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Biochar and Cd Alter the Degradation and Transport of Kasugamycin in Soil and Spinach

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Abstract: Biochar has been widely studied to reduce multiple contaminant sources in one matrix (e.g., several heavy metals in soils), yet less attention has been paid to accelerating pesticide degradation while in the presence of any heavy metals, such as when kasugamycin (KSM) and cadmium (Cd) are both present in soil. While KSM has low toxicity compared to other pesticides, it can be a potential health risk when applied to vegetable crops, especially when KSM is used or overapplied to achieve rapid reductions in insect and disease pressure. The degradation behavior of KSM (2 kg ha⁻¹) in the presence of Cd (20 mg kg⁻¹) and biochar (5% by wt.) when growing spinach (*Spinacia oleracea*) was studied. The biochar increased spinach shoot and root biomass by 51.0–54.8% and 24.4–39.0%, respectively, compared to the KSM treatment only. Compared to the treatments that did not receive biochar, the biochar application increased the KSM degradation in the soil by 8.4–68.4% and, subsequently, less KSM was absorbed by the spinach roots (18.0–48.4%) and shoots (33.0–33.2%). The KSM degradation rate, as a function of soil depth, was enhanced in the presence of Cd. The biochar also effectively decreased the KSM concentration with soil depth, reducing downward KSM migration. The KSM degradation, increased by the biochar, led to smaller organic moieties and some macromolecular organic phases. In soils that are contaminated with Cd and where vegetables are raised, biochar may be used as an environmentally friendly proponent for increasing KSM degradation, reducing KSM downward transport and, thus, protecting environmental and human health.

Keywords: biochar; deterioration; downward migration; pesticide; soil



Citation: Cui, L.; Ma, J.; Quan, G.; Yan, J.; Ippolito, J.A.; Wang, H. Biochar and Cd Alter the Degradation and Transport of Kasugamycin in Soil and Spinach. *Agriculture* **2023**, *13*, 2172. <https://doi.org/10.3390/agriculture13112172>

Academic Editors: Vinod Kumar and Pankaj Kumar

Received: 5 October 2023

Revised: 9 November 2023

Accepted: 10 November 2023

Published: 20 November 2023



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1. Introduction

Pesticides are widely used to protect crops and, consequently, to allow producers to maintain or increase yields. Globally, pesticide use increased from 1990 to 2020, with approximately 20,000 commercial products used for plant protection worldwide [1]. In 2022, the predicted pesticide volumes were 1.76×10^6 Mg for China, followed by USA and Brazil, with 4.08×10^5 and 2.05×10^5 Mg, respectively [2]. However, more than 90% of applied pesticides are lost or decomposed in the environment, which has led to an obvious waste of resources, along with a multitude of environmental problems, such as pesticide or organic pollution accumulation in sensitive ecosystems [3]. Some of the more environmentally friendly pesticides are naturally occurring, such as the naturally occurring antibiotic, kasugamycin (KSM; C₁₄H₂₅N₃O₉).

Specifically, KSM is an aminoglycoside antibiotic produced by two independently isolated strains of *Streptomyces kasugaensis*. It is composed of three structurally distinct

sub-components: a glycine imine, an unusual amino-sugar, kasugamine, and D-chiro-inositol, which is created by the fungus *Magnaporthe oryzae* [4]. Furthermore, KSM is an aminoglycoside antibiotic that exhibits considerable medical potential [5]. For example, KSM has been used to counteract some recalcitrant human diseases, such as by controlling *Mycobacterium tuberculosis*, inhibiting herpes simplex virus-2, and even showing promising activity reductions against COVID-19 [6]. However, KSM is sensitive to light and heat, and it is unstable under alkaline conditions. For example, the degradation half-life of KSM at pH 9.0 and 50 °C is only 7.28 h and, therefore, it has largely been limited in its practical application [7]. Fortunately, these extreme conditions typically do not occur in the environment. However, alkaline conditions do occur globally; thus, under these conditions KSM application is often widespread and excessive due to its known degradation rate.

Within agricultural systems, KSM has been used for over half a century to combat rice-sheath blight [8]. In addition, KSM has been widely used for pest and disease prevention in crops and vegetables, and for reducing blossom infections [9]. More specifically, Chen [10] reported that terminal residual levels of KSM in Chinese cabbage were not detected 14 days after application under field-test conditions, with degradation rates related to temperature and precipitation. Huang [11] showed that KSM inhibited the growth of some aquatic bacteria, but also stimulated the growth of other resistant bacteria. Zhang [12] found that KSM controlled plant diseases by disturbing the transport of secondary metabolites in plants. Although KSM has been proven to be helpful for pest and disease prevention, the presence of any residual KSM may be a threat to environmental and human health. Biochar might be a promising material to promote KSM degradation, thus reducing its fate and transport in the environment.

Biochar is a carbon-rich material that features low costs, a large specific surface area, and strong adsorption capacity. Biochars often contain abundant functional groups (e.g., lactone, phenolic, and carbonyl groups), negative charge due to the presence of Pi electrons, and pores ranging from the micro to the meso pore size, all of which can contribute to sorption and the physical entrapment of contaminants [13–15]. Thus, biochars have been applied widely to reduce soil inorganic and organic pollution [16,17]. Furthermore, biochar's physicochemical properties can improve soil quality and water relations [18,19] and contribute to carbon sequestration, and they have the proven potential to sorb pesticides, reducing their mobility and bioavailability and enhancing their degradation [20]. The land application of biochar, as an eco-friendly sorbent, has certainly emerged as a promising technology to minimize pesticide-associated health and environmental risks [21].

