PHYTOREMEDIATION AND ADVANTAGES OF WOODY SPECIES FOR PHYTOACCUMULATION OF METALLIC TRACE ELEMENTS.

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Abstract

Phytoremediation is a biotechnology using plant species to decontaminate various substrates. Whatever the process, the cost is generally lower than conventional treatments and the method better for the protection of ecosystems. The different techniques depend on the contaminated medium (atmosphere, water, soil) and the type of pollutant. Pollutants can be organic molecules generated by human activities such as pesticides, hydrocarbons, chlorated or halogenated solvents, and explosives. Pollutants can be also inorganic molecules, i.e. metallic trace elements (MTE), which are released in the environment as a result of human activities (essentially mines and various pesticides) or natural pollutions (volcanism, erosion, leaching).This review presents the different processes of phytoremediation and more particularly phytoaccumulation of MTE and emergence of woody species for this biotechnology.

Key words: *Cadmium, Gymnosperms,phytoextraction, tree species*

Résumé

La phytoremédiation est une biotechnologie qui utilise des plantes pour décontaminer différents substrats. Quel que soit le procédé, le coût est généralement plus faible que celui des traitements conventionnels et son application meilleure pour la protection des écosystèmes. Les différentes techniques dépendent du milieu contaminé (air, eau, sol) et du type de polluant. Les polluants peuvent être des molécules organiques produites par les activités humaines comme les pesticides, les hydrocarbures, les solvants chlorés et halogénés, les explosifs. Les polluants peuvent aussi être des molécules inorganiques, c'est-à-dire des éléments traces métalliques (ETM) qui sont libérés dans l'environnement à cause des activités humaines (essentiellement les exploitations minières, plusieurs pesticides) ou de pollutions naturelles (volcanisme, érosion, lessivage). Cette revue présente les différents procédés de phytoremédiation et plus particulièrement la phytoaccumulation des ETM et l'émergence d'espèces ligneuses pour cette biotechnologie..

Mots clés: *Cadmium, Gymnospermes, phytoextraction, arbres*

Introduction

The methods used today to decontaminate substrates consist in chemical or thermal treatments and are not without consequences on microorganisms and microfauna. Moreover, conventional remediation often requires a transfer of the medium (soil, water) to an adapted site for cleaning. The excavation is thus a very destructive method and does not help to protect ecosystems. Phytoremediation is an emerging alternative for decontamination with several advantages. Indeed, pollutants can often be removed directly on the contaminated site, a fact more favourable to maintain viability of ecosystems. Moreover, the cost is generally lower despite a longer time required to clean substrates by phytoremediation than by conventional technologies. Indeed, the price can be reduced by 50-75% and even by a factor of 10 to 100 according to the type of pollutant, the substrate, and the process of phytoremediation [1-4]. This lower cost can be explained by i) a decrease in transports because phytoremediation can be performed directly on the site, ii) a reduction of wastes since plants play the role of "cleaner", and iii) a recycling and valorization of plant biomass by producing energy for example [3].

I. Various tools for phytoremediation

There are four principal process of phytoremediation but all are not efficient for MTE storage.

1. Phytostabilization

This technique does not decontaminate soils but helps to confine pollution on the site and thus avoids leaching. Phytostabilization is usually a preliminary strategy before an effective decontamination and concerns essentially MTE. Selected plants called excluders release anionic organic acids from their roots via anionic channels, leading to the chelation and precipitation of cationic MTE in the medium [5, 6]. Among organic acid, the most cited are citrate, malate and oxalate [7-9]. Since excluders do not absorb MTE, they can grow on highly contaminated substrates (Fig. 1).
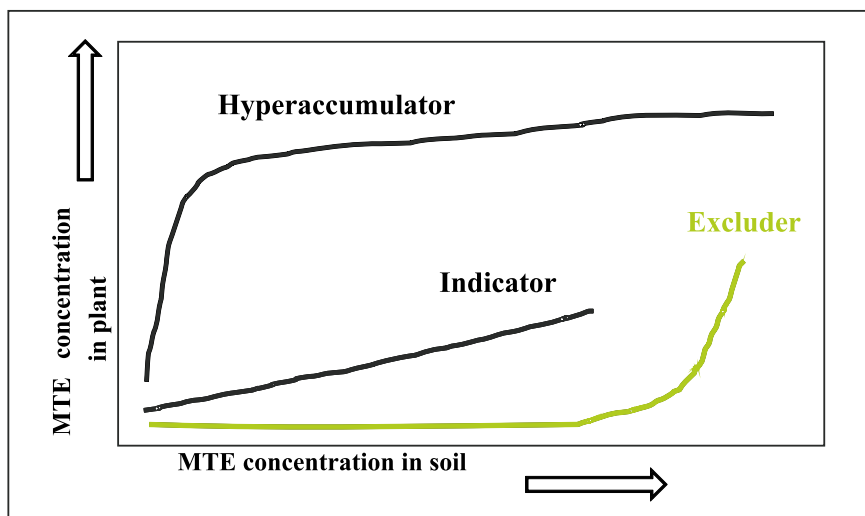

