



Phytoremediation of Heavy Metal Contaminated Soils: Mechanisms, Applications and Enhancement Strategies Using Brassica Species

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Abstract

*Environmental pollution continues to escalate at an alarming rate due to anthropogenic activities including urbanization, technological advancement, unsafe agricultural practices, and rapid industrialization. Heavy metals such as nickel (Ni) represent particularly persistent environmental contaminants that pose severe threats to ecosystems and human health through their toxicity, bioaccumulation potential, and capacity to induce oxidative stress via reactive oxygen species (ROS) generation. Bioremediation has emerged as an eco-friendly and cost-effective alternative to conventional remediation methods for heavy metal contamination. This comprehensive review examines phytoremediation as a promising bioremediation strategy, focusing particularly on the mechanisms of phytoextraction and the potential of Brassica species as effective hyperaccumulators. The analysis covers nickel toxicity in plants, including its detrimental effects on growth, physiology, photosynthesis, and antioxidant systems. Various enhancement strategies for phytoremediation are evaluated, including the application of organic amendments (compost, biochar), chelating agents (EDTA, citric acid), and microbial inoculants to improve metal uptake and plant tolerance. The review synthesizes current knowledge on the use of Brassica species—notably *B. juncea*, *B. napus*, and *B. oleracea*—for heavy metal remediation, highlighting their accumulation capacities, tolerance mechanisms, and the factors influencing their remediation efficiency. The integration of multiple approaches, including combined amendments and microbial-assisted phytoremediation, presents a viable path forward for effective and sustainable remediation of heavy metal-contaminated environments.*

1. Introduction

Pollution of the environment keeps on increasing at an alarming rate due to the activities of man such as urbanization, technological advancement, unsafe agricultural practices and rapid industrialization which degrades the environment. Heavy metals released into the environment are persistent due to their toxicity which poses a severe threat to organisms exposed to high levels of such pollutants. Metals are essential to the biological functions of plants and animals but at elevated levels, they interfere with metabolic reactions in systems of organisms. Toxic heavy metals such as nickel (Ni) which is not useful to plants, is capable of reducing plant growth due to reduced photosynthetic activities, plant

mineral nutrition, and reduced activity of essential enzymes (Nematian & Kazemeini, 2013). It is cytotoxic at low concentrations and could lead to cancer in humans (Dixit et al.). These toxic metals could accumulate in the body when consumed in contaminated food through the food chain and become health risks to living organisms (Tak, Ahmad, & Babalola, 2013). This causes oxidative stress, an unevenness involving the production of free radicals and the capacity of cells to eradicate them or repair the damage. This leads to base damage through formation of reactive oxygen species (ROS) which includes oxygen radicals (superoxide and hydroxyl) and non-radical derivatives of molecular oxygen (O₂) such as hydrogen peroxide (H₂O₂), as well as breakage of the DNA molecule (Ojuederie & Babalola, 2017). Heavy metal toxicity increases the production of ROS thereby

decreasing the antioxidant systems (glutathione, superoxide dismutase, etc.) which protect cells. If this condition continues, the normal functioning of the organism is affected and may invariably lead to cell death. Bioremediation is gradually being accepted as the standard practice for the restoration of heavy-metal-contaminated soils since it is more eco-friendly and cost effective compared to the conventional chemical and physical methods, which are often very expensive and ineffective when metal concentrations are low, in addition to producing significant amounts of toxic sludge (Ayangbenro & Babalola, 2017).

2. Bioremediation

Bioremediation is a technique used to remove environmental contaminants from the ecosystem. It utilizes the biological mechanisms inherent in microbes and plants to eradicate hazardous pollutants and restore the ecosystem to its original condition (Ayangbenro & Babalola, 2017). The basic principles of bioremediation involve reducing the solubility of these environmental contaminants by changing pH, the redox reactions and adsorption of contaminants from polluted environment. Bioremediation technologies are based on redox processes which focus on modifying the chemistry and microbiology of water by injecting selected reagents into contaminated water to enhance the degradation and extraction of various contaminants by in situ chemical oxidation/reduction reactions. Redox reactions involve chemically transforming harmful contaminants into innocuous or less toxic compounds that are more stable, less mobile or inert (Tandon & Singh, 2016). Redox reactions in contaminated soil sediments and groundwater are often affected by the physicochemical properties of the medium, but this can be manipulated by addition of organic and inorganic amendments such as composts and biochar (Bolan, Kunhikrishnan, & Naidu, 2013). The application of organic amendments such as compost in metal-contaminated soils could cause differences in the soil microbial population by changing pH, decreasing the solubility of heavy metals and increasing allochthonous microbial biomass and available nutrients (Albuquerque, De La Fuente, & Bernal, 2011).