Specifically, Jones [22] found that modified biochar promoted 10% greater simazine degradation in soil compared to soil not receiving biochar. Yang and Sheng [23] showed that the addition of wheat-straw biochar (1%; w:v) enhanced diuron sorption in water compared to a control. Biochar has been shown to reduce the negative effects of pesticides on certain microorganisms, all leading to the decreased phytotoxicity of 2,4-D in soils and plants [24]. Abas [25] found that biochar was effective in removing soil chlorantraniliprole mass (by 90% to 99%), a promising use of biochar for removing chlorantraniliprole in agricultural runoff. Cui [26] found that biochar promoted 2,4,6-trichlorophenol degradation into a combination of benzene derivatives and low-molecular-weight organic compounds. Unfortunately, the above studies did not focus attention on organic compound degradation in the presence of heavy metals.

Research has focused attention on biochar's use for improving plant growth in the presence of heavy metals. In a study of the application of wheat-straw biochar (5% by wt.), green pepper and eggplant biomass were significantly increased (16.9% to 519.9%), while the Cd concentration in both plants was decreased by 6.8% to 11.5% and by 15.1% to 15.4%, respectively [27]. Li [28] showed that the application of 30 Mg ha⁻¹ biochar to Cd-polluted soil increased eggplant growth and reduced Cd concentrations in roots, shoots, and fruits by 12.2%, 12.5%, and 18.5%, respectively, compared to a control. Pot experiments proved that a 0.5% to 1% (by wt.) application of an iron-based biochar inhibited Cd and As bioaccumulation in *Brassica chinensis* L. by 23.6–36.5% and 43.1–45.1%, respectively, compared to a

control [29]. Unfortunately, the above studies did not focus attention on biochar's ability to reduce both Cd and organic contaminant bioavailabilities simultaneously. One particular study, by Gao [30], found that biochar effectively reduced Cd and atrazine bioavailability in soils where pak choi was raised. The authors noted decreased plant stress, along with an increased rate of photosynthesis and antioxidant production.

The utilization of biochar to improve vegetable production and reduce pollution in soils containing both heavy metals (e.g., Cd) and residual pesticides (e.g., KSM) is yet to be fully explored. Thus, the objectives of this study were to (i) investigate the effectiveness of biochar in reducing the bioavailability and plant uptake of KSM, either in the absence or in the presence of excessive soil Cd, and (ii) identify the degradation pathways of KSM, in soil, when in the presence of biochar.

2. Materials and Methods

2.1. Experimental Materials and Design

Soil (0 cm to 20 cm depth) was collected in the Tinghu District (33°24'47.70" N, 120°17'43.85" E), Yancheng City, Jiangsu Province. The local climate was humid subtropical, with a mean annual temperature and precipitation of 14 °C and 1000 mm, respectively. The soil was described as a mainly coastal, salinized fluvo-aquic soil, according to the China soil-classification system (an Aridisol, according to the USDA Soil Taxonomic System) [31]. The soil was air-dried, stones and litter were removed, and then the soil was passed through a 5-millimeter sieve. Biochar was prepared by pyrolysis of wheat straw at 450 °C, with a hold time of 4 h, using a continuous anoxic production method at the Sanli New Energy Company, Shangqiu, Henan Province, China. Basic properties of soil and biochar were determined according to methods recommended by Lu [32] and IBI [33], and are presented in Table 1. Briefly, soil and biochar organic carbon contents were quantified using the dichromate oxidation method, cation-exchange capacity (CEC) was determined with the sodium-acetate-exchange method, cations (Ca, Mg, K, Na) determined via atomic absorption spectrometry (AAS; TAS-986, Persee, Beijing, China) following the NaOAc method, and biochar ash content was measured using the muffle-furnace method, with weight remaining determined using an electronic balance (to 0.0001 g; XPR105DR/AC, Mettler Toledo, Zurich, Switzerland) [32]. The pH was measured using a glass electrode in deionized water with soil-to-solution rate of 1:2.5. Total nitrogen (total N) was determined using the semi-micro-Kjeldahl procedure with Kjeldahl determination apparatus (UDK139, VELP, Usmate, Italy). Total phosphorus (total P) was measured using the NaOH fusion colorimetric method with P concentration determined via a spectrophotometer (TU-1901, Persee, Beijing, China). Total potassium (total K) was determined via NaOH fusion, with K concentration determined using AAS.

Table 1. Basic properties of soil and biochar at time of collection of both materials.

	pH	CEC (cmol kg ⁻¹)	EC (dS m ⁻¹)	OC (g kg ⁻¹)	Total N (g kg ⁻¹)	Total P (g kg ⁻¹)	Total K (g kg ⁻¹)	Ash (%)
Soil	8.7	2.4	4.1	6.5	0.7	0.4	7.3	NA
Biochar	10.4	10.6	4.4	650.2	6.3	20.4	16.9	28.6

CEC: cation-exchange capacity; EC: electrical conductivity; OC: organic carbon; NA: not applicable.

Wooden boxes (length × width × height = 1 × 0.5 × 0.5 m) were used as individual experimental units. Four treatments, replicated three times, were used: (1) KSM (C₁₄H₂₅N₃O₉, ≥90%, Sinopharm, China) (PZ1); (2) Cd and KSM (PZ2); (3) KSM and biochar (PZ3); and 4) KSM, Cd and biochar (PZ4). Wooden boxes were filled with 30 cm (compacting and watering every 10 cm) of soil containing either 0% (PZ1, PZ2) or 5% biochar (w:w; PZ3, PZ4; the biochar was mixed with soil before filling the box.). The soil bulk density in all boxes was similar to field conditions (1.41 g cm⁻³). The total amount of soil in each box was 60 kg. For the Cd treatments (PZ2, PZ4), 250 mL of 4.8 g L⁻¹ Cd (Cd(NO₃)₂·4H₂O, 99%, Sinopharm, China) was mixed with 500 g of soil. The soil was allowed to dry and was then spread

evenly over the top of the soil in the wooden boxes; the application rate was equivalent to 20 mg Cd kg^{-1} . All soils were then incubated outdoors and irrigated with tap water once per day in the summer, weekly in the spring and autumn, and monthly in winter, for three years.

