Evaluating the efficacy of nutrient solution reuse in hydroponics

by

Millicent Tetteh

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Major Professor
Manreet Bhullar, Ph.D.

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Abstract

Nutrient solution reuse in hydroponics is a sustainable strategy to improve resource efficiency. However, increased microbial risks, nutrient toxicity, and accumulation of allelochemicals can compromise system safety and crop yield. The first objective of this study they are not specifically tailored to hydroponic nutrient solutions. This review synthesizes current knowledge on the risks and opportunities of nutrient reuse in hydroponic systems, highlighting emerging treatment technologies and the urgent need for hydroponics-specific, science-based standards to ensure both sustainability and food safety to support production by small scale farmers. In the second objective, we evaluated the combined performance of slow sand filtration (SSF), biochar filtration, and UV-C disinfection for multi-cycle reuse of hydroponic nutrient solution in romaine lettuce production and assessed the impact of treated nutrient solutions use of plant growth and nutritional quality. Results showed that SU (SSF + UV-C) and SBU (SSF + Biochar + UV-C) consistently achieved ≥ 5 $\log_{10}$ reductions of *E. coli*, meeting World Health Organization (WHO) microbial safety criteria. Physically, turbidity remained <2 NTU, and SBU showed the highest light transmittance. Chemically, both treatments reduced ammonium-N, total N, and total P, with SBU showing the highest removals. However, nitrate levels remained relatively stable, calcium steadily accumulated across cycles, and pH in SU and C fell below Food and Agriculture Organization (FAO)/World Health Organization (WHO) guidelines. Micronutrient analysis revealed substantial declines in Fe, Zn, Cu, and Mn—up to 90–97% in SBU resulting in progressive micronutrient imbalance despite EC-based nutrient supplementation. Romaine lettuce grown in SU initially produced the highest biomass but declined by ~30% across cycles, while SBU showed even larger reductions (~45%). Chlorophyll, SPAD, and vitamin C decreased in all treatments, reflecting micronutrient depletion, salinity stress, and oxidative damage. The freshwater treatment also experienced reduced quality due to ammonium accumulation. Overall, combining SSF, biochar, and UV-C provides effective microbial inactivation and maintains physical water quality but progressively depletes essential micronutrients, limiting long-term crop performance. These findings highlight the potential of low-cost treatment systems for small-scale hydroponic growers while emphasizing the need for cycle-specific micronutrient supplementation to sustain yield and nutritional quality during extended nutrient-solution reuse.

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Lastly, I am thankful to God, my family, and my partner, Christian Mensah.

Dedication

I dedicate this to God Almighty, the Tetteh family, and my partner, Nana Kwame Arhin Mensah.

To everyone dreaming, keep going.

Big things can come from small places.

Preface

This is the original, unpublished work by T. Millicent.

Chapter1 Risks and Opportunities of Nutrient Reuse in Hydroponic Agriculture for Small Scale Producers: A Review

Abstract

Hydroponics is a form of soilless production that promises high yields, efficient use of land and water, and a year-round supply of fresh produce in urban areas. Central to its sustainability is the recirculation of nutrient solutions, which conserves resources and reduces effluent discharge. However, nutrient reuse also introduces new challenges. Imbalances in macro- and micronutrients, salt and ion accumulation, and the build-up of root exudates can impair plant growth and quality. Recirculating systems further increase the risk of microbial contamination by pathogens such as *Escherichia coli*, *Salmonella*, and *Listeria*, which are of particular concern for raw leafy greens. Several mitigation approaches, including physical, chemical, and biological treatments, have demonstrated effectiveness in reducing microbial loads and recovering nutrients, but no unified framework exists for their implementation. While the U.S Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) and international standards from the World Health Organization (WHO), Environmental Protection Agency (EPA), and the Food and Agriculture Organization (FAO) provide benchmarks for microbial and physicochemical water quality, they are not specifically tailored to hydroponic nutrient solutions. This review synthesizes current knowledge on the risks and opportunities of nutrient reuse in small-scale hydroponic systems, highlighting emerging treatment technologies and the urgent need for hydroponics-specific, science-based standards to ensure both sustainability and food safety, thereby supporting food production.

1.1 Introduction

Since the turn of the 20th century, urbanization has been a major driving force (Bisaga et al., 2019). Currently, 55% of the world's population lives in urban areas, with a predicted increase to 68% by 2050. This growing trend has led to significant transformations in agriculture, enabling increased productivity on an industrial scale to meet the rising food demand (Alkhaja et al., 2025; Maxwell & Slater, 2003; Satterthwaite et al., 2010). Despite their economic advantages, urban regions experience persistent food insecurity, unequal access to fresh produce, and vulnerability to supply chain disruptions (Coleman-Jensen et al., 2022; Losada-Rojas et al., 2021; Hecht et al., 2019). The dependence of urban areas on modern food production also affects food prices and availability. These challenges have intensified interest in *local food production systems* that can supply fresh, nutritious produce within cities (Russell & Heidkamp, 2011).

Urban agriculture is defined as the production, processing, distribution, and recycling of plants and animals in land and other spaces within the boundaries of urban and suburban regions (Artmann & Sartison, 2018; FAO, 2025; Zhu et al., 2024). They include rooftop gardens, traditional farming, hydroponics, aquaponics, and indoor farming. They are typically ground-based or integrated into buildings, with or without consideration for space efficiency (Goldstein et al., 2016). Urban agriculture makes a meaningful contribution to food availability and environmental sustainability; studies predict that it could supply up to 30% of the global urban population's vegetable demand (Kriewald et al., 2019; Martellozzo et al., 2014). It can save 14-15 kilowatt-hours of energy, sequester 100,000–170,000 tons of nitrogen, and prevent the runoff of 45–57 billion cubic meters of stormwater annually (Pradhan et al., 2023), thereby benefiting the environment.

However, small-scale, urban, and limited-resource growers face unique and persistent constraints that limit their ability to adopt or scale production. The most critical barriers include limited land availability (Nchanji, 2017), high costs of infrastructure and inputs, decreasing availability of water, and water pollution (Bisaga et al., 2019),, and competition for labor with other urban sectors (Hofmann, 2013; Zakir Hussain & Markus Hanisch, 2012). This has led to growing interest in closed-loop, resource-efficient agricultural models in urban agriculture settings, where water and nutrients are conserved and reused (Bisaga et al., 2019; Guiné, 2024; Manzoor et al., 2024; Sousa et al., 2024). Hydroponics is the soilless cultivation of crops in nutrient-rich solutions and inert substrates such as perlite and rockwool (Zimmermann & Fischer, 2020). In comparison to conventional farming, it leads to faster yields, offers year-round production, and reduces water use by up to 90% (Rajaseger et al., 2023a) and reduces land use (Gumisiriza et al., 2022).

To improve the circularity and reduce input costs, hydroponic systems often recirculate nutrient solutions (Asaduzzaman et al., 2022; Rufí-Salís et al., 2020; Son et al., 2020). However, recirculation can result in ion imbalances, nutrient deficiencies or toxicities, and the accumulation of allelochemicals that impair crop growth and quality (Fathidarehnijeh et al., 2024a; Maucieri et al., 2018). Furthermore, microbial risks from contaminated water, nutrient solutions, and implements such as seeds present another dimension that could compromise public health; therefore, treatment methods are needed to ensure system safety (Asaduzzaman et al., 2022). Because of these risks, nutrient solutions must be treated or sterilized before reuse to ensure system safety. This review examines the opportunities and risks associated with nutrient reuse in hydroponic systems and evaluates treatment strategies that support safe and sustainable production in small-scale urban agriculture.

1.2 Hydroponics

Hydroponics is defined as the process of growing crops in a soilless medium, typically using a nutrient solution. It can be traced back to ancient Egyptian, Babylonian, and Aztec civilizations (Fathidarehnijeh et al., 2024a). However, hydroponics was popularized by Professor William Gericke in the 1930s (Sharma et al., 2022). Compared to the ancient floating gardens, modern hydroponics has evolved into a highly efficient production system. It supports the growth of cucumbers and leafy greens such as kale, spinach, and lettuce, all of which are high-value crops. Market statistics estimate global vegetable consumption at 504 million metric tons in 2021 (Pandrc, 2022). Additionally, fruit consumption also reached 260 million metric tons, emphasizing the scale of the opportunity for hydroponically grown crops (Pandrc, 2022). This may be one of the reasons that accounts for the industry’s growth. The hydroponic industry is currently valued at $5 billion as of 2023 (Grand View Research, 2025). It is projected to grow at a compound annual growth rate (CAGR) of 12.4% through 2030 (Grand View Research, 2025; Sathyanarayana et al., 2022).

Hydroponics offers several advantages that make it well-suited for small-scale, urban, and limited-resource growers. Compared to traditional agriculture, hydroponics maximizes resource efficiency by using up to 90% less water (Rajaseger et al., 2023b) and requiring approximately 25% of the nutrients typically needed in open-field production (Khan et al., 2020). Hydroponics can also produce substantially higher yields, with studies reporting a 92% increase in crop productivity relative to the 77% increase observed under conventional farming practices (Maboko & Plooy, 2009). In addition, hydroponic systems support year-round production, improve crop uniformity and quality, optimize spatial use (especially in dense urban environments), and contribute to broader environmental sustainability goals (Baddadi et al., 2019; Fathidarehnijeh et al., 2024a; Rajaseger et al., 2023a). These combined benefits make hydroponics a compelling strategy for improving food security and local food access in urban settings.

1.2.1 Barriers and Limitations to Small-Scale Urban Farms

Despite its advantages, the adoption of hydroponics among small-scale urban growers is limited by several economic, technical, and infrastructural constraints. One of the most significant barriers is the high initial capital investment, which includes costs for structures, pumps, lighting, water treatment, and nutrient management systems. They can require up to 82 times more energy than conventional agriculture (Barbosa et al., 2015). Additionally, it requires technical expertise in system design, nutrient management, and maintenance; skills which may not be widely accessible to small growers (Kannan et al., 2022; Pomoni et al., 2023; Vourdoubas, 2015).

However, Bertello et al (2022), Ding et al (2022), Li et al (2021) recommend that the high cost associated with such enterprises can be offset through local material sourcing, government subsidies, or collaborative funding. Gumisiriza et al. (2022) designed a low-cost Kratky-based hydroponic system in Uganda using locally sourced materials and demonstrated that such systems can be profitable under smallholder conditions. Similarly, Folorunso et al.,

(2023) further reinstated the profitability of small scale hydroponics. it was confirmed that small scale hydroponic systems can achieve an estimated 10-year Internal Rate of Return (IRR) of 83%, indicating strong economic viability. Additional pilot studies in sub-Saharan Africa further demonstrate the success of low-cost hydroponics for leafy greens and tomatoes (Kookana et al., 2020; Zee et al., 2024).

With technology, Shin et al., (2024) developed SMART GROW, a low-cost automated monitoring system for pH, electrical conductivity (EC), and water levels explicitly designed for small-scale hydroponics. This system reduces labor requirements and supports growers with limited technical expertise. Furthermore, integrating hydroponic systems with renewable energy sources, such as solar, geothermal, and wind power, can significantly reduce energy costs. For example, the use of optimized renewable systems has been shown to decrease operational energy expenditures by up to 18% (Barbosa et al., 2015; Kaminski & Babbitt, 2024).

1.2.2 Classification of Hydroponics

Hydroponics can be grouped based on the method by which the nutrient solution is supplied to the plant roots (Hussain et al., 2014). Hydroponic systems can be classified into two types: solution culture and media culture. In solution culture, roots are suspended in a nutrient-rich solution, whereas in media culture, roots are supported by a substrate. Substrates can be organic, inorganic, or synthetic in nature (M. Enzo et al., 2007). The system adopted depends on the type of crop, environmental conditions, the scale of operation, and available resources (Velazquez-Gonzalez et al., 2022).

Table 1. Summary of Hydroponic System Designs and Recirculation Modes

Hydroponic System	**Characteristics**	**Recirculating systems**	**Reference**
Nutrient Film Technique (NFT)	Common Plant roots are submerged in a thin, aerated nutrient solution.	Closed/open loop system	(Fathidarehnijeh et al., 2024b; Griffith et al., 2023; Kozai,

	Suitable for small or urban farming operations		2019; Nitu et al., 2024; Wongkiew et al., 2017)
Deep Water Culture (DWC)	Less complex Plants' roots are continuously suspended in an aerated nutrient solution. Uses substantial amounts of nutrient solution. Common in commercial hydroponic systems	Closed/open loop system	(Hebbar et al., 2022; Kozai, 2019; Shohael et al., 2017; Singh et al., 2019; Vallotton, 2023)
Drip System	Uses tubes and emitters to deliver precise amounts of nutrient solution to plant roots	Closed/open loop system	(Cifuentes-Torres et al., 2021; Fathidarehnijeh et al., 2024b; Lal, 2001; Rosa-Rodríguez et al., 2020)
Aeroponics	Uses a misting spray to deliver nutrient solution to plant roots High maintenance	Open-loop system	(Santosh & Gaikwad, 2023)
Ebb and Flow (Flood and Drain)	Periodic flooding of plant roots with nutrient solutions. The regular repetition of this cycle facilitates the provision of nutrients and oxygen to the roots.	Closed	(Lal, 2001; Nicola et al., 2007)

Wick System	Simple Passive nutrient solution distribution to plant roots through capillary action	Closed, non-recirculating hydroponic system	(Ferrarezi & Testezlaf, 2016; Shrestha & Dunn, 2010)

Hydroponic systems can be classified as open-loop or closed-loop systems based on the method of recirculating nutrient solutions. In open-loop systems, the nutrient solution is used once and then discarded. It is easy to manage, but the discharge of nutrient solutions into the environment compromises the sustainability of this production system. In contrast, closed-loop hydroponic systems continuously recirculate the nutrient solution, allowing for repeated use of both water and dissolved nutrients. For small-scale urban growers, this system offers several advantages. Closed-loop systems can achieve up to 54.3% higher water productivity (Fayezizadeh et al., 2021), reduce fertilizer consumption by as much as 96% (Cifuentes-Torres et al., 2021; Fayezizadeh et al., 2021) and promote greater environmental sustainability (Fathidarehnijeh et al., 2024b; Fayezizadeh et al., 2021).

Despite these benefits, nutrient recirculation introduces important management challenges. Major concerns include the accumulation of root exudates, which can lead to autotoxicity and the formation of allelopathic or inhibitory compounds that negatively affect plant growth (Hosseinzadeh et al., 2017); the accumulation of ions that cause salinity issues and nutrient imbalances that impair uptake and plant physiology (Rouphael et al., 2006). Also the reuse of nutrient solution increases the risk of pathogen recurrence (Asaduzzaman et al., 2022). Collectively, these concerns have major effects on crop yield, quality, and food and system safety (Pardossi et al., 2002; Poulet et al., 2016)

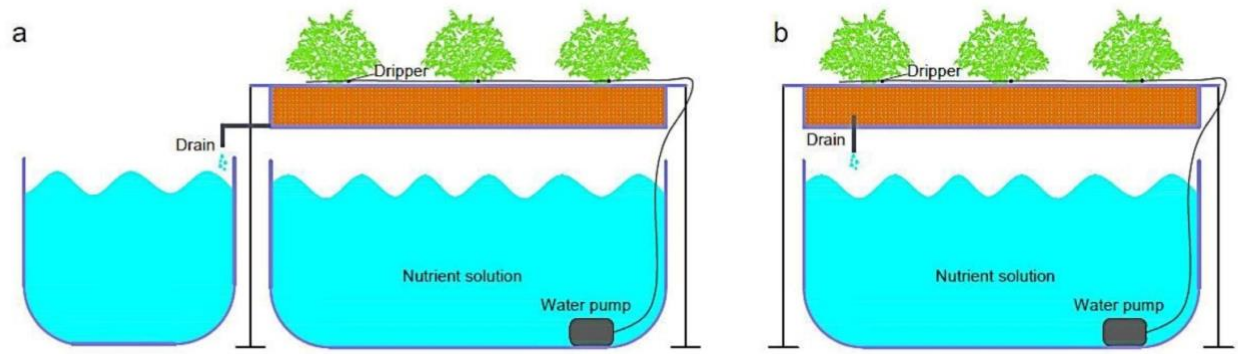

Figure 1. Illustration of (A) An Open-Loop Drip Hydroponic System And (B) A Closed-Loop Drip Hydroponic System (Fathidarehnijeh et al., 2024c)

1.3 Nutrient Imbalances and Chemical Risks Associated with Nutrient Solution Reuse

A standard management technique in hydroponics is adjusting the Electrical Conductivity (EC) of the nutrient solution to provide plants with adequate fertilizer ions to support growth. EC is a measure of the total amount of fertilizer salts or ions in a solution (Nemali, 2018) used to maintain nutrient levels in both fresh and recycled systems (Miller et al. 2020). However, for closed-loop hydroponics, EC alone remains insufficient to determine the actual nutrient profile. Over time, plant uptake, water quality, and the duration of reuse cause fluctuations in individual nutrient concentrations, leading to variations in nutrient availability (Tomasi et al., 2015). These shifts can create ionic imbalances (Liu et al., 2025a), that alter the ratios and bioavailability of essential elements; needed to support plant growth. Macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) are critical due to their central roles in metabolism and biomass formation (Carpanez et al., 2022, 2024). Micronutrients, including iron, boron, manganese, zinc, and copper, are also essential in trace quantities. In closed-loop hydroponics, these imbalances are caused by differences in nutrient uptake rates by plant roots, concentration changes due to evaporation, and ion desorption dynamics in the substrate (Lim et al., 2025). The disproportionate uptake or depletion of these nutrients can disrupt electrochemical balance in the root zone, leading to antagonistic ion interactions in which an increase in one ion suppresses uptake of another (Almeselmani, 2022; Modu et al., 2020).

In reused nutrient solutions, ions such as sodium (Na), calcium (Ca), and magnesium (Mg) may accumulate due to poor absorption, especially when growers rely on alkaline or lower-quality water sources (Kaushal et al., 2023; Liu et al., 2025a; Miller et al., 2020a; Sambo et al., 2019). This accumulation increases salinity, induces osmotic stress, and reduces growth and yield. elevated concentrations of bicarbonates, Na, Ca, or Mg can raise EC readings even when essential nutrients like N, P, or K have been depleted due to plant uptake. As a result, EC may appear within the optimal range despite the presence of underlying nutrient deficiencies. This challenge is especially problematic for small growers who depend primarily on EC rather than ion-specific monitoring. They may therefore misinterpret a "normal" EC value as evidence that nutrient levels are sufficient, even though the crop is functionally nutrient-limited. Nutrient imbalances caused by reuse affect plant morphology, decrease leaf area, lead to suboptimal plant nutrition, but can also increase plant root elongation (Liu et al., 2025b; Neocleous et al., 2017).

1.3.1 Chemical Risks and Accumulation

In addition to nutrient-related issues, chemical risks are a major concern in recycled hydroponic systems. Reuse conserves water and fertilizer inputs; it also promotes the accumulation of dissolved substances when the incoming water source is of poor quality or when solutions are reused for extended periods (Neocleous et al., 2017). Nutrient solutions contain macronutrients like nitrogen (N), phosphorus (P), and potassium (K). These come from calcium nitrate ($Ca\ (N_3)\ _2$), potassium nitrate (KNO_3), and mono-potassium phosphate (KH_2PO_4). In addition, micronutrients needed for plants include calcium (Ca), manganese (Mn), zinc (Zn), boron (B), iron (Fe), molybdate (Mo), chlorine (Cl), magnesium (Mg), copper (Cu), and sulfur (S) (Sangeetha & Periyathambi, 2024).

Under closed-loop operation, ions such as Na^+ and Cl^- can accumulate to levels that exceed crop tolerance thresholds, introducing osmotic stress, ion-specific toxicity, and nutrient disorders (Katsoulas et al., 2015; Neocleous et al., 2017; Pardossi et al., 2002) that affect crop growth and yield (Bar-yosef, 2008). Heavy metals are generally defined as elements with high atomic mass and density (typically >4–5 g cm^{-3}) and include both metals and metalloids (Edelstein & Ben-Hur, 2018; Vardhan et al., 2019). Heavy metals such as Cd, Cu, Pb, Mg, Ni, and Zn are major contaminants (Chrysargyris et al., 2021; Soudek et al., 2011) and present a considerable risk in recycled hydroponic systems. Once absorbed by plant membranes, they cause imbalances between reactive oxidative species and antioxidants (Soudek et al., 2011), leading to physiological toxic reactions that impede the absorption capacity of plants (Blanco et al., 2021; Mai et al., 2023; Meng et al., 2016).

Soudek et al. (2011) examined the behavior of heavy metals in hydroponic systems with garlic (*Allium sativum*), observing that cadmium accumulated in the roots, nickel inhibited root growth, and elevated copper levels facilitated amino acid exudation while diminishing chlorophyll content. High cobalt levels also stimulated carotenoid production in leaves. Similarly, in studies by Sipos et al. (2013) on energy grasses in hydroponic systems, it was found that zinc increased plant iron concentrations while, together with copper and cadmium, it altered chlorophyll content and transpiration rates and caused iron plaque formation on roots. Copper and cadmium also reduced growth and caused micronutrient imbalances in shoots. Beyond their effects on plant physiology, the consumption of produce grown in these systems may have detrimental effects on human health. Copper and zinc may affect kidney and liver function, nausea, vomiting, fatigue, intravascular hemolysis, drooling, death, and stiff muscles (Charkiewicz, 2024; Plum et al., 2010). Lead and cadmium are carcinogens, increasing the risk of cancer (Jomova et al., 2025).

1.4 Microbial Risks in Recirculating Systems

While there are concerns regarding plant health and yield associated with closed-loop hydroponics, microbial risks also exist as the probability of contamination increases due to recirculation. In conventional fresh produce production, microbial contamination can occur along the farm-to-fork continuum (Amin N. Olaimat et al., 2012; CDC, 2024; Organisation mondiale de la santé & Organisation des Nations Unies pour l'alimentation et l'agriculture, 2008). The hydroponic production system was considered low risk due to its reduced exposure to environmental conditions (Despommier, 2011). However, recent outbreaks (2021-23) associated with hydroponically grown produce have prompted fresh produce farmers to reassess risks (Ivey et al., 2025; Panayiota Xylia et al., 2021). These concerns are not unfounded, as leafy greens and sprouts, common in hydroponic systems, have long been associated with foodborne illness outbreaks, including those from soil-based farms (Amin N. Olaimat et al., 2012; Bennett et al., 2018; Carstens et al., 2019; Gina Riggio et al., 2019; Organisation mondiale de la santé & Organisation des Nations Unies pour l'alimentation et l'agriculture, 2008; Sela Saldinger et al., 2023a; Topalcengiz et al., 2023). The risk of contamination is compounded by the fact that most produce is eaten raw, with little to no processing to eliminate pathogens (Amin N. Olaimat et al., 2012; Panayiota Xylia et al., 2021). In 2021, *Salmonella* Typhimurium was found in packed leafy greens grown in an indoor hydroponic facility (McClure et al., 2023). It was a multistate outbreak reported in four states that affected 31 individuals and resulted in four hospitalizations (McClure et al., 2023). Similarly, the following year, in 2022, there was a massive recall of 633 cases of crunch, romaine, butter, and heads due to concerns of *Salmonella* contamination (US Food and Drug Authority, 2022). More recently, in 2023, hydroponically grown lettuce from Revolution Farms was recalled due to suspicion of *Listeria monocytogenes* contamination (US Food and Drug Authority, 2023). Although no illnesses were recorded in either recall, it highlights the possibility of contamination and associated risks present in hydroponic systems, including small-scale production systems.

Contaminated water and nutrient solutions, poorly sanitized equipment and infrastructure, and contaminated seeds are the commonly identified sources of contamination in hydroponic studies by Avila-Vega et al. (2014); Dankwa et al. (2020); Gina Riggio et al. (2019); Lopez-Galvez et al. (2016); Misra & Gibson (2021); Orozco et al. (2008); Sela Saldinger et al. (2023b, 2023c); and Xu & Warriner (2005).

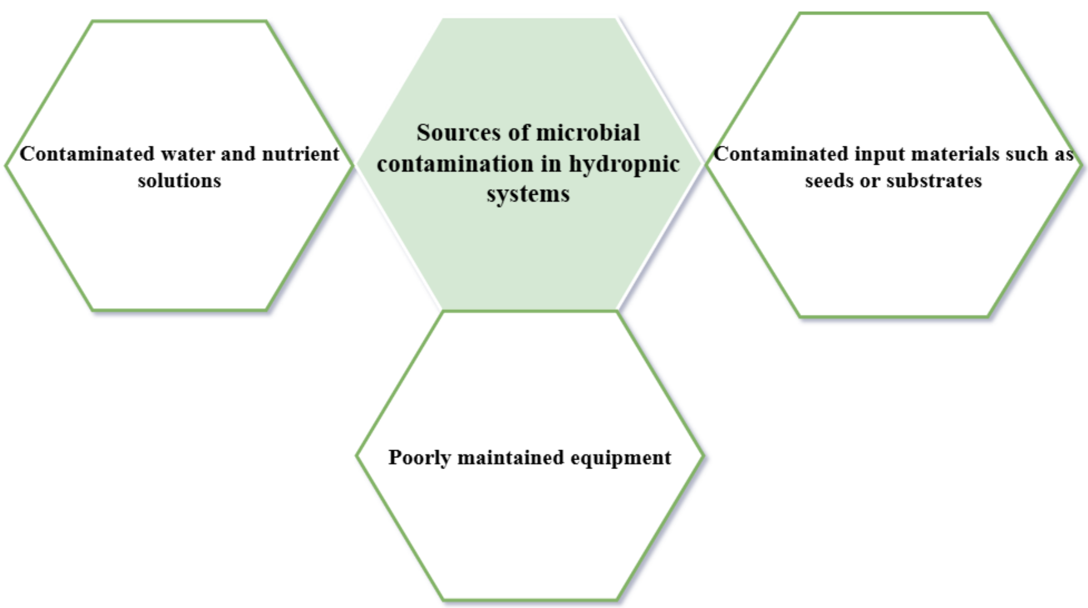

Figure 2.Sources of Contamination in Hydroponic Systems

In closed-loop hydroponic systems, where spent nutrient solution is recirculated, poor treatment and management increase the risk of microbial contamination (Gómez et al., 2019; H.-J. Kim et al., 2018). The recirculation of contaminated nutrient solution in these systems can facilitate the spread of pathogens (Gómez et al., 2019), which can lead to contamination of plants. Several research studies have shown the ability of pathogens such as *Escherichia coli*, *Salmonella,* and *Listeria monocytogenes* to survive and multiply in hydroponic systems (Dumitru Macarisin et al., 2014; Franz et al., 2007; Shaw et al., 2016).

