REVIEW ARTICLE

Application of Local Species for Sustainable Phytoremediation[#]

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ABSTRACT

Phytoremediation is green technology based on the application of plants to remediate contaminated media. This paper reviews five species of local plants used for phytoremediation in Thailand: *Pteris vittata* L., *Pityrogramma calomelanos* L., *Chrysopogon zizanioides* L., *Eichhornia crassipes* (Mart.) Solms, and *Pistia stratiotes* L. For each plant, its pollutant removal efficiency and mechanism is reviewed. The main mechanisms of phytoremediation, such as phytoextraction, rhizofiltration, phytostabilization, phytodegradation, rhizodegradation, and phyto-volatilization, are concisely described. Screening local plants for phytoremediation is a cost-effective and easy to manage approach to derive suitable plants that are resistant to harmful environmental conditions. To be suitable, plants should have a fast growth rate, produce a large biomass yield, have a high tolerance to the toxic effects of the pollutants, and have a good capacity for pollutant uptake. Moreover, applying the proper species for each contaminant enhances the removal efficiency and supports sustainable phytoremediation.

1. INTRODUCTION

The rapid global urbanization and the development of industrialization has significantly increased soil and water pollution. These pollutants include various organic and inorganic compounds, such as heavy metals, pesticides, petroleum waste, and radionuclides, that threaten the ecosystem and enhance human health risks (Singh and Pant, 2023). Phytoremediation is one of the most promising technologies for environmental cleanup. It is defined as using green plants to remove, contain, and render environmental toxicants (Hettiarachchi et al., 2019). Both soil and water pollution from various sources are found across Thailand, and phytoremediation is a developing approach within the country and so is reviewed in this manuscript.

Most of the research reviewed in this paper deals with the phytoremediation of soil and water contaminated with heavy metals. Phytoextraction refers to removing metals using a plant's ability to absorb metals from the soil or water into its roots and then translocate most of them to the aboveground biomass. Metal hyperaccumulator plants have developed several regulatory mechanisms to survive in a metal-contaminated environment, including heavy metal absorption, transportation, chelation, and detoxification (Memon et al., 2021). In Thailand, two kinds of ferns, *Pityrogramma calomelanos* L. and *Pteris vittata* L., have been shown to be arsenic (As) hyperaccumulators, while *Chrysopogon zizanioides* L., *Eichhornia crassipes* (Mart.) Solms, and *Pistia stratiotes* L. are candidate plant species for removing heavy metals from water. These five kinds of plants are common in Thailand, grow fast, and suit the climate.

Sustainable remediation projects are evaluated based upon their social, economic, and positive vs. negative environmental impacts (EnvironWiki, 2023). Sustainable phytoremediation is a promising technique for mitigating human environmental impacts and limiting adverse socio-economic impacts. However, to increase the efficiency of sustainable phytoremediation, it is necessary to raise the knowledge, risk assessment, public awareness, and acceptance of this technology among scientists, industry, stakeholders, government agencies, and non-governmental organizations (Latif et al., 2023).

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2. MECHANISM OF PHYTOREMEDIATION

The phytoremediation process can be separated into the six categories of phytoextraction, rhizofiltration, phytostabilization, phytodegradation, rhizodegradation, and phytovolatilization.

(1) Phytoextraction involves the absorption of toxic metals from the environment (topsoil, surface or ground water) by plant roots and then translocation to shoots and deposition at the vacuole, cell wall, cell membrane, and other inactive parts in the plant tissues (Kafle et al., 2022). Therefore, phytoextraction is an attractive method for the cleanup of heavy metal-contaminated sites by using the potential plants, which in Thailand includes *Chrysopogon zizanioides*, *Spirodela polyrhiza*, *Pistia stratiotes*, *Eichhornia crassipes*, and *Pennisetum purpureum* (Yang et al., 2020).

(2) Rhizofiltration is the adsorption or precipitation of contaminants that are in the solution surrounding the root zone onto plant roots or absorption into the roots. Plant roots excrete specific chemicals in the root environment, creating biogeochemical conditions that precipitate contaminants onto the roots or in the water body (Akhtar et al., 2017).

