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Research Paper

Effect of biochar amendment on soil microbial dynamics in heavy metal-contaminated soils under spinach cultivation

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ABSTRACT

The present study was conducted at SKUAST-K Shalimar during 2021-2022 to evaluate the effect of biochar amendments on soil microbiological properties under heavy metal contamination (Cd, Pb, and Cu). Pots were filled with soil spiked with heavy metals at double the threshold levels (5.2 ppm Cd, 100 ppm Pb, and 200 ppm Cu) and treated with biochar at rates of 0%, 1%, 3%, 5%, and 10% (w/w). Biochar was prepared through pyrolysis at 550°C using dal weed, deodar needles, and apple leaves. Spinach (Spinacia oleracea) was grown under these conditions, and soil microbial properties were analyzed post-harvest. Results indicated a significant improvement in the total microbial count (CFU/g) with biochar application. The pooled mean bacterial counts ranged from 4.466 × 10^7 CFU/g (T1 i.e., control with no biochar) to 14.50 × 10^7 CFU/g (T5: 10% biochar from dal weed), with dal weed biochar showing superior enhancement. Similarly, the pooled mean fungal counts varied between 1.60 × 10^5 CFU/g (T1) and 4.65 × 10^5 CFU/g (T5). Higher biochar concentrations and dal weed based biochar consistently enhanced microbial proliferation compared to control and other treatments.

Key words: Biochar, dal weed, heavy metal, microbial count, spinach

Heavy metal pollution in soils is a major environmental concern as it can adversely impact soil microbial communities and overall soil health (Azadi & Raiesi, 2021; Siles *et al*., 2022). Biochar, a carbon-rich material produced through pyrolysis of organic feedstocks, has emerged as a promising soil amendment for remediating heavy metal-contaminated soils (Dissanayake *et al*., 2022). Biochar can improve soil properties, such as pH, nutrient availability, and water-holding capacity, which in turn can promote the growth and activity of soil microorganisms (Moradi & Karimi, 2020). Several studies have investigated the effects of biochar amendments on microbial and fungal communities in heavy metal-polluted soils (Chen *et al*., 2023). Biochar has been shown to alleviate metal toxicity and enhance microbial community functions, leading to improved nutrient cycling and soil fertility (Azadi & Raiesi, 2021; Siles *et al*., 2022). The highly porous structure of biochar can also provide a favorable habitat for the growth of soil microorganisms (Zhu *et al*., 2022). However, the specific mechanisms by which biochar influences the microbial and fungal communities in heavy metal-contaminated soils are not fully understood (Zhu *et al*., 2022). Furthermore, the effectiveness of biochar in promoting microbial growth and activity may depend on the type and properties of the biochar used, as well as the characteristics of the heavy metal-polluted soil (Zhang *et al*., 2021; Narayanan & Ma, 2022). Careful selection of the appropriate biochar type is crucial for achieving the desired effects on soil microbiota (Dissanayake *et al*., 2022; Chen *et al*., 2023). This study aims to investigate the impact of biochar amendments on the microbial and fungal communities in heavy metal-polluted soils, with a focus on understanding the underlying mechanisms and the role of biochar properties in mediating these effects. The findings from this research will contribute to the development of effective biochar-based strategies for the remediation of heavy metal-contaminated soils and the restoration of soil ecosystem functions.

MATERIALS AND METHODS

Study area: The experiment was conducted at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar campus, during the 2021-2022 cropping season. The study employed a pot culture approach to assess the impact of biochar amendments on soil microbial properties in heavy metal-contaminated soils.

Experimental design: Pots were filled with soil artificially spiked with heavy metals—Cadmium (Cd), Lead (Pb), and Copper (Cu)—at concentrations double the threshold levels (5.2 ppm Cd, 100 ppm Pb, and 200 ppm Cu). Biochar was incorporated into the soil at five different rates: 0% (control), 1%, 3%, 5%, and 10% (w/w). The experiment followed a completely randomized design (CRD) with three replications.

Biochar preparation: Biochar was prepared from dal weed, deodar needle and apple leaves. These materials were air-dried at room temperature and then pyrolyzed in a muffle furnace (BDI-73) at a temperature of 550°C for 1 hour to produce biochar.

