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Review

Phytoremediation of heavy metals with several efficiency enhancer methods

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It is no doubt that the contamination of water, air and soil has worsened, and this occurs as a result of the increase in population. However, the need for remediation technologies has to be seriously considered. Phytoremediation is one of the remediation techniques with a relatively slow procedure and low efficiency. This review covers some of the biological, chemical, physical, physico-chemical and genetic methods, which were applied in parallel with phytoremediation, in an attempt to help increase the efficiency in the remediation of air, soil and water. These include lowering the pH and increasing the electrode potential (Eh), as well as using chelating agents and micro-organisms (arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR)).Among the introduced methods, an appropriate application of the PGPRs is one of the most useful and environmentally friendly techniques which is currently considered as a useful process in phytoremediation. As a result of the discovering of these new methods, multi-approaches have been executed for a faster and higher removal rate of the contaminants, with a consequent increase in the efficiency of phytoremediation, as compared to single techniques.

Key words: phytoremediation, heavy metal, plant growth promoting rhizobacteria, multi-functional method.

INTRODUCTION

Due to global industrialization and the increase in human population in the twentieth century, heavy metal contamination of soil, water and air has posed various uncompromising and fatal effects on humans and the stability of the ecosystem. Unlike organic contaminants, heavy metals are not biologically degradable, and therefore can remain in environmental bodies for a long time. The term 'heavy metal' has different definitions, but it is mostly used in the context of environmental pollution. Among others, Shaw et al. (2004) explained four criteria in distinguishing the groups of heavy metal: 1) Relatively abundant in the earth's crust; 2) reasonable extraction and usage; 3) having direct contact with people; and 4) toxic to humans. Another definition describes heavy metals as the metals which have a specific gravity of more than 4 (Anonymous, 1964; Nieboer and Richardson, 1980) or more than 5 (Lapedes, 1974; Nieboer and Richardson, 1980).

Most heavy metals are categorized as toxic and accessible, based on the classification of Wood (1974), and their concentrations in soil vary between 1 to 100,000 mg/kg (Blaylock and Huang, 2000). The plant toxicity of heavy metals differs according to plant species; for flowering plants the toxicity may appear as AS(III)~ Hg>Cd>Tl>Se(IV)>Pb>Bi~Sb (Fergusson, 1990). Never theless, it is important to highlight that many factors can influence this sequence. These include the properties of soil and the type of plants. There are generally four major soil remediation methods (Ward and Singh, 2004), namely:

1. Physical remediation: thermal desorption, cement kiln, air stripping and incineration;

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Abbreviations: Eh, Electrode potential; **AMF,** mycorrhizal fungi rhizobacteria; **PGPR;** plant growth promoting rhizobacteria; **TF,** translocation factor; **AF,** accumulation factor; **EDTA,** ethylenediaminetetraacetic acid; **EDDS,** S,S)-N,N´-ethylenediamine disuccinic acid; **ACC,** 1-aminocylopropane-1-carboxylate; **MerP,** mercuric ion binding protein; **MTs,** methallothioneins; **PCs,** phytochelatines; **PAHs,** polycyclic aromatic hydrocarbons; **PCBs,** polychlorinated biphenyls.

2. Chemical remediation: encapsulation, solvent extraction, neutralization, oxidation-reduction and precipitation;
3. Bioremediation: land farming, natural attenuation, biopiling, bioventing, bioaugmentation and bioreactor;
4. Phytoremediation: defined as the use of plants (including trees and grasses) to remove, destroy or sequester hazardous contaminants from various media such as soil, water and air (Prasad, 2003). Phytoremediation consists of the Greek word “phyto” which refers to plant, and the Latin suffix “remedium” which means curing or restoring. The main reason for the use of this technique was to collect the contaminants from the media and turn them into easily extractable form (plant tissues). Basically, the phytoremediation of contaminants is categorized under four major sub-groups (Khan, 2005; Ward and Singh, 2004), as follows:

a. Phytoextraction entails the use of plants to remove soil contaminants and transport them to above-ground plant tissues. Chaney (1983) proposed this technique as the most promising method for the remediation of contaminated soils.
b. Phytostabilization basically involves the mechanisms of plants, which immobilize or reduce the availability of soil contaminants, by plant roots or their associated bacteria.
c. Phytovolatilization requires plants to volatilize soil contaminants into the atmosphere.
d. Rhizofiltration involves the absorption of contaminants from waste water and aqueous waste streams by plant roots.

This natural and environmental friendly technology is cost-effective, aesthetically pleasant, soil organism-friendly, diversity enhancer, energy derivation from sunlight (Chaney et al., 2005; Huang et al., 2004; Susarla et al., 2002), and more importantly, it is able to retain the fertility status of the soil even after the removal of heavy metals (Kirkham, 2006). However, this relatively new technology poses some disadvantages which have limited its application. These include the necessary demands for nutritional materials and specific climatic conditions, as well as proper soil characteristics to maintain a normal plant growth. For instance, *Thlaspi praecox*, that is, the hyperaccumulator of cadmium (Cd), zinc (Zn) and lead (Pb), is a perennial plant which is native to Slovenia, where the roots survive during cold winters and is very sensitive to warm temperatures. In particular, one of the most important drawbacks in phytoextraction is the long time required in this method which has challenged the application of phytoremediation. Huang et al. (1997) justified that for an economical phytoextraction, plants should be able to accumulate at least 1% of the total heavy metal content present in the soil into their dry shoot biomass.

Neugschwandtner et al. (2008) estimated that in order to obtain the Czech threshold values for Cd (1 mg kg^{-1} soil) and Pb (220 mg kg^{-1} soil) in contaminated soils under maximum obtained remediation factors, 260 and 300 cropping seasons, would respectively be required. Obviously, operating the huge number of cropping seasons is definitely very costly and time-consuming.

