



# Zero-Valent Iron-Sand Filtration Reduces *Escherichia coli* in Surface Water and Leafy Green Growing Environments

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Surface water is used for irrigation by farmers. However, surface waters may be a source of bacterial foodborne pathogens which contaminate fresh produce intended for human consumption. Proposed but not finalized standards for microbial quality of irrigation water through the Produce Safety Rule (PSR) of the Food Safety Modernization Act (FSMA) in the US emphasize the need for effective reduction of levels of pathogens in surface water intended to be used on fruit and vegetable crops. This study evaluated a zero-valent iron (ZVI)-sand filtration system to reduce *E. coli* populations in pond water and those transferred to growing spinach plants in a field trial. Six filtration events were conducted with the same ZVI-sand or sand (S) laboratory filtration systems. Filtration systems were constructed by connecting 4 PVC pipes (1.25 L) together. ZVI-sand filters contained 50% ZVI/50% sand (0.43–0.60 mm particle size), while sand filters contained 100% sand (0.45–55 mm particle size). In each event, autoclaved pond water (PW) inoculated with *E. coli* (ca. 4 log CFU/ml)—8 L—was pumped (1 L/min) through each filter followed by uninoculated autoclaved PW (15 L) with samples taken throughout the filtering process for enumeration of *E. coli*. Data were fit to a linear model to determine reductions of *E. coli* levels. ZVI-sand filtration removed significantly ( $p < 0.05$ ) more *E. coli* (1.1 log CFU) compared to sand filtration. For ZVI-sand-filtered water, there was a statistically significant ( $p < 0.05$ ) difference of *E. coli* removal from early trials (trials 1–3, average removal 96%) than in later trials (trial 4–6, average removal 44%), suggesting that age of the ZVI-sand filters influences *E. coli* inactivation. Overall, ZVI-sand and sand filtration reduced *E. coli* populations by 70 and –10%, respectively, indicating that ZVI filtration lowered or inactivated *E. coli* populations while sand filters accumulated *E. coli*. Field trials showed that soil and spinach plants irrigated with ZVI-sand-filtered water had significantly lower *E. coli* levels than soils/plants irrigated with sand-filtered water or unfiltered control water. Overall, ZVI-sand filtration significantly reduced *E. coli* populations in water compared to sand filtration.

**Keywords:** *Escherichia coli*, irrigation, agricultural water, sand filtration, zero-valent iron

## INTRODUCTION

Population growth, climate change, and urbanization have focused more attention on the availability and microbial quality of water used for irrigation for fresh fruits and vegetables (USEPA, 2012). Proposed but not finalized microbial irrigation water standards in the Produced Safety Rule (PSR) developed by the U.S. Food and Drug Administration in the Food Safety Modernization Act (FSMA) state that generic *E. coli* levels cannot exceed 410 CFU/100 mL for any water sample or a geometric mean of more than 126 CFU/100 mL in irrigation water intended for the edible portion of crop (USFDA, 2018). Surface waters in the U.S. can contain bacterial foodborne pathogens like shiga-toxicogenic *E. coli* (STEC), *Salmonella* spp., and *Listeria monocytogenes* (Cooley et al., 2014; Weller et al., 2015; Cho et al., 2018; Callahan et al., 2019; Haymaker et al., 2019; Sharma et al., 2020). Several outbreaks related to the consumption of contaminated Romaine lettuce have been traced back to irrigation water containing the pathogen *E. coli* O157:H7 (CDC, 2018, 2019), indicating that surface irrigation water can serve as a vehicle for contamination of leafy greens. Improving irrigation water quality through targeted interventions may reduce the burden of foodborne illness related to contaminated produce in the U.S. Previous studies have shown that contaminated produce accounts for one-fourth of the total medical costs associated with foodborne illness in the US (USDA, 2019). Reducing levels of bacterial foodborne pathogens in surface water may allow more growers to use it for irrigation.

Almost 30% of farms in the United States have sales of less \$100,000/year. Cost-effective mitigation strategies improving the microbial quality of irrigation water may be of interest to fruit and vegetable farming operations with limited capital resources (Scharff, 2010). Survey results have shown that across the U.S., many farmers already use surface water as a primary irrigation water source and are interested in using additional sources of non-traditional irrigation water, provided that the microbial quality is acceptable at the point of use (Suri et al., 2019). Irrigation water withdrawals accounted for 42% of total freshwater withdrawals in the U.S., while 52% of irrigation water was obtained from surface water sources (Dieter et al., 2018).

Various studies have been conducted to develop and improve the methods to remove and inactivate microbial contamination from water. Membrane filtration technology has been considered a useful method for wastewater treatment but can be prone to biofouling, reducing effectiveness of filtration (Chang et al., 2002; Herzberg and Elimelech, 2007). Ultraviolet (UV) light is effective in inactivating microorganisms but requires an electrical power source (Gayan et al., 2012) and may have limited penetration in turbid water.

Zero-valent ion (ZVI)/sand filtration of agricultural irrigation water can improve its microbial quality without the addition of disinfectant chemicals. Permeable reactive barriers (PRBs) incorporated with bio-sand and ZVI filters have been previously utilized to remove chemical contaminants in groundwater (Henderson and Demond, 2007), based on its adsorption and potential inactivation of contaminants of concern in drinking water, including bromate (Xie and Shang, 2005),

chloropicrin (Pearson et al., 2005), haloacetic acids (Hozalski et al., 2001; Zhang et al., 2004), and *N*-nitrosodimethylamine (Odziemkowski et al., 2000) ZVI-based filter systems have reported to have a >5 log reduction of viruses in synthetic groundwater (You et al., 2005). The removal rate of viruses or bacteria through ZVI filters varies with contact time, particle size, solution pH (Zhang, 2003), dissolved oxygen (Lee et al., 2008), concentration of ions (Diao and Yao, 2009), and redox potential (Auffan et al., 2008; Bradley et al., 2011). Other workers have shown that modified zero-valent iron sand filters can reduce fecal coliform levels in contaminated river water by 1 log CFU (Bradley et al., 2011; George and Ahammed, 2019) and *E. coli* in drinking water by between 90.9 and 99.9% (Sizirici et al., 2019). In reclaimed water, ZVI filtration reduced concentrations of antibiotics (Kulkarni et al., 2019) and virus like particles (Chopyk et al., 2019). Several studies examining the inactivation of bacterial species have shown that *Listeria innocua* and *E. coli* in ZVI-filtered water applied to foliar died off more quickly than *E. coli* or *Listeria* spp. in sand-filtered water (Ingram et al., 2012; Marik et al., 2019).

