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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 **Effect of iron modified biochar on enzyme activity in rhizosphere soil of lettuce**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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APPROVE
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«___»________________2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Liu Jiacheng

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SUMMARY

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Cadmium (Cd) pollution of soil mainly comes from industrial exhaust and wastewater, mainly manifested in soil dysfunction, soil quality decline, endangering plant physiology and human health, with long-term, hidden and irreversible characteristics. Soil enzymes are biological catalysts in soil, and their main functions include promoting organic matter decomposition, nutrient conversion and energy metabolism. Cd has a certain influence on soil enzyme activity, which affects soil biochemical processes and fertility. Biochar is characterized by rich surface functional groups as well as high carbon content and pore structure, which plays an important role in improving and maintaining the soil organic carbon pool. In recent years, some metals such as iron oxides have been widely used in the modification of biochar adsorbent materials, which have all achieved better results.

In this study, we investigated the effect of Fe-modified biochar on the inter-root soil of lettuce through potting experiments. The iron-modified biochar had a significant amelioration effect on lettuce inter-root soil with or without Cd. The application of 1% and 3% Fe-modified biochar increased the soil pH by 11% and 13.3% compared with that of the original soil, and by 1.9% and 0.8% compared with that of the soil with 1% and 3% added original biochar. Relative to the original soil, the organic matter content of the soil with 1% and 3% Fe-modified biochar was increased by 26.6% and 60.9%, and the urease activity was elevated by 38.7%, respectively, 46.4%, and sucrase activity increased by 34.1% and 38.8%, respectively. In 2 mg/Kg Cd-contaminated soil, 1% and 3% Fe-modified biochar enhanced urease activity by 17% and 1.3%, sucrase activity by 11.9% and 2%, respectively, compared with 1% and 3% virgin biochar. It provides theoretical

support for the study of Fe-modified biochar in modifying the growth and development of lettuce.

Key words: cadmium; biochar; modification of iron; rhizosphere soil; soil enzymes; enzymatic activity; lettuce

TABLE OF CONTENTS

INTRODUCTION

The purpose of this study was to examine the effects of biochar and its modified form (iron-modified biochar) on specific aspects of interroot soil (urease and sucrase activity, pH, and organic matter content) of lettuce in the presence of the heavy metal contaminant cadmium. Through pot experiments, we created control and experimental groups, with the control group consisting of soils without any modified biochar, and the experimental group containing three soils with different cadmium contents mixed with different proportions of regular and iron-modified biochar, respectively. In each experimental group, the same number and size of lettuce seedlings were planted and the pH, activity of organic matter, urease and sucrase of the interroot soil were determined. An innovative aspect of the study was to examine the effects of biochar and its modification on the soil environment near the interroot zone of plants in cadmium-contaminated soil, which has not previously been reported. The practical significance of this study is that it provides important guidance for the remediation of soils contaminated with heavy metals, which can improve soil biochemical processes and promote soil ecological health in accordance with the concept of sustainable agriculture. Compared to the traditional method of using chemical fertilizers and pesticides, this method is more environmentally friendly and has less negative impact on the ecosystem. This research provides a scientific basis for sustainable farming practices, and the use of natural materials such as biochar for soil cultivation can reduce dependence on chemical fertilizers, reduce the environmental impact of agricultural activities and promote further research and application of biochar in related fields.

The relevance of the topic lies in highlighting a specific aspect (urease and sucrase activity, pH, organic matter content) of biochar and its modification (iron-modified biochar), which have not yet been studied (rhizosphere lettuce soil).

The purpose of the study was to study the effect of iron-modified biochar on the properties of Cd-contaminated lettuce rhizosphere soil and the activity of soil enzymes.

Taking into account this goal, the following **tasks** were set and completed:

– to determine the indicators of soil contaminated with cadmium after the application of different types and concentrations of biochar when growing lettuce: pH, organic matter content, urease and sucrase activity:

– to provide recommendations for the management of soils contaminated with cadmium and other heavy metals.

This research will help us better understand the effects of biochar on the soil ecosystem and plant growth, and will provide new ideas and methods for innovative pollution control and soil remediation strategies.

The object of the study are the properties of lettuce rhizosphere soil contaminated with cadmium, as well as primary and iron-modified biochar, lettuce rhizosphere soil with different levels of cadmium contamination.

The subject of the study are the changes in the cadmium-contaminated soil environment around lettuce roots when additives are added in the form of primary and iron-modified biochar.

Research methods: pot experiment, control and experimental groups were created. The control group had soil without any modified biochar added. The experimental group included three types of soils with different contents of cadmium (Cd) were mixed with soil CD 0 mg/kg, 1 mg/kg and 2 mg/kg, respectively, and then with conventional biochar (for example, 1%, 3%) and modified iron. Biochar (e.g., 1%, 3%) was mixed with each of the three soil types. The same number and size of lettuce seedlings were planted in each experimental group and soil rhizosphere pH, organic matter, urease and sucrase were measured.

The scientific novelty lies in the study of the effect of biological carbon and its modification on the soil environment near the plant rhizosphere in Cd-contaminated soils, which has not been previously reported.

The practical significance of the results obtained is that they have important guiding value in the rehabilitation of soils contaminated with heavy metals. Soil biochemical processes can be improved to improve the ecological health of the soil in line with the concept of sustainable agriculture. This method is more environmentally friendly and has less negative impact on the ecosystem than the traditional use of fertilizers and pesticides. This study provides a scientific basis for sustainable agricultural practices. By conditioning soils with materials such as biochar, dependence on chemical fertilizers can be reduced and the environmental impact of agricultural activities can be reduced. The results of the work will be taken into account in further research on the application of biochar in related fields, including optimization of the biochar preparation process, large-scale production and in-situ application.

Structure and scope of the qualification thesis. A qualification thesis consists of an introduction, three chapters, a conclusion, and a list of references.

CHAPTER 1
LITERATURE REVIEW

1.1 Cadmium pollution and hazards

Soil cadmium (Cd) contamination comes mainly from industrial activities, mining activities, transportation and air deposition. In addition, the widespread use of pesticides, agricultural fertilizers, sewage sludge and synthetic fertilizers can also lead to Cd pollution. These activities result in the release of cadmium into the environment, mainly through soil erosion and runoff, and the accumulation of cadmium in the soil. In addition, the World Health Organization (WHO) has previously expressed concern about the degradation of ecosystems in many countries due to cadmium (Cd) contamination in drinking water [1]. At the national scale, cadmium (Cd) is the main polluting heavy metal in soil at the national scale, and according to statistics, atmospheric deposition and livestock and poultry manure were the main sources of heavy metals into agricultural soils from 1999 to 2006, with livestock and poultry manure contributing about 55% of Cd. The latest research results also showed that the heavy metals in farmland soil in China in the last decade mainly came from the contribution of atmospheric deposition [2]. According to the data [3], Cd in soil is more importantly sourced from agricultural inputs in Northeast, North, East and Northwest China, while in Central, South and Southwest China, where the mining industry is more developed, it is influenced by more industrial activities. Research Report on Field Monitoring Experiments of Heavy Metals in Agricultural and Urban Soils in China (2000-2019) [4].

