

Remediation of Heavy Metals in Contaminated Soil: A Review of Effects, Mechanisms and Strategies

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Received: 20 October, 2024

Accepted: 22 December, 2024

Abstract: Contamination with heavy metals is a major problem that is increasing everywhere in the globe. This problem has drastically impacted the whole world including human and environmental health in the past 30 years. There are a number of strategies to remediate soil that is contaminated with heavy metals such as physical, biological and chemical, and these can be used individually and sometimes with one another to tackle this issue. Heavy metals such as lead (Pb) and arsenic (As) are often more effectively transmitted with the help of chemical and physical methods such as immobilization and soil cleaning due to low biological acid levels and strong soil binding. In contrast, metals such as cadmium (Cd) and zinc (Zn) are more mobile, and are suitable for biological approaches, including Phytoremediation & microbial therapy. Mercury (Hg) remains a challenge due to the conversion and complex transformations in environments to improve all methods. This review summarizes the toxic effects of heavy metal contaminated soil in different parts of earth. Microbes are used to change the concentration of heavy metals in soil to improve the ability of plants to grow in healthy way. This paper reviews about the mechanisms and strategies to remediate the contaminated soil. This paper discusses necessary technologies used for remediation of soil and also mentioning about the types and methods. There are so many microbes and plants that can be used as the best options for removing toxic heavy metals from soils because conventional and physical approaches can be expensive and ineffective in areas with low metal toxicity, where phytoremediation and bioremediation are considered as eco-friendly approaches.

Keywords: Soil, heavy metals, remediation, plants, microbes, contamination strategies, effects.

Introduction

Soil can be polluted by human made chemicals called as xenobiotics and sometimes by doing alteration in the environment of soil such as mining, smelters, industry, pesticides, fertilizers, sewage sludge, power stations and improper disposal of waste (Münzel et al., 2023; Lahori et al., 2024). These all are part of causing soil contamination (Abd-Alla, et al., 2012). This contamination of soil happens because of heavy metals, which can be known as highly toxic substances even at very low concentration, they are harmful for soil health that can pose serious threats to all the organisms and to our environment. These heavy metals get absorbed in food like fruits and vegetables and it has become a major health concern these days. The issues are arising drastically not only for human beings but also for ecosystem (Abd-Alla et al., 2000). The depletion of heavy metals such as nickel (Ni), zinc (Zn), chromium (Cr), arsenic (As), Copper (Cu), Cobalt (Co), Lead (Pb), Manganese (Mn), Mercury (Hg) and Aluminum (Al), are mainly found in heavy metal contaminated soil (Jin et al., 2018). These heavy metals cannot be biodegraded and by their

biological amplification, the concentration of these metals increases hundreds and thousands of times. After some period of time, they create major health problems for human beings and organisms (Li et al., 2015). Enzyme activities and cellular functions can be disturbed of animals, plants and humans due to high concentrations of heavy metals like lead, mercury, cadmium and arsenic. Heavy metals can also cause various health issues to human beings such as cardiovascular disease, cognitive impairment, damage of kidneys, cancer, chronic anemia, nervous system damage, and skin or bones problems (Järup, 2003). Metals that exist naturally in the soil cause no contamination, unlike the metals that occur due to anthropogenic activities with separate entities or those present in high concentrations (Ramos et al., 1994). Heavy metals are somewhat different because of the higher resistance to organic contamination such as chemically and biologically induced degradation, therefore heavy metals usually persist in the soil content for so many years for instance lead (Pb) has 150 to 5000 years long high persistence period and it has been documented to remain in soil for more than 150 years following sludge application (Kumar et al

., 1995) and similarly, the biological half-life of cadmium (Cd) is more than 18 years (Förstner, 1995). This review discusses the effects of heavy metals on the environment and how they can be effectively remediated using plants and microorganism. It further discusses various mechanisms utilized by these organisms for remediating heavy metal contamination.

Heavy Metal Effects on Humans, Plants and Soil

Anthropogenic activities have increased globally in the recent years which are causing major problem of contributing to the major sink of heavy metals in soil. These heavy metals alter the composition and activities of microbial communities in the soil (Xi et al., 2016). Exchangeable ions are present in heavy metals which are absorbed by inorganic substances on their surfaces. Several biochemical activities such as enzymes and microbial activities can be affected, if soil has high concentration of heavy metals, and it also affects the soil fertility and quality (Minnikova et al., 2017). Soil relative surface area, degree of complexation with ligand and soil, organic matter, density, and kind of charge in soil colloids (Norvell, 1984). Metals induce toxic effects on soil microbes, and the degree of impact varies due to physiochemical parameters, including pH, temperature, clay minerals, organic matter abundance, inorganic cations and anions, and metal chemical forms (Bååth, 1989).

