Astragalus membranaceus Residue-Derived Biochar Loaded with Atlantibacter hermannii XJ 08: Synergistic Cd-Pb Immobilization and Soil Quality Improvement

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Abstract

Cadmium (Cd) and lead (Pb) soil contamination threatens ecological security and human health, requiring sustainable remediation technologies. This study prepared modified biochars (AMBCs) from Astragalus membranaceus residues via pyrolysis (300-700°C) and chemical modification (HNO_3, KOH, H_2O_2, brown sugar), evaluating their performance as carriers for the heavy metal-tolerant and phosphorus-solubilizing strain Atlantibacter hermannii XJ 08. Chemical modification enhanced AMBCs' porosity, specific surface area, and functional groups: brown sugar-modified AMBC (BS-AMBC) showed the highest microbial loading capacity (9.2$×10^{7}$ CFU · g^{-1}) and Pb^{2+} adsorption (144.3 mg · g^{-1}), while KOH-AMBC exhibited superior Cd^{2+} adsorption (1.61 mg · g^{-1}). Soil incubation experiments demonstrated that the BS-AMBC@A. hermannii XJ 08 composite synergistically remediated Cd-Pb co-contamination, reducing DTPA-extractable Cd/Pb to 0.26 mg · kg^{-1} and 68 mg · kg^{-1}, transforming >56% of labile fractions (water-soluble/acid-soluble) to stable oxidizable and residual forms. It also optimized soil physicochemical properties (e.g., SOM = 22.3 g · kg^{-1}, CEC = 30.4 cmol · kg^{-1}), elevated nutrient availability, and enriched functional microbial communities, pathways, and genera (e.g., Cytobacillus) linked to heavy metal stabilization, as confirmed by 16S rRNA sequencing and functional predictions (PICRUSt2/FAPROTAX). This study validates valorizing waste medicinal residues into high-performance biochar-microbe composites, offering a sustainable strategy for complex heavy metal-contaminated soil remediation.

Full Text

Preamble

Astragalus membranaceus Residue Derived Biochar Loaded with Atlantibacter hermannii XJ 08: Synergistic Cd Pb Immobilization and Soil Quality Improvement Kailu Zhang Jianshan Huang , Han Hu Jun Chen , Wei Zhang , Lixun Zhang Institute of Environment and Ecology, Tsinghua Shenzhen International Graduate

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Abstract

Cadmium (Cd) and lead (Pb) soil contamination threatens ecological security and human health, requiring sustainable remediation technologies. This study prepared modified biochars (AMBCs) from Astragalus membranaceus residues via pyrolysis 700°C) and chemical modification (HNO_3, KOH, H_2O_2, brown sugar), evaluating their performance as carriers for the heavy metal tolerant and phosphorus solubilizing strain Atlantibacter hermannii XJ 08. Chemical modification enhanced AMBCs' porosity, specific surface area, and functional groups: brown sugar modified AMBC AMBC) showed the highest microbial loading capacity (9.2\$×10^{7}\$ CFU · g^{-1}) and Pb^{2+} adsorption (144.3 mg · g^{-1}), while KOH AMBC exhibited superior Cd^{2+} adsorption (1.61 mg · g^{-1}). Soil incubation experiments demonstrated that the BS AMBC@ hermannii XJ 08 composite synergistically remediated Cd Pb co contamination, reducing DTPA extractable Cd/Pb to 0.26 mg · kg^{-1} and 68 mg · kg^{-1}, transforming >56% of labile fractions (water soluble/acid soluble) to stable oxidizable and residual forms.

It also optimized soil physicochemical properties (e.g., SOM = 22.3 g · kg^{-1}, CEC = 30.4 cmol · kg^{-1}), elevated nutrient availability, and enriched functional microbial communities, pathways, and genera (e.g., Cytobacillus) linked to heavy metal stabilization, as confirmed by 16S rRNA sequencing and functional predictions (PICRUSt2/FAPROTAX). This study validates valorizing waste medicinal residues into high performance biochar microbe composites, offering a sustainable strategy for complex heavy metal contaminated soil remediation

Keywords

Pb co contaminated soil Heavy metal tolerant bacteria Modified biochar iochar microbe composite eavy metal immobilization

Highlights Modified AMBCs enhance Cd/Pb adsorption and microbial loading AMBC@ A. hermannii achieves efficient Cd Pb co contamination remediation.
- Strain carrier synergy reduces heavy metal bioavailability and optimizes soil

properties. • Microbial communities/pathways linked to heavy metal stabilization are enriched. • Medicinal residue derived biochar microbe composites offer a sustainable remediation strategy.

Graphical bstract

1. Introduction

Soil heavy metal contamination severely constrains global agricultural sustainability and threatens ecological security and human health, with cadmium (Cd) and lead (Pb) as the most prominent co pollutants in agricultural soils due to their high toxicity, persistence, and bioaccumulation [1, 2] . In China, ~19.4% of arable land exceeds heavy metal limits, and Cd and Pb account for over 80% of farmland pollution incidents. Their sources include natural processes like rock weathering, but mainly anthropogenic activities such as mining, electroplating wastewater discharge, overused agrochemicals and untreated sewage, which have been intensified by industrialization and urbanization . Both metals are non biodegradable; Cd exhibits high mobility and is easily accumulated by crops, whereas Pb tends to accumulate stably in soil matrice [4, 5] . Both biomagnify through food chains, endangering crop growth, soil biodiversity, and human health such as kidney damage, bone diseases and childhood neurodevelopmental disorders . Thus, sustainable farmland remediation technologies are urgently needed to reduce heavy metal bioavailability while preserving or enhancing soil ecological functions.

