# Intensive removal of PAHs in constructed wetland filled with copper biochar

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## ARTICLE INFO

## ABSTRACT

In this study, biochar-loading copper ions (Cu-BC), a novel composite for removing phenanthrene very efficiently from water, was prepared using the impregnation method. The performance of constructed wetlands (CWs) with these modified and original biochar as substrates was analyzed. CW with Cu-BC removed a large amount of phenanthrene (94.09 ± 3.02%). According to the surface characteristics analysis, Cu-BC can promote the removal of pollutants via complex absorption, hydrophobic adsorption, increasing the Lewis Pair and electrostatic attraction. Furthermore the higher nitrate removal rate in the treated system (91.11 ± 1.17%) was observed to have higher levels of bacterial metabolic diversity and denitrifier types. The phenanthrene accumulated in plants with this treatment system was enhanced by the role of copper in photosynthesis. It is able to boost the plant extraction of organic matter.

## 1. Introduction

Polyaromatic hydrocarbons (PAHs), consisting of two or more fused benzene rings in their aromatic structures, represent a kind of important organic contaminant. With high toxicity and carcinogenic properties, nearly sixteen PAHs compounds have been identified as primary hazardous chemicals by the Environmental Protection Agency (EPA) in the United States (Abdel-Shafy and Mansour, 2016; Bamforth and Singleton, 2005; Soltani et al., 2015). Recently, increasing amounts of PAHs in the environment are evident, mainly due to the anthropogenic processes such as incomplete burning of fossil fuels domestic heaters, fossil-fuelled power plants and vehicle exhaust fumes (Groh et al., 2019). These PAHs coming from atmospheric deposition, industrial discharge and urban runoff can potentially enter a treatment plant, which threatens people's health and water security (Douben, 2003; Ravindra et al., 2008; Zhang et al., 2004). It has also been widely reported that PAHs pollution in rivers and lakes poses a serious threat to the ecosystem and environment (Meng et al., 2019; Sverdrup et al., 2002). However, it is very difficult to remove PAHs from an aquatic ecosystem based on the properties of low solubility and bioavailability and the high hydrophobicity. And this creates huge challenges for sewage treatment. (Kang et al., 2019; Xu et al., 2006).

Constructed wetlands (CWs) are a type of promising artificial wastewater treatment system which can remove pollutants by physical, chemical and biological actions (Bezza and Chirwa, 2017). Due to the advantages of easy operation and maintenance, low cost, and good purification capacity, CWs are widely used to remove common pollutants such as chemical oxygen demand (COD), nitrogen and phosphorus (Ávila et al., 2015). However, only a few studies have examined the treatment performance of CWs for PAHs. As an important component of CWs, substrates can repair and capture the pollutants when wastewater flows through CWs, and provide various habitats for microbes (Fountoulakis et al., 2009). PAHs in artificial wastewater tend to be set by substrates due to high hydrophobicity (Makkar and Rockne, 2003). It can be rapidly intercepted by a substrate in the solid liquid interphase and then be absorbed or degraded by plants and microbes in CWs (Yang et al., 2018b). Based on this reason it is extremely important to enhance the adsorption content and ability of substrate.

Among numerous wetland substrates, biochar with the advantages of excellent structure properties and large surface area is increasingly used

as an environmental remediation material and has garnered more scientific attention (Shi et al., 2017). Produced by converting organic materials in an oxygen-deficient atmosphere, biochar has a strong affinity for apolar organic contaminants, such as PAHs. (Al-Wabel et al., 2013). It has been reported that biochar can effectively adsorb and immobilize PAHs by multiple processes and sorption mechanisms. Aqueous organic contaminants were remediated mainly by partitioning or adsorption and electrostatic interactions between organic contaminants and biochar (Verheijen et al., 2004). Of the numerous modification technologies now in use, metal ions as catalysts could provide a larger specific surface (Ahmad et al., 2014; Shukla et al., 2010a, 2010b). Metal ions on the biochar surface could serve as electron donors, and PAHs with benzene rings could accept electrons. These metal ions and PAHs could form π-complexation, which is able to enhance adsorption ability and selectivity (Moreno-Piraján et al., 2010). As a kind of non-polar compound, PAHs tend to access hydrophobic sites on biochar surfaces in the absence of H-bonding between water and O-containing functional groups (Ahmad et al., 2014). The loading of metal ions on the surface of the biochar could reduce H-bonding and weaken the electronegativity of biochar, consequently reducing the electrostatic repulsion between PAHs and biochar (Houben et al., 2013; Luo and Tu, 2018). The usage of biochar modified by metal ions in a CW not only promotes adsorption content and the capacity of the substrate, it also stimulates the activity of plants and microbes. Because copper nitrate is the best catalyst in the catalytic oxidation of dyeing and printing industry wastewater, copper was chosen as the active element for deposition on the biochar support (Oliveira et al., 2017).

In this study, a novel cost-effective Cu-BC composite was synthesized via immobilizing copper ions on the surface of *Arundo donax*-derived biochar, and a CW system was built containing substrates of Cu-BC to remove phenanthrene in wastewater. The main objectives of the present study are to: 1) evaluate the adsorption characteristics of Cu-BC composite; 2) evaluate the performance of a CW and its main components (substrate, plants, and microbes) on the removal of phenanthrene and common pollutants (COD, $NO_3$–N, $NH_4$–N, TP); and 3) study the roles of prepared biochar in pollutants' removal and promotion of the activities of plants and microbes.

## 2. Materials and methods

### 2.1. Preparation and characterization of biochar

The *Arundo donax* used to prepare biochar in the present study was collected from *Nansi Lake* wetland, Shandong province. The precursors were first sheared and smashed to 60 mesh before use. Then, they were mixed with $H_3PO_4$ (85 wt%) solution at an impregnation ratio of 2.0 (g $H_3PO_4$/precursor). After soaking for 24 h in the room temperature (25 °C), the materials were heated to 500 °C in a muffle furnace with a heating rate of 10 °C min$^{-1}$ under pure $N_2$ conditions for 1 h. Following this the materials were washed by distilled water after cooling to room temperature. Finally, the products were dried at 105 °C for 12 h and sieved through a 200 mesh using standard sieves (Model Φ200) for later characterization. The carbon product emerging from the above method is referred to as BC.

