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18 Abstract

19 A novel integrated removal strategy was developed to enhance the concurrent
20 elimination of copper (Cu), zinc (Zn), oxytetracycline (OTC), and enrofloxacin (ENR)
21 from the aqueous environments. The underlying adsorption mechanisms of spent
22 mushroom substrate (SMSB) and the *Herbaspirillum huttiense* strain (HHS1), and their
23 efficacy in removing Cu, Zn, OTC, and ENR were also examined. Results showed that
24 the SMSB-HHS1 composite stabilized 29.86% of Cu and 49.75% of Zn and achieved
25 removal rates of 97.95% for OTC and 59.35% for ENR through a combination of
26 chemisorption and biodegradation. Zinc did not affect Cu adsorption, and ENR did not
27 impact the adsorption of OTC on SMSB. However, the co-presence of OTC and ENR
28 modified the adsorption behaviors of both Cu and Zn. Copper and Zn enhanced the
29 adsorption of OTC and ENR by serving as bridging agents, facilitating the interaction
30 between the contaminants and SMSB. Conversely, OTC and ENR inhibited the
31 adsorption process of Cu by obstructing its interaction with the SMSB and occupying
32 the oxygen-containing functional groups. The -OH (3415 cm^{-1}) and C-O-C (1059 cm^{-1})
33 functional groups were identified as the principal active sites to form hydrogen bonds
34 and interact with Cu and Zn, leading to the formation of $\text{CuP}_4\text{O}_{11}$ and $\text{Zn}_4\text{CO}_3(\text{OH})_6\text{H}_2\text{O}$.
35 HHS1 also enhanced antibiotic removal through biodegradation, as evidenced by the
36 decrease of -C-O and increase of -C=O groups. This study underscores the innovative
37 potential of the SMSB-HHS1 composite, offering a sustainable approach to addressing
38 multifaceted pollution challenges in the aquatic environments.

39 **Keywords:** Heavy Metal, Antibiotics, Biochar, Adsorption, Biodegradation

40

41 1 Introduction

42 The burgeoning presence of veterinary antibiotics (VAs) and heavy metals (HMs) in
43 aquatic ecosystems has emerged as a formidable challenge, threatening both the
44 environmental and public health. Oxytetracycline (OTC) and enrofloxacin (ENR),
45 alongside HMs such as copper (Cu) and zinc (Zn), are particularly concerning due to
46 their widespread agricultural use and environmental persistence (Zhang et al., 2024a).
47 These substances can cause a range of deleterious effects, with HMs such as Cu and Zn
48 found to cause significant human health risks after entering drinking water or food
49 production systems, while VAs contribute to the propagation of antimicrobial resistance
50 (Liu et al., 2021; Komijani et al., 2021). It is therefore important to develop effective
51 and sustainable remediation technologies for reducing VAs and stabilizing HM
52 pollutants in the aquatic environment simultaneously.

53 Biochar, a product derived from organic materials such as fungus chaff, stands out in
54 environmental remediation for its impressive contaminant adsorption capabilities,
55 attributable to its large surface area and diverse functional groups. Tao et al. (2019)
56 reported that magnesium-modified biochar significantly improved the sorption of
57 sulfamethoxazole and cadmium in sediments, reducing their bioavailability and
58 phytotoxicity. Wang et al. (2020) pointed out that bamboo-derived biochar showed
59 excellent performance for synergistic removal of Cu^{2+} and tetracycline. Hydroxyl,
60 carboxyl, and phenolic groups on biochar provide crucial reactive sites, which facilitate
61 the binding of HMs through complexation, ion exchange, and precipitation,

significantly reducing the mobility and bioavailability of such HMs in aquatic systems (Meena et al., 2020; Cheng et al., 2021). Expanding on the versatility of biochar, Zhou et al. (2023) explored its interaction with VAs. They identified π - π interactions as pivotal in the adsorption process, where biochar's carboxyl, nitro, and ketone groups serve as electron acceptors, fostering interactions with aromatic VAs. This highlights the potential of biochar in tackling a spectrum of contaminants, a point further illustrated by Wang et al. (2021), who demonstrate biochar's efficacy in removing penicillin from water, achieving a removal rate of 93.3% and an adsorption capacity of 44.05 mg g⁻¹. Although these studies highlight biochar's application in environmental remediation, a gap remains in understanding the simultaneous interaction of biochar with HMs and VAs. Antibiotics can form complexes with HMs, which may influence the removal efficiency of pollutants. This interaction is crucial for devising effective treatment strategies. Krasucka et al. (2021) revealed the complexity of interactive dynamics between various pollutants, which can significantly influence remediation outcomes.

Simultaneously, the exploitation of bacterial strains for biodegradation presents a biological avenue for detoxifying polluted waters, leveraging their metabolic processes (Lou et al., 2019). *Burkholderia* sp. Z-90 can chelate HMs and decrease their mobility, and the bacteria strain *Providencia* sp. LLDRA6 is shown to absorb HMs including Pb, Cr, Cd, and Cu with high efficiencies (Li et al., 2020a). Moreover, a strain of *S. changzhouense*, named TC931, shows high efficacy at removing tetracycline at a rate

of 73.84% (Tan et al., 2021). Likewise, a strain isolated by Leng et al. (2016) showed that 44.43 mg L⁻¹ of tetracycline could be degraded within 4 days. Additionally, the application of bacterial strains like *Herbaspirillum huttiense* (HHS1) offers a biodegradation approach that complements the adsorptive properties of biochar. Zhao et al. (2020) show HHS1's effectiveness in degrading pollutants under suboptimal conditions, suggesting a synergistic potential when combined with biochar. A number of bacteria have been studied in regard to their ability to remove VAs or HMs, but few have been identified which can remediate both. However, the complimentary effects when combined with biochar into a microbially inoculated-biochar are promising, but thus far are poorly documented in the literature, and the mechanism of this type of remediation is not fully described.

Although the individual adsorptive capacities of biochar and the biodegradation potential of microbial strains like HHS1 are well-documented, the interplay between these two remediation strategies, particularly in the context of mixed contaminant scenarios, remains underexplored. The removal mechanisms of HMs and VAs combined are much more complicated than those in single systems (Wang et al., 2020). The existing literature provides limited insights into how adsorptive mechanisms of biochar and microbial degradation processes can be synergistically harnessed to enhance the removal of a complex mixture of antibiotics and heavy metals from the aquatic environments. This highlights a critical need for studies that investigate the integrated application of biochar and microbial action, particularly focusing on their

combined effects on contaminant removal efficiency.

Our study aims to address the complex challenge of mixed contaminant removal in the aquatic environments by hypothesizing that an integrated system utilizing spent mushroom substrate biochar (SMSB) and the bacterial strain *Herbaspirillum huttiense* (HHS1) can effectively remove a mixture of OTC, ENR, Cu, and Zn. The specific objectives are: (1) to investigate the concurrent adsorption processes of heavy metals (Cu and Zn) and veterinary antibiotics (OTC and ENR) on SMSB; (2) to delineate the role of functional groups of SMSB in immobilizing heavy metals and facilitating the degradation of VAs by HHS1 by employing isothermic and kinetic models, and other analysis (Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and X-ray diffraction (XRD)); (3) to evaluate the synergistic impact of adsorption by SMSB and degradation by HHS1 on the removal efficiency of the targeted contaminants.

By unveiling the synergistic potential of biochar and bacterial degradation, we identify the contributions and mechanisms of each component in the composite material in relation to the removal of HMs and VAs. This provides valuable insights for developing effective and sustainable water treatment technologies to address the growing challenge of mixed contamination.

2 Materials and methods

2.1 Raw Material and Chemicals

Oxytetracycline (purity > 99%) and ENR (purity > 98%) were purchased from

125 Biotopped (Beijing, China) and stored at -20 °C. Analytical-grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and
126 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ were obtained from Damao Chemical Reagent Factory (Tianjin, China).
127 High-performance liquid chromatography (HPLC) methanol and acetonitrile were
128 sourced from Sigma-Aldrich (St. Louis, MO). All solutions were prepared using
129 ultrapure water (18 M Ω cm). Lysogeny broth (LB) medium was prepared with 5 g L⁻¹
130 yeast extract, 10 g L⁻¹ tryptone, and 10 g L⁻¹ NaCl (pH = 7.0). Mineral medium (MM)
131 was composed of 1 g L⁻¹ (NH₄)₂SO₄, 1 g L⁻¹ KH₂PO₄, 1 g L⁻¹ K₂HPO₄, 0.5 g L⁻¹ NaCl,
132 0.05 g L⁻¹ FeCl₃·6H₂O, and 0.02 g L⁻¹ CaCl₂·2H₂O (pH = 7.0).