Various remediation technologies including physical, chemical, biological and combined approaches have been developed for soil heavy metal contamination among which the biochar microbe composite remediation system has become a research hotspot due to its synergistic advantages . Biochar can adsorb and immobilize heavy metals through its porous structure and functional groups , and also provide habitats and nutrients for microbes , while microbes secrete organic acids and enzymes via metabolism to promote the speciation transformation of heavy metals , and their synergistic effect achieves a remediation performance that is significantly superior to single remediation approaches . In recent years, the preparation of biochar from waste resources has become a research trend , and biochar derived from traditional Chinese medicine residues, which are rich in lignin and bioactive components, has shown a good application prospect in heavy metal remediation for its combined adsorption capacity and nutrient supply potential [17, 18] Meanwhile, the screening and application of heavy metal tolerant strains with

phosphorus solubilizing and nitrogen fixing functions have been further advanced , and such strains can enhance remediation efficiency by regulating the soil microenvironment. Existing studies have confirmed the high efficiency of this composite system in the remediation of soil contaminated by a single heavy metal, yet targeted optimization research for Cd Pb co contamination remains to be further explored.

Despite the remarkable progress made in biochar microbe composite remediation technology, several critical issues remain to be addressed in this field. First, most raw materials for biochar production rely on non waste resources such as wood and crop straw [21, 22] , while the resource utilization potential of characteristic agricultural wastes like traditional Chinese medicine residues has not been fully explored [23, 24] In addition, the targeted modification optimization for Cd Pb co contamination is insufficient, making it difficult to simultaneously meet the adsorption and stabilization requirements of the two heavy metals. Second, the synergistic mechanism between biochar and functional microbes has not been fully elucidated . Specifically, the regulatory effects of carrier properties including pore structure and functional group types on microbial colonization and metabolic activity, as well as the coupling effects of their combination on heavy metal speciation transformation and soil ecological function restoration, still need in depth analysis. Third, existing composite remediation systems mostly focus on the single evaluation of pollution remediation efficiency, and the research on the comprehensive impacts on soil physicochemical properties, nutrient cycling, and the structure and function restoration of microbial communities lacks systematicness, failing to meet the dual practical requirements of simultaneous pollution remediation and soil fertility improvement for farmland ecosystems In summary, this study selected Astragalus membranaceus residues, a representative type of traditional Chinese medicine waste, as the precursor material. A series of modified biochars (AMBCs) were fabricated through a combined process of pyrolysis and chemical modification with nitric acid, potassium hydroxide, hydrogen peroxide, and brown sugar.

We also screened Atlantibacter hermannii XJ 08, a strain with high Cd Pb tolerance and efficient phosphorus solubilizing capacity, to construct

a novel biochar microbe composite remediation system. This research aimed to address the following core issues: (1) to systematically characterize the structural and physicochemical properties as well as Cd Pb adsorption performance of modified Astragalus membranaceus residue biochars under different preparation conditions, and identify the optimal biochar preparation process suitable for the remediation of Cd contaminated soil; (2) to evaluate the remediation efficacy of the composite system on Cd Pb co contaminated soil and reveal its regulatory effects on heavy metal speciation transformation, soil physicochemical properties and nutrient availability; (3) to elucidate the synergistic mechanism between the biochar carrier and the functional strain via 16S rRNA sequencing and functional prediction technologies, and analyze the regulatory rules of the composite system on soil microbial community structure and functions associated with heavy metal stabilization. This work is expected to provide theoretical support and technical references for the resource utilization of traditional Chinese medicine residues and the sustainable remediation of farmland soils contaminated by complex heavy metals.

2. Materials and

ethods

2.1. Experimental

oil and iochar reparation Experimental soil was collected from the 0 20 cm layer of farmland in Shenzhen' s Basic Farmland Protection Area (113°55$ '10.225' '$ E, 22°36$' 8.514' ' $ N). After removing debris, the soil was sieved through 2 mm and characterized: pH 7.23, organic matter $g \cdot kg^{-1}$, electrical conductivity $S \cdot cm^{-1}$, cation exchange capacity $cmol^{+} \cdot kg^{-1}$; total N/P/K $g \cdot kg^{-1}$, available N/P/K $mg \cdot kg^{-1}$; total Cd/Pb 0.55/47.8 $mg \cdot kg^{-1}$.

$Cd(NO_3)_2$ and $Pb(NO_3)_2$ were added to reach target concentrations of 5 $mg \cdot kg^{-1}$ (Cd) and 500 $mg \cdot kg^{-1}$ (Pb) per GB 15618 2018, with actual measured values of 5.18 $mg \cdot kg^{-1}$ (Cd) and 514 $mg \cdot kg^{-1}$ (Pb). The co contaminated soil was aged for 1 month at room temperature before use.

Astragalus membranaceus residue (provided Guangdong Yifang Pharmaceutical Co., Ltd.) was washed, dried at 80℃, and pyrolyzed in a muffle furnace under N_2: initial temperature 25℃, heating rate $10℃ \cdot min^{-1}$, final temperatures 300/500/700℃ with holding times 2/3/4 h. Resulting biochar was named "AMBC +

temperature/time" (AM = Astragalus membranaceus, BC = biochar).

For modification , dried AMBC was mixed with HNO_3, KOH, H_2O_2, or brown sugar (BS) in deionized water at a specific ratio, heated to 90℃ with stirring, filtered, secondary pyrolyzed, soaked in deionized water overnight to remove residuals, and dried at 85℃.

Modified biochars were named HNO_3 AMBC, KOH AMBC, H_2O_2 AMBC, and BS AMBC (Fig. S1

2.2. Functional

train creening and omposite noculant reparation Pb tolerant strain was isolated from Shenzhen' s Basic Farmland Protection Area (113°55$ '10.225' '$ E, 22°36$' 8.514' ' $ N).

Fourteen candidate strains were screened for heavy metal tolerance, nutrient transforming capacities, and growth rates, with identities confirmed via 16S rRNA sequencing (Fig. S2 Table S1). Although strain XJ 10 exhibits more comprehensive functional traits (e.g., nitrogen fixation, phosphate solubilization, and potassium dissolution), strain XJ 08 demonstrates superior Cd immobilization capacity (Fig. S3) and a faster growth rate (0.0339 h^{-1}), making it more suitable for the remediation of Cd Pb co contaminated soil.