The copper biochar was prepared by the impregnation method. Firstly, 100 g activated biochar was submerged in 400 mL 2% $Cu(NO_3)_2$ solution for 24 h. Then, the samples were put into a drying oven at 120 °C for 2 h without the supernatant. Next, the materials were heated to 300 °C in a muffle furnace for 4 h. The material was cooled to room temperature, then washed and dried at 120 °C for 2 h in a vacuum oven. After that, the products were activated in a muffle furnace under 300 °C for 4 h. The end result was designated Cu-BC.

The Brunauer-Emmett-Teller surface area ($S_{BET}$) was identified by $N_2$ adsorption/desorption isotherm using a surface area analyzer (Quanta Chrome Corporation, USA) at 77 K. Surface morphology was determined using scanning electron microscope (SEM, Hitachi SU-8010, Japan). X-

ray photoelectron spectrometer (XPS) served to analyze the surface binding state and elemental speciation of the biochar (BC, Cu-BC before and after phenanthrene adsorption). All data were calibrated to the C1s peak of 283.9 eV. Fourier transform infrared (FTIR) was employed to explore the adsorption mechanism.

### 2.2. CW configuration and chemicals

Four vertical flow constructed wetlands (VFCWs) divided into two parallel groups with different biochar were located at Shandong University in Jinan, China (116.1°E, 36.5°N). The VFCWs reactors consisted of organic glass (75 cm in height, 25 cm in diameter). Group BC (systems I and II) was built with 40% biochar as produced above and 60% washed silica sand (v/v); group Cu-BC (systems III and IV) was added with 40% Cu-BC biochar as produced above and 60% washed silica sand (v/v), respectively. The schematic diagram of the experimental set-up is shown in Fig. 1. Substrate depth was 50 cm and water depth was retained at 15 cm above the substrate surface. Served as one type of typical plants that exist in wetland environments, the widely grown *Iris pseudacorus* was planted because it is inexpensive and effective in terms of survival and the planting density was 6 plants/unit. The phenanthrene (>99.5% purity) used in this experiment were purchased from Aladdin Reagent (Shanghai, China). The standard substitute (p-Terphenyl-d14 and 2-fluorobiphenyl) used for pretreatment recovery rate calculation of PAHs extraction was obtained from ANPEL Laboratory Technologies (Shanghai) Inc. Phenanthrene-d10 injected into the sample extracts were also obtained from ANPEL Laboratory Technologies (Shanghai) Inc.

### 2.3. Experimental procedure

The VFCWs units were seeded with activated sludge collected from *Everbright Water Wastewater Treatment Plant* (located in Jinan, Shandong 117.0°E, 36.7°N, China) for one month before it was formally operated. After that, each microcosm was fed with synthetic wastewater. The sample collection and analysis of experiment period were proceeded until the concentrations of pollutants in effluent tended to be stable for at least two weeks. The artificial wastewater consisted of glucose, $(NH_4)_2SO_4$, $KNO_3$, $KH_2PO_4$, $MgSO_4$, $CaCl_2$, $FeSO_4 \cdot 7H_2O$ and phenanthrene (0.013 mg/L). The hydraulic retention time (HRT) lasted 3 days during the experiment period. After each experimental cycle the wastewater was drained from the outlet at the bottom. Synthetic wastewater was then immediately added. Due to the high hydrophobicity and volatility of phenanthrene, it was dissolved into dichloromethane (chromatographically purity) at a high working concentration. The phenanthrene solution was stored in brown bottles with Teflon-lined lids at 4 °C. Before use, concentrated phenanthrene working solution was added to the influent water. To ensure the conditions remained consistent, the characteristics of the influent were periodically monitored during the experimental period.

### 2.4. Sample collection and analysis

The effluent samples were taken from the outlets every 3 days. After collection, the water samples were filtered through a 0.45 μm cellulose acetate membrane by syringe. The chemical oxygen demand (COD), ammonia nitrogen ($NH_4^+$-N), nitrate nitrogen ($NO_3^-$-N), and the total phosphorus (TP) of the effluents were measured by standard methods (Federation and Association, 2005). To test the concentration of phenanthrene, water samples after filtration were treated via the solid-phase extraction method. A solid phase extractor equipped with C18 extraction membrane was used to treat approximately 1000 mL of effluent samples with standard substitute. After the organic compounds were retained in the membrane, dichloromethane was utilized to elute and collect phenanthrene. The samples were added with internal standards (phenanthrene-d10) and were concentrated into 1 mL by parallel
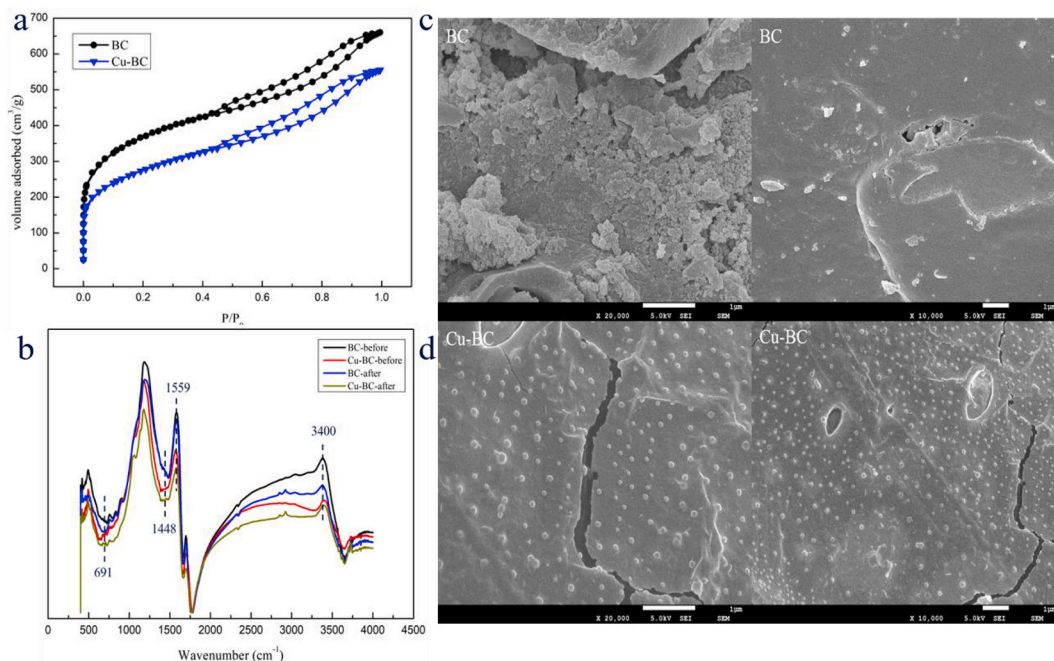
**Fig. 1.** N$_2$ adsorption and desorption isotherms of BC and Cu-BC (a), FTIR analysis of BCs before and after phenanthrene adsorption (b) and SEM images (c) of BC and (d) Cu-BC.