After three years, spinach seeds (*Spinacia oleracea* L. NO. 21; purchased from a local seed company) were planted in all boxes; the boxes remained outside. Initially, 2 g of seed was evenly placed in the soil in every box, and 50 plants were kept after plants reached the two-leaf stage. Boxes were usually irrigated weekly, based on natural precipitation events and visual inspection. Five months after sowing and one month before harvest, KSM (100 mg box^{-1} ; equivalent to 2 kg ha^{-1}) was sprayed on the leaf surfaces. Spinach-leaf and root samples were collected at 28 days after KSM application.

Spinach leaves and roots were washed three times with tap water and then three times with deionized water. A portion of each plant part was then stored at $-20 \text{ }^\circ\text{C}$ to reduce KSM degradation prior to analysis. Another portion of each plant part was placed in an envelope, dried for 30 min at $105 \text{ }^\circ\text{C}$ in an oven, removed, and then dried to constant weight in an oven at $60 \text{ }^\circ\text{C}$. Dried samples were crushed, mixed to homogenize, and then stored in polyethylene bags prior to chemical analysis.

Soil from depths of 0–5 cm was collected at 1 h, 1, 2, 7, 14, and 28 days after KSM application. Soil from depths of 0–5, 5–10, 10–15, 15–20, and 20–25 cm were also collected from all boxes at 14 and 28 days after KSM treatment. A portion of all soil samples was stored at $-20 \text{ }^\circ\text{C}$ to reduce KSM degradation prior to analysis. Another portion of all soil samples was air-dried, ground to pass through a 2-millimeter sieve, and stored for subsequent analyses. Some of the 2-millimeter-sieved, air-dried soil was further ground to pass through a 0.15-millimeter sieve and used for additional soil analyses. Soil pH and SOC were determined as described above. Total soil Cd content was determined by placing 0.5000 g soil in polyfluortetraethylene crucibles, adding 25 mL of a mixed solution of HF, HNO₃, and HClO₄ (10:2.5:2.5, V:V:V), and placing the crucibles on a hot plate. The mixtures were digested at $100 \text{ }^\circ\text{C}$ for 60 min, and then at $250 \text{ }^\circ\text{C}$ until the volume was reduced to approximately 2 mL. The solutions were then removed, cooled, brought to a final volume of 25 mL, and filtered through a 0.45-micrometer-membrane filter. The Cd concentration in these solutions was determined using AAS.

2.2. KSM Extraction and Chromatographic Analysis Methods

Extraction of KSM from plant samples: A 5.0000 g (accurate to 0.0001 g) spinach sample was placed into a 50 mL centrifuge tube, 20 mL of methanol (for HPLC, $\geq 99.9\%$, Sinopharm, Shanghai, China) was added, the mixture was homogenized at high speed (2500 rpm; Rotator, JOANLAB, Huzhou, China) for 1 min, and then the pH was adjusted to 5.7 with formic acid (0.5 mol L^{-1}). Samples were centrifuged for 5 min at 4000 rpm, passed through a $0.22 \text{ }\mu\text{m}$ nylon membrane filter, and then stored at $4 \text{ }^\circ\text{C}$ prior to analysis.

Extraction of KSM from soil samples: A 5.0000 g soil sample (accurate to 0.0001 g) was placed into a 50 mL centrifuge tube, 10 mL of 30% methanol (*v:v*, adjusted to pH = 5) was added, and then the mixture was sonicated for 30 min. Samples were then centrifuged for 5 min at 4000 rpm, followed by transferring the supernatant to a 50 mL volumetric flask. These steps were repeated three times, combining the supernatant in the 50 mL volumetric flask. The combined supernatant was then rotary-evaporated until nearly dry. Next, 2 mL of 30% methanol was added to redissolve the solid phase, the liquid was passed through a $0.22 \text{ }\mu\text{m}$ nylon membrane filter, and then it was stored at $4 \text{ }^\circ\text{C}$ prior to analysis. Both plant and soil KSM extractions and analyses followed those of Zhang et al. [34]. All samples were run in triplicate. The average KSM recovery from soil was 83.2–91.5%, with a standard deviation of 5.63–8.45%. The KSM recoveries were not performed on plant material.

All KSM filtrates were analyzed by high-performance liquid chromatography (HPLC, PerkinElmer Flexar-15) with a UV detector equipped with an Athena C18 column ($4.0 \text{ mm} \times 250 \text{ mm}$, $5 \text{ }\mu\text{m}$) with an isocratic mobile phase of 10:90 (MeOH:H₂O, V:V,

10 mmol L⁻¹ sodium heptanesulfonate buffer solution, pH = 3), column temperature of 25 °C, and flow velocity of 1 mL min⁻¹. All determinations were performed in triplicate.