(3) Phytostabilization can occur through the precipitation of heavy metals or reduction of the metal valency in the rhizosphere, with absorption and sequestration within root tissues, or adsorption onto root cell walls. This process uses metal-tolerant plant species to immobilize heavy metals belowground and so decrease their bioavailability, thereby preventing their migration into the ecosystem and reducing the likelihood of metals entering the food chain (Yan et al., 2020).

(4) Phytodegradation involves the plants' transformation or breakdown of organic contaminants through metabolic processes. The degradation of pollutants occurs inside or outside the plant through enzymes produced by roots, such as dehalogenases, nitroreductases, and peroxidases (Nedjimi, 2021). For example, it was reported that *Vetiveria zizanioides* could clean up 97% of trinitrotoluene from the soil (Das et al., 2010).

(5) Rhizodegradation refers to the breakdown of organic contaminants in rhizospheric soil using microorganisms. Exudates from plant roots, such as sugars, amino acids, and flavonoids, increase the microbial activity in the rhizosphere. The root exudates are a carbon and nitrogen source, providing a nutrient-rich environment for microorganisms and encouraging microbial activity (Latif et al., 2023).

(6) Phytovolatilization is the uptake of pollutants from the soil or water that are then converted into less toxic but volatile forms and are then released into the atmosphere via plant transpiration via the leaves or foliage system. This approach can be applied to the detoxification of organic pollutants and some heavy metals like selenium (Se), mercury (Hg), and As (Mahar et al., 2016).

3. SELECTION OF LOCAL SPECIES FOR PHYTOREMEDIATION

Appropriate selection of the plant species for the contaminants and geochemical site is crucial for effective phytoremediation. Therefore, the use of local species for phytoremediation is considered advantageous (already adapted) and they should possess the following characteristics:

(1) High tolerance to the toxic effects of the respective heavy metals and/or other pollutants.

(2) Good ability to uptake, translocate, or degrade the contaminants.

(3) High growth rate, high biomass production, and an extensive root system.

(3) High resistance to stressful environmental conditions, pests, weeds, and pathogens.

(4) Easy to cultivate and harvest.

(5) Low maintenance cost.

(6) Increase ecological value and support soil resilience.

Other factors, such as the temperature, pH, solar radiation, nutrient availability, and salinity, can greatly influence the phytoremediation potential and growth of the plant (Ali et al., 2020).

4. THE LOCAL PLANTS APPLIED TO CONTAMINATED SITES IN THAILAND

There are five principal plant species being utilized or well-studied for phytoremediation within Thailand, and these are discussed in turn as follows: (1) *Pteris vittata* L. (Chinese brake fern) and (2) *Pityrogramma calomelanos* L. (silverback fern).

The lead (Pb) tolerance and accumulation in ferns, including *Pteris vittata* (Figure 1) and *Pityrogramma calomelanos* (Figure 2), have been studied. The plants were collected from the Bo Ngam lead mine site. In hydroponic and pot experiments, *P. calomelanos* accumulated the highest concentration of Pb (root 14,161.1 mg/kg and frond 402.7 mg/kg) compared to *P. vittata*. The ferns were found to have a translocation factor of less than 1. When *P. vittata* and *P. calomelanos* were grown in contaminated mine soil for six months, *P. vittata* tolerated a higher soil Pb level (94,584-101,405 mg/kg) and accumulated more Pb in its frond (4,829.6 mg/kg) than *P. calomelanos* (3,605.1 mg/kg) (Soongsombat et al., 2009).

