

RESEARCH PAPER

Bioremediation of Heavy Metals in Tannery Effluent Contaminated Soil Using Biochar

Kiruthika¹, Deepika¹, Sinduja², Akhila² and Gowri Shankar^{1*}

¹Department of Biotechnology, St. Peter's College of Engineering and Technology, Avadi, Chennai, India

²Department of Environmental Sciences, National Agro Foundation, Research & Development Centre, Anna University Taramani Campus, Taramani, Chennai, India

*Corresponding author: gowrishankarba@spcet.ac.in (ORCID ID: 0000-0003-0612-3741)

Received: 29-03-2025

Revised: 22-05-2025

Accepted: 04-06-2025

ABSTRACT

This study examines how biochar can help remove heavy metals, like chromium (Cr), from soils contaminated by tannery effluent. Biochar is a carbon-rich material made by heating organic matter in the absence of oxygen. It has been found to improve soil health by enhancing its structure, water retention, and nutrient availability, while also reducing the levels of toxic metals in the soil. The incorporation of 10% biochar resulted in a substantial 90% reduction in Cr uptake by the plant, demonstrating its efficacy in sequestering heavy metals and limiting their bioavailability. Biochar not only stabilized Cr within the soil matrix but also improved soil health by enhancing structure, moisture retention, and nutrient availability. FTIR analysis confirmed the presence of various functional groups indicative of chemical transformations and interactions between biochar and soil contaminants. Peaks related to O-H, C≡C, C=C=N, and C=C=C stretching vibrations suggest the formation of active sites responsible for metal immobilization. The results underscore the potential of biochar to enhance phytoremediation performance and contribute to sustainable soil management strategies in heavy metal-contaminated environments.

HIGHLIGHTS

- ① Biochar application markedly reduced chromium uptake in contaminated soils.
- ② It enhanced soil health by improving structure, moisture retention, and nutrient availability.
- ③ FTIR analysis verified biochar-contaminant interactions, supporting heavy metal immobilization.
- ④ The presence of biochar boosted the phytoremediation efficiency of *Nerium oleander*.
- ⑤ Overall, biochar represents a sustainable soil management approach for heavy metal-contaminated environments.

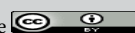
Keywords: Biochar, Phytoremediation, Heavy metal remediation, Chromium (Cr), *Nerium oleander*, Tannery effluent, FTIR spectroscopy, Soil amendment, Environmental sustainability, Contaminated soil

Soil is fundamental to agriculture and essential for the survival of humans and other living organisms. In recent years, the levels of heavy metals such as nickel, arsenic, and mercury in soil have been rising due to the discharge of various wastes from metal smelting factories, mining operations, the paint industry, coating processes, and sewage irrigation from farmland (Jiang *et al.* 2020). This increase significantly raises the risk of heavy metals entering the food chain, posing a serious threat to human health (Campos *et al.* 2020). When released improperly, this effluent contaminates both soil

and groundwater, leading to toxicity, acidification, and decreased fertility. Heavy metals, especially chromium, interfere with nutrient absorption and damage soil microorganisms, while organic pollutants and salts can block soil pores, resulting in poor water infiltration and compaction (Mandal *et al.* 2021). Over time, this contamination can cause toxins to accumulate in plants, diminishing

How to cite this article: Kiruthika, Deepika, Sinduja, Akhila and Shankar, G. (2025). Bioremediation of Heavy Metals in Tannery Effluent Contaminated Soil Using Biochar. *Int. J. Bioresource Sci.*, 12(01): 85-94.

Source of Support: IUCEA; **Conflict of Interest:** None



agricultural productivity and threatening human health (Li *et al.* 2023). Extended exposure can lead to irreversible soil degradation, rendering it unfit for cultivation. To combat these issues, strategies such as effluent treatment, chromium stabilization, wastewater recycling, and phytoremediation are essential for reducing harmful effects and restoring soil health. Therefore, alongside enforcing strict regulations on heavy metal discharges, effective remediation methods for already contaminated soil must be developed (Yang *et al.* 2021).

The presence of high levels of heavy metals in soils is a significant environmental issue, as it poses serious risks to agricultural productivity and human health. Among the heavy metals that raise the most concern regarding environmental impact and toxicity, chromium (Cr) is often regarded as one of the most dangerous pollutants resulting from human activities (Chen *et al.* 2021). Various industrial processes, including dye production, leather tanning, electroplating, and steel manufacturing, have significantly contributed to the excessive release of Cr into the environment, affecting nearby water systems and soil (S.-W.C. Chien *et al.* 2021). Additionally, Cr can be released directly into the environment from ultramafic sources like serpentine soils and sediments (Dilshara *et al.* 2025). The tanning industry is increasingly recognized as a major contributor to soil pollution, as it discharges large amounts of effluents and sludge containing a range of contaminants, including organic and inorganic nitrogen compounds, chromium, sulfides, suspended solids, and dissolved solids (Hasan 2021).

