

# Synergistic Effects of Biochar and Microbes on Soil Remediation

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**Abstract.** With the development of science and technology progress, while improving the quality of life has caused some pollution to the soil environment, this pollution has threatened the ecosystem and even human health. However, how to carry out soil remediation actively and effectively is the current problem faced. Several common soil remediation methods have their drawbacks that cannot be widely used. Therefore, research on the synergistic effect of biochar and microorganisms on soil remediation is urgent. The feature of microbial remediation technology without secondary contamination combined with biochar can be used as a high-quality amendment to assist in the remediation of soils contaminated with heavy metals and total petroleum hydrocarbons (TPHs). There have been many researches in the field of biochar in the past years, and its loose and porous characteristics and carbon stability provide suitable conditions for the survival and growth of microorganisms. Moreover, as a carrier, biochar can co-operate with microorganisms to absorb and degrade pollutants. The use of biochar combined with microorganisms in remediation not only reuses agricultural waste but also reduces some of the costs. In addition, the research on soil remediation in China started late, and there are large differences between the north and the south in China's vast territory, and the diversity of land also means the diversity of pollution. Therefore, there is an urgent need for the technology of biochar and microorganism to improve the soil environment. It is worth discussing how to realize this technology into the market at the present time when the government is eager to achieve energy saving and emission reduction and support the development of green enterprises. This paper will be divided into four main sections on this topic, starting with the current problems faced and the background of the research. The experimental mechanism of the different purification methods for organic and inorganic pollutants is discussed, in which organic pollutants represented by petroleum hydrocarbons and inorganic pollutants represented by heavy metals are analyzed. Soils contaminated by organic or inorganic pollutants are different in terms of purification mechanisms.

**Keywords:** Biochar, Microorganism, Soil Remediation, Petroleum Hydrocarbon, Heavy Metal.

## 1. Introduction

As a developing country, China is in urgent need of science and technology and economic development and is accelerating its development process. At the same time, more and more crude oil and mineral resources are being extracted and stored. This process inevitably results in oil pollution by run-off, leakage, dripping and spillage. The misuse of mineral resources after mining and the unreasonable treatment of industrial residues and industrial wastewater waste gas lead to soil contamination by heavy metals, which is basically irreversible [1]. And once the soil is polluted just rely on the method of cutting off the source of pollution and cannot restore soil health. Soil is a valuable non-renewable resource and is very expensive to remediate, so soil pollution and treatment is a problem faced by all mankind so far. Soil pollutants are mainly divided into inorganic and organic pollutants, with heavy metals as inorganic pollutants and petroleum hydrocarbons as organic pollutants. In the face of the two different types of pollutants, different soil cleaning mechanisms are

used. The biochar and microbial synergistic approach can be used to purify the soil with different mechanisms when facing two different pollutants at the same time.

The current main methods for managing soil pollution include physical remediation, chemical remediation, phytoremediation, and microbial remediation [2]. Different remediation methods have their different characteristics. Firstly, the advantage of physical remediation is usually better efficiency, but its remediation cost is high many soils are still not available for agricultural production after remediation, which is one of the important roles of the land. Secondly, the advantage of chemical remediation method is mainly good effect, treatment depth is not limited, but still face high costs and the use of many chemical agents in the remediation process is easy to produce secondary pollution. Finally, phytoremediation as a kind of bioremediation has the advantage of low cost and no secondary pollution, but its disadvantage is that it is time-consuming, and the degree of pollution should be strictly required. Other bioremediation also has the disadvantage of strict conditions of use and is not suitable for remediation of heavy metal pollution. However, microbial remediation technology is also widely used in soil remediation as a kind of bioremediation because of its significant remediation effect, no secondary pollution and low remediation cost [3].

