

Review Article

A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation

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Received 17 March 2011; Accepted 3 June 2011

Academic Editor: Hans-Jörg Bart

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Heavy metals are among the most important sorts of contaminant in the environment. Several methods already used to clean up the environment from these kinds of contaminants, but most of them are costly and difficult to get optimum results. Currently, phytoremediation is an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil and water. This technology is environmental friendly and potentially cost effective. This paper aims to compile some information about heavy metals of arsenic, lead, and mercury (As, Pb, and Hg) sources, effects and their treatment. It also reviews deeply about phytoremediation technology, including the heavy metal uptake mechanisms and several research studies associated about the topics. Additionally, it describes several sources and the effects of As, Pb, and Hg on the environment, the advantages of this kind of technology for reducing them, and also heavy metal uptake mechanisms in phytoremediation technology as well as the factors affecting the uptake mechanisms. Some recommended plants which are commonly used in phytoremediation and their capability to reduce the contaminant are also reported.

1. Introduction

Heavy metals are among the contaminants in the environment. Beside the natural activities, almost all human activities also have potential contribution to produce heavy metals as side effects. Migration of these contaminants into noncontaminated areas as dust or leachates through the soil and spreading of heavy metals containing sewage sludge are a few examples of events contributing towards contamination of the ecosystems [1].

Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. The chemical technologies generate large volumetric sludge and increase the costs [2]; chemical and thermal methods are both technically difficult and expensive that all of these methods can also degrade the valuable component

of soils [3]. Conventionally, remediation of heavy-metal-contaminated soils involves either onsite management or excavation and subsequent disposal to a landfill site. This method of disposal solely shifts the contamination problem elsewhere along with the hazards associated with transportation of contaminated soil and migration of contaminants from landfill into an adjacent environment. Soil washing for removing contaminated soil is an alternative way to excavation and disposal to landfill. This method is very costly and produces a residue rich in heavy metals, which will require further treatment. Moreover, these physio-chemical technologies used for soil remediation render the land usage as a medium for plant growth, as they remove all biological activities [1].

Recent concerns regarding the environmental contamination have initiated the development of appropriate technologies to assess the presence and mobility of metals in

soil [4], water, and wastewater. Presently, phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil. Phytoremediation is the use of plants to clean up a contamination from soils, sediments, and water. This technology is environmental friendly and potentially cost-effective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants [5]. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body [3].

Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soils. One of phytoremediation categories, phytoextraction, can be used to remove heavy metals from soil using its ability to uptake metals which are essential for plant growth (Fe, Mn, Zn, Cu, Mg, Mo, and Ni). Some metals with unknown biological function (Cd, Cr, Pb, Co, Ag, Se, Hg) can also be accumulated [5].

The objectives of this paper are to discuss the potential of phytoremediation technique on treating heavy metal-contaminated site, to provide a brief view about heavy metals uptake mechanisms by plant, to give some description about the performance of several types of plants to uptake heavy metals and to describe about the fate of heavy metals in plant tissue, especially on arsenic (As), lead (Pb), and mercury (Hg). This study is related to a research project that aims to identify potential plants in tropical country such as Malaysia which can uptake heavy metal contaminants from petrochemical wastewater.

2. Heavy Metals: Sources and Effect in the Environment

Heavy metals are conventionally defined as elements with metallic properties and an atomic number >20 . The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil [6]. Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni, and Co, while others have unknown biological function, such as Cd, Pb, and Hg [1].

Metal pollution has harmful effect on biological systems and does not undergo biodegradation. Toxic heavy metals such as Pb, Co, Cd can be differentiated from other pollutants, since they cannot be biodegraded but can be accumulated in living organisms, thus causing various diseases and disorders even in relatively lower concentrations [7]. Heavy metals, with soil residence times of thousands of years, pose numerous health dangers to higher organisms. They are also known to have effect on plant growth, ground cover and have a negative impact on soil microflora [8]. It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be transformed into nontoxic compounds [1].

2.1. Arsenic (As). Arsenic (atomic number 33) is a silver-grey brittle crystalline solid with atomic weight of 74.9, specific

gravity 5.73, melting point 817°C (at 28 atm), boiling point 613°C , and vapor pressure 1 mm Hg at 372°C [9]. Arsenic is a semimetallic element with the chemical symbol "As". Arsenic is odorless and tasteless. Arsenic can combine with other elements to form inorganic and organic arsenicals [10]. In the environment, arsenic is combined with oxygen, chlorine, and sulfur to form inorganic arsenic compounds. Inorganic arsenic compounds are mainly used to preserve wood. Organic arsenic compounds are used as pesticides, primarily on cotton plants [11].

Arsenic exists in the -3 , 0 , $+3$, and $+5$ valence oxidation states [9], and in a variety of chemical forms in natural waters and sediments [12]. Environmental forms include arsenious acids (H_3AsO_3 , H_3AsO_3 , $\text{H}_3\text{AsO}_3^{2-}$), arsenic acids (H_3AsO_4 , H_3AsO_4^- , $\text{H}_3\text{AsO}_4^{2-}$), arsenites, arsenates, methylarsenic acid, dimethylarsinic acid, and arsine. Two most common forms in natural waters arsenite (AsO_3^{3-}) and inorganic arsenate (AsO_4^{3-}), referred as As^{3+} and As^{5+} [9]. From both the biological and the toxicological points of view, arsenic compounds can be classified into three major groups. These groups are inorganic arsenic compounds, organic arsenic compounds, and arsine gas [13].

It is a hard acid and preferentially complexes with oxides and nitrogen. Trivalent arsenites predominate in moderately reducing anaerobic environments such as groundwater [9]. The most common trivalent inorganic arsenic compounds are arsenic trioxide, sodium arsenite, and arsenic trichloride [13]. Trivalent ($+3$) arsenates include $\text{As}(\text{OH})_3$, $\text{As}(\text{OH})_4^-$, $\text{AsO}_2\text{OH}^{2-}$, and AsO_3^{3-} [9]. Arsenite ($\text{As}(\text{OH})_3$, As^{3+}) is predominant in reduced redox potential conditions [12].

Arsenic is one of the contaminants found in the environment which is notoriously toxic to man and other living organisms [14]. It is a highly toxic element that exists in various species, and the toxicity of arsenic depends on its species. The pH, redox conditions, surrounding mineral composition, and microbial activities affect the form (inorganic or organic) and the oxidation state of arsenic. It is generally accepted that the inorganic species, arsenite [As^{3+}] and arsenate [As^{5+}], are the predominant species in most environments, although the organic ones might also be present [15].

In general, inorganic compounds of arsenic are regarded as more highly toxic than most organic forms which are less toxic [10, 14, 16, 17]. The trivalent compounds (arsenites) are more toxic than the pentavalent compounds (arsenates) [16, 17]. It has been reported that As^{3+} is 4 to 10 times more soluble in water than As^{5+} . However, the trivalent methylated arsenic species have been found to be more toxic than inorganic arsenic because they are more efficient at causing DNA breakdown [17]. Although As^{5+} tends to be less toxic compared to As^{3+} , it is thermodynamically more stable due to it predominates under normal conditions and becomes the cause of major contaminant in ground water [14]. Arsenate which is in the pentavalent state (As^{5+}) is also considered to be toxic and carcinogenic to human [18].

2.2. Lead (Pb). Lead (Pb), with atomic number 82, atomic weight 207.19, and a specific gravity of 11.34, is a bluish or silvery-grey metal with a melting point of 327.5°C and a

boiling point at atmospheric pressure of 1740°C. It has four naturally occurring isotopes with atomic weights 208, 206, 207 and 204 (in decreasing order of abundance). Despite the fact that lead has four electrons on its valence shell, its typical oxidation state is +2 rather than +4, since only two of the four electrons ionize easily. Apart from nitrate, chlorate, and chloride, most of the inorganic salts of lead²⁺ have poor solubility in water [19]. Lead (Pb) exists in many forms in the natural sources throughout the world and is now one of the most widely and evenly distributed trace metals. Soil and plants can be contaminated by lead from car exhaust, dust, and gases from various industrial sources.

Pb²⁺ was found to be acute toxic to human beings when present in high amounts. Since Pb²⁺ is not biodegradable, once soil has become contaminated, it remains a long-term source of Pb²⁺ exposure. Metal pollution has a harmful effect on biological systems and does not undergo biodegradation [7].

Soil can be contaminated with Pb from several other sources such as industrial sites, from leaded fuels, old lead plumbing pipes, or even old orchard sites in production where lead arsenate is used. Lead accumulates in the upper 8 inches of the soil and is highly immobile. Contamination is long-term. Without remedial action, high soil lead levels will never return to normal [20].

In the environment, lead is known to be toxic to plants, animals, and microorganisms. Effects are generally limited to especially contaminated areas [21]. Pb contamination in the environment exists as an insoluble form, and the toxic metals pose serious human health problem, namely, brain damage and retardation [5].

2.3. Mercury (Hg). Mercury is a naturally occurring metal that is present in several forms. Metallic mercury is shiny, silver-white, odorless liquid. Mercury combines with other elements, such as chlorine, sulfur, or oxygen, to form inorganic mercury compounds or salts, which are usually white powders or crystals. Mercury also combines with carbon to make organic mercury compounds [22]. Mercury, which has the lowest melting point (−39°C) of all the pure metals, is the only pure metal that is liquid at room temperature. However, due to its several physical and chemical advantages such as its low boiling point (357°C) and easy vaporization, mercury is still an important material in many industrial products [23]. As any other metal, mercury could occur in the soil in various forms. It dissolves as free ion or soluble complex and is nonspecifically adsorbed by binding mainly due to the electrostatic forces, chelated, and precipitated as sulphide, carbonate, hydroxide, and phosphate. There are three soluble forms of Hg in the soil environment. The most reduced is Hg⁰ metal with the other two forms being ionic of mercurous ion Hg₂²⁺ and mercuric ion Hg²⁺, in oxidizing conditions especially at low pH. Hg⁺ ion is not stable under environmental conditions since it dismutates into Hg⁰ and Hg²⁺. A second potential route for the conversion of mercury in the soil is methylation to methyl or dimethyl mercury by anaerobic bacteria [24].