Other disadvantages of phytoextraction are the limited tolerance of the plant (particularly encountered at high concentration of contaminants), lower efficiency over other non-biological remediation techniques and the limitations when the contaminated soil layer occasionally extends to the deeper profile (Wei et al., 2008; Wu et al., 2006). Another major issue is the handling and disposal of the contaminated plant waste which could be land filled, composted or incinerated (Keller et al., 2005; Rathinasabapathi et al., 2006). However, these methods could be responsible of the transfer of the contaminants to other compartments of the ecosystem (e.g. land filling increases the risk of groundwater contamination).

Generally, two approaches have been proposed to be employed in using plants to extract heavy metals from contaminated soils. First is the use of plants with extraordinary ability to accumulate the contaminants known as “hyperaccumulators”, and second is the use of tolerant plants (Peer et al., 2005) with a relatively higher accumulation ability as compared to most other plants (but with lower ability as compared to hyperaccumulators) and high biomass such as corn, rice, peas and Indian mustard. These are normally accompanied by other enhancement methods (e.g. using chelating agents) to increase the concentration of heavy metals in plant tissues (Do Nascimento and Xing, 2006). In total, there are 45 families and 400 species of introduced hyper-accumulator vascular plants (Reeves and Baker, 2000). The number of species with a high accumulation capacity and high biomass are rather rare because hyperaccumulators have a small above-ground biomass, slow growth and a long maturity phase (Zhou and Song, 2004). The term “hyperaccumulator” was first explained by Brooks et al. (1977) who related it to plants which could accumulate nickel (Ni) at a concentration of more than 1000 mg/kg dry weight in their leaves. This was followed by Baker and Brooks (1989) who defined this term by including the concentration of other heavy metals in shoots of hyperaccumulator plants as 100 mg/kg dry weight for Cd; 1,000 mg/kg dry weight for Ni, copper (Cu), cobalt (Co), Pb; 10,000 mg/kg dry weight for Zn and manganese (Mn) and 1 mg/kg dry weight for gold (Au). There are three other important characteristics (Wei et al., 2008; Wei et al., 2004) used in defining a plant as a hyperaccumulator. The first characteristic is the translocation factor (TF) which refers to the concentration of heavy metal in shoots divided by that in roots, and it should be higher than 1 (the concentration of contaminants in shoots should be higher than in roots). This particular criterion is especially important in phytoextraction since harvesting of the shoots is the main purpose. The second characteristic is the accumulation factor (AF), that is, the concentration of heavy metal in roots divided by that in soil which should be higher than 1. Tolerance is the third criterion, which is manifested by insignificant or no reduction in the shoot biomass of plants grown in contaminated sites. Although finding out the species which carry all the mentioned criteria is rather difficult, hyperaccumulators usually have a weak point in at least one of them.

INCREASING THE EFFICIENCY OF PHYTO-REMEDIATION

Increasing the bioavailability of heavy metals

One of the most critical points of phytoremediation is the phytoavailability of heavy metals in soil (Lombi et al., 2001). Based on the uptake by plants, heavy metals in soil could be classified into three groups which include "available" fractions (easily absorbable forms including free ions and chelating ions), "exchangeable" fractions (bound to organic matter, carbonates or Fe-Mn oxides) and "unavailable" fractions (residual forms which are most difficult to be absorbed) (Wei et al., 2008; Zhou and Song, 2004). Nevertheless, there are other techniques which can be used to increase the bioavailability of heavy metals such as decreasing pH by adding sulfuric acid or organic fertilizers (Roy and Singh, 2006; Warton and Matthiessen, 2005) or using chelating re-agents. Sappin-Didier et al. (2005) showed the increase in the accu-mulation of Cd in transgenic tobacco as pH decreased, whereas Singer et al. (2007) proved an increase in the Ni concentration of *Alyssum lesbiacum* which was paralleled with the increase in pH. The latter case showed a different impact strategy of pH under different situations on the accumulation of metal.

Chelating agents have been widely used by many researchers (Blaylock et al., 1997; Chiu et al., 2005; Marques et al., 2008b; Pastor et al., 2007). Synthetic chelating agents are shown to have the potential to increase the bioavailability of unavailable and exchangeable heavy metal fractions (Komarek et al., 2007; Sun et al., 2001). The most important application of the chelating reagent is related to phytoremediation of less bioavailable heavy metals such as lead (as only 0.1% of soil Pb is bioavailable) (Peer et al., 2005). The use of non-biodegradable or the least biodegradable chelating agents, such as ethylene-diaminetetraacetic acid (EDTA), can leach metals into the ground water (Santos et al., 2006) and create a new source of pollution by this residual chelating reagent (Wei et al., 2008). (S,S)-N,N´-ethylenediamine disuccinic acid (EDDS) is the biodegradable form of EDTA (Schowanek et al., 1997; Vandevivere et al., 2001) with 90% biode-gradability within 20 days (Dixon, 2004) and is a good substitute of EDTA (Tandy et al., 2006). Luo et al. (2006) showed that the application of hot EDDS (90°C) was much more efficient than the normal chelate solutions (25°C) in improving the uptake of heavy metals by plants. These authors also hypothesized that when hot water pre-treatment was used, the uptake of metal-EDTA was enhanced through physiological damage to the roots. However, the applicability of this experiment in big expanse of contaminated land is still being questioned, since spreading near boiling EDDS on soil surface can seriously injure plant shoots and further disrupt phyto-remediation process, unless long tubes are used to directly transfer hot solution to soil surface. Nevertheless, this method has its own economical and operational restrictions.

Another method is to increase the electrode potential (Eh) which can enhance the bioavailability of metals in soil solution (Zhou and Song, 2004). The adjustment of Eh is usually executed using farming techniques such as solar drying, balancing of organic materials or irrigation arrangement; however, the adjustment of Eh is generally complicated (Wei et al., 2008; Yang, 1998).