Degradation products of KSM were detected by liquid chromatography–triple-quadrupole mass spectrometry (LC-TQMS, Waters XevoTQS-micro, Massachusetts, USA) with a UV detector (215 nm) equipped with a Waters HPLC BEH HILIC column (2.1 mm × 100 mm, 1.7 μm) with isocratic mobile phases consisting of a mixture of 0.2% formic acid (A) and an aqueous 0.2% formic acid acetonitrile solution (B). Initial chromatographic conditions were created using a linear ramp up over 6 min to 90% A and 10% B. A second linear ramp was carried out over 7 min to 30% A and 70% B, and these conditions were maintained for 1 min, before moving back to initial conditions in 2 min and equilibrating the column for 8 min. The flow rate was 0.3 mL min⁻¹ and columns were kept at 40 °C.

Mass spectrometry was performed in a Waters XevoTQS-micro (Waters, Milford, MA, USA) triple-quadrupole mass spectrometer equipped with electrospray ionization (ESI) as the ionization source. Electrospray voltage was set at 3.0 kV and temperatures of the ion-transfer tube and vaporizer were both held at 600 °C.

2.3. Functional-Group Characterization of Biochar and Soil

Functional groups of raw biochar and soils were detected by Fourier-transform infrared spectroscopy (FTIR, NEXUS-670, NICOLET, Waltham, MA, USA).

2.4. Statistical Analysis

Data were expressed as means ± standard deviation. Significant differences between treatments were determined using analyses of variance (ANOVA) at a $p < 0.05$. When present, differences between means were determined using a Tukey post hoc test. All statistical analyses were carried out using SPSS version 22.0 (SPSS Institute, IBM, Armonk, NY, USA).

3. Results and Discussion

3.1. Effects of Biochar on pH and SOC

The biochar treatments increased the soil pH (Figure 1A,B; Figure S1A) and soil organic carbon (SOC; Figure 1C,D; Figure S1B) over time and depth more often than when no biochar was added (i.e., the PZ3 and PZ4 treatments compared to the PZ1 and PZ2 treatments). The biochar had a relatively high pH (10.4; Table 1), which is likely to have been due to the presence of oxides, hydroxides, and carbonate mineral phases [35]. The biochar also contained a significantly greater amount of C than the soil used in the study (expressed as OM, Table 1). Increases in soil OC (SOC) following biochar application have been shown by many others [36–38]. Increases in SOC via biochar application play an important role in soil oxidation/reduction and sorption/desorption processes and, in conjunction with changes in soil pH, they can affect soils' buffering capacity. This, in turn, affects pesticide sorption and desorption in soils [21]. Liang [3] showed that pesticide stability was related to pH, as in the case of benzoic imine bonds, which were relatively stable in neutral or alkaline solutions, but easily broken under acidic conditions.

