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Escherichia coli Reduction in Water by Zero-Valent Iron–Sand Filtration Is Based on Water Quality Parameters

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Abstract: Improving the microbial quality of agricultural water through filtration can benefit small farms globally. The incorporation of zero-valent iron (ZVI) into sand filters (ZVI–sand) has been effective in reducing *E. coli*, *Listeria* spp., and viruses from agricultural water. This study evaluated ZVI–sand filtration in reducing *E. coli* levels based on influent water type and the percentage of ZVI in sand filters. A ZVI–sand filter (50% ZVI/50% sand) significantly ($p < 0.001$) reduced *E. coli* levels in deionized water by more than 1.5 log CFU/mL compared to pond water over six separate trials, indicating that water type impacts *E. coli* removal. Overall reductions in *E. coli* in deionized water and pond water were 98.8 ± 1.7% and 63 ± 24.0% (mean ± standard deviation), respectively. Filters constructed from 50% ZVI/50% sand showed slightly more reduction in *E. coli* in pond water than filters made from a composition of 35% ZVI/65% sand; however, the difference was not statistically significant ($p = 0.48$). Principal component analysis identified that the turbidity and conductivity of influent water affected *E. coli* reductions in filtered water in this study. ZVI–sand filtration reduces *Escherichia coli* levels more effectively in waters that contain low turbidity values.

Keywords: *Escherichia coli*; zero-valent iron; sand; filtration; non-traditional water; pond; agricultural water; irrigation

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1. Introduction

Rapid urbanization, climate change, population growth, and water scarcity have focused more attention on agricultural water quality, specifically irrigation water intended for fresh fruits and vegetables that are to be consumed raw [1]. Growers are interested in the use of non-traditional irrigation water, including untreated surface waters, to supplement groundwater in order to reduce water scarcity [2,3]. However, multiple studies have shown that irrigation water from rivers, creeks, and ponds in the U.S. can contain varying levels of foodborne bacterial pathogens such as *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* [4–7]. Cooler seasons (lower water temperatures) were associated with a higher prevalence of *L. monocytogenes* compared to warmer seasons [4,6], and specific non-traditional water sources (ponds) had a lower prevalence of *Salmonella enterica* compared to rivers or creeks [6,7].

To prevent foodborne illness from the consumption of microbiologically contaminated produce, multiple criteria and standards for the microbiological quality of irrigation water have been established or recommended by multiple international, governmental, and industry organizations. Some of these are shown in Table 1.

Table 1. Selected current and proposed regulations and guidelines for the use of irrigation water in the growing and handling of edible produce.

International Agency or Organization	Water Use and Classification	Criteria (CFU/100 mL)	Reference
U.S. Food and Drug Administration (proposed to be implemented in 2022)	Water used during growing activities for covered produce	≤126 *E. coli* (geometric mean) ≤410 *E. coli* (statistical threshold)	FDA, 2018 [8]
	Water used for agricultural teas, sprout irrigation water, and postharvest washing	0 *E. coli*	
California Leafy Green Marketing Agreement (LGMA)	Type A agricultural water—unlikely to contain indicators of fecal contamination	0 *E. coli* (2 of 3 samples); ≤10 *E. coli* (remaining sample)	LGMA, 2020 [9]
	Type B agricultural water—water not subject to hydrogeologic filtration, U.S. EPA, or state-level treatment of water	≤126 *E. coli* (geometric mean); 0 *E. coli* when irrigation within 21 days of harvest ≤235 *E. coli* (single samples)	
World Health Organization	Water intended for: Crops likely to be eaten uncooked;	≤1000 *E. coli*	WHO, 1989; WHO, 2006 [10,11]
	Root crops;	≤10^3 *E. coli*	
	Leaf crops.	≤10^4 *E. coli*	
	Drip irrigation: unlikely to contact edible portion of crop	≤10^5 *E. coli*	
	Drip irrigation: likely to contact edible portion of crop	≤10^3 *E. coli*	
Tomato Good Agricultural Practices (T-GAP); U.S. state of Florida	Water to be used to irrigate tomatoes	≤126 *E. coli*	T-GAP, 2006 [12]
	Water to contact tomatoes at time of harvest	≤5% of samples ($n \geq 40$) positive for total coliforms	
European Food Safety Authority	Water intended for: Direct contact with edible portion of uncooked fruit/vegetable during preharvest/harvest;	≤100 *E. coli*	EFSA, 2017 [13]
	Indirect contact with edible portion of uncooked fruit/vegetable during preharvest/harvest;	≤1000 *E. coli*	
	Direct contact with edible portion of fruit/vegetable intended for cooking during preharvest/harvest;	≤1000 *E. coli*	
	Indirect contact with edible portion of cooked FFV during preharvest/harvest.	≤10,000 *E. coli*	

