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Original Article

Assessing the Relative Effectiveness of Organic Amendments in Alleviating Cadmium Toxicity

Muhammad Waqas*[1], Hamda Rafique[2], Muhammad Khizar Hayat[1], Abu Huraira[1], Mohsin Ali[3], Eiza Shahid[3], Muhammad Atiq ur Rahman[4], Aqsa Rasool[1]

[1] University of Agriculture Faisalabad, Sub-Campus Burewala, Vehari, Pakistan

[2] Superior University, Lahore, Pakistan

[3] University of Agriculture, Faisalabad, Pakistan

[4] Provincial Reference Fertilizer Testing Laboratory, Raiwind, Lahore, Pakistan

Corresponding author: zaibch767@gmail.com

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Abstract

Background: Cadmium (Cd) contamination in soils poses a significant threat to plant growth and human health, particularly in agricultural regions. Organic amendments, such as biochar and compost, have been explored as potential solutions to mitigate Cd toxicity by immobilizing the metal and enhancing plant growth.

Objective: This study aimed to evaluate the effectiveness of biochar and compost, individually and combined, in reducing Cd toxicity and promoting the growth of Spinacia oleracea in contaminated soils.

Methods: A controlled experiment was conducted in which Spinacia oleracea was grown in soil treated with 1% biochar, 1% compost, or a combination of both. Growth parameters, including root and shoot length, biomass, and leaf area, were measured. Physiological attributes, such as photosynthetic pigments and antioxidant enzyme activities, were assessed. Cd content, translocation, and bioaccumulation in plant tissues were analyzed using atomic absorption spectrophotometry. Statistical analysis was performed using SPSS 25.0, with significance set at $p<0.05$.

Results: The combined application of biochar and compost significantly increased root length (by 49.75%), shoot length (by 36.73%), and biomass (by 42.85%) in Spinacia oleracea. Cd content in roots and shoots was reduced by 40.41% and 51.16%, respectively, compared to control.

Conclusion: Biochar and compost, when combined, effectively mitigate Cd toxicity and enhance plant growth, offering a promising strategy for managing contaminated soils.

1 Introduction

Cadmium (Cd) contamination represents a severe threat to both plant and human health, primarily due to its pervasive presence in the environment and its ability to disrupt physiological and biochemical processes in plants. This contamination, which has escalated due to rapid industrialization and the discharge of untreated effluents, profoundly impacts soil fertility and plant development. In many developing regions, where freshwater scarcity is prevalent, the irrigation of vegetables with wastewater containing heavy metals is a common practice, leading to a significant accumulation of Cd in soils and crops. In Pakistan, for instance, approximately 26% of vegetables are irrigated with untreated wastewater, exacerbating the issue of heavy metal contamination in agricultural soils (1, 2). Cd, in particular, is known for its high toxicity to plants, animals, and humans, with industrial effluents, municipal sewage sludge, and phosphatic fertilizers being major contributors to its increased concentration in soils (2).

Upon entering plants, Cd disrupts essential metabolic processes, induces oxidative stress, and damages the photosynthetic apparatus, leading to a reduction in chlorophyll and carotenoid content and, ultimately, a decline in plant growth and productivity (3). The adverse effects of Cd on plant physiology are well-documented, with its accumulation in plants posing a significant risk to human health through bio-magnification (4). Various physio-chemical methods, such as precipitation, ion exchange, and reverse osmosis, have been employed for Cd remediation. However, these conventional approaches are often costly and inefficient for large-scale applications, particularly in the context of agricultural soils (2).

In contrast, phytoremediation has emerged as a promising, cost-effective strategy for the remediation of heavy metals in contaminated soils. Spinach (Spinacia oleracea), a widely cultivated hyperaccumulator, has demonstrated considerable potential in extracting heavy metals, including Cd, from contaminated soils. However, the practical application of spinach for phytoremediation is limited by its relatively slow growth and low biomass yield, which reduces its overall effectiveness in heavy metal extraction (5, 6). To enhance the growth and phytoremediation potential of spinach, the use of organic soil amendments such as biochar and compost has gained attention. These amendments are known to mitigate heavy metal toxicity through various mechanisms, including the adsorption and immobilization of metals, which reduces their bioavailability and toxicity to plants (7, 8).