Biochar is a product of pyrolysis of biomass obtained from sources such as crop residue, manure and solid wastes which can be used to stimulate microorganisms for bioremediation by making the environment more favorable (Ok, Uchimiya, Chang, & Bolan, 2015). Biochar has the ability to donate, accept or transfer electrons within their environments abiotically or through biological pathways (Saquing, Yu, & Chiu, 2016). It has been suggested by some researchers that biochar may also facilitate microbial electron shuttling processes since they display similar functional characteristics to soil redox-active organic matter (Yuan et al., 2017). Biochar acts by increasing the pH of contaminated soils thereby affecting the bioavailability of heavy metals for plant uptake. The mobility and toxicity of many elements, such as chromium, selenium, lead, arsenic, nickel and copper, rely basically on their oxidation states which, in turn, are

controlled by the redox reactions (Tandon & Singh, 2016). The effectiveness of bioremediation depends on several factors such as the nature of the organisms utilized, the prevailing environmental factors at the contaminated site, as well as the degree of the pollutants in that environment (Azubuike, Chikere, & Okpokwasili, 2016). Bioremediation can be achieved with the use of microorganisms (microbial bioremediation) which depends on the metabolic potential of the microorganisms to degrade environmental pollutants and change them to innocuous forms through redox processes (A. T. Jan, Azam, Ali, & Haq, 2014). It can also be carried out by plants which bind, extract and remediate pollutants from the environment (phytoremediation). The level of contaminated soil, the bioavailability of the metal contaminant, as well as the accumulation of metals as biomass by the plant, are critical to the success of phytoremediation as a means of eradicating heavy metals from contaminated sites using plants (Tak et al., 2013).

Bioremediation could be in-situ or ex-situ. In-situ bioremediation is an onsite cleanup process of contaminated environments which involves supplementing contaminated soils with nutrients to stimulate microorganisms in their ability to degrade contaminants, as well as add new microorganisms to the environment or improve the indigenous microorganisms to degrade specific contaminants using genetic engineering (Mani & Kumar, 2014). Utilization of natural microorganisms in the environment for in situ bioremediation is affected by the non-availability of suitable nutrient levels and/or environmental setting at the polluted location. Ex-situ bioremediation involves taking the contaminated media from its original site to a different location for treatment based on the cost of treatment, deepness of contamination, pollutant type and the extent of pollution, geographical locality and geology of the contaminated site (Azubuike et al., 2016).

3. Types of Bioremediation

Microorganisms are essential in remediation of heavy-metal-contaminated environments as they have a variety of ways to endure metal toxicity. The exploitation of microorganisms to sequester, precipitate, or change the oxidation state of numerous heavy metals has been widely studied. Bioremediation of heavy metals will be successful if a consortia of bacterial strains is utilized rather than using a single strain culture (Kang, Kwon, & So, 2016).

Phytoremediation deals with the cleanup of organic pollutants and heavy metal contaminants using plants and rhizospheric microorganisms (S. Jan & Parry, 2016). It is inexpensive, eco-friendly and an efficient means of restoration of polluted environments especially those that of heavy metals. Nonetheless, the level of soil contamination, the quantity of metal contaminant in the soil, as well as the ability of plants to aggressively take up metals from the soil, determine the success of phytoremediation at any polluted site. Plants utilized in phytoremediation are the hyperaccumulators with a very high heavy metal

accumulation potential and little biomass efficiency, and non-hyperaccumulators which possess lesser extraction capacity than hyperaccumulators, but whose total biomass yield is substantially higher and are fast-growing species (Abbaszadeh-Dahaji, Omidvari, & Ghorbanpour, 2016).

