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REVIEW

Plant growth regulators-assisted phytoextraction

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Abstract

Plant growth regulators (PRG)-assisted phytoremediation is a technique that could enhance the yield of heavy metal accumulation in plant tissues. So far, a small number of experiments have helped identify three groups of plant hormones that may be useful for this purpose: auxins, cytokinins, and gibberellins. Studies have shown that these hormones positively affect the degree of accumulation of metallic impurities and improve the growth and stress resistance of plants. This review summarizes the present knowledge about PGRs' impact on phytoextraction yield.

Additional key words: auxins, cytokinins, gibberelins, heavy metals, phytoremediation, pollutants

Introduction

Anthropogenic pollution of the environment is one of the most important factors contributing to the destruction of the biosphere. Processes such as power generation using fossil fuels, incineration, smelting and mining iron and nonferrous metals are sources of large emissions of many elements (Kabata-Pendias 2011). The increasing amounts of heavy metals in soils are also connected with waste disposal sites, sewage sludge treatment, agricultural fertilizers, application of pesticides, chemical disposal sites, and military shooting areas (Singh *et al.* 2009, Barbafieri and Tassi 2011). These metals and metalloids have great and complex impacts on the environment. Many of them are directly toxic to living organisms or indirectly promote degradation of the biosphere (Appenroth 2010). For example, the interaction between gaseous pollutants and atmospheric trace elements can result in the oxidation of SO_2 to SO_4^{2-}. This reaction may be catalyzed by Mn-rich dust. As stated in Kabata-Pendias (2011), the formation of HNO_2 aerosol from NO_2 is enhanced by Cu, Mo, and Zn. Moreover, acid rains decrease soil pH which favours the bioavailability of heavy metals and increase the strength of their toxic effects on biota (Kabata-Pendias 2011).

From all the 92 elements present in the Earth's crust, only 17 are known to be essential for normal plants growth, *i.e.*, carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium, magnesium, potassium, sulphur, chlorine, copper, boron, iron, manganese, molybdenum, nickel, and zinc. Another 12 are potentially beneficial but they are required only in trace amounts and by some plant species, *i.e.*, silver, cerium, chromium, fluorine, iodine, lanthanum, rubidium, tin, strontium, titanium, vanadium, and tungsten (Pilon-Smiths *et al.* 2009, Sarma 2011). Heavy metals (53 elements) are categorized into one group based on their density (> 5 g cm^{-3}) (Appenroth 2010). Some heavy metals, including Fe, Mn, Cu, Zn, and Ni are necessary for plant growth and functioning. They are involved in different enzymatic and redox reactions, carry electrons, or are involved in DNA and RNA metabolism (Zenk 1996, Miransari 2011). However, other heavy metals are not necessary for plant growth; in fact, their high content can adversely affect plant growth. Many elements (As, Be, Cd, Cu, Hg, Ni, Pb, Se, Tl, V, Zn, and some lanthanides and actinides) are considered

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Abbreviations: ABA - abscisic acid, BAP - 6-benzylaminopurine; EDDS - ethylenediamine-*N,N′*-disuccinic acid; EDTA - ethylenediaminetetraacetic acid; GA_3 - gibberellic acid; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; JA - jasmonic acid; NAA - naphthylacetic acid; NLMMOA - natural low molecular mass organic acids; NTA - nitrilotriacetic acid; PAA - phenylacetic acid; PGR - plant growth regulators.

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to be dangerous to the environment (Kabata-Pendias 2011). Their presence in the soil may cause a number of adverse changes in plants, *i.e.*, oxidative stress, damage of cellular structure, and decrease in growth rate (Bajguz and Hayat 2009, Israr *et al.* 2011).

Soil is one of the major sinks for contaminants in geochemical cycles and is also a natural buffer controlling the fluxes of chemical compounds to other environmental compartments. Good soil condition ensures its adequate productivity which sustains the biomass flow up the food web. The persistence of contaminants in soil is much greater than in the atmosphere, hydrosphere, or biota. The half-life times of trace elements for soils in the temperate climate are in the range from 75 to 380 years for Cd to 1 000 to 3 000 years for Ag, Cu, Ni, Pb, Se, and Zn (Kabata-Pendias 2011, Sarma 2011). The increasing amounts of trace elements and the decreasing soil pH are interrelated and are of great health and environmental concern.

