ISSN: 0973-4929, Vol. 21, No. (**1**) 2026, Pg. 231-242

Current World Environment

www.cwejournal.org

Biochar Amendment Reduces Nickel (II) Toxicity and Enhances Phytoextraction in *Zea mays L.*

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Abstract

The increasing anthropogenic release of heavy metals (HMs) threaten global biodiversity and sustainable agriculture. Phytoremediation of HMs in contaminated soil not only reduces metal bioavailability but also mitigates toxic effects on both plants and their consumers. Biochar is one such agent capable of reducing HMs toxicity. Thus, a pot experiment was conducted to investigate the toxicological effect of heavy metal Nickel (II) on seed germination and uptake efficiency in maize (*Zea mays* L.), an economically important agricultural crop. Soil samples were treated with graded concentrations of Nickel Chloride Hexahydrate ($NiCl_2.6H_2O$) at 100 mg kg^{-1} and 300 mg kg^{-1} and amended with rice-husk-derived biochar (produced at 400°C) at a dose of (1% w/w); a control was used for comparison. After 10 days of treatment, the concentration of Nickel (II) in soil, root and shoot was determined using the Dimethyl glyoxime (DMG) test. Scanning electron microscopy revealed that the high surface area and porous structure of biochar primarily decreased the bioavailability of Ni in the soil by physically trapping Ni (II) ions. The results showed that soil treated with biochar, increased seed germination by 14% at 100 mg kg^{-1} and doubled it at 300 mg kg^{-1} compared to untreated soil. Ni was primarily sequestrated in the roots for both sets of pots. However, biochar significantly lowered Ni accumulation by 25% and 6 % in roots at 100 mg kg^{-1} and 300 mg kg^{-1}, respectively. Furthermore, biochar enhanced the Bioconcentration factor (BCF) by more than two-fold, preventing the plants from being overwhelmed by Ni toxicity by limiting root to shoot transfer (Translocation Factor < 1). This allowed the maize to continue growing and performing phytoextraction over the 10-day experimental period. This study demonstrates that the phytoremediation of Ni-contaminated soil can be effectively achieved using maize and biochar, thereby preventing the transfer of toxicity into the food chain.

Article History
Received: 22 March 2026
Accepted: 04 May 2026

Keywords
Biochar;
Bioconcentration Factor;
Food Chain;
Heavy Metal;
Maize;
Nickel Toxicity;
Phytoremediation.

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Doi: http://dx.doi.org/10.12944/CWE.21.1.17

Introduction

Increasing anthropogenic environmental heavy metals (HMs) pollution is a serious global concern due to its adverse environmental effects. While HMs are natural components of soil, human activities have increased their concentration beyond permissible limits.[1] Waste from tanneries, mining, agricultural and metal industries, waste water irrigation, sewage sludge, and waste dumping contaminate both soil and groundwater. HM contamination frequently includes Arsenic, Cadmium, Chromium, Copper, Lead, Nickel, etc. The use of untreated sewage and wastewater leads to significant HM deposition in agricultural land; these metals are subsequently absorbed by crops and consumed by humans and other animals.[2] Long-term exposure of HMs is deleterious to biological systems- including plants, animals and humans- when concentrations exceed specific threshold levels.[3] Reducing HMs bioavailability is essential not only to mitigate health risks but also to facilitate the total removal of toxic metals from contaminated soil. Various soil remediation techniques, such as phytoextraction, chemical stabilization, and soil washing, have been extensively employed by researchers.[4] The integration of various natural and artificial amendments, including phosphate fertilisers, clay minerals, etc., enhances phytostabilization by promoting the root-mediated sequestration and the absorption of HMs.[5]

