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Nature and Science

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Current Trends and Impending Advances in Plant Assisted Remediation of Heavy Metals

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Abstract: Heavy metal pollution posed a severe threat to human health which subsequently affects the entire ecosystem. The presence and persistence of those toxic metals in the environment lead to the development of several remediation techniques. Conventional remediation techniques used include physical and chemical methods which are expensive and sometimes demand the expertise of highly trained personnel. Identifying a suitable way to remove heavy metals from the contaminated site is therefore, the dare needs. Among the recently identified safest, most innovative and environmental-friendly techniques for the remediation of heavy metals is phytoremediation. Many researchers explored the use of plants, especially those identified to accumulate high amount of metals. This review highlights the current and future developments in the application of phytoremediation technologies, including the use of molecular genetic engineering to modify various plants traits of interest. Manipulation of these traits could enhance phytoremediation ability of plants such as multiple metal accumulations, increasing biomass and tolerance as well as detoxification and transformation rates. Furthermore, the concerns about the potential transfer of contaminants through the food chains and the proper disposal of the biomass could be address by the process of metal recovery of commercial value called "Phytomining". Adequate application of profitable phytomining technique may increase acceptability and market value of phytoremediation in the future.
[Aminu S. U., Aliko A.A., Sabo A., Magama Y. and Ahmad M.S. **Current Trends and Impending Advances in Plant Assisted Remediation of Heavy Metals.** *Nat Sci* 2020;18(8):19-29]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 4. doi:10.7537/marsnsj180820.04.

Key word: Phytoremediation, Hyperaccumulation, Pollution, Phytomining,

1. Introduction

Since the Industrial Revolution, environmental pollution from industries and manufacturing sectors such as mining, electroplating, metal smelting, agriculture, municipal waste and fossil fuels has accelerated the heavy metal contamination of the aquatic and terrestrial biota (Kotrba *et al.,* 2004; Igwe and Abia, 2006; Liu *et al*., 2018). The concentration of heavy metals is reported to cause soil contamination in terrestrial, aqueous waste streams and groundwater, resulting in environmental and human health impact (Alkorta *et al*., 2004; Khan, 2005; Kramer, 2005; Kang, 2014; FOA, 2015; Saxena *et al*., 2019).

According to Gardea-Torresdey *et al.,* (2005), heavy metal is a name giving to some group of elements with relatively high atomic density more than 6gcm^{-3}. Examples of metal species include cadmium (Cd), Mercury (Hg), Lead (Pb), Arsenic (As), Chromium (Cr), Copper (Cu), Selenium (Se), Silver (Ag), Zinc (Zn), Nickel (Ni), e.t.c. Additionally, there are some few common metallic pollutants which include Aluminium (Al), Cobalt (Co), Caesium (Cs), Manganese (Mn), Molybdenum (Mo) Uranium (U), and Strontium (Sr) (McIantyre, 2003; Liu *et al*., 2018; Pandey and Bajpai 2019). Metals are present naturally in the earth's crust (Yang *et al.,* 2005). Rascio *et al.* (2011) reported that heavy metals like As, Cd, Hg, Pb and Se are non-essential elements as they are not involved in any known biological function. Essential elements required for growth and metabolism include Co, Cu, Fe, Mn, Mo, Ni, and Zn, though most metal ions required in higher concentration (Trace element) for normal plant functions, potentially become toxic to biota (Yang *et al*., 2005), thereby impacting their growth and functions. The general effect of various metals on plants are summarised by Gardea-Torresday *et al.* (2005) and Pal and Kumar (2011). Heavy metals accumulated in plant biomass on the other hand can be passed to human nutrition through food chain leading to toxicity. Human exposure to heavy metals may cause chronic and severe diseases. Toxic effects of some heavy metals on humans are described by Rai (2009) and Pal and Kumar (2011).

