



ELIMINATION OF HEAVY METALS FROM AGRICULTURAL SOIL AND WATER USING COMBINATION OF PLANTS AND MICROORGANISMS: A REVIEW

Nguyen Thi Lam

Ha Noi University of Natural Resources and Environment, Thanh Hoa Campus

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Abstract

Heavy metal contamination in soil and water has posed serious threat to natural ecosystems and human health through the food chain. There are many methods to remove heavy metals from soil and water. Compared with physicochemical methods, bioremediation is highly efficient, environmentally friendly, easy to operate and cost effective. Plants in combination with microorganisms have been widely used to remove toxic metals (Pb, Cd and Hg) from soil and water. It is more efficient, faster and suitable for areas with low concentrations of heavy metals. In Vietnam, bioremediation method has been studied and showed that it is a suitable and potential way for removing heavy metals from agricultural soils and water with low levels of contamination.

Keywords: Elimination; Heavy metals; Combination; Plant; Microorganisms; Agricultural soils and water

Corresponding author. Email: ntlam.ph@hunre.edu.vn

1. Introduction

Environmental protection and food safety are serious matters of urgency for each country [1, 2]. Many countries in the world are facing problems of toxic metal pollution in agricultural soil [2, 3]. Amongst heavy metals, Pb, Cd and Hg are undetectable toxic metals in the ecological role but clearly exhibit dangerous toxicity due to continuous accumulation in the environment, cumulative tendency biology in the biomass of the food chain poses a serious threat to plants, animals and humans even when exposed to low concentrations [1, 4]. Removing of these metals in soil and water to limit their effect on the quality of agricultural products is

therefore essential to maintaining a safe food chain and a healthy agricultural ecosystem. So far, a number of different physicochemical methods have been used to treat these pollutants in soil and water such as soil washing technology, on-site fixed/relocation technology and dynamoelectric treatment technology. With the tendency of soil and water pollution to occurring on a larger scale, these physiochemical measures are less effective, over cost. Especially, the area of cultivated land and water surface after treatment will not be used for agricultural cultivation. Bioremediation is performed through the use of microorganisms, plants or combination of both to restore the polluted environment to the initial

state [5 - 6]. This is a popular trend, an environmental method applied in the world, attracting the interest of many scientists by lower processing costs from 10 to 1000 times the traditional [7]. In particular, the combination of plants and microorganisms is not only to promote the removal of heavy metal [8] but also to increase activity and diversity microorganisms in the soil and water, maintaining a healthy ecosystem and thereby contributing to sustainable development.

This paper reviews the use of combined plants and microorganisms in bioremediation to remove toxic heavy metals (Pb, Cd and Hg) from agriculture soils and water in countries around the world and Vietnam in order to find the advantages, feasibility and application of this technology for Vietnam.

2. Pb, Cd and Hg pollution in agricultural soils and water

Agricultural practices with using chemical fertilizers, pesticides, etc. causes heavy metal contamination of water and soil. Chemical fertilizers, various fungicides, insecticides contains the salts of these toxic heavy metals. Furthermore, the slowly degradation characteristics of these heavy metals in the soil causes significant residues in the soil and attracted to the soil - water - plant - animal - human cycle, causing the discharge biology. The use of sludge containing heavy metals in different forms (i.e. free ion, carbonate, organic complex soluble and insoluble organic fertilizer) for poor agricultural land leads to the accumulation of large amounts of heavy metals in the soil and this can pose a serious problem. In addition, heavy metal contamination in agricultural soils and water may derive from industrial

wastewater, domestic effluent, urban spills and irrigation. These wastewater sources have a high heavy metal level; thus, the use of wastewaters for long-term irrigation will pollute the soil. As a result, the pollution of heavy metals (Pb, Cd and Hg) in soil and water using for agricultural production in countries around the world and Vietnam has become quite popular.

In the USA, about 100,000 or more croplands are damaged due to the significant level of metal contamination [9]. According to the US government data, coal mines contaminate more than 19,000 km of US stream water with heavy metal [10].

China has the fastest economic growth rate in the world, but it is also facing heavy metal contamination in agricultural land. About 20 million hectares of cultivated land (one-sixth of the total cultivated land) are contaminated of heavy metals, such as cadmium, mercury, lead (According results of the 2007 survey). Applying the Pi index to assess the level of heavy metal pollution in agricultural land (influenced by traffic), Zhang Hui (2017) found that Pi values of soil along Shenyang - Dalian Expressway were 0.98 - 1.51 (average 1.08) for Pb; 0.05 - 2.04 (average 0.99) for Cd. The results showed that agricultural land was low polluted by Pb and moderately polluted with Cd [11].