In Thailand, *P. vittata* and *P. calomelanos* were found in As contaminated land, such as the Ron Phibun District (Nakorn Si Thammarat Province) and Bannang Sata District (Yala Province) (Visoottiviseth et al., 2002). These areas in the Ron Phibun District to Ronna-Suangchan Mountain were part of a former tin mining area that resulted in As-contaminated soil in southern Thailand. The As concentrations in the topsoil ranged from 21 to 14,000 mg/g in Ron Phibun and from 540 to 16,000 mg/g in Bannang Sata. The researchers selected the plants based on the following criteria: high As tolerance, high bioaccumulation factor, short life cycle, high propagation rate, wide distribution, and large shoot biomass. The results indicated that the highest As concentrations in leaves of *P. calomelanos* and *P. vittata* were 8,350 and 6,030 mg/g, respectively, (Visoottiviseth et al., 2002).

The As uptake in most plants rarely exceeds 1 mg/kg. The WHO limits As in plants because plants do not readily translocate As from the roots to the shoots (Santra et al., 2013). Therefore, *P. calomelanos* and *P. vittata* are considered as As hyperaccumulators. With respect to the translocation and distribution of As in *P. vittata*, the As is believed to be taken up from the soil by the phosphate uptake system as As (V). However, within the root exudates and xylem sap of *P. vittata*, As was found in different forms of As, including As (III), As (V), mono-methyl arsenic acid, and dimethylarsinic acid. The reduction of As (V) to As (III) occurs in the frond of the fern (Fayiga and Saha, 2016). The presence of As (V) and As (III) induces plant antioxidant production higher antioxidant activity and results in a higher superoxide dismutase, catalase, and ascorbate peroxidase (APX), in *P. vittata* than in two non-hyperaccumulators, indicating the ability of *P. vittata* to get rid of reactive oxygen species and free radicals (Srivastava et al., 2005).

Moreover, increased ascorbate and glutathione (GSH) levels significantly increased the level of As uptake and translocation in *P. vittata* (Wei et al., 2010). Note that GSH is a precursor to phytochelatins that are involved in detoxifying As in *P. vittata* (Rosen, 2002). The other mechanism of As detoxification in *P. vittata* is the chelation of arsenite by ligands and binding with thiol groups, followed by sequestration away from the sites of metabolism in the cytoplasm, probably into the vacuole or cell wall (Pandey et al., 2015).

Figure 1. *P. vittata* (Photo courtesy of Assistant Professor Dr. Rossarin Pollawatn)

Figure 2. *P. calomelanos* (Photo courtesy of Emeritus Professor Dr. Thaweesakdi Boonkerd)

The effects of phosphorus fertilizer and rhizosphere microbe amendments on As accumulation by *P. calomelanos* were investigated in greenhouse and field experiments (Jankong et al., 2007). The greenhouse experiments used contaminated soils from Ron Phibun District of Nakhon Si Thammarat Province in Thailand with As concentrations of 136-269 mg/kg. The addition of phosphorus fertilizer significantly increased the plant biomass, rhizosphere microbes, and As accumulation in *P. calomelanos*. The enhanced phosphorus and level of rhizosphere bacteria increased the As phytoextraction, while rhizofungi significantly reduced the total As concentration in plants but increased the plant biomass (Upatham et al., 2014).

(3) *Chrysopogon zizanioides* L. (vetiver grass)

Vetiver grass (*Vetiveria zizanioides* has recently been reclassified as *Chrysopogon zizanioides*) is a tall, fast-growing perennial grass with a massive deep-penetrating root system (Figure 3) that originated from India. However, it is widely cultivated in tropical regions and in Thailand its cultivation was promoted for soil and water conservation in 1991 and then for slope stabilization, erosion control, and environmental improvement (Roongtanakiat, 2006). There are many ecotypes of vetiver grass due to ecological variation, and they show different tolerances/adaptations to different habitats. Nowadays, these cultivars of *C. zizanioides* are commonly found in all regions of Thailand.

