Original Research

# Biochar Based on *Boehmeria nivea* from Arsenic--Contaminated Soil and the Effect on Soil

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## Abstract

The utilization of remediating plants is an important factor that restricts phytoremediation. Ramie has good adsorption capacity for various heavy metals and is widely used in plant remediation. To achieve the multi-purpose development of heavy metal-contaminated ramie, research has been conducted on the preparation of biochar for using it as a raw material, and the feasibility of returning the prepared biochar to the soil has been explored. The results indicate that 300 °C is the appropriate pyrolysis temperature for preparing ramie biochar. Under these conditions, the biochar yield is  $42.90 \pm 2.29\%$ , significantly higher than other pyrolysis temperatures, and the arsenic concentration is also lower. Removing arsenic from ramie biochar is crucial for biochar to return to the soil, and  $85.62 \pm 1.36\%$  of arsenic can be removed through three rounds of 300 mM sodium hydroxide extraction. Adding modified biochar to heavy metal-contaminated soil can increase soil pH and organic matter content but does not significantly increase soil arsenic content. However, adding modified biochar did not significantly alter soil microbial community structure. A feasible plan for preparing reusable biochar using contaminated ramie was proposed in this study. The research results can directly serve the remediation of soil arsenic pollution using ramie and have reference significance for other plants to carry out heavy metal remediation.

Keywords: ramie, phytoremediation, soil microorganism, pyrolysis temperature, biochar

## Introduction

Heavy metal pollution is an important environmental issue worldwide. It threatens the survival and health of individual organisms and poses a significant threat to the ecosystem and human health through the food chain [1]. Until now, the heavy metals that pollute soil mainly included cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), arsenic (As), copper (Cu), zinc (Zn), nickel (Ni), etc. [2]. Many studies have been conducted on the toxicology and remediation of heavy metal pollution [3,4].

As is the 53rd most prevalent element in the Earth's crust and is considered one of the most carcinogenic and toxic "heavy metals," specifically As (III) [5, 6]. Studies have shown that over 50 countries are affected by As contamination and that As pollution is worsening [6]. To alleviate the toxicity of As pollution, various remediation strategies have been studied, among which bioremediation is considered a promising and environmentally-friendly option for the on-site reduction or stabilization of pollutants and for managing As contamination [6, 7]. Currently, the construction of a network between biological organisms

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and As pollution has become a research focus, including the use of organisms for As pollution control and the effective utilization of contaminated organisms.

In recent years, biochar, a carbon-neutral or carbonnegative product produced by the thermochemical transformation of plant- and animal-based biomass, has been widely used for the remediation of heavy metal pollution in soils [8]. The biochar production process is considered an efficient management method for disposing large amounts of organic waste, which has certain economic benefits. Research has shown the numerous advantages of applying biochar to improve soil quality, reduce the available fraction of heavy metals, promote plant growth, and inhibit the uptake and accumulation of heavy metals [8, 9]. The preparation of biochar is influenced by various factors, such as temperature and heating rate, and the effectiveness of biochar prepared under different conditions varies [8]. To control As pollution, biochars based on various organisms have been widely prepared, including Taraxacum mongolicum [5], bamboo, pine needles, and rice husks [8, 10]. Studies have shown that P and As have similar chemical properties. Soil P content is a critical factor in controlling the mobility of As, and P is often used to modify biochar [5, 8]. Although various types of biochar have been widely used to remediate soil contamination from heavy metals and reduce their mobility and bioavailability in soils in recent years, the negative effects and challenges for their application have also been emphasized, including biochar overapplication, toxic substances in biochar, activation of some heavy metals in soils by biochar, nonspecific adsorption, and the negative effects of biochar on soil microorganisms and plants [9]. Therefore, conducting more in-depth research on biochar and heavy metal adsorption is necessary to achieve effective utilization of biochar.