The concentration level of heavy metals cannot fully assess the harmful impacts and movement in soils. Heavy metals are extremely harmful for soils like once they got added in soil then there is possibility of disturbance of these organic compounds into kind of chemical forms including various types such as mobility, toxicity and bioavailability after being adsorbed by swift reactions in minutes and hours and then slow adsorptive reactions within days and years in the soil (Shiowatana et al., 2001). The characteristics specifically chemical characteristics, including metallic properties and bonding states are collectively influenced by environmental conditions. Such as pH levels, soil characteristics and organic matter compositions, availability of chelating exudates, and redox conditions frequently affect the toxicity and movement of metals in soil (Nyamangara 1998). Release of heavy metals into the environment is their toxicity makes them persistent, posing a significant threat to organisms exposed to elevated levels contaminants. Although in excess, metals are essential to the basic functions of both plants and animals. They interfere with the organisms systems. Plant growth may be hampered by hazardous heavy metals that are not good for plants, such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), zinc (Zn), uranium (U), selenium (Se), silver (Ag), gold (Au), nickel (Ni),

and arsenic (As). Fig 1 shows the Heavy metal contamination in soil according to percentage. Reduced photosynthetic activity, diminished plant mineral nutrition, and decreased functionality contribute to growth limitations to crucial enzymes (Kabata-Pendias, 2000).

Lead is one of the most toxic, harmful and dangerous organic compound among all the heavy metals because it causes dangerous effects on humans. The poisoning of lead damages the human brain seriously. It affects children and it can cause brain illnesses such as risk of lower IQ, impaired development, hyperactivity, mental deterioration, and shortened the attention span under age of six. Adults also face hazardous risks when they are exposed to lead such as anorexia, loss of memory, insomnia, nausea, and joints are highly affected and their reaction time gets decreased. Inhalation and ingestion are two main routes from where humans can get poisoned and when it accumulates in body, it affects brain dangerously. Gastrointestinal tract, kidneys and central nervous system also get affected but it depends on the level and duration of exposure (Ogoko, 2014). Figure 2 shows the effects on soil health, plants and on humans by heavy metals contaminated soil. Lead is usually found in fruits and vegetables through heavy metal contaminated soil and poisons the food chain. Crops including lettuce, carrot, corn, squash, beans, strawberries, apples and pica have lead accumulation. If lead is present in soil at 300ppm level then it is safe to eat in crops (Rosen, 2002).

Nickel is also a heavy metal, usually present in environment and comes from metal products and it is an ingredient of steel. It is usually found in a very low amount but it can also be hazardous, if its level of accumulation increases and it can cause various types of cancer, specifically if animals or humans live near the refineries. Soil get contaminated when Ni comes from different sources such as combustion of fossil fuels, metal plating, mining and metal plating industries (Khodadoust et al., 2004). Mercury damages kidney and it comes from coal combustion which is major source of contamination.

Mechanisms and Strategies to Remediate Contaminated Soil with Heavy Metals

Financial and technical implications and complexities have made soil clean-up a challenging task. Practical implementation of these conventional soil remediation methods faces several shortcomings and may implicate some level of hazard. Metal contamination in the environment can persist for a long time period, it depends on the type of metals and soil. Heavy metals can be remediated by using in-situ, ex-situ, on-situ, off-situ techniques including biological, chemical and

