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RECEIVED 07 December 2025

REVISED 12 January 2026

ACCEPTED 19 January 2026

PUBLISHED 11 March 2026

CITATION

Maldou C, El Janoussi Y, Al Salah DMM, Lahrouni M, Ouazzani N, Kurnaiwan TA, El Ghadraoui A, Elleuch A, Ammar A, Poté J and Aziz F (2026) Synergistic removal of various emergent pollutants in biochar-enhanced constructed wetlands: a pilot-scale nature-based solution for municipal wastewater treatment improvements.
Front. Environ. Chem. 7:1762724.
doi: 10.3389/fenvc.2026.1762724

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Synergistic removal of various emergent pollutants in biochar-enhanced constructed wetlands: a pilot-scale nature-based solution for municipal wastewater treatment improvements

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The primary drawback of reusing treated wastewater for irrigation is that most wastewater treatment plants (WWTPs) remain unable to remove most emerging pollutants detected in recent decades, necessitating a strategy to improve these WWTPs' performance. In this regard, the present study aims to evaluate the effectiveness of an enhanced pilot vertical-flow biochar-enriched constructed wetland for treating municipal wastewater pretreated with natural lagoons. Three key harmful micropollutants are addressed: heavy metals (trace elements), pharmaceutical residues, and antibiotic-resistant bacteria (ARB). The system utilizes a biochar layer to enhance treatment efficiency by increasing adsorption capacity and supporting beneficial microorganisms, in conjunction with *Phragmites australis* plants that facilitate oxygen transfer and nutrient uptake. To evaluate the treatment system's performance, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to measure heavy metal concentrations. A molecular approach using quantitative polymerase chain reaction (qPCR) was used to quantify antibiotic resistance genes (ARGs) in water samples before and after treatment, including β -lactamases TEM, CTX-M, and SHV. The performance obtained is remarkable for the well-known toxic metals, reducing their concentrations from $16.35 \pm 0.82 \mu\text{g L}^{-1}$ for arsenic, $0.0936 \pm 0.0047 \mu\text{g L}^{-1}$ for cadmium, and $0.6036 \pm 0.030 \mu\text{g L}^{-1}$ for lead to $2.75 \pm 0.14 \mu\text{g L}^{-1}$, $0.0014 \pm 0.000070 \mu\text{g L}^{-1}$, and $0.089 \pm 0.0045 \mu\text{g L}^{-1}$, respectively. Furthermore, the results showed a significantly reduced copy number of the 16S rRNA gene and of

the resistance-conferring genes. Pharmaceutical residues (doxycycline, ciprofloxacin, enoxacin, enrofloxacin, norfloxacin, ofloxacin, amoxicillin and ampicillin) were also effectively removed after treatment, with removal rates ranging from 63% to over 99% depending on the compound. For example, ibuprofen and ciprofloxacin were reduced by 96%–97% (from 7.310 to 238 ng L⁻¹ and from 2.300 to 83 ng L⁻¹, respectively). Even recalcitrant molecules such as carbamazepine and diclofenac have been reduced by more than 60%, confirming the high efficiency of the biochar-enriched artificial wetland system. These results highlight the considerable potential of this nature-based solution as a promising approach to addressing the global concern of enhancing WWTP removal of emergent pollutants. It therefore offers a sustainable, environmentally friendly alternative for wastewater treatment reuse in irrigation.

KEYWORDS

antibiotic-resistant bacteria, biochar, constructed wetland, heavy metals, pharmaceutical residues

1 Introduction

Over the past 30 years, environmental problems, particularly those related to chemical and biological water pollution, have become a major concern not only for society and public authorities but also for the entire industrial sector. All activities, whether domestic, agricultural, or industrial, generate wastewater or effluents containing undesirable pollutants that can harm the environment and human health. It is crucial to consider the environmental impact of the chosen technique, as some remediation methods can generate a decomposition product that is more harmful than the initial pollutant. Researchers and experts assert that there is no unique remediation solution applicable to all contaminants and sources of pollution; in fact, an effective strategy may involve the combined application of several techniques (Santos et al., 2021; Oscar Omondi and Caren Navalia, 2021). The challenge of treating emerging micropollutants in wastewater has received considerable attention in recent years, as conventional treatment processes often prove inadequate for their complete removal (Mustafa and Hayder, 2021; Thorslund et al., 2017). Heavy metals (trace elements), pharmaceutical residues and antibiotic resistance genes are categories of contaminants of particular concern due to their persistence, bioaccumulation potential and public health threats (Donde, 2017; Van Biervliet et al., 2020). Comprehensive reviews have highlighted the urgent need for innovative, sustainable, and cost-effective treatment technologies that can simultaneously treat multiple classes of micropollutants while remaining accessible to developing regions facing water scarcity challenges (Vymazal, 2022; Almuktar et al., 2018). Nature-based solutions, particularly built-up wetlands, have emerged as promising alternatives to conventional, energy-intensive treatment systems, offering both environmental and economic benefits (Atuga and Jembe, 2022).

Wetlands are among the most effective methods for decontaminating wastewater pollutants and are currently attracting the attention of environmentalists (Oscar Omondi and Caren Navalia, 2021). Pollutants are naturally removed from water resources by wetlands through various mechanisms, including biodegradation, sorption, phytostabilization, phytoextraction, and rhizofiltration. Constructed wetlands are designed to replicate these natural mechanisms in the removal and decomposition of pollutants

contained in wastewater (Almuktar et al., 2018; Brovelli et al., 2011). Engineering systems known as constructed wetlands are specifically designed to harness and maximize natural wastewater treatment processes. They significantly improve water quality while blending harmoniously into existing landscapes and ecosystems. These innovative systems have gradually gained popularity and recognition among environmental experts and communities for their deeply ecological and environmentally friendly nature. They also stand out for their exceptional ability to treat a wide range of wastewater sources, including urban effluents from cities, industrial discharges from manufacturing processes, and agricultural runoff and rainwater enriched with nutrients and loaded with various pollutants. Constructed wetlands are natural wastewater treatment systems that integrate physical, chemical, and biological processes into a single system, utilizing shallow water bodies with specific plant species anchored in them (Atuga and Jembe, 2022). These cutting-edge systems, developed and refined over decades, now offer a natural treatment technique that stands out for its modest construction costs, significant improvements in wastewater quality through a marked reduction in pollutant levels, and a straightforward, accessible method of operation and maintenance (Vymazal, 2022). Biochar (BC) is gaining increasing interest as a low-cost adsorbent, due to its rich surface functional groups and large surface area (El Barkaoui et al., 2023). BC produced by the pyrolysis of various raw materials, such as agricultural waste, is a carbon-rich material. Its low production cost is due to the low cost of the raw materials.

Numerous studies have highlighted the influence of pyrolysis conditions (temperature, duration, atmosphere and nature of the biomaterial) on the physicochemical properties of biochar. Pyrolysis temperature, in particular, plays a crucial role in determining the stability of carbon, porosity, and the presence of functional groups on the material's surface (Ahmad et al., 2014; Méndez et al., 2013; Cha et al., 2016). Previous research has shown that slow pyrolysis, performed at 400 °C–600 °C, results in a biochar with a good balance of yield, specific surface area and adsorption potential, while preserving the active functionalities necessary for interaction with organic and inorganic pollutants (Ahmad et al., 2014; Cha et al., 2016). In addition, biochar derived from lignocellulosic biomasses, such as olive pruning residues, has demonstrated effectiveness in water treatment processes and soil improvement, particularly due to

its high cation exchange capacity and long-term stability (Beesley et al., 2011; Inyang et al., 2012).

Despite several studies on the efficacy of biochar-enriched constructed wetlands for individual pollutant categories, comprehensive pilot-scale studies evaluating their synergistic performance against multiple classes of emerging contaminants, particularly heavy metals, pharmaceutical residues, and antibiotic resistance genes, remain limited, especially in contexts where treated water is intended for agricultural reuse. This lack of knowledge is particularly critical in Mediterranean and semi-arid regions, where water scarcity necessitates safe wastewater recycling practices that protect public health, environmental quality and safe agricultural reuse. Therefore, this study aims to evaluate the comprehensive treatment performance of a pilot-scale vertical-flow constructed wetland enriched with olive-derived biochar for the simultaneous removal of heavy metals (arsenic, cadmium, lead), pharmaceutical compounds, and antibiotic resistance genes from municipal wastewater intended for agricultural irrigation. The specific objectives are to: (1) characterize the physicochemical properties of locally produced olive biochar and evaluate its contribution to pollutant removal mechanisms; (2) quantify removal efficiencies for physicochemical parameters, trace metals, bacterial indicators, antibiotic resistance genes, and pharmaceutical residues over a 4-month operational period; and (3) assess the suitability of the treated effluent for agricultural reuse according to national and international water quality standards.

2 Materials and methods

2.1 Biochar generation

The biochar used in this study was produced from olive branch (*Olea europaea*) pruning residues by slow pyrolysis. The olive branches were collected locally, washed with distilled water to remove impurities, and air-dried for 7 days until the moisture content was less than 10%. The dried biomass was ground and sieved to obtain particles with a homogeneous size between 1 and 5 cm. The pyrolysis process was conducted in a pyrolysis furnace under a controlled atmosphere, with strict control over the oxygen supply. The pyrolysis temperature was set at 500 °C, which is considered optimal for producing a biochar with a favorable balance between thermal degradation and preservation of surface functional groups. After pyrolysis, the biochar was cooled under an inert nitrogen atmosphere to room temperature, then ground and sieved to a particle size of 2–5 mm for use in the constructed wetland system.

2.2 Biochar characterization

The mineral composition of the biochar was analyzed using X-ray fluorescence (XRF) with a portable XRF analyzer (Olympus NDT, Waltham, United States). The pH and electrical conductivity of the biochar were also measured (Lehman book). In addition, the morphology of the biochar was examined using scanning electron microscopy (SEM). The analysis was conducted at the Analysis and Characterization

Center of the Semailia Faculty of Sciences in Marrakech, utilizing a VEGA3 TESCAN instrument equipped with an EDAX detector for energy-dispersive spectroscopy (EDS). Fourier transform infrared spectroscopy (FTIR) was also used to identify the functional groups present in the biochar.

2.3 Pilot-scale constructed wetland description

The pilot-scale constructed wetland is built from a blue polyvinyl chloride cylindrical container with an estimated height of approximately 0.90 m, a volume of 200 L, and a surface area of 2.17 m² (Figure 1). It's a vertical-flow constructed wetland, meaning the water moves from the top to the bottom through several layers of material. The system features a multi-layered substrate configuration designed for optimal treatment performance. On the surface, a special wetland plant, *Phragmites australis*, was planted. This species is well-adapted to contaminated water and has a robust root system that facilitates the transfer of oxygen into the surrounding substrate. Just beneath the vegetation, a thick layer of small gravel ensures even distribution of the influent and supports plant root development. Next comes a sand layer, which acts as a physical filter by removing suspended solids and provides a large surface area for microbial growth, supporting biological treatment. Beneath the sand, a biochar layer is included to adsorb organic pollutants and foster the development of beneficial microbial communities. Below the biochar, a medium-gravel layer serves as a transition zone, preventing fine particles from migrating downward. Finally, the bottom of the system contains a large gravel layer that serves as the drainage zone, ensuring the smooth collection of the treated effluent. The selection of this specific layered configuration was based on treatment efficiency and local material availability. The variation in particle size creates distinct hydraulic retention zones that promote both physical and biological purification mechanisms. A drainage system is installed at the bottom of the unit to collect treated water. The system appears to be naturally ventilated, allowing oxygen diffusion and supporting aerobic treatment processes. The planted *Phragmites australis* enhances treatment by releasing oxygen in the rhizosphere, facilitating nutrient uptake, and providing a habitat for microorganisms essential for pollutant removal. The wetland operates under intermittent feeding conditions to maintain optimal oxygen levels and treatment efficiency throughout the operational cycle (Figure 1).