(2000-2019) [4] pointed out that the average concentration of cadmium in soil in China was 0.19 mg-kg -1 , compared with the background value of cadmium in soil (0.097 mg-kg -1), the cadmium content in farmland soil increased by one times (0.19 mg-kg -1), and the cadmium content in urban soil increased by two times (0.29 mg-kg -1). Cadmium concentrations in most of China's urban soils exceeded the standard limits in the soil pollution risk control standards for soil environmental quality agricultural land (GB15618-2018) to some extent [5].

The Agency for Toxic Substances and Disease Registry (ATSDR) states that four heavy metals, Hg, Pb, Cd, and As, are extremely harmful to plants and humans. In general, they may enter the plant system and contaminate the food chain, with dietary intake being the main route of Cd into the human body [6]. In human diet, the concentration of Cd is often influenced by the type of food as well as the level of environmental pollution. Cadmium levels in crops are higher than in meat, eggs, milk, dairy products and fish. In crop products, cadmium levels in cereals (rice, wheat, etc.) and root vegetables (green leafy vegetables, potatoes, etc.) are often higher than those in other crop products [7]. This is extremely dangerous for human health and the safety and quality of food quality. Long-term exposure to cadmium contamination may lead to chronic poisoning, damage to the nervous system, respiratory effects, digestive disorders and kidney damage. Once in the liver, cadmium binds to metallothionein and this complex is slowly released from the liver. Due to the slow release, its biological half-life can be at least ten years. Cadmium then enters the glomerulus and a large amount of cadmium is concentrated in the proximal renal tubules, resulting in nephrotoxic effects [8]. Cadmium pollution adversely affects the growth and development of plants, cadmium-affected plants usually grow short, root growth is impeded, and it also leads to the loss of green and yellow leaves, shortening of stalks, reduction of lateral roots and other symptoms. Lead to plant growth and yield decline and other symptoms, or even death. At the same time, cadmium pollution will also affect the quality of crops, such as nutritional value and taste.

1.2 Treatment of soil cadmium pollution

At present, the management of cadmium contamination in soil mainly covers three categories: physical, chemical and biological [9]. Physical methods, such as soil replacement, deep plowing, etc., can effectively remove cadmium from contaminated soil in a short period of time, but this method requires a large amount of manpower and material input, and the cost is high. What's more, the physical method is only a geographic transfer of pollution, and does not fundamentally solve the problem of soil pollution. Chemical treatment methods, on the other hand, regulate soil pH by adding lime, sulfide and other substances, aiming to reduce the bioavailability of cadmium, thus reducing its potential harm to plant and animal ecosystems. However, this approach carries certain risks because the application of chemical substances may bring in new sources of pollution, and their long-term impact on the environment is difficult to assess accurately and may trigger secondary pollution. In contrast, biological management methods have received increasing attention due to their environmental friendliness, low cost and sustainability. These include the cultivation of plants that are more tolerant to cadmium and the use of specific microorganisms to degrade or immobilize cadmium in the soil. These methods are not only environmentally friendly, but also have the potential for long-term soil remediation. However, bioremediation methods also face challenges, such as the fact that their effectiveness is highly influenced by environmental factors (e.g., temperature, humidity, soil type, etc.), and that in-depth studies are needed to determine the optimal conditions for plant growth as well as the optimal time window for remediation. Therefore, despite the many advantages of bioremediation methods, a variety of factors need to be considered in practical applications to ensure their effectiveness and sustainability [10].

1.3 Functions of biochar

Biochar is characterized by its porous nature, high specific surface area and abundant surface functional groups [11], which make it an excellent adsorbent. Its pore structure includes micropores, mesopores and macropores, which provide a large number of adsorption sites and increase the contact area between heavy metals and biochar. Meanwhile, the functional groups on the surface of biochar (e.g., hydroxyl, carboxyl, and amine groups) are able to form interactions such as electrostatic force, van der Waals force, and hydrogen bonding with the heavy metals, thus realizing the adsorption of heavy metals [12].

The function of heavy metal immobilization by biochar is mainly realized through three aspects: physical adsorption, chemical adsorption and biological adsorption [13].

1. Physical adsorption: Biochar, with its remarkably high porosity and expansive specific surface area, possesses an exceptional ability to physically adsorb a substantial amount of heavy metal ions. This exceptional adsorption capacity primarily hinges on the intricate pore structure and vast surface area inherent to biochar. Its pores, ranging from micro to meson, provide ample space for heavy metal ions to be trapped and retained. Moreover, the extensive surface area of biochar ensures maximum exposure to contaminants, thereby enhancing its adsorption efficiency.

2. Chemical adsorption: The surface of biochar is adorned with various functional groups that exhibit a remarkable reactivity towards heavy metal ions. These functional groups, such as carboxyl and hydroxyl moieties, can engage in chemical reactions with heavy metal ions, forming robust chemical bonds and facilitating adsorption. For instance, the carboxyl and hydroxyl groups present on the biochar surface have the propensity to establish ligand or ionic bonds with heavy metal ions. Consequently, this chemical interaction serves to immobilize the heavy metal ions firmly onto the biochar, effectively sequestering them from the environment. This process not only removes the contaminants but also prevents their further dissemination into the ecosystem.

3. Biosorption: Biochar harbors microorganisms that play a crucial role in the biosorption of heavy metal ions. Through their metabolic activities, these microorganisms can reduce heavy metal ions to metal particles, which are subsequently adsorbed onto the surface of the biochar. This reduction process transforms the contaminants into a less harmful state, facilitating their removal from the environment. Additionally, these microorganisms contribute to the adsorption of heavy metals by

producing extracellular polymers. These polymers, rich in functional groups, provide additional binding sites for heavy metal ions, further enhancing the biosorption capacity of biochar. This symbiotic relationship between biochar and microorganisms offers a natural and efficient method for the remediation of heavy metal-contaminated sites.

It has been shown that the adsorption of Cd2+ by three kinds of biochar increased with the increase of pH when the solution pH was 3-6, using three kinds of agricultural and forestry wastes, namely, rice and corn stover, as the raw materials [14], and preparing biochar by pyrolysis at 500°C. Coconut shell was used as raw material to make coconut shell-based biochar [15], which has good adsorption effect on heavy metal Cd contaminated water. The biochar prepared by using animal's manure pig stable manure [16] has morphological transformation capacity for heavy metals in soil. This biochar not only enhanced the adsorption capacity of heavy metals in the soil, but also increased the crop yield at the same time. This is mainly attributed to the improvement of soil physicochemical properties by biochar and its effective immobilization of heavy metals. In another study, researchers prepared biochar using lignite mixed with rice straw [17].