Due to the progressive pollution of the biosphere, increasing attention is given to technologies used for cleaning the soil, water, and atmosphere. Soil can be remediated by physical, chemical, and biological treatments (Kabata-Pendias 2011). A major disadvantage of most of these technologies is high cost and destruction of the soil matrix. Physical and chemical techniques are often very difficult to use and require complicated tools. In contrast, phytoremediation techniques based on plants and/or microorganisms are environmentally friendly and economically feasible (Table 1). They are also widely accepted by local communities and regulatory agencies (Barbafieri and Tassi 2011). Phytoextraction is therefore an interesting, promising technique which theoretically allows the complete removal of contamination without damaging the soil structure.

Phytohormones are active in very low concentrations and they have a powerful effect on plant growth and development (Machackova *et al.* 2008). The applications of different PGRs allow the development of a variety of *in vitro* techniques and help improve our understanding the plant life cycle from seeds to maturity. Nowadays, another application of PGRs is rising in the field of phytoremediation and this review is focused on the application of PGRs to enhance the effectiveness of phytoextraction of trace elements.

Plant strategies to survive high levels of heavy metals

Plants tolerant to the presence of high concentrations of metals and metalloids in the soil are classified as metallophytes (Masarovičová *et al.* 2010). To prevent the toxic effects of high amounts of trace elements in the soil, metallophytes have evolved two major strategies (Baker 1981, Masarovičová *et al.* 2010):

Exclusion: True exclusion occurs when plants avoid translocation of metals to the tissues (Kamal *et al.* 2004, Gosh and Singh 2005). This strategy is based on the modification of the pH in the rhizosphere by secretion of organic acids from roots which bind to the metals and decrease their bioavailability. Other mechanisms involve the accumulation of metals in cell walls or in mycorrhizal fungi, and slime production which constitute a mechanical barrier to metal uptake on the surface of roots. Beyond a certain threshold dose, this mechanism usually breaks down and the metal is taken up by roots (Baker 1981, Sanitá Di Toppi and Gabrielli 1999, Gosh and Singh 2005). Shoot exclusion is a process based on the uptake and accumulation of metals only in roots and the maintenance of a constant, low concentration in shoots as long as the metal content in the soil do not reach some critical dosage (Kamal *et al.* 2004). This mechanism was found in species such as *Silene maritima*, *Armeria maritima*, *Thlaspi praecox*, *T. arvense*, *Cousinia calocephala, Bromus tectorum,* and *Goldbachia laevigata* (Baker 1978, Szarek-Łukaszewska *et al*. 2004, Vogel-Mikuš *et al*. 2005, Hajiboland and Manafi 2007, Martin *et al.* 2012).

Accumulation: Considering the ability of plants to accumulate heavy metals in the tissues, metal indicators, metal accumulators, and hyperaccumulators can be distinguished among plants. Metal indicators are able to sequester metals in aboveground tissues and the metal content in the plant reflect the metal content in the soil (Baker and Walker 1990, Gosh and Singh 2005). Metal indicators are species characteristic for soil contamination with specific metals, *e.g*. *Agave sisalana* (Zn, Pb, Ni), *Betula pubescens* (Ni, Cu), *Cyperus articulatus* (Zn), *Ludwigia stolonifera* (Cd, Pb), *Sphaeranthus kirkii* (Pb) (Kozlov *et al.* 1995, Mganga *et al*. 2011).

Metal accumulators are plants that concentrate the respective metal in the aboveground tissues where the content is higher than in the soil (Baker and Walker 1990, Kamal *et al*. 2004). Nevertheless, the concentration of metals in the metabolically active parts is maintained at low level. This is possible due to sequestration of metals into old leaves, the leaf epidermis, epidermal secretory cells, vacuoles, and cell walls (Baranowska-Morek and Wierzbicka 2004). Metals can also be secreted in the form of crystals on the leaf epidermis (Sarret *et al*. 2006).