2.4 Description of study area

Sâada is a rural commune in the Marrakech prefecture, part of the Marrakech-Safi region (Figure 2). This commune is notable for its lack of a structured urban center. The rural commune of Sâada has three special infrastructure features. First, it has a modern separate sewerage system that effectively separates domestic wastewater from rainwater, thereby optimizing the management of different effluent types. Second, the entire municipal territory has a drinking water distribution system, ensuring a supply to all homes. Third, the municipality has wastewater treatment infrastructure,

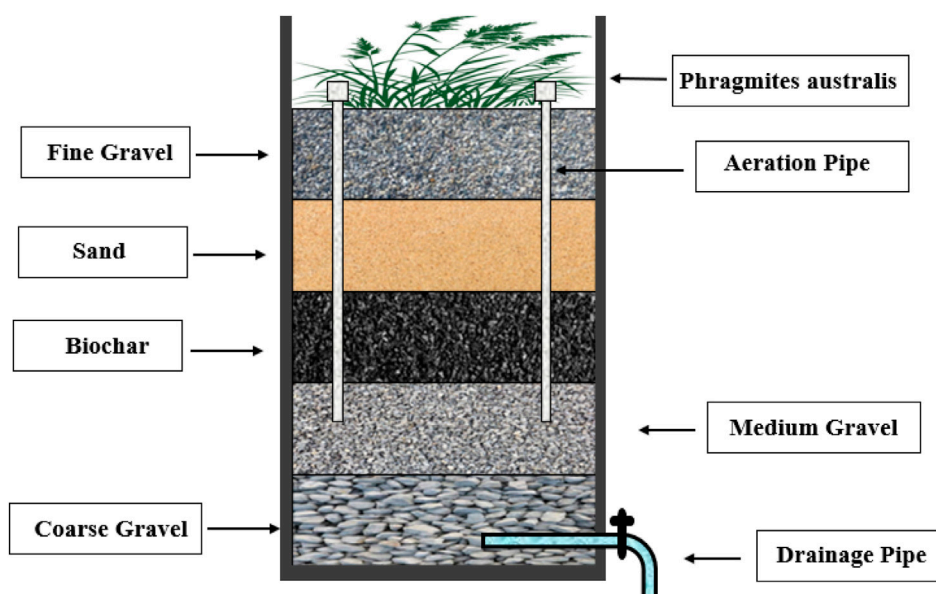


FIGURE 1
Pilot-scale constructed wetland.

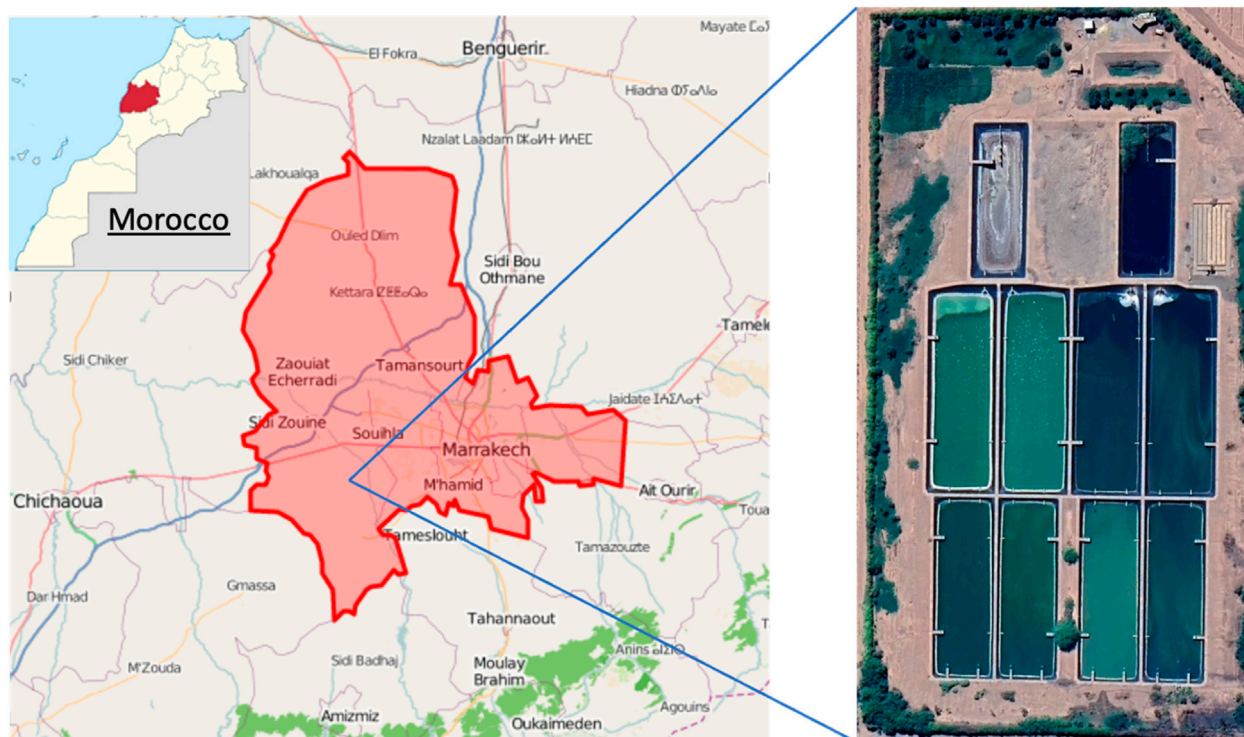


FIGURE 2
Sâada WWTP geographical location.

including a treatment plant located at a safe distance of 600 m from inhabited areas. This plant uses two complementary treatment technologies: natural lagooning and algal channels. In accordance with the principles of sustainable development, the plant's treated

water is reused for irrigation of the surrounding gardens, thereby contributing to the rational management of water resources.

Although officially considered a rural commune, Sâada gradually integrated into the urban dynamics of Marrakech

between 2004 and 2014, as the Marrakech metropolitan area expanded westward. This territorial evolution has been accompanied by remarkable population growth. The municipality had a total population of 39,071 in the 2004 census, and according to 2014 statistics, the population reached 67,085, an increase of more than 71% in a decade. This sustained population growth reflects the growing attractiveness of this peri-urban area (High Commission for Planning, 2004; High Commission for Planning, 2014).

2.5 Sampling

The municipal wastewater used in this study was collected weekly at the discharge point of the Sâada wastewater treatment plant, which employs secondary lagooning treatment. This treatment method produces relatively stable water quality, which is essential to maintaining test consistency. To evaluate the effectiveness of the biochar-enriched continuous-flow vertical planted filter (PS-VFCW) treatment system, wastewater samples were collected weekly at the inlet and outlet of the device. These samples were used to analyze physical and chemical parameters, including pH, conductivity, COD, BOD₅, nutrients, and ions, enabling monitoring of water quality over time. Additionally, samples for bacteriological analysis were collected every 2 weeks in sterile bottles, in accordance with current standards. To ensure the reliability of the results, all measurements were repeated three times, and some samples were stored at low temperatures for possible further analysis. All of these operations were carried out under strict storage and handling conditions in order to preserve the integrity of the samples until they were processed in the laboratory.

2.6 Pilot plant treatment performances

The pilot treatment system was continuously operated and monitored for 4 months to evaluate its medium-term performance under real-world conditions. During the entire experiment, the unit was supplied daily with pretreated municipal wastewater, ensuring a consistent hydraulic load representative of typical operational conditions. This regular feeding maintained optimal conditions for the growth and activity of the purifying biomass, while ensuring the proper functioning of the biochar-enriched vertical subsurface flow constructed wetland (PS-VFCW). The system's performance was assessed through weekly monitoring of physicochemical parameters and bimonthly analysis of bacteriological parameters, enabling a comprehensive evaluation of treatment efficiency throughout the study period.

2.7 Water physicochemical parameters

Water temperature, dissolved oxygen, pH, and turbidity are continuously measured using a Hanna Instruments HI98194 multiparameter meter (Hanna Instruments, Woonsocket, RI, United States). All measurements were performed in triplicate, and standard errors were <5% for ICP-MS analyses and <3% for spectrophotometric determinations. The various electrodes are calibrated regularly. Total suspended solids (TSS) are determined in accordance with AFNOR NF T90-105. After filtering a test sample

through microporous filters that have been dried and weighed beforehand, these filters are placed in an oven at 105 °C for 2 hours, then cooled in a desiccator. They are then weighed. The difference between wet and dry weight is used to evaluate suspended solids. Chemical oxygen demand (COD) is determined on raw and filtered samples (using filters with 0.45 µm pores) in accordance with AFNOR NF T90-101. COD is the oxidation of organic matter contained in the sample by an excess of potassium dichromate (K₂Cr₂O₇) in an acidic medium (H₂SO₄) and in the presence of silver sulfate (Ag₂SO₄) and mercury sulfate (HgSO₄). After 2 hours of mineralization at 150 °C, the optical density is read at 620 nm. The BOD₅ expresses the amount of oxygen (mg L⁻¹) required for the biological oxidation of biodegradable organic matter in 5 days at 20 °C and in darkness. It therefore represents the amount of biodegradable organic pollution generated by biomass respiration.

Total phosphorus (TP) is determined in accordance with standard AFNOR-NF-T90-023 (on a raw sample). It is measured after phosphorus mineralization in water in the presence of potassium persulfate, in an acidic medium, at 150 °C for 2 h. The various forms of phosphorus are thus converted into orthophosphates (PO₄³⁻), which are measured using the colorimetric method for orthophosphates described below. Orthophosphates (PO₄³⁻) are determined according to AFNOR NF T90-023 on a filtered sample using a colorimetric method. This method is based on the formation of a phosphomolybdic complex in a slightly acidic environment using ammonium molybdate and potassium antimony tartrate. Total nitrogen (total N) is determined according to AFNOR NF EN ISO 11905-1 on a sample by a peroxydisulfate oxidative digestion method. This method is based on converting all forms of nitrogen (free ammonia, ammonium, nitrites, nitrates, and organic nitrogen compounds) into nitrate under oxidative conditions, followed by determination by flow analysis, in which nitrate is reduced to nitrite.

2.8 Trace elements analysis by ICP-MS

Before conducting the analysis, 9.90 mL of the water samples was filtered through a 0.22-µm syringe filter and subsequently acidified with 100 µL of Suprapur® 65% HNO₃. The measurement of trace metals, specifically Arsenic, Cadmium, and Lead, in all samples was conducted using Inductively Coupled Plasma Mass Spectrometry (Agilent 7,700x series ICP-MS, Santa Clara, CA, United States). A collision/reaction cell (in Helium mode) and interference equations were employed to eliminate spectral interferences that could potentially skew the results. A multi-element standard solution (Merck IV, KGaA, Darmstadt, Germany) was used for calibration at various concentrations (0, 0.2, 1, 5, 20, 100, and 200 µg L⁻¹) (Thevenon et al., 2012; Thevenon et al., 2013). The certified reference material utilized was TMDA 51.4 (CRM Sales, Burlington, Canada). Metal contents in water samples were quantified in µg L⁻¹. The standard errors of the three replicate readings were less than 5%, and the chemical blanks for the technique constituted less than 1% of the sample signal.

2.9 Bacteriological analysis

The following incubation procedures were used to filter a volume of 100 mL of each water sample (in triplicate) was

TABLE 1 The primers used for ARGs and relevant bacterial markers quantification.

Target gene	Sequence (5'–3')	Amplicon size (bp)	T _m (°C)	Reference
bla _{TEM}	GCTGCCAACTTACTTCTGACAACG CTTTATCCGCCTCCATCC AGTCTA	247	62	Sidrach-Cardona et al. (2014)
bla _{SHV}	TCAGCGAAAAAAGACTCCTTG TCCCAGGATAAATAACACCGA	110	64	Xi et al. (2009)
bla _{CTX-M}	ATTCCRGCGAAYCAGCGGTGATAACC ACCGCGATATCGTTGGTG GTGCAT	227	68	Fujita et al. (2011)
<i>Enterococcus</i>	GGACGMAAGTCTGACCGA TTAAGAAACCGCCTGCGC	221	57	Ryu et al. (2013)
<i>E. coli</i>	GCAGCGCAATGACATTCTT ATCCTTCGGCGCGATTTTG	282	55	Gordillo et al. (2011)
16S rRNA	ACTCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	197	59	Ovreås et al. (1997)

serially diluted (10^{-5} and 10^{-6}) and filtered through 0.45 µm membrane filters (Sartorius Stedim Biotech, Germany) and placed on various selective culture media (Millipore, Germany): coliform bacteria: Endo agar was incubated for 24 h at 37 °C. *E. coli* was incubated for 4 h at 37 °C in Tryptone Soy Agar (TSA) medium before being moved to Tryptone Bile X-Gluc Agar (TBX) medium, which was incubated for 24 h at 44 °C. *Enterococci* were quantified by placing the filters on Slantez and Bartley agar plates for 24 h, then transferring the filters to Bile Esculin Azide agar (BEA) at 37 °C for 4 h. Finally, *Pseudomonas spp.* were grown on *Pseudomonas* Isolation Agar (PIA), incubated for 48 h at 30 °C. *Enterococcus* was quantified using two methods: first, direct inoculation on endo agar with incubation for 24 h at 37 °C, and second, deposition of the filters on Slanetz and Bartley agar for 24 h, followed by transfer to Bile Esculin Azide (BEA) agar at 37 °C for 4 h. Results were expressed as colony-forming units (CFU) per 100 mL (CFU 100 mL⁻¹).