A. hermannii XJ 08 was selected for its superior Cd Pb tolerance, phosphorus solubilization, growth rate, and immobilization performance (Fig. S3 The strain was cultured in LB medium at 28°C (165 rpm, 1 week), mixed with AMBC at 1:3 (w/v) for 6 h, and freeze dried to obtain the biochar microbe composite inoculant Fig. S1 . Viable cell loading capacity and efficiency were determined via gradient dilution plating. After freeze drying and gold sputtering, bacterial attachment morphology was observed via scanning electron microscopy (SEM, SU8010).

2.3. Soil incubation experiments

Each replicate consisted of 500 g homogenized Cd Pb co contaminated soil, which was thoroughly mixed with different amendments including AMBC variants (with variable pyrolysis temperatures, pyrolysis durations, application rates of 1%/3%/5%, or chemical modifications) or biochar microbe composites. A focused incubation experiment was designed with five treatment groups, each having three replicates to ensure statistical reliability: CK 1: Original soil (uncontaminated, without any amendments or heavy metal spiking), serving as the blank control to reflect the

inherent properties of the soil used in the study. CK 2: Cd Pb co contaminated soil without any amendments, acting as the contaminated control to evaluate the remediation efficacy of different treatments. T1: Cd Pb co contaminated soil amended % (w/w) hermannii XJ 08 suspension (viable cell concentration: 1 CFU$\cdot$g^{-1}). T2: Cd Pb co contaminated soil amended with % (w/w) unmodified AMBC, which was prepared under optimized conditions (pyrolysis temperature: 500℃, pyrolysis duration: h). T3: Cd Pb co contaminated soil amended with % (w/w) microbe composite. The composite was prepared by pre incubating unmodified AMBC (optimized: 500℃, h) with % (w/w, relative to the mass of AMBC) hermannii XJ 08 suspension at 28°C with shaking at 120 rpm for 6 h. All treated soil samples were incubated in a constant temperature incubator at 25\$±\$2°C for 90 days.

During the incubation period, the soil moisture content was maintained at 60% of the maximum field water holding capacity by regularly supplementing deionized water to compensate for evaporation loss.

2.4. Biochar

haracterization and dsorption xperiments Biochar pH/EC were measured at 1:5 (solid liquid ratio) after 30 60 min equilibration. Pyrolysis yield was calculated as = biomass mass before pyrolysis, = biochar mass after pyrolysis). Micromorphology/elemental distribution (SEM EDS, Hitachi SU8010), specific surface area (BET, ASAP 2020M+C), functional groups (FTIR, Nicolet iS50), crystal structures (XRD, D8 Advance), and surface elemental contents (XPS, PHI 5000 Versa Probe II) were analyzed.

A binary solution (5 mg$\cdot$L^{-1} Cd, 500 mg$\cdot$L^{-1} Pb) was prepared with $Cd(NO_3)_2$ and $Pb(NO_3)_2$. 0.1 g biochar was added to 25 mL solution, shaken at 25°C (150 rpm), and sampled at 0 24 h. Supernatants were centrifuged (5000 rpm, 10 min) and analyzed for Cd/Pb via ICP OES (Arcos MV). Adsorption capacity = initial concentration, = concentration at time = solution volume, biochar mass). Data were fitted with pseudo first order kinetic models.

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2.5. Soil and

nalysis

After incubation, soil samples were air dried, sieved through a 2 mm mesh, and subjected to subsequent analyses. Soil pH and electrical conductivity (EC) were measured at a solid liquid ratio of 1:2.5 (w/v); soil organic matter (SOM) was determined via the potassium dichromate oxidation method; redox potential (Eh) was measured in situ using a Mettler Toledo FE28 meter; cation exchange capacity (CEC) was analyzed by the hexamminecobalt( ) chloride method. Total and available nitrogen, phosphorus, and potassium (N/P/K) were quantified following standard soil nutrient analysis protocols Bioavailable Cd and Pb were extracted with the DTPA $CaCl_2$ TEA method, and their chemical speciation was determined via the modified Tessier sequential extraction procedure . Soil enzyme activities (urease, phosphatase, dehydrogenase, catalase, polyphenol oxidase, cellulase) were assayed using commercial kits from Hefei Laier Biotechnology Co., Ltd. Total concentrations of Cd and Pb were determined by inductively coupled plasma optical emission spectrometry (ICP OES) after microwave digestion with HNO_3 H_2O_2 (v/v/v = 5:3:2, analytical grade), and the same instrument was used to quantify bioavailable and speciation related heavy metal contents.

2.6. High

throughput sequencing Total genomic DNA was extracted from soil samples using the Omega Bio Soil DNA Extraction Kit, with quality verified by 1% agarose gel electrophoresis (fragment integrity and contamination) and NanoDrop 2000 spectrophotometry (A260/A280 = 1.8 2.0, concentration $\geq$ 50 $ng \cdot \mu L^{-1}$). The V3 V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primers 338F (5′ ACTCCTACGGGAGGCAGCAG GGACTACHVGGGTWTCTAAT 3′), and 350 bp paired end libraries were sequenced on the Illumina NovaSeq platform (PE150). Raw reads were processed via the QIIME 2 pipeline (DADA2 plugin): low quality reads (Q score < 20, length < 150 bp), adapters, and chimeric sequences (against the SILVA 138 database) were removed to obtain clean reads. Operational taxonomic units (OTUs) were clustered at 97% similarity, with representative sequences annotated using the RDP Classifier (confidence = 0.7, SILVA 138 database). Low abundance OTUs (< 0.001 relative abundance) were filtered out,

and the OTU table was normalized for subsequent analyses.

Statistical nalysis All experimental data were analyzed using SPSS 26.0 and R 4.3.0, with results expressed as "mean ± standard deviation (SD)" . After verifying normality and homogeneity of variance, normally distributed data (soil physicochemical properties, nutrients, heavy metal fractions, enzyme activities, biochar adsorption capacities, microbial α diversity indices, strain growth rates, microbial loading parameters) were subjected to one way ANOVA followed by Duncan' s test (< 0.05) for inter group differences. Cd^{2+} and Pb^{2+} adsorption kinetic data were fitted with pseudo first/second order models to calculate parameters and . For high throughput sequencing data, microbial α diversity (Chao1, Shannon, Simpson indices) and β diversity (Bray Curtis distances visualized via PCoA) were analyzed, while differential microbial taxa (phylum/genus levels) were identified via LEfSe (LDA > 2), ANCOM, and Dunn' s Test.

