

Review Article

Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods

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Soils polluted with heavy metals have become common across the globe due to increase in geologic and anthropogenic activities. Plants growing on these soils show a reduction in growth, performance, and yield. Bioremediation is an effective method of treating heavy metal polluted soils. It is a widely accepted method that is mostly carried out *in situ*; hence it is suitable for the establishment/reestablishment of crops on treated soils. Microorganisms and plants employ different mechanisms for the bioremediation of polluted soils. Using plants for the treatment of polluted soils is a more common approach in the bioremediation of heavy metal polluted soils. Combining both microorganisms and plants is an approach to bioremediation that ensures a more efficient clean-up of heavy metal polluted soils. However, success of this approach largely depends on the species of organisms involved in the process.

1. Introduction

Although heavy metals are naturally present in the soil, geologic and anthropogenic activities increase the concentration of these elements to amounts that are harmful to both plants and animals. Some of these activities include mining and smelting of metals, burning of fossil fuels, use of fertilizers and pesticides in agriculture, production of batteries and other metal products in industries, sewage sludge, and municipal waste disposal [1–3].

Growth reduction as a result of changes in physiological and biochemical processes in plants growing on heavy metal polluted soils has been recorded [4–6]. Continued decline in plant growth reduces yield which eventually leads to food insecurity. Therefore, the remediation of heavy metal polluted soils cannot be overemphasized.

Various methods of remediating metal polluted soils exist; they range from physical and chemical methods to biological methods. Most physical and chemical methods (such as encapsulation, solidification, stabilization, electrokinetics, vitrification, vapour extraction, and soil washing and flushing) are expensive and do not make the soil suitable for plant growth [7]. Biological approach (bioremediation) on

the other hand encourages the establishment/reestablishment of plants on polluted soils. It is an environmentally friendly approach because it is achieved via natural processes. Bioremediation is also an economical remediation technique compared with other remediation techniques. This paper discusses the nature and properties of soils polluted with heavy metals. Plant growth and performance on these soils were examined. Biological approaches employed for the remediation of heavy metal polluted soils were equally highlighted.

2. Heavy Metal Polluted Soils

Heavy metals are elements that exhibit metallic properties such as ductility, malleability, conductivity, cation stability, and ligand specificity. They are characterized by relatively high density and high relative atomic weight with an atomic number greater than 20 [2]. Some heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are required in minute quantities by organisms. However, excessive amounts of these elements can become harmful to organisms. Other heavy metals such as Pb, Cd, Hg, and As (a metalloid but generally

referred to as a heavy metal) do not have any beneficial effect on organisms and are thus regarded as the “main threats” since they are very harmful to both plants and animals.

Metals exist either as separate entities or in combination with other soil components. These components may include exchangeable ions sorbed on the surfaces of inorganic solids, nonexchangeable ions and insoluble inorganic metal compounds such as carbonates and phosphates, soluble metal compound or free metal ions in the soil solution, metal complex of organic materials, and metals attached to silicate minerals [7]. Metals bound to silicate minerals represent the background soil metal concentration and they do not cause contamination/pollution problems compared with metals that exist as separate entities or those present in high concentration in the other 4 components [8].

Soil properties affect metal availability in diverse ways. Harter [9] reported that soil pH is the major factor affecting metal availability in soil. Availability of Cd and Zn to the roots of *Thlaspi caerulescens* decreased with increases in soil pH [10]. Organic matter and hydrous ferric oxide have been shown to decrease heavy metal availability through immobilization of these metals [11]. Significant positive correlations have also been recorded between heavy metals and some soil physical properties such as moisture content and water holding capacity [12].

Other factors that affect the metal availability in soil include the density and type of charge in soil colloids, the degree of complexation with ligands, and the soil's relative surface area [7, 13]. The large interface and specific surface areas provided by soil colloids help in controlling the concentration of heavy metals in natural soils. In addition, soluble concentrations of metals in polluted soils may be reduced by soil particles with high specific surface area, though this may be metal specific [7]. For instance, McBride and Martinez [14] reported that addition of amendment consisting of hydroxides with high reactive surface area decreased the solubility of As, Cd, Cu, Mo, and Pb while the solubility of Ni and Zn was not changed. Soil aeration, microbial activity, and mineral composition have also been shown to influence heavy metal availability in soils [15].