Escherichia coli (*E. coli*) is a gram-negative bacterium and an indicator of fecal contamination in water due to its presence in human and animal waste (S. Cho et al., 2018; Leclerc et al., 2001; Nowicki et al., 2021). Most *E. coli* strains are harmless. However, pathogenic variants such as Enteropathogenic (EPEC), Enterohemorrhagic (EHEC), and Shiga-toxin producing strains (STEC) can cause serious illnesses ranging from diarrhea to sepsis and, in extreme cases, death (S. Cho et al., 2018; Gyles, 2007; Nataro & Kaper, 1998; Odonkor & Ampofo, 2013; World Health Organization, 2018). These strains have been implicated in waterborne outbreaks worldwide, including in developed countries (Probert et al., 2017; Rosenberg et al., 1977). Sawyer (2021) reported the first detection of Shiga toxin-producing *E. coli* (STEC) in hydroponic production systems; previously, only environmental *E. coli* had been detected in soilless systems. The aftermath of this has been several studies reporting the survival of Shiga toxin-producing *E. coli* (STEC) in hydroponic water (C.-P. Wang & Liao, 2023), detection on plant leaves, roots, and fruits (C.-P. Wang & Liao, 2023), and internalization as reported in spinach leaves by Dumitru Macarisin et al. (2014) and in lettuce leaves (Moriarty et al., 2019).

1.5 Mitigation strategies and interventions

The recirculation of nutrient solutions in closed-loop hydroponic systems may introduce microbial risk (European Food Safety Authority, 2013) that poses a threat to food and public health safety. Although various approaches exist for water treatment, a few have been adapted for use with hydroponic nutrient solutions, as shown in Table 2.

Table 2. Mitigation methods for microbial efficiency, adapted and modified from Velazquez-Gonzalez et al. (2022)

Method	Advantages	Disadvantages	Reference
Filtration -Sand Filters -Membrane, e.g., Reverse osmosis	Low cost Easy to operate. Effective pathogen removal	High space requirements Removal efficacy is dependent on the pathogen. Frequent clogging and seeping High maintenance requirements Expensive	(Itoh & Iwasaki, 2018; Raudales et al., 2014
Heat treatment -Pasteurization	Highly effective Precipitates are not generated	High capital costs High maintenance costs	(Ehret et al., 2001; Raudales et al., 2014)
Radiation -UV Radiation	Highly efficient Low space requirement	Efficiency is dependent on turbidity. Precipitation of Mn and Fe Expensive equipment	(Ehret et al., 2001; Raudales et al., 2014)
Chemical treatment -Ozone -Hydrogen peroxide -Chlorine	Highly effective Useful for cleaning irrigation systems Low-cost technique	High capital costs High maintenance costs Reduced efficiency at high organic matter levels, pH Chlorination may be ineffective against biofilms. Chelation of micronutrients	(Ehret et al., 2001; Raudales et al., 2014)

		Toxic byproducts formation	
Biological Processes Constructed wetlands. Biofilters Biological control agents such as *Pseudomonas fluorescens*	Effective pathogen removal	Complex processes	(Crini & Lichtfouse, 2019; Stewart-Wade, 2011).

Prenafeta-Boldú et al. (2017), studied the effectiveness of a full-scale horizontal slow sand filter for controlling phytopathogens in recirculating hydroponics, reported that the slow sand filter reduced the number of viable *Fusarium* propagules by 99.5%. Similarly, Lévesque et al. (2022) reports that electrochemical hyperchlorination, novel technologies such as Dimensionally stable anodes (DSA) and conventional techniques, including UV/ozone Advanced Oxidation Processes (AOPs), were all effective in reducing the phytopathogen *Fusarium oxysporum* by 4 logs.

Furthermore, Moore et al. (2025), Perez et al. (2024), and Zhang & Tu (2000) confirm the efficacy of UV-C disinfection in reducing pathogens such as *Phythium* and *Escherichia coli* levels in closed-loop and recirculating hydroponic production systems. However, UV-C disinfection could also affect native non-target bacteria in the rhizosphere (Zhang & Tu, 2000). In addition to these treatment strategies, most hydroponic farms can enhance their operations by implementing Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP), which include a solid food safety plan, Standard Operating Procedures (SOPs), a comprehensive record-keeping system, and detailed corrective actions to manage hazards (Rivero & Salvi, 2023) They can be intentional about system design, incorporating more hygienic system design and management to ensure all inputs are sterile, testing, and third-party audits to validate processes to reduce the risks of microbial contamination (Rivero & Salvi, 2023).

Beyond the threat of pathogens, the risk of autotoxicity from root exudate accumulation and nutrient toxicity from excess nutrients still exists. Malkawi et al. (2024) developed a two-step process involving forward osmosis and chemical precipitation to remove phosphorus present in high concentrations in waste nutrient solutions. Their results indicated that their approach exhibited phosphorus recovery of over 99%, with an additional advantage of water recovery of 90%. Similarly, studies by R. Chaudhuri & Balasubramanian (2025) to assess the nutrient removal from hydroponic effluent, a mixotrophic cultivation of microalgae was utilized, and their efficiency was reported to ensure nutrient removal by approximately 55%. Conversely, in reducing the risk of autotoxicity, Asao et al. (2008) reported that electrodegradation was a viable treatment in decomposing toxic root exudates. Lee et al. (2006) also noted that activated carbon eliminated phytotoxic organic acids, thereby reducing auto toxic effects associated with nutrient solution reuse. Hosseinzadeh et al. (2017) revealed that activated carbon adsorption was best, followed by ozonation treatments and ion exchange resins last. It is significantly important that mitigation methods address all facets of nutrient reuse, including microbial risk, nutrient toxicity, autotoxicity, and nutrient management techniques, as well as routine system monitoring parameters, to ensure an improved, standardized, and safe closed-loop hydroponic system, including small-scale production systems.

1.6 Policy, regulation, and standards

Under the United States Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR), farms that grow, harvest, pack, or hold covered produce are subject to the same food safety standards regardless of whether crops are grown hydroponically or conventionally. Farms that meet certain exemptions or qualified exemptions, such as small farms with limited annual sales or those selling primarily to local consumers, are partially exempt from the rule. Covered produce under PSR refers to "produce that is typically consumed raw and is a raw agricultural commodity" (US Food and Drug Authority, 2015). Water is a key component of nutrient solutions, and the practice of recycling increases the likelihood of contamination and hazards (Gómez et al., 2019; H.-J. Kim et al., 2018). Under the PSR, agricultural water is defined as "water used in covered activities on covered produce where water is intended to, or is likely to, contact covered produce or food contact surfaces, including water used in growing activities and harvesting, packing, and holding activities." (US Food and Drug Authority, 2015). Within the agricultural water provision (§112.40-112.50), the PSR categorizes agricultural water into two types: pre-harvest (production) water and post-harvest water. Pre-harvest water management involves irrigation and fertigation, activities that support plant growth. In contrast, post-harvest water is used for activities such as washing and cooling produce during and after harvest. This definition categorizes the nutrient solution in the hydroponic production system as pre-harvest water.

Drawing insight from this, the Controlled Environment Agriculture (CEA) Alliance, in collaboration with the International Fresh Produce Association, published the Commodity-Specific Food Safety Guidelines for Controlled Environment Agriculture in 2023. This guideline focuses on Good Agricultural Practices (GAP), as outlined in the PSR rule, for leafy green and herb producers. Unlike the PSR rule, it did not establish any specific standards for this production system; however, both focused primarily on microbial risks. Beyond this, water reuse in agricultural systems has been a long-standing practice. On a global scale, the World Health Organization (WHO) has developed comprehensive guidelines for the safe use of wastewater and excreta as recycled water in conventional agricultural systems. However, like Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR), the World Health Organization (WHO) standards only address microbial standards, hazards, and criteria to ensure the microbiological safety of water.

Table 3. World Health Organization health-based targets for recycled water for water use in agriculture (modified and adapted from Shoushtarian & Negahban-Azar 2020 World Health 2013)*)*

Exposure Scenario	Health-Based Target (DALY per Person Per Year)	*E. coli* (CFU/100 mL)	Log^{10}Pathogen Reduction Needed[a]	Number of Helminth Eggs Per Liter
Unrestricted irrigation				
Leaf crops (e.g., lettuce),	$\leq 10^{-6a}$	$\leq 10^{4}$	6	≤1[b, c]
			7	

Root crops (e.g., onion	$\leq 10^3$	7	≤ 1[b, c]
High-growing crops (drip),	$\leq 10^5$	5	Not recommended[d]
Low-growing crops (drip)	$\leq 10^3$	7	≤ 1[c]

Note - DALY = Disability- Adjusted Life Year

The Environmental Protection Agency (EPA) also sets standards for agricultural water reuse, categorizing it into two categories: water reuse for food crops and for non-food crops. Their standards extend beyond the microbial risk that may exist, and they provide recommendations for water treatment to ensure safety, along with some chemical limitations for water.

Table 4. Environmental Protection Agency guidelines for agricultural water reuse, adapted from US Environmental Protection Agency2012b)

Requirements	Agricultural Water Reuse Category	
	Food crops: crops which are consumed raw by humans	Processed food crops: crops that are processed before human consumption
Treatment	- Secondary[1] Filtration[2] Disinfection[3]	Secondary Filtration - Disinfection
Recycled water quality	- pH = 6.0–9.0 BOD5 ≤ 10 mg/L Turbidity ≤ 2 NTU Fecal Coliforms = 0/100 mL[4,5,6]	- pH = 6.0–9.0 BOD5 ≤ 30 mg/L Total suspended solids ≤ 2 NTU

	- Cl_2 residual ≤ 1 mg/L[7]	Fecal Coliforms ≤ 200/100 mL[4,5,6] - Cl_2 residual ≤ 1 mg/L[7]
Recycled water monitoring	- pH [weekly] - BOD5 [weekly] Turbidity [continuous] - Fecal Coliform [daily] Cl_2 residual [continuous]	pH [weekly] BOD5 [weekly] Total suspended solids [continuous] Fecal Coliform [daily] - Cl_2 residual [continuous]
Setback distances -	- 50 ft (15 m) to potable water supply wells 100 ft (30 m) when located in porous media	300 ft (90 m) to potable water supply wells 100 ft (30 m) to areas accessible to the public (if spray irrigation)

[1] Secondary treatment processes include activated sludge processes, trickling filters, rotating biological contractors, and stabilization pond systems. Secondary treatment should produce effluent in which both the BOD and SS do not exceed 30 mg/L. [2] Filtration means; the passing of wastewater through natural undisturbed soils or filter media such as sand and/or anthracite; or the passing of wastewater through microfilters or other membrane processes. [3] Disinfection means the destruction, inactivation, or removal of pathogenic microorganisms by chemical, physical, or biological means. Disinfection may be accomplished by chlorination, ozonation, other chemical disinfectants, UV, membrane processes, or other processes. [4] Unless otherwise noted, recommended coliform limits are median values determined from the bacteriological results of the last 7 days for which analyses have been completed. Either the membrane filter or fermentation tube technique may be used. [5] The number of fecal coliform organisms should not exceed 800/100 mL in any sample. [6] Some stabilization pond systems may be able to meet this coliform limit without disinfection. [7] This recommendation applies only when chlorine is used as the primary disinfectant. The total chlorine residual should be met after a minimum actual modal contact time of at least 90 min unless a lesser contact time has been demonstrated to provide indicator organism and pathogen reduction equivalent to those suggested in these guidelines. In no case should the actual contact time be less than 30 min

Standards set for microbial water quality are adequately provided; however, physicochemical standards have been addressed by the Food and Agriculture Organization (FAO) in their guidelines for water reuse. Recycled water is categorized based on its use:(A) Irrigation of crops likely to be eaten uncooked, sports fields, and public parks; (B) Irrigation of cereal crops, industrial crops, fodder crops, pasture, and trees; and (C) localized irrigation of crops in category B if exposure of workers and the public does not occur. (Food Agriculture Organization, 2006).

Table 5.FAO Water quality standards for irrigation, adapted from Food Agriculture Organization (2006)

Potential Irrigation Problem	Units	Degree of Restriction on Use		
		None	Slight to Moderate	Severe
Salinity				
EC^1_w	dS/m	<0.7	0.7–3.0	>3.0
or TDS	mg/L	<450	450–2000	>2000
Infiltration				
SAR = 0–3 and EC_w		>0.7	0.7-0.2	<0.2
3–6		>1.2	1.2–0.3	<0.3
6–12		>1.9	1.9–0.5	<0.5
12–20		>2.9	2.9–1.3	<1.3
20–40		>5.0	5.0–2.9	<2.9
Specific Ion Toxicity				
Sodium (Na)				
Surface irrigation	SAR	<3	3–9	>9
Sprinkler irrigation	me/L	<3	>3	
Chloride (Cl^-)				
Surface irrigation	me/L	<4	4–10	>10
Sprinkler irrigation	m^3/L	<3	>3	
Boron (B)	mg/L	<0.7	0.7–3.0	>3.0
Miscellaneous Effects				
Nitrogen (NO_3-N)	mg/L	<5	5–30	>30

Bicarbonate (HCO_3) me/L	<1.5	1.5–8.5	>8.5
pH	Normal range 6.5–8		

[1] Electric conductivity. [2] Total dissolved solids. SAR – Sodium Adsorption Ratio

Taken together, these standards provide essential benchmarks for microbial safety and thresholds to control salt and ion accumulation. Nevertheless, while Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR), sufficiently addresses microbial standards, the absence of guidelines explicitly tailored to chemical and physical quality in hydroponic production and nutrient reuse in closed-loop systems remains a significant gap.

Despite this, while they wait, small scale farmers with no access to advanced monitoring tools, should continue to adopt benchmarks from Food Safety Modernization Act (FSMA), World Health Organization (WHO), and Food and Agriculture Organization (FAO); implement and practice Good Agricultural Practices and Good Manufacturing Practices, such as worker safety; manage environmental factors to prohibit microbial growth and proliferation; schedule testing of water, nutrient solution, and implements; adopt hygienic design of operation; and participate in third-party audits when feasible (Kozai, 2019; Rivero & Salvi, 2023).

1.7 Future Directions

The future of hydroponics, particularly small-scale closed-loop systems, lies in the integration of accessible technology. This spans from the development of low-cost sensors to detect when plants lack nutrients, water, or other elements to the development of sensors that can detect phytopathogens, pathogens, and other microorganisms of concern (Al-Kodmany, 2018; Brigham, 2018; Rivero & Salvi, 2023; Sambo et al., 2019). Furthermore, the utilization of tools that allow hydroponic farmers to control and monitor water quality and the system as a whole would allow for a more optimized production system (Clercq, M et al., 2018; Rivero & Salvi, 2023) and potentially mitigate nutrient imbalances that may result from nutrient solution recirculation. Others are also looking to adopt AI into these systems to enable early detection of diseases and pests, as well as provide immediate advice, recommendations, and assistance to farmers. Although many of these initiatives are targeted towards hydroponic systems, including large commercial scale, the approaches could still be modified and adopted for small-scale hydroponic systems.

1.8 Conclusion

Hydroponic production offers a solution to address future food and water challenges by producing high-value crops with far less land and water than conventional agriculture. This circular approach is based on recirculating nutrient solutions, which makes water and fertilizer use more efficient and cuts down on pollution. However, this review highlights that nutrient reuse in small-scale systems also introduces significant risks, including shifts in nutrient balance, salt, and ion accumulation, autotoxicity from root exudates, and an increased potential for microbial contamination in systems where produce is consumed raw.

A growing body of research on mitigation technologies, including UV-C disinfection, slow sand filtration, and electrochemical treatments, as well as nutrient recovery processes, demonstrates that these risks can be managed and even transformed into opportunities for water and nutrient recycling, reduced inputs, and closed-loop sustainability. However, these advancements have not kept pace with the regulatory landscape. While the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR), World Health Organization (WHO) guidelines, and Environmental Protection Agency (EPA) and Food and Agriculture Organization (FAO) standards provide vital benchmarks for microbial and physicochemical quality, none are explicitly tailored to hydroponic nutrient solutions.

Moving forward, the opportunity lies in developing hydroponics-specific, science-based standards and monitoring protocols that integrate both microbial and chemical parameters, alongside the adoption of effective treatment methods. Such a framework would enable growers to reap the sustainability benefits of nutrient reuse while ensuring crop quality and consumer safety. Bridging this gap between practice, science, and policy will be crucial if hydroponic agriculture is to fulfill its potential as a resilient, resource-efficient food system for urban and small-scale farmers.

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Chapter2 UV-C Treatment of Reused Hydroponic Nutrient Solution in Deep Water Culture Systems: Implications for Microbial Safety, Plant Growth, Nutritional Quality of Romaine Lettuce.

Abstract

Water scarcity and rising production costs are increasing the need for affordable and sustainable hydroponic water-management solutions, especially for small-scale growers. While recirculating nutrient solutions conserve water and fertilizers, repeated reuse can lead to nutrient imbalances, salinity buildup, and microbial contamination that reduce crop performance. This study evaluated two low-cost, grower-friendly treatment strategies: slow sand filtration + UV-C (SU) and slow sand + biochar + UV-C (SBU) for multi-cycle reuse of hydroponic nutrient solution in deep-water culture romaine lettuce (*Lactuca sativa* var. *longifolia*) production. Treatments were compared with untreated recirculation (C) and a freshwater drain-to-waste system (FW) over six production cycles. SU and SBU consistently achieved ≥5 $\log_{10}$ reductions of *E. coli*, meeting World Health Organization (WHO) microbial safety criteria. Physically, turbidity remained <2 NTU, and SBU showed the highest light transmittance. Chemically, both treatments reduced ammonium-N, total N, and total P, with SBU showing the highest removals. However, nitrate levels remained relatively stable, calcium steadily accumulated across cycles, and pH in SU and C fell below Food and Agriculture Organization/World Health Organization guidelines. Micronutrient analysis revealed substantial declines in Fe, Zn, Cu, and Mn—up to 90–97% in SBU resulting in progressive micronutrient imbalance despite EC-based nutrient supplementation. Romaine lettuce grown in SU initially produced the highest biomass but declined by ~30% across cycles, while SBU showed even larger reductions (~45%). Chlorophyll, SPAD, and vitamin C decreased in all treatments, reflecting micronutrient depletion, salinity stress, and oxidative damage. The freshwater treatment also experienced reduced quality due to ammonium accumulation. Overall, combining SSF, biochar, and UV-C provides effective microbial inactivation and maintains physical water quality but progressively depletes essential micronutrients, limiting long-term crop performance. These findings highlight the potential of low-cost treatment systems for small-scale hydroponic growers while emphasizing the need for

cycle-specific micronutrient supplementation to sustain yield and nutritional quality during extended nutrient-solution reuse.

2.1 Introduction

The world population is projected to reach 9.7 billion by 2050, driving a 60% to 70% increase in food demand (Boretti & Rosa, 2019; Nations, 2014). It is estimated that the projected increase would affect agricultural water usage (Mancosu et al., 2015). However, the current landscape of climate change, drought, food insecurity, and decreasing arable land threatens the ability of agricultural output to meet this demand. As a result, controlled environmental systems such as hydroponics are gaining ground as alternative agricultural systems. They are characterized by their independence from factors that conventional farming systems depend on. For example, in hydroponic production systems, to increase resource efficiency, the nutrient solution is often recirculated (Fathidarehnijeh et al., 2024d; Rosa-Rodríguez et al., 2020; Rouphael & Colla, 2009).

Hydroponics is a cultivation method that utilizes nutrient-rich solutions to grow crops. It is used in the cultivation of crops such as lettuce, spinach, kale, peppers, and cucumbers. Nutrient solution delivers essential elements to plants through the root zone in hydroponic production systems (Nguyen et al., 2016). It has several advantages over soil-based farming, including reduced reliance on pesticides, higher yields, faster growth, and fewer soil-based diseases (Ezzahoui et al., 2021; Majid et al., 2021; Vidhya & Valarmathi, 2018). The hydroponic systems are commonly used for growing leafy greens including romaine Lettuce.

Romaine lettuce (*Lactuca sativa* var *longifolia)* is a popular crop well-suited for hydroponic systems (Grzegorzewska et al., 2023). They are a great source of minerals, vitamins, and phytochemicals such as flavonoids, carotenoids, and L-ascorbic acid (Shi et al., 2022; Yang et al., 2022). However, its nutritional content can be influenced by the environment and the type of cultivation system (Matysiak et al., 2023). Its vitamin C, sugar, and chlorophyll content tend to be higher in hydroponics compared to conventional farming(L. Wang et al., 2023). In hydroponic systems, its growing time is up to 30 days (Kovácsné Madar et al., 2019) as compared to 54-65 days in open-field production (Dufault et al., 2006).

In closed-loop hydroponic systems, nutrient solutions are used and reused. It offers the benefits of enhanced water nutrient use efficiency, (Fayezizadeh et al., 2021; Rosa-Rodríguez et al., 2020) while decreasing the impact of hydroponic production systems to environmental pollution (Rodríguez-Jurado et al., 2020). However, despite the advantages, the potential of excess ion accumulation leading to high salinity levels, nutrient toxicity, and nutrient imbalances affects crop yield and quality (Ko et al., 2013; Nakano et al., 2010; Pardossi et al., 2002; Rouphael et al., 2006). Additionally, recirculated nutrient solution may pose a microbial risk, especially as hydroponically grown produce has been linked to foodborne outbreaks and poses public health risks (Asaduzzaman et al., 2022; Panayiota Xylia et al., 2021; Shaw et al., 2016). It is therefore essential that the recycled nutrient solution be refreshed (Fathidarehnijeh et al., 2024b) to address this challenge. This means treating, changing, managing, or adding clean water to it. Mitigation strategies, including reverse osmosis (Miller et al., 2020b), pasteurization (Raudales et al., 2014), the use of biofilters (Stewart-Wade, 2011), and chlorine (Raudales et al., 2014), have been used in treating nutrient solution. However, the major drawbacks of high cost and maintenance, toxic byproduct formation, and complex processes associated with them serve as barriers to widespread adoption by the small scale hydroponic farmer (Amina et al., 2020; Raudales et al., 2014; Stewart-Wade, 2011; Velazquez-Gonzalez et al., 2022). Several nutrient solution management strategies also exist to help with crop growth, water, and nutrient use efficiency (Gumisiriza et al., 2022; Rouphael et al., 2016). They include regulating pH levels, electrical conductivity (EC)-based management strategies, nitrogen-based management (Fathidarehnijeh et al., 2024e) strategies, fertigation automatic control system (Steidle Neto et al., 2014), time-based adjustment of reused nutrient solution (Ko et al., 2013), and ion-specific nutrient management methods (W.-J. Cho et al., 2018; Jung et al., 2019).

Although successes have been reported, for the small-scale urban hydroponic farmer, these strategies tend to be costly, which affects their adoption into practice. Recognizing this gap, this study explored alternative treatment methods; characterized by how safe and easy, economically feasible, and effectiveness (Giannoccaro et al., 2019; Joint Research Centre (European Commission) et al., 2017; Naidoo & Olaniran, 2014).

Among the treatment methods, filtration methods, and UV-C could be the potential technologies that meet these requirements. Slow sand filtration is a simple, low-cost, low-maintenance water treatment method that uses physical, chemical, and biological mechanisms to remove suspended organic matter, lower turbidity, and reduce bacterial load (Bai et al., 2024; Logsdon et al., 2002). It requires the use of little to no chemicals, offers a simple design that facilitates its widespread adoption, and necessitates minimal operation and maintenance (Weixin Chen, 2017). Biochar filtration utilizes biochar, a substance produced from biomass through the pyrolysis process. It is a cost-effective material with high adsorption capabilities for removing metal ions, inorganic contaminants, and organic matter (Kaetzl et al., 2020a; Quispe et al., 2022). UV-C disinfection, a non-thermal, environmentally friendly method, has biocidal properties and emits germicidal radiation to inactivate pathogens by damaging the DNA and RNA (Pereira et al., 2023). It forms pyrimidine dimers that interfere with replication, transcription, and translation in microorganisms (A. Adhikari et al., 2020; Koutchma, 2019). This method is quick, residue-free, and does not alter the taste, odor, or composition of water (Olena Popovych et al., 2019).

While each of these treatment methods has shown promise individually in water treatment for hydroponic use (Fernandez, 2017; Kaetzl et al., 2020b; Prenafeta-Boldú et al., 2017), no study has assessed the combined performance of slow sand filtration, biochar filtration, and UV-C disinfection for treating recirculated nutrient solution for multi-cycle hydroponic water reuse under real-world production settings. However, evidence from the literature suggests that combining treatment methods can enhance their overall efficiency (Banach & Van Der Fels-Klerx, 2020).

Therefore, this study aimed to evaluate and validate the combined performance of slow sand filtration, biochar filtration, and UV-C disinfection for treating recirculated nutrient solution in a Deep-Water Culture (DWC) system. Specifically, the objectives were to: (a) assess microbial, physical, and chemical water quality outcomes of SSF + UV-C (SU) and SSF + Biochar + UV-C (SBU) across six reuse cycles; (b) determine whether treated recirculating nutrient solution meets Food and Agriculture Organization (FAO)/World Health Organization (WHO) agricultural water reuse standards; (c) identify advantages and limitations of each treatment approach to support practical adoption; and (d) evaluate the effects of treated nutrient solution reuse on romaine lettuce yield and nutritional quality.

2.2 Materials And Methods

This experiment was conducted in the Food Safety Lab at Kansas State University, Olathe. It involved growing romaine lettuce in a deep-water culture system with four treatments conducted over a six-cycle period. Nutrient solution was only treated between each cycle (after harvest, before the start of the next cycle). It followed a completely randomized design (CRD) with six cycles, four treatments, three replications, and 12 plants per experimental unit. The four nutrient solution treatments were:

1. Slow Sand Filtration (SSF) + UV-C disinfection
2. Slow Sand Filtration + Biochar Filtration + UV-C disinfection
3. Control (untreated nutrient solution)
4. Freshwater in a drain-to-waste or open loop system served as the negative control (FW).