1.1 Phytoremediation

The term Phytoremediation was coined from a Greek word "*pytho*" which means plant and a Latin root "*remedium*" meaning to correct evil

(Cunningham *et al*., 1996: cited in Gosh and sing, 2005). Plants have evolved adaptive mechanisms that enable them to accumulate and tolerate high levels of contaminants in their rhizosphere and with subsequent translocation to the above-ground tissues where the contaminants are metabolised, sequestered or volatilized (Yang *et al.,* 2005). As an emerging technology, phytoremediation uses various plant species and the plant associated microorganisms to remove pollutants from surroundings through containment, immobilization, extraction or detoxification to render them harmless and the possible transfer of the volatile compounds into the air (Denton, 2007; Kushwaha *et al*., 2018). Phytoremediation maintains the bio-properties and physical structure of the soil as it does not require the removal or excavation of the soil. Phytoremediation acceptance by both governmental agencies and industries is related to its cost effectiveness and environmental friendliness (Pilon-Smits, 2005; Yang *et al.,* 2005).

1.2 Basic Phytoremediation techniques

The process of remediation of metal contaminants using plant depends on the physiological mechanisms of plants and their assisted microflora (Ghosh and Singh 2005; Peter, 2011). This review describes four different plant-based remediation techniques involved for the decontamination of metal-polluted soil, sediment or water. These include, **phytoextraction**, in which plant roots absorb metals and translocate them to a harvestable biomass shoot; **rhizofiltration**, where plants roots are used to extract contaminants in various aquatic substrates; **phytovolatilization** which is a proves of removing certain metals by transport them to the aerial part where they released the pollutants into the air and **phytostabilization,** where the movement of the contaminants are prevented or restricted within the plant's root.

1.2.1. Phytoextraction

Kumar and Amit (2011) described phytoextraction as the process by which plants roots extract metal contaminants from soil and subsequently translocate them to their shoots. Then; both the roots and the above-ground shoots can be harvested and disposed of wisely. Apart from metal extraction, the established plants provide soil cover and prevent leaching and erosion. In addition, the continual harvest of the contaminated biomass results in total removal of the contaminant from the site (Jadia and Fulekar, 2009). Pandey and Bajpai (2019) explained that there is serious concern about the disposal of the harvested biomass but some researchers suggested that the volume of biomass for disposal could be reduced through incineration, and there is a possibility of recovering some valuable metals for re-use (Prasad and Freitas, 2003; Kumar and Amit, 2011)

1.2.2. Rhizofiltration

This technique is similar to phytoextraction because both methods involved the use of plants to extract, accumulate and detoxify the metals they absorbed from a contaminated substrate. However, Rhizofiltration is used to remove contaminants in an aqueous stream. Rhizofiltration techniques are applied in the treatment of groundwater, surface and wastewater (Prasad and Freitas, 2003; Pandey and Bajpai 2019). Rhizofiltration can either be applied in-situ or ex-situ. In-situ rhizofiltration, treatment system is mostly in the form of constructed wetland following pumping of the pollutant into the wetland for the plants to capture them, while ex-situ rhizofiltration systems are a closed hydroponic structure where polluted waters are pumped in, supplying the plant roots with contaminated water (Gosh and Singh, 2005; Jadia and Fulekar, 2009).

1.2.3. Phytovolatilization

Phytovolatilization is the process whereby plants take up water and nutrients from the soil through their roots and metabolise them into gaseous form and transpire the same compounds, or their metabolites through the leaves into the atmosphere (Pandey and Bajpai 2019). Phytovolatilization has been reported for the removal of Se and Hg from contaminated soil and they undergo a process called biomethylation though the volatile elements released by plants into the air which are less toxic and can easily migrate to another environment (Prasad and Freitas, 2003; Gosh and Singh, 2005).

1.2.4. Phytostabilisation

Phytostabilization technique uses plants roots and associated microflora to absorbs useful nutrients and release chemical substances which aid the plant's roots to adsorb contaminants and stabilise them onto their surfaces, thereby reducing pollutant mobility and bioavailability (Pandey and Bajpai 2019). Plants prevent mobility of pollutants from the surface to groundwater and the potential transfer to air and reducing runoff, erosion and subsequent transfer into the food chain (Pilon-Smits, 2005; Kumar and Pal, 2011). The applicability of this technique rests on the ability of roots to reduce the mobility and bioavailability of pollutant in soil and therefore, the method can be applied to remediate contaminated soil, sediment and sludges.

