Junk-greedy Greens: phytoremediation as a new option for soil decontamination

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Industrialization has led to the release of enormous quantities of toxic compounds into the environment. Industrial activities such as chemical works, garages and service stations, metal fabrication shops, paper mills , tanneries, textile plants, waste disposal sites and intensive agriculture are particularly guilty of polluting[1]. Pollutants can be categorized into two large classes: elemental and organic. Elemental compounds include heavy metals, like mercury and lead, non-metallic inorganic compounds such as arsenic, as well radionuclides like uranium. Organic contaminants consist of petroleum hydrocarbons, chlorinated solvents (PCBs), linear halogenated hydrocarbons (TCE) and explosives such as TNT[2]. There is increasing scientific evidence indicating the toxicological effects of these contaminants. Conversely, there is a growing impetus to reuse abandoned polluted sites in order to conserve remaining pieces of untouched land[1]. To balance these issues, governmental regulations have become increasingly strict in recent years in order to limit the release of pollutants and to adequately remediate polluted areas[3].

There are numerous options for the remediation of contaminated sites. Commonly used engineering techniques include excavation and landfilling, chemical treatment, vitrification and electrokinetics. These methods are extremely expensive, costing between $50 and $500 per ton of soil[4]. This financial burden probably plays a role in slowing down global efforts to eradicate pollution, particularly in developing countries where these techniques are clearly not affordable. As a result, it is highly desirable to develop more cost-effective remediation methods. Bioremediation technologies, which focus on living organisms as clean-up agents, are seen as an alternative with great potential for affordably remediating polluted sites. Bioremediation research is led mainly by microbiologists, who try to identify the appropriate bacteria to breakdown contaminants into harmless products. The amazing diversity of the prokaryotic world makes it an endless resource of metabolic pathways that can process organic compounds[1]. However, biologists have now recognized that plants also have qualities that can make them great remediating agents, and a new field called phytoremediation is receiving more attention from both academia and industry.

Phytoremediation is defined as the use of plants as well as microorganisms of the rhizosphere to remove or render harmless pollutants from contaminated sites[5,6]. The main advantage of phytoremediation is its low cost in comparison to engineering techniques, i.e. phytoremediation only costs about $5-$40 per ton of soil[4]. In addition, it is generally approved by the public, primarily because of its aesthetics and eco-friendly sustainability. Bioremediation using microorganisms is often performed on extracted soil, in the controlled environment of a bioreactor. It can also be used for in situ remediation, but it has often been found that the cleaning bacteria can compete with local microbes, and that keeping them at high concentrations requires the addition of a lot of nutrients[7]. In comparison, phytoremediation is easier to manage because it is an autotrophic system of large biomass that requires little nutrient input[1]. Moreover, plants offer protection against water and wind erosion, preventing contaminants from spreading[8].

Phytoremediation is a broad term that comprises several techniques used for water and soil decontamination. In this review, we will focus on four