Mercury is a persistent environmental pollutant with bioaccumulation ability in fish, animals, and human beings

[23]. Mercury salts and organomercury compounds are among the most poisonous substances in our environment. The mechanism and extent of toxicity depend strongly on the type of compound and the redox state of mercury [25].

Environmental contamination due to mercury is caused by several industries, petrochemicals, minings, painting, and also by agricultural sources such as fertilizer and fungicidal sprays [26]. Some of the more common sources of mercury found throughout the environment include but may not be limited to the household bleach, acid, and caustic chemicals (e.g., battery acid, household lye, muriatic acid (hydrochloric acid), sodium hydroxide, and sulfuric acid), instrumentation containing mercury (e.g., medical instruments, thermometers, barometers, and manometers), dental amalgam (fillings), latex paint (manufactured prior to 1990), batteries, electric lighting (fluorescent lamps, incandescent wire filaments, mercury vapor lamps, ultraviolet lamps), pesticides, pharmaceuticals (e.g., nasal sprays, cosmetics, contact lens products), household detergents and cleaners, laboratory chemicals, inks and paper coatings, lubrication oils, wiring devices and switches, and textiles. Though mercury use in many of the above items being produced now is restricted or banned, there are still some existing, older products in use [22].

Terrestrial plants are generally insensitive to the harmful effects of mercury compounds; however, mercury is known to affect photosynthesis and oxidative metabolism by interfering with electron transport in chloroplasts and mitochondria. Mercury also inhibits the activity of aquaporins and reduces plant water uptake [27].

Mercury and its compounds are cumulative toxins and in small quantities are hazardous to human health. The major effects of mercury poisoning manifest as neurological and renal disturbances as it can easily pass the blood-brain barrier and has effect on the brain [26].

3. Phytoremediation Technology

Phytoremediation techniques have been briefly depicted in many literatures or articles. The generic term “phytoremediation” consists of the Greek prefix *phyto* (plant), attached to the Latin root *remedium* (to correct or remove an evil) [28, 29]. Some definitions on phytoremediation that have been described by several researchers are listed in Table 1.

Generally, according to the above researchers, phytoremediation is defined as an emerging technology using selected plants to clean up the contaminated environment from hazardous contaminant to improve the environment quality. Figure 1 depicts the uptake mechanisms of both organics and inorganics contaminants through phytoremediation technology. For organics, it involves phytostabilization, rhizodegradation, rhizofiltration, phytodegradation, and phytovolatilization. These mechanisms related to organic contaminant property are not able to be absorbed into the plant tissue. For inorganics, mechanisms which can be involved are phytostabilization, rhizofiltration, phytoaccumulation and phytovolatilization.

TABLE 1: Definition of phytoremediation.

No.	Researchers	Definition of phytoremediation
(1)	[30]	The use of plants to improve degraded environments
(2)	[31]	The use of plants, including trees and grasses, to remove, destroy or sequester hazardous contaminants from media such as air, water, and soil
(3)	[24]	The use of plants to remediate toxic chemicals found in contaminated soil, sludge, sediment, ground water, surface water, and wastewater
(4)	[32]	An emerging technology using specially selected and engineered metal accumulating plants for environmental cleanup
(5)	[33]	The use of vascular plants to remove pollutants from the environment or to render them harmless
(6)	[3]	The engineered use of green plant to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water. This definition includes all plant-influenced biological, chemical, and physical processes that aid in the uptake, sequestration, degradation, and metabolism of contaminants, either by plants or by the free-living organisms that constitute the plant rhizosphere
(7)	[29]	Phytoremediation is the name given to a set of technologies that use different plants as a containment, destruction, or an extraction technique. Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water
(8)	[34]	Phytoremediation in general implies the use of plants (in combination with their associated microorganisms) to remove, degrade, or stabilize contaminants

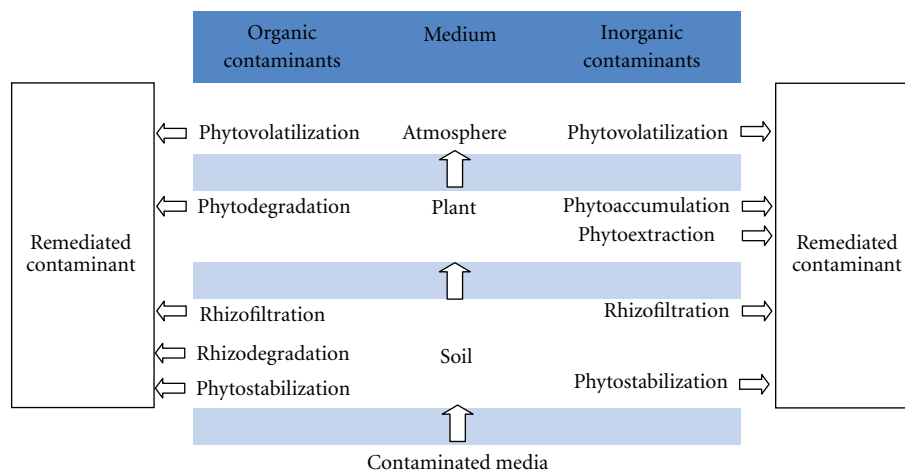


FIGURE 1: Uptake mechanisms on phytoremediation technology. Source: [35].

Based on Figure 1, some certain essential processes involved in phytoremediation technology [29, 31] are phytostabilization and phytoextraction for inorganic contaminants, and phytotransformation/phytodegradation, rhizofiltration, and rhizodegradation for organic contaminants.

The root plants exudates to stabilize, demobilize and bind the contaminants in the soil matrix, thereby reducing their bioavailability. These all are called as phytostabilization process. Certain plant species have used to immobilize contaminants in the soil and ground water through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone. This process is for organics and metals contaminants in soils, sediments, and sludges medium [29, 31].

Specific plant species can absorb and hyperaccumulate metal contaminants and/or excess nutrients in harvestable root and shoot tissue, from the growth substrate through

phytoextraction process. This is for metals, metalloids, radionuclides, nonmetals, and organics contaminants in soils, sediments, and sludges medium [29, 31].

Phytovolatilization process is the plants ability to absorb and subsequently volatilize the contaminant into the atmosphere. This process is for metal contaminants in groundwater, soils, sediments, and sludges medium. Since phytotransformation/phytodegradation process is the breakdown of contaminants taken up by plants through metabolic processes within the plant or the breakdown of contaminants externally to the plant through the effect of compounds produced by the plants. This process is for complex organic molecules that are degraded into simpler molecule contaminants in soils, sediments, sludges, and groundwater medium [29, 31].

Plant roots take up metal contaminants and/or excess nutrients from growth substrates through rhizofiltration

(=root) process, the adsorption, or, precipitation onto plant roots or absorption into the roots of contaminants that are in solution surrounding the root zone. This process is for metals, excess nutrients, and radionuclide contaminants in groundwater, surface water, and wastewater medium [29, 31].

The breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of the root zone is called rhizodegradation. This process uses microorganisms to consume and digest organic substances for nutrition and energy. Natural substances released by the plant roots, sugars, alcohols, and acids, contain organic carbon that provides food for soil microorganisms and establish a dense root mass that takes up large quantities of water. This process is for organic substance contaminants in soil medium [29, 31].

4. Mechanisms of Heavy Metal Uptake by Plant

Contaminant uptake by plants and its mechanisms have been being explored by several researchers. It could be used to optimize the factors to improve the performance of plant uptake. According to Sinha et al. [36], the plants act both as “accumulators” and “excluders”. Accumulators survive despite concentrating contaminants in their aerial tissues. They biodegrade or biotransform the contaminants into inert forms in their tissues. The excluders restrict contaminant uptake into their biomass.

Plants have evolved highly specific and very efficient mechanisms to obtain essential micronutrients from the environment, even when present at low ppm levels. Plant roots, aided by plant-produced chelating agents and plant-induced pH changes and redox reactions, are able to solubilize and take up micronutrients from very low levels in the soil, even from nearly insoluble precipitates. Plants have also evolved highly specific mechanisms to translocate and store micronutrients. These same mechanisms are also involved in the uptake, translocation, and storage of toxic elements, whose chemical properties simulate those of essential elements. Thus, micronutrient uptake mechanisms are of great interest to phytoremediation [37].

The range of known transport mechanisms or specialized proteins embedded in the plant cell plasma membrane involved in ion uptake and translocation include (1) proton pumps (“-ATPases that consume energy and generate electrochemical gradients), (2) co- and antitransporters (proteins that use the electrochemical gradients generated by “-ATPases to drive the active uptake of ions), and (3) channels (proteins that facilitate the transport of ions into the cell). Each transport mechanism is likely to take up a range of ions. A basic problem is the interaction of ionic species during uptake of various heavy metal contaminants. After uptake by roots, translocation into shoots is desirable because the harvest of root biomass is generally not feasible. Little is known regarding the forms in which metal ions are transported from the roots to the shoots [37].

Plant uptake-translocation mechanisms are likely to be closely regulated. Plants generally do not accumulate trace elements beyond near-term metabolic needs. And these

requirements are small ranging from 10 to 15 ppm of most trace elements suffice for most needs [37]. The exceptions are “hyperaccumulator” plants, which can take up toxic metal ions at levels in the thousands of ppm. Another issue is the form in which toxic metal ions are stored in plants, particularly in hyperaccumulating plants, and how these plants avoid metal toxicity. Multiple mechanisms are involved. Storage in the vacuole appears to be a major one [37].

Water, evaporating from plant leaves, serves as a pump to absorb nutrients and other soil substances into plant roots. This process, termed evapotranspiration, is responsible for moving contamination into the plant shoots as well. Since contamination is translocated from roots to the shoots, which are harvested, contamination is removed while leaving the original soil undisturbed. Some plants that are used in phytoextraction strategies are termed “hyperaccumulators.” They are plants that achieve a shoot-to-root metal-concentration ratio greater than one. Nonaccumulating plants typically have a shoot-to-root ratio considerably less than one. Ideally, hyperaccumulators should thrive in toxic environments, require little maintenance and produce high biomass, although few plants perfectly fulfill these requirements [38].

Metal accumulating plant species can concentrate heavy metals like Cd, Zn, Co, Mn, Ni, and Pb up to 100 or 1000 times those taken up by nonaccumulator (excluder) plants. In most cases, microorganisms bacteria and fungi, living in the rhizosphere closely associated with plants, may contribute to mobilize metal ions, increasing the bioavailable fraction. Their role in eliminating organic contaminants is even more significant than that in case of inorganic compounds [39, 40].