1.3 Advantages and disadvantages of phytoremediation

1.3.1 Advantages

The benefits of phytoremediation and its acceptance over other conventional remediation techniques is related to its cost-effectiveness and it is an elementary technology that does not require intensive human resources. It uses the natural plant ability as a solar-driving pump to remove a wide range of pollutants from the contaminated media. Another advantage is that it can be applied both *in-situ* and *ex-situ*. In-Situ applications are advantageous for it does not involve the removal of soil from the treatment site, as such reduces the rate of soil disturbance compared to other conventional remediation technique (Peter, 2011). Furthermore, heavy, noisy and expensive equipment is not required in the phytoremediation site nor are highly skilled workforces needed. Once the plants grow, they can maintain themselves, thereby leading to land restoration and habitat creation (Ghosh and Singh, 2005; Sureh *et al.,* 2004). Another advantage of phytoremediation is the production of low secondary waste bulk for disposal. However, in a broad-scale application, the biomass can be used to generate thermal energy. Also, contaminant that is not used up by plants like metals which are stored in the bulk biomass for disposal can potentially be recycled. This will further reduce the cost of phytoremediation when this biomass is used for the mining of commercially valuable metals (Sureh *et al.,* 2004; Ghosh and Singh, 2005).

1.3.2 Disadvantages

Despite its advantages, Phytoremediation also has certain limitations as is the case with many other methods. One of its major drawbacks is the plant growth and the duration required by plants to completely remediate a contaminated site. Climate change may also affect the activities of plants and as such, the performance of the plant in the remediation may reduce as a result of seasonal variations. Root length also is a limiting factor to the adequate performance of plants because they can only remediate sites with shallow and low-level contaminants. Another disadvantage of this method is the potential transfer of contaminants into the food chain and this may have an impact on human health (Sureh et al., 2004; Ghosh and Singh, 2005; Peter, 2011). Therefore, proper handling and disposal of harvested biomass are needed to prevent the contaminant from entering the food chain or going back to the soil (Agbontalor, 2007).

2.0 Current Trends in Phytoremediation of Heavy Metals

Research and interest in the field of plant application for the remediation of heavy metals have currently focused on the plant's ability to extract heavy metals from contaminated soils with much attention paid to the plants with hyper-accumulation capabilities. However, the area of metal volatilisations, containment and stabilisation by plants and studies on various enhanced phytoremediation techniques to maximise plant ability for better metal remediation are some of the current areas of research interest. This part therefore, aims to discuss the current trends associated with heavy metals phytoremediation techniques.

2.1 Metal Extraction and Hyper-accumulation

Plants use their roots to absorb and extract metals from a contaminated substrate and transfer these elements through the xylem to their harvestable tissues (Prasad and Freistas, 2003). Extraction and hyper-accumulation of heavy metals involve different processes including absorption, transportation and translocation through xylem and phloem tissues and subsequently hyper-accumulate those metals in harvestable biomass. Hyper-accumulation is a significant trend that requires large sinks for storing pollutants (Li *et al*., 2003; Suresh and Ravishankar, 2004).

2.1.1 Adsorption

Before adsorption, metal contaminants must be bioavailable for the plant to accumulate them. Bioavailability of metal can be enhanced by acidifying of the plant root zone by releasing root exudate to release the targeted metals into the soil solution for plant accumulation (Gosh and Singh, 2005). An article by Suresh and Ravishankar (2004) had demonstrated that the ability of *Brassica juncea* to concentrate high Cd, Ni, Pb and Si into their root tissue was 500 times greater when compared to those grown in an aqueous medium.

