ADVANCED OXIDATION PROCESSES FOR PATHOGEN CONTROL IN WASTEWATER FOR CROP PRODUCTION

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The Water Research Commission of South Africa financially supported the research.

The contents of this work have not been submitted in any form to another university, and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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DECLARATION 1: PLAGIARISM

I, Barnabas Oginga Oluoch, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

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Barnabas Oginga Oluoch

Date: 25th October 2024

DECLARATION 2: PUBLICATIONS

The following published and submitted manuscripts form part of the research presented in this thesis.

Publication – Chapter 2 of this thesis

Barnabas Oluoch*, Takudzwa Mandizvo, William Musazura, Taruvinga Badza, Benton Otieno, Stephen Ojwach, Alfred Odindo. A Review of Pathogen Removal from Municipal Wastewater using Advanced Oxidation Processes: Agricultural Application, Regrowth Risks, and New Perspectives. Published *https://doi.org/10.1016/j.heliyon.2024.e39625*

Conceptualization, investigation, data curation, formal analysis, methodology, validation, visualization, writing – original draft, review & editing for the above-listed manuscript were conducted in their entirety by Barnabas Oluoch with the technical advice from Prof. A.O. Odindo, Prof S.O Ojwach, and the rest of co-others. All tables, figures, and graphs were produced by Barnabas Oluoch unless otherwise referenced in the respective publications.

Publication – Chapter 3 of this thesis

Barnabas Oluoch*, Alfred Odindo, Stephen Ojwach, Benton Otieno, and William Musazura. Municipal Anaerobic Filter Effluent Treatment Using Advanced Oxidation Processes for Potential Use in Unrestricted Crop Production. Under review in *"Journal of Environmental Science and Health, Part A (Submission ID: 243564857)".*

Investigation, data curation, formal analysis, methodology, validation, visualization, writing – original draft, review & editing for the above-listed manuscript were conducted in their entirety by Barnabas Oluoch with the technical advice from Prof. A.O. Odindo, Prof S.O Ojwach, and the rest of co-others. All tables, figures, and graphs were produced by Barnabas Oluoch unless otherwise referenced in the respective publications.

Publication – Chapter 5 of this thesis

Barnabas Oluoch*, Takudzwa Mandizvo, William Musazura, Taruvinga Badza, Benton Otieno, Stephen Ojwach, Alfred Odindo. Irrigation Effects of Municipal Effluents Treated with Advanced Oxidation Processes; Bioaccumulation and Potential Health Risks. Under review in *"Journal of Hazardous Materials (HAZMAT-D-24-19590)."*

Investigation, data curation, formal analysis, methodology, validation, visualization, writing – original draft, review & editing for the above-listed manuscript were conducted in their entirety by Barnabas Oluoch with the technical advice from Prof. A.O. Odindo, Prof S.O Ojwach, and the rest of co-others. All tables, figures, and graphs were produced by Barnabas Oluoch unless otherwise referenced in the respective publications.

DECLARATION 3: LIST OF CONFERENCE PRESENTATIONS

1. Barnabas Oluoch*, Alfred Odindo, Stephen Ojwach, Benton Otieno, and William Musazura (2023). Reduction of pathogens in domestic wastewater using advanced oxidation processes for unrestricted crop production. "*1st International Water Association - Non-Sewered Conference*." 15th -18th of October 2023. Emperors Place, Johannesburg, South Africa.
2. Barnabas Oluoch*, Alfred Odindo, Stephen Ojwach, Benton Otieno, and William Musazura (2023). Reduction of pathogens in domestic wastewater using advanced oxidation processes for crop production. *"The 24th WaterNet/WARFSA/GWPSA Symposium."* 25th -27th of October 2023. Hotel Verde, Zanzibar, United Republic of Tanzania.

ABSTRACT

The persistence of pathogens in wastewater significantly limits its value for unrestricted crop production. Thus, stringent quality standards have been set to ensure safe water resources for sustainable crop management practices and portable uses. Consequently, there is a pressing need for further treatments in wastewater to ensure safe and unrestricted use for crop production. The present study sought to understand the efficiency of advanced oxidation processes (AOPs) as a post-treatment step for the removal of pathogens contained in municipal secondary effluent to achieve standards for unrestricted crop production that is safe for human consumption. Municipal secondary effluent was subjected to selected AOPs (ozonolysis, UV photolysis, and TiO_2-photocatalysis) to reduce pathogens in domestic wastewater and prevent regrowth upon storing the treated effluent. Ozonolysis and TiO_2-photocatalysis achieved a 6.4-log reduction in pathogens, while UV-photolysis achieved a 6-log reduction. After four days of storage, ozonolysis-treated (O-TE) samples showed no pathogen regrowth, while TiO_2-photocatalysis-treated effluent (Ti-TE) had regrowth of *E. coli* and Total coliforms at 2.5-log and 2.7-log, respectively. UV-photolysis-treated effluent (UV-TE) had a regrowth rate of 0.5-log for *E. coli* and 2.2-log for Total coliforms. Nutrient mineralization dynamics were also examined upon soil irrigation with AOP-treated effluents. Significant reduction in the ammonium-N levels across UV-TE, O-TE, and Ti-TE treatments was noted, which did not translate to notable changes in the nitrate-N levels. P mineralization was relatively slower across UV-TE and O-TE than in the controls (untreated wastewater (RW) and municipal tap water (MTW)); however, total P mineralization was achieved after day 70. Significant reduction of ortho-P in Ti-TE irrigated soils was confirmed, indicating effective phosphorus binding by TiO_2. Moreover, the effects of AOP-treated effluents on crop physiological functions with respect to photosynthetic rates and capacity and potential health risks to consumers was also investigated following the irrigation. The study found significant phytotoxicity effects on Swiss chard and lettuce irrigated with AOP-treated effluents, with Ti-TE showing the highest stress levels, followed by O-TE and UV-TE. While no pathogens were detected on the leaves across all the treatments, TiO_2 agglomerates were found on crops irrigated with Ti-TE. High TiO_2 levels were recorded upon quantification, suggesting potential health risks from consuming crops irrigated with Ti-TE. Overall, Ozonolysis was the most promising method, meeting standards for unrestricted crop production with minimal impact on soil nutrient mineralization and mild phytotoxicity. Additionally, irrigation with O-TE posed no health risks to consumers, as no pathogens were found on the edible parts (crop leaves).

Keywords: Advanced oxidation processes; Pathogen reduction; Unrestricted wastewater reuse. Crop production; Wastewater treatment

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DEDICATION

To my beloved late parents, James Oluoch Agutu and Elizabeth Atieno Oluoch.

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ABBREVIATION

AF-	Anaerobic Filter
AOPs-	Advanced Oxidation Processes
CAS-	Alumino-Copper Silicate
DEWATS-	Decentralized Wastewater Treatment System
EPA-	Environmental Protection Agency
EU-	European Union
GAC-	Granular activated carbon
GCN-	Graphitic Carbon Nitride
IDS-	Iminodisuccinic acid
MTW-	Municipal Tap Water
O-TE-	Ozone Treated Effluent
PMS-	Peroxymonosulphate
PS-	Persulfate
RW-	Untreated secondary wastewater
SDG-	Sustainable Development Goals
Ti-TE-	TiO_2 photocatalysis Treated Effluent
UV-TE-	UV Treated Effluent
UV-	Ultraviolet radiation
UN-	United Nations
WHO-	World Health Organization

CHAPTER 1: GENERAL INTRODUCTION TO ADVANCED OXIDATION PROCESSES FOR PATHOGEN CONTROL IN WASTEWATER FOR CROP PRODUCTION

1.1 Background

Access to safe and clean water resources is essential for human survival and agricultural production (Okeyo et al., 2018; Pal et al., 2018). As rural-urban migration continues to increase, the discharge of large volumes of domestic wastewater into the environment has become a pressing issue, potentially contaminating water sources and agricultural lands (Bao and He, 2019; Qadir et al., 2020; Wang and Li, 2019). Domestic wastewater management is then critical in preserving water resources and resolving agricultural water shortages; hence, the need for efficient and safe wastewater treatment methods (Huibers and Van Lier, 2005; Velusamy et al., 2021).