Boehmeria nivea, commonly known as ramie or Chinese grass, belongs to the Urticaceae family and is widely cultivated as a perennial herb in Asian countries [11]. Field investigations and hydroponic experiments have concluded that ramie possesses a certain degree of constitutional tolerance to Pb, Zn, Cd, and As, which could be a good candidate for the phytoremediation of metal-polluted sites [12]. Under As stress, the toxic effects of As in the form of inhibited growth were apparent, and significant changes in chlorophyll, electrolyte leakage, and H2O2 were observed at the highest level of added As [13]. Ramie is also widely used to prepare biochar, serves as a catalyst [14], adsorbs pollutants [15], and improves soil quality [16]. Soil is the ultimate destination for heavy metals, and the essence of heavy metal pollution remediation is the redistribution of heavy metals. This study collected B. nivea from heavy metal-contaminated areas, and prepared ramie biochar. The adsorption performance of the biochar was further explored. This study applied biochar to As-contaminated soil after removing As to explore its impact on the soil. The research hypothesis that using biochar to redistribute heavy metals is feasible provides a reference for the scientific utilization of biochar.

## **Material and Methods**

## Preparation of Biochar

B. nivea plants were collected from Xikuangshan (E 111.499313°, N 27.784217°), Hunan Province, China, in June 2023. Xikuangshan is the most important antimony (Sb) mining site in the world [17, 18]. Hundreds of years after mining, the area has experienced serious heavy metal pollution with Cd, Sb, and As [17, 19]. Although heavy metal pollution is severe in this region, there are still many plants, and phytoremediation is widely used in the region [20, 21]. The aboveground parts of the ramie were harvested, and the leaves were removed for subsequent biochar preparation. The ramie stems were washed with tap water and crushed. Then the ramie powder underwent pyrolysis in a muffle furnace with a heating rate of 10 °C min<sup>-1</sup>. A series of pyrolysis temperatures (300, 400, 500, 600, and 700 °C) were set for the study, and the above powders were subjected to continuous pyrolysis for 2 hours at the pyrolysis temperature. The pyrolysis product was cooled to room temperature, dried in an oven at 105 °C for 12 hours, and ground with a 100-mesh sieve. The biochars at different pyrolysis temperatures were denoted as ZBC300, ZBC400, ZBC500, ZBC600, and ZBC700, and the ramie powder was denoted as ZM. The biochar was weighed at different temperatures, and the yield was calculated by dividing the weight of the biochar by that of the ramie powder before preparation.

# Determination of pH, Elemental Contents, and Leaching Toxicity

The pH of the biochar was measured using a pH meter (PHS-3C, LEICI, China) at a ratio of 1:20 in the water body. To further understand the properties and toxicity of the biochar preparation, the carbon (C), nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), Cd, Pb, Zn, Cu, and leaching As contents of the biochar were measured. The nutrient and heavy metal contents were determined according to the following steps: 0.1 g of the biochar sample was weighed and placed in a 100 mL conical flask. Subsequently, 6 mL of aqua regia solution was added, heated on an electric heating plate (PZ 28-2, Harry Gestigkeit, Germany), and maintained in a slightly boiling state for 2 h. After cooling to room temperature, the extraction solution was filtered with slow quantitative filter paper and collected in a 50 mL volumetric flask. The solution was diluted with ultrapure water and stored for testing. Inductively coupled plasma mass spectrometry (ICP-MS, NexION 350, PerkinElmer, USA) and atomic fluorescence spectrophotometry (AFS-6801, China) were used to determine the heavy metal and nutrient contents, respectively [22]. The As extraction content was determined using sulfuric and nitric acids at a ratio of 2:1. Two grams of biochar sample were weighed, 20 mL of nitric acid sulfuric acid mixture was added, and flipping oscillation was conducted for 18 h at a speed of 30 r/min. After centrifugation and filtration, As content was determined using an atomic fluorescence spectrophotometer. During the above experimental process, ramie powder was used as a control to measure all the indicators. All chemical substances and standard materials used in the experiments were purchased from the National Standard Material Resource Sharing Platform (http://www.gbwpt.cn/).