The evaluation of ZVI filtration and implementation for agricultural irrigation water remains limited. Contaminated irrigation water containing *E. coli* may introduce bacterial foodborne pathogens to soil, where they may persist and transfer to crops which grow close to the soil surface (Shah et al., 2019). Extended persistence of bacterial pathogens in soil may increase the likelihood of transfer of pathogens to produce crops. However, there is a lack of understanding on the persistence of *E. coli* present in sand or ZVI-effluent applied to agricultural soils. Sand filters are commonly used in the irrigation of many fruit and vegetable crops, especially for drip irrigation purposes. Our goal was to use existing agricultural practices and modify it in order to improve agricultural water quality used in production of fruits and vegetables.

Our work presented here is an evaluation of the use of ZVI-based filtration in laboratory and field trials. The objectives of this study are (1) to evaluate and compare the removal of inoculated *E. coli* from pond water via ZVI-sand and sand filtration; (2) to determine if *E. coli* removal is consistent using the same ZVI-sand and sand filters multiple times over the duration (50–60 days) of an agricultural growing season for leafy greens; (3) determine presence of *E. coli* TVS 353 on spinach plants in fields irrigated with ZVI and sand filtered water.

## MATERIALS AND METHODS

### Construction of ZVI-Sand and Sand Filter

Each filter system was constructed using 4 pieces of 5 cm (diameter) by 60 cm (length) of Charlotte PVC linked together with U shaped connectors (Figure 1). For construction of ZVI-sand filters, PVC pipes were filled with equal volumes (625 mL, measured in a 1 L graduated cylinder) of 0.43–0.60 mm ZVI particles (Peerless Metals, Detroit MI) and sand particles (0.45–0.55 mm) (Northern Filter Media, Muscatine, IA). Sand and ZVI particles were mixed well by adding material to a disinfected commercial bucket, and rotating the bucket on it's side for 5 min. Sand and ZVI particle were further mixed for 1 min to obtain a



**FIGURE 1 |** ZVI/sand and sand filtration for laboratory and field trials of pond water. Each white PVC tube (60 cm length  $\times$  5 cm diameter) is filled with either (1) a mixture of 50% ZVI/50% sand (0.43–0.55 mm—particle size) for ZVI/sand filtration or (2) 100% sand (0.45–0.55 mm particle size) for sand filtration. Autoclaved pond water inoculated with *E. coli* TVS 353 was pumped through either ZVI/sand or sand filters at 1 L/min.

distributed mixture. This mixture (1.18 L) was transferred to a graduated cylinder. Columns (PVC pipes) were packed one at a time with efforts made to mix particles uniformly, and packed columns were not disturbed after packing to minimize particle resettling. PVC pipes were sealed at the end with landscape fabric to prevent leakage of sand/ZVI particles. Once PVC pipes were filled, pipes were attached to U-shaped connectors (which did not contain sand or ZVI/sand) so that 4 PVC pipes containing 1.18 L of sand/ZVI were connected. Sand filters were constructed identically and filled with 100% sand particles (0.45–0.55 mm). The approximate porosity of both ZVI and sand filters was 0.6, and the volumetric flow rate was set at 1 L/min for both filters using a metering pump (Flex pro A4 ProSeries, Standard Peristaltic Metering Pump, Cole-Parmer, Vernon Hills, IL) with a residence time of 3 min. Prior to testing, 2 L of autoclaved pond water were added to saturate both ZVI and sand filters to minimize air gaps in the filtration systems and held for 7 days prior to filtration.

### Collection of Pond Water

Pond water samples were collected from the a pond located at the University of Maryland Wye Research and Education Center (Wye REC, Queenstown, MD). Prior to each sampling event, ~80 L of pond water was collected in sterile 20-L carboys (VWR, Philadelphia, NJ) using a Honda WX10TA pump (Sweponville NC). Pond water samples were stored at 4°C after collection and filtered within 1 week. Pond water characteristics were measured using a ProDSS multi-parameter water quality sonde/meter (YSI, Yellow Springs, OH) and are summarized in **Table 1** for the duration between trials 1–8. Prior to filtration, pond water was

sterilized by autoclaving. Autoclaved pond water contained no rifampicin-resistant *E. coli* TVS 353 when tested.

### *E. coli* Inoculum Preparation

*E. coli* strain TVS 353, originally recovered from an agricultural water source (Tomás-Callejas et al., 2011), was isolated from frozen stock onto MacConkey agar (Neogen, Lansing, MI) supplemented with 80  $\mu$ g/mL rifampicin (MACR) (Sigma-Aldrich, St. Louis, MO). Plates were incubated at 37 for 18–24 h and a single colony was transferred to 100 mL of tryptic soy broth containing 80  $\mu$ g/mL rifampicin (TSBR) and incubated for 18–24 h at 37. The culture was then diluted 1:100, and 23 mL of this dilution was added to 8 L of autoclaved pond water.

### ZVI-Sand and Sand Filtration

Autoclaved pond water (8 L) was inoculated with *E. coli* TVS 353 (4 log CFU/ml) mixed vigorously and then pumped through the ZVI-sand and sand filters, followed by 15 L of uninoculated pond water. This level was chosen to be 1-log greater than the maximum level of *E. coli* observed in pond water from Wye REC over a 12-month period (3 log CFU/mL) (Solaiman et al., submitted). For each trial, the first 2 L of the effluent from both ZVI-sand and sand filters were discarded because this volume was assumed to be contained in the filter before pumping. The remaining 23 L effluent was collected to determine *E. coli* TVS 353 populations in each liter of the effluent. Six separate filtration trials were conducted sequentially for the ZVI-sand and sand filters in laboratory experiments.

For the field trial, separate ZVI-sand and sand filters were constructed specifically for field trials as described above. Two filtration events were performed 7 days apart in the laboratory, and filtered water was transported to the field for irrigation.

