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Biochar and zero-valent iron sand filtration simultaneously removes contaminants of emerging concern and *Escherichia coli* from wastewater effluent

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Abstract

Advanced treated municipal wastewater is an important alternative water source for agricultural irrigation. However, the possible persistence of chemical and microbiological contaminants in these waters raise potential safety concerns with regard to reusing treated wastewater for food crop irrigation. Two low-cost and environmentally-friendly filter media, biochar (BC) and zero-valent iron (ZVI), have attracted great interest in terms of treating reused water. Here, we evaluated the efficacy of BC-, nanosilver-amended biochar- (Ag-BC) and ZVI-sand filters, in reducing contaminants of emerging concern (CECs), *Escherichia coli* (*E. coli*) and total bacterial diversity from wastewater effluent. Six experiments were conducted with control quartz sand and sand columns containing BC, Ag-BC, ZVI, BC with ZVI, or Ag-BC with ZVI. After filtration, Ag-BC, ZVI, BC with ZVI and Ag-BC with ZVI demonstrated more than 90% (> 1 log) removal of *E. coli* from wastewater samples, while BC, Ag-BC, BC with ZVI and Ag-BC with ZVI also demonstrated efficient removal of tested CECs. Lower bacterial diversity was also observed after filtration; however, differences were marginally significant. In addition, significantly ($p < 0.05$) higher bacterial diversity was observed in wastewater samples collected during warmer versus colder months. Leaching of silver ions occurred from Ag-BC columns; however, this was prevented through the addition of ZVI. In conclusion, our data suggest that the BC with ZVI and Ag-BC with ZVI sand filters, which demonstrated more than 99% removal of both CECs and *E. coli* without silver ion release, may be effective, low-cost options for decentralized treatment of reused wastewater.

Highlights

- The efficacy of BC, Ag-BC, and ZVI sand filtration, and their combinations, in removing contaminants from reused water was evaluated.
- Ag-BC, ZVI, BC with ZVI and Ag-BC with ZVI demonstrated > 90% removal of *E. coli*.
- BC, Ag-BC, BC with ZVI and Ag-BC with ZVI demonstrated efficient removal of selected contaminants of emerging concern.

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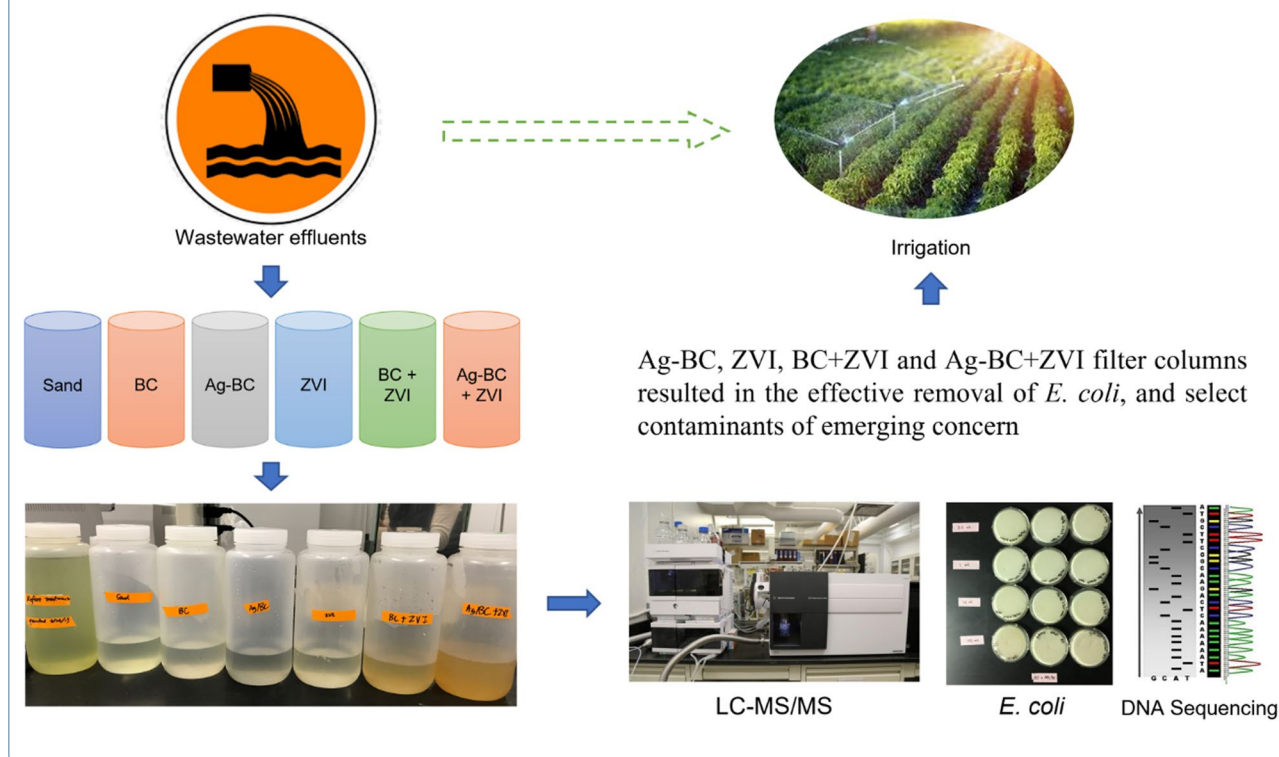
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Keywords Biochar, Zero-valent iron, Wastewater reuse, Contaminants of emerging concern, Recycled water, *E. coli*

Graphical Abstract



1 Introduction

Water scarcity is a critical issue currently affecting almost two-thirds of the global population (Mekonnen and Hoekstra 2016). Agricultural use of water accounts for more than 70% of global freshwater withdrawals worldwide (Dieter et al. 2018). Moreover, both irrigation water withdrawals and irrigated acreage increased by about 2% between 2010 and 2015 (Dieter et al. 2018), and Warziniack et al. (2022) recently predicted imminent increases in irrigation water use in the United States due to climate change. To meet expanding agricultural water demand, recycled wastewater is increasingly used to irrigate both food and non-food crops and landscape plants worldwide (Sapkota 2019; Wang et al. 2017). Utilizing recycled water in agricultural and landscape application has several associated benefits: reduction of pressure on freshwater sources; reduction of synthetic fertilizer use due to the presence of nutrients; and higher yields (among some crop types) compared to their freshwater-irrigated counterparts (Intriago et al. 2018). Therefore, recycled water has become one of the major, cost-effective alternative water sources for agricultural and landscape irrigation in

many regions worldwide. For instance, the Dan Region Reclamation Project in Israel can reclaim some 110–130 million cubic meters of wastewater effluent for unrestricted irrigation annually (Dillon 2002; Oren et al. 2007).