The increase of plant growth

As the biomass of plants (especially the biomass of shoot) has a critical role in the total metal removal, any physical (such as light and temperature adjustment), chemical (such as the use of fertilizers) or physico-chemical (such as adjustment of soil pH) methods could improve the efficiency of phytoremediation. Appropriate application of fertilizers (N, P, K) and irrigation also have beneficial effects (Wei et al., 2008); for instance, Jankong et al. (2007) found an increase in the biomass and accumulation of arsenic in silverback ferns (*Pityrogramma calomelanos*) fertilized with phosphorus. Meanwhile, Barrutia et al. (2009) observed an increase in the mean plant biomass and tolerance when treated with fertilizers in Pb, Cd and Zn contaminated soils. Hamlin and Barker (2006) discovered that nitrate fertilizers could be used to enhance the biomass of shoot and stimulate the accumulation of Zn. In spite of the presence of positive effects of fertilizers on metal accumulation, Marques et al. (2008a) showed a reduction in the accumulation of Zn in *Solanum nigrum* when amended with manure.

Decreased phytoremediation period

Another suggested approach is to accelerate the growth of plants, which consequently decreases phyto-remediation cycle, by providing specific demands of respective plant species (e.g. adjusting light, temperature and CO_2) (Wu et al., 2009 ; Wei et al., 2008). This could also be achieved by transferring the seedling to the field so as to decrease the duration of phytoremediation (as the accumulation of heavy metal in plant shoots at flowering stage is high) (Wei et al., 2008); however, this technique also has its own ambiguous applicability because of the restrictions in the sowing of individual seedlings in a vast area of contaminated land.

Biological methods

Hiltner (1904) was the first to describe the term ‘rhizosphere’. This microbial community is effective in tracing metal phytoavailability using different mechanisms, including the release of chelators, acidification and redox changes (Abou-Shanab et al., 2003). Generally, beneficial rhizospheric micro-organisms include free-living as well as symbiotic rhizobacteria and mycorrhizal fungi.

Arbuscular mycorrhizal fungi (AMF)

The term “mykorrhiza” was first used by Frank in 1885 and it was related to the modified root structure of forest trees. More than 80% of terrestrial plant diversity has a symbiotic association with mycorrhizae fungi (Sylvia, 2005). The principal role of mycorrhizal fungi is to improve the uptake of phosphorus and mineral nutrients for plants (Chen et al., 2006) and enhance the number and length of root branch (Padilla and Encina, 2005). However, the alleviation mechanisms of AMF on the phytoremediation of metal is not clear (Jankong and Visoottiviseth, 2008). Some researchers did not find any change in the concentration of heavy metals with the presence of AMF (e.g. Vogel-Mikus et al., 2006), while others found an increase in some metal concentrations in plant tissues (e.g. Marques et al., 2008a; Whitfield et al., 2003) and some others observed a decrease (e.g. Xu et al., 2008; Zhang et al., 2009).

Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) have initially been used in agriculture and forestry to increase plant yield, as well as growth and tolerance to diseases. In addition, they have recently been used in environmental remediation, particularly to overcome plant stress under flooded, high temperature and acidic conditions (Lucy et al., 2004). This group of microbes can be divided into two main groups, based on their relationships, namely free living (ePGPR) which live outside the plant cells and symbiotic (iPGPR) which live inside the plant cells and produce nodules (Gray and Smith, 2005). PGPR can promote the growth of plants using direct and indirect mechanisms. Direct mechanisms include lowering the production levels of ethylene through synthesis of 1-aminocylopropane-1-carboxylate (ACC) deaminase in plants (Reed and Glick, 2005; Safronova et al., 2006; Saleem et al., 2007), providing bioavailable phosphorus for plant uptake and atmospheric nitrogen fixation for plant use, sequestering trace elements like iron using siderophores (Glick et al., 1995) and production of plant hormones like gibberellins, cytokinins and auxins (Glick et al., 1999). Indirect impact of PGPR is usually achieved by increasing the plant tolerance to diseases (Guo et al., 2004). It is crucial to highlight that because the efficient use of PGPR is limited to slight and moderately contaminated sites (Wu et al., 2006), the most important limiting factor for the application of PGPR is their tolerance to the concentration of heavy metal. Based on the amount and the type of the organic compounds, which are mostly exuded from plant roots (Myers et al., 2001) as well as the amount and the type of heavy metals (Sandaa et al., 1999), the PGPR population between plants could be different among the same species in the contaminated soils, or even between the different growing stages of an individual plant. Wu et al. (2006) explained the increasing population of PGPR on the rhizosphere of *Brassica juncea* grown in Pb-Zn mine tailing by seedling stage > flowering stage > tillering stage.

PGPR in terrestrial plants: The alteration of rhizospheric microbial complex in the uptake of essential elements, such as Mn^{+2} and Fe^{+3} (Barber and Lee, 1974), and the efficiency of phytoremediation (O'Connell et al., 1996) have been well documented. Hasnain and Sabri (1997) showed an improvement in the growth of *Triticum aestivum* seedling in different Pb concentrations when their seeds were inoculated with two *Pseudomonas* strains as compared to the uninoculated control. The safety of their usage is one of the most important considerations which should be taken into account before deciding on whether to use PGPR for phytoremediation purposes. For example, *Burkholderia cepacia* is a multi-drug resistant PGPR with health risk potentials (Lee et al., 2008), but at the same time, it has been shown to have special abilities in increasing the efficiency of phytoremediation (Table 1). A number of new researches carried out in relation to the effects of PGPRs on the growth of plants and/or heavy metal concentrations in contaminated soils are summarized in Table 1.

PGPR in aquatic plants: Aquatic plants are relatively new approved organisms for remediation purposes; these include rhizofiltration, phytofiltration, and constructed wetlands (Abou-Shanab et al., 2007; Bennicelli et al., 2004; Zurayk et al., 2001). These aspects of phytoremediation have attracted more attention because of the increase in water pollution. Due to the new approach, most of the current research still focuses on the wetland hyperaccumulator species. Nonetheless, the availability of information on the effects of rhizospheric or rhizoplanic bacteria on the uptake of metal by plants rooted in aquatic systems is rather scarce. So et al. (2003) demonstrated that bacterial species resistant to Cu^{2+} or Zn^{2+}, isolated from water hyacinths (*Eichhornia crassipes*), had led to an increase in the Cu^{2+} removal

Table 1. Some recent researches in relation to the effects of PGPRs on plants in heavy metal contaminated soils.

