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Effects of Silk-Worm Excrement Biochar Combined with Different Iron-Based Materials on the Speciation of Cadmium and Lead in Soil

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Abstract: A 56d incubation experiment was conducted to explore the effects of the silk-worm excrement biochar (500 °C, BC) combined with different iron-based materials (FeCl₃, FeSO₄, and reduced iron powder) on the speciation of cadmium (Cd) and lead (Pb) in a contaminated soil. Application rate of BC and iron-based materials is 1% (W/W) and 0.2% (W/W) of the soil, respectively. At the same time, the soil physicochemical properties, such as pH, cation exchange capacity (CEC), and the structure of soil, were determined in order to explore the influence mechanism of amendments to forms of Cd and Pb in soil. The results show that the stabilization effects on Cd is (BC + FeSO₄) > (BC + FeCl₃) > (BC + Fe) > (BC) and Pb is (BC + Fe) > (BC + FeSO₄) > (BC + FeCl₃) > (BC) at the end of incubation, compared with the effect of the control group. The treatment of (BC + FeSO₄) is the most effective in terms of the stabilization of Cd and Pb, which makes the percentages of organic-bound and residual Cd and Pb increase by 40.90% and 23.51% respectively. In addition, with different ways of treatment, the pH value and CEC of soil see a remarkable increase by 1.65–2.01 units and 2.01–2.58 cmol·kg^{−1} respectively. X-ray diffraction (XRD) patterns show that the soil imprisons Cd and Pb in different mineral phases. As such the treatment of (BC + FeSO₄) can significantly improve soil environment, increase soil pH value & CEC and exert a relatively good stabilization effect on both Cd and Pb.

Keywords: silkworm excrement; biochar; ferrous materials; soil contamination; stabilization

1. Introduction

Cadmium (Cd) and lead (Pb) are two typical potential toxic metals (PTMs) [1] that often compound with each other and cause soil pollution. PTMs firstly accumulate in the soil, and then lead to deterioration of the soil ecosystem and a decline in crop yields and quality [2]. Finally, it enters human body through the food chain and thus endangers humans' physical health [3,4]. As a noxious carcinogenic pollutant, Cd is classified as a category I human carcinogen [5,6]. Numerous studies on the health effects of Cd showed that its adverse effects on the bones and kidneys [7–10]. Pb is a potent human neurotoxin which when accumulated in the blood results in neurocognitive damage including intelligence quotient (IQ) deficits, behavioral problems and, at very high levels, death [11]. Therefore, it is particularly important to prevent PTMs from getting into plants so as to ensure food safety.

The toxicity of PTMs not only has a closed relation with their total amount, but also involves their speciation in soil [12]. The biological effectiveness of PTMs greatly depends on its speciation [13]. Once in the soil, PTMs are adsorbed by initial fast reactions (minutes, hours), followed by slow adsorption reactions (days, years) and are, therefore, redistributed into different chemical forms with varying bioavailability, mobility, and toxicity [14,15]. This distribution is believed to be controlled by reactions of PTMs in soils such as (i) mineral precipitation and dissolution, (ii) ion exchange, adsorption, and desorption, (iii) aqueous complexation, (iv) biological immobilization and mobilization, and (v) plant uptake [16]. According to Li et al. [17] and Sun et al. [18], soil in situ stabilization technology is used to reduce the activity of PTMs by adding some kinds of material to the soil contaminated by PTMs, which can transform these metals into a form that is difficult to exploit through a series of chemical reactions. By doing so, the biological effectiveness of PTMs can be reduced.

Cui et al. [19] reported that 10–40 t/hm² biochar dosage can significantly reduce the percentage of Cd inside wheat seed by 24.8%–44.2%, improve soil fertility [20], and reduce the proportion of CaCl₂ leaching Cd in soil by 10.1%–40.2%. Using the *Miscanthus* straw to make biochar to remediate Cd, Pb, and Zn in soil, the percentage of the bioavailability of Cd and Pb decrease by 71% and 92% respectively [21]. Moreover, biochar can also induce root growth of plants [22–25]. The stabilizers of ferric salt such as FeSO₄, Fe₂(SO₄)₃, and FeCl₃ are commonly used in soil stabilization [26,27]. The iron compound has been widely recognized as an antidote that can effectively reduce the mobility of As [28]. However, studies on the stabilization effect of Cd and Pb in soil by iron-based materials are relatively few and the results are divided. Studies have found that ferrihydrite can enhance the adsorption capacity of Cd in soil with the help of Fe(III)-reducing bacteria, and it is also possible to produce a mixed mineral phase of CdCO₃-FeCO₃-CaCO₃ [29,30]. Cui et al. [29,31] use phosphate, FeSO₄ and Fe₂(SO₄)₃ to stabilize Pb and As in soil, as they find that Pb and As cannot be stabilized by solely used one of the stabilizers. The percentage of the exchangeable content of Pb and As can only be reduced by using two stabilizers together. However, other studies have found that ferric salt can increase the dissolution of Cd and Pb in soil. Shao et al. [32] found that ferrous sulphate fertilization significantly increased Cd concentrations in rice grains. Warren and Alloway have shown that mixing ferrous sulfate with lime can increase the uptake of Cu, Zn and Pb by plant [33].

At present, many organic materials such as animal excrement, plant straw, etc. have already been used in the remediation of PTMs contaminated soil, however, studies on the remediation of PTMs contaminated soil by silk-worm excrement have rarely been reported [34]. Moreover, studies on the remediation of Cd and Pb contaminated soil by the combination of iron-based materials and biochar which is made from silk-worm excrement have been not reported at home and abroad. This research uses the silk-worm excrement biochar, ferric chloride (FeCl₃), ferrous sulfate (FeSO₄·7H₂O), and reduced iron powder (Fe) as materials to explore whether adding iron-based materials can increase the stabilization of Cd and Pb in soil under the condition of applying the silk-worm excrement biochar. Meanwhile, this is trying to explore the effects of combination of these materials on the speciation of Cd and Pb in composite contaminated soil and to analyze the effects of different treatments on soil physicochemical properties in soil. Through analysis, hopefully a material combination which has a good effects on stabilization of Cd and Pb can be found, and therefore can provide a new suggestion for Cd and Pb contaminated soil remediation and also open up a new way for agricultural waste disposal in Guangxi.

