

DOI: <http://dx.doi.org/10.5281/zenodo.3244176>

# Microbial-aided phytoremediation of heavy metals contaminated soil: a review

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Received: 13 April 2019; Revised submission: 28 May 2019; Accepted: 08 June 2019



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**ABSTRACT:** Anthropogenic exercises as well as industrial enterprise and agricultural practices contribute considerably to the degradation and contamination of environment that considerably affects the soil. The normal physical and chemical know-how soil washing used for soil remediation render the land useless as a medium for plant growth, as they take away all biological activities. Others are labor-intensive and have high maintenance value phytoremediation, a cheaper and sustainable in situ remediation technique was so thought of. This data can enable proposing solutions to issues of contamination and eventually convalescent sites and soils. However, plants don't have the aptitude to degrade several soil waste matters particularly the organic pollutant. It's so imperative to require advantage of the degrading ability of soil microorganisms. This review so focuses on phytoremediation techniques improved by microbial colonies.

**Keywords:** Heavy metal; Microbial; Phytoremediation; Soil; Contamination; Pollution.

## 1. INTRODUCTION

Soil contamination refers to reduced soil quality due to the presence of harmful substances ensuing from human act. This might hurt human health or the environment, or otherwise violate personal or public interests. It's usually tough to watch as a result of its effects are often times restricted or quenched by the natural functions of soils, in particular: storing, degrading or immobilising pollutants [1]. Soil is nominal top layer of the Earth's crust, shaped by mineral particles, organic matter, water, air and living organisms. Contaminations of agricultural soils refer to its accumulation of heavy metals and connected compounds that might be from natural or phylogenesis sources. This threatens food quality, food security, and environmental health [2]. Soil pollution produces modification within the diversity and abundance of biological soil populations [3]. This is often vital due to the role of soil organisms in plant growth and survival. Such elimination of soil organisms will result in issues with plant growth and survival. Crops raised on contaminated soil might contain harmful levels of pollutants that may be passed on to the animals and human that eat them [4]. The planet population has exceeded seven billion and is apace approaching eight billion.

This ever-increasing population has exerted tremendous chaos on the present natural resources and has created immeasurable quantity of wastes across the world. Once pollution is in manageable quantity, the terrestrial, aquatic and atmospherically ecosystems will dilute, degrade or absorb the contaminants naturally. The rising burden of pollutants needs extra measures to curb the damaging effects of pollution [5, 6]. The information regarding the potentials of various plants to soak up, accumulate and translocate metals underneath varied condition is important with respect to the selection of plants for effective and economical phytoremediation method on contaminated environment. Improvement of contaminated soil could also be terribly tough as a result of each soil pollutants and soil minerals carry tiny electrical charges that cause them to bond with one another. It's well-known that heavy metals can't be with chemicals degraded and want to be physically removed or be immobilized [7]. Historically, remediation of heavy metal-contaminated soils is either on-the-scene management or excavation, and sequent disposal to a lowland site [8]. However, this methodology of disposal just shifts the contamination downside elsewhere. Soil washing for removing contaminated soil is an alternate to excavation and disposal to lowland. This methodology is both expensive and produces a residue made in heavy metals, which is able to need additional treatment or burial. Moreover, these physico-chemical technologies used for soil remediation render the land useless as a medium for plant growth, as they take away all biological activities. Other technologies like vitrification, leaching, electrokinetics soil vapor extraction, thermal natural process, chemical process, etc., are labor-intensive and have high maintenance value [9, 10]. The objective of this review is therefore to put on view microbial assisted phytoremediation as a technological helpful alternative for cleaning contaminated soils.

## 2. HEAVY METALS IN SOIL-POLLUTION

The term "heavy metal" refers to those metals of the periodic tables whose specific weight is larger than  $5 \text{ g/cm}^3$  or have atomic number on top of twenty, usually excluding alkali and alkaline-earth metal parts [11]. The term is somewhat inexact once taking under consideration the actual properties ionic chemistry parts, properties that outline the composite ability and biological properties. We tend to used different terms like "toxic metal" or "trace element", none of them refers to the identical parts, ensuing equally unsatisfying. In any case, consistent with Tiller [11], it appears that the term "heavy metal" may be employed in globalizing thanks to seek advice from those metals classified as environmental pollutants. The metalloids, meanwhile, have characteristics intermediate between metals and non-metals consistent with their binding properties and ionization. Non-metals like As, Se or Sb may additionally be necessary environmental pollutants [12]. Among the heavy metals there are essential and non-essential parts, for living organisms, though the boundary between these 2 teams are not clearly outlined and therefore the list of biologically necessary parts will increase with time. Heavy metals, essential or not, will become virulent once their contribution is excessive and adversely have an effect on the expansion and copy of organisms, even cause death. The rise of heavy metals in soil additionally inhibits microbial catalyst activity and reduces the range of populations of flora and fauna, inflicting sterility and increasing erosion. The transfer of metals to man will occur through the inhalation of dust, food, water, air or skin (result of dermal absorption of contaminants from soil and water) [13]. Pharmacology effects of metals to humans, significantly of Cd, Zn, Hg and Pb, that represent a number

of the foremost dangerous, are well documented and there are references wherever you'll be able to get info regarding this [11]. All soils have heavy metals as results of geologic processes and edaphogenetic. The natural content of existing chemical parts in soil is termed native geochemical fund (GF) or fonds level [17], and represents a perfect state of affairs that ought to be identified to see the contamination by the presence of amounts of metals remarkably high [18]. The determination of GF in soils isn't a straightforward task, and its price varies geographically primarily supported the geologic material. Usually igneous and metamorphic rocks, that occupy ninety five of the earth's crust [19], have high amounts of Mn, Cr, Co, Ni, Cu and Zn, and represent a very important natural supply of heavy metals into soil [17]. The natural concentration of metals in soils derived from serpentinized immoderate basic rocks, as an example, becomes virulent to animals and plants as results of the high content of heavy metals from the bedrock from that they derive.

### 3. PHYTOREMEDIATION DEFINITIONS

The answer lies within the hands of nature itself; plants are the nature's best defence against all human-made pollution. The word phytoremediation originates by combining 2 words Phyto (Greek) which means plants and remedium (Latin) which means removal or correction of malicious. Generally, phytoremediation means removal, degradation or stabilization of pollutants by plants. At current time, plants have regained their former standing of importance due to their multifarious applications. The contaminants are removed from soil, water and sediments victimization plants. Some plant root systems have special uptake capabilities, and additionally the shoot systems are capable in translocation, accumulation and degradation of the contaminants. These options enable economical uptake and removal of harmful toxicants from the environment. Phytoremediation may be a star energy-driven method and doesn't need external energy, thus it's efficient and fewer (zero) polluting as compared with ancient ways. There are many definitions of phytoremediation given by varied researchers; few are compiled in Table 1.

**Table 1.** Definitions of phytoremediation.

| No. | Definition  | References |
|-----|---|------------|
| 1   | Phytoremediation is a set of techniques or processes where plants are used for extracting, containing, degrading/destroying or restraint contaminants from the medium (soil, water or sediments)                  | [20]       |
| 2   | The usage of plants for remediation of toxicants found in groundwater, contaminated soil, sludge, wastewater, surface water and sediments   | [21]       |
| 3   | Phytoremediation is a technology that makes use of plants to purify contamination from water, sediments or soil   | [22]       |
| 4   | The application of plants for extraction and sequestration followed by detoxification of the contaminants   | [23]       |
| 5   | A sustainable and green process in which live plants are used for removing or degrading contaminants from the environment   | [24]       |
| 6   | Phytoremediation involves treatment of ecological problems (bioremediation) using floras that reduce ecological contamination, avoiding the need to uncover the polluted substances and dispose of them elsewhere | [25]       |

### 4. THE CHARACTER AND RESULT OF HEAVY METALS

A contaminant is something that's gift within the environment in excess to its original concentration. Waste generation by phylogenesis activities is thus numerous in nature that it's tough to reason them

effectively. Contaminants that make nuisance in soil and water are typically industrial wastes, municipal solid wastes, agricultural runoffs and leachates (organic pollutants) and radioactive wastes. The organic pollutants, heavy metals and radioactive wastes are dealt here as they're doubtless the foremost problematic pollutants in terms of soil and water. They cause adverse effects on to the plants animals as well as groups of people and generally indirectly the natural composition of ecosystems [22, 26]. Heavy metals might cause negative impact on plant growth and soil microflora [27]. Arsenic is one major environmental waste matter that falls underneath the class of heavy metal having number thirty three. Arsenic is found within the environment as organic arsenic species, inorganic arsenic compounds and gas. Arsenic may be a terribly virulent component, and its toxicity is typically depends on the species. The inorganic compounds of arsenic are typically a lot of virulent than its organic counterparts. Arsenites are a lot of virulent in nature than arsenates as they're a lot of susceptible to cause polymer breakdown [28, 29]. Arsenates are found to be a lot of stable thermodynamically than arsenites; so, they cause groundwater contamination [30]. Arsenic compounds are cancerous in nature and cause dermatitis wherever the groundwater is contaminated.