In phytotransformation or phytovolatilization-based remediation, plants uptake pollutants and utilize specialized enzymes or metabolic processes to transform the contaminants into volatile derivatives, which are eventually discharged into the atmosphere.[6] In phytofiltration, plants are initially acclimated to contaminated water prior to field transfer for site-scale remediation, once the roots reach pollutant saturation, the plants are harvested.[7] In addition to other remediation techniques, phytoextraction is used to clean up the HMs from contaminated soil, where metals are absorbed by roots and translocated to the aerial parts of the plant.[8] These plants are subsequently harvested and safely disposed. Certain plant groups can contain or reduce HM pollution through phytoremediation, offering an environmentally sustainable and eco-friendly long-term solution for soil reclamation.[2] Chemical immobilisation involves the application of soil amendments to contaminated soil; that reacts with HMs to form less toxic, insoluble complexes, thereby reducing their bioavailability and effectively hindering their absorption by the plants. Among the amendments that adsorb HMs and decrease their bioavailability and uptake, biochar has proved to be cost-effective[9] due to its unique physicochemical properties.[10,11] Biochar is a stable, carbon-dense material derived from the pyrolysis of agricultural wastes, making it a sustainable and environmental friendly material.[12] Biochar's porous structure, containing over 60% carbon, makes it a good adsorbent that improves soil structure and quality.[13] Additionally, biochar has been found to enhance nutrient retention, water holding capacity, and microbial activity in the soil, creating a favourable environment for plant growth and remediation processes.[14,15]

Nickel is recognised as one of the 23 heavy metal pollutants posing a significant threat to both the environment and human health;[16-18] however, its stress effect on plants is not yet fully understood. The concentration of Ni in soil varies significantly, ranging from 0.2 to 450 mg kg^{-1}, depending primarily on the underlying soil formation processes.[19-21] Nickel has long been known as an important plant micronutrient.[22,23] While small concentrations of Ni are essential for normal plant growth, elevated concentrations cause deleterious effects on plant development and trigger Ni toxicity symptoms, such as inhibition of germination, reduced root and shoot growth, chlorosis, foliar necrosis, leaf spotting, etc.[24-26]

Ni reaches the soil from various sources,[27-30] Ni contamination in soil results in widespread exposure across plants and is consumers, affecting various stages of life cycles. According to the World Health Organization (WHO), the target value of Ni in soil is 35 mg kg^{-1}, while the permissible value in plants or crops is 10 mg kg^{-1} [1]. Addressing this issue requires innovative and sustainable solutions to reduce the toxic effects of Ni, especially Ni (II), among the four oxides of divalent Nickel.[31-33]

Experimental results demonstrated that biochar-amended soil achieves substantial removal efficiencies for Ni.[34] The addition of biochar to contaminated soil facilitates the sequestration of HMs, effectively reducing their bioavailability and limiting plant uptake. This process is governed by several complex factors, including specific metal type, soil physical and chemical properties, amount of biochar used, pyrolysis temperature, porosity, and surface area, etc.[9,10] Therefore, the remediation of HMs from polluted soil is a challenging process. The selection of appropriate HM-tolerant plant species is the most important criterion for successful phytoextraction.[35] Native plants are generally selected as a medium for phytoextraction because these plants are evolutionary products of their specific environment. One species used for this purpose is the economically important food crop maize (*Zea mays* L.). Assisted phytoextraction has become an increasingly prevalent technique for the management of contaminated sites. Rice husk biochar pyrolyzed at 400°C may enhance the plant's growth, development and phytoextracting ability of maize.[36] Rice husk biochar is particularly effective due to its high silica content. The optimum pyrolysis temperature creates negatively charged binding sites for Ni (II) via cation exchange capacity (CEC), providing additional sites for metal binding.[37,38] Therefore, in our study, we selected biochar derived from pyrolysis of rice husk at 400°C under oxygen-limited conditions as a soil amendment to improve phytoextraction by maize. Converting rice husk, an agricultural waste, into biochar is a multi-win strategy to preserve soil carbon, improve soil fertility, and immobilize organic and HMs pollutants.[39]

To evaluate the efficacy of biochar in the phytoremediation of Ni, a pot experiment was conducted using maize grown in soil contaminated with 100 mg kg^{-1} and 300 mg kg^{-1} of Ni, with a control set maintained for comparative analysis. The effect of biochar on Ni sequestration in various maize plant parts was also assessed. We expect biochar to increase the germination percentage and growth of maize under high Ni-stress conditions while simultaneously enhancing its bioaccumulating capacity.

Materials and Methods

Preparation of Soil Sample

Garden soil samples were collected, air-dried at room temperature, and passed through a 2mm sieve to remove debris.[40] Sandy clay loam soil, free from exogenous metal contamination, was used for the experiment. The soil used for treatment had a pH range between 7.0 and 8.0, and a water-holding capacity of 80% (Table 1).