2.10 DNA extraction and quantification of ARGs and relevant bacterial markers

Water samples were filtered through 0.45 µm pore size membrane filters (Merck Millipore, Germany) using a vacuum filtration apparatus. The filters were stored at –20 °C until DNA extraction. Genomic DNA was extracted directly from the frozen membrane filters using the PureLink™ Microbiome DNA Purification Kit (Invitrogen, United States), following the manufacturer's instructions. The extracted DNA was stored at –20 °C until further analysis. To minimize the effect of potential polymerase chain reaction (PCR) inhibitors, the DNA extracts were further diluted 10× before quantitative PCR (qPCR) analysis.

qPCR was conducted using diluted DNA. Quantification of 16S rRNA, bla_{SHV}, bla_{CTX-M}, bla_{TEM}, *E. coli* and *Enterococcus* was performed as previously described (Al Salah et al., 2020). The target genes were quantified with the MIC Real-Time PCR System and the SensiFAST SYBR No-Rox kit (Table 1). The primers used in this study are shown in Table 1. In each sample, the relative abundance of ARGs was calculated as a percentage of “copy number of a gene relative to 16S rDNA copy number.”

The authors used pre-constructed plasmids carrying the insert of interest to establish standard curves of known concentrations and

determine the concentration of the corresponding gene in the sample.

2.11 Pharmaceutical residues

Pharmaceutical residues in the water samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques. Samples were filtered using 0.45 µm membrane filters (Merck Millipore, Germany) and conditioned. Solid-phase extraction (SPE) was performed for pre-concentration, followed by evaporation under a moderate nitrogen stream, and reconstitution in the mobile phase. Analyses were performed using direct injection on an Agilent 1,290 Infinity II system connected to an Agilent 6495D triple quadrupole mass spectrometer (LC-MS/MS). Chromatographic separation was conducted using a reversed-phase C18 column at a controlled temperature of approximately 30 °C–40 °C. The mobile phase consisted of ultrapure water and an organic modifier (methanol or acetonitrile), along with volatile additives to enhance ionization efficiency. Detection was performed in electrospray ionization (ESI) mode using both positive and negative polarities, depending on the molecule, with Multiple Reaction Monitoring (MRM) applied for quantification and validation. Calibration curves were established using external standards and isotopically labeled internal standards to reduce matrix effects and ensure analytical precision. The limits of detection (LOD) for most target chemicals ranged from several nanograms per liter (ng L⁻¹), accompanied by an overall analytical uncertainty of less than 20%.

2.12 Statistical analysis

Statistical analyses were conducted using analysis of variance (ANOVA) in STATISTICA 9 (StatSoft, Inc., Tulsa, Oklahoma, United States), with significance set at the 5% level ($p < 0.05$). Further statistical analyses and data visualization were conducted utilizing R software (version 4. x; R Core Team, Vienna, Austria). When significant effects were identified, Tukey's Honest Significant Difference (HSD) *post hoc* test was used for mean comparisons, with $p < 0.05$ as the criterion for statistical significance.

TABLE 2 Chemical characteristics of biochar derived from olive industry residues.

Element	Weight %	Atomic %	Net int	Error %	Kratio	Z	A	F
C K	92.11	93.96	685.45	2.83	0.8939	1.0044	0.9662	1.0000
O K	7.89	6.04	26.09	15.77	0.0269	0.9471	0.3599	1.0000

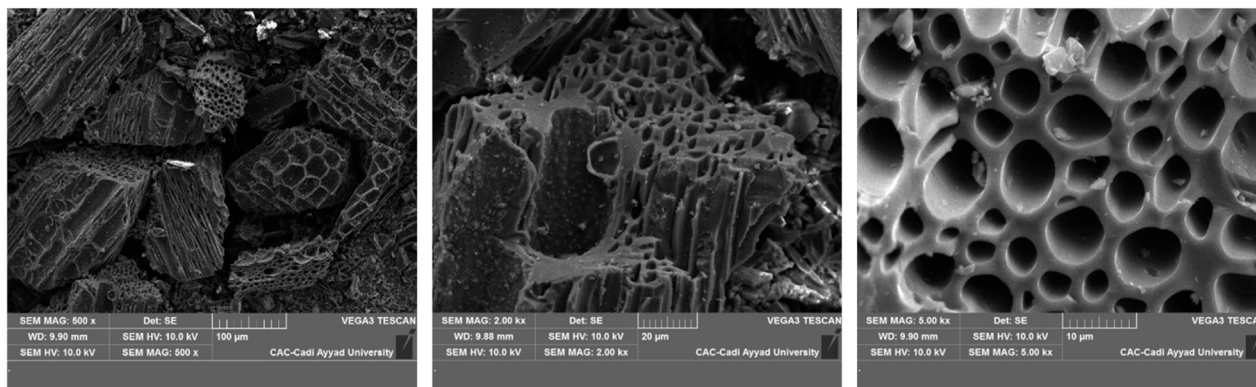


FIGURE 3
Surface morphology of biochar derived from olive industry residues observed by SEM.

3 Results and discussion

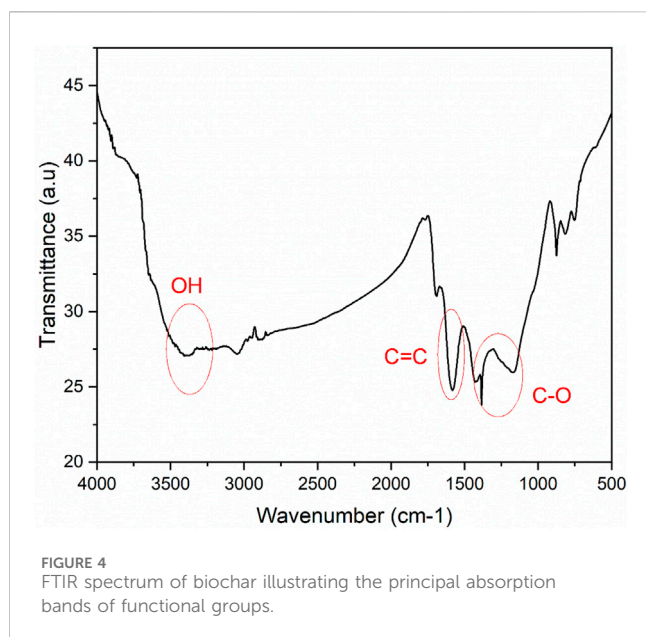
3.1 Biochar characterization

The results obtained show that the biochar used has an elemental composition dominated by carbon (92.11% by mass, 93.96% by atom) with a low oxygen content (7.89% by mass, 6.04% by atom), resulting in an exceptionally low O/C ratio of 0.086 (Table 2). The images obtained by scanning electron microscopy reveal a highly porous, heterogeneous structure with a complex morphology, characterized by pores of varying sizes, rough, irregular surfaces, and remnants of the biomass's original cellular structure. The biochar surface exhibits developed porosity, with residual tubular structures corresponding to the original plant's conducting vessels (Figure 3). These results are consistent with established relationships between pyrolysis conditions and biochar properties documented in the scientific literature (Weber and Quicker, 2018; Tomczyk et al., 2020; Ahmad et al., 2014), where pyrolysis temperature and biomass source are recognized as primary determinants of carbon content, O/C ratio, and surface functionality. While we did not experimentally vary pyrolysis conditions in this study, our biochar characteristics align with those reported for lignocellulosic biomass pyrolyzed at 450 °C–550 °C.

The carbon content of 92.11% observed in this study is higher than the typical range of 50%–85% reported for biochar produced from various feedstocks at moderate temperatures (Weber and Quicker, 2018), and approaches values of 85%–90% explicitly reported for high-temperature pyrolysis (>600 °C) of woody biomass (Tomczyk et al., 2020). This suggests efficient carbonization of our olive-branch feedstock at 500 °C, potentially due to the lignocellulosic composition and controlled pyrolysis

conditions. The low O/C ratio of 0.086, calculated from the results, is consistent with Spokas (2010), who showed that biochars with O/C ratios below 0.2 exhibit high stability and long-term carbon sequestration potential. This observation is also supported by the study by Lehmann and Joseph (2015), which revealed that a decrease in the O/C ratio is indicative of increased aromaticity and increased resistance to microbial degradation. The porous structure observed by SEM is consistent with the findings of Xiao et al. (2018), who characterized the multiscale structure of biochars and highlighted the importance of porosity for environmental applications. The heterogeneous morphology with residual tubular structures observed in our study is similar to that reported by Lehmann and Joseph (2015), who described how the original vascular structures of biomass are partially preserved during pyrolysis, creating a complex porous network. The absence of other detectable major elements (metals, sulfur, nitrogen) suggests a relatively pure biochar, which, according to Ahmad et al. (2014), is advantageous for remediation applications, as it minimizes the risk of secondary contamination.

The infrared spectroscopy results obtained for biochar showed that the spectrum exhibits several characteristic absorption bands, revealing the surface chemical composition and functional groups of the carbonized material (Figure 4). The broad band at around 3,400 cm⁻¹ corresponds to the stretching vibrations of hydroxyl groups (O-H) and associated hydrogen bonds (Tomczyk et al., 2020). This indicates a significant presence of oxygenated functional groups on the biochar surface, particularly phenolic and carboxylic groups. These are essential for adsorption at the biochar surface. These active sites allow electrostatic interactions and the formation of complexes with metal pollutants present in industrial wastewater. The intense peak at 1,580 cm⁻¹ is the most characteristic spectral signature of the biochar analyzed (Fidel et al.,



2017). This band corresponds to the C=C vibrations of aromatic rings and reflects the degree of aromaticity developed during pyrolysis (Harvey et al., 2012). The remarkably high intensity of this band indicates advanced carbonization, with the formation of polycondensed aromatic structures, which are essential for biochar stability in aqueous environments and its resistance to degradation during wastewater treatment (Zhang et al., 2015). These aromatic structures also promote the adsorption of organic pollutants through π - π interactions and van der Waals forces. The bands in the 1,000–1,300 cm^{-1} region reveal the complexity of the oxygenated functional groups (ethers, phenols, carboxyls) present on the surface (Keiluweit et al., 2010; Chia et al., 2012). The amide, amine, carboxylic (COOH), hydroxyl (OH), and lactone groups attached to the biochar surface play a critical role in determining its ability to adsorb various pollutants. Comparative analysis reveals a spectral profile consistent with biochars produced at moderate temperatures (400 °C–500 °C), maintaining an optimal balance between thermal degradation and preservation of surface functional groups (Crombie et al., 2013; Kloss et al., 2012). This pyrolysis temperature is particularly favorable for wastewater treatment applications as it maximizes the density of active sites while maintaining a developed porous structure.

3.2 Pilot plant treatment performances

3.2.1 Physicochemical parameters

The results obtained show that the constructed wetland system exhibits remarkable purification performances with removal rates ranging from 50.74% to 83.71% depending on the parameters (Table 3). The pH decreases slightly from 8.06 to 7.7, which is typical of organic decomposition and nitrification processes in wetlands (Vymazal, 2007). This final value remains optimal for microbial activity and complies with discharge standards. Additionally, a 50.74% reduction in electrical conductivity (from 2,114 to 1,041 $\mu\text{S cm}^{-1}$) was observed, comparable to the 40%–

60% reductions reported in similar systems. This decrease reflects the plants' ion absorption and the precipitation of salts in the substrate. The system's performance is consistent with findings from vertical subsurface flow constructed wetlands, where operational parameters such as temperature and hydraulic retention time significantly influence removal efficiency (Akratos and Tsihrintzis, 2007). An exceptional removal of 83.71% of suspended solids (133–21.66 mg L^{-1}) is noted, reaching the high end of the 75%–90% range documented by García et al. (2010). This performance results from sedimentation, mechanical filtration, and microbial flocculation. We observe a 75.70% reduction in COD (from 3,580.64 to 882.56 mg L^{-1}), which is notable given the very high initial load. This performance aligns with the 70%–80% reported by Vymazal (2011) for subsurface flow systems. The removal results from aerobic processes in the rhizosphere and anaerobic processes in the deep substrate. A remarkable removal for total phosphorus (77.75%) and orthophosphates (76.73%), significantly exceeding the typically observed 40%–60%. A recent study of Fang et al. (2013) reports similar performance (70%–80%) with modified substrates rich in calcium or iron. A removal of 68.86% of nitrates and 75.35% of nitrites, confirming active denitrification. These performances are consistent with the 50%–80% reported by Saeed and Sun (2012) in well-designed wetlands. The alternation of aerobic and anaerobic zones promotes the coupling of nitrification and denitrification. A recent study of Ilyas and Masih (2017) confirms that this spatial architecture optimizes nitrogen removal. A 61.44% reduction in sulfates (181.57–42.26 mg L^{-1}), indicating active bacterial reduction under anaerobic conditions. This performance is comparable to the 50%–70% documented by Wießner et al. (2005). Dong et al. (2012) emphasize that this reduction can mobilize iron-bound phosphorus, affecting long-term phosphorus retention. The results obtained show that this system meets or exceeds the average performance of constructed wetlands. In a recent meta-analysis, average rates of 45%–55% for conductivity, 80%–85% for TSS, 65%–75% for COD, and 45%–60% for nutrients were reported (Vymazal, 2018). The studied system particularly stands out for phosphorus removal (78% vs. 50% on average).