## Modification of Biochar Based on As Removal

Considering that the experimental ramie comes from mining areas and the biochar contains heavy metals, we selected biochar with the highest As content for the As removal experiments to modify the biochar. Based on previous reports, sodium oxalate [23] and sodium hydroxide [24] were selected for BC modification. Biochar (2 g) was weighed and placed in a 50 mL centrifuge tube. Then, different concentrations of sodium oxalate (25, 50, and 75 mM/L) or sodium hydroxide (100, 200, and 300 mM/L) solution were added at a solid-liquid ratio of 1:10 for the extraction experiments. The mixture was flipped, shaken for 12 h (70 rpm), and centrifuged for 10 minutes (8000 r/min). Subsequently, the biochar was separated from the extraction agent and dried at 60 ° C for 72 h. These steps were repeated thrice to remove As from the biochar, and the nutrient and heavy metal contents of the biochar were measured after each removal. Three replicates were used for each experiment, and pure water was used as the control.

# Effects on Soil Properties and Microbial Communities

The modified biochar with the highest As removal efficiency was used to explore its impact on soil. Heavy metal-contaminated soil was also obtained from Xikuangshan (E 111.49914°, N 27.769811°). Different proportions of biochar (0.5%, 1%, and 2%) were added to the heavy metal-contaminated soil, and no biochar was added as a control (CK). The experimental groups with different proportions of biochar were denoted as R 0.5, R 1, and R 2. Soil and biochar were mixed evenly after adding biochar, soil pH, soil redox potential (soil Eh), soil organic matter, and As content every seven days. The methods used to measure soil pH and As content were the same as those used to measure biochar. Soil Eh was measured in triplicate using an Eh meter according to the method reported by Honma et al. [25]. The soil organic matter (SOM) was measured using the potassium dichromate external heating method [4, 26].

Soil samples were collected on the 28th day to determine the composition of the soil microbial communities, including bacteria and fungi. Four soil samples were taken from the edge of the basin and one soil sample from the center; the five samples were mixed. DNA was extracted from 1.0 g of each soil sample using a DNA Extraction Kit (D5625-01) (Omega Bio-Tek, Norcross, GA, USA), according to the manufacturer's instructions [27]. The integrity and quality of the extracted DNA were assessed by agarose gel electrophoresis and a NanoDrop spectrophotometer (ND-1000, Thermo Fisher Scientific, Waltham, MA, USA). The primers used for bacterial DNA were 338F and 806R, targeting the V3-V4 region. The primers used for amplifying fungal DNA were ITS1F and ITS2F, which targeted the ITS region. Equal amounts of PCR amplicons were sequenced using an Illumina MiSeq platform at Majorbio Biotechnology Co., Ltd. (Shanghai, China).

#### Data Analysis

All experiments were repeated at least thrice. Differences between experimental treatments were compared using one-way analysis of variance, and 0.05 was set a p value. Statistical analysis was conducted using R 4.3.1, and drawings were performed using Origin 2024.

#### **Results and Analysis**

## Biochar Yield, Elemental Content, and Leaching Toxicity

As the pyrolysis temperature increased, the ramie biochar yield decreased continuously (Table 1). When the pyrolysis temperature is 300 °C, the yield of ramie biochar is  $42.90 \pm 2.29\%$ . When the pyrolysis temperature reaches 600 °C and 700 °C, the yield of ramie biochar is  $28.00 \pm 0.36\%$  and  $27.01 \pm 0.26\%$ , respectively, but the difference is insignificant (p > 0.05). The prepared ramie biochar is alkaline, and the pH of the biochar prepared under the pyrolysis temperature condition of 300 °C is significantly lower than that of other pyrolysis temperatures (Table 1).

As the raw material of ramie is heavy-metalcontaminated soil, the heavy metal content of ramie biochar is relatively high (Fig. 1). As the pyrolysis temperature increased, the As concentration in the biochar increased (Fig. 1A). The As concentration was  $6.4 \pm 0.46$  mg. kg<sup>-1</sup> in ramie raw materials, whereas the As concentration in biochar was  $43.97 \pm 1.51$  mg. kg<sup>-1</sup> when the pyrolysis temperature reaches 700 °C. When the pyrolysis temperature is below 500 °C, there is no significant difference in Cd concentration between ramie biochar and ramie raw materials (p > 0.05, Fig. 1B). However, when the pyrolysis temperature is 600 °C and 700 °C, the Cd concentration in biochar is  $0.12 \pm 0.03$  mg. kg<sup>-1</sup> and  $0.11 \pm$ 

Table 1. Differences in biochar yield and pH at different temperatures.