2.1.2 Transport and Translocation

Xylem loading plays a significant part in the transportation and translocation of metal ions in plants for distribution and detoxification, once into the plants before xylem uptake; solutes mostly form carbonate, sulphate or phosphate precipitate and immobilise into the roots apoplast and symplast chambers. Three routes govern movement from roots into the xylem: metal sequestration in the root, symplastic transport into the stele and lastly released into xylem vessels. However, the vacuole plays a vital role in the storage of metal ion, once inside the vacuole; they can be chelated by organic acids or phytochelatins (Gosh and Singh, 2005). Immediately after heavy metal uptake by roots and then get into the xylem, they could be stored in the roots or transported to the above-ground shoots (Suresh and Ravishankar, 2004).

One of the consequences of Phytochelatins (PC) and Metallothioneins (MT) is the transportation and translocation of metal ions in plants. PCs are a group of small peptides consisting of cysteine, glycine and glutamic acids. They function in multiple metal sequestration and heavy metal detoxification in plants. In many ways, MTs resemble PCs in terms of structure and function (Suresh and Ravishankar, 2004).

2.1.3 Hyper-accumulation

The goal of phytoremediation is to hyper accumulate toxic metal elements. The groups of plant species that exhibit this quality of accumulating excessively high amounts of metals are called hyperaccumulators (Li *et al.,* 2003; Ullah *et al*., 2015). The natural ability exhibited by some plants to uptake and accumulates a high concentration of metals in their tissues brought about the concept of hyperaccumulation. Hyperaccumulators can best be described as any plant species capable of extracting, concentrating and tolerating large amounts of one or more metals from the soil at concentration 100-1000-fold higher than non-hyper accumulators (Rascio and Navari-Izzo, 2011; Ullah *et al.,* 2015; Pandey and Bajpai, 2019). Usually accumulated metals are not retained in the root; however, they are translocated to the above-ground organs. More than 450 angiosperm families have been documented so far for heavy metals hyperaccumulation (Baker *et al*. 2000).

Different plants species such as Indian mustard (*Brassica juncea*), Alpine pennycress (*Thlaspi caerulescens*), Sunflower (Helianthus *annuus*) and Corn (Zea mays) are found to exhibit hyper-accumulation characteristics with cadmium and zinc (Caille *et al.,* 2004; Ullah *et al*., 2015). A plant known as alpine pennycress *Thlapsia caerulescens* with specific tolerance to metal contamination was reported to be a good phyto-remediator (Milner and Kochian, 2008). The search for the new high biomass metal hyperaccumulators is still on, a plant known as *Berkheya coddii* (Asteraceae) native to north Transvaal, South Africa, is reported to accumulate 3.7% in its dry biomass shows significant potentials in the area of phytoremediation (Salt *et al*., 1998).

Many aquatic plants such as water hyacinth and water velvet are investigated for their application in rhizo-filtration and extraction of metals. However, the potentiality of some aquatic plants such as *Azolla filliculoides*, *A. pinnata*, *Typha orientalis* and *Salvinia molesta* for the bio removal of heavy metals are also documented (Gosh and Singh, 2005).

Gardea-Torresdey *et al.* (2005) reported the ability of *Eichhornia crassipes* to accumulate >6,000 ppm cadmium and lead; more than 8,000 ppm of copper growing in water with the concentration of 5 ppm of these heavy metals. Duckweed was reported to effectively remove 76% of lead and 82% nickel from aqueous solution with 10 and 5 mg/l of each metal respectively (Gardea-Torresdey *et al*., 2005). On the other hand, aquatic *plants L. minima* and *Spirodela punctate* were found to remove 70-90 % of lead and zinc in a concentration of 1-8 ppm (Axtell *et al*., 2003). The effectiveness of water hyacinth (*Eichhornia crassipes*) in the removal of trace elements in waste streams was also indicated (Prasad and Freistas, 2003).