In Africa, mining activity is common and it causes metal wash, especially Hg pollution in agriculture soil. In addition, metal pollution in Africa may result from other commercialization subsistence practices, such as electroplating, leather tanning, vehicular emissions, oil and gas explorations, effluent discharge from power lines, intensive agriculture and sludge disposal. It possesses wide

distribution of Pb and other metals that pollute the soil, water sources and vegetation. However, the awareness about heavy metals is considerably low, especially for Cd, Pb and Hg [10].

In Vietnam, after a long period of war, the focus has been on agro-industrial development and urbanization, with little attention to environmental issues that have caused heavy metals pollution in agriculture soils and water. The first study by Tran Kong Tau and Tran Cong Khanh (1998) showed the heavy metal contamination in agricultural soil in the form of total and mobile in the 0 - 20 cm on surface layer in the alluvial soils of Red River and Mekong River [12]. Heavy metals (Pb, Cd and Hg) were also identified in alluvial soil in Hoc Mon District with Pb (0.48 - 1.05 mg/kg), Cd (1.25 - 3.75 mg/kg and Hg (0.049 - 0.512 mg/kg). Nguyen Ngoc Quynh, Le Huy Ba (2002) analyzed Pb, Cd and Hg from 126 rice cultivars and vegetables irrigated with water from discharged channels of Ho Chi Minh City and the results showed that Pb, Hg were found in some samples with low levels, compared to the standard of some European countries. However, Cd had a high accumulation in the soil, with a concentration of 9.9 - 10.3 mg/kg, exceeding the allowable level of 5 times. Meanwhile, analysis results of heavy metals pollution in paddy soil in the south of the Ho Chi Minh City showed that the Pb content was 14 - 85 ppm [13]. In the planning report of the hi-tech agricultural region in combination with processing in the economic areas in the South to 2010 and the vision to 2020 of the Institute of Planning and Investment (2005), Cd and Pb in water of Nhieu Loc canal - Thi Nghe were 16 times and 700 times, respectively, higher than the permitted level [14].

Evaluation of heavy metal pollution in agricultural land of 3 types of specialized land for rice, land for 2 rice - vegetable crops and specialized land for vegetable crops in Tien Du, Bac Ninh province showed that Pb and Cd content in all 3 types of land had not exceeded the maximum permissible threshold, according to QCVN 03: 2015. However, total Pb in some samples, such as TD16, TD30 was high (67.2 ppm and 63.3 ppm) in cropland belong to Minh Dao and Viet Doan communes [15]. A study on heavy metals in vegetable soils in Yen Nghia showed that total Pb and Cd concentrations in most of soil samples were lower than the allowable limit, except for the soil sample of mugwort (*Artemisia vulgaris*.L) and total Cd content was just reached the pollution threshold [16].

Nguyen Minh Hung (2019) investigated 90 soil samples on the total content of heavy metals in vegetable soil in Ho Chi Minh City and the results showed that heavy metal levels were 8.41 - 70.4 ppm (Pb); 0.04 - 1.92 ppm (Cd); and 0.18 - 0.59 ppm (Hg). Compared with the Vietnamese standard QCVN 03-MT:2015/BTNMT, most of the heavy metal levels were in the range below the allowable threshold, except Pb and Cd levels in few samples were higher than the allowable threshold. Evaluation of the pollution level of these heavy metals with Newmerow index showed that there were 85 samples with individual pollution index $P_i < 1$ (low pollution) and 5 samples with $P_i \geq 1$ (moderate pollution). The average pollution index of these 90 samples were 0.12 - 1.01 for Pb; 0.02 - 0.96 for Cd; 0.02 - 0.98 for Hg. This result proves that the soil quality is still quite safe because most of samples are not or lightly polluted. Of the 3 heavy metals, Pb

and Hg have $PI > 1.0$, indicating that some samples have been slightly contaminated with these metals. The environmental quality of the soil is possible pollution due to the Nemerow index of 0.769 ($0.7 \leq PI \text{ Nemerow} < 1.0$) [17]. Other study was conducted to determine the soil contamination of metals (Cd, Pb) in a Pb recycling area of Dong Mai village, Hung Yen Province, Vietnam. The analysis of 32 soil samples collected from seven different sites in the study area revealed that the contents of Cd and Pb in the soils ranged from 0.71 - 1.67 and 370 - 47,400 mg/kg, respectively. Compared with the Vietnamese standard QCVN 03-MT:2015/BTNM, all samples exceeded the allowable threshold for Pb many times and only some samples exceeded the allowable threshold for Cd [26].