2. Materials and Methods

2.1. Materials

The soil sample was collected from a polluted farmland near a lead-zinc mine in Chongzuo city, Guangxi, China. Basically, soil was collected from the surface layer (0–20 cm) of a cropland area. Sampling took place in 25 points over a 0.5-ha area, totaling an amount of 50 kg of soil. After removing

leaves, stones, and other miscellanies, the soil sample was air-dried, crushed, and sieved to 1 mm to conduct general analyses. Part of the soil was sieved to 0.15 mm to conduct the total content measurement of Cd and Pb in soil. The soil sample GBW07456 (GSS-27) was used as the quality control (QC) sample of the total content measurement of Cd and Pb, and purchased from Shide Jia Technology Co., Ltd., in Shenzhen, China. The soil sample GBW07459 (ASA-8) was used as the quality control (QC) sample of the pH and cation exchange capacity (CEC) measurement, and purchased from Aike Yingchuang Biological Technology Co., Ltd., in Beijing, China. The physicochemical properties of the experiment soil are shown in Table 1.

Table 1. The basic properties of the tested soil.

Item	pH	Cation Exchange Capacity ($\text{cmol}\cdot\text{kg}^{-1}$)	Total Lead ($\text{mg}\cdot\text{kg}^{-1}$)	Total Cadmium ($\text{mg}\cdot\text{kg}^{-1}$)
Paddy soil	6.76	10.86	1320.29	52.24
Control sample	8.60	13.42	0.61	41.57

Preparation of Silk-worm excrement biochar (BC): silk-worm excrement was collected from Guangxi Silk-Worm Technology Promotion Centre. The silk-worm excrement was placed in a ceramic crucible for weighing. The crucible was then heated in a muffle furnace (Nabertherm LT40, heating rate: $20\text{ }^{\circ}\text{C}/\text{min}$; ventilatory rate: $2\text{ mL N}_2/\text{min}$) at $500\text{ }^{\circ}\text{C}$ for 3 h, and then removed after cooling to room temperature. The crucible was then weighed to calculate the silk-worm excrement biochar yield at $500\text{ }^{\circ}\text{C}$ pyrolysis.

$$\text{Biochar yield (\%)} = (m_1/m_0) \times 100\%, \quad (1)$$

where m_0 (g) was the weight of silk-worm excrement before entering the muffle furnace, and m_1 (g) was the biochar weight of silk-worm excrement after charring.

The ash content was calculated by placing a fixed amount of biochar in a ceramic crucible, charring at $800\text{ }^{\circ}\text{C}$ in a muffle furnace, and recording the weight once a constant value was obtained.

$$\text{Ash content (\%)} = (M_1/M_0) \times 100\%, \quad (2)$$

where M_0 (g) was the biochar weight of silk-worm excrement before entering the muffle furnace, and M_1 (g) was the biochar weight of silk-worm excrement after charring.

Equation (1) was used to calculate yield rate which is 40.14%. Equation (2) was used to calculate ash content which is 32.57%. Then BC was sieved to 0.25 mm to conduct general analyses. The basic properties of BC are shown in Table 2.

Table 2. The basic properties of BC.

Material	pH	Cation Exchange Capacity ($\text{cmol}\cdot\text{kg}^{-1}$)	Alkaline Functional Groups ($\text{mmol}\cdot\text{g}^{-1}$)	Acid Functional Groups ($\text{mmol}\cdot\text{g}^{-1}$)
BC	10.20	71.58	4.02	0.59

2.2. Experimental Method

50 g soil samples through 1 mm sieves were put in fifteen 200 mL plastic jars respectively. Different materials were added into the soil samples as follows: ① control (CK); ② 1% (mass ratio) silk-worm excrement biochar (BC); ③ 1% silk-worm excrement biochar + 0.2% FeCl_3 (BC + FeCl_3); ④ 1% silk-worm excrement biochar + 0.2% $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ (BC + FeSO_4); ⑤ 1% silk-worm excrement biochar + 0.2% reduced iron powder (BC + Fe). Each treatment was replicated three times. The stabilizers and soil were fully mixed in accordance to the test setting. Then added distilled water to maintain a maximum moisture holding capacity of the soil at 40%. The plastic jars were covered with kraft paper and put in a $(25 \pm 1)\text{ }^{\circ}\text{C}$ biochemical incubator (LRH-70, Suzhou, China) to

allow incubation for 56d. The moisture contents of soil were kept using weighing method throughout the experiment every two days [35,36].

2.3. Items for Determination

pH of BC was measured with a pH meter (Mettler Toledo FE20 Plus) using “GB-T 12496.7-1999 pH measurements for wood-based activated carbon” [37] as a reference. Two grams of biochar were placed into a beaker, before adding 50 mL of ultrapure water and putting a glass evaporating dish over the beaker, and heating to boiling temperature for 5 min. Following that, the original 5 mL of filtrate was filtrated and discarded, and the remaining filtrate was cooled to room temperature for determining the pH value. The experiment was repeated three times.

The ash content of BC was determined using “GB-T12496.3-1999 ash content measurements for wood-based activated carbon” [38] as a reference. CEC of soil and BC were determined by a method described by Gillman and Sumpter [39]. Soil pH was determined using the “ISO soil quality-determination of pH” [40]. The determination of the content of surface functional groups was referred to Bohem method [41]. Tessier [42] five-step sequential extraction method was used to extract PTMs in soil at the end of incubation, extracting exchangeable fraction (F_1), carbonate bound fraction (F_2), Fe-Mn oxide fraction (F_3), bound organic fraction (F_4), and residual fraction (F_5) one by one. Cd and Pb in leaching solution and digestion liquid were filtered by 0.45- μ m membranes and measured by atomic absorption spectrophotometer (Agilent 55B AA, AUS). The soil samples before and after passivation were analyzed by using high power (4 KW) polycrystal X-ray diffractometer (DX-2700A, Liaoning, China).

2.4. Data Processing

IBM SPSS 21.0 statistical package program was applied to analyze data, and Origin Pro 8.0 were used to draw a data diagram.