Finally, there are plants that are able to concentrate metals in enormous amounts. The term ”hyperaccumulator” appeared for the first time in a publication by Brooks *et al.* (1974). Metal “hyperaccumulators” are plants that can accumulate greater metal concentrations in shoots than those usually found in non-accumulators (Baker and Walker 1990, Padmavathiamma and Li 2007). The phytoextraction is possible when the metal content in shoots is higher than 0.01 mg g^{-1}(d.m.) for Hg, 0.1 mg g^{-1}(d.m.) for Cd, 1 mg g^{-1}(d.m.) for Pb, Co, Cu, As, Se, and Ni, and 10 mg g^{-1}(d.m.) for Mn and Zn (Baker and Brooks 1989, Barbafieri and Tassi 2011). To date, there are more than 500 plant species known for their ability of heavy metal hyperaccumulation (Sarma 2011) including members of the *Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae* and *Lamiaceae* families (Padmavathiamma and Li 2007). About 75 % of them hyperaccumulate Ni, like *Alyssum bertolonii*, *Berkeya coddii*, and *Thlaspi goesingense*. Other hyperaccumulating species include *Thlaspi caerulescens* (Zn, Cd), *Sedum alfredii* (Zn, Cd), *Biscutella laevigata* (Pb, Tl), *Sesbania drummondii* (Pb), *Pteris vittata* (As), *Ipomoea alpine* (Cu), *Phytolacca americana* (Mn), and *Iberis intermedia* (Tl) (Padmavathiamma and Li 2007, Sarma, 2011, Tian *et al*. 2012). To become acquainted with the subject, the publication by Krämer (2010) is recommended.

Phytoremediation

Phytoremediation (Greek *phyto* meaning plant + Latin *remedium* meaning to clean or restore) is a universal, solar-driven, *in situ* treatment technique that uses selected or specialized plants and their associated microorganisms to extract, sequester, and/or detoxify nearly all pollutants from water, sediment, soil, and air (Cunningham and Berti 1993, Gosh and Singh 2005, Barbafieri and Tassi 2011, Sarma 2011). Pollutants can be divided into two major classes, organic and inorganic. The concentration of organic pollutants may be reduced by microbial or plant metabolism in such processes as rhizodegradation, phytodegradation, and phytovolatilization (Table 1). Contrarily, inorganics cannot be degraded and they accumulate up to harmful amounts with time. However, they can be stabilized or sequestered in harvestable plant tissues. The following techniques are applied: phyto-extraction (phytoaccumulation), phytostabilization, phytohydraulic control, and rhizofiltration (Table 1).

As every technology, phytoremediation has some limitations. It is generally slower than most other treatments and often requires several vegetation cycles to achieve a significant reduction of the contamination. The other limitation is the plants' ability to grow and survive under extreme conditions, such as high toxicity and low fertility, *etc*. When multiple pollution occurs, plants selected for phytoremediation should be able to overcome these limitations (Gosh and Singh 2005, Evangelou *et al*. 2007, Van Nevel *et al.* 2007).

Table 1. Types of phytoremediation (based on Gosh and Singh 2005, Padmavathiamma and Li 2007, Barbafieri and Tassi 2011, and Gawronski *et al*. 2011).

Contaminant	Phytoremediation technology	How it works?
Organic	rhizodegradation	Plant roots secrete organic compounds such as sugars, proteins, organic acids, and enzymes that support the growth of microorganisms capable to degrade pollutants. Enzymes in plants exudates are also responsible for the pollutant degradations. This process is much slower than phytodegradation.
	phytodegradation (phytotransformation)	Direct absorption and breakdown of organic compounds from soil or water by plants. This process can occur in both root and shoot tissues. In some cases, endophytic microorganisms may be involved. Degradation products may be subject to the lignification or mineralization (complete degradation).
Organic/inorganic	phytovolatilization	This process includes the uptake and volatilization of contaminants. As, Hg, and Se can be transformed into volatile organic compounds. Phytovolatilization has a restricted usage and is the most controversial of phyto-technologies because it only involves the transport of the contaminants from the soil or water to the atmosphere.
Inorganic	phytoextraction (phytoaccumulation)	This term refers to metal uptake, translocation and accumulation in the aboveground organs. This technique is based on hyperaccumulators, such as easy cultivated plant species having high biomass, tolerant to metal contamination, and able to accumulate the high amounts of pollutants.
	phytostabilization	Process based on the immobilization of contaminants through adsorption onto root surface, absorption and accumulation by roots, or precipitation within root zone and physical stabilization in soil. The main aim of phytostabilization is to reduce the mobility of contaminants to groundwater or air. Phystostabilization is less expensive and easier than other technologies.
	phytohydraulic control	Usage of plant (especially tree) ability to transport high amounts of water upward in the root zone and thus to prevent the contamination from leaching down or spreading horizontally.
	rhizofiltration (phytofiltration)	Extraction of metals and radionuclides from groundwater, surface water, and waste water. Contaminants are accumulated, absorbed, and precipitated in dense root net and can be removed by harvesting the plants.