Conversely, heavy metals may modify soil properties especially soil biological properties [16]. Monitoring changes in soil microbiological and biochemical properties after contamination can be used to evaluate the intensity of soil pollution because these methods are more sensitive and results can be obtained at a faster rate compared with monitoring soil physical and chemical properties [17]. Heavy metals affect the number, diversity, and activities of soil microorganisms. The toxicity of these metals on microorganisms depends on a number of factors such as soil temperature, pH, clay minerals, organic matter, inorganic anions and cations, and chemical forms of the metal [16, 18, 19].

There are discrepancies in studies comparing the effect of heavy metals on soil biological properties. While some researchers have recorded negative effect of heavy metals on soil biological properties [16, 17, 20], others have reported no relationship between high heavy metal concentrations and some soil (micro)biological properties [21]. Some of the inconsistencies may arise because some of these studies

were conducted under laboratory conditions using artificially contaminated soils while others were carried out using soils from areas that are actually polluted in the field. Regardless of the origin of the soils used in these experiments, the fact that the effect of heavy metals on soil biological properties needs to be studied in more detail in order to fully understand the effect of these metals on the soil ecosystem remains. Further, it is advisable to use a wide range of methods (such as microbial biomass, C and N mineralization, respiration, and enzymatic activities) when studying effect of metals on soil biological properties rather than focusing on a single method since results obtained from use of different methods would be more comprehensive and conclusive.

The presence of one heavy metal may affect the availability of another in the soil and hence plant. In other words, antagonistic and synergistic behaviours exist among heavy metals. Salgare and Acharekar [22] reported that the inhibitory effect of Mn on the total amount of mineralized C was antagonized by the presence of Cd. Similarly, Cu and Zn as well as Ni and Cd have been reported to compete for the same membrane carriers in plants [23]. In contrast, Cu was reported to increase the toxicity of Zn in spring barley [24]. This implies that the interrelationship between heavy metals is quite complex; thus more research is needed in this area. Different species of the same metal may also interact with one another. Abedin et al. [25] reported that the presence of arsenite strongly suppressed the uptake of arsenate by rice plants growing on a polluted soil.

3. Effect of Heavy Metal Polluted Soil on Plant Growth

The heavy metals that are available for plant uptake are those that are present as soluble components in the soil solution or those that are easily solubilized by root exudates [26]. Although plants require certain heavy metals for their growth and upkeep, excessive amounts of these metals can become toxic to plants. The ability of plants to accumulate essential metals equally enables them to acquire other nonessential metals [27]. As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly.

Some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress [28, 29]. An example of indirect toxic effect is the replacement of essential nutrients at cation exchange sites of plants [30]. Further, the negative influence heavy metals have on the growth and activities of soil microorganisms may also indirectly affect the growth of plants. For instance, a reduction in the number of beneficial soil microorganisms due to high metal concentration may lead to decrease in organic matter decomposition leading to a decline in soil nutrients. Enzyme activities useful for plant metabolism may also be hampered due to heavy metal interference with activities of soil microorganisms. These toxic effects (both direct and indirect) lead to a decline in plant growth which sometimes results in the death of plant [31].

The effect of heavy metal toxicity on the growth of plants varies according to the particular heavy metal involved in the process. Table 1 shows a summary of the toxic effects of specific metals on growth, biochemistry, and physiology of various plants. For metals such as Pb, Cd, Hg, and As which do not play any beneficial role in plant growth, adverse effects have been recorded at very low concentrations of these metals in the growth medium. Kibra [32] recorded significant reduction in height of rice plants growing on a soil contaminated with 1 mgHg/kg. Reduced tiller and panicle formation also occurred at this concentration of Hg in the soil. For Cd, reduction in shoot and root growth in wheat plants occurred when Cd in the soil solution was as low as 5 mg/L [33]. Most of the reduction in growth parameters of plants growing on polluted soils can be attributed to reduced photosynthetic activities, plant mineral nutrition, and reduced activity of some enzymes [34].