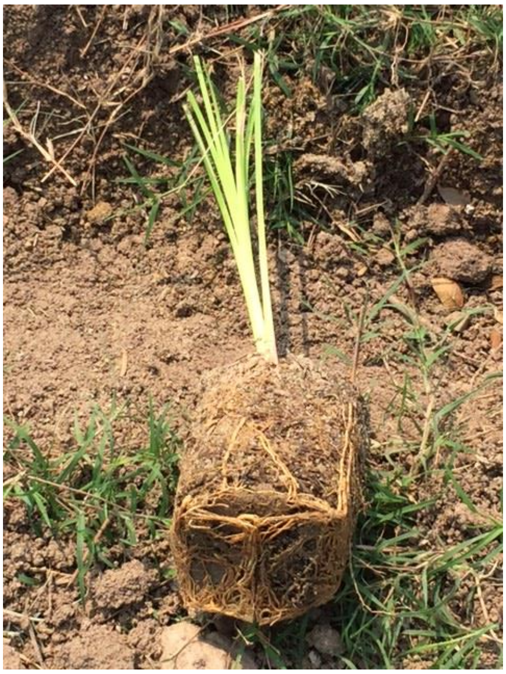

Figure 3. Sapling of *C. zizanioides*

Chrysopogon zizanioides has an excellent potential to remediate contaminated water and soil because this plant can tolerate high levels of heavy metals and pollutants. Moreover, its unique morphological and physiological characteristics include a high growth rate, adaptability to extreme environmental conditions, and a long root system. It can grow in soil across a very broad pH range of 3.3-12.5 and temperature range from 15-55°C. Moreover, it has a finely structured and vigorous root structure that can grow 3-4 m deep within the first year (Wikipedia, 2023a).

Various studies have demonstrated the successful application of this species as a remedy for removing pollutants and heavy metals in Thailand's contaminated or intoxicated soil and water (Roongtanakiat et al., 2007; Rotkittikhun et al., 2007). Therefore, the ability of *C. zizanioides* to uptake heavy metals from industrial wastewater has been investigated. Three vetiver ecotypes, Kamphaeng Phet-2, Sri Lanka, and Surat Thani, were cultured in four types of industrial wastewater sampling from a milk factory, a battery manufacturing plant, an electric lamp plant, and an ink manufacturing facility. The plants cultured in wastewater from a milk factory showed the highest plant growth and had the highest manganese (Mn), iron (Fe), zinc (Zn), and Pb removal efficiencies of 33.72%, 27.63%, 52.73%, and 8.94%, respectively. The *C. zizanioides* grown in wastewater from an ink manufacturing facility showed the highest copper (Cu) removal capacity of up to 87.5%, although the root growth was retarded as a Cu toxicity symptom (Roongtanakiat et al., 2007).

The Pb accumulation in the shoots and roots of four ecotypes of *C. zizanioides*; three from Thailand (Surat Thani, Songkhla, and Kamphaeng Phet) and one from Sri Lanka, was found to be similar in all four ecotypes. Plants accumulated higher Pb concentrations in the roots (4,100-5,900 mg/kg) than in the shoots (248-422 mg/kg). However, the application of 20% (w/w) pig manure was the most effective treatment (compared to inorganic fertilizer) to improve the biomass of vetiver grown in Pb-contaminated soils, which was due to the pig manure's increased electrical conductivity and reduced diethylenetriamine pentaacetate-extractable Pb concentration in the soils (Rotkittikhun et al., 2007).

Investigation of the mechanism of heavy metal uptake from sulphuric acid discharge in *C. zizanioides* and their subsequent metabolism revealed that the vetiver shoot displayed increased levels of amino acid metabolism, glutathione metabolism, TCA, and the urea cycle. The roots showed accumulated ornithine and oxaloacetate levels and downregulated phosphorlipid, phosphorylated metabolites, cellular respiration, glyoxylate, and amino acid metabolism. Organic acids and glutathione were secreted from the roots for rhizospheric metal chelation (Kiiskila et al., 2020).