Romaine lettuce (*Lactuca sativa* var. *Dragoon OG MTO*) seeds were purchased from Johnny's Selected Seeds Company (LOT number 100122, Wolcott, VT, USA) and were seeded in a pre-soaked rockwool cubes (Hydro farm, CA, USA) using a small nutrient film technique (NFT) desktop system (53.34 cm L x 33.02 cm W x 15.24 cm H; CropKing, Lodi, OH, USA) under a photoperiod of 16 hours, at a germination temperature of 22°C and a daily light integral (DLI) of 10 mol m-2·day-1 and LED grow lights (Model 10101-10135A; Root Farm, Marysville, OH, USA). Once the seedlings reached the five-true-leaf stage, the pH of the hydroponic solution was maintained between 5.7 and 5.9. At the same time, the electrical conductivity (EC) was adjusted to remain within the range of 1.0-1.2 mS/cm. Both parameters were monitored and adjusted every other day using calibrated pH and EC meters (H19811-51, Hanna Instruments, USA). Healthy, uniform seedlings were selected and transplanted into the deep-water culture system 15–16 days after germination.

In the deep-water culture system, each treatment consisted of three growing totes (129 x 20 x 15 inches wide; holding 22.5 gallons of distilled water) purchased from the local Home Depot, each with 12 planting holes. Each tote was equipped with two air stones (Paw fly, Guangzhou, China), timed to operate for 11 hours daily using an air pump (Eco Plus, China) and one water pump for mixing the nutrient solution (Eco Plus, China). The LED lights (Grow Star, ON, CA) were used to supply the lighting in all systems, with a photo period of 17 hours.

Nutrient Solution Preparation

Fertilizer stock solution was prepared using a two-part water-soluble fertilizer (Hydro Grow Leafy Green Fertilizer, CropKing, Lodi, OH, USA) at 97.5 g·L−1 and calcium nitrate CropKing, Lodi, OH, USA) at 75 g·L−1 as shown in Table 6.

Table 6. Composition of nutrients and their concentration in the two-part fertilizer of Crop King for leafy green vegetables

Nutrients	Composition	Concentration
Hydro-Grow (Part A)		
Macronutrients	Total Nitrogen (N)	4.3 %
	Available Phosphoric acid	9.3 %
	(P2O5)	35 %
	Soluble Potash (K2O)	3.9%
	Magnesium (Mg)	
Trace elements	Boron (B)	200 mg/kg
	Copper (Cu)	105 mg/kg
	Iron (Fe)	2100 mg/kg
	Manganese (Mn)	190 mg/kg
	Molybdenum (Mo)	42 mg/kg
	Zinc (Zn)	210 mg/kg
Calcium Nitrate Ca $(NO3)^2$ (Part B)		
Macronutrient	Total Nitrogen	15%
	Calcium	19%

To prepare the nutrient solution in the first cycle, growing totes were filled with 22.5 gallons of sterile distilled water, followed by adding an equal volume of stock solutions A and B (Table 6) to adjust the EC at 1.8 mS·cm-1 ±0.05. For subsequent cycles, distilled water was added to the total volume after treating the nutrient solution to bring the total volume to 22.5 gallons based on treatment. pH was maintained between 5.7 and 5.9, and the electrical conductivity (EC) was within the range of 1.75-1.8 mS/cm. EC and pH were monitored and adjusted twice weekly using an electrical conductivity (EC) meter (H19811-51, Hanna Instruments, USA) and a pH meter (H19811-51, Hanna Instruments, USA), and the devices were calibrated. Adjustments to pH were made using 10% sulfuric acid (H_2SO_4) to lower pH and 10% potassium hydroxide (KOH) to raise it. Nutrient levels were managed using Hydro-Gro Leafy Fertilizer (Part A) and calcium nitrate (Part B) twice a week. The composition and concentrations of the nutrients used are summarized in Table 6.

Romaine lettuce was grown for 5-6 weeks with samples collected on parameters collected on plant height, number of leaves, SPAD value on a biweekly basis. Lettuce was harvested after 28 days after transplant. Fresh shoot weight and root weight, vitamin c and chlorophyll content was collected after harvest. To study the potential of microbial risks, the nutrient solution was artificially spiked with *Escherichia coli* to evaluate the efficacy of UV-C treatment in reducing contamination.

Rifampicin resistant generic *Escherichia coli* Preparation and Inoculation

Sample Preparation

E. coli ATCC 25922 in cryovials containing 25–50% glycerol (Fisher Scientific, Hampton, New Hampshire) at −80°C were sourced from the Bhullar Food Safety Lab (Kansas State University). *E. coli* ATCC 25922 was selected for rifampicin resistance (80µg/mL) (Moore et al., 2025; Vlasselaer et al., 2024) to ensure only colonies of the inoculated *E. coli* strain with resistance were being counted on tryptic soy agar media. *E. coli* ATCC 25922 was cultured in a Tryptic Soy Broth (TSB) at 37°C for 24 h. The next day, the culture was transferred to tryptic soy agar plates containing 20µg/mL rifampicin. Colonies from the 20 µg/mL plates were transferred to TSB containing 40 µg/mL rifampicin and then plated onto TSA plates containing 40 µg/mL rifampicin. Colonies from this plate were transferred into TSB containing60 µg/mL rifampicin, then plated onto TSA media plates containing 60 µg/mL rifampicin. On the final day, colonies from this plate were transferred into TSB containing 80 μg/mL rifampicin, then plated onto TSA media plates containing 80 μg/mL rifampicin. Colonies from the final 80 μg/mL agar plates were used for subsequent subcultures.

Rifampicin-resistant *E. coli* ATCC 25922 was subcultured twice in Tryptic Soy Broth (TSB) containing 80 μg/mL rifampicin, with each incubation lasting 24 hours (TSB, Remel Inc., Lenexa, Kansas). After the second subculture, cells were centrifuged in 30 mL culture tubes at 10,846 g for ten minutes at 20°C. The supernatant was discarded, and 30 mL of 1× phosphate-buffered saline was added to each tube. Tubes were vortexed until the cell pellet was fully dissolved, and the centrifuging and washing process was repeated twice, resulting in a total of three washes. The viable count of the inoculum was determined by making serial dilutions in 0.1% buffered peptone water and plating on Tryptic Soy Agar (TSA, Remel, Inc., Lenexa, Kansas) with 80 μg of rifampicin per mL of agar. An 8.5 mL aliquot of the inoculum (average 9.24 log CFU mL^{-1}) was added to 22.5 gallons of nutrient solution in each growing tote to achieve the initial average target load of 5.20 log CFU/mL. A mixer was used to ensure uniform distribution.

2.2.1 Nutrient Solution Treatment

A custom designed equipment was used for the treatment process. It consisted of a commercial slow sand filtration chamber (Krystal Clear™ Sand Filter Pump, 925 GPH, Intex, Long Beach, CA 90810, USA), a biochar filtration chamber, and a UV-C disinfection apparatus (254 nm Minipure MIN-1 ultraviolet chamber, Atlantic Ultraviolet Corporation, New York, USA). The slow sand filtration chamber was filled with sand (Aqua Quartz Pool Filter Sand, Covia, Independence, OH, USA) and a gravel layer at the base (Gravel-14 - Fine Flint Media Tank, FreshWater Systems, Greenville, SC, USA). The biochar filtration chamber was filled with biochar, and the UV-C disinfection apparatus contained a UV lamp (34 W at 254nm). The inoculated recirculating nutrient solution (RNS) was treated with two different treatment combinations as described in Figure 3, that is, 1) Slow Sand Filtration + UV-C, and 2) Slow Sand Filtration + Biochar Filtration + UV-C.

For the Slow Sand Filtration + UV-C combination process, 22.5 gallons of inoculated recirculating nutrient solution from the growing totes were pumped through a hose into the Slow

Sand Filtration chamber, passed through the UV-C disinfection apparatus, collected in a 55-gallon container (lined with s sterile bag), and transferred using a peristaltic pump into the growing tote, as shown in Figure 3. For the Sand Filtration + Biochar Filtration + UV-C treatment, 22.5 gallons of recirculating nutrient solution from the growing totes were pumped through a hose into the slow sand filtration chamber, into the biochar filtration chamber, and through the UV-C disinfection apparatus and collected in a 55-gallon container (lined with sterile bag), and transferred using a peristaltic pump into the growing tote, as shown in Figure 3. Each treatment was applied to three independent replicates per cycle across six growing cycles. At the beginning of each treatment process, the equipment was disinfected with 5 ppm of free chlorine and then cleaned with 20–28 gallons of distilled water until the chlorine levels reached 0 ppm. All transfer pumps were sterilized using a 2-ppm chlorine solution. Spent nutrient solution (RNS) recirculated without treatment served as the positive control (C), while freshwater in a drain-to-waste or open loop system served as the negative control (FW).

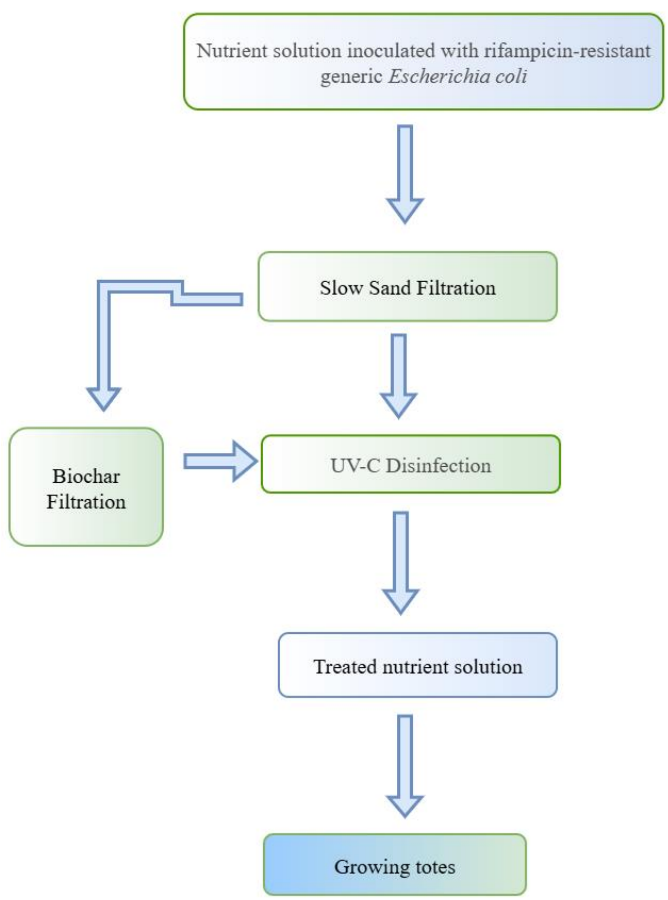

Figure 3. A Schematic Illustration of the Treatment System

2.2.2 Microbial Water Quality Analysis

2.2.2.1 Sample Collection and Enumeration of Rifampicin-resistant generic E. coli ATCC 25922

50 mL of recirculating nutrient solution was aseptically collected into sterile conical tubes. Water samples were collected from the growing totes before treatment and after each treatment step via sampling points installed along the treatment line and processed immediately. A 1/10 (w/v) dilution of the samples was prepared using 0.1% Buffered Peptone Water (BPW) (Alpha Biosciences, Baltimore, MD). The sample was homogenized, and then 1 mL of it was pipetted into 9 mL of 0.1% BPW tubes to create serial dilutions. From each dilution, 100 µL was spread onto petri dishes containing 18 mL of 80 µg/mL rifampicin Tryptic Soy Agar (TSA) (Fisher Scientific LLC, Illinois, USA) as described by Moore et al. (2025). Plates were incubated at 35°C for 24 ± 2 hours under aerobic conditions. After incubation, the plates were examined for the presence of colonies, which were then counted using a standard method. Counts were averaged and recorded as colony-forming units per mL (CFU/mL) of the sample.

2.2.3 Physical Water Quality Analysis

45 mL samples of the treated recirculating nutrient solution (tRNS) were taken from the growing totes after treatment and analyzed for electrical conductivity (EC), turbidity, absorbance, transmittance, and total dissolved solids (TDS).

2.2.3.1 Measurement of Turbidity (NTU)

Turbidity was measured using a portable turbidimeter (Model 2100Q; Hach Company, Loveland, CO, USA) according to U.S. Environmental Protection Agency (EPA) Method 180.1 (O'Dell, 1996). The instrument was calibrated using formazin standards (0, 20, 100, and 800 NTU) before analysis, and calibration was verified with an independent check standard. Samples were gently inverted before measurement to resuspend particles without introducing bubbles. Aliquots were transferred into clean turbidity vials, wiped, and inserted into the turbidimeter for measurement. Readings were recorded in nephelometric turbidity units (NTU).

2.2.3.2 Measurement of pH, EC (mS/cm), and TDS (mg/L)

pH, electrical conductivity (EC), and total dissolved solids (TDS) were recorded with a calibrated portable meter (H19811-51, Hanna Instruments, USA). The instrument was calibrated before use with manufacturer-supplied standard buffer solutions. Measurements were recorded directly from the nutrient solution, with values expressed as pH units, millisiemens per centimeter (mS/cm) for EC, and milligrams per liter (mg/L) for TDS.

2.2.3.3 Determination of Absorbance and Transmittance

Absorbance and transmittance were measured according to procedures described in "5910 UV-Absorbing Organic Constituents" (2017) using a UV–Vis spectrophotometer (G1-OS UV-Vis, Thermo Fisher Scientific, USA). Samples were analyzed in 1 cm pathlength quartz cuvettes. Calibration was done with distilled water before each set of measurements. Cuvettes were rinsed with the sample and handled by the frosted sides to avoid fingerprints. The absorbance was recorded in absorbance units (AU) at 254 nm. %Transmittance was calculated using Equation 1.

$$A = -\log_{10}\left(\frac{\%T}{100}\right) \qquad \text{Equation 1}$$

Where A is the absorbance and T is the transmittance of the sample tested.

2.2.4 Chemical Water Quality Analysis

15 mL samples were collected and sent to the Kansas State University Soil Testing Laboratory in Manhattan, KS, for analysis. The samples were tested for 13 parameters (see Table 7). The micronutrient removal ratesof the treatment method was estimated using Equation 2.

Equation 1: Micronutrient Removal Rates

$$\% \text{ Removal Efficiency} = \left(\frac{Ci-Cf}{Cf}\right) \times \mathbf{100}$$

Where Ci is the micronutrient concentration in Cycle 1 (ppm), and Cf is the final micronutrient concentration in Cycle 6 (ppm).

Table 7. Chemical Water Quality Sampling Parameters

Parameter	Units
Total nitrogen (N)	ppm
Nitrate (NO^3-N)	ppm
Chloride (Cl^-)	ppm
Calcium (Ca)	ppm
Total phosphorus (P)	ppm
Manganese (Mn)	ppm
Copper (Cu)	ppm
Ammonium (NH^4-N)	ppm
Sodium (Na)	ppm
Iron (Fe)	ppm
Zinc (Zn)	ppm

2.2.5 Measurement of Plant Height, Number of Leaves, and SPAD Value

Plant height, number of leaves, and SPAD value were measured biweekly on all twelve plants per experimental unit after transplanting. Plant height was measured from the base of the stem to the top of the canopy using a stainless-steel ruler. The number of true leaves was determined by counting true leaves on each plant. SPAD values were recorded from 3–4 fully expanded leaves by measuring the central point between the midrib and leaf margin. Measurements were averaged and recorded using a chlorophyll meter (SPAD-502; Minolta Corporation, Ltd., Osaka). The percentage change of plant height, number of leaves, and SPAD value was determined using Equation (3).

Equation 2. The percentage change of plant height, number of leaves and SPAD value recorded biweekly

$$\%\ change_{week} = \left(\frac{Value\ @cycle\ 6}{Value\ @\ cycle\ 1} - 1\right) \times 100$$

2.2.6 Harvest and Measurement of Yield Parameters

All yield parameters were measured on all twelve plants per experimental unit, after harvest, 28 days after transplantation. After harvest, the romaine lettuce plants were then separated into roots and shoots using scissors to determine their fresh shoot weight and fresh root weight, as measured by a weighing scale (OHAUS, Parsippany, NJ, USA). The percentage change of shoot and root weight was determined using Equation (4).

Equation 3.The percentage change of shoot and root weight

$$\% \text{ change} = \left(\frac{\boldsymbol{Weight\ at\ Cycle\ 6}}{\boldsymbol{Weight\ at\ cycle\ 1}} - \mathbf{1}\right) \times \mathbf{100}$$

2.2.7 Nutritional Quality

The postharvest quality was determined using the percentage changes in vitamin C, and chlorophyll content was determined using Equation 5.

Equation 4.The percentage change of Vitamin C and chlorophyll content

$$\% \text{ change} = \left(\frac{Value\ at\ Cycle\ 6}{Value\ at\ cycle\ 1} - 1\right) \times 100$$

2.2.7.1 Determination of Vitamin C Content

Vitamin C, also known as ascorbic acid (AA), was measured by following the procedure described by Desai & Desai(2019); Klimczak & Gliszczyńska-Świgło(2015). 2 g were collected from three plants per treatment, mixed with 20 ml of acid solution (2 N glacial acetic acid and 6% metaphosphoric acid) and homogenized using a benchtop homogenizer (Polytron PT 1600 E; Kinematica Inc., Bohemia, NY, USA). The samples were stored at -20°C for further analysis. On the day of analysis, samples were thawed and centrifuged twice at 12,000 rpm for 20 min at 4°C till clear. 1 mL of clarified extract was used for the analysis of ascorbic acid (AA). To oxidize AA to dehydroascorbic acid (DHAA), a drop of 0.2% of 2,6 6 2,6-dichloroindophenol (2,6-DCIP) solution was added, followed by incubation at room temperature for 1 hour. 2% thiourea solution was added to precipitate the excess 2,6-DCIP, and then 2% 2,4-dinitrophenylhydrazine

(2,4-DNPH) solution was added. Samples and standard solutions were incubated at 60°C for 3 hours in an oven (Heratherm™ Convection, ThermoFisher Scientific Inc., Asheville, NC, US) to allow ozone formation. After incubation, samples and standard solutions were cooled in an ice bath and reacted with 90% sulfuric acid. Absorbance was measured at 540 nm using a plate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA) by following the procedure described by Terada et al.(1978). Vitamin C was quantified using a standard calibration curve from ascorbic acid readings and expressed as mg·100 g-1 FW.

2.2.7.2 Determination of Chlorophyll content

Chlorophyll content was determined using procedures described by Wellburn(1994). Samples (0.3 g) were collected from three plant samples per treatment and homogenized with 10 ml of methanol. The homogenates were stored at 4°C for 24 hours. After this period, the homogenates were centrifuged at 10,000 rpm for 10 min at 4°C. Absorbance was measured at 653nm and 666nm using a plate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA). Total chlorophyll content was calculated using Equations 6, 7 & 8. The total chlorophyll content was expressed as mg/100 g FW.

Equation 6. Determination of chlorophyll a

Chlorophyll a (Chlo a): [15.65 x (Abs666) – (7.34 x (Abs653)]

Equation 5. Determination of chlorophyll b

Chlorophyll b (Chlo b): [27.05 x (Abs653) – (11.21 x (Abs666)]

Equation 6. Determination of total chlorophyll content

Total chlorophyll content = Chlorophyll a (Chlo a) + Chlorophyll b (Chlo b)

2.3 Statistical Analysis

The study was conducted using six consecutive growth cycles with Romaine Lettuce as the test crop. Each of the four treatments had three replicates within one cycle. Data were analyzed using R Studio (Version 2025.05.1+513; Posit PBC) with repeated-measures linear mixed-effects models fitted for each response variable. Treatment, cycle, and week, along with their interactions, were specified as fixed effects, while replicate was modeled as a random intercept to account for within-unit correlation. Models were fitted using restricted maximum likelihood (REML) estimation. Assumptions of normality and homoscedasticity were verified through diagnostic plots, and data were log- or square-root-transformed when necessary to meet model assumptions. Results are presented as estimated marginal means (EMMs) with corresponding 95% confidence intervals (CIs). When significant differences were detected ($p < 0.05$), pairwise comparisons were performed using Tukey-adjusted tests.

2.4 Results

2.4.1 Physical Water Parameters After Treatment and Reuse Cycles

Turbidity increased with cycle ($p < 0.001$) (Table 8), particularly from cycles 3–5 (Figure 4). No treatment differences were detected at cycles 1–2; however, from cycle 3, the Control group had higher turbidity values than the treated groups. Optical parameters showed inverse patterns: transmittance was highest in SSF + Biochar + UV-C and lowest in Control (Figure 5), while absorbance followed the opposite trend. Both parameters differed significantly by treatment and cycle ($p < 0.001$), with cycle 1 values distinct from later cycles (Table 8). TDS and EC exhibited parallel dynamics as shown in Table 8. Neither showed a main treatment effect, but both peaked at cycle four before declining in subsequent cycles ($p < 0.01$). At cycle four, EC values for control were highest compared to the other treatments.

Table 8. Summary of Type III ANOVA results for physical water quality parameters (turbidity, absorbance, transmittance, TDS, and EC) across treatments and reuse cycles

Source of variation	Turbidity (NTU)	Absorbance	Transmittance (%T)	EC (ms/cm)	TDS (mg/L)
Treatment	12.78^{***}	136.80^{***}	169.10^{***}	0.68^{ns}	0.89^{ns}
Cycle	3.62^{**}	11.40^{***}	12.03^{***}	6.91^{***}	8.29^{***}
Treatment*Cycle	2.56^{**}	2.86^{**}	2.98^{**}	2.47^{**}	2.73^{**}

Note: Values are F-ratios from Type III ANOVA

. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant

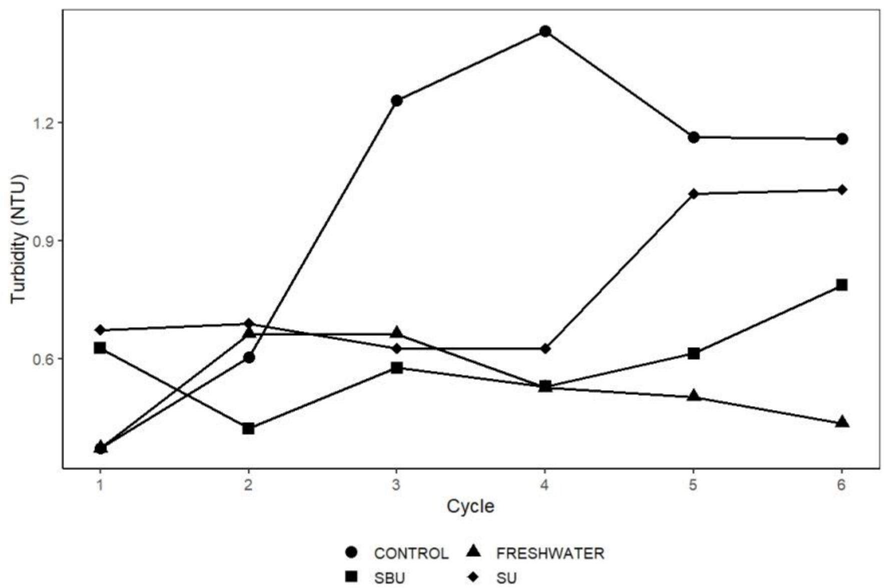

Figure 4. Turbidity (NTU) After Treatment for Each Reuse Cycle. Points Show Estimated Marginal Means (EMMs). Treatments Are Distinguished by Symbols: CONTROL, FRESHWATER, SBU= SSF + Biochar + UV-C, SU= SSF + UV-C.

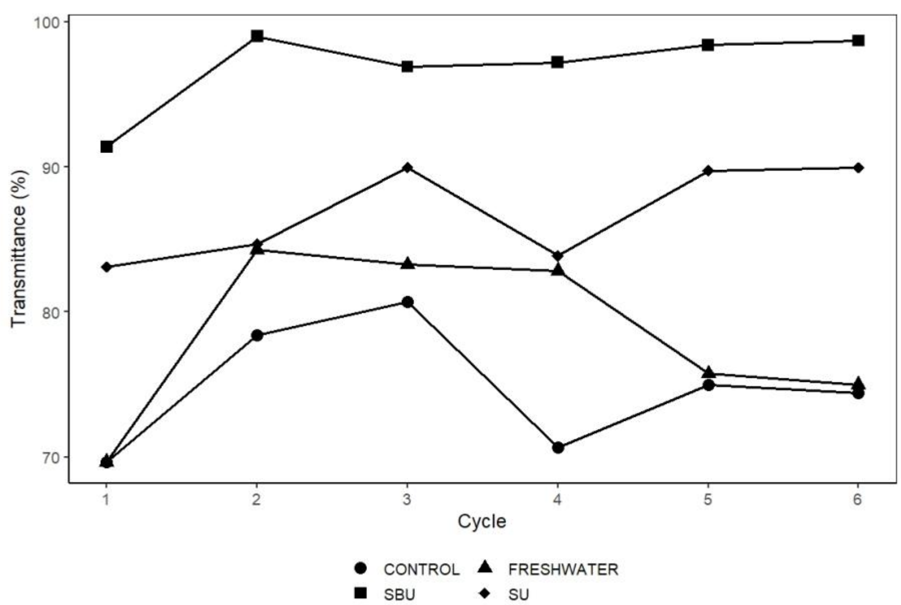

Figure 5.Transmittance (%) After Treatment for Each Reuse Cycle. Points Show Estimated Marginal Means (EMMs). Treatments Are Distinguished by Symbols: CONTROL, FRESHWATER, SBU= SSF + Biochar + UV-C, SU= SSF + UV-C.