133 2.2 Isolation of cultivation of HHS1

134 To isolate bacteria capable of degrading OTC and ENR, 1 g of VA-contaminated soil
135 from a poultry farm was added to 100 mL of MM containing 50 µg L⁻¹ of OTC. The
136 mixture was incubated at 15 °C, with shaking at 150 rpm to enrich low-temperature
137 resistant bacteria. After a 7-day incubation period, the enrichment culture was
138 transferred to 100 mL of fresh MM with an increased OTC concentration of 100 µg L⁻¹.
139 This process was repeated until the concentration of OTC in the MM reached 1 mg
140 L⁻¹.

141 The enrichment culture was then plated on the MM agar (containing 500 µg L⁻¹ OTC)
142 and incubated for three days. Single colonies were isolated and inoculated into fresh
143 MM solution (containing 1 mg L⁻¹ OTC). These cultures were incubated at 15 °C with
144 shaking at 150 rpm in the dark for 3 days, during which the concentration of OTC in
145 the solution was monitored. Bacteria exhibiting high OTC degradation efficiencies

were further screened for their ability to remove ENR. Ultimately, 11 strains capable of degrading both OTC and ENR were isolated.

Among these isolates, the strain HHS1 demonstrated a superior ability to remove both OTC and ENR. Strain HHS1 was identified by Sangon Biotech Co., Ltd (Shanghai, China). The genomic DNA of HHS1 was extracted using a Genomic DNA Extraction Kit and amplified using a PCR protocol targeting the 16s rRNA gene. The PCR amplicon was purified using the PCR clean up kit (Shanghai, China). The obtained sequences were aligned with those in the NCBI library (Leng et al., 2016). Results showed that the strain HHS1 was identified as *Herbaspirillum huttiense* strain. This strain is not only resistant to tetracycline but also promotes the growth of eucalypts (de Toledo Pocoli et al., 2005), highlighting its potential applicability in bioremediation and agricultural settings.

2.3 Preparation of SMSB

Spent mushroom substrate (SMS) was used as the feedstock for biochar preparation without further treatment. The SMS composition (sawdust: wheat bran: calcium sulfate: excipients=80:18.5:1:0.5, w:w:w:w) used in this study was sourced from Xiuyan county in the southern region of Northeast China, renowned for its mushroom production and significant output of SMS. Xiuyan county is also home to numerous poultry farms, where VA and HM contaminants have been detected frequently. The usage of SMSB for water and soil remediation in Xiuyan county not only addresses environmental contamination but also promotes resource re-utilization.

To prepare the SMSB, the SMS precursor was placed in a tube-shaped furnace for 10 minutes to ensure complete replacement of air with nitrogen gas. The subsequent heating procedure in the muffle furnace was as follows: the temperature was increased to 500 °C at a ramp rate of 5 °C/min and maintained for 4 hours. After the pyrolysis process, the furnace was allowed to cool down to room temperature (Zhang et al., 2021). The resulting biochar (SMSB) was then ground and sieved to a 100-mesh grade to ensure uniformity. To prepare the composite material, the SMSB was mixed with the bacterium and then incubated on a shaker at 15 °C and 120 rpm for 12 hours. Under these conditions, the fungus chaff-derived biochar with HHS1 was prepared.

2.4 Remediation experiment design

To evaluate the removal efficiency of OTC and ENR, as well as the adsorption efficiency of Cu and Zn using the SMSB-HHS1 composite, the composite was created with 2 % (w/v) SMSB and 10 % (w/v) HHS1 in 100 mL of water contaminated with Cu, Zn, OTC, and ENR over a period of three days. Samples were collected at the start of the experiment and subsequently at 24-hour intervals (0, 24, 48, and 72 hours) to quantify the concentrations of these pollutants.

2.5 Batch adsorption experiment

Batch kinetic and isothermal adsorption experiments were conducted to investigate the adsorption of Cu, Zn, OTC, and ENR on SMSB (0.2 wt%). The experimental scenarios included the adsorption of only Cu, only Zn, Cu+Zn, and a mixture of Cu+Zn+OTC+ENR (Table 1).

Table 1 Test for HM and VA adsorption

Adsorption test	Solution	pH
AT 1	Control	6.78±0.10
AT 2	Cu	5.75±0.12
AT 3	Zn	5.88±0.11
AT 4	Cu+Zn	5.82±0.15
AT 5	Cu+Zn+OTC+ENR	6.12±0.10
AT 6	OTC	6.16±0.05
AT 7	ENR	6.31±0.12
AT 8	OTC+ENR	6.28±0.10

The kinetic adsorption assays were carried out as described by Guo et al. (2019) and Joseph et al. (2020). Briefly, the initial concentrations of Cu and Zn were set to 100 mg L⁻¹ in a spiking solution using CuSO₄·5H₂O and ZnSO₄·7H₂O (Zhou et al., 2003). The samples were then incubated in a constant temperature shaker at approximately 200 rpm for 24 hours. Adsorption kinetics were measured at various time points: 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours. The models are represented by equations (1)-(2):

$$Q_t = Q_e[1 - \exp(-k_1 t)] \quad (1)$$

$$Q_t = \frac{k_2 Q_e^2 t}{1 + k_2 Q_e t} \quad (2)$$

where Q_e (mg g⁻¹) is the amount of HM or VA adsorbed at equilibrium, and k_1 and k_2 are the pseudo-first-order rate constant (h⁻¹) and pseudo-second-order rate constant (h⁻¹), respectively.

For the isothermal adsorption experiments, the initial concentrations of Cu and Zn were set to 10, 20, 40, 80, 160, 320, and 640 mg L⁻¹, with the pH ranging from 5.78 to 6.52. Residual Cu and Zn concentrations in the aqueous phase were measured after contacting with the SMSB for 6 h. The Freundlich model, an empirical model based on multilayer adsorption theory, and the Langmuir equations, based on single-layer

203 adsorption theory were used to fit the data as follows (Li et al., 2021):

$$Q_e = \frac{K_L Q_m C_e}{1 + K_L C_e} \quad (3)$$

$$q_e = K_f C_e^{1/n} \quad (4)$$

204 Where Q_e (mg g^{-1}) represents the amount of HM or VA adsorbed at equilibrium, Q_m
205 refers to the maximum adsorption capacity (mg g^{-1}), C_e is the concentration of HM or
206 VA at equilibrium (mg L^{-1}), K_L is the Langmuir constant relating to the affinity between
207 adsorbent and sorbate (L mg^{-1}), and k_f and n are the Freundlich adsorption constant and
208 linearity index (dimensionless), respectively. The obtained results were expected to
209 elucidate the adsorption mechanism of Cu and Zn and demonstrate the adsorption
210 effects in the presence of OTC and ENR.

211 The adsorption of OTC and ENR was investigated using similar kinetic and isothermal
212 methods as described for Cu and Zn. For isothermal adsorption, the initial
213 concentrations of OTC and ENR were set to 10, 20, 40, 80, 160, and 320 mg L^{-1} ,
214 considering their solubility. The centrifuge tubes containing the samples were wrapped
215 in aluminum foil to prevent photodegradation.

216 2.6 Characterizations of SMSB and HHS1

217 Heavy metals can react with VAs that contain functional groups such as carboxyl,
218 hydroxyl, and amino groups (Li et al., 2020a; Tabrizian et al., 2019). A FTIR was used
219 to identify the functional groups of extracellular polymeric substances and bacterial cell
220 surfaces so as to elucidate the mechanisms of absorption by microorganisms (Tan et al.,
221 2021; Cao et al., 2019). The SMSB and HHS1 samples were freeze-dried for FTIR

analysis (Nicolet 6700, Thermo Fisher Scientific, USA) (Zhang et al., 2021). The FTIR spectra of the prepared SMSB, over the range of 4000 cm^{-1} to 400 cm^{-1} , were obtained with 200 scans at a resolution of 4 cm^{-1} (Yuan et al., 2020). Additionally, the variations in surface functionality and mineral components of the SMSB samples were analyzed using XPS and XRD, respectively (Naggar et al., 2021), to analyze the mechanisms that control the adsorption of Cu, Zn, OTC, and ENR on SMSB. X-ray Photoelectron Spectroscopy measurements were conducted using a K-Alpha spectrometer (Thermo Scientific, USA) equipped with an Al $K\alpha$ X-ray source (6 kV) as the excitation source. The spectra binding energy was calibrated using the C1s peak of aliphatic carbons at 284.8 eV, and spectral interpretation was performed with Advantage software, employing the Gaussian-Lorentzian sum function for peak fitting (Guo et al., 2019). X-ray diffraction analysis was performed using an Ultima IV diffractometer (Rigaku, Japan) with Cu $K\alpha$ radiation over the 2θ range of $10 - 90^\circ$ (Li et al., 2020b), and target peaks were identified using Jade 6.0 software.