Nevertheless, the agricultural use of recycled water that is not adequately treated can pose potential food safety, public health and environmental risks (particularly in the case of vegetables eaten raw) due to the persistence of pathogens, heavy metals, and contaminants of emerging concern (CECs) (Chen et al. 2013; Panthi et al. 2019). In addition, recycled water used in the landscaping of parks, playgrounds and schoolyards may also result in exposure risks via direct human contact. As an important indicator of fecal contamination, *E. coli* has been used by many organizations to establish guidelines and standards for irrigation water quality. The World Health Organization (WHO) irrigation water guidelines recommend minimum monitoring parameters of no more than 1000 colony forming units (CFU) of *E. coli* per 100 mL for that used on root crops and drip irrigation of low-growing crops, no more than 10,000 CFU 100 mL⁻¹ for that used

on leaf crops and no more than 100,000 CFU 100 mL⁻¹ for drip irrigation of high-growing crops (WHO 2013). In addition, many countries have adopted *E. coli* based water quality guidelines for irrigation water applied to food crops (Table 1). Moreover, recent environmental toxicology and pharmacology studies suggest that chronic exposures to contaminants of emerging concern (CECs) (e.g., pharmaceuticals and personal care products (PPCPs), and endocrine-disrupting chemicals (EDCs)) may lead to long-term health risks (Ma et al. 2018). However, the specific risks associated with exposures to very low levels of these contaminants in recycled irrigation water remain unclear.

Nevertheless, to reduce the potential of such health risks, multiple treatment technologies are being employed to treat wastewater prior to reuse. Biochar (BC) is a carbon-rich solid derived from pyrolyzing biomass with little or no oxygen (Weber and Quicker 2018). As a potentially low-cost and environmentally-friendly alternative for water treatment, considerable research has been conducted on biochar-based materials for the removal of aqueous contaminants including chemicals and pathogens (Gwenzi et al. 2017). For example, Tong et al. (2019) recently reported that biochar is capable of adsorbing CECs in wastewater effluents. Moreover, Kaetzl et al. (2019) used on-farm biochar-based biofilters to remove pathogens from wastewater and Mohanty et al. (2013 and 2014) studied the efficacy of biochar in removing indicator bacteria during biofiltration of stormwater (Mohanty et al. 2013; Mohanty and Boehm 2014). As a strategy to improve the biocidal potency of biochar, members of our group recently developed a method to embed silver into biochar through its electron

storage capacity (ESC) (Xin et al. 2019 and 2020; Xin and Chiu 2020). Using wood-derived biochar with an ESC of 4.0 mmol g⁻¹, we produced silver-amended biochar (Ag-BC) that contained 27% nano-silver by mass (Xin et al. 2020; Xin and Chiu 2020). In preliminary tests, the Ag-BC was markedly more effective at inactivating *E. coli* than unmodified biochar (unpublished data, Figure S1).

In addition to biochar, zero-valent iron (ZVI) is another environmentally-friendly material that has shown promise in removing viruses, bacteria and CECs from surface and recycled waters (Kulkarni et al. 2019; Marik et al. 2019; Perini et al. 2014). We hypothesized that the combination of biochar-based materials and ZVI may work synergistically to simultaneously remove both chemical and microbial pollutants from recycled irrigation water, potentially representing a cost-effective and sustainable, farm-based solution to the irrigation water dilemma described above. To test this hypothesis, a laboratory-scale study was conducted to assess the effectiveness of biochar- and ZVI-sand filters (individually and in combination) in simultaneously removing CECs (e.g., atrazine, azithromycin, caffeine, ciprofloxacin, triclocarban and vancomycin) and bacteria (*E. coli* and total bacterial diversity) from wastewater effluent.

2 Materials and methods

2.1 Chemicals and reagents

All of the standard chemicals and their reference compounds were purchased from Toronto Research Chemicals (TRC, Ontario, Canada). Silver nitrate (purity > 99.9%) and sodium hydroxide (50% w/w) were purchased from Alfa Aesar (Haverhill, MA), respectively. Nitric Acid (HNO₃) (TraceMetal Grade) from Fisher

Table 1 World Health Organization and country-specific regulatory standards for fecal indicator bacteria in irrigation water

Country	<i>E. coli</i> criteria: colony forming units CFU 100 mL ⁻¹	References
WHO	Root crops and drip irrigation of low growing crops: ≤ 1000 Leaf crops: ≤ 10,000 Drip irrigation of high-growing crops: ≤ 100,000	(WHO 2013)
USA	Geometric mean ≤ 126 Statistical threshold value ≤ 410 Sprout irrigation: no detection	(FDA 2021)
EU	Class A (all food crops): ≤ 10 or below the detection limit Class B (food crops consumed raw, processed food crops and non-food crops): ≤ 100 Class C (food crops consumed raw, processed food crops and non-food crops): ≤ 1000 Class D (industrial, energy, and seeded crops): ≤ 10,000	(Rizzo et al. 2018)
Greece	Restricted irrigation: weekly median < 200 Unrestricted irrigation: < 5 (80% of samples), < (95% of samples)	(Shoushtarian and Negahban-Azar 2020)
France	< 250	(Becerra-Castro et al. 2015)
Italy	< 100	(Becerra-Castro et al. 2015)
Spain	< 100	(Becerra-Castro et al. 2015)
Jordan	< 100	(Becerra-Castro et al. 2015)
South Korea	No detection	(Jeong et al. 2016)

Chemical was used. All solvents (chromatographic grade) and chemicals were used as received from the commercial suppliers. All standard stock solutions were stored at -20°C .

Quartz sand (AGSCO Corporation, Pine Brook, NJ) was pre-sieved to a particle size range of 177–595 μm when purchased. The quartz sand was reported to contain 99.5% SiO_2 with a small amount (0.05%, w/w) of iron hydroxides (AGSCO Corporation, 2016). The biochar used in this study was Soil Reef Biochar (Soil Reef LLC, Berwyn, PA), which was produced through pyrolysis of Southern Yellow hardwood chips at 550°C . The properties of the biochar, including porosity, skeletal density, BET surface area, cation exchange capacity, and electron storage capacity, have been characterized in previous studies (Yi 2018; Saquing et al. 2016; Xin et al. 2020, 2019) and are summarized in Additional file 1: Table S1. The biochar used in this study was Soil Reef Biochar (Soil Reef LLC, Berwyn, PA), which was produced from pyrolyzed wood residues (Soil Reef LLC). Cast iron aggregate (zero-valent iron (ZVI)) was purchased from Peerless Metal Powders and Abrasives, Detroit, MI.