### Field Plot Setup

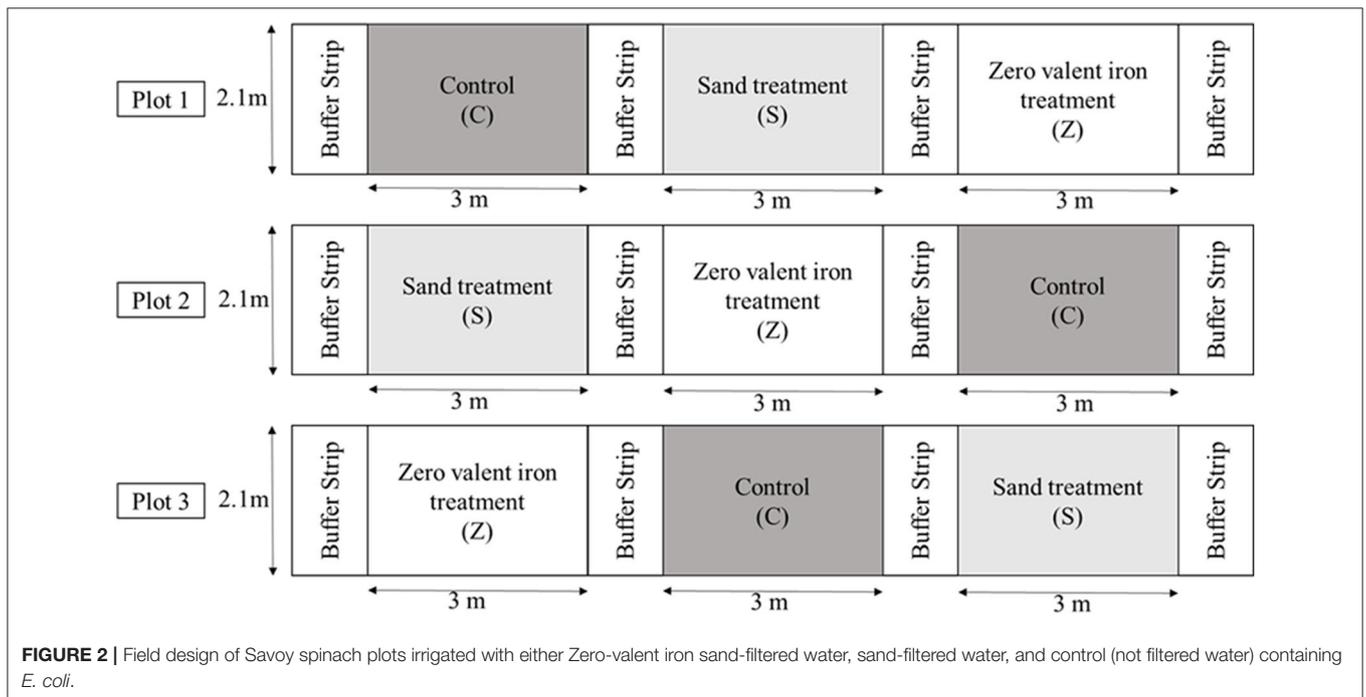
Butlertown silt loam soil was leveled in each field plot at the University of Maryland Wye REC. Approximately 22 kg of nitrogen (N) was applied to the entire plot area before making the planting beds, incorporated using a rototiller. Each plot was subdivided into three 3  $\times$  2.1 m subplots to apply irrigation water through either ZVI or sand filtration, each of which were divided by 2.1  $\times$  0.6 m buffer strip (**Figure 2**). Savoy type spinach was planted in each plot. Seeds were started in 50-cell tray flats in a greenhouse and then transplanted into plots. Plants were started 35 days before irrigation events occurred. Spinach plants were close to harvest when the two irrigation events occurred.

Autoclaved pond water was inoculated and filtered in the laboratory using the same ZVI/sand and sand filtration setup described previously. Filtered water or control (not filtered) was then transported to WyeREC. Carboys of ZVI-sand—and sand-filtered water were transported to the field and fed through drip tape to irrigate spinach plants.

Three replicate plots of spinach plants were irrigated with either ZVI/sand, sand, or control (not-filtered) water using drip tape (Rivulis Irrigation, Gvat, Israel). with 24 L of ZVI-sand, sand-filtered or control for each irrigation event. Each replicate plot was irrigated twice, 7 days apart. Drip tape for spinach plots was placed 1.5 cm below soil surface and irrigation emitters were

**TABLE 1** | Pond water quality characterization for dates during laboratory ZVI-sand or sand filtration trials to reduce inoculated *E. coli* levels.

| Date                         | Conductivity<br>(SPC $\mu\text{S/cm}$ ) | DO%      | ORP<br>(mV) | pH        | Pressure<br>(mmHg) | Temperature<br>( $^{\circ}\text{C}$ ) | Turbidity<br>(FNU) |
|------------------------------|---|----------|-------------|-----------|--------------------|---------------------------------------|--------------------|
| 8/8/2017                     | 177                                     | 123      | 72.9        | 7.6       | 761.9              | 31.0                                  | 4.7                |
| 8/21/2017                    | 36                                      | 71       | 83.9        | 7.9       | 762.8              | 23.5                                  | 4.1                |
| 9/11/2017                    | 60                                      | 161      | 60.3        | 8.0       | 765.9              | 27.9                                  | 5.6                |
| 9/25/2017                    | 100                                     | 177      | 92.9        | 8.6       | 768.0              | 22.1                                  | 6.1                |
| Average + standard deviation | 93 + 62                                 | 133 + 47 | 77.5 + 14.1 | 8.0 + 0.4 | 764.7 + 2.8        | 26.1 + 4.1                            | 5.2 + 0.9          |



30 cm apart. The drip tape flow rate was 0.75 L/min per 30.48 m of tape at 12 PSI. The planting bed was 75 cm on top and 10 cm high spinach plants were spaced 30 cm between the double rows and 15–30 cm between plants down each row with ~28 plants in each plot. Up to 2 L of water was collected at the end of the drip tape in sterile glass bottles, which were transported back to the laboratory in refrigerated coolers. Two irrigation events were performed 7 days apart. Pre- and post-filtered water samples were analyzed for *E. coli* as described below, as well as samples collected from the end of driplines.