Heavy metal uptake by plant through phytoremediation technologies is using these mechanisms of phytoextraction, phytostabilisation, rhizofiltration, and phytovolatilization as shown in Figure 2.

4.1. Phytoextraction. Phytoextraction is the uptake/absorption and translocation of contaminants by plant roots into the above ground portions of the plants (shoots) that can be harvested and burned gaining energy and recycling the metal from the ash [28, 39–42].

4.2. Phytostabilisation. Phytostabilisation is the use of certain plant species to immobilize the contaminants in the soil and groundwater through absorption and accumulation in plant tissues, adsorption onto roots, or precipitation within the root zone preventing their migration in soil, as well as their movement by erosion and deflation [28, 39–42].

4.3. Rhizofiltration. Rhizofiltration is the adsorption or precipitation onto plant roots or absorption into and sequestration in the roots of contaminants that are in solution surrounding the root zone by constructed wetland for cleaning up communal wastewater [28, 39–42].

4.4. Phytovolatilization. Phytovolatilization is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to

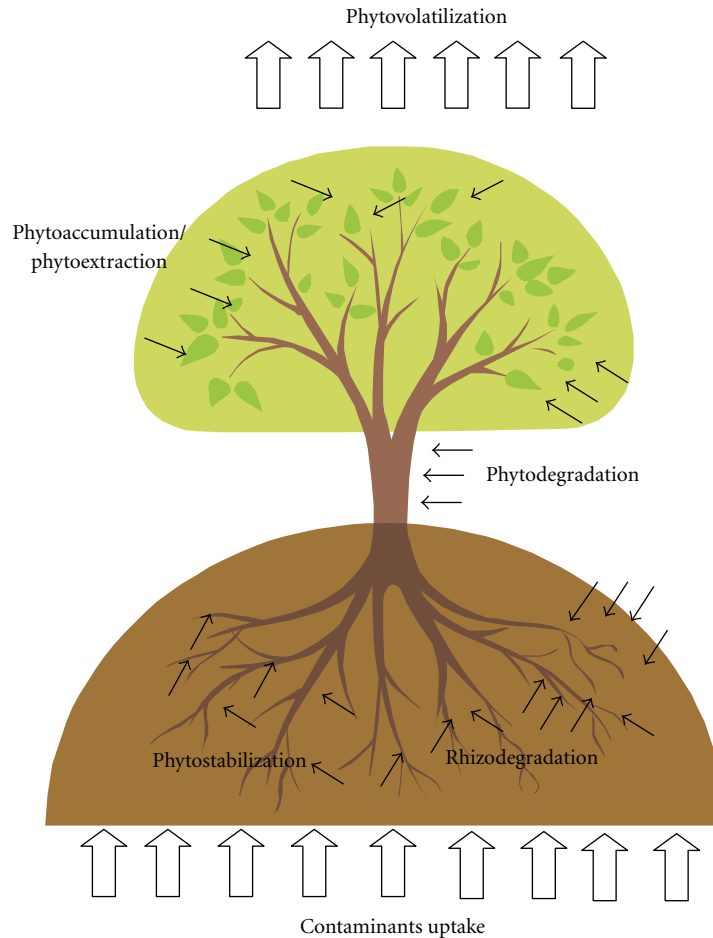


FIGURE 2: The mechanisms of heavy metals uptake by plant through phytoremediation technology.

the atmosphere from the plant. Phytovolatilization occurs as growing trees and other plants take up water along with the contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations [28, 39–42].

Plants also perform an important secondary role in physically stabilizing the soil with their root system, preventing erosion, protecting the soil surface, and reducing the impact of rain. At the same time, plant roots release nutrients that sustain a rich microbial community in the rhizosphere. Bacterial community composition in the rhizosphere is affected by complex interactions between soil type, plant species, and root zone location. Microbial populations are generally higher in the rhizosphere than in the root-free soil. This is due to a symbiotic relationship between soil microorganisms and plants. This symbiotic relationship can enhance some bioremediation processes. Plant roots also may provide surfaces for sorption or precipitation of metal contaminants [27].

In phytoremediation, the root zone is of special interest. The contaminants can be absorbed by the root to be subsequently stored or metabolised by the plant. Degradation of contaminants in the soil by plant enzymes exuded from the roots is another phytoremediation mechanism [43].

For many contaminants, passive uptake via micropores in the root cell walls may be a major route into the root, where degradation can take place [3].

5. Factors Affecting the Uptake Mechanisms

There are several factors which can affect the uptake mechanism of heavy metals, as shown in Figure 3. By having knowledge about these factors, the uptake performance by plant can be greatly improved.

5.1. The Plant Species. Plants species or varieties are screened, and those with superior remediation properties are selected [31]. The uptake of a compound is affected by plant species characteristic [44]. The success of the phytoextraction technique depends upon the identification of suitable plant species that hyperaccumulate heavy metals and produce large amounts of biomass using established crop production and management practices [24].

5.2. Properties of Medium. Agronomical practices are developed to enhance remediation (pH adjustment, addition of chelators, fertilizers) [31]. For example, the amount of lead absorbed by plants is affected by the pH, organic matter, and

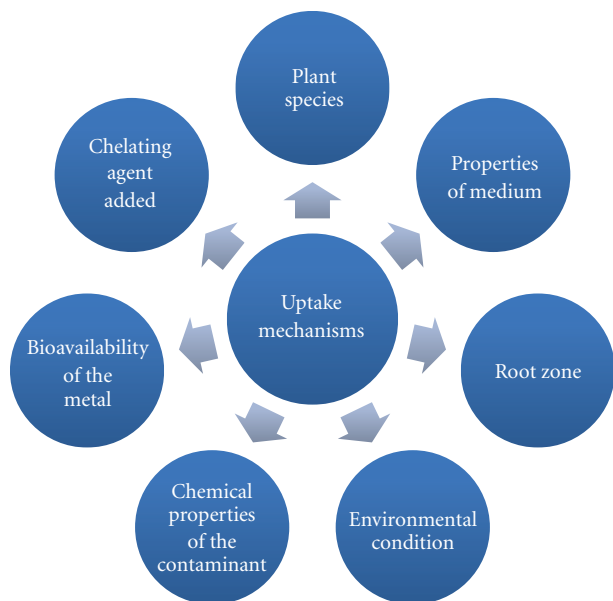


FIGURE 3: Factors which are affecting the uptake mechanisms of heavy metals.

the phosphorus content of the soil. To reduce lead uptake by plants, the pH of the soil is adjusted with lime to a level of 6.5 to 7.0 [20].

5.3. The Root Zone. The Root Zone is of special interest in phytoremediation. It can absorb contaminants and store or metabolize it inside the plant tissue. Degradation of contaminants in the soil by plant enzymes exuded from the roots is another phytoremediation mechanism. A morphological adaptation to drought stress is an increase in root diameter and reduced root elongation as a response to less permeability of the dried soil [43].

5.4. Vegetative Uptake. Vegetative Uptake is affected by the environmental conditions [44]. The temperature affects growth substances and consequently root length. Root structure under field conditions differs from that under greenhouse condition [43]. The success of phytoremediation, more specifically phytoextraction, depends on a contaminant-specific hyperaccumulator [45]. Understanding mass balance analyses and the metabolic fate of pollutants in plants are the keys to proving the applicability of phytoremediation [46].

Metal uptake by plants depends on the *bioavailability* of the metal in the water phase, which in turn depends on the retention time of the metal, as well as the interaction with other elements and substances in the water. Furthermore, when metals have been bound to the soil, the pH, redox potential, and organic matter content will all affect the tendency of the metal to exist in ionic and plant-available form. Plants will affect the soil through their ability to lower the pH and oxygenate the sediment, which affects the availability of the metals [47], increasing the bioavailability of heavy metals

by the addition of biodegradable physicochemical factors, such as chelating agents and micronutrients [34].

5.5. Addition of Chelating Agent. The increase of the uptake of heavy metals by the energy crops can be influenced by increasing the bioavailability of heavy metals through addition of biodegradable physicochemical factors such as chelating agents, and micronutrients, and also by stimulating the heavy-metal-uptake capacity of the microbial community in and around the plant. This faster uptake of heavy metals will result in shorter and, therefore, less expensive remediation periods. However, with the use of synthetic chelating agents, the risk of increased leaching must be taken into account [34]. The use of chelating agents in heavy-metal-contaminated soils could promote leaching of the contaminants into the soil. Since the bioavailability of heavy metals in soils decreases above pH 5.5–6, the use of a chelating agent is warranted, and may be required, in alkaline soils. It was found that exposing plants to EDTA for a longer period (2 weeks) could improve metal translocation in plant tissue as well as the overall phytoextraction performance. The application of a synthetic chelating agent (EDTA) at 5 mmol/kg yielded positive results [8]. Plant roots exude organic acids such as citrate and oxalate, which affect the bioavailability of metals. In chelate-assisted phytoremediation, synthetic chelating agents such as NTA and EDTA are added to enhance the phytoextraction of soil-polluting heavy metals. The presence of a ligand affects the biouptake of heavy metals through the formation of metal-ligand complexes and changes the potential to leach metals below the root zone [48].

6. Effectiveness of Heavy Metals Uptake by Plants

Several studies have described the performance of heavy metals uptake by plants. It is reported that phytoremediation technology is an alternative to treat heavy-metal-contaminated side which will be more admitted in order to remediate the environment. Table 2 lists some research done to remediate heavy metals from contaminated soil, while Table 3 lists some research conducted to remediate them from contaminated water and wastewater.

Based on the collected data from the phytoremediation research listed in Tables 2 and 3, the accumulation of heavy metals As, Pb, and Hg in plant tissue is summarized in respective, Figures 4, 5, and 6.

According to Figure 4, the highest accumulation of As in plant tissue (the researchers have not detailed which part it is, but it might be the whole plant) occurs in *Pteris vittata* L. species. It can reach more than 0.7 mg As/g dry weight of plant. In plant root, the highest accumulation of As is in *Populus nigra*, which can reach more than 0.2 mg As/g dry weight of plant root.