The role of arbuscular mycorrhizal fungi (AMF) associated with *C. zizanioides* grown in heavy metal-contaminated soils in phytoremediation greenhouse studies has been evaluated in several studies. The inoculation of AMF (*Rhizophagus intraradices* and *Glomus versiforme*) into Pb-contaminated soil increased the shoot and root dry weight of the *C. zizanioides* and increased both the Pb levels in the shoot and the root uptake levels (Bahraminia et al., 2016). Likewise, inoculation of a $HgCl_2$-contaminated soil with AMF (MykovamTM and *Glomus* sp.) increased the overall *C. zizanioides* weight more than the plants grown in the uninoculated soil for all tested $HgCl_2$ concentrations (Bretaña et al., 2019). The AMF improved the mineral uptake of the plants, reduced the contaminant toxicity, and increased the plant's tolerance to environmental stress in the associated hosts. The metals were absorbed through the fungal hyphae and then transported to the plant. Moreover, the presence of the AMF contributed to metal immobilization in the soil (Sahraoui et al., 2022).

(4) Eichhornia crassipes (Mart.) Solms (water hyacinth)

Eichhornia crassipes is a fast-growing free-floating macrophyte with a fibrous root system (Figure 4). This plant was first introduced to Thailand in 1901 by King Chulalongkorn the Great (Rama V), who brought it back from a visit to Indonesia and was grown in Sa Pathum Palace, a royal residence. However, due to its high growth rate and ease of dispersal, some water hyacinths colonized nearby canals, including Khlong Samsen, Klong Prem Prachakorn, and Khlong Phadung Krung Kasem (Ariyakanon, 2018), and then spread further. Nowadays, *E. crassipes* is widespread across the surface water in Thailand (Plant pest in Thailand, 2023). The optimum growth temperature of *E. crassipes* is 25-30°C with a maximum temperature of 33-35°C and its pH tolerance is estimated at pH 5.0-7.5. However, *E. crassipes* does not grow once the average salinity is greater than 15% (Wikipedia, 2023b).

Eichhornia crassipes has been studied for phytoremediation because of its rapid proliferation rate and high biomass production without showing many toxic symptoms (Malar et al., 2015). This plant is considered a multipurpose phytoremediator because it decontaminates inorganic nutrients, toxic metals, and persistent organic pollutants (Mishra and Maiti, 2017). It has been reported that *E. crassipes* is a promising plant species for the remediation of natural waterbodies/wastewater polluted with low levels of Zinc (Zn), chromium (Cr), copper (Cu), cadmium (Cd), Lead (Pb), silver (Ag), and nickel (Ni) (Priyanka et al., 2017). Evaluation of the heavy metal removal by *E. crassipes* in the Bueng Makkasan Pond (Central Thailand) revealed the accumulation of heavy metals (Zn, Mn, Cu, Cd, Pb, and Ni) in the roots of *E. crassipes* was significantly higher than in the shoots. This plant also absorbed nutrients (N, P, K, Ca, and Mg) from the wastewater (Chunkao et al., 2012). The high tolerance and affinity of *E. crassipes* for heavy metal accumulation are due to the high cellulose content and its functional groups, including amino ($-NH_2$), carboxyl (-COOH), hydroxyl (-OH), and sulfhydryl (-SH) groups (Patel, 2012).

The application of *E. crassipes* for wastewater treatment on a laboratory scale was evaluated using residential and surimi wastewater at 10-50% (v/v). The water parameters, including total Kjeldahl nitrogen (TKN) and total phosphorus (TP), biological oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS), in each wastewater were analyzed before and after phytoremediation. The removal efficiency of TKN, TP, BOD, COD, and TSS in 10% (v/v) residential wastewater ranged from 61.4 to 90.1%, while for 10% (v/v) surimi wastewater it was in the range of 62.3 to 90.6%, respectively, (Wattanapanich et al., 2020).

Three mechanisms have been described for the heavy metal removal from water by *E. crassipes*: root absorption, foliar absorption, and adsorption (Zhang et al., 2015). The root absorption uses carboxyl groups in the root systems that cause cation exchange inside the cell membrane and stimulate the movement of the heavy metals in the roots by an active absorption process. Foliar absorption occurs when contaminants are passively absorbed by stoma cells and cracks in the cuticle. A comparatively low amount of contaminant absorption takes place by foliar absorption. Finally, the adsorption process occurs at the rhizosphere of the plant, where *E. crassipes* has dense fibrous roots that help to trap suspended solids and bacteria. This offers proper attachment sites for the growth of fungus and bacteria; hence the contaminants get absorbed easily because of the ionic imbalance throughout the cell membrane (Aqdas and Hashmi, 2023).

