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KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Study of the Inhibition of Cadmium Uptake in Lettuce with Cassava Residue Biochar**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group
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APPROVE
Head of Biotechnology, Leather and Fur Department, Professor,
Dr. Sc., Prof.
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ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Chen Anqi

1. Thesis topic **Study of the Inhibition of Cadmium Uptake in Lettuce with Cassava Residue Biochar**

Scientific supervisor Ph.D., Assoc. Prof. Olena Okhmat
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WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
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8	Submission of bachelor's thesis to the department for review (14 days before the defense)		
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)		Similarity coefficient ____% Citation rate ____%
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I am familiar with the task:

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Abstract

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Cadmium (Cd), as a non-essential metal element in the process of plant growth, is widely present in different environments, and cadmium pollutants are an important member of inorganic pollutants in Chinese soil. Biochar can be used to remediate heavy metal contaminated soil, which can restore soil. After treatment with biochar, the mobility and bioavailability of heavy metal ions are reduced, the absorption efficiency of heavy metal ions by plants is reduced, and the final yield is improved. In this study, through pot experiments, the effects of cassava residue biochar on the growth of lettuce in heavy metal cadmium contaminated soil, MDA content, antioxidant enzyme POD, SOD activity, and the accumulation of heavy metal cadmium were studied. The experiment found that the application of cassava residue biochar significantly promoted the accumulation of lettuce biomass in cadmium contaminated soil; the application of cassava residue biochar effectively reduced the content of malondialdehyde (MDA) in lettuce leaves in cadmium contaminated soil, alleviating the level of oxidative stress; the application of cassava residue biochar increased the activity of peroxidase (POD) and superoxide dismutase (SOD) in lettuce leaves, which helps to clear the accumulation of reactive oxygen species caused by cadmium stress; the application of cassava residue biochar reduced the accumulation of cadmium in lettuce leaves and roots, especially in lettuce roots. The accumulation of cadmium has significantly decreased. The results ultimately showed that the application of cassava residue biochar in cadmium contaminated soil can significantly promote the accumulation of lettuce biomass and effectively reduce the absorption of cadmium by lettuce. The research results indicate that cassava residue biochar is an efficient cadmium blocker for lettuce.

Key words: Manioc waste, Biochar, Cadmium, lettuce, Heavy metal immobilization

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INTRODUCTION

Cadmium (Cd) contamination in soil is a significant environmental issue, particularly in agricultural regions where heavy metal pollution threatens food safety and ecosystem health. Cadmium, a non-essential and toxic metal for plants, enters the soil through industrial activities, wastewater irrigation, and the use of contaminated fertilizers. Its accumulation in crops poses serious risks to human health, making effective remediation strategies essential. Biochar, a carbon-rich material derived from biomass pyrolysis, has emerged as a promising solution for immobilizing heavy metals in soil due to its high adsorption capacity and ability to improve soil properties.

This study focuses on the application of cassava residue biochar to mitigate cadmium uptake in lettuce (Lactuca sativa) grown in contaminated soil. Cassava residue, a byproduct of starch and ethanol production, is an abundant agricultural waste that can be repurposed into biochar, aligning with sustainable waste management practices. The research aims to evaluate the effects of cassava residue biochar on lettuce growth, oxidative stress markers, antioxidant enzyme activity, and cadmium accumulation, providing insights into its potential as a low-cost and eco-friendly soil amendment.

The relevance of the topic lies in addressing the urgent need for sustainable remediation techniques to combat cadmium pollution in agricultural soils, ensuring food safety and environmental health.

The purpose of the study is to investigate the efficacy of cassava residue biochar in reducing cadmium uptake in lettuce and to elucidate the underlying mechanisms, thereby contributing to the development of practical solutions for heavy metal-contaminated farmland.

The objectives of the study are:

1. To assess the impact of cassava residue biochar on lettuce biomass production under cadmium stress.

2. To evaluate the biochar's role in alleviating oxidative stress by measuring malondialdehyde (MDA) content and antioxidant enzyme (POD, SOD) activities.

3. To determine the reduction in cadmium accumulation in lettuce tissues (leaves and roots) following biochar application.

4. To analyze the mechanisms by which cassava residue biochar immobilizes cadmium in soil and inhibits its uptake by plants.

The object of the study is lettuce (Lactuca sativa) grown in cadmium-contaminated soil treated with cassava residue biochar.

The subject of the study is the interaction between cassava residue biochar, cadmium, and lettuce plants, focusing on growth, physiological responses, and cadmium accumulation.

Research methods include:

- Pot experiments with controlled cadmium contamination and biochar application rates.

- Measurement of dry biomass, MDA content, and antioxidant enzyme activities (POD, SOD).

- Quantification of cadmium content in lettuce tissues using inductively coupled plasma atomic emission spectrometry (ICP-AES).

- Statistical analysis to evaluate treatment effects and correlations.

The scientific novelty of this study lies in the utilization of cassava residue biochar, an under-explored agricultural waste, for cadmium immobilization in soil, and the comprehensive analysis of its effects on plant physiology and cadmium uptake.

The practical significance of the results obtained is the potential application of cassava residue biochar as a sustainable and cost-effective soil amendment to reduce cadmium bioavailability in contaminated farmland, thereby improving crop safety and productivity. This research supports the circular economy by transforming agricultural waste into a valuable resource for environmental remediation.