In many rural and peri-urban areas without access to centralized treatment systems, decentralized wastewater treatment systems (DEWATS) are envisioned to address public health and environmental risk concerns associated with wastewater treatment (Bright-Davies et al., 2015). The DEWATS employs technologies like anaerobic digestion, constructed wetlands, and biological filters allowing for on-site wastewater treatment (Singh et al., 2019; Truyens, 2019; Varma et al., 2022). This approach reduces the pressure on off-site and centralized waterborne sanitation systems and improves the ability to deal with different types of wastewater found in urban areas (Truyens, 2019). However, these systems may not effectively remove all pathogens from the wastewater, which can subsequently impact agricultural lands (Naidoo and Olaniran, 2014).

In a recent South African research paper, a case study at eThekwini DEWAT system, it is revealed that alongside pathogens, there are about 24 micropollutants with anti-inflammatory drugs, antiretrovirals, and antibiotics having the highest concentrations in the raw wastewater. Most of these contaminants were still present in high concentrations in the Anaerobic Filter (AF) effluent and a slight reduction after the constructed wetlands. Regardless of the reduction, the effluent was still unsafe for discharge to streams or unrestricted agricultural applications, hence the post-treatment call (Arumugam et al., 2023; Späth et al., 2021).

Effluent standards for discharge or reuse cover a range of parameters, including organic compounds (such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS)), nutrient levels (nitrogen and phosphorus), and pathogen concentrations (Apollo and Aoyi, 2022; Arumugam et al., 2023; Buckley and Arumugam, 2016; Otieno et al., 2019a). Adhering to these standards is instrumental in preventing environmental degradation and safeguarding public health, particularly when treated wastewater is applied in activities such as agricultural irrigation, industrial processes, or aquifer recharge (Abdelghafour et al., 2020; Achag et al., 2021; Miller et al., 2020).

Adopting advanced oxidation processes (AOPs) for post-treatment of the DEWATS's effluent for agricultural use aligns with the urgent demand for innovative approaches to water management in the face of increasing water scarcity and pollution challenges (Alkhudhiri et al., 2019; Fund, 2015; Sdg, 2019; Taft, 2015). Advanced oxidation processes exhibit the potential to effectively degrade recalcitrant organic pollutants present in wastewater, including pathogens (Apollo and Aoyi, 2022; Otieno et al., 2019b). Moreover, using AOPs offers a promising pathway to mitigate the risks associated with applying untreated or partially treated wastewater in agriculture, safeguarding crop health and human well-being (Abd-Elhamid et al., 2021; Zhang and Shen, 2019).

Exploring these advanced treatment technologies aims to establish a systematic and reliable approach that optimizes water resources for sustainable crop production while ensuring minimal environmental impact. Furthermore, applying AOP-treated wastewater minimizes the reliance on conventional freshwater sources, providing a more resilient and resource-efficient approach to crop cultivation, especially in water-stressed regions (Taft, 2015). This integrated approach not only addresses multiple Sustainable Development Goals (SDGs) simultaneously but also fosters a more resilient and sustainable future for all, contributing significantly to improved sanitation, water conservation, food security, and overall health, as outlined in SDG 6, 2, and 3 (Gil et al., 2019; Guégan et al., 2018; Ortigara et al., 2018; Sdg, 2019).

1.2 Research problem

The exponential growth of urban populations in Africa has significantly strained conventional sewer systems, with increased production of domestic wastewater. In response, the adoption of DEWATS has emerged as a promising solution in reducing the burden on overloaded treatment infrastructure. Despite its potential, current DEWATS implementations fall short of meeting mandated discharge standards and enabling unrestricted reuse due to persistent concerns regarding the presence of pathogens, nutrient concentrations, organic pollutants, and micropollutants (Arumugam et al., 2023; Späth et al., 2021). Water in itself and the effluent’s nutrients are resources that need to be mined for proper waste management and water security. The currently used constructed wetlands as the polishing step has been reported to be land-intensive, expensive to construct, and produce non-complying effluent. Thus, a more advanced effluent polishing step is needed to promote a sustainable circular economy, potentially boosting livelihoods in low-income areas, reducing agricultural water demand, and limiting eutrophication and other forms of environmental pollution upon effluent discharge. Moreover, advanced effluent polishing steps are envisioned to mitigate the risk of pathogen regrowth in treated effluents, which can endanger public health if reused in agriculture.

1.3 Justification

Inadequately treated effluents can contaminate agricultural lands, potentially impacting the safety of food crops and posing risks to human health. Moreover, the environmental consequences of poorly treated wastewater include contamination of receiving waters, affecting ecosystems, potentially causing eutrophication, and spreading waterborne diseases. As a result, it is ideal to re-invent the existing treatment technologies and gain insight into new ideas to ensure that the effluent from DEWATS meets the necessary standards for unrestricted agricultural use and discharge. Advanced oxidation processes are considered an area of exploration.

1.4 Study aim

This study seeks to gain insights into how AOPs can enhance the efficiency and quality of wastewater treatment in replacing the land-intensive, expensive to construct, and non-complying wetlands to achieve standards for unrestricted crop production with produce that poses no health risks upon consumption.

1.5 Specific objectives

1. To determine the efficacy of the selected Advanced Oxidation Processes (ozonolysis, UV photolysis, and TiO_2 photocatalysis) on pathogen elimination and post-treatment regrowth.
2. To determine nitrogen and phosphorus release patterns in soils irrigated using e AOP-polished effluents.
3. To monitor the effects of the AOP-polished effluents upon irrigation on crop growth and the subsequent human health risks associated with their consumption.

1.6 Chapter outline

This thesis consists of 6 chapters:

Chapter 1

This chapter provides a general introduction and summary of the study's context, problem statement, aims, objectives, and justification.

Chapter 2

This chapter reviewed the state-of-the-art on the currently studied categories of advanced oxidation processes (AOPs) and their pathogen reduction potentials with a special focus on post-treatment regrowth. Also, new research perspectives were outlined.

Chapter 3

This chapter offered a bench experiment to optimize the efficacy of selected AOPs (ozonolysis, UV photolysis, and TiO_2 photocatalysis) for polishing the anaerobic filter (AF) effluent of DEWATS as an alternative to the constructed wetlands in meeting standards for unrestricted use for crop production.

Chapter 4

This chapter contributed to understanding nitrogen and phosphorus release patterns in soils irrigated with domestic wastewater reclaimed through the AOPs studied in Chapter 3.

Chapter 5

This chapter provided deeper insights into the extent to which AOPs-treated effluents can affect crop growth and subsequent health risks associated with the consumption of crops irrigated with such effluents.

Chapter 6

This chapter is a general conclusion on the qualities of AOPs-treated effluents, their effects on soil-irrigation scenarios, and their effects on crop growth. Also, it highlights subsequent health risks associated with the consumption of crops irrigated with such effluents. Key concerns that raised more questions were highlighted, and recommendations for further investigation were made.

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CHAPTER 2: A REVIEW OF PATHOGEN REMOVAL FROM MUNICIPAL WASTEWATER USING ADVANCED OXIDATION PROCESSES: AGRICULTURAL APPLICATION, REGROWTH RISKS, AND NEW PERSPECTIVES

Abstract

Pathogen removal in wastewater offers a chance to recover water and nutrients for crop production, reducing environmental contamination and public health risks. However, the risk of pathogens regrowing in treated effluents can endanger public health if reused in agriculture, attracting stringent reuse standards. While advanced oxidation processes (AOPs) promise to reduce pathogens, eliminating regrowth potential in AOP-treated effluents requires further scrutiny. This review aimed to summarize the available evidence on understanding pathogen reduction and regrowth potential in AOP-treated effluents, following best practices for scoping reviews, such as the preferred reporting items for systematic reviews and meta-analysis (PRISMA). This study covers recent pathogen studies under AOPs, current AOP investigations, the impact of AOP dosage and retention time on pathogen control, and challenges in reusing AOP-treated effluents for crop production. Additionally, it identifies areas needing improvement or complementary treatments for pathogen-free effluents with no regrowth potential. The review concludes by summarizing key findings and suggesting research areas for further exploration.

Keywords: Advanced oxidation processes; Pathogen reduction; Pathogen regrowth; Wastewater pathogens; Effluent reuse challenges; Sewage treatment

2.1 Introduction

Municipal wastewater treatment for reuse is a crucial strategy in mitigating water scarcity challenges (Fito and Van Hulle, 2021; Padder and Bashir, 2023). As global populations expand and urbanize, the demand for freshwater resources intensifies, placing immense pressure on water supplies (Mishra, 2023). By implementing efficient wastewater treatment processes at the municipal level, we can transform what was once considered a waste product into a valuable resource. When properly treated, wastewater can be safely reused for various non-potable purposes such as crop irrigation, industrial processes, and even replenishing aquifers (Chauhan and Kumar, 2020; Tzanakakis et al., 2023). This approach helps alleviate the strain on freshwater sources and promotes sustainability by reducing pollution and enhancing water resource management practices (Naidoo and Olaniran, 2014; Santos et al., 2023).