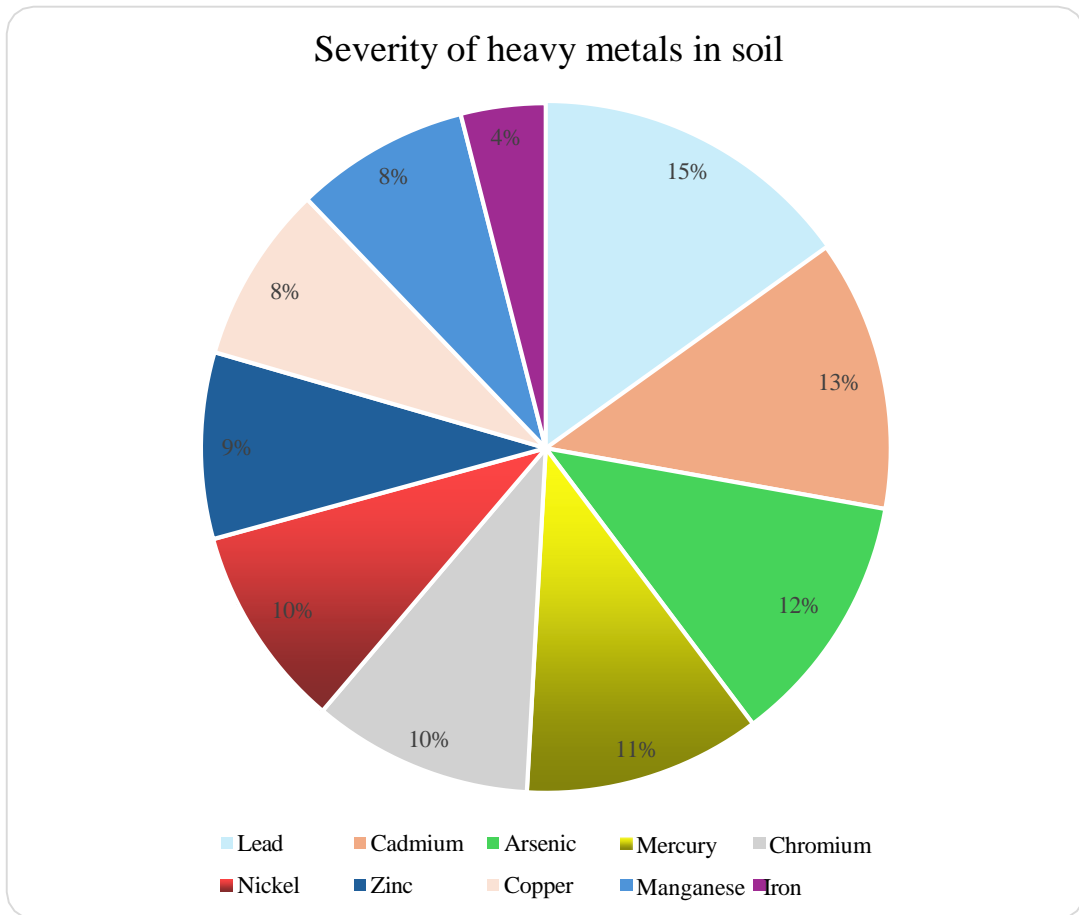


Fig. 1 Heavy metal contamination (%) in soil.