Tran Duc Ha (2018) studied heavy metals in sludge deposited in To Lich river, Hanoi and the results showed that with samples taken from Buoi sluice to Cau Moi, heavy metal levels reached 3.91 - 4.17 mg Pb/kg, 0.076 - 0.078 mg Cd/kg and 0.03 mg Hg/kg dry sludge [18]. Study the sources of heavy metal pollution to the soil, sludge samples were taken in 4 provinces of Ho Chi Minh City, Dong Nai, Binh Duong and Ba Ria Vung Tau to analyze the total heavy metal content. The results showed that the sludge had total heavy metal content ranging from 9.99 to 74.2 ppm Pb (average of 28,719 ppm Pb); 0.25 - 2.02 ppm Cd (average 0.627 ppm Cd); and 0.08 - 0.51 ppm Hg (average 0.18 ppm Hg). According to the national standard on sediment quality (QCVN 43:2017/BTNMT), the analyzed heavy metals (Pb, Cd and Hg) are at safe levels. However, there have been some samples with Hg level approaching or even exceeding the allowable threshold[24].

Research results show that the level of heavy metal contamination (Pb, Cd and mercury) in agricultural land and irrigation water is low to moderate. Therefore, it is necessary to study and choose appropriate, effective and sustainable treatment methods.

3. Effect of heavy metals (Pb, Cd and Hg) in soil and water on agricultural product quality and human health

Heavy metal pollution in agricultural soil and water have posed a risk to the yield and quality of crops and becomes a threat to animal and human health through the food chain even when exposed to low concentrations [4, 20]. Research by M. G. Kibra (2008) noted a significant decrease in height of rice planted on contaminated soil 1 mg Hg/kg; at the same time, the decrease in bud growth and cotton formation also occurred at this concentration of Hg in the soil [21]. For Cd, the development of shoots and roots in wheat occurs when Cd in the soil solution is as low as 5 mg/L. Study on the effects of heavy metals examined (Cd, Hg and Pb) on the growth of maize showed that the presence of these metals in the soil reduces the growth and protein content of maize. The toxicity of these metals occurs in the following order: $Cd > Hg > Pb$. This suggests that the combined effect of two or more heavy metals was only as harmful as the effect of most toxic heavy metals [22]. Exposure to Pb and cadmium in either the short or the long term can cause cancers in humans. Yadolah Fakhri et al. proved that Pb content in rice is 0.38 mg/kg d.w. which is higher than the national standard limit. Consumers of all age groups consuming rice containing this level of Pb have an increased risk of carcinogenesis ($ILCR > 10^{-3}$). The

age group of consumers of rice with Pb content had a significant carcinogenic risk (ILCR > 10⁻³) [23].

In Vietnam, research on this issue is still new, but some results suggest that areas using urban waste, sludge, sewage affect the plants, quality of the crop. According to Nguyen Dinh Manh (2000), vegetables grown in polluted areas (e.g. mining areas, waste land after coal mining, solid waste land) or vegetables are irrigated with contaminated water (e.g. urban wastewater, industrial wastewater) are contaminated with heavy metals. Studying heavy metals residues in agricultural products in the Ho Chi Minh City, Bui Cach Tuyen demonstrated the effect of heavy metal pollution on vegetable quality through correlation between heavy metals in water and water spinach was 0.73 with Pb and 0.94 with Cd; correlation coefficient between heavy metals in soil and vegetables was 0.12 with Pb and 0.99 with Cd.

Doan Chi Cuong et al., (2014) studied the risks to human health when using water spinach grown in Trung Son village. The concentration of heavy metals accumulated in water spinach was higher than in the soil, with Cd (0.0396 mg/kg) and Pb (1,656 mg/kg). Therefore, it is necessary to limit the use of water spinach in this area to avoid health risks [24].

Research on Hg, Pb and Cd content in the soil, water and some vegetables of Dong Anh district, Hanoi showed that Hg was less polluted in agricultural water, in soil and vegetables. Many soil and water samples were contaminated with Pb. There was a relatively close relationship between Pb pollution in soil and water and grown vegetables. Most Pb contaminated vegetable samples are

related to Pb contamination in soil or irrigation water which is contaminated with this element. The Cd content in the soil was at safe levels, but many water samples were contaminated with Cd [25].