For other metals which are beneficial to plants, "small" concentrations of these metals in the soil could actually improve plant growth and development. However, at higher concentrations of these metals, reductions in plant growth have been recorded. For instance, Jayakumar et al. [42] reported that, at 50 mgCo/kg, there was an increase in nutrient content of tomato plants compared with the control. Conversely, at 100 mgCo/kg to 250 mgCo/kg, reductions in plant nutrient content were recorded. Similarly, increase in plant growth, nutrient content, biochemical content, and antioxidant enzyme activities (catalase) was observed in radish and mung bean at 50 mgCo/kg soil concentration while reductions were recorded at 100 mgCo/kg to 250 mgCo/kg soil concentration [43, 44]. Improvements in growth and physiology of cluster beans have also been reported at Zn concentration of 25 mg/L of the soil solution. On the other hand, growth reduction and adverse effect on the plant's physiology started when the soil solution contained 50 mgZn/L [67].

It is worth mentioning that, in most real life situations (such as disposal of sewage sludge and metal mining wastes) where soil may be polluted with more than one heavy metal, both antagonistic and synergistic relationships between heavy metals may affect plant metal toxicity. Nicholls and Mal [70] reported that the combination of Pb and Cu at both high concentration (1000 mg/kg each) and low concentration (500 mg/kg) resulted in a rapid and complete death of the leaves and stem of *Lythrum salicaria*. The authors reported that there was no synergistic interaction between these heavy metals probably because the concentrations used in the experiment were too high for interactive relationship to be observed between the metals. Another study [71] examined the effect of 6 heavy metals (Cd, Cr, Co, Mn, and Pb) on the growth of maize. The result showed that the presence of these metals in soil reduced the growth and protein content of maize. The toxicity of these metals occurred in the following order: Cd > Co > Hg > Mn > Pb > Cr. It was also observed in this study that the combined effect of 2 or more heavy metals was only as harmful as the effect of the most toxic heavy metal. The researcher attributed this result to the antagonistic relationship which exists between heavy metals.

It is important to note that certain plants are able to tolerate high concentration of heavy metals in their environment. Baker [72] reported that these plants are able to tolerate these metals via 3 mechanisms, namely, (i) exclusion: restriction of metal transport and maintenance of a constant metal concentration in the shoot over a wide range of soil concentrations; (ii) inclusion: metal concentrations in the shoot reflecting those in the soil solution through a linear relationship; and (iii) bioaccumulation: accumulation of metals in the shoot and roots of plants at both low and high soil concentrations.

4. Bioremediation of Heavy Metal Polluted Soils

Bioremediation is the use of organisms (microorganisms and/or plants) for the treatment of polluted soils. It is a widely accepted method of soil remediation because it is perceived to occur via natural processes. It is equally a cost effective method of soil remediation. Blaylock et al. [73] reported 50% to 65% saving when bioremediation was used for the treatment of 1 acre of Pb polluted soil compared with the case when a conventional method (excavation and landfill) was used for the same purpose. Although bioremediation is a nondisruptive method of soil remediation, it is usually time consuming and its use for the treatment of heavy metal polluted soils is sometimes affected by the climatic and geological conditions of the site to be remediated [74].

Heavy metals cannot be degraded during bioremediation but can only be transformed from one organic complex or oxidation state to another. Due to a change in their oxidation state, heavy metals can be transformed to become either less toxic, easily volatilized, more water soluble (and thus can be removed through leaching), less water soluble (which allows them to precipitate and become easily removed from the environment) or less bioavailable [75, 76].

Bioremediation of heavy metals can be achieved via the use of microorganisms, plants, or the combination of both organisms.

4.1. Using Microbes for Remediation of Heavy Metal Polluted Soils. Several microorganisms especially bacteria (*Bacillus subtilis*, *Pseudomonas putida*, and *Enterobacter cloacae*) have been successfully used for the reduction of Cr (VI) to the less toxic Cr (III) [77–80]. *B. subtilis* has also been reported to reduce nonmetallic elements. For instance, Garbisu et al. [81] recorded that *B. subtilis* reduced the selenite to the less toxic elemental Se. Further, *B. cereus* and *B. thuringiensis* have been shown to increase extraction of Cd and Zn from Cd-rich soil and soil polluted with effluent from metal industry [82]. It is assumed that the production of siderophore (Fe complexing molecules) by bacteria may have facilitated the extraction of these metals from the soil; this is because heavy metals have been reported to simulate the production of siderophore and this consequently affects their bioavailability [83]. For instance, siderophore production by *Azotobacter vinelandii* was increased in the presence of Zn (II) [84].

TABLE 1: Effect of heavy metal toxicity on plants.