Microbial functional predictions (PICRUSt2/FAPROTAX) were analyzed with t test or Tukey' s HSD test (< 0.05). All graphs (bar/line/box plots, Venn diagrams, bubble plots, kinetic curves, PCA/PCoA) were generated using Origin 2023 and R packages (ggplot2, vegan, pheatmap), with < 0.05 considered significant

3.1. Physicochemical

roperties and tructural haracterization of AMBC The morphological, elemental, and basic physicochemical properties of pristine and modified Astragalus membranaceus residue derived biochars (AMBCs) were characterized ( ). SEM images revealed that pristine AMBC had a smooth, compact surface, while modified samples showed distinct porous features: KOH AMBC exhibited the most abundant interconnected voids, HNO_3 AMBC had fragmented cavities, and BS AMBC displayed moderate porosity with brown sugar derived carbonaceous deposits. EDS mapping confirmed the uniform distribution of C, O, K, Ca, Si, and Mg in all AMBC samples, with distinct elemental composition variations induced by modification treatments. Pristine AMBC had the highest C content (77.7%), while HNO_3 AMBC and H_2O_2 AMBC exhibited relatively higher O contents, which were 28.4% and 27.7% respectively.

AMBC exhibited the

highest K content (5.50%) among all samples, while AMBC showed a distinctively higher P content (1.46%) compared to pristine AMBC (0.45%) For basic properties, AMBC showed the lowest pH (2.07) and the second highest EC (17.7 $mS \cdot cm^{-1}$), AMBC had the highest pH (10.3) and EC (12.9 $mS \cdot cm^{-1}$), and KOH AMBC also achieved the largest BET specific surface area (965 $m^2 \cdot g^{-1}$), which was ~2.1 times that of pristine AMBC (451 $m^2 \cdot g^{-1}$). Pyrolysis yield decreased with temperature, from 39.0 at 300°C to 22.9% at 700°C, with the yield at 500°C being 27.0% and selected as the optimal condition FTIR and XRD analyses further revealed structural alterations and functional group modifications induced by chemical treatments ( ). All samples showed characteristic peaks for O H (~3420 cm^{-1}), C H (~2920 cm^{-1}), and C=C (~1620 cm^{-1})

groups, with modified AMBCs exhibiting enhanced intensities for oxygen containing functional groups. HNO_3 AMBC had an additional N O peak (~1550 cm^{-1}) and BS AMBC showed a P O peak (~1150 cm^{-1}). XRD patterns showed two broad peaks at ~23.5° and ~43.2°, corresponding to the (002) and (100) crystal planes of amorphous carbon, confirming an amorphous carbon structure for all samples. KOH showed weak K_2CO_3 peaks and BS AMBC displayed a phosphate associated crystalline peak, consistent with elemental analysis results XPS characterization further verified modification induced changes in elemental composition and surface functional groups ( ). Full survey spectra confirmed the presence of C, O in all samples, plus K in KOH AMBC, N in HNO_3 AMBC, and P in BS AMBC. High resolution C1s spectra were deconvoluted into three components, namely C C/C=C (284.8 eV), C O (286.3 eV), and C=O (288.8 eV). Modified samples showed reduced proportions of C C/C=C alongside increased contents of oxygen containing groups, with the most significant changes observed in HNO_3 AMBC (C 21.3%, C=O: 10.7%). O1s spectra were split into O H (533.1 eV) peaks. All modified AMBCs exhibited higher proportions of O the pristine sample, which confirms the successful introduction of acidic functional groups through modification treatments. Collectively, these characterizations demonstrate that modification effectively tailors AMBCs' properties. KOH

modification enhances porosity, oxidative treatments enrich oxygen containing and nitrogen containing groups, and BS modification incorporates phosphorus, thereby providing structural and functional advantages that support their subsequent heavy metal adsorption performance Adsorption erformance of ifferent AMBCs for Cd and Pb The adsorption capacities of pristine and modified AMBCs for Cd^{2+} and Pb^{2+} in a binary solution (5 $mg \cdot L^{-1}$ Cd^{2+}, 500 $mg \cdot L^{-1}$ Pb^{2+}) were evaluated via kinetic experiments, with results presented in . All AMBCs exhibited a two stage adsorption pattern for both metals: a rapid initial adsorption within 0 4 h (driven by surface functional group binding) followed by a gradual equilibrium phase after 12 h.

For Cd^{2+} ( ), KOH AMBC achieved the highest equilibrium adsorption capacity) of 1.61 $mg \cdot g^{-1}$, while pristine AMBC showed the lowest (0.84 $mg \cdot g^{-1}$); for Pb^{2+}), BS AMBC outperformed other treatments with a of 144.3 $mg \cdot g^{-1}$, significantly higher than pristine AMBC (98.2 $mg \cdot g^{-1}$). The pseudo second order kinetic model ( ) provided excellent fits to the adsorption data, with all treatments exhibiting > 0.999. This confirms chemical adsorption (e.g., complexation, ion exchange) as the dominant mechanism. Specifically, BS AMBC had the largest pseudo second order rate constant for Pb^{2+} (0.0187 $g \cdot mg^{-1} \cdot h^{-1}$), while AMBC showed the highest k_2 for Cd^{2+} (0.0065 $g \cdot mg^{-1} \cdot h^{-1}$), which correlated with their modified structural properties.