1.4 Modification of biochar

The adsorption properties and stability of virgin biochar may sometimes not meet the needs of a particular application. In order to improve the ability of biochar to adsorb and immobilize heavy metals, modified biochar with improved effects can be prepared by chemical modification. Modified biochar can improve the immobilization efficiency of cadmium-contaminated soils compared to the use of virgin biochar [18] and reduce the effectiveness and mobility of cadmium in the soil. Since cadmium is mainly bound to the surface of crystalline iron oxides in the soil, iron oxide-modified biochar materials have become the preferred choice for stabilizing cadmium-contaminated soils [19]. Iron-containing materials such as iron (hydro)oxides, iron sulfides, and zero-valent iron have been widely used for biochar modification, and biochar’s containing iron and iron oxides with larger surface area and more functional groups exhibit higher As(V) adsorption efficiency. This modification process can significantly enhance the ability to exhibit excellent adsorption and immobilization [20, 21], especially for the removal of certain heavy metals and organic pollutants. The experimental results showed that the adsorption of heavy metal chromium by modified biochar could reach up to 99.77% under specific preparation conditions and. This result demonstrates the great potential of modified biochar in the treatment of heavy metal pollution.

Iron-modified biochar has good adsorption properties for dye molecules and can effectively remove dyes from wastewater. This is mainly attributed to the abundant functional groups and high specific surface area on the surface of the iron-modified biochar, which provide a large number of adsorption sites for dye molecules. Meanwhile, the introduction of iron also enhanced the adsorption capacity of the biochar, making it easier for dye molecules to be adsorbed on the surface of the biochar [22]. The application of iron-modified biochar to cabbage planting soil resulted in a significant improvement in the growth of cabbage, with an increase in chlorophyll content and biomass [23]. This was mainly attributed to the adsorption and slow release of soil nutrients by iron-modified biochar, as well as the positive effects on soil microbial communities. In addition, iron-modified biochar can be used to remediate heavy metal-contaminated soil. By adsorbing and immobilizing heavy metal ions in the soil, it reduces their toxic effects on plants, thus protecting them from heavy metal stress. This remediation method is not only environmentally friendly and economical, but also sustainable, so it has a wide application prospect in agricultural production.

1.5 Functions of soil enzymes

Soil enzymes play a vital role in soil biochemical processes. They act as biocatalysts to promote oxidation and reduction reactions of certain inorganic compounds. There are more than 40 known soil enzymes, which are mainly categorized into oxidoreductases, hydrolases, transferases and laccases [24]. Among them, urease and sucrase are particularly important for their functions in lettuce inter-root soil. Urease in lettuce inter-root soil contributes to the rapid decomposition of urea, thus ensuring that lettuce can absorb and utilize nitrogen in a timely manner, and promoting the healthy growth of lettuce. Sucrase in the inter-root soil of lettuce can help decompose sucrose, increase the absorption rate of sugar in lettuce, and promote its growth and development. At the same time, sucrase also helps to improve the soil environment and provide an energy source for lettuce inter-root microorganisms. The effects of heavy metals on enzyme activity in soil are multifaceted [25]. First, heavy metals can directly bind to the active centers of enzymes, leading to changes in the structure and function of enzymes, thus reducing enzyme activity. Secondly, heavy metals can also induce oxidative stress and produce harmful substances such as free radicals and harmful oxides, which can further damage enzymes in soil. In addition, the type and concentration of heavy metals, as well as the type and nature of soil, can affect enzyme activity. For example, certain heavy metals may have an activating effect on enzyme activity at low concentrations, whereas they exhibit an inhibitory effect at high concentrations. Relevant studies have shown that if a certain amount of Cd and Pb is injected into the soil, it will have a significant inhibitory effect on urease, indicating that the effect of heavy metals on soil enzyme activity has practical application significance.

Biochar plays a crucial role in promoting soil urease activity. This is primarily due to biochar's expansive specific surface area, enabling it to adsorb a considerable amount of organic matter and nitrogen. When microorganisms in the soil decompose and transform biochar, they generate organic acids. These acids facilitate the release of nitrogen within the soil, subsequently bolstering the substrate supply for soil urease. As a result, this process enhances urease activity [26].

Furthermore, biochar contributes to improving the soil's physicochemical properties. It stabilizes the environment around the active zones of soil enzymes, leading to an increase in the number of active enzyme centers in the soil and strengthening the affinity between these active centers and the substrate. This, in turn, fosters an elevation in sucrase activity. The overall effect of biochar application is a marked improvement in soil fertility and enzyme activity, vital for healthy plant growth and soil sustainability [27].

1.6 Purpose and significance of this study

First of all, this experiment holds profound significance in helping us understand the profound effect of biochar on soil enzyme activity. This understanding paves the way for a deeper exploration of biochar's regulatory effect on the entire soil ecosystem. By carefully studying the impact of biochar on soil enzyme activity, we can gain valuable insights into how biochar influences crucial processes in the soil, such as the decomposition of organic matter, nitrogen conversion, phosphorus release, and other biochemical reactions. These processes are the lifeblood of the soil ecosystem, and understanding their dynamics is crucial to assessing the overall effect of biochar on soil health and fertility.

Delving deeper, this experiment not only sheds light on the biochemical processes but also helps to reveal the larger ecological implications of biochar application. By observing changes in soil enzyme activity, we can infer how biochar might affect the balance and diversity of soil microbiota, nutrient cycling, and ultimately, the sustainability of agricultural systems.

Secondly, the experiment serves as a valuable tool in exploring the relationship between biochar application and plant growth as well as yield. By focusing on the effects of iron-modified biochar on the inter-root soil enzyme activity of lettuce, we can gain a more nuanced understanding of how biochar improves the rhizosphere environment of plants. This understanding is crucial as it directly impacts plant health, nutrient uptake, and ultimately, crop yield. Such insights provide a scientific basis for the strategic application of biochar in agriculture, aiming to optimize crop production while minimizing environmental impact.

Lastly, this experiment contributes to the broader effort of developing new strategies for soil pollution management and remediation. Soil pollution, particularly heavy metal contamination, has become a pressing global issue. Finding cost-effective and environmentally safe treatment methods is imperative. In this context, biochar treatment for soil cadmium pollution emerges as a promising approach with a wide range of potential applications. By studying the effects of iron-modified biochar on soil enzyme activity and plant growth, we can not only assess its efficacy in remediating cadmium-contaminated soils but also draw parallels and lessons for the treatment of other heavy metal-polluted soils.

In conclusion, the experiment on the effect of iron-modified biochar on the inter-root soil enzyme activity of lettuce is a multifaceted endeavor. It not only advances our understanding of the intricate relationships between biochar, soil enzyme activity, and plant growth but also paves the way for innovative soil pollution treatment and remediation strategies. This holistic approach holds the potential to revolutionize sustainable agriculture practices and environmental conservation efforts alike.

Conclusions to chapter 1

1. Soil cadmium pollution mainly originates from activities such as industry, mining, transportation and atmospheric deposition, posing a serious threat to plant and human health.

2. For soil cadmium pollution, current treatment methods include physical, chemical and bioremediation technologies, among which biochar shows great potential in soil heavy metal adsorption and immobilization due to its porous nature, high specific surface area and abundant surface functional groups. Biochar can effectively reduce the bioavailability of cadmium in soil through physical adsorption, chemical adsorption and biosorption. In addition, Fe-modified biochar can further improve the adsorption and immobilization efficiency of Cd.