Heavy metal	Plant	Toxic effect on plant	Reference
As	Rice (<i>Oryza sativa</i>)	Reduction in seed germination; decrease in seedling height; reduced leaf area and dry matter production	[35, 36]
	Tomato (<i>Lycopersicon esculentum</i>)	Reduced fruit yield; decrease in leaf fresh weight	[37]
	Canola (<i>Brassica napus</i>)	Stunted growth; chlorosis; wilting	[38]
Cd	Wheat (<i>Triticum</i> sp.)	Reduction in seed germination; decrease in plant nutrient content; reduced shoot and root length	[33, 39]
	Garlic (<i>Allium sativum</i>)	Reduced shoot growth; Cd accumulation	[40]
	Maize (<i>Zea mays</i>)	Reduced shoot growth; inhibition of root growth	[41]
Co	Tomato (<i>Lycopersicon esculentum</i>)	Reduction in plant nutrient content	[42]
	Mung bean (<i>Vigna radiata</i>)	Reduction in antioxidant enzyme activities; decrease in plant sugar, starch, amino acids, and protein content	[43]
	Radish (<i>Raphanus sativus</i>)	Reduction in shoot length, root length, and total leaf area; decrease in chlorophyll content; reduction in plant nutrient content and antioxidant enzyme activity; decrease in plant sugar, amino acid, and protein content	[44]
Cr	Wheat (<i>Triticum</i> sp.)	Reduced shoot and root growth	[45, 46]
	Tomato (<i>Lycopersicon esculentum</i>)	Decrease in plant nutrient acquisition	[47, 48]
	Onion (<i>Allium cepa</i>)	Inhibition of germination process; reduction of plant biomass	[49]
Cu	Bean (<i>Phaseolus vulgaris</i>)	Accumulation of Cu in plant roots; root malformation and reduction	[50]
	Black bindweed (<i>Polygonum convolvulus</i>)	Plant mortality; reduced biomass and seed production	[51]
	Rhodes grass (<i>Chloris gayana</i>)	Root growth reduction	[52]
Hg	Rice (<i>Oryza sativa</i>)	Decrease in plant height; reduced tiller and panicle formation; yield reduction; bioaccumulation in shoot and root of seedlings	[32, 53]
	Tomato (<i>Lycopersicon esculentum</i>)	Reduction in germination percentage; reduced plant height; reduction in flowering and fruit weight; chlorosis	[54]
Mn	Broad bean (<i>Vicia faba</i>)	Mn accumulation shoot and root; reduction in shoot and root length; chlorosis	[55]
	Spearmint (<i>Mentha spicata</i>)	Decrease in chlorophyll a and carotenoid content; accumulation of Mn in plant roots	[56]
	Pea (<i>Pisum sativum</i>)	Reduction in chlorophylls a and b content; reduction in relative growth rate; reduced photosynthetic O ₂ evolution activity and photosystem II activity	[57]
	Tomato (<i>Lycopersicon esculentum</i>)	Slower plant growth; decrease in chlorophyll concentration	[58]
Ni	Pigeon pea (<i>Cajanus cajan</i>)	Decrease in chlorophyll content and stomatal conductance; decreased enzyme activity which affected Calvin cycle and CO ₂ fixation	[59]
	Rye grass (<i>Lolium perenne</i>)	Reduction in plant nutrient acquisition; decrease in shoot yield; chlorosis	[60]
	Wheat (<i>Triticum</i> sp.)	Reduction in plant nutrient acquisition	[61, 62]
	Rice (<i>Oryza sativa</i>)	Inhibition of root growth	[63]

TABLE 1: Continued.

Heavy metal	Plant	Toxic effect on plant	Reference
Pb	Maize (<i>Zea mays</i>)	Reduction in germination percentage; suppressed growth; reduced plant biomass; decrease in plant protein content	[64]
	Portia tree (<i>Thespesia populnea</i>)	Reduction in number of leaves and leaf area; reduced plant height; decrease in plant biomass	[65]
	Oat (<i>Avena sativa</i>)	Inhibition of enzyme activity which affected CO ₂ fixation	[66]
Zn	Cluster bean (<i>Cyamopsis tetragonoloba</i>)	Reduction in germination percentage; reduced plant height and biomass; decrease in chlorophyll, carotenoid, sugar, starch, and amino acid content	[67]
	Pea (<i>Pisum sativum</i>)	Reduction in chlorophyll content; alteration in structure of chloroplast; reduction in photosystem II activity; reduced plant growth	[68]
	Rye grass (<i>Lolium perenne</i>)	Accumulation of Zn in plant leaves; growth reduction; decrease in plant nutrient content; reduced efficiency of photosynthetic energy conversion	[69]

Hence, heavy metals influence the activities of siderophore-producing bacteria which in turn increases mobility and extraction of these metals in soil.