Lead with atomic number eighty two may be extremely virulent component that is non-biodegradable and remains within the environment for awfully very long time and accumulates within the soil and remains immobile. Sources of lead embrace natural sources, industrial sites, leaded fuels and orchards wherever the employment of insecticide takes place [22, 31]. The harmful effects of lead are unfold across a good vary of organisms like humans, animals, plants and microbes. In terms of human adverse impacts of contaminants on the environment health, lead causes major adverse impacts like slowness and brain harm [32]. Mercury is another heavy metal that's notoriously virulent and is accessible in soil in 3 soluble forms. It's a virulent component with a high bioaccumulation potential in living organisms like groups of people, fish and different animals. Mercury is found in naturally moreover as by phylogenesis activities within the environment. Mercury pollution within the environment is caused by mining, organic compound, painting industries, additionally from fertilizers, medical instruments, etc. [33]. Typically terrestrial plants aren't terribly sensitive to the adverse impacts of mercury, however it's been found that mercury interferes with electron transport in mitochondria and chloroplasts and adversely affects aerophilic metabolism and chemical action. Mercury acts as associate degree matter of aquaporin activities and causes reduction in water uptake in plant. In groups of people, the virulent impacts of mercury embrace medical specialty and excretory organ disorders [33]. As virulent metal-like species can't be degraded, there's a demand of physical removal or transformation to lesser virulent or non-toxic compounds.

## 5. MECHANISMS OF PHYTOREMEDIATION

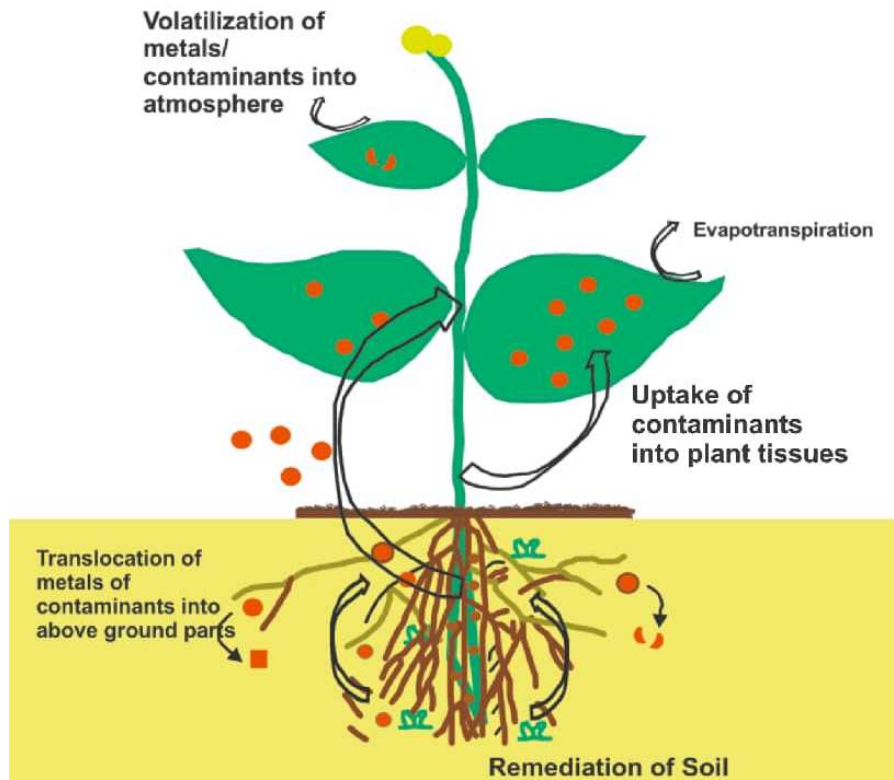
Phytoextraction: the employment of plants to get rid of contaminants from soils. Pollutant-accumulating plants are utilized to move and concentrate contaminants (metals or organics) from the soil into the above-ground shoots; the term is generally refers to metal removal from soils. In some cases, roots may be harvested moreover [34]. Phytoextraction involves the cultivation of upper plants that concentrate and translocate soil contaminants in their on top of ground tissues that may be harvested at the tip of the expansion amount [36]. It's the foremost effective among many phytoremediation ways, though technical difficulties are

there for its applications [35]. Choice of appropriate plant species is crucial for effective phytoextraction and biomass derived from shoot of a phytoremediator crop plant ought to be capable of depositing metal (oid) species at concentration 50–500 times on top of those within the contaminated soil substrate [46]. The known natural hyperaccumulators plants are alpine weed (*Thlaspi caerulescens L.*) capable of hyperaccumulating  $Zn^{2+}$ , and infrequently  $Cd^{2+}$  and  $Ni^{2+}$  [36], the snakelike endemic bush *Alyssum sp.*, Indian mustard *Brassica juncea (Brassicaceae)* and *Astragalus racemosus (Leguminosae)*. The Asian sedum herb *Alfredii (Crassulaceae)* has gained increased attention thanks to higher accumulation rate of metal, Cd, and Pb [37, 38]. Plants ideal for phytoextraction besides having associate degree inherent capability to tolerate and hyperaccumulate metals ought to possess multiple traits like (1) high and quick growing biomass; (2) extensively branched root systems; (3) ability to grow outside their space of collection; (4) comparatively simple to cultivate; and (5) attainable repulsive to herbivores to avoid the escape of accumulated metals to the organic phenomenon [39]. Sadly, most of the naturally hyperaccumulating plants have slow growth, poor biomass, and sometimes robust association with a selected surround, so limiting the phytoextraction potential [40]. However, non-hyperaccumulator plants having higher rate of growth and biomass might be changed or designed to realize the above-named attributes. to extend the potential of phytoextraction, factors limiting element accumulation in plants need to be resolved, which can embrace mobilization of poorly out there stuff within the soil, root uptake, sequestration by metal-complex formation and deposition in vacuoles for detoxification at intervals roots, translocation to symplast, economical vascular tissue loading, distribution and storage within the surface organ and tissues, and eventually expulsion of accumulated metal to less metabolically active cells, e.g., trichomes [41]. Two approaches are presently being explored to enhance or modify the metal accumulating plants: the traditional breeding and gene-splicing. Though variety of reports exist on productive crop breeding [42-45] yielding improved metal accumulator plants, the foremost constraint in developing such hybrid is sexual incompatibility between the taxa. Transgenic plants have opened new avenues in phytoremediation technology by expressing the specified factor and overcoming the constraints obligatory by sexual incompatibility.

**Phytostabilization:** the employment of plants to scale back the bioavailability of pollutants within the environment. Plants stabilize pollutants in soils, therefore rendering them harmless and reducing the danger of additional environmental degradation by natural process of pollutants into the bottom water or by mobile unfold [46].

**Phytovolatilization:** a variant of phytoextraction is phytovolatilization (Fig. 1), wherever the stuff isn't primarily targeted in surface tissues, however instead reworked by the plant into volatilisable and fewer virulent type before cathartic into the atmosphere [35].

Phytovolatilization is extremely a lot of promising for mercury (Hg) and element (Se) within which metals are regenerate to a volatile type for unleash and dilution into the atmosphere [47]. This methodology is advantageous over different phytoremediation ways because it removes metal (loid) from a site while not the necessity of harvest/disposal of contaminated plants.



**Figure 1.** Schematic representation of phytovolatilization where metals are volatilized by the process of evaporation by plants.

Phytodegradation: this methodology is additionally referred to as phytotransformation that refers to uptake of contaminants with the following breakdown, mineralization or metabolization by plants itself through varied internal accelerator reaction and metabolic processes [48]. Afterward several of those uptaken substances might even be metabolized into dioxide and water by catalyst complexes concerned within the plant metabolic cycle [49]. The perfect plant to be used of phytodegradation ought to have (1) extremely developed system that has the flexibility to secrete a substantial quantity of catalyst for degradation of the xenobiotics, (2) tolerance to the xenobiotics at a degree found in soil, (3) quick growth, and (4) a comparatively high biomass. Another study according the degradation of assorted nitroaromatic compounds by nitroreductase secreted by plants [50]. In another report, laccases are shown to be helpful for the degradation of a range of persistent environmental pollutants as well as alkenes, bisphenol A, and artificial dyes. The presence of plant derived enzymes capable of degrading environmentally dangerous xenobiotics therefore may be with success exploited for the event of future phytoremediation ways.