To meet these criteria, a variety of methods and techniques have been developed and updated to remove and inactivate pathogenic microorganisms in irrigation water intended for produce that is to be consumed raw or with minimal processing [9,14–17]. The use of chemical disinfection (sodium hypochlorite, peroxyacetic-acid-based sanitizers) remains a cost-effective method to disinfect surface irrigation water less than 21 days before harvest for a large portion of the leafy green crops grown in the U.S. [9]. However, concerns have emerged about the long-term effects of the accumulation of chlorine (hypochlorite) disinfectant byproducts (DBPs) in soils. DBPs such as chlorates, which can have negative human health effects, have been shown to accumulate and bioaccumulate in edible crops [14,15]. Membrane filtration technology is used effectively to reduce microorganisms in various settings, but this method can result in the development of bacterial biofilms, which can negatively affect filtration performance [16,17].

Sand filtration is a cost-effective and commonly used method to improve the microbial quality of drinking water [18,19], and has been used for agricultural water in order to prevent sediment from clogging drip irrigation lines [20]. Modifying sand filters by adding zero-valent iron (ZVI or Fe^0) has been shown to reduce viral pathogens and bacteria

in filtered water. In iron–sand filtration, ZVI is oxidized to Fe^{2+} and/or Fe^{3+} by water and dissolved oxygen, generating various iron oxides and hydroxides [21]. A redox transformation of ZVI can also result in degradation or adsorption of chemical pollutants. Several previous studies have demonstrated that ZVI can reduce the level of a multitude of chemicals in water, including bromate [22], chloropicrin [23], haloacetic acids [24,25], *N*-nitrosodimethylamine [26], and several classes of antibiotics [27].

Adding ZVI to sand may be a practical strategy to improve the microbiological quality of irrigation water for growers and producers who use sand filtration on a regular basis during irrigation of their crops. Marik et al. (2019) reported reductions in 2 log CFU *Escherichia coli* in surface water in over twenty filtration events using ZVI–sand filters [28]. Kim et al. (2020) found that ZVI–sand filtration reduced *Escherichia coli* levels by a significantly greater extent than sand filtration in inoculated surface water [29]. Sand filters containing iron-oxide-coated sand reduced *E. coli* levels by 1 log CFU compared to sand filters without iron oxide [30]. Other ZVI/sand filtration research has shown a reduction of human viral surrogates and virus-like particles and in agricultural or treated wastewater [31,32].

The reduction in bacteria or viruses in agricultural water by ZVI–sand filtration may be affected by the contact time with iron particles, which is influenced by iron particle size and loading in the filter [33], as well as influent water quality parameters such as pH [34] and dissolved oxygen [35]. However, those studies did not evaluate if different levels of ZVI can affect *E. coli* reduction levels by filtration, or evaluate longitudinal aspects of the effectiveness of ZVI–sand filtration. Evaluating specific factors in surface irrigation water that affect bacterial inactivation can help identify conditions where utilization of ZVI–sand filtration by small-scale farmers can optimally improve irrigation water quality.

The objectives of this study were to: (1) determine the effect of different water types (pond vs. deionized water) on *E. coli* removal by ZVI–sand filtration, (2) evaluate water quality parameters that affect *E. coli* reduction by ZVI-sand filtration, and (3) assess the effect of ZVI loading (35% vs. 50%) on *E. coli* removal.

2. Materials and Methods

2.1. Construction of ZVI and Sand Filter

Each filter system was made using 10 cm (diameter) by 30 cm (length) pieces of Charlotte polyvinyl chloride (PVC) with a 2.36 L interior volume. Columns were packed following the same procedures that were used previously [29]. To construct 50% ZVI–sand filters, each filter was filled with 1.18 L of ZVI particles (0.43–0.60 mm size, Peerless Metals, Detroit MI) and 1.18 L of sand (0.45–55 mm, Northern Filter Media, Muscatine, IA) using graduated cylinders. Filters consisting of 35% ZVI–sand filters were constructed by adding 0.83 L of ZVI particles to 1.53 L of sand particles. ZVI and sand particles were mixed in a sterile plastic bag. PVC columns were fitted with landscape cloth, which was used at the top and bottom of the cylinder to avoid leakage of particles.