2.7 HM and VA analyses

The concentration of Cu and Zn in the filtrate was measured using an inductively coupled plasma optical emission spectrometry (ICP-OES, 5100, Agilent, Co., USA) after the mixture had been filtered through a $0.45\text{ }\mu\text{m}$ nylon membrane filter (Zhang et al., 2024b). The extraction of VAs from soils followed the procedures outlined by Wang et al. (2019a), and VA levels were determined by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS, 1290 Infinity II, Agilent).

Briefly, 1 mL of the water sample was added to 1.5 mL microfuge tubes with the sorbents (3 mg of Na₂EDTA and 40 mg of primary-secondary amine). The samples were vortexed, followed by centrifugation at 8000 g for 10 minutes at 4°C. Subsequently, the supernatant was filtered through a 0.22 µm membrane filter prior to analysis by UPLC-MS/MS.

2.8 BET analysis

The specific surface area of the biochar was determined using the Brunauer-Emmett-Teller (BET) method (Sahoo et al., 2021). Prior to analysis, biochar samples were degassed under vacuum at 300°C for 24 hours to remove adsorbed gases and moisture. The BET surface area was then measured using nitrogen gas adsorption at liquid nitrogen temperature (77 K) with a BET surface area analyzer (ASAP 2460, Micromeritics, USA).

2.9 Statistical analysis

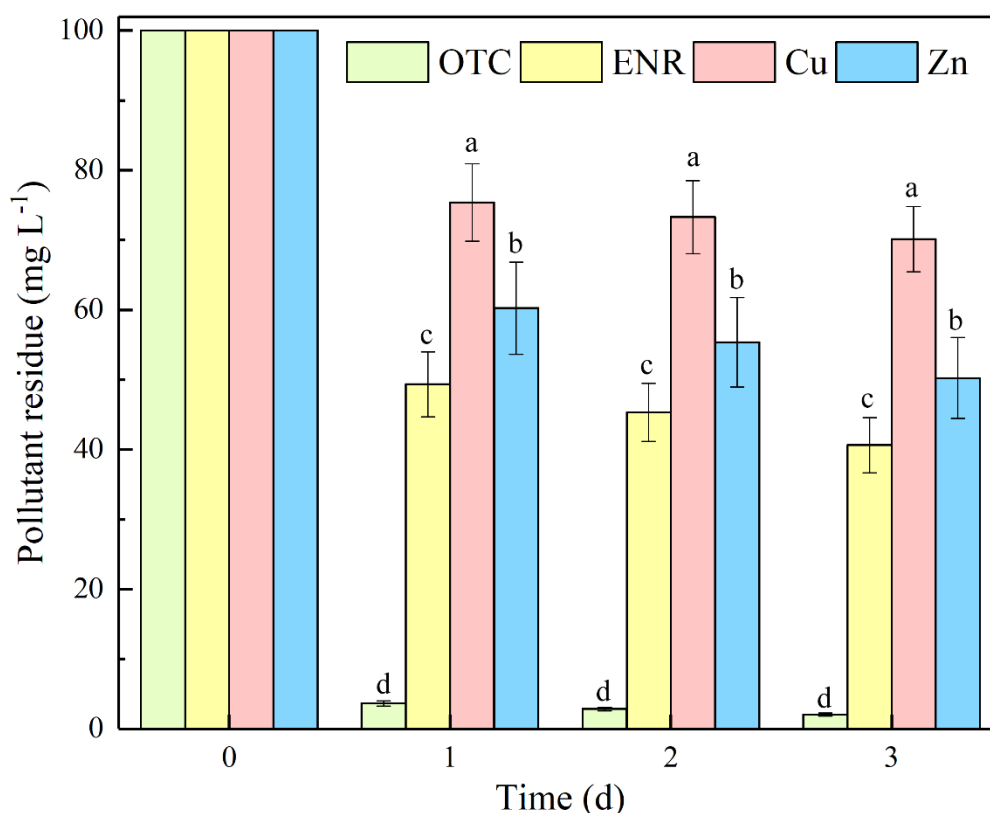
Statistical differences between samples were determined using SPSS. 19.0 (SPSS, Inc., Chicago, IL, USA), and Origin 2017 Lab was utilized to analyze the pollutant removal. All measurements were performed in triplicate.

3 Results

3.1 Treatment efficiency of HMs and VAs by fungus chaff-derived biochar with HHS1

The efficacy of SMSB-HHS1 for the co-removal of HMs and VAs from the contaminated wastewater are shown in Fig.1. The SMSB-HHS1 composite demonstrated substantial removal capabilities for Cu and Zn in the aqueous solution.

264 Following a three-day treatment period, the concentrations of Cu and Zn were reduced
 265 from 100 to 70.14 and 50.25 mg L⁻¹, respectively, corresponding to removal efficiencies
 266 of 29.86% and 49.75%, respectively. In terms of VA removal, the levels of OTC and
 267 ENR reduced to 2.05 and 40.65 mg L⁻¹, respectively, achieving removal rates of 97.95%
 268 for OTC and 59.35% for ENR.



269
 270 **Fig.1** VA and HM residue after treated by SMSB-HHS1

271 3.2 Thermal kinetics of adsorption of heavy metals and antibiotics

272 3.2.1 Heavy metal adsorption

273 Figure 2 illustrates the dynamic adsorption processes of Cu and Zn on SMSB, with the
 274 fitted kinetic parameters presented in Table 2. Adsorption experiments were conducted
 275 for single Cu, single Zn, both Cu and Zn, and a solution containing Cu, Zn, OTC, and

ENR, with the corresponding kinetic parameters aforementioned.

Copper and Zn were rapidly adsorbed onto the SMSB over time, followed by a slower increase in adsorption capacity until equilibrium was reached after 4 hours of treatment (Fig. 2). The highest value of rate constant k_1 (3.185 h^{-1}) was observed in the Cu-only solution (AT 2) while k_1 of Cu in Cu+Zn solution (AT 4) was lower at 0.580 h^{-1} . The result suggested that Cu in the Cu-only solution reached adsorption equilibrium more quickly, whereas the presence of Zn delayed the equilibration time of Cu in the Cu+Zn solution. The adsorption capacities for single Cu and Zn in solution were 49.717 mg g^{-1} and 58.988 mg g^{-1} , respectively. In contrast, the adsorption capacities for Cu and Zn containing OTC and ENR (AT 5) were only 18.212 and 13.038 mg g^{-1} , respectively.

Zinc could not influence the adsorption capacity of Cu, but the co-existence of OTC and ENR greatly affected Zn adsorption on the surface of SMSB. Chemical equilibration of Zn in AT 5 was achieved faster than that in the Zn-only solution (AT 3), with a relatively high kinetic rate constant (k_2) of $0.300 \text{ g mg}^{-1} \text{ h}^{-1}$. The result shows that Zn could quickly reach its maximum adsorption capacity in the mixture containing Cu, Zn, OTC, and ENR.

The adsorption of Cu reached an equilibrium in the absence of OTC and ENR. However, the equilibrium of Cu adsorption was not observed in the presence of OTC and ENR.

The amounts of OTC and ENR adsorbed on the SMSB increased as their C_e increased.

Tables 2 and 3 present the calculated kinetic and thermodynamic model parameters for Cu and Zn. When the OTC and ENR concentrations were 0 mg L^{-1} , the pseudo-second-

order model better fitted the adsorption of Cu (in AT 2 and AT 4) and Zn (in AT 3 and AT 5) ($R^2 > 0.998$), implying that chemisorption dominated the adsorption process. However, the adsorption of Cu in AT 5 and Zn in AT 4 was better fitted by the first-order pseudo model.