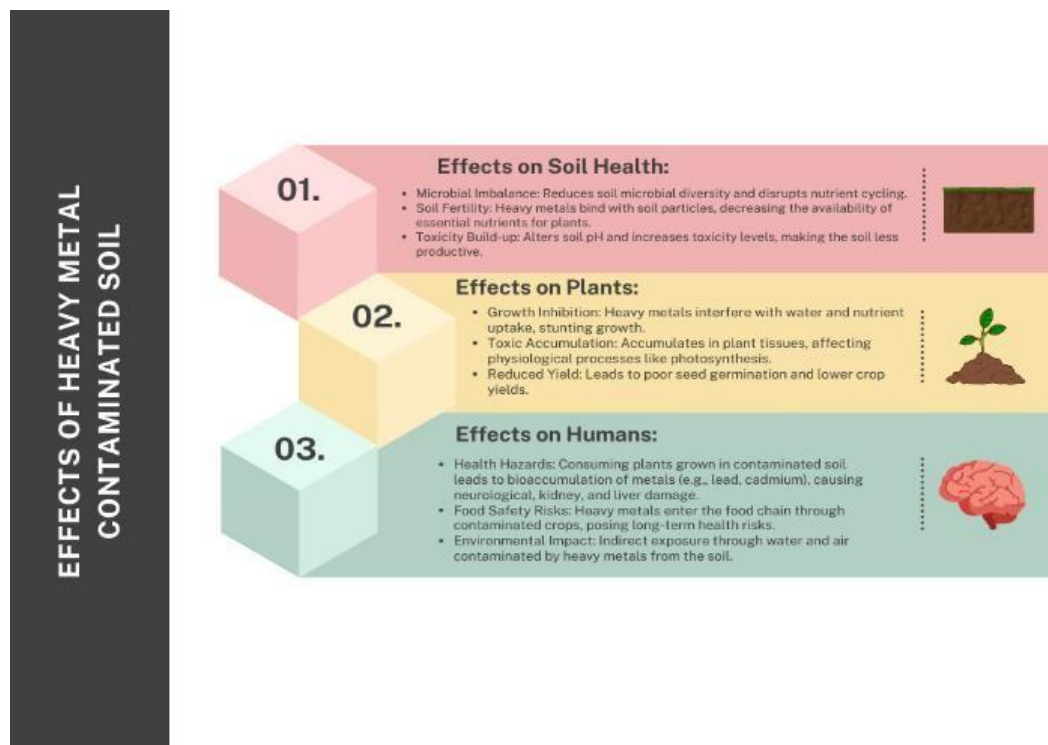


Fig. 2 Contaminated soil with heavy metals have effects on soil health, plants and on humans.

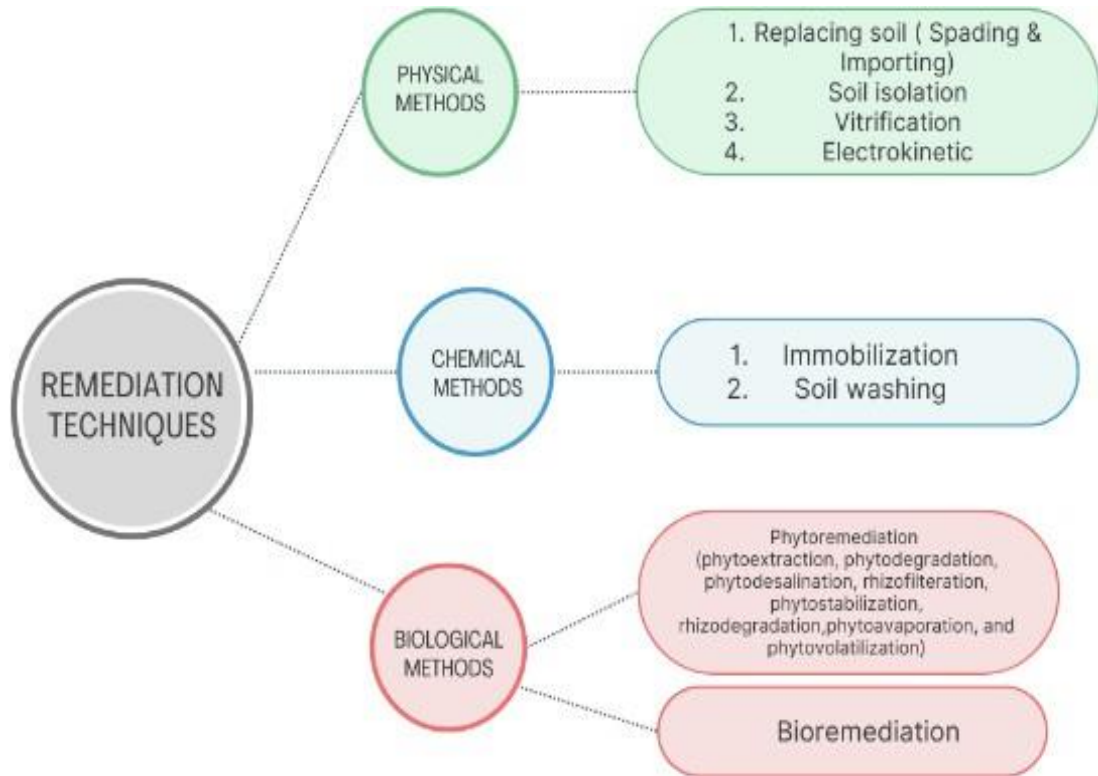


Fig. 3. Remediation techniques including physical, chemical and biological methods.

Table 1. Remediation mechanisms for specific heavy metal contamination in soil.