Azolla filiculoides was also reported to absorb up to 10,000ppm cadmium, 9000ppm copper and nickel while up to 6500ppm of zinc. Report on the use of *Azolla pinnata* in the treatment of industrial effluents containing heavy metals, with a record of about 94% of the Hg and Cd inhibition in the effluent (Rai, 2008). On a study conducted on 12 wetland species, Prasad and Freistas (2003) reported the use of *Polygonum hydropiperoides* Michx (smartweed) as the best heavy metal phytoremediator for their past growth and high plant density (Gosh and Singh, 2005). However, despite the success documented with the aquatic plants in metal accumulation, their potentials in rhizofiltration are restricted for their slow-growing roots and low biomass production. In an attempt to bridge the limitations, researchers proposed terrestrial plants as more suitable candidates for rhizofiltration because of their robust fibrous root system with a large surface area.

The root of sunflower (*Helianthus annuus*), was found to reduce the level of Cr, Mn, Cd, Ni, and Cu in water within 24 hours (Dushenkov *et al*., 1997). A similar result was obtained with Uranium, Sr, Pb, and Zn (Salt et al. 1995). Rascio and Navari-Izzo (2011) reported that, despite the research demonstrating the possible application of hyper-accumulator plants for the remediation of heavy metals, there is a limit to their potential in phytoremediation. Because most of them tend to be metal selective and have not been established for all metals of interest. The proposed use of trees could offer a solution to the problems mentioned above because they meet some of the requirements limiting the phyto-extraction ability of plants due to their extensive deep root system and high biomass (Pulford and Watson, 2003).

The use of trees species such as poplar willow, sycamore, birch, and alder growing on contaminated land is gaining attention. Interest in these studies focused on metal uptake, distribution and tolerance mechanism. In recent years, significant consideration has been giving to *Salix* spp., more than Ten known species are potential phyto-remediators with varying abilities (Pulford and Watson, 2003). Pulford and Watson (2003) reported *Salix viminalis* to have the highest metal accumulating ability: under field trial, Cadmium attained a transfer factor of 3.4 on contaminated soil. Another willow *Salix acmophylla* was tested for hyper-accumulation of Pb, Ni, and Cu (Ali *et al.,* 2003).

2.1.4 Detoxification and Transformation

Detoxification of heavy metals once it accumulates within the plant cell is a unique characteristic of some plants. For heavy metals detoxification in plants, the metals must be distributed to apoplast tissues like trichomes and cell walls (Gosh and Singh, 2005). Yang *et al.* (2005) reported the ability of the cell wall to play a role in detoxification of Ni, Zn and Cd in hyper-accumulation of plants, though mechanisms of detoxification by cell wall are still not well understood. Some experiments reported that phytochelatins (PC) in the root of *B. juncea* can be produced when exposed to Pb; this suggests that the PCs may be used in Pb detoxification. Cadmium detoxification in the vacuole was also achieved by the association with PCs (Salt *et al*., 1995). Moreover, Meagher (2000) reported the transformation of certain toxic elements like selenium, arsenic, chromium and mercury into a less toxic form as a promising phase in phytoremediation.

2.2. Metal Volatilization

A remediation technology alternative to phyto-extraction is phyto-volatilization in which the metal contaminants are not only accumulated in the above-ground biomass, but the accrued metal or metalloid species in the environment are biologically transformed into less toxic gaseous within the plants and subsequently released into air in a process known as phyto-volatilization (Kortab *et al.,* 2009; Kramer, 2005). Metal contaminants reported being volatilized includes As, Hg and Se with much attention paid to selenium volatilisation. Hg and Se are very toxic and there is great concern about the safety of volatilized forms of these elements into the atmosphere (Prasad and Priestas, 2003). However, an assessment study regarding the safety of volatilisation of mercury and selenium shows that the advantage of dispersion to air supersedes the potential risk perceived. This is because elemental Hg in the atmosphere poses less risk than other forms of Hg in the soil (Kortab *et al*., 2009).

