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BEATA SMOLIŃSKA
KATARZYNA KRÓL
Instytut Podstaw Chemii Żywności
Politechnika Łódzka

PHYTOEXTRACTION OF HEAVY METAL CONTAMINATED SOILS

Review: **Joanna Leszczyńska, Ph.D.**

This work presents the problem of soil pollution by heavy metals and its negative influence on the living organisms. In this paper the phytoremediation technique of cleaning soil polluted by heavy metals has been described. The use of plants to clean up soils polluted by heavy metals is more effective when the process is enhanced by different compounds. Some chemical enhancements like EDTA as well as natural substances, like low molecular weight organic acids (for example citric acid) can be used in the assisted phytoextraction.

This paper presents the latest reports about EDTA and citric acid assisted phytoextraction of lead, cadmium and zinc polluted soils. Moreover, the risks of using these chelators in assisted phytoextraction have been described.

Introduction

Heavy metals are natural constituents of the Earth's crust but human activities have drastically altered their geochemical cycles and biochemical balance in the biosphere [1]. In the last decades heavy metal pollution of soils is increasingly becoming a global problem with increasing development of industrial or mining activity, irrigation of waste water and the application of sewage sludge [2]. Because the soil-plant system is the elementary constructive unit of the geosphere and biosphere, heavy metal pollution of soil has an important influence not only on the yield and quality of crops, but also on the quality of atmospheric and aquatic environment [3]. Heavy metals are non-biodegradable and therefore display long-term persistence in aquatic and terrestrial ecosystems. There are potentially harmful to all biota and tend to accumulate in the food chain so that heavy metal contamination represents one of the most pressing threats to water and soil resources and to human health [4, 5, 6].

Heavy metal bioaccumulation in the food chain can be especially highly dangerous to human health. These metals enter the human body mainly through two routes namely: inhalation and ingestion, and with ingestion being the main route of exposure to these elements in human population. Heavy metals intake by human populations through the food chain has been reported in many countries with this problem receiving increasing attention from the public as well as governmental agencies, particularly in developing countries [7].

Heavy metal-contaminated soil is one of the widespread global problems. Removal of this persistent pollutant is necessary but very difficult. The remediation of large volumes of such soil by conventional physicochemical technologies previously developed for small, heavily contaminated sites would be very expensive. Phytoremediation of heavy metal-contaminated soil is an emerging technology that aims to extract or inactivate metals in soils. It has attracted attention in recent years for the low cost of implementation and environmental benefits. Moreover, the technology is likely to be more acceptable to the public than other traditional methods [8, 9, 10].

Heavy metals in soil and their influence on biota

Heavy metals, such as lead, cadmium and zinc, are important environmental pollutants, particularly in areas with high anthropogenic pressure. Their presence in the atmosphere, soil and water, even in traces, can cause serious problems to all organisms [11]. Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food quality, crop growth and soil flora, fauna and terrestrial animals health [7].

Lead

According to the Environmental Protection Agency (EPA), lead (Pb) is the most common heavy metal contaminant in the environment [12]. Human activities such as mining, smelting, burning of fossil fuels, dumping of municipal sewage sludge, and the manufacture of pesticides and fertilizers are the primary causes [4].

This element is a biologically nonessential, highly toxic to humans as well as animal reproduction and development, and known to adversely affect plant seed germination, nutrient assimilation, photosynthesis and growth [12, 13].

The concentrations of lead in soil depends mostly on soil exposure on pollution. In natural soils, which are not directly contaminated by lead, concentration of this element is rather low (geochemical background). In Poland, the mean values of lead concentrations for non-polluted sites are in the level of 25-40 ppm. The mobility of lead in soil is low. Lead is seldom present in soil solution in form of Pb^{2+} ion. This element forms complex compounds, like $PbOH^{+}$ and $Pb(OH)_4^{2-}$, which stimulate sorption and desorption processes [4].

Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. In many plants, Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human [14]. The introduction of Pb into the food chain may also affect human health [7].

Zinc

Zinc is one of the most common elements in the world as it naturally occurs in the earth's crust and is found in air, soil, water, and is present in most foods. Zinc can also enter the environment from a variety of human activities such as mining, steel production, coal burning, and burning of waste [4].

Zinc in soils is one of the most mobile element. The most common forms of its presence in soil are complexes, like $ZnOH^+$, $ZnHCO_3^+$, $Zn(OH)_3^-$ and ZnO_2^- [4]. Total zinc content of soils is largely dependent on the composition of the parent rock materials, but also on Zn application in fertilizers, pesticides and waste materials.