3.1 Phytoextraction/Phytoaccumulation

Phytoextraction involves the uptake and movement of metal pollutants in the soil through plant roots into above-ground components of the plants, based on the mechanism of hyperaccumulation. Hyperaccumulator plants take up metals in large quantities from contaminated soils, then transport and accumulate them in organs above the ground at concentrations from 100 to 1000 times higher than those found in non-hyperaccumulating species without suffering any apparent phytotoxic effect (Rascio & Navari-Izzo, 2011), hence they are very suitable for phytoremediation. These plants are usually found growing in areas with long-lasting metal contamination in soil over time and produce abundant biomass that can be easily harvested. Following criteria for hyperaccumulator plants based on metal concentrations in dried foliage (Cd 100, Co, Cu, Cr 300, Pb, Ni 1000, Zn 3000 and Mn ($\mu\text{g/g}$ respectively). Based on these criteria, about 500 taxa have been identified as hyperaccumulators of some metals and the popular ones are representatives of the following families: *Brassicaceae*, *Caryophyllaceae*, *Violaceae*, *Fabaceae*, *Euphorbiaceae*, *Lamiaceae*, *Asteraceae*, *Cyperaceae*, *Poaceae*, *Cunouniaceae* and *Flacourtiaceae* (Van der Ent, Baker, Reeves, Pollard, & Schat, 2013).

In recent times, much interest has been placed on the use of sunflower (*Helianthus annuus*) for phytoremediation of organic pollutants and heavy metals due to the plant's ability to take up heavy metals from the environment. The site of accumulation of these heavy metals differs from one plant to another. Some authors have reported accumulation of heavy metals mainly in the roots of sunflower with little movement from the roots to the above ground mass, while others reported effective movement from the roots to above ground mass (Adesodun et al., 2010). Recent studies showed that distribution of heavy metals in organs of sunflower is selective for each metal since 59% Pb accumulated in the leaves and as low as 1% accumulated in the seeds. Similar observations were made for Zn and Cd which accumulated 47% and 79% in the leaves of sunflower, respectively. Hyperaccumulator plants such as certain species within the *Brassica* genus (*Brassica napus*, *Brassica juncea* and *Brassica rapa*) are fast growers with high biomass (Angelova, Ivanova, Ivanov, Perifanova-Nemska, & Uzunova, 2016). An ornamental hyperaccumulator plant *Micranthemum umbrosum* (J.F. Gmel.) S.F. Blake was able to remove a higher percentage of As (79.3–89.5% from 0 to 1.0 g mL^{-1}) compared to Cd (60–73.1% 0.3 to 30.0 g mL^{-1} Cd). The results revealed the efficiency of *M. umbrosum* as an efficient accumulator of Cd and a hyper-accumulator of As toxicity. Despite the benefits of

phytoextraction, its effectiveness can be hampered if the heavy metal concentration is very high, few biomass is produced by the plant or its growth rate is slow, which will hinder metal uptake (Islam, Ueno, Sikder, & Kurasaki, 2013). In such cases the phytoextraction process can be enhanced by using chelators such as citric acid and EDTA which increase mobility of soil heavy metals, or the use of organic supplements such as chicken manure which has been proven to increase growth of the species *Rorippa globosa* and decreased soil-extractable Cd and concentrations of Cd (Paz-Ferreiro, Lu, Fu, Méndez, & Gascó, 2014). The type of soil present at a polluted site and the degree of metal contamination determines the rate at which hyperaccumulating plants can remediate that site. Therefore, research must be designed to identify hyperaccumulating plants that are fast-growing with the ability to accumulate abundant biomass and be tolerant to several metals. Research towards identification of hyperaccumulating plants should focus on evaluation of the effect of metal stress on beneficial microorganisms within the rhizosphere and crops, and developing better applications of bioremediation technologies for remediating metals from contaminated soils. The efficiency of phytoextraction is based on several factors which include:

The choice of plant used, (b) the degree of plant tolerance to higher concentrations of heavy metals and (c) the capacity of plants to drastically take up heavy metals and move them from the roots to exposed surfaces which are essential for the phytoextraction process. Phytoextraction can be commercially viable; besides removal of heavy metals from the soil, it also produces biomass with added value. Phytoextraction is the most preferred method used by plants for remediation of polluted environments as it is enhanced by plant growth promoting rhizobacteria (PGPR) associated with the plant roots (Jutsz & Gnida, 2015).