As reported by Prasad and Priestas (2003), some Brassica genus and other wetland plants have the natural ability to volatilize up to 40g Se per hectare per day into air. Toxicity of Volatile Se compounds such as dimethyl selenide was reported to be 1/600 to 1/500 as toxic as inorganic forms of Se found in the soil (DeSouza *et al*., 2000). The insertion of some genes promotes the absorption of elemental Hg and methyl mercury (*MeHg*) from the soil and release volatile, non-toxic Hg (O) into the atmosphere (He *et al*., 2001; Ruiz *et al.,* 2003; Prasad, 2004). Heaton *et al*. (1998) also suggest that the release of volatile Hg (O) into the air could be insignificant to the atmospheric pool.

2.3. Metal Stabilisation

Traditionally, toxicity of metals is reduced from the contaminated site through an in-place activation, which involves the use of soil amendment to mobilise metals in the soil matrix. Phyto-stabilization is a modified version of in-place activation which employs the use of plants to immobilise contaminants through the sorption, precipitation, complexation or metal balance reduction in the root rhizosphere. Contrary to other phytoremediation techniques, phyto-stabilisation techniques are aimed at stabilising metal at a site to reduce the risk of exposure to human health and the environment (Prasad and Preista, 2003).

Various features needed for effective plant selection for phyto-stabilisation include fast growth, dense canopies and root systems and should also be easier to establish. They should also be able to tolerate a high level of metal in the soil. Therefore, for phytostabilization of contaminated soils, three different cultivars from different grasses: two *Arostis tenius* and one *Festuca rubra* are reported for Pb, Zn and Cu (Salt *et al*., 1995; Prasad and Preista, 2003).

In an experiment to reduce leaching of metal from contaminated soil into water, three week old *B. juncea* seedling growing in a sand-Perlite mixture containing 625ug/g Pb was reported to significantly reduce Pb level in the leachate from 740ug/mL, in the absence of plant to 22ug/mL in the presence of plant (Salt *et al*., 1995).

2.4. Enhanced Phytoremediation Techniques

2.4.1 Genetic Engineering

Progress has been made in the field of genetic engineering to enhance the ability of plants through modification of various plant functions for phytoremediation. However, biotechnological techniques have been reported to be useful in developing plants with better characteristics of phytoremediation such as ability to accumulate multiple metals, tolerance to detoxification and transformation (McIntyre, 2003; Pandey and Bajpai 2019). Advances through selective breeding and direct insertion of a metal hyper-accumulator gene into high biomass species have been reported by many researchers (Clemens *et al.,* 2002; Rascio and Navari-Izzo, 2011). Most hyper-accumulator plants are disadvantaged by their slow growth and small biomass which affect their potential. The breeding practice was proposed to improve the plant capability of metal extraction, but the success of breeding was limited due to sexual incompatibility between the parents (Yang *et al*., 2005).

Ruiz *et al.* (2003) reported the transformation of both *merA* and *merB* genes into the chloroplast genome of tobacco. Introduction of PCs and MTs into transgenic plants for enhanced metal tolerance, accumulation and distribution were also reported (Mejare and Malin, 2001). Suresh and Ravishanker (2004) stated that overexpression of genes involved in PC synthesis encoding y-gluthamylcystein synthase (*gsh1*), glutathione synthase (*gsh2*) and PC synthase (*pcs*) increased Cd tolerance in various heterologous expression systems effectively.

Field trials using transgenic *Brassica juncea* (Indian mustard) have shown the overexpressing genes involved in sulphur (S)/Selenium (Se) metabolism to enhanced Se accumulation and tolerance (Baneulos *et al*., 2007). Accumulation and volatilization using engineered selenocysteine methyltransferase (SMT) gene from Se hyper-accumulator *A. bisulcatus* to Se non-tolerance *B. juncea* shows that SMT of transgenic plant growing in contaminated soil accumulated 65% more Se than the wild type (Van *et al*., 2004; Kortab *et al*., 2009; Zhao *et al*., 2009).

Furthermore, studies on the complex plants' association with microorganisms within the root rhizosphere could allow for the genetic modification of plants and their microbial symbionts to increase mobilisation of the metal species of concern. An example is shown in the engineering of *M. haukui* with phytochelatin synthase *AtPCS1* and metallothionein *MTL4* gene colonising nodules of *Adiantum sinicum* which resulted in an increase mobilisation and Cd2+ accumulation in plants tissues (Ike *et al*., 2007).