3. The possible effects of Fe-modified biochar on inter-root soil enzyme activities of lettuce, especially urease and sucrase, which play key roles in soil biochemical processes, are discussed.

4. Biochar and its modified forms have a positive impact on improving soil environment and promoting healthy plant growth, provide new ideas and methods for soil cadmium pollution management, and are of great significance in promoting sustainable agricultural development and environmental protection.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE STUDY

2.1 Experimental materials

2.1.1 Test materials

The test vegetable chosen for our study was Italian lettuce, a variety renowned for its fresh taste and nutritional value. We sourced the seeds from Beijing Sheng Hua De Feng Seed Co. For the soil component of our experiment, we carefully selected the topsoil from the greenhouse at Qilu University of Technology. This particular soil had not been used for any plant cultivation in the past six months, ensuring a clean and unbiased testing environment.

We conducted a thorough analysis of the soil's physicochemical properties, revealing a pH level of 6.5, which indicates a slightly acidic soil condition, ideal for the cultivation of Italian lettuce.

The soil was also found to be rich in organic matter, containing 18.12 g/kg, which is essential for maintaining soil fertility and promoting healthy plant growth.

Additionally, the soil contained significant levels of total nitrogen (1.02 g/kg), total phosphorus (0.93 g/kg), and total potassium (17.31 g/kg).

Furthermore, the soil had adequate levels of basic alkaline nitrogen (43.3 mg/kg), fast-acting phosphorus (11.3 mg/kg), and fast-acting potassium (96.8 mg/kg). we prepared two types of biochar in our laboratory: virgin and iron-modified biochar. Both types of biochar were carefully stored in our laboratory until they were ready to be incorporated into the soil for our experiment.

2.1.2 Main equipment

Table 2.1 – **Main experimental instruments and manufacturers**

Instrument Name	Model	Manufacturer
Ultra-clean bench	FD-01	Suzhou Purification Equipment Factory
Autoclave	MJ3760D	Jinan Deqiang Instrument Qingdao Haier
Refrigerator (4℃, -80℃)	BCD-539WT	Shanghai Meiyan Instrument
Spectrophotometer	UV-1500	Sartorius Scientific Instruments Co.
Electronic balance	JA2003	Shanghai Zhicheng Analytical Instrument Co.
Oven	ZXRD-A70880	METTLER TOLEDO
pH meter	S400-K	International Co.

2.2 Experimental methods

2.2.1 Treatment of Cd-contaminated soil

The above test soil underwent a grinding and sieving process to achieve uniformity in soil particle size, ensuring consistency in our experimental conditions. Following the preparation of the soil, we sprayed the pre-prepared cadmium solution evenly across the soil surface. It was imperative to distribute the cadmium solution as uniformly as possible to mimic real-world cadmium contamination scenarios accurately. To further enhance the homogeneity of cadmium distribution within the soil, we thoroughly mixed the contaminated soil, making sure that the cadmium ions were dispersed evenly. This mixing process was essential to guarantee reliable and repeatable test results. After mixing, the contaminated soil was placed in a controlled environment to allow sufficient time for the cadmium ions to adsorb onto the soil particles and reach an equilibrium state.

2.2.2 Potting experimental setup

The experiment was meticulously conducted from March to April 2024 within the state-of-the-art greenhouse facilities of Qilu University of Technology. Five distinct treatments for each soil sample contaminated: no biochar addition (labeled as "no BC"), the inclusion of 1% virgin biochar (denoted as "BC1%"), 3% virgin biochar ("BC3%"), 1% Fe-modified biochar ("BC-Fe1%"), and 3% Fe-modified biochar ("BC-Fe3%").

A comprehensive setup of fifteen soil treatments was implemented, with three replications per treatment, totaling 45 individual treatments. Soil from each treatment was carefully packed into plastic pots (with a bottom diameter of 14cm, a top diameter of 20cm, and a height of 16cm) according to a predetermined mass of 5kg. After thoroughly mixing the biochar and soil, lettuce seeds from the same batch, carefully selected for their uniformity in size and dimensions, were planted. The potted plants were then relocated to an incubator, where they were exposed to controlled conditions of 25℃, a 16-hour light cycle, and an 8-hour darkness cycle. This day-night cycle was maintained for 40 days, allowing for optimal plant growth and development. Throughout the cultivation process, general cultivation standards were adhered to unless specific requirements dictated otherwise [28].

2.2.3 Sample collection and processing

We collected lettuce inter-root soil by employing the soil shaking method as described in reference [29]. The process began cautiously: lettuce plants were carefully lifted out of the soil while large soil particles were lightly shaken off the roots. This initial step required delicacy to preserve the soil structure and microbial communities attached to the fibrous roots. Following this, we meticulously collected the inter-root soil—the soil that clung tightly to the fibrous roots—using a fine brush.

This soil, rich in microbial life and organic matter, was carefully placed into sterile self-sealing plastic bags to maintain its integrity and prevent contamination. Immediately after collection, the samples were rushed back to the laboratory for further processing. In the controlled environment of the lab, we took great care to air-dry the soil and remove any impurities that might have been introduced during the collection process. This cleaning step was crucial to ensure the accuracy and reliability of subsequent data analysis.

Once the soil was properly prepared, it underwent milling to achieve a fine, uniform consistency. This milling process was followed by screening the soil through 1 mm and 0.15 mm sieves to remove any remaining large particles or debris. After processing, the soil was divided into two portions. One part was stored in a refrigerator at -80 °C to preserve the enzymatic activity for future enzyme activity assays. The low temperature ensures that any biochemical reactions within the soil are slowed down, maintaining the soil's biological integrity for future testing. The second portion of the soil was air-dried and stored under controlled conditions for the determination of soil physicochemical properties, as described in reference [30]. This drying process removes any residual moisture, allowing for more accurate measurements of the soil's chemical composition and physical characteristics.

Throughout this entire process, strict attention to detail and careful handling were paramount to ensure the quality and reliability of the soil samples. From the initial collection to the final storage, each step was carefully executed to guarantee that the soil samples would provide valuable insights into the health and fertility of the soil surrounding the lettuce plants.

2.2.4 Soil pH and organic matter determination

Ph Determination

Soil pH was determined using a Mettler-Toledo pH meter [31], and an appropriate amount of inter-root soil sample of lettuce was accurately weighed in a beaker using an electronic balance according to the soil-water ratio of 1:2.5.

Deionized water was added to the beaker to mix the soil and water thoroughly. Wait for some time to allow the acids and alkalis in the soil to fully dissolve in the water. Then, insert the electrode of the Mettler-Toledo pH meter into the soil suspension, making sure that the electrode is in full contact with the suspension. After the pH meter has stabilized, record the displayed pH value.