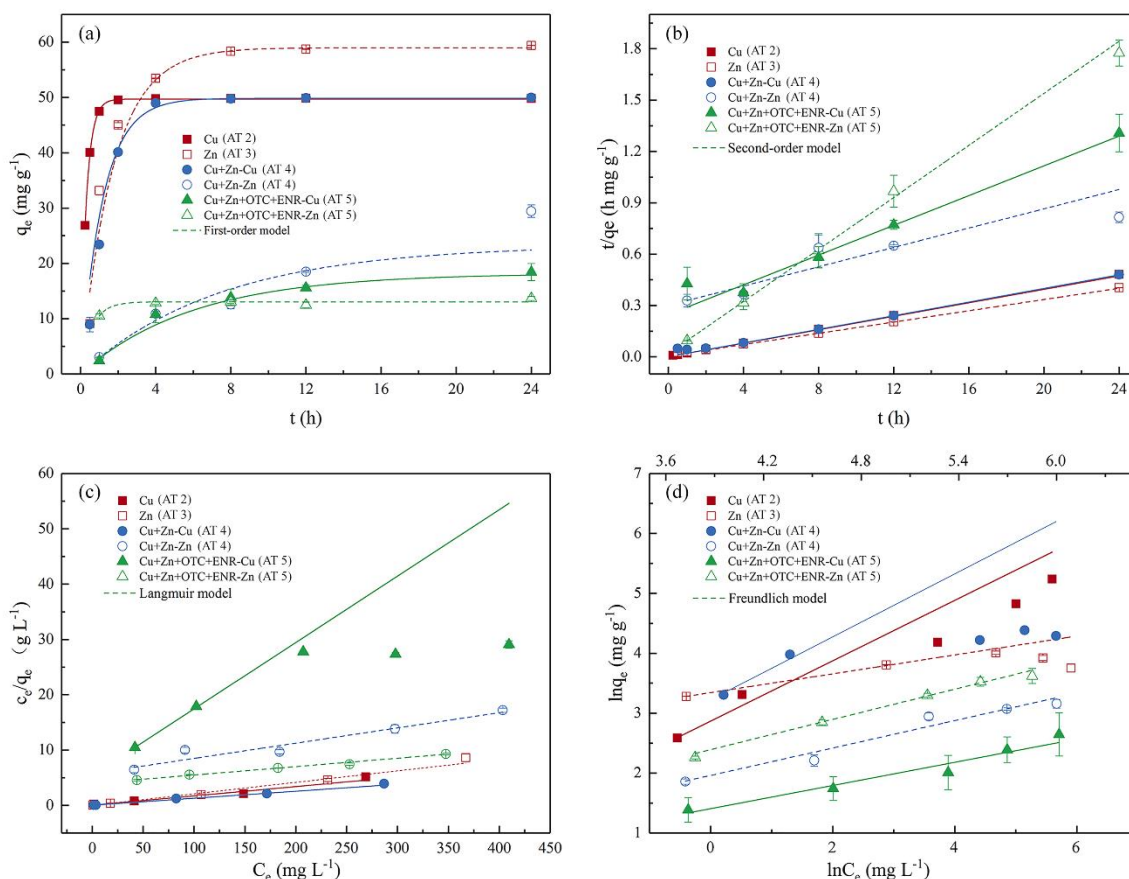


Fig. 2 Adsorption isotherms of Cu and Zn by SMSB with an adsorbent dosage of 2 g L⁻¹: (a) First-order kinetic model, (b) Second-order kinetic model, (c) Langmuir model, and (d) Freundlich model

Table 2 Kinetic parameters of Cu and Zn adsorption on SMSB

Heavy metal	First-pseudo order			Second-pseudo order		
	$q_e /$ ($\text{mg} \cdot \text{g}^{-1}$)	$k_1 /$ (h^{-1})	R^2	$q_e /$ ($\text{mg} \cdot \text{g}^{-1}$)	$k_2 /$ ($\text{g} \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$)	R^2
Cu	49.717	3.185	0.995	50.865	0.267	0.999
Zn	58.988	0.580	0.950	60.827	0.045	0.999

Cu (Cu+Zn)	49.858	0.847	0.910	50.226	0.248	0.9998
Zn (Cu+Zn)	23.328	0.134	0.990	35.423	0.003	0.878
Cu (Cu+Zn+OTC+ENR)	18.212	0.167	0.990	23.004	0.008	0.956
Zn (Cu+Zn+OTC+ENR)	13.038	1.633	0.853	13.156	0.300	0.998

Table 3 Langmuir and Freundlich adsorption isotherm parameters of Cu and Zn adsorption on SMSB

Heavy metal	Langmuir			Freundlich		
	$q_m /$ ($\text{mg} \cdot \text{g}^{-1}$)	$K_L /$ ($\text{L} \cdot \text{mg}^{-1}$)	R^2	$K_f /$ ($\text{mg}^{1-n} \cdot \text{g}^{-1} \cdot \text{L}^{-n}$)	$1/n$	R^2
Cu	59.137	0.810	0.971	17.585	0.504	0.919
Zn	48.170	1.821	0.993	28.285	0.158	0.990
Cu (Cu+Zn)	79.051	0.467	0.992	25.152	0.525	0.854
Zn (Cu+Zn)	36.337	0.005	0.911	0.669	0.610	0.987
Cu (Cu+Zn+OTC+ENR)	8.331	0.022	0.989	0.553	0.516	0.953
Zn (Cu+Zn+OTC+ENR)	65.833	0.004	0.994	0.809	0.672	0.989

3.2.2 Antibiotic adsorption

Figure 3 demonstrates that the adsorption of OTC and ENR was better fitted by the pseudo-second-order model ($R^2 > 0.904$) than the pseudo-first-order model (Table 4, $R^2 > 0.491$). The maximum adsorption capacities for OTC and ENR in each treatment reached within 4 h. It was observed that k_1 of OTC in AT 5 was the largest with a value of 5.425 h^{-1} , suggesting that the adsorption of OTC in AT 5 could more quickly reach equilibrium compared to other treatments.

The adsorption of Cu and Zn with and without the presence of OTC and ENR fitted well to the Langmuir model (except Zn in AT 3, Fig. 3). The Langmuir model, indicative of monolayer adsorption, was also more suitable for describing the adsorption of OTC

318 and ENR on SMSB when these antibiotics were present individually (Table 5). Once
319 Cu and Zn were added to the system, multiple-layer adsorption occurred as reflected by
320 the Freundlich model.

321 The adsorption capacity of OTC in the OTC+ENR solution (AT 8) by SMSB was
322 similar to that in the OTC-only solution (AT 6), suggesting that the presence of ENR
323 could not influence the absorption capability of SMSB for OTC. The BET result
324 indicated that VA adsorption decreased the surface area of SMSB (Table 6). The surface
325 area of SMSB decreased to $76.92 \text{ m}^2 \text{ g}^{-1}$ after adsorbing Cu (AT 1) and $82.10 \text{ m}^2 \text{ g}^{-1}$
326 after adsorbing Zn (AT 2). However, the BET surface area dropped to $9.80 \text{ m}^2 \text{ g}^{-1}$ after
327 absorbing Cu, Zn, OTC, and ENR simultaneously (AT 5), and from 105.32 (AT 1) to
328 $18.30 \text{ m}^2 \text{ g}^{-1}$ when SMSB was used to adsorb OTC and ENR simultaneously (AT 8).
329 These changes implied that the presence of OTC and ENR influenced the adsorption
330 behaviors of Cu and Zn.

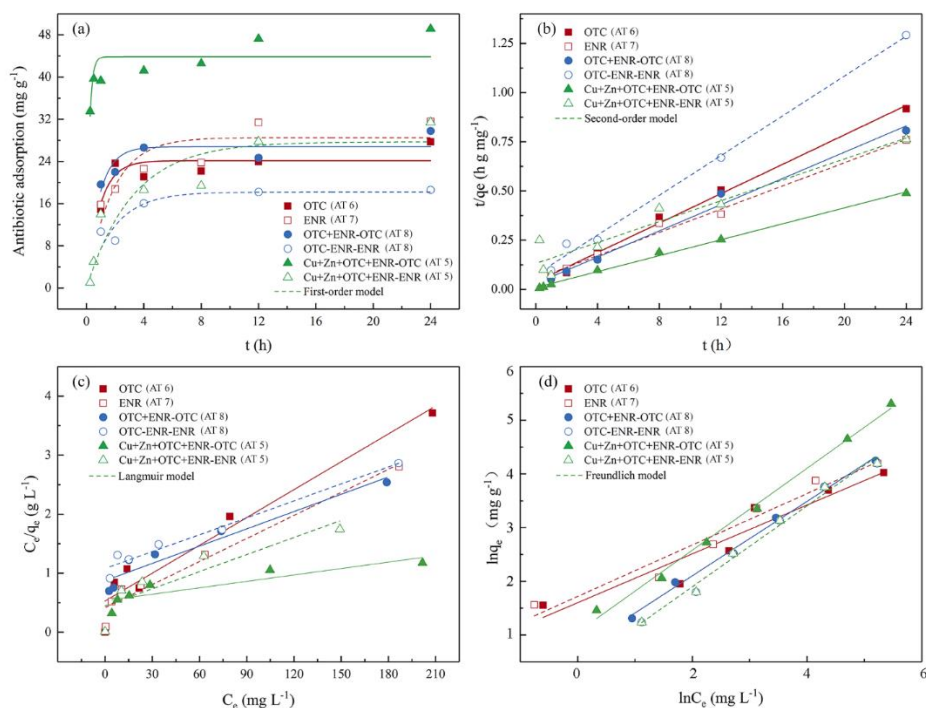


Fig. 3 Adsorption isotherms of OTC and ENR on SMSB with an adsorbent dosage of 2 g L⁻¹: (a) First-order kinetic model, (b) Second-order kinetic model, (c) Langmuir model, and (d) Freundlich model