As can be seen in Figure 5, several plants could accumulate Pb in their tissue of more than 50 mg/g dry weight of plant. Among those species are species of *Brassica campestris* L, *Brassica carinata* A. Br., *Brassica juncea* (L.) Czern. and

TABLE 2: Phytoremediation study on soil medium.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(1)	[16]	Greenhouse pot experiment (6, 10, and 16 days)	Phytoextraction (soil was added to aqueous solution and was dried overnight in an oven at 120°C, cooled, and transferred to the pot)	Aqueous solution containing 0.1041 g of sodium arsenate heptahydrate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$), the mixture which contained 50 mg/kg of As (wet weight)	<i>Leersia oryzoides</i> (rice-cut grass)—terrestrial plant	The increase in plant size is matched by a decrease in shoot arsenic concentration. The data show that 12, 13, and 13 mg/m ² of arsenic were absorbed by the shoots at 6, 10, and 16 weeks, respectively. Since the SRQ and PECs all exhibit the same downward trend after 6 wks, it is suggested that periodic mowing of <i>Leersia oryzoides</i> grown for phytoextraction purposes on contaminated land could maintain the high arsenic uptake at 6 week.
(2)	[33]	Laboratory (pot experiment) (90 days)	Fly ash and soil mixtures	Pb as lead nitrate, Zn as zinc sulfate, Ni as nickel sulfate, Mn as manganese chloride, and Cu as copper sulfate (1000 ppm concentration each (Spiked))	<i>Scirpus littoralis</i> —semiaquatic	The metal content ratios BO/soil (B/S) were higher than shoot/soil ratios (T/S) for all the metals, the highest being for Ni. Metal ratios BO/water (B/W) were also higher than shoot/water (T/W) ratios, but the B/W ratio was maximum for Zn. All the metals except Ni showed negative correlation with nitrogen but they were all nonsignificant. However, P uptake showed positive correlations with all the metals, and all were significant at 1% confidence limit.
(3)	[49]	Field study (90 days)	Soil (agricultural land area)	(Cu, Cd, Cr, Zn, Fe, Ni, Mn, and Pb)	Wheat (<i>Triticum aestivum</i> L.)—terrestrial Indian mustard (<i>Brassica campestris</i> L.)—terrestrial	Analyses of effluents and soil samples have shown high metal content than the permissible limit except Pb. Analyses of plant samples have indicated the maximum accumulation of Fe followed by Mn and Zn in root > shoot > leaves > seeds. Maximum increase in photosynthetic pigment was observed between 30 and 60 days while protein content was found maximum between 60 and 90 days of growth period in both plants.

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(4)	[5]	Laboratory (65 days)	Phytoextraction (soil)	Pb by using standard Pb solutions (75 mg Pb/1 kg soil)	Creeping zinnia (<i>Alternanthera phylloxeroides</i>)—aquatic Moss rose (<i>Sarvitalia procumbens</i>)—terrestrial Alligator weed (<i>Portulaca grandiflora</i>)—aquatic	<i>Alternanthera phylloxeroides</i> shows the highest lead content in its tissues. This might be caused by it forming long stolons, a massive fibrous root system, and large surface area which benefits the accumulation of lead. Efficiency process 30–80%.
(5)	[34]	Literature review	Soil	Cd, Cr, Cu, Ni, Pb, and Zn	<i>Brassica juncea</i> (Indian mustard), <i>Brassica rapa</i> (field mustard), and <i>Brassica napus</i> (rape)—terrestrial	<i>Brassica rapa</i> exhibited the highest affinity for accumulating Cd and Pb from the soil, either with/without additional use of mobilizing soil amendments. Two Brassica species (<i>Brassica napus</i> and <i>Raphanus sativus</i>) were moderately tolerant when grown on a multi-metalcontaminated soil. The distribution of heavy metals in the organs of crops decreased in the following order: leaves > stems > roots > fruit shell > seeds.
(6)	[50]	Laboratory—pot experiment (12 days)	Agropeat and half strength Hoagland solution	Arsenic (As) as of sodium (meta-) arsenite (50 uM, 150 uM and 300 uM)	<i>Brassica juncea</i> var. Varuna and Pusa Bold—terrestrial	Increase/decrease of antioxidant enzymes activities showed not much changes at the given concentrations. The data presented indicates the differential responses in both the varieties and also that the increased tolerance in P. Bold may be due to the defensive role of antioxidant enzymes, induction of MAPK, and upregulation of PCS transcript which is responsible for the production of metal-binding peptides.

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(7)	[51]	Field study (trials to extract heavy metals from two contaminated soils, one calcareous (5 years) and one acidic (2 years))	Phytoextraction (soil)	Cd and Zn	Willow (<i>Salix viminalis</i>)—terrestrial	Salix had performed better on the acidic soil because of larger biomass production and higher metal concentrations in shoots. Addition of elemental sulphur to the soil did not yield any additional benefit in the long term, but application of an Fe chelate improved the biomass production. Cd and Zn concentrations were significantly higher in leaves than stems. On both soils, concentration in shoots decreased with time.
(8)	[52]	Laboratory (26 days)	Sludge-amended soils	Cd and Zn	<i>Raphanus sativus</i> L.	This study has shown that clear evidence of as ludge-driven plateau response in metal uptake by plants will only be obtained when studies have found a good hyperbolic relationship between soil solution metal concentration with increasing sludge application rate and can link this to a plateau response in plant uptake of metals.

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(9)	[3]	Laboratory—lysimeter pot (March 1995–September 1995)	Soil	Zn as ZnSO ₄ (50, 1,500, 2,000 µg/g (ppm) Zn, and 2,000 µg/g (ppm), and 0 µg/g (ppm) (control) received nutrient only)	Hybrid poplar (<i>Populus sp.</i>)—terrestrial	At levels of zinc above 1,000 µg/g (ppm) in nutrient added, leachate levels were always below 100 µg/g (ppm) in samples as the zinc addition; these levels increased the following day and then decreased sharply the second day after the zinc addition, to concentrations less than 100 µg/g (ppm). The zinc concentration steadily decreased as the plants apparently reabsorbed the zinc as the nutrient was cycled through the pots on subsequent days. The root tissues showed much higher concentrations of accumulated and sequestered metal than did the above ground parts. Leachate analyses for zinc indicate that initially plants subjected to both levels of zinc were removing up to 70% of the zinc from the leachate. The plants receiving 160 µg/g Zn had grown considerably and were almost the same size as the controls (no zinc), but some of the mature leaf blades were rolled; the mean zinc removal rate for these plants was 50% of the zinc in the leachate. The plants receiving 600 µg/g Zn were smaller than the controls, their color was a darker green, most of the mature leaf blades were rolled, and the mean zinc removal rate was about 30% of the zinc in the leachate. The willows were able to remove approximately 9.5% of the available lead and about 1% of the total arsenic from the contaminated soil. The less mature poplars removed about 1% of the available lead and 0.1% of the total arsenic from the same soil. In the sand experiment, the willows took up about 40% of the administered lead and 30 to 40% of the administered arsenic.
		Laboratory (April 1996, 2 months)	Soil	Zn (160 µg/g Zn, 600 µg/g Zn, and 0 µg/g Zn (control))	Eastern gamagrass (<i>Tripsacum dactyloides</i>)—terrestrial	
			Soil	Pb and As (up to 1000 µg/g Pb and up to 200 µg/g As)	Hybrid willow (<i>Salix sp.</i>) and hybrid poplar (<i>Populus sp.</i>)—terrestrial	

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(10)	[53]	Field (1976–2001)	(Soil)	Nonessential (Cd, Ni, Pb) and essential heavy metals (Cu, Fe, Mn, Zn). The tetrasodium salt of EDTA was applied at rates of 0, 0.5, 1, 2 g EDTA salt/kg surface (25 cm depth) soil	Sunflower (<i>Helianthus annuus</i> L.) and Hybrid poplar (<i>Populus deltoides Marsh. x P. nigra</i> L.)—terrestrial	For sunflower, the 1.0 g/kg rate of chelate addition resulted in maximal removal of the three nonessential heavy metals (Cd, Ni, Pb). Uptake of the essential heavy metals by sunflower was little affected by the EDTA. The leaves of sunflower grown with 1.0 g EDTA Na ₄ · 2H ₂ O/kg soil accumulated more Cd, Ni, and Pb than leaves of sunflower grown without the EDTA salt. Removal of the non-essential heavy metals by sunflower was greater at the higher plant density compared to the lower one.
(11)	[54]	Laboratory	18 different phytoremediation treatments. I. parcel: mine waste without fly ash. Control and untreated plot. 3 test plants. II. mine waste + fly ash without liming. Control and untreated plot. 3 test plants. III. mine waste + fly ash + liming. Control and untreated plot. 3 test plants.	As, Cd, Mo, Pb, Zn (soil) and As, Cd, Pb, Ni, Zn (water)	Grasses (mixture of selected species), sorghum (<i>Sorghum bicolor</i> L.) and Sudan grass (<i>Sorghum sudanense</i>)—terrestrial	The chemical risks of the Gyöngyösorszi spoils were assessed. The major contaminants of the waste mine were identified: Pb, Zn, Cd, As. The concept of the integrated phytoremediation was successfully applied to vegetate Gyöngyösorszi spoil. The biomass production was different, depending on the technology variant. The highest biomass production was achieved, when multilevel revitalization was also applied. The integrated phytoremediation treatments not only produced high biomass, but also decreased the heavy metal content in the plants.

