well under pollution, which indicated tolerance to the contaminant levels set during the early stress-sensitive growth period. The authors have reported that As, Cu, Pb, and Zn accumulated more in the leaves than in the stems and branches, suggesting that the removal of the entire aboveground biomass, including the leaves, would enhance the phytoextraction potential for these contaminants using *Eucalyptus* spp., especially having in mind that eucalypts are evergreens. Similarly, Coupe et al. [160] investigated the potential of *E. camadulensis* for the phytoextraction of Pb and Zn due to the high bioconcentration factors for these elements and great ability for root/shoot allocation. The uptake and distribution to the aboveground parts in eucalypts was higher compared to the other two species, one of which was *Brassica juncea*, a hyperaccumulator plant with a great ability for Pb and Zn uptake [175, 176].

Further, Mughini et al. [161] pointed out important positive pair-wise correlations between heavy metal average contents (Cd and Pb, Cd and Cu, Cd and Zn, Pb and Cu, Pb and Zn, and Cu and Zn) detected in leaves, stems and branches suggesting that clone selections based on the uptake of these contaminants may notably improve the potential for the remediation of abovementioned pairs. Characteristics such as high yield, tolerance to the presence of metals in soil, reallocating and partitioning of metals in aboveground tissues classify eucalypts and paulownia species side by side with poplars and willows, which were confirmed in numerous researches as great phytoextractors for various trace elements. Wang et al. [162] conducted a pot experiment with six different species, including *Betula alnoides, Alnus nepalensis,* and *Eucalyptus globulus*, for the phytoremediation of mining-spoiled substrate. The obtained differences in growth and lead/zinc uptake differed within plant species, their parts and the kind of metals. For all three species, it was confirmed that they were fast growing, highlighting *A. nepalensis* as a species with the most intensive growth and biomass yield, together with the highest obtained accumulations of Pb and Zn in aboveground tissues. As a conclusion, the authors stated that *A. nepalensis* and *B. alnoides* could serve as appropriate species for the reforestation of mine tailing areas with high levels of Pb and Zn.

The possibility for heavy metal uptake from contaminated soils was assessed in three researches with *B. pendula* plants. Bojarczuk et al. [177] have tested the effect of aluminum-polluted soil and fertilization on the growth and physiology of silver birch (*Betula pendula* Roth.) seedlings. Treatments with fertilizers have a beneficial effect on plant growth, not only in unpolluted soil, but also in soils with high Al content. The authors have suggested that lower Ca/Al ratio in polluted soils may contribute to reduced membrane permeability and to the leakage of some cations from the cytosol. The obtained accumulations for Al and other trace elements lead to possible implications in the phytostabilization of heavy metals, due to the reduced allocation of elements to aboveground parts. In other studies regarding the same species, Bojarczuk et al. [174] have indicated that young seedlings of *B. pendula* are suitable plant material for the recultivation of soils highly contaminated with Cu and Pb, especially in pretreatments with ectomycorrhiza and fertilizers. Cu and Pb were mostly isolated in the roots of the silver birch, with lower translocation to them stem and leaves. Furthermore, the efficiency of the ectomycorrhiza/plant community in the amelioration of the Cu and Pb toxic effects on birch seedlings may be enhanced by providing propagules of heavy metals tolerant ectomycorrhiza fungi, able to restrict allocation of the metals from the roots to the aboveground parts. Similar to these observations, Evangelou et al. [132] found that birch is most suitable for biomass production combined with phytostabilization of soils with high Cd and Zn, but low Pb concentrations.

The only limitation is the fact that birch cannot be coppiced, so it is not useful for short rotation, in contrast to the eucalypts and black locusts which are suitable for methods of biomass production. It is very often that the results of field experiments are confirmed by pot experiments under controlled conditions. Obtained accumulations of trace elements might be the same in both pot and field experiments during short periods of growth, while metal concentrations in the vegetative tissues of plants grown in pots are always higher than those from the field after a longer growth period. This can be explained by the limited volume of soil available to the roots and thus their better efficiency. *Betula* and *Salix* species grown in (pot) field conditions may be useful for phytoextraction as they are able to mobilize reasonably high concentrations of metals to their aboveground parts. Theriault et al. [178] suggested that *Betula papyrifera* (white birch) might play a key role in the phytoextraction of Zn and Ni in a mining-reclaimed region.

This was a dominant species in Northern Ontario after land reclamation, with high bioaccumulation and translocation factor for Zn and Ni, particularly due to the lower bioavailability of these metals in the soil. *Betula pendula* plants were also used for the monitoring of uranium mining dumps [179] on the basis of a significant correlation between heavy metal content of foliage and soil. Promising results were found for the accumulation of Mn in the foliage (284–1724 mg kg^{-1}) with a high accumulation ratio, which indicated the following heavy metal absorption capability of *B. pendula* in order: Cd > Mn > Zn > Pb > Cu > Ni > Fe. The evaluation of heavy metal uptake and application of *Paulownia fortunei* for revegetation on heavy metal-polluted sites was carried out in two Chinese provinces with intense mining activities, which generate a significant quantity of dust, slag, and tailings every year, and contaminate the surrounding areas over several square kilometers [153, 166]. The effect of the paulownia plant rhizosphere on soil properties was studied by Wang et al. [166], who found that immobility and bioavailability of heavy metals were enhanced, with an evident change in the microenvironment of the rhizosphere. Paulownia plants exhibited the threshold limit for lead uptake, by accumulating up to 2700 mg kg^{-1} in leaves during revegetation.

These results are very similar to those obtained from the research of Zhao et al. [153], who have also reported that Pb concentrations in leaves of *P. fortunei* exceeded the hyperaccumulation threshold limit (>1000 mg kg^{-1}). Along with a high uptake of Pb, a significant uptake of Zn has been found in leaves (over 1300 mg kg^{-1}), pointing to prospective features of this species for phytoextraction purposes. Higher metal concentration in the leaves than in the branches and trunks imply that metal pollution might be removed by combining pulping management and harvesting. Short rotation coppicing has been proven as a useful and desirable operation for the dendroremediation of contaminated soils [180].

Such a method of harvesting plant biomass is cheap and efficient in the removal of pollutants, but highly dependent on a time scale for the removal of significant amounts of metals. Since the time needed for remediation of contaminated localities may be very long, even up to 150 years for the remediation to environmentally acceptable levels of pollutants, different methods and approaches must be reconsidered in order to decrease the duration of soil rehabilitation. According to the results obtained by Luo et al. [164], who subjected *Eucaliptus globulus* plants to different coppice systems in order to verify its viability as an alternative to the Cd phytoremediation practice in field scale, the replanting treatment should be recommended as a suitable method which may shorten the phytoremediation time and its efficiency. It is even more expensive to establish and implement replanting systems into practice, but in the long term it would be much faster to remediate soil for agriculture production.