CHAPTER 1
LITERATURE REVIEW

1.1 Current Status of Cadmium Contamination in Soil

Cadmium (Cd), a non-essential metal element for plant growth, is widely present in various environmental settings. Soil pollution is one of the most pressing environmental challenges in China, with heavy metal contamination being the predominant type. Among inorganic pollutants in soil, cadmium stands out as a particularly significant concern[1]. The primary sources of cadmium in soil are anthropogenic activities[2]. Based on the pathways of contamination, cadmium pollution can be broadly categorized into two types: irrigation with cadmium-laden wastewater and application of cadmium-containing solid waste.

Irrigation with cadmium-contaminated wastewater typically originates from industrial effluents discharged by facilities such as mining operations, galvanizing plants, dye and paint manufacturers, and rubber factories. When such wastewater is applied to farmland without proper treatment, it leads to cadmium accumulation in the soil. As a result, crops grown in these areas often contain cadmium concentrations several times higher than safety limits[3].

Application of cadmium-bearing solid waste involves the use of contaminated sludge or fertilizers with excessive cadmium levels. Improper or prolonged application of these materials can result in the gradual buildup of cadmium in the soil, eventually leading to significant contamination[5].

1.2 Impact of Cadmium Pollution on Plants

1.2.1 Effects on Plant Growth

The presence of cadmium during the process of plant growth can have profoundly adverse effects. At certain concentrations, cadmium can hinder seed germination and inhibit plant development. It may cause root deformation and necrosis, lead to curling and wilting of leaves, and disrupt normal physiological processes within the plant, ultimately preventing healthy growth[7].

1.2.2 Effects on Photosynthesis

Cadmium contamination can alter the structure of chloroplasts within plant leaves, thereby reducing their photosynthetic capacity[8]. It also inhibits stomatal opening and suppresses chlorophyll synthesis, which impairs the plant's gas exchange efficiency 9. Additionally, cadmium damages photosynthetic pigments, preventing the plant from carrying out normal photosynthesis.

1.2.3 Effects on Mineral Nutrient Uptake

Cadmium contamination adversely affects the plant's ability to absorb, transport, and utilize essential nutrients through its root system. In severe cases, it can even interfere with water uptake. Studies have shown that under high levels of cadmium stress, cadmium competes with essential mineral elements such as calcium, magnesium, potassium, and phosphorus in the soil, thereby hindering the roots' ability to absorb adequate amounts of these nutrients[11]. Furthermore, cadmium disrupts the activity of nitrate reductase, inhibiting the absorption and transport of nitrate ions[12], and may also impair the root's capacity to assimilate ammonia and nitrogen[13].

1.2.4 Oxidative Damage

Cadmium may cause oxidative stress in plants either directly or indirectly by promoting the formation of reactive oxygen species (ROS), which disrupts normal plant growth and development[14]. While some plants are capable of producing antioxidant enzymes to counteract cadmium-induced stress, these defense mechanisms often become ineffective under conditions of high cadmium concentrations[16].

1.2.5 Defense Mechanisms Against Cadmium Toxicity in Plants

To mitigate the toxic effects of cadmium on plants, researchers both in China and abroad have proposed a variety of strategies, which can be broadly categorized into two approaches: tolerance and avoidance. The tolerance strategy involves enhancing a plant's resistance to cadmium through breeding techniques. Plants achieve cadmium tolerance by binding cadmium ions with peptides, amino acids, or proteins, thereby storing and sequestering cadmium to reduce its toxicity. Hyperaccumulator plants, in particular, can chelate cadmium ions within their tissues, rendering them non-toxic[17]. The avoidance strategy focuses on restricting cadmium uptake by plants. This can be achieved by modifying the physicochemical properties of the soil to reduce cadmium bioavailability or by introducing external substances that chelate cadmium and neutralize its toxic effects. Studies have shown that soil pH significantly influences cadmium accumulation in vegetables. When cadmium levels in the soil are low, lower pH values lead to higher cadmium accumulation in edible plant parts. Conversely, when soil cadmium levels are high, maintaining a higher pH can help reduce cadmium uptake in plant tissues[20].

1.3 Cassava Residue

1.3.1 Overview of Cassava Residue

Cassava, also known as manioc, is a starchy tuber crop widely cultivated in tropical and subtropical regions. It serves as a food source, livestock feed, and raw material for various by-products. Cassava ranks as the fifth most important global source of starch. The by-products left after starch extraction or ethanol production from cassava are collectively referred to as cassava residue. The primary component of cassava residue is lignocellulose[21].

1.3.2 Reutilization of Cassava Residue

Discarded cassava residue is commonly used for composting; however, this process still leaves behind a large amount of solid waste, which can contribute to secondary pollution[22]. Some studies have explored the production of bioethanol from cassava residue, but the process requires specific pretreatment conditions to soften the material and ensure sufficient contact between cellulase and the fibrous substrate. Unfortunately, this softening process is technically demanding and involves the use of large quantities of organic solvents, making it unsuitable for widespread application[23].

Recent research has shown that cassava residue can be converted into biochar with excellent properties. Cassava residue biochar improves the physicochemical characteristics of soil, enhances nutrient content, and increases the soil's water retention capacity. It also stimulates microbial activity in the soil, showing particularly notable effects in the remediation of acidic soils[24]. This type of biochar typically contains a high level of carbon and is rich in essential nutrients such as nitrogen, phosphorus, and potassium, all of which contribute to improved soil conditions and enhanced plant productivity[25].

1.4 Biochar

1.4.1 Overview of Biochar

Biochar is a carbon-rich, stable material produced through the pyrolysis of biomass-such as crop residues, woody materials, and animal manure—under oxygen-limited or anoxic conditions at temperatures below 800°C. It is essentially a form of black carbon, with carbon (C) as its principal component. Most biochars typically contain 60% to 80% carbon by weight[26].