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(12)	[55]	Field (1995–1997)	Soil	Ni, Cu, Cd, Zn	Willow (<i>Salix spp.</i>)—terrestrial	One group of willow had relatively low Ni and Cu in the bark and high Cd and Zn in the wood, with a good survival rate and biomass production. The second group of willow had relatively high Ni and Cu in the bark and low Cd and Zn in the wood and performed poorly in terms of survival and biomass production. The use of the freeacid form of EDTA and exposure time of one to two weeks before harvesting increased the concentration of metals translocated to plant tissues. It is found no significant difference in heavy metal concentrations in higher and lower soil horizons between EDTA treated and untreated soils. Exposing plants to EDTA for a longer period (2 weeks) could improve metal translocation in plant tissue as well as the overall phytoextraction performance.
(13)	[8]	Laboratory (15 May and 25 September 2002)	Phytoextraction (soil)	Cu, Pb, Zn	Fescue (<i>Festuca arundinacea</i> Schreb.), Indian mustard (<i>Brassica juncea</i> (L.) Czern.), and willow (<i>Salix viminalis</i> L.)—terrestrial	The decrease of mean Hg concentration from 29.17 $\mu\text{g g}^{-1}$ at 0–10 cm horizon to 20.32 $\mu\text{g g}^{-1}$ at 10–40 cm horizon demonstrated the anthropogenic origin of the mercury in the soil. Preliminary results show that all crops extracted mercury, with Hg plant concentration reaching up to 0.479 $\mu\text{g g}^{-1}$ in wheat. The mercury concentration in the plants accounted for less than 3% of mercury concentration in the soil. The Hg concentrations in the plants were similar or even higher than that of the bioavailable Hg in the soils. Mercury extraction yields reached up to 719 mg/ha for barley.
(14)	[24]	Field experiment (3 years)	Phytoextraction (soil content with Hg)	Hg (mean Hg content of the soil was 29.17 $\mu\text{g/g}$ for the 0–10 cm horizon and 20.32 $\mu\text{g/g}$ for 10–40 cm horizon with less than 2% of the total Hg being bioavailable)	Three agriculture crop plants: <i>Triticum aestivum</i> (wheat)—terrestrial <i>Hordeum vulgare</i> (barley)—terrestrial <i>Lupinus luteus</i> (yellow lupin)—terrestrial	

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(15)	[56]	Pot experiment (20 weeks)	Soil from waste deposits of the lead smelter	Pb	<i>Agrostis capillaris</i> —terrestrial	Inoculation with indigenous or nonindigenous AMF in this experiment did not decrease Pb uptake by the host in comparison with nonmycorrhizal plants grown in contaminated soil. It can be concluded that 13 months of subculturing in an inert substrate did not affect development of <i>G. intraradices</i> PH5 isolated from the waste deposits of a Pb smelter in contaminated soil of its origin. The interaction of the fungus with the host plant was changed: the ability of the lineage cultured without HM to support plant growth in Pb-contaminated soil was decreased, while translocation of Pb from plant roots to shoots increased. It appears that EDTA is necessary for Pb extraction due to the low soil Pb bioavailability. Soil amendments like EDTA are necessary because they mobilize soil Pb, making it available to plant roots. It may not be advisable to apply EDTA in the environment, because EDTA mobilizes metals, which may leach into surrounding property of groundwater. The presence of other metals that compete for EDTA may increase the amount of EDTA required for Pb remediation.
(16)	[38]	Field and greenhouse experiments	Phytoextraction (As- and Pb-contaminated soil)	Arsenic (As) and lead (Pb)	Chinese Brake Ferns (<i>Pteris vittata</i>)—terrestrial Indian Mustard (<i>Brassica juncea</i>)—terrestrial	

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(17)	[27]	Laboratory experiment, using chamber (6 weeks)	Phytostabilization (mercury-contaminated soil used in this experiments was obtained from a chemical factory located in the southeast part of Poland, which has been in operation for over 50 years)	Hg	Species <i>Festuca rubra</i> (red fescue)—terrestrial <i>Poa pratensis</i> (meadow grass)—terrestrial <i>Armoracia lapathifolia</i> (horseradish)—terrestrial <i>Helianthus tuberosus</i> (Jerusalem sunflower)—terrestrial <i>S. viminalis</i> (willow)—terrestrial	The highest concentrations of mercury were found at the roots, but translocation to the aerial part also occurred. Most of the plant species tested displayed good growth on mercury contaminated soil and sustained a rich microbial population in the rhizosphere. An inverse correlation between the number of sulfur amino acid decomposing bacteria, and root mercury content was observed. These results indicate the potential for using some species of plants to treat mercury-contaminated soil through stabilization rather than extraction.
(18)	[57]	Field (July and October)	Phytoextraction and phytostabilisation (soil)	Zn, Cu, Cr and Cd	Two poplar clones (<i>Populus deltoides</i> x <i>maximowiczii</i> -clone Eridano and <i>P. x euramericana</i> -clone I-214)—terrestrial	Leaf, stem, root and woody cutting biomasses of treated plants were significantly greater than those in the controls in both clones, except for stem biomass at the beginning of October. Among the four heavy metals (Zn, Cu, Cr, and Cd), only Zn, Cu, and Cr concentrations in plants differed consistently between clones or soil treatments, while Cd levels were always below the detection limits.
(19)	[58]	Field study and laboratory experiment (2002-2003 (field study), 3 months for laboratory experiment)	Soil	Fe, Zn, Pb, Cu, Ni, Cr, Mn	<i>Brachythecium populeum</i>	The results obtained from this study on <i>B. populeum</i> lead to the inference that physiological/bio-chemical analysis of epiphytic bryophytes can serve as cost-effective indicators/monitors for the environmental quality of any area, and on the basis of this information appropriate steps can be taken to improve the air quality of an area.

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(20)	[59]	Pot experiment and field trial (2004-2005 for pot experiment, and 2005 field trial)	Phytoextraction and phytostabilization (soil)	As, Co, Cu, Pb, and Zn	Three poplar species (<i>Populus alba</i> , <i>Populus nigra</i> , <i>Populus tremula</i>) and <i>Salix alba</i> —terrestrial	Trace element concentrations were much higher in roots than in above-ground tissues, with particularly high concentrations in fine roots. The highest accumulations were measured in <i>P. nigra</i> and <i>S. alba</i> . In wood, the highest concentrations of Cu and Zn were in <i>S. alba</i> . <i>Salix alba</i> foliage contained highest concentrations of As, Cu, Pb, and Zn; leaf Zn concentration exceeded those of wood by almost 6 times. The overall removal of trace elements was only significantly higher in <i>P. alba</i> than in <i>S. alba</i> ; <i>P. alba</i> .
(21)	[60]	Pot experiment and field trial (2 years (2004-2005) for pot experiment and field trial on May-September 2005)	Phytoextraction and phytostabilization (soil (Pyrite ore contains mainly pyrite (FeS ₂), lesser amounts of chalcopyrite (CuFeS ₂), sphalerite (ZnS), magnetite (Fe ₃ O ₄), and various trace elements))	As, Co, Cu, Pb and Zn	<i>P. alba</i> L. (white poplar)—terrestrial <i>P. nigra</i> L. (black poplar)—terrestrial <i>P. tremula</i> L. (European aspen)—terrestrial <i>Salix alba</i> L. (white willow)—terrestrial	The result shown that establishment of <i>Populus</i> and <i>Salix</i> species at the site is achievable through ripping of the surface, minimal tillage, some mixing of the wastes with imported soil, irrigation and fertilisers. Potentially, the elevated concentrations of Pb, As and other elements could be leached from the remediated wastes towards groundwater or other receptors, and these fluxes could also be influenced by soil amendments, changes in the rhizosphere or both. Immobilisation of trace elements in both coarse and fine roots may reduce leaching, particularly of Cu and Zn but also As and Pb.

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(22)	[61]	Greenhouse	Phytoextraction and phytostabilization (soil)	Six sediment-derived soils with increasing field Cd levels (0.9–41.4 mg/kg)	Two willow clones (<i>Salix fragilis</i> “Belgisch Rood” and <i>Salix viminalis</i> “Aage”)—terrestrial	No growth inhibition was observed for both clones for any of the treatments. Dry weight root biomass and total shoot length were significantly lower for <i>S. viminalis</i> compared to <i>S. fragilis</i> for all treatments. Willow foliar Cd concentrations were strongly correlated with soil and soil water Cd concentrations. Both clones exhibited high accumulation levels of Cd and Zn in above ground plant parts. Cu, Cr, Pb, Fe, Mn, and Ni were found mainly in the roots. Bioconcentration factors of Cd and Zn in the leaves were the highest for the treatments with the lowest soil Cd and Zn concentration.
(23)	[62]	Laboratory and field	Rhizobox experiment was used to investigate the short-term effect of willow roots on metal availability in oxic and anoxic sediment. Longer-term effects were assessed in a field trial (soil)	Cd, Zn, Cu, and Pb	Willow (<i>Salix spp.</i>)—terrestrial	The rhizobox trial showed that Cd, Zn, and Cu extractability in the rhizosphere increased while the opposite was observed for Pb. The field trial showed that Cu and Pb, but not Cd, were more available in the root zone after water and ammonium acetate (pH 7) extraction compared with the bulk sediment. Sediment in the root zone was better structured and aggregated and thus more permeable for downward water flows, causing leaching of a fraction of the metals and significantly lower total contents of Cd, Cu, and Pb.

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(24)	[63]	Pot experiment	Phytoextraction (soil)	As (as Na_2HAsO_4), Cd (as CdCl_2), Pb (as $\text{Pb}(\text{CH}_3\text{COO})_2$), and Zn (as $\text{Zn}(\text{CH}_3\text{COO})_2$) (100 mg As/kg, 40 mg Cd/kg, 2000 mg Pb/kg, and 2000 mg Zn/kg)	<i>Salix spp.</i> —terrestrial	Although As and Cd uptake slightly increased in Suchdol-Zn soil compared to Suchdol-Pb soil, the element removal from soil was significantly higher in Suchdol-Pb soil due to a significant reduction of aboveground biomass yield in Suchdol-Zn soil. The yield reduction decreased the uptake of plant-available elements by biomass; thus higher plant-available portions of As and Cd were found in Suchdol-Zn soil.
(25)	[64]	Field survey: from 12 As-contaminated sites (September to November 2003)	Field study: contaminated soil	As	Samples of 24 fern species belonging to 16 genera and 11 families as well as their associated soils were collected—terrestrial	<i>Pteris multifida</i> and <i>P. oshimensis</i> can (hyper-) accumulate As in their fronds with high concentrations. Total As concentrations in soils associated with <i>P. multifida</i> and <i>P. oshimensis</i> varied from 1262 to 47,235 mg/kg, but the DTPA-extractable As concentrations were relatively low, with a maximum of 65 mg/kg. Although As concentrations in the fronds of <i>P. oshimensis</i> were comparatively lower than those of <i>P. multifida</i> , its high above ground biomass makes it more suitable for phytoremediating As-contaminated soils.