While our study did not include a side-by-side parallel control wetland without biochar, the extensive literature on conventional constructed wetlands provides robust comparative benchmarks. The physicochemical removal efficiencies we observed (50.7%–83.7% across parameters) align with or exceed the upper range of conventional CW performance reported in recent meta-analyses: Vymazal (2018) reported average rates of 45%–55% for conductivity, 80%–85% for TSS, 65%–75% for COD, and 45%–60% for nutrients in standard systems. Our system's particularly high phosphorus removal (77.8%) compared to the 50% average for conventional wetlands suggests a significant contribution from biochar through enhanced adsorption and precipitation mechanisms. These literature-based comparisons effectively demonstrate biochar's role in enhancing performance.

3.2.2 Bacteriological parameters

The results showed remarkably high removal efficiency for the majority of the bacterial indicators studied. Tertiary treatment using a biochar-enhanced constructed wetland significantly reduces bacterial concentrations compared to secondary lagooning (Table 4).

TABLE 3 Average CW removal, input, and outlet efficiency (mean of three replicates $\pm$ standard deviation).

Parameter	Mean inlet ($A \pm a$)	Mean outlet ($A \pm a$)	Mean removal
pH	8.06 ± 0.24	7.7 ± 0.23	-
Conductivity ($\mu\text{S cm}^{-1}$)	$2,114 \pm 63$	$1,041 \pm 31$	50.74%
TSS (mg L^{-1})	133 ± 4.0	21.66 ± 0.65	83.71%
COD (mg L^{-1})	$(3.58 \pm 0.11) \times 10^3$	$(8.83 \pm 0.26) \times 10^2$	75.70%
P total (mg L^{-1})	9.46 ± 0.28	3.652 ± 0.11	77.75%
PO_4^{3-} (mg L^{-1})	7.37 ± 0.22	1.79 ± 0.054	76.73%
NO_3^- (mg L^{-1})	15.14 ± 0.45	3.37 ± 0.10	68.86%
NO_2^- (mg L^{-1})	8.39 ± 0.25	2.61 ± 0.078	75.35%
SO_4^{2-} (mg L^{-1})	181.57 ± 5.4	42.26 ± 1.3	61.44%

TABLE 4 Bacterial load before and after treatment with an enhanced biochar-based constructed wetland.

Organism	Media	Lagoon (CFU 100 mL^{-1})	CW (CFU 100 mL^{-1})	Mean removal
<i>E. coli</i>	TBX	4.63×10^4	9.67×10^3	79.1%
<i>Enterococcus</i>	Endo	2.24×10^8	9.57×10^6	95.7%
<i>Enterococcus</i>	BEA	1.46×10^8	9.40×10^6	93.6%
<i>Pseudomonas</i>	Pseud. sel. Agar	2.07×10^7	1.10×10^7	46.9%

TB: Specific bacterial indicator (*Escherichia coli*), Endo: *Enterococcus* (*Enterococcus* medium), BEA: *Enterococcus* (BEA, agar - Bile Esculin Azide), Pseudo: *Pseudomonas*, CW: constructed wetland.

E. coli decreased from 4.63×10^4 CFU 100 mL^{-1} at the outlet of the lagooning system to 9.67×10^3 CFU 100 mL^{-1} after passing through the constructed wetland, indicating improved treatment. The Endo (*Enterococcus*) indicator performed exceptionally well, decreasing from 2.24×10^8 CFU 100 mL^{-1} to 9.57×10^6 CFU 100 mL^{-1} , representing an effective elimination rate of 95.7%. The BEA indicator (*Enterococcus* on BEA agar) followed a similar trend, with a reduction from 1.46×10^8 to 9.40×10^6 CFU 100 mL^{-1} , corresponding to an effectiveness of 93.6% in eliminating bacteria. Finally, the *Pseudomonas* indicator exhibits the lowest removal efficiency, with a reduction from 2.07×10^7 to 1.10×10^7 CFU 100 mL^{-1} , corresponding to 46.9% removal. Our results show that the system we designed can be remarkably efficient at reducing indicator bacteria and, therefore, a number of pathogens as well. The removal efficiency of 79.1% for *E. coli* is significantly higher than the results reported by Decamp and Warren (2000), which showed an average removal rate of *E. coli* of 41%–72% for microcosms in their underground flow wetlands. Our findings are similar to the 96.6%–98.9% achieved by these same authors for their improved pilot systems, confirming the efficiency of tertiary treatment using biochar-constructed wetlands. The most notable aspect of this study is the exceptional performance achieved with enterococci. The 95.7% removal efficiency of *Enterococcus* on Endo agar significantly exceeds the data reported by Zurita and Carreón-Álvarez (2015), who reported average reductions of 2.34–2.44 log units for total coliforms and 3.44–3.74 log units for *E. coli* in their constructed wetland hybrid systems. Although our system achieves a 1.37 log-unit reduction, the removal percentages (>95%) demonstrate that it is remarkably efficient at removing these critical indicators of fecal

contamination. The consistency between the two methods of detecting enterococci (Endo: 95.7% and BEA: 93.6%) strengthens the reliability of these exceptionally high results. This achievement far exceeds the data reported by Giacomán-Vallejos et al. (2015), who reported reductions in total and fecal coliforms of 70%–83% and 65%–78%, respectively, in their planted systems after 24 h of exposure, and 80%–82% and 86%–91% after 48 h. These findings suggest that biochar has a major positive effect on bacterial removal. The lower effectiveness observed for *Pseudomonas* (46.9%) is in line with the properties of these environmental bacteria, which are less directly linked to fecal contamination and are naturally more resistant to the removal mechanisms of constructed wetlands. This performance is still acceptable for this type of indicator.

The improvement provided by biochar can be attributed to several well-documented mechanisms in the literature. Biochar offers a high specific surface area, promoting the adsorption of contaminants and providing favorable attachment sites for beneficial microorganisms involved in purification processes. The study by Odedishemi Ajibade et al. (2021) confirms that the average COD and nitrogen removal efficiencies in a vertical planted filter with 1% biochar were $89.1\% \pm 5.6\%$ and $90.2\% \pm 3.1\%$, respectively, demonstrating the benefits of biochar for improving overall purification performance. A comparative study by Headley et al. (2013) found that fecal and total coliforms were reduced by 96.3% and 93.9%, respectively, in wetlands with groundwater flow, results comparable to ours for enterococci. The pioneering study of Oron et al. (1988) had already demonstrated the potential of constructed wetlands, reporting a 99% reduction in total coliforms and demonstrating their effectiveness in removing bacteria. This

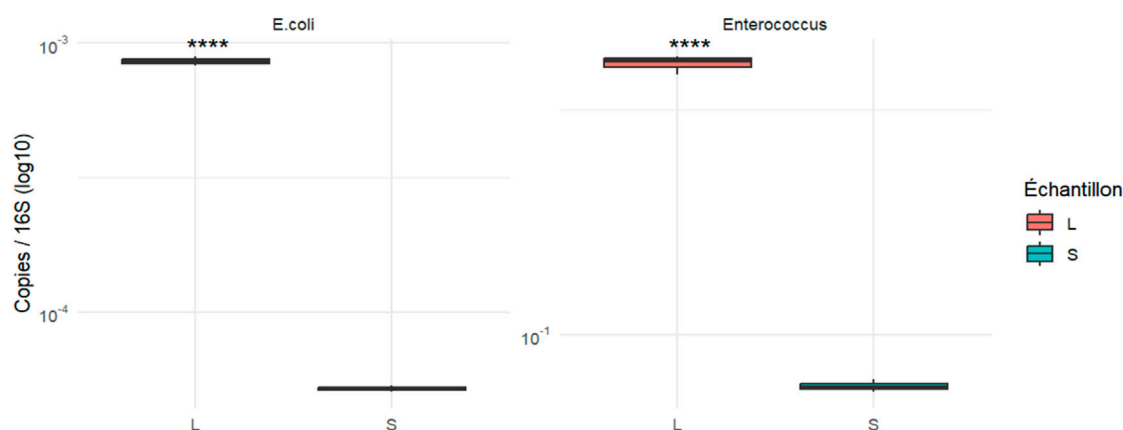


FIGURE 5
Bacterial concentration comparative analysis (*E. coli* and *Enterococcus*) between lagoon water and constructed wetland.

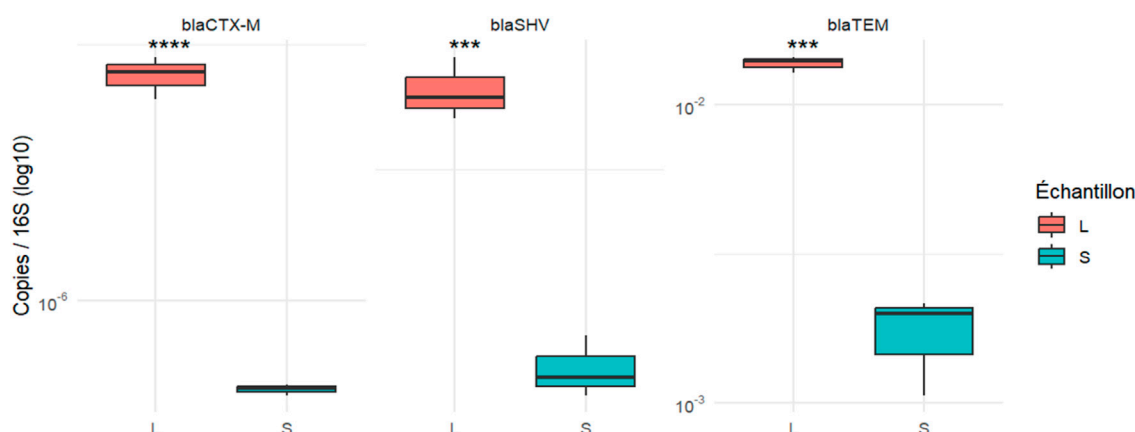


FIGURE 6
Identification of genes conferring resistance to β -lactams (*blaCTX-M*, *blaSHV*, *blaTEM*) between water from lagooning systems and constructed wetland.

highlights the system's increased effectiveness in eliminating bacteria and pathogens when used as a supplementary treatment step.

While the enhanced bacterial removal observed in our system is consistent with biochar's documented properties of providing microbial attachment surfaces and adsorbing contaminants, we did not perform direct microbial community analysis on the biochar surface in this study. Therefore, our attribution of improved bacterial removal to biochar-mediated mechanisms remains partially inferential, supported by precedent in the literature but requiring system-specific validation through microscopy, microbial DNA sequencing of biochar-colonizing communities, and parallel non-biochar controls in future work.

3.2.3 Quantification of ARGs and relevant bacterial markers

Current wastewater treatment plants cannot fully remove ARGs, and achieving a significant reduction in ARGs typically comes at a high cost. However, here we expand on how this cost-effective and

environmentally friendly system increases efficiency by reducing the occurrence of ARGs. The results show that biochar (substrate S) is remarkably effective at removing marker bacteria and antibiotic resistance genes (ARGs) (Figures 5, 6). The data reveal statistically significant differences ($p < 0.0001$) for all parameters analyzed, with reductions in abundance ranging from 1 to 4 orders of magnitude depending on the type of microorganism or gene targeted. Comparative analysis of the two substrates reveals exceptionally high efficiency in removing indicator bacteria. For *E. coli*, abundance decreased from approximately 10^{-3} copies/16S ($\log_{10}$) in the control (L) to less than 10^{-5} copies/16S ($\log_{10}$) in the biochar system (S), representing a reduction of more than 2 orders of magnitude. Similarly, for *Enterococcus*, a similar decrease of approximately 2–3 orders of magnitude was observed. Biochar's intrinsic properties can explain it. As shown in Figure 5, the sorption process is attributed to multiple physicochemical mechanisms, including physical sorption, intra-particle diffusion, ion exchange, metal bridges, and electrostatic interactions (Laishram et al., 2025), which collectively contribute to the observed removal efficiencies.