2.2. Influent Water Collection and Characteristics

Water was collected from the Wye pond located at the University of Maryland Wye Research and Education Center (Wye REC, Queenstown, MD, USA). Pond water was collected in sterile 20 L carboys and stored at 4 °C for up to 7 days before use. DI water was collected at the USDA-ARS Environmental Microbial and Food Safety Laboratory. Pond water and DI water quality characteristics (turbidity, pH, DO, ORP, and conductivity) were measured using a ProDSS multiparameter water quality sonde/meter (YSI, Yellow Springs, OH, USA) at each trial before filtration events. Each parameter was measured in triplicate and mean values were calculated (Table 2).

Table 2. Summary of microbial reductions and associated water quality parameters of influent water for each filtration trial.

			Explanatory Variables				
	Reduction (%)	**Time (Days)**	**Turbidity(FNU)**	**pH**	**DO(%)**	**ORP(mV)**	**Conductivity (SPC µS/cm)**
DI 50% ZVI	99.74%	0	0	8.37	95.7	−12	3.6
	99.94%	5	0	8.49	96.1	−18.9	3.6
	99.76%	13	0	9.81	92.0	107.0	9.4
	99.13%	20	0	8.36	94.8	95.1	9.3
	99.21%	27	0	9.90	99.2	−25.0	7.1
	95.58%	35	0	9.40	96.6	−23.2	7.4
Mean ± standard deviation			0	9.05 ± 0.73	95.7 ± 2.3	20.5 ± 62.6	6.75 ± 2.60
Pond 50% ZVI	98.99%	0	4.3	8.51	47.9	26.0	172.3
	85.04%	5	1.9	8.21	93.2	13.0	156.1
	61.96%	13	19.6	7.87	97.1	206.43	209.2
	48.19%	20	4.4	8.61	93.9	178.1	188.1
	40.23%	27	160.9	8.41	79.1	45.0	151.9
	43.93%	35	47.7	8.96	85.2	72.7	353.3
Mean ± standard deviation			39.8 ± 61.7	8.42 ± 0.36	82.7 ± 18.2	90.2 ± 82.0	205.1 ± 75.5
Pond 35% ZVI	55.82%	0	8.9	9.29	80.9	−11.4	138.5
	40.71%	7	33.8	9.29	90.8	1.3	159.2
	31.19%	14	50.4	7.54	82.5	77.4	363.3
	−46.69%	16	47.7	8.96	85.2	72.7	353.3
Mean ± standard deviation			35.2 ± 18.9	8.77 ± 0.83	84.8 ± 4.3	35.0 ± 46.5	253.5 ± 121.2

2.3. Inoculum Preparation

A non-pathogenic, rifampicin-resistant *E. coli* strain, TVS 353, previously isolated from agricultural water [36], was cultured from frozen stock onto MacConkey agar (Neogen, Lansing, MI, USA) supplemented with 80 µg/mL rifampicin (Sigma-Aldrich, St. Louis, MO, USA) (MACR). For each filtration trial, a single colony of *E. coli* TVS 353 was inoculated into 10 mL tryptic soy broth (Neogen, Lansing, MI, USA) supplemented with 80 µg/mL rifampicin (TSBR) and grown for 18–24 h. Overnight populations were determined by serial dilution in phosphate-buffered saline (Sigma-Aldrich, St. Louis, MO, USA) (PBS), spiral-plated onto MACR using an easySpiral Dilute (Interscience, Woburn, MA, USA), and incubated at 37 °C for 18–24 h. Colony counts were determined and recorded. *E. coli* TVS 353, suspended in PBS, was diluted into 2 L of either DI or pond water to achieve a level of 4 log CFU/mL. Specific populations in the inoculated waters were determined using the method described above. In order to measure *E. coli* TVS 353 populations in sample fractions, bottles were homogenized by shaking for one minute, either spiral- or spread-plated onto MACR, and counts were determined as described above.