Conventional wastewater treatment methods currently used by the municipalities are reported as not fully amenable to emerging contaminants and pathogens (Bracamontes-Ruelas et al., 2022; Späth et al., 2021). The inadequacy of these methods in eliminating pathogens and preventing their regrowth threatens public health and the environment (Lourenço and Nunes, 2020; Naidoo and Olaniran, 2014; Singh et al., 2019). Pathogens, including bacteria, viruses, and protozoa, can persist in treated wastewater, potentially contaminating receiving waters and endangering human health when used for irrigation or recreational purposes (Pal et al., 2018; Partyka and Bond, 2022). Pathogens' persistence and potential regrowth in the treated effluents are significant concerns that warrant a solution and further research, especially given the limited studies reporting on this issue.

Unlike conventional wastewater treatment methods, advanced oxidation processes (AOPs) employ various mechanisms that have been considered an alternative area of exploration for potentially eliminating pathogens in wastewater treatment (Abbasi Hassan Abadi et al., 2023; Fiorentino et al., 2018; Ganesh Kumar and Kanmani, 2022). Advanced oxidation processes employ highly reactive oxygen species (ROS) like hydroxyl radicals (•OH) to degrade organic matter, including pathogens, by attacking their cell membranes, DNA, and proteins (Ahmed et al., 2020a; Ahmed et al., 2021; Ahmed et al., 2022; Guo et al., 2020). These processes also generate reactive intermediates like Fe^{3+} and Fe^{2+} species through mechanisms such as Fenton reactions, further contributing to pathogen inactivation (Kim et al., 2019; Wang and Wang, 2020; Wang et al., 2022).

Additionally, AOPs can produce disinfection by-products (DBPs) with antimicrobial properties, enhancing pathogen elimination (Lei et al., 2021; Mayer and Ryan, 2019; Shao et al., 2023). Furthermore, AOPs can facilitate indirect pathogen degradation mechanisms like generating secondary oxidants such as sulfate radicals ($SO_4{\cdot}{-}$) through persulfate activation (Brienza and Katsoyiannis, 2017; Ike et al., 2018; Ushani et al., 2020). These secondary oxidants participate in radical chain reactions, increasing AOPs' oxidative potential for increased pathogen inactivation (Ike et al., 2018). Moreover, the synergistic effects of ROS, reactive intermediates, and DBPs contribute to comprehensive pathogen elimination, making AOPs valuable tools for ensuring safe wastewater effluent (Alaton et al., 2019; Fiorentino et al., 2018; Ike et al., 2018).

While pathogen-free effluents can be used effectively in urban landscaping (Maryam and Büyükgüngör, 2019), industrial processes (Jodar-Abellan et al., 2019), and recreational water bodies (Angelakis et al., 2018), their primary importance lies in agriculture due to water and nutrient demand for crop production (Musazura and Odindo, 2021; Valdes Ramos et al., 2019). According to the United Nations Sustainable Development Goals (SDGs), prioritizing the use of pathogen-free effluents from AOPs in agriculture aligns with broader goals of promoting sustainable water management, enhancing food production, and safeguarding public health (Gil et al., 2019; Guégan et al., 2018; Obaideen et al., 2022; Ortigara et al., 2018; Sdg, 2019) thereby presenting opportunities towards achieving SDG 6, 2 and 3 respectively. This strategic allocation of treated effluents emphasizes the importance of technological advancements to address pressing challenges in key sectors such as agriculture, where water quality and availability are crucial for global food supply, environmental stewardship, and ecological balance (Dinar et al., 2019; Mishra et al., 2021; Tzanakakis et al., 2020).

Despite the potential of AOPs in reducing the initial pathogen concentrations and recalcitrant organics, there is a lack of comprehensive, up-to-date studies that assess AOPs' effectiveness in eliminating regrowth in the context of agricultural application standards (Ogundele et al., 2023; Periasamy and Sundaram, 2013). Existing studies have reported varying outcomes and methodologies, making it almost impossible to make unwavering decisions on the overall efficacy of AOPs in suppressing pathogen regrowth (Abdel-Wahed et al., 2022; Ahile et al., 2021; Gholikandi and Amiri, 2022). Therefore, an evidence-based approach is necessary to evaluate the potential and efficacy of AOPs in this critical area of water resources recovery through wastewater treatment.

This study presents a state-of-the-art review focusing on the application of AOPs for pathogen elimination in effluents from conventional wastewater treatment methods. A special focus will be on evaluating the AOPs in terms of bacterial regrowth following treatment. A comprehensive analysis of existing literature over the past five years of research will allow us to consolidate and synthesize the latest available evidence, providing a clear and unbiased assessment of AOPs' effectiveness in pathogen removal. By merging data from multiple studies and applying rigorous statistical techniques, this review aims to uncover prospective opportunities for current AOP studies by clarifying the true impact of AOPs on pathogens across diverse wastewater treatment scenarios.

This review's findings will be beneficial in informing wastewater treatment practices, guiding policy decisions, and future studies regarding wastewater standards and reuse alternatives. The review will offer insights into (a) pathogens of focus in recent AOP studies, (b) AOPs currently studied, (c) the effects of AOPs dosage levels and retention time on pathogen reduction and regrowth, and (d) challenges regarding the reuse of AOP-treated effluents on crop production. Additionally, it will highlight stages where improvements or complementary treatments may be necessary to ensure pathogen-free effluents with zero regrowth. Ultimately, this review endeavors to serve as a significant step toward enhancing the safety and sustainability of wastewater treatment processes, safeguarding both public and environmental health in this era of increasing urbanization and environmental challenges.

2.2 Methodology

The existing published material supporting the study's broad research question was organized using a systematic review technique. The five proposed stages were observed: (i) identifying the research objective, (ii) search method, (iii) study choice, (iv) processing, and (iv) reporting findings (Gwara et al., 2020).

2.2.1 Research question(s)

The population, interventions, context, outcomes, and study design (**PICOS**) technique was used to establish the objective of the current study (Iacovidou et al., 2017). The **"population"** was defined as wastewater pathogens (bacteria, viruses, protozoans, and helminths). The **"intervention"** compared the application and efficiency of different AOPs in the **"context"** of wastewater treatment. Pathogen reduction and regrowth were defined as the **"outcomes."** The **"research designs"** were classified as quantitative or hybrid (mixed qualitative and quantitative).

2.2.2 Sources of data and search strategy

The search was conducted using both the Web of Science, including all its databases, and the Scopus databases. These databases were chosen to be comprehensive and cover all aspects of wastewater treatment. **"Title-Abstract-Keyword"** search was employed in Scopus while **"all fields"** for Web of Science databases. The review was confined to peer-reviewed publications published in English between January 1, 2018, and August 24, 2023. The time confinement enabled this study to retrieve all the most recent studies on advanced oxidation of wastewater. The search strategy included broad keywords to ensure no articles were left out (**Table 2.1**).

Table 2.1: Keyword-driven database search technique with Boolean operators.

Database	**Search Strategy**	**Search Results**
Web of Science	ALL FIELDS ("AOPs" AND "wastewater" AND "Treatment" AND "pathogen" OR "*E. coli*" or "coliforms" AND "Reduction" OR "elimination" OR "regrowth" AND "2024" or "2023" OR "2022" OR "2021" OR "2020" OR "2019" (Publication Years) AND "Article" (Document Types))	98120
Scopus	TITLE-ABS-KEY ("AOPs" AND "wastewater" OR "sewage" OR "effluent" AND "Treatment" AND "pathogen" OR "*E. coli*" OR "coliforms" AND "Reduction" OR "elimination" OR "regrowth")	28

2.2.3 Citations management, screening, and eligibility criteria

In the **"RIS"** format, the downloaded citations were exported into a group library in the Endnote (https://endnote.com/downloads). With the help of **"find duplicates"** within the Endnote library, duplicates were detected and deleted. The remaining citations were then title-screened manually to eliminate irrelevant citations. The product of the Endnote screening was converted from **"•enlx"** to **"XML"** format, saved, and then exported to Covidence (www.covidence.org), a web-based software where abstract screening, full-text screening, and data extraction of complete articles were performed (Babineau, 2014; Kellermeyer et al., 2018). Studies with **"Restricted Access"** during full-text screening were requested through the University of KwaZulu Natal's library and later incorporated into the review process. Generally, studies were eligible for data extraction only if written in English, peer-reviewed, focused on pathogen reduction or regrowth in municipal wastewater, explicitly addressing the advanced oxidation processes, had quantitative data on pathogens, and were original research papers and not review articles. Related citations were then combined as a study with the help of the **“Merge as study”** icon in Covidence.