Organic matter content measurement:

The organic matter content of the lettuce inter-root soil was determined by the potassium dichromate oxidation-external heating method [32, 33], in which a certain concentration of potassium dichromate-sulphuric acid solution was used to oxidize the organic matter (mainly carbon) in the lettuce inter-root soil under externally heated conditions (usually an oil bath temperature of 180°C and boiling for 5 min). The carbon in the organic matter is oxidized to carbon dioxide while the dichromate ions are reduced to trivalent chromium ions.

The organic carbon content is determined by titrating the remaining potassium dichromate, and since this method only oxidizes about 90% of the organic carbon compared to the dry burning method, the measured organic carbon content needs to be multiplied by a correction factor of 1.1.

2.2.5 Determination of soil enzyme activity

Determination of urease activity

Inter-root soil urease activity of lettuce was determined by sodium phenol-sodium hypochlorite colorimetric method [34]. Inter-root soil samples of lettuce were placed in test tubes and 10 ml (10%) of urease solution was added to the test tubes to ensure that the soil samples were in full contact with the urease. The test tubes were incubated in a thermostat for 24 hours. A certain amount of reaction solution was removed at regular intervals. Phenol-sodium hypochlorite solution was added to the removed reaction solution and color change was observed. The amount of indophenol produced was measured using spectrophotometric colorimetry at 578 nm. Based on the measured indophenol content, the urease activity can be calculated.

Determination of sucrase activity

Sucrase activity of inter-root soil of lettuce was determined by colorimetric method of 3,5-dinitrosalicylic acid [35]. Lettuce inter-root soil samples (0.1 g) were added to 15 μl of toluene and the tubes were placed in a water bath at 37°C for 15 min. Phosphate buffer (250 μL) and sucrase (750 μL) were added. The tubes were placed in a water bath (37°C) for 24 h and centrifuged at 10,000 g for 5 min at 4°C. The supernatant (200 μL) was taken in another test tube, DNS (500 μL) was added and placed in a water bath at 95°C for 5 min. After 10 dilutions with distilled water, the intensity of the colored product was measured by at 510 nm.

Conclusions to chapter 2

1. Italian lettuce was selected as the test vegetable in this study and the soil was not used for any plant cultivation in the last six months to ensure a clean and fair experimental environment. The experimental soil had ideal pH and rich organic matter, as well as moderate amounts of nutrients such as nitrogen, phosphorus and potassium. The experiment also prepared two types of biochar materials, virgin biochar and iron-modified biochar, for soil amendment.

2. The experimental methodology mainly included the treatment of cadmium-contaminated soil, potting experimental setup, sample collection and processing, determination of soil pH and organic matter content, as well as the determination of soil enzyme activity.

3. The determination of these enzyme activities will help us to gain insight into the role of biochar in soil ecosystems and its potential effects on plant growth. In addition, this study aims to evaluate the practical application of biochar as a soil amendment in the remediation of heavy metal-contaminated soils and to provide a scientific basis for further research and practice in related fields

CHAPTER 3
EXPERIMENTAL PART

3.1 Analysis of the results

The inter-root soil pH of lettuce under 15 soil modes is shown in Fig. 2.1.

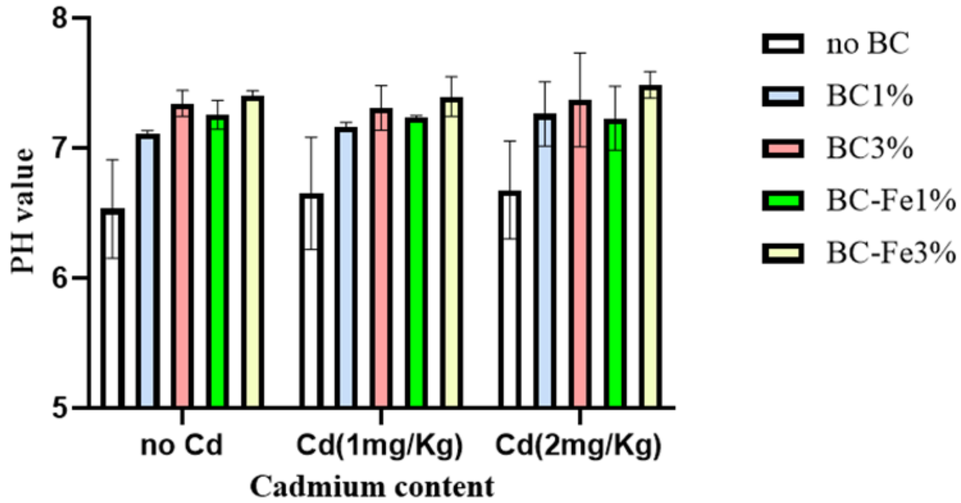

Figure 2.1 – Soil pH at different Cd concentrations

The results showed that at the same cadmium concentration, different treatments had significant effects on lettuce inter-root soil pH, with the BC-Fe3% group being the highest and the no BC group being the lowest. As can be seen from the graphs, regardless of cadmium concentration, the inter-root soil pH of lettuce in the treatment groups with the addition of biochar and iron-modified activated charcoal was generally higher than that of the 'no BC' group without the addition of any treatments, with the highest pH of 6.7 for the no BC group and the lowest pH of 7.1 for the biochar treatment group, and the iron-modified biochar treatment group, which showed a stronger effect of pH enhancement than that of the ordinary biochar treatment group. Further comparison of different percentages of BC and BC-Fe treatments revealed that as the addition of biochar or Fe-modified activated carbon increased (from 1% to 3%), lettuce inter-root soil pH also showed a certain degree of increase. It is noteworthy that the increase in pH was relatively small in the BC1% and BC3% treatment groups. With the increase of cadmium concentration (from 0 mg/Kg to 2 mg/Kg), the pH value of lettuce inter-root soil in the no BC group showed an increasing trend from 6.53 to 6.67. And with the increase of cadmium concentration (from 0 mg/Kg to 2 mg/Kg), the pH value of lettuce inter-root soil in the three treatment groups, except the BC-Fe1% group, showed an increasing trend.