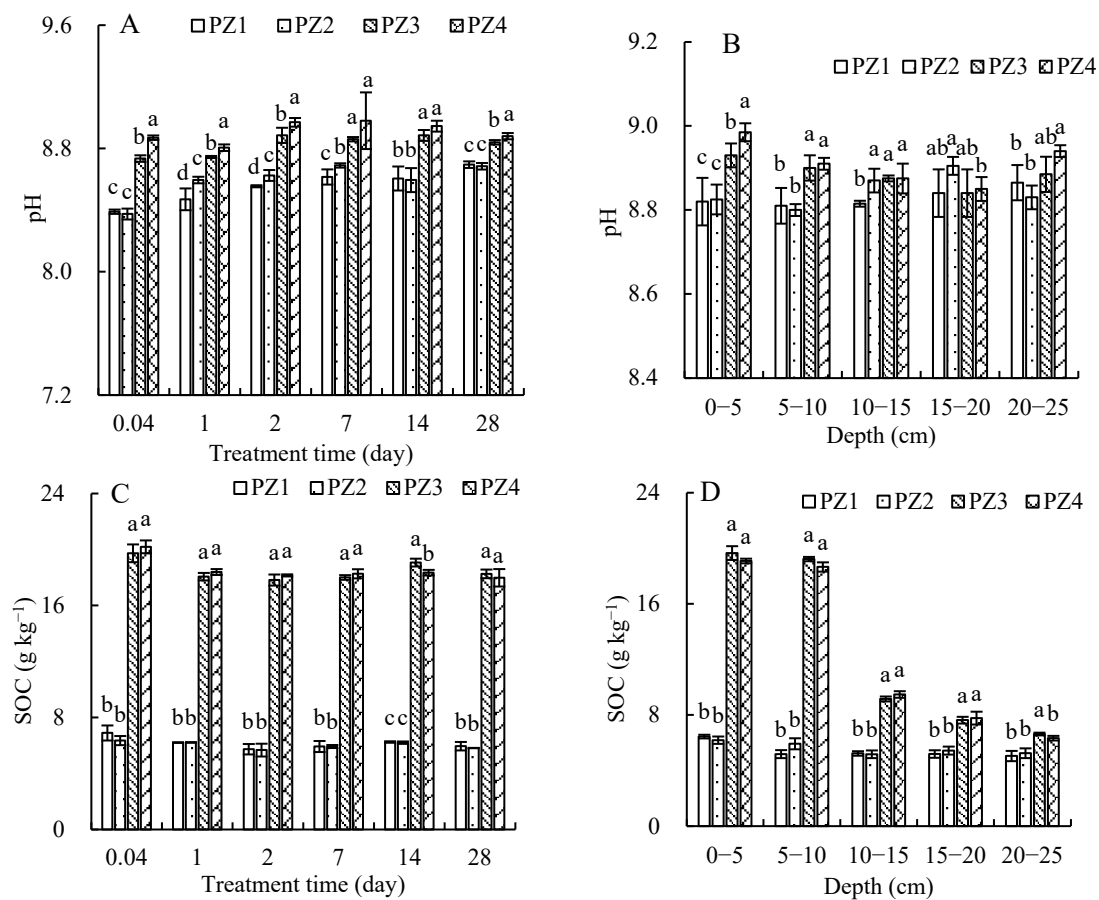


Figure 1. Changes in soil pH and soil organic carbon (SOC). (A) Soil pH (0–5 cm in depth) as a function of time; (B) soil pH as a function of soil depth, obtained at 14 days after KSM application; (C) SOC as a function of time; (D) SOC as a function of soil depth, obtained at 14 days after KSM application. Treatment PZ1 = 100 mg box⁻¹ was sprayed on spinach leaves in unamended soil; soil containing PZ2 = 20 mg Cd kg⁻¹ added to an unamended soil surface, and 100 mg box⁻¹ sprayed on spinach leaves; soil amended with PZ3 = 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves; and soil containing PZ4 = 20 mg Cd kg⁻¹ added to the surface of a soil amended with 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves. Different lower-case letters above error bars (error bars: the standard deviation of the mean; $n = 3$; $p < 0.05$, determined via a Tukey post hoc test) indicate statistically significant differences between treatments within a particular time (A,C) or depth (B,D).

3.2. Effects of Biochar on KSM Distribution in Soil and Spinach

The biochar's effects on the spinach-shoot and -root biomass are presented in Table 2. When biochar was applied (PZ3 and PZ4 treatments), both the shoot and the root biomass significantly increased compared to when biochar was not applied (PZ1 and PZ2 treatments). The addition of Cd, either with or without biochar, had little effect on the spinach-shoot or -root biomass (i.e., PZ2 vs. PZ1 and PZ4 vs. PZ3 treatments).

The KSM concentrations in the soils and in the spinach roots and shoots are shown in Figure 2. The soil KSM concentrations in the PZ3 and PZ4 treatments were decreased by 17.3–62.7% and 8.4–68.4% compared to the PZ1 and PZ2 treatments over the 28 d of the study (Figure 2A). Similarly, You [39] observed a decrease in soil metalaxyl (a pesticide) bioavailability in the presence of biochar and associated this reduction with the biochar's sorption capacity. When comparing the Cd treatments (e.g., PZ2 versus PZ1, or PZ4 versus PZ3), it appears that Cd also played a slight role in decreasing the presence of the soil KSM. However, based on the data in Figure 2A, it appears that the biochar application increased the KSM degradation to a greater degree than the Cd.

Table 2. Spinach-shoot and -root biomass. Treatment PZ1 = 100 mg box⁻¹ (equivalent to 2 kg ha⁻¹) was sprayed on spinach leaves in unamended soil; soil containing PZ2 = 20 mg Cd kg⁻¹ added to an unamended soil surface, and 100 mg box⁻¹ sprayed on spinach leaves; soil amended with PZ3 = 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves; and soil containing PZ4 = 20 mg Cd kg⁻¹ added to the surface of a soil amended with 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves. Different lower-case letters within a column indicate statistically significant differences between treatments, as determined by a Tukey post hoc test ($p < 0.05$, error = the standard deviation of the mean; $n = 3$).

Treatment	Shoot Biomass	Root Biomass
(g box ⁻¹)		
PZ1	76.7 ± 1.2 b	11.8 ± 1.0 ab
PZ2	74.0 ± 2.3 b	10.7 ± 0.8 b
PZ3	108.9 ± 4.2 a	14.7 ± 2.2 a
PZ4	117.3 ± 5.6 a	14.9 ± 3.4 a

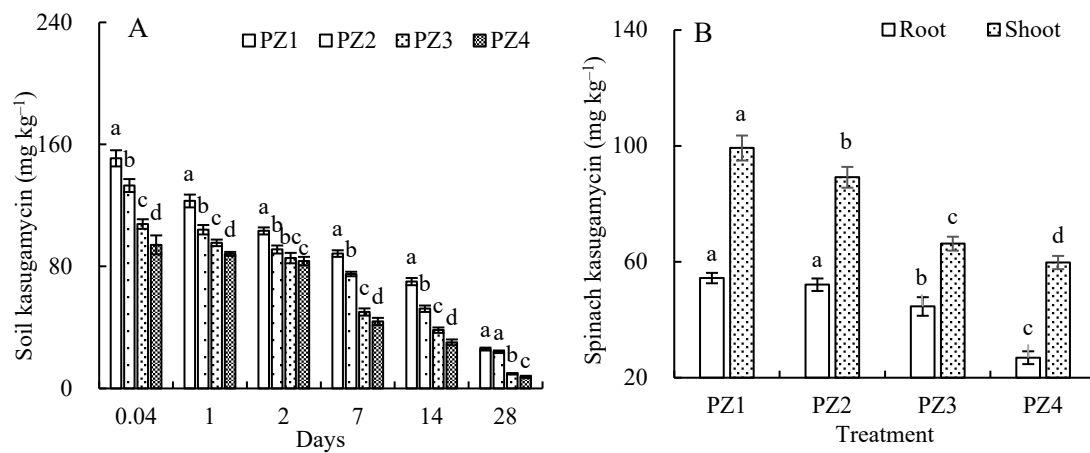


Figure 2. KSM concentrations in (A) soil (0–5 cm in depth) as a function of time and (B) spinach roots and shoots at the end of the study (28 days after KSM application). Treatment PZ1 = 100 mg box⁻¹ (equivalent to 2 kg ha⁻¹) was sprayed on spinach leaves in unamended soil; soil containing PZ2 = 20 mg Cd kg⁻¹ added to an unamended soil surface, and 100 mg box⁻¹ sprayed on spinach leaves; soil amended with PZ3 = 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves; and soil containing PZ4 = 20 mg Cd kg⁻¹ added to the surface of a soil amended with 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves. Different lower-case letters above error bars (error bars: the standard deviation of the mean; $n = 3$; $p < 0.05$, determined via a Tukey post hoc test) indicate statistically significant differences between treatments.