## 6. ASSISTANCE OF MICROORGANISMS IN PHYTOREMEDIATION

Plants don't have the aptitude to degrade several soil pollutants. It's so imperative to require advantage of the degrading ability of soil organisms. Organic toxins containing carbon like the hydrocarbons found in hydrocarbon and different fuels will solely be softened by microbic processes [51]. Dependent root colonizing organism through metal sequestration will increase metal tolerance in plants. The remediation by plant victimization the degrading ability of soil organisms is termed phytodegradation. This helps U.S. to know



integrated activity patterns between plants and microbes [52]. Some soil microbes like the arbuscular mycorrhizal fungi (AMF) secrete conjugated protein known as glomalin. This may form complexes with metals. Microbial organisms at intervals in the rhizosphere will participate in phytoremediation by protecting the plants from the virulent result of the contaminants whereas the plants reciprocally offer the microbial processes the boost they have to get rid of organic pollution from the soil a lot more quickly. Plants expel organic materials that function as food for microbes therefore enjoying a key role in deciding the scale and health of soil microbial population. Bioaugmentation permits a rise in biodegradation of contaminated sites by the introduction of single strains or assemblages of microorganisms with the specified chemical action capabilities [53]. Microbial assemblages are found to be economical since every partner will accomplish completely different components of the catabolic degradation [54].

Microbial association and mutualism at the basis zone or rhizosphere of the wetland plants play a very important role within the accumulation of metals. Several fascinating studies are drained this side. It absolutely was according that, once rhizosphere microorganisms were stifled with antibiotics, plants accumulated lower concentrations of metals; on the contrary once grown up axenically with additional microorganisms, accumulated a lot of those metals than axenic controls [55, 56]. Plants like the genus *Scirpus robustus* and *Polygonum monspeliensis* were found to accumulate lower concentrations of Se and Hg once they were treated with antibiotics than their traditional counterparts [56]. Similarly, mycorrhizae (symbiotic fungi related to roots), by increasing the spongy extent of root hairs, assist plants either absorb metals [57] or defend plants by proscribing the uptake of metals by restraining them. Therefore periphyton generally related to fresh soil plants (as an example, *Phragmites australis*) facilitate in sweetening and therefore the ability to accumulate and retain metals [58]. Microbial community plays a significant role in phytoremediation of soil plants. Community diversity and structure of microorganisms, their accelerator activity, and microbial-mediated edaphic processes (C and N mineralization, decomposition) principally rely on metal(s) concentration(s) of the basis zone of soil plants [59] that additionally facilitate plants to develop mechanisms to ameliorate toxicity of metals and to tolerate and/or resist multiple metal sequestration in an exceedingly complicated contaminated environment [59]. However, metal concentration plays a vital role in alteration in species composition, density, and biomass reduction of microorganisms [60-62]. It's according that metals like Cd, Cr, Mo, Ni, Pb, and metal shift the microorganism community with increase within the diversity of Gram positive microorganisms with members from *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia*, and *Chlorobi* teams in snake-like soils [63, 64]. However, few microorganism teams stay unchanged to sure metals with higher concentrations. As an example, actinobacterial community diversity remained unaffected with extra inputs of Pb and metal in an exceedingly Pb/Zn contaminated tract soil, community diversity became reduced. Curiously, several hyperaccumulators won't follow definite strategy to amass specific microorganisms proof against explicit metal(s) around their roots. Plants like *Alyssum bertolonii*, *A. serpyllifolium subsp. lusitanicum*, *Sebertia acuminata*, or *Thlaspi caerulescens subsp. calaminaria* are shown to host higher proportions of Cd-, Ni-, or Zn-resistant microorganisms within the rhizosphere compared to non-hyperaccumulating plants or non-vegetated soil [65-67]. These plants bit by bit develop resistance to a

collection of metals. Likewise, higher proportions of various Ni-tolerant microorganisms were found within the rhizosphere of *Alyssum serpyllifolium* subsp. *lusitanicum* once the plants are exposed to high Ni concentrations [67]. A synergistic result between plant roots and their associated microorganism is therefore evident. Production of metabolites by microorganism is increased by the indirect offer of necessary substrates within the root exudates provided by plants. On the opposite hand, microorganism at the basis zone (plant growth promoting rhizobacteria, PGPR) might facilitate within the production of phytohormones (such as auxin (IAA), cytokinins, and ethylene) [68]. Further, development, physiology, and exudation of root also are excited by the weathering agents that improves nutrient uptake by plants [68].

## 7. PHYTOEXTRACTION WITH ENDOPHYTIC MICROBES

Researchers meted out many experiments on the appliance of endophytic microorganism and mycorrhizal fungi within the phytoextraction of pollutants [69]. Endophytes are the dependent microbes inhabiting within the internal plant structure and are able to facilitate plant growth and increase resistance of plants against infectious agent and drought [70]. It's been recently according that the endophytic dependent microorganism *Methylbacterium populum* that lives at intervals poplar will mineralize one,3,5- trinitro-1,3,5-triazacyclohexane (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). However, the success rate of phytoextraction of heavy metals using endophytic microorganism remains slow due to the dearth of correct strains with heavy metal resistance and detoxification capacities [71]. Besides endophytes, the arbuscular mycorrhizal (AM) fungi also are identified to be concerned within the uptake of parts into plants [69] and are according to be gift in mutualistic association within the roots of plants growing on markedly contaminated soil [72-74]. Therefore, mycorrhizal fungi may be applied for important phytoextraction by up many attributes like increased metal tolerance, increased biomass production, and larger metal concentration in plant structure [75]. In brief, the goal of phytoextraction is to scale back the presence of trace parts in soils through their uptake and accumulation by plants; in distinction, phytostabilization aims to attenuate the mobile and bioavailable fraction of metals by combining the employment of metal-tolerant plants and soil amendments and therefore reduces natural process through soil. In each processes the “mobility and bioavailability of trace parts within the soil, particularly within the rhizosphere wherever root uptake and exclusion takes place, is a vital issue moving their outcome and success” [68].

## 8. MICROBIAL AND PLANT CONTRIBUTIONS IN PHYTOREMEDIATION

Microbial-assisted phytoextraction optimizes the synergistic result of plants and microorganisms and has been used for the cleaning-up of soils contaminated by metals. Plant translocates and sequesters pollutions like heavy metals. Plants will store several contaminants in biomass that may later be harvested, whereas microbial assemblages can even convert contaminants like heavy metals to stable and/or less virulent type. They will facilitate the uptake of pollutants like heavy metals by plant roots. Microorganisms that reside on or at intervals aerial plants tissue will facilitate to stabilize and/or rework contaminants that are translated which can limit the extent of volatilization [76]. Plant root exudates like enzymes, amino acids, aromatics, easy sugars, and aliphatics stimulate the expansion of root-associated microorganisms; on the opposite hand,



microbes will cut back the phytotoxicity of the stuffs within the soil or augments the capability of the plant to degrade contaminant. Ability of plant root to increase deeper into soil, permitting access to water and air and so dynamic the concentration of dioxide, the pH, diffusion potential, oxidation-reduction potential, oxygen concentration, and wet content of the soil, could lead on to associate degree environment which will higher able to support high micro-biomass [77]. This increased element uptake by plants may be ascribed to a rise in root absorption ability associate degreeed/or to a sweetening of trace metal bioavailability within the rhizosphere, mediate by microorganisms. Plants will increase biodegradation through the transfer of oxygen to the rhizosphere and therefore unleash of soluble exudates that offer nutrient sources for micro-organisms [78]. Thus, plants enhance microbes' growth and thus the associated contaminant-degradation processes. Organism contribution in restraint parts or facilitating plant absorption plants might considerably contribute to removal through uptake in biomass [79]. Microbial assemblages improve plant health and growth, suppress disease-causing microbes, and increase nutrient availableness and assimilation [80].

### 9. MECHANISMS OF MICROBES IN PHYTOREMEDIATION OF CONTAMINATED SOIL

Microbial inoculants will improve waste matter removal through varied mechanisms. Some has the potential to provide metal chelating siderophores that might improve metal bioavailability [81]. Moreover, they manufacture biosurfactants (rhamnolipids) that may enhance the solubility of poor soluble organic compounds and therefore the quality of heavy metals [82]. Formation of biofilm is another mechanism by that microbic inoculants assist plants in remediation of contaminated soils [83]. Additionally, these microbes will rework metals into bioavailable and soluble forms through the action of organic acids, biomethylation, and oxidation-reduction processes [83]. Numerous soil microbes have the flexibility to secrete plant hormones like indole-3-acetic acid (IAA), cytokinins, gibberellins (GAs), and sure volatiles that promote plant growth by neutering root design. The microbic plant growth stimulatory actions result from the manipulation of the complicated and balanced network of plant hormones that directly are chargeable for growth and root formation.