The total content of heavy metals in soil samples collected in the Dong Mai Pb recycling area in Chi Dao Commune, Van Lam District, Hung Yen Province, northern Vietnam exceeded the allowable limits. The serious contamination of soil with Pb and its contamination with Cd and Hg endanger the health and livelihoods of local residents. This metal contamination in food and vegetable crops, especially the high Pb contents in rice grains (*Oryza sativa*) and the shoots of water spinach (*Ipomoea aquatica*), was higher than the safe limit [18].

The correlation between the concentration of heavy metals in the soil, water and the concentration of heavy metals in foods was quite close. Soil and water contaminated with heavy metals in concentrations above the allowable threshold, become toxic to plants and microorganisms living in soil and water. When plants are grown on land and water contaminated with these heavy metals, plants absorb and accumulate heavy metals (Pb, Cd and Hg) in parts of the food that can affect animal and human health, as follows:

Pb is a highly toxic element for human health. Lead is toxic to the central nervous system, peripheral nervous system, affecting the enzyme system. People with lead poisoning will have disorders of the hematopoietic system. Depending on the degree of poisoning can cause abdominal pain, joint pain, kidney inflammation, high blood pressure, brain stroke, severe poisoning can cause death. After entering the body, lead is less excreted, but

accumulates over time and then causes toxicity. Lead accumulates in bones, inhibits calcium metabolism by inhibiting vitamin D metabolism [26]. Affects photosynthesis and growth, chlorosis, inhibit enzyme activities and seed germination, oxidative stress. Denatures nucleic acid and protein, inhibits enzymes activities and transcription

Cadmium is toxic to the liver, kidneys, lung and prostate cancer, lymphocytosis, microcytic hypochromic anemia, testicular atrophy, brain and bones disease. Depending on the level of exposure, symptoms such as vomiting may appear. Severe exposure can lead to death [26]. Impacts of Cd on plants as Chlorosis, decrease in plant nutrient content, growth inhibition, reduced seed germination [26, 27]. For microorganisms, Cd damage nucleic acid, denature protein, inhibit cell division and transcription, inhibits carbon and nitrogen mineralization [26, 27].

Hg is a very toxic metal that can cause brain and liver damage if exposed by inhalation or ingestion. It can attack the central nervous system and endocrine system, affect teeth, mouth and can cause birth defects,... Inorganic mercury affects mainly the kidneys, while methyl mercury mainly affects the central nervous system. After poisoning, patients are irritable, emotional, digestive disorders, nervous disorders, gingivitis, tremors. Severe poisoning of Hg can lead to death [28]. Hg affects antioxidative system, photosynthesis, enhances lipid peroxidation, induces genotoxic effect, inhibits plant growth, yield, nutrient uptake and homeostasis, oxidative stress [26, 27]. For microorganisms, Hg decreases population size, denatures protein, disrupts cell membrane, inhibits enzyme function [26, 27].

In short, the presence of heavy metals (Pb, Cd and Hg) in soil and water is closely related to their uptake in crop. They are not useful for plants and capable of reducing plant growth due to the reduction of photosynthetic activities, plant mineral nutrition and activity of essential enzymes. These heavy metals are cytotoxic at low concentrations and could lead to cancer in humans [4, 5].

4. Combination of plants and microorganisms for heavy metal removal from soil and water

According to Rufes et al., (2007) [292] traditional treatment methods of contaminated soil and water are soil washing, soil spraying, solidification/place, fixed solids displacement, localisation, transfer vitrification, thermal desorption, thermal treatment, electrolysis and burial. However, according to Vijayaraghavan and Yeoung (2008) these methods have the following limitations as applied only on a small scale, a small number of inorganic and organic compounds; excess handling should increase the amount of buried waste, thereby increasing the risk of airborne and water pollution; requires high levels of technology and the high of treating, so it is difficult to accept in developing countries and not friendly with environmental [30]. Compared with these pollution treatment methods, bioremediation is an innovative technique for the removal of heavy metal ions from polluted areas, using living organisms to reduce and/or recover heavy metal pollutants into less hazardous forms, using the activities of algae, bacteria, fungi, or plants [26].