The growth of certain plants in soil contaminated by tannery waste can lead to the accumulation of high levels of chromium in their edible parts (Dilshara 2025). Prolonged consumption of these metal-accumulating plants, such as *Zea mays L.*, *Allium sativum L.*, and *Brassica napus L.* (Bousdra *et al.* 2023), may pose significant health risks, especially when their consumption results in metal concentrations exceeding toxicity thresholds, even for essential micronutrients like copper, manganese, and zinc. Therefore, it is crucial to restore these heavy metal-laden soils using innovative and cost-effective technologies before they can be safely utilized for agriculture. Various methods have been employed to reduce or eliminate (Dhaliwal *et al.* 2020)

heavy metals in soil, including physical, chemical, and biological treatments. The physical method primarily involves the removal of contaminated soil and replacing it with soil from uncontaminated areas, which can be expensive and requires long-term management (Rahman *et al.* 2023). On the other hand, chemical remediation utilizes techniques such as vitrification, chemical leaching, chemical fixation, and electrokinetic remediation to extract heavy metals from the soil, but these methods can also be quite costly (Dhaliwal *et al.* 2020). Bioremediation is a commonly employed method for cleaning contaminated soils, which involves using native or introduced microorganisms and/or organic materials like compost, animal manure, and plant residues to detoxify or convert toxic metal forms into less harmful ones (McGrath *et al.* 2020; Wao *et al.* 2023; Yang *et al.* 2021). Some biochar, influenced by the source of biomass feedstock and the production conditions, can be a porous carbon material characterized by a high cation exchange capacity and alkaline properties (Nath *et al.* 2022). This allows heavy metals in the soil to be adsorbed through the complexation of metal ions with various functionalities present on the outer and inner surfaces of biochar. Moreover, heavy metals may exchange with metal cations such as Ca^{2+} and Mg^{2+} found in the biochar, or they might react with oxygen-containing functional groups within the biochar (Bandara *et al.* 2020). The use of biochar in soil can also reduce the mobility of heavy metals by changing the redox state. It's important to note that the effectiveness of soil remediation with biochar relies on specific characteristics of the biochar itself, such as its surface area, metal content, and alkalinity. These traits can vary greatly depending on the type of biomass used and the pyrolysis or gasification conditions employed. Consequently, the efficiency of heavy metal remediation in soil using biochar may differ based on the unique properties of the biochar being utilized. Various metals, metal oxides, or inorganic materials such as Fe, Mg, Al, P, and clay can be incorporated to create biochar composite. Applying post-treatment to biochar with metals, metal oxides, or metal hydroxides can enhance the properties of the biochar composite, owing to its superior ability for complexation, electrostatic interaction, and ion exchange with heavy metals. Several studies have been carried out to explore why biochar composites demonstrate greater efficiency



in removing heavy metals from the soil (Kapoor *et al.* 2024). Findings indicate that a higher formation of metal-ligand complexes, resulting from reactions between functional groups and heavy metals, accounts for the increased rate of physical and chemical adsorption of metals on the surfaces of biochar composites compared to standard biochar. The electron-donating characteristics of doped metals can also help convert toxic heavy metal ions into less harmful intermediate ions (Mandal *et al.* 2020). Additionally, when heavy metal ions replace metal ions in the biochar structure, they can immobilize those heavy metals. All of these factors can improve the effectiveness of soil remediation using biochar composites compared to using biochar alone. Therefore, a thorough understanding of the remediation reaction mechanisms is crucial for enhancing the separation efficiency of heavy metals from contaminated soil.

This study aimed to investigate the use of waste woody biochar derived from bioenergy agricultural waste as a soil amendment to immobilize bioavailable chromium and reduce contamination in soils impacted by tannery effluent. The results indicate that this method could be an effective and environmentally friendly way to manage contamination in soils polluted by tannery effluents. Utilizing biochar may help mitigate the negative effects of tannery waste on soil health. This approach offers a sustainable solution for tackling soil pollution caused by tannery effluents, presenting a promising green strategy for soil remediation.

MATERIALS AND METHODS

Characterization of Biochar and Amendments

The soil employed in this study was obtained from areas in proximity to tannery industries. The biochar was produced from agricultural waste byproducts, including manure, coconut shells, and woody residues. In the agricultural context, this biochar was generated by pyrolyzing the woody biomass of *Ficus religiosa* along with agricultural waste byproducts in a closed reactor at a temperature of 450°C. The biochar produced in this reactor was subsequently air-dried, ground using a blender, and sieved to achieve a particle size of less than 1 mm prior to its use in the experiments. Both the soil and biochar samples were air-dried and sieved to pass through a

1-mm sieve. The pH and electrical conductivity (EC) of the soil and biochar were evaluated in a 1:16 soil-to-water ratio suspension using a digital pH meter and an EC meter, respectively. Soil organic matter was quantified using the Walkley-Black (Alovisi *et al.* 2024) Wet Oxidation Method. To ascertain the total chromium concentration, a portion of the soil samples was digested in aqua regia, and the digested samples were analysed using atomic absorption spectroscopy. The characterization of biochar was summarized in table 1.

Incubation Study

The dried soil sample was obtained to analyze its physical and chemical characteristics, such as pH, electrical conductivity (EC), organic matter (OM), heavy metals (HM), boron, phosphorus, sulfur, calcium, magnesium, potassium, nitrogen, and zinc. Six incubation containers were set up, each filled with 500g of the finely sieved soil sample. Biochar was incorporated in quantities of 5g and 10g into the respective containers, which were then incubated for 24 hours. Both sets of sample containers were given 20 milliliters of distilled water, thoroughly mixed, and left to incubate for 4-5 days. Soil samples were extracted from each incubation container on the 0th, 5th, 10th, 15th, 20th, 25th, and 30th days to evaluate EC, pH, OM, and HM, with the goal of determining the reduction levels of heavy metals.

Pot Experiment

The experiment was employed with two types of soil: untreated soil (control) and soil treated with three different amounts of BC treatments (Chen *et al.* 2022) Dry biochar was mixed with a mass fraction of 5, 10, and 15% (w/w) to enrich the soil. Before being used, the amended soils were well mixed in sizable plastic containers. About 250g of biochar amendment soil was placed inside plastic pots that were 14.5 cm in diameter and 11.5 cm in height. After that, the pots were kept in a darkroom for two weeks so the soil combinations could acclimate. Every therapy was carried out three times. The pots were moved to an outdoor greenhouse after the equilibration phase. Twenty-five *Nerium* seeds were planted in each pot, and the plants were nurtured for nine weeks. Three times a week, 30 cm³ of tap water was used to irrigate each pot.