Heavy metals	Frequency found	Safe limit	Remediation mechanism	References
Copper	50-150 mg/kg	20-40 mg/kg	<i>Desulfovibrio desulfuricans</i> (bioremediation)	(Yue et al., 2015)
Mercury, cadmium, and zinc	1-10 mg/kg 3-8 mg/kg 100- 300 mg/kg	0.1 mg/kg 1 mg/kg 50-100 mg/kg	<i>Escherichia coli</i> (bioremediation)	(Wuana and Okieimen 2011; Lerebours et al., 2016)
Nickel	50-200 mg/kg	30-50 mg/kg	<i>Phyllanthus serpentinus</i> (phytoremediation)	(Chaney et al., 2010; Beesley et al., 2015)
Lead, cadmium, Copper, zinc	100-200 mg/kg 3-8 mg/kg 50-150 mg/kg 100- 300 mg/kg	20-30 g/kg 1 mg/kg 20-40 mg/kg 50-100 mg/kg	Acid solutions or chelators (Soil washing)	(Chen and Li 2018)
Lead	100-200 mg/kg	20-30 mg/kg	<i>Bacillus subtilis</i> X3 (bioremediation)	(Qiao et al., 2019)

remediation techniques are physical, chemical and biological. Figure 3 indicated the remediation techniques including physical, chemical and biological methods. Soil replacement, soil isolation, vitrification, and electrokinetics are examples of physical remediation techniques; phytostabilization, phytoevaporation, and phytoextraction are examples of biological techniques; and immobilization and soil washing are examples of chemical techniques. According to a number of studies conducted in Pakistan and India, crop irrigation using untreated wastewater causes heavy metals to accumulate in the soil (Jan et al., 2014). About thirty percent of Pakistan's wastewater is utilized directly to irrigate 32,500 hectares of crops, while sixty-four percent is released straight into water bodies without any initial treatment. In Pakistan, untreated wastewater is used to irrigate about 26% of the crops grown (Ensink et al., 2004). Due to multiple human activities, heavy metals that are naturally present in the Earth's crust are released into the soil, causing the levels of heavy metals in soil to rise to dangerous levels. The weathering of underlying bedrock produces soils that naturally contain heavy metals, which can be found in rocks in a variety of chemical forms, including ores, from which they can be extracted as minerals (Fuge et al., 1991).

Heavy metals typically appear in ore forms as oxides of Se, Al, Mn, and Sb and as sulfides of Pb, Co, Fe, As, Pb-Zn, Ag, and Ni. As oxide and sulfide ores, these metals are often extracted or mined from soils. Metal sulphides (As, Hg, Pb, and Cd) typically coexist in soils with sulphides of Cu (chalcopyrite, CuFeS_2), and Fe (pyrite, FeS_2). Thus, these heavy metals are typically obtained as byproducts of various hydrometallurgical processes following mining or as part of exhaust fumes in pyrometallurgical operations. For instance, because Cd and the Zn ore sphalerite co-occur (Cao et al., 2004). Cd is mostly acquired as a by-product of the Zn refining process. Table 1 shows the remediation mechanisms for specific heavy metal contamination in soil. As a result, a sizable quantity of heavy metals are annually transferred from the tainted Earth's crust aquifer to various environmental compartments, including soil, water and air.

Physical Methods

Contaminated soil can be remediated by replacing soil that is considered as physical remediation. Partly contaminated soil can be replaced by non-contaminated soil. In the early ages, excavation, off-site disposal and replacement of soil were the common methods to clean up contaminated sites. This method helps in diluting the concentration of heavy metal in soils and it improves the functionality of soil (Yao et al., 2012).

Replacement of soil includes two methods, one is spading and other one is importing. Soil spading involves the digging deeply into the contaminated sites to dilute the concentration of heavy metals. While importing new soil involves introducing clean soil into polluted soil with heavy metals. The extra soil is either covered on the surface or it can be blended to reduce the metal content (Douay et al., 2008). This approach minimizes the impact on the environment and effectively isolates the polluted soil and habitat. But this technique is expensive because of high labour work and good for highly contaminated soils with small areas. Costs for long distance transport of excavated soil may be substantially higher and this technique may not be applicable to agricultural sites because soil may lose its fertility.

Soil isolation: It is another method to remediate the heavy metal contaminated soil by removing and separating contaminants from uncontaminated soil, but for complete remediation there is still need of other additional engineering procedures (Zheng et al., 2004). Soil isolation technology prevents heavy metal contamination of groundwater when other cleanup approaches are not cost-effective or physically possible. In some circumstances, toxic areas are temporarily isolated to avoid transportation during site assessment and remediation.