2.4. Filtration and Recovery of E. coli TVS 353

Filters were flushed with 2 L of DI water or pond water before testing. Inoculated water samples were pumped (Flex-Pro A4 ProSeries, standard peristaltic metering pump,

Cole-Parmer, Vernon Hills, IL, USA) vertically up through filters to avoid preferential flow, at a rate of 0.5 L/min. Approximately 1.2 L of water was pumped through the filter. For each trial, pond water (2 L) or DI water (2 L) was inoculated with 4 log CFU/mL *E. coli* TVS 353 and pumped through filters, followed by 8 L of uninoculated pond water or DI water. Each 2 L of filtered effluent was collected to determine *E. coli* TVS 353 populations via serial dilution and spiral plating, as described previously, on to MACR media. Six filtration trials were performed with both inoculated DI and pond water through 50% ZVI–sand filters, while four filtration trials were performed for pond water through 35% ZVI–sand filters. After each filtration event, valves receiving influent water and controlled effluent flow were closed so that filters did not lose water and remained wet.

2.5. Statistical Analysis

For statistical analysis, *E. coli* TVS 353 levels from each 2 L of filtered effluent were transformed to log CFU/mL and plotted against the volume of effluent (X) in the equation below. Data from each trial for each water type/filter were used to fit a linear regression model, leading to Equation (1):

$$Y = \alpha_1 + \alpha_2 X + \varepsilon \quad (1)$$

where α_1 (y-intercept) and α_2 (slope) are fitted parameters, and ε is the error term.

In Equation (2), dummy variable D_1 and D_2 were used, which were both set to 0 if the data originated from deionized water passed through 50% ZVI–sand filters; D_1 was 1 and D_2 was 0 if the data originated from pond water passed through 50% ZVI–sand filters; and D_1 was 0 and D_2 was 1 if the data originated from pond water passed through 35% ZVI–sand filters. That is:

$$\begin{cases} D_1 = 0 \; and \; D_2 = 0, \; if \; data \; come \; from \; DI \; 50\% \; \text{ZVI} \\ D_1 = 1 \; and \; D_2 = 0, \; if \; data \; come \; from \; Pond \; 50\% \; \text{ZVI} \\ D_1 = 0 \; and \; D_2 = 1, \; if \; data \; come \; from \; Pond \; 35\% \; \text{ZVI} \end{cases} \quad (2)$$

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

$$Y = \alpha_1 + \alpha_2 X + (\alpha_3 + \alpha_4 X) \times D_1 + (\alpha_5 + \alpha_6 X) \times D_2 + \varepsilon \quad (3)$$

where α_1, α_2, α_3, α_4, α_5, and α_6 are the fitting coefficients and D_1 and 2 are dummy variables. Three different equations were acquired based on the different water type and ZVI ratio:

$$Y_{DI \; 50\% \; \text{zvi}} = \alpha_1 + \alpha_2 X + \varepsilon \quad (4)$$

$$Y_{pond \; \text{ZVI} \; 50\%} = (\alpha_1 + \alpha_3) + (\alpha_2 + \alpha_4) X + \varepsilon \quad (5)$$

$$Y_{pond \; \text{ZVI} \; 35\%} = (\alpha_1 + \alpha_5) + (\alpha_2 + \alpha_6) X + \varepsilon \quad (6)$$

The null hypothesis was that coefficient α_I is equal to zero and the alternative hypothesis was that α_I is not zero for the linear regression model. Coefficients α_1, α_3, and α_5 are y-intercepts, and α_2, α_4, and α_6 are slopes for fitting the linear regression model. *E. coli* reductions (log- and percentage-based) in inoculated pond and DI water effluents were calculated by comparing the area under the curve (AUC) generated from *E. coli* recovered in effluents to the initial inoculum level for each trial.

For this study, principal component analysis (PCA) was used to characterize variations in water quality parameters and reduce dimensionality. R version 3.53 was used to compute PCA [37]. PCA, which can characterize explanatory variables, was applied to this study. For the PCA analysis, water quality parameters (turbidity, pH, DO, ORP, and conductivity) measured from deionized and pond water before filtration and inoculation with *E. coli* were used from Table 3. Water quality parameters, along with the time variable, were

applied to PCA analysis as explanatory variables. Principal components 1 (PC1) and 2 (PC2) accounted for 34.9% and 23.8% of the variance, respectively.