2.5 Treatment After Phytoextraction

Trees grown on degraded and underused lands can bring both aesthetic and economic improvements. Phytoextraction could improve soil quality of mostly moderately contaminated lands within realistic time scales. However, a biomass with a higher content of heavy metals, grown during phytoextraction, is potentially hazardous, and regarded as a "waste mass." The disposal of such material must be carried out with special care in order to minimize heavy metal reentry into the environment. At the same time, crop biomass can bring some economic return [30]. The first step after successful phytoextraction is to reduce crop volume and weight of harvested biomass for easier and safer disposal, and if possible, to utilize obtained energy during this process. One of the most important benefits of dendroremediation is that tree biomass, after phytoextraction, can be used for different purposes. Although a number of crop disposal methods have been proposed, such as composting, compaction, incineration, ashing, pyrolysis, direct disposal, and liquid extraction, it seems that the energetic utilization of biomass by incineration or gasification is the most feasible [181].

Šyc et al. [182] investigated composting pretreatment of wood biomass, followed by incineration and fractional ash disposal. The success of this process depends on which heavy metal is present in the ash, but nevertheless the process can significantly reduce biomass weight and heavy metal leach ability compared to usual landfill biomass disposal. After combustion, most heavy metals remain in the bottom ash, but on the negative side, some heavy metals such as Cd can partly be volatilized by stack emissions, thus reentering into the atmosphere [183, 184]. It seems that high volatility of heavy metals in the flue gas presents a serious restrain of the biomass combustion process after phytoextraction. For example, Delpanque et al. [185] concluded that the combustion of *Salix* wood after the phytoextraction of Cd and Zn should occur only if combustion boilers are equipped with suitable filters, in order to reduce air pollution to an acceptable level. On the other hand, higher volatilization and gasification of heavy metals during thermal biomass treatments provide a possibility that bottom ash could be recycled and used as a fertilizer. All these technologies must be assessed based on each individual site characteristics. Thus, sustainability and economic valorization of phytoextraction are greatly correlated with the further development of appropriate methods for the efficient treatment of biomass yield.

2.6 Feasibility and Duration

The general phytoextraction efficiency on each specific site depends on biomass production and the bioconcentration factor (ratio of metal concentration in the aboveground plant parts to metal concentration in the soil). It is widely accepted that, for a reasonable duration of successful phytoextraction, the bioconcentration factor should be higher than 1, or in many cases much higher. It is very difficult to give precise predictions of phytoextraction durations on each specific site. For example, Dickinson and Pulford [30] state that the number of years needed to reduce soil Cd concentrations by 5 mg kg^{-1} using *Salix viminalis* could range from 3 to 33, 7 to 67, and 14 to 133 years in different soil depths (10, 20, and 40 cm, respectively), depending on the rate of metal uptake into aboveground tissues. Delpanque et al. [185] determined that *Salix* could reduce Cd in a contaminated dredged sediment landfill site from 2.39 to 2 mg kg^{-1} in 19 years. Many different variables have significant impact on the duration of phytoextraction, assessed on the basis of different hydroponic or soil tests and experiments. Pollution is often characterized by a heterogeneous spatial dispersion of heavy metals in soil [186]. Heavy metal uptake in trees is mostly confined to the roots [28, 130, 187], so the duration of the process could be significantly reduced if root bole could be periodically removed along with aboveground parts. Microbiological activity in contaminated soil is often seriously depressed, so bacterial activity is not as supportive to plant growth as a result of Glick [188].

Macronutrient content in contaminated soil has a significant impact on heavy metal availability and uptake [47], and it can change during the years, thus affecting heavy metal uptake. Specific physicochemical properties of soil highly affect the success of phytoextraction depending on soil pH values, texture, cation exchange capacity, mineral composition, and the composition of organic matter [38–43]. It is possible that the uptake ratio can change during different years in relation to many abiotic and biotic factors. For example, climate change will be one of the main driving forces in future yield performance and stability [189]. Low water availability is one of the main environmental factors affecting plant growth and yield in different regions of the world [190]. We can hypothesize that, in long-scale phytoextraction trials, climate shift will significantly change their duration. All mentioned parameters have a significant impact on both metal uptake and plant biomass productivity, thus affecting the duration of successful site remediation. In general, on heavily contaminated sites, with high concentrations of heavy metals, phytoextraction with fast-growing trees would last unrealistically long. Nevertheless, it is widely accepted by scientific community that fast-growing trees had shown high potential for the phytoextraction of low- to moderately contaminated sites, especially if economic valorization of produced biomass is possible.

References

1. Crawford RL (2006) Bioremediation. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) Prokaryotes, vol 1. Springer, Singapore, pp 850–863
2. Pilon-Smiths E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39
3. Susarla S, Medina VF, McCutcheon SC (2002) Phytoremediation: an ecological solution to organic chemical contamination. Ecol Eng 18:647–658
4. Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3:1–18