PGPR	Plant	Heavy metal	Effect(s)	References
Bradyrhizobium sp., *Pseudomonas* sp., *Ochrobactrum cytisi*	*Lupinus luteus*	Pb, Cu, Cd,	Decreased the metals accumulation. However, plant biomass was increased.	Dary et al. (2010)
Bacillus subtilis, *Bacillus cereus*, *Flavobacterium* sp. , *Pseudomonas aeruginosa*	*Orychophragmus violaceus*	Zn	Increased shoot biomass and Zn accumulation.	He et al. (2010)
Ralstonia metalidurans	*Maize*	Cr	Increased the accumulation of Cr in shoots by a factor of 5.2.	Braud et al. (2009)
Pseudomonas aeruginosa		Cr, Pb	Increased the uptake by shoots by a factor of 5.4 and 3.8, respectively.	
Achromobacter xylosoxidans strain Ax10	*Brassica juncea*	Cu	Increased the length of roots and shoots, fresh and dry weight significantly and extensively improved the Cu uptake of *B. juncea* plants as compared to the control.	Ma et al. (2009)
Brevibacterium Halotolerans	*Zea mays*	Pb, Zn,Cu	Demonstrated the highest concentrations of Pb, Zn, Cu with the PGPR strain.	Abou-shanab et al. (2008)
Bacillus subtilis, Bacillus pumilus Pseudomonas pseudoalcaligenes Brevibacterium halotolerans		Cr	Demonstrated the highest concentration of Cr with mixed PGPR strains.	
Pseudomonas tolaasii ACC23	*Brassica napus*	Cd	The Cd content per plant was not increased significantly; but the growth of roots was increased by 83% and shoots by 94%.	Dell'Amico et al. (2008)
Pseudomonas fluorescens ACC9			The Cd content per plant was increased to 72% and the growth of the roots and shoots was by 56% and 64% respectively.	
Pseudomonas aeruginosa	Black gram plants	Cd	Lessened the accumulation of cadmium in plants; showed extensive rooting and enhanced plant growth.	Ganesan (2008)
Burkholderia sp. J62	Maize and tomato	Cd, Pb	Increased the biomass of maize and tomato plants significantly; the increased Pb and Cd contents in tissues varied from 38% to 192% and from 5% to 191%, respectively.	Jiang et al. (2008)
Pseudomonas fluorescens G10, Microbacterium sp. G16	Rape	Pb	Increased root elongation of inoculated rape seedlings and total Pb accumulation as compared to the control plants.	Sheng et al. (2008)
Pseudomonas aeruginosa	Mustard and pumpkin	Cd	Demonstrated improved growth and branched rooting expansively, reduced cadmium uptake of pumpkin by 59.22% in roots and 47.40% in shoots; reduction in the uptake of Cd by 52.44% and 36.89% in roots and shoots of mustard, respectively.	Sinha and Mukherjee (2008)
Burkholderia cepacia	Sedum alfredii	Cd, Zn	Increased plant growth with Zn treatment up to 110%; increased Cd and Zn uptakes up to 243% and 96.3%, respectively.	Li et al. (2007)

Table 1. Contd.

Bradyrhizobium sp. RM8	Green gram var .K851	Ni, Zn	Increased plant growth and decreased uptake of heavy metals by plant.	Wani, et al. (2007)
Pseudomonas sp. RJ10 *Bacillus sp. RJ16*	Rape (Brassica napus)	Cd	Increased uptake of Cd by plant, and significantly enhanced shoot and root dry weight. Increased shoot and root Cd content from 11% to 136% and 20% to 27% compared to control; Increase shoot and root dry weight significantly.	Sheng et al. (2006)
Azotobacter chroococcum HKN-5 *Bacillus megaterium HKP-1* *Bacillus mucilaginosus HKK-1*	Brassica juncea	Pb, Zn, Cu	Increased the removal of Pb, Zn, Cu by 92%, 38% and 36%, respectively.	Wu et al. (2006)
Bacillus subtilis SJ-101	Brassica juncea	Ni	Approximately increased the accumulation of Ni by 1.5 fold; Increased plant biomass.	Zaidi et al. (2006)
V. paradoxus 2C-1, 2P-1, 2P-4, 3C-2, 3C-3, 3C-5, 3P-3, 5C-2, 5P-3 *Flavobacterium sp. 5P-4* *Rhodococcus sp. 4N-4*	Indian mustard (Brassica juncea)	Cd	Increased the length of roots significantly (specially strain 5C-2).	Belimov et al. (2005)
Azospirillum lipoferum 137, *Arthrobacter mysorens 7,* *Agrobacterium radiobacter 10*	Barley cultivar Tselinnyi-5	Pb, Cd	Increased growth of plants and uptake of nutrients; prevented the accumulation of Pb and Cd.	Belimov et al. (2004)

capacity of this plant species. Xiong et al. (2008), who worked on *Sedum alfredii* Hance (a terrestrial plant), in an aqueous medium with rhizospheric bacteria, suggested that rhizospheric bacteria appeared to protect the roots against heavy metal toxicity. The number of bacteria on the root surface of terrestrial plants is approximately 10^7 cell/cm^2 (Kennedy et al., 1998), but this was found to decrease to 10^6 cell/cm^2 in aquatic plants (Fry and Humphrey, 1978). The difference in the population of bacteria could be attributed to several factors, such as the variability of oxygen flux around the roots of aquatic plants, which might change the equations of phytoremediation in the different media.