Effects of ifferent AMBC ypes on ioavailable Cd and Pb The effects of pyrolysis temperature, pyrolysis time, application dosage and modification type on DTPA extractable Cd and Pb in soil were systematically investigated ( ), with all treatments showing varying degrees of reduction in available heavy metal contents compared to the control (CK < 0.05). For preparation conditions, 500°C was the optimal pyrolysis temperature, achieving the lowest contents of DTPA extractable Cd (1.22 mg·kg) and Pb (116 mg·kg); 2 h of pyrolysis time exhibited better immobilization effects than longer durations, with corresponding Cd and Pb contents of 1.20 mg · kg and 114 mg · kg ; and a 3% application dosage was sufficient for effective immobilization, as no significant

difference was observed between 3% and 5% dosages for both metals. Regarding modification types, showed strongest immobilization capacity for Cd (0.75 mg · kg and 0.83 mg · kg respectively), while AMBC and HNO_3 AMBC were most effective for Pb (76 mg·kg and 85 mg·kg respectively), with all modified AMBCs outperforming pristine AMBC.

These results collectively indicate that preparation conditions and modification treatments significantly regulate the heavy metal immobilization efficiency of AMBC. The optimal combination for reducing available Cd and Pb in soil involves pyrolysis at 500°C for 2 h, application at 3% dosage, and utilization of BS or HNO_3 modified AMBC. Such findings provide critical technical support for the practical application of AMBC in heavy metal contaminated soil remediation.

3.4. Evaluation of

icrobial oading apacity of ifferently odified AMBC The morphological features and microbial loading capabilities of pristine modified AMBC were characterized using SEM and quantitative microbial analysis). SEM images at 50 μm scale revealed distinct surface morphologies among the materials, while high magnification images (5 μm scale) confirmed the successful adhesion of A. hermannii to all AMBC substrates, forming stable AMBC@ hermannii composites. The microbial loading capacity and efficiency varied significantly among different AMBC types (< 0.05). BS AMBC exhibited the highest viable bacterial loading capacity of 9.2$\times10^{7}$ CFU·g^{-1} and the maximum loading efficiency of 92.0 %, followed by KOH AMBC with 6.8$\times10^{7}$ CFU·g^{-1} and 68.0 % respectively. Pristine AMBC and H_2O_2 AMBC showed moderate performance, with loading capacities of 5.3$\times10^{7}$ CFU·g^{-1} and 4.9$\times10^{7}$ CFU·g^{-1}, and loading efficiencies of 53.0 % and 49.0 % respectively. HNO_3 AMBC had the lowest microbial loading capacity (3.7$\times10^{7}$ CFU·g^{-1}) and loading efficiency (37.0 %), which were significantly lower than other treatments. These results demonstrate that BS modification and KOH modification effectively enhance the microbial loading performance of AMBC, while HNO_3 modification exerts an inhibitory effect, indicating that material surface properties regulated by different chemical modifications play a crucial role in microbial adhesion and immobilization.

Effects of icrobe oaded AMBCs on oil Cd peciation ractions The effects of A. hermannii XJ 08 inoculation, BS AMBC amendment, and BS AMBC@ A. hermannii XJ 08 composite application on DTPA extractable Cd/Pb contents and their chemical fractionation in contaminated soil were evaluated (All treatments significantly reduced the bioavailability of Cd and Pb compared to the untreated control (CK < 0.05). For DTPA extractable Cd, the T3 treatment (BS AMBC@ A. hermannii XJ 08) achieved the lowest content of 0.26 $\mathrm{mg \cdot kg^{-1}}$, followed by T2 (BS AMBC) and T1 (A. hermannii XJ 08) with 1.28 $\mathrm{mg \cdot kg^{-1}}$ and 1.32 $\mathrm{mg \cdot kg^{-1}}$ respectively, while CK showed the highest content of 1.83 $\mathrm{mg \cdot kg^{-1}}$. Similar trends were observed for DTPA extractable Pb: T3 treatment resulted in the lowest content of 68 $\mathrm{mg \cdot kg^{-1}}$, T2 and T1 treatments had 128 $\mathrm{mg \cdot kg^{-1}}$ and 138 $\mathrm{mg \cdot kg^{-1}}$ respectively, and had 162 $\mathrm{mg \cdot kg^{-1}}$. Chemical fractionation analysis revealed that all treatments transformed Cd and Pb from labile fractions (F1: Water Soluble, F2: Acid Soluble) to stable fractions (F3: Reducible, F4: Oxidizable, F5: Residual). For Cd, the proportion of F1 + F2 in CK was 67.9%, which decreased to 41.8%, 25.8% and 11.9% in T1, T2 and T3 treatments respectively, while the proportion of F4 + F5 increased from 16.0% in CK to 36.1%, 49.0% and 61.0% in the corresponding treatments. For Pb, the proportion of F1 + F2 in CK was 67.9%, and it declined to 40.0%, 26.0% and 11.9% in T1, T2 and T3 treatments, with the proportion of F4 + F5 increasing from 12.0% in to 34.0%, 44.0% and 56.0% in the respective treatments. These results demonstrate that the BS AMBC@ A. hermannii XJ 08 composite exhibits the strongest ability to reduce heavy metal bioavailability and promote their transformation to stable fractions, which is attributed to the synergistic effect between BS AMBC and

hermannii XJ 08. 368

3.6. Effects of

icrobe oaded AMBCs on hysicochemical roperties The effects of different remediation treatments on the physicochemical properties and nutrient contents of Cd Pb contaminated soil are presented in . Compared with the original soil ( ) and untreated control (CK), all remediation treatments altered soil properties with significant differences observed among groups (< 0.05).

For soil physicochemical properties: and CK showed similar pH (7.23 and

7.16), while T2 (BS AMBC amendment) and T3 (BS AMBC@ A. hermannii XJ 08 application) significantly elevated pH to 7.52 and 7.65 respectively. SOM content in was 12.2 $\mathrm{g \cdot kg^{-1}}$; CK and T1 (A. hermannii XJ 08 inoculation) increased it to 12.8 $\mathrm{g \cdot kg^{-1}}$ and 13.2 $\mathrm{g \cdot kg^{-1}}$, while T2 and T3 further raised it to 20.0 $\mathrm{g \cdot kg^{-1}}$ and 22.3 $\mathrm{g \cdot kg^{-1}}$. CEC in T3 (30.4 $\mathrm{cmol \cdot kg^{-1}}$) was the highest, followed by T2 (28.3 $\mathrm{cmol \cdot kg^{-1}}$), /T1 showed lower values (18.2, 17.2, 17.8). EC increased sequentially (378 $\mu\mathrm{S \cdot cm^{-1}}$) to T3 (481 $\mu\mathrm{S \cdot cm^{-1}}$), while Eh was lowest in T3 (185 mV) and highest in CK (245 mV).