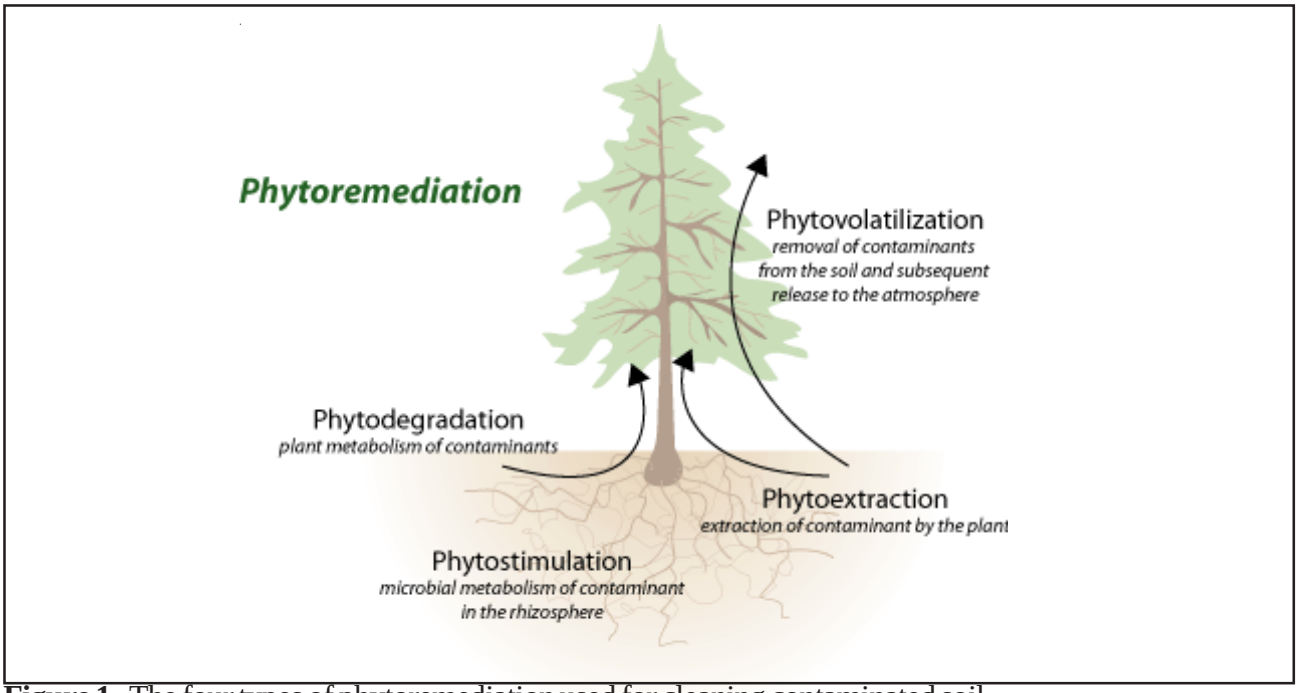

Figure 1. The four types of phytoremediation used for cleaning contaminated soil

main subgroups of techniques used for soil remediation:

1. *Phytoextraction:* the uptake of contaminants in plant roots and their concentration in harvestable tissues
2. *Phytovolatization:* the uptake of contaminants by plants and their subsequent release into the atmosphere in a volatile form.
3. *Phytodegradation:* biodegradation of pollutants by plant enzymes.
4. *Phytostimulation:* biodegradation of pollutants by plants, facilitated by microorganisms in the rhizosphere[6].

In general, phytoextraction and phytovolatization are considered as the main options for the removal of heavy metals and other elemental compounds, whereas phytodegradation and phytostimulation are applied mostly to organic contaminants[2]. We will review examples of these techniques, moreover, we will discuss the importance of understanding the natural mechanisms used by plants to perform these actions and the strategies being developed to enhance the efficiency of phytoremediation systems. Numerous challenges need to be overcome for phytoremediation to become a commercially viable technology, especially with regard to the generation of plants that have good remediating qualities without compromising their biomass yield and growth rate[9]. We will highlight some recent advances in molecular genetics that provide potential tools to solve these problems.

Phytoextraction

Phytoextraction is the best solution for the removal of contaminants that cannot be degraded. For this reason, it is used mainly to extract dangerous elemental compounds. During the last century, the amount of toxic metals released has reached over 1,350,000 t for zinc, 783,000 t for lead and 939,000 t for copper. Elemental compounds and radionuclides cause DNA damage, which is thought to be the cause of their carcinogenic effects in animals and humans[6]. Contrarily to organic compounds, elemental contaminants are immutable, which means that they cannot be degraded by any biological or physical process[2]. As a result, toxic inorganic compounds can only be removed from the site or converted into a biologically inert form[3]. Phytoextraction involves the uptake of contaminants

from the soil, followed by translocation and accumulation in the shoot. Once the plants have grown to their full size, the above-ground tissue is harvested using conventional farming machinery and the contaminant is permanently removed from the site. Typically, the harvested biomass will be incinerated or composted. In the case of metals, another alternative is to recycle the compound. However, because the cost of such an operation often surpasses the value of the metal itself, recycling is not commonly done[1].

There are two important factors to consider when evaluating the potential of a plant as phytoextractor: bioconcentration and biomass production. The former is defined as the ratio between the concentration of the pollutant in the shoot and its concentration in the soil. It serves as an indicator of the capacity of a plant to accumulate toxic compounds. Biomass production is also critical in order for phytoextraction to be commercially viable because it decreases the number of crops required to complete the remediation of a given site[10].

