

Phytoremediation: A Green Technology to Remove Environmental Pollutants

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Received September 8, 2012; revised December 10, 2012; accepted December 27, 2012

ABSTRACT

Land, surface waters, and ground water worldwide, are increasingly affected by contaminations from industrial, research experiments, military, and agricultural activities either due to ignorance, lack of vision, carelessness, or high cost of waste disposal and treatment. The rapid build-up of toxic pollutants (metals, radionuclide, and organic contaminants in soil, surface water, and ground water) not only affects natural resources, but also causes major strains on ecosystems. Interest in phytoremediation as a method to solve environmental contamination has been growing rapidly in recent years. This green technology that involved “tolerant plants” has been utilized to clean up soil and ground water from heavy metals and other toxic organic compounds. Phytoremediation involves growing plants in a contaminated matrix to remove environmental contaminants by facilitating sequestration and/or degradation (detoxification) of the pollutants. Plants are unique organisms equipped with remarkable metabolic and absorption capabilities, as well as transport systems that can take up nutrients or contaminants selectively from the growth matrix, soil or water. As extensive as these benefits are, the costs of using plants along with other concerns like climatic restrictions that may limit growing of plants and slow speed in comparison with conventional methods (*i.e.*, physical and chemical treatment) for bioremediation must be considered carefully. While the benefits of using phytoremediation to restore balance to a stressed environment seem to far outweigh the cost, the largest barrier to the advancement of phytoremediation could be the public opposition. The long-term implication of green plant technology in removing or sequestering environmental contaminations must be addressed thoroughly. As with all new technology, it is important to proceed with caution.

Keywords: Phytoremediation; Green Technology; Pollutants; Contaminants; Toxic Metals

1. Green Technology

The success of green technology in phytoremediation, in general, is dependent upon several factors. First, plants must produce sufficient biomass while accumulating high concentrations of metal. In some cases, an increased biomass will lower the total concentration of the metal in the plant tissue, but allows for a larger amount of metal to be accumulated overall. Second, the metal-accumulating plants need to be responsive to agricultural practices that allow repeated planting and harvesting of the metal-rich tissues. Thus, it is preferable to have the metal accumulated in the shoots as opposed to the roots, for metal in the shoot can be cut from the plant and removed. This is manageable on a small scale, but impractical on a large scale. If the metals are concentrated in the roots, the entire plant needs to be removed. Yet, the necessity of full plant removal not only increases the costs of phytoreme-

diation, due to the need for additional labor and plantings, but also increases the time it takes for the new plants to establish themselves in the environment and begin accumulation of metals. **Table 1** lists some of the common pollutant accumulating plants found by phytoremediation researchers.

The availability of metals in the soil for plant uptake is another limitation for successful phytoremediation. For example, lead (Pb^{2+}), an important environmental pollutant, is highly immobile in soils. Lead is known to be “molecularly sticky” since it readily forms a precipitate within the soil matrix. It has low aqueous solubility, and, in many cases, is not readily bioavailable. In most soils capable of supporting plant growth, the soluble Pb^{2+} levels are relatively low and will not promote substantial uptake by the plant even if it has the genetic capacity to accumulate the metal. In addition, many plants retain Pb^{2+} in their roots via absorption and precipitation with only minimal transport to the aboveground harvestable

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Table 1. Selected pollutant accumulating plants.

SCIENTIFIC NAME	COMMON NAME
<i>Armeria maritima</i>	Seapink thrift
<i>Ambrosia artemisiifolia</i>	Ragweed
<i>Brassica juncea</i>	Indian mustard
<i>Brassica napus</i>	Rape, Rutabaga, Turnip
<i>Brassica oleracea</i>	Flowering/ornamental kale and cabbage, Broccoli
<i>Festuca ovina</i>	Blue/sheep fescue
<i>Helianthus annuus</i>	Sunflower
<i>Thalspi rotundifolium</i>	Pennycress
<i>Triticum aestivum</i>	Wheat (scout)
<i>Zea mays</i>	Corn

plant portions. Therefore, it is important to find ways to enhance the bioavailability of Pb^{2+} or to find specific plants that can better translocate the Pb^{2+} into harvestable portions [1].

Although there are some challenges associated with the phytoremediation, it remains a very promising strategy and feasible alternative. However, in many situations, soil contamination may have unique factors that require special evaluation. Some plants may only accumulate these essential elements and prevent all others from entering. For plants termed as “hyperaccumulators” can extract and store extremely high concentrations (in excess of 100 times greater than non-accumulator species) of metallic elements [2]. It is believed that these plants initially develop the ability to hyperaccumulate non-essential metallic compounds as a means of protecting themselves from herbivorous predators that would experience serious toxic side effects from ingestion of the hyperaccumulator’s foliage [3].

1.1. Plants as Phytoremediators

The principal application of phytoremediation is for lightly contaminated soils and waters where the material to be treated is at a shallow or medium depth and the area to be treated is large. This will make agronomic techniques economical and applicable for both planting and harvesting. In addition, the site owner must be prepared to accept a longer remediation period. Plants that are able to decontaminate soils does one or more of the following: 1) plant uptake of contaminant from soil particles or soil liquid into their roots; 2) bind the contaminant into their root tissue, physically or chemically; and 3) transport the contaminant from their roots into growing shoots and prevent or inhibit the contaminant from leaching out of the soil.

Moreover, the plants should not only accumulate, degrade or volatilize the contaminants, but should also grow quickly in a range of different conditions and lend themselves to easy harvesting. If the plants are left to die *in situ*, the contaminants will return to the soil. So, for complete removal of contaminants from an area, the plants must be cut and disposed of elsewhere in a non-polluting way. Some examples of plants used in phytoremediation practices are the following: water hyacinths (*Eichornia crassipes*); poplar trees (*Populus* spp.); forage kochia (*Kochia* spp); alfalfa (*Medicago sativa*); Kentucky bluegrass (*Poa pratensis*); Scirpus spp, coontail (*Ceratophyllum demersum* L.); American pondweed (*Potamogeton nodosus*); and the emergent common arrowhead (*Sagittaria latifolia*) amongst others [4].

Four heavy metal concentrations in soils (Cu, Cr, As, and Pb) were examined to see if removal through the process of phytoremediation was possible. Tomato and mustard plants were able to extract different concentrations of each heavy metal from the soils. The length of time that the soils were exposed to the contaminants affected the levels of heavy metals accumulation. Today, many institutions and companies are funding scientific efforts to test different plants' effectiveness in removing wide ranges of contaminants. Scientists favor *Brassica juncea* and *Brassica olearacea*, two members of the mustard family, for phytoremediation because these plants appeared to remove large quantities of Cr, Pb, Cu, and Ni from the soil [5].

1.2. Grasses as Potential Phytoremediators

1.2.1. Vetiver Grass (*Vetiveria zizanioides* L.)

Vetiver (*Vetiveria zizanioides* L.) belongs to the same grass family as maize, sorghum, sugarcane, and lemon grass. It has several unique characteristic as reported by

the National Research Council [6]. Vetiver grass is a perennial grass growing two meters high, and three meters deep in the ground. It has a strong dense and vertical root system. It grows both in hydrophilic and xerophytic conditions. The leaves sprout from the bottom of the clumps and each blade is narrow, long and coarse. The leaf is 45 - 100 cm long and 6 - 12 cm wide.

Vetiver grass is highly suitable for phytoremediation application due to its extraordinary features. These include a massive and deep root system, tolerance to extreme climatic variations such as prolonged drought, flood, submergence, fire, frost, and heat waves. It is also tolerant to a wide range of soil acidity, alkalinity, salinity, sodicity, elevated levels of Al, Mn, and heavy metals such as As, Cr, Ni, Pb, Zn, Hg, Se, and Cu in soils [7]. The roots of vetiver are the most useful and important part. Its root system does not expand horizontally, but penetrates vertically deep into the soil, whether it is the main, secondary or fibrous roots. The horizontal expansion of the vetiver grass root system is limited up to only 50 cm. The root vertical penetration extends up to 5 meters. Normally, yield levels of the leaves is 15 - 30 tons·ha⁻¹ (15,000 - 30,000 kg·ha⁻¹) while vetiver grass roots can produce a dry matter yield of about 1428.6 to 2142.9 kg·ha⁻¹ [8].

Various uses of vetiver grass are known worldwide. In South Africa, it was used effectively to stabilize waste and slime dams from Pt and Au mines [9]. In Australia, vetiver grass was used to stabilize landfill and industrial waste sites contaminated with heavy metals such as As, Cd, Cr, Ni, Cu, Pb, and Hg [7]. In China, vetiver grass was planted in large scale for pollution control and mine tail stabilization [10]. In Thailand, vetiver grass is found widely distributed naturally in all parts of the country. It has been used for erosion control and slope stabilization. Vetiver hedges had an important role in the process of captivity and decontamination of pesticides, preventing them from contaminating and accumulating in crops [11]. When compared with other plants, vetiver grass is more efficient in absorbing certain heavy metals and chemicals due to the capacity of its root system to reach greater depths and widths [7]. As confirmed by Roongtanakiat and Chairaj [12], vetiver grass was found to be highly tolerant to an extremely adverse condition. Therefore, vetiver grass can be used for rehabilitation of mine tailings, garbage landfills, and industrial waste dumps which are often extremely acidic or alkaline, high in heavy metals, and low in plant nutrients.

1.2.2. Cogon Grass (*Imperata cylindrica* L.)

Cogon grass, generally occurs on light textured acid soils with clay subsoil, and can tolerate a wide range of soil pH ranging from strongly acidic to slightly alkaline [13]. It is hardy species, tolerant of shade, high salinity, and

drought. It can be found in virtually any ecosystem, especially those experiencing disturbances [8]. It is a perennial grass up to 120 cm high with narrow and rigid leaf-blades.