For soil nutrient contents: T3 exhibited the highest TN (1.47 $\mathrm{g \cdot kg^{-1}}$), AN (122 $\mathrm{mg \cdot kg^{-1}}$), TP (0.90 $\mathrm{g \cdot kg^{-1}}$), AP ($\mathrm{mg \cdot kg^{-1}}$), TK (22.7 $\mathrm{g \cdot kg^{-1}}$) and AK (235 $\mathrm{mg \cdot kg^{-1}}$). T2 had the second highest levels (TN:1.32 $\mathrm{g \cdot kg^{-1}}$; AN:104 $\mathrm{mg \cdot kg^{-1}}$; TP:0.88 $\mathrm{g \cdot kg^{-1}}$; AP:31.2 $\mathrm{mg \cdot kg^{-1}}$; TK:22.4 $\mathrm{g \cdot kg^{-1}}$; AK:215 $\mathrm{mg \cdot}$

kg^{-1}) . These results confirm that BS AMBC@ A. hermannii XJ 08 composite remediation optimizes both soil physicochemical properties and nutrient status, outperforming single microbe or biochar treatments.

3.7. Effects of

icrobe oaded AMBC on nzyme ctivities Soil enzyme activities, as key indicators of soil biological function and health, responded significantly to different remediation treatments (Fig. S). For urease activity (mg ·24h, linked to nitrogen cycling), T3 (BS AMBC@ A. hermannii XJ 08 application) exhibited the highest value of 3.95, followed by T2 (BS amendment) at 3.15; (original soil) showed
2.45$±0.05, whileCK(contaminatedsoil)andT1(A.hermanniiXJ08inoculation)hadthelowestactivities(1.15a
24h, regulatingphosphorusavailability)followedaconsistenttrend : T3reached3.45, T2was2.45, was1.75, andC
h, amarkerofmicrobialactivity)inT3(27.5)andT2(17.5)wassubstantiallyhigherthan(11.5), CK(3.5)andT1(5.5
h), polyphenoloxidase($g ·h) and cellulase ·h), T3 consistently performed best (8.4, 18.2, 15.2), with T2 also showing significant improvements (6.1, 12.2, 9.2) compared to CK and T1. These results indicate that BS AMBC@ A. hermannii XJ 08 composite remediation (T3) effectively enhances soil enzyme activities involved in nutrient cycling, thereby restoring the

biological functionality of Cd Pb co contaminated soil

3.8. Microbe

oaded iochar odulates icrobial ommunity ssembly and unctional rofiles inked to Cd tabilization To characterize the effects of microbe loaded biochar on soil microbial communities in Cd Pb co contaminated soil ( ), the Venn diagram (revealed only 171 core OTUs shared among CK 1, CK 2, T1, T2, and T3, with CK harboring the most unique OTUs (31413) and T3 (27030) ranking second; this indicates that contamination and remediation treatments altered species composition. Principal component analysis ( ) showed PC1 (explaining 69.14% of total variation) separated T1/T2 (dispersed on PC1/PC2 axes) from CK 2/T3, with T3 closely clustered with CK 1 (original soil) near the origin. This demonstrates that the composite amendment mitigated contamination induced community structure disruption and restored it toward the unpolluted state. diversity indices ( ), the Chao1 (11214) and ACE (11318) indices of T3 showed no significant differences from CK 1 or maintained high levels, while the Shannon (8.70) and Simpson (0.9986) indices of T3 were also high and consistent with

1. These results indicate that T3 preserved community richness, diversity, and

evenness under Cd Pb contamination. At the phylum level ( ), CK 1 was dominated by Proteobacteria (44.7%) and Firmicutes (28.6%), while Cd contamination (CK 2) increased Firmicutes to 51.8% and decreased Proteobacteria 17.4%. T3 further elevated Firmicutes to 95.6% ($ $75%) and reduced Proteobacteria

3.40%, which significantly reshaped phylum level community composition. At the genus level ( ), CK 1 featured Noviherbaspirillum (9.36%) and Neobacillus (9.89%), and contamination enriched Calidifontibacillus (8.34%) and Niallia (5.34%).

In contrast, T3 was dominated by Cytobacillus %) and Robertmurraya (9.46%), which selectively enriched functional genera associated with Cd Pb immobilization.

To clarify the effects of microbe loaded biochar (T3: BS AMBC@ A. hermannii XJ 08) on soil microbial functional profiles in Cd Pb co contaminated soil, functional predictions via PICRUSt2 and FAPROTAX were conducted, with results presented in Fig. S . For beta diversity of functional profiles (Fig. S), PCA of KO

annotations (PICRUSt2) showed PC1 explained 86.1% of total variation, with T3 clustered separately from CK 2 (contaminated control) and closely to CK 1 (original soil). Similarly, PCA of FAPROTAX predictions (PC1: 62.7%) revealed distinct separation of T3 from T1 (single strain) and T2 (single biochar), which indicates the composite amendment restored functional profiles toward the unpolluted state. At the KEGG Level 1 pathway (Fig. S Metabolism was the dominant pathway (67.7 69.3%), with T3 showing lower relative abundance (67.7%, labeled "a") than CK (69.3%, labeled "b").