2.2 Site description and sample collection

Wastewater effluent samples were collected from a tertiary wastewater treatment plant in the Mid-Atlantic region that primarily received residential wastewater. Since the PPCPs of the selected site were at the highest concentrations among all of the tested wastewater treatment plants in our previous work (Panthi et al. 2019), the wastewater effluents from this site were chosen for the column treatments in the present study. The treatment processes at this plant are as follows: (1) primary treatment: screens, grinders and grit chamber; (2) secondary treatment: activated sludge reactor/aeration tank and sedimentation tank/secondary clarifier; and (3) tertiary treatment: chlorine or ultraviolet (UV) disinfection. Chlorine is applied from March to November and the resulting chlorinated effluent is utilized for groundwater recharge via spray irrigation of rye grass fields. In the winter months, UV disinfection is used instead of chlorine, and the resulting effluent is discharged into a surface water body.

We completed six individual sampling events from February to May 2019. One sample was collected in February when UV disinfection was used and the other five were collected during the period in which chlorination was used for disinfection. The effluent was collected from a spigot into a 20 L Nalgene[®] polypropylene bottle (Thermo Fisher Scientific, Waltham, MA, USA). Water was allowed to run for at least 1 min before sample collection. Environmental parameters (Additional file 1: Table S2) were measured during sampling using a ProDSS

digital sampling system (YSI, Yellow Springs, OH, USA). Immediately after sample collection, the water samples were quenched with 1 mL 10% sodium thiosulfate per 1 L of wastewater effluent (Alfa Aesar, Heysham, England), in accordance with EPA method 1604 (US EPA, 2002). Bottles were capped and immediately transferred to coolers containing icepacks for the following filtration experiments.

2.3 Filter column preparation

The quartz sand was chemically treated with 0.3 M sodium citrate and 0.1 M sodium dithionite solution to remove iron hydroxides which could affect the inertness of the sand (Mehra and Jackson 1958). Soil Reefed Biochar (BC) was sieved to the 250–500 μm particle size range. ZVI was sieved to the same particle size range as BC (250–500 μm). Silver-amended biochar (Ag-BC) was made by loading Ag⁺ ions from AgNO_3 onto Reduced Soil-Reefed Biochar in an anaerobic glove box ($2.0 \pm 0.5\%$ H_2 in 98% N_2 , $\text{PO}_2 < 25$ ppm, Coy, MI). The silver loading on all Ag-BC used in this study was 1.0 mmol g^{-1} (Xin et al. 2020; Xin and Chiu 2020).

All filter columns were acrylic and 113 mL in capacity and were fabricated by the College of Engineering Machine Shop at the University of Delaware. As shown in Fig. 1, each experimental column contained the granular medium/media to be evaluated (BC, Ag-BC, ZVI, BC with ZVI or Ag-BC with ZVI) sandwiched between two layers of cleaned sand. Each treatment medium layer consisted of a uniform 1:1 (v/v) mixture of the treatment material and sand. Glass wool was used on both ends of each column to stabilize and evenly distribute water flow and to prevent media particles from leaving the column on the effluent end. A control column was similarly prepared that contained cleaned sand only.

The mass of materials in each column was chosen to ensure the same retention time ($t=0.8$ min) for each treatment medium layer (Fig. 1). This was determined by measurement of the bulk densities and porosity of each treatment material (and sand) to ensure the same volumes of treatment media in each column.

2.4 Column treatment procedure

Each column was first degassed with a continuous flow of CO_2 for at least 20 min to eliminate oxygen and fill the column with CO_2 . A peristaltic pump (Cole-Parmer Masterflex Peristaltic Tubing Pumps L/S series) was used to control water flow rates. CO_2 was removed by flushing the column with 1 mM NaOH solution for three pore volumes at a low flow rate of 10 mL min^{-1} . Wastewater samples were then passed through each column at a constant flow rate of 25 mL min^{-1} , for a total of six pore volumes. The first three pore volumes of column effluents

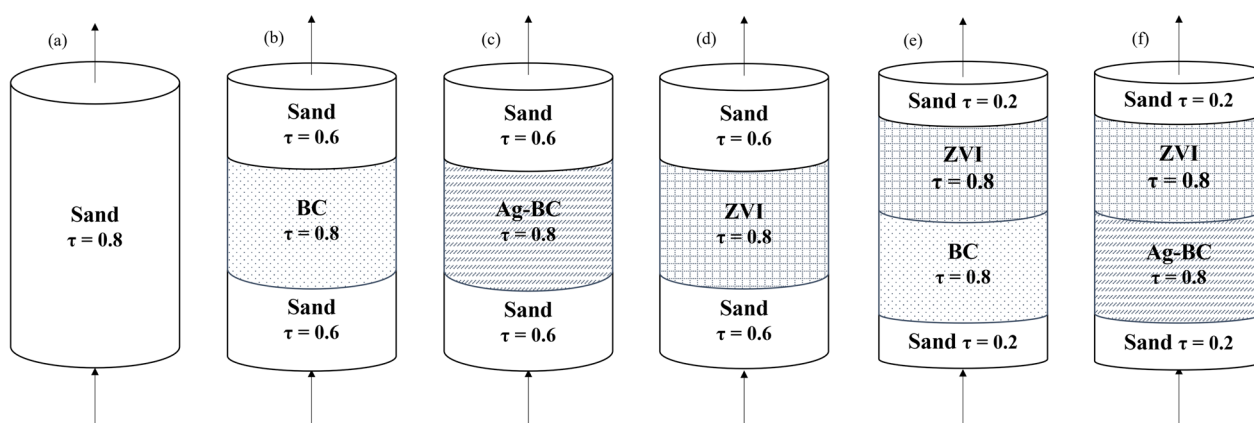


Fig. 1 Filter column setups: **a** Control column: sand; **b** Biochar (BC) sand column; **c** Nanosilver amended biochar (Ag-BC) sand column; **d** Zero valent iron (ZVI) sand column; **e** BC with ZVI sand column (BC + ZVI); **f** Ag-BC with ZVI column (Ag-BC + ZVI)

were discarded, and treated samples were then collected in separate 1L Nalgene[®] polypropylene bottles from each column and split into two portions (sub-samples) for *E. coli*, bacterial diversity and chemical analysis. The untreated and treated sub-samples were analyzed for *E. coli* and total bacterial communities immediately after treatments, while the rest of the sub-samples for chemical analysis were frozen at -80°C until analysis.