Table 3. The reduction in *E. coli* TVS 353 in (a) deionized water filtered through 50% ZVI/50% sand filters (ZVI 50% DI), (b) in pond water filtered through 50% ZVI–sand filters (ZVI 50% pond) or (c) in pond water filtered through 35% ZVI–sand filters (ZVI 35% pond) in each individual trial as shown by percentage and log reduction. *E. coli* reductions were calculated using the area under the curve (AUC) method.

		Reduction in *E. coli* by Filtration					
		ZVI 50% DI		**ZVI 50% Pond**		**ZVI 35% Pond Water**	
Trial	**Time (Days)**	**Pct (%)**	**Log CFU/mL**	**Pct (%)**	**Log CFU/mL**	**Pct (%)**	**Log CFU/mL**
1	0	99.74	2.28	98.99	1.85	55.82	0.34
2	5	99.94	3.16	85.04	0.78	40.71	0.17
3	13	99.76	2.42	61.96	0.34	31.19	0.06
4	20	99.21	2.04	48.19	0.26	−46.69	−0.26
5	27	99.13	2.00	40.23	0.18	- [1]	-
6	35	95.58	1.20	43.93	0.19	-	-
Mean ± standard deviation		98.8 ± 1.7	2.12 ± 0.64	63.0 ± 24.0	0.60 ± 0.65	20.3 ± 45.7	0.08 ± 0.25

[1] Trials 5 and 6 were not conducted for ZVI 35% pond water.

3. Results

E. coli levels were reduced more significantly ($p < 0.001$) when inoculated deionized (DI) water was passed through a 50% ZVI filter compared to when pond water was passed through either 50% ZVI or 35% ZVI filters (Figure 1).

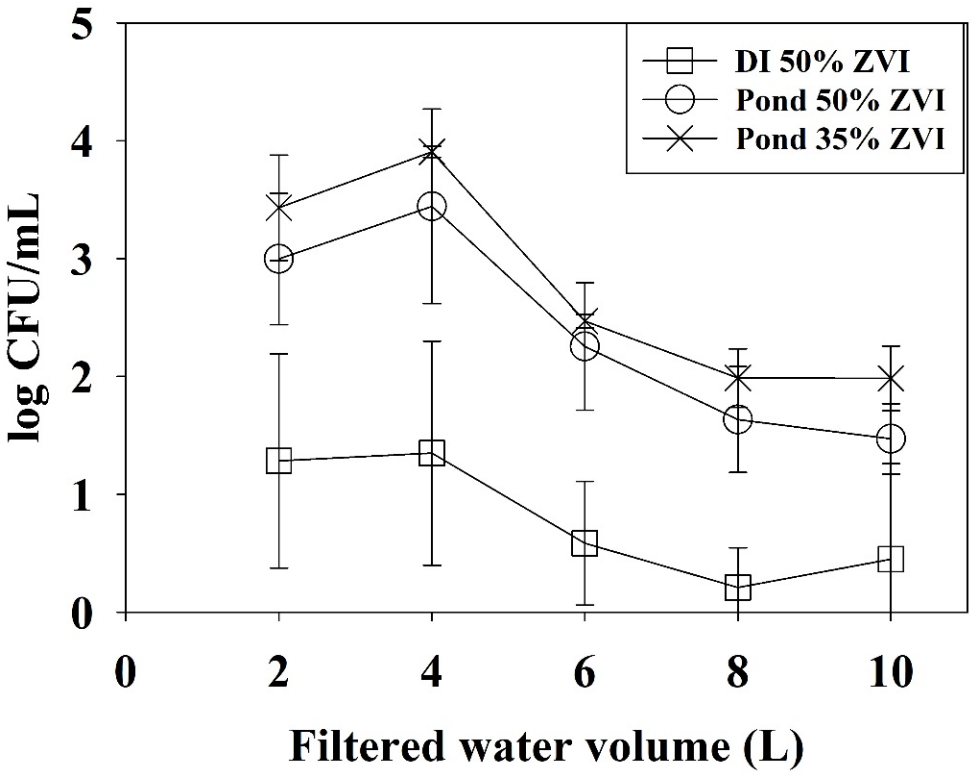

Figure 1. Mean *E. coli* reductions in effluents after filtration of pond water through 50% ZVI–sand filters (x); pond water through though 35% ZVI–sand filters (squares); and deionized water through 50% ZVI–sand filters (open circles). *E. coli* levels were determined from every 2 L aliquot of effluent water after filtration.