Hyperaccumulators

Phytoextraction has attracted increasing interest within the scientific community following the discovery of *hyperaccumulator* plant species. Hyperaccumulators are plants that have an innate capacity to absorb metal at levels 50-500 times greater than average plants[4]. They are often found in metal-rich regions where this trait probably gives them a competitive advantage[1]. Hyperaccumulators have a bioconcentration factor greater than one, sometimes reaching 50-100. Furthermore, they always have efficient root-to-shoot transport system and have enhanced tolerance to metals, indicating increased capacity for detoxification[10]. So far, more than 400 species of natural metal hyperaccumulators have been identified[10]. The best known hyperaccumulator is the pennycress *Thiaspi caerulescens*. This small plant can absorb zinc from the soil at a rate exceeding 40 kg per hectare per year[1]. Because it is a small and selfing diploid plant that can easily grow under lab conditions, *T.caerulescens* is used as a model for the study of metal hyperaccumulation. Hyperaccumulators are great tools for the study of phytoextraction. However, a problem with most hyperaccumulator species is that they do not have a sufficient biomass and growth rate to be successfully employed in the phytoremediation industry[9]. Many researchers in the field consider that the best way to work around this problem is to transfer the appropriate characteristics of hyperaccumulators into high biomass plants[4,9]. To do so, it is essential to understand how these plants manage to tolerate and accumulate such high quantities of metals.

Four processes are believed to be crucial for hyperaccumulation: root uptake of metals, root-to-shoot transport, complexation with chelating molecules and compartmentalization into the vacuole[10]. Increased uptake of metals in hyperaccumulators involves differences in the expression of metal transporters in the roots. For instance, Zn and Cd accumulation in *T. caerulescens* involves genes coding for metal transporters, ZNT-1 and ZNT-2, which are highly expressed in root tissue. The expression of these genes is almost completely unaffected by internal Zn concentrations[10]. Furthermore, it takes 50 times more Zn in the soil to downregulate ZNT-1 in *T. caerulescens* than in a related non-accumulator species, *T. arvense*[9].

Uptake of metal in root cells is of great importance, but for phytoextraction to occur, transportation in the shoot must also be efficient. In nickel hyperaccumulators such as *Alyssum lesbiacum*, exposure to the element triggers the release of histidine, which acts as a chelator that detoxifies nickel. This enhances root tolerance to the metal, but most importantly it increases the rate of Ni uptake into the xylem for transport to the shoot[9,10]. Once in the shoot, metals accumulate in the cells. What cell processes allow hyperaccumulators to tolerate such high concentrations of metal in their tissue? It is believed that hypertolerance is associated with the presence of high-affinity chelating molecules in the cytoplasm. Phytochelatins, for instance, are cysteine- and gluthanione- rich compounds that can sequester numerous metals such as Ag, Cd, Cu and Ni and thus protect cells form their harmful effects on surrounding proteins. In *T. caerulescens*, Zn is believed to be complexed with histidine in root cells and organic acids in the shoot[10]. Finally, complexed metals are transported and kept in the vacuole, which accounts for a great part of plant hypertolerance to metals.

Genes coding for vacuolar ion transport proteins

have be identified in several hyperaccumulator species. In *T. caerulescens*, the gene ZPT-1 codes for a transporter that belongs to the cation diffusion facilitator family. ZPT-1 is homologous to ZAT, an *Arabidopsis* gene that confers Zn tolerance when overexpressed. In *T. caerulenscens*, ZPT-1 is expressed mainly in leaves, and is not downregulated by high Zn concentrations[10].

Improving phytoextraction may involve the genetic transfer of hyperaccumulator traits into high biomass plants. Because hyperaccumulation often involves the action of multiple genes, a judicious strategy to transfer these traits is somatic breeding. Somatic breeding consists of fusing together protoplasts of two different species in order to combine their respective genetic material. For example. Brewer et al. generated a somatic hybrid between the zinc hyperaccumulator *Thiapsi caerulescens* and the high-biomass *Brassicae napus* (canola). The team successfully obtained a hybrid that was highly metal resistant while keeping the high growth rate and biomass of *B. napus*.

Another approach to improve the performance of phytoextraction systems is the use of transgenic plants, possibly expressing genes taken from bacteria or animals. There are some trace elements, such as lead and mercury, that no plants have been shown to tolerate or accumulate. However, several trace element detoxification systems have been extensively characterized in yeast and bacteria. Of course, it is not possible to predict how stably and efficiently animal and bacterial enzymes will behave in plants, but there are already promising projects that show the potential of transgenic plants for phytoextraction[9]. One of these projects addresses the problem of iron acquisition. In all plants except grasses, the insoluble ferric form (Fe^{3+}) present in the soil must be reduced to the more soluble ferrous form (Fe^{+}). This is mediated by a ferric reductase located in the root plasma membrane. Then the ferrous form is taken up into cells by a ferrous transporter. To increase iron acquisition, biologists transformed tobacco with two ferric reductase genes from yeast under constitutive promoters. The leaves of the transformants contained 50 times more iron than untransformed tobacco (Singh et al. 2003).