Phytoremediation is a technology that using super-accumulating plants to treat soil and water contaminated by

heavy metals (Pb, Cd and Hg) through phytoextraction, phytostabilization and phytovolatilization mechanisms [22]. Many studies in the world have proven effective use of terrestrial plants and aquatic plants to treat Pb, Cd and Hg pollution in soil and water medium [31, 32]. More than 400 species of “Hyperaccumulation” have been discovered of 45 families in the world [33]. In that, there are several plant species that have been shown to be super-accumulation for heavy metals (Pb, Cd and Hg) such as *Helianthus annuus* of the *Asteraceae* family [34, 35], *Minuartia vern* of the Carnation family *Viola boashanensis* of the *Violaceae* family phytoextraction Pb, Cd. These Hyperaccumulation plants have accumulation capacity of heavy metals from 10 to 500 times higher than ordinary plant species [36], or accumulation of more than 10 mg / kg Hg, 100 mg / kg Cd and 1000 mg / kg Pb [37]. This result showed that phytoremediation is considered to be a new approach to clean up contaminated soil and water. However, this technology has certain constraints such as time-consuming, biomass, root depth, soil chemistry and pollution levels, tree age, pollutant concentration, impact of mats polluted vegetation and climatic conditions [38].

The microbial bioremediation can be done by using natural microorganisms (native or extraneous) to remove heavy metals via some approach methods, such as bioaugmentation, biostimulation and bioattenuation. Bioremediation can be carried out in soil, sediment and water through biological processes and it can be performed ex situ or in situ [15, 39]. Microbial bioremediation has also been studied and widely used in the world. There are many species of

microorganisms (including bacteria, root fungi, molds, yeasts) such as *Bacteria Pseudomonas veronii* bacteria for removing Cd [40], *Aspergillus fumigatus* fungi for removing Pb, *Sacharomyces cerevisiae* yeast for removing Pb, Cd [41], *Spirogyra* spp. and *Cladophora* spp. algae for removing Pb (II). The advantage of microbial bioremediation is that it improves contaminated soils and water with low cost, environmentally friendly and easiest to operate. The limitation of this technology is that to remove metals requires a lot of time, the efficiency of the treatment depends on the identification of the species, the species of microorganism composition and character of the soil and apply on the soil surface because microorganisms cannot reach deeper in the soil.

Using plants and microbes separately for the remediation of contaminated soil and water is not very much effective and sufficient. Therefore, the combination of plants and microorganisms will be an appropriate solution to overcome limitations mentioned previously [39]. Microorganisms associated with plant root system also play significant role in plants mediated heavy metal remediation technologies. Such microbial community can be classified into two major groups: mycorrhizal fungi and plant growth promoting rhizobacteria (PGPR). These microorganisms in rhizosphere provide a critical link between plant and soil. Mycorrhizal fungi form major component of rhizosphere and show mutualistic association with most plants. Mycorrhizal fungi, such as arbuscular mycorrhizal (AMF) can benefit plant in terms of: improve nutrient absorption through extensive extra radical hyphal networks, which explore the soil absorb nutrient and

translocate them to the roots; modify root system resulting in a more extensive length and increased branching and therefore enhanced nutrient absorption capacity of roots; change the chemical composition of root exudates and influences soil pH thus quantitatively affecting the microbial populations in the rhizosphere; improve soil structure; Regulate hormones; and tolerance and protection against biotic and abiotic stress such as soil-borne plant pathogens, insect herbivores and high levels of heavy metals [42]. On the basis of relationship with plants, PGPR communities can be divided into two groups: symbiotic bacteria and free-living rhizobacteria. These microorganisms are able to enhance plant growth through various mechanisms [42], such as allowing plants to develop longer roots during early stages of growth by reducing ethylene production; nitrogen fixation; specific enzymatic activity; supply bioavailable phosphorous and other trace element for plant uptake; production of phytohormones such as auxins, cytokinins and gibberellins; increase plant tolerance against flooding, salt stress and water deprivation; produce siderophores (have played key role in solubilizing unavailable forms of heavy metals bearing minerals by complexation reaction [43]). Depending on the different microorganisms apply different mechanisms for growth and metal tolerance, accumulation and metabolism in plants. It can so be beneficial to design the process of phytoremediation in combination with appropriate microorganisms consortium, which may include AMF and PGPR [44].