Figure 1. Strategies of plant responses when MTE concentration increases in the soil: excluders limit MTE absorption, hyperaccumulators extract MTE from soil and store them in their tissues, indicators accumulate MTE as a function of MTE concentration in soil but are less tolerant than hyperaccumulators (modified from [3]).

In order to improve this process, amendments are sometimes added with cation chelators such as EDTA, CDTA, DTPA, phosphate compounds, citric acid [10-12]. However, they generate a supplemental cost and their leaching can be important [13]. Moreover, some of them can have adverse effects on microfauna and microorganisms. Indeed, it was shown that EDTA can increase mortality of nematodes [14].

2. Phytodegradation

This process concerns only organic pollutants because metallic ions are not biodegradable. The degradation of pollutants involves several families of enzymes with different sites of cleavage. The less toxic by-products are then translocated to the vacuole [15].
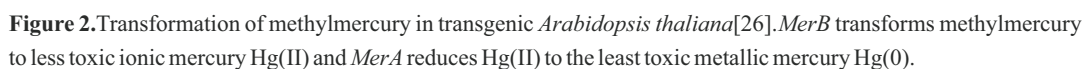
Most enzymes participating to phytodegradation belong to glutathione-S-transferases (GST) and cytochrome P450 monooxygenases (P450).

For example, 48 genes encoding GST and more than 300 genes encoding P450 were identified in *Arabidopsis thaliana*,

indicating the important number of isoforms and the possibility to degrade more or less efficiently various pollutants such as pesticides, petroleum hydrocarbons, or explosives [16-19]. GSTscatalyze conjugation between electrophilic xenobiotic and glutathione [20].The *S*-glutathionylated products are tagged for vacuolar import byATP binding cassette (ABC) transporters, which selectively transportGSH conjugates [16,18]. P450 enzymesuse electrons from NADPH to catalyzeoxidation of organic compounds [17]. Other enzymes are required to transform xenobiotic such as nitroreductases, nitrilases, phosphatases, dehalogenases, laccases, andperoxidases[21].

Phytodegradationhelps to clean air, water or soil. For air decontamination, several plant species can directly absorb gases via their stomata but the most efficient process is the injection of contaminated air in an artificial substrate containing plants. In this case, phytodegradation occurs at the root level. For aquatic depollution, contaminated water is filtered through beds containing selected hydrophytes and helophytes. For example, *Myriophyllumaquaticum* can be used to remove antibioticsor pesticides [22, 23].Soil and / or sludgedepollution can be done directly on the site or after the removal of polluted substrate and the subsequent storage on an artificial site for phytodegradation. In both cases, plants are selected according to the pollutant(s) to transform and the type of soil. For example, after 120 days on soil contaminated with *polycyclic* aromatic *hydrocarbons* (PAH), *Mirabilis jalapa* was shown able to degrade 60 % of the pollutant [24].In salt marsh polluted with PAH*, Juncusrosmarinus*removed 97 % of these compounds after one year [25].

3. Phytovolatilization

The process is not frequent since only selenium, mercury and few organic pollutants can be volatilized. Methylmercury is extremely toxic and is the primary source of human mercury poisoning from consumed fish. The surexpressionof bacterial genes *MerA* (mercuricreductase)and *MerB*(organomercuriallyase)in *Arabidopsis thaliana* allowed to convert methylmercury into metallic mercury Hg(0) and to increase the phytovolatilization by a factor of 50 (Fig. 2)[26].

$$R - CH_2 - Hg^+ + H^+ \xrightarrow{MerB} Hg(II) + R - CH_2$$

$$Hg(II) + NADPH + OH^- \xrightarrow{MerA} Hg(0) + NADP^{++} + H_2O$$

Figure 2. Transformation of methylmercury in transgenic *Arabidopsis thaliana*[26].*MerB* transforms methylmercury to less toxic ionic mercury Hg(II) and *MerA* reduces Hg(II) to the least toxic metallic mercury Hg(0).

