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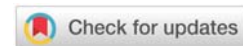
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Review Article

Chelate-assisted phytoextraction using Brassicaceae plants

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Abstract

In these last decades, excessive metal concentration pose serious contamination in soils. Therefore, it is urgent to develop and adopt a new strategy and technology to remove soil contaminants. Here, the phytoextraction was considered as a recently developed approach to clean up metal-polluted soils in that the plants are used to translocate the toxic metals from the contaminated soils. Therefore, this mini-review provides a perfection of recent knowledge related to the heavy metal phytoextraction from soils and their accumulation in plants. It has been reported that EDTA enhances the availability of heavy metal to plants and thus increases the accumulation in their shoots. In addition, fundamental and basic advancement has been made on the molecular aspects and the physiological regarding tolerance and metals phytoaccumulation in plants. A multidisciplinary strategy is warranted to make phytoextraction a plausible trade technology to remediate metal-polluted soils. Hence, the purpose is to integrate soil-related and biological progress to suggest research needs and future direction.

Introduction

The civilization of mankind and the agriculture sustainability rely on two precious bio-resources namely land and water [1,2]. So, the two last ones have been subjected to an hyper exploration and they were severely polluted by kinds of contaminants having its origin from various anthropogenic studies [2,3].

The potentially toxic elements can occur naturally in soils at various amounts depending on the source rock geochemical compositions and soil formation processes like weathering sedimentation and volcanic eruptions. Meanwhile, anthropogenic activities such mining and smelting operations, industrial production, oil and gas production, agricultural activities and military practices can also result in elevated soil contaminant amounts. Indeed Figure 1 illustrates the potentially toxic element sources in soil ecosystems [4,5].

It is crucial to note that the majority of contaminants get accumulated in plants and either directly or indirectly, find their way into food web thus cause severe consequences [3]. In addition, the plants undergo several abiotic stresses such as salt, drought and heavy metal witch are known among the most limiting ones [6-10]. Indeed, it is crucial to signal that

as much as one-half of the world irrigated areas are affected by the high salinity level [7,8,10,11], the drought and also the excessive soil metal amount [6,7,12]. The last cited one pose significant hazard to human, plant, animal and health, hence to the ecosystem [7,13,14]. We indicate in this case that soil contamination by toxic metal has often resulted from human activities, such as those related to application of sewage sludge to agricultural soils, industrial emissions, mining, leakage and/ or disposal of industrial wastes and also the pesticide use [12,15]. It is basic also to signal that the phytoextraction is one of the metal phytoremediation technologies as illustrated in Table 1 [16].

Due to the potential toxicity and the high metals persistence, those facts constitute a serious environmental problem that requires an affordable solution [15,17]. Hence, phytoextraction seems to be the most promising technique and has received increasing attention from researchers since it was proposed by various research teams such as [18] as a technology for reclaiming metal polluted soils [3,7,12].

The metal phytoextraction from the soil relies on the use of plants to extract and translocate metals to their harvestable parts [3,16,19,20]. The phytoextraction aim consists on the reduction of the metal concentration in contaminated soils to regulatory