concentrator before being measured (Eichelberger et al., 1988).

Based on the previous study, 50% of DO was consumed in the upper 10 cm of the vertical subsurface flow constructed wetlands. Most oxygen was consumed by degraded organic matter above 40 cm, and was mostly consumed by nitrification reaction in the lower part (below 40 cm) (Liu et al., 2016). Five parallel samples of substrates at different depths (0–10 cm, 10–40 cm, 40–50 cm) below the water-sediment interface were collected and mixed for measurement purposes. The PAH GC/MS analysis method for substrate was executed according to EPA 8270C (Spasojević et al., 2015). Phenanthrene in substrate was extracted by accelerated solvent extraction with dichloromethane-methanol mixture (1:1). The plant samples' aboveground parts (stems) and underground parts (roots) were collected, respectively. Subsequent to this, they were dried and ground into small pieces. Ultrasound extraction technique was used to extracate the organic substrates of plants. Then, the mixtures were shaken for 1 h, and centrifuged at 1600 rpm for 10 min.

Next, the water phase was removed, and the silica gel column which contained neutral chlorine dioxide, silica gel, and Na$_2$SO$_4$ served to purify the dichloromethane layer (He and Chi, 2016). Before being measured, the organic solvents added with phenanthrene-d10 were concentrated into 1 mL. All the samples with phenanthrene were measured by a gas chromatography/mass spectrometry (GC/MS) system. The PAHs GC/MS analytical procedure was conducted according to EPA 525.2 on a Shimadzu (Japan) QP2020 GC/MS with a Rxi 5Sil MS column, working in SIM mode. The pretreatment recovery rates for water analyses ranged from 65% to 105%, 70%–95% for the substrate, and 50%–96% for the plant.

### 2.5. Microbial analysis

In order to study the roles of copper biochar in wetland microbes, extraction of community DNA from media samples collected at three substrate layers (0–10 cm, 10–40 cm, 40–50 cm) of VFCWs was done using MOBIO PowerSand™ DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). To obtain the desired microbial community for each substrate, Illumina high-throughput sequencing was done. To ensure high-quality sequence reads, any sequences shorter than 250 base pairs (bp) in length and with a quality score lower than 30 were removed from the pyrosequencing-derived data sets. The UCLUST method was used to choose the representative sequences for all operational taxonomic units (OTUs) with a similarity threshold value of 97%. A number of Alpha diversity measures were evaluated by Mothur software including the abundance-based coverage estimator (ACE), terminal richness estimation (Chao1), the Shannon index and Simpson index.

## 3. Results and discussion

### 3.1. Characterization of biochar

In order to investigate the textural property of prepared biochar and the copper modified biochar, isotherms of adsorption–desorption of N$_2$, FTIR analysis and SEM images were determined and shown in Fig. 1.

Based on the SEM images and N$_2$ adsorption and desorption isotherms illustrated in Fig. 1, it could be observed that the pore diameters of biochar produced by *Arundo donax* precursor were micro-mesoporous structures, which was consistent with the results of pore size distributions (Fig. S1). The surface morphology characterized by the SEM images and textural parameters (Table S1) of the carbon materials revealed that the loading of copper ions decreased the BET surface area and porous volume mainly by covering the micropores. However, the proportion of mesoporous pores rose. This phenomenon is documented in other studies (Hu et al., 1999; Moreno-Piraján et al., 2010; Shukla et al., 2010b). However, biochar in this study was successfully produced and even the modified biochar had a much higher surface area and porous volume.

Added to this, FTIR analysis of biochar before and after phenanthrene adsorption was performed. FTIR spectra (400–4000 cm$^{-1}$) of the BCs are presented in Fig. 1b. The spectra reveal the peaks at 1559, 3400–3000 and 1625-1430 cm$^{-1}$, respectively, corresponding to C=O (carboxyl, lactone groups or conjugate carbonyl group), C–H and C=C of the aromatic nucleus. These three characteristic absorption regions were characteristic regions of the aromatic nucleus (Abbas et al., 2017; Min et al., 2004). Once treated with phenanthrene adsorption, biochar modified by copper recorded the strongest stretching vibration compared to the original carbon, which is attributed to the higher content of phenanthrene loading in Cu-BC. It could be concluded that

biochar modified by copper ions was successfully prepared and it had excellent potential for adsorbing pollutants.