Biochar	Yield (%)	pН
ZBC300	$42.90 \pm 2.29$ a	$9.42\pm0.46\ b$
ZBC400	$33.48\pm0.80\ b$	$10.36\pm0.12\ a$
ZBC500	$29.97\pm0.96\ c$	$10.28\pm0.14~\text{a}$
ZBC600	$28.00\pm0.36\ d$	$10.10\pm0.04~a$
ZBC700	$27.01 \pm 0.26 \ d$	$10.34\pm0.06\pm a$

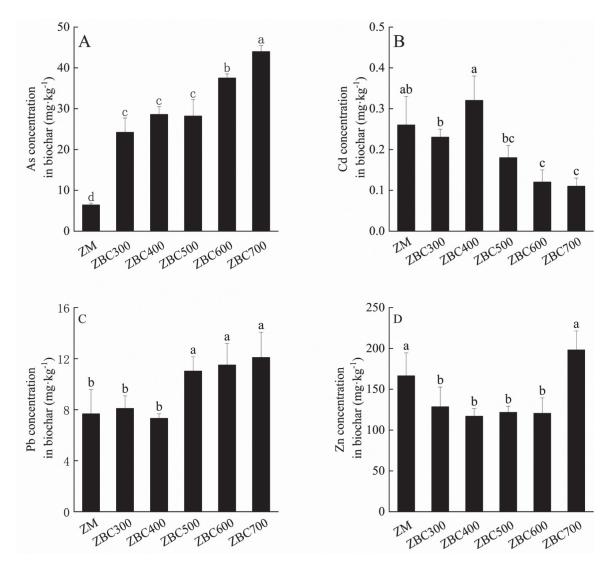


Fig. 1. The As concentration (A), Cd concentration (B), Pb concentration (C) and Zn concentration (D) in different ramie biochar.

0.02 mg. kg<sup>-1</sup>, respectively, which were significantly lower than those of the ramie raw materials (p < 0.05). For the Pb concentration, when the pyrolysis temperatures are 300 °C and 400 °C, there is no significant difference between biochar and ramie raw materials (p > 0.05). However, when the pyrolysis temperature exceeds 500 °C, the Pb concentration in ramie biochar is significantly higher than in ramie raw materials (p < 0.05, Fig. 1C). When the pyrolysis temperature is 700 °C, there is no significant difference in Zn concentration between biochar and ramie raw materials (p > 0.05); however, at other pyrolysis temperatures, the Zn concentration in biochar is significantly lower than in ramie raw materials (p < 0.05, Fig. 1D). The concentrations of Ca, K, P, Mg, and Mn increased with increasing pyrolysis temperature (Fig. 2). The concentrations of Na and Fe in the different ramie biochars showed irregular changes with increasing pyrolysis temperature. Considering the biochar yield, the contents of various elements decreased during biochar preparation (Table 2). For the As content, the loss rate of As is the highest (34.978%) when the pyrolysis temperature is 500 °C.

# The As Leaching Toxicity

The As leaching toxicity of ramie raw materials is  $0.63 \pm 0.11$  mg. kg<sup>-1</sup> (Fig. 3A). The leaching toxicity of biochar prepared at a pyrolysis temperature of 300 °C is the highest  $(2.6 \pm 0.22 \text{ mg. kg}^{-1})$ , significantly higher than that of biochar prepared under other temperature conditions (p < 0.05). The correlation coefficients between the leaching toxicity of As and the concentrations of various elements in the biochar were relatively high; however, except for the significant correlation coefficients between Pb and Ca concentrations, the other correlation coefficients were insignificant (p > 0.05, Fig. 3B). The leaching toxicity of As was positively correlated with the Cd concentration and negatively correlated with the concentrations of other elements. The biochar with the highest As leaching toxicity (ZBC300) was selected for the next step of As removal experiments.