Table 4 Kinetic parameters of OTC and ENR adsorption on SMSB

Veterinary antibiotic	Pseudo-first order			Pseudo-second order		
	q _e / (mg·g ⁻¹)	k ₁ / (h ⁻¹)	R ²	q _e / (mg·g ⁻¹)	k ₂ / (g·mg ⁻¹ ·h ⁻¹)	R ²
OTC	24.152	1.085	0.579	26.731	0.038	0.994
ENR	28.535	0.567	0.650	33.841	0.017	0.988
OTC (OTC+ENR)	26.785	1.150	0.584	29.967	0.037	0.987
ENR (OTC+ENR)	18.197	0.523	0.678	19.794	0.035	0.993
OTC (Cu+Zn+OTC+ENR)	43.859	5.425	0.491	49.407	0.044	0.997
ENR (Cu+Zn+OTC+ENR)	27.725	0.310	0.842	37.651	0.005	0.904

Table 5 Langmuir and Freundlich adsorption isotherm parameters of OTC and ENR adsorption on SMSB

Veterinary antibiotic	Langmuir			Freundlich		
	$q_m /$ ($\text{mg}\cdot\text{g}^{-1}$)	$K_L /$ ($\text{L}\cdot\text{mg}^{-1}$)	R^2	$K_F /$ ($\text{mg}^{1-n}\cdot\text{g}^{-1}\cdot\text{L}^{-n}$)	$1/n$	R^2
OTC	63.573	0.030	0.928	4.958	0.455	0.882
ENR	76.805	0.031	0.958	5.573	0.479	0.948
OTC (OTC+ENR)	102.459	0.011	0.920	2.046	0.693	0.993
ENR (OTC+ENR)	104.603	0.009	0.962	1.476	0.754	0.987
OTC (Cu+Zn+OTC+ENR)	276.243	0.007	0.740	2.866	0.763	0.994
ENR (Cu+Zn+OTC+ENR)	103.627	0.022	0.746	3.401	0.646	0.997

338 **Table 6** BET surface area of the SMSB (0.2%wt) after 6 h treatment

Group	Concentration (mg L^{-1})	BET ($\text{m}^2 \text{g}^{-1}$)
Adsorption test 1' (AT 1')	0	105.32
AT 2'	100 for Cu	76.92
AT 3'	100 for Cu	82.10
AT 4'	100 for both Cu and Zn	79.88
AT 5'	100 for Cu, Zn, OTC, and ENR	9.80
AT 6'	100 for OTC	26.64
AT 7'	100 for ENR	54.73
AT 8'	100 for both OTC and ENR	18.30

339 3.3 FTIR, XRD and XPS analyses of adsorption

340 Figure 4 illustrates FTIR spectra of SMSB and HHS1 before and after absorbing the
341 contaminants. After the adsorption of HMs and VAs onto SMSB, the stretching
342 vibrations of $-\text{OH}$ and $-\text{C}-\text{O}-\text{C}$ at approximately 3415 cm^{-1} and 1059 cm^{-1} exhibited
343 both red- and blue-shifts (Fig. 4 (a)). These shifts suggested the formation of H-bonds
344 between SMSB and the single contaminants, as well as coordination between these
345 groups and Cu and Zn. The FTIR spectra also showed peaks around 1617 cm^{-1} ,
346 corresponding to $-\text{C}=\text{C}$ or $-\text{C}=\text{O}$ vibrations, which influenced the variations of $-\text{COOH}$.
347 A new peak at about 599.63 cm^{-1} assigned to $\text{Cu}-\text{O}$ significantly increased after the

in deionized water

X-ray diffraction analysis was performed to explore the crystal structure variations of SMSB before and after adsorption (Fig. 5), and XPS was used to identify the chemical elements and speciation on the surface of SMSB (Fig. 6). The XRD data indicated significant changes in the crystalline structure of SMSB after exposure to Cu and Zn. Before adsorption, SMSB had several diffraction peaks, corresponding to silica (SiO_2) and calcium carbonate (CaCO_3), suggesting the presence of these compounds within the pores or on the surfaces of SMSB. New substances with great crystal structure, such as $\text{CuP}_4\text{O}_{11}$ and $\text{Zn}_4(\text{CO}_3)(\text{OH})_6\cdot\text{H}_2\text{O}$ were formed after the adsorption of Cu and Zn (Fig. 5 (a)). Oxytetracycline and ENR are mainly composed of carbon, hydrogen, oxygen, and nitrogen. As such, no new crystal structure formed on the surface of SMSB (Fig. 5 (b)).

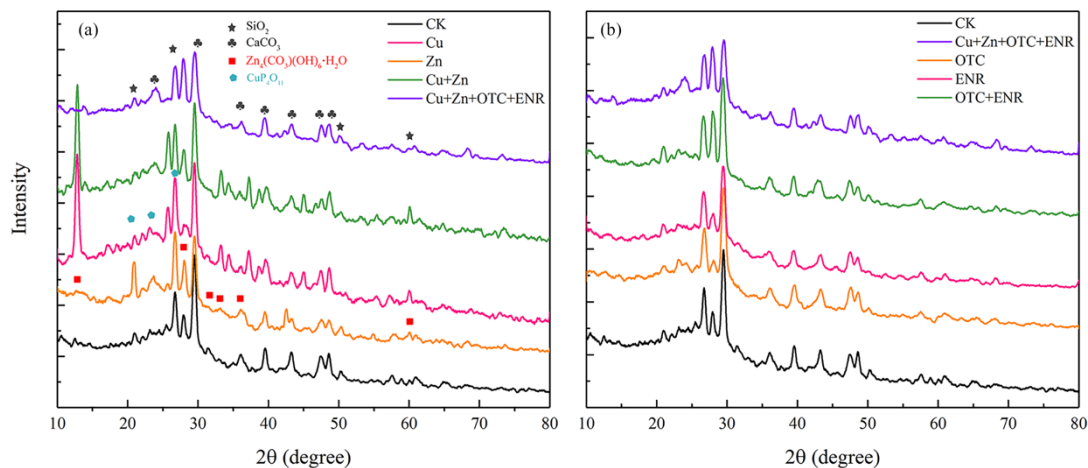


Fig. 5 XRD patterns of SMSB before and after HM and VA adsorption: (a) HMs and (b) VAs

As for XPS, the peaks belonged to Zn2p, Cu2p, C1s, O1s, N1s, and F1s (Fig. 6). The

peaks of Cu2p and Zn2p were observed to be modified by the Cu and Zn solutions, which proved that Cu and Zn successfully adsorbed onto the surface of the SMSB (Fig. 6). N1s and F1s discovered inferred that OTC and ENR could adsorbed on SMSB surface as well (Fig. 7). Although the intensities of Cu2p and Zn2p increased after absorbing Cu and Zn, Zn2p exhibited weak intensity in solution containing Cu, Zn, OTC, and ENR simultaneously. These results were consistent with the first-order model (Fig. 2 (a)). As for Cu2p, the characteristic peaks of the Cu 2p_{3/2} and Cu 2p_{1/2} were around 933.31 eV (49.7%) and 954.40 eV (12.4%) respectively, which was not present before adsorption. The peak of 963.10 eV was assigned to shake-up satellites and was only found in AT 4, AT 6, and AT 7.