Treatment combinations: Twelve treatment combinations with control were established, as follows:

Treatment Code	Treatments
T_1	Soil + Heavy metals (Cd, Pb, Cu) (Control)
T_2	Soil + Heavy metals (Cd, Pb, Cu) + 1% Biochar (Dal weed)
T_3	Soil + Heavy metals (Cd, Pb, Cu) + 3% Biochar (Dal weed)
T_4	Soil + Heavy metals (Cd, Pb, Cu) + 5% Biochar (Dal weed)
T_5	Soil + Heavy metals (Cd, Pb, Cu) + 10% Biochar (Dal weed)
T_6	Soil + Heavy metals (Cd, Pb, Cu) + 1% Biochar (Deodar needles)
T_7	Soil + Heavy metals (Cd, Pb, Cu) + 3% Biochar (Deodar needles)
T_8	Soil + Heavy metals (Cd, Pb, Cu) + 5% Biochar (Deodar needles)
T_9	Soil + Heavy metals (Cd, Pb, Cu) + 10% Biochar (Deodar needles
T_{10}	Soil + Heavy metals (Cd, Pb, Cu) + 1% Biochar (Apple leaves)
T_{11}	Soil + Heavy metals (Cd, Pb, Cu) + 3% Biochar (Apple leaves)
T_{12}	Soil + Heavy metals (Cd, Pb, Cu) + 5% Biochar (Apple leaves)
T_{13}	Soil + Heavy metals (Cd, Pb, Cu) + 10% Biochar (Apple leaves)

Crop establishment: Spinach (*Spinacia oleracea*) seeds were sown in pots containing soil treated with heavy metals, biochar, and recommended doses of fertilizers. The crop was maintained until the maturity stage, following standard agronomic practices for irrigation, weeding, and pest management.

Microbiological analysis: Post-harvest, soil samples were collected from each pot to evaluate the microbiological properties using standard methods.

Total viable bacteria (CFU/g of soil): This is determined using dilution technique (Gerdemann and Nicolson, 1963). Nutrient agar medium is used which is first autoclaved for 15 minutes & then is allowed to cool but not solidify. This medium is then poured on petriplates & allowed to solidify. 1 ml of dilution is poured & spread evenly on these plates & hence allowed to incubate at 28 ^{0}C for 24-48 hours. The bacterial colonies hence formed are then counted.

Total viable fungi (CFU/g of soil): For the determination of total viable fungi in soil, potato dextrose agar is used, which is first autoclaved at 121 ^{0}C for about 15 minutes. The agar medium is then poured onto the petriplates & allowed to solidify. 1 ml of dilution is poured & spread evenly on these plates & then counted.

RESULTS AND DISCUSSION

The effect of biochar amendment on total microbial count (CFU/g × 10^{-7}) in soil under heavy metal (Cd, Pb, and Cu) contamination was significant across the different treatments for both 2021 and 2022. The results, summarized in Table 1 and Fig. 1, indicated a positive response of microbial count to increasing biochar application rates, irrespective of the biochar source. The maximum number of total microbial count was recorded in **T5** (14.30 ± 0.40 in 2021, 14.70 ± 0.50 in 2022, pooled mean: 14.50 ± 0.28), followed by **T13** (13.20 ± 0.40 in 2021, 13.70 ±0.40 in 2022, pooled mean: 13.45 ± 0.35) and **T1** (control) recording the lowest value (4.13 ± 0.35 in 2021, 4.80 ± 0.40 in 2022, pooled mean: 4.46 ± 0.47).

Table 2: Effect of biochar amendment on total microbial count (CFU/g ×10^{-7}) in soil under the influence of heavy metal (Cd +Pb + Cu) contamination

Treatment	2021	2022	Pooled mean
T1	4.13 ± 0.35	4.80 ± 0.40	4.46 ± 0.47
T2	7.20 ± 0.30	7.40 ± 0.30	7.30 ± 0.14
T3	10.10 ± 0.50	10.50 ±0.10	10.30 ± 0.28
T4	12.20 ± 0.70	12.60 ± 0.40	12.40 ± 0.28
T5	14.30 ± 0.40	14.70 ± 0.50	14.50 ± 0.28
T6	5.20 ± 0.20	5.70 ± 0.20	5.45 ± 0.35
T7	8.40 ± 0.30	8.80 ± 0.60	8.60 ± 0.28
T8	10.40 ± 0.50	11.10 ± 0.40	10.75 ± 0.50
T9	12.80 ± 0.30	13.30 ± 0.60	13.05 ± 0.35
T10	6.30 ± 0.50	6.90 ± 0.30	6.60 ± 0.42
T11	9.30 ± 0.40	9.60 ± 0.40	9.45 ± 0.21
T12	11.83 ± 0.30	11.80 ± 0.60	11.81 ± 0.02
T13	13.20 ± 0.40	13.70 ±0.40	13.45 ± 0.35
C.D	0.698	0.715	0.711