The porous structure of biochar captures pollutants through surface adsorption and void-filling, thereby significantly improving contaminant retention in constructed systems (Karki, 2024).

Figure 6 illustrates the variable effectiveness across the resistance gene type analyzed. The blaCTX-M gene shows the most significant reduction, from approximately 10^{-4} copies/16S ($\log_{10}$) in the control to less than 10^{-7} copies/16S ($\log_{10}$) with biochar, representing a reduction of roughly 3–4 orders of magnitude. The blaSHV gene also shows a significant decrease of approximately 2–3 orders of magnitude, whereas blaTEM shows a more moderate, but still statistically significant, reduction of roughly 1–2 orders of magnitude. Several factors can explain this variation in efficacy: (1) Gene location and mobility, blaCTX-M genes are frequently carried on highly mobile plasmids that can transfer between bacterial species, whereas blaTEM genes may be more commonly chromosomally encoded in certain host bacteria. The differential susceptibility of plasmid-bearing versus chromosomal-gene-bearing bacteria to the biochar-wetland environment could influence removal rates. (2) Host bacterial diversity and susceptibility: different ARGs are associated with different bacterial hosts (Ejaz et al., 2021). The wetland's environmental conditions (oxygen gradients, nutrient availability, pH, and presence of plant root exudates) may differentially affect these host populations. For instance, if blaCTX-M is predominantly found in more sensitive Enterobacteriaceae, while blaTEM is in more resilient environmental bacteria, removal rates would differ accordingly. (3) Gene copy number variations: some bacteria carry multiple copies of certain resistance genes, affecting the gene-to-cell ratio and consequently the qPCR-measured gene abundance relative to 16S rRNA. (4) Extracellular DNA stability: following bacterial cell lysis in the wetland, different ARG sequences may exhibit variable stability in the extracellular environment. Differences in DNA secondary structure, nucleotide composition, or association with protective proteins could influence adsorption to biochar surfaces and degradation rates. (4) Selective pressure dynamics: the biochar-enriched wetland may create microenvironments where certain antibiotic residues are preferentially adsorbed or degraded, potentially generating differential selective pressures that favor retention or elimination of specific resistance genes.

Based on our qPCR methodology (which quantifies both intracellular and extracellular DNA) and the parallel reduction in bacterial indicators (*E. coli* and *Enterococcus*), we conclude that ARG removal primarily occurs through: (1) physical filtration and retention of bacteria harboring these genes within the biochar porous structure and sand matrix, (2) inactivation of bacteria due to unfavorable conditions in the wetland substrate, and (3) potential adsorption of extracellular DNA fragments onto biochar surfaces via electrostatic interactions with positively charged surface sites. The fact that bacterial abundance decreased by 2–3 log units while corresponding ARGs decreased by 1–4 log units suggests that both cell-associated and extracellular ARG removal mechanisms are operational. Figure 7 illustrates the overall role of the constructed wetland system in both the removal and potential reloading dynamics of antibiotic resistance genes throughout the treatment process.

The precise mechanisms underlying these differential removal rates require further investigation through metagenomic analysis, gene-specific tracking studies, and assessment of horizontal gene

transfer dynamics within the wetland to distinguish between bacterial death, gene loss, and potential gene transfer events.

3.2.4 Metallic trace elements analysis by ICP-MS

The results show that the vertical planted filter system enhanced with biochar (CW) is highly effective at removing heavy metals (Table 5). For arsenic, the constructed wetland achieves a removal rate of 91% (residual concentration of $2.75 \mu\text{g L}^{-1}$) compared to 46.5% for lagooning ($16.36 \mu\text{g L}^{-1}$), an improvement of 83.2%. The results are even more spectacular for cadmium and lead, with removal efficiencies of 99.998% and 99.84% respectively, by the constructed wetland, compared to 99.85% and 98.95% for lagooning. These results demonstrate that incorporating biochar into vertically planted filters enables extremely low residual concentrations, particularly for cadmium ($0.001 \mu\text{g L}^{-1}$) and lead ($0.089 \mu\text{g L}^{-1}$). These results are consistent with recent scientific literature, which confirms the exceptionally high efficiency of biochar-enhanced planted filter systems in removing heavy metals. Wang et al. (2022) report that biochar layered in planted filter systems can achieve removal efficiencies of 96.6% and 98.6% for cadmium and 97.2% and 98.9% for lead, which closely match the performance observed in our study of 99.998% and 99.84%, respectively. Similarly, Fu et al. (2022) confirm that planted filter systems can achieve removal rates exceeding 98.5% for lead, cadmium, copper, zinc, and iron, thereby validating our observations. Several well-documented synergistic mechanisms can explain biochar's superior effectiveness. Liu et al. (2022) emphasize that modified biochar is attracting considerable attention in the scientific community for its effectiveness in adsorbing and immobilizing heavy metal ions, thanks to its high specific surface area and microporous structure. Qiu et al. (2022) demonstrate that biochar generally improves metal retention through increased adsorption surface area and favorable surface chemical properties. Regarding the stability of adsorbed metals, our biochar exhibits a relatively stable pH (7–8 range) that buffers the system. The pH of treated effluent remained between 7.70 and 8.06 throughout the monitoring period, minimizing desorption risks associated with pH fluctuations. The high removal efficiencies (>98% for Cd and Pb) suggest strong binding mechanisms, likely involving both surface complexation with oxygen-containing functional groups (carboxyl, hydroxyl, phenolic groups identified by FTIR) and precipitation as insoluble metal hydroxides or carbonates in the alkaline biochar environment. However, we acknowledge that sudden pH drops, for example, from acidic industrial discharges mixed with the municipal wastewater, could potentially remobilize adsorbed metals. We therefore recommend continuous pH monitoring and pre-treatment pH adjustment (to the 6–9 range) for influents outside this optimal range to ensure long-term metal stability in the biochar matrix.

3.2.5 Pharmaceutical residues

According to the results, the biochar-enhanced wetland system exhibits a wide range of removal efficiencies for pharmaceutical active ingredients (APIs) and their metabolites. Based on performance patterns, compounds can be grouped into three categories: Group A ($\geq 90\%$ removal - Excellent performance): Five compounds achieved exceptional removal: ibuprofen showed 96%–97% efficiency (from 7,310/6,590 to 238 ng L^{-1}), ciprofloxacin achieved 96% reduction (from 2,300 to 83 ng L^{-1}), atenolol showed 94%–95% elimination (from 232/243 to 12 ng L^{-1}), azithromycin

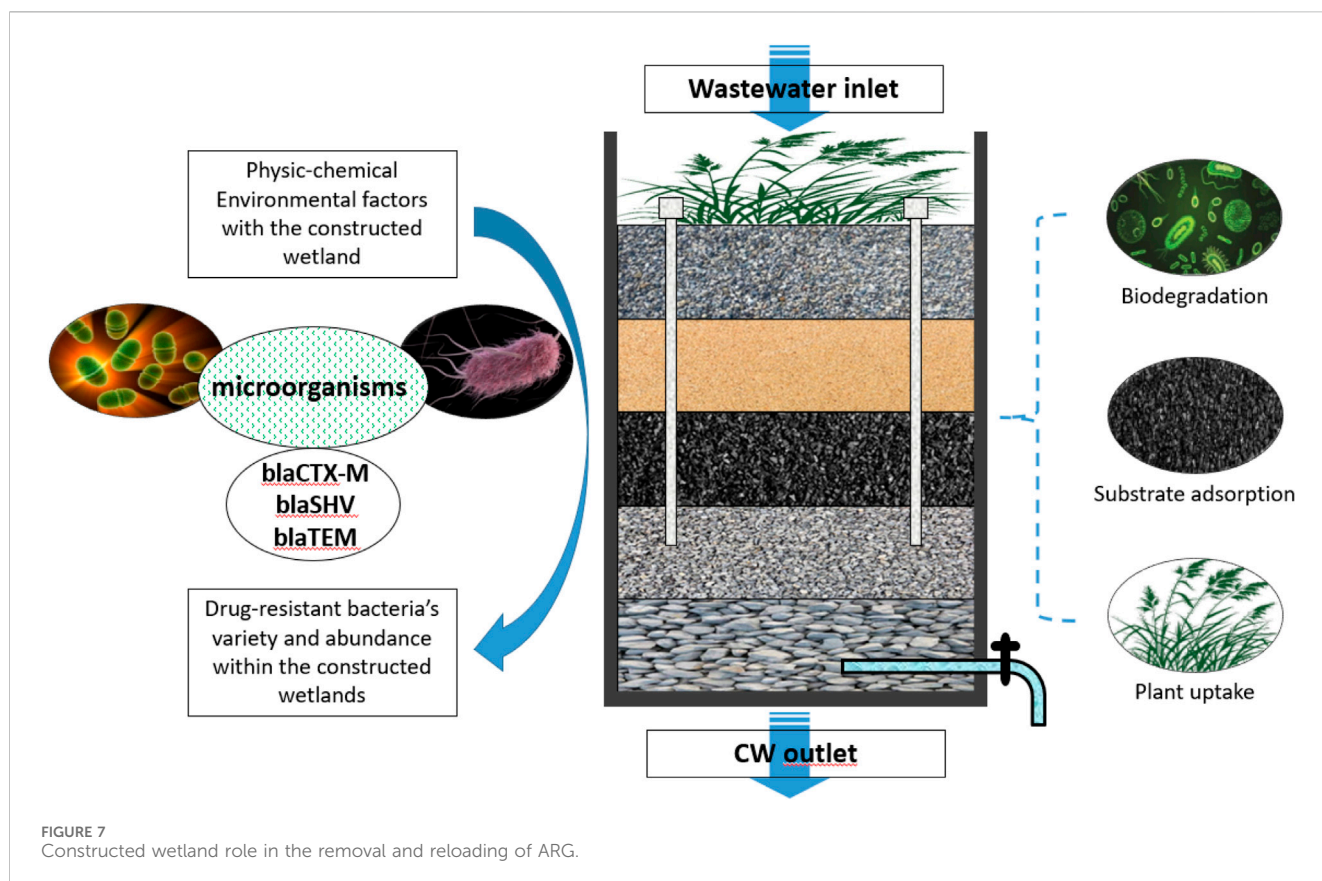


TABLE 5 Performance in the removal of heavy metals using lagooning and constructed wetland (calculated using 5% standard error for ICP-MS data).

Samples	As ($\mu\text{g L}^{-1}$)	Cd ($\mu\text{g L}^{-1}$)	Pb ($\mu\text{g L}^{-1}$)
TMDA-61.3	30.5658 $\pm$ 1.5	61.2409 $\pm$ 3.1	57.3719 $\pm$ 2.9
Lag	16.3561 $\pm$ 0.82	0.0936 $\pm$ 0.0047	0.6036 $\pm$ 0.030
CW	2.7509 $\pm$ 0.14	0.0014 $\pm$ 0.000070	0.0896 $\pm$ 0.0045

exceeded 94% efficiency (from 337 to $<20 \text{ ng L}^{-1}$), and paracetamol showed near-complete elimination ($>99.9\%$) with residual concentrations below detection limits. Group B (60%–80% removal - Satisfactory performance): Diclofenac (66%–67% elimination: from 1,450/1,480–488 ng L^{-1}), carbamazepine (63%–64% reduction: from 800/865 to 312 ng L^{-1}), naproxen (62%–65% efficiency: from 1,290/1,360–481 ng L^{-1}), irbesartan (67%–69% elimination: from 1,410/1,520 to 467 ng L^{-1}), and sulfamethoxazole (70% elimination: from 417 to 123 ng L^{-1}) all showed satisfactory removal efficiencies. Group C (40%–70% removal - Moderate performance): Hydrochlorothiazide (60%–63% reduction: from 377/350 to 139 ng L^{-1}) and mefenamic acid (64%–67% efficiency: from 1,260/1,370–448 ng L^{-1}) demonstrated moderate removal efficiencies.

Erythromycin, metoprolol, and trimethoprim were already below detection limits in the influent, while citalopram (from 18 ng L^{-1} to $<10 \text{ ng L}^{-1}$), clarithromycin (from 64–71 ng L^{-1} to $<10 \text{ ng L}^{-1}$), and

propranolol (from 36–37 ng L^{-1} to $<10 \text{ ng L}^{-1}$) were all totally removed below detection thresholds. The concentration of valsartan acid increased significantly (from 133/145 to 1,140 ng L^{-1}), indicating a significant deviation that warrants further research. This might be due to desorption, metabolic transformation, or analytical interference.