3.2 Advantages of Bioremediation:

- Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contamination increase in number when contaminant is present; when the contaminant degraded, the biodegradative population declines.
- Less energy is required as compared to other technologies.
- Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.
- Bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products.
- Instead of transferring contaminants from one environment medium to another, for e.g. from land to water or air, the complete destruction of target pollutants

is possible (Kulshreshtha, Agrawal, Barar, & Saxena, 2014).

4. Nickel in Plants

Nickel is an essential microelement for normal plant growth and development and part of several biological functions. It is an integral part of several enzymes such as glyoxalase-I and urease required for nitrogen metabolism in higher plants (Mustafiz et al., 2014). Ni being a vital element contributes significant role in nitrogen assimilation as well as helps plants against numerous biotic and abiotic stresses. Therefore, Ni deficiency in plants may lead to affect negatively and symptoms such as retarded plant growth, senescence, reduced N metabolism and reduced Fe uptake can be seen in Ni-deficient plants. Although Ni is an essential element, it may cause toxicity at higher concentrations and could lead to several deleterious alterations in plants. Excessive Ni results in Ni toxicity and symptoms like leaf chlorosis may appear with addition to growth inhibition, reduced photosynthesis and respiration, and mineral nutrition disorders, sugar transport and water relations. Ni toxicity also plays a prominent role in inhibition of plant root growth, but it is difficult to show a comprehensive mechanism because Ni toxicity affects several metal ions and metabolic pathways (Shahzad et al., 2018).

5. Ni Toxicity and plant growth and physiology

Excessive Ni concentration also influences structural and anatomical dynamics of plants. Higher concentrations of Ni have been shown to reduce vegetative growth such as plant height and overall biomass production in several agricultural crops. Ni stress has detrimental effects on ultrastructure of leaves and thickness of mesophyll cells, size of vascular bundles, vessel diameter and width of leaf epidermal cells of wheat. Excessive Ni decreased volume of intercellular spaces, palisade and mesophyll sponge in *Brassica oleracea* plants. In addition to the disruption of general metabolic processes, Ni toxicity is also known to reduce the plasticity of cell walls. Elevated levels of Ni in the soil altered various physiological process in plants and toxicity symptoms including chlorosis and necrosis were observed in numerous plant species especially in rice (*Oryza sativa* L.). Membrane functionality disruption and ion balance in cytoplasm are the major consequences of Ni toxicity in plants (Saad, Kobaiissi, Robin, Echevarria, & Benizri, 2016). Several researchers have reported that Ni stress disturbs water relations such as osmosis and diffusion. Leaf water potential, stomatal conductance, transpiration rate and total moisture contents were decreased under Ni stress and a strong correlation with Ni toxicity and leaf area was found. Reduced leaf area of *Cajanus cajan* plants grown in sand under NiCl (1 mM). From the fact that Ni is essential for optimal growth and development of plants at lower concentrations, nonetheless higher Ni concentrations can affect the physiology and anatomy of plant that ultimately determines the fate of plants (Shahzad et al., 2018).

5.1. Nickel toxicity and antioxidant enzyme activities

Maintaining metabolic functions and stress conditions is crucial for plants to survive under stressed conditions. Therefore, balance between generation and scavenging of oxidative indices is required, which is achieved by enzymatic and non-enzymatic antioxidants (Wu, Tito, & Giraldo, 2017). Hence, ability of plants to cope oxidative stress is characterized by the degree of antioxidant activities (Shakeel Ahmad Anjum et al., 2015). However, activities of these antioxidants may vary with the duration, crop species and plant parts under any stress conditions (Shakeel A Anjum et al., 2017).