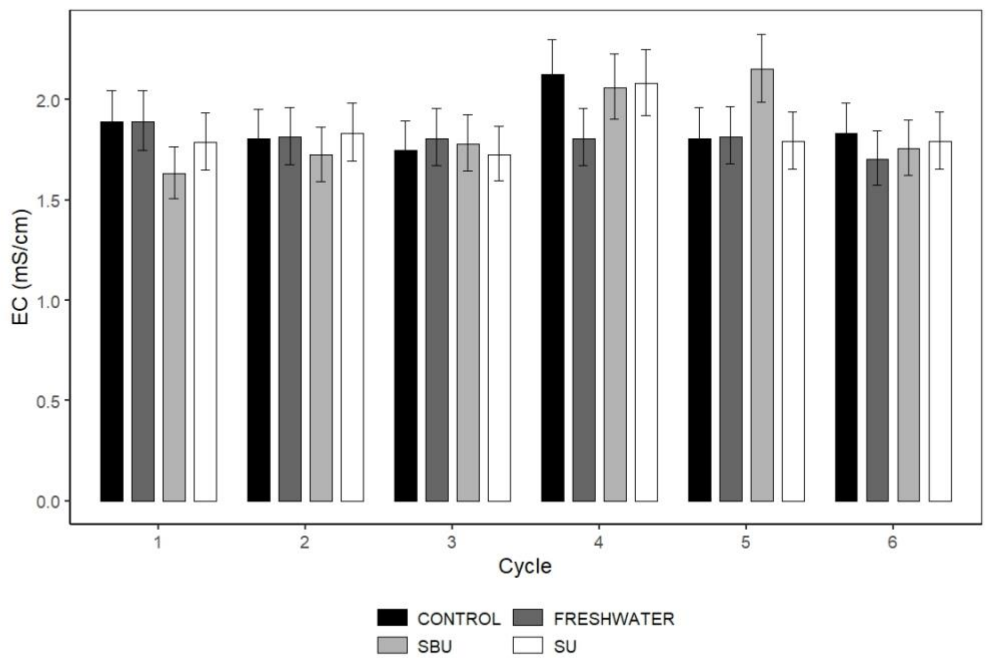

Figure 6.EC (mS/cm) After Treatment for Each Reuse Cycle. Bars Represent Estimated Marginal Means (EMMs) with 95% Confidence Intervals from a Linear Mixed-Effects Model: Treatment × Cycle + (1 | Replicate). Treatments: CONTROL, FRESHWATER, SBU= SSF + Biochar + UV-C, SU= SSF + UV-C.

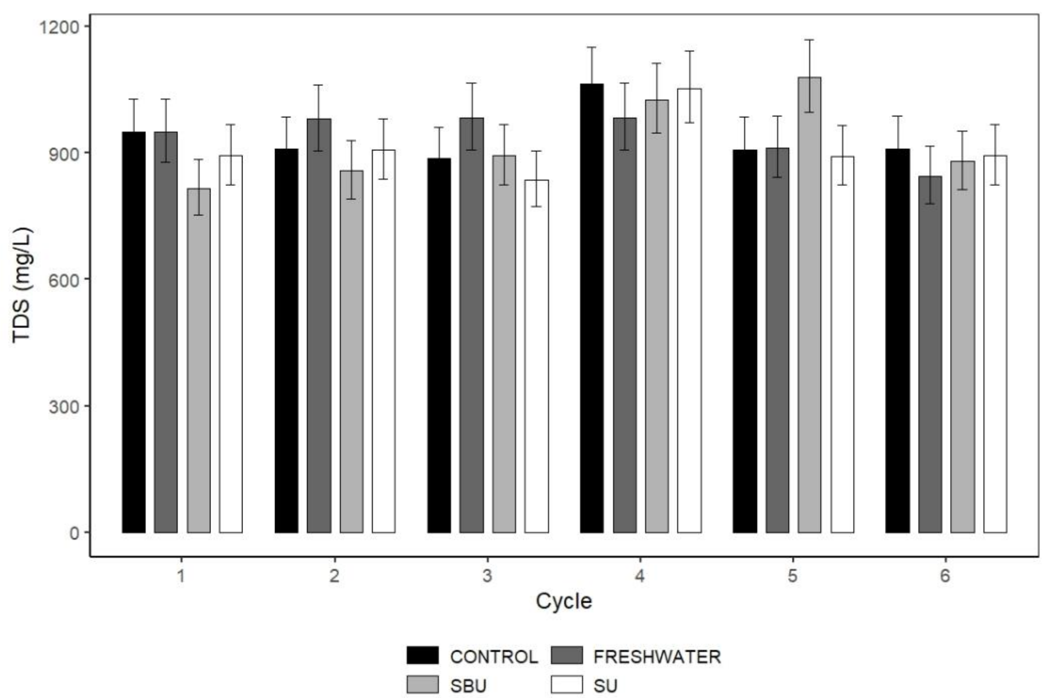

Figure 7. TDS (mg/L) after Treatment for Each Reuse Cycle. Bars Represent Estimated Marginal Means With 95% Confidence Intervals from a Linear Mixed-Effects Model: Treatment × Cycle + (1 | Replicate). Treatments CONTROL, FRESHWATER, SBU= SSF + Biochar + UV-C, SU= SSF + UV-C.

2.4.2 Treatment Effects on *Escherichia coli* Log_{10} Reduction

Treatment significantly influenced *E. coli* log_{10} reduction ($p < 0.001$), whereas cycle and the treatment × cycle interaction were not significant (Table 9). Across all six reuse cycles, SSF + UV-C (SU) and SSF + Biochar + UV-C (SBU) achieved (4.3-5.2) log_{10} reductions, while the log reduction in the Control remained <0.60 log_{10} (Figure 8). Post hoc comparisons detected no difference between SU and SBU ($p > 0.05$), though SU showed slightly higher mean reductions.

Table 9. Summary of ANOVA (Type III) on Log reduction (CFU/mL) of *Escherichia coli*

Source of variation	Log Reduction (CFU/mL)
Treatment	343.22^{***}
Cycle	2.19^{ns}
Treatment * Cycle	0.35^{ns}

Note. Values are F-ratios from Type III ANOVA.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant.

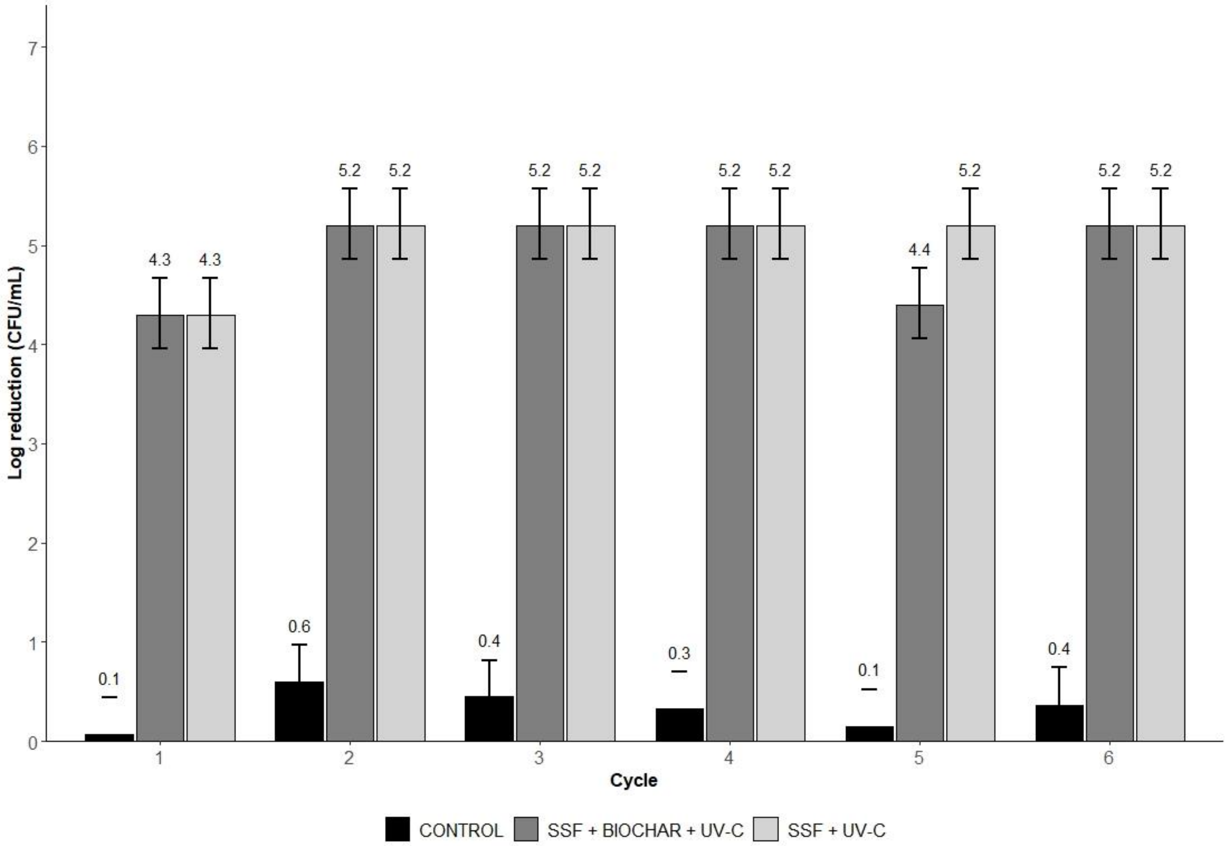

Figure 8. Log_{10} Reduction of *Escherichia coli* Across Six Reuse Cycles. Values Represent Estimated Marginal Means (EMMS) ± 95% Confidence Intervals from Linear Mixed-Effects Models. Treatments are distinguished by grayscale shading: Control, SSF + UV-C (SU), and SSF + Biochar + UV-C (SBU).

2.4.3 Chemical Water Parameters After Treatment

Tables 10 and 11 summarize chemical parameters across treatments and reuse cycles. pH was significantly affected by treatment, cycle, and their interaction ($p < 0.001$; Table 10). Post hoc tests ranked treatments as SSF + Biochar + UV-C > SSF + UV-C = Control > Freshwater, with Freshwater exhibiting the lowest pH across cycles.

Among nutrient parameters, chloride (Table 10) and ammonium-N (Table 10) were significantly influenced by treatment and cycle ($p < 0.001$), while nitrate-N, total P, and total N were not (Table 10). Chloride concentrations were higher in cycles 1–2 than in later cycles, with SSF + Biochar + UV-C consistently greater than the other treatments. Ammonium-N declined by

>93% across treated groups and Control but increased by 267% in Freshwater from cycle 1 to 6. In contrast, nitrate-N, total P, and total N showed no significant treatment or cycle effects, although modest declines (−45% for nitrate-N; −32% for total P; −43% for total N) were observed in SSF + Biochar + UV-C.

Sodium was significantly influenced by treatment, cycle, and their interaction ($p < 0.001$; Table 10). Freshwater had lower sodium than other treatments, while sodium levels decreased in cycles 5–6 compared to cycles 1–4. From cycle 1 to 6, sodium declined by 22% in SSF + Biochar + UV-C and 12% in SSF + UV-C but increased by 10% in Control.

Table 10. Estimated Marginal Means of pH, chloride (Cl^{-}) and sodium (Na) across treatments and reuse cycles.

Treatment	C	FW	SU	SBU
		pH		
Cycle				
1	6.7^{Ad}	6.7^{Ad}	6.93^{Bd}	8.03^{Bd}
2	5.83^{Ac}	5.83^{Ac}	6.37^{Bc}	7.4^{Bc}
3	6.3^{Ab}	5.83^{Ab}	6.23^{Bb}	6.3^{Bb}
4	5.67^{Aa}	5.8^{Aa}	5.63^{Ba}	6.77^{Ba}
5	6.26^{Abc}	5.87^{Abc}	6.1^{Bbc}	6.8^{Bbc}
6	5.99^{Aab}	5.67^{Aab}	5.93^{Bab}	6.83^{Bab}
		Cl^{-}		
1	7.45^{Ac}	7.45^{Ac}	15.81^{Ac}	17.36^{Bc}
2	$12.02^{A\,Ac}$	22.09^{Ac}	8.40^{Ac}	14.70^{Bc}
3	5.29^{Abc}	23.00^{Abc}	2.23^{Abc}	8.63^{Bbc}
4	2.77^{Aa}	0.82^{Aa}	0.60^{Aa}	3.74^{Ba}
5	0.65^{Aab}	0.80^{Aab}	3.13^{Aab}	40.85^{Bab}
6	0.11^{Aa}	0.10^{Aa}	0.1^{Aa}	14.00^{Ba}
		Na		

1	57.13^{Bb}	57.13^{Ab}	54.27^{Bb}	54.97^{Bb}
2	53.25^{Bb}	67.13^{Ab}	50.57^{Bb}	49.26^{Bb}
3	53.84^{Ba}	71.97^{Aa}	48.69^{Ba}	48.85^{Ba}
4	68.00^{Ba}	14.25^{Aa}	59.38^{Ba}	60.02^{Ba}
5	60.70^{Ba}	3.20^{Aa}	46.89^{Ba}	44.45^{Ba}
6	60.74^{Ba}	2.24^{Aa}	47.92^{Ba}	43.00^{Ba}
		p-value		
	pH	Cl^-	Na	
Treatment	***	***	***	
Cycle	***	***	***	
Treatment*Cycle	***	**	***	

Note. Values are estimated marginal means. C =Control; SU = Slow Sand Filtration (SSF) + UV-C; SBU = SSF + Biochar + UV-C; FW = Freshwater.

Uppercase letters within a column indicate significant differences between treatments ($p < 0.05$). Lowercase letters (a, b, ab, bc) denote significant differences among cycles. Lowercase italic letters (x, y, z, xy, yz) indicate significant differences for treatment × cycle interactions (Tukey's HSD). Groups not sharing a letter differ significantly at $\alpha = 0.05$* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant

Table 11. Estimated Marginal Means of ammonium-N (NH_4-N), nitrate-N($N0_3$-N), total P, total N, across treatments and reuse cycles.

Treatment	C	FW	SU	SBU
		NH_4-N		
Cycle				
1	1.7^{A}	1.7^{B}	1.40	1.02^{A}
2	1.92^{A}	1.80^{B}	2.62^{A}	1.38^{A}
3	0.37^{A}	3.53^{B}	0.80^{A}	0.33^{A}
4	0.22^{A}	4.47^{B}	0.25^{A}	0.91^{A}
5	0.06^{A}	7.97^{B}	0.07^{A}	1.06^{A}
6	0.08^{A}	6.19^{B}	0.05^{A}	0.07^{A}
		$N0_3$-N		
1	87.37	87.34	78.72	81.36

2	81.55	23.3	80.28	66.36
3	74.69	82.80	76.04	66.30
4	90.37	120.0	99.68	85.61
5	74.37	138.82	80.88	44.07
6	72.89	126.40	77.79	45.03
		Total N		
1	89.04	89.04	91.54	89.23
2	81.70	18.29	86.20	65.18
3	72.24	90.91	72.88	65.08
4	108.18	147.45	117.91	102.68
5	87.02	186.09	91.68	55.05
6	76.95	128.14	77.14	51.19
		Total P		
1	21.23	21.23	24.13	23.90
2	18.41	8.0	22.06	16.69
3	17.44	24.79	21.62	17.88
4	25.43	28.61	27.76	22.34
5	19.86	42.81	21.96	19.84
6	18.38	34.87	22.22	16.30
		p-value		
	NH_4-N	$N0_3$-N	Total N	Total P
Treatment	***	ns	Ns	*
Cycle	Ns	ns	Ns	**
Treatment * Cycle	*	ns	Ns	ns

Note. Values are estimated marginal means. C =Control; SU = Slow Sand Filtration (SSF) + UV-C; SBU = SSF + Biochar + UV-C; FW = Freshwater.

Uppercase letters within a column indicate significant differences between treatments ($p < 0.05$). Lowercase letters (a, b, ab, bc) denote significant differences among cycles. Lowercase italic letters (x, y, z, xy, yz) indicate significant differences for treatment × cycle interactions (Tukey's HSD). Groups not sharing a letter differ significantly at $\alpha = 0.05$

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant

2.4.3 Micronutrient levels after treatment

Micronutrient concentrations were significantly influenced by treatment, cycle, and in several cases, their interaction (Tables 12-13). Calcium decreased across all treatments over the reuse period, with the highest declines in Control (−49.2%) and SU (−36.5%), and a more modest reduction in SBU (−15.3%). Concentrations were higher in later cycles (4–6) than in earlier ones. Among the trace metals, treatment effects were consistent: Freshwater had the highest concentrations, SBU the lowest, and Control and SU intermediate. Manganese declined slightly across cycles (−6 to −8%), with cycle 1 consistently higher than later cycles. Copper decreased by ~100% in SBU but increased in SU (+59.2%), Control (+98.1%), and Freshwater (+56.2%). Zinc showed sharp reductions in SBU (−91.9%) and SU (−53.5%), while Control (+44.6%) and Freshwater (+35.8%) increased. Iron declined in SBU (−97.4%) but increased modestly in the other treatments.

Overall, micronutrient patterns showed that SBU consistently reduced Mn, Cu, Zn, and Fe over successive cycles, while Control and Freshwater often exhibited stable or increasing concentrations. SU behaved in an intermediate manner, with moderate declines in some metals and increases in others.

Table 12. Estimated Marginal Means of zinc (Zn), iron (Fe), and copper (Cu) across treatments and reuse cycles.

Treatment	C	FW	SU	SBU
		Zn		
Cycle				
1	0.16^{Bb}	0.16^{Cb}	0.16^{Ba}	0.16^{Ab}
2	0.16^{Ba}	0.08^{Ca}	0.18^{Ba}	0.02^{Aa}
3	0.17^{Bc}	5.8^{Cc}	0.18^{Bc}	0.04^{Ac}
4	0.21^{Bb}	0.19^{Cb}	0.23^{Bb}	0.01^{Ab}
5	0.19^{Bab}	0.22^{Cab}	0.19^{Bab}	0.01^{Aab}
6	0.2^{Bb}	0.21^{Cb}	0.20^{Bb}	0.01^{Ab}

		Fe		
1	1.33^{Cc}	1.33^{Cc}	1.42^{Bc}	1.39^{Ac}
2	1.34^{Ca}	0.85^{Ca}	1.09^{Ba}	0.01^{Aa}
3	1.42^{Cab}	1.34^{Cab}	0.86^{Bab}	0.2^{Aab}
4	2.05^{Cbc}	1.77^{Cbc}	1.07^{Bbc}	0.01^{Abc}
5	1.8^{Cabc}	1.85^{Cabc}	0.72^{Babc}	0.02^{Aabc}
6	1.95^{Cabc}	1.81^{Cabc}	0.66^{Babc}	0.04^{Aabc}
		Cu		
1	0.06^{BCb}	0.06^{Bb}	0.07^{Cb}	0.07^{Ab}
2	0.06^{BC}	0.03^{Ba}	0.08^{Ca}	0.01^{Aa}
3	0.06^{BCab}	0.06^{Bab}	0.08^{Cab}	0.02^{Aab}
4	0.09^{BCb}	0.07^{Bb}	0.11^{Cb}	0.01^{Ab}
5	0.09^{BCb}	0.09^{Bb}	0.09^{Cb}	0.01^{Ab}
6	0.12^{BCb}	0.09^{Bb}	0.11^{Cb}	0^{Ab}
		p-value		
	Zn	Fe	Cu	
Treatment	***	***	***	
Cycle	***	***	**	
Treatment * Cycle	***	***	***	

Note. Values are estimated marginal means. C =Control; SU = Slow Sand Filtration (SSF) + UV-C; SBU = SSF + Biochar + UV-C; FW = Freshwater.

Uppercase letters within a column indicate significant differences between treatments ($p < 0.05$). Lowercase letters (a, b, ab, bc) denote significant differences among cycles. Lowercase italic letters (x, y, z, xy, yz) indicate significant differences for treatment × cycle interactions (Tukey's HSD). Groups not sharing a letter differ significantly at α = 0.05

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant

Table 13.Estimated Marginal Means of calcium (Ca) and manganese (Mn) across treatments and reuse cycles.

Treatment	C	FW	SU	SBU
		Mn		
Cycle				
1	0.15^{Bb}	0.15^{Cb}	0.14^{Bb}	0.13^{Ab}
2	0.11^{Ba}	0.09^{Ca}	0.12^{Ba}	0.03^{Aa}
3	0.09^{Ba}	0.13^{Ca}	0.11^{Ba}	0.02^{Aa}
4	0.08^{Ba}	0.15^{Ca}	0.12^{Ba}	0.04^{Aa}
5	0.06^{Bab}	0.21^{Cab}	0.10^{Bab}	0.05^{Aa}
6	0.07^{Ba}	0.19^{Ca}	0.07^{Ba}	0.13^{Ab}
		Ca		
1	150.97^{Bab}	150.97^{Aab}	147.14^{Bab}	143.40^{ABab}
2	152.52^{Ba}	88.39^{Aa}	160.65^{Ba}	140.39^{Aba}
3	169.43^{Bab}	136.24^{Aab}	160.55^{Bab}	153.74^{ABab}
4	223.54^{Bc}	178.97^{Ac}	211.30^{Bc}	202.41^{ABc}
5	203.72^{Bbc}	141.95^{Abc}	182.60^{Bbc}	164.28^{ABbc}
6	225.18^{Bbc}	141.24^{Abc}	200.86^{Bbc}	165.31^{ABbc}

p- value		
	Mn	Ca
Treatment	***	***
Cycle	***	***
Treatment * Cycle	**	Ns

Note. Values are estimated marginal means ± SE. C =Control; SU = Slow Sand Filtration (SSF) + UV-C; SBU = SSF + Biochar + UV-C; FW = Freshwater.

Uppercase letters within a column indicate significant differences between treatments ($p < 0.05$). Lowercase letters (a, b, ab, bc) denote significant differences among cycles. Lowercase italic letters (x, y, z, xy, yz) indicate significant differences for treatment × cycle interactions (Tukey's HSD). Groups not sharing a letter differ significantly at $\alpha = 0.05$

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant

2.4.4 Fresh Shoot and Root Weight

Both shoot and root biomass of romaine lettuce were significantly affected by Treatment, Cycle, and their interaction (all $p < 0.05$; Table 14). For shoot biomass, SU-grown romaine lettuce outperformed all other treatments, while Freshwater produced the lowest values; Control and SBU did not differ. Root biomass followed a similar trend, with SU > SBU > (Control = Freshwater).

Table 14. Estimated Marginal Means of Fresh Shoot Weight (FSW) and Fresh Root Weight (FRW) of romaine lettuce for treatment across each cycle.

Treatment	C	FW	SU	SBU
		FSW		
Cycle				
1	120.11^{Bd}	120.11^{Ad}	144.64^{Cd}	125.78^{Bd}
2	100.00^{Bc}	84.58^{Ac}	107.30^{Cc}	105.69^{Bc}
3	65.86^{Bb}	66.44^{Ab}	87.22^{Cb}	85.81^{Bb}
4	85.14^{Bb}	70.31^{Ab}	90.50^{Cb}	88.92^{Bb}
5	69.44^{Ba}	33.30^{Aa}	70.81^{Ca}	69.03^{Ba}
6	80.64^{Bb}	79.40^{Ab}	101.50^{Cb}	68.75^{Bb}
		FRW		
	8.0^{Ab}	8.0^{Ab}	8.41^{Bb}	8.28^{Abb}
	6.42^{Aa}	5.03^{Aa}	7.01^{Ba}	7.13^{Aba}
	5.44^{Aa}	4.41^{Aa}	8.2^{Ba}	6.16^{Aba}
	5.89^{Aa}	5.78^{Aa}	6.14^{Ba}	6.76^{Aba}
	5.45^{Aa}	7.81^{Aa}	5.71^{Ba}	6.10^{Aba}
	5.15^{Aa}	4.87^{Aa}	7.12^{Ba}	5.10^{Aba}

p-value	FSW	FRW
Treatment	**	***
Cycle	***	***

Treatment*Cycle	**	***

Note. Values are estimated marginal means C =Control; SU = Slow Sand Filtration (SSF) + UV-C; SBU = SSF + Biochar + UV-C; FW = Freshwater.
Uppercase letters within a column indicate significant differences between treatments ($p < 0.05$). Lowercase letters (a, b, ab, bc) denote significant differences among cycles. Lowercase italic letters (*x*, *y*, *z*, *xy*, *yz*) indicate significant differences for treatment × cycle interactions (Tukey's HSD). Groups not sharing a letter differ significantly at $\alpha = 0.05$
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant.

As shown in Figures 9 and 10, biomass in both tissues was highest in Cycle 1 and declined in subsequent cycles ($p < 0.05$, Table 14). The decline was more pronounced for shoots, with reductions of 29.8% (SU), 45.3% (SBU), and 32.9% (Control) from Cycle 1 to 6, compared to root reductions of 15.0% (SU), 38.4% (SBU), and 35.6% (Control), which stabilized after the initial drop.

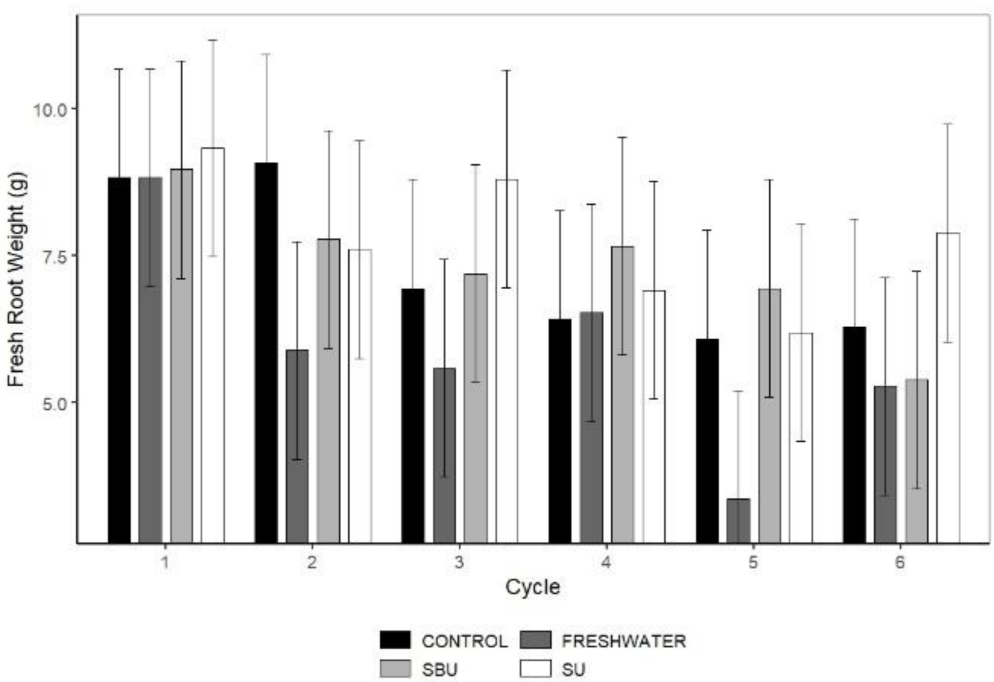

Figure 9. Fresh root weight (g) by treatment and cycle. Bars represent estimated marginal means with 95% confidence intervals from a linear mixed-effects model: Treatment × Cycle + (1 | Replicate) Treatments: CONTROL, FRESHWATER, SBU= SSF + Biochar + UV-C, SU= SSF + UV-C.