2.2.4 Data classification, summary, and reporting

The present review employed a quantitative-analytical technique in extracting data from the articles that met the inclusion criteria (Abujayyab et al., 2022). The extracted data from the reviewed studies included (a) the type of AOP used, (b) the AOP operating conditions (dosage, retention time, and temperature), (c) the number of pathogens eliminated or regrown after treatment, (d) the type of pathogen studied, (e) the treatment scale based on the reactor volume, (f) author, (g) DOI number, and (h) year of publication. The quantitative data in the graphs were extracted using the **“web plot digitizer”** (https://automeris.io/WebPlotDigitizer), a web-based tool for extracting data from plots, images, and maps. The extracted data were compiled in the **“data extraction template”** within the Covidence software, and then using the **“Export”** icon, it was sent into Microsoft Excel 2016.

2.2.5 Data standardization and presentation

In the diverse nature of research, different scientists present their results in various ways. During data extraction in this review, it was observed that pathogen data were reported in CFU/100 mL, MPN/100 mL, CFU/mL, and MPN/mL, which made it difficult to compare the findings. All extracted data was then standardized into MPN/mL units to enhance suitable data comparison.

2.3 Results and Discussions

2.3.1 Summary of the identified studies

Following the search criteria (**Table 2.1**), 98148 articles were identified, 28 in Scopus and 98120 in Web of Science. The **"find duplicates"** function in EndNote identified and eliminated 21 duplicates. The remaining 98127 articles were manually title-screened within the Endnote following the eligibility criteria in section (**2.2.3**). A total of 97926 articles didn't meet the eligibility criteria and were thus deemed irrelevant and excluded from the study. As a result, 201 articles were considered eligible for abstract and full-text screening and were exported to **"covidence" (**www.covidence.org**)**. Subsequently, 135 articles did not meet the abstract inclusion criteria, leaving only 66 articles for further screening. Only 17 of the 66 full-text screened articles fulfilled the inclusion criteria (**Figure 2.1**).

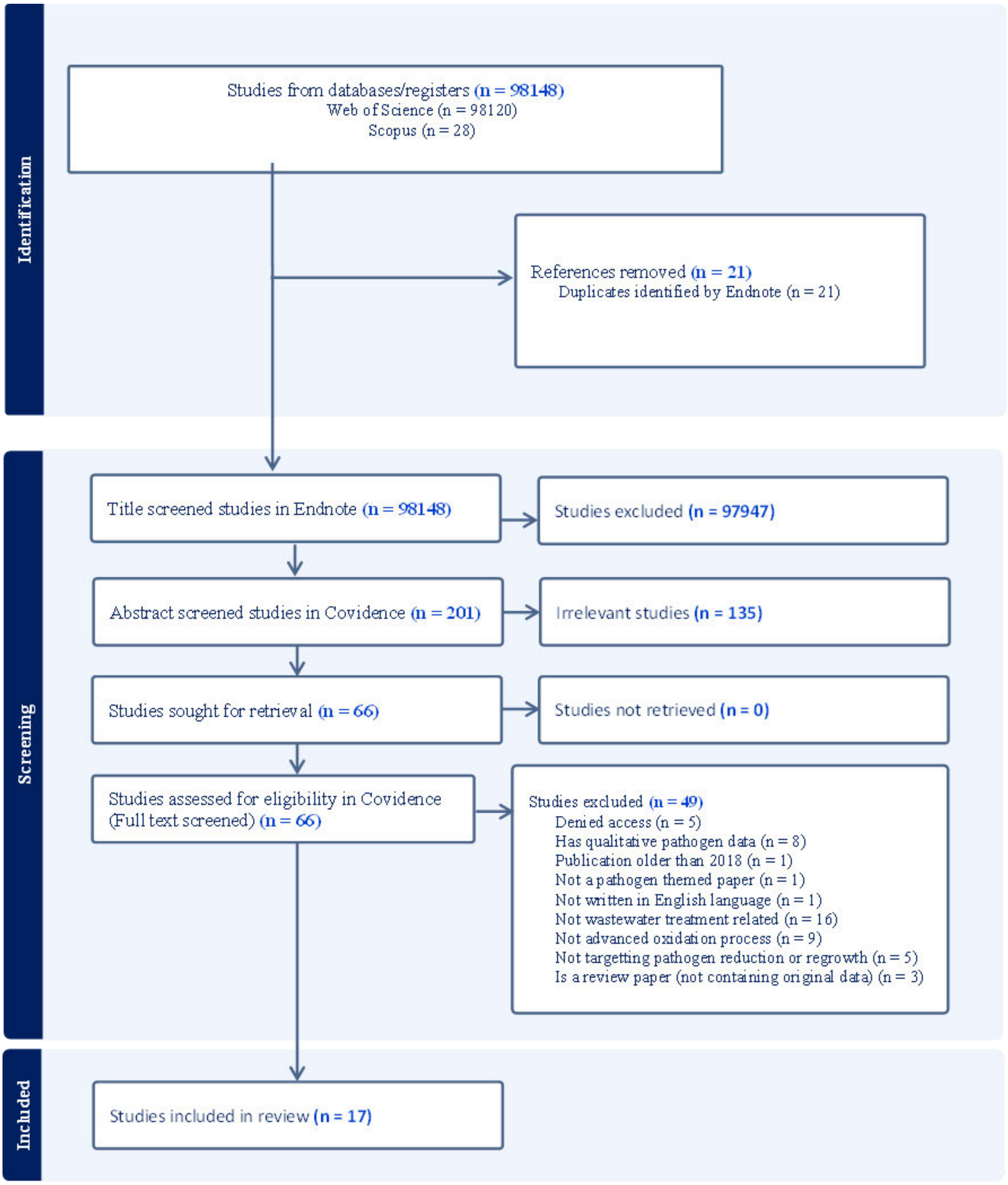

Figure 2.1: The flow chart summary of search results, screening, and study inclusion.

During data extraction, it was observed that many articles included multiple AOP studies, with each study treated independently during the review process. Thus, in total, 67 AOP studies were extracted from the 17 articles (**Table2.2**).

Table 2.2: Summary of the extracted studies with details on the author(s)' identity, article title, publication year, and the corresponding DOI number.