As a carbon-based solid adsorbent, biochar exhibits a high degree of heterogeneity. Variations in pyrolysis temperature during its production result in differing physical properties and structures. Key characteristics such as porosity, specific surface area, and ash content significantly influence the physicochemical behavior of biochar[27]. Depending on the source materials used, the pore size of biochar generally ranges from 0.9 to 50 nanometers[28].

1.4.2 Mechanisms of Heavy Metal Immobilization by Biochar

Biochar has the capability to immobilize heavy metals in soil. This is primarily achieved through mechanisms such as electrostatic adsorption, ion exchange, precipitation, and complexation, which enable the capture and stabilization of heavy metal ions.

Biochar typically contains oxygen-containing functional groups—such as carboxyl groups (–COOH)—that can bind heavy metal ions through ion exchange processes[29]. In addition, biochars that release cations like calcium and magnesium can further exchange with metal ions in the soil[30]. The abundance of functional groups in biochar also provides numerous active sites for metal complexation[31]. As a result, soluble metal ions are transformed into insoluble forms, reducing their mobility and bioavailability in the soil.

Biochar can also influence the solubility and bioavailability of heavy metal ions by altering the physicochemical properties of soil. Generally alkaline in nature, biochar increases soil pH upon application, which in turn reduces the solubility of heavy metals. In addition, biochar exhibits cation exchange capacity; when introduced into soil, it can release mineral elements such as sodium, potassium, calcium, and phosphorus, which then participate in the adsorption of metal ions[32]. Moreover, biochar can release dissolved organic carbon, thereby enriching the soil's organic carbon content and further reducing the mobility of heavy metals[33].

1.4.3 Application of Biochar in the Remediation of Heavy Metal-Contaminated Soil

Studies have shown that biochar, as an ideal adsorbent, holds significant promise for the remediation of soils contaminated with heavy metals. Biochar exhibits high adsorption capacity and efficiency for heavy metal pollutants present in soil. Venegas et al.[25]produced biochar from materials such as sludge, bark, and grapevine residues, and found that it effectively adsorbed elements including Pb, Zn, Cd, Ni, and Cu. An Zengli et al.[34]prepared rice straw biochar under low-temperature conditions (300–400°C), which resulted in a greater number of adsorption sites, thereby enhancing its ability to immobilize heavy metals in soil. Similarly, Jiang Tianyu et al.[35] demonstrated that biochar can effectively adsorb cadmium (Cd) from contaminated soil.

The effectiveness of biochar in remediating heavy metal-contaminated soils depends on both the type of biochar used and the application rate. When biochar is applied to soil, it induces changes in soil properties such as pH, organic carbon content, cation exchange capacity, and electrical conductivity[36]. These changes contribute to the reduced mobility and bioavailability of heavy metal ions, thereby lowering their uptake by plants and ultimately improving crop yields. Furthermore, biochar often contains minerals such as carbonates and phosphates, which not only aid in the immobilization of heavy metals but also provide essential nutrients and trace elements to crops[37]. Successful remediation of heavy metal-contaminated soils requires a comprehensive approach that considers the characteristics of both the crop and the biochar. It is also necessary to assess the extent of soil contamination and carefully evaluate the interactions between biochar, soil, and heavy metal ions in order to select the most appropriate type and dosage of biochar for effective remediation.

1.5 Objectives and Significance of This Study

Soil pollution is one of the major environmental threats facing China, with heavy metal contamination being a key component. Among heavy metals, cadmium is one of the most common and hazardous contaminants. This study aims to investigate the effects of cassava residue biochar—produced from agricultural waste—on the growth of lettuce and cadmium uptake under cadmium-contaminated conditions, using varying application rates of the biochar. By utilizing agricultural waste as a resource, this research provides technical support for the safe use of cadmium-contaminated farmland and offers a theoretical foundation for the development of low-cost and environmentally friendly soil remediation technologies.

Conclusions to chapter 1

1. Cadmium, a non - essential metal for plants, is prevalent in the environment. In China, soil pollution, especially heavy metal contamination, is a serious environmental issue, with cadmium being a major concern among soil inorganic pollutants. Anthropogenic activities are the main sources of cadmium in soil. Cadmium pollution can be mainly divided into two types: irrigation with cadmium - laden wastewater from industries like mining, galvanizing, dye - making, and rubber manufacturing, which causes cadmium accumulation in soil and makes crops' cadmium levels exceed safety limits; and application of cadmium - containing solid waste such as contaminated sludge or high - cadmium fertilizers, leading to gradual soil cadmium buildup and contamination.

2. Cadmium pollution exerts extensive and profound impacts on plants, hindering seed germination and plant development, causing root deformation and

necrosis, leaf curling and wilting, and interfering with normal physiological processes. It reduces photosynthetic capacity by altering chloroplast structure, inhibiting stomatal opening and chlorophyll synthesis, and damaging photosynthetic pigments. Cadmium also affects the absorption, transport, and utilization of essential mineral elements like calcium, magnesium, potassium, and phosphorus in plant roots, and interferes with nitrate reductase activity to inhibit nitrate ion absorption/transport and root assimilation of ammonia and nitrogen. Additionally, it directly or indirectly promotes the formation of reactive oxygen species (ROS), triggering oxidative stress—while some plants produce antioxidant enzymes to counteract this, defense mechanisms often fail under high cadmium concentrations. Plant defense strategies against cadmium toxicity primarily include tolerance and avoidance: tolerance enhances resistance through breeding, such as binding cadmium ions with peptides, amino acids, or proteins for storage and sequestration, with hyperaccumulator plants chelating cadmium in tissues to render it non-toxic; avoidance reduces cadmium bioavailability by modifying soil physicochemical properties (e.g., low soil cadmium levels lead to higher cadmium accumulation in edible plant parts under acidic conditions, while maintaining alkaline pH reduces plant tissue uptake under high cadmium levels) or introducing external substances to chelate and neutralize cadmium toxicity.