5. Pajević S, Borišev M, Nikolić N, Krstić B, Pilipović A, Orlović S (2009) Phytoremediation capacity of poplar (*Populus* spp.) and willow (*Salix* spp.) clones in relation to photosynthesis. Arch Biol Sci 61:239–247
6. González-Oreja JA, Rozas MA, Alkorta I, Garbisu C (2008) Dendroremediation of heavy metal polluted soils. Rev Environ Health 23:1–12
7. Prasad MNV (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol 50:686–700
8. Marmiroli M, Pietrini F, Maestri E, Zacchini M, Marmiroli N, Massacci A (2011) Growth, physiological and molecular traits in *Salicaceae* trees investigated for phytoremediation of heavy metals and organics. Tree Physiol 31(12):1319–1334
9. Baker AJM (1981) Accumulators and excluders—strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654
10. Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1: 81–126
11. Marchiol L, Sacco P, Assolari S, Zerbi G (2004) Reclamation of polluted soil: phytoremediation potential of crop-related Brassica species. Water Air Soil Pollut 158:345–356
12. Dickinson NM, Baker AJM, Doronila A, Laidlaw S, Reeves RD (2009) Phytoremediation of inorganics: realism and synergies. Int J Phytoremediation 11:97–114
13. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77:229–236
14. Zacchini M, Pietrini F, Scarascia Mugnozza G, Iori V, Pietrosanti L, Massacci A (2009) Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. Water Air Soil Pollut [Internet] 197(1–4):23–34. http://link.springer.com/10.1007/s11270-008-9788-7
15. McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282
16. Singh NP, Santal AR (2015) Phytoremediation of heavy metals: the use of green approaches to clean the environment. In: Ansari A, Singh S, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants, vol 2. Springer International Publishing, Cham, pp 115–129
17. Bolan NS, Park JH, Robinson B, Naidu R, Huh KY (2011) Phytostabilization: a green approach to contaminant. Adv Agron 112:145–204
18. Kotrba P (2013) Transgenic approaches to enhance phytoremediation of heavy metal-polluted soils. In: Gupta DK (ed) Plant-based remediation processes. Springer, Berlin, pp 239–272
19. Singh OV, Jain RK (2003) Phytoremediation of toxic aromatic pollutants from soil. Appl Microbiol Biotechnol 63:128–135
20. Maestri M, Marmiroli M, Visioli G, Marmiroli N (2010) Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. Environ Exp Bot 68:1–13
21. Lai HY, Chen ZS (2009) In-situ selection of suitable plants for phytoremediation of multi-metals contaminated sites in central Taiwan. Int J Phytoremediation 11:235–250
22. Komives T, Gullner G (2006) Dendroremediation: the use of trees in cleaning up polluted soils. In: Mackova M et al (eds) Phytoremediation rhizoremediation, theoretical background. Focus on biotechnology. Springer, Dordrecht, pp 23–31
23. Pulford I, Watson S (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int [Internet] 29(4):529–540. http://linkinghub.elsevier.com/retrieve/pii/S0160412002001526
24. Laureysens I, Blust R, Temmerman L, Lemmens C, Ceulemans R (2004) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. Environ Pollut 131:485–494
25. Turnau K, Henriques FS, Anielska T, Renker C, Buscot F (2007) Metal uptake and detoxification mechanisms in Erica andevalensis growing in a pyrite mine tailing. Environ Exp Bot 61:117–123

26. Chen J, Chen Y, Shi ZQ, Su Y, Han FX (2015) Phytoremediation to remove metals/metalloids from soils. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants, vol 2. Springer, Cham, pp 297–304
27. Pahdey VC, Bajpai O, Singh N (2016) Energy crops in sustainable phytoremediation. Renew Sust Energy Rev 54:58–73
28. Greger M, Landberg T (1999) Use of willow in phytoextraction. Int J Phytoremediation 1:115–123
29. Robinson BH, Mills TM, Petit D, Fung L, Green S, Clothier B (2000) Natural and induced cadmium accumulation in poplar and willow: implications for phytoremediation. Plant Soil 227:301–306
30. Dickinson NM, Pulford ID (2005) Cadmium phytoextraction using short-rotation coppice *Salix*: the evidence trail. Environ Int 31(4):609–613
31. Unterbrunner R, Puschenreiter M, Sommer P, Wieshammer G, Tlustos P, Zupan M et al (2007) Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environ Pollut [Internet] 148(1):107–114. http://www.ncbi.nlm.nih.gov/pubmed/17224228
32. Hammer D, Kayser A, Keller C (2003) Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. Soil Use Manag 19(3):187–192
33. Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256:265–272
34. Migeon A, Richaud P, Guinet F, Chalot M, Blaudez D (2009) Metal accumulation by woody species on contaminated sites in the North of France. Water Air Pollut 204(1–4):89–101
35. Guerra F, Gainza F, Perez M, Zamudio F (2011) Phytoremediation of heavy metals using poplars (*Populus* spp.): a glimpse of the plant responses to copper, cadmium and zinc stress. In: Golubev IA (ed) Handbook of phytoremediation. Nova Science, New York, pp 387–413
36. Logan TJ (1990) Chemical degradation of soil. In: Lal R, Stewart BA (eds) Advances in soil science. Soil degradation. Springer, New York, pp 187–223
37. Borišev M, Pajević S, Nikolić N, Krstić B, Župunski M, Kebert M et al (2012) Response of *Salix alba* to heavy metals and diesel fuel contamination. Afr J Biotechnol 11(78): 14313–14319
38. Hough RL, Tye AM, Crout NMJ, McGrath SP, Zhang H, Young SD (2005) Evaluating a 'free ion activity model' applied to metal uptake by Lolium perenne L. grown in contaminated soils. Plant Soil 270:1–12
39. Ross SM (1994) Retention, transformation and mobility of toxic metals in soils. In: Ross SM (ed) Toxic metals in soil-plant systems. Wiley, New York, pp 63–152
40. Ross SM (1994) Toxic metals in soil–plant systems. Wiley, Chichester
41. Kayser A, Wenger K, Keller A, Attinger W, Felix HR, Gupta SK, Schulin R (2000) Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. Environ Sci Technol 34:1778–1783
42. Angelova V, Ivanov K, Ivanova R (2003) Effect of chemical forms of lead, cadmium and zinc in polluted soils on their uptake by tobacco. J Environ Prot Ecol 4(2):316–327
43. Farrag K, Senesi N, Nigro F, Petrozza A, Palma A, Shaarawi S, Brunetti G (2012) Growth responses of crop and weed species to heavy metals in pot and field experiments. Environ Sci Pollut Res 19:3636–3644
44. Farrag K, Senesi N, Rovira PS, Brunetti G (2012) Effects of selected soil properties on phytoremediation applicability for heavy-metal-contaminated soils in the Apulia region, Southern Italy. Environ Monit Assess 184:6593–6606
45. Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C (2002) Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J 32:539–548
46. Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53(366):1–11
47. Borišev M, Pajević S, Nikolić N, Orlović S, Župunski M, Pilipović A, Kebert M (2016) Magnesium and iron deficiencies alter Cd accumulation in *Salix viminalis* L. Int J Phytoremediation 18(2):164–170