The roots can penetrate to a soil depth of about 58 cm in alluvial soil. More than 80 percent of shoots can originate from rhizomes less than 15 cm below the soil surface. The average number of shoots of cogon grass was about 4.5 million per hectare, producing 18,500 kg·ha⁻¹ of leaves and rhizomes (11,500 kg of leaves and 7000 kg of rhizomes) [13].

1.2.3. Carabao Grass (*Paspalum conjugatum* L.)

Carabao grass is a vigorous, creeping perennial grass with long stolons and rooting at nodes. Its culms can ascend to about 40 to 100 cm tall, branching, solid, and slightly compressed where new shoots can develop at every rooted node. Under a coconut plantation, a yield of about 19,000 kg·ha⁻¹ of green materials was obtained. It grows from near sea-level up to 1700 m altitude in open to moderately shaded places. It is adapted to humid climates and found growing gregariously under plantation crops and also along stream banks, roadsides, and in disturbed areas. This grass can adapt easily to a wide range of soils [14].

2. Phytoremediation as a Cleansing Tool: An Overview

Phytoremediation is described as a natural process carried out by plants and trees in the cleaning up and stabilization of contaminated soils and ground water. It is actually a generic term for several ways in which plants can be used for these purposes. It is characterized by the use of vegetative species for *in situ* treatment of land areas polluted by a variety of hazardous substances [15].

Garbisu [16] defined phytoremediation as an emerging cost effective, non-intrusive, aesthetically pleasing, and low cost technology using the remarkable ability of plants to metabolize various elements and compounds from the environment in their tissues. Phytoremediation technology is applicable to a broad range of contaminants, including metals and radionuclides, as well as organic compounds like chlorinated solvents, polychlorobiphenyls, polycyclic aromatic hydrocarbons, pesticides/insecticides, explosives and surfactants. According to Macek [17], phytoremediation is the direct use of green plants to degrade, contain, or render harmless various environmental contaminants, including recalcitrant organic compounds or heavy metals. Plants are especially useful in the process of bioremediation because they prevent erosion and leaching that can spread the toxic substances to surrounding areas [18].

Several types of phytoremediation are being used today. One is phytoextraction, which relies on a plant's

natural ability to take up certain substances (such as heavy metals) from the environment and sequester them in their cells until the plant can be harvested. Another is phytodegradation in which plants convert organic pollutants into a non-toxic form. Next is phytostabilization, which makes plants release certain chemicals that bind with the contaminant to make it less bioavailable and less mobile in the surrounding environment. Last is phytovolatilization, a process through which plants extract pollutants from the soil and then convert them into a gas that can be safely released into the atmosphere [19]. Rhizofiltration is a similar concept to phytoextraction, but mainly use with the remediation of contaminated groundwater rather than the remediation of polluted soils. The contaminants are either absorbed onto the root surface or are absorbed by the plant roots. Plants used for rhizofiltration are not planted directly *in situ*, but are acclimated with the pollutant first. Until a large root system has developed, plants are hydroponically grown in clean water rather than in soil. Once a large root system is in place, the water supply is substituted for polluted water supply to acclimate the plant. After the plants become acclimated, they are planted in the polluted area. As the roots become saturated, they are harvested and disposed of safely.

Phytoremediation is a naturally occurring process recognized and documented by humans more than 300 years ago [2]. Since then, humans have exploited certain plant abilities to survive in contaminated areas and to assist in the removal of contaminants from the soil. However, scientific study and development of these plants' unique qualities were not conducted until the early 1980's [2]. At this time, it was recognized that certain species of plants could accumulate high levels of heavy metals from the soil while continuing to grow and proliferate normally [2]. Research has been slow and tedious due to scientists' incomplete understanding of the generalized cellular mechanisms of plants. However, the advent of new genetic technology has allowed scientists to determine the genetic basis for high rates of accumulation of toxic substances in plants [20]. Using genetic engineering, scientists may soon be able to exploit plants' characteristics that can provide faster and more efficient means of removing contaminants from the soil. Genetic engineering will also be crucial for the creation of transgenic plants that will be able to combine the natural agronomic benefits associated with plants (ease of harvest and rapid, expansive growth) with the remediation capabilities of bacteria—a traditional organism used in bioremediation [21].

Phytoremediation of heavy metals from the environment serves as an excellent example of plant-facilitated bioremediation process and its role in removing environmental stress. Traditionally, when an area becomes

contaminated with heavy metals, the area must be excavated and the soil should be removed and put to a landfill site [2]. This process is extremely expensive and, therefore not entirely appealing despite recent discoveries regarding phytoremediation [2]. Analysts have estimated the cost of cleaning one hectare of highly contaminated land at a depth of one meter. The estimated cost would range from \$600,000 to \$3,000,000 depending on the extent of the pollution and the toxicity of the pollutants [21]. The cost of phytoremediation could be as much as 20 times less expensive, making this practice far less prohibitive than conventional methods [2]. The ideal type of phytoremediator is a species that creates a large biomass, grows quickly, extensive root system, and can be easily cultivated and harvested [20]. The only problem is that natural phytoremediators often lack most of the qualities described above. Therefore, scientists have been forced to become very creative in developing effective transgenic phytoremediators.

Many human diseases result from the buildup of toxic metals in soil, making remediation crucial in protecting human health. Lead is one of the most difficult contaminants to be removed from the soil and one of the most dangerous. According to Lasat [2], the presence of Pb in the environment can have devastating effects on plant growth and can result in serious side effects—including seizures and mental retardation if ingested by humans or animals. Much of the global Pb contamination has occurred as a result of mining and iron smelting activities [22]. Phytoremediation of Pb contaminated soil involves two of the aforementioned strategies—phytostabilization and phytoextraction. It is believed that plants' ability to phytoextract certain metal is a result of its dependence upon the absorption of many metals such as Zn, Mn, Ni, and Cu [2].

2.1. Phytoremediation of Water Pollutants

In 2005, Cortez [23] conducted a study to assess pollution and survey the potential plants that can be used as phytoremediators of heavy metals in Nueva Ecija, Philippines. Water and plant samples were taken near the dumpsites, which is about 500 m away from the creek. Results of the water analysis showed that the dumpsite and Panlasian Creek were slightly polluted with considerable amount of phosphate. Results of the plant chemical analysis showed that kangkong (*Ipomea aquatic*) and Hydracharitaceae (*Ottelia alismoides* L.) were both efficient in phytoremediating Pb. Analysis of the plants further suggests that the concentrations of Pb in morning glory (*Ipomea violacea* L.) and hydracharitaceae (*Ottelia alismoides* L.) was about 210% more than the concentration of Pb in the water [23].

Xia and Ma [24] in 2005 investigated the potential of

water hyacinth (*Eichhornia crassipes*) in removing a phosphorus pesticide ethion. The disappearance rate constants of ethion in culture solutions were -0.01059 , -0.00930 , -0.00294 and -0.00201 for the non-sterile planted, sterile planted, non-sterile unplanted and sterile unplanted treatment, respectively. The accumulated ethion in live water hyacinth plant decreased by 55% - 91% in shoots and 74% - 81% in roots after the plant growing 1 week in ethion free culture solutions, suggesting that the plant uptake and phytodegradation might be the dominant process for ethion removal by the plant. Given the promising result of the study, water hyacinth could be utilized as an efficient, economical and ecological alternative to accelerate the removal and degradation of agro-industrial wastewater polluted with ethion.

Letachowicz *et al.* [25] conducted a study on the phytoremediation capacity on heavy metals accumulation in different organs of *Typhya latifolia* L. The concentrations of Cd, Pb, Cu, Ni, Mn, Zn, and Fe were determined in different organs of *Typhya latifolia* from seven water bodies in the Nysa region in Poland. The *Typhya latifolia* species that can absorb heavy metals can be used as bio-indicator of pollutants is a macrohydrophyte and is widely present in the entire lowland and lower mountain sites. It is linked with nutritious water and organic or inorganic mineral bottom sediments. *Typhya latifolia* is a strongly expansive species because it can control water space due to intensive growth of rhizomes and often creates almost mono-species group, though it can also be found in various groups of rushes.

2.2. Phytoremediation Species in Coastal Water

The Philippines is blessed to have relatively high mangrove diversity having 35 species [26] including five major families, namely: Avicenniaceae; Arecaceae; Combretaceae; Lythraceae; and Rhizophoraceae [27]. Though Philippines has high mangrove diversity, it was reported that there was a drastic decline of mangrove resources from 450,000 hectares in 1918 to 120,000 hectares in 1995. The decrease of the mangrove forests was due to human activities, such as fish pond conversion, human settlement, and salt production [28]. However, with the alarming rate of mangrove forest degradation, Philippines strived to continue greater conservation of mangroves and reforestation of the coastal areas [29].

Mangroves are higher plants, which are found mostly in the intertidal areas of tropical and subtropical shorelines and show remarkable tolerance to high amounts of salt and oxygen poor soil. The mechanisms of mangrove to keep the salt away from the cytoplasm of the cell were through the excretion of salt in their salt glands found in the leaves and roots and through storage of salts in the mature leaves, bark and wood [26]. Mangroves developed unique body features in order to cope up with harsh

environment. There are different types of roots, such as prop roots in *Rhizophora*, pencil-like pneumatophores in *Avicennia*, and cone-like pneumatophores in *Sonneratia* that have large lenticels to permit gas exchange. The leaves of mangroves have characteristics to survive from desiccation and conserve water like the presence of thick epidermis, waxy cuticle, and presence of hypostomata [26]. Mangrove ecosystem is exposed to different pollutants such as heavy metal, sewage wastes, pesticides and petroleum products. Heavy metal accumulation in the mangrove sediment can result in biological and ecological effects. Even though, mangrove trees may have the immunity against the toxic effects of the heavy metals, but the animals thriving in the ecosystem are vulnerable to the negative effects of heavy metals [30].