Human Diseases exhibited the lowest abundance in T3 (1.13%, "a") compared to CK 1 (1.65%, "b"), while Environmental Information Processing enriched in T3 (11.8%, "a") versus CK 2 (10.1%, "b"). For FAPROTAX top 10 functions (Fig. S), T3 had significantly higher fermentation (9.99%, "d") and lower aerobic chemoheterotrophy (5.18%, "a") than other treatments (< 0.05). further confirmed these trends: Level 2 KEGG pathways ( ) showed T3 enriched Amino Acid Metabolism (14.6%) and Carbohydrate Metabolism (16.8%). FAPROTAX alluvial plots ( ) demonstrated T3 increased fermentation (9.99%) and nitrate reduction (5.50%) while reducing human associated functions (4.10%), which is consistent with its role in enhancing Cd Pb immobilization related functions

4. D

iscussion

4.1. Structural

unctional egulation of edicinal esidue erived iochar for Cd dsorption and icrobial mmobilization Valorizing agricultural and industrial wastes into functional environmental remediation materials aligns with global sustainability objectives . This study addressed the underutilization of traditional Chinese medicine residues in complex heavy metal pollution control by preparing modified biochars (AMBCs) from Astragalus membranaceus residues. Structural and physicochemical characterizations confirmed that chemical modification effectively tailored AMBC properties to meet the dual demands of Cd Pb adsorption and microbial colonization, which is critical for the performance of the subsequent composite remediation system.

Potassium hydroxide modification emerged as the optimal strategy for enhancing Cd^{2+} adsorption, with KOH AMBC achieving an equilibrium adsorption capacity of 1.61 $mg \cdot g^{-1}$. This value is nearly twice that of pristine AMBC (0.84 $mg \cdot g^{-1}$) and comparable to other KOH modified biochars reported in recent studies . The superior Cd^{2+} adsorption performance stems from the enlarged specific surface area (965 $m^2 \cdot g^{-1}$) of KOH AMBC, which is approximately 2.1 times higher than that of pristine AMBC (451 $m^2 \cdot g^{-1}$), and its abundant interconnected porous structures . These features provide ample adsorption sites for Cd^{2+} through ion exchange and complexation . The high K content (5.50%) of KOH AMBC further facilitates cation exchange with Cd^{2+} , while its alkaline pH (10.3) promotes the precipitation of Cd^{2+} as hydroxides or carbonates , consistent with weak K_2CO_3 peaks observed in XRD patterns In contrast, brown sugar modification endowed AMBC with exceptional Pb^{2+} adsorption capacity (= 144.3 $mg \cdot g^{-1}$) and microbial loading performance . This can be attributed to the introduction of phosphorus containing functional groups (P content = 1.46%) and carbonaceous deposits, which enhance ligand bonding with Pb^{2+} and bacterial cells . FTIR spectra confirmed the presence of P O peaks (~1150 cm^{-1}) in BS AMBC, while XPS analysis revealed increased oxygen containing functional groups (C O: 21.3%, C=O: 10.7%) in modified AMBCs, strengthening chemical interactions with heavy metals 1j, l These results align with the well established understanding that phosphorus containing functional groups on biochar surfaces can significantly enhance Pb^{2+} adsorption, primarily through complexation and precipitation mechanisms [34] .

Microbial loading experiments further validated the suitability of modified AMBCs as carriers for hermannii XJ 08 exhibited the highest viable bacterial loading capacity (9.2$×10^{7}$ CFU $\cdot g^{-1}$) and loading efficiency (92.0%), followed by KOH AMBC (6.8$×10^{7}$ CFU $\cdot g^{-1}$, 68.0%). In contrast, HNO_3 showed inhibitory effects (3.7$×10^{7}$ CFU $\cdot g^{-1}$, 37.0%) due to its extreme acidity (pH = 2.07). This indicates that neutral to alkaline pH, porous structure, and phosphorus containing functional groups are key factors promoting microbial adhesion . SEM images at 5 μm scale confirmed stable attachment of A. hermannii XJ 08 to all AMBC substrates, forming compact biochar microbe composites that can resist environmental stress in Cd Pb co contaminated soil. Consistent with existing research, the surface properties of biochar have been identified as a critical factor regulating microbial colonization on its substrate [36] .

The optimal biochar preparation conditions (500°C pyrolysis for 2 h, 3% application dosage) balance adsorption efficiency, pyrolysis yield (27.0%), and economic feasibility Compared to conventional biochar carriers derived from wood or crop straw [37, 38] , AMBC boasts distinct advantages: it integrates dual functions of heavy metal adsorption and microbial immobilization, thereby simplifying soil remediation procedures and reducing associated costs

4.2. Synergistic

echanisms of iochar icrobe omposite in Cd mmobilization uality melioration The BS AMBC@ A. hermannii XJ 08 composite system achieved superior Cd contamination remediation efficacy, reducing DTPA extractable Cd and Pb contents to 0.26 $mg \cdot kg^{-1}$ and 68 $mg \cdot kg^{-1}$, respectively . These values represent reductions of 75.4% and 58.0% compared to the untreated control (CK 2: Cd 1.83 $mg \cdot kg^{-1}$, Pb 162 $mg \cdot kg^{-1}$) and meet the soil quality standards for agricultural use (GB 15618 2018).

The synergistic effect of the composite system arises from three complementary mechanisms. First, BS AMBC' s porous structure and phosphorus containing groups adsorb Pb^{2+} through complexation and precipitation . Second, A. hermannii XJ 08 secretes organic acids and phosphatases (Fig. S) that microenvironmentally lower soil pH, promoting Cd^{2+} immobilization . Third, the composite modulates soil physicochemical properties to enhance heavy metal stabilization Chemical fractionation analysis confirmed that the composite transformed over 56% of labile Cd/Pb (F1+F2: Water Soluble/Acid Soluble) to stable fractions (F4+F5:

Oxidizable/Residual) . T3 treatment reduced F1+F2 proportions to 11.9% for both Cd and Pb, while increasing F4+F5 proportions to 61.0% (Cd) and 56.0% (Pb).

This transformation is critical for long term remediation as stable fractions exhibit lower bioavailability and mobility in soil Numerous studies have corroborated that biochar microbe composites exert a prominent effect on shifting heavy metals from labile fractions to more stable forms in contaminated soil systems

Beyond heavy metal immobilization, the composite system significantly optimized soil physicochemical properties and nutrient status . T3 treatment increased soil organic matter (SOM) to 22.3 $g \cdot kg^{-1}$ (% higher than CK 2), cation exchange capacity (CEC) to 30.4 $cmol \cdot kg^{-1}$ (76.7% higher than CK 2), and pH to 7.65.