Treatment	As (%)	Cd (%)	Pb (%)	Zn (%)	Ca (%)	K (%)	Mg (%)	Na (%)	P (%)	Fe (%)	Mn (%)
ZBC300	17.01	63.05	53.86	37.83	12.86	1.61	12.13	1.43	10.54	12.33	18.82
ZBC400	26.08	55.00	66.69	4.10	11.75	2.40	12.05	2.01	13.61	1.03	14.01
ZBC500	34.97	79.08	55.20	6.10	11.17	4.50	9.69	7.84	8.78	5.43	14.50
ZBC600	18.83	89.42	55.17	7.11	21.24	4.78	16.94	4.68	7.72	8.92	20.52
ZBC700	7.88	87.14	56.69	2.31	27.70	7.88	17.16	2.87	5.06	8.66	20.84

Table 2. Loss of heavy metals and nutrients from biochar at different temperatures.

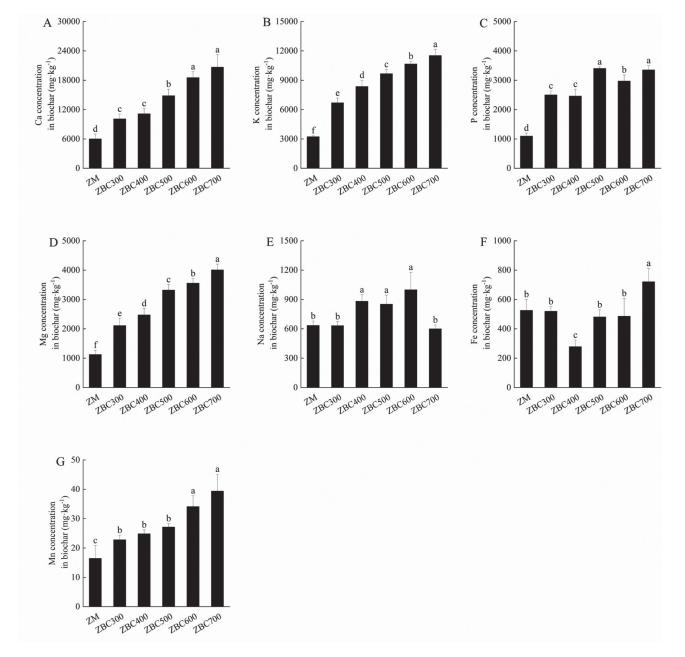


Fig. 2. The concentration of Ca (A), K (B), P (C), Mg (D), Na (E), Fe (F) and Mn (G) in different ramie biochar.

# Arsenic Removal Efficiency

For the first time, no significant difference was observed in the removal efficiency of As from biochar with different concentrations of sodium oxalate (Fig. 4A). The second removal efficiency of As from biochar by 75 mM sodium oxalate reached  $39.40 \pm 2.89\%$ , significantly higher than the other two experiment levels (p < 0.05). The As removal rate reached  $56.36 \pm 1.49\%$  after three consecutive removals by 75 mM sodium oxalate. Different

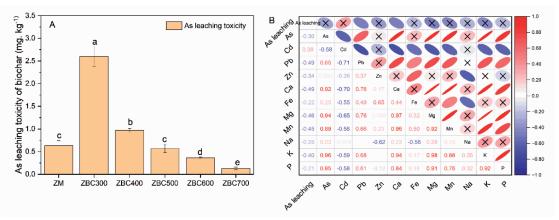


Fig. 3. As leaching toxicity (A), and correlation between As leaching toxicity and the elemental content (B).

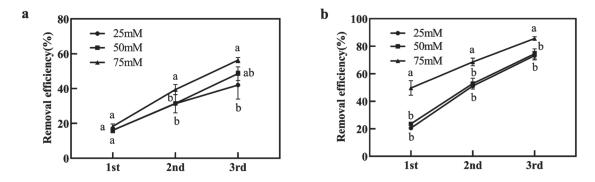


Fig. 4. Removal efficiency of As from biochar by different concentrations of sodium oxalate (A) sodium hydroxide (B).

from sodium oxalate, the first removal efficiency of As from biochar by 300 mM sodium hydroxide reached 49.66  $\pm$  5.31%, significantly higher than the other two sodium hydroxide concentrations (p < 0.05, Fig. 4B). In the second and third removal processes, the removal of As by 300 mM NaOH was more significant (p < 0.05). After continuous removal of 300 mM sodium hydroxide, the removal rate of As in biochar reached 85.62  $\pm$  1.36%. Owing to the best removal effect of 300 mM NaOH on As in the biochar, the biochar removed three times based on this concentration was used for subsequent soil experiments.