Few studies were conducted about phytoremediation potential of mangroves and other wetland plant species. However, those researchers paved the way to explore more species of mangroves particularly the native species present in the area, for their feasibility to accumulate heavy metals. Zheng *et al.* [31] studied the different metal concentrations of Cu, Ni, Cr, Zn, Pb, Cd, and Mn in *Rhizophora stylosa* at Yingluo Bay, China. The study showed less pollution due to relatively low concentration of metals especially Pb, Mn, Zn and Cd.

MacFarlane and Burchett [32] examined the cellular distribution of Cu, Pb and Zn in grey mangrove, *Avicennia marina* (Forsk.) using scanning electron microscope X-ray microanalysis and atomic absorption spectroscopy. They reported that metals mostly accumulate in plants' cell walls. Their study showed that certain parts of mangroves have the ability to control the entrance of heavy metals in other parts of the plants. The laboratory research of MacFarlane and Burchett [33] contributed information on the accumulation, growth effect, and toxicity of Cu, Pb and Zn in grey mangrove, *Avicennia marina* (Forsk.). Accumulation of the different metals occurred at varying concentrations in the roots and leaf tissue. In the roots, Pb accumulated lesser than the other metals while high concentration of Zn was found in the leaf tissue. The effects of excessive Cu and Zn on young mangrove were reductions in seedling height, leaf number, total biomass, and root growth. The germination of mangrove was inhibited at $800 \mu\text{g}\cdot\text{g}^{-1}$ Cu and $1000 \mu\text{g}\cdot\text{g}^{-1}$ Zn. The Pb showed only little negative effects in the growth of the plant due to low absorption of this metal.

Cheng [34] cited heavy metals can be absorbed by plants using their roots, or via stems and leaves, and stored the metals into different plant parts. Moreover, the distribution and accumulation of heavy metals in the plants depend on plant species and chemical factors. The *Avicennia marina*, a salt-excretive mangrove, and *Rhizophora stylosa*, a salt-exclusion mangrove, have dif-

ferent accumulation potential of different heavy metals. In terms of Pb absorption, *A. marina* was able to accumulate more concentrations of heavy metals than in *R. stylosa*. However, the purification processes of plants were affected by different factors such as heavy metal concentration, plant species, and exposure duration.

Sari *et al.* [35] conducted an *in-situ* experiment on the bioaccumulation of Pb in two mangrove species, *Avicennia alba* and *Rhizophora apiculata* using hydroponics culture. The mangroves were grown in 0%, 15% and 30% salinity and 0.03, 0.3, and 3 mg·L⁻¹ of Pb concentration. They observed that both mangroves had significantly lower Pb accumulation in leaves than in roots. They claimed that the mobility of Pb in the aerial part of the plant can be related to its mechanism associated with the accumulation of sodium in the salt glands found in the leaves. Saenger and McConchie [36] evaluated the accumulation trend of Pb in the tissues, barks and woods, old and young leaves and fruits of different mangrove species. They discovered that Pb concentrated more in the bark than in other tissues of mangroves because of atmospheric Pb due to vehicle exhausts from nearby major roads.

Shete *et al.* [37] revealed in their study entitled, "Bioaccumulation of Zn and Pb in *Avicennia marina* (Forsk.) from urban areas of Mumbai (Bombay), India," that the mangrove species can bioaccumulate and survive despite heavy metal contamination. Results showed that mangroves have greater uptake of heavy metals. Variations on the concentrations of Zn were found from the different plant parts while high accumulation of Pb was focused in the roots. They found out that Pb concentrations were present in the leaves and roots. Kamaruzzaman *et al.* [38] studied the cumulative partitioning of Pb and Cu in the *Rhizophora apiculata* in the Setiu mangrove forest, Terengganu. Results showed increasing concentration of Cu and Pb from the leaf, bark, root, and sediments. The study by Pahalawattaarachchi *et al.* [39] reported the absorption, accumulation, and partitioning of eight different metals specifically Cu, Cd, Cr, Fe, Mg, Ni, Pb and Zn by mangrove species, *Rhizophora mucronata* (Lam.) at Alibag, Maharashtra, India. They revealed that Cu, Mn and Fe showed limited mobility due to their accumulation in the roots while other metals (Cd, Zn, Ni and Pb) were concentrated in the aerial part of the plant. They concluded that *Rhizophora mucronata* (Lam.) was more capable of phytostabilization rather than phytoextraction because of low uptake capacity of different metals.

Nazli and Hashim [40] revealed that *Sonneratia caseolaris* was a potential phytoremediation species for selected heavy metals in Malaysian mangrove ecosystem. The study assessed the concentrations of Cd, Cr, Cu, Pb and Zn in *Sonneratia caseolaris*. Results showed that both roots and leaves of *Sonneratia caseolaris* accumu-

lated and exceeded the general normal upper range of Cu and Pb in plants. In Iran, Parvaresh *et al.* [41] studied the bioavailability of different heavy metals (Ni, Cu, Cd, Pb and Zn) in the sediments of Sirik Azini creek. The outcome of their research revealed no heavy metal pollution was found in the area due to low geo-accumulation index of Pb in the sediment. They assessed that the concentration of heavy metals particularly Pb in the leaves were higher than the concentration of Pb in the sediment.

Qui *et al.* [42] studied the different accumulation and partitioning of seven trace metals, namely, As, Cd, Cr, Cu, Hg, Pb and Zn, in mangroves and sediments from three estuarine wetlands of Hainan Island, China. They analyzed the sediment samples and found out that the heavy metals present in the area were still at relatively low levels. Furthermore, Pb analysis of mangroves showed that this metal was found mostly in the branches of the different mangroves. Zhang *et al.* [43] investigated the physiological response of *Sonneratia apetala* (Buch) to the addition of wastewater nutrients and heavy metals (Pb, Cd, and Hg). They planted mangroves in four different treatments: 1) control, which has only salted water; 2) normal concentration of wastewater nutrients and heavy metals; 3) five times the normal treatment; and 4) ten times the normal treatment. Results revealed that growth of mangrove increased with increasing levels of wastewater pollution. The study showed that mangroves were potential phytoremediator in wetland ecosystem.

The research of Nirmal *et al.* [44] entitled, "An assessment of the accumulation potential of Pb, Zn, and Cd by *Avicennia marina* (Forssk) in Vamleshwar Mangroves, Gujarat, India," reported that sediments in the area are below critical soil concentration for heavy metals. *A. marina* possesses the capacity to uptake selected heavy metals, Pb, Zn and Cd, via its roots and storing them in their leaves without any sign of complications. The concentrations of heavy metals in the *A. marina* were in normal range except for Pb. The roots of mangrove contained the highest concentration of heavy metal except for Cd. Furthermore, *A. marina* had the capacity to uptake metals via its roots and accumulates them in their leaves without any sign of injury. The study showed *Avicennia marina* as a potential phytoremediation species for selected heavy metals in many mangrove ecosystems. Subramanian [45] cited that mangroves generally have low concentration of heavy metal. Kathiresan and Bingham [27] mentioned that mangroves can tolerate metal pollution because they were poor accumulators of heavy metals. A study on the metal uptake of *Rhizophora mangle* in Sepetiba Bay, Rio de Janeiro, Brazil showed that only one percent of the total heavy metals concentration in the sediment accumulated in the mangrove [27]. In their experiment, they used young *Bruguiera gymnorhiza* and artificially synthesized wastewater treatments

with different levels of Cu, Cd, Cr, Ni, and Zn. The control treatment showed higher biomass and growth than the plants treated with wastewater.

2.3. Phytoremediation of Soil Pollutants

Phytoremediation is a cleanup technology for metal contaminated soils, specifically Pb. In order for this type of remediation strategy to be successful, it is necessary to utilize metal accumulating plants to extract environmentally toxic metals from the soil, such as Pb, Ni, Cr, Cd and Zn. Certain plants have been identified not only to accumulate metals in the plant roots, but also to translocate the accumulated metals from the root to the leaf and to the shoot. While many plants performed this function, some plants, known as “hyperaccumulators”, can accumulate extremely high concentrations of metals in their shoots (0.1% to 3% of their dry weight) [46]. The metal-rich plant material can then be harvested and removed from the site without extensive excavation, disposal costs, and loss of topsoil that is associated with traditional remediation practices.

Bioremediation process would be extremely slow because the rate of bioremediation is directly proportional to growth rate while the total amount of bioremediation is correlated with a plant's total biomass. No plant has been discovered yet capable of meeting all the ideal criteria of an effective phytoremediator. These criteria are fast growing, deep and extensive roots, high biomass, easy to harvest and hyperaccumulators of a wide range of toxic metals. A Pb absorption study by Huang and Cunningham [47] cited corn as a perfect phytoremediator due to its large biomass, fast rate of growth, and the existence of extensive genomic knowledge of this crop. Introduction of hyper accumulating genes as well as genetic information would better prepare these species to deal with diverse climatic conditions [19]. The mobilization of metal contaminants, both in the soil and the plant, is another important factor influencing the success of phytoremediation. The amount of soluble Pb^{2+} in the soil appears to be a key factor to the enhancement of Pb^{2+} uptake by plants [48].