Bioremediation can also occur indirectly via bioprecipitation by sulphate reducing bacteria (*Desulfovibrio desulfuricans*) which converts sulphate to hydrogen sulphate which subsequently reacts with heavy metals such as Cd and Zn to form insoluble forms of these metal sulphides [85].

Most of the above microbe assisted remediation is carried out *ex situ*. However, a very important *in situ* microbe assisted remediation is the microbial reduction of soluble mercuric ions Hg (II) to volatile metallic mercury and Hg (0) carried out by mercury resistant bacteria [86]. The reduced Hg (0) can easily volatilize out of the environment and subsequently be diluted in the atmosphere [87].

Genetic engineering can be adopted in microbe assisted remediation of heavy metal polluted soils. For instance, Valls et al. [88] reported that genetically engineered *Ralstonia eutropha* can be used to sequester metals (such as Cd) in polluted soils. This is made possible by the introduction of metallothionein (cysteine rich metal binding protein) from mouse on the cell surface on this organism. Although the sequestered metals remain in the soil, they are made less bioavailable and hence less harmful. The controversies surrounding genetically modified organisms [89] and the fact that the heavy metal remains in the soil are major limitations to this approach to bioremediation.

Making the soil favourable for soil microbes is one strategy employed in bioremediation of polluted soils. This process known as biostimulation involves the addition of nutrients in the form of manure or other organic amendments which serve as C source for microorganisms present in the soil. The added nutrients increase the growth and activities of microorganisms involved in the remediation process and thus this increases the efficiency of bioremediation.

Although biostimulation is usually employed for the biodegradation of organic pollutants [90], it can equally be used for the remediation of heavy metal polluted soils.

Since heavy metals cannot be biodegraded, biostimulation can indirectly enhance remediation of heavy metal polluted soil through alteration of soil pH. It is well known that the addition of organic materials reduces the pH of the soil [91]; this subsequently increases the solubility and hence bioavailability of heavy metals which can then be easily extracted from the soil [92].

Biochar is one organic material that is currently being exploited for its potential in the management of heavy metal polluted soils. Namgay et al. [93] recorded a reduction in the availability of heavy metals when the polluted soil was amended with biochar; this in turn reduced plant absorption of the metals. The ability of biochar to increase soil pH unlike most other organic amendments [94] may have increased sorption of these metals, thus reducing their bioavailability for plant uptake. It is important to note that, since the characteristics of biochar vary widely depending on its method of production and the feedstock used in its production, the effect different biochar amendments will have on the availability of heavy metals in soil will also differ. Further, more research is needed in order to understand the effect of biochar on soil microorganisms and how the interaction between biochar and soil microbes influences remediation of heavy metal polluted soils because such studies are rare in literature.

4.2. Using Plants for Remediation of Heavy Metal Polluted Soils. Phytoremediation is an aspect of bioremediation that uses plants for the treatment of polluted soils. It is suitable when the pollutants cover a wide area and when they are within the root zone of the plant [76]. Phytoremediation of heavy metal polluted soils can be achieved via different mechanisms. These mechanisms include phytoextraction, phytostabilization, and phytovolatilization.

4.2.1. Phytoextraction. This is the most common form of phytoremediation. It involves accumulation of heavy metals

in the roots and shoots of phytoremediation plants. These plants are later harvested and incinerated. Plants used for phytoextraction usually possess the following characteristics: rapid growth rate, high biomass, extensive root system, and ability to tolerate high amounts of heavy metals. This ability to tolerate high concentration of heavy metals by these plants may lead to metal accumulation in the harvestable part; this may be problematic through contamination of the food chain [7].

There are two approaches to phytoextraction depending on the characteristics of the plants involved in the process. The first approach involves the use of natural hyperaccumulators, that is, plants with very high metal-accumulating ability, while the second approach involves the use of high biomass plants whose ability to accumulate metals is induced by the use of chelates, that is, soil amendments with metal mobilizing capacity [95].