Biochar, in particular, has been recognized as an effective soil remediation agent due to its large surface area, high cation exchange capacity, alkaline pH, and water retention properties (9). However, while biochar is effective in sorbing heavy metals, it may not provide sufficient nutrients to support optimal plant growth, necessitating its combined use with nutrient-rich amendments like compost. Compost not only enriches the soil with essential nutrients but also fosters the growth of beneficial microorganisms, further enhancing the phytoremediation potential of plants (10). The combined application of biochar and compost has shown promise in improving soil properties, reducing Cd bioavailability, and promoting plant growth, making it a viable strategy for the remediation of Cd-contaminated soils.

This study seeks to investigate the combined effects of biochar and compost on the growth and phytoremediation potential of Spinacia oleracea in Cd-contaminated soils. By examining the influence of these organic amendments on plant growth parameters, photosynthetic attributes, and Cd uptake and translocation, this research aims to provide insights into the effectiveness of biochar and compost as a combined amendment strategy for mitigating Cd toxicity in agricultural soils and enhancing sustainable agricultural practices (11, 12).

2 Material and Methods

The study was conducted in a controlled environment at the College of Agriculture, University of Sargodha, situated at 32.08° North latitude and 72.67° East longitude, with an elevation of 193 meters above sea level. Soil samples were collected from a designated field within the college premises, air-dried, and sieved to achieve a homogenous texture. The soil's physicochemical properties, including pH, electrical conductivity, organic matter content, texture, moisture content, and nutrient levels (nitrogen, phosphorus, and potassium), were analyzed to establish baseline characteristics. Biochar was produced through the pyrolysis of sawdust and garden waste residues under limited oxygen conditions at 500°C for three hours. The resulting biochar was then cooled, ground, and sieved to obtain particles ranging from 0.2 to 0.4 mm in size. Compost was prepared in large earthen pots with perforations, containing a mixture of vegetable peels, poultry manure, and soil, and was subjected to periodic mixing to facilitate aerobic microbial degradation over five to six months.

The experimental design involved the application of biochar (1%) and compost (1%) to the soil, which was then incubated for one month to ensure adequate amendment integration. A control group with untreated soil was maintained for comparison. Spinacia oleracea, commonly known as spinach, was selected as the test plant species due to its hyperaccumulative properties. Two varieties, Desi palak (V1) and Kanta palak (V2), were chosen for their distinct growth characteristics. The experiment was conducted from December to February 2023, with ambient temperatures ranging from 4°C to 10°C. Seeds were sown in pots filled with treated and untreated soils, and the plants were allowed to grow for 60 days under natural light conditions.

Upon reaching maturity, plants were carefully uprooted, washed with distilled water, and divided into root and shoot sections. Growth parameters, including root and shoot length, fresh and dry weight, and leaf area, were measured using standard techniques. The leaf area was quantified using a leaf area meter. Biomass measurements were taken immediately for fresh weight, while dry weight was determined after oven-drying the samples at 75°C for 48 hours until a constant weight was achieved. Photosynthetic pigments, gas exchange attributes, and water use efficiency were assessed using established protocols, with measurements taken for chlorophyll, carotenoids, lycopene, and anthocyanin content. Antioxidant activities, including superoxide dismutase, peroxidase, and catalase, were also evaluated.

To determine cadmium bioavailability, soil samples were subjected to diethylene triamine pentaacetic acid (DTPA) extraction following ISO 14870 standards. Plant samples were digested using a mixture of perchloric acid, sulfuric acid, and nitric acid, and cadmium content was analyzed using an atomic absorption spectrophotometer. The translocation factor (TF) and bioaccumulation factor (BAF) were calculated to assess the movement and accumulation of cadmium within plant tissues, using established formulas. Data on growth, physiological parameters, cadmium uptake, and antioxidant activities were subjected to statistical analysis using SPSS version 25.0. Descriptive statistics, one-way analysis of variance (ANOVA), and post-hoc tests were performed to determine significant differences between treatment groups, with a p-value of less than 0.05 considered statistically significant.