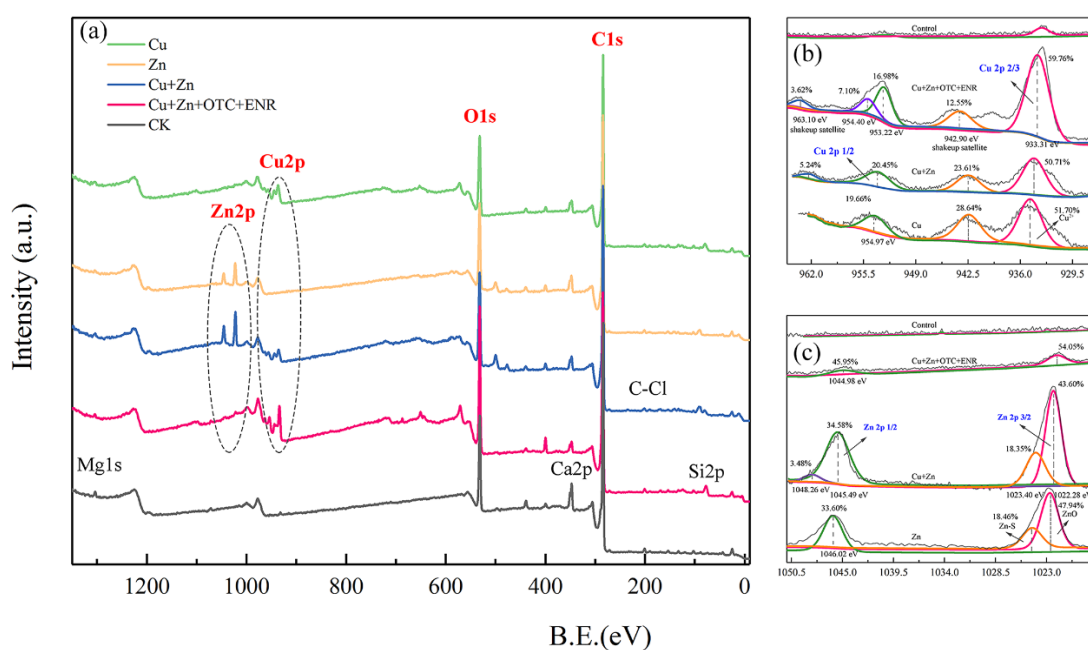


Fig. 6 XPS of the SMSB before and after HM adsorption: (a) wide-scan XPS spectrum of SMSB, (b) Cu2p and (c) Zn2p of the SMSB surface

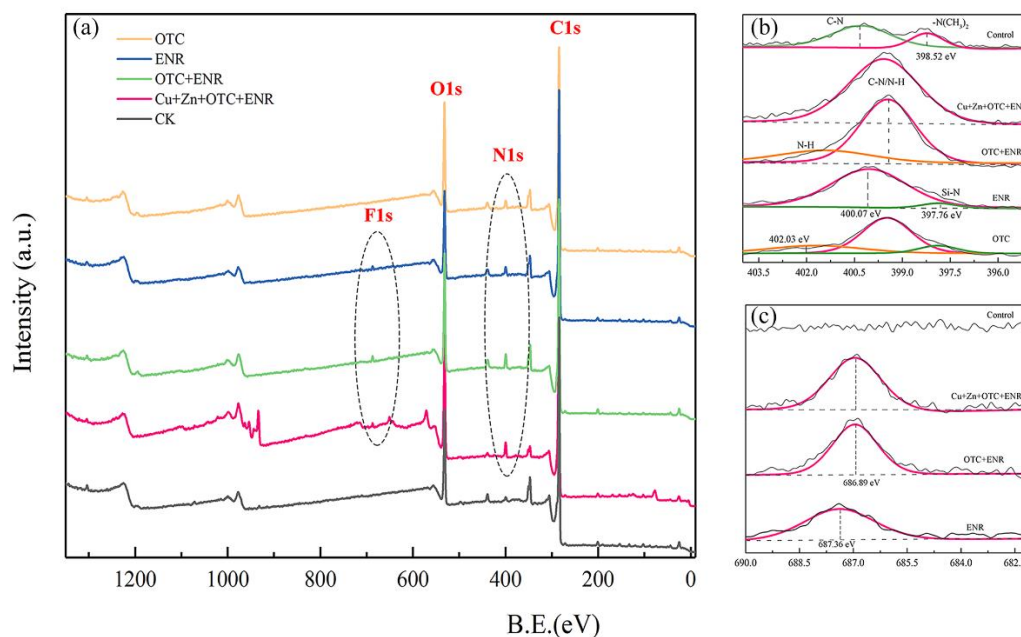


Fig. 7 N1s and F1s bonding states of SMSB before and after treating VAs as determined by XPS, respectively: (a) wide-scan XPS spectrum of SMSB, (b) N1s, and (c) F1s of the SMSB surface

Peaks of C1s and O1s were seen in Fig. 8. The relative content of C–C bond in CK was 59.9%, and that of –C–O–C bonds were 30.6%, and the percentage of O–C=O or inorganic –C–O (CO_3^{2-}) was lower than 10%. The molar ratio of C–C bond ratio after absorbing Cu, Zn, OTC, and ENR was 4.3% higher than that before adsorption, while the ratios of –C–O–C and –O–C=O bonds reduced. After the co-adsorption of OTC, ENR, Zn, and Cu, in comparison with single OTC and ENR adsorption, the relative ratio of the –C–O–C for OTC decreased from 47.4% to 23.8%, and for ENR the relative ratio decreased from 25.8% to 23.8%. That said, oxygen-containing functional groups were occupied by HMs and VAs gradually.

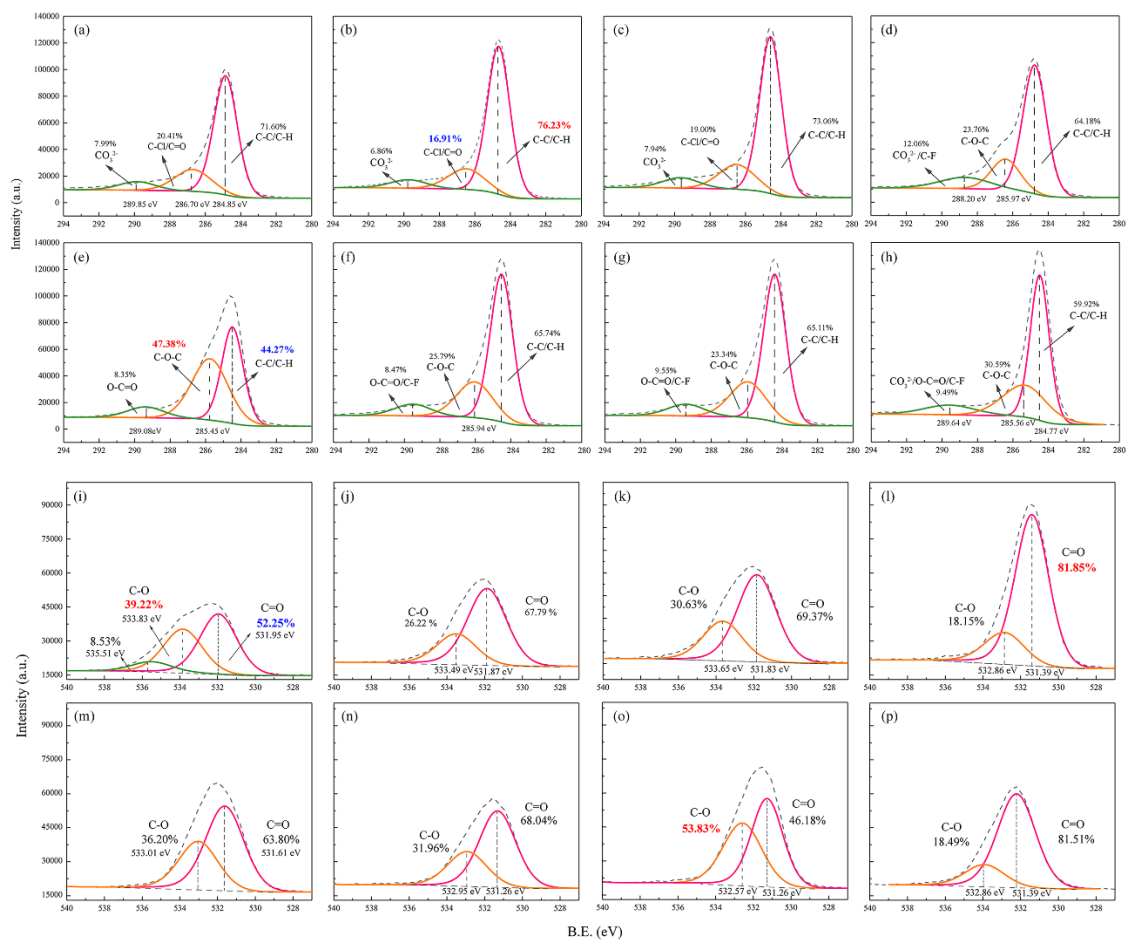


Fig. 8 C1s and O1s bonding states and their relative percentages (%) on the surface of SMSB after treating HMs and VAs as determined by XPS, respectively

Fig. 8 (a)-(h) showed C1s bonding states of SMSB before and after HM and VA adsorption: (a) Cu, (b) Zn, (c) Cu+Zn, (d) Cu+Zn+OTC+ENR, (e) OTC, (f) ENR, (g) OTC+ENR, and (h) control. Fig. 8 (i)-(p) showed O1s bonding state before and after HMs and VAs treatments: (i) Cu, (j) Zn, (k) Cu+Zn, (l) Cu+Zn+OTC+ENR, (m) OTC, (n) ENR, (o) OTC+ENR, and (p) control.