The symbiotic association between plants and microorganisms in the rhizosphere of plants or endophytic association between them is the suitable

solution to improve remediation of soil and water pollution from the environment. Plant - microbe interactions enhance the plant health but also help them in well acclimatization in environment. In plant - microbial interactions, plant roots help microorganisms to reach deeper in soil and improve aeration and nutrients supply and the endophytic microorganisms allow degradation of pollutants within the plants [44].

In addition, genetic engineering can be used to improve the remediation efficiency of microorganisms and plants and also increase the plant - microbial interactions to degrade those toxic substances which were impossible to degrade by naturally occurring bacteria and plants [45]. Great successes have been achieved in the development of transgenic plants with enhanced heavy metal accumulating capacity but majority of genes have been transferred from other plants or organisms.

Combination of microorganisms and plant species can provide effective measures which plant species can provide effective measures for heavy metal decontamination. It offered several advantages that are attractive to site owners, regulatory agencies and the public for the elimination of heavy metals from agricultural soil. However, several obstacles need to be overcome for commercial application of such treatment systems [46] such as commercially cost - effective mass - production and formulation of microbial inoculums; microbial inoculum should be relatively universal for various plants and soils and its effectiveness should be relatively easy to evaluate; effectiveness of the microbial consortium to function in natural conditions; knowledge of

possible interactions between plants and associated soil microorganisms in the natural environment.

5. Some results from using the combination of plants and microorganisms to remove Pb, Cd, Hg from soil and water

Combination of plants and microorganisms in bioremediation offers several advantages that are attractive to site owners, regulatory agencies and the public for elimination of heavy metals from agricultural soil and water. This combination is demonstrated by scientists with fungi capable of reducing the toxicity of heavy metals to plants and affecting their accumulation and transport when combined.

Mohammad Rezvani et al. (2015) in a greenhouse experiment barley plants were inoculated with AM species, *Glomus mosseae* and grown in polluted soil of cadmium (Cd) and lead (Pb). The results indicated that mycorrhizal barley absorbed significantly higher amounts

of Pb. AM species also significantly decreased Cd by barley showing the alleviating effects of *G. mosseae* on the stress of such heavy metals [47]. Millar and Bennett (2016) also demonstrated that symbiosis with fungi under abiotic pressure as heavy metals were considered to be effective medium for plant growth [48]. According to a study by Sun et al. (2017) with the addition of *Mucor circinelloides*, the Pb accumulation in *Solanum nigrum* L. was 45.77 % higher than that of single plant treatments [49]. Reports of Li et al., (2017a) also showed that the effects of *Aspergillus aculeatus* on Bermudagrass showed a relative growth rate of 2.9 and 10 times. The relative standardized respiratory rate was higher at 12.5 % and 19.4 % at concentrations of 150 mg/kg and 250 mg/kg Cd compared with non - vaccinated animals [50]. Some results of combining plants and microorganisms to remove heavy metals (Pb, Cd and Hg) in soils and water are summarized in the Table 1 below.

Table 1. Some reported use the combination of plants and microorganisms to remove Pb, Cd and Hg from soils

Plants	Combinated microorganism	Pollutants	Effective	Reference
Cabbage (<i>Brassica rapa</i> L.)	<i>Aspergillus niger</i> and <i>A.flavus</i>	Hg	Hg-resistant indigenous fungi can mobilize mercury in the soil and serve as potential bioremediation agents for contaminated agricultural soil.	[51]
Arabidopsi thailiana	<i>Mucor circinelloides</i> (MC) and <i>Trichoderma asperellum</i> (TA)	Cd, Pb	Tolerance to Cd and Pb with 40.19 - 117.50 % higher root length and 58.31 - 154.14 % shoot fresh weight of plant.	[52]
Rice	<i>Enterobacter</i> sp. (S2) <i>K. michiganensis</i> (S8)	Cd	Enhanced cadmium accumulating plant growth promoting rhizobacterium	[53]
Brassica Juncea	The bacterial strains of <i>pseudomonas aeruginosa</i> , <i>alcaligenes feacalis</i> and <i>bacillus subtilis</i>	Cd	Compared with control treatment, soil inoculation with bacterial isolates significantly increased the mount of soluble heavy metals in soil by 50 %.	[54]