### 3.2. Pollutants removal performance in different CW units

Based on the well prepared Cu-BC, laboratory-scale CW systems with modified substrate were built. Throughout the experimental stage, the influent COD, $NH_4^+$-N, $NO_3^-$-N and phenanthrene were approximately $104.8 \pm 7.5$ mg/L, $7.7 \pm 0.58$ mg/L, $10.0 \pm 3.7\%$ mg/L, and $12.8 \pm 1.7$ mg/L, respectively. The concentrations of contaminants in wastewater (COD, $NH_4^+$-N, $NO_3^-$-N and phenanthrene) are depicted in Fig. 2. During the experiment the concentrations of these pollutants reached a steady state in the effluent. An excellent COD removal performance in the Cu-BC system was observed with the higher average removal rate of $75.33 \pm 1.72\%$, while CW filled with BC only realized $51.15 \pm 1.27\%$ removal efficiency. Having an adequate carbon source supply is one of the most important conditions for denitrification and $NO_3^-$-N degradation (Ge et al., 2012). The average $NO_3^-$-N removal rates of Cu-BC and BC system were $91.11 \pm 1.17\%$ and $89 \pm 2.61\%$, respectively. In CW, COD and nitrate were mainly removed by the oxidation reaction with oxygen of air and water. Copper ions could act as a catalyst to improve the oxidation and conversion rates for this reaction (Hu et al., 1999). What is more, copper ions fixed in biochar could be kept in a dispersed but stable state to enhance the pollutants' surface concentration, which is favored by the catalytic reaction (Kumar et al., 2018). However, the greater consumption of nitrate contributed to the higher average concentration of ammonia in the effluent of the Cu-BC system. Another possible factor leading to the higher ammonia concentration was the inhibiting effect on nitrifying bacteria of trace copper ions (Ouyang et al., 2016).

Fig. 2d shows that the phenanthrene removal efficiency of Cu-BC was $94.09 \pm 3.02\%$, which was higher than the removal efficiency of BC ($89.83 \pm 2.55\%$). It could be concluded that the treated system filled with modified biochar could definitely improve the organic pollutant removal performance. The phenanthrene concentration of the output decreased dramatically over steady state. Also, phenanthrene amounts fixed by wetland substrate and plants were measured at the end of this study.
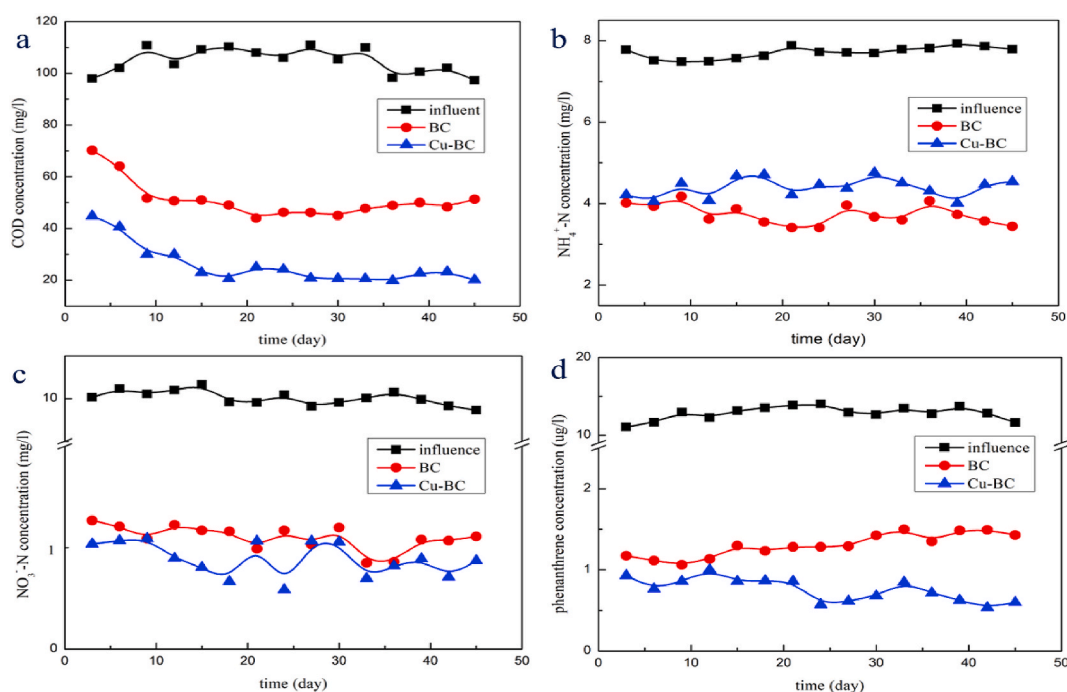
Fig. 3 shows phenanthrene accumulation in leaves and roots of plants and different depths of substrate. There was a slight increase in the total amount of phenanthrene immobilized on the substrates. It could be assumed that the role of modified biochar did not merely facilitate the adsorption intensity and capacity. More importantly, biochar could boost the following biological processes in CW. In the superficial layer of the substrate, the Cu-BC system had accumulated more phenanthrene than the BC system. Favorable surface properties of the modified biochar in the superficial layer could quickly intercept phenanthrene when the waste water flowed through. The higher concentration of organic matter could lead to a more efficient biological oxidation reaction. In the deep layer, nitrate could also be used as an electron acceptor for some PAHs-degraders except oxygen (Rockne and Strand, 1998).

Phenanthrene amounts in leaves and roots of plants in the treated system were 20.81% and 38.32%, respectively, these amounts being higher than in the untreated system. Plants play an important role in the removal of pollutants in the CW due to extraction and degradation during metabolism. For the treated system, copper could participate in photosynthesis and promote the growth of leaves, which could boost the plant extraction of organic matter (Mehta et al., 2006). In the substrate's superficial layer, the mutual effect between plant roots, biochar and microbes were most intense (Mani and Kumar, 2014). Root exudates released by plants could play a role in the degradation of PAHs by boosting the break of benzene. In the rhizosphere, root-associated enzymes and organic matter of biochar could increase the activity and density of root-associated microorganisms (Fismes et al., 2002). In addition, the amounts of pollutant collected in the leaves were 3.6 and 4.1 times more than that of roots in the treated system and untreated system, respectively. Intensive transpiration may be the main factor leading to the better fixing effects of pollutants in plants' leaves (Li et al., 2014).