2.5 *E. coli* analysis

E. coli was chosen as a model microorganism because it is one of the most commonly used indicator organisms to infer the presence of fecal contamination in water (Holcomb and Stewart 2020). The control, original wastewater effluents (untreated samples) and filter-treated samples were enumerated for *E. coli* by standard membrane filtration according to EPA Method 1604 (*E. coli* and TC). Briefly, tenfold dilutions of each water sample were prepared, and 10 mL of each dilution (representing 0.1 mL, 1 mL, and 10 mL of each sample), and 100 mL of each original water sample, were filtered through $0.45\ \mu\text{m}$, 47 mm cellulose ester membrane filters (Pall Corporation, Ann Arbor, MI, USA). Membrane filters were aseptically placed onto MI agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for the quantification of *E. coli*. Plates were incubated at 35°C for 24 h and then counted. Blue colonies under ambient light were recorded as *E. coli* (Allard et al. 2019).

2.6 Bacterial community profiling with 16S rRNA gene sequencing

Untreated and filter-treated wastewater samples were immediately filtered through $0.2\ \mu\text{m}$, 47 mm filters (Pall Corporation, Port Washington, NY, USA) using sterile filter funnels (Thermo Fisher Scientific, Waltham,

MA, USA). All of the filters were transferred aseptically into lysing matrix B tubes (MP Biomedicals, Solon, OH, USA). The tubes were stored at -80°C until DNA extraction was performed. Total DNA was extracted from the stored filters in lysing matrix tubes following previously published protocols (Chopyk et al. 2017). Briefly, after adding 1000 μl of ice-cold 1X PBS buffer, samples underwent enzymatic and mechanical lysis of cells. Then, the samples were purified using the QIAamp DSP DNA mini kit 50, v2 (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. The V3V4 hypervariable region of the 16S rRNA gene was then PCR-amplified and sequenced on an Illumina HiSeq using a dual-indexing method developed at the Institute for Genome Sciences (Chopyk et al. 2017).

Quality sequences (demultiplexed, chimera trimmed, high quality and read length) from QIIME were clustered de-novo using VSEARCH and taxonomies were assigned using the Greengenes database (at 97% confidence threshold). Statistical analysis of sequencing reads was performed in R Statistical computing software (v.0.99.473) using package phyloseq (v. 1.19.1) (McMurdie and Holmes 2013), vegan (v. 2.4.5) (Bu et al. 2020), MetagenomeSeq (v. 1.16.0) (Paulson et al. 2013). Statistical tests were carried out using ANOVA (analysis of variance) and Tukey's HSD (Honestly Significant Difference) post hoc test on a 95% confidence level to measure variation among the samples within each group. Values of p less than 0.05 were considered statistically significant. Beta diversity was estimated using vegan v. 2.4.5 and phyloseq packages. Diversity was calculated using Principal Coordinate Analysis (PCoA) on Bray–Curtis dissimilarity. Distances were tested for significance using ANOSIM (analysis of similarities on 999 permutations) tests between groups of samples. Reads

were visualized using the ggplot2 package (v. 2.2.1). A total of 1,269,115 reads were obtained from 42 samples. The average number of reads per sample was 30,217.02 ($\pm 28,129.36$). Goods coverage index was computed, and all the samples were above a 0.95 cutoff value.

2.7 Sample preparation and LC–MS/MS analysis for organic CECs

Frozen samples were allowed to thaw overnight at room temperature. 200 mL aliquots of thawed samples were extracted as previously described (Sapkota et al. 2007). In accordance with our previous study (Panthi et al. 2019), we chose six organic CECs (atrazine, azithromycin, caffeine, ciprofloxacin, triclocarban and vancomycin), which were frequently detected at high concentrations at the tested wastewater treatment plant. After spiking samples with a $10 \mu\text{g mL}^{-1}$ internal standard mixture, the samples were extracted using Oasis HLB (500 mg) cartridges (Waters Corp; Milford MA) that were conditioned with 5 mL of methanol, followed by 5 mL of HPLC grade water. After loading the samples, the cartridges were eluted with 5 mL of a 50:50 methanol/acetone mix followed by 3 mL of methanol with 0.1% formic acid. The extracts were dried under gentle nitrogen flow at 45°C and reconstituted with 1 mL of a 90:10 Water: Methanol mixture and transferred to 1.5 mL autosampler vials.

The extracted samples were analyzed using an Agilent 1290 Infinity II HPLC coupled with an Agilent 6470 QQQ triple-quadrupole mass spectrometer (MS/MS). Details regarding the instrumental conditions have been described previously (Panthi et al. 2019).

2.8 ICP-MS analysis of silver and iron

The original wastewater effluents (untreated samples) and filter-treated samples were acidified for silver and iron analysis through the addition of 0.25 mL of 15.8 M HNO_3 to a 200 mL sample to dissolve metal oxides and aggregates, if present. This brought the pH of all samples to between 2.4 and 2.7. A 0.5-mL acidified sample was then placed in a 15 mL centrifuge tube containing 4.5 mL of 2% HNO_3 (i.e., tenfold diluted). The mixture was passed through a $0.22\text{-}\mu\text{m}$ filter into a 15 mL centrifuge tube. These acidified, diluted, and filtered samples were then analyzed using an Agilent 7500 inductively coupled plasma mass spectrometer (ICP-MS) for silver ions (Xin et al. 2020). The same procedure was used for iron analysis except that the dilution factor was 20.