3. Cassava residue, a by-product from starch extraction or ethanol production of cassava (a starchy tuber crop widely grown in tropical and subtropical regions as a food source, livestock feed, and the world's fifth most important starch

resource), is mainly composed of lignocellulose. While traditionally used for composting, this practice often leads to solid waste and secondary pollution, and attempts to produce bioethanol from it require technically demanding pretreatment with large organic solvents to soften lignocellulose for cellulase interaction, limiting its wide application. Recent research, however, has shown that converting cassava residue into biochar offers promising benefits: this biochar improves soil physicochemical properties (e.g., nutrient content, water retention), enhances microbial activity—especially in acidic soil remediation—and, with its high carbon content and rich nitrogen, phosphorus, and potassium, boosts soil fertility and plant productivity.

4. Biochar, a carbon-rich (60–80% by weight) and stable material produced via pyrolysis of biomass (e.g., crop residues, wood) under oxygen-limited conditions below 800°C, is a heterogeneous carbon-based adsorbent with properties like porosity and specific surface area influenced by pyrolysis temperature and source materials (pore size typically 0.9–50 nm). It immobilizes soil heavy metals through mechanisms such as electrostatic adsorption, ion exchange (via oxygen-containing groups like –COOH and released cations like Ca^{2+} /Mg^{2+}), precipitation, and complexation, transforming soluble ions into insoluble forms; additionally, its alkaline nature raises soil pH, its cation exchange capacity releases minerals (Na^{+} , K^{+} , etc.), and dissolved organic carbon reduces metal mobility. As an ideal adsorbent for heavy metal-contaminated soil remediation, biochar shows high adsorption capacity for pollutants like Pb, Zn, Cd, and Cu (e.g.,

sludge/bark/grapevine residue biochar by Venegas et al. , low-temperature rice straw biochar by An Zengli et al. , and Cd-adsorbing biochar by Jiang Tianyu et al.). Its effectiveness depends on type and application rate, as it modifies soil pH, organic carbon, cation exchange capacity, and electrical conductivity to reduce metal bioavailability and plant uptake, while supplying nutrients (carbonates, phosphates) to crops. Successful remediation requires integrating crop and biochar characteristics, evaluating contamination severity, and assessing biochar-soil-metal interactions to optimize biochar selection and dosage.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

This research, through the resource utilization of agricultural waste, provides technical support for the safe utilization of cadmium-contaminated farmland and offers a theoretical basis for low-cost and green remediation technologies/

2.1 Experimental Materials

2.1.1 Test Materials

The test crop used in this experiment was lettuce, and the seeds were of the variety *Shanghai Ai Kang Qing*, supplied by Zhejiang Sancheng Seed Co., Ltd.

The test soil was sourced from the surface layer of uncontaminated soil at the greenhouse base of Qilu University of Technology, with no detectable heavy metal pollution. The basic physiological indicators of the soil are shown in Table 2.1.

Table 2.1 – **Basic physiological indicators of soil**

Soil Component	Content
Organic Matter	22.3 g • kg^{-1}
Cation Exchange Capacity	14.6 cmol • kg^{-1}
Available Phosphorus (P)	0.35 mg • kg^{-1}
Nitrate Nitrogen	562 mg • kg^{-1}
Ammonium Nitrogen	274 mg • kg^{-1}
pH	7.38

The cassava residue biochar used in the experiment was prepared in-house, with a pyrolysis temperature of 500°C. Its physical and chemical properties are listed in Table 2.2.

Table 2.2 – Physical and Chemical Properties of Biochar

Sample	N (%)	C (%)	H (%)	S (%)	O (%)	H/C	O/C	(O+N)/C
BC500	2.305	45.974	2.002	0.038	10.515	0.044	0.229	0.279

2.1.2 Major Equipment

The spectrophotometer used was model UV-1500, purchased from Shanghai Meixi Instruments. The high-speed refrigerated centrifuge was model Centrifuge 5424R, purchased from Eppendorf. The constant-temperature shaking incubator was model DHZ-2001A, purchased from Jiangsu Peiying. The electronic balance was model JA2003, purchased from Sartorius Scientific Instruments Co., Ltd. The drying oven was model ZXRD-A70880, purchased from Shanghai Zhicheng Analytical Instrument Co., Ltd. Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) was supplied by PerkinElmer.

2.2 Experimental Design

2.2.1 Preparation of Cadmium-Contaminated Soil

The soil was first air-dried in a shaded, cool location. Cadmium chloride hydrate ($CdCl_2 \cdot 2.5H_2O$) was then added and thoroughly mixed into the soil. The treated soil was stored for 14 days under dark conditions at 25°C and 20% humidity to simulate cadmium contamination. Three levels of cadmium concentration were prepared: 0 mg/kg (control), 1 mg/kg, and 2 mg/kg. Cassava residue biochar was added to the soil at three application rates: 0%, 1%, and 3%.

2.2.2 Pot Experiment Setup

Lettuce seeds were used as the experimental material. Seeds of similar size and full maturity were selected for further processing: they were rinsed several times with distilled water and then disinfected with 75% ethanol before being sown in cadmium-free soil. Each pot was sown with 20 seeds. After germination, the seedlings were cultivated for one additional week, and those with uniform growth were transplanted into new pots for the experiment. Each pot contained 9 seedlings, and each treatment was replicated three times.