Only forms of zinc which are soluble or may be solubilised are bioavailable. Therefore, it is important to distinguish between the total quantities of metal and the amounts which can be converted into more soluble forms. Soil organic matter is an important soil constituent which forms complexes with zinc. Reactions between zinc and soil organic matter have been widely studied because of the frequent occurrence of zinc deficiency in organic soils [15].

Zinc is an essential trace element (micronutrient) required in small but critical amounts by both plants and animals (including humans). Plants absorb Zn primarily as a divalent cation, which acts either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a large number of enzymes [15].

Cadmium

Cadmium levels in the environment vary widely. In soils it is derived from both natural and anthropogenic sources. Natural sources include underlying bedrock or transported parent material such as glacial till and alluvium. Anthropogenic input of cadmium to soils occurs by aerial deposition and sewage sludge, manure and phosphate fertilizer application [16].

The Cd^{2+} ion is present in rocks, mostly in basic magmatic ones, where its concentration is on the level 0.03-0.22 ppm. In soils Cd is much less mobile than in air and water. The major factors governing cadmium speciation, adsorption and distribution in soils are pH, soluble organic matter content, hydrous metal oxide content, clay content and type, presence of organic and inorganic ligands. Cadmium is present in soils in form of Cd^{2+} ion, as well as in form of complexes, like $CdOH^+$, $CdHCO_3^-$, $CdCl^-$ and $Cd(OH)_4^{2-}$. It can also form chelates with soil organic substance [4].

This element can be take up and accumulate by plants. In plant tissues Cd is translocated through cation transporters to all parts of plant and causes stunted growth, with poor rooting and pale leaves [17]. Metabolic impairments by interaction of Cd^{2+} ions with the sulphydryl group of enzymes, hormonal imbalance, and reduced iron and mineral uptake account for the cadmium effects in plants [18, 19].

Phytoremediation

Conventional technologies suitable for water and soil remediation used *in situ* and *ex situ* are: soil flushing, pneumatic fracturing, solidification/stabilization, vitrification, electrokinetics, chemical reduction/oxidation, soil washing, and excavation, retrieval, and off-site disposal. These technologies are cost-prohibitive and processes often generate secondary waste. Compared to existing physical and chemical methods of soil remediation, the use of plants is cost-effective and less disruptive for the environment [20, 21].

Phytoremediation can be defined as the use of plants, including trees and grasses, to remove, destroy, or sequester hazardous contaminants from media, such as soil, water, and air. It is being investigated and/or used commercially to treat a variety of contaminants [20, 22].

The basic idea of phytoremediation technique is the use of these plant species, which accumulate extraordinarily high metal levels in their tissues, have a high biomass and short vegetation period. The data presented by McGrath and Zhao (2003) [23] pointed 400 plant species, including members of the *Asteraceae*, *Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae*, that have been identified as a natural metal hyperaccumulators. Metal hyperaccumulators are highly attractive model organisms, because they have overcome major physiological obstacles limiting metal accumulation in shoots and metal tolerance. Hyperaccumulators have often been isolated from nature in areas of high contamination or high metal concentration [21]. Metal hyperaccumulating plants occur on metal-rich soils and accumulate metals in their aboveground tissues, to concentrations between one and three orders of magnitude higher than surrounding plants grown at the same place [24]. Unfortunately hyperaccumulating plants are characterized by slow growth and limited biomass production. Consequently, nowadays, fast-growing, high biomass plant species that accumulate moderate levels of metals in their shoots are actively being tested for their metal phytoremediation potential [25].

Depending on the contaminants, the site conditions, the level of clean-up required, and the types of plants, phytoremediation technology can be used for containment or removal purposes. There are four different plant-based technologies of phytoremediation, each having a different mechanism of action for remediating metal-polluted soil, sediment, or water:

- phytostabilization, where plants stabilize, rather than remove contaminants by plant roots metal retention;

- phytofiltration, involving plants to clean various aquatic environments;
- phytovolatilization, utilizing plants to extract certain metals from soil and then release them into the atmosphere by volatilization;
- phytoextraction, in which plants absorb metals from soil and translocate them to harvestable shoots where they accumulate [26, 27].

Phytoextraction

Phytoextraction, also called phytoaccumulation, refers to the uptake and translocation of metal contaminants in the soil by plant roots into above-ground components of the plants [21].