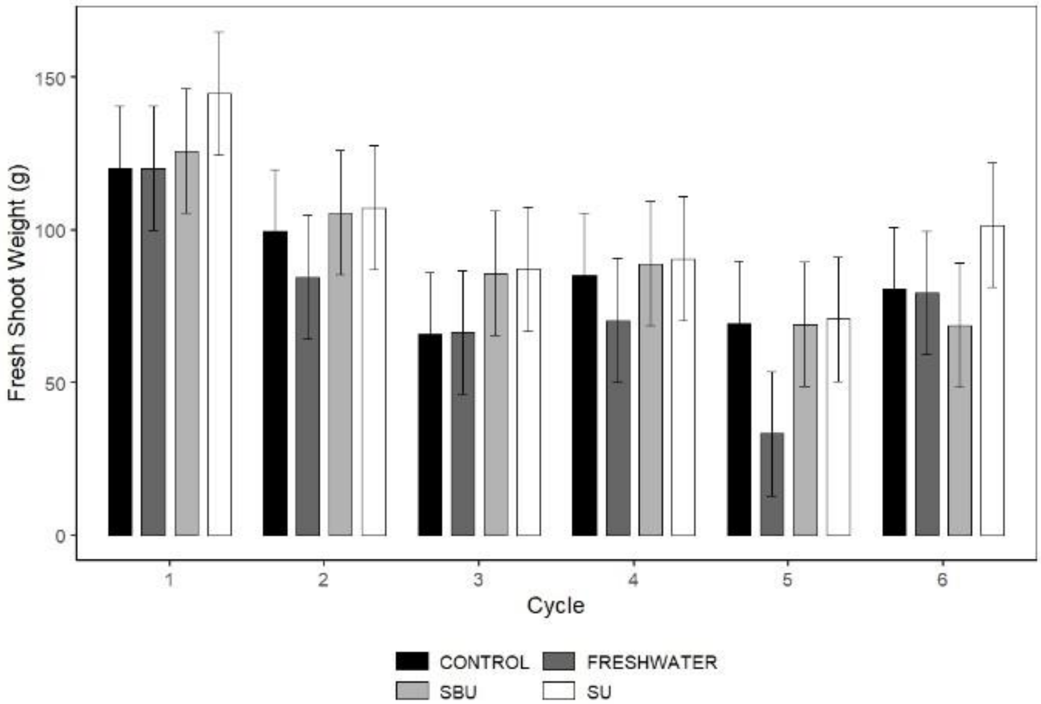

Figure 10. Fresh shoot weight (g) by treatment and cycle. Bars represent estimated marginal means with 95% confidence intervals from a linear mixed-effects model: Treatment × Cycle + (1 | Replicate). Treatments: CONTROL, FRESHWATER, SBU= SSF + Biochar + UV-C, SU= SSF + UV-C.

2.4.5 SPAD (Soil and Plant Analyzer Development) Values, Plant Height, and Number of Leaves

Romaine lettuce grown with Freshwater had the highest SPAD values, while SU-grown romaine lettuce had the tallest plants and the highest leaf numbers. SBU and Control were intermediate and like each other, as shown in Figures 11, 12, and 13 from the post hoc test in Table 15.

Table 15. Estimated Marginal Means for SPAD Value, Plant Height(cm), and Number of leaves of romaine lettuce for each treatment across each cycle.

Treatment	Week	C	FW	SU	SBU
			SPAD Value		
1	2	43.33^{Aa}	43.33^{Ba}	43.54^{Aa}	44.59^{Aa}
2		46.36^{Abc}	45.46^{Bbc}	47.19^{Abc}	50.63^{Abc}

3		48.66^{Abc}	45.67^{Bbc}	48.60^{Abc}	48.86^{Abc}
4		49.61^{Ac}	49.66^{Bc}	50.89^{Ac}	49.67^{Ac}
5		45.40^{Abc}	53.86^{Bbc}	43.33^{Abc}	46.55^{Abc}
6		46.04^{Ab}	49.43^{Bb}	52.96^{Ab}	43.63^{Ab}
1	4	46.08^{Aa}	46.08^{Ba}	39.45^{Aa}	43.32^{Aa}
2		47.78^{Abc}	46.50^{Bbc}	48.41^{Abc}	51.26^{Abc}
3		46.55^{Abc}	43.02^{Bbc}	50.36^{Abc}	48.71^{Abc}
4		48.50^{Ac}	46.68^{Bc}	48.38^{Ac}	48.78^{Ac}
5		36.13^{Abc}	60.54^{Bbc}	43.96^{Abc}	47.77^{Abc}
6		47.08^{Ab}	51.94^{Bb}	46.24^{Ab}	34.84^{Ab}
Plant Height					
1	2	5.6^{Ba}	5.6^{Aa}	7.30^{Ca}	5.58±^{Ba}
2		9.03^{Bd}	9.74^{Ad}	9.30^{Cd}	8.80^{Bd}
3		8.29^{Bc}	7.96^{Ac}	8.55^{Cc}	8.83^{Bc}
4		8.68^{Bd}	9.51^{Ad}	9.24^{Cd}	9.18^{Bd}
5		9.22^{Bb}	5.99^{Ab}	9.76^{Cb}	9.06^{Bb}
6		9.48^{Bd}	9.23^{Ad}	8.89^{Cd}	9.76^{Bd}
1	4	13.69^{Ba}	13.69^{Aa}	14.43^{Ca}	13.48^{Ba}
2		14.62^{Bd}	14.81^{Ad}	14.11^{Cd}	14.07^{Bd}
3		14.05^{Bc}	14.82^{Ac}	13.72^{Cc}	14.13^{Bc}
4		14.50^{Bd}	14.33^{Ad}	14.83^{Cd}	15.00^{Bd}
5		13.55^{Bb}	8.04^{Ab}	14.64^{Cb}	14.32^{Bb}
6		15.09^{Bd}	15.3^{Ad}	15.80^{Cd}	15.11^{Bd}
Number of Leaves					
1	2	6.64^{Ba}	6.64^{Ba}	8.26^{Ba}	7.15^{Aa}
2		7.40^{Bbc}	9.27^{Bbc}	8.32^{Bbc}	7.92^{Abc}
3		8.32^{Bd}	8.35^{Bd}	8.93^{Bd}	8.6^{Ad}
4		9.00^{Bd}	10.9^{Bd}	9.15^{Bd}	9.23^{Ad}
5		7.74^{Bb}	7.30^{Bb}	8.47^{Bb}	7.77^{Ab}
6		8.02^{Bc}	7.76^{Bc}	7.91^{Bc}	7.88^{Ac}
1	4	13.2^{Ba}	13.2^{Ba}	14.3^{Ba}	13.62^{Aa}

2	15.4^{Bbc}	16.3^{Bbc}	14.8^{Bbc}	14.23^{Abc}
3	16.5^{Bd}	18.6^{Bd}	18.3^{Bd}	17.73^{Ad}
4	16.2^{Bd}	15.2^{Bd}	17.8^{Bd}	17.57^{Ad}
5	14.5^{Bb}	10.9^{Bb}	16.3^{Bb}	15.20^{Ab}
6	15.2^{Bc}	14.4^{Bc}	16.3^{Bc}	15.71^{Ac}

p- value

	SPAD value	Plant Height	Number of leaves
Treatment	***	***	***
Cycle	***	***	***
Week	ns	***	***
Treatment*Cycle	***	***	***
Treatment* Week	ns	ns	*
Cycle*Week	ns	***	***
Treatment*Cycle * Week	***	***	***

Note. Values are the estimated marginal means. C =Control; SU = Slow Sand Filtration (SSF) + UV-C; SBU = SSF + Biochar + UV-C; FW = Freshwater.

Uppercase letters within a column indicate significant differences between treatments ($p < 0.05$). Lowercase letters (a, b, ab, bc) denote significant differences among cycles. (Tukey's HSD). Groups not sharing a letter differ significantly at $\alpha = 0.05$

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant.

Treatment and Cycle significantly influenced all traits, with Week having a significant effect on height and leaf number (Table 15). Across cycles, all traits were lowest in Cycle 1 and improved thereafter ($p < 0.05$). SPAD values declined under SU (21.6% in Week 2 and 17.2% in Week 4) and under Control (6.24% in Week 2 and 2.16% in Week 4) but increased under SBU (2.15% in Week 2 and 19.6% in Week 4). Plant height rose across cycles, with increases of 69.2% (Week 2) and 10.3% (Week 4) under Control, 21.8% (Week 2) and 9.5% (Week 4) under SU, and 46.8% (Week 2) and 9.5% (Week 4) under SBU. Leaf numbers followed a similar pattern, with changes of +20.8% (Week 2) and +15.1% (Week 4) under Control, −4.29% (Week 2) and +14.0% (Week 4) under SU, and +10.2% (Week 2) and +15.4% (Week 4) under SBU. Week effects were consistent for height and leaf number, which increased from Week 2 to Week 4, while SPAD showed only a marginal decline over time ($p = 0.06$).

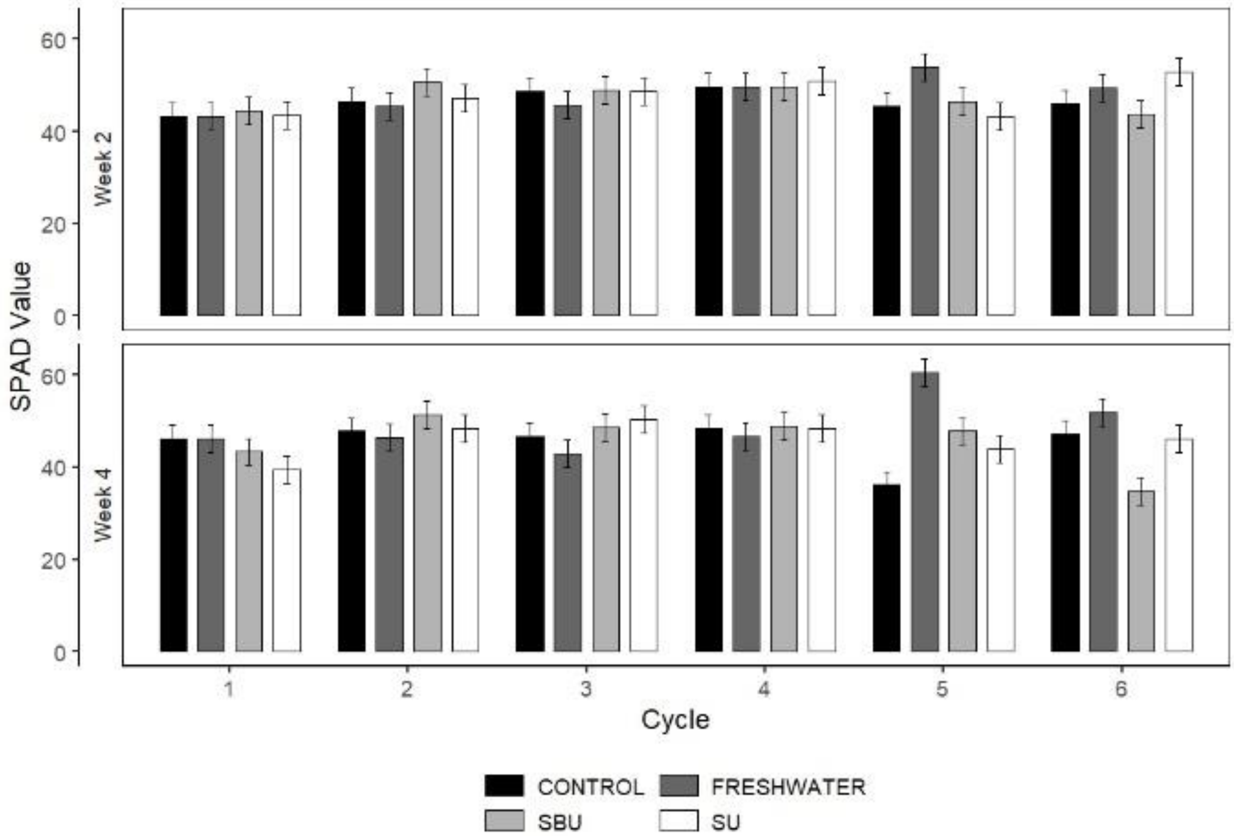

Figure 11.SPAD Value by treatment and cycle. Bars represent estimated marginal means with 95% confidence intervals from a linear mixed-effects model. Treatments: CONTROL, FRESHWATER, SBU= SSF + Biochar + UV-C, SU= SSF + UV-C.

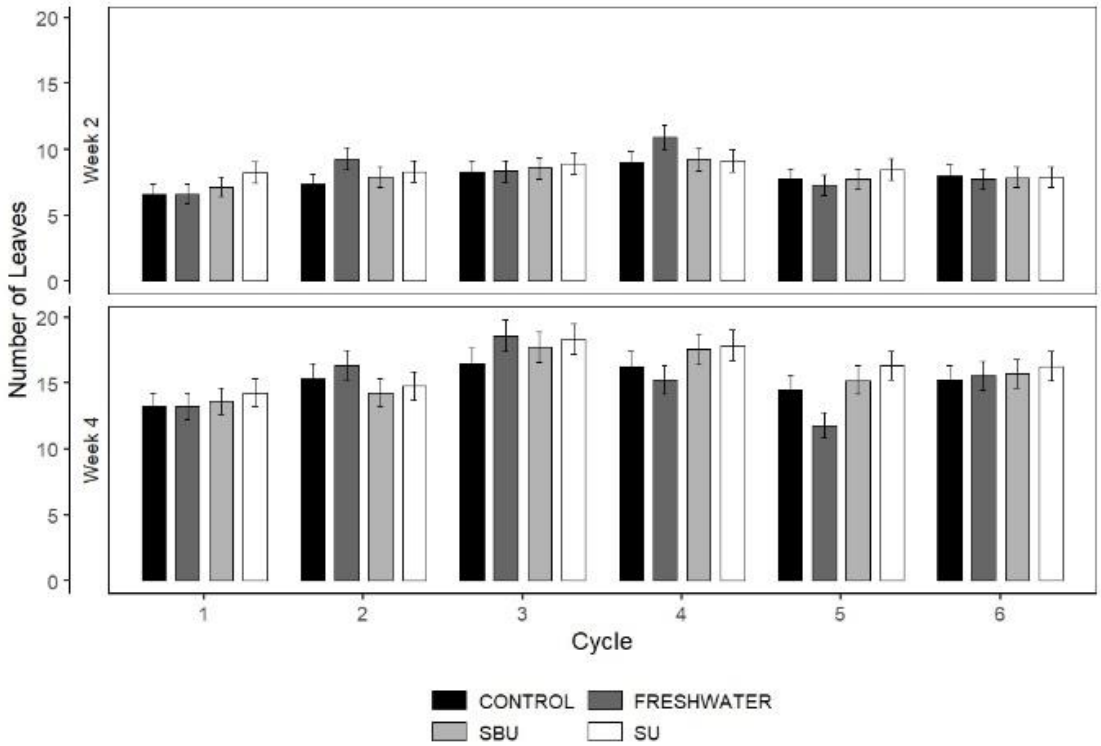

Figure 12.Number of leaves by treatment and cycle. Bars represent estimated marginal means with 95% confidence intervals from a linear mixed-effects model: Treatments: CONTROL, FRESHWATER, SBU= SSF + Biochar + UV-C, SU= SSF + UV-C.

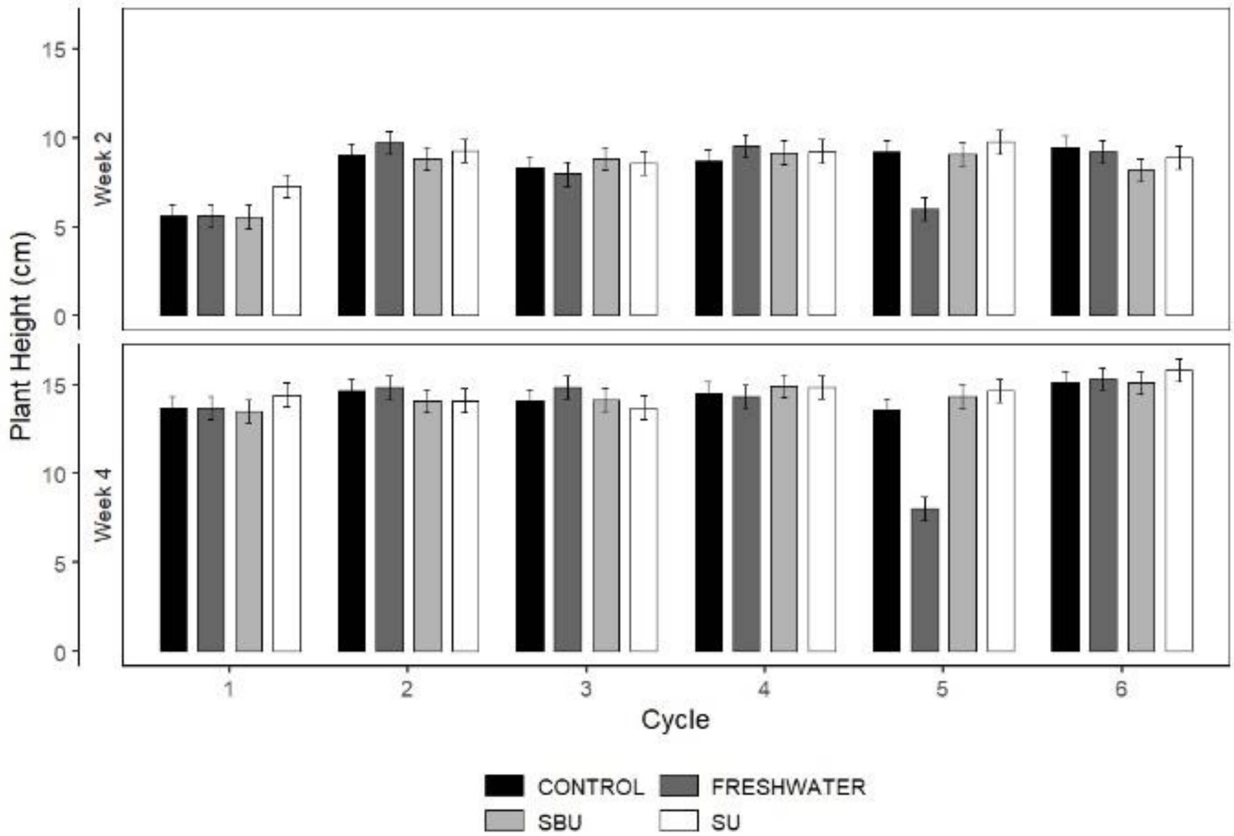

Figure 13. Plant height(cm) by treatment and cycle. Bars represent estimated marginal means with 95% confidence intervals from a linear mixed-effects model: Treatments: CONTROL, FRESHWATER, SBU = SSF + Biochar + UV-C, SU = SSF + UV-C.

2.4.6 Nutritional Quality

2.4.6.1 Vitamin C and Chlorophyll Content

Both compounds were significantly affected by Cycle, while Treatment and the Treatment × Cycle interaction were not significantly different (Table 16).

Table 16. 17Estimated Marginal Means for Vitamin C(mg/100g) FW and Chlorophyll content(mg/100g) FW of romaine lettuce for each treatment across each cycle.

Treatment	C	FW	SU	SBU
		Vitamin C (mg/100g) FW		

Cycle				
1	21.64^{b}	21.42^{b}	27.87^{b}	14.61^{b}
2	15.62^{a}	11.14^{a}	12.11^{a}	17.22^{a}
3	12.95^{ab}	14.70^{ab}	15.09^{ab}	14.40^{ab}
4	17.02^{ab}	13.58^{ab}	16.21^{ab}	15.14^{ab}
5	15.48^{ab}	33.50^{ab}	16.67^{ab}	15.93^{ab}
6	15.50^{ab}	23.24^{ab}	18.61^{ab}	11.83^{ab}
		Chlorophyll (mg/100g) FW		
	84.89^{b}	84.90^{b}	75.44^{b}	73.87^{b}
	73.64^{b}	76.04^{b}	79.38^{b}	79.46^{b}
	41.64^{a}	46.25^{a}	37.24^{b}	37.61^{a}
	34.05^{a}	38.00^{a}	32.74^{b}	35.71^{a}
	41.20^{a}	58.71^{a}	29.65^{b}	41.05^{a}
	31.23^{a}	58.10^{a}	41.86^{b}	36.00^{a}

p-value

	Vitamin C	Chlorophyll
Treatment	ns	Ns
Cycle	**	***
Treatment*Cycle	ns	Ns

Note. Values are estimated marginal means. C =Control; SU = Slow Sand Filtration (SSF) + UV-C; SBU = SSF + Biochar + UV-C; FW = Freshwater.

Lowercase letters (a, b, ab, bc) denote significant differences among cycles. (Tukey's HSD). Groups not sharing a letter differ significantly at $\alpha = 0.05$.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant.

Vitamin C content was higher in Cycle 1 than in Cycle 2 ($p < 0.05$) and declined over subsequent cycles, as seen in Figure 14, with overall reductions from Cycle 1 to 6 of 33.2% in SU, 19.0% in SBU, and 28.4% in Control. Chlorophyll followed a similar cycle-dependent pattern as shown in Figure 15, with higher concentrations in Cycles 1–2 compared to Cycles 3–6 ($p < 0.05$). Declines were more pronounced for chlorophyll than Vitamin C, with losses of 63.2% in Control, 51.4% in SBU, and 44.5% in SU between Cycle 1 and 6.

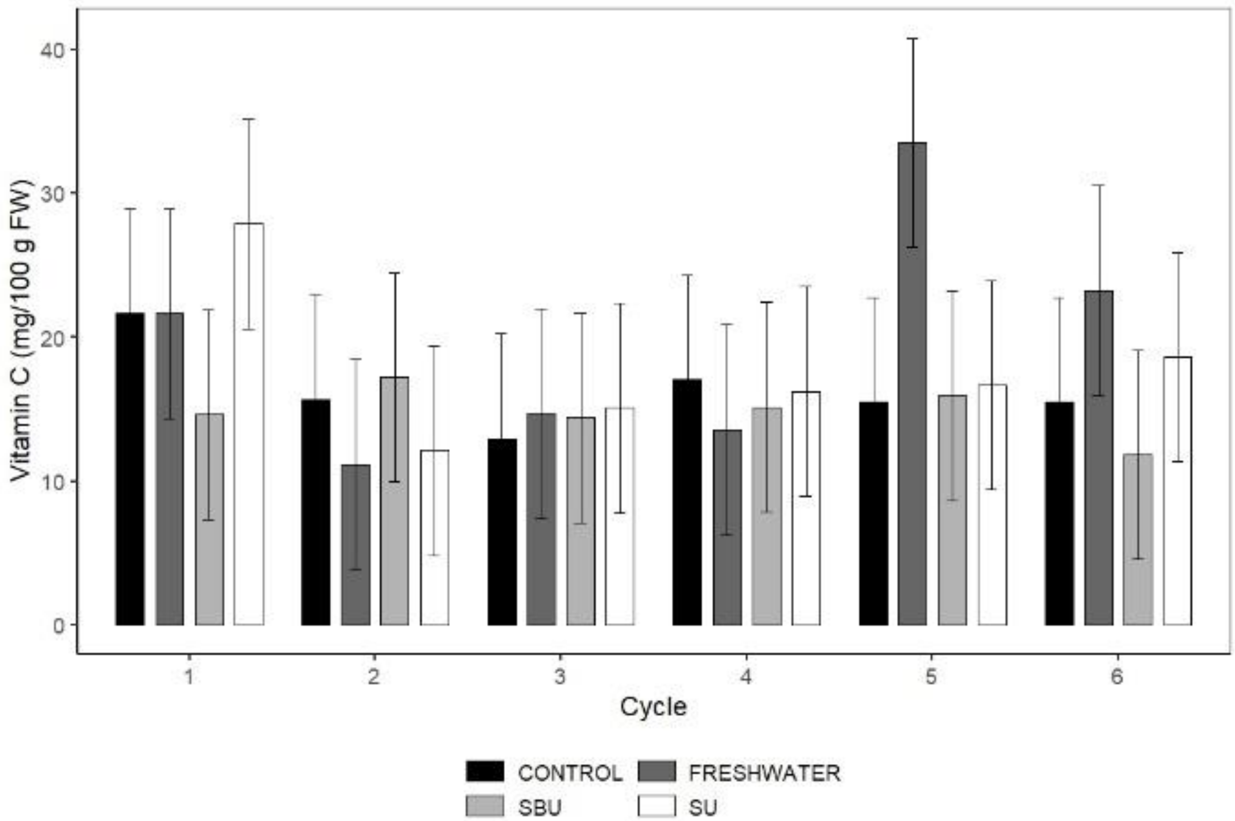

Figure 14. Vitamin C (mg/100g) FW by treatment and cycle. Bars represent estimated marginal means with 95% confidence intervals from a linear mixed-effects model Treatment: CONTROL, FRESHWATER, SBU = SSF + Biochar + UV-C, SU = SSF + UV-C.