Two main amendment techniques have been used to increase the bioavailability of Pb in soils and the mobility of Pb within plant tissue by lowering soil pH and adding synthetic chelates. Soil pH is a significant parameter in the uptake of metal contaminants because soil pH value is one of the principal soil factors controlling metal availability [49]. Maintaining a moderately acidic pH in the soil may be attained through the use of ammonium containing fertilizer or soil acidifiers. By this, Pb metal bioavailability and plant uptake can increase [50-52]. In a study performed by Cholpecka *et al.* [53] on metal contaminated soils in southwest Poland, reported that soil

samples with pH of less than 5.6 contained relatively more metals in the exchangeable form than in soil samples with pH greater than 5.6. In addition, at lower pH, the Pb in soil has a greater potential to translocate from a plant's roots into its shoots. Synthetic chelates, such as ethylenediaminetetraacetic acid (EDTA), have been shown to aid in the accumulation of Pb^{2+} in the plant tissue. EDTA and other chelates have been used in soils and nutrient solutions to increase the solubility of metal cations and the translocation of Pb into shoots [54].

The physiological and biological mechanisms involved in Pb uptake of plants involving root to shoot transport of Pb may require some time to develop and become functional. Since plant species can differ significantly in Pb uptake and translocation, the success of using plants to extract Pb from contaminated soils requires the following: 1) the identification of Pb accumulating plants that can survive in the presence of contaminants; 2) the measurement of the concentration of pollutant in the soil, and 3) knowledge of chemistry (availability or speciation) of the metal in the soil matrix. The combination of soil amendment and foliar fertilizer application to plants capable of absorbing and translocation of Pb may be an effective means of remediating an area with varying levels of Pb concentrations.

Other model of phytoremediators includes various varieties of transgenic trees. Trees are ideal in the remediation of heavy metals because they can withstand higher concentrations of pollutants due to their large biomass. As such, they can accumulate large amounts of the contaminants in their systems because of their size capable of reaching huge area and great depths due to their extensive root systems. Furthermore, they can stabilize an area, prevent erosion, and minimize spread of contaminant because of their perennial presence. They can also be easily harvested and removed from the area with minimal risk, effectively taking with them a large quantity of the pollutants that were once present in the soil [19].

3. Phytoremediation, Is It Good or Bad?

Earlier discussion has illustrated many advantages and disadvantages of transgenic phytoremediation. The primary advantages of using plants in bioremediation are as follows: it is more cost-effective; more environmentally friendly; and more aesthetically pleasing than conventional methods. The conventional methods are usually expensive and environmentally disruptive [55]. Plants also offer a permanent, *in situ*, nonintrusive, self-sustaining method of soil contaminant removal. More importantly, contaminants can be removed much more easily through the harvest of plants than from the soil itself.

More benefits are derived through phytoextraction. It

enables scientists to reclaim and recycle usable materials, including a wide variety of precious metals from the soil [21]. Also, its potential benefits are extremely high and extremely attractive to scientists and businessmen alike [21]. Furthermore, phytoextraction is economical because only solar energy must be present to maintain the system [55]. Finally, the greatest advantage of this technology is that it utilizes the inherent agronomic benefits of plants [56]. These benefits include high biomass, extensive root systems that both stabilize the ecosystem by preventing contaminant to spread through leaching as well as reaching a large volume of contaminated soil and a greater ability to withstand adverse environmental conditions and interspecies competition than bacteria [56]. As extensive as these benefits are, the possible costs of using plants for bioremediation should not be ignored. Some concerns voiced out in response to phytoremediation include its slow speed in comparison to mechanical methods such as soil excavation and climatic restrictions that may limit growing many species of plants, and the unknown long-term environmental costs [19]. Also, potential danger might exist for animals that live in the areas in which phytoremediators are grown, especially if these animals typically feed on plants being used for phytoremediation [21].

Moreover, concerns have been raised regarding the potential for contaminants to move up the food chain more quickly. This problem may occur if toxic materials are sequestered in consumable sources such as plants [57]. Finally, issues with the disposal of these toxic materials still remain. Once contaminants have been extracted from the soil by the plants, we are still faced with the dilemma of what to do with these contaminants. It seems that the end result remains the same. This involves the removal of contaminants to a landfill location where the plants would eventually biodegrade and the contaminants could enter the soil system once again [57].

4. Case Study: Phytoremediation Research in the Tropics

4.1. Phytoremediation of Lead Contaminated Soils

The global problem concerning contamination of the environment as a consequence of human activities is increasing. Most of the environmental contaminants are chemical by-products such as Pb. Lead released into the environment makes its way into the air, soil and water. Lead contributes to a variety of health effects such as decline in mental, cognitive, and physical health of the individual. An alternative way of reducing Pb concentration from the soil is through phytoremediation. Phytoremediation is an alternative method that uses plants to clean up contaminated area. Hence, Paz-Alberto *et al.*

[58] conducted a study in the Philippines. The objectives of this study were 1) to determine the survival rate and vegetative characteristics of three grass species such as vetiver grass, cogon grass, and carabao grass grown in soils with different Pb levels; and 2) to determine and compare the ability of three grass species as potential phytoremediators in terms of Pb accumulation by plants. The three test plants: vetiver grass (*Vetiveria zizanioides* L.); cogon grass (*Imperata cylindrica* L.); and carabao grass (*Paspalum conjugatum* L.) were grown in different individual plastic bags containing soils with 75 mg·kg⁻¹ (37.5 kg·ha⁻¹) and 150 mg·kg⁻¹ (75 kg·ha⁻¹) of Pb, respectively. The Pb contents of the test plants and the soil were analyzed before and after experimental treatments using an atomic absorption spectrophotometer. This study was laid out following a 3 × 2 factorial experiment in a completely randomized design [58].

Results of the study (**Table 2**) revealed that on the vegetative characteristics of the test plants, vetiver grass registered the highest whole plant dry matter (33.85 - 39.39 Mg·ha⁻¹). Carabao grass had the lowest herbage mass production of 14.12 Mg·ha⁻¹ and 5.72 Mg·ha⁻¹ from soils added with 75 and 150 mg·Pb·kg⁻¹, respectively. Vetiver grass also had the highest percent plant survival which meant it best tolerated the Pb contamination in soils. Vetiver grass registered the highest rate of Pb absorption (10.16 ± 2.81 mg·kg⁻¹). This was followed by cogon grass (2.34 ± 0.52 mg·kg⁻¹) and carabao grass with the mean Pb level of 0.49 ± 0.56 mg·kg⁻¹. Levels of Pb among the three grasses (shoots + roots) did not vary significantly with the amount of Pb added (75 and 150 mg·kg⁻¹) to the soil. Vetiver grass yielded the highest biomass; it also has the greatest amount of Pb absorbed (roots + shoots). This can be attributed to the highly extensive root system of vetiver grass with the presence of an enormous amount of root hairs. Extensive root system denotes more contact to nutrients in soils, therefore more likelihood of nutrient absorption and Pb uptake. The efficiency of plants as phytoremediators (**Table 3**) could be correlated with the plants' total biomass. This implies that the higher the biomass, the greater the Pb uptake. Plants characteristically exhibit remarkable capacity to absorb what they need and exclude what they do not need. Some plants utilize exclusion mechanisms, where there is a reduced uptake by the roots or a restricted transport of the metals from roots to shoots. Combination of high metal accumulation and high biomass production results in the most metal removal in the soil [58]. The study indicated that vetiver grass possessed many beneficial characteristics to uptake Pb from contaminated soil. It was the most tolerant and could grow in soil contaminated with high Pb concentration. Cogon grass and carabao grass are also potential phytoremediators since they can absorb small amount of Pb in soils, although cogon

Table 2. Levels of Pb absorbed 1) by whole plants (roots + shoots) and estimated total uptake of Pb 2) by vetiver grass, cogon grass and carabao grass.

Grasses	Amount of Pb added ($\text{kg}\cdot\text{ha}^{-1}$)		Mean ($LSD_{0.05} = 17.2$)
	37.5	75	
1. Levels of Pb in whole plants			
	($\text{mg}\cdot\text{kg}^{-1}$)		($\text{mg}\cdot\text{kg}^{-1}$)
Vetiver grass	11.84 ± 2.94	8.47 ± 1.59	10.16 ± 2.81a [§]
Cogon grass	2.00 ± 0.19	2.68 ± 0.54	2.34 ± 0.52b
Carabao grass	0.40 ± 0.32	0.58 ± 0.25	0.49 ± 0.56c
Mean ($LSD_{0.05} = 1.5$)	4.75 ± 2.5x [†]	3.91 ± 2.6x	($LSD_{0.05} = 1.8$)
2. Plant uptake of Pb			
	($\text{kg}\cdot\text{ha}^{-1}$)		($\text{kg}\cdot\text{ha}^{-1}$)
Vetiver grass	29.71 ± 8.71	33.78 ± 10.02	31.74 ± 9.01a [§]
Cogon grass	1.93 ± 0.48	2.69 ± 0.19	2.33 ± 0.53b
Carabao grass	0.19 ± 0.06	0.34 ± 0.03	0.27 ± 0.03b
Mean ($LSD_{0.05} = 5.6$)	13.95 ± 2.91x [†]	12.27 ± 3.32x	($LSD_{0.05} = 6.8$)

[§]Means in respective columns (1 and 2) with the same letter(s) are not significantly different at 5% level of significance. [†]Means in respective rows (1 and 2) with the same letter(s) are not significantly different at 5% level of significance.

Table 3. Estimated removal (%) of Pb by three grasses from soils amended with varying levels of Pb.

Grasses	Amount of Pb added ($\text{kg}\cdot\text{ha}^{-1}$)		Mean ($LSD_{0.05} = 17.2$)
	37.5	75	
	(%)		
Vetiver grass	79.2	45.1	62.2
Cogon grass	5.1	3.6	4.4
Carabao grass	0.5	0.5	0.5

grass is more tolerant to Pb-contaminated soil compared with carabao grass. The important implication of the findings of this study is that vetiver grass can be used for phytoextraction on sites contaminated with high levels of heavy metals, particularly Pb [58].