5.2. Nickel toxicity on photosynthesis

Nickel toxicity may hinder photosynthesis by inhibiting activities of key enzymes of Calvin cycle such as Rubisco, 3-phosphoglycerate kinase, fructose-1,6-bisphosphate, aldolase and NAD- and NADP-dependent phosphoglyceraldehyde dehydrogenase. Photosynthetic inhibition in *C. cajan* leaves following several days of incubation with 1 mM NiCl₂ solution. These findings suggest that Ni stress might have targeted the key enzymes of photosynthesis. In some cases, accumulation of ATP and NADPH would be resulted from the inhibition of Calvin cycle reactions, later it develops a pH gradient across the thylakoid membranes, ultimately blocking the activity of PSII. It further triggers stomatal closure limiting the photosynthesis through blockage of CO₂ uptake. These devastating effects of Ni toxicity on several other metabolic processes would amplify direct inhibition of photosynthesis. In crux, Ni-toxicity results in photosynthetic inhibition by disturbing normal functioning of Calvin cycle and electron transport chains. Due to inefficient biochemical pathway reactions, excessive production of ATP and NADPH may result in overall photosynthetic inhibition (Shahzad et al., 2018).

Phytoremediation is a promising technique for heavy metal remediation as it involves use of hyper accumulating plants for the uptake of metals from soil and their deposition in harvestable plant parts (Kamran et al., 2014). About 48 members of Brassicaceae family can accumulate up to 3% of Ni in their leaves. For instance, hyper accumulator plants of Brassicaceae *Alyssum caricum* Dudley and *Thlaspi oxyceras* (Boiss.) Hedge can accumulate 12273 and 13778 mg/kg of Ni in leaves respectively. However, this range is wider in genus *Alyssum* L. than rest of the hyperaccumulator plants of this family (ALTINÖZLÜ, KARAGÖZ, Polat, & ÜNVER, 2012).

6. Brassica and its role in phytoremediation

The genus *Brassica* (Brassicaceae) includes more than 30 species plus several varieties and hybrids. Among these are several important species in agriculture, used for human consumption, animal fodder, condiments, oil production, biofuel, among others. Common vegetables used for human consumption are included in this genus, including several

Brassica oleracea varieties (like cabbages, broccoli, cauliflower and Brussel sprouts). They are considered a source of many nutrients such as carotenoids, tocopherols, different essential elements, carbohydrates and amino acids. Several *Brassica* species are known metal accumulators and have been evaluated as potential phytoextraction plants. The fact that some of these plants can accumulate relatively high amounts of toxic metals, without visible symptoms, and are also food crops, leads to potential contamination of the food chain and this has to be taken into account in any phytoremediation process. The potential use of *Brassica* species in phytoremediation (mainly phytoextraction) stems from its intrinsic tolerance to heavy metals and considerable above-ground biomass production (Mourato et al., 2015).

Brassica juncea (Indian mustard) is important in oil production, has medicinal properties and is used as a condiment. It is a tolerant plant to heavy metals, grows fast and produces large amount of above-ground biomass. Due to these characteristics, this species has been the target of several studies to evaluate its phytoremediation potential. However, the presence of heavy metals has been reported to reduce the amount of oil produced by these plants (Ahmad et al., 2015).

Brassica napus (rapeseed) is consumed as a vegetable but its main use is as a source of oil, being one of the largest sources of edible oil in the world (Mourato et al., 2015). The by-products from oil production are used in animal feed.

Brassica oleracea varieties include very common vegetables used for human consumption, like cabbages, broccoli, cauliflower and Brussel sprouts among others. They are an important source of many nutrients, of compounds with antioxidative activity and of other bioactive compounds such as some glucosinolates that are recognized as beneficial for human health (Gonçalves, Alegria, & Abreu, 2013).

Brassica carinata is mainly cultivated in Ethiopia as an oil crop, although it has also received some attention as a source for biofuel production. Both *B. rapa* and *B. nigra* are also used for the oil content of its seeds. There is an increasingly large body of literature describing the effects of heavy metals in different *Brassica* species, confirming the relatively high tolerance that they have towards this abiotic stress, however the exact mechanism for this tolerance remains to be fully clarified (Kumar, Mahajan, & Yadav, 2012).