Three replicate plots of spinach for each irrigation event (sand, ZVI, and control) were evaluated. No rainfall was recorded in the 7 days between the first and second irrigation events during this field trial. Control (non-filtered water) was collected and stored in the same manner as ZVI-sand- and sand-filtered water.

## Microbial Analysis

For laboratory experiments, each liter ( $n = 24$ ) of filtered water was collected in sterile glass or plastic bottles and shaken for 20 s before 1 mL of from each liter was used to make serial dilutions

in 0.1% peptone water. Volumes of serial dilutions (50  $\mu\text{L}$ ) were spiral plated (WASP2 spiral plater (Microbiology International, Frederick, MD), in duplicate, onto MACR. Plates were incubated at 37 $^{\circ}\text{C}$  for 18–24 h before enumeration. For field experiments, the 24 L effluent from both ZVI-sand and sand filtration events were collected and analyzed for *E. coli* TVS 353 as previously described. Water samples in bottles collected from ends of driplines in the field study were shaken for 20 s by hand and serial dilutions were plated on MACR as described previously.

Three soil samples from each replicate plot were collected from the soil surface (0–3 cm) after irrigation events, and transported back to the laboratory in refrigerated coolers. Samples were homogenized by hand for 30 s and 20 g was measured into sterile 24 oz. filtered Whirlpak bags (Nasco, Ft Atkinson, WI). For each soil sample, 80 mL of Buffered Peptone Water (BPW, Neogen, Lansing, MI) were added and soil was homogenized by hand. The homogenate (50  $\mu\text{L}$ , in duplicate) was spiral plated onto MACR and incubated for 18–24 h at 37. To determine moisture levels in soil samples, ~5 g of the original homogenized sample was aliquoted to 2 oz. Whirl-pak bags and analyzed using a TrueDry CV9 (Decagon Devices, In., Pullman,

WA). An additional soil sampling was conducted 7 days after the second irrigation event to determine if *E. coli* TVS 353 populations changed after that time period.

Each replicate field plot was divided into four zones, and one spinach plant from each zone was collected using sterile scissors immediately after irrigation. Spinach samples were collected in 92 oz. Whirl-pack bags and transported back to the laboratory in coolers. For each plant, 500 mL TSBH was added and then hand-massaged for 1 min before being incubated for 18–24 h at 37°C. Following incubation, enrichments were streaked for isolation on MACR using a 10  $\mu$ L loops and plates which were incubated at 37 for 18–24 h. Presumptive violet-colored colonies from MACR plates were isolated on tryptone bile x-glucuronide (TBX, Neogen, Lansing, MI) and incubated at 37 for 18–24 h. Teal-colored colonies on TBX indicated the confirmed presence of *E. coli* TVS 353.

## Statistical Analysis

For statistical analysis, *E. coli* TVS 353 counts were log-transformed (log CFU/mL), and populations in each liter of filtered effluents were plotted and fit to a linear regression model to compare the overall reductions achieved by ZVI-sand and sand filtration using R version 3.4.3 (R Core Team, 2017).

For statistical analysis, Equation 1 was used:

$$(Y) = \alpha_1 + \alpha_2 * (\text{filtered water } (l)) \quad (1)$$

Where  $\alpha_1$  is the y-intercept value,  $\alpha_2$  is the slope value, and (Y) is the log CFU/ml of sand-filtered water in the specific liter [filtered water (l)] of effluent of sand-filtered water. A variable T can be applied, which is set to 0 if evaluating sand filtration, and T = 1 if ZVI/sand filtration was evaluated, leading to Equation 2:

$$T = \begin{cases} 0, & \text{if data originates from sand filter} \\ 1, & \text{if data originates from ZVI filter} \end{cases} \quad (2)$$

By combining Equations (1) and (2), the following equation is derived:

$$Y = \alpha_1 + [\alpha_2 * (\text{filtered water}(l))] + \alpha_3 + [\alpha_4 * (\text{filtered water}(l))] \times T \quad (3)$$

Where  $\alpha_3$  is the y-intercept and  $\alpha_4$  is slope value for ZVI-sand filtered water. Two different Equations were obtained:

$$(Y_A) = \alpha_1 + [\alpha_2 * \text{filtered water } (\text{liter})_A] \quad (4)$$

$$(Y_B) = (\alpha_1 + \alpha_3) + [(\alpha_2 + \alpha_4) * \text{filtered water } (\text{liter})_B] \quad (5)$$

Where  $Y_A$  is the log CFU/ mL of *E. coli* TVS 353 for sand-filtered water and  $Y_B$  is the log CFU/ml for *E. coli* TVS 353 for ZVI-sand

filtered water for specific liter [filtered water (l)] of the effluent. Equations used for these regression models are based on those described for biophase dose-response time models (Gabrielsson et al., 2019).

The null hypothesis for the linear regression modeling is that all coefficients  $\alpha_i$  are equal to zero; the alternative hypothesis is that  $\alpha_i$  values are not zero. Percentage reductions of *E. coli* populations in inoculated water by ZVI-sand- and sand-filtration were determined by calculating the area under the curve (AUC) for each ZVI-sand or sand filtration trial using the linear regression equation for each trial and comparing to the population of the initial *E. coli* inoculum introduced to filters.