3. Results and Discussion

3.1. The Effect of Different Treatments on Cd and Pb in Soil

3.1.1. The Effect of Different Treatments on Cd in Soil

At the end of incubation, the influence of different treatments on the speciation of Cd in soil is shown in Figure 1. After the four treatments (BC, BC + FeCl_3 , BC + FeSO_4 and BC + Fe), the percentages of the Cd (F_1) of each treatment decreased by 17.77%, 33.81%, 39.53%, and 24.40% respectively, the percentages of Cd (F_2) of each treatment decreased by 1.29%, 11.56%, 12.52%, and 14.43% respectively, the percentage of Cd (F_3) of each treatment increased by 0.45%, 3.80%, 18.85%, and 8.56% respectively, the percentages of Cd (F_4) of each treatment increased by 35.68%, 58.75%, 47.69%, and 29.13%, and the percentages of Cd (F_5) of each treatment increased by 13.10%, 32.89%, 39.38%, and 27.02% respectively, compared with the percentages of these chemicals in the CK (F_1 : 31.11%, F_2 : 21.28%, F_3 : 9.47%, F_4 : 7.23%, and F_5 : 30.92%).

The exchangeable fraction and carbonate bound fraction are greatly affected by the environment, because they are easy to be transferred and converted by plants [43]. Fe-Mn oxide fraction can be greatly influenced by soil pH value and redox conditions. While the bound organic and residual fraction are stable forms that cannot be easily absorbed by plants. As shown in Figure 1, different treatments can effectively reduce the percentages of Cd (F_1 and F_2) and increase those of the Cd (F_4 and F_5) at the end of incubation, so as to achieve the purpose of reducing Cd bioavailability. The effect of different treatments on speciation of Cd is not consistent. Among them, the treatment of (BC + FeSO_4) has the best effect, causing an increase of 40.90% in the percentages of the Cd (F_4 and F_5), and of 28.44% in the percentages of Cd (F_1 and F_2). The stabilization effect is (BC + FeSO_4) > (BC + FeCl_3) > (BC + Fe) > (BC), which indicates that adding iron-based materials can increase the stabilization effect

of Cd in soil. Some studies mention that adding iron-based materials can promote the formation of hydroxylated metal ions and promote the formation of hydroxide precipitate of Cd, Pb, Zn, and other PTM ions in soil [44].

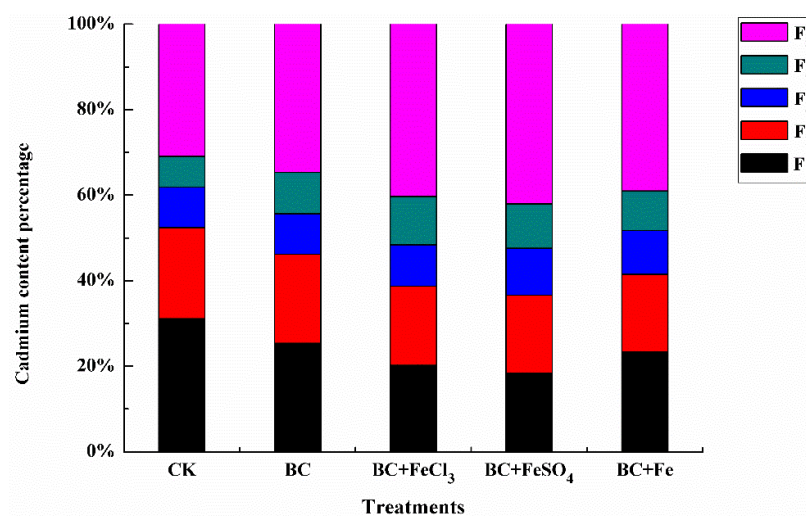


Figure 1. The effect of different treatments on chemical form of Cd in soil.

3.1.2. The Effect of Different Treatments on Pb in Soil

At the end of incubation, the influence of different treatments on the speciation of Pb in soil is shown in Figure 2. After the four treatments (BC, BC + FeCl₃, BC + FeSO₄ and BC + Fe), the percentages of the Pb (F₁) of each treatment decreased by 41.87%, 30.68%, 38.79%, and 51.84% respectively, the percentages of Pb (F₂) of each treatment increased by 24.13%, 7.48%, 12.17%, and 1.88% respectively, the percentage of Pb (F₃) of each treatment increased by 0.17%, 0.93%, 0.70%, and 8.00% respectively, the percentages of Pb (F₄) of each treatment increased by 13.46%, 31.77%, 31.53%, and 31.09% respectively, and the percentages of Pb (F₅) of each treatment increased by 23.95%, 17.51%, 19.67%, and 29.77% respectively, compared with the percentages of these chemicals in the CK (F₁: 6.13%, F₂: 49.40%, F₃: 20.53%, F₄: 7.76%, and F₅: 16.18%).

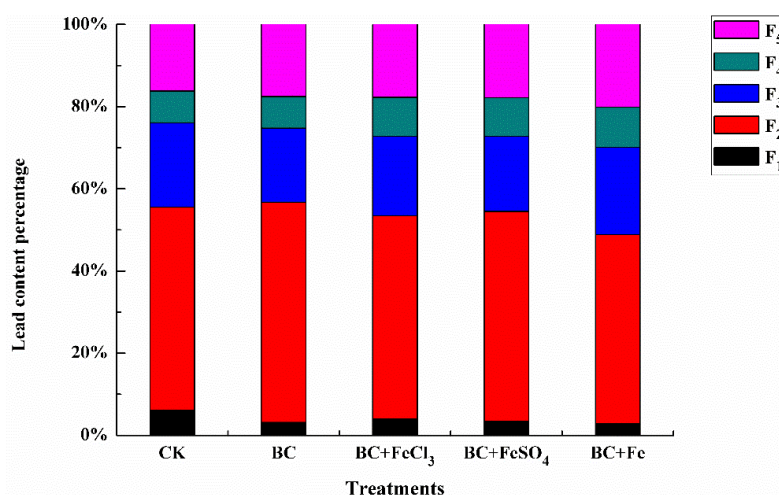


Figure 2. The effect of different treatments on chemical form of Pb in soil.