Genetically-engineered approaches

As a result of the development in biological science, genetic modification methods have attracted the attention of many scientists. Higher efficiency in the remediation by plants is achieved mostly by an increase in the tolerance and/or accumulation capacity of transgenic plants. The earliest research by Misra and Gedamu (1989) showed an increase in cadmium tolerance of transgenic tobacco plants (*Nicotiana tobacum*) expressing a yeast metallothionein gene. In addition, Hsieh et al. (2009) found an increase in mercury (Hg) accumulation and tolerance of *Arabidopsis thaliana* when mercuric ion binding protein (MerP), originated from transposonTnMERI1 of transposon TnMERI1 *Bacillus megaterium* strain MB1, was expressed in the transgenic plants. Transgenic plants usually contain some beneficial enzymes like ACC deaminase (Grckho et al., 2000; Nie et al., 2002) and gamma-glutamylcysteine synthetase (Han et al., 2000), which in turn improve the tolerance of plants to stress and increase the ratio between plant growth and shoot/root. Transgenic plants, with selected genes, have also been shown to have higher abilities to biodegrade organic contaminants in their tissues (Doty et al., 2000; Kawahigashi et al., 2003).

Along with genetically engineered plants, the

the role of transgenic PGPR is considerable. The transgenic PGPRs usually have a higher ability to degrade organic contaminants (Barac et al., 2004; Monti et al., 2005) and exude heavy metal binding components, such as methallothioneins (MTs) and phytochelatines (PCs), which are useful in phytoremediation and bioremediation of contaminants (Wu et al., 2006).

Since all the research mentioned in the earlier section were merely confined to laboratory studies and field applications of transgenic organisms were also highly restricted, the use of this approach is therefore restricted in most countries; thus, they could not be considered as possible tools in phytoremediation of contaminated lands in the near future.

Multi-functional methods to improve phytoremediation

As each described method has its own advantages and disadvantages, new approaches have been focusing on multi-improvement methods. Lin et al. (2009) found a better efficiency of the low dose EDTA with a medium soil nutrient level on the accumulation of Pb in sunflower. Vaxevanidou et al. (2008) showed a 10% increase in the extraction of Pb with bacteria (*Desulfuromonas palmitatis)* and EDTA, as compared to the amendment ofEDTA alone. However, in the same study, a 30% reduction was observed for the extraction of Zn, with the presence of bacteria and EDTA, as compared to only EDTA. Similarly, Di Gregorio et al. (2006) showed a 56% increase in the efficiency of the EDTA-led phytoextraction by *B. juncea,* which was combined with an application of Triton X-100 and *Sinorhizobium* sp. Pb002 inoculums. More processes for the multi-function removal of contaminants are currently being used in removing organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). For instance, a multi-process which includes physical (volatilization), photo-chemical (photooxidation) and microbial remediation (contaminant degrading bacteria, PGPRs) processes was employed by Huang et al. (2004). In their study, the average efficiency for the removal of 16 priority PAHs, using the multi-process remediation system, was found to be 100% more than land-farming, 50% more than using bacteria alone and 45% more than phytoremediation alone.

CONCLUSION

The increase of heavy metal pollution in the environment has led many researchers to focus on developing fast, economical and more efficient remediation technologies. It is no doubt that phytoremediation is an environmentally friendly technique, but the removal process is rather slow with lower efficiency as compared to many other techniques. Thus, some other remediation techniques, which are paralleled to or in sequence with phyto-remediation, have been suggested to increase the potential for remediation. This review has highlighted some phytoremediation efficiency enhancer methods, including the recent studies which showed higher abilities when multiple techniques were used to increase the concentration and speed of pollution removal. At the same time, it is important to note that plants have a focal role in this system, and that the entire accompanying techniques are for higher and faster bioaccumulation of contaminants in plant tissues.

REFERENCES

Abou-Shanab RAI, Delorme TA, Angle JS, Chaney RL, Ghanem K, Moawad H, Ghozlan HA (2003). Phenotypic characterization of microbes in the rhizosphere of *Alyssum murale*. Int. J. Phytoremediation 5: 367-79

Abou-Shanab RAI, Angle JS, van Berkum P (2007). Chromate-tolerant bacteria for enhanced metal uptake by *Eichhornia crassipes* (Mart.). Int. J. Phytoremediation 9: 91-105

Abou-Shanab RAI, Ghanem K, Ghanem N, Al-Kolaibe A (2008). The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World J. Microb. Biot. 24: 253-262

Anonymous (1964). Encyclopedia of chemical science. Van Norstrand, Princeton. p. 533.

Baker AJM, Brooks RR (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. T. Biorecovery. 1: 81-126

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert J (2004). Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat. Biotechnol. 22: 583-588

Barber DA, Lee RB (1974). The effect of microorganisms on the absorption of manganese by plants. New Phytol. 73: 97-106

Barrutia O, Epelde L, García-Plazaola JI, Garbisu C, Becerril JM (2009). Phytoextraction potential of two *Rumex acetosa* L. accessions collected from metalliferous and non-metalliferous sites: Effect of fertilization. Chemosphere, 74: 259-264

Belimov AA, Kunakova AM, Safronova VI, Stepanok VV, Iudkin L, Alekseev IU V, Kozhemiakov AP (2004). Employment of associative bacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. Mikrobiologiia, 73: 118-25

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR (2005). Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Biol. Biochem. 37: 241-250

Bennicelli R, Stepniewska Z, Banach A, Szajnocha K, Ostrowski J (2004). The ability of *Azolla caroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. Chemosphere, 55: 141-146

Blaylock MJ, Huang JW (2000). Phytoextraction of metals. In: Raskin I, Ensley B (eds) Phytoremediation of toxic metals: Using plants to clean up the environment. Wiley-Interscince, New jersey, pp. 53-88

Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol. 31: 860-865

Braud A, Jezequel, K, Bazot, S , Lebeau T (2009). Enhanced phytoextraction of an agricultural Cr^- and Pb^-contaminated soil by bioaugmentation with siderophore-producing bacteria. Chemosphere, 74: 280-286.