Another project aimed at increasing plant tolerance to cadmium. Metallothionins (MTs) are another class of cysteine-rich proteins that have high affinity to cations such as Cd, Cu and Zn[6]. The gene corresponding to the metal-binding domain of a mouse MT was overexpressed in tobacco, which led to increased accumulation and resistance of the plants to Cd[2].

Phytovolatization

Another option for the remediation of certain toxic elemental compounds is to convert them into a less harmful volatile form. Phytovolatization is the uptake of contaminants by the roots followed by their conversion to volatile compounds and their subsequent release into the atmosphere[6]. For instance, the Indian mustard *Brassicae Juncea* can naturally extract selenium for the soil, where it is often present as the highly toxic selenocyanate, and convert part of it to dimethylselenide, a volatile form that is 500 to 700 times less toxic than selenate or selenite[11]. Although they have not been identified so far, it would certainly be interesting to study the genes involved in this process in order to increase the efficiency of Se volatization.

Phytovolatization systems have also been developed for the removal of mercury, in what is probably the most successful use of transgenic plants for phytoremediation. The form of mecury that can bioaccumulate and cause neurodegenerative diseases in fish is methylmercury (CH_3Hg). Industrial effluents usually release ionic mercury (Hg(II)), but it is eventually converted to CH_3Hg by sedimentary bacteria[12]. Some mercury-contaminated sites contain bacteria that convert CH_3Hg to the much less toxic elemental mercury (Hg(0)), which is diffused out of the bacteria and is released into the atmosphere. The enzymes responsible for this process are an organomercurial lyase (MerB) and a mercuric reductase (MerA). MerB first converts CH_3Hg to Hg(II), which is then reduced to the volatile Hg(0) in a NADPH-dependent fashion. This pathway was introduced in *Arabidopsis thaliana* by transferring MerA and MerB in front of a constitutively active promoter. The resulting transgenic plants were able to grow with concentrations of CH_3Hg 50 times greater than control plants[12].

Using fast-growing trees such as yellow poplar and willow as phytovolatization systems could be an

efficient and cost-effective option for phytoremediation. Trees have a long life-span, they have a deep and extensive root system that gives them a powerful hydraulic pull and stabilizes the soil, and they produce large amount of litter which might increase the bioavailability of metal[9,1]. A promising development in this regard is the transfer of MerA to yellow poplar. Primary results indicate that transgenic poplars were able to volatilize 10 times more mercury than control plantlets[9].

Phytodegradation

Phytodegradation offers great hope for the remediation of sites contaminated by organic compounds. Contrary to elemental contaminants, organic compounds can be chemically degraded into harmless products, and even mineralized, i.e. broken down into CO_2 and H_2O molecules[2]. The idea of using plants to perform such processes first appeared when it was observed that organic pollutants disappeared more quickly from vegetated soils than from barren soil[13]. Phytodegradation is defined as the breakdown of pollutants either by metabolic processes inside plant tissues, or by plant enzymes secreted in the soil[6]. The two following examples demonstrate the potential of phytodegradation for extremely toxic compounds such as explosives and halogenated hydrocarbons. 2,4,6-Trinitrotoluene (TNT) is one of the most dangerous and persistent explosives. Its use and disposal has led to the contamination of numerous sites worldwide, but the current means available to clean up these sites are so expensive that few of them have been remediated[14]. Numerous plants species are able to degrade TNT in their tissue, but this process greatly affects their growth and development, and that prevents their use for large-scale phytodegradation projects[2]. A soil bacteria, *Enterobacter cloacae*, was found to be able to use nitrate ester explosives as its source of nitrogen. Two enzymes identified in this bacterium are able to perform the denitrification reaction: PETN reductase and nitroreductase[7,14]. Both reductases use NADPH as a source of electrons to reduce TNT into less harmful compounds. In two independent studies, the genes coding for these enzymes, *onr* and *nfsl* respectively, were introduced in tobacco plants under the control of constitutive promoters. In both cases, the transgenic plants were resistant to TNT concentrations that severely affected the development of wild-type plants[7,14].