Phytoextraction

Phytoextraction can be applied in the treatment of soil or water contaminated with heavy metals, metalloids, and radionuclides (Padmavathiamma and Li 2007). The time required for phytoextraction depends on the type and extent of contamination, the duration of growing season, the climate, the biomass yield, and the capacity of the plants for metal accumulation (Padmavathiamma and Li 2007). Phytoextraction undoubtedly has numerous advantages, but the most important challenge in improving the efficiency of phytoextraction is to increase the accumulation of metals in plants (Wu and Tang 2009, Sarma 2011). Besides, plants favourable to phyto-remediation need to be fast growing, having a high biomass, tolerant to pollution, and with a high translocation factor (*i.e.*, the ratio of the metal concentration in shoots to the metal concentration in roots). It is worth mentioning that the contaminant must be in a plant-available form, which may be problematic, *e.g.*, with Pb pollution (Vogel-Mikuš *et al.* 2005, Padmavathiamma and Li 2007, Sarma 2011). Generally, phytoextraction is applicable to areas with low-to-moderate metal contamination (Barbafieri and Tassi 2011, Sarma 2011).

Continuous phytoextraction: This is a long-term technique, which is based on the use of natural hyperaccumulating plants. These plants are grown in contaminated soil during the growth season (several weeks or months) after which they are harvested and disposed of. This growing cycle is repeated until the contamination will be satisfactorily reduced (Padmavathiamma and Li 2007).

Chelate-assisted phytoextraction: In contrast to continuous phytoextraction, which relies on natural metal hyperaccumulators, assisted phytoextraction involves tolerant plant species and supplementary agents to increase the contaminant bioavailability and accu-mulation (Gosh and Singh 2007, Barbafieri and Tassi 2011). The best known assisted phytoextraction method is phytoextraction using synthetic complexing agents such as ethylenediaminetetraacetic acid (EDTA), ethylenediamine-*N*,*N'*-disuccinic acid (EDDS), nitrilo-triacetic acid (NTA), organic acids, or ion competitors to enhance metal mobility and bioavailability (Tassi *et al.* 2004, Gosh and Singh 2007, Barbafieri and Tassi 2011). Chelating agents increase the bioavailability of metals by direct metal chelation. As it was proposed by Sarret *et al.* (2001), the highly stable metal-EDTA chelates can be taken up by plants from the soil solution and totally or partially dissociated in the plant tissues. The effectiveness of these methods depend on the type of chelating compound, the amount of chelator which was applied, the type of metal and its content in the soil, the soil itself, and the plant species (Evangelou *et al.* 2007). The major advantage of this technique is a higher accumulation of metals in the plant tissues, which can be over 100 times higher than without chelators (Evangelou *et al.* 2007). However, there is a potential risk of leaching metals to groundwater (Padmavathiamma and Li 2007). Complexing agents also have negative effects on the soil microorganisms (Barbafieri and Tassi 2011). Moreover, EDTA and its degradation products are persistent in soil (Yuan and VanBriesen 2006, Evangelou *et al.* 2007, Hadi *et al.* 2010).