Statistical Analyses

Statistical analyses were carried out to compare the average results of different BC application rates on the growth of plants and the accumulated concentrations of Cr in plant tissues using a one-way analysis of variance (ANOVA) followed by Fisher's test ($p < 0.05$) for multiple comparisons. Mean separation procedure (least significant different test) and group comparison contrast were used after performing the ANOVA for complete randomized design (CRD). All statistical analyses were carried out using statistical software package.

RESULTS AND DISCUSSION

Initial Characteristics of Soil

The analysis results of a soil sample as shown in table 2 details various physicochemical parameters compared against their permissible limits where applicable. The pH of the soil is within the acceptable range, indicating a neutral to slightly alkaline nature. Electrical conductivity is well below the maximum limit, suggesting low salinity. Organic matter content is reported at 0.95%, contributing to soil fertility, although no specific limit is mentioned. Total nitrogen, phosphorous, and potassium levels reflect nutrient availability, with potassium just above the lower limit of the permissible range, indicating adequate nutrient status. Heavy metals such as chromium and lead are reported as Below Detectable Limit (BDL), which indicates safe concentrations in terms of soil contamination and toxicity.

Chromium and Lead Study Result

The sequence of reduction in Cr (97.1 mg/L – 22.80 mg/L) and Pb (85.01 mg/L – 26.38 mg/L) was observed to be effective with the T_2 upto 30th day. Recent results reveal that the high electronegativity of biochar can facilitate the electrostatic attraction of positively charged ions (Ahmad *et al.* 2016; Ahmad *et al.* 2018). This shows that the biochar of 10 g helps in remediation of Cr and Pb in soil. As compared to lead, the chromium contamination is reduced more.

Details of Pot Experiment

Particulars	Description
Crop name	Kanar

Scientific name	Nerium oleander
Variety	Petite Pink
Soil type	Well-drained sandy or clay soils
Duration	30 days
Date of transplanting	04/03/2025
Date of harvesting	16/04/2025

Table 1: Characterization of biochar

Parameters analyzed	Values
pH	8.3
Electrical conductivity (dS/cm)	0.18
Colour	Black
Total organic carbon (%)	44.4
Total nitrogen (%)	0.21
Total phosphates as P_2O_5 (%)	0.18
Cadmium	BDL
Chromium	BDL
Lead	BDL
Nickel	BDL

Table 2: Heavy metal uptake in plant and initial characteristics of the soil before incubation and incorporation of biochar into the soil

Parameter	Unit	Permissible limit	Obtained value
pH	—	7-7.9	7.5
Electrical Conductivity	dS/cm	4	0.6
Organic Matter	%	—	0.95
Total Nitrogen	mg/kg	—	60.1
Total Phosphorous	mg/kg	—	21.3
Total Potassium	mg/kg	400-3510	412.6
Total Chromium	mg/kg	2.0 ppm	BDL
Total Lead	mg/kg	10 ppm	BDL

BDL- Below deductive limit.

1. Effect of biochar on pH in chromium contaminated pot experiment with *Nerium*

The application of biochar led to a gradual increase in soil pH over a 30-day period in chromium-contaminated pots planted with *Nerium*. At the start, all treatments, including the control, showed similar pH levels (6.9–7.1), but over time, biochar-amended soils displayed a consistent rise in pH compared to the control. By Day 30, the highest pH was observed in T2(10% biochar) at ~7.6, followed by T3 (~7.), T1 (~7.45), and the control (~7.2). This trend indicates that biochar raises soil pH in a



dose-dependent manner, improving soil conditions under chromium stress. Among the treatments, T2 was the most effective, making it the best option for enhancing soil health and potentially reducing chromium toxicity in *Nerium*-grown soils. (Ambika *et al.* 2022), (Yuan *et al.* 2011), (Wardle *et al.* 2008) and (Fowles *et al.* 2007) suggested alkaline biochar may increase the pH of acidic soil to a more neutral pH.

2. Effect of biochar on EC (dS m^{-1}) in the chromium-contaminated pot experiment with *Nerium*.

EC (Electrical Conductivity) levels, indicating soluble salt content in soil, were initially low ($0.1\text{--}0.3 \text{ dS m}^{-1}$) across all treatments. By Day 10, T1 showed a rise to $\sim 0.75 \text{ dS m}^{-1}$, while T2 and T3 remained lower at ~ 0.5 and $\sim 0.3 \text{ dS m}^{-1}$, respectively. On Day 20, EC increased in all groups, with T2 nearing 1.0 dS m^{-1} , followed by T1 and the control, while T3 stayed relatively stable. By Day 30, T2 peaked at $\sim 2.0 \text{ dS m}^{-1}$, followed by T1 (~ 1.7), control (~ 1.2), and T3 (~ 0.9). Generally, the addition of biochar and modified biochar increases the EC content of soil since biochars contain organic matter and nutrients (Dume *et al.* 2016). Overall, these results indicate that higher biochar dosages can significantly influence salinity buildup in soil, with T3 showing the most consistent salinity control.