Several works have been published comparing the performance of several *Brassica* species to toxic concentrations of heavy metals. (Mourato et al., 2015) made an extensive study regarding the heavy metal tolerance of different species (including several varieties of *B. campestris*, *B. rapa*, *B. napus*, *B. oleracea* and *B. carinata*), confirming that they have high levels of tolerance mainly to Zn, and less to Pb and Cd, the metals under study. (Purakayastha et al., 2008) also analyzed the phytoextractive capacity of five different species (*B. juncea*, *B. campestris*, *B. carinata*, *B. napus* and *B. nigra*) to four heavy metals, Zn, Cu, Ni, and Pb. They concluded that *B. carinata* performed better in relation

to Zn, Ni and Pb, and would thus be a better candidate for phytoremediation purposes, than the other species, especially *B. juncea*, usually considered very tolerant. In relation to Cu, *B. juncea* presented the highest uptake of this metal. On the other hand, the tolerance of three *Brassica* species to a multi-contaminated soil and, based on a calculated tolerance index, concluded that the tolerance order was *B. juncea* > *B. carinata* > *B. oleracea*. They also observed that these *Brassica* species behaved as Zn and Pb excluders, able to maintain an almost constant level of these metals in the shoots, up to a certain level of toxicity. In yet another study comparing the phytoextraction behavior of *B. juncea*, *B. napus* and *B. carinata*, these species demonstrated similar potential for both Cd and Zn. The accumulation of different metals under study (Cd, Cr, Cu, Ni, Pb and Zn) was more pronounced in shoots than in roots as is typical of an accumulator species. *B. napus* and *B. juncea* accumulated more Cu in roots than in shoots under Cu stress for 7 and 14 days in hydroponic solution (Feigl et al., 2013). Naturally the concentrations of the heavy metal under study and the duration of the effect are very important experimental parameters. In addition, not only plant species but also the specific variety/cultivar can have different behavior in regard to heavy metal uptake and effects. Some authors perform the experiments in hydroponics because it is easier to control all other conditions, besides the variable under study. Even so, different hydroponic techniques, nutrient concentrations and pH can also affect plant development. The experiments in soil are usually more complex as the number of variables affecting heavy metal uptake also increase (like soil type, pH, organic matter etc.). Some authors use soil contaminated over years of anthropogenic activity while others spike the soil and perform the experiment after a few days or weeks of stabilization and this can have a large impact on the results obtained. Other parameters like the age of the plant at contamination are also critical to the effects usually measured. Within the same species there is variability in the uptake capacity of different varieties, and even of different accessions (Mourato et al., 2015).

7. Techniques for Improving Heavy Metal Uptake and Tolerance

In order to enhance the availability of heavy metals to allow the effective use of phytoextraction processes, several chemical chelating agents have been proposed to increase the uptake of metals by plants and the translocation from roots to shoots but without affecting plant growth (Gupta, Huang, & Corpas, 2013). Several studies have been performed in *Brassica* species, with different chelating agents (Neilson & Rajakaruna, 2015). The addition of citric acid to the contaminated medium led to increased uptake and amelioration of *B. napus* in relation to heavy metal (Shakoor et al., 2014). (S,S)-ethylenediamine-N,N'-disuccinic acid (EDDS) was even more effective than NTA in facilitating the phytoextraction of a multi-contaminated soil. This EDTA-

isomer had the added advantage of a rapid degradation in the soil, reducing the potential leaching into ground waters.