Hyperaccumulators accumulate 10 to 500 times more metals than ordinary plant [96]; hence they are very suitable for phytoremediation. An important characteristic which makes hyperaccumulation possible is the tolerance of these plants to increasing concentrations of these metals (hypertolerance). This could be a result of exclusion of these metals from the plants or by compartmentalization of these metal ions; that is, the metals are retained in the vacuolar compartments or cell walls and thus do not have access to cellular sites where vital functions such as respiration and cell division take place [76, 96].

Generally, a plant can be called a hyperaccumulator if it meets the following criteria: (i) the concentration of metal in the shoot must be higher than 0.1% for Al, As, Co, Cr, Cu, Ni, and Se, higher than 0.01% for Cd, and higher than 1.0% for Zn [97]; (ii) the ratio of shoot to root concentration must be consistently higher than 1 [98]; this indicates the capability to transport metals from roots to shoot and the existence of hypertolerance ability [7]; (iii) the ratio of shoot to root concentration must be higher than 1; this indicates the degree of plant metal uptake [7, 98]. Reeves and Baker [99] reported some examples of plants which have the ability to accumulate large amounts of heavy metals and hence can be used in remediation studies. Some of these plants include *Haumaniastrum robertii* (Co hyperaccumulator); *Aeollanthus subcaulis* (Cu hyperaccumulator); *Maytenus bureaviana* (Mn hyperaccumulator); *Minuartia verna* and *Agrostis tenuis* (Pb hyperaccumulators); *Dichapetalum gelonioides*, *Thlaspi tatrense*, and *Thlaspi caerulescens* (Zn hyperaccumulators); *Psycotria vanhermanni* and *Streptanthus polygaloides* (Ni hyperaccumulators); *Lecythis ollaria* (Se hyperaccumulator). *Pteris vittata* is an example of a hyperaccumulator that can be used for the remediation of soils polluted with As [100]. Some plants have the ability to accumulate more than one metal. For instance, Yang et al. [101] observed that the Zn hyperaccumulator, *Sedum alfredii*, can equally hyperaccumulate Cd.

The possibility of contaminating the food chain through the use of hyperaccumulators is a major limitation in phytoextraction. However, many species of the Brassicaceae family which are known to be hyperaccumulators of heavy metals contain high amounts of thiocyanates which make

them unpalatable to animals; thus this reduces the availability of these metals in the food chain [102].

Most hyperaccumulators are generally slow growers with low plant biomass; this reduces the efficiency of the remediation process [103]. Thus, in order to increase the efficiency of phytoextraction, plants with high growth rate as well as high biomass (e.g., maize, sorghum, and alfalfa) are sometimes used together with metal chelating substances for soil remediation exercise. It is important to note that some hyperaccumulators such as certain species within the *Brassica* genus (*Brassica napus*, *Brassica juncea*, and *Brassica rapa*) are fast growers with high biomass [104].

In most cases, plants absorb metals that are readily available in the soil solution. Although some metals are present in soluble forms for plant uptake, others occur as insoluble precipitate and are thus unavailable for plant uptake. Addition of chelating substances prevents precipitation and metal sorption via the formation of metal chelate complexes; this subsequently increases the bioavailability of these metals [7]. Further, the addition of chelates to the soil can transport more metals into the soil solution through the dissolution of precipitated compounds and desorption of sorbed species [13]. Certain chelates are also able to translocate heavy metal into the shoots of plants [73].

Marques et al. [7] documented examples of synthetic chelates which have successfully been used to extract heavy metals from polluted soils. Some of these chelates include EDTA (ethylenediaminetetraacetic acid), EDDS (SS-ethylenediamine disuccinic acid), CDTA (*trans*-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid), EDDHA (ethylenediamine-di-*o*-hydroxyphenylacetic acid), DTPA (diethylenetriaminepentaacetic acid), and HEDTA (N-hydroxyethylenediaminetriacetic acid). EDTA is a synthetic chelate that is widely used not only because it is the least expensive compared with other synthetic chelates [105] but also because it has a high ability to successfully improve plant metal uptake [106–108]. Organic chelates such as citric acid and malic acid can also be used to improve phytoextraction of heavy metals from polluted soils [109].