The KSM's degradation by the biochar, in turn, led to significant differences in KSM concentration within the spinach roots and shoots. The KSM concentrations in the roots and shoots of the PZ3 and PZ4 treatments were significantly decreased, by 18.0–48.4% and 33.0–33.2%, respectively, compared to the PZ1 and PZ2 treatments (Figure 2B). In the case of the Cd, however, KSM was present at significantly lower concentrations when the biochar and Cd were co-applied (PZ4) compared to the Cd alone (PZ2). In the soil treated with Cd–KSM–biochar, the Cd could have formed a bridge between the negative sites on the biochar and the negative sites on the KSM, helping to further hold the KSM in place for degradation to occur, a phenomenon proven by Awan [40]. You [39] observed a decrease in the uptake of metalaxyl by lettuce (*Lactuca sativa*) in the presence of wood-waste-derived biochar. These authors also found that the biochar amendment effectively decreased the shoot concentrations of metalaxyl and their metabolites by 57.7–86.3%, similar to the observations in the current study. The uptake of organic pollutants varies among different plants, and plants' capacity to absorb KSM also varies with biochar application [41].

The migration and distribution of the KSM concentrations at different soil depths are shown in Figure 3A. The KSM soil concentration decreased with increasing soil depth, indicating that soils do play a role in reducing the downward migration of KSM. However, when KSM was added to the soils that received biochar, the KSM concentration present at the soil surface was greatly reduced, and the KSM concentration changes with depth were less dramatic than with the absence of biochar. These findings suggest that biochar leads to either KSM degradation and/or strong KSM sorption. In either case, the KSM concentrations at different soil depths were reduced in the presence of biochar.

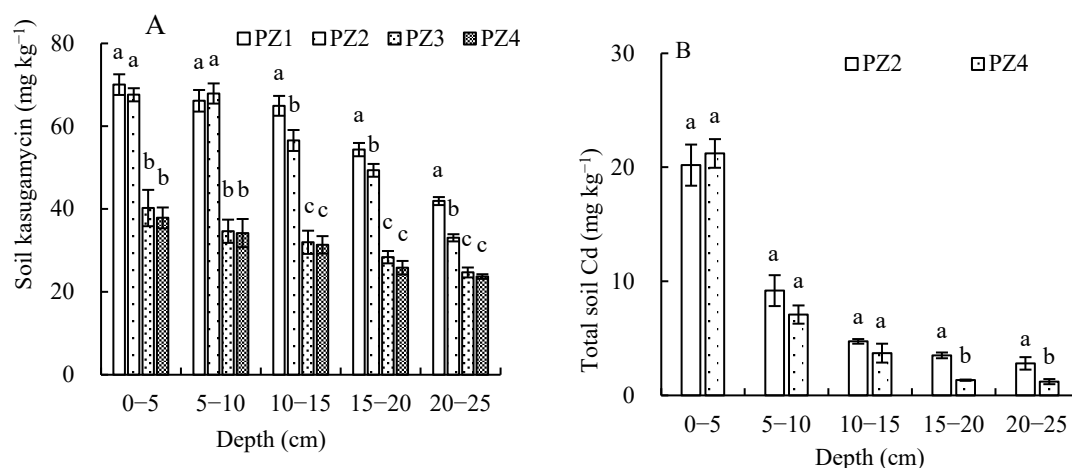


Figure 3. Soil KSM (A) and Cd (B) concentrations by soil depth at the end of the study (14 days after KSM application). Treatment PZ1 = 100 mg KSM box⁻¹ (equivalent to 2 kg ha⁻¹) was sprayed on spinach leaves in unamended soil; soil containing PZ2 = 20 mg Cd kg⁻¹ added to an unamended soil surface, and 100 mg box⁻¹ sprayed on spinach leaves; soil amended with PZ3 = 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves; and soil containing PZ4 = 20 mg Cd kg⁻¹ added to the surface of a soil amended with 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves. Different lower-case letters above error bars (error bars: the standard deviation of the mean; *n* = 3; *p* < 0.05, determined via a Tukey post hoc test) indicate statistically significant differences between treatments within a particular depth.

The KSM degradation rate, according to soil depth, tended to increase in the presence of Cd without (PZ1 versus PZ2) or with biochar (PZ2 versus PZ4) (Figure 3A). Specifically, the downward transport of KSM was reduced by the presence of Cd for the PZ2-versus-PZ1 treatments at the soil depths of 10–15, 15–20, and 20–25 cm. Compared to when no biochar was applied, the presence of the biochar reduced the spinach-leaf and -root Cd concentrations and reduced the soil's Cd bioavailability (Tables S1 and S2). Furthermore, the downward leaching of the Cd was reduced by the biochar application (Figure 3B). These results support the contention that the Cd may have formed a bridge between the negative sites on the biochar and KSM, to hold the KSM in place and to enhance the KSM degradation.

Similarly, Gao [42] showed that swine biochar could co-sorb Cd and glyphosate. Other works have shown that a modified biochar (polyacrylic acid, Fe and Co) could quickly (4 h) sorb 73% of Pb²⁺ while concomitantly degrading 81% of the quinclorac present in contaminated water [43]. Yu [44] found that the application of 1% biochar to soil decreased the total plant contents of chlorpyrifos and carbofuran by 10% and 25% of a control, respectively. The authors noted that the biochar was particularly effective in reducing the phytoavailability of both pesticides due to its high affinity for and ability to sequester pesticide residues.