There was no statistical difference ($p = 0.48$) in reduction in *E. coli* levels between pond water filtered through 50% ZVI–sand and pond water filtered through 35% ZVI–sand filters. Results obtained from the linear regression analysis are summarized below. The regression model had a R^2 value of 0.74. Coefficient α_1 (1.61 log CFU/mL) is the y-intercept for DI water filtered through 50% ZVI–sand filters (Equation (4)). Coefficient α_3 (2.2 log CFU/mL) represents the difference between y-intercept value α_1 (DI water passed through 50% ZVI–sand filters) and the y-intercept value for pond water filtered through 50% ZVI–sand filters ($\alpha_1 + \alpha_3$, Equation (5)).

In practical terms these findings show *E. coli* levels in the first 2 L of effluent of DI passed through a 50% ZVI–sand filter were 2.2 log CFU/mL lower than *E. coli* levels in the first 2 L of effluent of pond water passed through a 50% ZVI–sand filter. Similarly, coefficient α_5 (2.58 log CFU/mL) is the difference between α_1 and the y-intercept value for pond water filtered through 35% ZVI–sand filters ($\alpha_1 + \alpha_5$, Equation (6)). Practically, *E. coli* levels in the first 2 L of effluent of DI passed through a 50% ZVI–sand filter were 2.58 log CFU/mL lower than pond water passed through 35% ZVI–sand filters. Coefficient α_1, α_3, and α_5 were all statistically significant ($p < 0.001$). Of the three calculated slope values, α_2 (0.14), α_4 (0.10), and α_6 (0.10), only α_2—the slope coefficient for DI water filtered through 50% ZVI–sand filters—was statistically significant ($p < 0.001$), indicating that the rate of *E. coli* decline in deionized water passed through 50% ZVI–sand filters was significantly different than *E. coli* in pond water passed through 35% or 50% ZVI–sand filters.

Table 3 shows the reduction in *E. coli* in each combination of water type and ZVI concentration by individual experiment trials. *E. coli* reductions were determined by the AUC. As Table 3 shows, deionized water and pond water have different water quality parameters, which influenced the reduction efficacy of *E. coli* by ZVI–sand filtration.

At least a 2-log reduction (99%) of *E. coli* levels in deionized water filtered through 50% ZVI–sand filters was achieved in the first five trials, resulting in a mean reduction of 98.8% ± 1.7% compared to levels of *E. coli* in influent water. In comparison, *E. coli* in pond water filtered through 50% ZVI–sand filters was reduced by 63.0% ± 24.0%. The level of *E. coli* in pond water filtered through 35% ZVI–sand filters was reduced by 20.3% ± 45.7%. The comparatively high levels of reductions in *E. coli* in deionized water filtered through 50% ZVI–sand filters are in contrast to the pond water being passed through 50% ZVI–sand filters. In pond water passed through a 50% ZVI–sand filter, only trial one showed a 1.85-log reduction in *E. coli* levels, with trials 2–6 (0.19–0.78-log reduction) showing decreasing effectiveness in reducing *E. coli* in pond water.

The most dramatic difference in water quality parameters between pond water and deionized water were observed with turbidity and conductivity values. Figure 2 presents the principal component analysis (PCA) of the percentage reduction in *E. coli* with regard to water quality factors and time, based on which day each trial occurred compared to the first trial.

PC1 and PC2 accounted for 34.9% and 23.8% of the variance, respectively. Trials with lower *E. coli* reductions were correlated with higher conductivity, ORP, and turbidity values. Higher pH and DO values were measured in deionized water compared to pond water (Table 3) and were associated with larger reductions in *E. coli*.

Values for pH and dissolved oxygen values were positively correlated; however, these two variables were negatively correlated with conductivity. Conductivity was positively correlated with ORP (oxidation–reduction potential) and turbidity. ORP and turbidity were extremely well-correlated, but ORP contributed less to PC1 than turbidity. The time variable contributed more than any other variable to PC2 but was not correlated with any water quality factor. Overall, trials with *E. coli* reductions greater than 90%, as determined by the area under the curve (AUC) method, were associated with higher pH and DO values.