A field survey was conducted by Bautista [59] to identify phytoremediators present in the selected cities in the province of Nueva Ecija, Philippines. The plants found in the heavy traffic area of Cabanatuan City were the “balite” (*Ficus bengalensis*) and the “espada” (*Sanasavi-
era trifasciata*). In the heavy traffic area of San Jose City the most common plants are the Bougainvillea (*Bougainvillea* sp.) and the Cherry Pink plant. The Indian tree (*Polyalthia longifolia*) and the bougainvillea (*Bougainvillea* sp.) were the most common plants found along the traffic islands of the Science City of Muñoz. In Cabanatuan City, the balite absorbed 2.822 ppm of Pb, while espada absorbed 2.352 ppm of Pb; in San Jose City, the cherry pink plant absorbed 4.803 ppm, while the bougainvillea absorbed 1.521 ppm of Pb; and in the Science City of Muñoz, the Indian tree absorbed 0.217 ppm, and

the bougainvillea absorbed 0.528 ppm, respectively. Results of the chemical analysis proved that all of the plants along the traffic islands of the three selected cities of Nueva Ecija were phytoremediators of Pb. They were the most effective phytoremediator of Pb among the plants in the traffic area within the three selected cities.

As discussed previously, there are several different methods through which phytoremediation can occur. However, in order to maximize the success of a phytoremediation strategy, it is critical to have significant metal bioavailability at a contaminated site as well as a large quantity of plant biomass with high rates of growth. Metal contaminants that are not soluble, may limit the success of phytoremediation. In most Pb contaminated soils usually less than 0.1% of the total Pb present is bioavailable for plant uptake. The plants grown in a contaminated soil accumulated less Pb in both the roots and shoots than the plants grown hydroponically in a solution with a similar Pb concentration. The difference in uptake was because the Pb in the solution was much more bioavailable to the plants.

It should be noted that while hydroponic tests do not reflect accurately the accumulation potential in terrestrial applications, these tests could be valuable in the screening for Pb accumulating plant species and tolerance levels. The second limitation in Pb phytoextraction is the poor translocation of the metal from the roots to the harvestable shoots. In the plants that do translocate Pb, translocation is less than 30% [22].

Research has been conducted in the field to improve both the uptake and translocation of Pb through induced hyperaccumulation, which involves soil pH adjustments or the application of synthetic chelates. In general, the more biomass that the plant has, the more metal can be accumulated since the metal uptake is a function of the overall biomass [60]. The use of fertilizers can help facilitate rapid plant establishment and growth. For most Pb-contaminated soil, P availability is very low due to the precipitation of Pb-P precipitation. Thus, a foliar P fertilizer spray applied topically to the plant's leaves and stem increases phosphorous content in the plant, while not confounding the Pb-P binding problem in the soil. In a study reported by Huang *et al.* [50], soil to which phosphate fertilizer was added directly showed diminished Pb bioavailability, presumably due to Pb-P precipitation, in contrast to hydroponic uptake. Furthermore, although the foliar P application decreases Pb²⁺ concentration in shoots by 55% and root-Pb²⁺ concentration by 20%, the total amount of Pb²⁺ accumulation increased by 115% in shoots and 300% in the roots. This is the result of the large increase in biomass production made possible by overcoming phosphate limitations. These results further emphasize the relationship between Pb²⁺ accumulation and plant biomass in Pb²⁺ phytoextraction.

4.2. Phytoremediation Potential of Some Plant Species from Mining Sites

The focus of this study were on the accumulation of heavy metals in plants most commonly found in mine tailings of Victoria, Manlayan, Benguet, Philippines and identification of the different plant species within the area of the study. These plant species were assumed to be potential phytoremediation species [61].

The heavy metals extracted from the plants in the mine tailing were Cu, Cd, Pb and Zn. The fourteen plant species that were identified within the study were: *Eleusine indica* L.; *Amaranthus spinosus* L.; *Alternanthera sessilis* L.; *Portulaca oleracea* L.; *Fimbristylis meliacea* L., Vahl, *Mikania cordata* ((Burm. F.) B. I. Robins; *Polygonum barbatum* L.; *Achyranthes aspera* L., *Blumea* sp., *Cyperus alternifolius* L.; *Crassocephalum crepidioides* (Benth.) S. Moore; *Cyperus compactus* Retz.; *Desmodium* sp. and *Muntingia calabura* L. These plants absorbed certain metals at low and high levels. Among the plants species,

A. spinosus was found to have almost all the metals extracted in large amounts particularly Pb. The other plant species with high concentration of Pb were *A. sessilis*, *Desmodium* sp., *P. oleracea*, and *A. aspera*. *E. indica* has the highest concentration of Zn together with *M. cordata*, *C. compactus*, *F. meliacea* and *A. spinosus*. In contrast, Cd was found in trace amount in soil, but high in the following species: *C. crepidioides*, *P. oleracea*, *A. sessilis*, and *C. alternifolius*. Nickel was found high only in *A. sessilis* and *Blumea* sp. but trace amount in *Desmodium* sp. and *F. meliacea*. Also, high Cu concentrations were found in *A. spinosus* and *P. oleracea*.

In this study, the phytoremediation potential was dependent on population within species. The potential of the surveyed species mentioned for phytoremediation was remarkable and promising because of the presence of heavy metals suspected to have accumulated in the soil. Root system of these plants showed higher root to shoot ratios compared to other plants found in the area indicating high translocation of metals to the shoot. These species also plays an important role in the phytostabilization of metals to reduce leaching and run off. Also, these may be transformed to less toxic forms. These typical plants have dense root systems which can be effective for phytostabilization and elimination of contaminants such as Pb, Cd, Zn, As, Cu, and Ni in mine tailing sites.

A similar study conducted in Poland was worth including in this section. Wislocka *et al.* [62] studied the bioaccumulation of heavy metals by selected plants from uranium mining dumps in the Sudety Mountains, Poland. They found out that the investigated plants from the uranium dumps in the Sudety Mountains grew on acidic soils with an unfavorable C/N ratio. However, the nutrient status as well as relatively high CEC, and organic matter of the soil allowed the growth of spontaneous vegetation. Contamination by heavy metals (Pb, Zn, Cu, Cd and Ni), being associated with the mineral assemblage of the spoil material, was found to be significant within all dumps. All plants examined (*Salix caprea*, *Betula pendula* and *Rubus idaeus*) accumulated high amounts of heavy metals, but in general *R. idaeus* showed lower concentration of heavy metals (except Mn) in its leaves. However, Pb was accumulated to a similar degree in both trees and *R. idaeus*. Among all the heavy metals analyzed in the three species, Cd exhibited the greatest accumulation rate and the Cd accumulation ratio was several times higher for *S. caprea*, in comparison to the other two species. *B. pendula* and *R. idaeus* exhibited higher accumulation rates for Mn than *S. caprea*. However, the potential use of *R. idaeus* in monitoring metal concentration in the environment requires further investigation. The significant positive correlation between Pb in soil and leaves of the same tree suggest that *S. caprea*

should be employed for monitoring Pb in the environment.

4.3. Phytoremediation Potential of Selected Plants for Mutagenic Agents

Research and development has its own benefits and inconveniences. One of the inconveniences is the generation of enormous quantity of diverse toxic and hazardous wastes and its eventual contamination to soil and groundwater resources. Ethidium Bromide (EtBr) is one of the commonly used substances in molecular biology experiments. It is highly mutagenic and moderately toxic substance in DNA-staining during electrophoresis. Interest in phytoremediation as method to solve chemical contamination has been growing rapidly in recent years. The technology has been utilized to clean up soil and groundwater from heavy metals and other toxic organic compounds in many countries like the United States, Russia and most of European countries. Phytoremediation requires somewhat limited resources and is very useful in treating a wide variety of environmental contaminants. It is in this context that Uera *et al.* [63] conducted a study aimed to assess the potential of selected tropical plants as phytoremediators of EtBr.

This study used tomato (*Solanum lycopersicum*), mustard (*Brassica alba*), vetiver grass (*Viteveria zizanioides*), cogon grass (*Imperata cylindrical*), carabao grass (*Paspalum conjugatum*) and talahib (*Saccharum spontaneum*) to remove EtBr from laboratory wastes. The six tropical plants were planted in individual plastic bags containing 10% EtBr-stained agarose gel. The plants were allowed to establish and grow in the soil for 30 days. Ethidium Bromide content of the test plants and the soil were analyzed before and after soil treatment. Ethidium Bromide contents of the plants and soils were analyzed using an UV VIS spectrophotometer.

Results showed a highly significant ($p \leq 0.001$) difference in the ability of the tropical plants to absorb the EtBr from the soils. Mustard registered the highest absorption of EtBr ($1.4 \pm 0.12 \mu\text{g}\cdot\text{kg}^{-1}$) followed by tomato

and vetiver grass with average uptake of 1.0 ± 0.23 and $0.7 \pm 0.17 \mu\text{g}\cdot\text{kg}^{-1}$ EtBr, respectively. Cogon grass, talahib, and carabao grass had the least amount of EtBr absorbed ($0.2 \pm 0.6 \mu\text{g}\cdot\text{kg}^{-1}$). Ethidium bromide content of the soil planted with mustard was reduced by 10.7%. This was followed by tomato with an average reduction of 8.1%. Only 5.6% reduction was obtained from soils planted to vetiver grass. Soils planted to cogon grass, talahib and carabao grass had the least reduction of 1.52% from its initial EtBr content (Table 4 and Figure 1). Mustard had the highest potential as phytoremediator of EtBr in soil. However, the absorption capabilities of the other test plant may also be considered in terms of period of maturity and productivity. Uera *et al.* [63] recommended that a more detailed and complete investigation of the phytoremediation properties of the different plants tested should be conducted in actual field experiments. Plants should be exposed until they reach maturity to establish their maximum response to the toxicity and mutagenicity of EtBr and their absorbing capabilities. Different plant parts should be analyzed individually to determine the movement and translocation of EtBr from soil to the tissues of the plants. Since this study has an increased amount of EtBr application should be explored in future studies. It is suggested therefore that a larger, more comprehensive exploration of phytoremediation application in the management of toxic and hazardous wastes emanating from biotechnology research activities should be considered especially on the use of vetiver grass, a very promising tropical perennial grass.