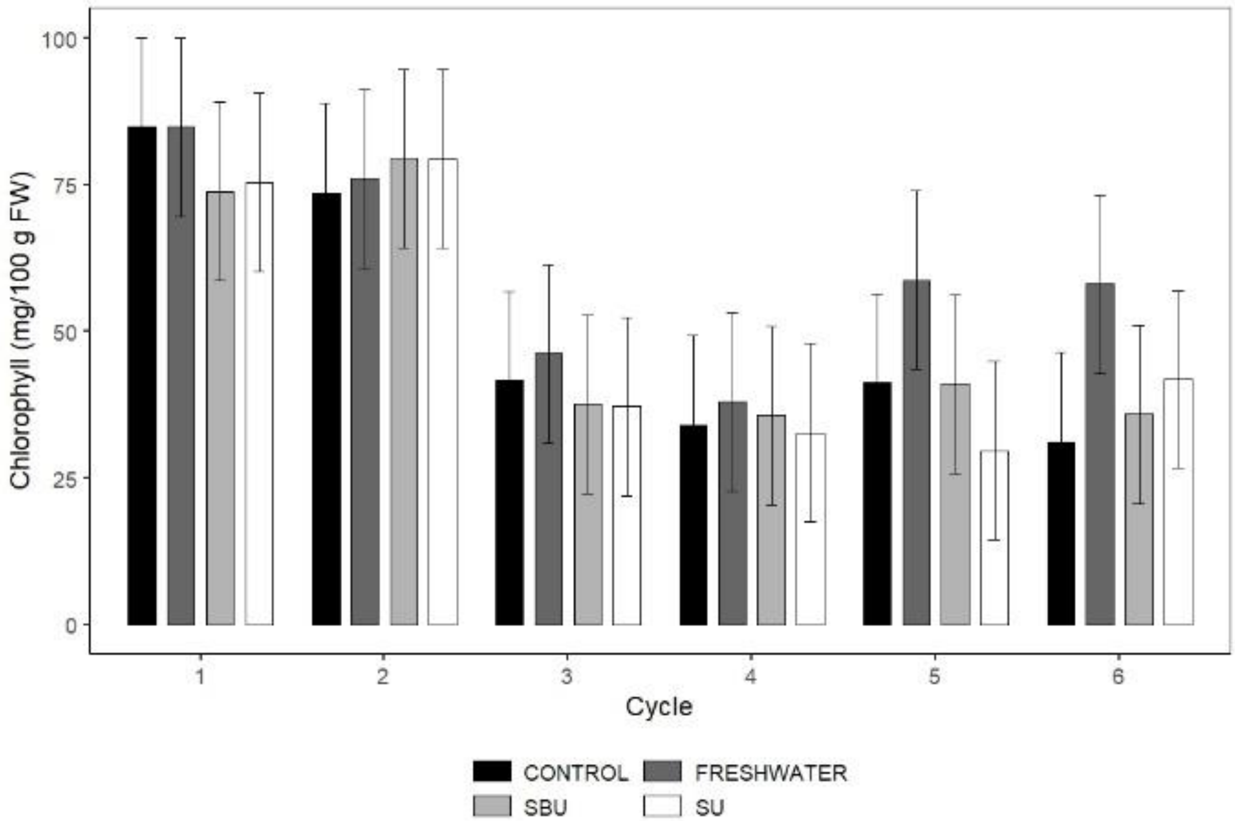

Figure 1515. Chlorophyll (mg/100g) FW by treatment and cycle. Bars represent estimated marginal means with 95% confidence intervals from a linear mixed-effects model: Treatment: CONTROL, FRESHWATER, SBU = SSF + Biochar + UV-C, SU = SSF + UV-C.

2.5 Discussion

This study evaluated the combined effectiveness and efficacy of slow sand filtration, biochar filtration, and UV-C disinfection in treating nutrient solution in a deep-water culture hydroponic systems to produce safely treated recirculating nutrient solution for continued use by small scale growers. Agricultural water reuse offers several benefits, including reducing dependence on freshwater, lowering costs, mitigating the environmental impact of pollution, and producing a nutrient-rich product that supports the environment, while also enhancing crop production. In turn, agricultural water reuse creates a system that is both reliable and sustainable (US Environmental Protection Agency, 2023). Currently, there are no internationally recognized standards for recirculating nutrient solutions in hydroponics. Therefore, water quality and microbial safety in this study were benchmarked against the guidelines of the Food and Agriculture Organization (FAO), World Health Organization (WHO), European Union (EU), and US Environmental Protection Agency (EPA) for the reuse of agricultural water. and scientific recommendations from research on nutrient solution recycling.

2.5.1 Log reduction of *Escherichia coli*

Across all cycles, both SU and SBU treatments achieved (4.3-5.2) $\log_{10}$ reductions in rifampicin-resistant *E. coli.* These reductions correlate with the reported performance of individual components in several studies: SSF (1.3–1.7 $\log_{10}$) through schmutzdecke-mediated removal (Kaetzl et al., 2020a; Trikannad et al., 2023), biochar filtration (1.4–2.3 $\log_{10}$) via high-surface-area adsorption that allows for bacterial attachments (Bui et al., 2024) and UV-C disinfection ($\geq$ 4 $\log_{10}$) through DNA damage and replication inhibition (Adeniyi & Jimoh, 2024; H.-J. Kim et al., 2023; C.-P. Wang & Liao, 2023; Yokoyama & Mizutani, 2014). The consistently high reductions in this study compared to the others suggest additive or synergistic effects, similar to recommendations made by Banach & Van Der Fels-Klerx, (2020) that combining water treatments increases their efficiency in reducing microbial populations.

The 5.2 $\log_{10}$ reductions achieved in this study met the World Health Organization (WHO) standard for treated wastewater for agricultural use microbial criteria ($\leq$ 1 CFU/100 mL)World Health Organization2012)**.** This makes it suitable for growing crops that are eaten raw in hydroponic production systems. However, the 4.3 $\log_{10}$ reductions observed after treatment in cycles 1 and 5 suggest some variability in treatment performance across reuse cycles, which may reflect fluctuations in operational stability. These could be attributed to filter conditions, differing biofilter maturation, and turbidity (Collivignarelli et al., 2021; Harold Wright & Kenan Ozekin, 2025; Langenbach et al., 2009; Maiyo et al., 2023)

2.5.2 Physical, Chemical, and Micronutrient Levels

Turbidity remained below 2 NTU in all treated recirculating nutrient solution (tRNS), well within the Food and Agriculture Organization (FAO)/World Health Organization (WHO) (≤ 5 NTU) and European Union (EU) Class A (≤ 2 NTU) limits set by ; Joint Research Centre (European Commission) et al.(2017). Higher transmittance and lower absorbance in SBU compared to SU indicate that biochar contributed to the removal of fine particulates and chromophoric organic matter, consistent with prior findings by (Broadley et al., 2012). Electrical conductivity (EC) and total dissolved solids (TDS) values in both treatments fell within the FAO "slight to moderate" salinity range for irrigation (ECw 0.7–3.0 dS m^{-1}; TDS 450–2000 mg L^{-1}), indicating some suitability for hydroponic recirculation. However, for lettuce hydroponics, EC values outside approximately 1.2–1.8 mS cm^{-1} can impair water uptake and nutrient intake by plants (Afonso et al., 2023); however, in practice, salinity can be further managed through dilution or nutrient solution adjustments, or novel methods such as the use of biostimulants, such as amino acids, to mitigate saline stress (Arif et al., 2020; İkiz et al., 2024). pH in SBU-treated recirculating nutrient solution fell within the acceptable range set by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) of 6-8.5. In contrast, nutrient solution treated with SU, C (spent nutrient solution), and freshwater (drain to waste system) fell below the recommended range. Although many hydroponic systems require a near pH of 5.5–6.5 to operate, a low pH can increase the mobilization of toxic metals such as manganese, potentially leading to toxicity (C. Wang et al., 2014).

Both treatments (SU and SBU) reduced ammonium-N, nitrate-N, total phosphorus, and total nitrogen compared to the positive control, with SBU showing the highest removal rate (96.2% NH_4-N, 44.7% NO_3-N, 31.8% total P, and 42.6% total N), similar to the average removal of 71% and 57% for nitrogen-ammonium and phosphorus-phosphate by Bui et al. (2024), Blanco-Canqui(2019), Nabiul Afrooz & Boehm (2017), and Tian et al2016). These results correlate with the high sorption capacity of biochar for dissolved nutrients, attributed to its internal surface area and porosity (Blanco-Canqui, 2019). We attribute the differences in removal efficiencies observed in our findings, compared to other studies to the differential properties of biochar, the different influent composition, and the presence of filters, which in our case is SSF (Blanco-Canqui, 2019; Mohanty et al., 2018). However, nitrate-N concentrations remained above 30 mg L^{-1} in all treatments, placing the water in the Food and Agriculture Organization "severe restriction" category for irrigation and posing a risk for eutrophication if discharged (US Environmental Protection Agency, 2012a). Therefore, treated recirculating nutrient solution with such a profile should be utilized for the production of plant species with high nitrate uptake, such as leafy greens, incorporating legumes in hydroponic systems to reduce reliance on synthetic fertilizers (D-Andrade et al., 2025; Swarnalakshmi et al., 2020). Despite this, for the production of lettuce, in closed loop hydroponics, nitrate levels are recommended to be within 100-150 mg L^{-1} (Djidonou & Leskovar, 2019). Also altering nitrate concentrations in nutrient solutions and suspending nitrogen fertilization according to Breś et al.(2022); Tabaglio et al.(2020) is another nitrate-targeted mitigation step.

Chloride and sodium levels in SU and SBU met Food Agriculture Organization (FAO) thresholds (< 142 mg L^{-1} and < 69 mg L^{-1}, respectively), minimizing salinity hazard to crops. Micronutrient concentrations (Fe, Zn, Cu, Mn) also fell within recommended limits for agricultural reuse (FAO, 2023), reducing the risk of phytotoxicity, reduced crop growth, and chlorosis (Farshid Shoushtarian & Masoud Negahban-Azar, 2020; Helmecke et al., 2020). However, calcium levels in all treatments exceeded the Australian Guideline for Water Recycling range (10–74 mg L^{-1}), which could interfere with the uptake of other essential nutrients in sensitive crops (Water Quality Australia, 2008). However, it is essential to note that Na levels in freshwater (drain to waste) fell outside this range and, if released into the environment without treatment, contribute to freshwater salinization, leading to increased mobilization and concentration of other pollutants (Kaushal et al., 2023).

2.5.3 Plant Height and Leaf Number

Plant height and leaf number in romaine lettuce increased progressively across all treatments during repeated reuse of recirculating nutrient solutions. Similar observations have been reported in recirculating deep water culture systems, where taller lettuce plants were observed under summer conditions (Sublett et al., 2018). In the present study, nitrate concentrations in treated recirculating nutrient solutions ranged from 44.07 to 99.68 mg/L, with additional supplementation. This elevated nitrate supply likely contributed to increased shoot elongation through enhanced N-driven vegetative growth (Coleby-Williams, 2009). Interestingly, a decline in leaf number was observed in SU-grown romaine lettuce at early growth stages. This may reflect stress-induced inhibition of leaf initiation, possibly linked to salinity stress (Munns & Tester, 2008).

2.5.3 Root and Shoot Biomass

Romaine lettuce cultivated with treated recirculating nutrient solutions (SU and SBU) had on average, higher shoot and root biomass compared to the freshwater (drain to waste or open loop system). This finding contrasts with Méndez-Cifuentes et al.(2020) who reported that closed-loop systems achieved a 9.5% decline in biomass compared to open-loop systems. In romaine lettuce grown with SU-treated nutrient solution, shoot and root weights were initially highest but declined by approximately 29.8% and 15%, respectively, over six cycles. For SBU-treated nutrient solution-grown romaine lettuce, although its shoot weight was comparable to the control, shoot and root biomass still declined by 45.3% and 38.4%, respectively, as the cycles progressed. The trends differ from that of Miller et al.(2020), who reported that reverse osmosis-treated nutrient solution did not significantly affect crop biomass, suggesting that the type of treatment method has a strong influence on yield outcomes. On the other hand, the decreasing shoot and root biomass in the control (recycled nutrient solution without treatment)-grown romaine lettuce as the cycles progressed, was similar to his observations

The analysis of nutrients levels explained these patterns in both treated combinations (SU and SBU), nitrate concentrations remained within a sufficient baseline (44.07–99.68 mg L^{-1}), and additional supplementation likely supported the initially high biomass. However, as cycles progressed, calcium and magnesium concentrations increased, contributing to spikes in EC. Vought et al. (2024) states that EC management in recirculating nutrient solutions causes the underutilization of certain nutrients while the total ionic strength continues to rise, ultimately contributing to yield loss. Lettuce is a salt sensitive crop, therefore, in the control treatment, the accumulation of sodium (Na^+) and chloride (Cl^-) in the recycled, untreated nutrient solution likely induced salinity stress. Elevated Na^+ and Cl^- can disrupt osmotic balance and nutrient uptake, forcing plants to adjust osmotically and thereby negatively affecting growth (Chimi et al., 2024; Sonneveld & Voogt, 2009). It is therefore important that EC levels be kept at its original threshold. Thus, although the mechanisms differed, the common outcome was a progressive decline in biomass. These findings suggest that recycling nutrient solutions in hydroponics, while initially beneficial, may compromise crop yield potential over extended reuse periods if ion accumulation and nutrient balance are not carefully managed.

2.5.4 Vitamin C, Chlorophyll content, and SPAD (Soil and Plant Analyzer Development) value

Chlorophyll content declined significantly from Cycles 3 to 6 across all treatments, indicating that time-dependent stresses associated with repeated reuse were the primary drivers of pigment loss. SBU exhibited extreme reductions in Fe (−97%), Zn (−92%), Cu (−90%), and Mn (−75%), and SU showed moderate but consistent declines in these same trace metals. These micronutrients play essential roles in chlorophyll biosynthesis and photosynthetic electron transport; deficiencies therefore suppress pigment formation and stability (Broadley et al., 2012). This decline is consistent with known UV-C degradation of Fe-DTPA complexes, which reduces Fe bioavailability even after fertilizer supplementation (Albano & Miller, 2001; Arcas et al., 2025), and with biochar's strong sorption affinity for Fe, Zn, Cu, and Mn, Similarly, in the SBU treatment, biochar may have altered the availability of key micronutrients such as magnesium and iron. Despite nutrient supplementation, the adsorption properties of biochar could have limited their uptake, further contributing to reduced chlorophyll levels (Haraz et al., 2020) limiting their availability in recirculating systems.

In the control treatment, increased salinity, osmotic stress, and impaired nutrient uptake likely contributed to the inhibition of chlorophyll biosynthesis in the leaves (B. Adhikari et al., 2023; Breś et al., 2022). These stressors are known to disrupt nitrogen and magnesium assimilation, both of which are essential for chlorophyll formation. In the SU treatment, UV-C–induced destabilization of iron chelates reduced iron availability, a critical cofactor in chlorophyll synthesis (Albano & Miller, 2001; Arcas et al., 2025). This reduction in Fe bioavailability triggered micronutrient stress, which contributed to the observed decline in pigment. Similarly, in the SBU treatment, biochar may have altered the availability of key micronutrients such as magnesium and iron. Despite nutrient supplementation, the adsorption properties of biochar could have limited their uptake, further contributing to reduced chlorophyll levels (Haraz et al., 2020). The increase in calcium concentrations across cycles further amplifies chlorophyll suppression, as high Ca antagonizes Mg and Fe uptake and interferes with pigment formation (Fageria, 2001; Hawkesford et al., 2012). In the Control treatment, the accumulation of sodium and chloride induced osmotic stress and impaired nutrient uptake. Salinity stress is known to inhibit chlorophyll biosynthesis through reduced N and Mg assimilation(B. Adhikari et al., 2023; Breś et al., 2022; Munns & Tester, 2008). (In the Freshwater treatment, chlorophyll decline coincided with very high ammonium (NH_4^+) accumulation (Cycle 3–6), which is known to induce leaf chlorosis, suppress chlorophyll biosynthesis, and generate oxidative stress in leafy greens (Britto & Kronzucker, 2002)

Concurrently, while SPAD value, which stands for Soil and Plant Analyzer Development, is strongly correlated with chlorophyll, it is also influenced by leaf nitrogen status, thickness, and structure (Xiong et al., 2015). In freshwater-grown plants, higher SPAD values were consistent with elevated leaf nitrogen content. In Control and SU, SPAD value declined, consistent with the effects of salinity stress and reduced Fe availability. However, SPAD values increased in SBU across cycles, This pattern likely reflects the fact that SPAD is influenced not only by chlorophyll but also by leaf nitrogen content, thickness, and structure (Parry et al., 2014; Xiong et al., 2015). The increase in SPAD observed in SBU therefore suggests altered leaf morphology or nitrogen distribution rather than true improvement in pigment status and growth (Jagła et al., 2019; Ling et al., 2011)

Vitamin C levels decreased progressively across all treatments with each reuse cycle. Ascorbate is highly sensitive to environmental stress, and its synthesis depends on Fe-, Cu-, Mn-, and Zn-containing enzymes ((Gill & Tuteja, 2010; Smirnoff, 2018). The progressive decline in vitamin C is attributed to the micronutrient depletion which introduces stress. These stressors, as suggested by Akram et al.,(2017) and Senizza et al(2020) increase oxidative damage and impair antioxidant enzyme activity, thereby reducing the plant's capacity to regenerate vitamin C. In addition to oxidative stress, repeated cycles can also increase salinity and disrupt nutrient balances. Salinity stress reduces the efficiency of nitrogen and carbon assimilation, forcing plants to allocate carbon toward osmolyte production instead of the ascorbate biosynthetic pathway, similar to findings by Hasegawa et al., (2025) in tomato tissues. Since vitamin C is water-soluble and easily lost from the body, its decline in romaine lettuce suggests that normal metabolic, immune, and antioxidant functions may be affected, even when dietary intake appears sufficient (Carr & Vissers, 2013; Gallie, 2013; M. J. Kim et al., 2016).

Our findings indicate that recirculating nutrient solutions can support the production of romaine lettuce in hydroponic systems. However, repeated reuse introduces treatment-specific stresses in the crop. Although nutrient solutions were fertilized after each treatment cycle based on EC targets, the EC-based fertilization primarily corrects macronutrients (e.g., NO_3^-, Ca^{2+}, K^+, Mg^{2+}), while micronutrients contribute minimally to EC and therefore remain uncorrected unless specifically dosed. Thus, each reuse cycle followed a pattern of (1) micronutrient removal during treatment, (2) macronutrient restoration during fertilization, and (3) additional micronutrient depletion after subsequent treatment. This explains why chlorophyll, SPAD declined and vitamin C over time despite maintaining acceptable EC and nitrate levels: the long-term imbalance between restored macronutrients and progressively depleted micronutrients.

2.6 Conclusion

This study evaluated the combined effectiveness of slow sand filtration, biochar filtration, and UV-C disinfection for the safe and sustained reuse of hydroponic nutrient solution in deep-water-culture romaine lettuce production. Both SSF + UV-C (SU) and SSF + Biochar + UV-C (SBU) consistently achieved > 5 $\log_{10}$ reductions of *E. coli*, meeting microbial safety standards for agricultural water reuse; however, operational instability may affect system efficiency. Physical parameters, such as turbidity, transmittance, and salinity, were maintained within Food and Agriculture Organization (FAO) /World Health Organization (WHO) guidelines, confirming the suitability of treated recirculating nutrient solution (tRNS) for reuse in hydroponic systems. Chemically, SU and SBU effectively reduced ammonium-N, total N, and total P, with SBU achieving the highest removal. However, nitrate remained relatively stable, calcium continued to accumulate across cycles, and pH in SU and C frequently fell below recommended reuse thresholds. Most importantly, both treatments caused substantial declines in essential micronutrients particularly Fe, Zn, Cu, and Mn despite routine EC-based fertilization. Because

EC correction restores primarily macronutrients, repeated treatment–fertilization cycles generated a progressive micronutrient imbalance. These chemical shifts translated into biological impacts. Although SU initially supported the greatest biomass, both SU and SBU showed significant yield declines over six cycles. Chlorophyll, SPAD, and vitamin C consistently decreased, reflecting a combination of micronutrient depletion, salinity stress, and oxidative damage. Even the freshwater (drain-to-waste) system experienced quality losses due to ammonium accumulation, highlighting that all systems face stressors when reused over multiple cycles.

Overall, this work demonstrates that SSF, biochar, and UV-C constitute an effective, low-cost treatment strategy for microbial control and general water-quality improvement in small-scale hydroponics. However, long-term reuse without targeted micronutrient supplementation leads to hidden deficiencies that compromise pigment stability, antioxidative capacity, and yield. To sustain crop performance over multiple cycles, small-scale growers will require (1) cycle-specific micronutrient additions especially Fe, Zn, Mn, and Cu, (2) monitoring of Ca-driven salinity buildup, and (3) periodic partial solution replacement. Implementing these adjustments can enable growers to capitalize on the water-saving benefits of nutrient-solution reuse while minimizing productivity losses and preserving nutritional quality.

Given the growing importance of hydroponics and the reliance on recirculating nutrient solutions, organizations such as the Food and Agriculture Organization (FAO), US Environmental Protection Agency (EPA) and US Food and Drug Administration (FDA) should consider developing specific standards for the reuse of nutrient solutions. Establishing internationally recognized benchmarks would provide small scale farmers and regulators with clearer guidance for safe and sustainable hydroponic production.

2.7 References

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APPENDICES

Appendix A - Day-to-Day Data Logs

Experiment	Date	Replication	Treatment	Volume of water added	TEMP°C	D.O LEVEL(PPM)	Initial pH	pH adjustment added(ml)	FINAL pH	INITIAL EC (mS/cm)	Stock Solution ADDED	FINAL EC (mS/cm)
1	7/3/2024	1	Control	0	21.1	7.38	8.1	23 acid	5.8	0.68	0	0.68
1		2	Control	0	21.1	7.46	8.1	22 acid	5.8	0.59	0	0.59
1		3	Control	0	21.1	6.74	8	22 acid	5.9	0.6	0	0.6
1		1	SSF + UV	0	21.1	6.34	8.1	23 acid	5.9	0.66	0	0.66
1		2	SSF + UV	0	21.1	7.4	8	23 acid	5.8	0.6	0	0.6
1		3	SSF + UV	0	21.2	8.56	7.9	17 acid	5.8	0.6	0	0.6
1		1	SSF +BIOCHAR + UV	0	21.2	6.01	7.9	17 acid	5.8	0.65	0	0.65
1		2	SSF +BIOCHAR + UV	0	21.2	5.86	8.1	23 acid	5.8	0.6	0	0.6
1		3	SSF +BIOCHAR + UV	0	21.2	6.89	8.1	21 acid	5.8	0.63	0	0.63
1	7/5/2024	1	Control	0	20.8	4.71	7.1	15 acid + 20 base	5.9	0.85	0	0.85
1		2	Control	0	20.9	6.79	6.9	4 acid	5.8	0.61	0	0.61
1		3	Control	0	21.1	6.63	6.9	4 acid	5.9	0.61	0	0.61
1		1	SSF + UV	0	21	6.08	7.1	5 acid	5.9	0.65	0	0.65
1		2	SSF + UV	0	20.9	6.58	7	7 acid	5.8	0.61	0	0.61
1		3	SSF + UV	0	20.8	6.6	6.9	4 acid	5.7	0.61	0	0.61

1		1	SSF +BIOCHAR + UV	0	20.9	5.86	7	4 acid	5.8	0.57	0	0.57
1		2	SSF +BIOCHAR + UV	0	21	6.84	7	6 acid + 1 base	5.8	0.61	0	0.61
1		3	SSF +BIOCHAR + UV	0	20.9	6.03	7	6 acid	5.7	0.63	0	0.63
1	7/8/2024	1	Control	0	20.5	6.94	6.1	4 acid + 16 base	5.9	0.86	45 each	0.99
1		2	Control	0	20.7	5.85	6.4	2.5 acid	5.9	0.61	175 each	1
1		3	Control	0	20.8	5.31	6.8	6 acid + 9.5 base	5.7	0.61	0	0.61
1		1	SSF + UV	0	20.9	6.14	6.9	5 acid + 6.5 base	5.9	0.66	130 each	0.99
1		2	SSF + UV	0	20.7	6.12	6.4	4.5 acid	5.8	0.62	175 each	0.99
1		3	SSF + UV	0	20.6	4.93	6.1	1.5 acid + 7.5 base	5.8	0.62	50 each	0.86
1		1	SSF +BIOCHAR + UV	0	20.8	6.09	6.8	3.5 acid + 2 base	5.7	0.66	155 each	0.99
1		2	SSF +BIOCHAR + UV	0	20.7	6.04	6.6	1 acid + 3.5 base	5.9	0.62	170 each	1
1		3	SSF +BIOCHAR + UV	0	20.7	4.87	6.7	3 acid + 8.5 base	5.8	0.64	100 each	0.89
1	7/12/2024	1	Control	0	20.2	7.14	6.2	9 base	5.8	1	375 each	1.83
1		2	Control	500	20.8	6.48	6.8	0.5 acid + 12 base	5.9	0.98	405 each	1.8
1		3	Control	2000	20.6	4.79	6.7	0.9 acid + 10 base	5.8	0.64	215 each	1.8

1		1	SSF + UV	0	20.8	6.77	6.2	12 base	5.8	0.97	415 each	1.8
1		2	SSF + UV	460	20.5	6.36	6.4	11 base	5.8	1	350 each	1.8
1		3	SSF + UV	0	20.5	4.85	6.4	13 base	5.7	0.79	475 each	1.84
1		1	SSF +BIOCHAR + UV	0	20.7	6.79	6.2	10 base	5.7	0.99	430 each	1.8
1		2	SSF +BIOCHAR + UV	500	20.6	6.73	6.4	13 base	5.8	0.99	425 each	1.8
1		3	SSF +BIOCHAR + UV	1350	20.5	4.86	6.6	0.5 acid + 17 base	5.8	0.82	480 each	1.8
1	7/15/2024	1	Control	1030	20.2	5.98	6.3	12.5 acid + 22 base	5.7	1.87	0	1.87
1		2	Control	950	20.6	6.19	6.3	2 acid	5.8	1.78	16 each	1.81
1		3	Control	860	20.7	6.57	6.5	3 acid + 3 base	5.8	1.13	0	1.13
1		1	SSF + UV	970	20.5	5.19	6.3	2 acid	5.9	1.78	16 each	1.8
1		2	SSF + UV	2500	20.5	6.23	6.3	2 acid	5.7	1.72	55 each	1.84
1		3	SSF + UV	0	20.5	6.67	6.2	2 acid	5.8	1.79	10 each	1.81
1		1	SSF +BIOCHAR + UV	0	20.5	6.49	6.2	2 acid	5.9	1.78	16 each	1.8
1		2	SSF +BIOCHAR + UV	1000	20.6	6.83	6.4	2 acid	5.8	1.82	0	1.82
1		3	SSF +BIOCHAR + UV	400	20.6	5.93	6.4	3 acid	5.8	1.8	0	1.8
1	7/19/2024	1	Control	540	20.3	7.43	5.2	2 base	5.9	1.87	0	1.87
1		2	Control	900	20.4	6.26	5.3	2 base	5.7	1.8	0	1.8