One of the main features of microbial remediation technology is that the metabolic activity of various microorganisms can be used to transform the remaining contaminants in the soil into non-toxic and harmless substances or to completely decompose and metabolize them into carbon dioxide and water to bring the soil to a healthy and usable state. Cured microbial treatment technology is a type of bioremediation, which means that microorganisms are immobilized on selected carriers, so that they can multiply rapidly and in large numbers in a suitable living environment and maintain high biological activity [4]. However, the key issue in cured remediation is the selection of curing carriers and the screening of specific microorganisms. Currently, the commonly used carrier materials are non-renewable resources and restrictions prevent further promotion of cured microbial remediation technology. However, biochar made from agricultural waste biomass is a good choice as an immobilization carrier. If agricultural waste is prepared into biochar and applied to immobilized microorganisms, agricultural waste biomass can be further applied with low price and abundant resources and has the importance of reducing environmental pollution. Biochar can play a great role in the environmental treatment of heavy metals, organic pollutants, carbon sink reduction and soil improvement and remediation [3]. The consequences of soil pollution are serious. The harmful substances in the soil will accumulate continuously, and the harmful substances will enter the plants planted in the soil, which will directly endanger human health through the food chain. Besides, the harmful substances in the soil will pollute the surface water and groundwater through the infiltration of rainwater. Therefore, the study of the synergistic effect of biochar and microorganisms on soil environmental purification is of great significance to the whole ecosystem and human health.

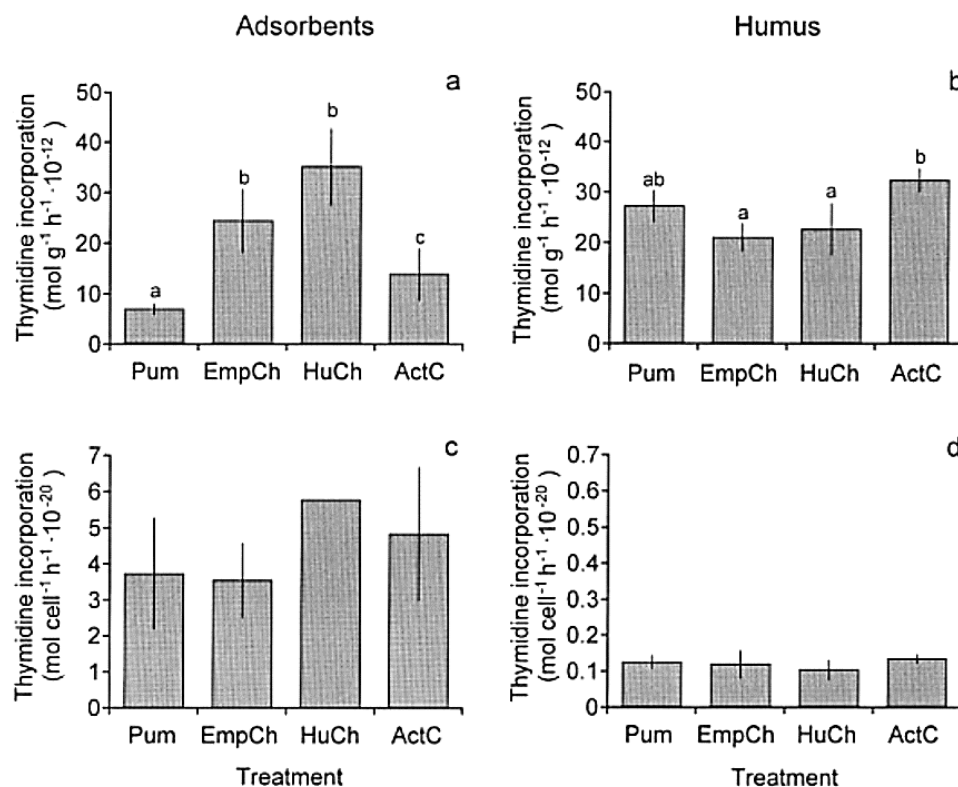
To sum up, in this paper, we will focus on the current situation of soil decontamination and discuss the synergistic approach of microbial and biochar decontamination. Firstly, we will analyze the mechanism of biochar and microbial synergism in the purification of petroleum hydrocarbons, an organic pollutant, and heavy metals, an inorganic pollutant. Secondly, a comparison of the purification effect of petroleum hydrocarbons and heavy metals will be made based on experiments. Finally, the future development of this purification will be discussed.