3. Effect of biochar on Organic carbon (%) in chromium contaminated pot experiment with *Nerium*

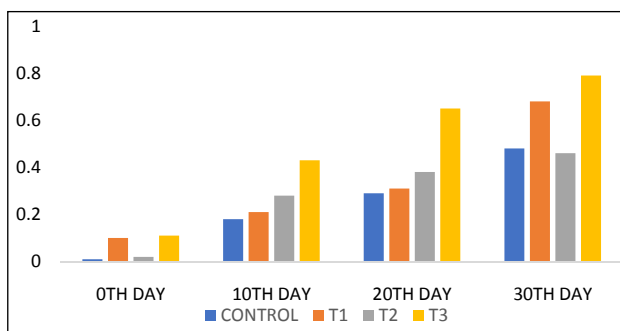


Fig. 1: Shows organic carbon content for different treatments measured on Day 0, Day 10, Day 20, and Day 30. The x-axis indicates the sampling days, while the y-axis represents the organic carbon percentage

As shown in Fig. 1, biochar application led to a steady increase in organic carbon content across all treatment groups. The results of the study clearly demonstrate that biochar application

positively influences the physicochemical properties of chromium-contaminated soil. Among these improvements, the increase in organic carbon content was particularly notable, contributing to enhanced soil structure and fertility. High organic carbon in soils treated with biochar has been also reported by (Lehmann *et al.* 2007). (Solomon *et al.* 2007) and (Liang *et al.* 2006) also revealed the higher organic C and total N at the ancient terra preta compared with adjacent soils. In addition to boosting organic carbon levels, biochar significantly raised soil pH, which can help reduce the mobility and toxicity of heavy metals like chromium. The impact on electrical conductivity (EC), as shown in earlier figures, varied depending on the level of biochar applied—highlighting the importance of optimizing application rates. Overall, the findings, including those illustrated in Fig. 1, emphasize the role of biochar as an effective soil amendment for improving soil health and resilience under heavy metal stress conditions.

Effect of biochar on plant parameters in chromium contaminated pot experiment with *Nerium*

The application of biochar (BC) significantly influenced the growth parameters of *Nerium* plants cultivated in chromium-contaminated soil.

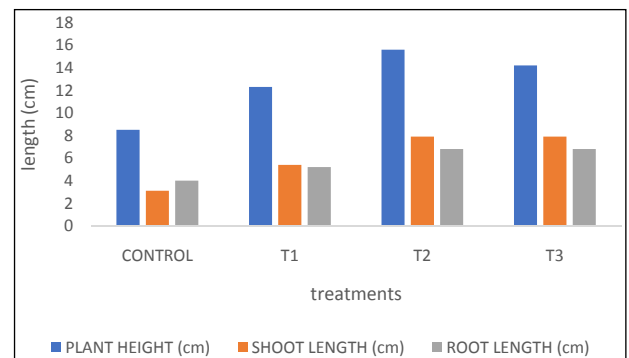


Fig. 2: The results show a progressive increase in plant growth parameters (plant height, shoot length, and root length) as the biochar concentration increased, with the 10% biochar treatment (T2) showing the highest values for all parameters

As illustrated in Fig. 2, there was a clear improvement in plant height, shoot length, and root length with increasing concentrations of biochar. The control group, which received no biochar, recorded the lowest values for all parameters—plant height (8.5 cm), shoot length (3.1 cm), and root length (4.0 cm)—

highlighting the detrimental effects of chromium stress on plant development. With the addition of biochar, plant growth improved noticeably. The addition of BC to TWS had mostly contributed to the promotion of plant growth while enhancing the fertilizing effects. Moreover, these findings agree with those of recent studies (Houben *et al.* 2013) confirming higher plant productivity when BC is applied, likely resulting from the immobilization of Cr in TWS. Among the treatments, T2 (10% BC) demonstrated the most pronounced enhancement across all growth metrics, achieving the highest values: 15.6 cm in plant height, 7.9 cm in shoot length, and 6.8 cm in root length. T3 (15% BC) showed nearly comparable shoot and root lengths to T2 but exhibited a slightly lower plant height (14.2 cm), suggesting that increasing biochar beyond 10% may not further enhance plant growth. T1 (5% BC) also led to moderate improvements compared to the control.

Table 3: Table shows the effects of biochar amendments on plant parameters (plant height, shoot length, and root length) in a pot experiment with *Nerium* plants grown in chromium-contaminated soil. The experiment involved different treatments where biochar (BC) was added to the soil at varying concentrations: 0% (control), 5% (T1), 10% (T2), and 15% (T3)

Treatments	Plant height (cm)	Shoot length (cm)	Root length (cm)
Control	8.5	3.1	4
T ₁	12.3	5.4	5.2
T ₂	15.6	7.9	6.8
T ₃	14.2	7.9	6.8
Mean	4	4	4
SEd	1	1	1
CD	1	1	1

Control- unamended soil, T₁ – 5% BC-amended soil (Biochar), T₂ – 10% BC-amended soil, T₃ – 15% BC-amended soil.

Chromium accumulation in *Nerium* plant (Pot experiment)

The influence of biochar on plant biomass and chromium (Cr) accumulation in *Nerium* grown in Cr-contaminated soil was evident from the observed data. As detailed in Table 4, there was a progressive increase in plant biomass with increasing biochar application rates. The highest biomass was recorded in T2 (10% biochar) at 30.3 g per plant, followed by

T3 (15%) with 28.9 g, T1 (5%) with 22.9 g, and the lowest in the control at 15.9 g. These results suggest that biochar played a significant role in alleviating Cr-induced stress, potentially by immobilizing heavy metals, improving soil nutrient retention, and enhancing overall soil structure. Chromium uptake also followed a similar trend, increasing alongside biomass. According to (Girish *et al.* 2010), the amount of Cr adsorbed to soils increased with an increase in pH and CEC of soils T2 again showed the highest Cr uptake (3030 µg/plant), followed by T3 (2890 µg/plant), T1 (2290 µg/plant), and the control (1590 µg/plant). The data in Table 4 indicate a strong correlation between biomass accumulation and Cr uptake, reflecting the dual benefit of biochar in promoting plant growth and enhancing phytoextraction capacity. However, the slightly lower values in T3 compared to T2 hint at a possible saturation point, beyond which additional biochar does not further enhance effectiveness.