3 Results

3.1 Water quality of wastewater effluents

Concentrations of tested CECs and *E. coli* in original wastewater effluents (unfiltered samples) are shown in

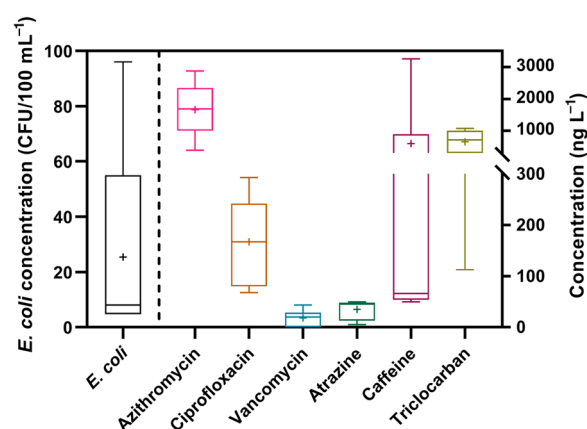


Fig. 2 Concentrations of *E. coli* and contaminants of emerging concern (CECs) in original wastewater effluents

Fig. 2. Notably, azithromycin was detected at an average concentration higher than 1000 ng L^{-1} . Ciprofloxacin and vancomycin were also found in wastewater effluents with average concentrations of 167.5 and 18.0 ng L^{-1} , respectively. Atrazine was found in the wastewater effluents at an average concentration of 34.9 ng L^{-1} while triclocarban was detected at an average concentration of 659.9 ng L^{-1} . The presence of *E. coli* in original wastewater effluent samples (untreated) ranged from 5 to $96 \text{ CFU } 100 \text{ mL}^{-1}$ with a mean concentration of $26 \text{ CFU } 100 \text{ mL}^{-1}$.

3.2 Removal of CECs

The average removal efficiencies of the six CECs from wastewater effluent samples are shown in Fig. 3. Removal efficiencies by the control sand column were generally low, ranging from 2.5 to 47.5%. Meanwhile, the ZVI columns performed better than sand alone, but poorer than the other column treatments. The other four columns demonstrated high removal efficiencies with more than 99% removal of CECs from the wastewater effluent samples. However, the removal efficiencies of triclocarban by the six columns were all below 90%, relatively lower than that of the other compounds.

3.3 Removal of *E. coli*

The removal efficiencies of *E. coli* in wastewater effluents by the five filter columns compared to that of the sand control column are shown in Fig. 4. Four columns, Ag-BC, ZVI, BC+ZVI and Ag-BC+ZVI, achieved more than 90% removal of *E. coli*. In contrast, the removal efficiency of the biochar column was lower, approximately 60%, only slightly higher than that of the sand column. Levels of *E. coli* in the treated wastewater effluents were reduced to $<10 \text{ CFU}/100 \text{ mL}$ by Ag-BC, ZVI, BC+ZVI and Ag-BC+ZVI.

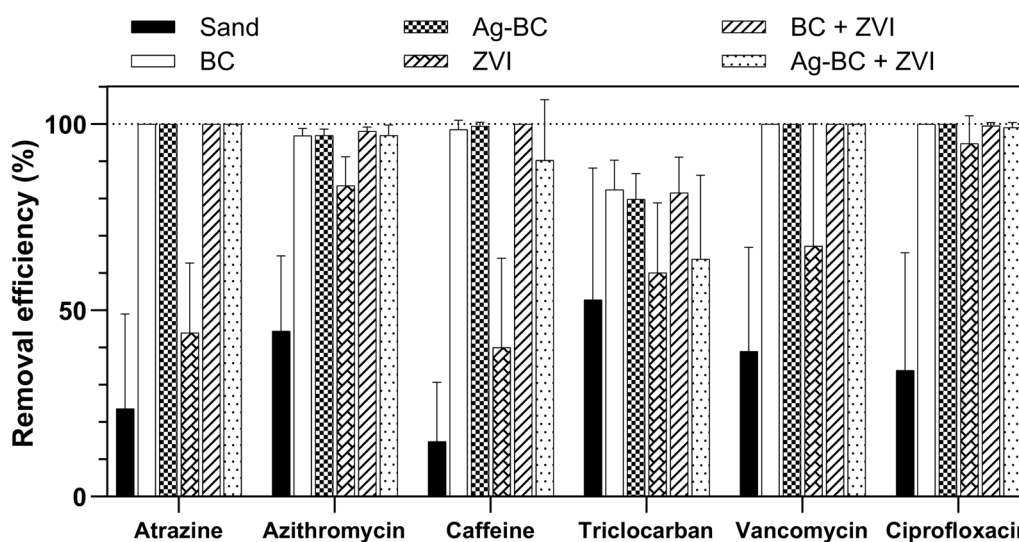


Fig. 3 Average removal efficiencies of CECs from wastewater effluents by the five different column setups compared to the control sand column

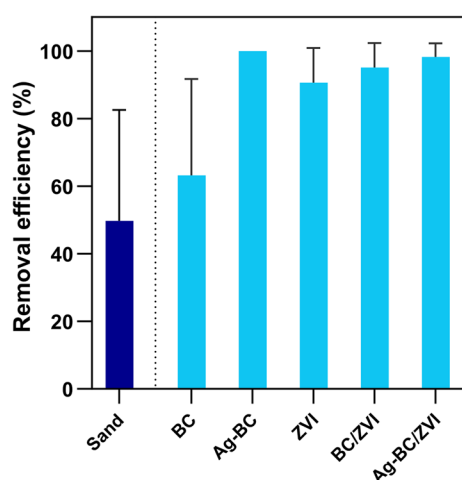


Fig. 4 Removal efficiencies of *E. coli* from wastewater effluents by five different filter columns compared to the control sand column. BC biochar, Ag-BC nanosilver amended biochar, ZVI zero-valent iron, BC + ZVI biochar in combination with zero-valent iron, Ag-BC + ZVI nanosilver amended biochar in combination with zero-valent iron

3.4 Changes in bacterial diversity and community composition

Alpha diversity measures (the Observed number of species and Shannon diversity index) demonstrated a significant effect (ANOVA p -value < 0.05) of the various filter treatments on bacterial diversity (Fig. 5a). Specifically, the difference in Shannon indices between untreated samples and a few filter-treated samples were marginally significant: BC treated (p =0.07), ZVI treated (p =0.08), Ag-BC + ZVI treated (p =0.06), and BC + ZVI (p =0.07).

In addition, time of water sampling had a significant effect (p -value < 0.05) on bacterial alpha diversity. Overall, alpha diversity gradually increased from February to late May. Beta diversity analysis on Bray–Curtis dissimilarity distances showed significant temporal variations in bacterial diversity (ANOSIM R =0.69; p -value < 0.001) (Fig. 5b) rather than variations attributed to different treatments.

3.5 Iron and silver ions in treated samples

The total concentrations of silver in the filter-treated samples are shown in Fig. 6a. Silver was not detected in original wastewater samples or any filter-treated samples except those from columns containing only Ag-BC. However, the Ag-BC + ZVI columns exhibited minimal silver concentrations in the filter-treated samples.