Vitrification: The procedure was found to be highly effective in a cleaning heavy metal contaminated waste materials and large amounts of soil. Vitrification is easiest approach to remediate heavy metals from contaminated soil (Trifunović, 2021). It can be used to treat the majority of soils polluted with inorganic heavy metals and organic pollutants. The temperature during this method is very important for the immobilization of heavy metals in soil sample (Navarro et al., 2013). This technique is easily applicable however it has limitation that it is costly because of high energy requirement.

Electrokinetic: Soil is remediated by an approach which is cost-effective and called as electrokinetic. Liu et al., 2018). In order to do this, a method for producing a suitable electric field gradient on both sides of an electrolytic tank filled with contaminated soil must be developed. To reduce heavy metal pollution from soil, heavy metals can be readily separated via electric seepage, electrophoresis, or electro-migration. Studies demonstrated that an electro-kinetic field improved Pb, As, and Cs dissolution and reduced soil pH to about 1.5 (Mao et al., 2016). It increased overall compound's solubility and bioavailability. The soil which has very low permeability can easily be used for this remediation technique and it can work efficiently (Hanson et al., 1992).

Chemical Methods

Contaminated soil with heavy metals can be remediated by chemical methods such as immobilization technique, encapsulation, and soil washing. Immobilization is the method that works as adding immobilizing of chemicals to remediate the contaminated soil in order to minimize the mobility, bioavailability and bioaccessibility of heavy metals. Heavy metals can be immobilized by adsorption processes, complexation and bioavailability. Heavy metals are re-disturbed from soil solution to solid particles as a result of these activities, which stops their mobility and bioavailability in soil. Bio solids and animal manures are the two major organic amendments utilized to immobilize heavy metals. Heavy metals are typically present in bio-solids, which are treated and stabilized solid organic residual by-products; however, improvements in sewage and wastewater treatment methods are effectively lowering the levels of heavy metals in bio-solids. The detrimental effects of using bio-solids as a source of heavy metal contamination have been documented in a number of research (Cele and Maboeta, 2016). Benefits of immobility is that it is quick and simple to use, reasonably priced, and effective against a wide range of inorganic contaminants. This method is a temporary solution for remediation and it requires permanent monitoring in it. Apart from these techniques we can further discuss about soil washing. It is chemical method which can be used for organic and inorganic extractants and reagents (Guo et al., 2012). These reagents and extractants can easily leach out heavy metals from contaminated soil. Previously, it has proven as an alternative for leaching out heavy metals by using suitable extractants to some extent and it is conventional method for clean-up strategies. Heavy metals have been mobilized and extracted from contaminated soil using a variety of reagents, including organic acids, humic compounds, surfactants, cyclodextrins, and synthetic chelating agents (EDTA, EDDS) (Kulikowska et al., 2015). Other soil washing chemicals include high concentrations of salt, chloride solution such as iron (III) chloride and calcium chloride (Makino et al. 2008).

Biological Methods

Phytoremediation: The efficacy of phytoremediation, a method of employing plants to remove heavy metals from contaminated locations, depends on the amount of contaminated soil, the bioavailability of the metal contaminant, and the accumulation of metals as biomass by the plant (Tak et al., 2012). Locoweed is a plant that can help in removing high amounts of selenium. This selenium is usually present in the tissues of plants. Plants have been tested and utilized to alleviate heavy metal contamination in wetlands

and soils since the 1970s. Phytoremediation is the technology that is going on peak and is emerging technique that uses different plants to extract, contain, degrade or immobilize the heavy metal contaminations from soil and water (Glick, 2003). Plants have the ability to eliminate and stabilize heavy metal pollutants as well as break down or degrade organic pollutants. Sites contaminated with organic contaminants are remedied using slightly different techniques than those used to phytoremediate metal contamination. This technique is economically viable and it is more likely to be accepted by people as it provides aesthetical pleasure. The procedures for remediating the heavy metals contamination from soil include phytoextraction, phytodegradation, phytodesalination, rhizofiltration, phytostabilization, rhizodegradation, phytoavaporation, and phytovolatilization (Awa and Hadibarata, 2020). Dicotyledons such as *Thlaspi caerulescens* can help in reducing the concentration of cadmium and zinc, *Alyssum sp.* can help in reducing Nickel, *Brassica junica* helps in minimizing lead and zinc. Grasses like *Vetiveria zizanioides* help in lowering Zn species are *Paspalum notatum*, *Stenotaphrum secundatum*, *Pennisetum glaucum* and *Vetiveria zizanioides* (Wuana and Okieimen 2011).