Brooks RR, Lee J, Reeves RD (1977). Detection of nickliferous rocks by analysis of herbarium species of indicator plants. J. Geochem. Explor. 7: 49-77.

Chaney RL (1983). Plant uptake of inorganic waste constituents. In: JF Parr PBM, JM Kla (ed) Land Treatment of Hazardous Waste. Noyes

Data Corporation, Park Ridge, NJ, USA, pp. 50-76

Chaney RL, Angle JS, McIntosh MS, Reeves RD, Li YM, Brewer EP, Chen KY, Roseberg RJ, Perner H, Synkowski EC, Broadhurst CL, Wang S, Baker AJ (2005). Using hyperaccumulator plants to phytoextract soil Ni and Cd. Z. Naturforsch [C]. 60: 190-198

Chen BD, Zhu YG, Smith FA (2006). Effects of arbuscular mycorrhizal inoculation on uranium and arsenic accumulation by Chinese brake fern (*Pteris vittata* L.) from a uranium mining-impacted soil. Chemosphere, 62: 1464-73

Chiu KK, Ye ZH, Wong MH (2005). Enhanced uptake of As, Zn, and Cu by Vetiveria zizanioides and Zea mays using chelating agents. Chemosphere, 60: 1365-75

Dary M, Chamber-Pérez MA, Palomares AJ, Pajuelo E (2010). In situ phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J. Hazard. Mater. 177: 323-330

Dell'Amico E, Cavalca L, Andreoni V (2008). Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. Soil Biol. Biochem. 40: 74-84

DI Gregorio S, Barbafieri M, Lampis S, Sanangelantoni AM, Tassi E, Vallini G (2006). Combined application of Triton X-100 and *Sinorhizobium* sp. Pb002 inoculum for the improvement of lead phytoextraction by *Brassica juncea* in EDTA amended soil. Chemosphere, 63: 293-299

Dixon N (2004). Biodegradable alternatives to chemical Octel's experience in the chelant market Proceeding of EU sustainable chemicals management conference.

Do Nascimento CWA, Xing B (2006). Phytoextraction: A review on enhanced metal availability and plant accumulation. Sci. Agr. 63: 299-311

Doty SL, Shang TQ, Wilson AM, Tangen J, Westergreen AD, Newman LA, Strand SE, Gordon MP (2000). Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1. PNAS, 97: 6287-6291

Fergusson JE (1990). The heavy elements: chemistry, environmental impact and health effects. Pergamon Press, Oxford

Frank AB (1885). Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. Dtsch. Bot. Ges. 3: 128-145

Fry JC, Humphrey NCB (1978). Techniques for the study of bacteria epiphytic on aquatic macrophytes. In: Lovelock DW, Davies R (eds) Techniques for the Study of Mixed Populations academic Press, London, pp. 1-29

Ganesan V (2008). Rhizoremediation of cadmium soil using a cadmium-resistant plant growth-promoting rhizopseudomonad. Curr. Microbiol. 56: 403-407.

Glick B, Karaturovíc D, Newell P (1995). A novel procedure for rapid isolation of plant growth promoting Pseudomonas. Can. J. Microbiol. 41: 533-536

Glick B, Patten C, Holguin G, Penrose D (1999). Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London.

Gray EJ, Smith DL (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol. Biochem. 37: 395-412

Grckho V, Filby B, Glick B (2000). Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb, and Zn. J. Biotechnol. 81: 45-53

Guo J, Qi H, Guo Y, Ge H, Gong L, Zhang L (2004). Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. Biol. Control, 29: 66-72

Hamlin RL, Barker AV (2006). Influence of ammonium and nitrate nutrition on plant growth and zinc accumulation by Indian mustard. J. Plant Nutr. 29: 1523-1541

Han KH, Meilan R, Ma C, Strauss S (2000). An *Agrobacterium tumefaciens* transformation protocol effective on a variety of cottonwood hybrids (genus Populus). Plant Cell. Rep. 19: 315-320

Hasnain S, Sabri AN (1997). Growth stimulation of *Triticum aestivum* seedlings under Cr-stresses by non-rhizospheric pseudomonad strains. Environ. Pollut. 97: 265-273

He CQ, Tan GE, Liang X, Du W, Chen YL, Zhi GY, Zhu Y (2010). Effect of Zn-tolerant bacterial strains on growth and Zn accumulation in *Orychophragmus violaceus*. Appl. Soil Ecol. 44: 1-5

Hiltner L (1904). Über neuere Erfahrungen und Probleme auf dem Gebiet der Berücksichtigung der Gründüngung und Brache. Arb Deutsch Landwirt Ges. 98: 59-78.

Hsieh JL, Chen CY, Chiu MH, Chein MF, Chang JS, Endo G, Huang CC (2009). Expressing a bacterial mercuric ion binding protein in plant for phytoremediation of heavy metals. J. Hazard. Mater.161: 920-925

Huang JW, Chen J, Berti WR, Cunningham SD (1997). Phytoremediadon of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. Environ. Sci. Technol. 31: 800-805

Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM (2004). A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ. Pollut. 130: 465-476

Jankong P, Visoottiviseth P (2008) Effects of arbuscular mycorrhizal inoculation on plants growing on arsenic contaminated soil. Chemosphere. 72: 1092-1097

Jankong P, Visoottiviseth P, Khokiattiwong S (2007). Enhanced phytoremediation of arsenic contaminated land. Chemosphere, 68: 1906-1912

Jiang CY, Sheng XF, Qian M, Wang QY (2008). Isolation and characterization of a heavy metal-resistant *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere, 72: 157-164.

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y (2003). Transgenic rice plants expressing human CYP1A1 exude herbicide metabolites from their roots. Plant Sci. 165: 373-381

Keller C, Ludwig C, Davoli F, Wochele J (2005). Thermal treatment of metal-enriched biomass produced from heavy metal phytoextraction. Environ. Sci. Technol. 39: 3359-67

Kennedy AC (1998). The rhizosphere and spermosphere. In: Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (eds) Priciples and Applications of Soil Microbiology. Prentice-Hall, Upper Saddle, NJ

Khan AG (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J. Trace Elem. Med. Biol. 18: 355-64

Kirkham MB (2006) Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. Geoderma. 137: 19-32

Komarek M, Tlustos P, Szakova J, Chrastny V, Ettler V (2007). The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere, 67: 640-51

Lapedes DN (1974). Dictionary of of scientific and technical terms. McGraw Hill, New York p. 674.