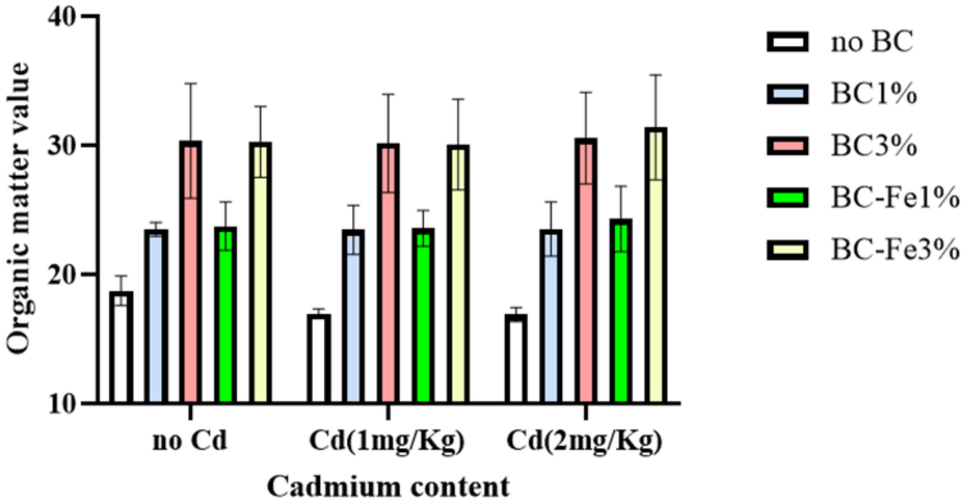

Figure 2.2 – Organic matter content of soils with different Cd contents

The organic matter content of lettuce inter-root soil under 15 soil modes is shown in Fig. 2.2. The results showed that at the same Cd concentration, the different treatments had significant effects on the organic matter content of the lettuce inter-root soil, with the NO BC group having the lowest organic matter content. Overall, the organic matter content of lettuce inter-root soil showed an increasing trend with the increase of BC and its modified form (BC-Fe) addition. At 0 mg/Kg (no Cd), 1 mg/Kg and 2 mg/Kg cadmium (Cd) concentrations, the organic matter content of lettuce inter-root soils supplemented with BC and BC-Fe (either 1% or 3%) was generally higher at the same Cd concentration as compared to the no BC group. With increasing Cd concentration (from 0 mg/Kg to 2 mg/Kg), the organic matter content of the lettuce inter-root soil in the no BC group showed a decreasing trend from 18.7 mg/Kg to 16.9 mg/Kg. The organic matter content of the BC3% treatment group was generally higher than that of the 1% BC treatment group at the same Cd concentration, and the organic matter content of the BC-Fe3% treatment group was generally higher than that of the BC-Fe1% treatment group. Fe1% treatment group. Iron-modified biochar (BC-Fe) did not significantly increase the inter-root soil organic matter content of lettuce compared to regular biochar (BC) at the same additive level (either 1% or 3%). This suggests that iron modification itself may not be the main factor affecting the organic matter content, and that the differences in organic matter content between the BC and BC-Fe treatment groups were not significant at different Cd concentrations. The differences in organic matter content between the BC1% and BC-Fe1% (23.492mg/Kg~24.301mg/Kg), BC3% and BC-Fe3% (30.160mg/Kg~ 31.407mg/Kg).

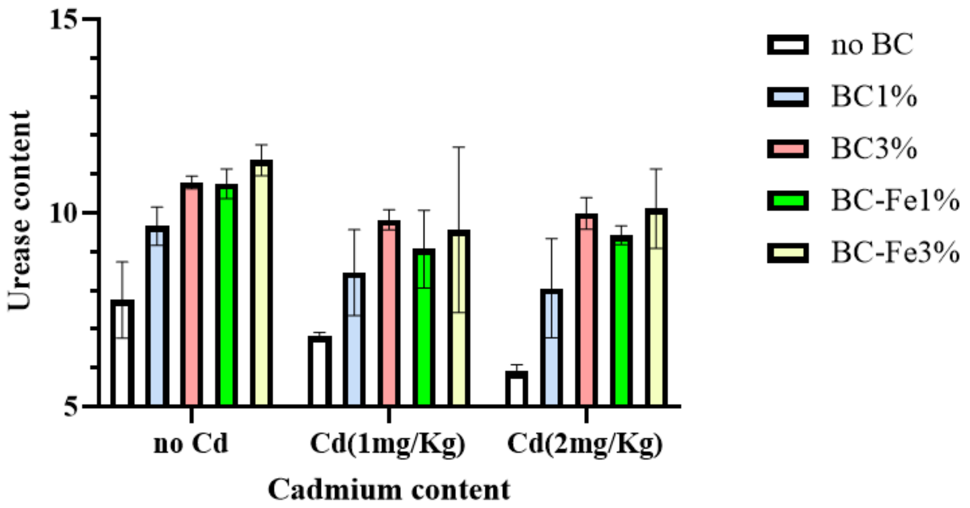

Figure 2.3 – Urease activity in soils with different Cd contents

The inter-root soil urease activities of lettuce under 15 soil patterns are shown in Fig. 2.3. The results showed that at the same Cd concentration, different treatments significantly affected soil urease activity, with the lowest organic matter content in the no BC group. Overall, the organic matter content of lettuce inter-root soils spiked with BC and BC-Fe (either 1% or 3%) was generally higher than that of the no BC group at the same Cd concentration at 0 mg/Kg (no Cd), 1 mg/Kg, and 2 mg/Kg of Cadmium (Cd). The urease content was generally higher in the BC3%

treatment group than in the BC1% treatment group at the same Cd concentration, and similarly, the urease content was generally higher in the BC-Fe3% treatment group than in the BC-Fe1% treatment group. In most cases, iron-modified biochar (BC-Fe) was more effective than ordinary biochar (BC) in enhancing the urease activity of lettuce inter-root soils at the same additive level (either 1% or 3%). As the cadmium concentration increased (from 0 mg/Kg to 2 mg/Kg), the inter-root soil urease activity of lettuce in the no BC group showed a significant decreasing trend from 7.75 IU/g to 5.91 IU/g. A similar trend was observed in the BC1% treatment group, and the enhancement of urease activity obtained by the change in the BC content from 1% to 3% became greater. As the cadmium concentration changed from 1 mg/Kg to 2 mg/Kg, the urease activity increased in the BC-Fe treatment group, while no similar trend was observed in the BC treatment group.