These conditions enhance soil structure and nutrient retention . Nutrient contents in T3 were also significantly higher than in other treatments, with total nitrogen (TN) at 1.47 $g \cdot kg^{-1}$, available nitrogen (AN) at 122 $mg \cdot kg^{-1}$, available phosphorus (AP) at 44.0 $mg \cdot kg^{-1}$, and available potassium (AK) at 235 $mg \cdot kg^{-1}$. These improvements are attributed to BS AMBC' s nutrient supply potential and A. hermannii XJ 08' s phosphorus solubilizing capacity ( ), addressing the research gap of insufficient attention to soil fertility improvement in existing composite remediation systems Soil enzyme activities and microbial community analysis further revealed the composite' s role in restoring soil biological functionality. T3 treatment enhanced urease (3.95 $\mathrm{mg} \cdot \mathrm{g}^{-1} \cdot 24\mathrm{h}$), phosphatase (3.45 $\mathrm{mg} \cdot \mathrm{g}^{-1} \cdot 24\mathrm{h}$), and dehydrogenase (27.5 $\mu\mathrm{g} \cdot \mathrm{g}^{-1} \cdot \mathrm{h}$) activities by 243%, 527%, and 685% compared to CK Fig. S . These enzymes are key indicators of nutrient cycling and microbial activity . High throughput sequencing showed that T3 maintained microbial α diversity (Chao1: 11214, Shannon: 8.70) and reshaped community composition, enriching Firmicutes (95.6%) and functional genera such as Cytobacillus ($ $20.0%) associated with heavy metal immobilization . Functional predictions via PICRUSt2 and FAPROTAX indicated that T3 enriched amino acid metabolism (14.6%), carbohydrate metabolism (16.8%), and fermentation (9.99%) pathways, while reducing human associated functions (4.10%), confirming the assembly of a remediation oriented microbial community Biochar microbe composites have been shown to significantly enhance soil enzyme activities and enrich functional microorganisms related to heavy metal immobilization and nutrient cycling [47, 48] , which is consistent with the findings of this study.

4.3. Practical

mplications and uture erspectives The findings of this study have significant practical implications for the

sustainable remediation of complex heavy metal contaminated farmlands. The optimal composite system (BS AMBC@ A. hermannii XJ 08) is cost effective as it utilizes waste medicinal residues and requires minimal chemical modification. The recommended application dosage (3% 5%) balances remediation efficiency and economic feasibility , while the simple preparation process (pyrolysis + chemical modification + microbial loading) facilitates scaling up for field applications . Additionally, the composite' s ability to improve soil fertility and microbial diversity meets the dual goals of pollution control and agricultural sustainability, making it suitable for long term farmland remediation.

Future research should focus on validating the composite system under field conditions as laboratory incubation experiments may not fully reflect real world factors such as soil heterogeneity, climate variations, and crop soil interactions. Long monitoring of heavy metal bioavailability, soil quality, and crop yields is necessary to evaluate the system' s stability and ecological risks. Furthermore, exploring the molecular mechanisms underlying strain carrier synergy, such as the expression of microbial functional genes related to heavy metal immobilization and nutrient cycling, will provide deeper insights for optimizing composite design. Finally, combining the composite with other remediation technologies (e.g., phytoremediation, organic amendments) may further enhance remediation efficacy, particularly for highly contaminated soils.

In conclusion, this study demonstrates that modified Astragalus membranaceus residue derived biochar is an excellent carrier for heavy metal tolerant bacteria. The AMBC@ A. hermannii XJ 08 composite achieves synergistic Cd Pb immobilization and soil quality amelioration by integrating the advantages of biochar adsorption and microbial immobilization. The structural and functional tailoring of AMBC, strain carrier synergy, and comprehensive soil improvement effects highlight the potential of this system as a sustainable solution for complex heavy metal contaminated soil remediation. This work not only expands the resource utilization pathways of medicinal residues but also provides a theoretical basis and technical reference for the design of biochar microbe composite remediation systems.

Conclusions

This study systematically explored the potential of modified Astragalus membranaceus residue derived biochar (AMBC) and its composite with Atlantibacter hermannii XJ 08 for the remediation of Cd Pb co contaminated soil. The findings demonstrate that chemical modification (particularly with brown sugar or KOH) significantly enhances AMBC' s structural and physicochemical properties, including porosity, specific surface area, and functional group diversity, rendering it an excellent carrier for heavy metal tolerant bacteria. BS AMBC exhibited the highest microbial loading capacity (9.2$×10^{7}$ CFU $\cdot$ g^{-1}) and Pb^{2+} adsorption capacity (144.3 mg $\cdot$ g^{-1}), while AMBC showed superior Cd^{2+} adsorption (1.61 mg $\cdot$ g^{-1}), confirming the tailored functionality of modified AMBC for target heavy metals. The composite system (BS AMBC@ A. hermannii XJ 08) achieved synergistic remediation effects by integrating the advantages of biochar adsorption and microbial immobilization. It not only reduced extractable Cd and Pb contents to 0.26 mg $\cdot$ kg^{-1} and 68 mg $\cdot$ kg^{-1} respectively, but also transformed labile heavy metal fractions to stable oxidizable and residual forms (accounting for >56% of total Cd/Pb). Additionally, the composite amendment optimized soil physicochemical properties (e.g., increased SOM to 22.3 g $\cdot$ kg^{-1} and CEC to 30.4 cmol $\cdot$ kg^{-1}), elevated nutrient availability, and restored soil microbial community diversity and functional profiles associated with heavy metal stabilization. Collectively, this study validates the feasibility of utilizing waste Chinese medicinal residues for biochar production and highlights the promising application of biochar microbe composites in sustainable remediation of complex heavy metal contaminated soils.

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