One major disadvantage of using chelates in phytoextraction is the possible contamination of groundwater via leaching of these heavy metals [110]. This is because of the increased availability of heavy metals in the soil solution when these chelates are used. In addition, when chelates (especially synthetic chelates) are used in high concentrations, they can become toxic to plants and soil microbes [106]. In general, solubility/availability of heavy metals for plant uptake and suitability of a site for phytoextraction are additional factors that should be considered (in addition to suitability of plants) before using phytoextraction for soil remediation [26].

4.2.2. Phytostabilization. Phytostabilization involves using plants to immobilize metals, thus reducing their bioavailability via erosion and leaching. It is mostly used when phytoextraction is not desirable or even possible [98]. Marques et al. [7] argued that this form of phytoremediation is best applied when the soil is so heavily polluted so that using

plants for metal extraction would take a long time to be achieved and thus would not be adequate. Jadia and Fulekar [111] on the other hand showed that the growth of plants (used for phytostabilization) was adversely affected when the concentration of heavy metal in the soil was high.

Phytostabilization of heavy metals takes place as a result of precipitation, sorption, metal valence reduction, or complexation [29]. The efficiency of phytostabilization depends on the plant and soil amendment used. Plants help in stabilizing the soil through their root systems; thus, they prevent erosion. Plant root systems equally prevent leaching via reduction of water percolation through the soil. In addition, plants prevent man's direct contact with pollutants and they equally provide surfaces for metal precipitation and sorption [112].

Based on the above factors, it is important that appropriate plants are selected for phytostabilization of heavy metals. Plants used for phytostabilization should have the following characteristics: dense rooting system, ability to tolerate soil conditions, ease of establishment and maintenance under field conditions, rapid growth to provide adequate ground coverage, and longevity and ability to self-propagate.

Soil amendments used in phytostabilization help to inactivate heavy metals; thus, they prevent plant metal uptake and reduce biological activity [7]. Organic materials are mostly used as soil amendments in phytostabilization. Marques et al. [113] showed that Zn percolation through the soil reduced by 80% after application of manure or compost to polluted soils on which *Solanum nigrum* was grown.

Other amendments that can be used for phytostabilization include phosphates, lime, biosolids, and litter [114]. The best soil amendments are those that are easy to handle, safe to workers who apply them, easy to produce, and inexpensive and most importantly are not toxic to plants [113]. Most of the times, organic amendments are used because of their low cost and the other benefits they provide such as provision of nutrients for plant growth and improvement of soil physical properties [7].

In general, phytostabilization is very useful when rapid immobilization of heavy metals is needed to prevent groundwater pollution. However, because the pollutants remain in the soil, constant monitoring of the environment is required and this may become a problem.

4.2.3. Phytovolatilization. In this form of phytoremediation, plants are used to take up pollutants from the soil; these pollutants are transformed into volatile forms and are subsequently transpired into the atmosphere [115]. Phytovolatilization is mostly used for the remediation of soils polluted with Hg. The toxic form of Hg (mercuric ion) is transformed into the less toxic form (elemental Hg). The problem with this process is that the new product formed, that is, elemental Hg, may be redeposited into lakes and rivers after being recycled by precipitation; this in turn repeats the process of methyl-Hg production by anaerobic bacteria [115].

Raskin and Ensley [116] reported the absence of plant species with Hg hyperaccumulating properties. Therefore,

genetic engineered plants are mostly used in phytovolatilization. Examples of transgenic plants which have been used for phytovolatilization of Hg polluted soils are *Nicotiana tabacum*, *Arabidopsis thaliana*, and *Liriodendron tulipifera* [117, 118]. These plants are usually genetically modified to include gene for mercuric reductase, that is, merA. Organomercurial lyase (merB) is another bacterial gene used for the detoxification of methyl-Hg. Both merA and merB can be inserted into plants used to detoxify methyl-Hg to elemental Hg [119]. Use of plants modified with merA and merB is not acceptable from a regulatory perspective [119]. However, plants altered with merB are more acceptable because the gene prevents the introduction of methyl-Hg into the food chain [120].

Phytovolatilization can also be employed for the remediation of soils polluted with Se [7]. This involves the assimilation of inorganic Se into organic selenoamino acids (selenocysteine and selenomethionine). Selenomethionine is further biomethylated to dimethylselenide which is lost in the atmosphere via volatilization [121]. Plants which have successfully been used for phytovolatilization of soils polluted with Se are *Brassica juncea* and *Brassica napus* [122].