As shown in Figure 2, the percentages of Pb (F₁) in different treatments significantly decrease while the percentages of other fractions to some extent increase at the end of incubation, which indicates the Pb in soil is transformed from the exchangeable fraction to more stable fractions. Different treatments have certain passivation effects on Pb in soil, and the treatment of (BC + Fe) has the best

effect among all treatments. The percentages of Pb (F_4 and F_5) increase by 30.2% and the Pb (F_1 and F_2) decrease by 8.43% compared with those percentages of these chemicals in the control sample. The stabilization effect is $(BC + Fe) > (BC + FeSO_4) > (BC + FeCl_3) > (BC)$, which indicates that adding iron-based materials can increase the stabilization effect of Pb in soil. Research show that after $FeSO_4$ is oxidized to $Fe_2(SO_4)_3$ in soil, $FeSO_4$ and $Fe_2(SO_4)_3$ would generate Fe_2O_3 precipitation [45], and iron oxide has a strong metal ion adsorption and enrichment ability [46].

3.2. The Effect of Different Treatments on Soil Property and Soil Crystal Structure

3.2.1. The Effect of Different Treatments on Soil pH Value

As shown in Figure 3, soil pH values of the treatments with stabilizer were significantly higher than that of CK (pH: 6.81). The pH values of the four treatments (BC, BC + $FeCl_3$, BC + $FeSO_4$ and BC + Fe) significantly increased by 1.96, 1.94, 1.65, and 2.01 units when compared with the control sample at the end of incubation. The difference of soil pH by the treatment of (BC) and iron-based materials is not significant, but there are significant differences with the treatment of blank control. This indicates that the soil pH can be significantly increased by adding the silk-worm excrement biochar. The reason why the pH value of soil got increased after adding stabilizers was that silk-worm excrement contains alkaline matter (Na, K, Ca, Mg, and $CaCO_3$) [47,48], which can increase the pH value of soil [49]. And this result is consistent with previous ones [50,51].

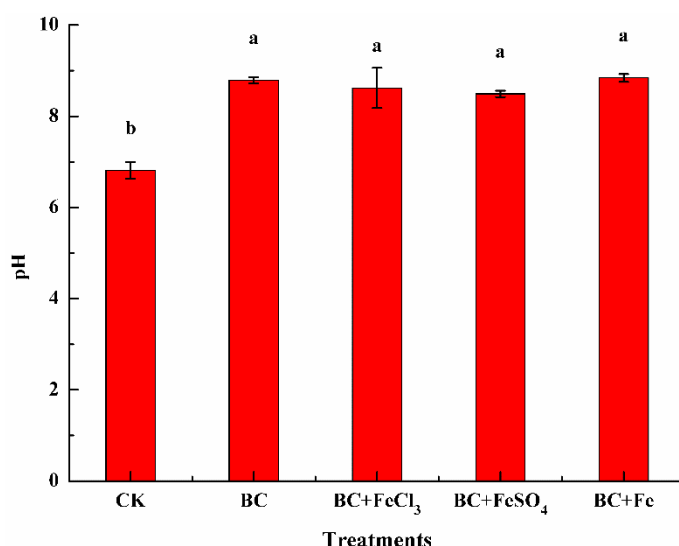


Figure 3. The effect of different treatments on soil pH. Note: different letters for the Figure 3 indicates that the differences are statistically significant ($P < 0.05$).

3.2.2. The Effect of Different Treatments on Soil CEC

As shown in Figure 4, soil CEC of the treatments with stabilizer were significantly higher than that of CK (CEC: $10.15 \text{ cmol} \cdot \text{kg}^{-1}$). The content of soil CEC after the four treatments (BC, BC + $FeCl_3$, BC + $FeSO_4$ and BC + Fe) significantly increased by 2.13, 2.16, 2.01, and 2.58 $\text{cmol} \cdot \text{kg}^{-1}$ respectively compared with that of the control sample at the end of incubation. CEC refers to the amount of cationic ions in the soil that can be absorbed by the soil colloid [52]. Its amount can reflect the soil system buffering ability, which is also an important basis for evaluating synthetical fertility and soil improvement. The soil CEC got increased after the use of stabilizer, which means that the fertility of the soil also got increased. The difference of soil CEC by the treatment of (BC) and iron-based materials is not significant, but there are significant differences with the treatment of blank control. This indicates that the soil CEC can be significantly increased by adding the silk-worm excrement biochar. Due to the micropore structure of biochar, it can adsorb a lot of mineral elements, so causing the application of biochar to increase the soil CEC [53]. In addition, the increase of soil CEC may be

related to relatively high content of ash of silk-worm excrement biochar in this study. Singh et al. [54] reported that because insect feces tend to have high ash content, biochar made from them has higher CEC than charcoal or straw, which leads to a significant increase in soil CEC by the application of silk-worm excrement biochar compared with CK.

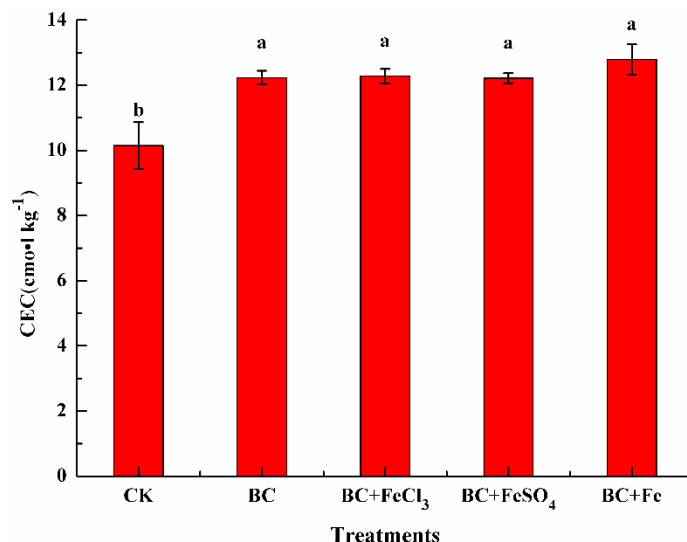


Figure 4. The effect of different treatments on soil cation exchange capacity (CEC). Note: different letters for the Figure 4 indicates that the differences are statistically significant ($P < 0.05$).