Table 4: Comparison of the biomass of *Nerium* plants and accumulated concentrations of Cr in plant tissues due to the influence of different biochar application rates were observed in table 4

$$\text{Biomass (g)} = \frac{\text{Cr concentration } (\mu\text{g/g})}{\text{Cr uptake } (\mu\text{g/plant})}$$

Treatments	Cr content in above ground biomass (µg g ⁻¹)	Cr content in root biomass (µg g ⁻¹)	Biomass of <i>Nerium</i> (g)	Cr uptake (µg/plant)
Control	1160	400	15.9	1590
T ₁	1770	520	22.9	2290
T ₂	2350	680	30.3	3030
T ₃	2210	680	28.9	2890
Mean	800	400	24.5	1200
SEd	200	100	2.48	300
CD	2	1	2.8	3

Bioconcentration factor (BCF), the Translocation factor (TF) and Enrichment factor (EF) for *Nerium* in pot culture experiment

Biochar application significantly affected chromium uptake, accumulation, and biomass production in *Nerium oleander* grown in contaminated soil. As biochar concentration increased, key



phytoremediation indices Bioconcentration Factor (BCF), Translocation Factor (TF), and Enrichment Factor (EF) also rose, peaking at 10% biochar (T2). BCF increased from 4.0 (control) to 6.8 (T2 and T3), TF rose from 2.90 to 3.46 in T2, and EF from 11.6 to 23.5 in T2 before slightly declining in T3 which is illustrated in Fig. 4. These findings suggest that moderate biochar levels enhance chromium uptake and internal movement within the plant, while higher concentrations may favor metal immobilization in the roots. In conclusion, T2 emerged as the most effective treatment, striking a balance between promoting plant growth and maximizing chromium extraction, emphasizing the importance of optimizing biochar dosage for efficient phytoremediation.

Table 5: Presents the calculated values of Bioconcentration Factor (BCF), Translocation Factor (TF), and Enrichment Factor (EF) of *Nerium oleander* grown in chromium-contaminated soil amended with different concentrations of biochar (T1, T2, and T3), compared to the untreated control. All three indices showed an increasing trend with the application of biochar, peaking in treatment T2

Treatments	Bio-concentration factor (BCF)	Translocation factor (TF)	Enrichment factor (EF)
Control	4.0	2.90	11.6
T ₁	5.2	3.40	17.7
T ₂	6.8	3.46	23.5
T ₃	6.8	3.25	22.1

Table 6: Significant increase in peak position which was obtained in FTIR spectra

Peak position	Group	Class	Peak details
3584-3700	O-H stretching	Alcohol	Medium, sharp
2100-2140	C≡C stretching	Alkyne	Weak
2000	C=C=N stretching	Ketenimine	Medium
1900-2000	C=C=C stretching	Allene	Medium

Effects of BC on the growth of plants

Fig. 5 illustrates the changes in chromium reduction in *Nerium* plants cultivated in soil amended with biochar (BC) which was analysed through FTIR. The introduction of BC significantly enhanced chromium reduction, particularly with a 10% BC application. In contrast, the lowest reduction of

chromium was noted in *Nerium* plants grown in soil with only 5% BC. It is an established fact that low organic matter and Cr accumulation may disturb the soil structure and results in reduced crop growth (Ruttens *et al.* 2006) Six weeks post-sowing of *Nerium* seeds, symptoms of chromium toxicity became evident in the aerial parts of the plants in the BC-amended soil. The chromium levels in plants grown in soil with a 10% BC amendment were reduced by 90% compared to those receiving 5% and 15% BC applications. These results are consistent with recent research (Houben *et al.* 2020), which suggests that the application of BC enhances plant productivity, likely due to the immobilization of chromium.



Control 5% BC 10% BC 15% BC

Fig. 3: Representative image showing differences in *Nerium* plant growth 9 weeks after sowing. From left to right: BC-unamended soil, 5 % BC amended soil, 10 % BC-amended soil, 15 % BC-amended soil

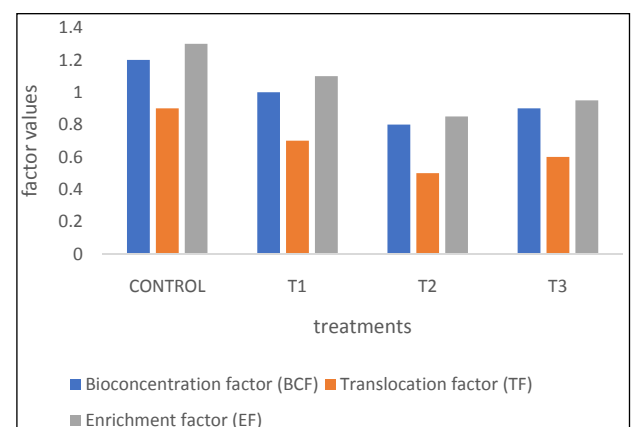


Fig. 4: Bioconcentration factor (BCF), translocation factor (TF), and enrichment factor (EF) of *Nerium oleander* under different biochar treatments (Control, T1, T2, T3) in chromium-contaminated soil. Bars represent mean values of each parameter across treatments

Effect of biochar on uptake of Cr in *Nerium* plants

Biochar has proven effective in reducing chromium (Cr) uptake and toxicity in *Nerium oleander*, a plant known for its phytoremediation potential. With

biochar application, especially at 15%, As shown in Fig. 6 FTIR analysis revealed noticeable shifts in functional groups like carboxyl, hydroxyl, and phenolic moieties, indicating their role in binding and immobilizing Cr. These chemical interactions, supported by FTIR spectral changes, highlight biochar's ability to decrease metal mobility in soil. Notably, a 10% biochar concentration led to a significant 90% reduction in Cr uptake, also limiting its movement from roots to shoots and easing phytotoxic symptoms such as chlorosis and stunted growth.