The total concentrations of iron in the original wastewater effluent and filter-treated samples were also measured, as shown in Fig. 6b. The original wastewater effluents (i.e., column influent) had a relatively high Fe content in the February samples, approximately ten times the secondary drinking water standard (5.4 μ M) in the United States. However, the iron concentration decreased precipitously after chlorination began in the wastewater treatment plants from March. In treated samples from columns containing ZVI (i.e., ZVI, BC + ZVI, Ag-BC + ZVI columns), however, elevated levels of iron on the order of a few hundred μ M were observed.

4 Discussion

The potential presence of bacteria and CECs (e.g., antimicrobials and pesticides) in reused wastewater may pose environmental, food safety and public health

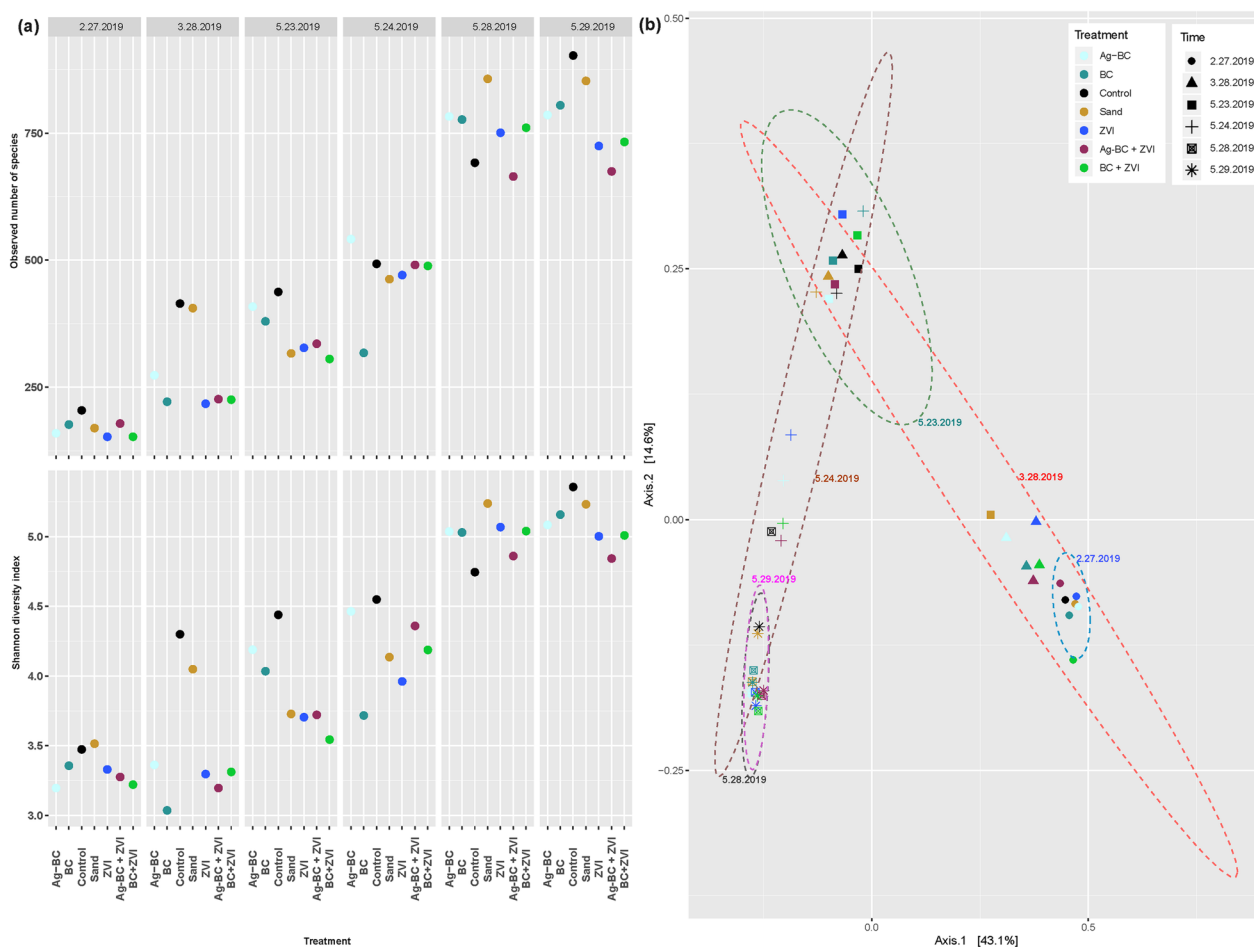


Fig. 5 Diversity analysis and relative abundance of bacterial communities present in the untreated and filter-treated water samples. **a** Alpha diversity plots showing Observed number of species and Shannon diversity indices between untreated and filter-treated samples. **b** Principal coordinate plot of beta diversity based on Bray Curtis indices

risks if these waters are used for agricultural irrigation. However, low-cost, environmentally-friendly filter technologies that incorporate materials such as biochar and zero-valent iron could effectively remove these contaminants. Here, we compared the efficacy of five types of ZVI- and BC-sand filters in reducing concentrations of CECs (azithromycin, ciprofloxacin, vancomycin, atrazine, caffeine and triclorcarban) and bacteria in wastewater effluents from a treatment plant that was previously surveyed as part of a longitudinal water quality study conducted by members of our CONSERVE Center (<http://conservewaterforfood.org/>) (Chopyk et al. 2020; Malayil et al. 2021; Panthi et al. 2019; Solaiman and Micallef 2021; Zhu et al. 2021). With the removal of CECs and *E. coli* to under detection limit, our data suggest that two types of sand filters, one containing BC with ZVI and the other containing Ag-BC with ZVI, may potentially be effective

and low-cost options for decentralized wastewater treatment.

Specifically, the biochar filter column demonstrated more than 99% removal of atrazine, azithromycin, caffeine, vancomycin and ciprofloxacin from the wastewater effluents and the highest removal rate of triclorcarban comparing to the other filter columns. While this result was consistent with previous reports of biochar being a good adsorbent material regarding heavy metals and organic pollutants (Gwenzi et al. 2017), biochar alone showed low efficacy in removing *E. coli* compared to other columns. Many earlier studies have found that biochar type can largely impact *E. coli* removal capacities (Guan et al. 2020; Mohanty et al. 2014), which may explain why we observed low removal efficiencies of *E. coli* by BC filtration in the present study.