## **2. Mechanism of microbial coupled biochar for soil remediation**

### **2.1. The role of biochar**

The porous, weak alkaline and water-locked nature of biochar creates a good living environment for indigenous microorganisms and promotes their growth and metabolism. The strong adsorption performance of biochar can adsorb and gather pollutants and reduce the spread of pollution. The complex pore structure of biochar has a fixed effect on large molecule petroleum hydrocarbon pollutants and can use its strong polarity to effectively remove petroleum hydrocarbon pollutants. The addition of biochar can significantly improve the adsorption and degradation ability of petroleum

hydrocarbon and improve the soil quality. The research for the addition of biochar into petroleum hydrocarbon contaminated soil as remediation agent has made initial development, which can accelerate the remediation efficiency and shorten the remediation time. The progress of remediation of petroleum hydrocarbon contaminated soil using only biochar is slow. When using microorganisms alone, the survival environment of microorganisms in the contaminated soil is poor and limited. Therefore, the use of microorganisms immobilized with biochar phase could improve the remediation efficiency. Under the same treatment conditions, the reed biochar immobilized microorganism group showed a significant increase in oil degradation rate than the experimental group using reed biochar alone [4].



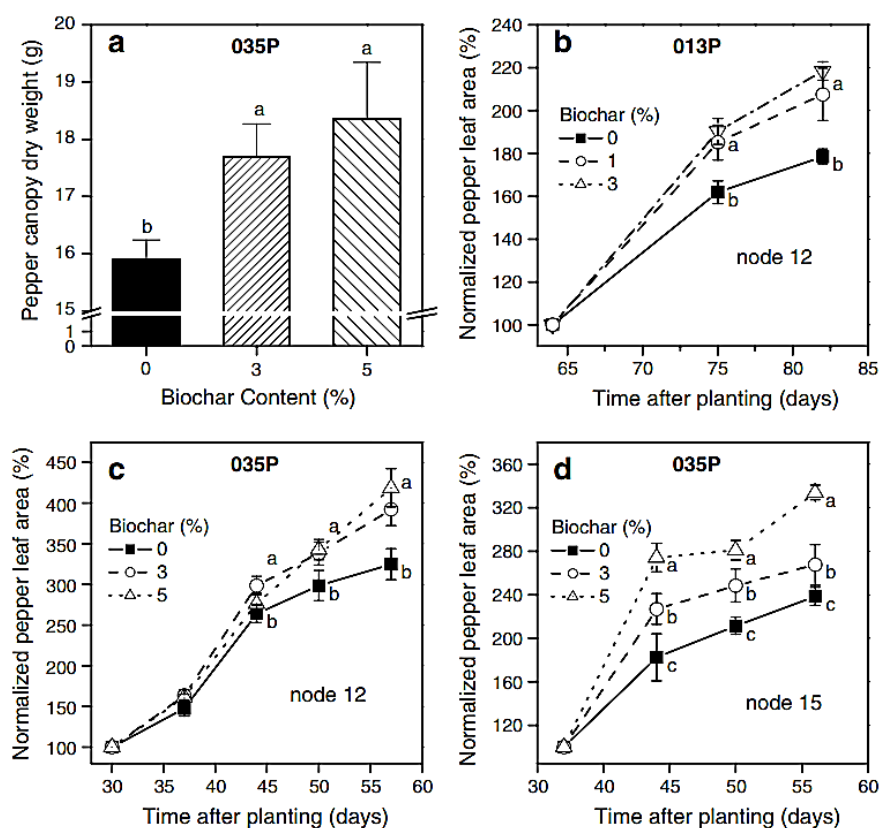
**Figure 1.** Bacterial growth rates in adsorbents and humus [5]

The weak alkalinity of biochar itself provides functional groups and free radicals for more active microbial life activity and better degradation. Pietikainen et al. found that weakly alkaline biochar made microbial life activity more active and improved substrate uptake by microorganisms [5], as shown in Figure 1. In addition, the addition of biochar to the soil can sustainably reduce the amount of soil nutrient leaching, and the effect of moisture and fertilizer fixation effectively reduces fertilizer waste, facilitates microbial growth, and improves the soil environment. Biochar has different void sizes and distribution patterns, which determine its differences in improving soil pore structure, oxygen sparging and water and fertilizer retention properties [6].