Importantly, the conventional CW studies without biochar reported in Table 6 serve as effective reference controls, demonstrating that biochar addition provides substantial performance improvements: conventional wetlands typically achieve 40%–60% removal for most pharmaceuticals (Ilyas et al., 2020), while our biochar-enhanced system exceeded 90% for five priority compounds and maintained 60%–80% efficiency even for moderately recalcitrant molecules. This systematic comparison with published recalcitrant CW performance data clearly quantifies biochar's additive benefit, showing improvement factors of 1.3–3.2 $\times$ depending on the pharmaceutical compound and its physicochemical properties.

Our system achieved 96%–97% removal of ibuprofen, compared to 85%–90% typically reported for commercial activated carbon systems (Całus-Makowska et al., 2024), and 63%–64% for the highly recalcitrant carbamazepine, versus $<20\%$ in conventional wastewater treatment systems (Chand et al., 2022). We attribute this superior performance to: (1) the unique combination of micro- and mesopores in olive-branch biochar produced at 500 $^{\circ}\text{C}$, creating diverse adsorption sites for molecules of varying sizes; (2) the synergistic wetland environment that combines adsorption with biodegradation and photodegradation processes; and (3) the presence of *Phragmites australis* roots that maintain aerobic

TABLE 6 Comparative performance of olive-branch biochar vs. commercial adsorbents and other biochars for pharmaceutical removal.

Pharmaceutical	Our study (olive biochar, 500 °C)	Commercial activated carbon	Other biochar studies	Reference
Ibuprofen	96%–97%	85%–90%	70%–85% (various feedstocks)	Calus-Makowska et al. (2024)
Ciprofloxacin	96%	80%–88%	65%–80% (wood biochar)	Panghal et al. (2024)
Carbamazepine	63%–64%	30%–50%	<20% (conventional CW)	Chand et al. (2022)
Diclofenac	66%–67%	75%–85%	40%–60% (rice husk biochar)	Calus-Makowska et al. (2024)
Sulfamethoxazole	70%	80%–90%	50%–65% (mixed feedstock)	Chand et al. (2022)

conditions in the rhizosphere, preventing biochar surface fouling and supporting active microbial communities that contribute to pharmaceutical degradation.

Mechanistically, the high specific surface area and heterogeneous pore structure of our biochar (as evidenced by SEM imaging, Figure 3) provide diverse adsorption sites that simultaneously accommodate different pollutant classes. The presence of both hydrophobic aromatic structures (evidenced by the strong C=C peak at $1,580\text{ cm}^{-1}$ in FTIR spectra, Figure 4) and polar functional groups (O-H at $3,400\text{ cm}^{-1}$, and oxygenated groups at $1,000\text{--}1,300\text{ cm}^{-1}$) enables parallel removal pathways: π - π interactions for aromatic pharmaceuticals like ciprofloxacin and ibuprofen, electrostatic attraction for ionized compounds under the system's pH conditions (7.70–8.06), and physical entrapment within the porous structure for larger molecules.

These findings align with research showing that biochar's qualities, including its ability to enhance microbial activity, benefit plant growth, nitrogen cycling, and pollution removal in artificial wetlands. According to Karki (2024), biochar may effectively remove nutrients, heavy metals, and emerging pollutants, including pharmaceuticals and pathogens, due to its high specific surface area, porosity, and chemical stability. The removal methods include complexation, ion exchange, precipitation, and pore filling, as detailed in Calus-Makowska et al. (2024), in which the kinetic behavior of removing diclofenac, carbamazepine, sulfamethoxazole, and ibuprofen from water was evaluated using activated carbon and biochar as adsorbents.

The exceptional efficiency for ibuprofen (96%–97%) is consistent with Panghal et al. (2024) findings that vertical flow constructed wetlands assisted by biochar showed high removal of caffeine, ibuprofen, and amoxicillin, while the 63%–64% efficiency observed for carbamazepine significantly exceeds typical performance reported by Chand et al. (2022) where this recalcitrant molecule generally shows removal efficiencies <20% in conventional systems, with compound-dependent variations showing ibuprofen and naproxen >70%. The physicochemical properties of target molecules play a crucial role in removal processes. Hydrophobic compounds, such as ibuprofen ($\log K_{ow} = 3.97$) and diclofenac ($\log K_{ow} = 4.51$), exhibit enhanced adsorption onto biochar aromatic surfaces through hydrophobic interactions and π - π bonding. In contrast, more polar or ionizable compounds, such as hydrochlorothiazide, show lower removal, potentially due to reduced affinity for the predominantly hydrophobic biochar surface. Molecular size also influences removal: larger

pharmaceutical molecules may experience size-exclusion effects or preferential adsorption in mesopores, while smaller molecules access the full pore network, including micropores. Yu et al. (2023) demonstrated that biochar applications in constructed wetlands involve complex interactions among photodegradation, biodegradation, phytoremediation, and sorption processes, thereby explaining the superior performance achieved through multiple removal mechanisms optimized by biochar addition. The study by Ilyas et al. (2020) supports our findings, showing that removal efficiencies for the 34 most-studied pharmaceutical products range from 21% to 93%. Our results consistently fall at the higher end of this range, confirming the tested system's competitiveness relative to conventional wastewater treatment technologies.

An important limitation is that we did not perform detailed Brunauer–Emmett–Teller (BET) surface area or pore size distribution characterization of our biochar. Future studies should include a complete N_2 adsorption-desorption isotherm analysis to establish quantitative structure-performance relationships between biochar pore distributions (micropores, mesopores, and macropores) and pharmaceutical removal efficiency for molecules of different sizes and properties.

With performance exceeding 90% for five priority molecules and satisfactory efficiencies (60%–80%) for four other compounds, these results demonstrate the remarkable effectiveness of biochar-enhanced constructed wetlands to eliminate an extensive range of pharmaceutical residues, proving this innovative method as a practical and sustainable solution for tertiary treatment of wastewater loaded with pharmaceutical micropollutants.

3.3 System longevity and operational considerations

A critical practical consideration for implementing biochar-enhanced constructed wetlands is the operational lifespan of the biochar layer and the need for replacement or regeneration. Our results showed consistent removal efficiencies throughout the 4-month monitoring period, with no significant decline in performance across any pollutant class, suggesting that the biochar layer had not reached saturation under the applied loading conditions. The high carbon stability of our olive-branch biochar, indicated by the low O/C ratio (0.086) and high aromaticity (strong FTIR peak at $1,580\text{ cm}^{-1}$), suggests resistance to microbial degradation and chemical oxidation, key factors in long-term

performance stability (Spokas, 2010). Based on biochar stability models and considering our hydraulic loading rate, we conservatively estimate that the biochar layer could maintain effective pollutant removal performance for 12–18 months before requiring replacement or regeneration. Several factors support extended biochar functionality in the wetland environment: (1) Physical protection; the biochar layer's position within the multi-layer substrate matrix (beneath sand and vegetation layers) protects from direct sunlight (preventing photo-oxidation) and from rapid washout during high-flow events. (2) Microbial colonization benefits; rather than diminishing performance, the progressive colonization of biochar surfaces by beneficial wetland microorganisms may actually enhance removal capacity through biodegradation mechanisms that complement physical adsorption. Biochar's porous structure provides an ideal habitat for microbial biofilm development, creating synergistic biological-physical treatment. (3) Self-regeneration potential; under anaerobic conditions in deeper substrate zones, microbially-mediated reductive dissolution of certain adsorbed metals and degradation of organic pollutants may provide partial *in-situ* regeneration of adsorption sites, extending functional lifespan beyond expectations based solely on sorption capacity. (4) Regeneration strategies; when replacement becomes necessary due to gradual saturation (which would manifest as declining removal efficiency trends), the spent biochar need not be disposed of as waste. Potential regeneration strategies include: thermal regeneration through controlled pyrolysis to remove adsorbed organics while maintaining the carbon structure; chemical regeneration using gentle solvent washes for specific pollutant classes; or alternative beneficial uses, such as soil amendment for non-food landscaping applications, where the accumulated nutrients (N, P) and stable carbon provide agronomic value. (5) Economic and sustainability implications; the projected 12–18-month operational lifespan translates to relatively low maintenance requirements and costs. Given that olive pruning waste is an abundant, low-cost feedstock in Mediterranean regions, replacing biochar with this waste represents a minor operational expense relative to the energy costs of conventional tertiary treatment technologies (e.g., ozonation, advanced oxidation processes). Life cycle assessments should be conducted to compare the full environmental footprint (including the energy required for biochar production) with alternative treatment intensification approaches. (6) Monitoring indicators for replacement scheduling; to optimize biochar replacement timing and avoid premature or delayed replacement, we recommend monitoring the following early warning indicators: declining pharmaceutical removal efficiency trends (specifically for well-removed compounds like ibuprofen as sentinel indicators), increasing heavy metal concentrations in effluent approaching 50% of regulatory limits, and periodic pilot-scale sorption capacity tests using biochar samples extracted from the system.

3.4 Potential reuse in irrigation

The evaluation of the potential for the reuse of treated water in irrigation requires a rigorous comparison of the residual concentrations against the international and national standards in force. Table 6 presents a comparative summary

of the PS-VFCW system's performance relative to the main standards for the reuse of treated wastewater in irrigation. The system demonstrates strong compliance across multiple parameter categories: (1) Heavy metals compliance: The biochar-enriched vertical planted filter system achieves heavy metal concentrations largely compliant with the strictest regulatory thresholds. For arsenic, the final concentration of $2.75 \mu\text{g L}^{-1}$ represents only 2.75% of the maximum limit of $100 \mu\text{g L}^{-1}$ recommended by the FAO, while cadmium ($0.0014 \mu\text{g L}^{-1}$) and lead ($0.089 \mu\text{g L}^{-1}$) reach 0.014% and 0.0018% of their limit values, respectively (Ayers and Westcot, 1985). (2) Physicochemical parameters compliance: The physicochemical parameters comply with the Moroccan standards outlined in Joint Decree No. 1275–01 (Ministry of Spatial Planning Water and Environment, 2002) of 17 October 2002. The suspended solids (21.7 mg L^{-1}) abide by the 30 mg L^{-1} limit for localized irrigation. At the same time, the nutrient concentrations demonstrate the system's ability to produce an effluent suitable for fertigation, a practice highly valued in the Mediterranean context of water scarcity (Cirelli et al., 2012). (3) Bacteriological considerations: The bacteriological results, with reductions exceeding 79% in fecal contamination indicators, reinforce the potential for agricultural valorization, although additional disinfection is recommended to meet the World Health Organization, (2006) criteria for unrestricted irrigation. (4) Emerging contaminants: The near-total elimination of pharmaceutical residues and the substantial reduction of antibiotic resistance genes (1–4 orders of magnitude) add a particularly relevant health security dimension in the face of concerns related to emerging contaminants, an aspect often overlooked in conventional standards but gradually becoming an additional quality criterion (Hamoda et al., 2004). (5) Overall assessment: All these results position the PS-VFCW system as a tertiary treatment solution for producing high-quality irrigation water, capable of meeting current regulatory requirements and emerging environmental protection and public health criteria, thereby aligning with the objectives of the circular economy and integrated water resource management. However, as discussed in Section 3.6, long-term monitoring of crop uptake, soil health, and ecotoxicological impacts is recommended for comprehensive safety assurance.

3.5 Ecotoxicological considerations and health risk assessment for irrigation reuse

While the treated effluent meets current regulatory chemical standards for irrigation (Table 7), the long-term ecological and human health implications of applying water containing trace pharmaceutical residues and residual antibiotic resistance genes to agricultural systems warrant careful consideration: (1) Pharmaceutical bioaccumulation risks; although our system achieved 60%–97% removal of target pharmaceuticals, residual concentrations of 83–488 ng L^{-1} remain for several compounds. Certain pharmaceuticals, particularly lipophilic compounds such as ciprofloxacin and ibuprofen, can bioaccumulate in plant tissues when present in irrigation

TABLE 7 Comparison of the performance of the PS-VFCW system with the standards for the reuse of treated wastewater in irrigation.