### 10. MICROBIAL CHOICE AND PLANT EFFECTUALITY IN PHYTOREMEDIATION

Improvement of biomass production is most significant for the appliance of phytoextraction technology that ends up in the next metal extraction or total metal yield. As an example, immunisation of rhizobacteria genus *Pseudomonas fluorescens* biotype F, isolated from heavy metal contaminated soil, helped to enhance the expansion of helianthus plants (*Helianthus annuus*) and their tolerance to salt in soil [84]. Microorganism production of IAA and siderophores compete necessary roles to develop tolerance towards salt [85]. Few studies recommend that application of transgenic plants together with rhizospheric PGPR improve plant biomass which will facilitate in phytoextraction [86]. *Pseudomonas putida* HS-2 (isolated from Ni-contaminated soil) applied to the transgenic canola (*Brassica napus*) showed trends of upper accumulation of total Ni per plant. However, Kuffner et al. [87] according that rhizobacterial strains that were found to extend Cd/Zn uptake and accumulation and consequently growth of pussy willow were neither phytohormone-producing strains nor siderophore producers. Application of bioremediation practices rely on

the detoxification of virulent metals and xenobiotics through metabolism. It's according that among varied molecules, proteins like hemoprotein P450, phytochelatins, and metallothioneins are important biomolecules during this method. Augmenting the expression of those biomolecules might facilitate to enhance the potency of bioremediating agent [88-90]. Genetic supplementation by making transgenic plants to extend remediation potential of extremely virulent component is an alternate approach during this technology. It's been shown that tobacco plants carrying MerA factor from *E. coli* (encoding mercurous reductase) will mobilize mercury 5–8 times on top of management counterpart [91]. Similarly, over expressing 2 microorganism genes (encoding salt enzyme (arsC) and  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -ECS)) within the tiny weed *Arabidopsis thaliana* considerably increased the buildup of arsenic in leaves [92]. Reduction of salt to arsenite is catalyzed by the arsC, whereas  $\gamma$ -ECS catalyzes the primary step within the synthesis pathway of phytochelatins, increasing the pool of thiol compounds as well as phytochelatins, in the course of the body of the plant. When detoxification of arsenite by thiol compounds forming arsenic-protein thiolates, could also be hold on and/or partitioned off within the bodily cavity sanctionative arsenic to accumulate at larger amounts within the leaves of those transgenic plants [92].

Phytoremediation method, thus, could also be improved victimization plant-associated microorganisms that alter the solubility, availability, and transport of trace parts and nutrients by reducing soil pH scale, secretion of chelators and siderophores, or oxidation-reduction changes. Element (Se) phytoremediation (accumulation and volatilization) by Indian mustard (*Brassica juncea*) was simplest within the presence of plant growth promoting rhizobacteria [55]. out there information suggests that microorganism like *Azotobacter chroococcum* (N<sub>2</sub>-fixer), *Eubacterium megaterium* (P-solubilizer), and *Eubacterium mucilaginosus* (K-solubilizer) and *Eubacterium sp.* RJ16 will decrease soil pH scale, in all probability by evacuation low weight molecular acids, enhancing the bioavailability of heavy metals like Cd and metal for plants [93]. It's been seen that the presence of various rhizobacteria related to 3 plants, *Alyssum murale*, *A. serpyllifolium subsp. lusitanicum*, *Thlaspi caerulescens*, increased the potentiality of heavy metal accumulation to their bodies [67, 94]. Rhizosphere actinobacteria European black alder living in mutualism with N<sub>2</sub>-fixing *Frankia* were found to tolerate over 2.0 mm Ni together with the rise yield of the plant [95]. Likewise, a microorganism mixture of microorganism *Microbacterium saperdae*, *Pseudomonas monteilii*, and *Enterobacter cancerogenus* helped in higher metallic element extraction by plants like *T. caerulescens*. For waste treatment in wetlands, establishing a dense stand of vegetation is a lot of necessary than choosing a specific species. Any species which will grow well may be chosen. However, for storm water wetlands, native plant species work best. Choosing native, native plant species for soil restoration is needed because the plants are tailored to the native climate, soils, and close plant and animal communities, and are seemingly to try and do well. As an example, Bulrushes (*Scirpus sp.*) are wide employed in treating biodegradable pollution and wastewaters thanks to their ability to face up to high levels of nutrients, establish simply and noninvasive nature. Like that, point (*Sagittaria sp.*) and hydrophytic plant (*Pontederia cordata*) could also be employed in agricultural wetlands. The potency of water plant (*Eichhornia crassipes*) for nutrient uptake and their rapid climb rate have place them to use for several years in improvement up municipal and industrial waste [96].

## 11. METHODS OF INOCULATING PLANTS WITH MICROBIAL INOCULANTS

Plants to be used as phytoremediator to wash contaminated soils might be inoculated with microbial assemblages via quite a variety of techniques. These ways might include:

- (1) Seed inoculation,
- (2) Soaking plant roots with microbial suspension, once the basis of grass was soaked with a suspension of associate degree endophytic *Massilia* sp. (Pn2) the identical was found to own been translocated to the plant shoots [97].
- (3) Painting plant leaves with microbial suspension [98-100]. Afzal et al. [101] discovered the cells of *Burkholderia phytofirmans* PsJN within the internal tissue of the shoot and root once the plant was inoculated via leaf painting. Root formation strategy was found to be the best formation methodology for circumventing the danger of plant organic contamination [99]

## 12. PHYTOREMEDIATION: YESTERDAY, TODAY AND TOMORROW

In recent years we've got determined an increased interest in hyperaccumulators, though despite information gained on molecular/cellular uptake mechanisms of designated trace parts [102], their translocation to individual surface organs and detoxification, we tend to still must house the matter of terribly restricted biomass of those plants. Initial studies involved non-woody plants, however thanks to their low biomass, considerably contributory to increased prices of usage of those plants, interest was quickly shifted to incorporate additionally woody plants [103]. Different aspects of enhancing the phytoremediation potential are connected, e.g. with modification of contaminated substrate to facilitate natural action of metals/metalloids from soil by the plant system [104], the appliance of microorganisms [105]. This latter side appears to be of explicit interest, because it is connected with the increased demand for energy from renewable sources, crucial significantly in recent years. Designated plant taxa from genus *Populus* or *Hamamelis* genus species are characterized by a big increment in biomass, particularly in areas with position water levels, and at the identical time comparatively high capability to soak up heavy metals/metalloids [106]. Renewable energy sources (RES) are enjoying associate degree more and more necessary role within the generation of primary energy within the international organization. Within the years 2001-2009, generation of energy from renewable sources increased from 10.6% to 18.3%. Biomass became the most supply of renewable energy. New objectives were per this package regarding the employment of renewable energy and greenhouse emission emissions. It absolutely was assumed that by the year 2020 the share of renewable energy would increase to twenty the troubles (an important increase within the use of non-forest biomass in energy generation) within the total balance of energy consumption within the EU. In such a case biomass from phytoremediation, with the appliance of extra measures limiting additional heavy metal transport to the environment, may considerably increase the number of biomass needed to satisfy the stipulations of the directive. In recent years, studies on phytoremediation have centered on the employment of microorganism and mycorrhizal fungi moreover as genetic modifications represented in one among the points below [107, 108]. Designated microorganism strains, i.e. plant growth-promoting rhizobacteria (PGPR) like *Azospirillum*, *Rhizobium*, *Enterobacter* or *Arthrobacter*, could also be wont to increase plant growth [109].

These organisms are capable of cooperating with plants by reducing the adverse effects of virulent substances on their growth, stimulation of nutrient transport needed for applicable plant growth or formation of such compounds [110, 111]. The presence of PGPR within the rhizosphere of plants employed in phytoremediation is especially essential, as they need a positive result on the event of the basis system (stimulation of growth and therefore additionally uptake of nutrients from soil) and limiting plant ageing processes by gas inhibition, i.e. interference of its production by ACC-deaminase activity from microorganism (ACC-1-aminocyclopropane-1-carboxylate) [112]. PGPR are capable of manufacturing several phytohormones, e.g. gibberellins, cytokinins or indole-3-acetic acid (IAA) [113]. For this reason, it should be assumed that these organisms within the close to future are necessary subjects of studies on enhancing resistance of plants growing in areas contaminated with metals/metalloids, moreover as maintaining or increasing their growth (preventing a discount of biomass underneath conditions adverse for plant growth). Another fascinating cluster of growth promoting organisms, at the identical time enhancing potency of heavy metal uptake from contaminated areas, contains endophytic microorganism (Gram-positive and Gram-negative) moreover as siderophoreproducing microorganism (*Pseudomonas putida*, *Eubacterium megaterium* or *Ralstonia metallidurans*). The previous are organisms colonising plant tissues having a positive result on plant growth and enhancing tolerance to the presence of virulent trace parts. They exhibit many important traits, e.g. they promote the uptake of nutrients needed for applicable plant growth and that they have a positive result on the capability to limit the adverse result of pathogens [114]. A fair a lot of fascinating side of recently undertaken analysis is connected with the pertinency of low molecular chelators made by fungi, microorganism and plants, exhibiting high affinity to choose metal ions (Al, Cd, Cu, Fe or Zn). Siderophore-producing microorganisms (SPB) also are capable of stimulating plant growth, yielding a rise of biomass and increased resistance to the presence of heavy metals. They exhibit a capability to extend the number of metals absorbed by plant tissues or enhance plant tolerance by stimulating growth of individual plant organs [115]. Within the close to future, various studies on phytoremediation are seemingly to specialize in the appliance of recent specialized organisms, which can which is able to, promote plant growth and development and at the identical time will defend plants against the adverse result of heavy metals gift within the soil. Moreover, such studies conducted in place can create it attainable to develop best tips for the appliance of plants designated for growing in contaminated areas so as to realize the best attainable potency of heavy metal uptake from soil.