Bioconcentrating of heavy metals by plants is well established. Plants growing in metal-contaminated and polluted terrestrial ecosystems take up toxic heavy metals and bind them, thus helping in both environmental decontamination and recovery of metals [20, 28]. Phytoextraction is the use of plants to absorb contaminants from soil into plant roots, in many cases eventually to be translocated and concentrated in shoots or other aboveground organs. Phytoextraction is most commonly used for the recovery of metals [20, 29]. For plant roots, in order to take up metals from soil, the soil-bound metal must first be solubilized. This could be accomplished by secretion of metal-chelating molecules or metal reductases by the plant roots. Once solubilized, the metal ions may enter roots via extracellular or intracellular pathways, with the intracellular pathways generally requiring the presence of an ion channel or a metal transport protein in the plasma membrane of the root cell. It has been speculated that some of these channels may be nonspecific, so that different metals can use them. Once metal ions reach the root, they can either be stored in the vacuoles in the root, often in the chelated form, or transported to the shoots [20].

Regardless of the plant used, phytoextraction process also depends on soil factors, such as cation exchange capacity, pH or organic matter content, which determine for example metal speciation [25, 30]. All of these soil parameters affect on the bioavailability of heavy metals to plant roots.

Assisted Phytoextraction

To overcome the problem of low bioavailability of heavy metals for plant roots some of enhancements, natural of chemical, have been used. In chemically enhanced phytoextraction, chelating agents are used almost exclusively as the mobilizing agents. Chelates desorb toxic metals from solid phases by forming strong water-soluble complexes, which can be removed from soil by plants through the phytoextraction process [29].

Wu et al. [31] reported that many synthetic chelators capable of inducing phytoextraction might form chemically and microbiologically stable complexes with heavy metals, threatening soil quality and groundwater contamination. Several chelating agents, such as EDTA (ethylenediaminetetraacetic acid), EGTA (ethylene glycol-O,O′-bis-[2-amino-ethyl]-N,N,N′,N′,-tetra acetic acid), EDDHA (ethylene diaminedi-o-hyroxyphenylacetic acid), EDDS (ethylenediaminedisuccinate) and citric acid, have been found to enhance phytoextraction by mobilizing metals and increasing metal accumulation [26, 32, 33].

Unfortunately, chelator application in chemically assisted phytoextraction may also have potentially environmental risks. Firstly, some chelators themselves are usually phytotoxic, and increasing metal solubility by them may be also phytotoxic to non-hyperaccumulator plants, thus plant growth may be inhibited, and the chance of success with chemically assisted phytoextraction may be lowered [34]. Secondly, chelators cause possible leaching of metal chelates to groundwater, which may have toxic effects on soil microorganisms and soil microfauna, thus affect soil ecosystem stability and function [35, 36]. Therefore, environment-friendly and biodegradable chelators should be developed and used to assist phytoextraction [37].

In chemically enhanced phytoextraction, chelating agents are used almost exclusively as the mobilizing agents. Chelates desorb toxic metals from solid phases by forming strong water-soluble complexes, which can be removed from soil by plants through the phytoextraction process [38]. There are a lot of chemical substances, which can be used as a chelators during chemical assisted phytoextraction of soils polluted by lead, cadmium and zinc.

Recently, one of the common used chelator is ethylenediaminetetraacetic acid, EDTA. It is a synthetic chelating agent, which is very effective at complexing metals in soil, however it is non-selective chelator, therefore it can form a strong complex with a variety of metals. Application of this compound to soil polluted by heavy metals increase the metal solubility by dissolving the metal bounds with the soil particles. At the same time the increase of bioavailability and accumulation of pollutants in aboveground parts of plants are observed [39, 40].

Several authors have dealt with the enhanced uptake of heavy metals by addition of EDTA, which depends on the plant and heavy metal study [9]. The study of Luo et al. (2005) [41] pointed that the plant available zinc in soil increased 5 times after application of 5 mmol kg^{-1} of EDTA and the amount of plant available cadmium increased over 114-fold, in the same experimental conditions. The significant increase of plant available lead, zinc and cadmium were observed by Lai and Chen (2005) during the experiment where EDTA in concentration 5 mmol kg^{-1} was applicated to the soil [42]. Unfortunately, these studies showed that the EDTA chelator had the negative effect on plant growth. In investigation conducted by Luo et al. (2005) [41] the 40 % decrease of plant biomass of corn (Zea *mays*), as well as disease syndromes like chlorosis and necrosis, were observed. Moreover, the study showed that the metal uptake is increased by the application of EDTA, but heavy metals amount mobilized in the soil is higher than the uptake.