48. Cieslinski G, Neilsen GH, Hogue EJ (1996) Effect of soil cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruit of strawberry plants (*Fragaria* x *ananassa* Duch.). Plant Soil 180:267–276
49. Vidal M, Santos MJ, Abrao T, Rodriguez J, Rigol A (2009) Modeling competitive metal sorption in a mineral soil. Geoderma 149:189–198
50. Trakal L, Komárek M, Száková J, Tlustoš P, Tejnecký V, Drábek O (2012) Sorption behavior of Cd, Cu, Pb, and Zn and their interactions in phytoremediated soil. Int J Phytoremediation 14:806–819
51. Xin J, Huang B, Yang Z, Yuan J, Dai H, Qiu Q (2010) Responses of different water spinach cultivars and their hybrid to Cd, Pb and Cd-Pb exposures. J Hazard Mater 175:468–476
52. Hernández-Allica J, Garbisu C, Barrutia O (2007) Becerril JM EDTA-induced heavy metal accumulation and phytotoxicity in cardoon plants. Environ Exp Bot 60:26–32
53. Zhang GP, Fukami M, Sekimoto H (2000) Genotypic differences in effects of cadmium on growth and nutrient compositions in wheat. J Plant Nutr 23:1337–1350
54. Borišev M, Pajević S, Nikolić N, Pilipović A, Krstić B, Orlović S (2009) Phytoextraction of Cd, Ni, and Pb using four willow clones (*Salix* spp.). Pol J Environ Stud 18(4):553–561
55. Meers E, Lesage E, Lamsal S, Hopgood M, Vervaeke P, Tack FMG et al (2005) Enhanced phytoextraction: I. Effect of EDTA and citric acid on heavy metal mobility in a calcareous soil. Int J Phytoremediation 7(2):129–142
56. Meers E, Vandecasteele B, Ruttens A, Vangronsveld J, Tack FMG (2007) Potential of five willow species (*Salix* spp.) for phytoextraction of heavy metals. Environ Exp Bot 60:57–68
57. Derome J, Lindroos AJ (1998) Effect of heavy-metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of the Harjavalta Cu-Ni smelter, SW Finland. Environ Pollut 99:225–232
58. Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Phys 17:21–34
59. Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J. Article ID 756120. http://dx.doi.org/10.1155/2015/756120
60. Yadav SK (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76:167–179
61. Nikolić N, Kojić D, Pilipović A, Pajević S, Krstić B, Borišev M et al (2008) Responses of hybrid poplar to cadmium stress: photosynthetic characteristics, cadmium and proline accumulation, and antioxidant enzyme activity. Acta Biol Cracov Bot 50(2):95–103
62. Dai H-P, Shan C, Wei Y, Liang J-G, Yang T-X, Sa W-Q et al (2012) Subcellular localization of cadmium in hyperaccumulator *Populus* × *canescens*. Afr J Biotechnol 11:3779–3787
63. Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88:1707–1719
64. Castiglione S, Franchin C, Fossati T, Lingua G, Torrigiani P, Biondi S (2007) High zinc concentrations reduce rooting capacity and alter metallothionein gene expression in white poplar (*Populus alba* L. cv. Villafranca). Chemosphere [Internet] 67(6):1117–1126. http://www.ncbi.nlm.nih.gov/pubmed/17223164
65. Macovei A, Ventura L, Donà M, Faè M (2010) Effect of heavy metal treatments on metallothionein expression profiles in white poplar (*Populus alba* L.) cell suspension cultures. Ann Univ Oradea Fascicula Biol Tom. XVII(2):274–279.
66. Salt DE, Rauser WE (1995) MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol 107:1293–1301
67. Bittsánszky A, Kömives T, Gullner G, Gyula G, Kiss J, Heszky L et al (2005) Ability of transgenic poplars with elevated glutathione content to tolerate zinc(2+) stress. Environ Int 31:251–254
68. Sanitá di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130
69. Ivanova L, Ronzhina D, Ivanov L, Stroukova L, Peuke A, Rennenberg H (2009) Chloroplast parameters differ in wild type and transgenic poplars overexpressing gsh1 in the cytosol. Plant Biol 11:625–630

70. Salamanca EJP, Medera-Parra CA, Avila-Williams CA, Rengifo-Gallego AL, Rios DA (2015) Phytoremediation using terrestrial plants. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants, vol 2. Springer, Cham, pp 305–319
71. Pilipović A, Orlović S, Nikolić N, Borišev M, Krstić B, Rončević S (2012) Growth and plant physiological parameters as markers for selection of poplar clones for crude oil phytoremediation. Šumarski list 136(5–6):273–281
72. Nikolić N, Borišev M, Pajević S, Arsenov D, Župunski M, Orlović S, Pilipović A (2015) Photosynthetic response and tolerance of three willow species to cadmium exposure in hydroponic culture. Arch Biol Sci 67(4):1411–1420. doi:10.2298/ABS150421120N
73. Küpper H, Parameswaran A, Leitenmajer B, Trtílek M, Šetlík I (2007) Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. New Phytol 175(4):655–674
74. Katanić M, Pilipović A, Orlović S, Krstić B, Kovačević B, Pekeč S (2008) The influence of lead on the in vitro growth and concentration of photosynthetic pigments in shoots of the white poplar (*Populus alba*) clones. Lesnícky čas For J 54(Suppl 1):29–36
75. Klang-Westin E, Perttu K (2002) Effect of nutrient supply and soil cadmium concentration on cadmium removal by willow. Biomass Bioenergy 23:415–426
76. Donovan LA, Dudley SA, Rosenthal DM, Ludwig F (2007) Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers. Oecologia 152(1):13–25
77. Becerril JM, Gonzales-Marua C, Munoz-Rueda A, de Felipe MR (1989) Changes induced by cadmium and lead in gas exchange and water relations of clover and lucerne. Plant Physiol Biochem 27:913–918
78. Dickmann DI, Kuzovkina J (2014) Poplars and willows of the world, with emphasis on silviculturally important species. In: Isebrands JG, Richardson J (eds) Poplars and willows: trees for society and the environment. FAO and CABI, Croydon, pp 8–92
79. Kuzovkina YA, Vietto L (2014) An update on the cultivar registration of *Populus* and *Salix* (Salicaceae). Skvortsovia 1(2):133–148
80. Argus GW (1997) Infrageneric classification of New World *Salix* L. (Salicaceae). Syst Bot Monogr 52:1–121
81. Argus GW (2010) Salix. In: Flora of North America Editorial Committee (ed) Flora of North America, vol 7, Magnoliophyta: Salicaceae to Brassicaceae. Oxford University Press, New York, pp 23–51
82. Lauron-Moreau A, Pitre FE, Argus GW, Labrecque M, Brouillet L (2015) Phylogenetic relationships of American willows (Salix L., Salicaceae). Plos One 10(4):e0121965
83. Eckenwalder JE (2010) Populus. In: Flora of North America Editorial Committee (ed) Flora of North America North of Mexico. Oxford University Press, New York, pp 23–162
84. Rae AM, Street NR, Rodríguez-Acosta M (2007) *Populus* trees. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 7. Forest Trees. Springer, Berlin, pp 1–28
85. Gaudet M, Pietrini F, Beritognolo I, Iori V, Yacchini M, Massaci A et al (2011) Intraspecific variation of physiological and molecular response to cadmium stress in *Populus nigra* L. Tree Phys 31:1309–1318
86. Hamzeh M, Dayanandan S (2004) Phylogeny of *Populus* (Salicaceae) based on nucleotide sequences of chloroplast TRNT-TRNF region and nuclear rDNA. Am J Bot 91:1398–1408
87. Vyslouzilova M, Tlustos P, Szakova J, Vysloužilová M, Tlustoš P, Száková J (2003) Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant Soil Environ 49(12):542–547
88. Tlustoš P, Száková J, Vysloužilová M, Pavlíková D, Weger J, Javorská H (2007) Variation in the uptake of arsenic, cadmium, lead, and zinc by different species of willows *Salix* spp. grown in contaminated soils. Cent Eur J Biol 2(2):254–275
89. Cloutier-Hurteau B, Turmel MC, Mercier C, Courchesne F (2014) The sequestration of trace elements by willow (*Salix purpurea*)—which soil properties favor uptake and accumulation? Environ Sci Pollut Res 21:4759–4771