4.3. Combining Plants and Microbes for the Remediation of Heavy Metal Polluted Soils. The combined use of both microorganisms and plants for the remediation of polluted soils results in a faster and more efficient clean-up of the polluted site [123]. Mycorrhizal fungi have been used in several remediation studies involving heavy metals and the results obtained show that mycorrhizae employ different mechanisms for the remediation of heavy metal polluted soils. For instance, while some studies have shown enhanced phytoextraction through the accumulation of heavy metals in plants [124–126], others reported enhanced phytostabilization through metal immobilization and a reduced metal concentration in plants [127, 128].

In general, the benefits derived from mycorrhizal associations—which range from increased nutrient and water acquisition to the provision of a stable soil for plant growth and increase in plant resistance to diseases [129–131]—are believed to aid the survival of plants growing in polluted soils and thus help in the vegetation/revegetation of remediated soils [132]. It is important to note that mycorrhiza does not always assist in the remediation of heavy metal polluted soils [133, 134] and this may be attributed to the species of mycorrhizal fungi and the concentration of heavy metals [7, 132]. Studies have also shown that activities of mycorrhizal fungi may be inhibited by heavy metals [135, 136]. In addition, Weissenhorn and Leyval [137] reported that certain species of mycorrhizal fungi (arbuscular mycorrhizal fungi) can be more sensitive to pollutants compared to plants.

Other microorganisms apart from mycorrhizal fungi have also been used in conjunction with plants for the remediation of heavy metal polluted soils. Most of these microbes are the plant growth-promoting rhizobacteria (PGPR) that are usually found in the rhizosphere. These PGPR stimulate plant growth via several mechanisms such as

production of phytohormones and supply of nutrients [138], production of siderophores and other chelating agents [139], specific enzyme activity and N fixation [140], and reduction in ethylene production which encourages root growth [141].

In general, PGPR have been used in phytoremediation studies to reduce plant stress associated with heavy metal polluted soils [142]. Enhanced accumulation of heavy metals such as Cd and Ni by hyperaccumulators (*Brassica juncea* and *Brassica napus*) has been observed when the plants were inoculated with *Bacillus* sp. [143, 144]. On the other hand, Madhaiyan et al. [145] reported increased plant growth due to a reduction in the accumulation of Cd and Ni in the shoot and root tissues of tomato plant when it was inoculated with *Methylobacterium oryzae* and *Burkholderia* spp. Thus, this indicates that the mechanisms employed by PGPR in the phytoremediation of heavy metal polluted soils may be dependent on the species of PGRP and plant involved in the process. Although studies involving both the use of mycorrhizal fungi and PGPR are uncommon, Vivas et al. [146] reported that PGPR (*Brevibacillus* sp.) increased mycorrhizal efficiency which in turn decreased metal accumulation and increased the growth of white clover growing on a heavy metal (Zn) polluted soil.

5. Conclusion

Plants growing on heavy metal polluted soils show a reduction in growth due to changes in their physiological and biochemical activities. This is especially true when the heavy metal involved does not play any beneficial role towards the growth and development of plants. Bioremediation can be effectively used for the treatment of heavy metal polluted soil. It is most appropriate when the remediated site is used for crop production because it is a nondisruptive method of soil remediation. Using plants for bioremediation (phytoremediation) is a more common approach to bioremediation of heavy metal compared with the use of microorganisms. Plants employ different mechanisms in the remediation of heavy metal polluted soils. Phytoextraction is the most common method of phytoremediation used for treatment of heavy metal polluted soils. It ensures the complete removal of the pollutant. Combining both plants and microorganisms in bioremediation increases the efficiency of this method of remediation. Both mycorrhizal fungi and other PGPR have been successfully incorporated in various phytoremediation programmes. The success of the combined use of these organisms depends on the species of microbe and plants involved and to some extent on the concentration of the heavy metal in soil.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

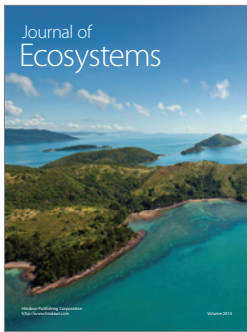
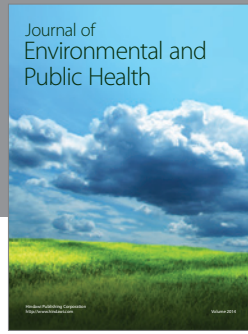
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