Preparation of Contaminated Soil and Pot Experiment:

Nickel Chloride Hexahydrate *($NiCl_2.6H_2O$)* solutions were prepared by dissolving salts equivalent to 100 mg and 300 mg of Ni in 1litre of distilled water, respectively. Prior to sowing, the Ni solution was added to the soil. To ensure uniformity, the air-dried soil was thoroughly mixed with the metal salts through repeated agitation before being distributed into three replicate pots per treatment. Pots were arranged in a completely randomized design, and metal concentration analyses were blinded to treatment identity. After 10 days of incubation, maize seedlings were raised in a 2-inch layer of soil contaminated with Ni (100 mg and 300 mg kg^{-1}).[41-43] Experimental controls were established using three pots of uncontaminated soil. In addition, specific soils were amended with 1% (w/w) biochar.[44] Each concentration set and biochar treatment was executed in three replicates.

Maize seeds (VNR 4226) were purchased from the Bidhan Chandra Krishi Viswavidyalaya (BCKV), West Bengal, India. This variety is partially Ni-tolerant and commonly cultivated by local farmers. The seeds were surface sterilised for 2 minutes in ethanol, followed by 10 minutes in sodium hypochlorite solution (0.75% of Cl)[45] and subsequently washed six times with distilled water.[46] The plants were grown under natural light at ambient temperature and harvested 10 days after sowing. The number of germinated seeds was recorded daily to determine growth parameters, including the germination percentage. Seeds were considered germinated upon the visible protrusion of a 2mm plumule.[47] The equation for determining germination percentage is as follows (1)[48]:

Preparation of Biochar

1kg of rice husk was collected from rice fields post-harvest. After being cut and washed, the husk was air-dried under direct sunlight for 48 hours. The samples were then ground and sieved to obtain a particle size of 0.25 mm. The ground rice husk biochar was prepared by slow pyrolysis in a high-temperature programmable Muffle Furnace (Labocon, LMF-1200). Biochar was produced by heating the biomass at a rate of 10°/min to a peak temperature of 400°C for 2 hours under oxygen-limited conditions.[49,38]

$$\textbf{Germination percentage (GP)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100 \quad ....(1)$$

Characterization of Biochar

Different characterization techniques were employed to analyse the surface morphology and modification feasibility of the rice husk biochar. To ensure the presence of appropriate functional groups required for HM sequestration, Fourier transform infrared spectroscopy (FTIR) analysis was conducted using a Shimadzu FTIR 8400 instrument, over a spectral range of 4000 to 400cm^{-1} (Fig. S1). Additionally, the surface morphological structure of biochar was characterised using a Carl Zeiss SIGMA 300 Scanning Electron Microscope (SEM). Biochar pH, CEC, and surface area were not measured, thereby limiting comparison with other studies.

Table 1: Physio-chemical properties of soil from different heavy metal concentrations.

Treatment	pH	Electrical conductivity (µS cm^{-1})	Organic matter (%)
Control	6.86 ± 0.08	359.70 ± 49.10	0.39 ± 0.00
100 mg kg^{-1}	7.10 ± 0.01	689.67 ± 108.67	0.44 ± 0.01
100 mg kg^{-1} +Biochar	7.87 ± 0.00	1198.74 ± 44.94	0.50 ± 0.00
300 mg kg^{-1}	7.20 ± 0.18	605.89 ± 127.09	0.43 ± 0.02
300 mg kg^{-1} +Biochar	7.36 ± 0.13	892. 85 ± 123.46	0.46 ± 0.01

Impact of Biochar Amendment on the Bioavailability and Tissue Specific Accumulation of Heavy Metals In Maize:

The study assessed the distribution of HMs by analysing their accumulation levels within the soil and plant parts. The concentration of Nickel in the digested soil, roots and shoots extracts was determined by the Dimethylglyoxime (DMG) test.[50,51] The absorbance of resulting mixture was measured at 540 nm using a JASCO V-630 UV-Vis spectrophotometer. Standard curve was prepared using $NiCl_2$ solutions ranging from 0.1 to 10 mg L^{-1} ($R^2 = 0.9758$).