The study of *nfsl*-expressing plants indicates that transgenic root and shoot tissue analyzed by HPLC contained no TNT and minute amounts of ADNT, its degradation product. On the other hand, wild-type plants grown on TNT-medium contained high concentrations of TNT. This suggests that *nfsl*-expressing plants readily reduces TNT to ADNT and/or may conjugate it and its transformation products to chelating molecules before sequestering them in the vacuole in an unextractable form. Because ADNT is also a carcinogenic compound, it is desirable that its stays sequestered in the plant rather than excreted back into the soil[14]. Plants that naturally degrade TNT appear to be able to reduce it to CO_2 and ammonium or nitrate[2]. In order to diminish the production of dangerous down-products such as ADNT, it would certainly be interesting to express genes involved in the complete mineralization of TNT in *onl* and *nfsl* transgenic plants .

Trichoroethylene (TCE) is a halogenated compound used in the industry as a degreasing agent. It is one of the most widespread organic pollutants and it is particularly hard to remove because of its high mobility[2,1]. While rhizospheric bacteria have long been known to degrade TCE, it is only recently that the direct role of plant enzymes in this process has been discovered. In experiments using isotopic-labeling, Gordon et al.[15] were able to show that hybrid poplar cell cultures were able to absorb TCE present in the growth medium and subsequently degrade it to trichloroethanol, trichloroacetate and finally to CO_2. The same group also conducted field trials showing that poplar trees grown on soil injected with TCE were capable of the same degradation reactions, volatilizing up to 90 percent of the TCE absorbed[15]. These data suggest that plants possess a very efficient oxidative degradation pathway for xenotopic chlorinated compounds such as TCE. In fact, among all living organisms, plants have the capacity to synthesize, rearrange and detoxify the most complex array of organic compounds, such as cellulose, lignin, flavanoids and other secondary metabolites[2]. For this

reason, there is great hope that future research will unravel the biochemistry of plant degradation of other organic pollutants.

Phytostimulation

Far more is known about the microbial pathways responsible for the breakdown of toxic organic compounds than of plant metabolic pathways. In addition, the symbiotic relationship that exists between plants and several soil microorganisms has been extensively studied. The rhizosphere, which is the zone of soil immediately surrounding the roots, provides the dynamic environment mediating plant-microbe exchanges[6]. An interesting use for phytostimulation is being developed for the remediation of polychlorinated biphenyls (PCBs). PCBs are among the most alarming contaminants because of their persistence in the environment, their carcinogenicity and and their general toxicity[2]. In order to be degraded by soil bacteria, PCBs must be co-metabolised with another carbon source. In laboratories where PCB bioremediation is performed in bioreactors, the co-metabolite of choice is the closely related compound biphenyl. However, biphenyl is highly toxic and therefore cannot be used for in situ remediation of contaminated soil[16]. The need for alternative co-metabolites has led several research groups to look for plant species capable of excreting phenols that can support PCB-degrading bacteria. By screening plants where these bacteria preferentially grew, a team was able to identify the mulberry *Morus rubra L.* as a promising candidate for phytostimulation studies involving PCB degradation[13].

Conclusion

Our society is increasingly concerned about land and water pollution and its potential effects on ecosystems and on human health. Consequently, considerable effort has been put into the development of cost-effective and efficient ways to clean up contaminated sites. Phytoremediation has gained considerable acceptance over the years, and its place in the environmental technology market is steadily growing[1]. Despite its relatively slow rate of action and limitations related to environmental conditions necessary for plant growth, it is considered a low cost, environmentally sound technology that could in certain cases replace current engineering practices. Recent research has widened the possibilities for phytoremediation. However, much of the present data on the performance of phytoremediating transgenic plants are based on observations made in laboratories, often on agar media, rather than in the field. Therefore, it is now important to confirm the performance of phytoremediation systems on large-scale contaminated sites. In fact, a few papers already report cases where transgenic plants appeared efficient in the lab, but did not differ significantly from controls under field conditions[9]. The bioavailability of the contaminants on real contaminated sites appears to be a major factor in the discrepancy between lab and field conditions. A better understanding of soil properties and of physiochemical factors influencing the solubility of toxic compounds will likely allow the improvement of on-site plant performances in the future[6]. Furthermore, we need to gain more knowledge about the molecular mechanisms that allow plants to remediate polluted soils, particularly with regard to hyperaccumulation and hypertolerance. It is the identification of novel genes involved in the acquisition and the homeostasis of toxic compounds, as well as an understanding of the way they are regulated that will encourage real improvement in phytoremediation systems.

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