Author(s)	Title	SE	PY	DOI
Sareh Abbasi Hassan Abadi	Evaluation of Lemna minor and cyanobacteria effect in aerated and non-aerated conditions on biological oxygen demand (BOD), dissolved chemical oxygen (COD), total coliform, and fecal coliform of municipal and industrial wastewater	2	16-Jun-21	doi.org/10.1080/03067319.2021.1933463
Ali B. Abou Hammad	Nanoceramics and novel functionalized silicate-based magnetic nanocomposites as substitutional disinfectants for water and wastewater purification	1	6-May-20	doi.org/10.1007/s11356-020-09073-9
Yelitza Aguas	Reclamation of Real Urban Wastewater Using Solar Advanced Oxidation Processes: An Assessment of Microbial Pathogens and 74 Organic Microcontaminants Uptake in Lettuce and Radish	2	25-Jul-19	doi.org/10.1021/acs.est.9b00748
Edwar Aguilar-Ascon	Removal of *Escherichia coli* from Domestic Wastewater using Electrocoagulation	2	15-Mar-19	doi.org/10.12911/22998993/105331
Yunus Ahmed	Efficient inactivation of antibiotic-resistant bacteria and antibiotic-resistance genes by photo-Fenton process under visible LED light and neutral pH	5	3-May-20	doi.org/10.1016/j.watres.2020.115878
Somaye Akbari	Superior visible light-mediated catalytic activity of a novel N-doped, $Fe_3O_4^-$incorporating MgO nanosheet in the presence of PMS: Imidacloprid degradation and implications on simultaneous bacterial inactivation	1	15-Jul-22	doi.org/10.1016/j.apcatb.2022.121732
Olufemi Oluseun Akintunde	Disinfection and Photocatalytic Degradation of Organic Contaminants Using Visible Light-Activated GCN/Ag_2CrO_4 Nanocomposites	1	25-Aug-22	doi.org/10.3390/catal12090943
Débora Antonio da Silva	Combined AOP/GAC/AOP systems for secondary effluent polishing: Optimisation, toxicity, and disinfection	6	15-May-21	doi.org/10.1016/j.seppur.2021.118415
Ilaria Berruti	UV-C Peroxymonosulfate Activation for Wastewater Regeneration: Simultaneous Inactivation of Pathogens and Degradation of Contaminants of Emerging Concern	1	12-Aug-21	doi.org/10.3390/molecules26164890
®Antonino Fiorentino	Disinfection of urban wastewater by a new photo-Fenton-like process using Cu-iminodisuccinic acid complex as catalyst at neutral pH	7	13-Aug-18	doi.org/10.1016/j.watres.2018.08.024
®Irene García-Fernández	Disinfection of urban effluents using solar TiO_2 photocatalysis: A study of the significance of dissolved oxygen, temperature, type of microorganism and water matrix	1	13-Apr-18	doi.org/10.1016/j.cattod.2014.03.026
Gagik Badalians Gholikandi	Disinfection process intensification of treated municipal wastewater employing peroxymonosulfate-UV advanced oxidation process and simultaneous amoxicillin micropollutant removal	3	20-Feb-22	doi.10.5004/dwt.2022.28349
Stefanos Giannakis	Solar light and the photo-Fenton process against antibiotic-resistant bacteria in wastewater: A kinetic study with a Streptomycin-resistant strain	3	24-Oct-18	doi.org/10.1016/j.cattod.2017.10.033
P. Ganesh Kumar	Removal of persistent organic pollutants and disinfection of pathogens from secondary treated municipal wastewater using advanced oxidation processes	24	1-Jan-22	doi.org/10.2166/wst.2022.308
Qianlinglin Qiu	Removal of antibiotic-resistant microbes by Fe (II)-activated persulfate oxidation	1	18-Apr-20	doi.org/10.1016/j.jhazmat.2020.122733
Andrea Di Cesare	Combination of flow cytometry and molecular analysis to monitor the effect of UVC/H_2O_2 vs UVC/H_2O_2/Cu-IDS processes on pathogens and antibiotic-resistant genes in secondary wastewater effluents	2	16-Jul-20	doi.org/10.1016/j.watres.2020.116194
Idil Arslan Alaton	Elimination of antibiotic resistance in treated urban wastewater by iron-based advanced oxidation processes	5	10-Sep-19	doi.10.5004/dwt.2019.24929

SE; *Studies extracted*
PY; *Publication year*
®; Studies with pathogen regrowth data

2.3.2 Characteristics of the included studies

2.3.2.1 Widely applied AOPs

The analysis of the extracted literature revealed a predominant focus on three main AOP-based effluent treatments: ozone-based AOPs, photolysis and photocatalytic-based AOPs, and combined/hybrid AOPs. Among the 67 studies analyzed, 11 were identified as ozone-based AOPs, 20 focused on photolysis-based AOPs, and 21 delved into combined/hybrid AOPs, showcasing the extensive research attention directed toward these treatment methods. The remaining 15 studies, categorized as "other AOPs" (**Figure 2.2**), contained diverse AOP studies with low entries that could not be scientifically comparable. They included peroxidation, peroxyfenton, electrocoagulation, and iron-activated persulfate.

These findings suggest that ozone-based, photolytic and photocatalytic-based, and combined/hybrid AOPs were explored more than the other AOPs. Their operational parameters, treatment mechanisms, and environmental impacts have been extensively studied in the past five years, making them suitable candidates for detailed analysis and comparisons. This level of scrutiny and research focus underscores the significance of these AOPs in contemporary wastewater treatment strategies, highlighting their potential for further advancements and optimization in sustainable effluent treatment practices.

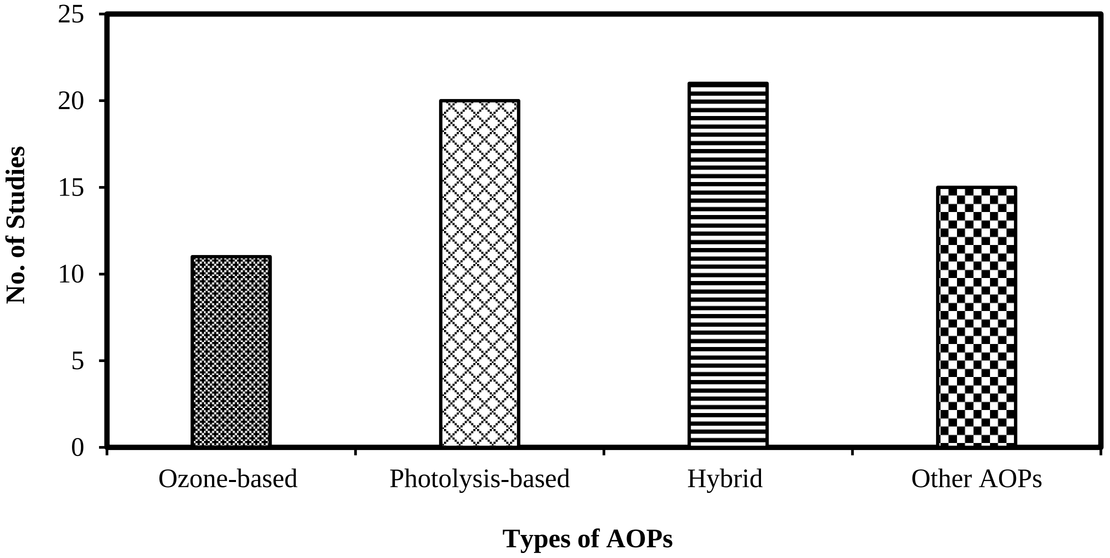

Figure 2.2: Grouped data of the AOPs studied.

2.3.2.2 Pathogens commonly studied

A comprehensive analysis of pathogen data revealed a total of 113 data points. *Escherichia coli* and total coliforms stood out with 35 and 60 data entries, respectively, indicating a significant emphasis on these pathogens. Additionally, *Enterococcus spp* was examined in 10 data points, with *Salmonella spp* and *Pseudomonas aeruginosa* scoring 2 entries each, suggesting less attention than *E. coli* and total coliforms. Notably, some pathogens were relatively under-studied, such as *S. enterica, S. aureus, E. faecalis,* and *Listeria monocytogenes*, each represented by a single entry in the data (**Figure 2.3**). Only pathogens with large data entries above 10 (*E. coli* and total coliforms) were used in the study to draw unbiased comparisons.

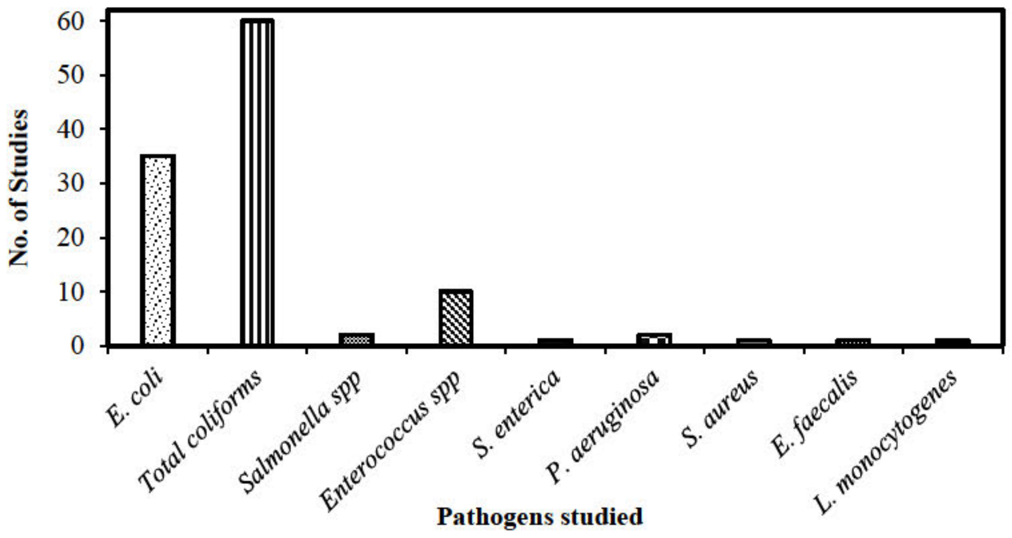

Figure 2.3: Number of research studies extracted corresponding to pathogen species.