### 3.3. Characterization of substrates microorganisms

Microbial communities in different depths of substrates in Cu-BC and BC system were measured. The Shannon diversity index, Chao and ACE species richness, and Simpson species diversity, at a cutoff level of 3%,
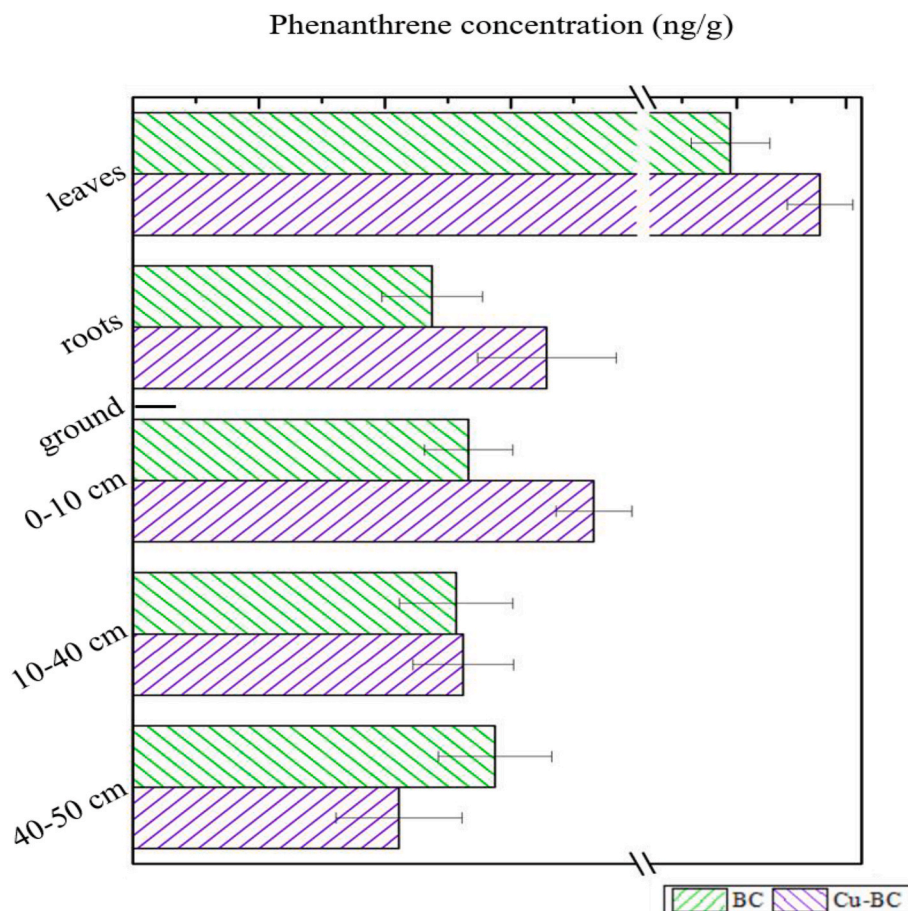


**Fig. 2.** Influent and effluent water quality of different VFCW systems, throughout the experiment period: (a) COD; (b) $NH_4^+$-N; (c) $NO_3^-$-N and (d) phenanthrene (during the aeration phase).

## Phenanthrene concentration (ng/g)



**Fig. 3.** Contents of phenanthrene in leaves and roots of plants and of substrate in different depth of CWs.

were also calculated for these samples, in order to investigate the abundance and diversity of the bacterial community for each group (Table S2). It could be concluded that in the shallow parts of substrates, species richness and diversity were the highest due to the affluent root exudates. The values of ACE and Chao 1 showed that the community richness of microbes in shallow parts of substrates was higher than in the other layers.

The different bacterial compositions of various depths of substrates at the phylum and class level were also observed, as shown in Fig. 4. At the phylum level [Fig. 4a], the main groups of the whole substrates in Cu-BC system were *Proteobacteria* (average 42.62%), *Bacteroidetes* (average 13.40%), *Chloroflexi* (average 12.93%), *Actinobacteria* (average 9.73%) and *Firmicutes* (average 5.09%). Specifically, the average relative abundance of the phylum *Proteobacteria*, *Bacteroidetes* and *Firmicutes* in the whole substrates of CW system with Cu-BC were about 11.22%, 36.18% and 20.05%, respectively. These numbers were higher than in a CW with BC. These three phyla were also crucial for the degradation of phenanthrene (Kanaly et al., 2000; Toledo et al., 2006). The bacterial community distribution at the class level indicated the occurrence of *Gammaproteobacteria*, *Anaerolinean*, *Deltaproteobacteria*, *Bacteroidia*, *Actinobacteria* and *Alphaproteobacteria* as shown in Fig. 4b. The increasing abundance of *Gammaproteobacteria*, *Anaerolinean*, *Deltaproteobacteria*, *Bacteroidia* was noted.

The distribution of the bacterial community indicated that the relative abundance of *Pseudomonas*, *Gordonia sp*, *Bacillus* and *Desulfovibrio* with a degradation capability for refractory organics was significantly increased. The relative abundance of *Gordonia sp*, *Pseudomonas* and *Bacillus* in the Cu-BC system were 563.7%, 20.31% and 11.69% higher than of BC system, respectively. The very large amounts of *Gordonia sp* could enhance the removal of PAHs in the treated system, due to the

favorable bioremediation of the environment contaminated by aromatic hydrocarbons (Franzetti et al., 2009). The genus *Bacillus* is known to be able to degrade aromatic compounds and to excrete enzyme catalase, and *Pseudomonas* could effectively degrade PAHs (Arun et al., 2008; Baran et al., 2004). It has been reported that the oxidation of PAHs with bacterial enzyme can be promoted by copper due to its role of mediator and catalyst (Zeng et al., 2016). Certified as typical phyla for the denitrification process, the greater abundance of *Bacteroidetes*, *Proteobacteria* and *Firmicutes* strains led to better nitrate removal rates in the treated system (Shen et al., 2013). As a kind of PAHs-degrader which could metabolize PAHs by utilizing nitrate as the electron acceptor, *Thiobacillus* was also be observed in both CW systems.