2.4.2. Biostimulation and Bioaugmentation

Another promising approach to enhance phytoremediation is the possibility of manipulations of soil settings to improve metal availability, hence increasing plant uptake, or stabilising the metals in the soil, and so decrease plant uptake. Chelating agents, which are molecules that could form bonds with metal ions, are used, such as EDTA (ethylenediaminetetraacetic acid). When applied to Pb contaminated soil, EDTA increases the amount of bioavailable lead in the soil and enhances accumulation in plants (Gosh and Singh, 2005). Addition of chelates to lead-contaminated soils increases accumulation and absorptions of Pb in *Zea mays* and *Pisum sativun* tissues increased dramatically from less than 500mg kg^{-1} to more than 10,000mg kg^{-1}. Ethylene diamine tetraacetic acid was reported as the best active chelate for increased lead availability (Huang *et al.,* 1997; Gosh and Singh, 2005).

The concept of bio-stimulation involved the addition of either microbial products, such as bio-surfactants or enzymes directly to the substrate as an amendment and they could be introduced alone or in combination with microbial inoculants. The addition of nutrients enrichment is called fertilisation; a bioremediation approach like phosphorus and nitrogen that are applied to plants in farms is added to the contaminated environment to stimulate the growth of local populations of microorganisms that can degrade pollutants (Thieman and Palladino, 2009).

The studies of the interaction between plants and microorganisms and their role in the decontamination process are an important advance in phytoremediation. The root microbial association can increase plant performance, nutrient absorption and soil quality. Some bacteria can enhance plant growth, metal and P uptake (Kavamura and Esposito, 2010; Ullah et al., 2015).

Bio-augmentation on another hand involves the introduction of microorganisms (including genetically modified) that possessed biodegradation potential into the contaminated environment to support the native microbes with bio-degradative processes. Some microorganisms in the plant rhizosphere can tolerate and detoxify heavy metals; some can produce chelating agent which may complex lead, gold and uranium in plants thereby increasing metal availability and translocation to the shoot (Kavamura and Esposito, 2010). In a study involving arbuscular mycorrhizal fungi, Khan (2005) reported the effect of chitosan and fungus on copper, zinc and lead accumulation by *Elsholtzia splendens* growing in soil contaminated with copper smelt factory ash. Mycorrhizal plants showed an increased metal uptake with no sign of toxicity.

Despite the promising advances documented by many researchers, the potential applications of bio-augmentation and bio-stimulation are yet to gain public acceptance. Particularly the concern about using genetically engineered microbes due to the belief that these organisms, when introduced into contaminated soil, may affect the natural ecology of the environment and could probably pose a risk to the environmental health if they persist after the remediation of the contaminated soil (Peter, 2011). There is also concern about the mobilisation of metals because they can easily percolate into the groundwater as a result of EDTA addition to the soil (Cooper *et al*., 1999).

3.0. Future Development

Much progress in the application of phytoremediation technologies for the remediation of a heavy metal contaminated sites has been made in recent years with success in the scientific research gaining public acceptance because the techniques are cost-effectiveness, easy to maintain and environmentally friendly. However, slow growth rate of the plants, low biomass production and the rate at which plants concentrate and accumulate heavy metals and the way biomass is harvested and disposed of need to be improved for the technology to remain the best available option for remediation of heavy metals are still among other limitations of the current phytoremediation techniques (Sureh*, et al.,* 2004; Ghosh and Singh, 2005; Peter, 2011).

3.1. Genetic Engineering to Enhance Phytoremediation

Several biotechnological advancements have been made in the field of phytoremediation through maximising plants ability to phytoremediate specific contaminants. Jiang *et al.* (2010) proposed that future research in phytoremediation should focus on studying different plant flora to identify species that can characteristically accumulate more than one type of metal at high concentrations than the traditional plant species used in phytoremediation. Identifying novel genes and their transfer from natural hyper-accumulators and into fast-growing metal hyper-accumulators could provide openings for enhancing phytoremediation effectiveness (Saxena *et al*., 2019).