The extensive focus on *E. coli* and total coliforms in wastewater management reflects their role as reliable indicators of fecal contamination and potential health risks (Holcomb and Stewart, 2020; Li et al., 2021). These organisms are crucial markers for assessing wastewater's microbiological quality and efficacy of treatment processes (Devane et al., 2020; Holcomb and Stewart, 2020). Regulatory standards, such as those set by the Environmental Protection Agency (EPA), mandate limits on *E. coli* and Total coliform levels in treated wastewater, driving the need for thorough monitoring and compliance (Adams et al., 2023; Schellenberg et al., 2020; Summerlin et al., 2021). Beyond regulatory requirements, the presence of these bacteria in untreated or inadequately treated wastewater poses notorious significant health concerns, including gastrointestinal illnesses and infections, necessitating comprehensive research and mitigation strategies (Hong et al., 2018; Tariq and Mushtaq, 2023). Furthermore, studying *E. coli* and total coliforms provides insights into treatment efficiency, helping identify areas for improvement and optimization of protocols to enhance overall water quality (Shaikh and Birajdar, 2024; Waqar and Ali).

2.3.2.3 Pathogen regrowth study

Out of the 67 extracted datasets, only 3 studies provided additional information regarding the pathogen regrowth (**Figure 2.4**). Monitoring pathogen regrowth after AOP treatment of effluent ensures the effectiveness and reliability of AOPs in eliminating pathogens from wastewater (Fiorentino et al., 2018), which is important in safeguarding public health and environmental safety. Additionally, assessing regrowth potential provides insights into the stability and microbial dynamics of treated effluents during storage and distribution, helping to identify potential risks of pathogen resurgence in reclaimed water systems (Drigo et al., 2021). Moreover, it aids in developing targeted mitigation strategies and optimizing treatment processes to minimize health hazards associated with effluent reuse, particularly in agricultural irrigation, where exposure to pathogens can directly impact food safety and human health (Al-Hazmi et al., 2022; Fiorentino et al., 2018).

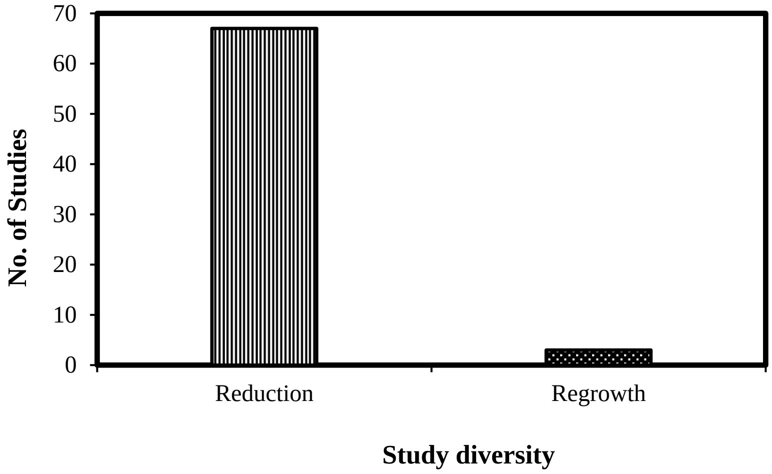

Figure 2.4: Reported studies on pathogen reduction and regrowth information.

2.3.3 Scrutiny of the potential of studied AOPs in pathogen removal

2.3.3.1 Ozone-based AOPs

Ozone-based AOPs offer various mechanisms for pathogen elimination in wastewater treatment. Their ability to directly oxidize pathogens, generate ROS, and induce secondary reactions makes them valuable tools in ensuring water safety and meeting regulatory standards for microbial quality. **Table 2.3** presents the potential of various ozone-based AOPs in reducing microbial contaminants (*E. coli* and total coliforms). Among these processes, ozone combined with adsorption (Granular Activated Carbon) (O_3-GAC- O_3) at a dosage of 24.5 mg.L^{-1} O_3 with a retention time of 60 min showed moderate potential, reducing *E. coli* by 6.31E+03 MPN.mL^{-1} and total coliforms by 6.17E+03 MPN.mL^{-1} (da Silva et al., 2021). Another promising approach was $O_3/H_2O_2/MnO_2$ treatment at 13 mg.L^{-1} O_3, 50 mg.L^{-1} MnO_2 and 10 mg.L^{-1} H_2O_2 for 10 minutes, which significantly reduced total coliforms by 8.13E+04 MPN.mL^{-1}. Notably, O_3/peroxymonosulfate (PMS) significantly reduced 1.00E+05 MPN.mL^{-1} of total coliforms using a lower ozone dosage of 13 mg.L^{-1} O_3 and 3 mg.L^{-1} PMS over 60 min (Ganesh Kumar and Kanmani, 2022). Catalytic ozonation, such as O_3/Fe^{2+}, O_3/Fe_2O_3, O_3/MnO_2, under different ozone dosages with varying retention times, also displayed varying degrees of efficacy in reducing microbial contaminants (**Table 2.3**).

Ozone (O_3) alone and O_3 with catalysts exhibit differing potentials in reducing microbial contaminants. O_3 alone, at a dosage of 13 mg.L^{-1} over 40 min, significantly reduced microbial counts, with *E. coli* and total coliforms by 1.95E+05 MPN.mL^{-1} and 6.17E+04 MPN.mL^{-1}, respectively (Ganesh Kumar and Kanmani, 2022). However, when ozone is combined with catalysts such as Fe^{2+}, Fe_2O_3, or MnO_2, varying degrees of improvement in microbial reduction are observed (**Table 2.3**). For instance, O_3/Fe^{2+} at a dosage of 13 mg.L^{-1} O_3 and 2.5 mg.L^{-1} Fe^{2+} over 60 min reduced *E. coli* by 7.76E+03 MPN.mL^{-1}, indicating a notable enhancement compared to O_3 alone (Ganesh Kumar and Kanmani, 2022). Similarly, O_3/Fe_2O_3 at 13 mg.L^{-1} O_3 and 25 mg.L^{-1} Fe_2O_3 over 30 min reduced *E. coli* by 1.17E+04 MPN.mL^{-1}, showcasing a moderate improvement (Ganesh Kumar and Kanmani, 2022). Additionally, O_3/MnO_2 at 13 mg.L^{-1} O_3 and 50 mg.L^{-1} MnO_2 dosage over 15 min exhibited the highest enhancement, reducing *E. coli* by 4.17E+04 MPN.mL^{-1}, surpassing the efficacy of O_3 alone (Ganesh Kumar and Kanmani, 2022).

Significant drawbacks have been noted, such as the potential formation of by-products when certain catalysts are employed. In ozone combined with Fenton processes (O_3/Fe^{2+} or O_3/Fe_2O_3), the presence of iron ions (Fe^{2+}/Fe^{3+}) or iron oxide as catalysts can lead to the formation of iron-based precipitates, necessitating additional filtration steps and potentially increasing operational complexities and costs (Lai et al., 2021; Ruan et al., 2021). Additionally, the stability and longevity of catalysts such as Fe_2O_3 and MnO_2 under continuous use need careful consideration, as degradation over time can impact the overall efficiency and performance of the AOP (Hamad et al.; Wang et al., 2020).

Table 2.3: Summary of the studied ozone-based AOPs on log reduction of pathogens (MPN.mL^{-1}).

Ozone-based AOPs	**Dosage**	**Retention time**	***E. coli***	**Total coliforms**
O_3-GAC-O_3	24.5 mg.L^{-1} O_3	60 min	6.31E+03	6.17E+03
O_3	13 mg.L^{-1} O_3	40 min	---	1.95E+05
O_3	13 mg.L^{-1} O_3	60 min	---	6.17E+04
O_3	24.5 mg.L^{-1} O_3	26 min	6.31E+03	6.31E+03
O_3/PMS	13 mg.L^{-1} O_3, 3 mg.L^{-1} PMS	60 min	---	1.00E+05
O_3/PS	13 mg.L^{-1} O_3, 1 mg.L^{-1} PS	60 min	---	3.98E+05
O_3/Fe_2O_3	13 mg.L^{-1} O_3, 25 mg.L^{-1} Fe_2O_3	30 min	---	1.17E+04
$O_3/H_2O_2/Fe^{2+}$	13 mg.L^{-1} O_3 + 10 mg.L^{-1} Fe^{2+} +10 mg.L^{-1} H_2O_2	10 min	---	3.98E+04
$O_3/H_2O_2/Fe_2O_3$	13 mg.L^{-1} O_3 + 50 mg.L^{-1} Fe_2O_3 +10 mg.L^{-1} H_2O_2	10 min	---	1.91E+03
$O_3/H_2O_2/MnO_2$	13 mg.L^{-1} O_3 + 50 mg.L^{-1} MnO_2 +10 mg.L^{-1} H_2O_2	10 min	---	8.13E+04

GAC; Granular activated carbon
PMS; Peroxymonosulphate
PS; Persulfate

2.3.3.2 Photolytic and photocatalytic-based AOPs

Photolytic and photocatalytic-based AOPs are crucial in pathogen elimination within wastewater treatment contexts. Photolysis involves the breakdown of compounds induced by light, particularly ultraviolet (UV) radiation (Wypych, 2020), whereas photocatalysis involves the use of catalysts alongside UV (Brooms et al., 2018). When applied in AOPs, photolysis can result in the formation of reactive oxygen species (ROS), primarily hydroxyl radicals (•OH), which are highly effective in disinfecting water by damaging microbial structures and disrupting cellular functions (Brooms et al., 2018; Huang et al., 2020).