3.3. KSM and Degradation Products

The concentrations of pesticides in soil tend to diminish with biochar application, both of which affect pesticide sorption, mineralization, and degradation [45]. The current study was not a biochar-rate study, but the degradation-product results should still provide evidence pointing towards biochar acting as a sorbent or a degrader of KSM. The soil (0–5 cm) KSM and degradation products' chromatogram full-scan mass spectra, after 1 day following the PZ4 treatment, are shown in Figure S2. The mass spectra show a partly protonated molecule $[M+4H]^+$ (m/z 384) and the loss of O from a protonated molecule due to in-source collision-induced dissociation (m/z 368). It appears that the KSM was quickly degraded in the soil and produced an abundance of low-molecular-weight organic moieties or larger molecular molecules (Table 3, Figure S4). Slack [46] showed that apple-blossom blight-control efficacy was significant with KSM usage, but stated that KSM is vulnerable to photodegradation. If photodegradation had been dominant in the current study, differences in the presence of KSM would not have been observed with or without biochar (or Cd), yet differences existed. In a chili-field study, Lu [7] showed that the degradation rate of KSM was relatively fast and that, upon degradation, smaller molecular substances were created. In the current study, and based on the degradation-product findings, the results suggest that the biochar did, in fact, lead to a more rapid KSM degradation. Others have shown similarly rapid degradation results when using biochar to degrade other pesticides (e.g., atrazine; Jablonowski [47]; Qiu [48]).

Table 3. KSM-soil-degradation products 1 day after application to spinach leaves (PZ4).

$\frac{C_{14}H_{25}O_9N_3}{C_8H_{13}O_4N_3}$	$\frac{C_{14}H_{29}O_8N_3}{C_7H_{13}ON_3}$	$\frac{C_{14}H_{27}O_7N_3}{C_7H_{14}ON_2}$	$\frac{C_{12}H_{24}O_8N_2}{C_6H_{14}O_2N_2}$	$\frac{C_{13}H_{21}O_7N}{C_6H_{10}O}$	$\frac{C_8H_{17}O_8N}{C_3H_9N}$
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3.4. Functional-Group Characteristics of Raw Biochar and Soil

Soil and raw biochar functional groups were detected using FTIR (Figure 4) and XPS (Figure S3), with the functional groups for the band assignments provided in Table S3. A broad-spectrum band at 3419 cm^{-1} was the result of -OH stretching vibration (ν_{O-H}), which could have been due to soil and biochar organic carbon [49]. The stretching of -CH₂- or -CH₃ in aliphatics or alicyclics (ν_{C-H}) in peaks at $2950\text{--}2860\text{ cm}^{-1}$ was mainly present in the soil, with these peaks slightly increasing with the biochar application. A peak at 1632 cm^{-1} represented the stretching of C=O groups associated with aromatic rings, or -COOH ($\nu_{C=O}$), which were abundant in the soil regardless of the biochar application. A red shift (i.e., the wavenumber was shifted in the soil compared to the raw biochar) of the stretching appeared at 1020 cm^{-1} in the biochar and 1087 cm^{-1} in the soil with biochar, which is likely to have been due to the presence of aromatic C-O (ν_{C-O}). The aliphatic CH₂ deformation (773 cm^{-1}) was decreased after the biochar was applied to the soil. The biochar contained Si-O functional groups, as shown at 466 cm^{-1} . The XPS data also show functional groups of biochar C1s, i.e., C-C (284.8 eV), C-O (286.1 eV), and C=O (287.0 eV), and four peaks for the O1s were C=O at 532.5 eV, O-H at 530.3 eV, O-C=O at 532.8 eV, and C=OO at 533.8 eV. The above data support the fact that biochar contains abundant oxygen-containing functional groups, as shown by our research group in the past [50]. Sanchez-Hernandez [51] showed that biochar can inactivate metabolites of organophosphorus pesticides, which they mainly attributed to the abundant functional groups in biochar. Zhang [52] proved that organophosphorus pesticide-degradation mechanisms can be ascribed to biochar's high surface area and porosity, as well as to strong π - π interaction and H-bonding between biochar surfaces and organophosphorus pesticides. Our findings, in conjunction with those observed by others, suggest that biochar has the potential to sorb, sequester, and degrade pesticides.

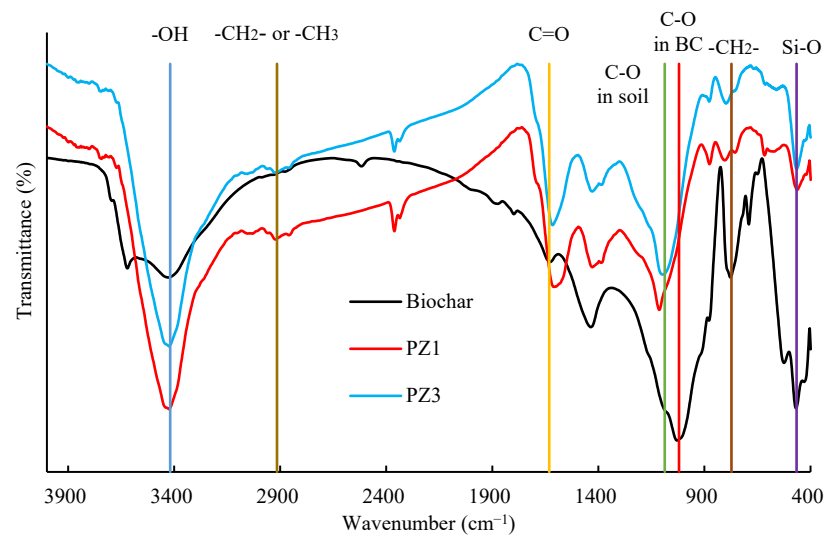


Figure 4. FTIR of raw biochar and soil treatment 1 day following KSM application to spinach leaves. Note: PZ1, 100 mg box⁻¹ (equivalent to 2 kg ha⁻¹) was sprayed on spinach leaves in unamended soil; soil amended with PZ3, 5% biochar (*w:w*), and 100 mg box⁻¹ sprayed on spinach leaves.