3.2.3. The Effect of Different Treatments on the Soil Crystal Structure

The effect of different treatments on soil crystal structure is shown in Figure 5. It can be seen from the figure that Cd in soil crystal mainly exists in the forms of $\text{Cd}(\text{OH})\text{NO}_3$ and $\text{Cd}(\text{OH})_2$. Pb mainly exists in the forms of Pb_5O_4 and $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$. This indicates that Cd and Pb are fixed in the soil in different mineral phases. However, the mineral phase is relatively less. It is speculated that, this phenomenon may be because only a handful of PTM is stabilized in the form of crystals in soil. The content of Pb_5O_4 mineral phase treated by (BC + Fe) and (BC + FeSO_4) at the number 1 is higher than others. This indicates that the majority of Pb_5O_4 is fixed in the form of soil crystals, and the soil treated by (BC + Fe) and (BC + FeSO_4) has more crystal of Pb_5O_4 . The differences of crystal structures after different treatments are not distinct when compared with the crystal structure of the control sample. This shows that there is no significant change in soil crystal structure by adding different stabilizers. The content of $\text{Cd}(\text{OH})\text{NO}_3$, $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$ and $\text{Cd}(\text{OH})_2$ mineral phase treated by (BC + FeCl_3), (BC + FeSO_4) and (BC + Fe) are higher than that treated by BC at the number 2, 3, and 4, which indicates that it is feasible to apply iron-based materials to the stabilization of Cd and Pb. In addition, mineral phase of $\text{Cd}(\text{OH})\text{NO}_3$ treated by (BC + FeSO_4) at the number 2 is higher than (BC + Fe), which indicates that the treatment of (BC + FeSO_4) has a better stabilizing effect on Cd in soil than (BC + Fe). In summary, the treatment of (BC + FeSO_4) has a better promotion of conversion of Cd and Pb into mineral phases, which means that Cd and Pb eventually exist in the soil in a form of low bioavailability.

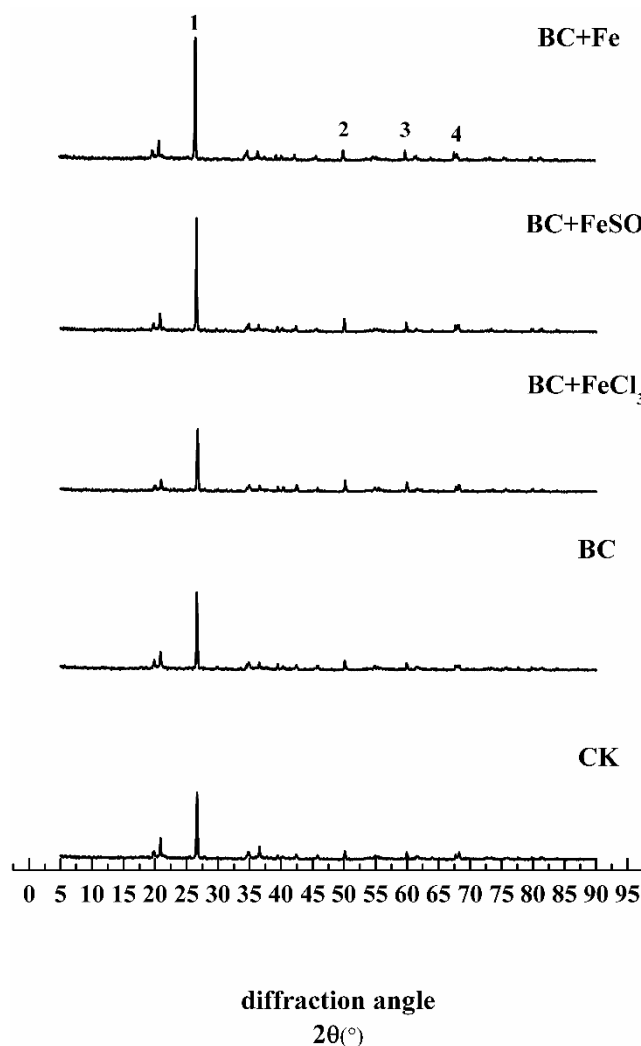


Figure 5. X-ray diffraction (XRD) patterns of the soil under different treatments. 1: Pb_5O_4 , 2: $\text{Cd}(\text{OH})\text{NO}_3$, 3: $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$, 4: $\text{Cd}(\text{OH})_2$, BC: biochar, CK: control sample.

3.3. Correlation between the Speciation of PTMs and Soil Properties

The results of correlation among the speciation of Cd and Pb, the pH value and CEC are shown in Table 3. The pH value and CEC were significantly negatively correlated with the content of exchangeable fraction and carbonate bound fraction Cd ($P < 0.01$), and positively correlated with the content of bound organic and residual fraction Cd ($P < 0.05$); the pH value and CEC were significantly negatively correlated with the content of exchangeable fraction and carbonate bound fraction Pb, and significantly positively correlated with the content of bound organic and residual fraction Pb. This indicates that the exchangeable and carbonate bound Cd fraction and Pb were respectively gradually converted to the bound organic and residual fraction Cd and Pb after adding stabilizers. This process is mainly achieved through increasing the pH value and CEC. In the process of remediating soil contaminated by PTMs, soil pH value is a very important factor [55]. Soil pH value directly controls the solubility of metal hydroxide, carbonate, and phosphate, the hydrolysis of PTMs, the formation of ionic radius, the dissolution of organic matter, and the charge property of soil surface [56–58]. With the increasing pH value, the functional groups, such as the phenols, hydroxyl groups and carbonyl groups are separated from soil organic matter. In this way, their affinity for metal cation increases, and thus they can reduce the mobility of PTMs ions [13]. The exchange adsorption is also one of important reasons for the reduction of PTMs activities [59]. The bigger the number of cation exchange, the stronger the retention of PTMs [60,61]. As the content of soil CEC increases,

the adsorption and exchange ability on cationic in soil is enhanced, and the content of the available PTMs in soil can be reduced by exchangeable reaction [62].

Table 3. Correlation analysis among the speciation of typical potential toxic metals (PTMs), pH value and CEC.