While EDTA itself has also been reported to increase the uptake of heavy metals from contaminated soils by *Brassica* species, there are concerns regarding the environmental persistence of this chelating agent and its strong chelating capacity (Mourato et al., 2015). Another strategy to improve soils contaminated with heavy metal is the use of organic amendments to reduce the bioavailability of the metals to the plants and/or to improve plant growth. The usage of *B. juncea* for phytoextraction at a multi-contaminated site with and without added amendments (cow manure and compost). They concluded that plant growth and metal uptake was highly dependent on soil pH, although the added amendments improved plant growth. They also calculated that between a minimum of 1150 years for Cu and a maximum of 360,000 years for Pb would be necessary for the effective clean-up using *B. juncea* as a phytoextractor obviously making this technique completely unsuitable. *B. napus* plants where they reported that the addition of municipal solid waste compost improved the extraction of heavy metals by those plants, however they calculated that more than 1000 years would be necessary to clean up a contaminated site, also making this plant unsuitable for phytoextraction purposes (Brunetti, Farrag, Rovira, Nigro, & Senesi, 2011). The behavior of canola (*B. napus*) in a multi-contaminated soil and concluded that although it presented moderate tolerance to the heavy metals in the soil (including high levels of Cd, Cr, Cu, Ni, Pb and Zn), it was not adequate for phytoremediation purposes.

Possibly the combined toxicity of all these metals overcome the defense and tolerance mechanisms of the plant. In their study with *B. napus* var. *oleifera*, confirmed the relatively high tolerance of this plant to another multi-contaminated soil (Mourato et al., 2015). The effect of the application of biochar in *B. napus* plants growing in contaminated soil. Biochar is reported to reduce the availability of heavy metals to plants and to increase the soil quality allowing improved plant growth. The authors confirmed that the addition of 5% and 10% biochar to the soil reduced the uptake of the metals and significantly improved rapeseed growth compared to the control. These results lead to the conclusion that while the addition of biochar makes unfeasible the use of rapeseed for phytoextraction due to the reduced metal uptake, it could be an effective strategy for phytostabilization. Other strategies to improve the phytoremediation capacity of *Brassica* species include the inoculation of plants with certain bacteria with the aim to promote the growth of the plant in a contaminated medium and thus improving the metal uptake capacity (Houben, Evrard, & Sonnet, 2013).

8. Phytoremediation by different amendments

Phytoremediation is a green, sustainable remedial option that can be adopted to remediate soil that is contaminated with a mixture of organic and metal contaminants (Chirakkara & Reddy, 2015a). The capability of the plants to

uptake contaminants, survive in contaminated soil and the low bio-availability of the contaminants in the soil are some of the limiting factors that influence phytoremediation efficiency. Phytoremediation can be enhanced either by increasing the capability of contaminant uptake by the plant or amending the soil to increase the bioavailability of the contaminants. Plant uptake of contaminants can also be increased by the use of transgenic plants (Bhargava & Srivastava, 2014) or the inoculation of engineered entophytic bacteria (Bell, Joly, Pitre, & Yergeau, 2014). By improving the growth of the plants, their water and nutrient uptake will be increased, which leads to increased contaminant uptake.

Plant uptake of contaminants will also be affected by ion competition at the soil and plant level (Chirakkara & Reddy, 2015a). The addition of organic matter to the soil can improve the soil and increase the plant biomass in phytoremediation (Masciandaro, Macci, Peruzzi, Ceccanti, & Doni, 2013). Biochar can immobilize metals in the soil through the surface sorption of the metals and also improve the biological activity in the soil (Paz-Ferreiro et al., 2014). It can release essential nutrients that are beneficial for plant growth (Uchimiy, Lima, Klasson, & Wartelle, 2010). The biochar amendment of the soil can improve its water holding capacity and porosity and improve the soil structure to promote better plant growth (Glaser, Wiedner, Seelig, Schmidt, & Gerber, 2015).

These properties make biochar a suitable option to amend soil in order to enhance phytoremediation. Only a limited number of researchers have addressed the combination of biochar application and phytoremediation for the remediation of metal contaminated soils (Fellet, Marmiroli, & Marchiol, 2014). The addition of compost to the soil can improve plant growth as well as increase soil microbial activity (Chirakkara & Reddy, 2015b). Compost is expected to immobilize the metal contaminants in the soil, thus plant germination and growth are expected to improve in composted soil (Alvarenga, de Varennes, & Cunha-Queda, 2014). A number of researchers in the past decade have examined the combination of phytoremediation with a compost application to the soil (Marchand et al., 2016). Most of these studies concentrate on the enhanced biodegradation of organic compounds using the combined method.