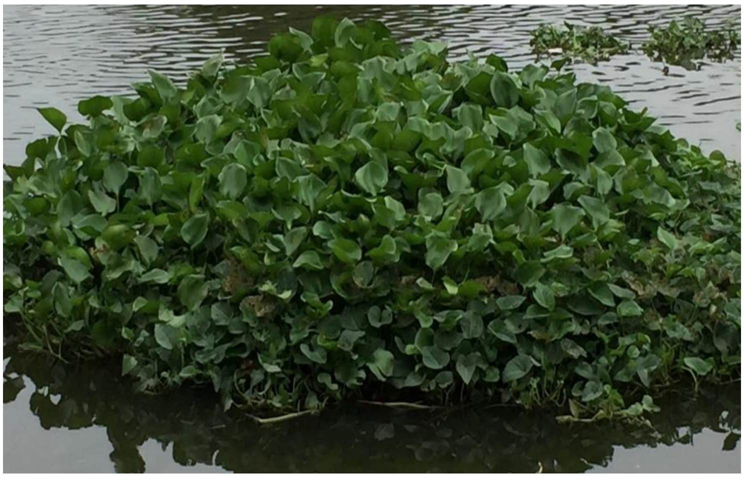

Figure 4. *E. crassipes*

(5) *Pistia stratiotes* L. (water lettuce)

Pistia stratiotes is a free-floating and perennial aquatic macrophyte (Figure 5). It was first discovered from the Nile near Lake Victoria in Africa, but is now widely distributed in tropical and subtropical fresh waterways, especially in slow-moving rivers, lakes, and ponds, including across Thailand. The optimal growth temperature of this species is 22-30°C, but it can endure extreme temperatures of up to 35°C. The optimal pH of this is plant is slightly acidic water (pH 6.5-7.2) (Wikipedia, 2023c).

Currently, *P. stratiotes* is distributed in every part of Thailand and is commonly found in standing surface water, including lakes and ponds (BGO Plant Databases, 2023). It can remove several heavy metals from water due to its extensive root systems (Farnese et al., 2014). The advantages of *P. stratiotes* are that it is fast-growing with a large biomass, can cover large water surfaces, and requires an uncomplicated harvesting process (Pang et al., 2023).

Thirteen species of aquatic plants collected from unpolluted water around Bangkok, Thailand, including *Lemna minor*, *Typha angustifolia*, and *P. stratiotes*, were tested for their heavy metal accumulation in the laboratory. The results showed that *P. stratiotes* did not tolerate a high concentration of Cd (10 mg/L). However, at low Cd concentrations (0.1 and 1.0 mg/L), it accumulated 182.09 and 2,554.52 mg/kg DW, respectively, (Bunluesin et al., 2004). The removal capacity of chlorpyrifos from the water by the two aquatic plants, *P. stratiotes* and *L. minor*, collected from the ponds at Kasetsart University, Bangkok, Thailand, was investigated in the laboratory. An initial chlorpyrifos concentration of 0.1 mg/L was reduced to an undetectable level from 4 and 5 days after culturing with *L. minor* and *P. stratiotes*, respectively, and to 82.0±1.7% and 87.0±2.1%, respectively, at an initial chlorpyrifos concentration of 0.5 mg/L (Prasertsup and Ariyakanon, 2011).

Both *E. crassipes* and *P. stratiotes* could decolorize and detoxify five different textile dyes: CI Direct Blue 201, Cibacron Blue, Cibanone Gold Yellow, Vat Green, and Moxilon Blue. The decolorization mechanisms of Direct Blue 201 dye by *E. crassipes* and *P. stratiotes* were based on biosorption and intracellular enzyme activities (Ekanayake et al., 2021). In addition, phyto-remediation of industrial sewage sludge with wetland plants on a pilot scale revealed *E. crassipes*, *Salvinia molesta*, and *P. stratiotes* as promising candidates for the removal of Cu, Cr, Cd, Ni, and Zn (Kodituwakku and Yatawara, 2020).