The temperature of biochar preparation affects the efficiency of microbial decomposition of petroleum hydrocarbons. The bacterium bran char at different temperatures are rich in functional group structure. With the increase of pyrolysis temperature, the total content of functional groups decreases, where the organic matter is decomposed to remove the bound water and the hydroxyl peak is gradually weakened. The stronger the aromaticity and stability of bacterium bran char, the better the ability of adsorption of organic matter. When the preparation temperature of bacterium bran char is higher, the pH value and organic carbon increase. And the nutrients required for the growth and metabolism of microorganisms are abundant, followed by the aromaticity, pore volume increases, and stability is enhanced, which can provide a living place for microorganisms. Meanwhile, the adsorption ability of petroleum hydrocarbons is enhanced, and the uptake ability of microorganisms increases, which accelerates the degradation of petroleum hydrocarbons [3].

## 2.2. The synergy of microorganisms and biochar

The rich carbon and inorganic salts of biochar provide sufficient carbon source and inorganic substances for the survival of microorganisms. At the same time, biomass has a large specific surface area and porous characteristics microorganisms can be adsorbed on the surface through the adsorption of biochar. On the other hand, microorganisms can enter the biochar and have sufficient living space, which improves the stability of biochar and the abundance of microorganisms. At the same time, the porosity is conducive to air circulation and improves air quality. The porous characteristic enables biochar to hold water, and microorganisms cannot reproduce without water. Graber et al. showed that biochar can weaken the harshness of the environment and provide favorable conditions for microbial survival, which can cause changes in the structure and increase the abundance of soil microbial communities and affect the biodegradation of organic pollutants in soil [7]. In addition, as shown in Figure 2, the added biochar also has a certain effect on the plants themselves.



**Figure 2.** Effects of biochar on (a) pepper canopy dry weight (035P), (b) pepper leaf area (013P), (c) pepper leaf area (035P) and (d) pepper leaf area (035P). [7]

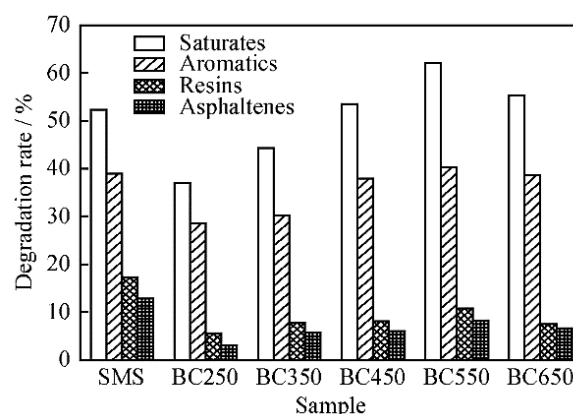
Biochar can change the presence of heavy metals in soil in several ways. Some researchers have found that the application of biochar made from rice straw to soil can increase soil pH and effectively reduce the effectiveness of heavy metals Pb and Cd in soil [8], slowing down their toxic effects on soil. For example, biochar can reduce the mobility of heavy metals in soil through adsorption or solidification. The addition of biochar to soil can promote the conversion of heavy metals from the more mobile valence state to the less mobile valence state. Microbially induced calcium carbonate precipitation (MICP) is achieved by using urease produced by microorganisms to decompose urea, producing ammonium and carbonate ions, and when  $\text{Ca}^{2+}$  is present in the system, carbonate-based minerals can be formed through a series of chemical reactions to achieve co-precipitation of  $\text{Ca}^{2+}$  with heavy metal ions. The MICP of urea hydrolysis is based on a series of biochemical reactions by high urease producing bacteria. Catalyzed by urease, 1 mol of urea is hydrolyzed into 1 mol of ammonia and 1 mol of carbamate, which is rapidly decomposed into ammonia and carbonic acid, which are hydrolyzed and equilibrated to produce carbonate, ammonium salt and hydroxide ions. Due to the absorption of large amounts of calcium ions by the negatively charged cell walls of

microorganisms, calcium carbonate deposits are formed. Urease produced by microbial metabolism can convert urea into a nitrogen source, which can be used by the microorganisms themselves, i.e., catalyzing the hydrolysis of urea to produce ammonium and carbonate. Microorganisms can convert urea to soluble inorganic carbon and ammonium. In the presence of  $\text{Ca}^{2+}$ , non-homogeneous and amorphous  $\text{CaCO}_3$  is formed on the cell wall due to local supersaturation of calcium carbonate.  $\text{CaCO}_3$  gradually wraps around the cell and kills it. Amorphous  $\text{CaCO}_3$  can eventually be converted into more stable calcite. Polar groups in the molecular chains of bioorganisms regulate crystal growth through a series of interactions. In addition to urease, bacterial carbonic anhydrase promotes the hydration of  $\text{CO}_2$  and thus the production of calcium carbonate. Carbonic anhydrase has been shown to be the main promoter of calcite deposition during MICP. Biochar with a pyrolysis temperature of  $300\text{ }^\circ\text{C}$  promotes the growth and reproduction of bacterial strains, and proper incorporation of BC300 can significantly increase  $\text{CaCO}_3$  production and facilitate MICP [1].