In contrast to the biochar sand filter columns, the ZVI column demonstrated higher removal of *E. coli*, as was reported in our previous studies (Kulkarni et al. 2019;

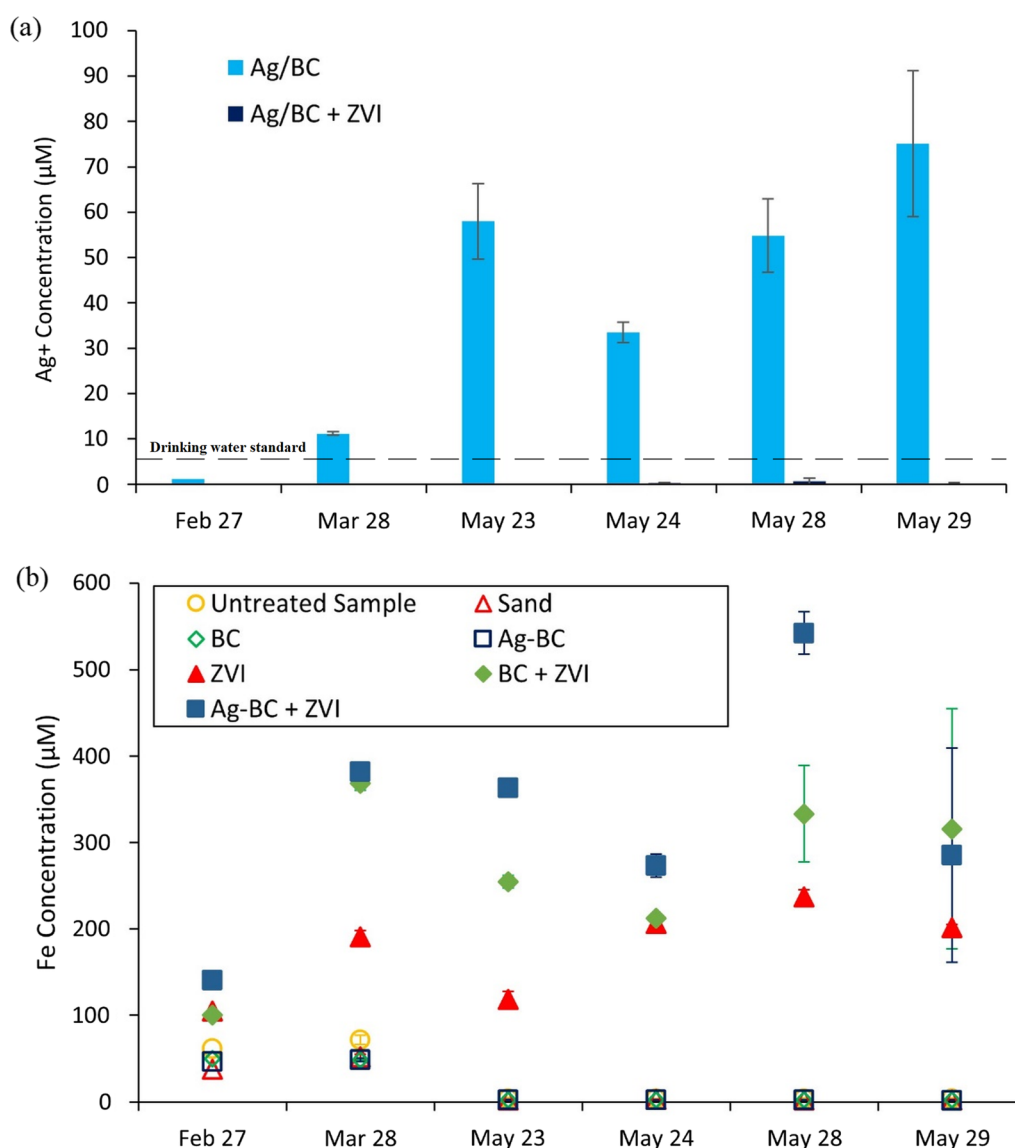


Fig. 6 **a** Silver concentrations in effluents from Ag-BC and Ag-BC + ZVI filter columns, for all six experiments carried out. Other columns with no silver as part of the treatment material had no silver ions in their effluents (data not shown). **b** Iron concentration in effluents from all five columns and the sand only control column, for all six experiments. Error bars are for duplicate analysis of the samples

Marik et al. 2019). However, the ZVI column showed a relatively lower rate of removal of the selected CECs than other columns. This was probably due to a lower adsorption capacity of organic adsorbates. Other groups have reported that ZVI can oxidize some organic pollutants, including antibiotics (quinolones, sulfonamides, and tetracyclines) and triclosan in contaminated water (Wu et al. 2020). In our previous study, where a commercial biosand filter (HydrAid® BioSand Water Filter, Native-Energy, Burlington, VT, USA) was adapted through the addition of ZVI to achieve a 50:50 sand/ZVI mixture (with a particle size range of 400 to 625 µm), we observed

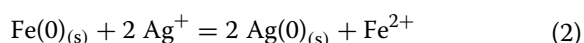
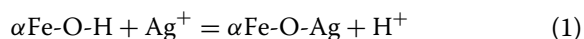
significant reductions in the concentrations of azithromycin, ciprofloxacin, oxolinic acid, penicillin G, sulfamethoxazole, linezolid, pipemidic acid and vancomycin (Kulkarni et al. 2019). Evaluating the mechanisms driving contaminant removal was beyond the scope of the studies of Kulkarni et al. (2019); however, based on other previous reports, the reduction of organic contaminants by ZVI could occur through reductive or oxidative degradation, coagulation, sedimentation or adsorption (Fu et al. 2014; Sun et al. 2016).

With the above-mentioned attributes of both ZVI- and BC-sand based filters, our composite BC + ZVI column

demonstrated higher removal capacities (than ZVI or BC columns alone) for both the selected CECs and *E. coli*. This was potentially due to a greater adsorption capacity of BC for the selected CECs via the combined column, as well as a higher removal efficacy of *E. coli* by the ZVI, in the combined BC + ZVI column.

The Ag-BC column demonstrated similar removal efficiencies of CECs compared to the BC column. This was potentially due to the significant Brunauer–Emmett–Teller (BET) surface areas of both BC and Ag-BC (165 and 94 m²g⁻¹, respectively) (Xin et al. 2020), resulting in comparable adsorption rates for the tested organic micropollutants. Furthermore, the Ag-BC column was the most effective in removing *E. coli* from wastewater effluents, with removal to no detection of *E. coli* for all time points. This may be attributed to Ag⁺ produced from the nano-silver within the Ag-BC column during wastewater filtration that can render bacteria including *E. coli* either nonculturable or inactivated (Woo et al. 2008). The antibacterial activity of Ag⁺ has also been shown during the treatment of drinking water with a chitosan-coated biochar filter combined with a nanosilver composite (Hu et al. 2019).