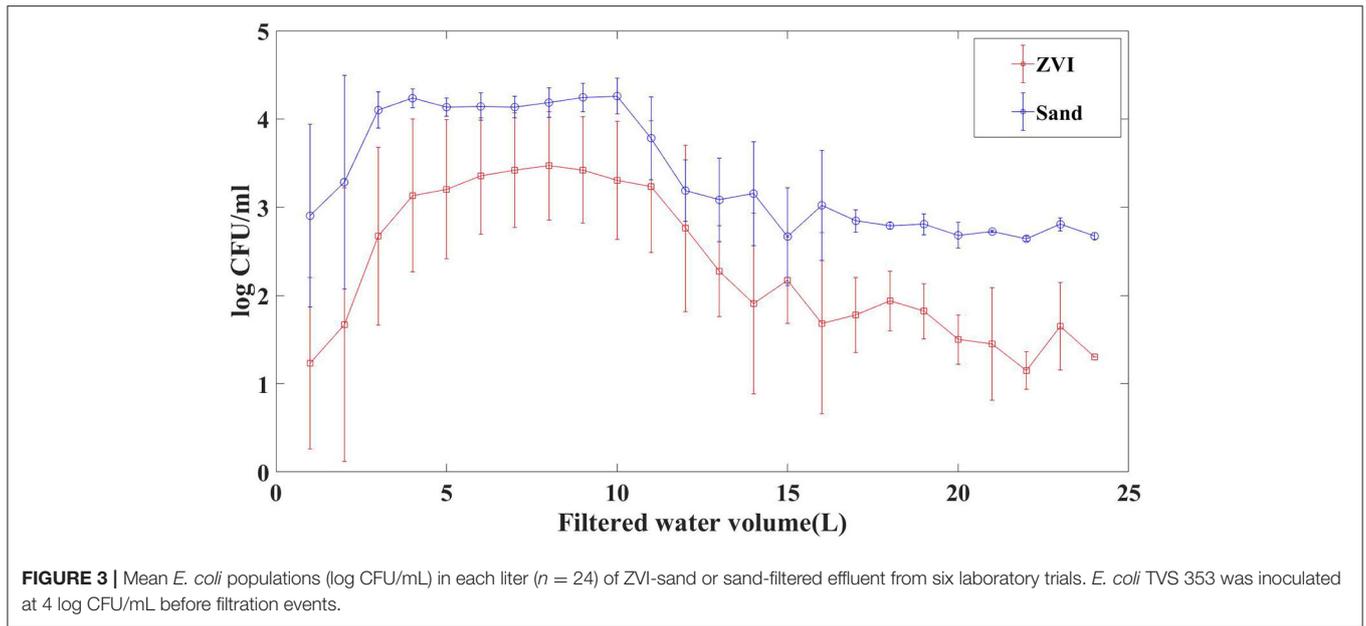
The Kruskal-Wallis and pairwise Wilcoxon rank sum tests was used to determine differences in *E. coli* populations introduced to soils through control (unfiltered), ZVI- or sand-filtered water after the first and second irrigation events, and 7 days after the second irrigation event. These non-parametric tests were used because there were <20 observations and a normal distribution of these values during these experiments could not be assumed. In all cases, *p*-values of 0.05 were defined as statistically significant.

## RESULTS AND DISCUSSION

### Laboratory Study of ZVI and Sand Filtration

Average populations of *E. coli* TVS 353 recovered from each liter of 24 L of ZVI-sand or sand-filtered effluent over 6 trials are shown in **Figure 3**. These data show that ZVI-sand filtration was more effective at removing *E. coli* than sand filtration. ZVI-sand filtered water contained significantly ( $p < 0.001$ ) lower populations of *E. coli* than sand filtered water. For both ZVI-sand and sand filtration, the first 2 L of effluent contained relatively low *E. coli* TVS 353 levels for both filters. Populations of *E. coli* were relatively constant between liters 3–11 for both ZVI-sand and sand-filtered effluents (**Figure 3**). The concomitance and similar duration (~8 L) of the high *E. coli* population plateaus suggests both ZVI-sand and sand filters performed like plug-flow reactors. Therefore, the ~1-log lower *E. coli* population in ZVI-sand filter effluent was most likely due to removal of *E. coli* by ZVI rather than retarded transport, which would have shifted the maximum *E. coli* population to a later time. **Table 2** shows the reduction of *E. coli* TVS 353 by ZVI-sand and sand filtration in each of the six individual trials. The reduction of *E. coli* by ZVI-sand and sand filtration were varied over 6 trials. Mean reductions of *E. coli* TVS 353 were  $70 \pm 35\%$  by ZVI-sand and  $-10 \pm 87\%$  by sand filtration. ZVI-sand filtration reduced *E. coli* populations more effectively in trials 1–3 (mean reduction: 96%) compared to trials 4–6 (mean reduction: 44%) (**Table 2**), indicating that the age of the ZVI-sand filtration media may influence its efficacy in reducing *E. coli* populations in surface waters.

The highest populations of *E. coli* were detected after 8 L (3.47 log CFU/ml) and 10 L (4.26 log CFU/ml) in ZVI-sand- and sand-filtered effluents, respectively. Trials 2–4 of sand filtration had an average *E. coli* reduction of 57% (**Table 2**). However, sand filtration also showed a negative reduction of *E. coli* in trials 5–6, indicating that *E. coli* cells may be accumulating in the sand filters from trial to trial, leading to diminished or no reduction of *E. coli* populations. The gradual diminished efficacy of sand filtration



**FIGURE 3 |** Mean *E. coli* populations (log CFU/mL) in each liter ( $n = 24$ ) of ZVI-sand or sand-filtered effluent from six laboratory trials. *E. coli* TVS 353 was inoculated at 4 log CFU/mL before filtration events.

**TABLE 2 |** The reduction of *E. coli* TVS 353 by ZVI-sand and sand filtration in each of the six individual trials as shown by percentage or log CFU/ml reduction.

| Trial | Time (days) | Reduction of <i>E. coli</i> by filtration |            |             |            |
|-------|-------------|---|------------|-------------|------------|
|       |             | ZVI-sand filter                           |            | Sand filter |            |
|       |             | Percentage                                | log CFU/mL | Percentage  | log CFU/mL |
| 1     | 0           | 98  | 1.70       | -26         | -0.1       |
| 2     | 7           | 92  | 1.08       | 52          | 0.32       |
| 3     | 14          | 98  | 1.79       | 86          | 0.86       |
| 4     | 28          | 38  | 0.21       | 35          | 0.19       |
| 5     | 42          | 81  | 0.71       | -50         | -0.17      |
| 6     | 49          | 15  | 0.07       | -155        | -0.41      |

**TABLE 3 |** Statistical and estimated coefficients for model to evaluate the use of ZVI-sand and sand for average inactivation of *E. coli* from Equations (7) and (8).

| $\alpha_1$ | <i>p</i> -value | $\alpha_2$ | <i>p</i> -value | $\alpha_3$ | <i>p</i> -value | $\alpha_4$   | <i>p</i> -value | $R^2$ |
|------------|-----------------|------------|-----------------|------------|-----------------|--------------|-----------------|-------|
| 4.167      | <0.001          | -0.063     | <0.001          | -1.120     | <0.001          | <b>0.011</b> | 0.553           | 0.36  |

If *p*-value in bold is higher than 0.05, the constant  $\alpha_i$  is not a significant constant.