The amount of *Thiobacillus* in the treated system was 79.33% higher than that in the untreated system (Kuppusamy et al., 2016). It has been found that ammonia-oxidizing bacteria were susceptible to copper. The higher concentration of $NH_4^+$-N in Cu-BC system was caused by the inhibition of copper on ammonia oxidation (Ouyang et al., 2016). As a kind of ammonia-oxidizing bacteria, *Nitrosomonas* was found to decrease in the treated CW system. It could be concluded that compared to the slightly higher adsorption ability of modified substrates, the enhanced mutual effect between biochar, plant and microbes was the main factor for promoting the removal of phenanthrene.

### 3.4. Mechanism of phenanthrene removal in CW with modified adsorbent

#### 3.4.1. Phenanthrene adsorption mechanism on biochar

In order to further explore the single adsorption mechanism of biochar, the O1s, C1s and Cu2p XPS spectra before and after phenanthrene adsorption of BCs is demonstrated in Fig. 5. The new Cu2p peak appeared at 934.08 eV of modified biochar, indicating the existence of
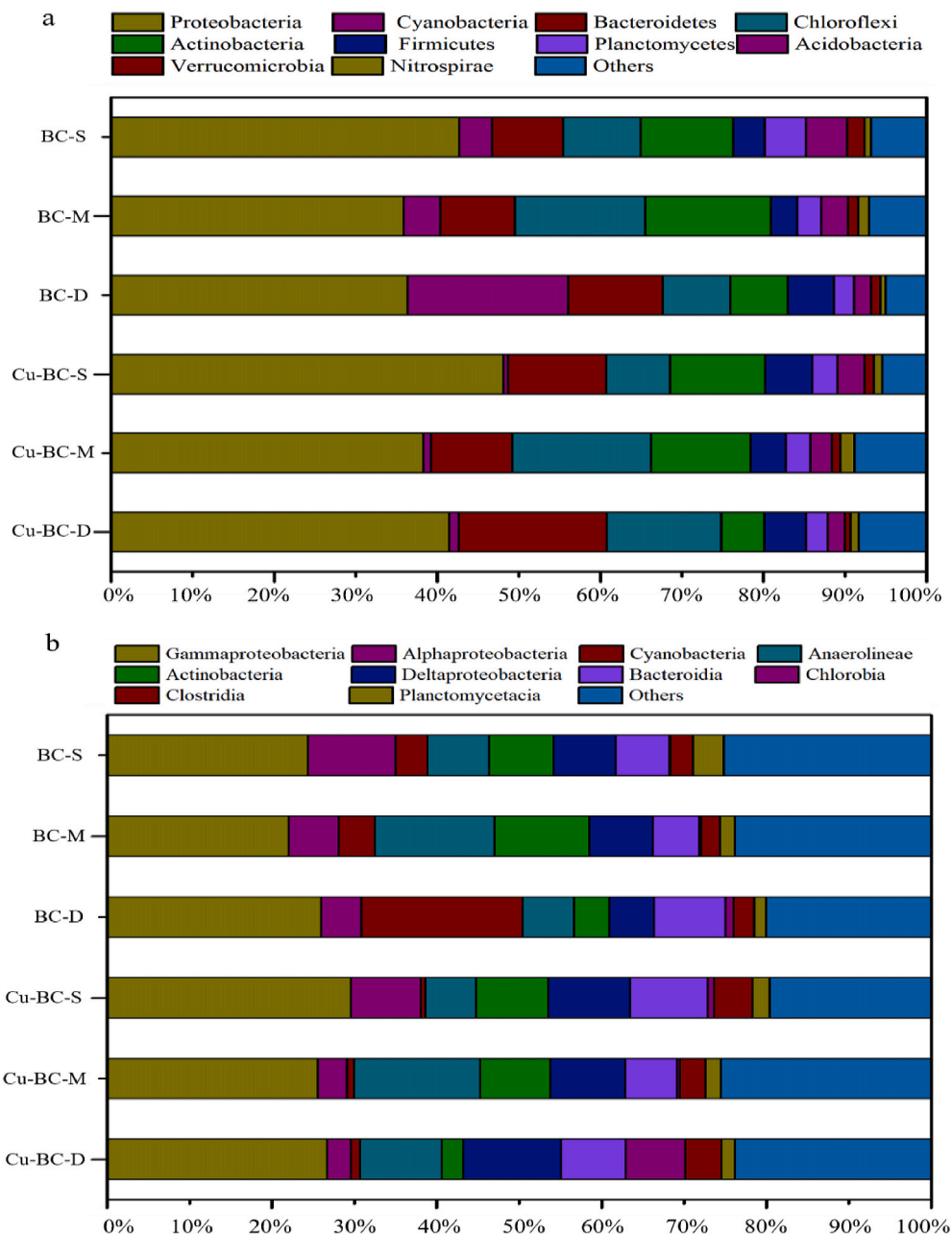
**Fig. 4.** Bacterial community composition of different depths of substrates at phylum level (a) and class level (b) as revealed by high-throughput sequencing analyses in CWs.

copper. The peaks corresponding to Cu2p1/2 and Cu2p3/2 were observed at 954.32 and 933.83 eV, respectively, which corresponded to the standard spectrum diagram of $Cu^{2+}$. Cu2p3/2 satellite peak which is characteristics of CuO was clearly observed at 944.33 eV (Fig. 5d) (Boruban and Esenturk, 2018). The peak at 533.58 eV in the O1s spectrum (Fig. 5b) was assigned to the $O^{2-}$ ion in CuO while those at 531.68, 535.30, and 537.28 eV corresponded to the O=C, O–C–O or C–OH and O=C–O bonds of the BC, respectively (Chen et al., 2009). The C 1s were classified as the following groups: graphitic carbon at 284.68 eV; hydroxyl or ether groups at 285.88 eV; and carboxyl groups at 288.98 eV (Huang et al., 2011). The increase of O=C groups and decrease of $O^{2-}$ ions and CuO may be caused by the combination of copper and phenanthrene. The $O^{2-}$ ion in CuO was released and this part may combine with carbon in the biochar surface. Thus, the XPS analysis indicated that copper ions participated in phenanthrene adsorption, which could

promote the ability of biochar to adsorb phenanthrene.