### 13. MECHANISMS OF MICROBIAL REMEDIATION OF METAL-POLLUTED SOILS

Microorganisms will detoxify metals by valence transformation, extracellular chemical precipitation, or volatilization. In fact, some microorganisms will enzymatically cut back a range of metals in metabolic processes that aren't associated with metal assimilation [115]: some microorganism get energy for growth by coupling the reaction of easy organic acids and alcohols, hydrogen, or aromatic compounds, to the reduction of Mn(IV). Microorganism uses a terminal negatron acceptor could also be helpful for removing metal from contaminated sites. The reduction of the virulent selenate and selenite to the insoluble and far less virulent elemental element could also be exploited to reinforce removal of those anions from contaminated sites [116].

The a lot of virulent style of metallic element, Cr(VI), can even be detoxified by bacterially mediate reduction and therefore the accelerator mechanism chargeable for the reduction of Cr(VI) to Cr(III) is presently being studied and will ultimately result in an ad bioremediation method [117]. Another natural reduction method currently being developed for industrial applications is that the transformation of mercurous particle, Hg(II), to volatile metal-like mercury, Hg(0). Microorganisms can even enzymatically cut back different metals like vanadium, molybdenum, gold, silver and copper, however reduction of those metals has not been studied extensively [115].

#### **14. DISADVANTAGES OF MICROBIAL REMEDIATION OF METAL POLLUTED SOIL**

Though it's true that microorganisms that use metals as terminal negatron acceptors or cut back them as a detoxification mechanism may be of use for the removal of those pollutants from the environment [116], it's under no circumstances less true that once considering the remediation of a metal-polluted soil, metal-accumulating plants supply various blessings over microbial processes since plants will truly extract metals from the contaminated soils, in theory rendering them clean (metal-free soils). In fact, though a good number of microorganism, fungal, protista and plant systems are capable of concentrating virulent metals from their surroundings, to date no efficient method exists to retrieve tiny pollutant from the soil [117]. Therefore, and in regard to the bioremediation of heavy metals, microorganisms are principally used to treat industrial waste streams, with the organisms either immobilized onto completely different support matrixes or in an exceedingly free-living state, basined in treatment tanks or other forms of reactor vessels. Afterward, the metal-loaded biomass may be either disposed of fittingly or, counting on their concentrations, treated to recover the metals. Within the environment, as is that the case for the in place bioremediation systems, microorganism aren't effective as a permanent, large-scale resolution to heavy metal-polluted areas, since this means the final word removal of the contaminated biomass from the location. As a consequence, application of microbic bioremediation to the in place removal of heavy metals from contaminated soils is principally restricted to metal immobilization by precipitation or reduction [118].

#### **15. MECHANISMS OF MICROBES IN THE ASSISTED PHYTOREMEDIATION**

Microbes related to phytoextraction plant-assisted bioremediation has been primarily involved with the degradation of organic and inorganic pollutants (Table 2) and therefore the use of microorganisms to enhance the plant-metal uptake from soils has hardly been investigated. Roots will use rhizospheric organisms (mycorrhizal fungi or root-colonizing bacteria) to extend the bioavailability of metals [119]. However, it's believed that plant uptake of sure mineral nutrients like metallic element and Mn could also be expedited by rhizospheric microorganisms [120]. Similar results could also be found for non-essential heavy metals. Many strains of eubacterium and genus *Pseudomonas* increased the entire quantity of Cd accumulated by mustard seedlings [121]. From these studies, it may be all over that by populating the rhizosphere with designated microorganisms throughout the phytoextraction, it ought to be attainable to reinforce uptake of heavy metals from soils. Plant growth promoting rhizobacteria (PGPR) are the heterogeneous category of microorganism strains that may be found within the plant rhizosphere. PGPRs will improve plant growth by direct or indirect

ways [122]. The precise mechanism behind the improved plant growth is ambiguous. These PGPRs have a special ability to grow in heavy metal contaminated environment [123, 124]. Heavy metals contamination is that the results of technological development, occurring at important concentrations within the environment [123]. Due to the environmental persistence, toxicity and skill to be incorporated into food chains, these industrial wastes as well as heavy metal are new threat and challenge [125]. Uses of rhizospheric microorganisms (bacteria/fungi etc.) are usually thought of as safe, value effective and reliable technique, for elimination of heavy metals from environmental compartments [123]. Rhizospheric microorganism will survive underneath the heavy metal contaminated sites, and might increase plant growth and metal tolerance [122]. Moreover, rhizospheric microorganisms will enhance biomass production and tolerance of plants to heavy metals in stress environment. In recent years, studies regarding rhizobacteria and their interactions with hyperaccumulating or accumulating plants have attracted the eye of many investigators [124]. These microorganisms will promote plant growth by manufacturing siderophore production, indole carboxylic acid production, phosphate solubilisation and compound production. Recent studies have unconcealed that these PGPRs might promote plant growth and defend plants against heavy metals toxicity in heavy metal-contaminated soils [123].

**Table 2.** Examples of soil contaminants that could be removed from soil by microbial-assisted phytoremediation practice.

| Plant                      | Microbes   |
|----------------------------|--|
| <i>Helianthus annuus</i>   | <i>Micrococcus</i> sp. MU1 and <i>Klebsiella</i> sp. BAM1        |
| <i>Polygonum pubescens</i> | <i>Enterobacter</i> sp. JYX7 and <i>Klebsiella</i> sp. JYX10     |
| <i>Zea mays</i>            | <i>Azotobacter chroococum</i> and <i>Rhizobium leguminosarum</i> |
| <i>Vigna unguiculata</i>   | <i>Scutelospora reticulata</i> , <i>Glomus phaseous</i>          |
| <i>Solanum nigrum</i>      | <i>Pseudomonas</i> sp. LK9                                       |
| <i>Brassica napus</i>      | <i>Acinetobacter</i> sp. Q2BJ2                                   |

## 16. CONCLUSIONS

Contaminants of soil might be organic or inorganic within the hydrosoluble fraction adsorbed onto particles or dissolved. Microbial-assisted phytoremediation take away, destroy, sequester, or cut back the concentrations or virulent effects of stuff in contaminated soils. Production of siderophores, biosurfactants, formation of biofilms, organic acids production, biomethylation, and oxidation-reduction processes and plant growth hormones stimulation are mechanisms utilized by microbes in phytoremediation. The amount of obtainable degrading microbes and therefore the physical and chemical properties of pollutants determine the success of microbial assisted phytoremediation. Exceptional pollutant tolerance, ability to quickly grow on degraded land, ability to grow outside their space of assortment, and speedy biomass production are necessary plant characteristics to be thought of within the selection of plant for phytoremediation.

**Author Contributions:** This work was carried out in collaboration between all authors. S.A.A. designed the study, wrote the protocol. U.J.J.I. managed the literatures, gathered the initial data and performed preliminary



data review. O.P.A. and J.D.B. managed the literature searches and produced the initial draft, also produced all original figures. All authors read and approved the final manuscript.