Index	The Speciation of Cd				
	F ₁	F ₂	F ₃	F ₄	F ₅
pH	−0.816 **	−0.729 **	0.059	0.705 **	0.142
CEC	−0.792 **	−0.839 **	0.126	0.630 *	0.225

Index	The Speciation of Pb				
	F ₁	F ₂	F ₃	F ₄	F ₅
pH	−0.929 **	−0.323	0.156	0.692 **	0.919 **
CEC	−0.860 **	−0.207	0.325	0.651 **	0.861 **

Note: * and ** indicate respectively that the correlation is statistically significant ($p < 0.05$ and $p < 0.01$).

4. Conclusions

(1) Adding iron-based materials can increase the stabilization effect of Cd and Pb in soil under the condition of applying the silk-worm excrement biochar.

(2) The speciation of Cd and Pb in soil could be changed after the application of BC and iron-based materials. In doing so, the speciation of Cd and Pb can be transformed into fraction of low bioavailability. The treatment of (BC+FeSO₄) has a good effect on the stabilization of Cd and Pb in soil relative to other treatments. In addition, XRD patterns showed that Cd and Pb were respectively immobilized on the soil in the form of Cd (OH)NO₃, Cd (OH)₂, Pb₅O₄, and Pb₃(CO₃)₂(OH)₂.

(3) The soil pH value and CEC could be significantly improved after the application of BC and iron-based materials; the pH value and CEC were significantly negatively correlated with the content of exchangeable fraction and carbonate bound fraction Cd, and positively correlated with the content of bound organic and residual fraction Cd; the pH value and CEC were significantly negatively correlated with the content of exchangeable fraction and carbonate bound fraction Pb, and significantly positively correlated with the content of bound organic and residual fraction Pb. The increase of soil pH and CEC are important factors affecting the speciation transformation of Cd and Pb in soil.

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References

1. Race, M.; Ferraro, A.; Fabbicino, M.; La, A.M.; Panico, A.; Spasiano, D.; Tognacchini, A.; Pirozzi, F. Ethylenediamine-*N,N'*-Disuccinic Acid (EDDS)-Enhanced Flushing Optimization for Contaminated Agricultural Soil Remediation and Assessment of Prospective Cu and Zn Transport. *Int. J. Environ. Res. Public Health* **2018**, *15*, 543. [[CrossRef](#)] [[PubMed](#)]
2. Gurung, B.; Race, M.; Fabbicino, M.; Komínková, D.; Libralato, G.; Siciliano, A.; Guida, M. Assessment of metal pollution in the Lambro Creek (Italy). *Ecotoxicol. Environ. Saf.* **2018**, *148*, 754–762. [[CrossRef](#)] [[PubMed](#)]
3. Raicevic, S.; Kaludjerovicradovic, T.; Zouboulis, A.I. In situ stabilization of toxic metals in polluted soils using phosphates: Theoretical prediction and experimental verification. *J. Hazard. Mater.* **2005**, *117*, 41–53. [[CrossRef](#)] [[PubMed](#)]

4. Shyleshchandran, M.N.; Mohan, M.; Ramasamy, E.V. Risk assessment of heavy metals in Vembanad Lake sediments (south-west coast of India), based on acid-volatile sulfide (AVS)-simultaneously extracted metal (SEM) approach. *Environ. Sci. Pollut. Res.* **2018**, *25*, 7333–7345. [[CrossRef](#)] [[PubMed](#)]
5. Gustavsson, P. Strategies for Primary Prevention of Occupational Cancer. In *Occupational Cancers*; Anttila, S., Boffetta, P., Eds.; Springer: London, UK, 2014; pp. 565–572.
6. Arito, H. Risk assessment of hazardous substances revisited. *Ind. Health* **2015**, *53*, 193–195. [[CrossRef](#)] [[PubMed](#)]
7. Chen, X.; Zhu, G.; Jin, T.; Lei, L.; Liang, Y. Bone mineral density is related with previous renal dysfunction caused by cadmium exposure. *Environ. Toxicol. Pharmacol.* **2011**, *32*, 46–53. [[CrossRef](#)] [[PubMed](#)]
8. Horiguchi, H.; Aoshima, K.; Oguma, E.; Sasaki, S.; Miyamoto, K.; Hosoi, Y.; Katoh, T.; Kayama, F. Latest status of cadmium accumulation and its effects on kidneys, bone, and erythropoiesis in inhabitants of the formerly cadmium-polluted Jinzu River Basin in Toyama, Japan, after restoration of rice paddies. *Int. Arch. Occup. Environ. Health* **2010**, *83*, 953–970. [[CrossRef](#)] [[PubMed](#)]
9. Ke, S.; Cheng, X.Y.; Zhang, N.; Hu, H.G.; Yan, Q.; Hou, L.L.; Sun, X.; Chen, Z.N. Cadmium contamination of rice from various polluted areas of China and its potential risks to human health. *Environ. Monit. Assess.* **2015**, *187*, 408. [[CrossRef](#)] [[PubMed](#)]
10. Wallin, M.; Sallsten, G.; Fabricius-Lagging, E.; Öhrn, C.; Lundh, T.; Barregard, L. Kidney cadmium levels and associations with urinary calcium and bone mineral density: A cross-sectional study in Sweden. *Environ. Health* **2013**, *12*, 22. [[CrossRef](#)] [[PubMed](#)]
11. Harvey, P.J.; Taylor, M.P.; Handley, H.K. Widespread Environmental Contamination Hazards in Agricultural Soils from the Use of Lead Joints in Above Ground Large-Scale Water Supply Pipelines. *Water Air Soil Pollut.* **2015**, *226*, 178. [[CrossRef](#)]
12. Han, C.; Wang, L.; Gong, Z.; Huaxia, X.U. Chemical forms of soil heavy metals and their environmental significance. *Chin. J. Ecol.* **2005**, *24*, 1499–1502.
13. Santona, L.; Castaldi, P.; Melis, P. Evaluation of the interaction mechanisms between red muds and heavy metals. *J. Hazard. Mater.* **2006**, *136*, 324–329. [[CrossRef](#)] [[PubMed](#)]
14. Shiowatana, J.; McLaren, R.G.; Chanmekha, N.; Samphao, A. Fractionation of Arsenic in Soil by a Continuous-Flow Sequential Extraction Method. *J. Environ. Qual.* **2001**, *30*, 1940–1949. [[CrossRef](#)] [[PubMed](#)]
15. Buekers, J. Fixation of Cadmium, Copper, Nickel and Zinc in Soil: Kinetics, Mechanisms and Its Effect on Metal Bioavailability. Ph.D. Thesis, Katholieke Universiteit Leuven, Leuven, Belgium, 2007.
16. Levy, D.B.; Barbarick, K.A.; Siemer, E.G.; Sommers, L.E. Distribution and Partitioning of Trace Metals in Contaminated Soils near Leadville, Colorado. *J. Environ. Qual.* **1992**, *21*, 185–195. [[CrossRef](#)]
17. Li, H.; Ye, X.; Geng, Z.; Zhou, H.; Guo, X.; Zhang, Y.; Zhao, H.; Wang, G. The influence of biochar type on long-term stabilization for Cd and Cu in contaminated paddy soils. *J. Hazard. Mater.* **2016**, *304*, 40–48. [[CrossRef](#)] [[PubMed](#)]
18. Sun, Y.; Sun, G.; Xu, Y.; Liu, W.; Liang, X.; Wang, L. Evaluation of the effectiveness of sepiolite, bentonite, and phosphate amendments on the stabilization remediation of cadmium-contaminated soils. *J. Environ. Manag.* **2016**, *166*, 204–210. [[CrossRef](#)] [[PubMed](#)]
19. Cui, L.; Pan, G.; Li, L.; Yan, J.; Zhang, A.; Bian, R.; Chang, A. The reduction of wheat cd uptake in contaminated soil via biochar amendment: A two-year field experiment. *Bioresources* **2012**, *7*, 5666–5676. [[CrossRef](#)]
20. Sohi, S.P.; Krull, E.; Lopez-Capel, E.; Bol, R. Chapter 2—A Review of Biochar and Its Use and Function in Soil. *Adv. Agron.* **2010**, *105*, 47–82.
21. Houben, D.; Evrard, L.; Sonnet, P. Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (*Brassica napus* L.). *Biomass Bioenergy* **2013**, *57*, 196–204. [[CrossRef](#)]
22. Biederman, L.A.; Harpole, W.S. Biochar and its effects on plant productivity and nutrient cycling: A meta-analysis. *Glob. Change Biol. Bioenergy* **2014**, *6*, 172–175. [[CrossRef](#)]
23. Jeffery, S.; Verheijen, F.G.A.; Velde, M.V.D.; Bastos, A.C. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agric. Ecosyst. Environ.* **2011**, *144*, 175–187. [[CrossRef](#)]