90. Sommerville AHC (1992) Willows in the environment. Proc Roy Soc Edinb B Biol Sci 98:215–244
91. Bedell JP, Capilla X, Girya C, Schwartzb C, Morelb JL, Perrodina Y (2009) Distribution, movement and availability of Cd and Zn in a dredged sediment cultivated with *Salix alba*. Environ Exp Bot 67:403–414
92. Mleczek M, Rutkowski P, Rissmann I, Kaczmarek Z, Golinski P, Szentner K et al (2010) Biomass productivity and phytoremediation potential of *Salix alba* and *Salix viminalis*. Biomass Bioenergy 34(9):1410–1418
93. Dickinson NM, Riddell-Black (2014) Phytoremediation and carbon sequestration of degraded lands. In: Isebrands JG, Richardson J (eds) Poplars and willows: trees for society and the environment. CABI, Boston
94. Lunáčková L, Masarovičová E, Král'Ová K, Streško V (2003) Response of fast growing woody plants from family Salicaceae to cadmium treatment. Bull Environ ContamToxicol 70(3):576–585
95. Riddell-Black D (1994) Heavy metal uptake by fast growing willow species. In: Aronsson O, Perttu K (eds) Willow vegetation filters for municipal wastewaters and sludges. A biological purification system. Swedish University Agricultural Sciences, Uppsala, pp 145–151
96. Landberg T, Greger M (1994) Can heavy metal tolerant clones of Salix be used as vegetation filters on heavy metal contaminated land? In: Aronsson P, Perttu K (eds) Willow vegetation filters for municipal wastewaters and sludges. A biological purification system. Swedish University Agricultural Sciences, Uppsala, pp 133–144
97. Landberg T, Greger M (1996) Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. Appl Geochem [Internet] 11(1–2):175–180. http://www.sciencedirect.com/science/article/pii/0883292795000828.
98. Lyyra S, Lima A, Merkle SA (2006) In vitro regeneration of *Salix nigra* from adventitious shoots. Tree Physiol 26(7):969–975
99. Lux A, Masarovicovà E, Liskova D, Sottnikova-Stefanovicova A, Lunackova L, Marcekova M (2002) Physiological and structural characteristics and in vitro cultivation of some willows and poplars. In: Proceedings of the Cost Action 837, Bordeaux, 25-27 April 2002
100. Capuana M (2011) Heavy metals and woody plants—biotechnologies for phytoremediation. iForest Biogeosci For [Internet] 4(1):7–15. http://www.sisef.it/iforest/?doi=10.3832/ifor0555-004
101. Confalonieri M, Balestrazzi A, Bisoffi S, Carbonera D (2003) In vitro culture and genetic engineering of *Populus* spp.: synergy for forest tree improvement. Plant Cell Tissue Organ Cult 72:109–138
102. Di Lonardo S, Capuana M, Arnetoli M, Gabbrielli R, Gonnelli C (2011) Exploring the metal phytoremediation potential of three *Populus alba* L. clones using an in vitro screening. Environ Sci Pollut Res 18:82–90
103. Watson C, Pulford ID, Riddell-Black D (1999) Heavy metal toxicity responses of two willow (*Salix*) varieties grown hydroponically: development of a tolerance screening test. Environ Geochem Health 21:359–364
104. YangW, Zhao F, Zhang X, Ding Z, Wang Y, Zhu Z et al Variations of cadmium tolerance and accumulation among 39 *Salix* clones: implications for phytoextraction. Environ Earth Sci [Internet] 2015;73(7):3263–3274. http://link.springer.com/10.1007/s12665-014-3636-4
105. Lux A, Šottníková A, Opatrná J, Greger M (2004) Differences in structure of adventitious roots in *Salix* clones with contrasting characteristics of cadmium accumulation and sensitivity. Physiol Plantarum 120:537–545
106. Vassilev A, Perez-Sanz A, Semanem B, Carleer R, Vangronsveld J (2005) Cadmium accumulation and tolerance of two *Salix* genotypes hydroponically grown in presence of cadmium. J Plant Nutr 28:2159–2177
107. Luković J, Merkulov L, Pajević S, Zorić L, Nikolić N, Borišev M et al (2012) Quantitative assessment of effects of cadmium on the histological structure of poplar and willow leaves. Water Air Soil Pollut [Internet] 23(6):2979–2993. http://link.springer.com/10.1007/s11270-012-1081-0