Metal Uptake in Plant

To evaluate the phytoextraction potential of maize, the bioconcentration factor (BCF) and translocation factor (TF) were determined. The value of BCF of hyperaccumulator plants is greater than 1, indicating that the plant is a potential accumulator for the HMs.[52] A translocation factor (TF) greater than 1 signifies the plant's potential as a hyperaccumulator and metal transport to the shoots; conversely, TF<1 indicates a strategy of phytostabilization, where HMs are effectively sequestered within the root system.[53]

Both bioconcentration Factor and translocation factor were calculated as by the equation (2) and (3), respectively.[54]

$$\textbf{BCF} = \frac{\text{Cplant}}{\text{Csoil}} \quad ....(2)$$

$$\textbf{Translocation factor (TF)} = \frac{\text{Cshoot}}{\text{Croot}} \quad ....(3)$$

Where C_{plant} – metal concentrations in the tissues of plant parts, i.e. shoot and root, C_{soil} – initial concentration of metal in the soil. In the case of TF, Cshoot is the heavy metal content in the aerial part and Croot is the total heavy metal content in the underground.

Statistics

All tests were performed in triplicate. Significant differences in plant germination and accumulation parameters with reference to the application of biochar in Ni-contaminated soil were evaluated using Student's t-test. Results were summarised by the mean and standard deviation.

Results

Characterization of Biochar

The detailed surface morphology of the rice husk biochar has been demonstrated through SEM images, suggesting a high potential for HM adsorption. At a magnification of ×1080, the biochar exhibits several irregular voids and a diverse composition, a well-developed porous and rough texture in the first image (Fig. 1A), resulting from the pyrolysis of lignocellulose precursor. These characteristics point to a large surface area, which is advantageous for adsorption and facilitates physical sequestration of Ni.[55] Sharp, ridge-like structures that seem to protrude from the surface of the biochar are highlighted in the second image (Fig. 1 B), which has a greater magnification of ×1860. These sharp edges probably improve the biochar's reactive surface characteristics and mechanical stability, and act as a secondary sink for heavy metals, where ions are immobilised to mitigate phytotoxicity.

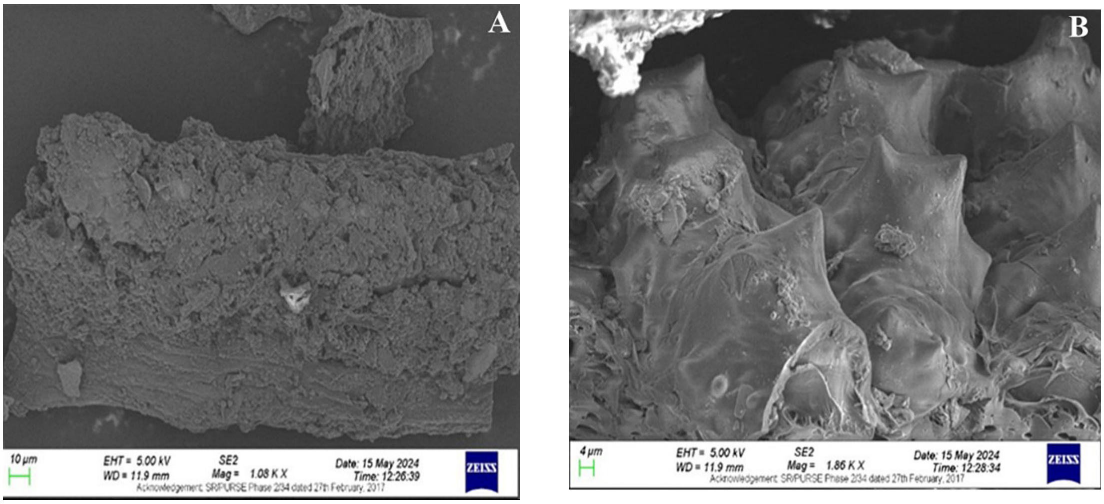

Fig. 1: Scanning electron microscope (SEM) images of 0.25 mm sieved biochar particles. SEM images from the same sample were taken (A) at ×1080 magnification and (B) at ×1860 magnification.

Effect on Germination Percentage

Germination percentage was higher in the control setup, which is 73% as compared to soil contaminated with Ni (33%) at varying concentrations ($p < 0.005$). Although germination percentage increased to about 48±7.70 percent (Fig. 2) when contaminated soil was amended with biochar. A significant effect of biochar was observed on the germination of maize growing at 300 mg kg^{-1} concentration of Ni ($p = 0.03$).