*3.4.2. Conceptual model for the removal of phenanthrene based on mutual impacts of different CW units*

Based on the results, there were significant correlations between functional microbial abundance, substrates and plants in the CWs with modified biochar. A conceptual model for the removal of phenanthrene using biochar amendment combined with copper is shown in Fig. 6. Organic matter was first intercepted by substrates when the wastewater flowed through the CW system. Enhanced phenanthrene removal was realized based on the better adsorption by modified biochar, and an acceleration of the biological reactions of plants and microbes.

Referring to substrate adsorption, a pollutant could be fixed by adsorption in the carbonized fraction, and then be partitioned in a non-carbonized fraction (Ahmad et al., 2014). The electron donor acceptor
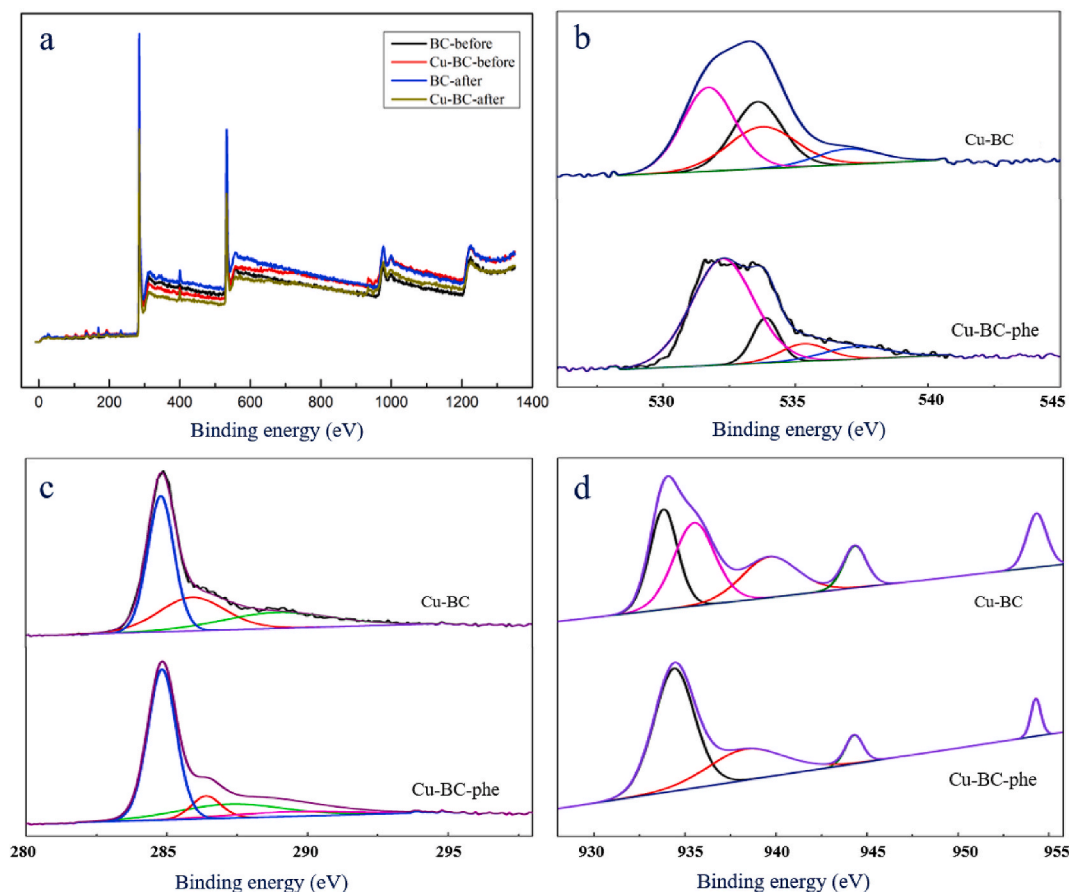
**Fig. 5.** XPS spectra for BC and Cu-BC before and after phenanthrene uptake: survey spectra (a), O1s (b), C1s (c) and Cu2p (d).

(EDA) process occurred between copper ions (π-electron acceptor) and π-electrons in phenanthrene (π-electron donors). One study identified that these EDA interactions were the key mechanisms for PAH sorption to biochar (Zhang et al., 2014). As a kind of non-polar compound, phenanthrene tends to access hydrophobic sites on biochar surfaces in the absence of H-bonding between water and O-containing functional groups (Ahmad et al., 2012). $Cu^{2+}$ can form an inner-sphere-type complexation, which drives a fraction of oxygen-containing groups to the interior while the hydrophobic sites are sent to the outside (Luo and Tu, 2018). This increase in hydrophobic adsorption by adding copper ions to the biochar surface constituted one sorption mechanism and led to a successful removal performance. In addition, electrostatic attraction/repulsion between organic contaminants and biochar is another possible adsorption mechanism (Ahmad et al., 2014). In brief, the application of copper modified biochar realized notable pollutants' removal in the CW by complex absorption, hydrophobic adsorption and electrostatic attraction.