Selenium can be converted into dimethylselenide, a compound 500-700times less toxic than inorganic Se species. It was reported for example in *Characanescens*, *Brassica juncea*and the hybrid poplar *Populustremula* x *alba*[27-29].

II. Phytoextraction or phytoaccumualtion of MTE

1. Benefits from phytoaccumulation

Approximately 10 billion ha are contaminated by MTE in Europe and the volume of contaminated soils in USA is estimated to 200 billion m^3 [30]. Conventional treatments cause an alteration of the site and represent a high cost comprised between 10 and 1000 $ per m^3[3].Phytoextraction takes a longer time for decontaminate the site but is cheaper (around 0.05 $ per m^3). The lower price can be explained by the valorization of biomass after harvest (Fig. 3). Indeed, biomass can lead to the production of gases or energy, and minerals (included MTE) contained into the ash can be separated and recycled.
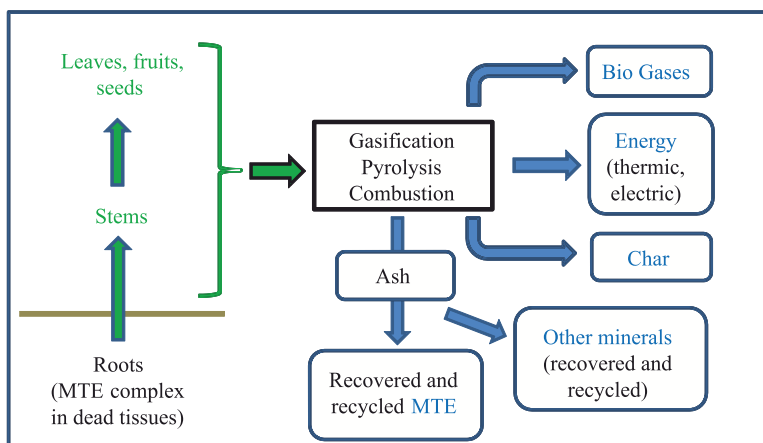
Figure 3.
Transformation of biomass after MTE phytoextraction (modifiedfrom [3]).

2. Steps of phytoaccumulation in plants

2.1. Cell wall adsorption

A part of MTE does not penetrate cells and is adsorbed onto cell walls of epidermis, essentially at the primary cell wall level. Primary cell wall is composed of 35 % pectins,30 % cellulose, 30 % hemicellulosesand 5 % glycoproteins [31]. It was estimated that 70 to 90 % of MTE present in the cell wall arebound to pectins and more particularly to homogalacturonans[32-34]. Indeed most MTE are divalent cations and can replace calcium (Ca^{2+}) bound to the carboxylic function ofgalacturonic acids. Less frequently, rhamnogalaturonanscan scavenge MTE as reported for grapevine and sugar beet exposed to Pb [35].

2.2. Membrane transporters

Not adsorbed MTE penetrate cytoplasm by membrane transporters. Essential MTE such as copper, zinc or iron use more or less specific transporters (Fig.4). However, non-essential MTEsuch as cadmium or leadcompete with essential MTE or non-metallic cationsat the transport level and / or use non-selective cationchannels [36].

Figure 4.
Main families of MTE transporters on the plasma membrane. CNGC: Cyclic Nucleotide-Gated Channel, ZIP: Zinc regulated transporter – Iron regulated transporter-like Protein, IRT: Iron Regulated Transporter, YSL: Yellow Stripe Like, Nramp: Natural Resistance Associated-Macrophage Protein, COPT: COPper Transporter.
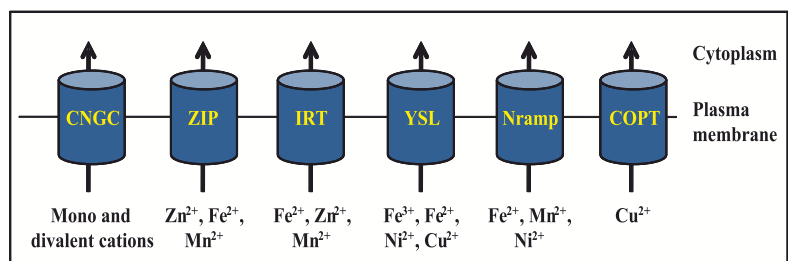
In root epidermic cells, MTE can be transported to other tissues by symplast and apoplast or can be stored in the vacuole. The vacuolar storage involvestonoplasttransporters such as cation diffusion facilitators or ABC transporters (Fig. 5) [37-39].
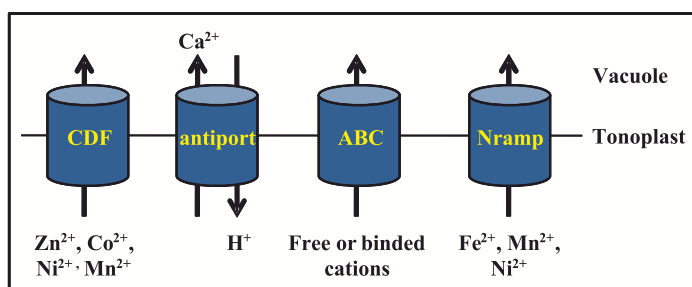