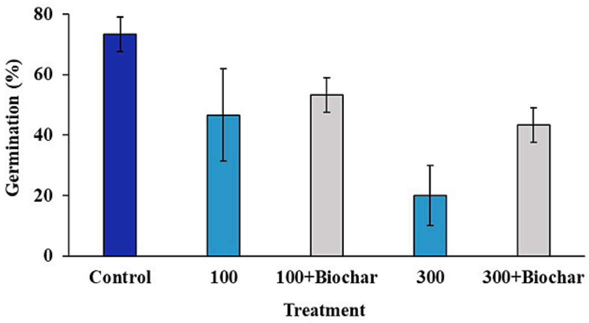

Fig. 2: Germination of potted maize seeds across different Ni concentrations in soil treatments 100 to 300 mg kg^{-1} with and without biochar. The error bar represents standard deviation.

Nickel Accumulation in Soil and Plant Parts

Various concentrations of Ni were used in a pot experiment, treated with and without biochar. Bioavailable Ni was significantly reduced in the soil treated with biochar ($p < 0.005$) than without the application of biochar (Fig. 3). At 100 mg kg^{-1} biochar, the accumulation of Ni in roots was significantly reduced ($p < 0.01$), but had no significant effect on Ni deposition in roots at higher concentrations ($p = 0.6$). It has no significant effect on Ni accumulation in the shoot across all treatments (Fig. 4).

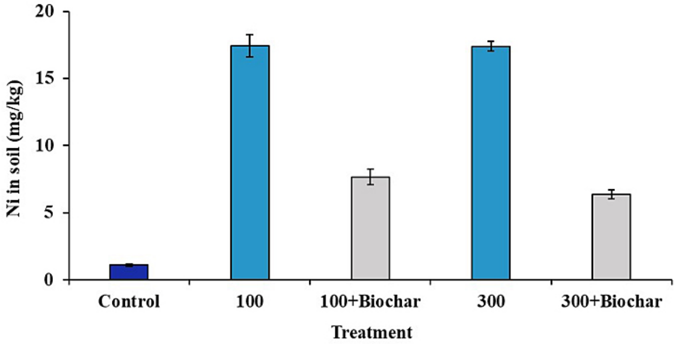

Fig. 3: Bioavailable concentrations of Nickel (mg kg^{-1}) across different Ni treatments in soil 100 and 300 mg kg^{-1} with and without biochar. The error bar represents the standard deviation.

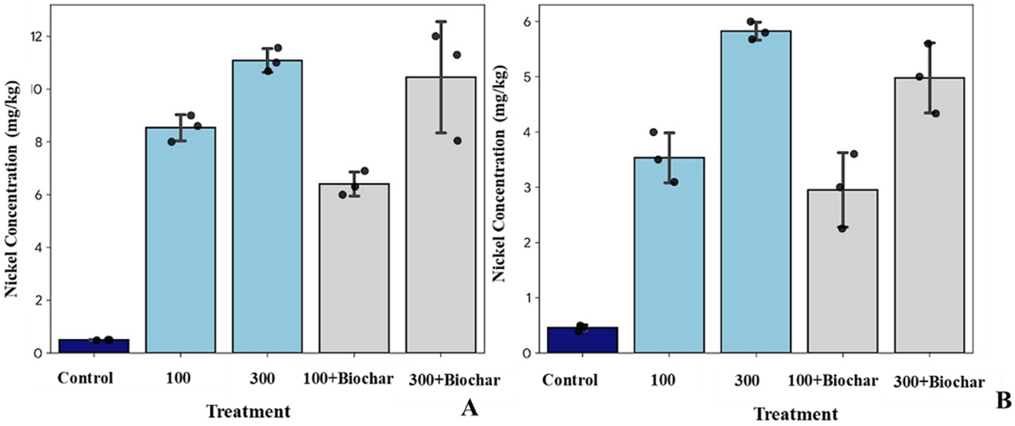

Fig. 4: Accumulated Ni concentration in maize plant parts, (A) root and (B) shoot across different Ni soil treatments 100 to 300 mg kg^{-1} with and without biochar. The error bar represents standard deviation.