TABLE 2: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants name and type	Result
(26)	[65]	Field survey (contaminated site since 1976; the sample was taken in 2006)	Soil	Cu, Pb, Cd, and Zn	<i>Paulownia fortunei</i> (seem) Hems	In the rhizosphere and bulk soils of <i>P. fortunei</i> , all physico-chemical properties increased with the revegetation time. The total contents of Cu, Pb, Cd, and Zn also consistently increased with the re-vegetation time; moreover, rhizosphere soils accumulated more heavy metals than bulk soils with the revegetation time. In the rhizosphere soils of <i>P. fortunei</i> , the immobility and bioavailability of heavy metals were enhanced. In the rhizosphere microenvironment, pH, OM, and EC were important factors affecting the distribution of heavy metal fractions. Among different heavy metal fractions, the exchangeable and organically bound fractions were easily available for <i>P. fortunei</i> , but carbonate, Fe–Mn oxide, and residual fractions were not easily available for <i>P. fortunei</i> .
(27)	[4]	Greenhouse pot experiment (August–September 2002)	Soil was sampled in two sites: contaminated soil was taken near road with heavy traffic and clean soil was taken from park protected from the road by buildings	Ag, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Se, Sr, Th, Ti, Tl, U, V and Zn	Wheat <i>Triticum vulgare</i> , sort Umanka—terrestrial	Concentrations of Ag, Cd, Cu, Pb, Sb, and Zn in the initial contaminated soil were 3–6 times higher than those in the initial clean soil. In particular, contents of Cu, Mo, Ni, Pb, Sb and Zn in roots of the wheat grown in the contaminated soil were higher than those in the roots of the plants grown in the clean soil. Moreover, all the elements except Pb transferred more easily from roots to leaves.
(28)	[66]	Field experiment (155 days (May–November))	Soil (agricultural soil)	Cd, Cr, Pb, As, and Hg	Rice (<i>Oryza sativa</i> L.)—terrestrial	The results showed the rice grain contained significantly lower amounts of five metals than straw and root in all sampling sites. Rice root accumulated Cd, As, and Hg from the paddy soil. The rice plant transported As very weakly, whereas Hg was transported most easily into the straw and grain among studied heavy metals.

No. (7), (10), (12), (18), (22), (23) adapted from no. (20). Phytoremediation Bibliography, Annotated Bibliography on Phytoremediation prepared by Mark Coleman, Biological Scientist, USDA Forest Service Southern Research Station and Ronald S. Zalesny Jr., Research Plant Geneticist, USDA Forest Service North Central Research Station May 1, 2006.

TABLE 3: Phytoremediation study on water medium (hydroponic).

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants	Result
(1)	[67]	Field study (October–July 2005)	Water of Tasik Chini	Cd, Cu, and Pb	Five aquatic plant species, <i>Lepironia articulata</i> , <i>Pandanus helicopus</i> , <i>Scirpus grossus</i> , <i>Cabomba furcata</i> , and <i>Nelumbo nucifera</i> —aquatic	The highest concentration of heavy metals among the aquatic plants and plant parts was found in the roots of <i>S. grossus</i> . The concentrations of Cd in the leaves and stems of submerged aquatic plant, <i>C. furcata</i> , were higher than that in the leaves and stems of emergent aquatic plant and floating leaf plant. The concentration of Cu in the stem of <i>C. furcata</i> was greater than that in the leaf, while the concentration of Cd was more in the leaf than that in the stem. The heavy metal contents of the aquatic plants were in descending order of Pb > Cu > Cd. The highest internal translocation was found in <i>P. helicopus</i> , while the lowest internal translocation was found in <i>S. grossus</i> .
(2)	[68]	Laboratory (pot experiment)/14 days	Hydroponic	As and Se as Na ₂ HAsO ₄ ·7H ₂ O and Na ₂ SeO ₃ /0, 0.73, 2.5, 4.27, 5.00 mg/L	Chinese brake fern (<i>Pteris vittata</i> L.)—terrestrial	At low levels of Se, As enhanced both Se uptake and the translocation of Se from roots to fronds. At higher levels of Se, As suppressed the uptake of Se. These results suggest that As serves to both stimulate and suppress Se uptake. The result is also in agreement with the well-known fact that Se is an element with both beneficial and toxic properties. The effect can change from beneficial to toxic based on the concentration of Se in plants.

TABLE 3: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants	Result
(3)	[47]	Laboratory and Field study: wetland-pond system (Laboratory scale: 3 days cultivative and 84 hours exposure)	Field study: contaminated soil. Medium of laboratory scale experiment: L 0.1% Hoagland solution	Field study: Zn, Cu, Cd, and Pb. Laboratory: ZnCl ₂ , CuCl ₂ , CdCl ₂ , and Pb(NO ₃) ₂ (mixture of 20 μmol Zn, 0.5 μmol Cu, 1.5 μmol Cd, and 1.5 μmol Pb/L)	<p><i>Potamogeton natans</i> L.-aquatic <i>Lemna gibba</i> L.-aquatic <i>Alisma plantago-aquatica</i> L.-aquatic <i>Sagittaria sagittifolia</i> L.-aquatic <i>Juncus effusus</i> L.-aquatic <i>Lemna minor</i> L.-aquatic <i>Elodea canadensis</i> Michx.-aquatic <i>Lythrum salicaria</i> L.-aquatic <i>Phalaris arundinacea</i> L.-aquatic <i>Impatiens parviflora</i> DC.-terrestrial <i>Urtica dioica</i> L.—terrestrial <i>Filipendula ulmaria</i> L.-aquatic <i>P. natans</i>-aquatic A. <i>plantago-aquatica</i>-aquatic <i>F. ulmarina</i>-aquatic</p>	<p>The aquatic plants seem to have a higher metal accumulation capacity in shoots than terrestrial plants. This may be due to the capacity of aquatic plants to take up by shoot directly from the water. When submersed and free-floating plants are actively growing and accumulating metals directly from the water, they will function as an effective filter in stormwater treatment. Emergent plants in general mediate the binding of these metals in the sediment. Also, the terrestrial plants have the capacity to bind Cd and Zn to their roots, and; therefore, they can mediate a good stabilization of these metals in soil.</p>
(4)	[32]	Laboratory (15 days)	Hydroponic	Pb as (Pb(NO ₃) ₂)	Indian mustard (<i>Brassica juncea</i> var. <i>megarrhiza</i>)—terrestrial	<p><i>Brassica juncea</i> is one plant which accumulates high levels of Pb and other heavy metals. The results indicate that lead nitrate obviously inhibits the root, hypocotyls, and shoot growth of <i>Brassica juncea</i> at the concentration of 10⁻³ M Pb²⁺. <i>Brassica juncea</i> has the ability to accumulate Pb primarily in its roots, transport, and concentrate it in its hypocotyls and shoots in much lesser concentrations.</p>

TABLE 3: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants	Result
(5)	[30]	Laboratory (5 days)	Phytofiltration (water)	Mercury as HgCl ₂ (0, 0.05, 0.5, 1, 2.5, 5, 10 mg/L)	Indian mustard (<i>Brassica juncea</i>)—terrestrial	Roots-concentrated Hg 100–270 times (on a dry weight basis) above initial solution concentrations. Mercury was more toxic to plants at 5 and 10 mg/L. The plants translocated little Hg to the shoots, which accounted for just 0.7–2% of the total Hg in the plants. Most Hg volatilisation occurred from the roots. Volatilised Hg was predominantly in the Hg(0) vapour form. Volatilisation was dependant on root uptake and absorption of Hg from the ambient solution. Efficiency process >95%. The results show that not only internalized, but also surface-adsorbed arsenic (mostly arsenate) contributes significantly to the total amount of arsenic uptake in aquatic macrophyte <i>S. polyrhiza</i> L. The arsenic uptake in <i>S. polyrhiza</i> L. occurred through the phosphate uptake pathway as well as by physicochemical adsorption on Fe plaques of plant's surfaces. The arsenate uptake in the plant is related to the Fe ion and phosphate concentrations in culture medium while DMAA was not.
(6)	[69]	Laboratory	Hydroponic	Arsenate (As(V)) and dimethylarsinic acid (DMAA)	Duckweed (<i>Spirodela polyrhiza</i> L.)—aquatic	

TABLE 3: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants	Result
(7)	[2]	Laboratory (the contact times of 25–200 min were selected for the metal solutions (Co = 1.00 mM) with 2.0 g biomass/l at the obtained optimal pHs for each metal ion from the previous study)	Adsorption (water)	The Hg^{2+} , Cr^{3+} , Cr^{6+} and Cu^{2+} stock solutions were prepared by dissolving their corresponding salts, viz. $HgCl_2$, $CrCl_3 \cdot 3H_2O$, $K_2Cr_2O_7$, $CuCl_2$ (analytical grade from Merck) in distilled water (pH values were almost 7.0, 5.0, 3.0 and 6.0 for Hg^{2+} , Cr^{3+} , Cr^{6+} and Cu^{2+} , respectively)	<i>Lemna minor</i> —aquatic	The potentiometric titration can be useful to study the pretreatment process of biomass (<i>L. minor</i>) using the acidic and alkali agents, the Qmax and KL values to remove Hg(II), Cr(III), Cr(VI), and Cu(II) from the aqueous solution by the activated <i>L. minor</i> at the alkali solution and by $CaCl_2/MgCl_2/NaCl$ with 1 : 1 : 1 molar ratio were higher than those for the reference one at the same conditions, the removal percents of metal ions by no. ACS <i>L. minor</i> was higher than ACS one at the pre-treatment pHs before 7.0, but it was higher by ACS biomass than no. ACS one at the pre-treatment pHs after 7.0.
(8)	[70]	Laboratory (seedling 2 weeks and treatment 2 weeks)	Hydroponic	Hg and Au (0, 50, 100, and 200 μM Hg (as Hg $(CH_3COO)_2$) and 0 and 50 μM Au (as $KAuCl_4$) in hydroponics)	<i>Chilopsis linearis</i> (Cav.) sweet—terrestrial	The data showed that Au equimolar to Hg reduced the Hg toxicity. The concentration of Au and Hg in shoots indicated that <i>C. linearis</i> absorbed and translocated both Au and Hg at higher concentrations, compared to reported data. The data showed that the treatments produced structural alterations in both the vascular cylinder and the cortex. At the highest concentration, Hg produced a breakdown of the spongy parenchyma.