4 Discussion

4.1 Influence of co-contaminants on adsorption dynamics

The adsorption of VAs increased with the increasing contact time due to the readiness

412 of adsorption sites for interactions with the adsorbate molecules attaining the maximum
413 adsorption at 240 min (Meghani et al., 2023). The kinetic rate constant (k_2) of OTC
414 increased in the Cu+Zn+OTC+ENR solution, indicating that the addition of Cu and Zn
415 accelerated the stabilization processes of OTC and ENR. The adsorption kinetics were
416 best described by the pseudo-second-order model, suggesting that chemisorption is a
417 dominant mechanism governing the adsorption process (Meghani et al., 2023). Chang
418 et al. (2018) pointed that the pseudo-second-order model was based on the conception
419 that the rate-limiting step may involve valence forces by sharing or exchanging
420 electrons between the adsorbent and adsorbate. According to this assumption, the
421 adsorption rate limiting step of OTC and ENR by HHS1 could be connected to
422 chemisorption, which likely occurred via surface complexation reactions at a specific
423 adsorption site (Song et al., 2018). The pseudo-second-order kinetic model indicated a
424 strong interaction between the adsorbent and adsorbates, driven by the functional
425 groups on the surface of the adsorbents (Laddha et al., 2023). This strong surface
426 interaction is responsible for the higher adsorption capacity and removal efficiency
427 observed for OTC and ENR. After the adsorption sites were occupied by OTC and ENR,
428 the adsorption rate of Cu became low and the adsorption process gradually stabilized.
429 Under these conditions, Cu and Zn adsorption was affected. A similar observation was
430 reported by Guo et al. (2019). Furthermore, the adsorption data were well-described by
431 the Freundlich isotherm model, suggesting that the adsorption of OTC and ENR onto
432 the adsorbent occurs via multilayer adsorption with heterogeneous binding sites. This

finding indicates the potential for strong interactions, including chemisorption, between the VA molecules and the adsorbent surface, which likely contributes to the effective contaminant removal observed.

4.2 Characterization of adsorption mechanisms of HMs and VAs using FTIR, XRD, and XPS analysis

It has been reported that Zn had low affinity with functional groups (e.g. -OH and -COOH) in biochar (Yang et al., 2021), suggesting that SMSB preferably stabilized Cu in Cu+Zn solution. Adsorption of Cu and Zn was achieved by occupying adsorption sites on the SMSB. Thus, stabilization of Cu was inhibited in the presence of Zn (Figs. 1-2). The addition of OTC and ENR also slowed down the adsorption process of Cu. These phenomena were related to two mechanisms. On one hand, the coexistence of Cu and Zn can accelerate the adsorption of OTC and ENR through the “bridging” role (Yao et al., 2020). On the other hand, the presence of OTC and ENR can slow down the adsorption of Cu and Zn by hindering the collision between HMs and SMSB. The competition effect was enhanced, and the adsorption capacity and enhancement were decreased (Wang et al., 2020).

The complexation capacity of carboxyl functional groups for HMs is higher than that of π - π^* bonds (Gao et al., 2020). Carboxyl functional groups (1617 cm^{-1}) on the surface of SMSB were observed (Fig. 4). HMs were thus prone to being stabilized in this study. The peak of stretching vibration of -OH shifted (blue shift) after adsorption with Cu, Zn, OTC, and ENR. It was also noted that red shifts occurred with a peak of 1436 cm^{-1}

¹, which suggested that coordination effects increased average masses of vibration systems of –OH and –C=O, and (metal–O)–H and C=(O–metal) formed. In contrast, the elastic coefficient of –C–O–C became larger once the oxygen atom was coordinated with Cu and Zn, leading to an increase of wavenumber of –C–O–C and blue shifts (Yao et al., 2020). Clear changes confirmed that interactions between the oxygen-containing functional groups of the SMSB/HHS1 and HMs/VA molecules occurred. Moreover, the 1616 cm⁻¹ adsorption band generated blue shift, which could be interpreted as π - π interactions between Cu/Zn/OTC/ENR and SMSB (Gao et al., 2020). The π -bonds (C=C) in biochar can facilitate the interaction between the adsorbent and adsorbate molecules via electrostatic and π - π interactions enabling efficient chemisorption of the target contaminants on the biochar surface (Fu et al., 2021; Wang et al., 2021). Copper and Zn had stronger affinity to biochar (via coordination) than OTC and ENR (via π - π stacking and H-bonding) (Li et al., 2017). Under these conditions, Cu and Zn, instead of OTC and ENR, were preferentially oriented towards and fixed onto the surface of SMSB. The conjugate ability reduced, which gave an explanation for the fact that pollutants aforementioned were absorbed on SMSB and HHS1 steadily.

As magnesium and calcium were added as the nutrients for *Lentinus edodes*, and silicon was bonded to C and O elements, Mg2p, Ca2p, and Si2p were found on the surface of SMSB (Fig. 6). Co-presence of SiO₂ and CaCO₃ in raw SMSB was helpful in improving the adsorption of HMs (Sakhiya et al., 2021). Available Cu decreased after reacting with calcium by ion exchange, and CuP₄O₁₁ and Zn₄CO₃(OH)₆H₂O, which were not

475 discovered before adsorption, verified the occurrence of stabilization of Cu and Zn
476 directly. This suggests that phosphorous and carbonate substances on the SMSB
477 precipitated with Cu and Zn in solution directly. When only Cu was absorbed on the
478 surface of SMSB, the percentages of Cu 2p_{3/2} and Cu 2p_{1/2} changed to 51.70%
479 (935.59 eV) and 19.66% (954.97 eV). In terms of binary contaminants of heavy metal-
480 antibiotic, the percentages of Cu 2p_{3/2} and Cu 2p_{1/2} varied to 59.76% (933.7 eV) and
481 16.98% (953.61 eV), respectively after the addition of OTC, ENR, and Zn in solution
482 (Fig. 6 (b)). More importantly, strong coordination species were confirmed to shift to
483 high bond energy when organic molecules coexisted with Cu. A new shakeup satellite
484 appeared at about 942.90 eV, which was representative of a new coordination state of
485 Cu-antibiotics (Ling et al., 2013). Abundant oxygen-containing functional groups on
486 the surface of the biochar were able to react with Cu to form organic-bound Cu, thus
487 increasing oxidizable fractions (Su et al., 2016; Zhang et al., 2020). Likewise, a new
488 satellite band at about 1023 eV was found in Zn2p spectra as well (Fig. 6 (c)), which
489 meant that Zn might form complex with OTC or ENR (Huang et al., 2017). Under these
490 conditions, Cu and Zn could be stabilized. For C1s, the relative –C–O content decreased
491 as the –C–C/C=C increased, indicating the C–O functional groups were occupied after
492 contaminant adsorption and Cu²⁺ was adsorbed on the SMSB by surface complexation
493 (Fig. 8). The surface complexation between Cu²⁺ and oxygen-containing functional
494 groups played a role in the removal of Cu²⁺ (Meghani et al., 2023). A previous study
495 suggested that some of the oxygen-containing functional groups (–C=O, C–O, and –

OH) on biochar could combine with Cu(II), and OTC/ENR-Cu complexes might be formed (Li et al., 2020b). Due to the presence of Cu and Zn, areas of -C=O and O-C=O also showed obvious decrease in this study. Based on O1s analysis, a decrease of -C=O indicated that the HM and VA adsorption on SMSB were attached to the -C=O groups. Similar loss of -C=O groups was also mentioned by OTC pathways. Wang et al. (2019b) also noted that oxygen-containing functional groups in biochar adsorbed HMs and OTC via hydrogen bonding and π - π interaction. These results inferred that the amounts of oxygen-containing functional groups on the surface of SMSB played essential roles in absorbing Cu, Zn, OTC, and ENR in the soil. Nevertheless, the oxygen functional groups decreased, resulting in a reduction of the available adsorption sites on SMSB as the adsorption process progressed. Subsequently, the adsorption capacity diminished and eventually stabilized (Fig. 2).