4.4. Phytoremediation Potential of Selected Tropical Plants for Acrylamide

Environmentally hazardous and health risk substances in animals and humans in the environment have increased as a result of continuing anthropogenic activities. Examples of these activities are food processing, laboratory, food production, industrial and other relative activities that use various forms of acrylamide. All acrylamide in

Table 4. Levels of EtBr in soils and relative reduction of EtBr in soils after 30 days.

Plants (Treatments)	Initial Level in Soil ($\mu\text{g}\cdot\text{kg}^{-1}$)	Final Level in Soil ($\mu\text{g}\cdot\text{kg}^{-1}$)	Percent Reduction in Soil
Tomato	19.7	18.1 ± 0.17	8.12b [§]
Mustard	19.7	17.6 ± 0.23	10.66a
Vetiver grass	19.7	18.6 ± 0.23	5.58b
Talahib	19.7	19.4 ± 0.15	1.52c
Carabao grass	19.7	19.4 ± 0.20	1.52c
Cogon grass	19.7	19.4 ± 0.21	1.52c

[§]Means in column followed by a common letter(s) are not significantly different from each other at $p \leq 0.05$.

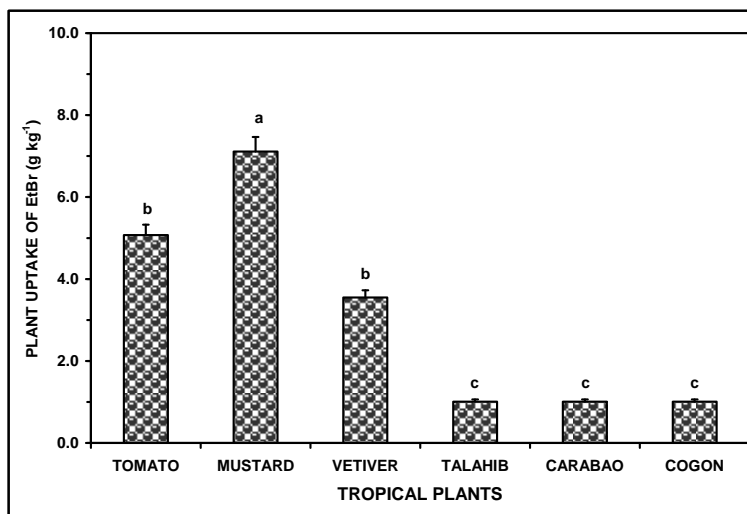


Figure 1. Average uptake of EtBr by the different tropical plants. Uptake of EtBr by different tropical plants are significantly different ($p \leq 0.05$) when superscripts located at top of bars are different.

the environment are man-made. It is the building block for the polymer, polyacrylamide, which is considered to be a non-toxic additive. However, if the polymerization process is not perfect and complete, the polyacrylamide may still contain acrylamide which is toxic and may pose risks and hazards to the environment. Another form of acrylamide may pose danger as well in the environment is the acrylamide monomer, also a very toxic organic substance that could affect the central nervous system of humans and is likely to be carcinogenic.

Phytoremediation could be a tool to somehow absorb this neurotoxic agent and lessen the contamination in the soil. This technology could lessen the soil and water contamination by acrylamide thereby limiting the exposure of animals and humans. This technique may also help solve the problem of disposing of contaminated acrylamide waste materials. Thus, Paz-Alberto *et al.* [63] conducted a study 1) to evaluate phytoremediation potentials of some selected tropical plants in acrylamide contaminated soil; and 2) to compare the performance of tropical plants in absorbing acrylamide through accumulation in their roots, stems, and leaves. The 200 grams polyacrylamide gel (PAG) was poured in each pot and mixed thoroughly with the soil by stirring manually. The soil was watered with 1,000 ml water and the test plants were transplanted after three days. Plant samples were collected at 45 days and 60 days after being planted onto PAG contaminated soil. The mustard and pechay were collected after 45 days of exposure while vetiver grass, hogweeds, snake plant, and common sword fern were collected after 60 days of exposure.

Among the plants tested, the highest concentration of acrylamide was absorbed by the whole plant of mustard ($6512.8 \text{ mg}\cdot\text{kg}^{-1}$) compared with pechay ($3482.7 \text{ mg}\cdot\text{kg}^{-1}$), fern ($2015.4 \text{ mg}\cdot\text{kg}^{-1}$), hogweeds ($1805.3 \text{ mg}\cdot\text{kg}^{-1}$),

vetiver grass ($1385.4 \text{ mg}\cdot\text{kg}^{-1}$) and snake plants ($887.5 \text{ mg}\cdot\text{kg}^{-1}$). Results of the study regarding the acrylamide absorption of the whole plants of mustard and pechay conformed to previous findings of other studies (Figure 2). Two members of *Brassica* family, *Brassica juncea* L. (mustard) and *Brassica chinensis* L. (pechay) were found to be effective in removing wide ranges of contaminants. Mustard, pechay, and fern plants had 60% survival rate while hogweeds had 80% survival rate. Snake plant and vetiver grass had 100% survival rate.

All the test plants planted in soil without acrylamide had survival rate of 100%. The 100 percent survival rate of vetiver grass and snake plant was due to the tolerance of these plants to acrylamide (Table 5). These findings could be attributed to the extraordinary features of vetiver grass such as its massive and deep root system and heavy biomass including its highly tolerance to extreme soil conditions like heavy metal toxicities and high metal concentration.

Results of the study proved that all the test plants are potential phytoremediators of acrylamide. However, mustard and pechay were the most effective as they absorbed the highest acrylamide concentrations in their roots, shoots and the whole plants. On the other hand, vetiver grass and snake plant had the highest uptake of acrylamide even though these plants did not absorb the highest acrylamide concentration. Therefore, these two plants can be considered as the best phytoremediator of acrylamide because they are perennial plants with heavier biomass with long, dense and extended root system. As such, these plants are capable of absorbing acrylamide in the soil for a long period of time.

As preventive measures and for application purposes, vetiver grass and snake plants could be planted along and around the wastewater treatment ponds of laboratories

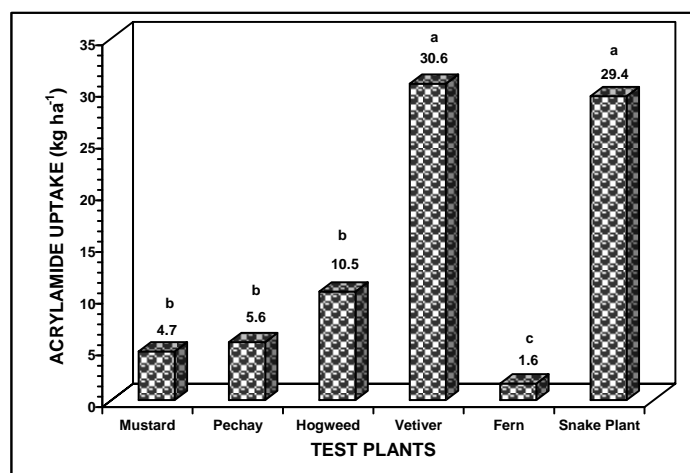


Figure 2. Comparative amount of acrylamide uptake among the different tropical plants. Acrylamide uptakes among the different tropical plants are significantly different ($p \leq 0.05$) when superscripts located at top of bars are different.

Table 5. Survival rate and weight of test plants at harvest.

Test Plants	Survival Rate (%)	Weight at Harvest (g)
Mustard (<i>Brassica juncea</i> L.)	60	1.3
Pechay (<i>Brassica chinensis</i> L.)	60	2.9
Hogweed (<i>Portulaca oleracea</i> L.)	80	10.5
Vetiver (<i>Vetiveria zizaniodes</i> L.)	100	39.7
Fern (<i>Nephrolepis cordifolia</i> L.)	60	1.4
Snake plant (<i>Sansevieria trifasciata</i> Prain)	100	59.6

using polyacrylamide gel. These plants can prevent further migration of pollutants to the environment aside from making the ponds more resistant to soil erosion. Further studies are suggested to evaluate acrylamide contaminations from laboratory washing, primary treatment pond, and seepage ponds that have earth dikes. Vetiver grass and snake plants are recommended for further phytoremediation studies for longer period of time to test the reduction of acrylamide in soil. Moreover, the outcome of acrylamide accumulation in the plants is also recommended for further study in conjunction with labeled-carbon tracer to determine its effects on the plants.

5. Outlook

Phytoremediation using “green plants” has potential benefits in restoring a balance in stressed environment. It is an emerging low cost technology, non-intrusive, and aesthetically pleasing using the remarkable ability of green plants to metabolize various elements and compounds from the environment in their tissues. Phytoremediation technology is applicable to a broad range of contaminants, including metals and radionuclides, as well as organic compounds like chlorinated solvents, polycyclic aromatic hydrocarbons, pesticides, explosives,

and surfactants. However, phytoremediation technology is still in its youthful development stages and full scale application is still inadequate. As with all new technology, it is important to proceed with caution.