TABLE 3: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants	Result
(9)	[71]	Laboratory (30 days)	Phytoextraction (water)	Mercury as HgSO_4 (0, 0.5 and 2 mg/L)	Water hyacinth (<i>Eichornia crassipes</i>)—aquatic lettuce (<i>Pistia stratiotes</i>)—aquatic Zebra rush (<i>Scirpus tabernaemontani</i>)—semi aquatic Taro (<i>Colocasia esculenta</i>)—aquatic	The higher the mercury concentration, the greater the amount of mercury removed by the plants. The largest uptake and accumulation capability is for water lettuce, followed by water hyacinth, taro and rush, respectively.
(10)	[72]	Laboratory—(pot experiment (10 days))	Hydroponic	As and Se (0, 150, or 300 μM of arsenat ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) in the presence of 0, 5 or 10 μM of selenat (Na_2SeO_4))	<i>Pteris vittata</i> L.—terrestrial	Application of 5 μM Se enhanced As concentration by <i>P. vittata</i> fronds by 7–45%. At 5 μM , Se acted as an antioxidant, inhibiting lipid peroxidation (reduced by 26–42% in the fronds) via increased levels of thiols and glutathione (increased by 24% in the fronds). The results suggest that Se is either an antioxidant, or it activates plant protective mechanisms, thereby alleviating oxidative stress and improving arsenic uptake in <i>P. vittata</i> .

TABLE 3: Continued.

No.	Researcher	Research scale and duration	Uptake mechanisms and media (substrate)	Contaminant or parameter and concentration	Plants	Result
(11)	[45]	Laboratory (72 hours (for kinetics of Arsenic uptake), 3 days (effects of plant density, Plant re-use, and plant age), 10 days (groundwater remediation))	The groundwater was collected from a location which may have been contaminated from application of arsenical herbicides in the past.	As (pH 7.0, total As of 46 $\mu\text{g/L}$, As^{3+} of 1.6 $\mu\text{g/L}$, and total P of 20 $\mu\text{g/L}$)	Chinese Brake fern (<i>Pteris vittata</i> L.) plants—terrestrial	Chinese brake fern was efficient in taking up arsenic from a contaminated groundwater and was capable of reducing arsenic concentrations in the groundwater. One plant was sufficient to reduce arsenic in 600 mL groundwater to below 10 $\mu\text{g/L}$ in 3 days. Young fern plants were more effective in arsenic removal than old fern plants of similar size. Ferns can be reused to remove arsenic from groundwater, but at a slower rate given the interval between exposures and nutritional status. The more resistant clones produced more biomass in the glasshouse and field and had higher metal concentrations in the wood. The less resistant clones had greater concentrations of Cu and Ni in the bark and produced less biomass in the glasshouse and field. Significant relationships were found between the response of the same clones grown in the short-term glasshouse hydroponics system and in the field. The efflux of arsenate was much higher (by about 9-fold) than that of arsenite. This may be because most of arsenite inside the cells was complexed with thiol compounds. The high As-accumulating Azolla (<i>A. caroliniana</i>) released approximately two times more As than the low-As accumulating Azolla (<i>A. filiculoides</i>). It appears that the amount of As efflux was proportional to the amount of As accumulation in the two strains of Azolla.
(12)	[73]	Laboratory	Hydroponic	Cu and Ni	<i>Salix viminalis</i> clones and the basket willow Black Maul (<i>S. triandra</i>). <i>S. burjatica</i> "Germany", <i>S.x dasyclados</i> , <i>S. candida</i> and <i>S. spaethii</i> —terrestrial	
(13)	[74]	Laboratory (10 days cultivate and 7 days exposure)	Nutrient solution	As (0, 5, 10, 20, 40 and 80 μM)	Azolla: <i>A. caroliniana</i> and <i>A. filiculoides</i> —aquatic	

No. (12) adapted from no. (20). Phytoremediation Bibliography, Annotated Bibliography on Phytoremediation prepared by Mark Coleman, Biological Scientist, USDA Forest Service Southern Research Station and Ronald S. Zalesny Jr., Research Plant Geneticist, USDA Forest Service North Central Research Station, May 1, 2006.

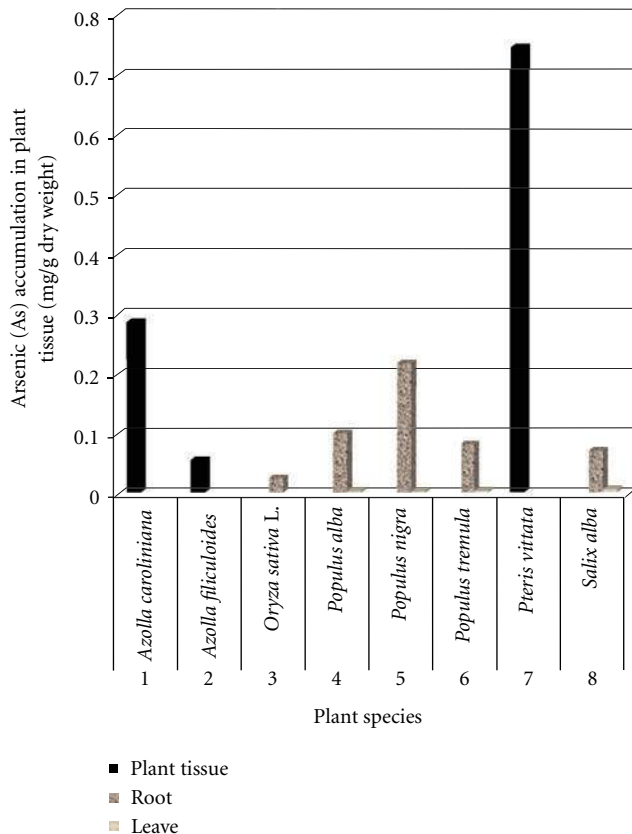


FIGURE 4: As accumulation in plant tissue.

Brassica nigra (L.) Koch that could accumulate more than 100 mg Pb/g dry weight.

Figure 6 shows that accumulated Hg in *Brassica juncea* L. Czern. is much higher than in other species of plants. It could reach more than 1 mg Hg/g dry weight of plant, while the other plants only accumulate less than 0.2 mg Hg/g dry weight.

7. Advantages of Phytoremediation

Phytoremediation techniques may also be more publicly acceptable, aesthetically pleasing, and less disruptive than the current techniques of physical and chemical process [38]. Advantages of this technology are its effectiveness in contaminant reduction, low-cost, being applicable for wide range of contaminants, and in overall it is an environmental friendly method. Figure 7 simplifies some advantages of phytoremediation technology.

The major advantages of the heavy metal adsorption technology by biomass are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials [2]. Phytoremediation as possibly the cleanest and cheapest technology can be employed in the remediation of selected hazardous sites [29]. Phytoremediation encompasses a number of different methods that can lead to contaminant degradation [24].

Phytoremediation is a low-cost option and inexpensive approach for remediating environmental media, particularly

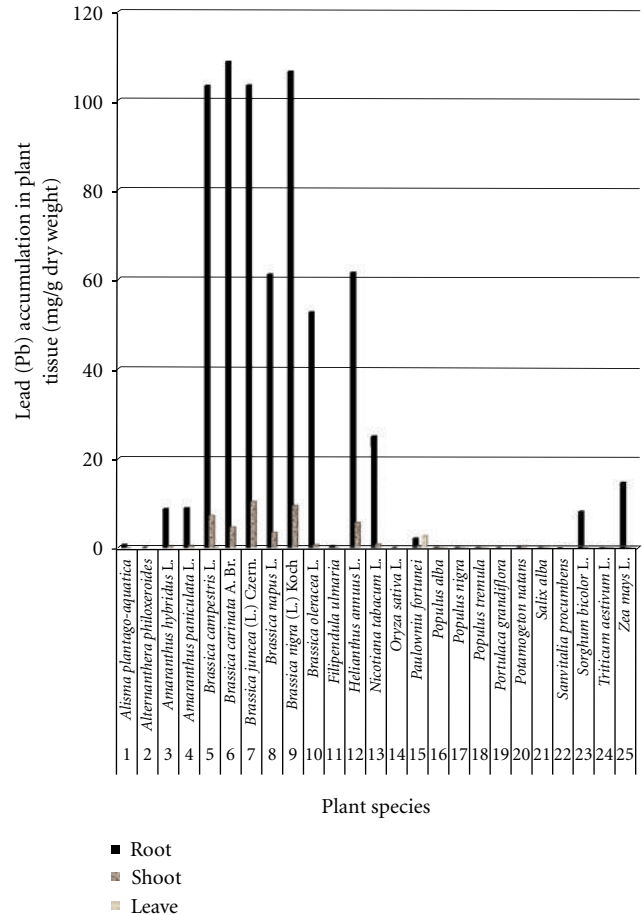


FIGURE 5: Pb accumulation in plant tissue.

suitable to large sites that have relatively low levels of contamination [34]. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites [29]. Phytoremediation potentially offers unique, low cost solutions to many currently problems of soil contamination [32, 75]. It is inexpensive (60–80% or even less costly) than conventional physicochemical methods, since it does not require expensive equipment or highly specialized personnel. It is cost-effective for large volumes of water having low concentrations of contaminants and for large areas having low to moderately contaminated surface soils [46].

It is applicable to a wide range of toxic metals and radionuclides [32] and also useful for treating a broad range of environmental contaminants, including organic and inorganic contaminants [46].