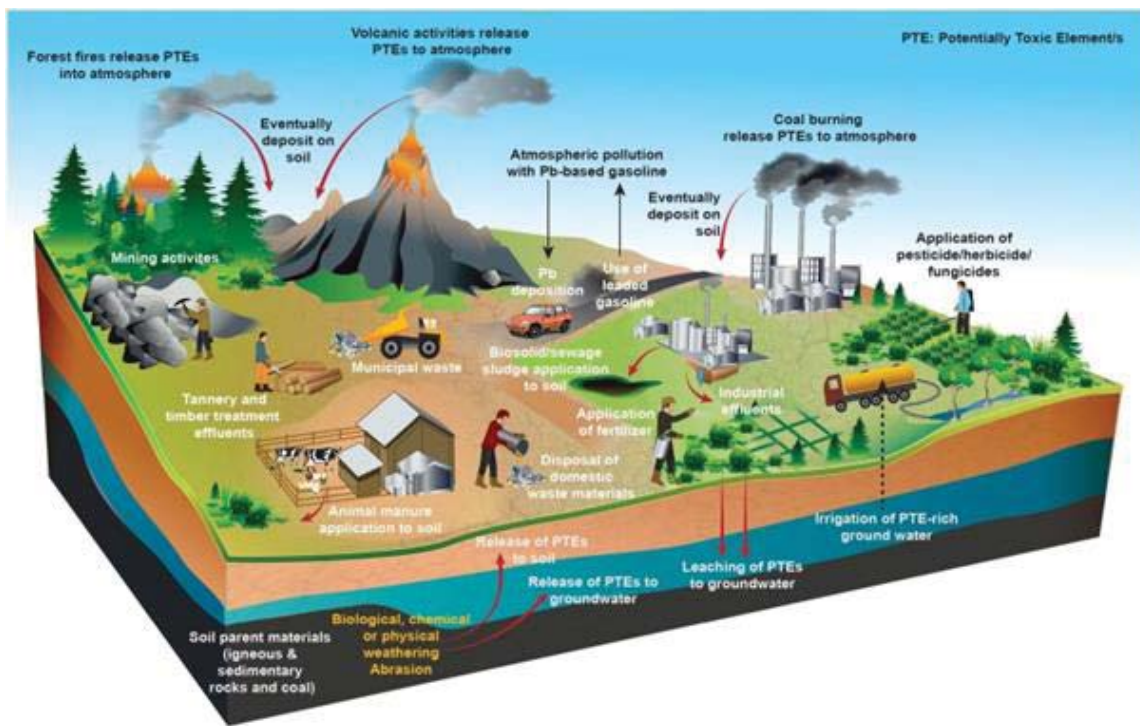


Figure 1: Illustration of the plausible toxic element sources in soil [4].

levels within a reasonable time frame. This extraction process depends directly on the able duality of the selected plants to grow and accumulate metals under the specific climatic and soil conditions of the site being remediated [16,20].

In another case, it is fundamental to note that the Brassicaceae plant family represents hundreds of plant families reported so far for their potential use in the remediation of varied environmental contaminants including toxic metals and metalloids. The most of the members of the Brassicaceae plant family well represent the metal hyperaccumulation among 0.2% of all angiosperms and thus, have key role in phytoremediation technology. Many of the plant species within Brassicaceae family such as *Alyssum*, *Arabidopsis*, *Berkheya*, *Bornmuellera*, *Cardamine*, *Cochlearia*, *Crambe*, *Peltaria*, *Stanleya*, *Thlaspi* including oilseed Brassicas grow fast, yield high biomass and are well adapted to a range of environmental conditions [19,20,21].

Metal extraction process

The heavy metal extraction process is due to two approaches that have currently been used to reach this purpose: the first one results in the exploration of plants with exceptional, natural metal-accumulating capacity, so called hyperaccumulators [22], and the second one is the use of high-biomass crop plants with a chemically enhanced phytoextraction method [6,12,21-25]. So, the basic properties of those two phytoextraction strategies of metals from soils are illustrated as followed in Table 2. In addition, the analysis of the Table 2 content and based on some of other's investigations give birth to a fundamental conclusion summarized on the fact that the natural metal-accumulating capacity seem to be the most

Table 1: Illustration of the phytoremediation technologies and their significances.

Metal phytoremediation technologies	
phytoextraction	Exploration of plants to remove metals from soils and transportation and also their concentration in above-ground biomass
Phytostabilization	the use of plants to minimize metal mobility in contaminated soil through accumulation by roots or precipitation within the rhizosphere
phytovolatilization	Exploitation of plants to turn volatile chemical species of soil metals

Table 2: Basic properties of the two phytoextraction strategies of metals from soils.