Plants	Combinated microorganism	Pollutants	Effective	Reference
Ryegrass and Seduce alfredii	Microbacterium sp. KL5 and Candida tropicalis C10	Cd	The results of metal phytoextract (36.1% Cd) were obtained by interplanting Ryegrass and Seduce alfredii with regular re-inoculation with Microbacterium sp. KL5 and Candida tropicalis C10 in the contaminated soil.	[55]
Zea mays. L	Micrococcus sp. TISTR2221	Cd	Micrococcus sp. TISTR2221 significantly promoted the root length, shoot length and dry biomass; and increased cadmium accumulation in the roots and shoots of Zea mays. L	[56]
Ferns	Mycorrhizal arbuscular fungi	Pb	Combining mycorrhizal arbuscular fungi and ferns for removing Pb from the soil and indicated that the Pb accumulated up to 834.63 mg/kg in the root and 121.19 mg/kg in the stalk-leaves of the fern	[57]

Genetic engineering is also used to increase the ability of combining plants and microorganisms in the treatment of heavy metals Pb, Cd and Hg. Reports have been made on the use of genetically engineered Eschericia coli strain M109 and Pseudomonas putida containing the merA gene to effectively eradicate Hg from contaminated soils and sediments.

Introduction of Brevibacterium casei MH8a into soil with Sinap is significantly increased Cd accumulation by 208 % in the plant shoots [58]. Some report was made on the use of genetically modified plants and microorganisms used to improve the bioremediation efficiency of heavy metals (Pb, Cd and Hg) are summarized in Table 2.

Table 2. Genetic engineering of plant and/or microbes for the improvement of bioremediation heavy metals

Gen	Genne source	Host plant/ microbe	Pollutants	Remarks	Reference
PaMT1	Pisolithus albus	Eucalyptus tereticornis	Cd	High PaMT1 expression levels increased in the presence Cd	[59]
MerRTPAB	PDU58-Serratia marcescens	E.coli K12	Hg	Expression of Mer operon enables transport of Hg ²⁺ into the cell, cleavage of organic C - Hg bonds and reduction of Hg ²⁺ into Hg (0)	[60]
Gcsgs	Enterobacter sp. CBSB1	Brassica juncea	Cd and Pb	Gcsgs gene introduced into Enterobacter sp. CBSB1 upgraded the phyto remediation efficacy of B. juncea	[61]

Gen	Genne source	Host plant/ microbe	Pollutants	Remarks	Reference
ScYCF1	Saccharomyces cerevisiae	Populus alba	Cd and Pb	increased Cd accumulation in the aerial tissues and increased Cd and Pb accumulation in the roots	[62]

The above results have shown that the technology combining plants and microorganisms has been widely studied and highly appreciated in the world. It is gained much attention for elimination heavy metals (Pb, Cd and Hg) pollution from agriculture soil and water in situ or out situ with native plants and microorganisms, or with genetically modified organisms and plants. Most results have been reported under laboratory and greenhouse conditions for contaminated agriculture soils, showing that inoculation of beneficial microbes particularly plant growth promoting bacteria and/or mycorrhizae may stimulate heavy phytoextraction or phytostabilization. Only a few studies have demonstrated the effectiveness of the microbial assisted plant bioremediation of toxic metals in the field conditions. Therefore, it is necessary to continue to study the mechanism of metal - microbes - plant interactions and to improve the performance and processing time through use of beneficial microbes as inoculants for microbial assisted phytoremediation under field conditions. Simultaneously, emphasis should be placed when developing bioremediation systems using plant - associated bacteria, to choose wild type bacteria, or bacteria enhanced using natural gene transfer, to avoid the complications of national and international legislation restricting and monitoring the use of genetically modified microbes. However, with a global political shift towards sustainable

and green bioremediation technologies, the use the combination of plants and microorganisms to remove toxic heavy metals in agriculture soil and water may provide an efficient, economic and sustainable green remediation technology for environment and sustainable agriculture development.

6. Application prospects in Vietnam

The results of using bioremediation in the world and in Vietnam for the treatment of heavy metals, which is overcome the limitations of the individual application of plants or microorganisms and demonstrate superiority of the process of treating heavy metals in soil and water acceleration faster and more efficiently.