1		3	Control	290	20.7	6.34	5.6	1 base	5.9	1.13	350 each	1.81
1		1	SSF + UV	950	20.6	5.89	5.3	2.5 base	5.7	1.79	0	1.79
1		2	SSF + UV	0	20.5	5.5	5.4	2 base	5.7	1.79	0	1.79
1		3	SSF + UV	500	20.5	6.55	5.4	2 base	5.8	1.82	0	1.82
1		1	SSF +BIOCHAR + UV	0	20.5	5.86	5.5	3 base	5.7	1.81	0	1.81
1		2	SSF +BIOCHAR + UV	1000	20.5	5	5.5	0.5 base	5.7	1.8	0	1.8
1		3	SSF +BIOCHAR + UV	170	20.6	6.14	5.7	0	5.7	1.79		1.79
1	7/22/2024	1	Control	1000	20.2	7.28	5.4	1 base	5.8	1.86	0	1.86
1		2	Control	560	20.5	6.31	5.4	4 base	5.8	1.76	10 of each	1.8
1		3	Control	710	20.9	7.1	5.1	5 base	5.8	1.78	10 of each	1.8
1		1	SSF + UV	980	19.6	7.56	5.3	3 base	5.8	1.77	10 of each	1.8
1		2	SSF + UV	0	20.3	6.72	5.4	2 base	5.8	1.79	0	1.79
1		3	SSF + UV	290	20.4	6.54	5.6	2 base	5.8	1.79	0	1.79
1		1	SSF +BIOCHAR + UV	790	20.3	6.67	5.5	0.5 base	5.8	1.81	0	1.81
1		2	SSF +BIOCHAR + UV	1000	20.6	6.01	5.5	2 base	5.8	1.78	10 of each	1.8
1		3	SSF +BIOCHAR + UV	1000	20.5	6.15	5.6	2 base	5.9	1.79	0	1.79
1	7/26/2024	1	Control	1690	20.5	6.53	4.9	4 base + 0.5 acid	5.8	1.81	0	1.81

1		2	Control	2950	20.5	5.29	5	4 base	5.9	1.76	30 of each	1.84
1		3	Control	200	21	6.39	5.3	2.5 base	5.8	1.79	0	1.79
1		1	SSF + UV	2900	20.6	5.84	5.3	4 base	5.8	1.75	50 of each	1.82
1		2	SSF + UV	4800	20.5	7.23	5	4 base	5.8	1.76	30 of each	1.81
1		3	SSF + UV	2800	20.7	6.54	5.3	3 base	5.8	1.77	40 of each	1.83
1		1	SSF +BIOCHAR + UV	1670	20.6	5.97	5.1	4.5 base	5.7	1.58	40 of each	1.85
1		2	SSF +BIOCHAR + UV	2000	20.6	5.36	5	4.5 base	5.8	1.75	40 of each	1.82
1		3	SSF +BIOCHAR + UV	1000	20.6	6.57	5.4	2 base	5.7	1.77	20 of each	1.8
1	7/29/2024	1	Control	1800	19.9	6.84	5.2	4 base	5.9	1.75	50 of each	1.85
1		2	Control	0	20.4	5.27	5.1	4 base	5.7	1.74	50 of each	1.82
1		3	Control	960	20.9	6.05	5.2	4 base	5.8	1.74	45 of each	1.84
1		1	SSF + UV	2300	20.4	5.48	6.2	1.5 acid	5.8	1.72	50 of each	1.81
1		2	SSF + UV	0	20.4	5.74	5.5	2 base	5.9	1.69	50 of each	1.79
1		3	SSF + UV	1000	20.7	6.09	5.3	4 base	5.7	1.76	45 of each	1.84
1		1	SSF +BIOCHAR + UV	1540	20.9	5.67	5.1	4.5 base	5.7	1.77	30 of each	1.83
1		2	SSF +BIOCHAR + UV	1550	20.5	5.29	5.3	3 base	5.9	1.77	50 of each	1.83
1		3	SSF +BIOCHAR + UV	1370	20.8	6.18	5.2	4 base	5.9	1.77	50 of each	1.85

1	8/2/2024	1	Control	2670	20.2	5.88	5.9	0	5.9	1.8	0	1.8
1		2	Control	2000	20.3	5.45	6.2	2 acid	5.8	1.74	45 of each	1.84
1		3	Control	3000	20.4	5.93	5	5 base	5.8	1.7	30 of each	1.8
1		1	SSF + UV	3000	20.3	5.81	6.3	2 acid	5.8	1.71	70 of each	1.8
1		2	SSF + UV	3000	20.2	6.53	6.1	1 acid	5.9	1.73	40 of each	1.81
1		3	SSF + UV	2000	20.3	6.13	5.8	0	5.9	1.76	20 of each	1.81
1		1	SSF +BIOCHAR + UV	1800	20.2	5.9	5.8	0	5.8	1.74	40 of each	1.83
1		2	SSF +BIOCHAR + UV	2000	20.4	6.12	6.2	2 acid + 2 base	5.8	1.73	45 of each	1.83
1		3	SSF +BIOCHAR + UV	2400	20.3	6.38	5.2	4 base	5.8	1.79	0	1.79
1	8/5/2024	1	Control	1590	20.3	6.2	5.8	0	5.8	1.58	100 of each	1.86
1		2	Control	1990	20.4	6.32	6	0.5 acid	5.8	1.75	20 of each	1.8
1		3	Control	1000	20.5	5.72	5.9	0	5.9	1.69	50 of each	1.8
1		1	SSF + UV	2000	20.4	5.6	5.9	0	5.9	1.75	20 of each	1.79
1		2	SSF + UV	3000	20.3	6.42	5.8	0	5.8	1.62	70 of each	1.85
1		3	SSF + UV	1900	20.3	6.48	5.9	0	5.9	1.71	50 of each	1.81
1		1	SSF +BIOCHAR + UV	1990	20.2	5.14	5.9	0	5.9	1.72	50 of each	1.81
1		2	SSF +BIOCHAR + UV	3000	20.3	6.45	6	1 acid	5.8	1.75	20 of each	1.8

1		3	SSF +BIOCHAR + UV	1900	20.5	5.96	5.9	0	5.9	1.72	50 of each	1.82
2	8/30/2024	1	Control	2500	20.2	6.22	6.7	2.8 acid	5.8	1.87	0	1.87
2		2	Control	4000	20.4	6.31	6.7	3.8 acid	5.8	1.78	15 eacg	1.8
2		3	Control	4400	20.5	6.18	6.6	4.2 acid	5.9	1.79	15 each	1.83
2		1	SSF + UV	4900	20.6	6.15	6.9	5.5 acid	5.9	1.63	110 each	1.85
2		2	SSF + UV	9000	20.8	7.28	6.7	10 acid + 6 base	5.7	1.63	110 each	1.83
2		3	SSF + UV	5000	21	6.78	6.9	4.4 acid	5.9	1.53	75 each	1.8
2		1	SSF +BIOCHAR + UV	6300	20.4	7.6	7.9	14 acid	5.9	1.43	200 each	1.82
2		2	SSF +BIOCHAR + UV	7000	21.2	6.48	7.9	14.5 acid	5.8	1.43	220 eaxh	1.86
2		3	SSF +BIOCHAR + UV	9900	21.1	7	7.5	13.5 acid	5.8	1.51	150 each	1.8
2		1	FRESHWATER	22.5 galloons								
2		2	FRESHWATER	22.5 galloons								
2		3	FRESHWATER	22.5 galloons								
2	9/3/2024	1	Control	260	20.7	6.94	6.1	0.5 acid	5.9	1.9	0	1.9
2		2	Control	750	20.7	6.93	6.1	1 acid	5.9	1.82	0	1.82
2		3	Control	250	21	6.96	6.1	1 acid	5.9	1.81	0	1.81
2		1	SSF + UV	530	20.9	6.37	6.3	1.5 acid	5.9	1.85	0	1.85

2		2	SSF + UV	520	20.8	6.81	6.1	1 acid	5.8	1.87	0	1.87
2		3	SSF + UV	750	20.7	6.47	6.1	2 acid + 0.5 base	5.7	1.83	0	1.83
2		1	SSF +BIOCHAR + UV	740	20.9	6.55	6.6	3.5 acid	5.7	1.85	0	1.85
2		2	SSF +BIOCHAR + UV	480	20.9	6.38	6.2	1.5 acid	5.8	1.89	0	1.89
2		3	SSF +BIOCHAR + UV	220	20.9	6.16	6.2	1 acid	5.9	1.8	0	1.8
2		1	FRESHWATER									
2		2	FRESHWATER									
2		3	FRESHWATER									
2	9/6/2024	1	Control	0	21.5	6.33	6	0.5 acid	5.8	1.88	0	1.88
2		2	Control	20	21.5	6.47	6	0.5 acid	5.7	1.82	0	1.82
2		3	Control	1000	21.2	6.39	5.8	0	5.8	1.81	0	1.81
2		1	SSF + UV	0	20.8	6.18	6	1 acid	5.7	1.86	0	1.86
2		2	SSF + UV	10	21.5	6.56	5.9	0	5.9	1.87	0	1.87
2		3	SSF + UV	800	20.9	6.21	5.8	0	5.8	1.8	0	1.8
2		1	SSF +BIOCHAR + UV	0	21.5	6.71	6	0.5 acid	5.7	1.85	0	1.85
2		2	SSF +BIOCHAR + UV	2000	21.2	6.61	6	0.5 acid	5.8	1.89	0	1.89
2		3	SSF +BIOCHAR + UV	0	21.3	6.93	6.1		5.8	1.8	0	1.8
2		1	FRESHWATER									

2		2	FRESHWATER									
2		3	FRESHWATER									
2	9/9/2024	1	Control	0	20.6	6.68	5.9	0	5.9	1.87	0	1.87
2		2	Control	880	20.5	7.01	6	1 acid	6	1.82	0	1.82
2		3	Control	0	20.5	6.37	5.9	0	5.9	1.79	0	1.79
2		1	SSF + UV	1000	20.3	7.32	5.9	0	5.9	1.87	0	1.87
2		2	SSF + UV	0	20.8	6.29	6	1 acid	5.9	1.87	0	1.87
2		3	SSF + UV	0	20.5	6.74	5.9	0	5.9	1.79	0	1.79
2		1	SSF +BIOCHAR + UV	790	20.8	7.12	5.9	0	5.9	1.85	0	1.85
2		2	SSF +BIOCHAR + UV	1000	20.6	5.9	5.9	0	5.9	1.85	0	1.85
2		3	SSF +BIOCHAR + UV	0	20.8	6.71	6	1 acid	5.8	1.82	0	1.82
2		1	FRESHWATER	`1000	20.8	6.77	6.4	3 acid	5.8	1.92	0	1.92
2		2	FRESHWATER	`1000	20.8	6.27	6.6	5 acid	5.7	1.87	0	1.87
2		3	FRESHWATER	0	20.8	6.28	6.3	3 acid+ 1 base	5.6	1.84	0	1.84
2	9/13/2024	1	Control	1000	21.2	6.87	6.1	1 acid	5.7	1.81	0	1.81
2		2	Control	900	21.4	6.48	5.9	0	5.9	1.79	0	1.79
2		3	Control		21.3	5.81	6	1.5 acid + 1 base	5.7	1.78	10 of each	1.8
2		1	SSF + UV	1200	21.4	6.76	5.9	0	5.9	1.84	0	1.84
2		2	SSF + UV	1250	21.3	6.16	5.8	0	5.8	1.86	0	1.86
2		3	SSF + UV	700	21.2	6.59	5.9	0	5.9	1.81	0	1.81

2		1	SSF +BIOCHAR + UV	900	24.1	6.4	6	1 acid	5.9	1.83	0	1.83
2		2	SSF +BIOCHAR + UV	1300	21.3	5.18	5.8	0	5.8	1.8	0	1.8
2		3	SSF +BIOCHAR + UV	1200	21.3	6.54	5.9	0	5.9	1.77	15 each	1.8
2		1	FRESHWATER	0	21.5	7.15	5.8	0	5.8	1.9	0	1.9
2		2	FRESHWATER	900	21.5	6.31	5.9	0	5.9	1.84	0	1.84
2		3	FRESHWATER	0	21.1	6.31	5.9	0	5.9	1.86	0	1.86
2	9/16/2024	1	Control	1000	21.1	6.39	5.9	0	5.9	1.86	0	1.86
2		2	Control	1500	21.2	6.67	6	1.5 acid	5.7	1.75	20 of each	1.81
2		3	Control	1500	21.1	6.3	5.9	0	5.9	1.77	15 of each	1.8
2		1	SSF + UV	1500	21.2	6.89	5.9	0	5.9	1.79	0	1.79
2		2	SSF + UV	2500	20.1	6.02	5.9	0	5.9	1.84	0	1.84
2		3	SSF + UV	1000	20.9	6.72	5.9	0	5.9	1.76	20 each	1.81
2		1	SSF +BIOCHAR + UV	1000	20.8	6.65	5.9	0	5.9	1.85	0	1.85
2		2	SSF +BIOCHAR + UV	2000	21.1	5.35	5.7	0	5.7	1.75	20 of each	1.81
2		3	SSF +BIOCHAR + UV	1000	21.1	7.35	6	1.5 acid	5.9	1.78	10 each	1.8
2		1	FRESHWATER	2000	21.4	6.89	5.8	0	5.8	1.91	0	1.91
2		2	FRESHWATER	1000	20.3	6.48	5.9	0	5.9	1.79	0	1.79

2		3	FRESHWATER	1000	21	6.22	5.9	0	5.9	1.71	30 each	1.83
2	9/20/2024	1	Control	1400	21.3	6.53	6	1.5 acid +3 base	5.7	1.84	0	1.84
2		2	Control	3900	21.4	6.93	5.8	0	5.8	1.74	25 of each	1.79
2		3	Control	2000	21.2	5.76	5.7	0	5.7	1.76	15 of each	1.79
2		1	SSF + UV	2000	21.3	6.93	5.8	0	5.8	1.73	30 of each	1.79
2		2	SSF + UV	3000	21.3	6.67	5.8	0	5.8	1.76	25 of each	1.82
2		3	SSF + UV	2000	21	6.54	5.9	0	5.9	1.77	15 of each	1.8
2		1	SSF +BIOCHAR + UV	2000	21.3	5.76	5.7	0	5.7	1.78	15 of each	1.81
2		2	SSF +BIOCHAR + UV	1200	21.3	6.23	5.7	0	5.7	1.74	30 of each	1.8
2		3	SSF +BIOCHAR + UV	1700	21.3	6.51	5.8	0	5.8	1.76	25 of each	1.82
2		1	FRESHWATER	500	21.4	5.99	5.8	0	5.8	1.85	0	1.85
2		2	FRESHWATER	900	21.4	6.2	5.4	2.5 base	5.9	1.82	0	1.82
2		3	FRESHWATER	2000	21.1	6.85	5.9	0	5.9	1.89	0	1.89
2	9/23/2024	1	Control	1000	20.9	6.13	5.9	0	5.9	1.8	0	
2		2	Control	1000	20.9	5.19	6.3	1 base	5.7	1.69	25 of each	1.82
2		3	Control	270	20.9	6.32	5.9	0	5.9	1.74	40 of each	1.81
2		1	SSF + UV	1000	20.9	7.05	6.2	3 acid + 2 base	5.7	1.71	55 of each	1.82
2		2	SSF + UV	730	21	5.74	5.7	0	5.7	1.75	25 of each	1.8

2		3	SSF + UV	1000	20.7	6.77	6	1 base + 2 acid	5.7	1.74	30 of each	1.81
2		1	SSF +BIOCHAR + UV	1000	20.1	7.13	5.6	2 base	5.7	1.75	20 of each	1.79
2		2	SSF +BIOCHAR + UV	1000	21	5.93	5.8	0	5.8	1.72	40 of each	1.81
2		3	SSF +BIOCHAR + UV	1900	20.9	6.48	5.9	0	5.9	1.76	25 of each	1.81
2		1	FRESHWATER	1850	21.2	6.22	5.3	1.5 acid+6.5base	5.9	1.83	0	1.83
2		2	FRESHWATER	1000	21	6	5.6	2 base	5.7	1.78	15 of each	1.8
2		3	FRESHWATER	2000	20.8	6.78	5.8	0	5.8	1.84	0	1.84
2	9/25/2024	1	Control	540	21.1	6.07	5.8	0	5.8	1.78	20 of each	1.8
2		2	Control	2000	21.3	6.28	6.5	3 acid+1 base	5.8	1.67	80 of each	1.8
2		3	Control	2000	21.1	6.16	5.8	0	5.8	1.82	0	
2		1	SSF + UV	1000	21.2	6.96	5.7	0	5.7	1.69	80 of each	1.8
2		2	SSF + UV	1000	21.2	6.86	5.7	0	5.7	1.78	20 of each	1.81
2		3	SSF + UV	1000	21	6.55	5.8	0	5.8	1.78	20 of each	1.82
2		1	SSF +BIOCHAR + UV	840	21.2	7.13	5.6	2 base	5.8	1.76	30 of each	1.8
2		2	SSF +BIOCHAR + UV	1000	21.2	5.41	5.6	2 base	5.9	1.78	20 of each	1.8
2		3	SSF +BIOCHAR + UV	920	21.1	5.93	5.7	0	5.7	1.77	20 of each	1.8

2		1	FRESHWATER	1000	21.3	6.58	5.5	3 base	5.8	1.77	20 of each	1.8
2		2	FRESHWATER	1000	21.2	6.38	5.6	1 base	5.8	1.81	0	1.81
2		3	FRESHWATER	1000	21.2	6.07	5.7	0	5.7	1.81	0	1.81
2												
2	9/27/2024	1	Control	900	21.1	6.27	5.8	0	5.8	1.79	10 of each	1.8
2		2	Control	1000	21.5	6.94	5.9	0	5.9	1.75	20 of each	1.8
2		3	Control	1000	21.1	6.43	5.8	0	5.8	1.77	20 of each	
2		1	SSF + UV	1500	7.06	21.1	5.6	2 base	5.8	1.82	0	1.82
2		2	SSF + UV	1000	21.1	6.72	5.7	0	5.7	1.78	10 of each	1.8
2		3	SSF + UV	400	20.9	6.88	5.8	0	5.8	1.78	10 of each	1.8
2		1	SSF +BIOCHAR + UV	1000	21.2	7.35	5.7	0	5.7	1.79	5 of each	1.8
2		2	SSF +BIOCHAR + UV	1000	21.2	5.88	5.8	0	5.8	1.78	10 of each	1.8
2		3	SSF +BIOCHAR + UV	250	6.57	21.1	5.6	2 base	5.8	1.78	10 of each	1.81
2		1	FRESHWATER	1000	21.3	6.68	5.4	2 base	5.7	1.78	20 of each	1.8
2		2	FRESHWATER	0	21.2	6.38	5.6	2 base	5.8	1.77	25 of each	1.81
2		3	FRESHWATER	1000	21	6.29	5.6	2 base	5.8	1.79	10 of each	1.82
3	10/12/2024	1	Control	2000	20.2	6.12	5.1	3 base	5.8	1.85	0	1.85
3		2	Control	5000	20.1	6.91	6.1	1 acid	5.9	1.85	0	1.85
3		3	Control	3000	20.4	6.12	6	1 acid	5.7	1.84	0	1.84
3		1	SSF + UV	4000	20	7.03	6.2	3 acid	5.7	1.88	0	1.88

3		2	SSF + UV	5000	19.2	6.61	6.2	3 acid	5.7	1.83	0	1.83
3		3	SSF + UV	7000	19.9	6.56	6.3	2 acid	5.8	1.86	0	1.86
3		1	SSF +BIOCHAR + UV	5000	19.3	7.34	7.8	16 acid	5.8	1.58	110 of each	1.81
3		2	SSF +BIOCHAR + UV	5000	20.4	5.45	7.1	10 acid	5.8	1.84	0	1.84
3		3	SSF +BIOCHAR + UV	3500	19.3	6.12	7.2	11 acid	5.8	1.81	0	1.81
3	10/14/2024	1	Control	1000	20.8	6.27	5.5	1.5 base	5.7	1.82	0	1.82
3		2	Control	0	20.9	6.05	5.9	0	5.9	1.75	30 of each	1.81
3		3	Control	0	20.8	6.45	5.9	0	5.9	1.79	5 of each	1.8
3		1	SSF + UV	0	20.2	7.14	5.8	0	5.8	1.82	0	1.82
3		2	SSF + UV	0	20	6.61	6	0.5 acid	5.8	1.75	25 of each	1.8
3		3	SSF + UV	0	20.2	6.05	5.8	0	5.8	1.73	25 of each	1.8
3		1	SSF +BIOCHAR + UV	0	19.9	7.06	6.4	1.5 acid	5.8	1.71	35 of each	1.8
3		2	SSF +BIOCHAR + UV	2000	20	6.63	5.9	0	5.9	1.75	25 of each	1.8
3		3	SSF +BIOCHAR + UV	0	20.2	6.5	6	0.5 acid	5.9	1.77	20 of each	1.8
3		1	FRESHWATER	22.5 gallons	23.4	6.16	9.4	13 acid	5.8	0.66	600 of each	1.84
3		2	FRESHWATER	22.5 gallons	23.5	6.48	9.3	14.5 acid	5.9	0.68	600 of each	1.82

3		3	FRESHWATER	22.5 gallons	23.2	6.34	9.4	14.5 acid	5.9	0.65	650 of each	1.84
3	10/18/2024	1	Control	600	21.3	5.89	5.3	3 base	5.8	1.81	0	1.81
3		2	Control	1850	21.3	6.87	5.8	0	5.8	1.82	0	1.82
3		3	Control	400	21.4	6.42	5.8	0	5.8	1.84	0	1.84
3		1	SSF + UV	1000	21	6.36	5.8	0	5.8	1.83	0	1.83
3		2	SSF + UV	1000	21	6.06	5.8	0	5.8	1.83	0	1.83
3		3	SSF + UV	1400	21.4	6.42	5.8	0	5.8	1.79	5 of each	1.8
3		1	SSF +BIOCHAR + UV	990	20.4	6.58	6	0.5 acid	5.8	1.81	0	1.81
3		2	SSF +BIOCHAR + UV	600	21.6	7.01	5.8	0	5.8	1.77	20 of each	1.8
3		3	SSF +BIOCHAR + UV	400	21.4	6.42	5.8	0	5.8	1.84	0	1.84
3		1	FRESHWATER	0	21.5	6.16	6.5	6 acid	5.9	1.86	0	1.86
3		2	FRESHWATER	0	21.5	6.41	6.4	5 acid + 1.5 base	5.8	1.83	0	1.83
3		3	FRESHWATER	0	21.3	6.37	6.5	5.5 acid	5.9	1.78	20 of each	1.86
3	10/21/2024	1	Control	0	20.7	6.05	5.4	3 base	5.8	1.79	5 of each	1.8
3		2	Control	0	20.7	6.5	5.8	0	5.8	1.78	20 of each	1.83
3		3	Control	0	20.5	6.71	5.8	0	5.8	1.81	0	1.81
3		1	SSF + UV	0	20.7	6.28	5.8	0	5.8	1.81	0	1.81
3		2	SSF + UV	0	20.6	6.44	5.8	0	5.8	1.79	5 of each	1.8
3		3	SSF + UV	0	20.5	6.33	5.8	0	5.8	1.78	20 of each	1.83

3		1	SSF +BIOCHAR + UV	0	20.1	6.81	5.9	0.5 acid	5.8	1.8	0	1.8
3		2	SSF +BIOCHAR + UV	1000	20.9	6.41	5.8	0	5.8	1.77	25 of each	1.8
3		3	SSF +BIOCHAR + UV	0	20.1	6.91	5.8	0	5.8	1.76	25 of each	1.82
3		1	FRESHWATER	0	21	6.2	6.1	1 acid	5.8	1.87	0	1.87
3		2	FRESHWATER	0	20.7	6.37	6	0.5 acid	5.9	1.84	0	1.84
3		3	FRESHWATER	0	20.4	6.04	6	0.5 acid	5.9	1.91	0	1.91
3	10/25/2024	1	Control	1000	20.2	6.21	5.5	1base	5.8	1.81	0	1.81
3		2	Control	2000	21	6.32	5.7	0	5.7	1.81	0	1.81
3		3	Control	2000	20.9	6.11	5.8	0	5.8	1.8	0	1.8
3		1	SSF + UV	1000	21	5.96	5.8	0	5.8	1.8	0	1.8
3		2	SSF + UV	3000	20.9	6.01	5.8	0	5.8	1.79	5 base	1.8
3		3	SSF + UV	1000	20.8	6.57	5.8	0	5.8	1.82	0	1.82
3		1	SSF +BIOCHAR + UV	2000	20.2	6.21	5.8	0	5.8	1.81	0	1.81
3		2	SSF +BIOCHAR + UV	1000	21	6.41	6.1	1 acid	5.9	1.82	0	1.82
3		3	SSF +BIOCHAR + UV	2000	20.3	6.27	5.8	0	5.8	1.81	0	1.81
3		1	FRESHWATER	0	21.1	6.32	6	1 acid	5.9	1.88	0	1.88
3		2	FRESHWATER	0	21	6.14	6.3	3.5 acid	5.9	1.84	0	1.84
3		3	FRESHWATER	0	20.9	6.06	6.2	3 acid	5.8	1.89	0	1.89

3	10/28/2024	1	Control	1000	21	6.21	5.6	1 base	5.7	1.78	15 of each	1.81
3		2	Control	1000	21	6.21	5.9	0	5.9	1.77	25 of each	1.8
3		3	Control	1000	20.9	6.24	5.9	0	5.9	1.76	30 of each	1.81
3		1	SSF + UV	1000	20.9	6.16	5.8	0	5.8	1.78	20 of each	1.8
3		2	SSF + UV	0	20.9	6.01	5.8	0	5.8	1.74	30 of each	1.8
3		3	SSF + UV	1000	20.9	5.99	5.8	0	5.8	1.8	0	1.8
3		1	SSF +BIOCHAR + UV	1000	20.8	6.08	5.7	0	5.7	1.75	25 of each	1.8
3		2	SSF +BIOCHAR + UV	1000	20.9	6.73	6.3	2 acid	5.8	1.78	20 of each	1.83
3		3	SSF +BIOCHAR + UV	0	20.9	6.93	5.8	0	5.8	1.8	0	1.8
3		1	FRESHWATER	0	21.5	5.97	6.2	2.5 acid	5.9	1.86	0	1.86
3		2	FRESHWATER	0	21	6.06	5.9	0	5.9	1.84	0	1.84
3		3	FRESHWATER	0	20.9	6.11	6	0.5 acid	5.9	1.87	0	1.87
3	10/31/2024	1	Control	0	20.8	5.99	5.9	0	5.9	1.82	0	1.82
3		2	Control	2000	20.7	6.06	6	1 acid	5.9	1.78	20 of each	1.8
3		3	Control	1000	20.7	6.21	5.9	0	5.9	1.81	0	1.81
3		1	SSF + UV	1000	20.9	6.16	6	0.5 acid	5.8	1.77	20 of each	1.8
3		2	SSF + UV	2000	20.7	5.91	5.7	0	5.7	1.79	5 of each	1.8
3		3	SSF + UV	1000	20.9	5.99	5.8	0	5.8	1.77	20 of each	1.8
3		1	SSF +BIOCHAR + UV	1000	20.2	6.14	5.6	1.5 base	5.7	1.77	15 of each	1.8