#### Effects of the Modified Biochar on Soil Properties

The application of modified biochar to heavy metalcontaminated soil affected soil pH, soil Eh, soil organic matter, and As concentrations (Fig. 5). Compared with the control, the application of modified biochar resulted in varying degrees of increasing soil pH. With the extension of the use time of modified biochar, the soil pH also showed a decreasing trend, and the difference with the control was reduced (Fig. 5A). After adding 0.5% modified biochar, the change in soil Eh was significantly different from that of the control group and the other two biochar treatment groups and lower than the control group. For all groups, the soil Eh did not show significant changes over time (Fig. 5B). After adding modified biochar, the SOM concentration underwent regular changes. The higher the proportion of modified biochar added, the higher the soil organic matter content (Fig. 5C). After adding the modified biochar, the As concentration in the soil increased. There was a significant increase in the soil As concentration with the addition of 1% and 2% modified biochar. As the usage time of the modified biochar increased, the soil As concentration also increased slowly (Fig. 5D).

# Effects of Modified Biochar on the Soil Microbial Community

The structure of the soil microbial community changed with the addition of the modified biochar (Fig. 6). Regardless of whether biochar was added or the proportion of biochar added, the abundance of bacteria was higher than fungi. In the CK group, 1055 bacterial species were detected, and the number of species was much higher than in the group treated with biochar. The number of bacterial species shared by all the groups was 533, which was much higher than the number of species unique to each group. The main bacterial compositions included Chloroflexi, Actinobacteria, Proteobacteria, and WPS-2, and there were no significant differences among the different groups. The number of fungal species shared by all the groups was 53, less than the number of species unique to the CK and R\_1 groups (74 in both groups).

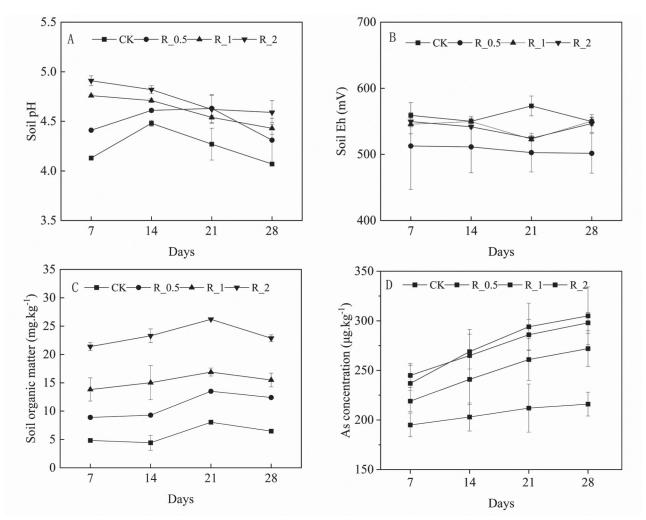


Fig. 5. The effect of modified biochar on soil pH (A), soil Eh (B), soil organic matter (C) and As concentration (D).

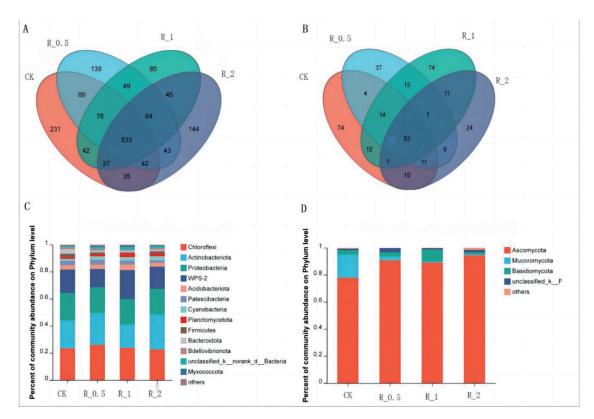


Fig. 6. Venn diagram and species composition for phylum level. A: Venn diagram of bacterial; B: Venn diagram of fungal; C: bacterial species composition for phylum level; D: fungal species composition for phylum level.