Figure 5. Main families of MTE transporters on the tonoplast.CDF: Cation Diffusion Facilitators.

2.3. MTE chelators

MTE can be transported as free molecules but they generally form complex with various ligands according to the type of MTE. Indeed, it was shown that histidine has a higher affinity for Ni than for other MTE[40]. According to the plant species, Ni can be scavenged by other ligands such as nicotianamine (which can also form other complexes with Zn and Fe) or organic acids (which can form other complexes with Pb and Al) [41-43].Various MTE including essential cations (Cu, Zn…) can be transported by metallothioneins and other chaperones proteins [44, 45]. Cystein-rich oligopeptides can also transport MTEsince cysteine exhibits a sulfhydryl function with high affinity for metallic cations. It was shown for example that glutathionecan form complex with Cd, Zn, Pb, As or Cr and that phytochelatinscan scavenge essentially Cd and As, and with a lower affinity Pb, Zn, Cu, Ag or Hg [46, 47]. Phytochelatins are synthesized from glutathione and are composed of 2 to 11 Glutamate-Cysteinmotifs with a C-terminal Glycine residue (Fig. 6).Phytochelatin synthase, the last enzyme of phytochelatin synthesis pathway, is activated only in presence of MTE[48].
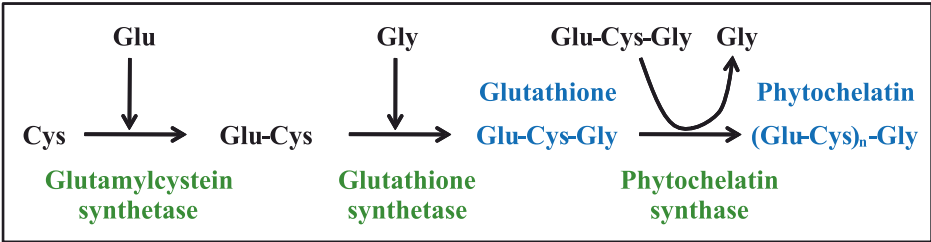

Figure 6. Glutathione and phytochelatin biosynthesis pathway.

III. MTE hyperaccumulators

Between450 to 500 plant species have been identified as hyperaccumulatorsbecause they can store 50 to 500 times more MTE than other plant species [49]. Hyperaccumulators are mainly herbaceous species and are found in 45 botanical families, 25 % of them belonging to the family of Brassicaceae. They can store high quantities of one or two MTE but 80 % of these species hyperaccumulateNi[49]. Hyperaccumulation is interpreted as an adaptation to extreme conditions in order to limit plant competition and / or a mechanism of defense against herbivores[50].

Hyperaccumulators generally accumulate more MTE in aerial parts than in root system except Cd, Pb and Hg which are more often concentrated in roots [51].The minimal concentration of MTE for hyperaccumulators is recorded in aerial parts since roots are difficult to harvest. The minimal content for most MTE is 1 g. kg^{-1} dry weight (DW) but this value can be lower or higher for several MTE(Table 1). Among hyperaccumulators, Thlaspicaerulescens(Brassicaceae) is one of the most efficient species and can store39.6 g Zn and 1.8 g Cd per kg DW[50].

MTE	Concentration (g. kg^{-1} DW)
Fe, Mn, U, Zn	10
Al, As, Co, Cr, Cu, Ni, Pb, Se	1
Cd	0.1
Hg	0.01

Table 1
Minimal concentration of MTE in aerial parts to consider that a plant species is a hyperaccumulator[52].

IV. Woody species as a new tool for MTE phytoaccumulation

1. Most studies are related to Angiosperms

Few woody species are actually identified as hyperaccumulators: *Sesbaniadrumondii*(Fabaceae) forPb and Hg, *Cassia siamea*(Fabaceae) for Fe, Ni and Zn, *Salix dasyclados*(Salicaceae) for Cd and Zn,and more recently *Averrhoacarambola* (Oxalidaceae) for Cd [53 - 57].