Table 2.4 shows the evaluated photolytic and photocatalytic-based AOPs and their potential to reduce bacterial load in wastewater. Among the tested AOPs, solar/H_2O_2 treatment at a dosage of 20 mg.L^{-1} with a retention time of 20 min resulted in a reduction of *E. coli* by 5.01E+01 MPN.mL^{-1} and total coliforms by 3.98E+01 MPN.mL^{-1}. Conversely, a similar solar/H_2O_2 treatment at 20 mg.L^{-1} of H_2O_2 with an extended retention time of 90 min achieved a notable reduction of total coliforms by 1.00E+06 MPN.mL^{-1}. Further experiments using solar-photo-Fenton processes demonstrated varying potentials. At 20 mg.L^{-1} H_2O_2 and 10 mg.L^{-1} Fe^{2+} within 10 min, reduced *E. coli* by 1.00E+01 MPN.mL^{-1} and total coliforms by 3.98E+01 MPN.mL^{-1}. Conversely, 20 mg.L^{-1} H_2O_2 and 1 mg.L^{-1} Fe^{2+} with a 60-min retention time significantly reduced total coliforms by 1.00E+06 MPN.mL^{-1}.

Comparing the efficiency of various catalysts in photocatalytic-based AOPs in reducing bacterial contaminants revealed distinct performance variations. UV-C/H_2O_2 and UV/Fe^{2+} were the most efficient, significantly reducing *E.coli* and total coliforms within relatively short treatment periods (**Table 2.4**). UV-C/H_2O_2, notably, achieved reductions of approximately 7.24E+07 MPN.mL^{-1} for total coliforms in just 20 min, showcasing a high potential in bacterial deactivation. In contrast, Solar/Fe^{2+} with 1 mg.L^{-1} Fe^{2+} and UV/PS with 3 mmol.L^{-1} PS exhibited comparatively lower potential, achieving moderate reductions within longer treatment durations.

The efficacy of photolytic and photocatalytic-based AOPs in pathogen elimination depends on several factors, such as the intensity and wavelength of the light source, the presence of catalysts, the duration of exposure, and pathogen type (Reis-Mansur et al., 2019; Wypych, 2020). UV light with wavelengths in the germicidal range (around 254 nm) shown potential in inactivating pathogens by damaging their nucleic acids and proteins (Horton et al., 2020). Additionally, introducing photocatalysts can enhance ROS generation and extend the range of targetable pathogens (Zhou et al., 2021), making photolytic and photocatalytic-based AOPs diverse tools for wastewater disinfection in various settings.

Table 2.4: Summary of the studied photolytic and photocatalytic-based AOPs on log reduction of pathogens (MPN.mL^{-1}).

Photolysis-based AOPs	Catalyst dosage	Retention time	*E. coli*	Total coliforms
solar/H_2O_2	20 mg.L^{-1}	20 min	5.01E+01	3.98E+01
solar/H_2O_2	20 mg.L^{-1}	90 min	---	1.00E+06
Solar-photo-Fenton	20mg.L^{-1} H_2O_2,10mg.L^{-1} Fe^{2+}	10 min	1.00E+01	3.98E+01
solar photo-Fenton	20 mg.L^{-1} H_2O_2, 1 mg.L^{-1} Fe^{2+}	60 min	---	1.00E+06
Solar/Fe^{2+}	1 mg.L^{-1}	60 min	---	3.16E+00
UV/Fe^{2+}	15 mg.L^{-1}	15 min	---	2.75E+06
UV/Fe_2O_3	75 mg.L^{-1}	20 min	---	1.17E+04
UV/Fe^{2+}	2.8 mg.L^{-1}	60 min	4.90E+00	---
UV/H_2O_2	340.2 mg.L^{-1}	60 min	---	2.00E+06
UV/H_2O_2	31.9 mg.L^{-1}	60 min	---	6.31E+03
UV/H_2O_2	31.9 mg.L^{-1}	20 min	---	2.95E+00
UV-A/H_2O_2	50 mg.L^{-1}	20 min	---	2.75E+03
UV-C/ H_2O_2	50 mg.L^{-1}	20 min	---	7.24E+07
UV/O_3	13 mg.L^{-1}	30 min	---	2.00E+05
UV-C/PMS	0.5 mmol.L^{-1}	15 min	---	1.00E+02
UV/PS	3 mmol.L^{-1}	30 min	---	3.89E+00
UV/PMS	0.06 mmol.L^{-1}	30 min	---	3.98E+05
UV/PMS	0.5 mmol.L^{-1}	40 min	---	2.82E+00
UV/PS	3 mmol.L^{-1}	60 min	---	6.92E+03
UV/TiO_2	10 mg.L^{-1}	30 min	---	4.90E+05

PMS; Peroxymonosulphate
PS; Persulfate

2.3.3.3 Combined AOPs (Hybrid)

Combined AOPs, also known as hybrid AOPs, offer a promising approach for pathogen elimination in water and wastewater treatment (Babuponnusami et al., 2023; Fedorov et al., 2022). These hybrid systems integrate multiple AOPs with other treatment methods to enhance the removal efficiency of pathogens (Dhangar and Kumar, 2020). Among the tested methods, UV-A/Fe^{2+}/H_2O_2 treatment with 1 g.L^{-1} Fe^{2+} and 20 mg.L^{-1} H_2O_2 for 80 minutes reduced total coliforms by 6.61E+04 MPN.mL^{-1} (**Table 2.5**). These results highlight the potential of these hybrid AOPs in microbial disinfection, with varying capabilities based on the specific treatment parameters.

In contrast, some combinations showed limited potential against microbial contaminants. For instance, UV coupled to graphitic carbon nitride (GCN) and silver chromate (UV/GCN/Ag_2CrO_4) treatment with 1 mg Ag_2CrO_4.mL^{-1} effluent for 60 minutes yielded negligible reductions in *E. coli* and total coliforms. Similarly, UV/MnO_2/H_2O_2 treatment at 50 mg.L^{-1} MnO_2 and 10 mg.L^{-1} H_2O_2 for 10 minutes did not demonstrate significant microbial reduction (**Table 2.5**).

The hybrid AOPs exhibited notable strengths and limitations. On the positive side, these methods demonstrate high efficacy in reducing bacterial contaminants like *E. coli* and total coliforms, with some processes achieving substantial reductions even within relatively short treatment times (Ahmad et al., 2020; Alaton et al., 2019; Berruti et al., 2021; Fiorentino et al., 2018; Kumar and Kanmani, 2022). The diversity of AOP combinations allows for tailored approaches, providing versatility in addressing various water treatment challenges (Dhangar and Kumar, 2020; Kumar and Kanmani, 2022). However, challenges such as cost implications due to expensive reagents or catalysts (Jafarinejad, 2019), complexity in implementation requiring specialized equipment and expertise (Coha et al., 2021), and potential formation of harmful by-products or residuals necessitate careful consideration when selecting and designing AOP-based treatment strategies.

Table 2.5: Summary of the studied hybrid AOPs on log reduction of pathogens (MPN.mL^{-1}).