Natural low molecular mass organic acids (NLMMOA), such as citric acid, oxalic acid, or malic acid, are natural constituents of root exudates (Evangelou *et al.* 2007). These compounds are secreted in larger quantities especially during stress and can increase the heavy metal solubility both directly and indirectly (Jones and Darrah 1995). Directly by acidification of rhizo-sphere, chelation, and oxidation-reduction reactions with heavy metals, and indirectly by interactions with soil microbiota, rhizosphere physical properties, and by modification of root growth (López-Bucio *et al.* 2000, Evangelou *et al.* 2007). Compared to synthetic chelators, NLMMOA are completely and rapidly biodegradable. They also makes continuous use possible (Evangelou *et al.* 2007). The use of NLMMOA in the case of some metals like U gives significantly better results than the use of synthetic chelators, wherease with other metals, for example Pb, the efficiency is low (Huang *et al.* 1998, Evangelou *et al.* 2006). It depends strongly on the plant species which may be also considered as a disadvantage of this technique (Huang *et al.* 1998).

Ion competition is a phenomenon well known in plant nutrition. Competition occurs, for example, in the case of P and As. Due to the similarity of the chemical properties between P and As, both elements are absorbed into the plant by the phosphate transport system (Meharg and Hartley-Whitaker 2002), thus in the absence of phosphorus, absorption of As should be increased (Santos *et al.* 2008). These assumptions have been confirmed experimentally by Wang *et al.* (2002) and Santos *et al.* (2008), who stated that in P-starved plants the uptake of As was higher compared to the control, and by Tu *et al.* (2004), who found that the addition of P in hydroponic cultures decreased As uptake. However, Tassi *et al.* (2004) found that addition of ammonium phosphate to the As contaminated soil can increased As uptake by increasing the water-soluble As concentration.

Phytoextraction assisted by plant growth regulators

Phytoextraction that is aided by phytohormone treatments can be called PGR-assisted phytoextraction (Barbafieri and Tassi 2011). There are five classical phytohormones: auxins, cytokinins, gibberellins, ethylene, and abscisic acid (ABA). In addition, there is also a class of "new plant hormones" which includes brassinosteroids, salicylic acid, jasmonates, and stringolactones. In addition, oligosaccharins, systemin, polyamines, and nitric oxide have been shown to possess activities similar to those of plant hormones (Machackova *et al*. 2008, Chen *et al*. 2009). The use of plant growth-promoting substances improves phytoextraction by increasing both the shoot and root growth and the biomass yield, and enhances the effectiveness of antioxidation systems in plants, thereby facilitating the greater acquisition and accumulation of trace elements (Liphadzi *et al*. 2006, Israr *et al*. 2011). However, hyperaccumulation mechanisms have not yet been fully understood (Yang *et al*. 2005, Verbruggen *et al*. 2009). Hyperaccumulation of metals in the plant depends on the efficient operation of the various mechanisms in the different parts of the plant. These mechanisms include the transport of metals to the root cells by plasma membrane, xylem loading and the translocation of metals to the upper parts of plants, and the detoxification and sequestration of metals in tissues. A good insight into the molecular mechanisms of heavy metal hyperaccumulation can be found in publications by Yang *et al*. (2005) and Verbruggen *et al*. (2009). How the exogenous phytohormones affect the heavy metal accumulation yield is a matter of conjecture, but we can guess that they work through these mechanisms.

The use of PGR (see Table 1 Suppl.) may result in benefits without risk of leaching metals or other negative environmental impacts (López *et al*. 2005). Moreover, the chelate- and PGRs-assisted methods are often combined and used together to enhance phytoextraction efficiency (López *et al*. 2005, Hadi *et al*. 2010).

Auxins: Auxins are involved in cell division and elongation, growth, maturation, and organ differentiation. Auxins have an important impact on tropisms and are directly involved in cation uptake and cation fluxes (Vamerali *et al*. 2011). Their most marked effect is the maintenance of apical dominance. Auxins, together with cytokinins, have the ability to manage key events in plant morphogenesis. The most commonly detected natural auxin is indole-3-acetic acid (IAA), but endogenous 4-chloro-IAA, indole-3-butyric acid (IBA), and weak auxin phenylacetic acid (PAA) also occur (Machackova *et al*. 2008).