Plant tolerance to heavy metal stress is a complex process that includes a series of metabolic adjustments. These include the activation of detoxification and antioxidant defence systems and related protective metabolic pathways, the transformation of metabolic pathways in the plant, and the content change of many substances to reconstruct metabolic balance. The metabolic changes in *P. stratiotes* to alleviate the phytotoxicity effect of a Zn and Cd combination at different concentrations was evaluated in the laboratory. The results revealed that *P. stratiotes* increased heat dissipation in the leaf photosynthetic apparatus to reduce the damage caused by excess excitation energy to the photosystem II (PS II) reaction center and safeguard the balance between absorption and utilization of light energy. The plant also increased the APX activity and the oxidized ascorbic acid (i.e., dehydroascorbic acid) content in the leaves to enhance the antioxidant capacity of the ascorbate-glutathione (AsA-GSH) cycle and maintain the stability of the reduced glutathione (GSH) and oxidized glutathione (GSSG) contents. Finally, the plant increased the levels of carnosol, and substances related to lipid metabolism (including the cutin, suberine, and wax biosynthesis pathway), to maintain cell stability and increase resistance to the combined stress of Zn and Cd (Li et al., 2022).

Figure 5. *P. stratiotes*

5. FUTURE PERSPECTIVES FOR PHYTO-REMEDIATION

Phytoremediation is an eco-friendly technology with great potential to decontaminate soil and water. To date, some mechanisms of the plant's ability to uptake heavy metals or organic pollutants have been explicitly studied at the cellular level. However, clarification of the mechanism related to specific genes and enzymes in plants is missing yet is essential to increase our knowledge and phytoremediation efficiency.

- Many successful cases of phytoremediation have been reported. However, most of these studies are at the greenhouse or small pilot scale. Actual real-world application studies, such as in wastewater treatment systems in factories, municipalities, and canteens, are lacking yet are critical for achieving a better environment for the next generation.
- The interaction between microorganisms in the rhizosphere and plants is complex, but poorly resolved. Therefore, it is necessary to understand it in more detail to improve metal uptake. Moreover, it will be related to discovering new metabolites and mechanisms for optimizing the degradation of organic pollutants.
- Current research has established that the use of nanotechnology and transgenic plants can enhance the phytoremediation efficiency. However, if these technologies are to be implemented in the environment, their potential adverse environmental impact and suitable and comprehensive regulatory systems must also be developed.
- After the phytoremediation process, the appropriate management of harvested plants should be carefully applied. The correct disposal of harvested plants needs to be clarified to avoid subsequent environmental (human health and ecological) risks.
- The contribution and coordination of scientists, local communities, industrial sectors, and government authorities through educational programs is required to enact long-term sustainable phytoremediation in Thailand.

6. CONCLUSION

The pollutant removal efficiency of five local plant species in Thailand and their uptake mechanism was reviewed. Two kinds of fern, *P. vittata* and *P. calomelanos*, were found in As-contaminated soil and found to be As hyperaccumulators. *C. zizanioides is* generally found in all regions of Thailand and is tolerant to extreme environmental conditions (pH and temperature) and has a long root system. It can be applied for heavy metal removal from soil and wastewater. *E. crassipes* is widespread in the surface water across Thailand and has a suitable optimum growth temperature of 25-30 °C and pH tolerance at

5.0-7.5. It can be applied to remove some heavy metals and nutrients from water. *P. stratiotes* is mostly found in standing surface water, including lakes and ponds, in every part of Thailand. It can be applied to remove heavy metals, chlorpyrifos, and textile dyes from contaminated water. Because these five plant species can uptake high concentration of pollutants, proper management of the plants after harvesting should be carefully applied. This process could then lead to sustainable phytoremediation.

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