### 3. Synergistic remediation of soil by microorganisms and biochar

#### 3.1. Remediation of petroleum hydrocarbon contaminated soils

Shi Lifang modeled the removal of petroleum hydrocarbons from soil using biochar, a form of chemisorption [2]. Four treatment groups were set up in the experiment. CK is the control group, that is, the natural degradation of petroleum hydrocarbon and its components under suitable conditions. In the soil samples, corn straw biochar was added as T1 treatment group, reed straw biochar was added as T2 treatment group, and pine needle biochar was added as T3 treatment group. The proportion of biochar added was 5% of the weight of air-dried soil. Due to the differences in properties and structures of biochar derived from biomass, the remediation effects of soil contaminated with petroleum hydrocarbons are different. Under the same pyrolysis temperature and preparation conditions, in the corn straw biochar, reed straw biochar and pine needle biochar, the removal rate of petroleum hydrocarbon by reed straw biochar increased the fastest, and the removal rate was greater than that of the other three kinds, so reed straw biochar had the best effect on soil remediation. Adding biochar to the soil can continuously reduce the amount of soil nutrients leaching and promote the growth of plant cells. Each biochar has different void size and distribution rules, which determines its different performance in improving soil pore structure, oxygen dredging, water and fertilizer conservation. And as shown in Table 1, there are different methods for repairing petroleum hydrocarbon polluted soil.



**Figure 3.** Degradation rate of four components of petroleum hydrocarbons by microorganisms immobilized by fungus chaff and fungus chaff charcoal [4]

Zhang Bofan constructed the adsorption model of bacterial bran and bacterial bran charcoal for petroleum hydrocarbon [3]. For the saturated and aromatic components, the adsorption firstly increased and then decreased with the increase of temperature. The highest adsorption efficiency of BC450 was 42.22% and 30.98%, respectively. For colloid and asphaltene, high temperature bacterial bran adsorption is more favorable. However, due to the high temperature, the pore is blocked, the

pore is distorted, and the adsorption site is reduced. The adsorption effect of BC550 is higher than that of BC650. In general, BC550 has the highest adsorption rate for total petroleum hydrocarbon. Degradation rate of four components of petroleum hydrocarbon by immobilized microorganisms of bacterial bran and bacterial bran carbon. For saturated and aromatic components, BC550 had the best degradation effect of 62.04% and 40.31%, respectively, and for cyclic refractory aromatic hydrocarbons, SMS had the best degradation effect of 17.26% and 12.91%, respectively. Adsorption and degradation experiments show that BC550 has both adsorption and degradation abilities, as shown in Figure 3. Among them, degradation plays a dominant role in the pollution removal effect. The adsorption capacity plays an important role in the removal of pollution [4]. The results show that among the different components of total petroleum hydrocarbons, the removal rate of saturated hydrocarbons is the highest, the removal rate of aromatic hydrocarbons is lower than that of saturated hydrocarbons, and the removal rate of non-hydrocarbon substances is the lowest.