24. Wang, Y.; Zhang, L.; Yang, H.; Yan, G.; Xu, Z.; Chen, C.; Zhang, D. Biochar nutrient availability rather than its water holding capacity governs the growth of both C3 and C4 plants. *J. Soils Sediments* **2016**, *16*, 801–810. [[CrossRef](#)]
25. Haefele, S.M.; Konboon, Y.; Wongboon, W.; Amarante, S.; Maarifat, A.A.; Pfeiffer, E.M.; Knoblauch, C. Effects and fate of biochar from rice residues in rice-based systems. *Field Crops Res.* **2011**, *121*, 430–440. [[CrossRef](#)]
26. Seidel, H.; Görsch, K.; Amstätter, K.; Mattusch, J. Immobilization of arsenic in a tailings material by ferrous iron treatment. *Water Res.* **2005**, *39*, 4073–4082. [[CrossRef](#)] [[PubMed](#)]
27. Subacz, J.L.; Barnett, M.O.; Jardine, P.M.; Stewart, M.A. Decreasing arsenic bioaccessibility/bioavailability in soils with iron amendments. *J. Environ. Sci. Health Part A Toxic/Hazard. Subst. Environ. Eng.* **2007**, *42*, 1317–1329. [[CrossRef](#)] [[PubMed](#)]
28. Feng, B. Research on groundwater arsenic removal technology. *Public Technol.* **2010**, *24*, 21–23. (In Chinese)
29. Xenidis, A.; Stouraiti, C.; Papassiopi, N. Stabilization of Pb and As in soils by applying combined treatment with phosphates and ferrous iron. *J. Hazard. Mater.* **2010**, *177*, 929–937. [[CrossRef](#)] [[PubMed](#)]
30. Muehe, E.M.; Obst, M.; Hitchcock, A.; Tyliczszak, T.; Behrens, S.; Schröder, C.; Byrne, J.M.; Michel, F.M.; Krämer, U.; Kappler, A. Fate of Cd during Microbial Fe(III) Mineral Reduction by a Novel and Cd-Tolerant Geobacter Species. *Environ. Sci. Technol.* **2013**, *47*, 14099. [[CrossRef](#)] [[PubMed](#)]
31. Cui, Y.; Du, X.; Weng, L.; Van Riemsdijk, W.H. Assessment of In Situ Immobilization of Lead (Pb) and Arsenic (As) in Contaminated Soils with Phosphate and Iron: Solubility and Bioaccessibility. *Water Air Soil Pollut.* **2010**, *213*, 95–104. [[CrossRef](#)]
32. Shao, G.; Chen, M.; Wang, D.; Xu, C.; Mou, R.; Cao, Z.; Zhang, X. Using iron fertilizer to control Cd accumulation in rice plants: A new promising technology. *Sci. China Life Sci.* **2008**, *51*, 245–253. [[CrossRef](#)] [[PubMed](#)]
33. Warren, G.P.; Alloway, B.J. Reduction of arsenic uptake by lettuce with ferrous sulfate applied to contaminated soil. *J. Environ. Qual.* **2003**, *32*, 767–772. [[CrossRef](#)] [[PubMed](#)]
34. Qiu-Jun, L.I.; Da-Rong, L.I.; Wang, Y.H.; Feng, Z.Y.; Ning, X.J.; Li-Xiang, W.U. Effects of Three Kinds of Organic Materials on Physicochemical Properties and Available Heavy Metals in Soil. *J. Soil Water Conserv.* **2013**, *6*, 036.
35. Lu, H.; Li, Z.; Fu, S.; Méndez, A.; Gascó, G.; Paz-Ferreiro, J. Effect of Biochar in Cadmium Availability and Soil Biological Activity in an Anthrosol Following Acid Rain Deposition and Aging. *Water Air Soil Pollut.* **2015**, *226*, 164. [[CrossRef](#)]
36. Zhang, P.; Zhang, C.; Pan, L.; Yang, W.; Li, C.; Xu, G.; Li, F.; Lu, M. Effects of Amendments and Water Conditions on the Chemical Speciation of Cd and Pb in Contaminated Paddy Soil in a Mining Area. *J. Soil Contam.* **2016**, *25*, 717–726. [[CrossRef](#)]
37. SBTs. GB-T 12496.7-1999 Test methods of wooden activated carbon—determination of pH [S]. *China Stand. Press Beijing* **1999**. (In Chinese)
38. SBTs. GB-T 12496.3-1999 Test methods of wooden activated carbon—determination of ash content [S]. *China Stand. Press Beijing* **1999**. (In Chinese)
39. Gillman, G.P.; Sumpter, E.A. Modification to the compulsive exchange method for measuring exchange characteristics of soils. *Soil Res.* **1986**, *24*, 61–66. [[CrossRef](#)]
40. ISO. *Soil Quality—Determination of Ph*; ISO: Geneva, Switzerland, 1994.
41. Boehm, H.P. Some aspects of the surface chemistry of carbon blacks and other carbons. *Carbon* **1994**, *32*, 759–769. [[CrossRef](#)]
42. Tessier, A.; Campbell, P.G.C.; Bisson, M. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* **1979**, *51*, 844–851. [[CrossRef](#)]
43. Zhu, Q.; Wu, J.; Wang, L.; Yang, G.; Zhang, X. Effect of Biochar on Heavy Metal Speciation of Paddy Soil. *Water Air Soil Pollut.* **2015**, *226*, 429. [[CrossRef](#)]
44. Adriano, D.C.; Wenzel, W.W.; Vangronsveld, J.; Bolan, N.S. Role of assisted natural remediation in environmental cleanup. *Geoderma* **2004**, *122*, 121–142. [[CrossRef](#)]
45. Wu, B. The immobilization remediation of Cadmium, Lead and Arsenic in contaminated soils. Level of Master Thesis, Central South University, Changsha, China, 2014. (In Chinese)
46. Tsuji, M.; Tamaura, Y. Thermodynamic study of M^+/H^+ exchanges on cryptomelane-type manganic acid. *Solvent Extr. Ion Exch.* **2000**, *18*, 187–202. [[CrossRef](#)]