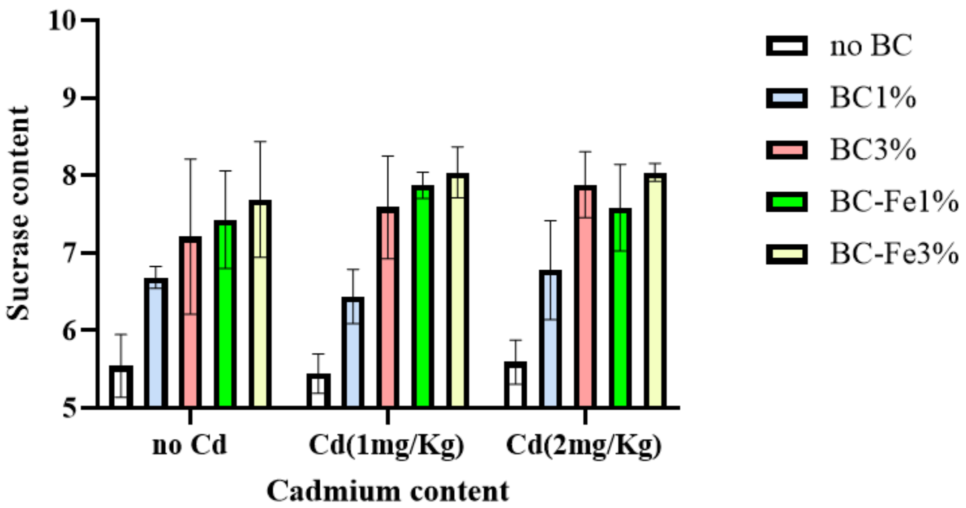

Figure 2.4 – Sucrase activity in soils with different Cd contents

The inter-root soil sucrase activities of lettuce under 15 soil patterns are shown in Fig. 2.4. The results showed that at the same Cd concentration, the different treatments had a significant effect on soil sucrase activity, with the lowest organic matter content in the no BC group. Overall, the sucrase activity of lettuce inter-root soils supplemented with BC and BC-Fe (either 1% or 3%) was generally higher than that of the no BC group at the same cadmium concentration at 0 mg/Kg (no Cd), 1 mg/Kg and 2 mg/Kg of cadmium (Cd), with the highest enzyme activity under the BC-Fe 3% treatment. With increasing cadmium concentration (from 0 mg/Kg to 2 mg/Kg), sucrase content in the no BC group did not change significantly (5.52 ± 0.06 U/g). At 1mg/Kg Cd concentration, with the increase of Cd contamination, the enzyme content elevation under BC-Fe treatment was higher than that of lettuce inter-root soil under BC treatment, but the relative relationship was similar to that at 0mg/Kg. BC and BC-Fe additions were still able to increase the enzyme activity to a certain extent, and it is worth noting that at 2mg/Kg Cd concentration, the degree of elevation of sucrase activity of lettuce inter-root soil under BC-Fe treatment did not change significantly (552±0.06 U/g). The degree of sucrase activity enhancement was not obvious, especially under BC-Fe1% treatment, and even decreased. However, the soil sucrase activities under BC treatments were all increased, by about 0.3 U/g.

3.2 Discussion

The greater the amount of biochar added, the more obvious the effect of enhancing the pH value of the lettuce inter-root soil and the corresponding increase in the organic matter content of the lettuce inter-root soil. This suggests that biochar may enhance the activity of microorganisms in lettuce inter-root soil by improving the physical and chemical properties of lettuce inter-root soil (e.g., improving soil aeration, water retention, pH, etc.), and thus promote the decomposition and re-synthesis of organic matter. It is worth noting that the effects of different ratios of BC and BC-Fe additions on the pH adjustment of lettuce inter-root soil varied, and the Fe-modified biochar did not significantly increase the organic matter content of lettuce inter-root soil compared with that of ordinary biochar, which suggests that the effect of Fe modification on the direct enhancement of the organic matter content of lettuce inter-root soil may be more limited.

It is noteworthy for the changes in enzyme activity that when the soil cadmium content was elevated, the regular biochar treatment group may have required a higher level of biochar in order to have an increase in soil urease activity. As cadmium concentration increased, ordinary biochar was significantly more effective than iron-modified biochar in increasing inter-root soil sucrase activity. And at the same cadmium concentration, increasing the content of iron-modified biochar was not as effective as ordinary biochar in enhancing sucrase activity. It may be because although the iron-modified biochar increased the adsorption capacity of heavy metals, it may have a certain effect on microbial activities or enzyme activities in the soil due to the introduction of iron ions.

Biochar and its modified forms have potential applications in soil remediation and fertility enhancement, but the specific effects are influenced by many factors. The importance of choosing appropriate remediation materials for different enzyme species under different pollution levels is also exposed. In actual soil remediation projects, the addition ratio of biochar or iron-modified activated carbon can be precisely adjusted to achieve an ideal soil acid-base balance and good soil organic matter content

Conclusions to chapter 3

1. Iron-modified biochar showed stronger effect in increasing pH. The addition of biochar and its iron-modified form equally increased the organic matter content of the inter-root soil of lettuce, with limited effect of iron modification in directly enhancing the soil organic matter content.

2. Addition of biochar and its Fe-modified form significantly increased urease and sucrase activities. At lower cadmium concentrations, iron-modified biochar showed better results in enhancing urease activity, but as cadmium concentration increased, plain biochar was more effective in increasing sucrase activity. This may be due to the introduction of iron ions to the soil microbial activity or enzyme activity while increasing the heavy metal adsorption capacity of iron-modified biochar.

3. Biochar and its modified forms have potential applications in soil remediation and fertility enhancement, but the specific effects are influenced by a variety of factors. In actual soil remediation projects, suitable remediation materials should be selected for different pollution levels and different enzyme species, and the addition ratio of biochar or iron-modified activated carbon should be precisely adjusted to achieve ideal soil acid-base balance and good soil organic matter content.

CONCLUSIONS

In this paper, we conducted an in-depth exploration into the effects of soil amendments, specifically virgin biochar and iron-modified biochar, on various soil properties and enzyme activities in the context of heavy metal Cd pollution. Through meticulous soil simulation experiments and potting trials, we were able to observe and document significant changes in the soil environment around lettuce roots.

1. Our findings reveal that under conditions of cadmium stress, the introduction of both virgin biochar and iron-modified biochar led to a notable increase in the pH of the soil surrounding the lettuce roots. This increase was directly proportional to the quantity of biochar added, indicating a clear relationship between biochar application and soil pH enhancement. This observation is crucial as soil pH plays a pivotal role in nutrient availability and plant health.

2. Furthermore, our study demonstrates that the addition of both types of biochar significantly boosted the organic matter content of the soil around the lettuce roots, even in the presence of cadmium stress. Interestingly, there was no discernible difference in the efficacy of virgin biochar and iron-modified biochar in terms of increasing organic matter content. This suggests that both forms of biochar are equally beneficial in enriching soil organic matter, a key factor in soil fertility and plant growth. Additionally, we found a positive correlation between soil organic matter content and the amount of biochar added, highlighting the potential of biochar as a soil enhancer.

3. Our results also indicate that, regardless of cadmium stress, both virgin and iron-modified biochar had a significant positive effect on the urease activity in the soil surrounding the lettuce roots. Notably, iron-modified biochar exhibited a stronger influence on urease activity compared to virgin biochar. This finding suggests that the iron modification process enhances the biochar's ability to stimulate urease activity, which is essential for nitrogen cycling in soil. Moreover, as the concentration of cadmium increased, the impact of iron-modified biochar on urease

activity became even more pronounced, indicating its potential to mitigate the negative effects of heavy metal pollution on soil enzymes.

4. Additionally, our study shows that both virgin and iron-modified biochar significantly boosted sucrase activity in the soil around the lettuce roots, regardless of cadmium stress. However, an interesting observation was made regarding soil cadmium content: at 1 mg/kg of cadmium, iron-modified biochar resulted in higher sucrase activity compared to 2 mg/kg. Conversely, virgin biochar showed higher sucrase activity at 2 mg/kg compared to 1 mg/kg. Furthermore, when comparing the two types of biochar at the same cadmium concentrations, iron-modified biochar consistently demonstrated higher sucrase activity. This finding suggests that iron-modification not only enhances the biochar's ability to stimulate soil enzyme activity but also makes it more resilient to cadmium stress.

In conclusion, our study provides valuable insights into the beneficial effects of biochar amendments, particularly iron-modified biochar, on soil properties and enzyme activities in the presence of cadmium pollution. These findings have important implications for soil remediation efforts and sustainable agriculture practices in contaminated areas.