4.3 Contributions of adsorption and degradation on VA removal

The interplay between OTC, ENR, Cu, and Zn in water treatments utilizing SMSB reveals intriguing insights into the removal mechanisms of antibiotics. Notably, the co-presence of these elements and compounds significantly enhances the removal efficiency of OTC on SMSB surfaces, as evidenced by the reduction of OTC concentration from 100 mg L^{-1} to 2.72 mg L^{-1} (Table 7). This phenomenon suggests a synergistic effect that facilitates OTC adsorption, underlining the potential of SMSB in treating antibiotic-contaminated waters.

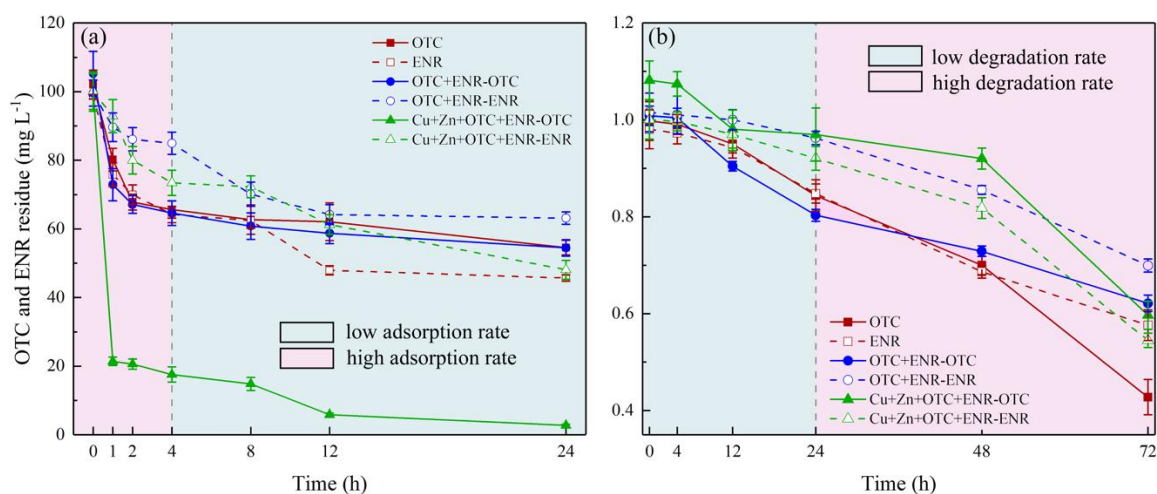
The observed adsorption rates of OTC and ENR are noteworthy, with averages of 9.63

mg h⁻¹ and 6.18 mg h⁻¹, respectively, within the initial 4 hours (Fig. 9). This rapid adsorption indicates that SMSB acts almost instantaneously, a characteristic beneficial for immediate reduction of antibiotic levels in aquatic environments. Furthermore, the degradation rates of OTC and ENR increase significantly after 24 hours, indicating a shift in the dominant removal mechanism. This shift points towards the microbial action of HHS1, which starts to degrade the antibiotics more extensively, using them as sources of carbon and nitrogen. Specifically, HHS1 was able to reduce OTC concentrations from 997.50 µg L⁻¹ to 427.76 µg L⁻¹, showing its potential in biodegradation processes. The sequential removal dynamics, where adsorption plays a crucial role in the initial stages followed by pronounced biodegradation, suggest a comprehensive approach to VA removal from aquatic systems. The initial findings, including the substantial decrease in OTC and ENR concentrations, align with those shown in Fig. 2, confirming the effectiveness of the combined adsorption and biodegradation strategy. This integrated approach not only highlights the efficiency of SMSB in adsorbing antibiotics but also showcases the critical role of biodegradation by HHS1 in the longer-term treatment of contaminated water, offering a promising avenue for enhancing water treatment methodologies.

538 Table 7 OTC and ENR residues (mg L^{-1}) in wastewater after treated by SMSB and

539 HHS1

Treatment		SMSB				
Time	OTC	ENR	OTC	ENR	OTC	ENR
(h)			(OTC+ENR)	(OTC+ENR)	(Cu+Zn+OTC+ENR)	(Cu+Zn+OTC+ENR)
0	100.00	100.00	100.00	100.00	100.00	100.00
1	80.35	76.01	73.11	89.65	21.40	92.86
2	68.94	70.05	67.27	86.31	20.60	80.00
4	65.71	64.87	64.76	85.09	17.56	73.43
8	62.53	62.81	60.97	70.33	14.84	72.29
12	62.26	48.06	58.91	64.20	5.84	61.26
24	54.46	45.83	54.74	63.20	2.72	48.09
Treatment		HHS1				
Time	OTC	ENR	OTC	ENR	OTC	ENR
(h)			(OTC+ENR)	(OTC+ENR)	(Cu+Zn+OTC+ENR)	(Cu+Zn+OTC+ENR)
0	997.48	980.50	1008.43	1015.40	1081.92	1000.48
4	990.82	972.38	1004.24	1010.28	1074.29	996.84
12	950.33	943.25	904.37	1000.38	980.79	970.38
24	843.89	848.01	803.09	962.71	969.93	920.61
48	699.61	686.39	788.879	855.50	920.19	817.98
72	427.76	576.19	621.19	699.25	596.66	545.00



540

541 **Fig. 9** OTC and ENR residues treated by (A) SMSB absorption and (B) HHS1

542 degradation

To assess the practical applicability of this biochar-microbial system, our laboratory findings were compared with studies conducted in the real-world aquatic environments. Zhang et al. (2023) prepared a novel magnetized biochar via hydrothermal carbonization and pyrolysis activation of starch-rich rice waste. Results showed that 2 g L⁻¹ of the biochar showed 96.52% removal of OTC in contaminated water. Enaime et al. (2020) found that addition of biochar increased 24% removal efficiency of Zn in storm water effluent. These results indicate that the SMSB-HHS1 composite has the promising potential for HM-VA water treatment applications.

Overall, the SMSB-HHS1 composite effectively removes HMs and VAs from contaminated water through a combination of chemisorption and biodegradation mechanisms. The -OH and -C-O-C groups in the SMSB form hydrogen bonds and coordinate with Cu and Zn, leading to the formation of stable complexes. HHS1 enhances VA removal through biodegradation, as evidenced by changes in functional group vibrations, including the disappearance of -C-O peaks and the appearance of -C=O peaks. The SMSB provides a high surface area and reactive sites for initial adsorption of HMs and VAs, while HHS1 subsequently degrades VAs, improving their removal efficiencies. This integrated system offers an effective and sustainable solution for environmental remediation, highlighting its potential for practical applications.

5 Conclusion

A novel integrated strategy using SMSB and HHS1 was developed for the concurrent removal of Cu, Zn, OTC, and ENR from water. The SMSB-HHS1 composite stabilized

29.86% of Cu and 49.75% of Zn, and removed 97.95% of OTC and 59.35% of ENR through a synergistic mechanism of chemisorption and biodegradation. The presence of Cu and Zn significantly enhanced the adsorption of OTC and ENR onto the SMSB. Conversely, the presence of OTC and ENR appeared to decelerate the adsorption of Cu. The –OH and –C–O–C groups on SMSB were identified as key active sites for binding HMs and VAs, with Cu and Zn coordinating with SMSB to form stable compounds such as $\text{CuP}_4\text{O}_{11}$ and $\text{Zn}_4\text{CO}_3(\text{OH})_6\text{H}_2\text{O}$, thereby stabilizing these HMs. The composite's high surface area and reactive sites facilitated the initial adsorption of HMs and VAs, with subsequent degradation by HHS1 improving overall VA removal efficiencies. This study demonstrates the promising potential of integrating SMSB adsorption with HHS1 biodegradation as a sustainable and effective approach for remediating HM-VA contamination in aquatic environments.

Statements and Declarations

- Ethics approval and consent to participate

Not applicable

- Consent for publication

Written and signed consent to publish the case was obtained from the patient.

- Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

- Competing interests

585 The authors declare that they have no competing interests.

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590 ● Author contributions

591 All authors contributed to the study conception and design. Original and revised
592 draft preparation, material preparation, data collection and analysis were performed
593 by Xiaorong Zhang. Original and revised drafts were reviewed by Richard A.
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