Bioremediation: Bioremediation is a technique that is used for removing heavy metal toxicity from contaminated soil to reduce the effects of these contaminants instead of using plant technique. This process involves using microorganisms to increase the capacity of phyto- extraction of plants. It is economical comparing to physical and chemical methods. It takes less time period for remediation and helps in enhancing the growth of plants. Heavy metals can easily be up taken and translocation can be easy. However, it depends on the microorganism specie, soil type and also on the type of metals but public acceptance is extremely high in this method. This strategy can be possible in two ways, one is in- situ and other one is ex-situ. In-situ method of clean up contaminated soil with heavy metals is called in-situ bioremediation. It calls for adding nutrients to contaminated soil to encourage various microorganisms to consume or break down the toxins and pollutants of heavy metals. It also calls for introducing new microorganisms into the environment or using genetic engineering to enhance existing microorganisms to degrade particular contaminants (Rayu et al., 2012), while ex-situ bioremediation is a method that requires the clean up by moving the contaminated media from its original place to a different one for treatment, depending on the factors including treatment cost, depth of contamination, type of pollutant, extent, and geographic location (Jan et al., 2014).

Conclusion

Soil contamination is now global concern and it needs to be remediated by using various techniques, methods and strategies such as physical, chemical and biological. These mechanisms required strong background knowledge of effects, and impacts on soil, plants or food chain and also on human beings. Physical remediation includes replacement of soil (spading and importing), soil isolation, vitrification, and electrokinetics. Chemical remediation includes immobilization and soil washing. Biological remediation includes phytoremediation (phytoextraction, phytodegradation, phytodesalination, rhizofiltration, phytostabilization, rhizodegradation, phytoevaporation, and phytovolatilization), and bioremediation. Future directions for heavy metal soils focus on the integration of physical, chemical and biological methods to improve efficiency and sustainability. A combination of techniques such as phytochemicals and soil changes (such as biochar and nanomaterials) can improve metal remediation and at the same time restore soil fertility. Advances in genetic engineering and microbial vaccines provide promising equipment to support soil detoxification and healthy plant growth. These integrated approaches aim to ensure safe harvest production and protect human and animal health in polluted environments. This review discusses the effects of heavy metals on the environment and how they can be effectively remediated using plants and microorganisms. It further discusses various mechanisms utilized by these organisms for remediating heavy metal contamination. These strategies are essential to tackle soil contamination because heavy metals are toxic compounds, and they harm fertility of soil, affect the growth of plants and are hazardous to human health.

References

- Abd-Alla, M. H., Morsy, F. M., El-Enany, A. W. E., Ohyma, T., (2012). Isolation and characterization of a heavy-metal-resistant isolate of *Rhizobium leguminosarum* bv. viciae potentially applicable for biosorption of Cd^{2+} and Co^{2+} . *International Biodeterioration & Biodegradation* **67**, 48-55.
- Abd-Alla, M. H., Omar S. A., Karanxha, S., (2000). The impact of pesticides on arbuscular mycorrhizal and nitrogen-fixing symbioses in legumes. *Applied Soil Ecology* **14**(3): 191-200.
- Awa, S. H., Hadibarata, T. (2020). Removal of heavy metals in contaminated soil by phytoremediation mechanism: a review. *Water, Air, & Soil Pollution* **231**(2): 47.
- Bååth, E., (1989). Effects of heavy metals in soil on microbial processes and populations (a review). *Water, Air, and Soil Pollution* **47**: 335-379.
- Beesley, L., Moreno, J. E., Fellet, G., Carrijo, L. and Sizmur, (2015). Biochar and heavy metals. *Biochar for environmental management*, Routledge: 563-594.
- Cao, X., Leena, Q. M., Cong, T. (2004). Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). *Environmental Pollution* **128**(3): 317-325.
- Cele, E. N., Maboeta M. (2016). A greenhouse trial to investigate the ameliorative properties of biosolids and plants on physicochemical conditions of iron ore tailings: Implications for an iron ore mine site remediation. *Journal of environmental management* **165**: 167-174.
- Chaney, R. L., Broadhurst, C. L., (2010). Phytoremediation of soil trace elements. *Trace elements in soils*: 311-352.
- Chen, W., H. Li (2018). Cost-effectiveness analysis for soil heavy metal contamination treatments. *Water, Air, & Soil Pollution* **229**: 1-13.
- Douay, F., et al. (2008). Impact of a smelter closedown on metal contents of wheat cultivated in the neighbourhood. *Environmental Science and Pollution Research* **15**: 162-169.
- Ensink, J. H., Mahmood, T., Hoek, W. V. D., Sally, L., (2004). A nationwide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one? *Water policy* **6** (3): 197-206.
- Förstner, U. (1995). Non-linear release of metals from aquatic sediments. Biogeochemistry of pollutants in soils and sediments: *Risk assessment of delayed and non-linear responses*: 247-307.
- Fuge, R., Glover, S. P., Pearce, N. J., Perkins, W. T., (1991). Some observations on heavy metal concentrations in soils of the Mendip region of north Somerset. *Environmental Geochemistry and Health* **13**: 193-196.
- Glick, B. R. (2003). Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnology advances* **21**(5): 383-393.