3		2	SSF +BIOCHAR + UV	0	20.8	6.43	6.4	1.5 acid	5.9	1.78	15 of each	1.83
3		3	SSF +BIOCHAR + UV	2000	20.3	6.21	5.7	0	5.7	1.79	5 of each	1.8
3		1	FRESHWATER	0	21.2	7.02	5.9	0	5.9	1.87	0	1.87
3		2	FRESHWATER	0	20.9	6.61	6	0.5 acid	5.9	1.84	0	1.84
3		3	FRESHWATER	1000	20.6	6.13	6	0.5 acid	5.9	1.94	0	1.94
4	11/4/2024	1	Control	1000	20.8	6.13	6.2	1 acid	5.7	1.78	10 of each	1.8
3		2	Control	2000	21	6.11	6.3	2 acid	5.9	1.74	30 of each	1.8
3		3	Control	3000	20.8	5.57	6.1	1 acid	5.9	1.8	0	1.8
3		1	SSF + UV	2000	20.9	6.05	6.3	2 acid	5.8	1.75	30 of each	1.8
3		2	SSF + UV	2000	20.7	5.97	6	4 base + 1.5 acid	5.9	1.76	25 of each	1.8
3		3	SSF + UV	1000	20.6	5.98	5.8	0	5.8	1.78	10 of each	1.8
3		1	SSF +BIOCHAR + UV	980	20.1	7.01	5.5	2.5 base	5.8	1.79	5 of each	1.8
3		2	SSF +BIOCHAR + UV	2000	21	6.14	6.4	2 acid	5.8	1.77	15 of each	1.8
3		3	SSF +BIOCHAR + UV	1000	20.3	6.37	5.6	2 base	5.7	1.75	30 of each	1.8
3		1	FRESHWATER	0	21.1	7.12	5.6	1 base	5.7	1.86	0	1.86
3		2	FRESHWATER	0	209.9	6.61	5.8	0	5.8	1.85	0	1.85
3		3	FRESHWATER	0	20.7	6.13	5.8	0	5.8	1.89	0	1.89
4	13/11/24	1	Control	4000	21		6.4	2.5 acid	5.9	1.8	0	1.8

4		2	Control	4000	20.3		6.3	2 acid	5.9	1.78	10 of each	1.83
4		3	Control	4000	21.1		6.2	1.5 acid	5.9	1.76	20 of each	1.8
4		1	SSF + UV	4000	20		6.3	1.5 acid	5.8	1.77	30 of each	1.84
4		2	SSF + UV	5000	20		6.2	1.5 acid	5.8	1.61	95 of each	1.8
4		3	SSF + UV	5000	20		5.9	0	5.9	1.81		
4		1	SSF +BIOCHAR + UV	4100	20.3		6.3	1.5 acid	5.8	1.76	25 of each	1.82
4		2	SSF +BIOCHAR + UV	7000	20		6.4	1.5 acid	5.9	1.75	25 of each	1.8
4		3	SSF +BIOCHAR + UV	5000	20		6	1 acid	5.8	1.75	25 of each	1.8
4		1	FRESHWATER	22.5 gallons	23.1		8.6	25.4 acid	5.8	0.3	850 of each	1.82
4		2	FRESHWATER	22.5 gallons	23		6.9	42 base	5.8	0.2	850 of each	1.88
4		3	FRESHWATER	22.5 gallons	21.6		7	45 base	5.7	0.2	850 of each	1.9
4												
4	11/15/2024	1	Control	0	22.6		5.7	0	5.7	1.73	30 of each	1.82
4		2	Control	0	22		5.9	0	5.9	1.75	30 of each	1.8
4		3	Control	0	22.1		6.1	1 acid	5.9	1.74	20 of each	1.8
4		1	SSF + UV	0	22.1		5.9	0	5.9	1.76	20 of each	1.81
4		2	SSF + UV	0	21.7		5.9	0	5.9	1.72	30 of each	1.8
4		3	SSF + UV	0	22.1		5.8	0	5.8	1.72	30 of each	1.81

4		1	SSF +BIOCHAR + UV	0	22		6	0.5 acid	5.8	1.73	30 of each	1.81
4		2	SSF +BIOCHAR + UV	0	22.1		5.8	0	5.8	1.69	50 of each	1.8
4		3	SSF +BIOCHAR + UV	0	22.1		5.9	0	5.9	1.66	60 of each	1.85
4		1	FRESHWATER	0	22.4		6.1	1 acid	5.9	1.82	0	1.82
4		2	FRESHWATER	0	22		6.4	2.5 acid	5.9	1.84	0	1.84
4		3	FRESHWATER	0	21.8		6.1	2 acid	5.9	1.9	0	1.9
4				0								
4	11/19/2024	1	Control	0	21		5.9	0	5.9			
4		2	Control	2000	21.4		6.2	1 acid	5.9			
4		3	Control	0	21.2		5.9	0	5.9			
4		1	SSF + UV	1000	21.5		6.2	1 acid	5.8			
4		2	SSF + UV	1000	21.3		5.9	0	5.9			
4		3	SSF + UV	1000	21.2		5.5	3 base	5.7			
4		1	SSF +BIOCHAR + UV	2000	21.3		5.9	0	5.9			
4		2	SSF +BIOCHAR + UV	0	21.4		6	0.5	5.9			
4		3	SSF +BIOCHAR + UV	1000	21.3		5.8	0	5.8			
4		1	FRESHWATER	0	21.6		6	1 acid	5.8			
4		2	FRESHWATER	0	21.4		6	1.5 acid	5.9			
4		3	FRESHWATER	0	21		6.2	2 acid	5.9			

4												
4	11/22/ 2024	1	Control	1000	22		5.9	0	5.9	1.82	0	1.82
4		2	Control	1000	22		5.9	0	5.9	1.81	0	1.81
4		3	Control	0	22.1		5.9	0	5.9	1.8	0	1.8
4		1	SSF + UV	1000	22.2		5.9	0	5.9	1.8	0	1.8
4		2	SSF + UV	1000	22		6	0.5	5.9	1.8	0	1.8
4		3	SSF + UV	1000	21.9		5.4	3.5 base	5.7	1.79	10 of each	1.81
4		1	SSF +BIOCH AR + UV	1000	22.1		5.9	0	5.9	1.78	20 of each	1.81
4		2	SSF +BIOCH AR + UV	1000	22.2		5.8	0	5.8	1.8	0	1.8
4		3	SSF +BIOCH AR + UV	2000	22.2		5.2	2 base	5.7	1.86	0	1.86
4		1	FRESHW ATER	0	22.4		6.2	1 acid	5.7	1.59	50 of each	1.8
4		2	FRESHW ATER	0	22.1		5.9	0	5.9	1.86	0	1.86
4		3	FRESHW ATER	0	21.9		6	1 acid	5.9	1.91	0	1.91
4												
4	11/25/ 2024	1	Control	1000	21.4		5.9	0	5.9	1.81	0	1.81
4		2	Control	2000	21.7		5.9	0	5.9	1.79	5 of each	1.8
4		3	Control	1000	21.6		5.9	0	5.9	1.8	0	1.8
4		1	SSF + UV	1000	21.7		5.9	0	5.9	1.79	10 of each	1.8
4		2	SSF + UV	1000	21.5		5.9	0	5.9	1.8	0	1.8
4		3	SSF + UV	1000	21.3		5.6	2 base	5.7	1.79	5 of each	1.8

4		1	SSF +BIOCHAR + UV	1000	21.7		5.9	9	5.9	1.81	0	1.81
4		2	SSF +BIOCHAR + UV	1000	21.7		6.1	1.5 acid	5.9	1.8	0	1.8
4		3	SSF +BIOCHAR + UV	2000	21.6		5.3	4 base	5.7	1.83	0	1.83
4		1	FRESHWATER	0	21.8		6	1 acid	5.8	1.92	0	1.92
4		2	FRESHWATER	0	21.7		5.9	0	5.9	1.87	0	1.87
4		3	FRESHWATER	2000	21.3		6	0.5 acid	5.9	1.9	0	1.9
4												
4	11/29/2024	1	Control	0	21.8		6.2	1 acid	5.7	1.79	0	1.79
4		2	Control	2000	22		5.7	0	5.7	1.72	30 of each	1.83
4		3	Control	1000	22		6	1 acid	5.7	1.76	30 of each	1.83
4		1	SSF + UV	2000	22		6.2	2.5 base	5.7	1.78	10 of each	1.81
4		2	SSF + UV	2000	21.9		5.9	0	5.9	1.77	20 of each	1.83
4		3	SSF + UV	1000	21.6		5.6	2 base	5.7	1.78	10 of each	1.81
4		1	SSF +BIOCHAR + UV	2000	21.9		6	1 acid	5.8	1.8	0	1.8
4		2	SSF +BIOCHAR + UV	2000	21.9		6.3	1.5 acid	5.9	1.77	30 of each	1.8
4		3	SSF +BIOCHAR + UV	1000	22		5.3	4 base	5.9	1.82	0	1.82
4		1	FRESHWATER	1000	22		5.9	0	5.9	1.93	0	1.93
4		2	FRESHWATER	0	22		5.8	0	5.8	1.88	0	1.88

4		3	FRESHWATER	0	21.9		5.9	0	5.9	1.9	0	1.9
4												
4	12/2/2024	1	Control	2000	21.8		6.4	1.5 acid	5.9	1.8	0	1.8
4		2	Control	2000	22		6.1	0.5 acid	5.9	1.8	0	1.8
4		3	Control	2000	21.8		5.9	0	5.9	1.83	0	1.83
4		1	SSF + UV	2000	21.6		6.2	1 acid	5.9	1.76	20 of each	1.81
4		2	SSF + UV	2000	21.7		5.9	0	5.9	1.79	5 of each	1.81
4		3	SSF + UV	1000	21.4		5.9	0	5.9	1.79	5 of each	1.8
4		1	SSF +BIOCHAR + UV	2000	21.8		6.1	1 acid	5.9	1.76	20 of each	1.81
4		2	SSF +BIOCHAR + UV	3000	21.5		6.4	4.5 acid	5.9	1.8	0	1.8
4		3	SSF +BIOCHAR + UV	2000	21.8		5.6	3.5 acid	5.7	1.79	5 of each	1.81
4		1	FRESHWATER	0	21.9		5.8	0	5.8	1.91	0	1.91
4		2	FRESHWATER	1000	22		5.7	0	5.7	1.96	0	1.96
4		3	FRESHWATER	1000	21.6		5.9	0	5.9	1.93	0	1.93
4												
4	12/6/2024	1	Control	2000	21.5		6.5	2.5 acid	5.7	1.76	20 of each	1.82
4		2	Control	4000	21.9		6.2	2 acid	5.8	1.76	20 of each	1.81
4		3	Control	2000	21.8		5.9	0	5.9	1.79	5 of each	5.9
4		1	SSF + UV	2000	21.7		6.2	1.5 acid	5.8	1.77	20 of each	1.82
4		2	SSF + UV	3000	21.6		5.3	4 base	5.7	1.78	10 of each	1.81

4		3	SSF + UV	3000	21.4		6.2	2 acid	5.9	1.77	20 of each	1.81
4		1	SSF +BIOCHAR + UV	2000	21.7		6.1	1 acid	5.7	1.55	55 of each	1.89
4		2	SSF +BIOCHAR + UV	3000	21.8		6.4	2.5 acid	5.8	1.74	30 of each	1.81
4		3	SSF +BIOCHAR + UV	2000	21.8		5.7	0	5.7	1.83	0	1.83
4		1	FRESHWATER	2000	21.9		5.5	2 base	5.7	1.91	0	1.91
4		2	FRESHWATER	2000	21.8		5.5	3 base	5.7	1.92	0	1.92
4		3	FRESHWATER	2000	21.6		5.9	0	5.9	1.96	0	1.96
4												
4	12/9/2024	1	Control	3000	21.9		5.7	0	5.7	1.8	0	1.8
4		2	Control	2000	22.1		5.9	0	5.9	1.74	30 of each	1.8
4		3	Control	2000	22		6.1	1 acid	5.7	1.77	20 of each	1.8
4		1	SSF + UV	2000	22		5.9	0	5.9	1.78	15 of each	1.81
4		2	SSF + UV	2000	21.9		5.3	4.5 base	5.8	1.77	20 of each	1.8
4		3	SSF + UV	2000	21.6		5.9	0	5.9	1.77	20 of each	1.81
4		1	SSF +BIOCHAR + UV	2000	22		5.7	0	5.7	1.88	0	1.88
4		2	SSF +BIOCHAR + UV	2000	21.9		6.1	1 acid	5.8	1.76	20 of each	1.81
4		3	SSF +BIOCHAR + UV	2000	22		6.1	1 acid	5.7	1.79	5 of each	1.8
4		1	FRESHWATER	0	22		1.87	0	1.87	1.87	0	1.87

4		2	FRESHWATER	2000	22		1.9	0	1.9	1.9	0	1.9
4		3	FRESHWATER	0	22		1.96	0	1.96	1.96	0	1.96

Appendix B - Nutrient Analysis Data of Nutrient Solution after Treatment

ID	Treatment	Rep	Cycle	Zn/ppm	Total N/ppm	Total P	NH_4-N	NO_3-N	Ortho P/ppb	Cl	Ca	Mg	Na	K	Cu	Fe	Mn	S
1	CONTROL	1	1	0.15	80.05	15.56	1.5	85.49	16600	0.54	159.29	38.67	60.61	205.2	0.05	1.08	0.16	166.09
1	CONTROL	2	1	0.18	97.03	24.43	2.07	90.37	26700	19.17	153.04	45.16	57.41	195.14	0.07	1.5	0.14	155.62
1	CONTROL	3	1	0.16	90.87	25.1	1.53	86.24	24400	18.4	138.35	41.96	53.49	200.77	0.06	1.42	0.16	154.28
2	SSF + UV-C	1	1	0.16	87.9	22.67	1.17	68.56	23800	15.13	149.31	48.46	55.2	164.95	0.07	1.42	0.12	165.66
2	SSF + UV-C	2	1	0.17	94.54	27.83	1.39	84.92	26100	13.77	149.67	45.99	53.47	195.96	0.07	1.52	0.15	164.3
2	SSF + UV-C	3	1	0.16	92.3	22.25	1.66	83.78	22900	18.94	139.67	39.74	54.14	178.19	0.06	1.32	0.14	148.92
3	SSF + BIOCHAR + UV-C	1	1	0.16	92.68	23.77	1.1	83.05	23900	20.08	146.34	42.93	57.49	172.69	0.06	1.38	0.12	154.78
3	SSF + BIOCHAR + UV-C	2	1	0.16	86.3	24.53	0.87	77.32	23600	15.73	140.44	43.86	51.77	173.02	0.08	1.42	0.12	155.76
3	SSF + BIOCHAR + UV-C	3	1	0.16	88.84	23.42	1.09	83.86	24100	16.56	140.5	40.63	55.72	178.78	0.06	1.37	0.14	154.35
4	FRESHWATER	1	1	0.15	80.05	15.56	1.5	85.49	16600	0.54	159.29	38.67	60.61	205.2	0.05	1.08	0.16	166.09
4	FRESHWATER	2	1	0.18	97.03	24.43	2.07	90.37	26700	19.17	153.04	45.16	57.41	195.14	0.07	1.5	0.14	155.62
4	FRESHWATER	3	1	0.16	90.87	25.1	1.53	86.24	24400	18.4	138.35	41.96	53.49	200.77	0.06	1.42	0.16	154.28
1	CONTROL	1	2	0.13	79.91	12.56	2.48	81.25	13300	13.47	158.37	37.63	56.85	168.71	0.04	1.08	0.08	170.86
1	CONTROL	2	2	0.17	82.25	21.67	1.28	80.4	22300	8.97	144.41	45.03	51.61	142.81	0.07	1.47	0.11	151.96
1	CONTROL	3	2	0.18	82.95	22.78	2.11	82.99	22100	14.31	152.11	42.99	51.37	174.88	0.07	1.47	0.14	161.82
2	SSF + UV-C	1	2	0.21	91.22	23.39	2.7	86.2	24000	7.51	172.3	51.31	53.62	151.49	0.09	1.32	0.12	182.83

2	SSF + UV-C	2	2	0.18	88.17	25.39	1.26	82.33	24200	7.65	155.24	44.55	47.65	172.76	0.09	1.16	0.14	168.17
2	SSF + UV-C	3	2	0.15	79.64	18.04	4.35	72.91	17800	10.26	152.12	35.53	50.52	130.38	0.06	0.79	0.11	156.34
3	SSF + BIOCHAR + UV-C	1	2	0.01	53.4	11.34	1.19	53.85	11400	19.17	115.35	38.04	50.39	145.45	0.01	0	0.02	151.14
3	SSF + BIOCHAR + UV-C	2	2	0.02	70.18	19.42	0.84	69.6	20000	10.82	157.33	46.44	46.95	164.16	0.01	0.01	0.04	176.42
3	SSF + BIOCHAR + UV-C	3	2	0.02	73.88	20.96	2.31	77.91	19900	15.24	149.21	42.66	50.47	173.65	0.01	0.01	0.03	167.37
4	FRESHWATER	1	2	0.14	89.13	22.07	1.57	87.97	21200	20.59	148.36	37.22	67.79	159.68	0.05	1.25	0.13	154.82
4	FRESHWATER	2	2	0.15	95.40	24.46	3.81	92.27	23200	21.29	136.71	39.08	66.31	172.83	0.05	1.29	0.14	152.13
4	FRESHWATER	3	2	<0.01	0.72	0.24	0.68	0.73	430	24.57	32.58	15.16	67.30	8.56	<0.01	<0.01	0.01	67.58
1	CONTROL	1	3	0.13	66.23	11.88	0.02	67.6	12600	11.04	164.06	39.23	57.14	158.47	0.05	1.14	0.06	185.86
1	CONTROL	2	3	0.19	76.41	22.31	0.81	80.59	22000	1.07	185.82	49.35	52.38	123.83	0.07	1.67	0.09	193.11
1	CONTROL	3	3	0.18	74.5	19.87	0.62	75.89	20000	9	156.73	43.88	52.08	156.18	0.07	1.46	0.11	173.4
2	SSF + UV-C	1	3	0.18	73.64	21.82	0.31	74.88	21600	1.19	176.01	51.62	52.91	123.8	0.09	0.82	0.09	190.31
2	SSF + UV-C	2	3	0.17	69.19	20.96	0.44	69.16	20000	1.57	139.47	39.71	40.4	131.92	0.07	0.79	0.1	155.87
2	SSF + UV-C	3	3	0.18	75.96	22.09	2.13	84.87	22600	4.97	165.43	45.2	53.34	151.67	0.07	0.97	0.13	173.56
3	SSF + BIOCHAR + UV-C	1	3	0.05	68.2	16.21	0.36	69.4	16600	13.04	143.13	44.8	50.36	170.43	0.03	0.32	0.04	184.46
3	SSF + BIOCHAR + UV-C	2	3	0.03	61.58	18.14	0.24	63.01	19000	5.01	165.12	48.08	48.28	147.15	0.01	0.18	0	196.28
3	SSF + BIOCHAR + UV-C	3	3	0.05	65.62	19.44	0.41	66.64	20200	9.59	150.76	42.88	47.92	158.17	0.02	0.1	0.03	173.36

4	FRESHWATER	1	3	5.8	80.80	24.02	1.33	75.60	23500	23.05	134.38	42.10	73.46	159.71	0.06	1.43	0.13	160.59
4	FRESHWATER	2	3	5.8	89.63	24.34	4.47	81.74	23800	23.55	130.70	40.66	71.84	166.40	0.06	1.28	0.13	157.56
4	FRESHWATER	3	3	5.8	103.73	26.04	5.62	91.84	24900	22.41	140.83	41.10	70.63	174.95	0.06	1.31	0.13	156.70
1	CONTROL	1	4	0.15	94.88	17.66	0.11	69.75	10319	5.28	212.73	50.38	73.83	167.91	0.06	1.61	0.02	237.74
1	CONTROL	2	4	0.25	114.55	32.7	0.19	99.29	28550	0.43	251.75	67.36	65.72	129.09	0.12	2.51	0.11	258.2
1	CONTROL	3	4	0.23	116.47	28.37	0.4	102.06	23498	4.96	205.77	58.13	64.63	177.81	0.1	2.02	0.11	229.38
2	SSF + UV-C	1	4	0.22	113.43	27.06	1.06	87.9	21480	0.19	234.21	67.2	66.46	136.47	0.11	1.12	0.09	248.72
2	SSF + UV-C	2	4	0.24	125.11	30.46	0.08	112.06	26272	0.47	198.21	56.69	51.67	184.32	0.11	1.11	0.17	202.53
2	SSF + UV-C	3	4	0.22	115.52	25.95	0.03	100.52	23176	1.32	200.34	54.81	60.48	153.17	0.1	0.98	0.11	200.97
3	SSF + BIOCHAR + UV-C	1	4	0.02	101.62	19.67	2.3	94.05	19606	12.08	191.72	57.44	60.88	190.58	<0.01	0.01	0.06	232.19
3	SSF + BIOCHAR + UV-C	2	4	0.01	98.84	23.79	1.56	73.01	19377	0.88	224.57	65.05	61.82	158.35	<0.01	0.01	0.04	264.14
3	SSF + BIOCHAR + UV-C	3	4	0.01	107.79	23.81	0.01	91.35	22213	3.34	189.75	54.16	57.41	177.21	<0.01	0	0.03	217.19
4	FRESHWATER	1	4	0.12	76.93	14.00	<0.01	66.66	14580	4.02	167.93	39.70	57.40	130.09	0.04	1.26	0.03	191.28
4	FRESHWATER	2	4	0.23	177.45	40.58	7.05	133.04	38104	<0.1	158.04	40.07	3.42	279.63	0.09	2.04	0.20	114.32
4	FRESHWATER	3	4	0.24	234.84	40.63	12.85	194.32	41497	<0.1	212.35	40.14	3.61	286.73	0.09	2.00	0.22	114.92
1	CONTROL	2	5	0.14	86.08	13.93	0.01	75.41	11028	1.3	201.89	45.22	63.65	132.24	0.06	1.48	0.03	211.66
1	CONTROL	2	5	0.22	84.99	25.2	0.04	70.33	21069	0.24	227.74	59.29	58.22	95.22	0.11	2.16	0.09	226.15
1	CONTROL	3	5	0.2	90.06	23.53	0.2	77.36	15617	0.54	181	50.23	54.99	133.44	0.09	1.76	0.08	201.01
2	SSF + UV-C	1	5	0.16	86.22	24.25	0.05	75	21982	2.09	197.1	55.57	51.46	106.55	0.08	0.61	0.06	213.31

2	SSF + UV-C	2	5	0.2	99.72	21.36	0.03	86.8	24528	10.26	181.74	51.09	44.03	163.86	0.1	0.81	0.12	188.9
2	SSF + UV-C	3	5	0.2	89.63	20.44	0.16	81.26	21868	1.02	167.17	45.71	45.33	121.74	0.09	0.75	0.11	169.48
3	SSF + BIOCHAR + UV-C	1	5	0.02	72.54	18.28	4.51	59.1	16589	17.84	150.08	44.71	41.11	214.2	0.01	0.03	0.05	210.94
3	SSF + BIOCHAR + UV-C	2	5	0.01	66.42	20.69	0.49	53.01	15587	8.19	178.91	52.15	45.92	135.48	<0.01	0.02	0.05	216.62
3	SSF + BIOCHAR + UV-C	3	5	0.01	34.63	20.65	0.07	27.2	20523	422.43	162.12	46.52	46.43	495.38	<0.01	0.01	0.06	197.35
4	FRESHWATER	1	5	0.24	199.68	43.59	8.46	157.41	43493	<0.1	169.45	39.58	4.35	266.03	0.09	1.83	0.19	117.49
4	FRESHWATER	2	5	0.22	174.64	39.39	7.54	125.55	38578	1.26	123.73	36.90	2.75	287.40	0.09	1.83	0.22	106.34
4	FRESHWATER	3	5	0.23	184.80	45.69	7.91	135.35	39211	1.35	133.54	37.52	2.62	280.52	0.09	1.90	0.23	101.49
1	CONTROL	2	6	0.15	83.60	14.67	0.16	72.99	13494	<0.1	216.80	47.44	64.12	132.78	0.09	1.63	0.05	196.77
1	CONTROL	2	6	0.22	69.74	19.33	0.05	66.36	19955	<0.1	250.09	62.88	58.83	79.55	0.15	2.26	0.07	219.48
1	CONTROL	3	6	0.23	78.15	23.14	0.04	79.32	23216	<0.1	207.58	55.73	59.82	146.06	0.12	1.97	0.09	190.35
2	SSF + UV-C	1	6	0.19	69.41	18.30	0.06	68.09	18716	<0.1	219.79	59.13	54.62	97.42	0.10	0.68	0.04	210.50
2	SSF + UV-C	2	6	0.19	78.03	23.85	0.05	79.52	22958	<0.1	190.12	51.13	43.92	131.83	0.09	0.65	0.08	189.62
2	SSF + UV-C	3	6	0.23	84.74	25.10	0.05	86.92	25332	<0.1	191.03	49.82	45.57	131.35	0.13	0.65	0.10	163.96
3	SSF + BIOCHAR + UV-C	1	6	0.02	39.69	17.51	0.05	27.30	16448	25.41	154.44	44.12	40.84	182.43	0.00	0.06	0.05	191.64
3	SSF + BIOCHAR + UV-C	2	6	0.01	58.91	16.12	0.05	59.38	18319	17.60	187.79	52.09	48.57	138.92	0.00	0.03	0.03	190.89
3	SSF + BIOCHAR + UV-C	3	6	0.01	57.37	15.35	0.10	56.07	16134	5.73	152.93	44.20	39.82	190.93	0.00	0.02	0.04	184.91

4	FRESHWATER	1	6	0.20	120.50	34.01	3.09	117.36	35291	<0.1	141.37	35.11	2.28	239.97	0.10	1.86	0.20	89.72
4	FRESHWATER	2	6	0.21	128.83	34.08	7.91	130.42	32805	<0.1	141.79	34.91	2.19	234.72	0.09	1.79	0.19	88.90
4	FRESHWATER	3	6	0.21	135.52	36.58	8.39	131.94	36628	<0.1	137.61	34.82	2.24	243.66	0.09	1.78	0.19	87.76