Although the use of EDTA as a chelator in chemical assisted phytoextraction can be effective in cleaning soil polluted by lead, cadmium and zinc [40, 41] its presence in the soil can lead to leaching of heavy metals to groundwater. As a consequence EDTA or EDTA-heavy metal complexes can occur at higher concentration in rivers water, causing their pollution.

The different way of enhancing the metal bioavailability in soils is amendment of substances which are natural or made of natural components and which has no potential negative influence on the plant growth. In this group of substances is a group of natural low molecular weight organic acids (NLMWOA) and compost.

Low molecular weight organic acids in soil are produced by plant roots, microorganisms, or during the degradation of soil organic matter. The acids which belong to the NLMWOA are simple carboxylic acids exuded by plant roots, like for example citric, oxalic, tartaric or acetic acids [43]. These acids have the potential to enhance metal mobility in soil profiles by reducing soil pH and forming complexes with heavy metals [43, 44]. The exudation of organic compounds by roots may influence the solubility of ions in soil. In the directly way of transforming the ions into the soil solution the acidification, chelation, precipitation and redox reactions in the rhizosphere are observed. The solubility of ions in soil can be also increased indirectly by effecting the microbial activity, changing physical properties in the rhizosphere or root growth dynamics [45].

There are research papers where authors used NLMWOA, mostly citric acid, to enhance the phytoextraction of lead, zinc and cadmium polluted soils. Quartacci et al. (2005) [46] pointed that application of 10 mmol kg^{-1} of citric acid into soil contaminated by cadmium increased the heavy metal uptake by Indian mustard (*Barssica juncea*) of about 1.5-3 times, depending on the method of experiment. The very important factor is that citric acid had no negative influence in the plant growth and plant biomass. In the case of lead phytoextraction by Indian mustard (*Barssica juncea*) Wu et al. (2004) proved that addition of citric acid in concentration 3 mmol kg^{-1} to soil polluted by lead increased heavy metal uptake over twice. In the experiments conducted for soil polluted by zinc and cadmium the authors did not observe any significant changes in metal uptake. Huang et al. (1998) [48] stated that citric acid is advantageous in the use of chelate assisted phytoextraction, because it is easily biodegradable and rapidly degrades to carbon dioxide and water. Depending on the concentration of heavy metals in soil, concentration of chelator and plant spices the influence of citric acid on the phytoextraction process can be observed.

Conclusions

Assisted phytoextraction has been described as a promising technique to remediate the heavy metal contaminated sites. The laboratory and field experiments carried out with the use of chelating agents showed that chelators have the potential to increase the heavy metal bioavailability in soils. Moreover, the application of chelators in many studies increased the heavy metal uptake and their translocation form roots to shoots of the plant. Unfortunately, the common used chelators, like EDTA or citric acid, can indirectly caused water pollution by transferring the chelator-heavy metal complexes to the groundwater. This can be lessened by reducing the concentrations of chelating agents applicated to soils, but in the same time the phytoextraction process can be limited. All of the advantages and disadvantages of using chelator induced phytoextraction should be considered before starting cleaning sites polluted by heavy metals with the use of plants.

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FITOEKSTRAKCJA WSPOMAGANA GLEB ZANIECZYSZCZONYCH METALAMI CIĘŻKIMI

Streszczenie

W pracy zaprezentowany został problem zanieczyszczenia gleb metalami ciężkimi, wraz z zagrożeniami dla organizmów żywych, jakie niosą wysokie koncentracje metali w glebach. Zastosowanie roślin wyższych w procesie oczyszczania gleb zanieczyszczonych metalami ciężkimi jest bardziej efektywne, podczas stosowania substancji wspomagających proces. Obok związków chemicznych, stosowanych w procesie fitoekstrakcji wspomaganej, takich jak EDTA, na uwagę zasługują substancje naturalne, takie jak m.in. naturalne kwasy organiczne o niskiej masie cząsteczkowej (m.in. kwas cytrynowy).

W pracy przedstawiono najnowsze doniesienia literaturowe dotyczące fitoekstrakcji wspomaganej gleb zanieczyszczonych ołowiem, cynkiem i kadmem. Ponadto przedstawiono potencjalne ryzyko środowiskowe stosowania substancji wspomagających proces.