Seven common fungi were shared by adding different proportions of modified biochar groups. Ascomycota had the highest proportion among all groups and was much higher than the other fungal phyla. The addition of the modified biochar increased the proportion of Ascomycota. For both bacteria and fungi, adding modified biochar did not significantly alter the soil microbial diversity index.

## Discussion

#### Reuse of Phytoremediation Plants

Reusing remediation plants has become an important limiting factor in the application of plant remediation in heavy metal pollution control [28]. Developing remediation plants offsets economic costs and facilitates the redistribution of heavy metals. Utilizing tissues not enriched in heavy metals is a relatively simple development strategy [29]. However, it is often unsuitable for tissues with transport functions, such as roots and stems. The work on *Pteris vittate*, a highly enriched plant for As and is widely used in As pollution control, provided 995–53,050 US\$/hm<sup>2</sup> per year to a phytoremediation project with phenolic extract [30]. As a traditional economic crop, ramie has a wide range of uses, but evaluation of its properties is required before its utilization.

# Feasibility of Preparing Biochar from Heavy Metal-Contaminated Plants

Research has shown that biochar prepared from ramie has good adsorption capacity for various heavy metals [31]. However, the ramie biochar was made from non-heavy metal-contaminated ramie as a raw material. Pyrolysis temperature is a key factor in ramie biochar preparation. The investigation of the adsorption-reduction mechanisms of ramie biochars to Cr(VI) showed that low-temperature biochar had better adsorption capability owing to its abundant carboxyl and hydroxyl groups [32]. This study observed the highest biochar yield and lowest As concentration at a pyrolysis temperature of 300 °C, making it a good pyrolysis temperature. Biochar prepared at this pyrolysis temperature was also the easiest for As removal through extraction. The removal efficiency of As for this biochar reached  $85.62 \pm 1.36\%$  when treated with 300 mM sodium hydroxide, and the modified biochar has almost achieved the reuse of biochar. At the same time, acidification is the main reason for biochar aging, and sodium hydroxide treatment is beneficial for the reactivation of biochar [33]. Modified biochar can be applied to soil and further developed by referring to other biochar application pathways.

## Cycle and Enhancement Effects of Ramie Biochar on Phytoremediation

Recycling is an important strategy for achieving sustainable development of plant remediation, and

biochar is an important carrier for achieving material cycling. Although most studies have confirmed the ability of biochar to absorb heavy metals and enhance plant resistance, the damage it causes to soil ecosystems has also received attention. Directly applying ramie biochar from the heavy metal remediation area to the soil without removing heavy metals showed that biochar significantly increased soil pH, soil organic matter, available potassium content, and available phosphorus content [26]. The results of this study are consistent with the above results, and the modified biochar significantly increased the soil pH and soil organic matter content. Due to the absence of heavy metals removed from the ramie biochar, adding ramie biochar to the soil significantly increased the heavy metal content, reaching more than seven times that of the control. In this study, although adding modified biochar increased the soil As content, the increase was insignificant, confirming the necessity of removing heavy metals from biochar. Soil microorganisms play an important role in plant remediation and are widely used as indicators to evaluate the impact of exogenous substances on the soil [22]. In the present study, adding modified biochar did not cause significant changes in the soil microbial structure, especially in terms of bacterial composition. This may be because of the rich microbial diversity in heavy metal-contaminated soil, which is beneficial for resisting the impact of external substances on the soil. Research suggests that adding modified biochar is feasible from the perspective of microorganisms. Therefore, a plant remediation cycle centered on ramie can be established, where ramie plants are used to adsorb heavy metals, and ramie biochar can be repeatedly applied to soil after modification to improve soil and enhance plant remediation effects.

## Conclusions

This study aimed to prepare and modify biochar based on heavy metal-contaminated ramie as a raw material for plant remediation. The study has determined that the appropriate pyrolysis temperature for the preparation of biochar is 300 °C. They concluded that removing heavy metals from biochar is crucial for its reuse, and that 300 mM sodium hydroxide is suitable for removing As from biochar. By removing heavy metals from ramie biochar, modified biochar improves soil properties and does not damage the soil microbial structure. This study confirms the feasibility of using ramie to control soil As pollution.

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## **Conflict of Interest**

The authors declare no conflicts of interest.

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