Composting can improve plant growth and organic contaminant degradation by ryegrass (*Lolium perenne*) (Chirakkara & Reddy, 2015a). Nutrient solutions were applied to the soil to improve the growth of plants for phytoremediation (Hasan et al., 2015). Even though NPK fertilizer application is expected to improve remediation due to improved plant biomass, there are instances of reduced concentration of metals in plants produced in fertilized soils due to increased yield (Habiba et al., 2015). The bioavailability of metals can decrease in the presence of organic contaminants and become a limiting factor in the phytoremediation of mixed contaminated soils (Chirakkara, Cameselle, & Reddy, 2016). The addition of chelating agents or surfactants to the soil can increase the metal bioavailability by desorbing it from the particle surfaces or by complex

formation. Research shows that the mobility of metals in the soil can be considerably increased by the addition of chelating agents. A number of natural and synthetic chelating agents are available, although their effectiveness varies with plant and soil types. The chelating agent EDTA is commonly used for increasing plant metal uptake of metals (Zaier et al., 2014). Surfactants are the other group of chemicals that can assist in increasing the water solubility of contaminants, so they can be more easily accumulated by plants (Chirakkara & Reddy, 2015a). These are biodegradable soil amendments for enhancing the bioavailability of the contaminants. Most of the phytoremediation studies involving surfactants are conducted on soil contaminated solely with organic contaminants (Chirakkara & Reddy, 2015a). There are also instances where surfactants were used to improve the bioavailability of metal contaminants (Chirakkara et al., 2016). Use of surfactants in co-contaminated soils can improve both phytoextraction and phytodegradation (Sun et al., 2013).

9. Conclusion

The escalating problem of heavy metal contamination in terrestrial ecosystems demands effective, sustainable, and economically viable remediation strategies. Phytoremediation, particularly through phytoextraction using hyperaccumulator plants, represents a promising green technology that aligns with ecological restoration principles. Among the various plant species investigated for this purpose, Brassica species demonstrate significant potential due to their relatively high metal tolerance, substantial biomass production, and capacity to accumulate heavy metals in harvestable parts. The success of phytoremediation depends on multiple factors, including plant selection, metal bioavailability, soil characteristics, and environmental conditions.

Nickel, while essential in trace amounts, becomes highly toxic at elevated concentrations, disrupting various physiological and biochemical processes in plants, including photosynthesis, mineral nutrition, antioxidant systems, and overall growth. The use of Brassica species for nickel phytoremediation shows considerable promise, though their efficiency can be enhanced through strategic interventions. Organic amendments such as compost and biochar improve soil conditions and plant growth while modifying metal bioavailability. Chelating agents increase metal mobility and uptake, though their environmental persistence requires careful consideration. Microbial inoculants, particularly plant growth-promoting rhizobacteria, enhance plant tolerance and metal accumulation through various mechanisms.

Future research should focus on developing integrated phytoremediation systems that combine the strengths of multiple approaches—selecting superior hyperaccumulator genotypes, optimizing amendment applications, and harnessing beneficial plant-microbe interactions. Additionally, attention must be given to the safe disposal or utilization of metal-laden biomass to prevent secondary contamination. While phytoremediation may not provide

immediate solutions for heavily contaminated sites, it offers a sustainable, cost-effective approach for moderate contamination and can be effectively combined with other remediation technologies. The continued advancement of phytoremediation strategies will contribute significantly to global efforts in environmental restoration and sustainable land management, ultimately protecting ecosystem health and human well-being from the threats posed by heavy metal pollution.

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12. Conflict of Interest:

Authors declare no conflict of interest.

13. Authors Contribution:

Hamna Bashir and Amina Rashir: Writing Original Draft; Muhammad Ashir Hameed, Abdul Qadir: Supervision, Writing reviews and corrections.

14. SDGS Addressed

SDG 15 (Life on Land) by remediating contaminated ecosystems and SDG 3 (Good Health and Well-being) by reducing human exposure to toxic heavy metals.

Other strongly connected goals include SDG 12 (Responsible Consumption and Production) through the use of eco-friendly remediation methods and SDG 6 (Clean Water and Sanitation) by preventing groundwater contamination.

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