Phytoextraction strategies	
Natural phytoextraction	Chemically assisted phytoextraction
-Plants naturally hyperaccumulate metals -Slow growing ability, low biomass production	- Plants are normally metal excluders -Fast growing capacity, high biomass plants
-Natural capacity to extract high amount of metals from contaminated soils	-Synthetic chelators and organic acids are used to enhance metal uptake
-Efficient transfer of metals from roots to shoots	-Chemical amendments increase the metal transfer from roots to shoots
-High tolerance; survival with high concentrations of metals in tissues	-Low metal tolerance; the increase in absorption leads to plant death
-No environmental drawback regarding leaching of metals	-Risk of leaching of metal chelates to groundwater

one because of the negative repercussion of the chemically enhanced phytoextraction method [26-28].

Metal hyperaccumulation and tolerance in plants

The metal hyperaccumulation capability is a rare phenomenon in plants. Occurring ≈400 vascular plant species

in which vast majority of those ones discovered so far was being Ni hyperaccumulators. The plant species having the ability to accumulate Zn, Cd, Pb, Cu and Co are much less numerous [23]. The hyperaccumulation concept has been extended to a plant growing in its natural habitat in that those metal concentrations have been canned in the dry matter of any above ground tissue. This more detailed definition includes plants which accumulate metals in their aerial tissues other than leaves, that might be useful to phytoextraction as well, and disqualify any species that hyperaccumulate metals under synthetic conditions like massive metals addition to the soil or the nutrient solution [13].

The correlation and also the relationship between metal hyperaccumulation and tolerance is still a subject of discussion. Some authors suggest the non-correlation between those traits, while others suggest that hyperaccumulators possess a high degree of metals tolerance. [6] compiled a number of studies in that the metal accumulation meaning tolerant and non-tolerant plants had been compared [29]. Those funding's led to conclude that there is no pattern regarding accumulation and tolerance [6]. Both shoot and root concentrations are equally variable even when only one particular metal is [7,30,31]. However, at least in some cases, it is clear that the increased tolerance give birth to greater metal amount. Plants may use two strategies to deal with high metal amounts adjacent to their roots: (i) exclusion mechanisms by which the uptake and/or root to shoot transport of metals are restricted; and (2i) internal tolerance mechanisms which immobilize, compartmentalize or detoxify metals in the symplasm through production of metal binding compounds. Given that the phytoextraction purpose is to maximize metal accumulation efficiency in plant tissues, mechanisms of internal tolerance are likely to be crucial and [6,19,32].

Stress-associated protein provides tolerance to heavy metals

The boom in industrialization over the past few decades has led to the onset of long-term pollution by heavy metals as well as serious environmental and also ecological problems for humans, plants and animals. In addition, we signal that some heavy metals have a crucial role during various physiological processes in plants [33,34]; Yet, when present in excessive concentrations in the soil, they can inactive biomolecules, block functional proteins, or displace other essential metal ions and, hence, become toxic causing serious ecological problems. We recently showed that overexpression of LmSAP, a member of the stress-associated protein (SAP) gene family isolated from *Lobularia maritima*, in transgenic plants led to enhanced tolerance to metal stresses (Cd, Cu, Mn and Zn). Indeed, LmSAP expression increased after 12h of treatment with those metals, suggesting its involvement in the plant response to heavy metal stress [34]. LmSAP transgenic tobacco plants subjected to these stress conditions were healthy, experienced higher seedling survival rates, and had longer roots than non-transgenic plants. However, they exhibited higher tolerance towards cadmium and manganese than towards copper and zinc [7,30,33,35,36].

Chemical amendments upgrades metal phytoextraction

The solubility and availability of metal are both dependent on the soil characteristics and are strongly influenced by pH [4] and also the level of complexation with soluble ligands [37,38]. So, the metals exist in soil under various organization types: in solution as ionic or organically complexed species; on exchange sites of reactive soil components; complexed with organic matter; occluded in Fe, Al, and Mn oxides and hydroxides; entrapped in primary and secondary minerals [37]. Most metals in soils exist in unavailable forms, thus soil conditions have to be altered to elicit phytoextraction since the phenomenon, depends on a relatively abundant source of soluble metal to enable significant metal uptake and translocation to shoots. In addition, some metals have their extraction rate limited by their inherently low solubility. In such a case, the organic compounds may be used as amendments to upgrade phytoextraction. Such substances can chelate metal ions, therefore modifying the metals availability in soils [4,37,38].