Parameter	PS-VFCW concentration	FAO/WHO standards	Moroccan standard	Compliance
Heavy metals				
Arsenic ($\mu\text{g L}^{-1}$)	2.75	<100	<100	Conforms
Cadmium ($\mu\text{g L}^{-1}$)	0.0014	<10	<10	Conforms
Lead ($\mu\text{g L}^{-1}$)	0.089	<5,000	<5,000	Conforms
Physicochemical parameters				
TSS (mg L^{-1})	21.66	—	<30	Conforms
Nitrates (mg L^{-1})	3.37	<50	<50	Conforms
Nitrites (mg L^{-1})	2.61	<3	—	Conforms
Sulfates (mg L^{-1})	42.26	<500	—	Conforms
Bacteriological indicators				
<i>E. coli</i> (CFU 100 mL ⁻¹)	9.67×10^3	< 10^3 *	< 10^3 **	Disinfection recommended
Enterococci	93.6% reduction	—	—	Excellent performance
Total coliforms	95.7% reduction	—	—	Excellent performance
Emerging contaminants				
Pharmaceutical residues	>90% removal	No standard	No standard	Excellent performance
Resistance genes (ARGs)	1–4 log reduction	No standard	No standard	Excellent performance

water, with bioconcentration factors ranging from 0.1 to 10, depending on the compound and crop species (Christou et al., 2017). Leafy vegetables and root crops may accumulate different pharmaceutical classes through distinct uptake pathways. While current residual concentrations are well below acute phytotoxicity thresholds, chronic low-level exposure effects on sensitive plant species require monitoring. (2) Human health exposure pathways; consumption of crops irrigated with pharmaceutical-containing water represents a potential human exposure route, though studies indicate that pharmaceutical concentrations in edible plant tissues are typically 10–1,000 fold lower than irrigation water concentrations (Malchi et al., 2014). Nevertheless, daily dietary exposure from multiple crops could result in cumulative effects, particularly for compounds with long elimination half-lives in humans. Of particular concern are antibiotics such as ciprofloxacin (83 ng L^{-1} residual), which, even at trace levels, may contribute to the selection of antimicrobial resistance in environmental and gut microbiomes. (3) Antimicrobial resistance transmission; despite achieving 1–4 log reductions in ARGs, residual antibiotic resistance genes remain detectable in treated effluent. The potential for horizontal gene transfer from environmental bacteria to human pathogens via the soil-plant-human pathway, though low, represents a recognized biosafety concern. Agricultural workers handling irrigation equipment and consumers of raw vegetables may face the highest exposure risks. The persistence of ARGs in

agricultural soils following irrigation with treated wastewater requires long-term monitoring to assess the balance between accumulation and degradation.

4 Conclusion

This study demonstrates the effectiveness of a pilot vertical-flow constructed wetland enhanced with biochar (PS-VFCW) for treating municipal wastewater contaminated with a range of micropollutants. The addition of a layer of biochar produced from olive industry waste significantly enhanced elimination mechanisms through adsorption and microbial support. The treated wastewater was characterized by a mix of pollutants, including heavy metals, pharmaceutical residues, and antibiotic-resistant bacteria and genes. Elimination efficiency monitored during the treatment period demonstrated the PS-VFCW's ability to adapt to load variations and provide exceptionally high elimination efficiencies of 83.71%, 75.70%, 77.75%, 76.73%, 68.86%, 75.35%, and 61.44% for TSS, COD, total phosphorus, orthophosphates, nitrates, nitrites, and sulfates, respectively. The system also proved highly effective at removing toxic metals, reducing arsenic concentrations from 16.35 to $2.75 \mu\text{g L}^{-1}$, cadmium from 0.0936 to $0.0014 \mu\text{g L}^{-1}$, and lead from 0.6036 to $0.089 \mu\text{g L}^{-1}$, with removal rates of 98% or higher. The system significantly reduced ARGs and indicators by 1–4 orders of magnitude, depending on the targeted microorganism. In addition, complete removal of residual pharmaceutical molecules

(doxycycline, ciprofloxacin, enoxacin, enrofloxacin, norfloxacin, ofloxacin, amoxicillin, and ampicillin) was observed after treatment. The quality of the water treated by PS-VFCW meets Moroccan irrigation standards; the treated effluent could be safely reused for agricultural irrigation, contributing to a circular economy. Therefore, the proposed treatment strategy is a sustainable, environmentally friendly, and nature-based solution that could be scaled up for industrial-scale advanced municipal wastewater treatment, while maintaining the proportions and configurations used in this study.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

CM: Data curation, Investigation, Writing – original draft. YE: Data curation, Investigation, Writing – review and editing. DA: Formal Analysis, Validation, Writing – review and editing. ML: Investigation, Validation, Writing – review and editing. NO: Conceptualization, Supervision, Writing – review and editing. TK: Conceptualization, Validation, Writing – review and editing. AyE: Formal Analysis, Investigation, Writing – review and editing. AmE: Conceptualization, Investigation, Writing – review and editing. AA: Validation, Writing – review and editing. JP: Funding acquisition, Supervision, Writing – review and editing. FA: Funding acquisition, Methodology, Project administration, Supervision, Writing – review and editing.

References

- Ahmad, M., Rajapaksha, A. U., Lim, J. E., Zhang, M., Bolan, N., Mohan, D., et al. (2014). Biochar as a sorbent for contaminant management in soil and water: a review. *Chemosphere* 99, 19–33. doi:10.1016/j.chemosphere.2013.10.071
- Akratos, C. S., and Tsihrintzis, V. A. (2007). Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. *Ecol. Eng.* 29 (2), 173–191. doi:10.1016/j.ecoleng.2006.06.013
- Al Salah, D. M. M., Ngweme, G. N., Laffite, A., Otamonga, J.-P., Mulaji, C., and Poté, J. (2020). Hospital wastewaters: a reservoir and source of clinically relevant bacteria and antibiotic resistant genes dissemination in urban river under tropical conditions. *Ecotoxicol. Environ. Saf.* 200, 110767. doi:10.1016/j.ecoenv.2020.110767
- Almuktar, S. A. A. N., Abed, S. N., and Scholz, M. (2018). Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. *Environ. Sci. Pollut. Res.* 25 (24), 23595–23623. doi:10.1007/s11356-018-2629-3
- Atuga, G., and Jembe, T. (2022). “Constructed wetlands’ application for flower farms wastewater treatment in developing countries: case study in Kenya,” in *Circular economy and sustainability* (Elsevier), 257–269. doi:10.1016/B978-0-12-821664-4.00004-2
- Ayers, R. S., and Westcot, D. W. (1985). “Water quality for agriculture,” in *FAO irrigation and drainage paper 29 Rev. 1*. Rome: Food and Agriculture Organization of the United Nations.
- Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J. L., Harris, E., Robinson, B., and Sizmur, T. (2011). A review of biochars’ potential role in the remediation, revegetation and restoration of contaminated soils. *J. Instrum.* 159 (12), 3269–3282. doi:10.1016/j.envpol.2011.07.023
- Brevelli, A., Carranza-Diaz, O., Rossi, L., and Barry, D. A. (2011). Design methodology accounting for the effects of porous medium heterogeneity on hydraulic residence time and biodegradation in horizontal subsurface flow constructed wetlands. *Ecol. Eng.* 37 (5), 758–770. doi:10.1016/j.ecoleng.2010.04.031
- Całus-Makowska, K., Grosser, A., Grobelak, A., Bialek, H., and Siedlecka, E. (2024). Kinetic study of the simultaneous removal of ibuprofen, carbamazepine, sulfamethoxazole, and diclofenac from water using biochar and activated carbon adsorption, and TiO₂ photocatalysis. *Desalination Water Treat.* 320, 100817. doi:10.1016/j.dwt.2024.100817
- Cha, J. S., Park, S. H., Jung, S.-C., Ryu, C., Jeon, J.-K., Shin, M.-C., et al. (2016). Production and utilization of biochar: a review. *J. Industrial Eng. Chem.* 40, 1–15. doi:10.1016/j.jiec.2016.06.002
- Chand, N., Suthar, S., Kumar, K., and Singh, V. (2022). Removal of pharmaceuticals by vertical flow constructed wetland with different configurations: effect of inlet load and biochar addition in the substrate. *Chemosphere* 307, 135975. doi:10.1016/j.chemosphere.2022.135975
- Chia, C. H., Gong, B., Joseph, S. D., Marjo, C. E., Munroe, P., and Rich, A. M. (2012). Imaging of mineral-enriched biochar by FTIR, Raman and SEM-EDX. *Vib. Spectrosc.* 62, 248–257. doi:10.1016/j.vibspec.2012.06.006
- Christou, A., Agüera, A., Bayona, J. M., Cytryn, E., Fotopoulou, V., Lambropoulou, D., et al. (2017). The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: the knowns and unknowns of the fate of antibiotics and antibiotic resistant bacteria and resistance genes – a review. *Water Res.* 123, 448–467. doi:10.1016/j.watres.2017.07.004
- Cirelli, G. L., Consoli, S., Licciardello, F., Aiello, R., Giuffrida, F., and Leonardi, C. (2012). Treated municipal wastewater reuse in vegetable production. *Agric. Water Manag.* 104, 163–170. doi:10.1016/j.agwat.2011.12.011
- Crombie, K., Mašek, O., Sohi, S. P., Brownsort, P., and Cross, A. (2013). The effect of pyrolysis conditions on biochar stability as determined by three methods. *GCB Bioenergy* 5 (2), 122–131. doi:10.1111/gcbb.12030
- Decamp, O., and Warren, A. (2000). Investigation of *Escherichia coli* removal in various designs of subsurface flow wetlands used for wastewater treatment. *Ecol. Eng.* 14 (3), 293–299. doi:10.1016/S0925-8574(99)00007-5