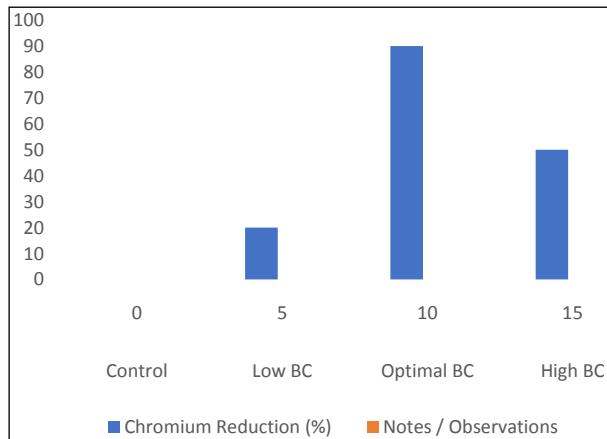


Fig. 5: Here's the graph showing the effect of biochar (BC) on the accumulation of Chromium (Cr) in *Nerium* plants after 9 weeks. Hence proving that Cr concentration in plants decreased as BC percentage increased, with the lowest at 10%

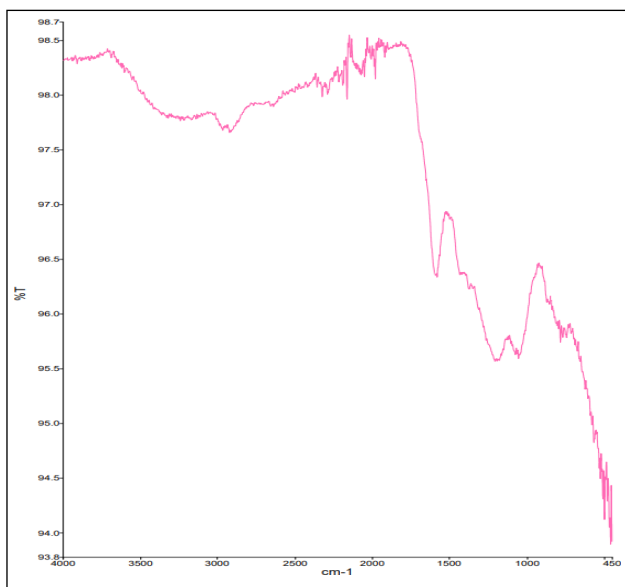


Fig. 6: FTIR Spectra of *Nerium* plant with 10% applied biochar

The reduction in leaf area could be related to structural abnormalities, chlorosis, and/or tissue

necrosis because of Cr toxicity (Stanton *et al.* 2014) but the addition of biochar gave better results in terms of improved plant growth and physiological attributes. This improvement might be associated to high surface area of biochar that have sorbed most of the Cr and lowered its availability and toxicity for plant growth and development (Lucchinia *et al.* 2014), (Wang *et al.* 2015). In conclusion, biochar enhances soil quality and plant health by effectively immobilizing heavy metals at the molecular level, making it a valuable tool in phytoremediation strategies.

CONCLUSION

The 90% reduction in chromium uptake with 10% biochar application demonstrates biochar's strong potential as a sustainable solution for heavy metal remediation. By effectively immobilizing Cr in the soil, biochar minimizes its movement into plant systems and the broader environment, reducing contamination risks. Beyond heavy metal stabilization, biochar also enhances soil health by improving structure, water retention, and nutrient availability. In *Nerium oleander*-based phytoremediation, biochar boosts the plant's metal tolerance while locking contaminants within the soil. FTIR analysis revealed the presence and strengthening of functional groups such as O-H (alcohols), C≡C (alkynes), C=C=N (ketenimines), and C=C=C (allenes), indicating changes in chemical bonding due to biochar's interaction with soil compounds. These molecular-level modifications highlight biochar's role in metal binding and soil chemistry alteration. In conclusion, integrating 10% biochar into Cr-contaminated soils offers an effective, eco-friendly approach to reduce heavy metal bioavailability, enhance plant resilience, and promote sustainable land restoration.

REFERENCES

- Ahmad, M., Ok, Y.S., Kim, B.Y., Ahn, J.H., Lee, Y.H., Zhang, M., Moon, D.H., Al-Wabel, M.I. and Lee, S.S. 2016. Impact of soybean stover- and pine needle-derived biochars on Pb and As mobility, microbial community, and carbon stability in a contaminated agricultural soil. *J. Environ. Manage.*, **166**: 131-139.
- Ahmad, Z., Gao, B., Mosa, A., Yu, H., Yin, X., Bashir, A., Ghozeisi, H. and Wang, S. 2018. Removal of Cu (II), Cd (II) and Pb (II) ions from aqueous solutions by biochars derived from potassium-rich biomass. *J. Clean Prod.*, **180**: 437-449.