**Conflict of Interest:** The authors declare no conflict of interest.

## REFERENCES

1. Ana PP, Natalia RE. Status of local soil contamination; A report by the Joint Research Centre (JRC) in collaboration with the European Information and Observation Network (Eionet) National Reference Centres for Soil, 2018.
2. Wu Q, Leung JYS, Geng X, Chen S, Huang X, Li H, et al. Heavy metal contamination of soil and water in the vicinity of an abandoned e-waste recycling site: implications for dissemination of heavy metals. *Sci Total Environ.* 2015; 506-507: 217-225.
3. Alori E, Fawole O. Phytoremediation of soils contaminated with aluminium and manganese by two arbuscular mycorrhizal fungi. *J Agric Sci.* 2012; 4: 246-252.
4. Khan S, Afzal M, Iqbal S, Khan QM. Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. *Chemosphere.* 2013; 90: 1317-1332.
5. Glick BR. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol Adv.* 2003; 21(5): 383-393.
6. Glick BR. Using soil bacteria to facilitate phytoremediation bacteria. *Biotechnol Adv.* 2010; 28: 367-374.
7. Kroopnick PM. Vapor abatement costs analysis methodology for calculating life cycle costs for hydrocarbon vapour extracted during soil venting. In: Wise DL, Trantolo DJ, eds. *Remediation of hazardous waste.* Marcel Dekker, New York, 1994: 779-790.
8. Parker R. Environmental restoration technologies. *EMIAA yearbook.* 1994: 169-171.
9. Danh LT, Truong P, Mammucari R, Tran T, Foster N. Vetiver grass, *Vetiveria zizanioides*: a choice plant for phytoremediation of heavy metals and organic wastes. *Int J Phytorem.* 2009; 11: 664-691.
10. Haque N, Peralta-Videa JR, Jones GL, Gill TE, Gardea-Torresdey JL. Screening the phytoremediation potential of desert broom (*Baccharis sarothroides* Gray) growing on mine tailings in Arizona, USA. *Environ Pollut.* 2008; 153: 362-368.
11. Tiller KG. Heavy metals in soils and their environmental significance. *Adv Soil Sci.* 1989; 9: 113-141.
12. Paulo JC, Pratas FJ, Varun M, D'Souza R, Paul MS. Phytoremediation of soils contaminated with metals and metalloids at mining areas: potential of native flora. In: *Environmental Risk Assessment of Soil Contamination.* InTech Open, 2014: 485-517.
13. Chehregani A, Malayer B. Removal of heavy metals by native accumulator plants. *IJAB.* 2007; 9(3): 462-465.
14. Adriano DC. Trace elements in terrestrial environments: biogeochemistry, bioavailability and risks of metals. 2nd edn. Springer-Verlag New York, Berlin Heidelberg, 2001.
15. Rubio C, González Weller D, Martín-Izquierdo RE. El zinc: oligoelemento esencial. *Nutrición Hospit.* 2007; 22(1): 101-107.
16. Adams A, Raman A, Hodgkins D. How do the plants used in phytoremediation in constructed wetlands, a sustainable remediation strategy, perform in heavy-metal contaminated mine sites. *Wat Environ J.* 2013; 27(3): 373-386.
17. Ross SM. Sources and forms of potentially toxic metals in soil-plant systems. In: Ross SM, ed. *Toxic*

- metals in soil-plant systems. John Wiley & Sons, 1994: 3-25.
18. Gough LP. Understanding our fragile environment, lessons from geochemical studies. USGS Circular 1105, United States Government Printing Office, Washington, DC, 1993.
  19. Mitchell RL. Trace elements in soil. In: Bear FE, ed. Chemistry of the soil. Reinhold Publishing Corporation, New York; Chapman and Hall, London; 1964: 320-368.
  20. EPA. (U.S. Environmental Protection Agency), Introduction to Phytoremediation. National Risk Management Research Laboratory, EPA/600/R-99/107, 2000, <http://www.clu-in.org/download/remed/introphyto.pdf>
  21. Rodriguez L, Lopez-Bellido FJ, Carnicer A, Recreo F, Tallos A, Monteagudo JM. Mercury recovery from soils by phytoremediation. In: Book of environmental chemistry. Springer, Berlin, 2005: 197-204.
  22. Tangahu BV, Sheikh Abdullah SR, Basri H, Idris M, Anuar N, Mukhlisin M. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng. 2011; 2011: ID 939161.
  23. Ismail S. Phytoremediation: a green technology. Iran J Plant Physiol. 2012; 3(1): 567-576.
  24. Cameselle C, Chirakkara RA, Reddy KR. Electrokinetic-enhanced phytoremediation of soils: status and opportunities. Chemosph. 2013; 93(4): 626-636.
  25. Abioye OP, Ijah UJJ, Aransiola SA. Phytoremediation of soil contaminants by biodiesel plant *Jatropha curcas*. Chapter 4. In: Baudh K. et al., eds. Phytoremediation potential of bioenergy plants. Springer Nature Singapore Pte Ltd. 2017.
  26. Pehlivan E, Özkan AM, Dinç S, Parlayici S. Adsorption of Cu<sup>2+</sup> and Pb<sup>2+</sup> ion on dolomite powder. J Hazard Mater. 2009; 167(1-3): 1044-1049.
  27. Roy S, Labelle S, Mehta P. Phytoremediation of heavy metal and PAH-contaminated brown field sites. Plant Soil. 2005; 272(1-2): 277-290.
  28. Ampiah-Bonney RJ, Tyson JF, Lanza GR. Phytoextraction of arsenic from soil by *Leersia oryzoides*. Int J Phytoremed. 2007; 9(1): 31-40.
  29. Vaclavikova M, Gallios GP, Hredzak S, Jakabsky S. Removal of arsenic from water streams: an overview of available techniques. Clean Techn Environ Policy. 2008; 10(1): 89-95.
  30. Chutia P, Kato S, Kojima T, Satokawa S. Arsenic adsorption from aqueous solution on synthetic zeolites. J Hazard Mater. 2009; 162(1): 440-447.
  31. Traunfeld JH, Clement DL. Lead in garden soils. Home and garden. Maryland Cooperative Extension, University of Maryland, 2001, <http://www.hgic.umd.edu/media/documents/hg18.pdf>
  32. Cho-Ruk K, Kurukote J, Supprung P, Vetayasuporn S. Perennial plants in the phytoremediation of lead contaminated soils. Biotechnol. 2006; 5(1): 1-4.
  33. Resaee A, Derayat J, Mortazavi SB, Yamini Y, Jafarzadeh MT. Removal of mercury from chlor-alkali industry wastewater using *Acetobacter xylinum* cellulose. Am J Environ Sci. 2005; 1(2): 102-105.
  34. Aransiola SA, Ijah UJJ, Abioye OP. Phytoremediation of lead polluted soil by *Glycine max* L. Appl Environ Soil Sci. 2013: ID 631619.
  35. Kramer U. Phytoremediation: novel approaches to cleaning up polluted soils. Curr Opin Biotechnol. 2005; 16: 133-141.
  36. Milner MJ, Kochian LV. Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. Ann Bot. 2008; 102: 3-13.
  37. Lu L, Tian S, Yang X, Wang X, Brown P, Li T. Enhanced root-to-shoot translocation of cadmium in the hyperaccumulating ecotype of *Sedum alfredii*. J Exp Bot. 2008; 59: 3203-3213.
  38. Deng D, Deng J, Li J, Zhang J, Hu M, Lin Z. Accumulation of zinc, cadmium, and lead in four

- populations of *Sedum alfredii* growing on lead/zinc mine spoils. *J Integr Plant Biol.* 2008; 50: 691-698.
39. Seth CS. A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. *Bot Rev.* 2012; 78: 32-62.
  40. Chaney RL, Angle JS, McIntosh MS, Reeves RD, Li YM, Brewer EP. Using hyperaccumulator plants to phytoextract soil Ni and Cd. *Z Naturforsch C.* 2005; 60: 190-198.
  41. Clemens S, Palmgren MG, Kraemer U. A long way ahead: understanding and engineering plant metal accumulation. *Trends Plant Sci.* 2002; 7: 309-315.
  42. Gleba D, Borisjuk NV, Borisjuk LG, Kneer R, Poulev A, Skarzhinskaya M, et al. Use of plant roots for phytoremediation and molecular farming. *Proc Nat Acad Sci.* 1999; 96: 5973-5977.
  43. Dushenkov S, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I, Ensley B. Phytoremediation: a novel approach to an old problem. In: Wise DL, ed. *Global environmental biotechnology.* Elsevier, Amsterdam, 1997: 563-572.
  44. Alkorta I, Hernandez-Allica J, Becerril JM, Amezaga I, Albizu I, Garbisu I. Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as Zn, Cd, Pb and arsenic. *Rev Environ Sci Biotechnol.* 2004; 3: 71-90.
  45. Nehnevajova E, Herzig R, Erismann KH, Schwitzguebel JP. In vitro breeding of *Brassica juncea* L to enhance metal accumulation and extraction properties. *Plant Cell Rep.* 2007; 26: 429-437.
  46. Carlos G, Alkorta I. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Biores Technol.* 2001; 77: 229-236.
  47. Bhargava A, Carmona F, Bhargava M, Srivastava S. Approaches for enhanced phytoextraction of heavy metals. *J Environ Manage.* 2012; 105: 103-120.
  48. Spaczynski M, Seta-Koselska A, Patrzylas P, Betlej A, Skorzynska-Polit E. Phytodegradation and biodegradation in rhizosphere as efficient methods of reclamation of soil contaminated by organic chemicals (a review). *Acta Agrophys.* 2012; 19: 155-169.
  49. McCutcheon SC, Schnoor JL. *Phytoremediation, transformation and control of contaminants.* Wiley, Hoboken, NJ, 2003.
  50. Boyajian GE, Carreira LH. Phytoremediation: a clean transition from laboratory to marketplace? *Nat Biotechnol.* 1997; 15: 127-128.
  51. Adesodun JK, Atayese MO, Agbaje TA, Osadiaye BA, Mafe OF, Soretire AA. Phytoremediation potentials of sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with zinc and lead nitrates. *Water Air Soil Pollut.* 2010; 207: 195-201.
  52. Bell TH, Joly S, Pitre FE, Yergeau E. Increasing phytoremediation efficiency and reliability using novel omics approaches. *Trends Biotechnol.* 2014; 32: 271-280.
  53. Lebeau T. Bioaugmentation for in situ soil remediation: how to ensure the success of such a process. In: Singh A, et al., eds. *Bioaugmentation, biostimulation and biocontrol.* Springer, Berlin, 2011: 129-186.
  54. Rahman KS, Rahman T, Lakshmanaperumalsamy P, Banat IM. Occurrence of crude oil degrading bacteria in gasoline and diesel station soils. *J Basic Microbiol.* 2002; 42: 284-291.
  55. de Souza MP, Chu D, Zhao M, Zayed AM, Ruzin SE, Schichnes D, Terry N. Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. *Plant Physiol.* 1999; 119(2): 565-574.
  56. Stout LM, Dodova EN, Tyson JF, Nusslein K. Phytoprotective influence of bacteria on growth and cadmium accumulation in the aquatic plant *lemna minor*. *Water Res.* 2010; 44(17): 4970-4979.
  57. Meharg AA, Cairney JW. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated

- environments. *Adv Ecol Res.* 2000; 30: 69-112.
58. Lakatos G, Kiss M, Mezzaros I. Heavy metal content of common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) and its periphyton in Hungarian shallow standing waters. *Hydrobiologia.* 1999; 415: 47-53.
  59. Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. *Ecotoxicol Environ Saf.* 2000; 45: 198-207.
  60. Baath E. Effects of heavy metals in soil on microbial processes and populations. *Water Air Soil Pollut.* 1989; 47: 335-379.
  61. Baath E, Diaz-Ravina M, Bakken LR. Microbial biomass, community structure and metal tolerance of a naturally Pb-enriched forest soil. *Microb Ecol.* 2005; 50: 496-505.
  62. Chander K, Dyckmans J, Joergensen R, Meyer B, Raubuch M. Different sources of heavy metals and their long-term effects on soil microbial properties. *Biol Fertil Soils.* 2001; 34(4): 241-247.
  63. Mengoni A, Grassi E, Barzanti R, Biondi EG, Gonnelli C, Kim CK, et al. Genetic diversity of bacterial communities of serpentine soil and of rhizosphere of the nickel-hyperaccumulator plant *Alyssum bertolonii*. *Microb Ecol.* 2004; 48: 209-217.
  64. Akerblom S, Baath E, Bringmark L, Bringmark E. Experimentally induced effects of heavy metal on microbial activity and community structure of forest mor layers. *Biol Fertil Soils.* 2007; 44: 79-91.
  65. Schlegel C, von Neumann CP, Neumeyer F, Richter A, Strauch S, de Boer J, et al. Depopulation of 180 Tam by Coulomb excitation and possible astrophysical consequences. *Phys Rev C Nucl Phys.* 1994; 50: 2198-2204.
  66. Lodewyckx C, Mergeay M, Vangronsveld J, Clijsters H, van der Lelie D. Isolation, characterization, and identification of bacteria associated with the zinc hyperaccumulator *Thlaspi caerulescens* subsp. *calaminaria*. *Int J Phytorem.* 2002; 4: 101-115.
  67. Becerra-Castro C, Monterroso C, Garcia-Leston M, Prieto-Fernandez A, Acea MJ, Kidd PS. Rhizosphere microbial densities and trace metal tolerance of the nickel hyperaccumulator *Alyssum serpyllifolium* subsp. *lusitanicum*. *Int J Phytorem.* 2009; 11: 525-541.
  68. Kidd P, Barcelob J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shileve S, et al. Trace element behaviour at the root-soil interface: implications in phytoremediation. *Environ Exp Bot.* 2009; 67: 243-259.
  69. Doty SL. Enhancing phytoremediation through the use of transgenic and endophytes. *New Phytol.* 2008; 179: 318-333.
  70. Taghavi S, van der Lelie D, Hoffman A, Zhang YB, Walla MD, Vangronsveld J, et al. Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. *PLoS Genet.* 2010; 638(6): e1000943.
  71. Luo S, Wan Y, Xiao X, Guo H, Chen L, Xi Q. Isolation and characterization of endophytic bacterium LRE07 from cadmium hyperaccumulator *Solanum nigrum* L and its potential for remediation. *Appl Microbiol Biotechnol.* 2011; 89: 1637-1644.
  72. Khade HW, Adholeya A. Arbuscular mycorrhizal association in plants growing on metalcontaminated and noncontaminated soils adjoining Kanpur tanneries, Uttar Pradesh, India. *Water Air Soil Pollut.* 2009; 202: 45-56.
  73. Javaid A. Importance of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. In: Khan MS, Zaidi A, Goel R, Musarrat J, eds. *Biomangement of metalcontaminated soils.* Springer, New York, 2011.
  74. Miransari M. Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. *Biotechnol*

- Adv. 2011; 29: 645-653.
75. Vamerli T, Bandiera M, Mosca G. Field crops for phytoremediation of metal-contaminated land: a review. *Environ Chem Lett.* 2010; 8: 1-17.
  76. Bell TH, Joly S, Pitre FE, Yergeau E. Increasing phytoremediation efficiency and reliability using novel omics approaches. *Trends Biotechnol.* 2014; 32: 271-280.
  77. Lin X, Li P, Li F, Zhang L, Zhou Q. Evaluation of plant microorganism synergy for the remediation of diesel fuel. *Contam Soil Bull Environ Contam Toxicol.* 2008; 81: 19-24.
  78. Robinson B, Fernández JE, Madejón P, Marañón T, Murillo JM, Green S, Clothier B. Phytoextraction: an assessment of biogeochemical and economic viability. *Plant Soil.* 2003; 249: 117-125.
  79. Guittonny-Philippe A, Masotti V, Höhener P, Boudenne JL, Viglione J, Laffont-Schwob I. Constructed wetlands to reduce metal pollution from industrial catchments in aquatic Mediterranean ecosystems: a review to overcome obstacles and suggest potential solutions. *Environ Int.* 2014; 64: 1-16.
  80. Babalola OO. Beneficial bacteria of agricultural importance. *Biotechnol Lett.* 2010; 32: 1559-1570.
  81. Visca P, Imperi F, Lamont IL. Pyoverdine siderophores: from biogenesis to biosignificance. *Trends Microbiol.* 2007; 15: 22-30.
  82. Zhang X, Xu D, Zhu C, Lundaa T, Scherr KE. Isolation and identification of biosurfactant producing and crude oil degrading *Pseudomonas aeruginosa* strains. *Chem Eng J.* 2012; 209: 138-146.
  83. Ullah A, Heng S, Munis MFH, Fahad S, Yang X. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. *Environ Exp Bot.* 2015; 117: 28-40.
  84. Raab A, Schat H, Meharg AA, Feldmann J. Uptake, translocation and transformation of arsenate and arsenite in sunflower (*Helianthus annuus*): formation of arsenic-phytochelatin complexes during exposure to high arsenic concentrations. *New Phytol.* 2005; 168(3): 551-558.
  85. Prasad MVN. Aquatic plants for phytotechnology. In: Singh SN, Tripathi RD, eds. *Environmental bioremediation technologies*. Springer, Germany, 2007.
  86. Farwell AJ, Vesely S, Nero V, Rodriguez H, Shah S, Dixon DG, Glick BR. The use of transgenic canola (*B. napus*) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site. *Plant Soil.* 2006; 288: 309-318.
  87. Kuffner M, Puschenreiter M, Wieshammer G, Gorfer M, Sessitsch A. Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. *Plant Soil.* 2008; 304: 35-44.
  88. Cobbett CS. Phytochelatin and their role in heavy metal detoxification. *Plant Physiol.* 2000; 123: 825-833.
  89. Cobbett C, Goldsbrough P. Phytochelatin and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu Rev Plant Biol.* 2002; 53: 159-182.
  90. Gillam EMJ. Engineering cytochrome P450 enzymes. *Chem Res Toxicol.* 2008; 21: 220-231.
  91. Ke HY, Sun JG, Feng XZ, Czako M, Marton L. Differential mercury volatilization by tobacco organs expressing a modified bacterial merA gene. *Cell Res.* 2001; 11: 231-236.
  92. Doucleff M, Terry N. Pumping out the arsenic. *Nat Biotechnol.* 2002; 20: 1094-1095.
  93. Sheng X, Xia JJ. Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. *Chemosphere.* 2006; 64: 1036-1042.
  94. Cloutier-Hurteau B, Sauve S, Courchesne F. Influence of microorganisms on Cu speciation in the rhizosphere of forest soils. *Soil Biol Biochem.* 2008; 40: 2441-2451.
  95. Wheeler CT, Hughes LT, Oldroyd J, Pulford ID. Effects of nickel on *Frankia* and its symbiosis with *Alnus glutinosa* (L.). *Gaertn. Plant Soil.* 2001; 23: 81-90.