The largest barrier to the advancement of phytoremediation, however, may be public opposition to genetic modification in general. Because all natural hyperaccumulator species are small in size, genetic modification can be used to introduce this technology to other species or to increase the biomass of the natural hyperaccumulators in order to create effective phytoremediators. This public opposition was the same fears that surround the issue of genetic modification of crops, and includes concerns regarding decreased biodiversity, the entry of potentially harmful genes into products consumed by humans, and the slippery slope created by introducing and transferring novel, foreign DNA between non-related species. Nonetheless, the benefits of using phytoremediation to restore balance to a stressed environment seem to far outweigh the costs.

REFERENCES

- [1] N. Kumar, V. Dushenkov, H. Motto and I. Raskin, “Phytoextraction: The Use of Plants to Remove Heavy Metals

- from Soils,” *Environmental Science and Technology*, Vol. 29, No. 5, 1995, pp. 1232-1238.
[doi:10.1021/es00005a014](https://doi.org/10.1021/es00005a014)
- [2] M. M. Lasat, “Phytoextraction of Metals from Contaminated Soil: A Review of Plant/Soil/Metal Interaction and Assessment of Pertinent Agronomic Issues,” *Journal of Hazardous Substance Research*, Vol. 2, No. 5, 2000, pp. 1-25.
- [3] J. A. Pollard and A. J. Baker, “Deterrence of Herbivory by Zinc Hyperaccumulation in *Thlaspi caerulescens*,” *New Phytologist*, Vol. 135, No. 4, 1997, pp. 655-658.
[doi:10.1046/j.1469-8137.1997.00689.x](https://doi.org/10.1046/j.1469-8137.1997.00689.x)
- [4] Freshwater Management Series No. 2, “Phytoremediation: An Environmentally Sound Technology for Pollution Prevention, Control and Remediation: An Introductory Guide to Decision-Makers. United Nation Environment Program 2000,” 2000.
- [5] “Sunflowers Bloom in Tests to Remove Radioactive Metals from Soil and Water,” *Wall Street Journal*, Vol. 6, No. 2, 1996, pp. 23-28.
- [6] National Research Council (NRC), “Lead in the Human Environment,” National Academy of Sciences, Washington DC, 1980, p. 525.
- [7] P. N. Truong and D. Baker, “Vetiver Grass System for Environmental Protection. Royal Development Projects Protection,” Technical Bulletin No. 1998/1, Pacific Rim Vetiver, Office of the Royal Development Projects Board, Bangkok, 1996.
- [8] A. Mekonnen, “Handbook on Vetiver Grass Technology—From Propagation to Utilization,” GTZ IFSP S/GONDER, Ethiopia, 2000.
- [9] C. Knoll, “Rehabilitation with Vetiver,” *African Mining*, Vol. 2, 1997, p. 43.
- [10] H. Chen, “Chemical Methods and Phytoremediation of Soil Contaminated with Heavy Metals,” Vol. 41, American Chemical Society, Washington DC, 1998, pp. 229-234.
- [11] P. N. Truong, “Stiffgrass Barrier with Vetiver Grass. A New Approach to Erosion and Sediment Control,” *2nd International Vetiver Conference*, 18-22 January 1995, Pechaburi.
- [12] N. Roongtanakiat and P. Chairaj, “Vetiver Grass for Remedying Soil Contaminated with Heavy Metals,” 2010. http://www.google.com/ Roongtanakiat+ N+and+Chairaj+ R=2001&meta=&aq=o&aqi=&aql=&oq=&gs_rfai=&fp=da1b4ba80a870679
- [13] E. R. R. L. Johnson and D. G. Shilling, “Cogon Grass. Plant Conservation Kirchner, A. 2001. Mine-Land Restoration: Phytoremediation of Heavy-Metal Contaminated Sites—A Critical View,” International Ecological Engineering Society, 2002.
http://www.iees.ch/EcoEng011/EcoEng011_R2.html
- [14] L. Mannerje, “Paspalum Conjugatum,” 2004.
<http://www.fao.org/ag/AGP/AGP/doc/GBASE/DATA/PF000492.HTM>
- [15] M. Y. Sykes, B. J. Vina and S. Abubakr, “Biotechnology: Working with Nature to Improve Forest Resources and Products,” *International Vetiver Conference*, 4-8 February 1999, Chiang Rai, pp. 631-637.
- [16] C. Garbisu, “Phytoremediation: A Technology Using Green Plants to Remove Contaminants from Polluted Areas,” *Reviews on Environmental Health*, Vol. 17, No. 3, 2002, pp. 173-188. [doi:10.1515/REVEH.2002.17.3.173](https://doi.org/10.1515/REVEH.2002.17.3.173)
- [17] T. Macek, et al, “Phytoremediation: Biological Cleaning of a Polluted Environment,” *Reviews on Environmental Health*, Vol. 19, No. 1, 2004, pp. 63-82.
[doi:10.1515/REVEH.2004.19.1.63](https://doi.org/10.1515/REVEH.2004.19.1.63)
- [18] United States Environmental Protection Agency (USEPA), “Hazard Summary. Lead Compounds,” 2004.
<http://www.epa.gov/ttn/atw/hlthef/lead.html>
- [19] S. Clemens, M. G. Palmgren and U. Kramer, “A Long Way Ahead: Understanding and Engineering Plant Metal Accumulation,” *Trends in Plant Science*, Vol. 7, No. 7, 2002, pp. 309-314. [doi:10.1016/S1360-1385\(02\)02295-1](https://doi.org/10.1016/S1360-1385(02)02295-1)
- [20] A. S. Moffat, “Plants Proving Their Worth in Toxic Metal Cleanup,” *Science*, Vol. 9, No. 2, 1995, pp. 302-303.
[doi:10.1126/science.269.5222.302](https://doi.org/10.1126/science.269.5222.302)
- [21] J. W. Huang and S. D. Cunningham, “Lead Phytoextraction: Species Variation in Lead Uptake and Translocation,” *The New Phytologist*, Vol. 134, No. 1, 1996, pp. 75-84. [doi:10.1111/j.1469-8137.1996.tb01147.x](https://doi.org/10.1111/j.1469-8137.1996.tb01147.x)
- [22] P. C. C. Cortez, “Assessment and Phytoremediation of Heavy Metals in the Panlasian Creek,” An Unpublished High School Thesis, University Science High School, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, 2005.
- [23] H. Xia and X. Ma, “Phytoremediation of ethion by water hyacinth (*Echhornia crassipes*) from water. Bioresour Technology. College of Food Sciences, Biotechnology and Environmental Engineering, Zhejiang Ghongshang University, Hangzhou, 2005.
- [24] B. Letachowicz, J. Krawczyk and A. Klink, “Accumulation of Heavy Metals in Organs of *Typha latifolia*,” *Polish Journal of Environmental Studies*, Vol. 15, No. 2a, 2006, pp. 407-409.
- [25] J. H. Primavera, R. B. Sadaba, M. J. H. L. Leбата and J. P. Altamirano, “Handbook of Mangroves in Philippines-Panay,” SEAFDEC Aquaculture Department, Iloilo, 2004.
- [26] K. Kathiresan and B. L. Bingham, “Biology of Mangroves and Mangrove Ecosystems,” 2011.
<http://faculty.wvu.edu/ bingham/mangroves.pdf>
- [27] Philippines Environment Monitor, “Resources and Ecosystems,” 2012.
<http://siteresources.worldbank.org/INTPHILIP PINES/Resources/PEM05-ch2.pdf>
- [28] J. B. Long and C. Giri, “Mapping the Philippines’ Mangrove Forests Using Landsat Imagery,” 2011.
www.mdpi.com/1424-8220/11/3/2972/pdf
- [29] P. J. Hogarth, “The Biology of Mangroves and Seagrasses,” Oxford University Press, Oxford, New York, 2007, p. 217.
- [30] W. Zheng, X. Chen and P. Lin, “Accumulation and Biological Cycling of Heavy Metal Elements in *Rhizophora stylosa* Mangroves in Yingluo Bay, China,” 2012.