Although the addition of silver in biochar composite filters used to treat water demonstrates promising antibacterial activity, the release of Ag⁺ in irrigation water may pose a potential risk of secondary contamination. There are no strict governmental regulations regarding concentrations of silver in irrigation water, but the high Ag⁺ concentrations in Ag-BC column effluents may represent a potential concern for food safety and human health (Hadrup and Lam 2014; Kittler et al. 2010). To circumvent this problem, we tested the addition of ZVI to the Ag-BC column in the present study. In the combination Ag-BC + ZVI filter column, minimal Ag⁺ was detected in the treated water samples, indicating that more than 99% of the Ag⁺ formed from the combined Ag-BC was rapidly sequestered within the column, either via adsorption to iron hydroxides on the ZVI surface (Eq. (1)) or via reduction back to elemental silver by ZVI (Eq. (2)),

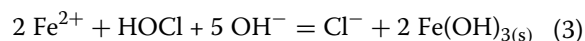


where $\alpha\text{Fe-O-H}$ represents surface iron hydroxide sites available for Ag⁺ sorption.

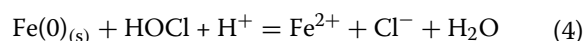
Therefore, the addition of ZVI to Ag-BC as a reductant can not only enhance *E. coli* removal but also sequester Ag⁺ and prevent its release.

However, the addition of ZVI to sand-based filter columns may result in elevated iron ions in filtered water. The decrease of iron ions in the original wastewater

effluents after chlorination comparing to that after UV disinfection is presumably due to the oxidation of the soluble Fe²⁺ by residual chlorine to the insoluble ferric hydroxide, as shown in Eq. (3).



However, the subsequent elevated iron ions in the ZVI filter-treated wastewater effluent samples indicate that ZVI was the source of the iron. The iron concentrations likely increased after chlorination began in the wastewater treatment plant after winter, suggesting that the iron was formed via oxidation of ZVI by residual chlorine in the original wastewater effluent samples, as shown in Eq. (4).



Therefore, the addition of ZVI to BC or Ag-BC filter columns, while beneficial in terms of *E. coli* removal and the control of silver release from the columns, may result in elevated levels of iron during the treatment of wastewater effluent. Elevated iron concentrations in resulting irrigation water may be not represent a potential health hazard for consumers of irrigated crops; however, this may have downstream impacts in terms of plant and soil health that need to be taken into consideration.

In addition to studying CEC and *E. coli* removal efficiencies of the sand-based filters, we also evaluated their impact on overall bacterial diversity in the wastewater effluents. Comparing the bacterial diversity indices, water samples treated with BC, ZVI, Ag-BC + ZVI, and BC + ZVI had marginally significantly ($p < 0.09$) lower alpha diversity indices compared to the untreated samples, indicating that these four treatments have potential effects on the reduction of overall bacterial diversity in wastewater effluents. Furthermore, we observed seasonal variations in the bacterial diversity of the original wastewater effluents, which is consistent with previous studies demonstrating seasonal variability in bacterial populations of water systems (Wilhelm et al. 2014) with higher bacterial diversity occurring during summer months compared to winter months (Wilhelm et al. 2014).

4.1 Limitations

While this study yielded promising findings regarding the efficacy of multiple combined filter treatments in the removal of CECs and bacteria, there are several limitations. First, the raw wastewater was obtained from one treatment plant on six different dates. This allowed us to evaluate some seasonal variability during the study; however, a longer and larger study that tested wastewater samples recovered from multiple sites over time would further understanding regarding the effectiveness of the

combined filter columns on removing CECs and bacteria from diverse recycled water samples. Second, while this study tested for a diverse suite of CECs, other important CECs including per- and polyfluoroalkyl substances (PFAS) were not tested. Given that understanding and addressing PFAS contamination in the environment is a major priority of the current U.S. Environmental Protection Agency Administration, future studies testing irrigation water treatment technologies should incorporate PFAS testing. Finally, longer-term and larger-scale evaluations will be necessary to demonstrate the cost-effectiveness and feasibility of the proposed treatments.

5 Conclusions

In the present study, we evaluated BC and ZVI-amended sand filter columns, including BC, Ag-BC, and ZVI individually, as well as their combinations (BC+ZVI and Ag-BC+ZVI) in the simultaneous removal of CECs, *E. coli* and diverse bacterial communities from wastewater effluents intended for water reuse. The concentrations of the six selected CECs in the original wastewater effluent samples were as high as 2,880 ng L⁻¹ while that of *E. coli* ranged from 5 to 96 CFU 100 mL⁻¹. After treatment, the Ag-BC, ZVI, BC+ZVI and Ag-BC+ZVI filter columns demonstrated more than 90% removal of *E. coli*. Meanwhile, the BC, Ag-BC, BC+ZVI and Ag-BC+ZVI demonstrated more than 99% removal of the selected CECs, except triclocarban. However, the BC+ZVI and Ag-BC+ZVI columns performed the best and were highly effective at removing both CECs and *E. coli*, while reducing the average relative abundance of selected bacterial species. These combined filters may serve as promising low-cost solutions to improving the quality of recycled wastewater effluents prior to their use in downstream irrigation application.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s42773-023-00240-y>.

Additional file 1: Figure S1. A preliminary batch *E. coli* inactivation experiment to compare the efficacy of BC, Ag-BC, and ZVI. Each 125 mL borosilicate glass reactor contained 100 mL of 10 mM bicarbonate solution adjusted to pH 7.1. The initial *E. coli* concentration, [*E. coli*]₀, was 3.6E6 CFU/mL in all reactors. **Table S1.** Physical and chemical properties of Soil Reef Biochar (BC). **Table S2.** Environmental parameters of wastewater effluents measured on-site in 2019.

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Author contributions

LZ collected samples, processed and analyzed samples, wrote the first draft of the manuscript. SC, OEA, SL and LM performed laboratory analysis and contributed to manuscript writing. SP and HAC collected water samples. SM, MS, KEK, EFM, PCC, AS, ARS contributed to data interpretation, reviewed and edited the manuscript. All authors reviewed and edited the manuscript and approved the final version.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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