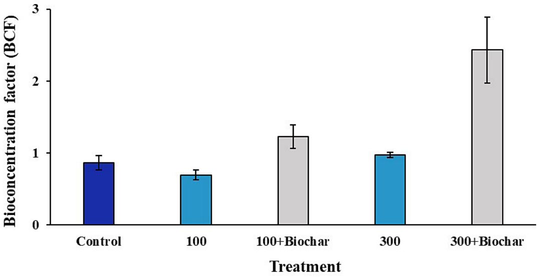

Fig. 5: Bioconcentration of Ni in maize plant across different soil treatments from 100 to 300 mg kg^{-1} with and without biochar. The error bar represents standard deviation.

BCF of the maize plant growing in control soil was 0.8. In comparison, at a concentration of 100 mg kg^{-1}, BCF was 0.6 without biochar, which was enhanced to 1.2 with the application of biochar. At a higher concentration of Ni at 300 mg kg^{-1}, BCF doubled to 2.4 from 0.9 by the addition of biochar.

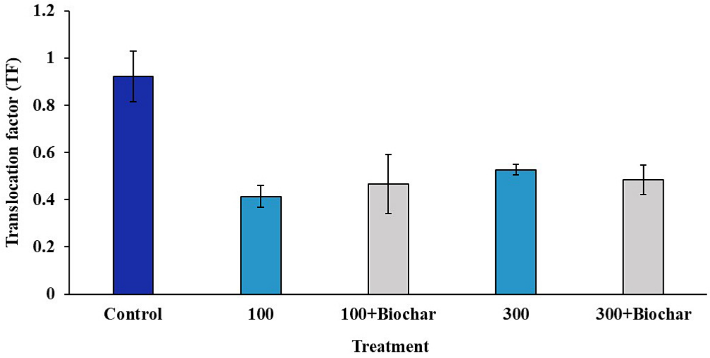

Fig. 6: Translocation of Ni in maize plant across different soil treatments 100 to 300 mg kg^{-1} with and without biochar. The error bar represents standard deviation.

BCF significantly increased with application of biochar at both 100 mg kg^{-1} (Ni 95% CI [0.5315, 0.8560]; Ni+Biochar 95% CI [0.8275, 1.6292], Cohen's d = 4.3431, p = 0.02) and at 300 mg kg^{-1} (Ni 95% CI [0.8834, 1.0610]; Ni+Biochar 95% CI [1.2977, 3.5631], Cohen's d = 4.5088, p = 0.03) (Fig. 5). In all cases TF remains <1 (Fig. 6); biochar has no significant effect on TF.

Discussion

Ni is an essential metal needed for the growth of plants, but become highly toxic when plants are grown in soil with excessive Ni concentrations.[20] One of the signs of Ni toxicity is the inhibition of germination and a delay in germination time.[25] Some authors suggest that elevated Ni concentration in the growth medium impairs the germination of rice seeds by triggering Ni stress; this restricts the hydrolysis process and delays the mobilisation of reserve protein within the endosperm.[20] The phytotoxic impact of excessive Ni can significantly reduce the germination percentage.[56] In our study, treatment with Ni at 100 mg kg^{-1} and 300 mg kg^{-1} decreased the germination percentage compared to the control set. At optimum concentrations, Ni is known to improve seed germination in many species.[57,58] Therefore, a high percentage of maize seeds germinated in the control set. According to the WHO, the regulatory limit of Ni in soil is 35 mg kg^{-1} and the permissible value in crops is 10 mg kg^{-1} [1] In this study, bioavailable Ni in soil did not exceed the WHO permissible limit in all treatment cases; however, at the higher concentration, i.e. 300 mg kg^{-1}, the Ni concentration in the roots exceeded the permissible limit. Seminal work on Ni uptake kinetics shows that the bioavailability of Ni in soil and its uptake by plants is influenced by several factors, including the soil Ni concentration,[56] soil or soil solution acidity,[59] and plant species,[20] etc. Nearly half of the accumulated Ni is retained within the roots,[56] which is attributed to its sequestration at the cation exchange sites of the vessel walls and xylem parenchyma; this process effectively immobilises the metal[56]. The soil used in our experiment was slightly alkaline (pH: 7.6), which may result in immobilisation of Ni, thereby reducing its bioavailability to the plant.[20]

The addition of biochar further increases soil alkalinity (pH: 8.0). This reduces metal solubility by promoting precipitation into hydroxides or carbonates and enhancing binding with soil particles.[60] This mechanism represents an indirect metal immobilisation by biochar amendments, which reduces the bioavailability of Ni and renders the soil less toxic to plants. This finding is supported by several studies.[61-63]

Although maize is often cited as a potential hyperaccumulator,[64,65] the BCF of maize in all our experimental cases remained <1, without biochar application. Biochar amendments improved plant growth and enhanced the phytoextraction of Ni; consequently, the BCF of the biochar-treated maize plants at both concentrations increased to >1.