In hydroponics, the effect of 1 to 100 µM IAA for 100 to 200 µM Pb was frequently tested. The 10 to 100 µM IAA treatment enhanced Pb accumulation in the roots of *Medicago sativa* by about 20 - 40 % (López *et al*. 2005). In *Sedum alfredii*, a 10 - 100 µM IAA treatment significantly increased Pb accumulation in the shoots of both hyperaccumulating (HA) and non-hyperaccumulating (NHA) ecotypes, but 2.7-times more in the HA than in the NHA. However, this treatment had no effect on the root Pb accumulation of both the ecotypes (Liu *et al*. 2007).. In *Picris divaricata*, 10 - 100 µM IAA increased the Pb content in leaves by about 28.4 to 37.3 %, but not in roots (Du *et al*. 2011). Wang *et al.* (2007) tested the effects of 250 and 500 µM IAA on *Zea mays* grown under 20 µM Pb and observed that the Pb content in roots increased with increasing concentrations of IAA but the Pb content in shoots showed an inverse tendency. In an experiment with *Helianthus annuus* grown at 2.5 µM Pb and 15 µM Zn, an application of 0.001 to 1 nM IAA had no significant effect on the Pb content in both shoots and roots (Fässler *et al*. 2010).

In a pot experiment with *Z. mays*, the application of IBA or naphthylacetic acid (NAA) increased Pb uptake by 41.2 and 127.4 %, respectively, whereas NAA also increased Zn uptake by 59.5 % (Fuentes *et al*. 2000). In *H. annuus* growing in polluted soil or after spraying with a composed sludge (3 or 6 mg dm^{-3}), 17, or 34 µM IAA caused high accumulation of Mn and Ni in leaves. When plants were grown in a composted sludge, they also accumulated Cd and Pb (Liphadzi *et al*. 2006). *Z. mays* grown in a mixture of clay, sand, and humus with addition of 500 mg Pb kg^{-1} showed a significant (4-fold) increase in Pb accumulation in leaves, stems, and roots when foliar spray with 10 µM IAA was used (Hadi *et al*. 2010). When *Raphanus sativus* was grown in a mixture of metalloid-polluted pyrite cinders, the enhancement of accumulation of Co, Cu, and Pb in shoots was observed when 1 mg kg^{-1} IBA was added to the substrate. When IBA was added to the substrate together with foliar spray, the concentrations of As and Zn in shoots was the highest. The same treatment resulted in the highest concentration of As, Co, Cu, and Zn in roots (Vamerali *et al*. 2011).

Cytokinins: Cytokinins promote cell division and differentiation, leaf expansion, bud formation, accumulation of chlorophyll, and the conversion of etioplasts to chloroplasts. Cytokinins control development and senescence, enhance plant resistance to salinity, low temperatures, and drought, act both in synergy and antagonism with other plant hormones, and play a role in the regulation of seed development, dormancy, and germination (Tassi *et al*. 2008). As cytokinins can increase stomatal opening (Pospíšilová *et al*. 2000), they can also enhance the efficiency of phytoextraction. Water taken up by roots carries dissolved mineral nutrients and soluble contaminants. The greater the rate of transpiration, the more contaminants will be transferred from soil to plants. In addition, the enhanced evolution of ethylene and ABA in plant tissues as a result of environmental stresses causes a decrease in stomatal conductance (Lipiec 2012) and the use of cytokinins may counteract these changes.

Tassi *et al*. (2008) examined the effects of kinetin and the commercially available mixtures of cytokinins, gibberellins, auxins, vitamins, and microelements *Cytokin* (*Miler Chemical & Fertilizer Corporation*, Hanover, USA) and *Phytagro* (*Miler Chemical & Fertilizer Corporation*, St. Louis, USA) on *H. annuus* grown in Pb and Zn contaminated soil. All compounds increase Pb and Zn accumulation in sunflower leaves and so phytoextraction efficiency. Cassina *et al*. (2012) tested *H. annuus* and *Brassica juncea* grown in Hg contaminated soil and under *Cytokin* treatment. *Cytokin* induces a 16 % decrease of Hg content in all tissues, except in the roots of *B. juncea* (no significantly different), as well as 53 and 68 % decrease of Hg content in shoots and roots of *H. annuus*. In *Pennisetum glaucum* grown in a soil mixed with solid tannery waste, 6-benzylaminopurine (BAP) increased the metal (Cr>Fe>Cu >Cd>Na>Zn) extraction potential (Bareen *et al*. 2012).