Lee CS, Lee HB, Cho YG, Park JH, Lee HS (2008). Hospital-acquired *Burkholderia cepacia* infection related to contaminated benzalkonium chloride. J. Hosp. Infect. 68: 280-282

Li WC, Ye ZH, Wong MH (2007). Effects of bacteria on enhanced metal uptake of the Cd/Zn-hyperaccumulating plant, *Sedum alfredii*. J. Exp. Bot. 58: 4173-4182

Lin C, Liu J, Liu L, Zhu T, Sheng L, Wang D (2009). Soil amendment application frequency contributes to phytoextraction of lead by sunflower at different nutrient levels. Environ. Exp. Bot. 2(3): 410-416

Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001). Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. J. Environ. Qual. 30: 1919-1926.

Lucy M, Reed E, Glick BR (2004). Applications of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek, 86: 1-25

Luo CL, Shen ZG, Baker A, Li XD (2006). A novel strategy using biodegradable EDDS for the chemically enhanced phytoextraction of soils contaminated with heavy metals. Plant Soil. 285: 67-80

Ma Y, Rajkumar M, Freitas H (2009). Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J. Environ. Manage. 90: 831-837

Marques APGC, Oliveira RS, Rangel AOSS, Castro PML (2008a). Application of manure and compost to contaminated soils and its effect on zinc accumulation by *Solanum nigrum* inoculated with arbuscular mycorrhizal fungi. Environ. Pollut. 151: 608-620.

Marques APGC, Oliveira RS, Samardjieva KA, Pissarra J, Rangel AOSS, Castro PML (2008b). EDDS and EDTA-enhanced zinc accumulation by *Solanum nigrum* inoculated with arbuscular mycorrhizal fungi grown in contaminated soil. Chemosphere, 70: 1002-1014.

Misra S, Gedamu L (1989). Heavy-metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants. Theor. Appl. Genet. 78: 161-168

Monti MR, Smania AM, Fabro G, Alvarez ME, Argarana CE (2005). Engineering *Pseudomonas flseudomons* for biodegradation of 2, 4-dinitrotoluene. Appl. Environ. Microbiol. 71: 8864-8872.

Myers RT, Zak DR, White DC, Peacock A (2001). Landscape level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Sci. Soc. Am. J. 65: 359-367.

Neugschwandtner RW, Tlustos P, Komárek M, Száková J (2008). Phytoextraction of Pb and Cd from a contaminated agricultural soil using different EDTA application regimes: Laboratory versus field scale measures of efficiency. Geoderma, 144: 446-454.

Nie L, Shah S, Burd G, Dixon D, Glick B (2002). Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2. Plant. Physiol. Biochem. 40: 355-361

Nieboer E, Richardson DHS (1980). The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environmental Pollution. Series B, Chem. Phys.1: 3-26

O'Connell KP, Goodman RM, Handelsman J (1996). Engineering the rhizosphere expressing a bias. Trends Biotechnol. 14: 83-88

Padilla IMG, Encina CL (2005). Changes in root morphology accompanying mycorrhizal alleviation of phosphorus deficiency in micropropagated *Annona cherimola* Mill. Amsterdam. Plants. Sci. Hortic. 106: 360-369

Pastor J, Aparicio AM, Gutierrez-Maroto A, Hernandez AJ (2007). Effects of two chelating agents (EDTA and DTPA) on the autochthonous vegetation of a soil polluted with Cu, Zn and Cd. Sci. Total Environ. 378: 114-118.

Peer W, Baxter I, Richards E, Freeman J, Murphy A (2005) Phytoremediation and hyperaccumulator plants. In Molecular Biology of Metal Homeostasis and Detoxification. In: Tamas M, Martinoia E (eds), Springer, Berlin, Topics in Current Genetics, 14: 299-340.

Prasad MNV (2003). Phytoremediation of Metal-Polluted Ecosystems: Hype for Commercialization. Russ. J. Plant Phys. 50: 686-700

Rathinasabapathi B, Ma LQ, Srivastava M (2006). Arsenic hyperaccumulating ferns and their application to phytoremediation of arsenic contaminated sites. In: Silva JATd (ed) Floriculture, Ornamental and Plant Biotechnology. Global Science Books, London, pp. 304-311

Reed M, Glick B (2005). Growth of canola (Brassica napus) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can. J. Microbiol. 51: 1061-1069.

Reeves RD, Baker AJM (2000). Matal accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals - Using plants to clean up the environment. John Wiley & Sons, New York, pp. 193-229.

Roy S, Singh SB (2006). Effect of soil type, soil pH, and microbial activity on persistence of clodinafop herbicide. Bull. Environ. Contam. Toxicol. 77: 260-266

Safronova V, Stepanok V, Engqvist G, Alekseyev Y, Belimov A (2006). Rootassociated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol. Fertil. Soils, 42: 267-272

Saleem M, Arshad M, Hussain S, Bhatti AS (2007). Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J. Ind. Microbiol. Biotechnol. 34: 635-648

Sandaa RA, Torsvik V, Enger Ø (1999). Analysis of bacterial communities in heavy metal-contaminated soils at different levels of resolution. FEMS Microbiol. Ecol. Eng. 30: 237-51.