In the CW environment, plants extracted phenanthrene during the process of metabolism. Copper could participate in photosynthesis and promote the growth of leaves, which could boost the plants' ability to extract organic matter. It follows that organic matter was mainly removed by the biological oxidation reaction with oxygen of air and water. Copper ions could act as a catalyst to improve the oxidation and conversion rates for this reaction (Hu et al., 1999). In the rhizosphere, root exudates released by plants could participate in the degradation of PAHs by boosting the break of benzene. For microbes, the oxidation of PAHs with bacterial enzyme can be promoted by copper due to its role of mediator and catalyst. It emerged that phenanthrene could be degraded by the anaerobic process with nitrate serving as the terminal electron receptor (Yang et al., 2018a). The mutual impact shared by plant roots, biochar and microbe were most intense in the superficial layer. In this context, root-associated enzymes and organic matter of biochar could increase the activity and density of root-associated microorganisms. Aerobic microbial degradation and photo-biodegradation were greatly boosted by copper.

## 4. Conclusions

Enhanced removal of phenanthrene and other kinds of pollutants was observed in this work by filling copper modified biochar into a CW. Phenanthrene could be effectively adsorbed by copper biochar based on the processes of complex absorption, hydrophobic adsorption, and electrostatic attraction. A larger concentration of organic matters in substrates accelerated the biological oxidation reaction. Distribution of the bacterial community indicated that the relative abundance of *Pseudomonas*, *Gordonia sp*, *Bacillus* and *Desulfovibrio* with a degradation capability for PAHs was significantly increased. Also, the biological accumulation of CW plants was improved by the participation of copper in photosynthesis. Finally, due to the higher levels of bacterial metabolic diversity and many types of denitrifiers, the treated system made possible a higher nitrate removal rate. However, with the operation of CW system, the substrate is prone to saturating by the gradual accumulation of suspended solid (SS), biological film, plant residue, etc. In consideration of the sustainable operation of CW, it is quite meaningful to evaluate the pollutants accumulation till saturation of CWs and find solutions to the problems of sustainable operation for the further study.

## Credit author statement

**Xiaotong Shen:** Conceptualization, Methodology, Writing-Original draft preparation. **Jian Zhang:** Supervision, Validation, Project administration. **Huijun Xie:** Conceptualization, Methodology, Writing-
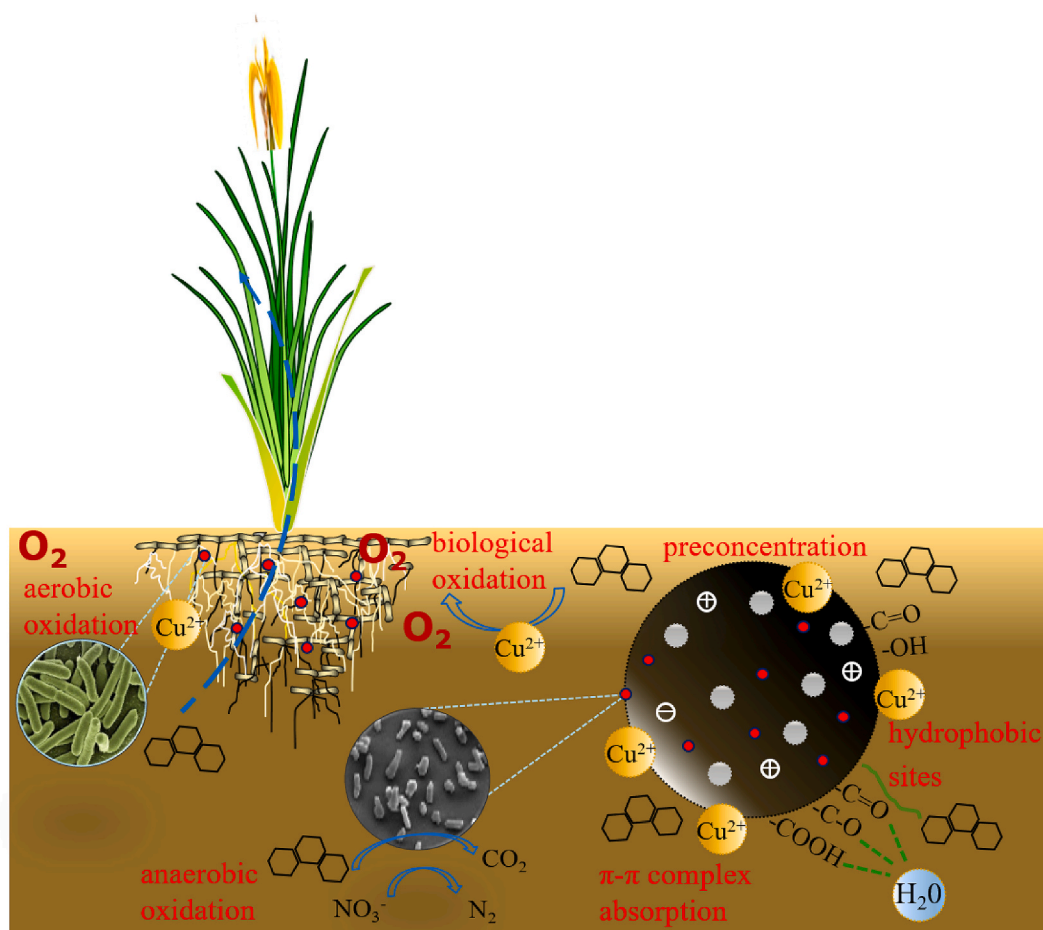
**Fig. 6.** Conceptual model of phenanthrene removal using biochar amendment combined with copper.

Reviewing and Editing. **Zhen Hu:** Conceptualization. **Shuang Liang:** Supervision, Project administration. **Huu Hao Ngo:** Writing - Review and Editing. **Wenshan Guo:** Writing - Review and Editing. **Xinhan Chen:** Methodology. **Jinlin Fan:** Visualization. **Congcong Zhao:** Visualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.111028.

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