108. Cocozza C, Maiuro L, Tognetti R (2011) Mapping cadmium distribution in roots of *Salicaceae* through scanning electron microscopy with x-ray microanalysis. iForest 4:113–120
109. Vaculík M, Konlechner C, Langer I, Adlassnig W, Puschenreiter M, Lux A, Hauser MT (2012) Root anatomy and element distribution vary between two Salix caprea isolates with different Cd accumulation capacities. Environ Pollut 163:117–126
110. Sottnikova A, Lunackova L, Masarovicova E, Lux A, Stresko V (2003) Changes in the rooting and growth of willow and poplars induced by cadmium. Biol Plant 46:129–131
111. Cosio C, Vollenweider P, Keller C (2006) Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.). Environ Exp Bot [Internet] 58(1–3):64–74. http://linkinghub.elsevier.com/retrieve/pii/S009884720500105X
112. Yang W, Wang Y, Zhao F, Ding Z, Zhang X, Zhu Z et al (2014) Variation in copper and zinc tolerance and accumulation in 12 willow clones: implications for phytoextraction. J Zhejiang Univ Sci B [Internet] 15(9):788–800. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4162880&tool=pmcentrez&rendertype=abstract
113. Dos SantosUtmazian MN, Wieshammer G, Vega R, Wenzel WW (2007) Hydroponic screening for metal resistance and accumulation of cadmium and zinc in twenty clones of willows and poplars. Environ Pollut [Internet] 148(1):155–165. http://linkinghub.elsevier.com/retrieve/pii/S0269749106005872
114. Zhivotovsky OP, Kuzovkina JA, Schulthess CP, Morris T, Pettinelli D, Ge M (2010) Hydroponic screening of willows (*Salix* L.) for lead tolerance and accumulation. Int J Phytoremediation 13:75–94
115. Klang-Westin E, Eriksson J (2003) Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. Plant Soil 249(1):127–137
116. Wieshammer G, Unterbrunner R, Garcia TB, Zivkovic MF, Puschenreiter M, Wenzel WW (2007) Phytoextraction of Cd and Zn from agricultural soils by *Salix* ssp. and intercropping of *Salix caprea* and *Arabidopsis halleri*. Plant Soil [Internet] 298(1–2):255–264. http://link.springer.com/10.1007/s11104-007-9363-9
117. Dos SantosUtmazian MND, Wenzel WW (2007) Cadmium and zinc accumulation in willow and poplar species grown on polluted soils. J Plant Nutr Soil Sci Fur Pflanzenernahrung Und Bodenkd [Internet] 170(2):265–272. <Go to ISI>://000246417400011
118. Pietrini F, Zacchini M, Iori V, Pietrosanti L, Ferretti M, Massacci A (2010) Spatial distribution of cadmium in leaves and its impact on photosynthesis: examples of different strategies in willow and poplar clones. Plant Biol (Stuttg) [Internet] 12(2):355–363. http://www.ncbi.nlm.nih.gov/pubmed/20398241
119. Vollenweider P, Cosio C, Günthardt-Goerg MS, Keller C (2006) Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.). Environ Exp Bot [Internet] 58(1–3):25–40. http://linkinghub.elsevier.com/retrieve/pii/S0098847205001048
120. Kabata-Pendias A, Pendias H (2001) Trace elements in soils, 3rd edn. CRC Press, Boca Raton, p 413
121. Almeida A-AF, Valle RR, Mielke MS, Gomes FP (2007) Tolerance and prospection of phytoremediator woody species of Cd, Pb, Cu and Cr. Braz J Plant Physiol 19:83–98
122. Vandecasteele B, Meers E, Vervaeke P, De Vos B, Quataert P, Tack FMG (2005) Growth and trace metal accumulation of two *Salix* clones on sediment-derived soils with increasing contamination levels. Chemosphere 58(8):995–1002
123. Liu Y, Chen CG, Zhang J, Shi X, Wang R (2011) Uptake of cadmium from hydroponic solutions by willows (*Salix* spp.) seedlings. Afr J Biotechnol 10(72):16209–16218
124. Nissim WG, Hasbroucq S, Kadri H, Pitre FE, Labrecque M (2015) Potential of selected Canadian plant species for phytoextraction of trace elements from selenium-rich soil contaminated by industrial activity. Int J Phytoremediation [Internet] 17(8):745–52. http://www.tandfonline.com/doi/full/10.1080/15226514.2014.987370
125. Pulford ID, Riddell-Black D, Stewart C (2002) Heavy metal uptake by willow clones from sewage sludge-treated soil: the potential for phytoremediation. Int J Phytoremediation [Internet] 4(1):59. http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=7262728&site=ehost-live

126. Borišev M, Krstić B, Orlović S, Pilipović A, Nikolić N, Pajević S, Župunski M (2013) Antagonism between Cd and Ni in willows phytoextraction test. In: 13th international scientific conference “Sakharov Readings 2013: Environmental Problems of XXIst Century”, May 16-17th, Minsk, The Book of Abstracts
127. Pajević S, Borišev M, Nikolić N, Luković J, Župunski M, Arsenov D, Orlović S (2014) Phytoextraction of elevated heavy metals in soil by using fast growing trees (*Salix* sp. and *Populus* sp.). In: The international bioscience conference IBSC 29-30 September 2014, Phuket, Proceedings, pp 13–18
128. Hrynkiewicz K, Dabrowska G, Baum C, Niedojadlo K, Leinweber P (2012) Interactive and single effects of ectomycorrhiza formation and Bacillus cereus on metallothionein MT1 expression and phytoextraction of Cd and Zn by willows. Water Air Soil Pollut 223(3): 957–968
129. Kuzovkina J (2014) Stress tolerance in North American willow species. In: Isebrands JG, Richardson J (eds) Poplars and willows: trees for society and the environment. CABI, Boston
130. Watson C, Pulford ID, Riddell-Black D (2003) Screening of willow species for resistance to heavy metals: comparison of performance in a hydroponics system and field trials. Int J Phytoremediation 5:351–365
131. Hermle S, Gunthardt-Goerg MS, Schulin R (2006) Effects of metal-contaminated soil on the performance of young trees growing in model ecosystems under field conditions. Environ Pollut 144:703–714
132. Evangelou M, Robinson B, Günthardt-Goerg M, Schulin R (2013) Metal uptake and allocation in trees grown on contaminated land: implications for biomass production. Int J Phytoremediation 15:77–90
133. Jensen JK, Holm PE, Nejrup J, Larsen MB, Borggaard OK (2009) The potential of willow for remediation of heavy metal polluted calcareous urban soils. Environ Pollut [Internet] 157(3):931–937. http://dx.doi.org/10.1016/j.envpol.2008.10.024
134. Li J-T, Baker AJM, Ye Z-H, Wang H-B, Shu W-S (2012) Phytoextraction of Cd-contaminated soils: current status and future challenges. Crit Rev Environ Sci Technol [Internet] 42(20):2113–2152. http://www.scopus.com/inward/record.url?eid=2-s2.0-84867723299&partnerID=tZOtx3y1
135. Kirkham MB (2006) Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. Geoderma 137:19–32
136. Vervaeke P, Luyssaert S, Mertens J, Meers E, Tack FM, Lust N (2003) Phytoremediation prospects of willow stands on contaminated sediment: a field trial. Environ Pollut [Internet] 126(2):275–282. http://linkinghub.elsevier.com/retrieve/pii/S0269749103001891
137. Algreen M, Trapp S, Rein A (2014) Phytoscreening and phytoextraction of heavy metals at Danish polluted sites using willow and poplar trees. Environ Sci Pollut Res [Internet] 21(15):8992–9001. http://link.springer.com/10.1007/s11356-013-2085-z
138. Katanić M, Kovačević B, Đorđević B, Kebert M, Pilipović A, Klašnja B et al (2015) Nickel phytoremediation potential of white poplar clones grown *in vitro*. Rom Biotechnol Lett 20:10085–10096
139. Kovačević B, Miladinović D, Orlović S, Katanić M, Kebert M, Kovinčić J (2013) Lead tolerance and accumulation in white poplar cultivated i*n vitro*. SEEFFOR 4:3–12
140. Bojarczuk K (2004) Effect of toxic metals on the development of poplar (*Populus tremula* L. × *P. alba* L.) cultured *in vitro*. Pol J Environ Stud 13:115–120
141. Nikolić N, Borišev M, Krstić B, Pajević S, Pilipović A, Orlović S. (2011) Tolerance of *Populus deltoides* to soil contamination. In: Proceedings of the “STREPOW” international workshop; 2011 Feb 23-24; Andrevlje, Novi Sad, Serbia, pp 231–236.
142. Migeon A, Richaud P, Guinet F, Blaudez D, Chalot M (2012) Hydroponic screening of poplar for trace element tolerance and accumulation. Int J Phytoremediation 14:350–361
143. Pilipović A, Orlović S, Nikolić N, Galić Z, Klašnja B, Borišev M, Krstić B (2011) The effect of heavy metal contamination on physiological parameters of five poplar (*Populus* sp.) clones. In: Proceedings of the “STREPOW” international workshop; 2011 Feb 23-24; Andrevlje, Novi Sad, Serbia; pp 237–243