- Jan, A. T., Azam, M., Ali, A., Mohd, Q., Haq, R., (2014). Prospects for exploiting bacteria for bioremediation of metal pollution. *Critical Reviews in Environmental Science and Technology* **44**(5): 519-560.
- Järup, L. (2003). Hazards of heavy metal contamination. *British medical bulletin* **68**(1): 167-182.
- Jin, Y., Luan, Y., Ning, Y., Wang, L., (2018). Effects and mechanisms of microbial remediation of heavy metals in soil: a critical review. *Applied Sciences* **8**(8): 1336.
- Kabata-Pendias, A. (2000). Trace elements in soils and plants, CRC press.
- Khodadoust, A. P., Reddy, K. R., Maturi, K.,(2004). Removal of nickel and phenanthrene from kaolin soil using different extractants. *Environmental Engineering Science* **21**(6): 691-704.
- Kulikowska, D., Gusiatin, Z. M., (2015). Humic substances from sewage sludge compost as washing agent effectively remove Cu and Cd from soil. *Chemosphere* **136**: 42-49.
- Kumar, P. N., Dushenkov, V., Motto, H., Rasken, I., (1995). Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental science & technology* **29**(5): 1232-1238.
- Li, J., Yu, H., Laun, Y., (2015). Meta-analysis of the copper, zinc, and cadmium absorption capacities of aquatic plants in heavy metal-polluted water. *International Journal of Environmental Research and Public Health* **12**(12): 14958-14973.
- Liu, L., Wei, L., Song, W., Guo, M., (2018). Remediation techniques for heavy metal-contaminated soils: Principles and applicability. *Science of the total environment* **633**: 206-219.
- Norvell, W. (1984). Comparison of chelating agents as extractants for metals in diverse soil materials. *Soil Science Society of America Journal* **48**(6): 1285-1292.
- Nyamangara, J. (1998). Use of sequential extraction to evaluate zinc and copper in a soil amended with sewage sludge and inorganic metal salts. *Agriculture, ecosystems & environment* **69**(2): 135-141.
- Ogoko, E. (2014). Evaluation of polycyclic aromatic hydrocarbons, total petroleum hydrocarbons and some heavy metals in soils of Nnpc Oil Depot Aba Metropolis, Abia State, Nigeria. *IOSR J Environ Sci Toxicol Food Technol* **8**(5): 21-27.
- Qiao, W., Zhang, Y., Xia, H., Luo, Y., Liu, S., Wang, S., Wang, W., (2019). Bioimmobilization of lead by *Bacillus subtilis* X₃ biomass isolated from lead mine soil under promotion of multiple adsorption mechanisms. *Royal Society open science* **6**(2): 181701.
- Ramos, L., Hernandez, L. M., Gonzalez, M. J., (1994). Sequential fractionation of copper, lead, cadmium and zinc in soils from or near Donana National Park, *Wiley Online Library*.
- Rayu, S., Karpouzas, D. G., Singh, B. K., (2012). Emerging technologies in bioremediation: constraints and opportunities. *Biodegradation* **23**: 917-926.
- Shiowatana, J., McLaren, R. G., Chanmekha, N., Samphao, A., (2001). Fractionation of arsenic in soil by a continuous-flow sequential extraction method. *Journal of environmental quality* **30**(6): 1940-1949.
- Tak, H. I., Ahmed, F., Babalola, O. O., (2012). Advances in the application of plant growth-promoting rhizobacteria



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