96. Espinoza-Quinones FR, Modenes AN, Costa IL Jr, Palacio SM, Szymanski N, Trigueros DEG, et al. Kinetics of lead bioaccumulation from a hydroponic medium by aquatic macrophytes *Pistia stratiotes*. *Water Air Soil Pollut*. 2009; 203: 29-37.
97. Liu J, Liu S, Sun K, Sheng YH, Gu YJ, Gao YZ. Colonization on root surface by a phenanthrene-degrading endophytic bacterium and its application for reducing plant phenanthrene contamination. *PLoS One*. 2014; 9: e108249.
98. Afzal M, Yousaf S, Reichenauer TG, Kuffner M, Sessitsch A. Soil type affects plant colonization, activity and catabolic gene expression of inoculated bacterial strains during phytoremediation of diesel. *J Hazard Mater*. 2011; 186: 1568-1575.
99. Sun K, Liu J, Gao Y, Sheng Y, Kang F, Waigi MG. Inoculating plants with the endophytic bacterium *Pseudomonas* sp. Ph6-gfp to reduce phenanthrene contamination. *Environ Sci Pollut Res* 2015; 22(24): 19529-19537.
100. Zhu X, Ni X, Liu J, Gao YZ. Application of endophytic bacteria to reduce persistent organic pollutants contamination in plants. *Soil Air Water*. 2014; 42: 306-310.
101. Afzal M, Khan S, Iqbal S, Mirza MS, Khan QM. Inoculation method affects colonization and activity of *Burkholderia phytofirmans* PsJN during phytoremediation of diesel contaminated soil. *Int J Biodeter Biodegrad*. 2013; 85: 331-336.
102. Jabeen R, Ahmad A, Iqbal M. Phytoremediation of heavy metals: physiological and molecular mechanisms. *Bot Rev*. 2009; 75: 339-364.
103. Yadav R, Arora P, Kumar S, Chaudhury A. Perspectives for genetic engineering of poplars for enhanced phytoremediation abilities. *Ecotoxicology*. 2010; 19: 1574-1588.
104. Mleczek M, Kozłowska M, Kaczmarek Z, Magdziak Z, Goliński P. Cadmium and lead uptake by *Salix viminalis* under modified Ca/Mg ratio. *Ecotoxicology*. 2012; 20: 158-165.
105. Weyens N, Schellingen K, Dupae J, Croes S, van der Lelie D, Vangronsveld J. Can bacteria associated with willow explain differences in Cd accumulation capacity between different cultivars? *J Biotechnol*. 2010; 150: 291-292.
106. Adegbidi HG, Volk TA, White EH, Abrahamson LP, Briggs RD, Bickelhaupt DH. Biomass and nutrient removal by willow clones in experimental bioenergy plantations in New York State. *Biomass Bioenerg*. 2001; 20: 399-411.
107. Miransari M. Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. *Biotechnol Adv*. 2011; 29: 645-653.
108. Prasad A, Kumar S, Khaliq A, Pandey A. Heavy metals and arbuscular mycorrhizal (AM) fungi can alter the yield and chemical composition of volatile oil of sweet basil (*Ocimum basilicum* L.). *Biol Fertil Soils*. 2011; 47: 853-861.
109. Farina R, Beneduzi A, Ambrosini A, de Campos SB, Lisboa BB, Wendisch V, et al. Diversity of plant growth-promoting rhizobacteria communities associated with the stages of canola growth. *Appl Soil Ecol*. 2012; 55: 44-52.
110. Jha Y, Subramanian RB, Patel S. Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. *Acta Physiol Plant*. 2011; 33: 797-802.
111. Tang S, Liao S, Guo J, Song Z, Wang R, Zhou X. Growth and caesium uptake responses of *Phytolacca americana* Linn. and *Amaranthus cruentus* L. grown on caesium contaminated soil to elevated CO<sub>2</sub> or inoculation with a plant growth promoting rhizobacterium *Burkholderia* sp. D54, or in combination. *J*



- Hazard Mater. 2012; 198: 188-197.
112. Saleem M, Arshad M, Hussain S, Bhatti AS. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *J Ind Microbiol Biotechnol.* 2007; 34: 635-648.
  113. Ma Y, Prasad MNV, Rajkumar M, Freitas H. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. *Biotechnol Adv.* 2011; 29: 248-258.
  114. Rajkumar M, Noriharu A, Freitas H. Endophytic bacteria and their potential to enhance heavy metal phytoextraction. *Chemosphere.* 2010; 77: 153-160.
  115. Lovley DR. Dissimilatory metal reduction. *Annu Rev Microbiol.* 1993; 47: 263-290.
  116. Garbisu C, Alkorta I. Bioremediation: principles and future. *J Clean Technol Environ Toxicol Occup Med.* 1997; 6: 351-366.
  117. Ow DW. Heavy metal tolerance genes: prospective tools for bioremediation. *Resour Conserv Recycling.* 1996; 18: 135-149.
  118. Summers AO. The hard stuff: metals in bioremediation. *Curr Opin Biotechnol.* 1992; 3: 271-276.
  119. Raskin I, Smith RD, Salt DE. Phytoremediation of metals: using plants to remove pollutants from the environment. *Curr Opin Biotechnol.* 1997; 8: 221-226.
  120. Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ. Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant Soil.* 1991; 130: 179-198.
  121. Salt DE, Prince RC, Pickering IJ, Raskin I. Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.* 1995; 109: 1427-1433.
  122. Dell'Amico E, Cavalca L, Andreoni V. Improvement of *Brassica napus* growth under cadmium stress by cadmium resistant rhizobacteria. *Soil Biol Biochem.* 2008; 40: 74-84.
  123. Barakat MA. New trends in removing heavy metals from industrial wastewater. *Arab J Chem.* 2011; 4: 361-377.
  124. Burd GI, Dixon DG, Glick BR. Plant growth promoting bacteria that decrease heavy metal toxicity in plants. *Can J Microbiol.* 2000; 46: 237-245.
  125. Kumar V, Upadhyay N, Kumar V, Sharma S. A review on sample preparation and chromatographic determination of acephate and methamidophos in different samples. *Arab J Chem.* 2015; 8(5): 624-631.
  126. Prapagdee B, Chanprasert M, Mongkolsuk S. Bioaugmentation with cadmium-resistant plant growth-promoting rhizobacteria to assist cadmium phytoextraction by *Helianthus annuus*. *Chemosphere.* 2013; 92: 659-666.
  127. Jing YX, Yan JL, He HD, Yang DJ, Xiao L, Zhong T, et al. Characterization of bacteria in the rhizosphere soils of *Polygonum pubescens* and their potential in promoting growth and Cd Pb Zn uptake by *Brassica napus*. *Int J Phytoremed.* 2014; 16: 321-333.
  128. Hadi F, Fano A. Effect of diazotrophs (*Rhizobium* and *Azobactor*) on growth of maize (*Zea mays* L.) and accumulation of lead (Pb) in different plant parts. *Pak J Bot.* 2010; 42: 4363-4370.
  129. Chen L, Luo S, Li X, Wan Y, Chen J, Liu C. Interaction of Cd hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas* sp. Lk9 on soil heavy metals uptake. *Soil Biol Biochem.* 2014; 68: 300-308.
  130. Zhang Y, He L, Chen Z, Wang Q, Qian M, Sheng X. Characterization of ACC deaminase-producing endophytic bacteria isolated from copper-tolerant plants and their potential in promoting the growth and copper accumulation of *Brassica napus*. *Chemosphere.* 2011; 83: 57-62.
  131. Liang X, He CQ, Ni G, Tang GE, Chen XP, Lei YR. Growth and Cd accumulation of *Orychophragmus violaceus* as affected by inoculation of Cd tolerant bacterial strains. *Pedosphere.* 2014; 24: 322-329.

132. Sheng XF, Xia JJ, Jiang CY, He LY, Qian M. Characterization of heavy metal-resistant endophytic bacteria from rape *Brassica napus* roots and their potential in promoting the growth and lead accumulation of rape. *Environ Pollut*. 2008; 156: 1164-1170.
133. Wężowicz K, Turnau K, Anielska T, Zhebrak I, Gołuszka K, Błaszowski J, Rozpądek P. Metal toxicity differently affects the *Iris pseudacorus*-arbuscular mycorrhiza fungi symbiosis in terrestrial and semi-aquatic habitats. *Environ Sci Pollut Res*. 2015; 22: 19400-19407.
134. Srivastava S, Verma PC, Chaudhary V, Singh N, Abhilash PC, Kumar KV, et al. Inoculation of arsenic-resistant *Staphylococcus arlettae* on growth and arsenic uptake in *Brassica juncea* (L.) Czern. Var. R-46. *J Hazard Mater*. 2013; 262: 1039-1047.
135. He CQ, Tan GE, Liang X, Du W, Chen YL, Zhi GY, Zhu Y. Effect of Zn tolerant bacterial strains on growth and Zn accumulation in *Orychophragmus violaceus*. *Appl Soil Ecol*. 2010; 44: 1-5.