**TABLE 4 |** Statistical and estimated coefficients for model to evaluate the longevity and comparisons of *E. coli* survival in effluent by use of ZVI-sand and sand for trials 1-6.

|         | $\alpha_1$ | <i>p</i> -value | $\alpha_2$ | <i>p</i> -value | $\alpha_3$    | <i>p</i> -value | $\alpha_4$    | <i>p</i> -value | $R^2$ |
|---------|------------|-----------------|------------|-----------------|---------------|-----------------|---------------|-----------------|-------|
| Trial 1 | 4.188      | <0.001          | -0.079     | 0.131           | -2.444        | 0.001           | <b>0.066</b>  | 0.365           | 0.556 |
| Trial 2 | 4.388      | <0.001          | -0.048     | 0.157           | -1.746        | <0.001          | <b>0.080</b>  | 0.098           | 0.494 |
| Trial 3 | 3.141      | <0.001          | -0.007     | 0.900           | -2.036        | 0.009           | <b>0.134</b>  | 0.088           | 0.304 |
| Trial 4 | 4.119      | <0.001          | -0.041     | 0.304           | <b>-0.653</b> | 0.234           | <b>0.032</b>  | 0.567           | 0.107 |
| Trial 5 | 4.350      | <0.001          | -0.077     | <0.001          | -0.822        | 0.007           | <b>-0.026</b> | 0.202           | 0.771 |
| Trial 6 | 4.516      | <0.001          | -0.081     | <0.001          | <b>-0.252</b> | 0.444           | <b>-0.037</b> | 0.111           | 0.691 |

If  $\alpha_i$  is in bold, the *p*-value is not a significant ( $p < 0.05$ ) constant.

on reducing *E. coli* levels may be due to the adsorption and mechanical trapping of the *E. coli* cells in the sand filter in early trials, and those cells being released from the filter during later

trials, leading to a higher number of cells in the effluent compared to the initial inoculum level (4 log CFU/ml). It is possible the conditions inside the sand filter supported *E. coli* growth because

of sufficient moisture and organic material introduced through pond water. During sand filtration, *E. coli* populations in effluent were generally higher than the initial inoculum between liters 3–10. *E. coli* populations in ZVI-sand filters did not exceed the inoculum level in effluent collected in liters 3–10, potentially indicating that ZVI-sand filters may suppress the growth or physiologically stress *E. coli* populations. Neither ZVI-sand or sand filters were back-flushed during these experiments.

Examining the  $\gamma$ -intercept values  $\alpha_1$  and  $\alpha_3$  indicates a significant ( $p < 0.05$ ) difference between sand-filtration and ZVI-sand filtration (Table 3), which shows approximately a 1-log CFU/ml difference between *E. coli* levels in ZVI-sand- and sand-filtered water. The lack of statistical differences between the coefficient  $\alpha_2$  and  $\alpha_4$  (slope values) of the survival curve of *E. coli* in ZVI-sand- and sand-filtered effluents indicates that the majority of the reduction in *E. coli* populations occurs at the outset of ZVI-filtration.

Table 4 shows the fitting coefficients determined by applying the linear model to each of the six individual trials. The differences (log CFU/mL *E. coli*) between sand and ZVI-sand  $\gamma$ -intercept ( $\alpha_3$ ) values for trials 1, 2, 3, and 5 ranged between  $-2.5$  and  $-0.8$  ( $p < 0.05$ ), indicating that ZVI-sand filtration resulted in significantly lower *E. coli* populations in effluents from these trials compared to sand filtration. Coefficients of  $\alpha_4$ —the differences between the slope values for ZVI-sand and sand effluents—for trial 1, 2, 3, and 5—were not statistically significant,

indicating that effluent release patterns through both ZVI and sand filters are similar.

Ingram et al. (2012) reported that ZVI filtration was more effective than sand filtration at inactivating *E. coli* O157:H12 in inoculated water. Several studies have shown that inactivation of bacterial cells has resulted from contact with ZVI particles (You et al., 2005; Lee et al., 2008; Ingram et al., 2012; Marik et al., 2019). The transition of iron from a zero-valent state ( $\text{Fe}^0$ ) to ferric iron ( $\text{Fe}^{3+}$ ) in the ZVI system leads to the formation of reactive oxygen species, causing the degradation of bacterial cells under oxidative stress conditions (Auffan et al., 2008; Lee et al., 2008). Other workers have suggested that increasing the influent volume may reduce *E. coli* reductions over time in ZVI-sand filtration systems (Ahmed and Davra, 2011). In our study, volume did not exceed 23 L during any trial, which may have extended the efficacy of the ZVI-sand filter and increased its longevity as compared to sand filtration.

Other research evaluating the longevity of ZVI-sand and sand filters, showed that both types of filters were effective over 13 trials (390 L of water), a 22-week period (Marik et al., 2019). In those experiments, a 35% ZVI-sand filter was effective consistently reducing *E. coli*, by  $2.27 \pm 0.27$  log CFU/ml and *L. monocytogenes* by  $2.53 \pm 0.31$  log CFU/ml when inoculated into pond water at ca. 7 log CFU/ml. In that work, ZVI-sand-filtration was significantly more effective in reducing *L. monocytogenes* compared to *E. coli*. The differences in longevity between the Marik et al. study and the current one presented here may be

**TABLE 5** | Summary of *E. coli* TVS 353 levels inoculated into autoclaved pond water (pre-filter), recovered after ZVI-sand and sand filtration (post-filter), or control (not filtered).

| Trial | Treatment type           |             |          |                      |             |          |                         |          |
|-------|--------------------------|-------------|----------|----------------------|-------------|----------|-------------------------|----------|
|       | ZVI/sand<br>(log CFU/ml) |             |          | Sand<br>(log CFU/ml) |             |          | Control<br>(log CFU/ml) |          |
|       | Pre-filter               | Post-filter | Dripline | Pre-filter           | Post-filter | Dripline | Not filtered            | Dripline |
| 1     | 4.63                     | 2.30        | 1.95     | 4.66                 | 4.01        | 3.40     | 4.21                    | 3.30     |
| 2     | 4.71                     | 1.00        | 0        | 4.67                 | 3.96        | 3.49     | 4.18                    | 3.79     |

After filtration and during irrigation of spinach plots, water samples were collected from driplines were either ZVI/sand-filtered, sand-filtered, or control waters were applied.