Adding a small number of microorganisms has a better repair effect than adding a large number of microorganisms. Biochar can improve the phenomenon that petroleum hydrocarbon pollutants damage microbial growth environment, restrict their growth and reproduction, and lead to the reduction of microbial activity and diversity. This is mainly due to changes in the physical and chemical properties of the soil. When immobilized microorganisms are added to the soil, it is beneficial to loosen the soil and enhance the flow of oxygen, so the chaff and biochar can form a buffer system to eliminate those adverse conditions on the soil. The microbial activity, soil ecotoxicity, enzyme activity and petroleum hydrocarbon degradation rate were compared during the remediation process, it can be seen that the overall degradation effect of immobilized group is better than that of free bacteria group, and both of them are better than that of adding biochar and fungal bran alone. This indicated that the bacterial strain could maintain high activity after immobilization with biochar and bacterial bran. It is beneficial to shield the toxic and harmful substances in the soil environment, and then increase the density of bacteria, and enhance the uptake and metabolism of petroleum hydrocarbons. In the first 35 days of restoration, the degradation rate of petroleum hydrocarbon by immobilized microorganisms in the bran was higher than that in the biochar immobilized group, but the degradation rate slowed down and was significantly lower than that in the BIM group at the later stage of restoration [2]. The carrier material can have better synergistic effect with microorganism. As a result, it can be concluded that immobilized microorganism is the best repair method.

**Table 1.** Different methods for repairing petroleum hydrocarbon polluted soil [2]

Number	A flower pot	Composition (500g soil)	Role
1	Natural restoration (CK)	No	Simulate the effect of natural repair
2	Bran alone (SMS)	10.72% bacterial chaff	Repair effect of fungus bran alone
3	Chaff-Free Bacteria (SMSB)	10.72%, 10% free bacteria, 10% immobilized microorganisms	Strengthening effect of bran and free bacteria
4	Immobilized microorganism of bran (SIM)	10% immobilized microorganisms	Repair effect of immobilized microorganism on fungal bran
5	Biochar alone (BC)	Bacterial chaff 5% carbon	Biochar alone repair effect
6	550°C bacterial bran carbon+free bacteria (BCB)	5% bacterial bran charcoal, 10% free bacteria	Enhancement of biochar and free bacteria
7	Immobilized microorganism with bacterial bran carbon at 550°C (BIM)	10% immobilized microorganisms	Remediation effect of biochar immobilized microorganisms

### 3.2. Remediation of heavy metal contaminated soils

Biochar also has remediation effect on heavy metal pollution [8]. Li Shuo constructed an experiment on remediation of heavy metals in soil by biochar and MICP [1]. The distribution order of Pb in soil before soil restoration is: carbonate bound state>residue state>commutable state>organic bound state>iron manganese oxidation state. After BC and MICP restoration, the distribution order of Pb in soil is: carbonate bound state>Commutable state>Residue state>Organic bound state>Iron manganese oxidation state. Heavy metal binding states in soil have a great impact on metal toxicity and biomagnification because exchangeable binding states have high bioavailability and mobility in soil.

Xiong Jing constructed an experiment on the effect of returning biochar to field [9]. The application of biochar in farmland soil can effectively reduce the available content of heavy metals in soil and the enrichment of heavy metals in agricultural products, and the reduction range is shown as follows: the available contents of Cd, Pb, Zn and Cu in soil were reduced by 4.5%-92%, 11.3%-79.6%, 25%-91% and 28.9-50.1%, respectively. The available contents of Cd, Pb, Zn and Cu in rice grains were reduced by 52.0% to 77.3%, 71.9% to 79.3%, 40.0% to 75.6% and 14.7%, respectively. The Cd content of rapeseed decreased by 10.8%-21.5%. Adding biochar to the soil also provides the soil with essential organic carbon and nutrients. Therefore, soil respiration, soil enzymes and microbial activities can be effectively enhanced. In addition, biochar can help improve plant root environment, saturated water conductivity and soil water retention, dilute the heavy metal content in plant tissues, and eliminate the toxicity of heavy metals to plants.

**Table 2.** The efforts of in-situ application on contaminants in field trials [9]

Type of biochar	Amount of biochar added(t/hm <sup>2</sup> )	Pollutants	Effects
Wheat straw biochar	10-14	Cb/Pb	The content of available Cd decreased by 0.2, 0.4, 0.5 mg/kg The content of available Pb decreased by 10.0, 8.9, 15.2 mg/kg
Crop straw biochar	0.75-22.5	Zn	The content of available Zn in small rice soil decreased by 0.3, 0.5, 0.5 mg/kg The content of available Zn in wheat soil decreased by 0.4, 0.5, 0.6 mg/kg
Oak biochar	20-40	Cd/Zn	The content of acid-soluble Cd decreased from 80% to 69% The content of acid-soluble Zn decreased from 21% to 19%
Broadleaved hardwood biochar	5-20	Ni/Zn	The residual Ni content increased from 51% to 61-66% The residual Zn content increased from 7% to 21-35%
Bagasse biochar	1.5-3.0	Cd/Cu/Pb	Exchangeable Cd content decreased by 0.04, 0.08, 0.08 mg/kg Exchangeable Cu content decreased by 9.8, 16.5, 17.0 mg/kg Exchangeable Pb content decreased by 18.6, 24.8, 29.6 mg/kg The Cd content of Chinese cabbage decreased by 61.6%, 71.5% and 75.5% The Cu content of Chinese cabbage decreased by 14.9%, 25.5%, 37.6%