47. Chao, X.U.; Lin, X.B.; Qi-Tang, W.U.; Tang, H.T.; Liao, Y.L.; Qin, X.B. Impacts of Biochar on Availability of Heavy Metals and Nutrient Content of Contaminated Soil Under Waterlogged Conditions. *J. Soil Water Conserv.* **2012**, *26*, 194–198.
48. Cao, X.; Harris, W. Properties of dairy-manure-derived biochar pertinent to its potential use in remediation. *Bioresour. Technol.* **2010**, *101*, 5222–5228. [[CrossRef](#)] [[PubMed](#)]
49. Fellet, G.; Marchiol, L.; Delle, V.G.; Peressotti, A. Application of biochar on mine tailings: Effects and perspectives for land reclamation. *Chemosphere* **2011**, *83*, 1262–1267. [[CrossRef](#)] [[PubMed](#)]
50. Ahmad, M.; Soo, L.S.; Yang, J.E.; Ro, H.M.; Han, L.Y.; Sik, O.Y. Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. *Ecotoxicol. Environ. Saf.* **2012**, *79*, 225–231. [[CrossRef](#)] [[PubMed](#)]
51. Uchimiya, M.; Lima, I.M.; Klasson, K.T.; Wartelle, L.H. Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. *Chemosphere* **2010**, *80*, 935–940. [[CrossRef](#)] [[PubMed](#)]
52. Chapman, H.D. *Cation-Exchange Capacity 1*; U.S. Salinity Laboratory: Riverside, CA, USA, 2011; Volume 44, p. 202.
53. Schulz, H.; Glaser, B. Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. *J. Plant Nutr. Soil Sci.* **2012**, *175*, 410–422. [[CrossRef](#)]
54. Singh, B.; Singh, B.P.; Cowie, A.L.; Krull, E.; Singh, B.; Joseph, S. Characterisation and evaluation of biochars for their application as a soil amendment. *Soil Res.* **2010**, *48*, 516–525. [[CrossRef](#)]
55. Ma, L.Q.; Choate, A.L.; Rao, G.N. Effects of Incubation and Phosphate Rock on Lead Extractability and Speciation in Contaminated Soils. *J. Environ. Qual.* **1997**, *26*, 801–807. [[CrossRef](#)]
56. Mustafa, G.; Singh, B.; Kookana, R.S. Cadmium desorption from goethite in the presence of desferrioxamine B and oxalic acid. Proceedings of 3rd Australian New Zealand Soils Conference, Sydney, Australia, 5–9 December 2004.
57. And, S.S.; McBride, M.; Hendershot, W. Lead Phosphate Solubility in Water and Soil Suspensions. *Environ. Sci. Technol.* **1998**, *32*, 388–393.
58. Sauvé, S.; McBride, M.; Hendershot, W. Soil Solution Speciation of Lead(II): Effects of Organic Matter and pH. *Soil Sci. Soc. Am. J.* **1998**, *62*, 618–621. [[CrossRef](#)]
59. Wang, S.; Xu, Y.; Norbu, N.; Wang, Z. *Remediation of Biochar on Heavy Metal Polluted Soils*; IOP Conference Series: Earth and Environmental Science; IOP Publishing: Bristol, UK, 2018; p. 042113.
60. Lehmann, J.; Gaunt, J.; Rondon, M. Bio-char Sequestration in Terrestrial Ecosystems—A Review. *Mitig. Adapt. Strateg. Glob. Change* **2006**, *11*, 403–427. [[CrossRef](#)]
61. Rees, F.; Simonnot, M.O.; Morel, J.L. Short-term effects of biochar on soil heavy metal mobility are controlled by intra-particle diffusion and soil pH increase. *Eur. J. Soil Sci.* **2014**, *65*, 149–161. [[CrossRef](#)]
62. Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, N.; Mohan, D.; Vithanage, M.; Lee, S.S.; Ok, Y.S. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2014**, *99*, 19–33. [[CrossRef](#)] [[PubMed](#)]