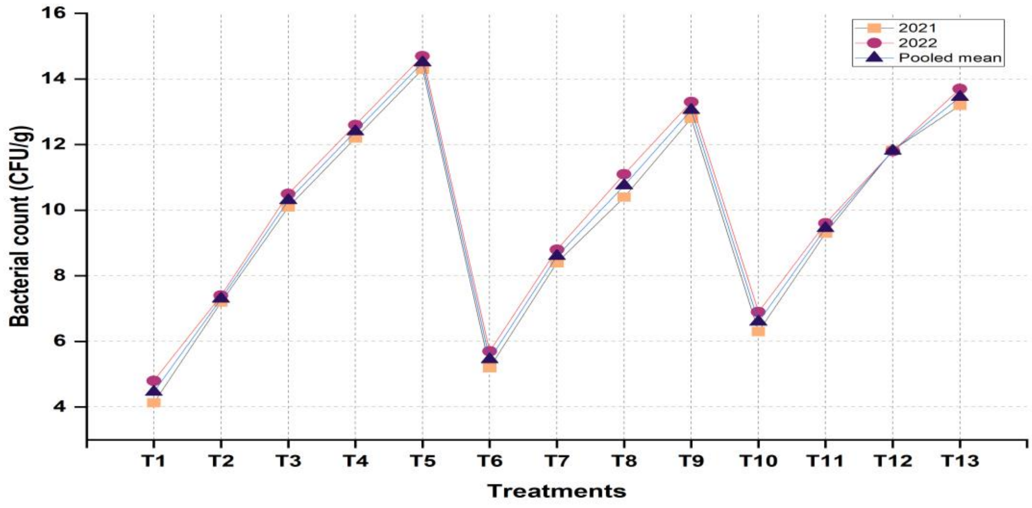

Fig. 1: Effect of biochar amendment on total microbial count (CFU/g ×10^{-7}) in soil under the influence of heavy metal (Cd +Pb + Cu) contamination

The effect of biochar amendment on total fungal count (CFU/g × 10^{-5}) in soil under heavy metal (Cd, Pb, and Cu) contamination was significant across the different treatments for both 2021 and 2022. The results, summarized in Table 2 and Fig. 2, indicated a positive response of fungal count to increasing biochar application rates, irrespective of the biochar source. The maximum number of total fungal count was recorded in **T5** (4.40 ± 0.20 in 2021, 4.90 ± 0.20 in 2022, pooled mean: 4.65 ± 0.35), followed by **T13** (4.10 ± 0.20 in 2021, 4.40 ±0.50 in 2022, pooled mean: 4.25 ± 0.21) and **T1** (control) recording the lowest value (1.50 ± 0.30 in 20211.70 ± 0.20 in 2022, pooled mean: 1.60 ± 0.14).

Table 2: Effect of biochar amendment on total fungal count (CFU/g ×10^{-5}) in soil under the influence of heavy metal (Cd +Pb + Cu) contamination

Treatment	2021	2022	Pooled mean
T_1	1.50 ± 0.30	1.70 ± 0.20	1.60 ± 0.14
T_2	2.10 ± 0.20	2.40 ± 0.30	2.250 ± 0.21
T_3	2.70 ± 0.30	3.00 ± 0.40	2.85 ± 0.21
T_4	3.40 ± 0.50	3.90 ± 0.40	3.65 ± 0.35
T_5	4.40 ± 0.20	4.90 ± 0.20	4.65 ± 0.35
T_6	1.80 ± 0.10	1.90 ± 0.40	1.85 ± 0.71
T_7	2.20 ± 0.30	2.50 ± 0.30	2.35 ± 0.21
T_8	2.90 ± 0.40	3.30 ± 0.20	3.10 ± 0.28
T_9	3.90 ± 0.20	4.20 ± 0.30	4.05 ± 0.21
T_{10}	1.90 ± 0.30	2.20 ± 0.40	2.05 ± 0.21
T_{11}	2.40 ± 0.20	2.80 ± 0.30	2.60 ± 0.28
T_{12}	3.10 ± 0.40	3.46 ± 0.25	3.28 ± 0.26
T_{13}	4.10 ± 0.20	4.40 ±0.50	4.25 ± 0.21
C.D	0.497	0.557	0.527