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Acknowledgements

This work has been carried out on behalf of the CYCLOLIVE-Prima project (ID 1977) and in the context of bilateral Moroccan-Swiss cooperation.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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- Donde, O. O. (2017). Wastewater management techniques: a review of advancement on the appropriate wastewater treatment principles for sustainability. *Environ. Manag. Sustain. Dev.* 6 (1), 40. doi:10.5296/emsd.v6i1.10137
- Dong, H., Qiang, Z., Li, T., Jin, H., and Chen, W. (2012). Effect of artificial aeration on the performance of vertical-flow constructed wetland treating heavily polluted river water. *J. Environ. Sci.* 24 (4), 596–601. doi:10.1016/S1001-0742(11)60804-8
- Ejaz, H., Younas, S., Abosalif, K. O. A., Junaid, K., Alzahrani, B., Alsrhani, A., et al. (2021). Molecular analysis of blaSHV, blaTEM, and blaCTX-M in extended-spectrum β -lactamase producing Enterobacteriaceae recovered from fecal specimens of animals. *PLOS ONE* 16 (1), e0245126. doi:10.1371/journal.pone.0245126
- El Barkaoui, S., Mandi, L., Aziz, F., Del Bubba, M., and Ouazzani, N. (2023). A critical review on using biochar as constructed wetland substrate: characteristics, feedstock, design and pollutants removal mechanisms. *Ecol. Eng.* 190, 106927. doi:10.1016/j.ecoleng.2023.106927
- Fang, Z., Song, H.-L., Cang, N., and Li, X.-N. (2013). Performance of microbial fuel cell coupled constructed wetland system for decolorization of azo dye and bioelectricity generation. *Bioresour. Technol.* 144, 165–171. doi:10.1016/j.biortech.2013.06.073
- Fidel, R. B., Laird, D. A., Thompson, M. L., and Lawrinenko, M. (2017). Characterization and quantification of biochar alkalinity. *Chemosphere* 167, 367–373. doi:10.1016/j.chemosphere.2016.09.151
- Fu, G., Zhou, S., Zhao, Y., Li, Z., Xu, Y., and Guo, Z. (2022). Removal efficiency of heavy metals such as lead and cadmium by different substrates in constructed wetlands. *Processes* 10 (12), 2502. doi:10.3390/pr10122502
- Fujita, S., Yosizaki, K., Ogushi, T., Uechi, K., Takemori, Y., and Senda, Y. (2011). Rapid identification of gram-negative bacteria with and without CTX-M extended-spectrum β -lactamase from positive blood culture bottles by PCR followed by microchip Gel electrophoresis. *J. Clin. Microbiol.* 49 (4), 1483–1488. doi:10.1128/JCM.01976-10
- García, J., Rousseau, D. P. L., Morató, J., Lesage, E., Matamoros, V., and Bayona, J. M. (2010). Contaminant removal processes in subsurface-flow constructed wetlands: a review. *Crit. Rev. Environ. Sci. Technol.* 40 (7), 561–661. doi:10.1080/10643380802471076
- Giácoman-Vallejos, G., Ponce-Caballero, C., and Champagne, P. (2015). Pathogen removal from domestic and swine wastewater by experimental constructed wetlands. *Water Sci. Technol.* 71 (8), 1263–1270. doi:10.2166/wst.2015.102
- Gordillo, R., Córdoba, J. J., Andrade, M. J., Luque, M. I., and Rodríguez, M. (2011). Development of PCR assays for detection of *Escherichia coli* O157:H7 in meat products. *Meat Sci.* 88 (4), 767–773. doi:10.1016/j.meatsci.2011.03.011
- Hamada, M. F., Al-Ghusain, I., and Al-Mutairi, N. Z. (2004). Sand filtration of wastewater for tertiary treatment and water reuse. *Desalination* 164 (3), 203–211. doi:10.1016/S0011-9164(04)00189-4
- Harvey, O. R., Kuo, L.-J., Zimmerman, A. R., Louchouart, P., Amonette, J. E., and Herbert, B. E. (2012). An index-based approach to assessing recalcitrance and soil carbon sequestration potential of engineered Black carbons (Biochars). *Environ. Sci. & Technol.* 46 (3), 1415–1421. doi:10.1021/es2040398
- Headley, T., Nivala, J., Kassa, K., Olsson, L., Wallace, S., Brix, H., et al. (2013). *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: effects of design and plants. *Ecol. Eng.* 61, 564–574. doi:10.1016/j.ecoleng.2013.07.062
- High Commission for Planning (HCP). (2004). General census of population and housing 2004. Kingdom of Morocco.
- High Commission for Planning (HCP). (2014). General census of population and housing 2014. Kingdom of Morocco.
- Ilyas, H., and Masih, I. (2017). The performance of the intensified constructed wetlands for organic matter and nitrogen removal: a review. *J. Environ. Manag.* 198, 372–383. doi:10.1016/j.jenvman.2017.04.098
- Ilyas, H., Masih, I., and Van Hullebusch, E. D. (2020). Pharmaceuticals' removal by constructed wetlands: a critical evaluation and meta-analysis on performance, risk reduction, and role of physicochemical properties on removal mechanisms. *J. Water Health* 18 (3), 253–291. doi:10.2166/wh.2020.213
- Inyang, M., Gao, B., Yao, Y., Xue, Y., Zimmerman, A. R., Pullammanappallil, P., et al. (2012). Removal of heavy metals from aqueous solution by biochars derived from anaerobically digested biomass. *Bioresour. Technol.* 110, 50–56. doi:10.1016/j.biortech.2012.01.072
- Karki, B. K. (2024). Amended biochar in constructed wetlands: roles, challenges, and future directions removing pharmaceuticals and personal care products. *Heliyon* 10 (21), e39848. doi:10.1016/j.heliyon.2024.e39848
- Keilweite, M., Nico, P. S., Johnson, M. G., and Kleber, M. (2010). Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environ. Sci. & Technol.* 44 (4), 1247–1253. doi:10.1021/es9031419
- Kloss, S., Zehetner, F., Dellantonio, A., Hamid, R., Ottner, F., Liedtke, V., et al. (2012). Characterization of slow pyrolysis biochars: effects of feedstocks and pyrolysis temperature on biochar properties. *J. Environ. Qual.* 41 (4), 990–1000–1000. doi:10.2134/jeq2011.0070
- Laishram, D., Kim, S., Lee, S., and Park, S. (2025). Advancements in biochar as a sustainable adsorbent for water pollution mitigation. *Adv. Sci.* 12 (19), 2410383. doi:10.1002/advs.202410383
- Lehmann, J., and Joseph, S. (2015). Biochar for environmental management: science, technology and implementation 0 éd. London, United Kingdom: Routledge. doi:10.4324/9780203762264
- Liu, Z., Xu, Z., Xu, L., Buyong, F., Chay, T. C., Li, Z., et al. (2022). Modified biochar: synthesis and mechanism for removal of environmental heavy metals. *Carbon Res.* 1 (1), 8. doi:10.1007/s44246-022-00007-3
- Malchi, T., Maor, Y., Tadmor, G., Shenker, M., and Chefetz, B. (2014). Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. *Environ. Sci. & Technol.* 48 (16), 9325–9333. doi:10.1021/es5017894
- Méndez, A., Terradillos, M., and Gascó, G. (2013). Physicochemical and agronomic properties of biochar from sewage sludge pyrolysed at different temperatures. *J. Anal. Appl. Pyrolysis* 102, 124–130. doi:10.1016/j.jaap.2013.03.006
- Ministry of Spatial Planning, Water and Environment (Morocco) (2002). *Jt. Order No. 1275-01 Oct. 17, 2002, Establishing Quality Standards Irrigation Water.*
- Mustafa, H. M., and Hayder, G. (2021). Recent studies on applications of aquatic weed plants in phytoremediation of wastewater: a review article. *Ain Shams Eng. J.* 12 (1), 355–365. doi:10.1016/j.asej.2020.05.009
- Odedishemi Ajibade, F., Wang, H.-C., Guadie, A., Fausat Ajibade, T., Fang, Y.-K., Muhammad Adeel Sharif, H., et al. (2021). Total nitrogen removal in biochar amended non-aerated vertical flow constructed wetlands for secondary wastewater effluent with low C/N ratio: microbial community structure and dissolved organic carbon release conditions. *Bioresour. Technol.* 322, 124430. doi:10.1016/j.biortech.2020.124430
- Oron, G., Deveg, A., and Porath, D. (1988). Nitrogen removal and conversion by duckweed grown on waste-water. *Water Res.* 22 (2), 184–184. doi:10.1016/0043-1354(88)90076-0
- Oscar Omondi, D., and Caren Navalía, A. (2021). “Constructed wetlands in wastewater treatment and challenges of emerging resistant genes filtration and reloading,” in *Inland waters—dynamics and ecology*. Editors A. Devlin, J. Pan, and M. Manjur Shah (IntechOpen). doi:10.5772/intechopen.93293
- Ovreås, L., Forney, L., Daae, F. L., and Torsvik, V. (1997). Distribution of bacterioplankton in meromictic Lake saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl. Environ. Microbiol.* 63 (9), 3367–3373. doi:10.1128/aem.63.9.3367-3373.1997
- Panghal, V., Singh, A., Arora, D., and Kumar, S. (2024). Biochar-modified constructed wetlands using *Eclipta alba* as a plant for sustainable rural wastewater treatment. *Environ. Sci. Pollut. Res.* 31 (11), 17299–17310. doi:10.1007/s11356-024-32144-0
- Qiu, M., Liu, L., Ling, Q., Cai, Y., Yu, S., Wang, S., et al. (2022). Biochar for the removal of contaminants from soil and water: a review. *Biochar* 4 (1), 19. doi:10.1007/s42773-022-00146-1
- Ryu, H., Henson, M., Elk, M., Toledo-Hernandez, C., Griffith, J., Blackwood, D., et al. (2013). Development of quantitative PCR assays targeting the 16S rRNA genes of enterococcus spp. and their application to the identification of enterococcus species in environmental samples. *Appl. Environ. Microbiol.* 79 (1), 196–204. doi:10.1128/AEM.02802-12
- Saeed, T., and Sun, G. (2012). A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manag.* 112, 429–448. doi:10.1016/j.jenvman.2012.08.011
- Santos, M., Melo, V. F., Monte Serrat, B., Bonfleur, E., Araújo, E. M., and Cherobim, V. F. (2021). Hybrid technologies for remediation of highly Pb contaminated soil: sewage sludge application and phytoremediation. *Int. J. Phytoremediation* 23 (3), 328–335. doi:10.1080/15226514.2020.1813077
- Sidrach-Cardona, R., Hijosa-Valsero, M., Martí, E., Balcázar, J. L., and Becares, E. (2014). Prevalence of antibiotic-resistant fecal bacteria in a river impacted by both an antibiotic production plant and urban treated discharges. *Sci. Total Environ.* 488–489, 220–227. doi:10.1016/j.scitotenv.2014.04.100
- Spokas, K. A. (2010). Review of the stability of biochar in soils: predictability of O:C molar ratios. *Carbon Manag.* 1 (2), 289–303–303. doi:10.4155/cmt.10.32
- Thevenon, F., Adatte, T., Poté, J., and Spangenberg, J. E. (2012). Recent human-induced trophic change in the large and deep perialpine Lake Lucerne (Switzerland) compared to historical geochemical variations. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 363, 37–47. doi:10.1016/j.palaeo.2012.08.010
- Thevenon, F., de Alencastro, L. F., Loizeau, J. L., Adatte, T., Grandjean, D., Wildi, W., et al. (2013). A high-resolution historical sediment record of nutrients, trace elements and organochlorines (DDT and PCB) deposition in a drinking water reservoir (Lake Brét, Switzerland) points at local and regional pollutant sources. *Chemosphere* 90 (9), 2444–2452. doi:10.1016/j.chemosphere.2012.11.002
- Thorslund, J., Jarsjö, J., Jaramillo, F., Jawitz, J. W., Manzoni, S., Basu, N. B., et al. (2017). Wetlands as large-scale nature-based solutions: status and challenges for research, engineering and management. *Ecol. Eng.* 108, 489–497. doi:10.1016/j.ecoleng.2017.07.012
- Tomczyk, A., Sokołowska, Z., and Boguta, P. (2020). Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. *Rev. Environ. Sci. Bio/Technology* 19 (1), 191–215. doi:10.1007/s11157-020-09523-3

- Van Biervliet, O., McInnes, R. J., Lewis-Phillips, J., and Tosney, J. (2020). Can an integrated constructed wetland in Norfolk reduce nutrient concentrations and promote *in situ* bird species richness? *Wetlands* 40 (5), 967–981. doi:10.1007/s13157-019-01247-7
- Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. *Sci. Total Environ.* 380 (1–3), 48–65. doi:10.1016/j.scitotenv.2006.09.014
- Vymazal, J. (2011). Constructed wetlands for wastewater treatment: five decades of experience. *Environ. Sci. & Technol.* 45 (1), 69–69. doi:10.1021/es101403q
- Vymazal, J. (2018). Does clogging affect long-term removal of organics and suspended solids in gravel-based horizontal subsurface flow constructed wetlands? *Chem. Eng. J.* 331, 663–674. doi:10.1016/j.cej.2017.09.048
- Vymazal, J. (2022). The historical development of constructed wetlands for wastewater treatment. *Land* 11 (2), 174. doi:10.3390/land11020174
- Wang, G., Yu, G., Chi, T., Li, Y., Zhang, Y., Wang, J., et al. (2022). Insights into the enhanced effect of biochar on cadmium removal in vertical flow constructed wetlands. *SSRN Electron. J.* doi:10.2139/ssrn.4165247
- Weber, K., and Quicker, P. (2018). Properties of biochar. *Fuel* 217, 240–261. doi:10.1016/j.fuel.2017.12.054
- Wießner, A., Kappelmeyer, U., Kusch, P., and Kästner, M. (2005). Influence of the redox condition dynamics on the removal efficiency of a laboratory-scale constructed wetland. *Water Res.* 39 (1), 248–256. doi:10.1016/j.watres.2004.08.032
- World Health Organization (WHO) (2006). “Guidelines for the safe use of wastewater, excreta and greywater,” in *Wastewater use in agriculture*, Volume 2. Geneva: WHO Press.
- Xi, C., Zhang, Y., Marrs, C. F., Ye, W., Simon, C., Foxman, B., et al. (2009). Prevalence of antibiotic resistance in drinking water treatment and distribution systems. *Appl. Environ. Microbiol.* 75 (17), 5714–5718. doi:10.1128/AEM.00382-09
- Xiao, X., Chen, B., Chen, Z., Zhu, L., and Schnoor, J. L. (2018). Insight into multiple and multilevel structures of biochars and their potential environmental applications: a critical review. *Environ. Sci. & Technol.* 52 (9), 5027–5047. doi:10.1021/acs.est.7b06487
- Yu, G., Huang, J., Chen, H., Chen, J., Ge, S., Liu, J., et al. (2023). Recent advances in the effects of biochar on constructed wetlands: treatment performance and microorganisms. *Separations* 10 (12), 593. doi:10.3390/separations10120593
- Zhang, H., Voroney, R. P., and Price, G. W. (2015). Effects of temperature and processing conditions on biochar chemical properties and their influence on soil C and N transformations. *Soil Biol. Biochem.* 83, 19–28. doi:10.1016/j.soilbio.2015.01.006
- Zurita, F., and Carreón-Álvarez, A. (2015). Performance of three pilot-scale hybrid constructed wetlands for total coliforms and *Escherichia coli* removal from primary effluent – a 2-year study in a subtropical climate. *J. Water Health* 13 (2), 446–458. doi:10.2166/wh.2014.135