This study adhered to ethical standards consistent with the Helsinki Declaration, ensuring the integrity and ethical conduct of the research. While the study did not involve human or animal subjects, ethical approval was sought and obtained from the institutional review board of the University of Sargodha for the experimental procedures involving environmental and plant research. Data collection and analysis were conducted with due diligence to ensure accuracy, reproducibility, and transparency in reporting the findings (1, 2).

3 Results

The results of the study demonstrate significant improvements in various growth parameters, physiological attributes, and cadmium (Cd) uptake in Spinacia oleracea due to the application of biochar and compost, both individually and in combination. The combined application of these amendments showed the most pronounced positive effects, indicating their potential in mitigating Cd toxicity and enhancing plant growth.

Table 1: Physiochemical Characteristics of Soil, Biochar, and Compost

Parameters	Soil	Biochar	Compost
Electrical Conductivity (ds/m)	1.4 ± 0.0	21.1 ± 1.2	3.4 ± 0.8
pH	7.3 ± 0.7	10.4 ± 0.0	8.1 ± 0.2
Texture	Loam	-	-
Moisture Content (%)	42.6 ± 1.5	7.1 ± 0.0	58.3 ± 2.6
Organic Matter (%)	0.4 ± 0.0	0.6 ± 0.0	64.1 ± 4.1
Potassium (g/kg)	0.2 ± 0.0	3.7 ± 0.0	31.6 ± 1.8
Nitrogen (%)	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
Phosphorus (mg/kg)	7.2 ± 0.4	281.3 ± 3.2	106.4 ± 12.0
Carbon (%)	-	63.5 ± 4.2	42.0 ± 1.0
Ash Content	-	528.1 ± 17.0	-
Ammonium (g/kg)	-	-	4.2 ± 0.0

The analysis of soil, biochar, and compost revealed that the biochar had a notably higher pH and electrical conductivity compared to the soil and compost. The compost, on the other hand, showed a substantially higher moisture content and organic matter percentage, indicating its potential for enhancing soil fertility and plant growth. The biochar exhibited higher potassium and carbon content, which are critical for improving soil structure and nutrient retention (1).

Table 2: Effect of Biochar and Compost on Growth Parameters of Spinacia oleracea

Variety	Treatment	Root Length (cm)	Shoot Length (cm)	Root Fresh Weight (g)	Root Dry Weight (g)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Leaf Area (cm^2)
V1	Control	10.5 ± 0.3	14.7 ± 0.5	4.2 ± 0.2	1.5 ± 0.1	7.2 ± 0.3	2.1 ± 0.2	25.3 ± 1.1
V1	Biochar	12.3 ± 0.4	16.5 ± 0.6	5.6 ± 0.3	1.8 ± 0.1	9.4 ± 0.4	2.6 ± 0.2	30.1 ± 1.3
V1	Compost	13.2 ± 0.4	17.1 ± 0.6	5.9 ± 0.3	1.9 ± 0.1	9.8 ± 0.4	2.7 ± 0.2	31.7 ± 1.3
V1	Combined	15.7 ± 0.5	20.1 ± 0.7	6.7 ± 0.3	2.2 ± 0.1	12.1 ± 0.5	3.0 ± 0.2	34.5 ± 1.5
V2	Control	9.8 ± 0.3	13.9 ± 0.5	3.9 ± 0.2	1.3 ± 0.1	6.8 ± 0.3	1.9 ± 0.2	22.7 ± 1.0
V2	Biochar	11.6 ± 0.4	15.8 ± 0.6	5.1 ± 0.3	1.6 ± 0.1	8.7 ± 0.4	2.4 ± 0.2	28.6 ± 1.2
V2	Compost	12.1 ± 0.4	16.4 ± 0.6	5.4 ± 0.3	1.7 ± 0.1	9.1 ± 0.4	2.5 ± 0.2	29.4 ± 1.2
V2	Combined	14.4 ± 0.5	18.9 ± 0.7	6.3 ± 0.3	2.0 ± 0.1	11.4 ± 0.5	2.9 ± 0.2	32.8 ± 1.4

The combined application of biochar and compost led to significant increases in all growth parameters measured, with Variety 1 (V1) showing greater enhancements compared to Variety 2 (V2). Root and shoot lengths, fresh and dry weights, and leaf area were all markedly higher in the combined treatment groups compared to the control and individual treatments, indicating the synergistic effect of biochar and compost in promoting plant growth under cadmium stress (2).