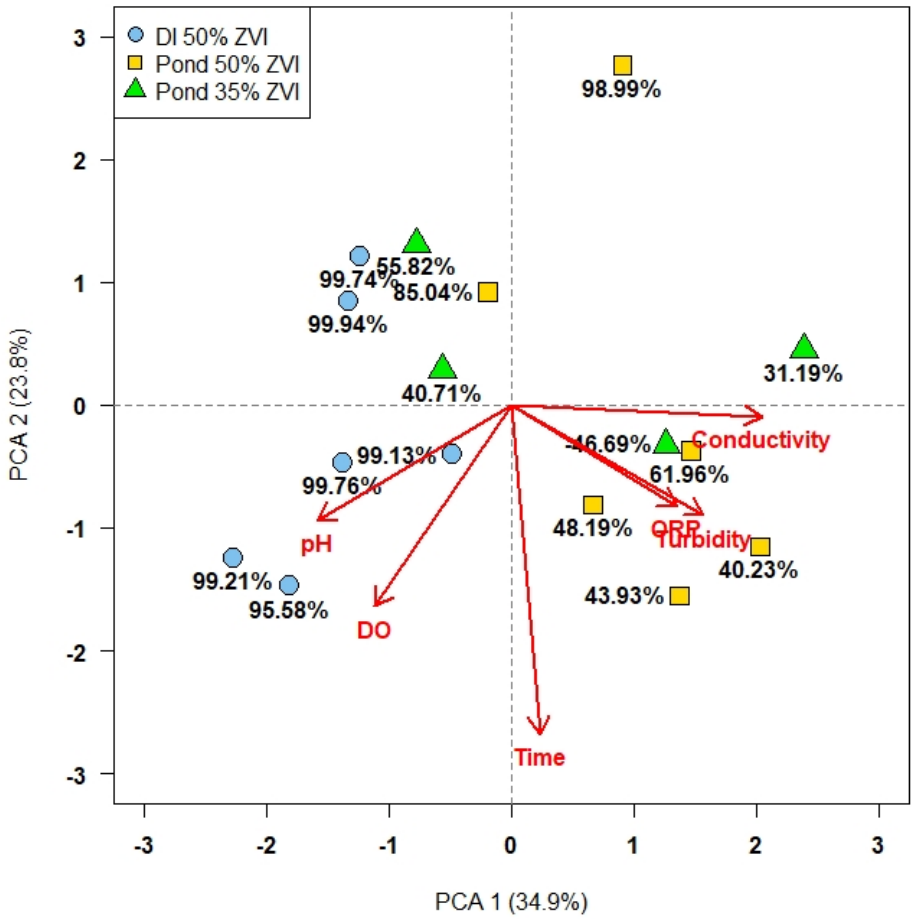

Figure 2. Principal component analysis (PCA) of reduction in *E. coli* by all ZVI-sand filtration combinations with water quality factors. Prior to PCA modeling, all variables were centered to the mean of each variable and scaled by their respective standard deviations. Percentage reductions in *E. coli* are plotted on the bottom (PC1) and leftmost (PC2) axes. The smaller the size of the angle between specific water quality variables, the more closely correlated they are; the length and direction of each vector line indicates how much each water quality variable contributes to either PC1 or PC2.

4. Discussion

Zero-valent iron filtration more effectively reduced levels of *E. coli* in deionized water than in pond water. *E. coli* levels in pond water were not reduced by ZVI/sand filtration in later trials. These results indicate that different water types, with different levels of water quality parameters (specifically turbidity and conductivity), influence the effectiveness of ZVI–sand filters in reducing *E. coli* levels. *E. coli* levels in pond water filtered through 35% ZVI–sand filters in trials 1–3 showed a mean reduction of 42%, with a negative reduction in *E. coli* occurring in trial 4. The lack of reductions in effluent may be due to viable *E. coli* cells being physically trapped but not inactivated in 35% ZVI–sand filters. These *E. coli* cells may emerge in later trials with the same filters as additional influent water dislodges these viable *E. coli* into the effluent, causing the level of *E. coli* in the effluent to exceed levels in the influent for that specific trial. Higher levels of turbidity and conductivity in pond water compared to deionized water may account for the lower levels of inactivation of *E. coli* in pond water during our study. In addition, the presence of other microbes in pond water may have affected the reduction in *E. coli* TVS 353 in pond water.