144. Nikolić N, Krstić B, Orlović S, Pajević S, Borišev M, Pilipović A (2009) Bioindication and phytoremediation of cadmium and nickel contaminated environment using poplars. Экологический Вестник 3/4(9/10): 6–12
145. Sebastiani L, Scebba F, Tognetti R (2004) Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoides* × *maximowiczii*) and I-214 (*P.* × *euramericana*) exposed to industrial waste. Environ Exp Bot 52:79–88
146. Di Baccio D, Tognetti R, Sebastiani L, Vitagliano C (2003) Responses of *Populus deltoides* × *P. nigra* (*P.* × *euramericana*) clone I-214 to high zinc concentrations. New Phytol 159:443–452
147. Laureysens I, De Temmerman L, Hastir T, Van Gysel M, Ceulemans R (2005) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture. II. Vertical distribution and phytoextraction potential. Environ Pollut 133:541–551
148. Simpson JA, Picchi G, Gordon AM, Thevathasan NV, Stanturf J, Nicholas I (2009) Short rotation crops for bioenergy systems. Task 30. Environmental benefits associated with short-rotation woody crops Technical Review No. 3 2009 IEA Bioenergy. http://www.ieabioenergytask43.org/Task_30_Web_Site/PDFs/Tech%20review%20No.%2003%20 for%20web.pdf. Accessed 9 Oct 2015
149. Dillen SY, Djomo SN, Al Afas N, Vanbeveren S, Ceulemans R (2013) Biomass yield and energy balance of a short rotation poplar coppice with multiple clones on degraded land during 16 years. Biomass Bioenergy 56:157–165
150. Rockwood DL, Naidu CV, Carter DR, Rahmani M, Spriggs TA, Lin C, Alker GR, Isebrands JG, Segrest SA (2004) Short-rotation woody crops and phytoremediation: opportunities for agroforestry? Agrofor Syst 61–62:51–63
151. Rédei K, Osváth-Bujtás Z, Veperdi I (2008) Black locust (Robinia pseudoacacia L.) improvement in Hungary: a review. Acta Silv Lign Hung 4:127–132
152. Mertens J, Van Nevel L, De Schrijver A, Piesschaert F, Oosterbean A, Tack FMG, Verheyen K (2007) Tree species effect on the redistribution of soil metals. Environ Pollut 149:173–181
153. Zhao X, Liu J, Xia X, Chu J, Wei Y, Shi S et al (2013) The evaluation of heavy metal accumulation and application of a comprehensive bio-concentration index for woody species on contaminated sites in Hunan, China. Environ Sci Pollut Res 21:5076–5085
154. Županski M, Borišev M, Orlović S, Arsenov D, Nikolić N, Pilipović A et al (2015) Hydroponic screening of black locust families for heavy metals tolerance and accumulation. Int J Phytoremediation 18(6):583–591
155. Rockwood DL, Rudie AW, Ralph SA, Zhu JY, Winandy JE (2008) Energy product options for eucalyptus species grown as short rotation woody crops. Int J Mol Sci 9:1361–1378
156. Silva I, Novais R, Jham G, Barros N, Gebrim F, Nunes F et al (2004) Responses of eucalypt species to aluminum: the possible involvement of low molecular weight organic acids in the Al tolerance mechanism. Tree Physiol 24:1267–1277
157. Assareh MH, Shariat A, Ghamari-Zare A (2008) Seedling response of three *Eucalyptus* species to copper and zinc toxic concentrations. Caspian J Environ Sci 6:97–103
158. Fine P, Rathod PH, Beriozkin A, Mingelgrin U (2013) Uptake of cadmium by hydroponically grown, mature *Eucalyptus camaldulensis* saplings and the effect of organic ligands. Int J Phytoremediation 15:585–600
159. Pietrini F, Iori V, Bianconi D, Mughini G, Massacci A, Zacchini M (2015) Assessment of physiological and biochemical responses, metal tolerance and accumulation in two eucalypt hybrid clones for phytoremediation of cadmium-contaminated waters. J Environ Manage 162:221–231
160. Coupe SJ, Sallami K, Ganjian E (2013) Phytoremediation of heavy metal contaminated soil using different plant species. Afr J Biotechnol 12(43):6185–6192
161. Mughini G, Alianiello F, Benedetti A, Gras L, Gras M, Salvati L (2013) Clonal variation in growth, arsenic and heavy metal uptakes of hybrid Eucalyptus clones in a Mediterranean environment. Agrofor Syst 87:755–766
162. Wang Y, Bai S, Wu J, Chen J, Yang Y, Zhu X (2015) Plumbum/zinc accumulation in seedlings of six afforestation species cultivated in mine spoil substrate. J Trop For Sci 27(2):166–175