**TABLE 6** | Water quality parameters measured before water samples were obtained (24 h before filtration) and immediately after sand and ZVI-sand filtration before irrigation of spinach plots.

| Water quality parameters                 | Trial 1                 |                 |                | Trial 2                 |                 |                |
|--|-------------------------|-----------------|----------------|-------------------------|-----------------|----------------|
|  | Pre-filtered pond water | Post            |                | Pre-filtered pond water | Post            |                |
|  |                         | Sand filtration | ZVI filtration |                         | Sand filtration | ZVI filtration |
| Conductivity ( $\mu\text{S}/\text{cm}$ ) | 112.5                   | 168.1           | 169.3          | 137.5                   | 157.6           | 121            |
| DO (%)                                   | 107.7                   | 104.3           | 84.1           | 105                     | 102.6           | 77.7           |
| ORP (mV)                                 | 168.7                   | 111.5           | 66.1           | 129.9                   | 139.5           | 115.9          |
| pH                                       | 8.45                    | 8.56            | 9.43           | 8.52                    | 8.2             | 9.33           |
| Pressure (mmHg)                          | 767.2                   | 762.9           | 762.9          | 764.2                   | 762.9           | 763.2          |
| Temperature ( $^{\circ}\text{C}$ )       | 17.7                    | 20.6            | 21.3           | 21.3                    | 19.7            | 19.9           |
| Turbidity (FNU)                          | 37.3                    | 76.8            | 13.5           | 36.4                    | 66.3            | –              |

due to different water preparation methods, inoculum levels, and ZVI to sand ratios used in the composition of the filters. The same source of ZVI was used in the Marik study and our current study; however their filtration system had a total volume of 1.25 L compared to 5 L in our current study. In our current study these ZVI-sand and sand filters were evaluated over 56 days, which estimates a growing season for leafy green crops; this is shorter than previous studies evaluating sand/iron filters for decontaminating drinking water (Bradley et al., 2011), which evaluated virus removal by sand/iron filters over a 12-month time period. Work examining the removal of MS-2 bacterial, an enteric viral surrogate, showed that iron-oxide containing biosand filters were able to effectively remove 6.5 log PFU after over 370 days (Bradley et al., 2011). The addition of iron-coated sand to biosand filters resulted in a 1-log increase in removal of *E. coli* compared to biosand filters without iron, improving the quality of drinking water (Ahammed and Davra, 2011). Previous work has shown that an increase in the concentration of humic acid, a component of natural organic material (NOM), measured as dissolved organic carbon (DOC), decreased the ZVI effectiveness in removing nitrobenzene by fouling ZVI binding sites (Yin et al., 2012); fulvic acid, another component of NOM, may also have the same affect (Qin et al., 2020). Neither the ZVI-sand or sand filtration systems were back-flushed during these experiments.

Previous studies have shown that internal oxidation reduction potential, influent pH, and alkalinity are major predictors of the performance of zero valent iron permeable reactive barriers (PRB) (Henderson and Demond, 2007). The relatively high pH of influent pond water (8.18) for this study (Table 1) may have been a factor in the reduced performance of ZVI after the 4th trial as well. The increased pH of pond water over trials, potentially due to the changes in trophic activity in ponds, may affect the bactericidal mechanism of ZVI. At pH values >4, ZVI particles become coated in various iron oxides (Noubactep, 2009). The combination of increased pH and accumulation of NOM in ZVI/sand filters may have decreased the ability of ZVI to strongly adsorb to *E. coli* cells by making the charge of the surface negative, which may have decreased the removal efficacy of the ZVI/sand filters. The accumulation of NOM and available soluble carbon in sand filters may have allowed a higher level of *E. coli* population to remain in the filter, which may partially explain the higher levels of *E. coli* in the effluent of sand filters for trials 5 and 6 compared to introduced amount. The use of autoclaved pond water may have reduced the number and diversity of microorganisms in forming the biological sand layer (schmutzdecke), which may have affected *E. coli* levels in the effluent and the filter. Marik et al. (2019) did not autoclave pond water from the same source and observed more consistent reductions of *E. coli* over a longer duration of time in ZVI/sand and sand filters. Since our study was intended to evaluate agricultural water used for irrigation, these 6 trials occurred over 56 days, which represented the growing season for various leafy green commodities (Table 1). This is considerably shorter than the evaluation period for biosand filters for drinking water disinfection, which can last for more than 1 year. Agricultural operations may present more opportunities

for changing or replacing filters between growing seasons than drinking water filters, which may be kept in use for longer periods of time.

## ZVI-Sand and Sand Filtration—Field Trials

Table 5 describes the reduction of *E. coli* TVS 353 populations by ZVI-sand, sand, or no (control) filtration for field trials. New ZVI and sand filters were constructed for field trials after laboratory trials. Initial populations of inoculated *E. coli* TVS 353 in pond water were 4.51 log CFU/ml. As in laboratory trials, ZVI-sand-filtration significantly ( $p < 0.05$ ) reduced *E. coli* TVS 353 levels by 2.01 log CFU/ml compared to 0.68 log CFU/ml by sand filtration. There were no significant differences between

**TABLE 7 |** *E. coli* TVS 353 population (log CFU/g) in soil samples after irrigation by ZVI-sand, sand filtration and control (no filtration) water.