However, no significant differences in shoot Ni concentrations were observed between the plants treated with and without biochar. Furthermore, the translocation factor was also lower than 1 in all cases of treatment except for the control set. Studies describe phytostabilization as stabilization and accumulation of HM by the root but outside in the soil and rhizosphere,[66] whereas phytoextraction refers to the accumulation of HMs inside root tissue.[67] Since most of the Ni is accumulated in the roots, here maize is referred to as a phytoextracting plant.

A similar result has been reported in studies with the hyperaccumulator *Alyssum murale* growing at Ni rich environment. Here, shoot Ni concentration was also not affected by the biochar amendments, despite the enhancement of germination and growth.[68,9,69,70]

The ultrastructural image of biochar prepared by the pyrolysis of rice husk at 400°C exhibits a highly heterogeneous surface characterised by micro and mesopores, with a rugged and fractured appearance. This structure is suitable for providing a high surface area for Ni binding sites and helps in mitigating Ni toxicity by CEC.[37,38] A study with cottonseed hull biochar obtained within a temperature range of 200-800°C demonstrated that the soil matrix controls metal sequestration (Nickel, Copper, Lead and Cadmium) through the surface functional groups of the biochar.[71] Thus, the prevalence of oxygen-containing functional groups in low- temperature biochar makes it a superior adsorbent for inorganic contaminants, facilitating sequestration through electrostatic attraction and precipitation.[39]

As expected, the addition of biochar improved phytoextraction of Ni in contaminated soil by maize (*Zea mays* L.). As an amendment, it improves the plant germination and survivability by restricting shoot Ni uptake and sequestering the majority of Ni within the maize roots. However, the effectiveness of biochar as a phytoremediating agent of heavy metals is influenced by several factors[39] and further studies are required to evaluate its long-term efficacy.

Conclusion

Although maize is considered a hyperaccumulator plant, the BCF of Ni in maize for both treatments (100 and 300 mg kg^{-1}) without biochar application was less than 1, with metals primarily sequestered in the roots of maize. Therefore, the present study indicates that maize (*Zea mays*. L) does not efficiently extract Ni from highly contaminated soil independently. Our findings suggest that assisted phytoremediation using biochar is a feasible approach for Ni-contaminated soil. Although heavy metal in maize roots decreased, biochar application resulted in an increase in the bioconcentration factor (BCF >1), especially at higher concentrations, but with limited translocation (TF < 1). The positive effect on germination percentage following biochar application indicates that using *Zea mays* L. in conjunction with biochar could be a suitable strategy for reclaiming soil contaminated with heavy metals like Ni.

Thermochemical conversion of rice husk into biochar offers a strategic approach for the productive repurposing of agricultural wastes, mitigating the disposal challenges. The application of cost-effective biochar for the remediation of HM-contaminated soil offers a dual environmental advantage by simultaneously immobilising contaminants and facilitating carbon sequestration.

Care should be taken when generalizing these findings to field application or to biochar derived from different feedstocks and production methods. A comprehensive evaluation of the long-term sustainability of these assisted phytoremediation techniques is essential for their transition from laboratory to field-scale application. Supplementing contaminated sites with biochar or chemical fertilizers can drive the continuous biomass production essential for the effective and large-scale extraction of toxic Ni from the environment.

Acknowledgement

The authors are thankful to Guru Nanak Institute of Pharmaceutical Science and Technology, West Bengal, for providing the necessary instruments and equipment for the experiment.

Funding Sources

This author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The authors will provide the primary data if and whenever required.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

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Not Applicable

Author Contributions

- **Munmun Kundu:** Conceptualization, Methodology, Data Collection, Analysis, Visualization, Writing- Original Draft
- **Souparna Chakrabarty:** Analysis, Visualization, Writing- Review and Editing
- **Swati Chakraborty:** Conceptualization, Supervision

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