Throughout the experiment, soil moisture was maintained to ensure optimal growth conditions for the lettuce. Each pot (with an actual diameter of 5 cm and height of 15 cm) was filled with 1 kg of soil. Nine treatment groups were established: Cd0-ck: no cadmium, no cassava biochar; Cd0-B1: no cadmium, 1% cassava biochar; Cd0-B3: no cadmium, 3% cassava biochar; Cd1-ck: 1 mg/kg cadmium, no biochar; Cd1-B1: 1 mg/kg cadmium, 1% cassava biochar; Cd1-B3: 1 mg/kg cadmium, 3% cassava biochar; Cd2-ck: 2 mg/kg cadmium, no biochar; Cd2-B1: 2 mg/kg cadmium, 1% cassava biochar; Cd2-B3: 2 mg/kg cadmium, 3% cassava biochar.

2.2.3 Sample Collection and Processing

According to the requirements of the measurement indicators, lettuce leaves were harvested and washed. A portion of the samples was stored in a refrigerator at 4°C, while another portion was subjected to enzyme deactivation at 105°C for 2 hours, then dried in an oven at 60°C until a constant weight was achieved. Lettuce roots were also collected and washed. Part of the root samples was immediately frozen in liquid nitrogen and stored at –80°C. The remaining portion was enzyme-deactivated at 105°C for 2 hours and then oven-dried at 60°C to a constant weight.

2.3 Experimental Methods

Statistical analysis for significance was performed using SPSS. Identical letters in the results indicate no statistically significant difference between groups ($p > 0.05$). Data in the text are expressed as mean ± standard error ($n = 3$).

2.3.1 Determination of Dry Weight of Lettuce Leaves and Roots

Lettuce leaves and roots were selected and soaked in 0.1 M EDTA-2Na solution for 10 minutes to remove surface-bound metals. They were then rinsed with deionized water and placed into paper bags. The samples were heat-treated at 105°C for 2 hours to inactivate enzymes, followed by drying in an oven at 60°C until a constant weight was reached. The dry weight was then measured using an analytical balance.

2.3.2 Determination of Malondialdehyde (MDA) Content in Lettuce Leaves

A 0.1 g sample of lettuce leaf tissue was placed in a mortar and ground thoroughly. Extraction was performed using 5% trichloroacetic acid (TCA) solution. The resulting extract was transferred to a centrifuge tube and centrifuged at 10,000 rpm for 10 minutes at 4°C. Then, 0.1 mL of the supernatant was pipetted and added to 0.3 mL of 0.5% thiobarbituric acid (TBA) solution. The mixture was vortexed for 1 minute to ensure homogeneity. The mixed solution was then heated in a water bath at 95°C for 30 minutes, with periodic shaking during the heating process. After the reaction was complete, the solution was removed and cooled to room temperature. It was then centrifuged again at 10,000 rpm for 1 minute. The final supernatant was collected and loaded into a microplate, and absorbance was measured at 532 nm and 600 nm using a microplate reader. The MDA content was calculated according to the literature method[38].

2.3.3 Determination of Antioxidant Enzyme Activity in Lettuce Leaves

Lettuce leaves were ground in phosphate buffer (pH 6.0) to form a homogenate. The homogenate was transferred to a centrifuge tube and centrifuged at 6000 rpm for 15 minutes at 4°C. The supernatant was collected and transferred into a 25 mL volumetric flask and stored at 4°C. The remaining residue in the centrifuge tube was re-extracted twice with the same phosphate buffer (pH 6.0), and the resulting supernatants were combined into the same volumetric flask and brought to a final volume of 25 mL. The final solution was preheated in a water bath at 37°C. Then, 0.1 mL of the enzyme extract was added, and the reaction time was recorded immediately using a stopwatch. The optical density (OD) was measured at 470 nm every minute. Enzyme activity was defined as the amount of enzyme causing an increase in OD_{470} of 0.01 per minute. A heat-inactivated enzyme extract was used as the blank control. Enzyme activity was expressed in units per minute per milligram (U/[min·mg]).

Lettuce leaves were ground in phosphate buffer (pH 7.8) to form a homogenate. The homogenate was centrifuged at 6000 rpm for 15 minutes at 4°C. Then, 0.01 mL of the supernatant was added to 4 mL of NBT reaction solution in a test tube and exposed to light at an intensity of 4000 lx for 10 minutes. After a color change occurred, the OD was measured at 560 nm. The enzyme activity was calculated using 0.01 mL of 50 mmol/L phosphate buffer (pH 7.8) as the blank. One unit of enzyme activity was defined as the amount required to inhibit 50% of the photochemical reduction of NBT, expressed as U/mg.

2.3.4 Determination of Cadmium Content in Lettuce Leaves and Roots

Lettuce leaves and roots were dried at 60°C to a constant weight, then ground into a fine powder. A measured amount of the powdered sample was placed into a digestion tube and digested using a mixture of nitric acid and perchloric acid. After digestion, the solution was diluted to a constant volume using 5% nitric acid (HNO_3). The cadmium content in the samples was then determined using inductively coupled plasma atomic emission spectrometry (ICP-AES).

Conclusions to chapter 2

Cassava residue can be converted into biochar with excellent functional properties. Cassava residue biochar improves the physicochemical properties of soil, enriches its nutrient content, and enhances its water retention capacity. It also stimulates microbial activity within the soil. Moreover, this biochar exhibits strong adsorption capacity and efficiency for heavy metal pollutants in soil. When applied to cadmium-contaminated soils in which lettuce is cultivated, cassava residue biochar not only adsorbs cadmium effectively but also helps regulate soil properties and nutrient composition. These benefits highlight its promising potential in environmental remediation and sustainable agriculture.