Table 3: Effect of Biochar and Compost on Cadmium Content, Translocation Factor (TF), and Bioaccumulation Factor (BAF) in Spinacia oleracea

Variety	Treatment	Cd Content in Roots (mg/kg)	Cd Content in Shoots (mg/kg)	Translocation Factor (TF)	Bioaccumulation Factor (BAF) in Shoots	Bioaccumulation Factor (BAF) in Roots
V1	Control	3.2 ± 0.1	4.5 ± 0.2	1.41	0.18	0.12
V1	Biochar	2.8 ± 0.1	3.8 ± 0.2	1.36	0.16	0.11
V1	Compost	2.5 ± 0.1	3.0 ± 0.2	1.20	0.15	0.09
V1	Combined	1.9 ± 0.1	2.2 ± 0.2	1.16	0.12	0.07
V2	Control	3.5 ± 0.1	4.9 ± 0.2	1.40	0.21	0.14
V2	Biochar	3.1 ± 0.1	4.2 ± 0.2	1.35	0.19	0.13
V2	Compost	2.7 ± 0.1	3.7 ± 0.2	1.37	0.17	0.11
V2	Combined	2.2 ± 0.1	2.8 ± 0.2	1.27	0.13	0.09

The results indicated that the combined application of biochar and compost significantly reduced the cadmium content in both roots and shoots of Spinacia oleracea. The translocation factor (TF) and bioaccumulation factor (BAF) were also markedly decreased in the combined treatment, with V1 showing lower Cd content and translocation compared to V2, suggesting a higher tolerance of V1 to cadmium stress (3).

Table 4: Effect of Biochar and Compost on Antioxidant Activities and Oxidative Stress Parameters in Spinacia oleracea

Variety	Treatment	Superoxide Dismutase (SOD) (%)	Peroxidase (POD) (%)	Catalase (CAT) (%)	Membrane Stability (%)	Malondialdehyde (MDA) (nmol/g)
V1	Control	32.4 ± 1.5	25.6 ± 1.2	45.8 ± 2.1	66.1 ± 2.5	5.8 ± 0.3
V1	Biochar	42.7 ± 2.0	34.1 ± 1.6	57.4 ± 2.7	73.4 ± 2.8	4.6 ± 0.2
V1	Compost	45.8 ± 2.2	36.5 ± 1.7	60.1 ± 2.8	75.6 ± 2.9	4.2 ± 0.2
V1	Combined	56.8 ± 2.6	44.2 ± 2.1	69.3 ± 3.2	81.1 ± 3.1	3.4 ± 0.2
V2	Control	29.7 ± 1.4	22.5 ± 1.1	42.1 ± 2.0	63.7 ± 2.4	6.2 ± 0.3
V2	Biochar	39.3 ± 1.9	32.4 ± 1.5	54.8 ± 2.6	71.5 ± 2.7	5.0 ± 0.2
V2	Compost	42.1 ± 2.0	34.8 ± 1.6	58.7 ± 2.7	74.3 ± 2.8	4.6 ± 0.2
V2	Combined	51.8 ± 2.4	41.6 ± 2.0	66.1 ± 3.1	79.4 ± 3.0	3.8 ± 0.2

The combined application of biochar and compost significantly enhanced antioxidant activities in Spinacia oleracea, with increases in superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities. Membrane stability also improved, while malondialdehyde (MDA) content, an indicator of lipid peroxidation, decreased significantly, particularly in V1, which showed higher antioxidant activity and better protection against oxidative stress compared to V2 (4).

Overall, the results clearly indicate that the combined application of biochar and compost effectively mitigated the detrimental effects of cadmium toxicity in Spinacia oleracea, enhancing plant growth, reducing cadmium uptake, and improving physiological and antioxidant responses. This suggests a strong potential for the combined use of these organic amendments in the remediation of cadmium-contaminated soils.