The research works mainly use the single species of plants such as water hyacinth, duckweed, water fern, vetiver, reed,... and some microorganisms such as mold (*Aspergillus.sp.*, *Penicillium.sp.*), the fungi of AMF roots isolated from soil and water medium to remove toxic heavy metals (Pb, Cd, Hg, ...). Study of Le Duc and Tran Thi Tuyet Thu (2000) showed that in the morning leaf, the Pb accumulation after 40 days and 60 days increased from 125 to 130 times compared with vegetable before experiment; in water hyacinth, Pb accumulation after 40 days and 60 days increased from 115 to 160 times than before experiment [63]. Dang Dinh Kim (2010) is using two species of Fern (*Pteris vittata* and *Pityrogramma calomelano*), Goose grass (*Eleusine indica*) and Vetiver (*Vetiveris zizanioides* (Linn) to treat Pb

pollution in soil. Results showed that the Vetiver still grow well in soil containing 1,400 - 2,530 mg Pb / kg, the ability of Pb extraction in grass contaminated soil from 87 % to 92.56 % after 90 days of experiment [64]. Another study by Le Nhu Kieu et al. (2010) is to identify some species of plants, including Butterfly needles (*Bidens pilosa. L.*), Ludwigia Octovalvis (*Ludwigia Hyssopifolia (G.don) Exell (Jussiaea linifolia Vahl)*), Nipa (*Nypa fruticans*), Sunflower (*Helianthus annuus*), Buffalo spinach (*Enydra fluctuans Lour*) which have the ability to accumulate Pb. However, these selected plants are wild plants without economic value. This study also selected some number of microorganisms that can accumulate Pb: 193.46 mg Pb by bacteria; yeast 234,19 mg Pb; mold mildew 203,64 mg Pb and 1 strain AMF 657,48 mg Pb. These results is just the announcement, detecting the species are able to accumulate, metabolize the metal more or less [65].

There is only a few research reports using plants and microorganisms to remove these toxic metals from agriculture soils, such as report of Le Nhu Kieu et al. (2011) on combination of five native plants and isolated microorganisms (*B. subtilis*; *Saccharomyces cerevisiae*; *Glomus australe* and *Gibberela sp* - in the formulation) in field trial. The result showed that the effect of that combination was markedly apparent in *Sunflower* (69.51 %), *Coconut water* (35.87 %), *Monosodium glutamate* (25.36 %) [65]. Application of microbial inoculants of *Bacillus licheniformis* and *Glomus intraradices* increased the accumulated amount of Pb, Cd and Hg in fruit 3.5 %, 4 % and 5 % respectively and 28 % and 30 % respectively in the roots, stems

and leaves of okra plants compared with the control without preparation. Using a combination of okra and with this microbial inoculants of *Bacillus licheniformis* and *Glomus intraradices* reduced heavy metals content (Pb, Cd, Hg) in soil by 26 - 30 % compared with control after 2 years of experiment [17].

Studies using microorganisms with plants in treatment of heavy metal on contaminated agriculture soil and water in Vietnam are still very limited. But these early results have also provided the basis for science and practice to advance further research and open up new possibilities for the use of this technology for the treatment of heavy metals (Pb, Cd and Hg) in agricultural soil and water because it combines two characteristics of plants and microorganisms, the ability of high biomass and super - accumulation mechanism to heavy metals successfully process in the environment. Moreover, with the cost of treatment is lower than traditional methods and environmentally friendly, the combination of plants and microorganisms is considered a possible solution in the future. Specifically, with the orientation of sustainable and safe agricultural development, it is necessary to focus on exploring indigenous multipurpose plants and native strains of microorganisms capable of combining together to remove metals Pb, Cd and Hg from agricultural soil, water and achieved results faster and more efficient clean - up of the polluted site, contribute to safe and sustainable agricultural production. Vietnam is recognized as one of 16 highly diverse countries in the world. Thus, the study of combining plants and microorganisms is a possible and promising solution in controlling and restoring heavy metal pollution in

agricultural land and water in Vietnam. Plant species and microorganisms are studied, selected, tested and replicated models of heavy metal treatment in agricultural land. They also have the prospect of commercialization in domestic and international markets.

7. Conclusion

Contamination of agricultural soils and surface water with heavy metals is becoming a serious environmental issue in the world and Vietnam. Finding economical and eco-friendly techniques to tackle this problem is on priority. Bioremediation is a great prospect in that the combination of two basic properties, the ability to increase biomass and super-absorb heavy metals, of plants and microorganisms will successfully treat heavy metals in the environment and increase the biomass of plants and microorganisms. Application of plant - microbe synergy to restore lands and water contaminated with heavy metals (Pb, Cd and Hg) is a highly efficient, cost - effective and environmentally - friendly technique. By using different biotech approaches, researchers have improved the efficiency and processing time of plants and microorganisms. This effect is a scientific and practical basis for research and application of this technology to remove heavy metals (Pb, Cd and Hg) in soil and water, contributing to sustainable agriculture in Vietnam.

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