- Almaroai, Y., Usman, A.A., Ahmad, M., Moon, D., Cho, J-S., Joo, Y., Jeon, C., Lee, S. and Ok, Y. 2014. Effects of biochar, cow bone, and eggshell on Pb availability to maize in contaminated soil irrigated with saline water. *Environ. Earth Sci.*, **71**: 1289–96.
- Ambika, S., Kumar, M., Pisharody, L., Malhotra, M., Kumar, G., Sreedharan, V., Singh, L., Nidheesh, P.V. and Bhatnagar, A. 2022. Modified biochar as a green adsorbent for removal of hexavalent chromium from various environmental matrices: Mechanisms, methods, and prospects. *Chem. Eng. J.*, **439**: 135716.
- Bandara, T., Franks, A., Xu, J., Bolan, N., Wang, H. and Tang, C. 2020. Chemical and biological immobilization mechanisms of potentially toxic elements in biochar-amended soils. *Critical Reviews in Environmental Science and Technology*, **50**(9): 903-978.
- Bashir, M.A., Naveed, M., Ahmad, Z., Gao, B., Mustafa, A. and Núñez-Delgado, A. 2020. Combined application of biochar and sulfur regulated growth, physiological, antioxidant responses and Cr removal capacity of maize (*Zea mays* L.) in tannery polluted soils. *Journal of Environmental Management*, **259**: 110051.
- Bashir, M.A., Wang, X., Naveed, M., Mustafa, A., Ashraf, S., Samreen, T. ... and Jamil, M. 2021. Biochar mediated-alleviation of chromium stress and growth improvement of different maize cultivars in tannery polluted soils. *International Journal of Environmental Research and Public Health*, **18**(9): 4461.
- Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B. and Sizmur, T. 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.*, **159**: 3269–82.
- Boudra, T., Papadimou, S.G. and Golia, E.E. 2023. The use of biochar in the remediation of Pb, Cd, and Cu-contaminated soils. The impact of biochar feedstock and preparation conditions on its remediation capacity. *Land*, **12**(2): 383.
- Campos Díaz de Mayorga, P. and Rosa Arranz, J.M. 2020. Assessing the effects of biochar on the immobilization of trace elements and plant development in a naturally contaminated soil.
- Chen, X., Dai, Y., Fan, J., Xu, X. and Cao, X. 2021. Application of iron-biochar composite in topsoil for simultaneous remediation of chromium-contaminated soil and groundwater: Immobilization mechanism and long-term stability. *Journal of Hazardous Materials*, **405**: 124226.
- Chirakkara, R.A. and Reddy, K.R. 2015. Biomass and chemical amendments for enhanced phytoremediation of mixed contaminated soils. *Ecological Engineering*, **85**: 265-274.
- Choppala, G., Bolan, N., Kunhikrishnan, A., Skinner, W. and Seshadri, B. 2015. Concomitant reduction and immobilization of chromium in relation to its bioavailability in soils. *Environ. Sci. Pollut. Res.*, **22**: 8969–78.
- Dai, L., Chen, Y., Liu, L., Sun, P., Liu, J., Wang, B. and Yang, S. 2022. Effect of biochar on the uptake, translocation and phytotoxicity of chromium in a soil-barley pot system. *Science of the Total Environment*, **826**: 153905.
- Dhaliwal, S.S., Singh, J., Taneja, P.K. and Mandal, A. 2020. Remediation techniques for removal of heavy metals from the soil contaminated through different sources: a review. *Environ. Sci. and Poll. Res.*, **27**(2): 1319-1333.
- Dilshara, P., Abeysinghe, B., Premasiri, R., Dushyantha, N., Ratnayake, N., Senarath, S. ... and Batapola, N. 2025. Transforming nickel toxicity into resource recovery through phytomining: opportunities and applications in Sri Lanka. *International Journal of Environmental Science and Technology*, pp. 1-20.
- Di Palma, L., Mancini, D. and Petrucci, E. 2012. Experimental assessment of chromium mobilization from polluted soil by washing.
- Fowles, M. 2007. Black carbon sequestration as an alternative to bioenergy. *Biomass- Bioenergy*, **31**(6): 426–432.
- Girish, C., Nanthi, B., Megharaj, M. and Zuliang, C. 2010. Sorption and mobility of chromium species in a range of soil types. 19th World Congress of Soil Science, Soil Solutions for a Changing World 1-6 August 2010, Brisbane, Australia.
- Gomez, J., Deneff, K., Stewart, C., Zheng, J. and Cotrufo, M. 2014. Biochar addition rate influences soil microbial abundance and activity in temper ate soils. *Eur. J. Soil Sci.*, **65**: 28–39.
- Hasan, S.M.M., Akber, M.A., Bahar, M.M., Islam, M.A., Akbor, M.A., Siddique, M.A.B. and Islam, M.A. 2021. Chromium contamination from tanning industries and Phytoremediation potential of native plants: A study of savar tannery industrial estate in Dhaka, Bangladesh. *Bulletin of Environmental Contamination and Toxicology*, **106**(6): 1024-1032.
- Hashem, M.A., Payel, S., Mim, S., Hasan, M. A., Nur-A-Tomal, M.S., Rahman, M.A. and Sarker, M.I. 2022. Chromium adsorption on surface activated biochar made from tannery liming sludge: A waste-to-wealth approach. *Water Science and Engineering*, **15**(4): 328-336.
- Houben, D., Daoulas, G., Faucon, M.P. and Dulaurent, A.M. 2020. Potential use of mealworm frass as a fertilizer: Impact on crop growth and soil properties. *Scientific Reports*, **10**(1): 4659.
- Houben, D., Evrard, L. and Sonnet, P. 2013. Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (*Brassica napus* L.). *Biomass Bioenergy*, **57**: 196–204.
- Jiang, Z., Li, J., Jiang, D., Gao, Y., Chen, Y., Wang, W. ... and Zhang, Y. 2020. Removal of atrazine by biochar-supported zero-valent iron catalyzed persulfate oxidation: reactivity, radical production and transformation pathway. *Environmental Research*, **184**: 109260.
- Kapoor, R.T. and Zdarta, J. 2024. Fabrication of engineered biochar for remediation of toxic contaminants in soil matrices and soil valorization. *Chemosphere*, 142101.