Phytoremediation is regarded as a new approach for the cleanup of contaminated soils, water, and ambient air [34]. Phytoremediation research can also contribute to the improvement of poor soils such as those with high aluminum or salt levels [75]. It is applicable to a range of toxic metals and radionuclides, minimal environmental disturbance, elimination of secondary air or water-borne wastes, and public acceptance [32]. Phytoextraction is considered as an environmentally friendly method to remove metals from

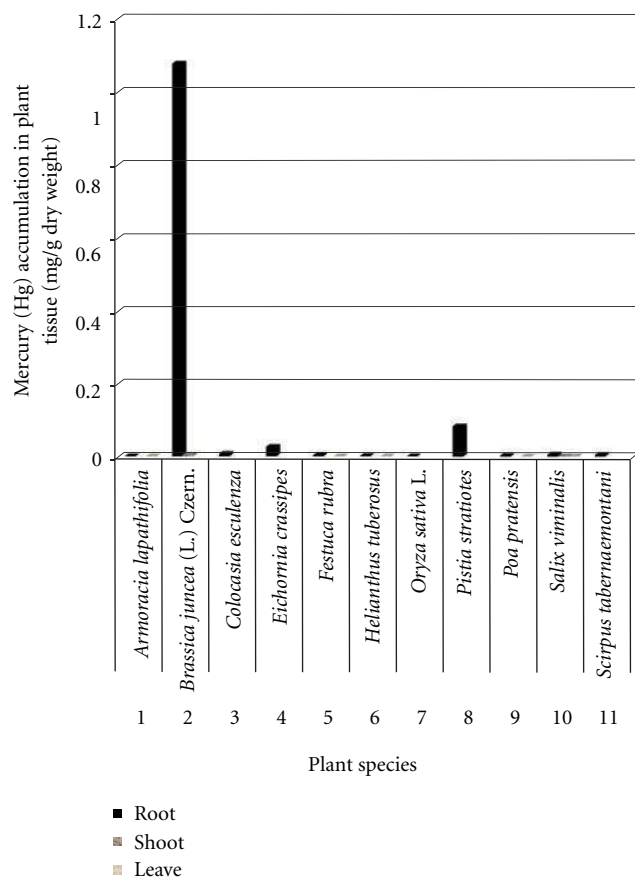


FIGURE 6: Hg accumulation in plant tissue.

contaminated soils in situ. This method can be used in much larger-scale clean-up operations and has been applied for other heavy metals [76]. It is an esthetically pleasing, solar-energy-driven cleanup technology and there is minimal environmental disruption and in situ treatment preserves topsoil. *In Situ* applications decrease the amount of soil disturbance compared to conventional methods. It can be performed with minimal environmental disturbance with topsoil left in a usable condition and may be reclaimed for agricultural use. The organic pollutants may be degraded to CO_2 and H_2O , removing environmental toxicity [46]. Phytoremediation can be an alternative to the much harsher remediation technologies of incineration, thermal vaporization, solvent washing, or other soil washing techniques, which essentially destroy the biological component of the soil and can drastically alter its chemical and physical characteristics as well as creating a relatively nonviable solid waste. Phytoremediation actually benefits the soil, leaving an improved, functional soil ecosystem at costs estimated at approximately one-tenth of those currently adopted technologies [3]. It is the most ecological cleanup technology for contaminated soils and is also known as a green technology.

Another advantage of phytoremediation is the generation of a recyclable metal-rich plant residue [32]. Phytoremediation could be a viable option to decontaminate heavy-metal-polluted soils, particularly when the biomass produced during the phytoremediation process could be

economically valorized in the form of bioenergy. The use of metal-accumulating bioenergy crops might be suitable for this purpose. If soils, contaminated with heavy metals, are phytoremediated with oil crops, biodiesel production from the resulting plant oil could be a viable option to generate bioenergy [34]. In large-scale applications, the potential energy stored can be utilized to generate thermal energy [46]. The success of the phytoextraction technique depends upon the identification of suitable plant species that can hyperaccumulate heavy metals and produce large amounts of biomass using established crop production and management practices [24].

8. Limitations of Phytoremediation Technology

On the other hand, there are certain limitations to phytoremediation system (Figure 8). Among them are being time-consuming method, the amount of produced biomass, the root depth, soil chemistry and the level of contamination, the age of plant, the contaminant concentration, the impacts of contaminated vegetation, and climatic condition.

Phytoremediation can be a time-consuming process, and it may take at least several growing seasons to clean up a site. The intermediates formed from those organic and inorganic contaminants may be cytotoxic to plants [46]. Phytoremediation is also limited by the growth rate of the plants. More time may be required to phytoremediate a site as compared with other more traditional cleanup technologies. Excavation and disposal or incineration takes weeks to months to accomplish, while phytoextraction or degradation may need several years. Therefore, for sites that pose acute risks for human and other ecological receptors, phytoremediation may not be the remediation technique of choice [29, 46]. Phytoremediation might be best suited for remote areas where human contact is limited or where soil contamination does not require an immediate response [38].

Under the best climatic conditions, with irrigation and fertilization, total biomass productivities can approach 100 t/ha/y. One of the unresolved issues is the tradeoff between toxic element accumulation and productivity. In practice, a maximum harvestable biomass yield of 10 to 20 t/ha/y would be likely, particularly for heavy metal accumulating plants. These values for productivity of biomass and heavy metal content would limit annual toxic element removal capacity between about 10 and 400 kg/ha/y, depending on the pollutant, plant species, climatic and other factors. For a target soil depth of 30 cm (4,000 t/ha), this amounts to an annual reduction from 2.5 to 100 ppm in soil toxic element levels. This is often an acceptable rate of contaminant removal, allowing site remediation over a few years to a couple of decades, particularly where the concentration of the contaminant can be lowered sufficiently to meet regulatory criteria. These values for productivity of biomass and heavy metal content would limit annual toxic element removal capacity between 10 and 400 kg/ha/y, depending on the pollutant, plant species, climatic and other factors [37].

The success of phytoremediation may be limited by factors such as growing time, climate, root depth, soil

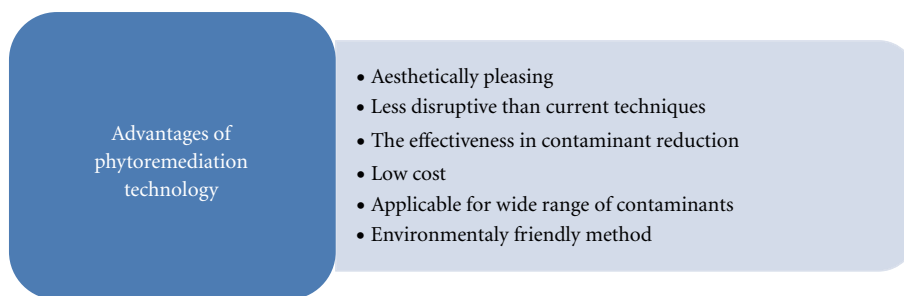


FIGURE 7: Advantages of phytoremediation technology.

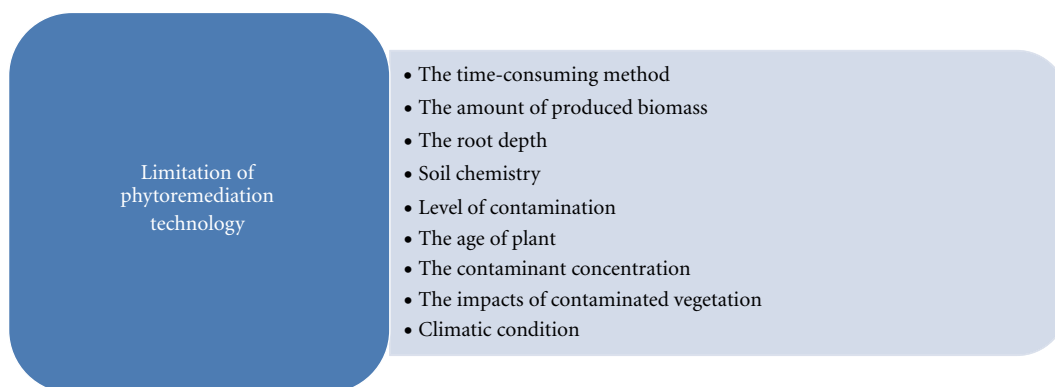


FIGURE 8: The limitation of phytoremediation technology.

chemistry, and level of contamination [38]. Root contact is a primary limitation on phytoremediation applicability. Remediation with plants requires that contaminants be in contact with the root zone of the plants. Either the plants must be able to extend roots to the contaminants, or the contaminated media must be moved to be within range of the plants [29]. Restricted to sites with shallow contamination within rooting zone of remediative plants, ground surface at the site may have to be modified to prevent flooding or erosion [46].

Age greatly affects the physiological activity of a plant, especially its roots. Generally, roots of a young plant display greater ability to absorb ions than do those of an old plant when they are similar in size. It is important to use healthy young plants for more efficient plant removal. However, this does not rule out the use of larger older plants whose larger size may compensate for their lower physiological activity as compared to smaller younger plants [45].

High concentrations of contaminants may inhibit plant growth and, thus, may limit application on some sites or some parts of sites. This phytotoxicity could lead to a remedial approach in which high-concentration waste is handled with expensive ex situ techniques that quickly reduce acute risk, while in situ phytoremediation is used over a longer period of time to clean the high volumes of lower contaminant concentrations [29]. A major limitation in the phytoremediation of toxic elements is the maximal level that can be accumulated by plants. Plants with the

highest levels of toxic metal contents, known as “hyperaccumulators”, generally exhibit, on a dry weight basis, from about 2000 ppm (0.2%) for more toxic elements (Cd, Pb) to above 2% for the less toxic ones (Zn, Ni, Cu) [75]. Restricted to sites with low contaminant concentrations, the treatment is generally limited to soils at one meter from the surface and groundwater within a few meters of the surface with soil amendments may be required [46].

Some ecological exposure may occur whenever plants are used to interact with contaminants from the soil. The fate of the metals in the biomass is a concern. Although some forms of phytoremediation involve accumulation of metals and require handling of plant material embedded with metals, most plants do not accumulate significant levels of organic contaminants. While metal-accumulating plants will need to be harvested and either recycled or disposed of in compliance with applicable regulations, most phytoremediative plants do not require further treatment or disposal [29]. Harvested plant biomass from phytoextraction may be classified as a hazardous waste, hence, disposal should be proper. Consumption of contaminated plant biomass is a cause of concern; contaminants may still enter the food chain through animals/insects that eat plant material containing contaminants [46].

Climatic or hydrologic conditions may restrict the rate of growth of plants that can be utilized. Introduction of nonnative species may affect biodiversity [46].

9. Conclusions

Heavy metals uptake, by plants using phytoremediation technology, seems to be a prosperous way to remediate heavy-metals-contaminated environment. It has some advantages compared with other commonly used conventional technologies. Several factors must be considered in order to accomplish a high performance of remediation result. The most important factor is a suitable plant species which can be used to uptake the contaminant. Even the phytoremediation technique seems to be one of the best alternative, it also has some limitations. Prolong research needs to be conducted to minimize this limitation in order to apply this technique effectively.

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