Santos FS, Hernández-Allica J, Becerril JM, Amaral-Sobrinho N, Mazur N, Garbisu C (2006). Chelate-induced phytoextraction of metal polluted soils with Brachiaria decumbens. Chemosphere, 65: 43-50

Sappin-Didier V, Vansuyts G, Mench M, Briat JF (2005). Cadmium availability at different soil pH to transgenic tobacco overexpressing ferritin. Plant Soil, 270: 189-197

Schowanek D, Feijtel TC, Perkins CM, Hartman FA, Federle TW, Larson RJ (1997). Biodegradation of [S, S], [R,R] and mixed stereoisomers of ethylene diamine disuccinic acid (EDDS), a transition metal chelator. Chemosphere, 34: 2375-2391

Shaw BP, Sahu SK, Mishra RK (2004). Heavy metal induced oxidative damage in terrestrial plants. In: Prasad MNV (ed) Heavy Metal Stress in Plants: From Biomolecules to Ecosystems, pp. 84-126

Sheng XF, Xia JJ (2006). Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere. 64: 1036-1042.

Sheng XF, Xia JJ, Jiang CY, He LY, Qian M (2008). Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ. Pollut. 156: 1164-1170.

Singer AC, Bell T, Heywood CA, Smith JA, Thompson IP (2007). Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant *Alyssum lesbiacum*: evidence of histidine as a measure of phytoextractable nickel. Environ. Pollut. 147: 74-82.

Sinha S, Mukherjee SK (2008). Cadmium-induced siderophore production by a high Cd-resistant bacterial strain relieved Cd toxicity in plants through root colonization. Curr. Microbiol. 56: 55-60

So LM, Chu LM, Wong PK (2003). Microbial enhancement of Cu^{2+} removal capacity of *Eichhornia crassipes* (Mart.). Chemosphere, 52: 1499-1503

Sun B, Zhao FJ, Lombi E, McGrath SP (2001). Leaching of heavy metals from contaminated soils using EDTA. Environ. Pollut. 113: 111-120

Susarla S, Medina VF, McCutcheon SC (2002). Phytoremediation: An ecological solution to organic chemical contamination. Ecol. Eng. 18: 647-658

Sylvia DM (2005). Mycorrhizal symbioses In: Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (eds) Principles and Applications of Soil Microbiology. Pearson, Prentice Hall, New Jersey, pp. 263-282.

Tandy S, Schulin R, Nowack B (2006). The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers. Chemosphere, 62: 1454-1463.

Vandevivere PC, Saveyn H, Verstraete W, Feijtel TC, Schowanek DR (2001). Biodegradation of metal-[S,S]-EDDS complexes. Environ. Sci. Technol. 35: 1765-1770.

Vaxevanidou K, Papassiopi N, Paspaliaris I (2008). Removal of heavy metals and arsenic from contaminated soils using bioremediation and chelant extraction techniques. Chemosphere, 70: 1329-1337

Vogel-Mikus K, Pongrac P, Kump P, Necemer M, Regvar M (2006). Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. Environ. Pollut. 139: 362-371

Wani PA, Khan MS, Zaidi A (2007). Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants. Chemosphere, 70: 36-45

Ward OP, Singh A (2004). Soil bioremediation and phytoremediation-An overview. In: Singh A, Ward OP (eds) Springer, Berlin, Applied bioremediation and phytoremediation. 1: 1-11

Warton B, Matthiessen JN (2005). The crucial role of calcium interacting with soil pH in enhanced biodegradation of metam-sodium. Pest. Manage. Sci. 61: 856-862

Wei SH, Teixeira da Silva JA, Zhou QX (2008). Agro-improving method of phytoextracting heavy metal contaminated soil. J. Hazard. Mater. 150: 662-668

Wei SH, Zhou QX, Wang X, Cao W, Ren LP, Song YF (2004). Potential of weed species applied to remediation of soils contaminated with heavy metals. J. Environ. Sci. (China) 16: 868-73

Whitfield L, Richards AJ, Rimmer DL (2003). Effects of mycorrhizal colonization on *Thymus polytrichus* from heavy metal-contaminated sites in north England. Mycorrhiza, 14: 47-54

Wood JM (1974). Biological cycles for toxic elements in the environment. Sci. 4129: 1049-1052

Wu CH, Wood TK, Mulchandani A, Chen W (2006a). Engineering plant-microbe symbiosis for rhizoremediation of heavy metals. Appl.

Environ. Microbiol. 72: 1129-1134
Wu SC, Cheung KC, Luo YM, Wong MH (2006). Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by *Brassica juncea*. Environ. Pollut. 140: 124-35.
Wu H, Tang S, Zhang X, Guo J, Song Z, Tian S, Smith DL (2009) Using elevated CO2 to increase the biomass of a Sorghum vulgare x Sorghum vulgare var. sudanense hybrid and Trifolium pratense L. and to trigger hyperaccumulation of cesium. J Hazard Mater 170: 861-70.
Xiong J, He Z, Liu D, Mahmood Q, Yang X (2008). The role of bacteria in the heavy metals removal and growth of *Sedum alfredii* Hance in an aqueous medium. Chemosphere, 70: 489-494
Xu P, Christie P, Liu Y, Zhang J, Li X (2008). The arbuscular mycorrhizal fungus *Glomus mosseae* can enhance arsenic tolerance in Medicago truncatula by increasing plant phosphorus status and restricting arsenate uptake. Environ. Pollut. 156: 215-220.
Yang SR (1998). The Rice Analecta of Yang Shouren. Liaoning Science and Technology Press, China, Shenyang
Zaidi S, Usmani S, Singh BR, Musarrat J (2006). Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere, 64: 991-997.
Zhang XH, Lin AJ, Gao YL, Reid RJ, Wong MH, Zhu YG (2009). Arbuscular mycorrhizal colonisation increases copper binding capacity of root cell walls of *Oryza sativa* L. and reduces copper uptake. Soil Biol. Biochem. 41: 930-935
Zhou QX, Song YF (2004). Remediation of contaminated soils principles and methods. Beijing: Sciences Press, China
Zurayk R, Sukkariyah B, Baalbaki R, Ghanem DA (2001). Chromium Phytoaccumulation from Solution by Selected Hydrophytes. Int. J. Phytoremediation 3: 335-350.