				The Pb content of Chinese cabbage decreased by 17.3%, 21.8% and 49.1%
Wheat straw biochar	20-40	Cd/Zn		The content of available Cd decreased by 20% and 85% The content of available Zn decreased by 35% and 91%
Wood chip biochar	2.4-4.8	Cd		The content of available Cd decreased by 4.5%, 6.2%, 4.9% and 10.5%
Sludge biochar	4.2	Cd		Cd content decreased by 27.8%
Corn stover biochar	2.5-5.0	Cd/Pb		The content of available Cd decreased by 0.3 and 0.4mg/kg The content of available Pb decreased by 7.2 and 10.8 mg/kg
Palm silk biochar	5-30	Cd/Pb		The maximum reduction of available Cd content was 1.3 mg/kg The maximum reduction of available Pb content was 30.8 mg/kg
Rice straw biochar	10-20	Cd		The content of available Cd decreased by 45%, 66% and 89%
Rice husk biochar	Mix with compound fertilizer 1:1 and apply in topdressing period	Cd/Pb/Cr/As		Cd content in rice grains decreased by 52.0% and 77.3% The grain Pb content of rice decreased by 71.9% and 79.3% As content in rice grains decreased by 40.0% and 75.6% respectively The grain Cr content of rice was reduced by 14.7%
Cotton straw biochar	5-20	Cd		Cd content in rapeseed decreased by 46.7%, 48.8% and 54.8%
Plant straw biochar	5-19	Cd		The Cd content of green vegetables decreased by 10.8 and 21.5%
biochar	20-40	Cd		Cd content decreased by 63.6% and 74.5%

Previous studies have shown that heavy metals complex with functional groups such as light and carboxyl groups on the surface of biochar. Thus, heavy metal ions in the soil can be migrated, adsorbed and fixed on the surface of biochar, as shown in Table 2. The adsorption of heavy metals by biochar can be divided into fast adsorption and slow adsorption [8]. A large number of studies have proved that biochar has a positive effect on the reduction of heavy metal content and has a strong adsorption capacity for heavy metals or other harmful substances in soil [10]. Chemical processes such as ion exchange, complexation with oxygen-containing functional groups, and precipitation with inorganic components can convert the available heavy metal content into a stable state, thereby reducing the bioavailability of heavy metals.

## 4. Conclusions

This article mainly discusses synergistic effects of biochar and microbes on inorganic and organic polluted soil. The organic pollutants are represented by petroleum hydrocarbons and the inorganic pollutants are represented by heavy metals. In the selection of biochar preparation materials, the main target was agricultural and forestry solid waste, and the biochar made from bacterium bran and reed straw were selected. These two types of biochar were the most effective materials for soil remediation using biochar in collaboration with microorganisms. In addition, it is important for this study to compare the experimental mechanism and remediation effect to find the best material for remediation and apply it to purify the soil environment. For example, as a kind of edible mushroom cultivation waste, mushroom bran has low production cost and high yield. The use of mushroom bran to prepare mushroom bran carbon is a kind of green recycling low carbon behavior. The reed straw is a kind of renewable plant with strong vitality, and it is a multi-purpose renewable biological resource as agricultural and forestry solid waste. The preparation of biochar from reed straw is a new use. In the future, if the use of biochar made from mushroom bran and reed straw can be widely used, the biochar made can be used to remediate soil pollution in the market. In the market environment where the government strongly supports the development of green industry, biochar made from mushroom bran and reed straw can be developed rapidly because of its low cost and easy access to raw materials. The promotion and application of this technology is of great significance for the development of green and low-carbon industries in China to achieve the goal of carbon neutrality.

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