However, woody species present two main advantages compared to herbaceous plants: i) they have a deeper root system improving MTE extraction from soil, and ii) aerial biomass is more important, allowing a higher quantity of MTE accumulated per plant compared to a herbaceous species, even if the woody species is not an hyperaccumulator[58, 59].

Most studies tend to focus on fast growth rate trees such as poplars and willows. They are not hyperaccumulators, as they do not store more MTE in roots than in aerial parts. However, several works showed that this definition depends of the MTE and the tree species. For example Zn and Cd contents in *Salix fragilis* and *Salix viminalis* are similar in both roots and aerial parts whereas Cr, Cu, De, Pb and Ni accumulated preferentially in the root system [60]. Other works on alders, birches and hybrid poplars and willows highlighted that Zn and Cd accumulated more in leaves whereas Cu accumulated mainly in trunk and branches [58, 61].

Several studies are related to hybrid tree species since they are often more vigorous and thus potentially more efficient for phytoaccumulation than their parents [62, 63]. According to literature, fast growth rate trees could even be more efficient than herbaceous hyperaccumulators. *Thlaspi caerulescens*, the most efficient Cd and Zn hyperaccumulator, produces a low biomass per year (Table 2). By contrast, hybrid poplars and willows exhibit lower Cd and Zn contents but their higher biomass allows to harvest an annual higher quantity of Cd and Zn compared to *T. caerulescens*[60, 62, 64, 65].

	Thlaspi caerulescens	*Populus sp.* and *Salix sp.* hybrids
Harvested biomass (t. ha^{-1}. y^{-1})	4	20
Cd content (g. kg^{-1} DW)	1.2	0.5
Zn content (g. kg^{-1} DW)	4.8	1.2
Harvested Cd (kg. ha^{-1}. y^{-1})	4.8	10
Harvested Zn (kg. ha^{-1}. y^{-1})	19.2	24

Table 2.
Comparaison of phytoextraction efficiency between *Thlaspi caerulescens* and poplar and willow hybrids. Cd and Zn contents correspond to the concentrations in aerial parts at the end of the season. Harvested quantities are total Cd and Zn accumulated in aerial parts at the end of the growing season.

2. Gymnosperms: new species to investigate

Little information is available about the capacity of phytoaccumulation by Gymnosperms despite the fast growth rate of several species. A comparison between Populus x canadensis and Larix olgensis concluded that Angiosperms would be better tools for phytoaccumulation[66]. However, this study was performed with a hybrid poplar and literature often shows that hybrids are more resistant than their parents. Thus a hybrid larch would have been a better choice to compare Angiosperms and Gymnosperms. Another work consisted in testing the ability of 34 species of conifers to accumulate Cr in leaves [67]. Results indicated that deciduous conifers (Larix, Taxodium, Ginkgo) were the most interesting species for phytoaccumulation since they had the highest ability to adsorb chromium ions. However, accumulation depends on the MTE since another work showed that Larix kaempferi excluded Ni instead of accumulating it [68].

Our laboratory initiated few years ago a research program on two species of Pinophytes, Douglas (Pseudotsugamenziesii) and a hybrid larch (Larixx eurolepis), in order to evaluate their capacity to accumulate MTE. Both species share a fast growth rate and are more and more used for forestry in temperate and cold-temperate zones. The originality of larch is its deciduous needleswhich allow to harvesteach year a part of stored MTE. The advantage of this hybrid is its more important resistance to biotic and abiotic stresses as well as its wider ecological amplitude compared to its parents, L. decidua and L. kaempferi[69]. Preliminary experiments indicated that hybrid larch was able to tolerate Cd concentration corresponding to a moderate polluted soil and to accumulate Cd in aerial parts close to the minimal required for hyperaccumulators (data not shown). The other study concerning Douglas showed that Cd content in aerial parts was low but experiments were performed with a low concentration of MTE in the soil. However, barks concentrated non negligible quantity of MTE and this process can be improved by modifying these barks. Indeed, harvested barks can be grafted with aminatedoligogalacturonans and used to filter water contaminated with various MTE such as Cd, Pb, Cr, Ni, Zn or U [70-72].

Conclusions

Phytoremediation and particularly MTE phytoaccumulation is a promising biotechnology to clean environment because it does not destroy contaminated sites and is not very expensive. Nevertheless, further studies are required since MTE phytoaccumulation depends on the plant species, the MTE, the soil composition and the climatic conditions. The efficiency of this process is thus a good compromise to find between these four factors to take into account.

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