1. Experimental Materials and Design

Test Materials: Lettuce (variety "Shanghai Ai Kang Qing") and surface soil from Qilu University of Technology (heavy metal-free). Cassava residue biochar (BC500) was prepared via pyrolysis at 500°C.

Cadmium-Contaminated Soil Preparation: Soil was spiked with $CdCl_2 \cdot 2.5H_2O$ to achieve 0 mg/kg (control), 1 mg/kg, and 2 mg/kg Cd levels, with biochar applied at 0%, 1%, and 3%.

Pot Experiment: Nine treatment groups (3 Cd concentrations × 3 biochar rates) with 3 replicates each. Lettuce dry weight, malondialdehyde (MDA) content, antioxidant enzyme activities, and Cd accumulation were measured.

CHAPTER 3
EXPERIMENTAL PART

3.1 Results and Analysis

3.1.1 Effects of Different Treatments on the Dry Weight of Lettuce Leaves and Roots

As shown in Fig. 2.1, compared with the control group Cd0 (no cassava residue biochar added), the experimental groups B1 and B3 (with 1% and 3% biochar, respectively) exhibited a notable increase in the dry weight of both lettuce leaves and roots. Specifically, the root dry weight increased by approximately 0.4 to 0.6 grams, indicating that cassava residue biochar has considerable potential to enhance lettuce biomass. Additionally, treatment groups with different cadmium concentrations (Cd1 and Cd2) were included in the experiment. The results showed that the dry weights of lettuce leaves and roots in these groups were not significantly different from those in the Cd0 control group, remaining around 0.8 grams. This suggests that, under the cadmium concentrations applied in this study, cadmium did not exert a significant negative impact on lettuce biomass.

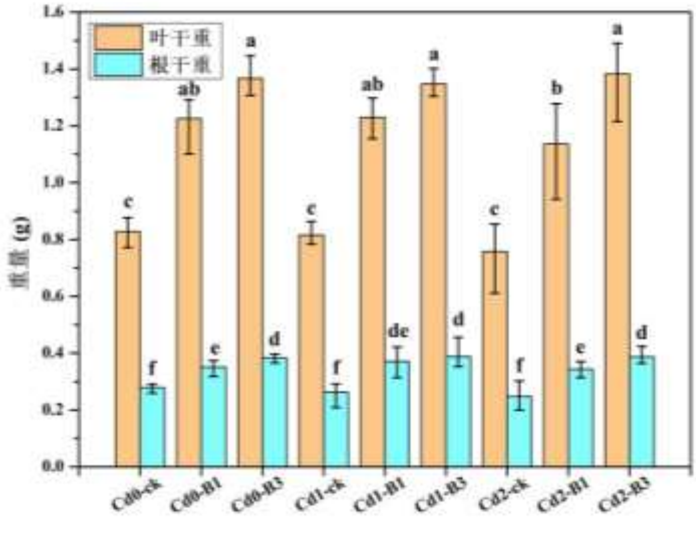

Figure 2.1 – Dry weight of lettuce leaves and roots under different treatments

3.1.2 Effects of Different Treatments on MDA Content in Lettuce Leaves

Malondialdehyde (MDA) is a toxic byproduct formed through lipid peroxidation of cell membranes. Its concentration in biological samples serves as an indicator of oxidative stress within organisms. As shown in Fig. 2.2, compared with the Cd0 group (no cadmium added), the MDA content in lettuce leaves significantly increased in the Cd1 and Cd2 groups, and the increase was more pronounced with higher cadmium concentrations. This indicates that the presence of cadmium induced oxidative stress in lettuce. However, after the application of cassava residue biochar, MDA levels in lettuce leaves decreased across all treatments, regardless of whether cadmium was present in the soil. Compared to the B0 group (no biochar), MDA content in the B1 and B3 groups was consistently lower. Specifically, MDA content decreased by 11–21% in the B0 group, 18–30% in the B1 group, and 23–40 % in the B2 group following biochar addition. Moreover, higher biochar application rates resulted in greater reductions in MDA content. These findings demonstrate that the addition of cassava residue biochar to soil effectively alleviates oxidative stress in lettuce and helps the plant resist cadmium-induced oxidative damage.