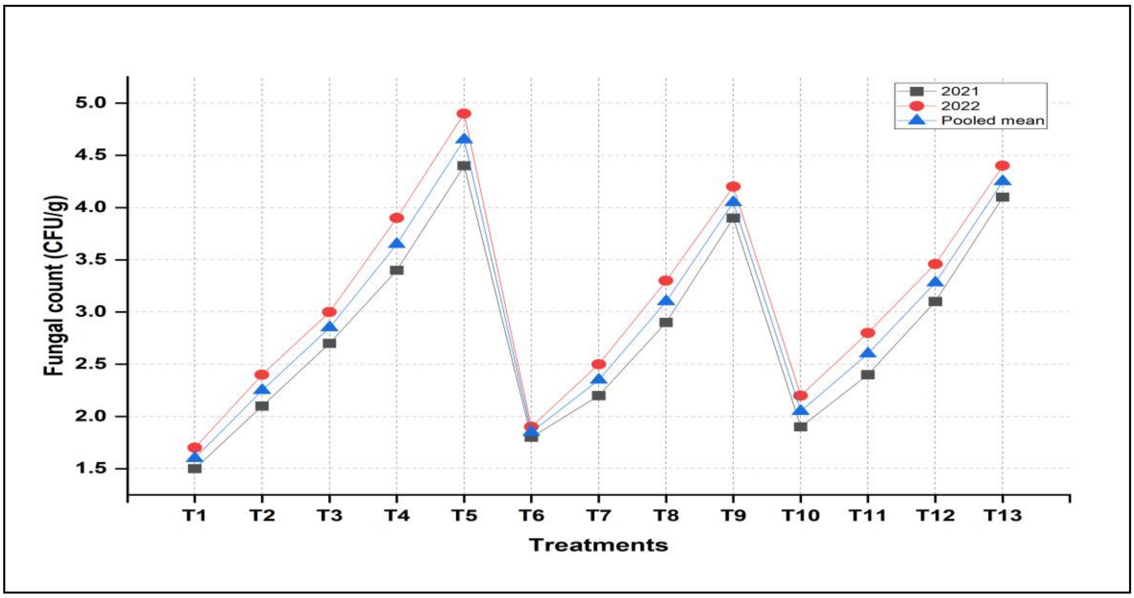

Fig. 2: Effect of biochar amendment on total fungal count (CFU/g ×10^{-5}) in soil under the influence of heavy metal (Cd +Pb + Cu) contamination

The observed increase in the total microbial count with biochar addition can be attributed to several mechanisms. Biochar can provide a favorable habitat for microorganisms and fungi by improving soil physical properties, such as increased porosity, water-holding capacity, and aeration (Brtnicky *et al*., 2019; Wang *et al*., 2019). Additionally, biochar can serve as a source of labile carbon and nutrients, stimulating microbial and fungal growth and activity (Grafmuller *et al*., 2024; Tarin *et al*., 2021). Furthermore, the ability of biochar to immobilize heavy metals and reduce their bioavailability in the soil may have contributed to the enhanced microbial and fungal population (Gomez-Díaz *et al*., 2014; Hou *et al*., 2015). Heavy metals can be toxic to soil microorganisms and fungi, and the mitigation of their adverse effects by biochar amendment may have created a more favorable environment for microbial proliferation (Ding *et al*., 2018; Lu *et al*., 2015). The differences observed among the biochar feedstocks can be attributed to the variations in their physicochemical properties, such as pH, surface area, and nutrient content, which can influence the soil microbial and fungal community structure and function (Liu *et al*., 2019; Chen *et al*., 2020; Xu *et al*., 2016). Overall, the results demonstrate the potential of biochar amendment to enhance the total microbial count and total fungal count in heavy metal-contaminated soils, with the highest application rate (10%) exhibiting the most pronounced effects. These findings suggest that biochar can be a valuable tool for improving soil microbial and fungal health and supporting sustainable agricultural practices in areas affected by heavy metal pollution.

CONCLUSION

The study demonstrates that biochar amendment significantly enhances the total microbial and fungal counts in heavy metal-contaminated soils. This improvement is attributed to biochar's ability to provide a conducive environment for microbial growth by improving soil physical properties, offering a source of labile carbon and nutrients, and immobilizing heavy metals to reduce their toxicity. Among the biochar sources tested, dal weed and apple leaf biochar showed the most pronounced effects, particularly at the highest application rate (10%), which consistently recorded the highest microbial and fungal counts. These findings highlight the potential of biochar as a sustainable amendment for mitigating the adverse effects of heavy metal contamination on soil health. The use of biochar not only enhances soil microbial activity but also supports better soil functionality, which is critical for sustainable agriculture in polluted environments. Further research on long-term field trials and optimization of biochar feedstocks and application rates will provide more insights into its broader application in agricultural systems.

CONFLIT OF INTEREST

All the authors affirm that there is no conflict of interest among them. All research activities comply with relevant legal, institutional and ethical standards.

AUTHOR CONTRIBUTION

All the authors contributed equally in the present study.

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