LIST OF REFERENCES

1. Shahid, Muhammad, Khalid, et al. Heavy metal stress and crop productivity. 2015: 1-25.
2. Peng H, Chen Y, Weng L, et al. Comparisons of heavy metal input inventory in agricultural soils in North and South China: A review. Sci Total Environ, 2019, 660: 776-786.
3. Chen Yali, Weng Li-Ping, Ma, Jie, et al. Progress of Source Analysis of Heavy Metal Pollution in Chinese Soil in the Past Ten Years. 2019, 38: 2219-2238.
4. Xuehong Yuan, Nandong Xue, Zhiguang Han, A meta-analysis of heavy metals pollution in farmland and urban soils in China over the past 20 years. J Environ Sci (China), 2021, 101: 217-226.
5. Changwei Zou, Yujie Jiang, Hong Huang, Advances in the distribution, exposure and health risk assessment of cadmium heavy metal. Journal of Ecotoxicology, 2022, 17(06): 225-243.
6. Huang Yun, Yuan Hong, Huang Zhijun, et al. Advances in the study of population health hazards associated with environmental heavy metal exposure. China Public Health, 2016.
7. Järup L, A Akesson, Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol, 2009, 238(3): 201-8.
8. Nordberg G, Jin T, Wu X, et al. Kidney dysfunction and cadmium exposure--factors influencing dose-response relationships. J Trace Elem Med Biol, 2012, 26(2-3): 197-200.
9. Huang Wei, Zhuang Ronghao, Liu Hui, et al. Progress of Cadmium Pollution in Agricultural Soil and Treatment Methods. Journal of Natural Science, Hunan Normal University, 2022, 45(01): 49-56.
10. Chen Jia, Bao Rong, Current status of cadmium contamination in soil and its prevention and control technology in China. Anhui Agricultural Bulletin, 2020, 26(24): 135-136+181.

11. Zhuang Ye, Wang Juan, A review of the effects of biochar and fertilizers on soil physical and chemical properties and crop growth. Journal of Water Resources and Construction Engineering, 2021, 19(04): 186-193.
12. Li Yibing, Li Han, Huang Wenxuan, et al. Preparation of biochar and its progress in enhancing electron transfer and catalytic performance. Environmental Science Research, 2021, 34(05): 1157-1167.
13. Liu JF, Zhu YB, Yang XS, et al. Research progress of heavy metal removal by biochar. Value Engineering, 2015, 34(22): 149-152.
14. Ji Mengyuan, Hu Yiwen, Liang Cheng, et al. Adsorption Characteristics of Agroforestry Waste-Based Biochar on Heavy Metals Lead and Cadmium. Journal of Ecology and Rural Environment, 2020, 36(01): 106-114.
15. Li Wenxin, Zhang Bing, Zhong Jianxin, et al. Adsorption performance and adsorption mechanism of coconut shell-based biochar on cadmium polluted water. China Petroleum and Chemical Standard and Quality, 2023, 43(21): 130-132.
16. Zhan Xiumei, Peng Jing, Wang Yue, et al. Role of biochar and charcoal-based fertilizer in improving the physicochemical properties of brown soil and increasing peanut yield. Journal of Plant Nutrition and Fertilizer, 2015, 21(06): 1633-1641.
17. Ding Yaling, Liu Biao, Dong Meng, et al. Preparation of biochar by mixing lignite and rice straw and its adsorption of heavy metals. Chemical Technology and Development, 2021, 50(Z1): 55-58.
18. Si T, Yuan R, Qi Y, et al. Enhancing soil redox dynamics: Comparative effects of Fe-modified biochar (N–Fe and S–Fe) on Fe oxide transformation and Cd immobilization. Environmental Pollution, 2024, 347: 123636.
19. Wei B, Zhang D, Jeyakumar P, et al. Iron-modified biochar effectively mitigates arsenic-cadmium pollution in paddy fields: A meta-analysis. Journal of Hazardous Materials, 2024, 469: 133866.
20. Lyu H, Zhao H, Tang J, et al. Immobilization of hexavalent chromium in

contaminated soils using biochar supported nanoscale iron sulfide composite. Chemosphere, 2018, 194: 360-369.

21. Wang S, Zhao M, Zhou M, et al. Biochar-supported nZVI (nZVI/BC) for contaminant removal from soil and water: A critical review. J Hazard Mater, 2019, 373: 820-834.
22. Zhou Shuhong, Zhang Bowen, Wei Jiahua, et al. Adsorption mechanism of several dyes on iron-modified biochar. Environmental Chemistry, 2023(11): 3928-3939.
23. Lin Changhua, Ma Chongjian, Qiu Yongfei, Effects of iron-modified biomass charcoal on cadmium uptake and yield and quality of Brassica napus in cadmium-contaminated soil. Guangdong Agricultural Science, 2019, 46(9): 7.
24. Wang, L. D., Wang, F. L., Guo, C. X., et al. Advances in soil enzymology. Soil, 2016, 48(01): 12-21.
25. Yan Wende, Tian Dalun, Relationship between soil enzyme activity and heavy metal content in manganese mine waste land in Xiangtan. Journal of Central South Forestry College, 2006(03): 1-4.
26. Dai Qunying, Study on the effect of biochar on urease activity. 2014.
27. Yu, Chuan-Yang, Research on the effect of biochar added sludge compost on soil improvement. Green Technology, 2017(08): 95-98+100.
28. Du Yongzhi, Zhao Chunhui, Feng Yanzhi, Exploration of Crop Planting Management Technology. Journal of Intelligent Agriculture, 2022, 2(11): 67-69.
29. Hao Yanru, Lao Xiurong, Sun Weihong, et al. Interactions between root systems and inter-root microenvironments in wheat/corn intercrops. Rural Ecology, 2003(04): 18-22.
30. Yao Yuhua, Wang Yulin, Yao Xiaohua, et al. Effects of different cropping patterns on the structure of inter-root soil microbial communities of barley. Northwest Journal of Agriculture, 2021, 30(04): 545-554.
31. Huang Xiang, Chen Gang, Hong Juan, et al. Comparative study on the

determination of soil pH in arable land. Hubei Agricultural Science, 2021, 60(19): 106-109.

32. Wang Yuan-peng, Huang Jing, Liu Kai-lou, et al. Evaluation and spatial variability of paddy soil fertility in typical county of northeast China. Journal of Plant Nutrition and Fertilizers, 2020, 26(2): 256-266.
33. Bao, Shidan, Agrochemical analysis of soils. 2000: Soil agrochemical analysis.
34. WANG Yugong, LIU Jingjing, LIU Yixi, et al. Determination of factors affecting soil urease activity by phenol-sodium hypochlorite colorimetric method. Soil Bulletin, 2019, 50(05): 1166-1170.
35. Cai M, Hu C, Wang X, et al. Selenium induces changes of rhizosphere bacterial characteristics and enzyme activities affecting chromium/selenium uptake by pak choi (Brassica campestris L. ssp. Chinensis Makino) in chromium contaminated soil. Environ Pollut, 2019, 249: 716-727.