Gibberellins: Gibberellins are diterpenoid compounds involved in flowering, seed germination, sex determination, stem elongation, leaf expansion, trichome, flower, and fruit development, and transition from the juvenile to adult phase in plant growth (Khan and Chaudhry 2006). Exogenously applied gibberellins protect the photosynthetic apparatus against heavy metal stress (Ouzounidou and Ilias 2005). The most widely used is gibberellic acid (GA_3).

Hadi *et al*. (2010) tested the possibility of gibberellins to enhance metal phytoextraction by *Z. mays*. A significant increase in Pb uptake by roots and its translocation through stem to leaves were observed. The content of Pb in leaves was about five times higher than that in the control without GA_3. The percentage of Pb in roots was 67 % of the total Pb accumulated in shoots when plants were treated only with GA_3 and 69 and 68 % when plants were treated with GA_3 + EDTA or IAA + EDTA, respectively (Hadi *et al*. 2010).

Other growth regulators: There are several compounds which have hormone-like effects on plants (Balakhnina *et al*. 2009). Little is known about the stringolactone exudates from plant roots, which induce hyphal branching in arbuscular mycorrhizal fungi. Mycorrhizal fungi support plant growth and development and can be useful in the phytoremediation process, as they enhance host plant tolerance to abiotic and biotic stresses (Chen *et al*. 2009).

In the studies on *Wolffia arrhiza*, Piotrowska *et al*. (2009) found that jasmonic acid (JA) acts in a concentration-dependent manner. A treatment with 100 μM JA results in an enhancement of heavy metal absorption and heavy metal toxicity, but at a concentration of 0.1 μM, JA protects plants by inhibiting heavy metal accumulation. JA of this concentration also activates the enzymatic and non-enzymatic antioxidant systems and suppresses oxidative destruction of cellular components.

Comparison of chelate-assisted and PGRs-assisted phytoextraction: In general, chelates increase the translocation factor which increases the accumulation of metals in the aboveground parts, however, different studies show different results. For example, López *et al*. (2005) found that a 10 - 100 μM IAA treatment enhances the accumulation of Pb mainly in roots and stems, whereas EDTA (0.2 mM) decreases Pb accumulation. When these agents were used together, they show dramatically increased Pb accumulation in leaves of about 2 800 %. Similar results were obtained by Hadi *et al*. (2010). In comparison to 1 μM GA_3 and IAA treatments, the use of 400 mg EDTA per kg of soil enhances the accumulation of Pb by about 2.4 times in leaves and by about two times in roots, however, the highest accumulation was achieved when both EDTA and phytohormones were used simultaneously (Hadi *et al*. 2010). On the other hand, in an experiment of Du *et al*. (2011), 10 - 100 μM IAA induced higher accumulation of Pb in leaves and roots compared to the 100 μM EDTA treatment. This effect probably occurs because the plant species used (*Picris divaricata*) is unable to take up the Pb-EDTA complex. These findings show that the efficiency of heavy metal uptake varies strongly depending on the plant species, the plant organ, and the treatment used (PGRs or chelators or both).

Conclusions

Phytoextraction assisted by plant growth regulators has many positive and desirable effects. Apart from increasing the degree of accumulation and translocation of heavy metals, PGRs also protect the photosynthetic apparatus from oxidative shock induced by heavy metals, increase root length, shoot growth, and biomass accumulation, enhance the transpiration rate, flowering, and yield.

The biggest challenge of this technique seems to be its efficiency. Compared to chelate-assisted phytoextraction, plant hormone stimulation produces an order of magnitude a lower degree of metal accumulation. These two methods are often combined together due to their synergistic action. Chelate agents cause a high metal accumulation rate, whereas PGRs reduce the negative effects of stress caused by pollution and even further increase the metal accumulation.

The manner of application of PGRs has also an impact on phytoextraction effectiveness. The most effective method of application is spraying or the direct addition of hormones to the substrate. Seed soaking in solutions of hormones usually does not result in increased accumulation.

The current state of knowledge of the use of PGRs in phytoremediation indicates the complexity of the research problem, many aspects of which still need to be discovered.

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