- Katenta, J., Nakiguli, C., Mukasa, P. and Ntambi, E. 2020. Removal of Chromium (VI) from tannery effluent using bio-char of Phoenix reclinata seeds. *Green and Sustainable Chemistry*, **10**(3): 91-107.
- Lehmann, J. 2007. Bio-energy in the black. *Frontiers in Ecology and the Environment*, **5**: 381-387.
- Li, Y., Awasthi, M.K., Sindhu, R., Binod, P., Zhang, Z. and Taherzadeh, M.J. 2023. Biochar preparation and evaluation of its effect in composting mechanism: A review. *Bioresource Technology*, **384**: 129329.
- Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizao, F.J., Petersen, J. and Neves, E.G. 2006. Black carbon increases cation exchange capacity in soils. *Soil Sciences Society of America J.*, **70**: 1719-1730.
- Lucchinia, P., Quilliam, R.S., DeLucad, T.H., Vameralia, T. and Jones, D.L. 2014. Does biochar application alter heavy metal dynamics in agricultural soil? *Agric. Ecosyst. Environ.*, **184**: 149-157.
- Mandal, S., Pu, S., Adhikari, S., Ma, H., Kim, D.H., Bai, Y. and Hou, D. 2021. Progress and future prospects in biochar composites: application and reflection in the soil environment. *Critical Reviews in Environmental Science and Technology*, **51**(3): 219-271.
- McGrath, S.P., Dunham, S.J. and Correll, R.L. 2020. Potential for phytoextraction of zinc and cadmium from soils using hyperaccumulator plants. In *Phytoremediation of contaminated soil and water* (pp. 109-128). CRC Press.
- Murad, H.A., Ahmad, M., Bundschuh, J., Hashimoto, Y., Zhang, M., Sarkar, B. and Ok, Y.S. 2022. A remediation approach to chromium-contaminated water and soil using engineered biochar derived from peanut shell. *Environmental Research*, **204**: 112125.
- Nath, H., Sarkar, B., Mitra, S. and Bhaladhare, S. 2022. Biochar from biomass: a review on biochar preparation its modification and impact on soil including soil microbiology. *Geomicrobiology Journal*, **39**(3-5): 373-388.
- Rafique, M.I., Usman, A.R., Ahmad, M., Sallam, A. and Al-Wabel, M.I. 2020. *In situ* immobilization of Cr and its availability to maize plants in tannery waste-contaminated soil: effects of biochar feedstock and pyrolysis temperature. *Journal of Soils and Sediments*, **20**: 330-339.
- Rahman, S., Rahman, I.M. and Hasegawa, H. 2023. Management of arsenic-contaminated excavated soils: A review. *Journal of Environmental Management*, **346**: 118943.
- S.-W.C. Chien, H.-H. Wang, Y.-M. Chen, M.-K. Wang, C.-C. Liu, 2021. Removal of heavy metals from contaminated paddy soils using chemical reductants coupled with dissolved organic carbon solutions, *J. Hazard. Mater.*, **403**: 1-8.
- Skrzypczak, D., Szopa, D., Mikula, K., Izydorczyk, G., Baśladyńska, S., Hoppe, V. ... and Witek-Krowiak, A. 2022. Tannery waste-derived biochar as a carrier of micronutrients essential to plants. *Chemosphere*, **294**: 133720.
- Solomon, D., Lehmann, J., Thies, J., Schafer, T., Liang, B., Kinyangi, J., Neves, E., Petersen, J., Luizao, F. and Skjemstad, J. 2007. Molecular signature and sources of biochemical recalcitrance of organic C in Amazonian dark earths. *Geochimica et cosmochimica Acta*, **71**: 2285-2298.
- Stanton, K.M. and Mickelbart, M.V. 2014. Maintenance of water uptake and reduced water loss contribute to water stress tolerance of *Spiraea alba* Du Roi and *Spiraea tomentosa* L. *Hortic. Res.*, **33**: 1-7.
- Wang, S., Gao, B., Li, Y., Mosa, A., Zimmerman, A.R., Ma, L.Q., Harris, W.G. and Migliaccio, K.W. 2015. Manganese oxide-modified biochars: Preparation, characterization and sorption of arsenate and lead. *Bioresour. Technol.*, **181**: 13-17.
- Wao, A.A. and Pandey, J. 2023. Restoration of contaminated agricultural soils by microbes. In *Integrative Strategies for Bioremediation of Environmental Contaminants, Volume Two* (pp. 273-295). Academic Press.
- Wardle, D.A., Nilsson, M.C. and Zackrisson, O. 2008. Fire-derived charcoal causes loss of forest humus. *Science*, **320**(5876): 629-629.
- Yang, Q., Wang, Y. and Zhong, H. 2021. Remediation of mercury-contaminated soils and sediments using biochar: a critical review. *Biochar*, **3**: 23-35.
- Younis, U., Danish, S., Datta, R., Alahmadi, T.A. and Ansari, M.J. 2024. Sustainable remediation of chromium-contaminated soils: boosting radish growth with deashed biochar and strigolactone. *BMC Plant Biology*, **24**(1): 115.
- Yuan, J.H., Xu, R.K. and Zhang, H. 2011. The forms of alkalis in the biochar produced from crop residues at different temperatures. *Bioresour. Technol.*, **102**(3): 3488-3497.
- Zhang, Q.C., Wang, C.C., Cheng, J.H., Zhang, C.L. and Yao, J.J. 2021. Removal of Cr (VI) by biochar derived from six kinds of garden wastes: Isotherms and kinetics. *Materials*, **14**(12): 3243.
- Dume, B., Mosissa, T. and Nebiyu, A. 2016. Effect of biochar on soil properties and lead (Pb) availability in a military camp in South West Ethiopia. *Afr. J. Environ. Sci. Technol.*, **10**(3): 77-85.
- Mandal, S., Pu, S., He, L., Ma, H. and Hou, D. 2020. Biochar induced modification of graphene oxide & nZVI and its impact on immobilization of toxic copper in soil. *Environmental Pollution*, **259**: 113851.
- Sinduja, M., Sathya, V., Maheswari, M., Dhevagi, P., Kalpana, P., Dinesh, G.K. and Prasad, S. 2022. Evaluation and speciation of heavy metals in the soil of the Sub Urban Region of Southern India. *Soil and Sediment Contamination: An International Journal*, **31**(8): 974-993.