163. Asensio V, Vega F, Singh B, Covelo E (2013) Effects of tree vegetation and waste amendments on the fractionation of Cr, Cu, Ni, Pb and Zn in polluted mine soils. Sci Total Environ 443:446–453
164. Luo J, Qi S, Peng L, Xie X (2015) Phytoremediation potential of cadmium-contaminated soil by *Eucalyptus globulus* under different coppice systems. Bull Environ Contam Toxicol 94:321–325
165. Doumett S, Lamperi L, Checchini L, Azzarello E, Mugnai S, Mancuso S, Petruzzelli G, Bubba M (2008) Heavy metal distribution between contaminated soil and Paulownia tomentosa, in a pilot-scale assisted phytoremediation study: influence of different complexing agents. Chemosphere 72:1481–1490
166. Wang J, Zhang CB, Jin ZX (2009) The distribution and phytoavailability of heavy metal fractions in rhizosphere soils of *Paulownia fortunei* (seem) Hems near a Pb/Zn smelter in Guangdong, PR China. Geoderma 148(3–4):299–306
167. Doumett S, Fibbi D, Azzarello E, Mancuso S, Mugnai S, Petruzzelli G, Del Bubba M (2010) Influence of the application renewal of glutamate and tartrate on Cd, Cu, Pb and Zn distribution between contaminated soil and Paulownia tomentosa in a pilot-scale assisted phytoremediation Study. Int J Phytoremediation 13:1–17
168. Gomes M, Marques TCLLS, Carneiro MML, Soares Â (2012) Anatomical characteristics and nutrient uptake and distribution associated with the Cd-phytoremediation capacity of Eucalyptus camaldulenses Dehnh. J Soil Sci Plant Nutr 12:481–496
169. Arriagada C, Herrera M, Ocampo J (2005) Contribution of arbuscular mycorrhizal and saprobe fungi to the tolerance of Eucalyptus globulus to Pb. Water Air Soil Pollut 166:31–47
170. Leite FP (2001) Nutritional relationships and chemical characteristics alterations in soils of the Rio Doce Valley caused by eucalyptus cultivation. PhD thesis, Federal University of Viçosa, Viçosa, 72 p. In Portuguese with English abstract.
171. Caparros S, Diaz MJ, Ariza J, Lopez F, Jimenez L (2008) New perspectives for *Paulownia fortunei* L. valorisation of the autohydrolysis and pulping processes. Bioresour Technol 99:741–749
172. Azzarello E, Pandolfi C, Giordano C, Rossi M, Mugnai S, Mancuso S (2012) Ultramorphological and physiological modifications induced by high zinc levels in *Paulownia tomentosa*. Environ Exp Bot 81:11–17
173. Kopponen P, Utriainen M, Lukkari K, Suntioinen S (2001) Clonal differences in copper and zinc tolerance of birch in metal-supplemented soils. Environ Pollut 112:89–97
174. Bojarczuk K, Kieliszewska-Rokicka B (2010) Effect of ectomycorrhiza on Cu and Pb accumulation in leaves and roots of silver birch (*Betula pendula* Roth.) seedlings grown in metal-contaminated soil. Water Air Soil Pollut 207:227–240
175. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668
176. Gisbert C, Clemente R, Navarro-Aviñó J, Carlos Baixauli C, Ginér A, Serrano R, Walker DJ, Pilar Bernal MP (2006) Tolerance and accumulation of heavy metals by *Brassicaceae* species grown in contaminated soils from Mediterranean regions of Spain. Environ Exp Bot 56:19–26
177. Bojarczuk K, Karolewski P, Oleksyn J, Kieliszewska-Rokicka B, Żytkowiak R, Tjoelker MG (2002) Effect of polluted soil and fertilisation on growth and physiology of silver birch (*Betula pendula* Roth.) seedlings. Pol J Environ Stud 11(5):483–492
178. Theriault G, Nkongolo K, Michael P (2014) Genetic and metal analyses of fragmented populations of Betula papyrifera (Marsh) in a mining reclaimed region: identification of population–diagnostic molecular marker. Ecol Evol 4(17):3435–3443
179. Wisłocka M, Krawczyk J, Klink A, Morrison L (2006) Bioaccumulation of heavy metals by selected plant species from uranium mining dumps in the Sudety Mts., Poland. Pol J Environ Stud 15(5):811–818
180. Rockwood DL, Cardellino R, Alker G, Lin C, Brown N, Spriggs T, Tsangaris S, Isebrands J, Hall R, Lange R, Nwokike B (2004) Fast-growing trees for heavy metal and chlorinated solvent phytoremediation. In: Magar VS, Kelley ME (eds) In situ and on-site bioremediation-2003. Proceedings of the seventh international in situ and on-site bioremediation symposium, Orlando, FL, June 2–5, 2003, Paper F-12, Battelle Press, Columbus

181. Sas-Nowosielska A, Kucharski R, Małkowski E, Pogrzeba M, Kuperberg JM, Kryński K (2004) Phytoextraction crop disposal—an unsolved problem. Environ Pollut 128:373–390
182. Šyc M, Pohořely M, Kameníková P, Habart J, Svoboda K, Punčochár M (2012) Willow trees from heavy metals phytoextraction as energy crops. Biomass Bioenergy 37:106–113
183. Keller C, Ludwig C, Davoli F, Wochele J (2005) Thermal treatment of metal-enriched biomass produced from heavy metal phytoextraction. Environ Sci Technol 39(9):3359–3367
184. Stals M, Carleer R, Reggers G, Schreurs S, Yperman J (2010) Flash pyrolysis of heavy metal contaminated hardwoods from phytoremediation: characterisation of biomass, pyrolysis oil and char/ash fraction. J Anal Appl Pyrolysis 89(1):22–29
185. Delpanque M, Collet S, Gratta FD, Schnuriger B, Gaucher B, Bert V (2013) Combustion of *Salix* used for phytoextraction: the fate of metals and viability of the process. Biomass Bioenerg 49:160–170
186. CL:AIRE (2004) Technical Bulletin TB7. Improving the reliability of contaminated land assessment using statistical methods: part 1. Basic principles and concepts. Rep. no., London
187. Punshon T, Dickinson NM (1999) Heavy metal resistance and accumulation characteristics in willows. Int J Phytoremediation 1:361–385
188. Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393
189. Kertész A, Madarász B (2014) Conservation agriculture in Europe. Int Soil Water Conserv Res 1(2):91–96
190. Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. Funct Plant Biol 30:239–264

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