4 Discussion

The findings of this study demonstrated that the combined application of biochar and compost significantly mitigated cadmium (Cd) toxicity in Spinacia oleracea, leading to marked improvements in growth parameters, physiological attributes, and antioxidant responses. These results are consistent with previous research that has highlighted the effectiveness of organic amendments in reducing the bioavailability of heavy metals in contaminated soils, thereby enhancing plant growth and stress tolerance. The improvements observed in root and shoot length, biomass accumulation, and leaf area in plants treated with biochar and compost underscore the synergistic effect of these amendments in promoting plant health under Cd stress (11-15).

The reduction in Cd content, translocation, and bioaccumulation in both root and shoot tissues, particularly in the combined treatment group, aligns with earlier studies that reported the ability of biochar to immobilize heavy metals and reduce their uptake by plants. Biochar's high surface area, cation exchange capacity, and alkaline pH contribute to its ability to adsorb heavy metals, thereby reducing their solubility and mobility in the soil (2). However, the study also revealed that compost, rich in organic matter and nutrients, played a crucial role in enhancing soil fertility and supporting plant growth, which biochar alone could not achieve to the same extent. The combined use of biochar and compost thus provided a balanced approach, offering both immobilization of heavy metals and improved nutrient availability, which collectively enhanced the overall growth and Cd tolerance of Spinacia oleracea (13,16).

Physiologically, the increased levels of photosynthetic pigments, such as chlorophyll and carotenoids, and improved gas exchange attributes observed in the combined treatment suggest that biochar and compost effectively mitigated the oxidative stress induced by Cd toxicity. The enhanced antioxidant enzyme activities, including superoxide dismutase, peroxidase, and catalase, further support the notion that these amendments helped maintain cellular homeostasis by scavenging reactive oxygen species (ROS), thereby protecting the plants from oxidative damage (14,17,18). This protective effect was particularly pronounced in Variety 1 (V1), which exhibited lower Cd accumulation and higher antioxidant activities compared to Variety 2 (V2), indicating a varietal difference in Cd tolerance that warrants further investigation.

The strengths of this study include the comprehensive assessment of both growth and physiological parameters, providing a holistic understanding of the effects of biochar and compost on Cd-stressed plants. The use of Spinacia oleracea, a known hyperaccumulator, also adds relevance to the findings, as it highlights the potential of these organic amendments in enhancing phytoremediation efforts. However, the study also had limitations. The experiment was conducted under controlled conditions, which may not fully replicate field conditions where multiple environmental factors could influence the effectiveness of the amendments. Additionally, the study focused solely on the effects of biochar and compost, without exploring the potential benefits of integrating other soil amendments or plant growth-promoting rhizobacteria, which have been shown to further enhance phytoremediation and plant growth (5).

Given these findings, future research should consider field trials to validate the effectiveness of biochar and compost under real-world conditions and explore the long-term impacts of these amendments on soil health and crop productivity. Moreover, investigating the interactions between biochar, compost, and other soil amendments could provide insights into optimizing amendment strategies for different crops and contamination scenarios. The study also recommends exploring the genetic basis of varietal differences in Cd tolerance, as this could inform breeding programs aimed at developing more resilient crop varieties for use in contaminated environments.

This study provided strong evidence that the combined application of biochar and compost is an effective strategy for mitigating Cd toxicity in Spinacia oleracea, enhancing plant growth, reducing heavy metal uptake, and improving physiological resilience to stress. These findings have significant implications for sustainable agriculture and environmental remediation, particularly in regions where soil contamination poses a major threat to food security and human health. The study contributes to the growing body of literature supporting the use of organic amendments in phytoremediation, offering practical solutions for managing heavy metal-contaminated soils while promoting healthy crop production (6).

5 Conclusion

The study concluded that the combined application of biochar and compost effectively mitigated cadmium toxicity in Spinacia oleracea, significantly enhancing plant growth, reducing cadmium uptake, and improving physiological resilience. These findings underscore the potential of these organic amendments as a sustainable strategy for managing heavy metal contamination in agricultural soils. In terms of human healthcare, this approach not only promotes safer crop production by reducing heavy metal accumulation in edible plants but also contributes to environmental health by improving soil quality, thus playing a crucial role in safeguarding food security and public health in contaminated regions.

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Disclaimers	
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