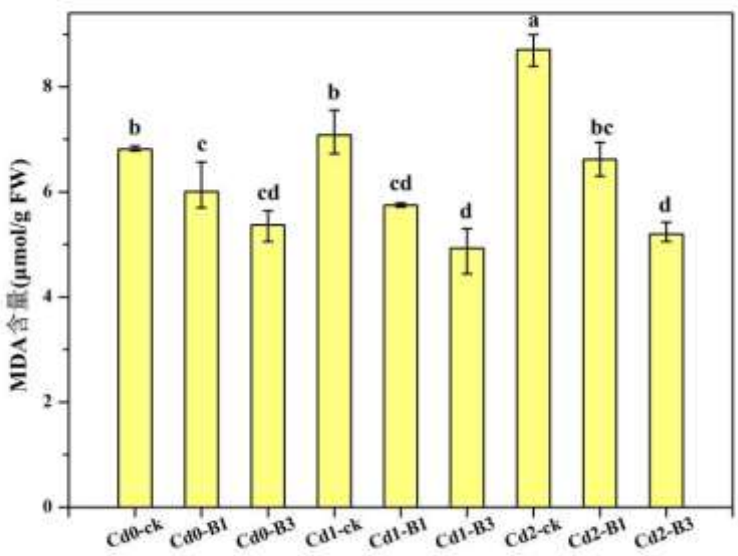

Figure 2.2 – MDA content of lettuce leaves under different treatments

3.2 Effects of Different Treatments on Antioxidant Enzyme Activity in Lettuce Leaves

The accumulation of cadmium within plant tissues can disrupt physiological and biochemical processes, leading to plant wilting and necrosis. Reactive oxygen species (ROS) play a pivotal role in regulating plant growth, responding to abiotic stress, mediating systemic signaling, triggering programmed cell death, and supporting various aspects of plant development[39]. An increase in intracellular ROS levels can cause lipid peroxidation, membrane rupture, electrolyte leakage, and DNA damage, ultimately disturbing the normal physiological and biochemical functions of plant cells. As ROS levels rise, the plant's antioxidant defense system is activated. A key component of this system is a group of antioxidant enzymes that scavenge ROS and mitigate cellular damage[40]. These enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GSH-Px), and ascorbate peroxidase (APX).

As shown in Fig. 2.3, in the control group without cassava residue biochar (ck), there was no significant difference in the activities of the antioxidant enzymes POD and SOD, regardless of whether cadmium was present in the soil. However, after the addition of cassava residue biochar, both the B1 and B3 groups exhibited significantly higher POD and SOD activities compared to the ck group. Moreover, the B3 group, which received a higher biochar dosage, showed a greater increase in enzyme activity. This suggests that under cadmium stress, the inherent capacity of lettuce cells to activate antioxidant defenses is limited. However, the introduction of cassava residue biochar can stimulate the antioxidant defense mechanism in lettuce, enhancing the activities of POD and SOD. This facilitates more effective scavenging of reactive oxygen species generated under cadmium stress, thereby reducing cellular damage in lettuce.

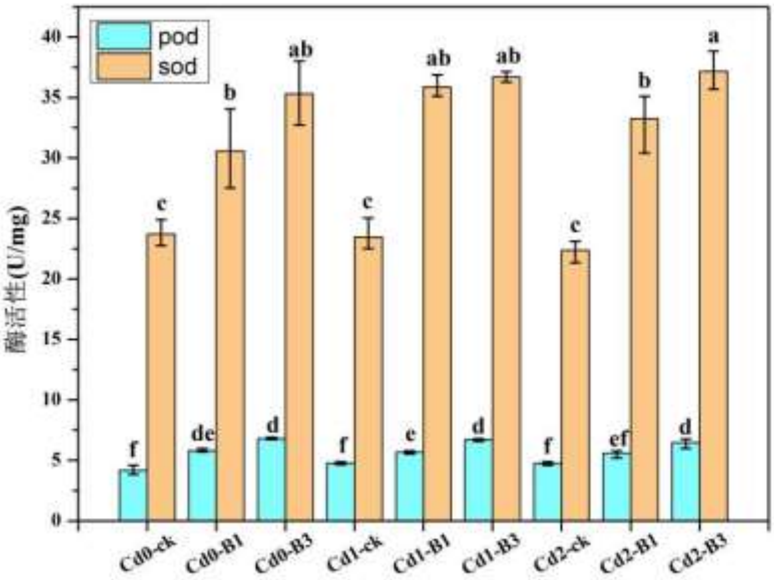

Figure 2.3 – Effects of biochar on lettuce leaves POD and SOD under cadmium stress

3.3 Effects of Different Treatments on Cadmium Accumulation in Lettuce Leaves and Roots

Lettuce has a high potential to absorb cadmium (Cd) from the soil and accumulate it in its leaves. Reducing cadmium accumulation in lettuce is essential for improving food safety for consumers. Studies have shown that biochar has the potential to reduce cadmium accumulation in plants[41]. As shown in Figure 2.4, lettuce grown in cadmium-contaminated soil accumulated substantial amounts of cadmium in both leaves and roots. In the groups without cassava residue biochar, when the cadmium concentration in soil was 1 mg/kg, the cadmium content in lettuce leaves and roots reached 0.218 mg/kg and 1.544 mg/kg, respectively. When the soil cadmium level increased to 2 mg/kg, the cadmium accumulation in the leaves and roots rose to 0.482 mg/kg and 2.618 mg/kg, respectively. These results indicate that lettuce roots tend to accumulate more cadmium than leaves, and that the accumulation is strongly correlated with the cadmium concentration in the soil.

After the addition of cassava residue biochar, the cadmium content in both lettuce leaves and roots decreased significantly, with a more pronounced reduction observed in the roots. In the group with 1 mg/kg of cadmium in the soil, the addition of 1% biochar reduced cadmium content in lettuce roots by approximately 0.25 mg/kg compared to the group without biochar. With a 3% biochar addition, the reduction reached around 0.4 mg/kg. Similarly, in the group with 2 mg/kg of soil cadmium, a 1% biochar addition resulted in a decrease of about 0.6 mg/kg in root cadmium content, while a 3% addition led to a reduction of approximately 0.8 mg/kg. These findings clearly demonstrate that the application of cassava residue biochar significantly reduces cadmium accumulation in both lettuce leaves and roots, with the effect being more substantial in root tissues.