| Treatment | Plot number    | Recovered <i>E. coli</i> (log CFU/g) |             |             |
|-----------|----------------|--------------------------------------|-------------|-------------|
|           |                | First                                | Second      | Third       |
| ZVI-sand  | Z1             | 0                                    | 0           | 0           |
|           |                | 0                                    | 1.38        | 1.09        |
|           |                | 0                                    | 0           | 0           |
|           | Z2             | 0                                    | 0           | 0           |
|           |                | 0                                    | 0           | 0           |
|           |                | 0                                    | 0           | 0           |
|           | Z3             | 0                                    | 0           | 0           |
|           |                | 0                                    | 0           | 0           |
|           |                | 0                                    | 0           | 0           |
|           | <b>Average</b> |                                      | <b>0</b>    | <b>0.15</b> |
| Sand      | S1             | 1.77                                 | 0           | 2.27        |
|           |                | 2.15                                 | 2.2         | 0           |
|           |                | 0                                    | 0           | 1.87        |
|           | S2             | 1.08                                 | 3           | 1.57        |
|           |                | 2.36                                 | 0           | 1.87        |
|           |                | 2.29                                 | 2.23        | 1.94        |
|           | S3             | 0                                    | 2.69        | 1.4         |
|           |                | 1.08                                 | 0           | 2.14        |
|           |                | 2.03                                 | 0           | 2.18        |
|           | <b>Average</b> |                                      | <b>1.42</b> | <b>1.12</b> |
| Control   | C1             | 2.14                                 | 0           | 1.09        |
|           |                | 2.14                                 | 1.55        | 2           |
|           |                | 0                                    | 2.31        | 0           |
|           | C2             | 0                                    | 2.86        | 0           |
|           |                | 0                                    | 2.72        | 1.7         |
|           |                | 1.08                                 | 2.32        | 0           |
|           | C3             | 0                                    | 0           | 1.57        |
|           |                | 1.56                                 | 0           | 1.57        |
|           |                | 2.56                                 | 0           | 1.4         |
|           | <b>Average</b> |                                      | <b>1.05</b> | <b>1.31</b> |

Soils samples were taken either immediately after irrigation events (first, second) or 7 days after the second irrigation event (third).

*E. coli* levels in sand-filtered and control (non-filtered) effluents (Table 5). *E. coli* populations were significantly lower in effluents of all water types when collected from the ends of driplines when compared to pre-filtration levels and levels before irrigation pumping commenced.

The pond water quality characteristics both before and after filtration for the field trials are summarized in Table 6. As expected, ZVI-sand filtration increased the pH of filtered water (9.3–9.4) compared to pre-filtered water (8.5) but sand filtration did not (8.5–8.5), (Zhang, 2003), compared to pre-filtered water in field irrigation trials. Other characteristics of the pond water (dissolved oxygen, oxidation reduction potential) might have influenced *E. coli* reductions. Previous filtration trials using nanoscale ZVI (nZVI) elevated pH values by 2–3 units (Zhang, 2003). In our study, the pH of ZVI-sand filtered water was greater than the acceptable range for agricultural irrigation (6.5–8.4), but may have been influenced by the relatively high pH of pond water before filtration (USEPA, 2012). ZVI-sand filtration significantly reduced dissolved oxygen percentage (DO%) and oxidation reduction potential (ORP) values in field trials. Turbidity values of pond water were lowered after ZVI-sand filtration compared to sand filtration in trial 1 could not be measured after ZVI-filtration in trial 2. Several previous studies confirmed that the reaction between ZVI and DO is one of the most rapid among the chemical reactions involving ZVI, especially when DO% values are relatively low (He et al., 2010; Johnson et al., 2013; Kocur et al., 2014). These conditions may not be applicable to the pond water since the DO% is relatively high (84%), and therefore more investigation is needed. Rapidly decreasing DO levels in ZVI-sand-filtered water might be an indicator of ZVI activity and may be used as an indicator of the longevity of the ZVI filter.

### Effect of ZVI and Sand Filtration on *E. coli* on Plants and in Soils

Populations of *E. coli* TVS 353 in soils irrigated by control, ZVI-sand- and sand-filtered water are shown in Table 7. Soils were analyzed after each of two irrigation events (7 days apart), and then 7 days following the 2nd irrigation event. ZVI-sand-irrigated soils contained significantly ( $p < 0.001$ ) lower levels of *E. coli* TVS 353 than control ( $p < 0.001$ ) or sand-filtered irrigated soils ( $p < 0.001$ ). There were no statistical differences ( $p = 0.31$ ) between *E. coli* levels in soils irrigated with sand-filtered or control water, indicating that *E. coli* persisted at lower levels in ZVI-sand-irrigated soils compared to sand-filtered or control irrigated soils.

In both irrigation trials, 8% (1/12) of spinach plants irrigated with ZVI-sand-filtered water contained *E. coli* TVS 353, while 25% (3/12) of plants irrigated with sand-filtered water contained *E. coli* TVS 353. In control-irrigated plots, 0% (0/12) and 33% (4/12) of the spinach plants in the first and second irrigation trials contained *E. coli* TVS 353, respectively. Previous studies have shown that ZVI-sand-irrigated leaves of plants showed lower survival or faster die-off of bacterial pathogens on foliar surfaces. Ingram et al. (2012) showed that leaves irrigated with

ZVI-filtered water had *E. coli* O157:H12 populations ca. 3–4 log CFU/g lower than leaves irrigated with sand-filtered water over 4 days in a field study. Marik et al. (2019) showed that die-off rates of *Listeria innocua* introduced to cucumber leaves through ZVI-sand filtration were significantly lower on days 3 and 4 compared to those introduced through sand filtration. However, die-off rates of *E. coli* TVS 353 on cucumber leaves irrigated with ZVI-sand- or sand-filtered water were similar over 4 days.

## CONCLUSIONS

ZVI-sand filtration was more effective than sand filtration in reducing *E. coli* inoculated in pond water. Results show that reductions of *E. coli* populations occurred at the outset of filtration events, and ZVI-sand-mediated reductions were greater in early trials (1–3) than later trials (4–6). In field trials, ZVI-sand-filtered water contained less *E. coli* than sand-filtered waters, and also introduced lower levels of *E. coli* to spinach leaves and soils than sand-filtered waters. ZVI-sand-filtration represents a potential practical solution for small-scale growers to expand their use of different sources of irrigation water beyond groundwater while attempting to help them comply with microbial water quality standards.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## AUTHOR CONTRIBUTIONS

SK, PK, RB, EH, CE, SA, and MN contributed to experimental design, planning, and execution. KK, PC, and MS contributed to all aspects of the research project. AS responsible for experimental planning. SK responsible for statistical analysis/manuscript writing. MS responsible for experimental coordination and manuscript writing. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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