Findings from several previous studies demonstrated that bacterial cells are inactivated from contact with ZVI particles [28,35,38]. Exposure to ZVI particles may incur oxidative stress lethal to bacterial cells during the transition of iron from a zero-valent state (Fe^0) to ferrous (Fe^{2+}) [35,39]. Other research evaluating the longevity (ability of ZVI–sand to reduce *E. coli* levels in water over time) of 35% ZVI–sand filters reported that *E. coli* levels

in inoculated pond water (ca. 7 log CFU/mL) were consistently reduced by an average of 2.3 log CFU/mL over 13 trials (390 L) [28]. Different physicochemical factors in the influent water and the original inoculum levels may result in varying levels of longevity between the results of Marik et al. (2019) and this study [28]. Previous studies reported that influent alkalinity, reduction potential, and pH were likely factors influencing the performance of ZVI permeable reactive barriers (PRB), with higher pH values in influent water diminishing the performance of ZVI filters [40]. The decreased ability of 50% ZVI–sand filters to reduce *E. coli* in pond water after the fourth trial may be due to higher turbidity levels in pond water compared to deionized water (Table 3), due to the organic load accumulating within the filter over time effectively reducing the removal and inactivation efficiencies. The results of the linear regression model are in agreement with Kim et al. (2020), where *E. coli* reduction by ZVI–sand filtration was reported to occur immediately after filtration [29].

The results of the principal component analysis showed *E. coli* reductions, regardless of influent water type and filter composition, were strongly related with water quality parameters. PC1 and PC2 explained 34.9% and 23.8% of the variability, respectively. Higher conductivity values may have led to the development of a passivation layer on the iron particles, decreasing the interaction between the surface area of iron particles in ZVI filters and *E. coli*, reducing the inactivation of *E. coli* [41]. Previous studies reported that iron hydroxides can form a passivation layer on the surface of iron particles. In addition, we hypothesize that organic matter (measured as turbidity) blocked iron surfaces from contacting *E. coli* cells, resulting in decreased *E. coli* reductions in trials conducted with pond water. Previous studies have also shown that reduction in *E. coli* in pond water decreased with repeated ZVI–sand filtration through the same system over time [29]. Many studies have shown that the initial pH values played a critical role in the removal of metals or inactivated microbes [42–44]. Kim et al. (2011) found that inactivated *E. coli* through nano-ZVI (nZVI) was greater under de-aerated conditions (low DO values) than under air-saturated conditions (higher DO values) [35,44]. These results indicate that reductions in *E. coli* through ZVI–sand filtration can be improved if turbidity and conductivity levels are actively lowered and managed during the maintenance of ZVI filtration, and if water quality is characterized in advance of ZVI–sand filtration.

ZVI–sand filtration inactivated or removed *E. coli* most efficiently from water containing lower turbidity values (deionized water) compared to pond water. Higher levels of conductivity and turbidity, and oxidation–reduction potential potentially negatively influenced *E. coli* reduction by ZVI–sand filtration. Reducing turbidity in water, as well as backflushing filters to remove organic matter before ZVI–sand filtration, may improve the removal of *E. coli* in surface water.

5. Conclusions

Zero-valent iron (ZVI)–sand filtration reduced *Escherichia coli* levels in different types of influent water. *E. coli* populations were reduced by larger amounts in DI when filtered through 50% ZVI/sand columns compared to pond water. The quantitative reduction in *E. coli* was dependent upon chemical parameters of the influent water. Higher turbidity, conductivity, and ORP levels in pond water compared to DI water may have contributed to the lower *E. coli* reductions observed in pond water. While ZVI filtration was effective in reducing *E. coli* in early trials with pond water, its effectiveness over time decreased, indicating additional modifications are needed to increase the longevity of effective filtration. These findings indicate that ZVI filtration can be used to reduce *E. coli* in surface waters to help small farmers and growers gain compliance with government and commercial irrigation water standards.

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experimental design, experimental procedures, data management, and manuscript preparation; K.E.K.—experimental design, experimental procedures, technical advice, and manuscript preparation; M.S.—experimental design, experimental procedures, data management, data analysis, and manuscript preparation. All authors have read and agreed to the published version of the manuscript.

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