The use of modern genetic approaches can also enhance the understanding of the biochemical processes involved in plant heavy metal uptake, transport, accumulation, resistance and tolerance through engineering the present plant species used in phytoremediation to find and choose the best plants that could possibly survive well in metal-stressed soils (Jiang *et al.,* 2010; Pandey and Bajpai 2019). Applications of genetically engineered plants for the enhanced metal phytoremediation in the laboratory have been reviewed (Goel *et al*., 2009; Kotrba *et al*., 2009; Vangronsveld *et al*., 2009; Chandra *et al*., 2015).

All the advances in genetic engineering are primarily investigated to enhance plant ability for the phytoremediation of heavy metals. However, most of the advances made with genetically modified plants are still in a laboratory test. Therefore, the future waits to see the full field applicability of those genetically engineered plant for phytoremediation of heavy metals.

3.2. Phyto-mining

Phytoremediation can potentially be applied in the extraction and recovery of metals for commercial value. Researches have demonstrated that metals can be mined using plants. The technique of extracting and recovering marketable metals using plant is called phytomining (Gardea-Torresdey *et al.,* 2005; Pandey and Bajpai 2019). Reports from the researchers showed that some plants could uptake gold and silver at high concentration (van der Ent *et al*., 2015). Although up till now, a successful application of proper commercial phyto-mining has not been documented (Rascio and Navari-Izzo, 2011; van der Ent *et al*., 2015). Gardea-Torresdey *et al.* (2005) revealed the capacity of alfalfa plant cultured in Agar-based media containing KAuCL4 or $AgNO_3$ was able to accumulate more than 370mg kg of Au and 120mg kg of Ag in their aerial part. A yield of 105 kg Ni ha^{-1} was achieved in Albania using *A. murale* (Bani *et al*., 2015). Previously, a pilot study carried out on a small area in California using *Streptanthus polygaloides* yielded 100 kg Ni ha^{-1} (Brooks *et al*., 1998). *A. bertolonii* yielded 72 kg Ni ha^{-1} in Italy (Robinson *et al*., 1997) and *Berkheya coddii* yeilded100 kg Ni ha^{-1} in South Africa (Robinson et al., 1997). In future, Phyto-mining may become a component of significant and incorporated revenue flow for Phyto-miners worth more than most food crops (van der Ent *et al*., 2015). However, Rascio and Navari-Izzo (2011) proposed the need to investigate strategies for metal recovery, to provide sufficient information on the pattern and the distribution of the hyper-accumulating species and to maximise their metal uptake ability to properly utilised plant species in the process of Phytomining (Jiang *et al*., 2015). The future establishment of commercial extraction of heavy metals using plants will subsequently reduce the cost of phytoremediation.

4.0. Conclusion

Pollution of the environment by heavy metals has been a major environmental problem needing urgent attention, as such different remediation technologies have been used to address the problem among which phytoremediation is included. Phytoremediation is a growing field of interest because it can be applied to remediate a variety of contaminants in various substrates. The method is cheaper and reduces the rate of damages to the environment.

Among the different phytoremediation techniques, attention was paid to phytoextraction and rhizofiltration, and the potential of some terrestrial and aquatic plants to accumulate a high number of contaminants in their biomass. *Thlaspi caerulescens, Brassica juncea and Helianthus annus,* are among the widely researched plant for phytoextraction, while several aquatic plants are also found for rhizofiltration such *as Azolla filliculoides, A. pinnata, Typha orientalis and Salvinia molesta*. Phytostabilzation and phytovolatilization have also been used effectively for metal removal. Moreover, the application of genetic engineering to enhance plants and some microbe's ability for phytoremediation showed a promising trend, though there is concern about the use of those genetically engineered organisms.

Finally, the possible re-use of plant biomass for a commercial metal extraction should be re-examined, as the research in this area could extensively lead to the reduction in the total cost of the phytoremediation process.

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