- <http://www.int-res.com/articles/meps/159/m159p293.pdf>
- [31] G. R. Macfarlane and M. D. Burchett, "Toxicity, Growth and Accumulation Relationships of Copper, Lead and Zinc in the Grey Mangrove *Avicennia marina* (Forsk.) Vierh," 2011.
[http://www.mendeley.com/research/toxicity-growth-and-accumulation-rel ationships-of-copper-lead-and-zinc-in-the-grey-mangrove-avicennia-mari-na-forsk-vierh/](http://www.mendeley.com/research/toxicity-growth-and-accumulation-rel-ationships-of-copper-lead-and-zinc-in-the-grey-mangrove-avicennia-mari-na-forsk-vierh/)
- [32] G. R. Macfarlane and M. D. Burchett, "Cellular Distribution of Copper, Lead and Zinc in the Grey Mangrove, *Avicennia marina* (Forsk.) Vierh," 2011.
<http://www.sciencedirect.com/science/article/pii/S0304377000001054>
- [33] S. Cheng, "Heavy Metals in Plants and Phytoremediation," 2012.
<http://ir.ihb.ac.cn/bitstream/152342/9658/1/Heavy%20metals%20in%20journals.pdf>
- [34] I. Sari, Z. Bin Din, and G. W. Khoon, "The Bioaccumulation of Heavy Metal Lead in Two Mangrove Species (*Rhizophora apiculata* and *Avicennia alba*) by the Hydroponics Culture," 2011.
[http://eprints.usm.my/1490/1/The_Bioaccumulation_Of_Heavy_Metal_Lead_In_Two_Mangrove_Species_\(Rhizophora_apiculata_and_Aviicennia_alba\)_by_The_Hydroponics_Culture.pdf](http://eprints.usm.my/1490/1/The_Bioaccumulation_Of_Heavy_Metal_Lead_In_Two_Mangrove_Species_(Rhizophora_apiculata_and_Aviicennia_alba)_by_The_Hydroponics_Culture.pdf)
- [35] P. Saenger and D. McConchie, "Heavy Metals in Mangroves Methodology, Monitoring and Management," 2011.
<http://www.frienvic.nic.in/bulletinwork/bulletin.htm>
- [36] A. Shete, V. R. Gunale and G. G. Pandit, "Bioaccumulation of Zn and Pb in *Avicennia marina* (Forsk.) Vierh. and *Sonneratia apetala* Buch. Ham. from urban Areas of Mumbai (Bombay), India," 2011.
<http://www.ajol.info/index.php/jasem/article/viewFile/55142/43614>
- [37] B. Y. Kamaruzzaman, M. C. Ong, K. C. A. Jalal, S. Shahbudin and O. Mohd Nor, "Accumulation of Lead and Copper in *Rhizophora apiculata* from Setiu Mangrove Forest, Terengganu, Malaysia," 2011.
www.jeb.co.in/journal_issues/200909_sep09_supp/paper_08.pdf
- [38] V. Pahalawattaarachchi, C. S. Purushothaman and A. Venilla, "Metal Phytoremediation Potential of *Rhizophora mucronata* (Lam.)," 2011.
[http://nopr.niscair.res.in/bitstream/123456789/.../IJMS%2038\(2\)%20178-183.pdf](http://nopr.niscair.res.in/bitstream/123456789/.../IJMS%2038(2)%20178-183.pdf)
- [39] M. F. Nazli and N. R. Hashim, "Heavy Metal Concentrations in an Important Mangrove Species, *Sonneratia caseolaris*, in Peninsular Malaysia," 2011.
www.tshe.org/ea/pdf/vol3s%20p50-55.pdf
- [40] H. Parvaresh, Z. Abedi, P. Farshchi, M. Karami, N. Khorasani and A. Karbassi, "Bioavailability and Concentration of Heavy Metals in the Sediments and Leaves of Grey Mangrove, *Avicennia marina* (Forsk.) Vierh, in Sirik Azini Creek, Iran," 2011.
<http://www.mendeley.com/research/bioavailability-concentration-heavy-m-etals-sediments-leaves-grey-mangrove-avicennia-marina-forsk-vierh-sirik-azini-creek-iran/>
- [41] Y. W. Qui, K. F. Yu, G. Zhang and W. X. Wang, "Accumulation and Partitioning of Seven Trace Metals in Mangroves and Sediment Cores from Three Estuarine Wetlands of Hainan Island, China," 2012.
<http://www.sklog.labs.gov.cn/atticle/B11/B11012.pdf>
- [42] J. E. Zhang, J. L. Liu, Y. Ouyang B. W. Lia and B. L. Zhao, "Physiological Responses of Mangrove *Sonneratia apetala* Buch-Ham Plant to Wastewater Nutrients and Heavy Metals," 2011.
<http://www.tandfonline.com/doi/abs/10.1080/15226511003671395#preview-physio>
- [43] I. J. Nirmal, P. R. Sajish, R. Nirmal, G. Basil, and V. Shailendra, "An Assessment of the Accumulation Potential of Pb, Zn and Cd by *Avicennia marina* (Forsk.) Vierh. in Vamleshwar Mangroves, Gujarat, India," 2011.
<http://notulaebiologicae.ro/nsb/article/viewFile/5593/5343>
- [44] A. N. Subramanian, "Persistent Chemicals," 2011.
<http://ocw.unu.edu/international-network-on-water-environment-and-health/unu-inweh-course-1-mangroves/Persistent-Chemicals.pdf>
- [45] US Environmental Protection Agency, "Lead-How Lead Affects the Way We Live and Breath. Office of Air Quality Planning and Standards," 2004.
<http://www.epa.gov/air/urbanair/lead/index.html>
- [46] J. W. Huang and S. D. Cunningham, "Lead Phytoextraction: Species Variation in Lead Uptake and Translocation," *New Phytologist*, Vol. 134, No. 1, 1996, pp. 75-84.
<http://www.lenntech.com/Pb-en.htm>
[doi:10.1111/j.1469-8137.1996.tb01147.x](https://doi.org/10.1111/j.1469-8137.1996.tb01147.x)
- [47] J. Wu, F. C. Hsu and S. D. Cunningham, "Chelate-Assisted Pb Phytoextraction: Pb Availability, Uptake, and Translocation Constraints," *Environmental Science and Technology*, Vol. 33, No. 11, 1999, pp. 1898-1904.
[doi:10.1021/es9809253](https://doi.org/10.1021/es9809253)
- [48] B. J. Alloway, "Cadmium. Heavy Metals in Soil. London," Blackie and Son, London, 1990.
- [49] J. W. Huang, J. Chen, W. R. Berti and S. D. Cunningham, "Phytoremediation of Lead-Contaminated Soils: Role of synthetic Chelates in Lead Phytoextraction," *Environmental Science and Technology*, Vol. 31, No. 3, 1997, pp. 800-805.
[doi:10.1021/es9604828](https://doi.org/10.1021/es9604828)
- [50] D. E. Salt, M. Blaylock, I. Chet, S. Dushenkov, B. Ensley, P. Nanda and I. Raskin, "Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment Using Plants," *Biotechnology*, Vol. 13, No. 5, 1995, pp. 468-474.
[doi:10.1038/nbt0595-468](https://doi.org/10.1038/nbt0595-468)
- [51] S. R. Smith, "Effect of Soil pH on Availability to Crops of Metals in Sewage Sludge Treated Soils, Cadmium Uptake by Crops and Implications for Human Dietary Intake," *Environmental Pollution*, Vol. 86, No. 1, 1994, pp. 5-13.
[doi:10.1016/0269-7491\(94\)90003-5](https://doi.org/10.1016/0269-7491(94)90003-5)
- [52] A. Cholpecka, J. R. Bacon, M. J. Wilson and J. Kay, "Heavy Metals in the Environment. Forms of Cadmium, Lead, and Zinc in Contaminated Soils from Southwest Poland," *Journal of Environmental Quality*, Vol. 25, No. 1, 1996, pp. 69-79.
[doi:10.2134/jeq1996.00472425002500010009x](https://doi.org/10.2134/jeq1996.00472425002500010009x)
- [53] M. Sadiq and G. Hussain, "Effect of Chelate Fertilizers

- on Metal Concentrations and Growth of Corn in a Pot Experiment,” *Journal of Plant Nutrition*, Vol. 16, No. 4, 1993, pp. 699-711. [doi:10.1080/01904169309364567](https://doi.org/10.1080/01904169309364567)
- [54] S. P. Bizily, C. L. Rugh, A. O. Summers and R. B. Meagher, “Phytoremediation of Methylmercury Pollution: merB Expression in Arabidopsis Thaliana Confers Resistance to Organomercurials,” *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 96, No. 12, 1999, pp. 6808-6813. [doi:10.1073/pnas.96.12.6808](https://doi.org/10.1073/pnas.96.12.6808)
- [55] S. Abdulla, “Tobacco Sucks up Explosives,” *Nature*, 2002. <http://www.nature.com/nsu/990429/990429-5.html>
- [56] A. Kirchner, “Mine-Land Restoration: Phytoremediation of Heavy-Metal Contaminated Sites—A Critical View,” International Ecological Engineering Society, 2002. http://www.iees.ch/EcoEng011/EcoEng011_R2.html
- [57] A. M. Paz-Alberto, G. C. Sigua, B. G. Bauí and J. A. Prudente, “Phytoextraction of Lead-Contaminated Soil Using Vetiver grass (*Vetiveria zizanioides* L.), Cogon grass (*Imperata cylindrica* L.) and Carabao grass (*Paspalum conjugatum* L.),” *Environmental Science and Pollution Research*, Vol. 14, No. 7, 2007, pp. 498-504. [doi:10.1065/espr2007.05.415](https://doi.org/10.1065/espr2007.05.415)
- [58] M. B. Bautista, “Phytoremediation Potential of Selected Plants on Lead in Nueva Ecija,” An Unpublished High School Thesis, University Science High School, Central Luzon State University, Science City of Muñoz, Nueva Ecija, 2006.
- [59] K. A. Gray, “The Phytoremediation of Lead in Urban, Residential Soils,” Northwestern University, Evanston, 2000.
- [60] J. R. Undan, R. T. Alberto, A. M. Paz-Alberto and C. T. Galvez, “Heavy Metals in Plant Species in Mine Tailings of Victoria in Manlayan, Benguet Province,” *CAS Faculty Journal*, Vol. 2, No. 1.
- [61] M. Wislocka, J. Krawczyk, A. Klink and L. Morrison, “Bioaccumulation of Heavy Metals by Selected Plant Species from Uranium Mining Dumps in the Sudety Mountains, Poland,” *Polish Journal of Environmental Studies*, Vol. 15, No. 5, 2006, pp. 811-818.
- [62] R. B. Uera, A. M. Paz-Alberto and G. C. Sigua, “Phytoremediation Potentials of Selected Tropical Plants for Ethidium Bromide,” *Environmental Science and Pollution Research*, Vol. 14, No. 7, 2007, pp. 505-509. [doi:10.1065/espr2007.02.391](https://doi.org/10.1065/espr2007.02.391)
- [63] A. M. Paz-Alberto, M. J. J. De Dios, R. T. Alberto and G. C. Sigua, “Assessing Phytoremediation Potentials of Selected Tropical Plants for Acrylamide,” *Journal of Soils and Sediments*, Vol. 11 No. 7, 2011, pp. 1190-1198. [doi:10.1007/s11368-011-0390-z](https://doi.org/10.1007/s11368-011-0390-z)