3.5. The Relationship between KSM, Biochar, Soil Cd, and Soil Properties

The biochar application improved the soil properties, decreased the soil KSM concentration and increased degradation, and reduced the plants' KSM uptake and transfer, with the direct or indirect pathways illustrated via PCA and correlation analysis (Figure 5, Table S4). The PCA results showed that the KSM concentration in the soil and plants was correlated with the soil properties (e.g., SOC, pH, soil Cd) in PC1 (81.7%). The factors of PC2 mainly included SOC and soil pH, which were mainly related to the biochar-application rates. The factors of PC1 and PC2 explained 95.5% of the variability for all the factors. Positive relationships between soil Cd, SOC (i.e., in this case, biochar application), soil KSM concentration with depth, and plant-root KSM content were observed (Table S4). In particular, and importantly for the current study, the biochar and soil heavy-metal concentrations were found to be key factors in reducing KSM concentrations in soils and plants (Figure S5). This is similar to the findings of others. For example, Mazarji [53], using a PCA analysis, found that the level of biochar amendment affected the degree of total PAH degradation in the presence or absence of heavy metals; the biochars also sorbed and prevented heavy-metal transport, leading to moderate PAH degradation. Kong [54] showed that biochar and heavy metals played an important role in mitigating organic toxicity with respect to seed germination and plant growth.

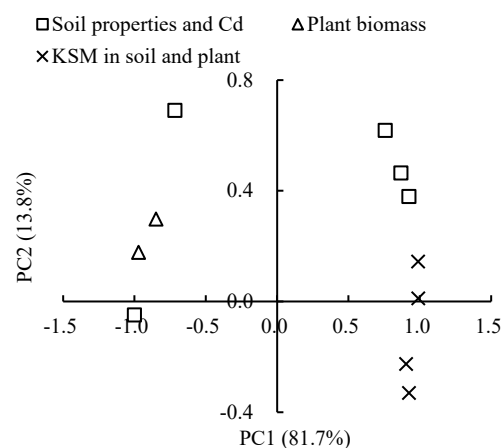


Figure 5. Principal component analysis as a function of soil properties and Cd presence, plant biomass, and KSM in both soils and plants.

4. Conclusions

Kasugamycin (KSM) is often used as an agricultural fungicide to control blight and blossom diseases; residual KSM in soils or plants can pose a risk to the environment and humans. In order to reduce the environmental/human health risk of KSM, accelerating its degradation post-application is important. Biochar was shown to effectively decrease the bioavailable KSM concentration, accelerate KSM degradation, and lessen KSM's downward transport in soil. The KSM degradation was further enhanced in the presence of both biochar and Cd, which is possibly attributable to the Cd forming a bridge between the negative sites on both the biochar and the KSM and, thus, further stimulating KSM sorption and degradation. In an equally important finding, the biochar increased the spinach biomass while decreasing the KSM concentration in the leaves and roots. It appears that biochar can act as both a sorbent and a degrader of KSM, reduce KSM uptake by plants, and in the future, potentially lessen pesticides' effects on the environment.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture13112172/s1>. Figure S1: Changes in soil (A) pH and (B) soil organic carbon (SOC) as a function of depth at the end of the study (28 days after KSM application); Figure S2: Chromatogram of soil KSM and degradation products after 1 day following treatment (soil containing PZ4; 20 mg Cd kg⁻¹ added to the surface of a soil amended with 5% biochar (wt:wt), and 100 mg box⁻¹ sprayed on spinach leaves) full-scan mass spectra; Figure S3: XPS peak fits of C1s (A), O1s and (B) and maps (C) from biochar; Figure S4: Possible KSM degradation products; Figure S5: Possible KSM degradation routes; Table S1: Total Cd concentration in spinach leaves and roots (mg kg⁻¹). Treatment PZ2 did not feature biochar, while treatment PZ4 featured the application of 5% biochar to soil; Table S2: Soil bioavailable Cd concentrations by depth (DTPA, mg kg⁻¹). Treatment PZ2 did not feature biochar, while treatment PZ4 featured the application of 5% biochar to soil; Table S3: Band assignments in FTIR spectra of biochar; Table S4. Band assignments in.

Author Contributions: Conceptualization, J.Y. and L.C.; methodology, L.C. and H.W.; software, J.M.; validation, L.C., G.Q. and J.M.; formal analysis, L.C.; investigation, J.M.; resources, L.C. and J.M.; data curation, J.M.; writing—original draft preparation, J.M. and H.W.; writing—review and editing, L.C., G.Q., J.M. and H.W.; visualization, H.W.; supervision, J.Y. and J.A.I.; project administration, J.Y.; funding acquisition, L.C., H.W. and J.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Natural Science Foundation of Jiangsu Province, China, grant number SBK2022020100, and National Natural Science Foundation of China, grant numbers 21677119, 22006127, and 41501339.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data generated for this study are presented within the manuscript and the Supplementary Files.

Acknowledgments: The FTIR was performed in Analytical and Testing Center of Yancheng Institute of Technology.

Conflicts of Interest: The authors declare no conflict of interest.

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