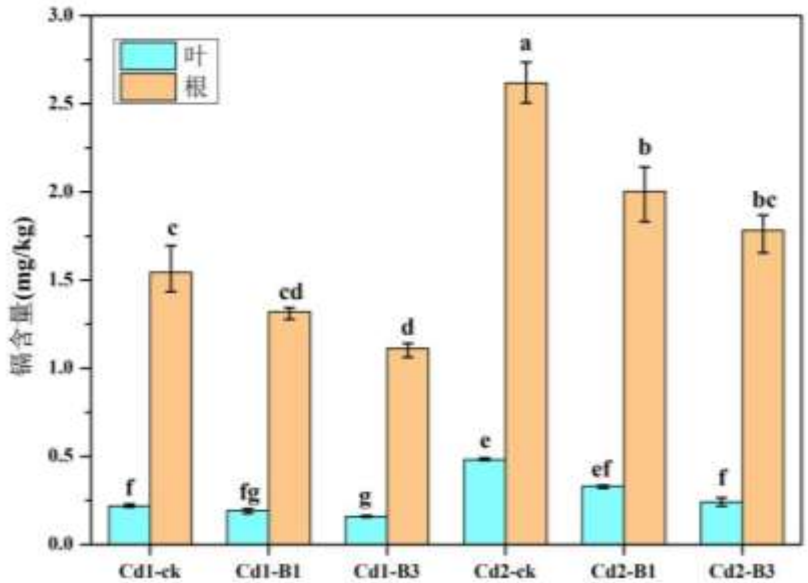

Figure 2.4 – Cd content of lettuce leaves and roots under different treatments

3.4 Analysis of the Mechanism by Which Cassava Residue Biochar Inhibits Cadmium Uptake in Lettuce

Studies have shown that biochar can act as a cadmium immobilization agent for plants, promoting plant growth by increasing soil pH and enhancing the plant's antioxidant capacity[42].

Experimental results from this study indicate that cadmium, in the absence of cassava residue biochar, did not significantly affect lettuce biomass. However, when cassava residue biochar was applied, lettuce biomass increased markedly. This suggests that cassava residue biochar can promote biomass accumulation and improve the overall growth of lettuce, regardless of whether the plants are under cadmium stress.

Experimental results also showed that under cadmium stress, the MDA content in lettuce leaves increased, indicating that cadmium exposure induces oxidative stress in the plants. However, when cassava residue biochar was applied, the MDA content significantly decreased. Under cadmium stress, the presence of cadmium may damage the cell membranes of lettuce leaf cells, reducing nutrient uptake efficiency and increasing lipid peroxidation. The application of cassava residue biochar helped limit cadmium uptake by the plants, thereby alleviating the degree of oxidative stress.

When exposed to heavy metal stress, plants typically activate their antioxidant defense systems, increasing the activity of antioxidant enzymes to mitigate the damage caused by reactive oxygen species (ROS). In this study, it was observed that under cadmium stress alone, the activities of antioxidant enzymes POD and SOD were not sufficiently activated. However, the addition of cassava residue biochar enhanced the activities of both POD and SOD in lettuce leaves, facilitating the removal of ROS accumulated as a result of cadmium stress. Therefore, cassava residue biochar plays a role in boosting antioxidant enzyme activity in lettuce, helping to reduce the physiological toxicity caused by cadmium.

Moreover, the experiment demonstrated that the addition of cassava residue biochar significantly reduced cadmium accumulation in both lettuce leaves and roots. This provides direct evidence that cassava residue biochar can effectively inhibit cadmium uptake in lettuce. In summary, the application of cassava residue biochar in cadmium-contaminated soil not only promotes lettuce biomass production but also reduces cadmium absorption. Thus, cassava residue biochar serves as an effective cadmium-blocking agent for lettuce.

Conclusions to chapter 3

Effect on Lettuce Biomass: 1% and 3% biochar significantly increased dry weights of lettuce leaves and roots (root dry weight +0.4–0.6 g), while Cd contamination (1–2 mg/kg) showed no significant biomass inhibition.

Effect on Oxidative Stress: Cd exposure increased MDA content in lettuce leaves (higher Cd concentrations led to greater increases), but biochar reduced MDA by 11–40%, with 3% biochar showing the highest efficacy.

Effect on Antioxidant Enzyme Activities:Biochar significantly enhanced superoxide dismutase (SOD) and peroxidase (POD) activities, with 3% biochar yielding the greatest increases, indicating improved ROS scavenging under Cd stress.

Effect on Cd Accumulation:Without biochar, Cd accumulation in roots and leaves increased with soil Cd concentration (roots accumulated more Cd than leaves). Biochar addition significantly reduced Cd content in both tissues, with roots showing more pronounced decreases (e.g., 3% biochar reduced root Cd by 0.8 mg/kg in the 2 mg/kg Cd group).

3. Mechanisms of Action

Soil Modification: Biochar elevates soil pH, organic carbon, and cation exchange capacity, reducing Cd bioavailability.

Enhanced Antioxidant Defense: Biochar stimulates SOD and POD activities, mitigating Cd-induced oxidative damage.

Inhibition of Cd Uptake: Biochar immobilizes soil Cd via adsorption and ion exchange, decreasing root uptake and translocation to shoots.

CONCLUSIONS

Based on pot experiments, this study investigated the effects of cassava residue biochar on lettuce growth, MDA content, antioxidant enzyme activities (POD and SOD), and cadmium accumulation in cadmium-contaminated soil. The main findings are as follows:

1. The addition of cassava residue biochar significantly increased biomass accumulation in lettuce grown in cadmium-contaminated soil.

2. The application of cassava residue biochar reduced MDA content in lettuce leaves, thereby alleviating oxidative stress caused by cadmium exposure.

3. Cassava residue biochar enhanced the activities of antioxidant enzymes POD and SOD in lettuce leaves, contributing to the removal of reactive oxygen species generated under cadmium stress.

4. The addition of cassava residue biochar reduced cadmium accumulation in both lettuce leaves and roots, with a particularly notable effect in root tissues.

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