At the same case, we note that as indicated by [39], DTPA and EDTA were noticeably effective in desorbing Pb whereas the natural organic acids solubilized very small amount of this metal from soil. Actually, the lower efficiency of LMWOA in making soil-Pb available to plants compared to synthetic chelates had already been demonstrated. The major drawback for using synthetic chelates (especially EDTA) in field conditions is their high persistence to biodegradation, with consequent high risks of metal leaching through soil profile. The heavy metal levels of in the soil solution after EDTA treatment can be much higher than plant's capabilities to absorb them. Indeed, the analysis of the fundings followed in Table 3 shows that EDTA and DTPA can solubilize up to 77 and 100% of Pb present in the soil samples, respectively. This was equivalent to bring 383 (EDTA) and 521 mg kg⁻¹ (DTPA) of Pb into the soil solution. Of course, such high values must be weighted against the use of these chelates for phytoextraction in the field [6,39].

The chelating agent and its use in Brassicaceae plants for phyto-extraction

Concerning the chelating agent and its use in Brassicaceae plants for phyto-extraction, various studies and investigations were described the effect of the heavy metal and the EDTA

Table 3: Dose for maximum desorption, estimated amount of metal solution, and recovery rate for doses of chelates versus concentrations of Cd and Pb in a contaminated soil.

Metal	Dose (mmol/Kg)	Solubility (mg/L)	Recovery (%)
EDTA			
Cd	20.2	28.1	57
Pb	18.9	383.3	77
DTPA			
Cd	13.4	38.6	77
Pb	14.8	521	104
Oxalic Acid			
Cd	15.7	16.9	34
Galic Acid			
Cd	15.3	12	2



application on plant growth and the phyto-extraction potential. Indeed, the pot experiment was conducted to evaluate the phyto-extraction capability of heavy metals by Sorghum. The last one was grown in soil artificially contaminated with a range of heavy metals at various concentrations like lead (300, 350 and 400 mg/kg), chromium (50,100 and 150 mg/kg) and cadmium (100, 150 and 200 mg/kg). In this case, 5 mM EDTA was applied, as chelating agent to the plants after 4 weeks of sowing. Plants were grown for a total of two months and fresh weight and dry weight of shoot and heavy metal accumulation were analyzed at 6 and 8 weeks after sowing. The findings revealed that the described heavy metals application and EDTA adversely affected shoot length, fresh weight and dry weight of the biological matrix used at both time intervals. Hence, heavy metals uptake increased with the increment of heavy metal by the used plant. The use of 5mM EDTA upgrades the uptake of heavy metal [40-42]. It has been reported that EDTA enhances the availability of heavy metal to plants and thus increases the accumulation in their shoots [43-45].

Final Remarks and future research outlook

Basic and fundamental advances have been made in those last decades in understanding the processes implicated in metal phytoextraction from the contaminated soils. In the case of the chemically-assisted phytoextraction, the metal chelates dynamics in the rhizosphere have to be examined, either to overcome the risks associated with the low synthetic chelators degradability, or to optimize the use of more biodegradable compounds. In addition, researchers and policy makers have also to look for into the chemical pools of metals in soils and identify which ones are the targets for phytoextraction. A complete understanding of plant metal tolerance will be crucial to develop strategies to ameliorate the plant metal accumulation capacity. This will have significant involvements for phytoremediation. Since most of the known hyperaccumulator species are slow-growing and have small biomass, expressing their metal-accumulating genes in fast-growing, high biomass plants, is a promising approach for developing plants that can be used as novel tools in phytoextraction.

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