Hybrid AOPs	Dosage	Retention time	*E. coli*	Total coliforms
Co-CAS	50 mg.L^{-1} CAS	60 min	5.01E+06	---
UV/Fe^{2+}/H$_2$O$_2$	2.8 mg.L^{-1} Fe^{2+}/340.2 mg.L^{-1} - H$_2$O$_2$	30 min	2.00E+06	---
UV/Fe^{2+}/H$_2$O$_2$	2.8 mg.L^{-1} Fe^{2+}/340.2 mg.L^{-1} - H$_2$O$_2$	60 min	7.41E+01	---
UV/N-doped MgO@Fe$_3$O$_4$	150 mg.L^{-1} catalyst	15 min	1.00E+06	---
UV/GCN/Ag$_2$CrO$_4$	1mg Ag$_2$CrO$_4$.mL^{-1} effluent	60 min	1.00E+00	6.31E+00
O$_3$/H$_2$O$_2$-GAC-O$_3$/H$_2$O$_2$	24.5 mg.L^{-1} O$_3$ + 75.3 mg.L^{-1} H$_2$O$_2$	60 min	6.31E+03	6.31E+03
UV/H$_2$O$_2$-GAC-UV/H$_2$O$_2$	24.5 mg.L^{-1} O$_3$ + 31.9 mg.L^{-1} H$_2$O$_2$	60 min	6.31E+03	6.31E+03
UV-C/H$_2$O$_2$/Cu^{2+}-IDS	50 mg.L^{-1} H$_2$O$_2$ + 0.5 mg.L^{-1} IDS	10 min	7.94E+02	---
UV-C/H$_2$O$_2$/Cu^{2+}	50 mg.L^{-1} H$_2$O$_2$ + 0.5 mg.L^{-1} Cu^{2+}	10 min	7.94E+02	---
UV-C/H$_2$O$_2$/Fe^{2+}	50 mg.L^{-1} H$_2$O$_2$ + 0.5 mg.L^{-1} Fe^{2+}	10 min	1.26E+03	---
H$_2$O$_2$/IDS-Cu^{2+}	50 mg.L^{-1} H$_2$O$_2$ + 0.5 mg.L^{-1} IDS	10 min	1.26E+03	---
UV/MnO$_2$/H$_2$O$_2$	50 mg.L^{-1} MnO$_2$ +10 mg.L^{-1} H$_2$O$_2$	10 min	---	2.51E+04
UV/H$_2$O$_2$/Fe^{2+}	5 mg.L^{-1} Fe^{2+} +10 mg.L^{-1} H$_2$O$_2$	10 min	---	2.82E+06
UV/H$_2$O$_2$/Fe$_2$O$_3$	50 mg.L^{-1} Fe$_2$O$_3$ +10 mg.L^{-1} H$_2$O$_2$	10 min	---	1.20E+04
UV/H$_2$O$_2$/TiO$_2$	35 mg.L^{-1} TiO$_2$ +10 mg.L^{-1} H$_2$O$_2$	10 min	---	1.17E+04
UV/O$_3$/Fe^{2+}	13 mg.L^{-1} O$_3$ + 10 mg.L^{-1} Fe^{2+}	10 min	---	2.40E+04
UV/O$_3$/Fe$_2$O$_3$	13 mg.L^{-1} O$_3$ + 25 mg.L^{-1} Fe$_2$O$_3$	10 min	---	2.40E+04
UV/O$_3$/MnO$_2$	13 mg.L^{-1} O$_3$ + 25 mg.L^{-1} MnO$_2$	10 min	---	2.40E+04
UV-C/H$_2$O$_2$/Cu^{2+}-IDS	50 mg.L^{-1} H$_2$O$_2$ + 6.2 mg.L^{-1} Cu^{2+}-IDS	20 min	3.50E+00	---
UV-A/Fe^{2+}/H$_2$O$_2$	1 g.L^{-1} Fe^{2+} + 20 mg.L^{-1} H$_2$O$_2$	80 min	---	6.61E+04
UV-A/Fe^{3+}/H$_2$O$_2$	1 g.L^{-1} Fe^{3+}+ 20 mg.L^{-1} H$_2$O$_2$	80 min	---	7.08E+03
UV/O$_3$/H$_2$O$_2$	9 mg/min O$_3$ + 20 mg.L^{-1} H$_2$O$_2$	30 min	---	1.00E+04

IDS; Iminodisuccinic acid,
CAS; Alumino-copper silicate
GCN; Graphitic carbon nitride

2.3.4 Potential challenges in preventing regrowth in AOP-treated effluents

Despite AOPs being powerful oxidants with strong antimicrobial properties in a wide range of pathogens, they face challenges in preventing pathogen regrowth in treated wastewater (Babuponnusami et al., 2023; Miller et al., 2020). Pathogens with adaptive mechanisms, such as those capable of upregulating stress response proteins like *E. coli* (Hews et al., 2019) and *Salmonella spp* (Aguas et al., 2019), demonstrated increased resilience to photolytic and photocatalytic-based AOPs (**Table 2.4**). Spore-forming pathogens like *Listeria monocytogenes* showed resistance to ROS (Abou Hammad et al., 2020). These pathogens can survive and proliferate in post-treated effluents due to their resistant structures and protective mechanisms (Michael-Kordatou et al., 2018; Vilela et al., 2022; Ye et al., 2022).

Moreover, AOPs can disrupt pathogen biofilms due to their oxidative effects (Duan et al., 2021; Luo et al., 2022). However, incomplete removal of disrupted biofilms can still occur, like in the case of photolytic and photocatalytic-based AOPs, contributing to regrowth risks (Monteoliva-García et al., 2020). During AOPs, such as peroxyfenton, peroxidation, and iron-activated persulfate, organic pollutants in wastewater are mineralized. As a result, some residual organic matter (ROM) were noted to remain after treatment, providing nutrients to surviving pathogens and leading to regrowth challenges (Babuponnusami et al., 2023; Chandra et al., 2018; Reis et al., 2019). Furthermore, the production of disinfection by-products (DBPs) by ozone-based AOPs, such as aldehydes (Chen and Wang, 2012) and organic acids (Tak and Vellanki, 2018) were noted. These DBPs are reported to have variable antimicrobial activity, potentially influencing microbial interactions and regrowth dynamics within treated effluents (Agbaba et al., 2016; Mayer and Ryan, 2019). Factors like temperature and pH influenced respective AOPs' efficacy in pathogen control and regrowth prevention (García-Fernández et al., 2015; Starling et al., 2021).

2.3.5 Challenges associated with the reuse of AOP-treated effluents

Recent AOP studies have focused extensively on optimizing catalysts to enhance effluent treatment efficiency (Ahmed et al., 2020b; Akbari et al., 2022; Fiorentino et al., 2018). However, the presence of persisting catalysts in AOP-treated effluents influences these treatment methods' efficacy, environmental impact, and reuse purposes (Asgari et al., 2022). Catalysts like TiO_2 (Afzal et al., 2019), iron-based catalysts (Vilela et al., 2022), metal-organic frameworks (MOFs) (Liu et al., 2022), and carbon-based materials play a significant role in AOPs by promoting ROS generation and facilitating contaminant degradation (Antonio da Silva et al., 2021). As a result, even after the treatment process, these catalysts can persist in the effluent, contributing to their continued reactivity upon reuse or discharge into the environment (Leifeld et al., 2018).

TiO_2 catalysts in photocatalytic AOPs persisted in the effluent and continued to exhibit photocatalytic activity, enabling the degradation of contaminants upon exposure to sunlight or UV radiation in natural water bodies (Kanan et al., 2019). Similarly, iron-based catalysts used in Fenton reactions were reported to persist in the effluent, retaining their ability to generate hydroxyl radicals (•OH) under suitable conditions (Matavos-Aramyan and Moussavi, 2017; Shaida et al., 2023). MOFs and carbon-based materials enhanced the persisting catalytic activity in treated effluents, offering sustained pollutant removal capabilities beyond the treatment facility (John et al., 2022; Xia et al., 2023). While these catalysts contribute to effective pollutant removal during treatment, their post-treatment presence highlights the importance of understanding their fate and potential impacts on receiving water bodies or upon reuse.

2.3.6 Concerns of AOP catalysts and by-products on crop production

Recent research findings highlight the diverse effects of residual AOP catalysts on soil biota and nutrient cycling processes. Metal nanoparticles from photocatalysis-based AOPs, such as Zn and Fe, are reported to accumulate in the soil over time, potentially impacting soil biota and nutrient cycling processes (Khan et al., 2021; Rajput et al., 2021). While some photocatalysts, like Zn, Fe, and Mn, contribute to nutrient accumulation and growth stimulation in plants, others, like Ag, facilitate the uptake of toxic elements or lead to residue accumulation in soil and plant tissues (Ulhassan et al., 2022). Furthermore, the soil accumulation of Zn, Fe, and Mn alters plant metabolism, influencing biochemical pathways and potentially causing oxidative stress (Ali et al., 2019; Zhang et al., 2020).

The Advanced Oxidation Process (AOP) generates by-products like ROS and residual chemicals that can significantly impact crops when effluents treated with AOP are used for irrigation (Akl et al., 2020; Chen et al., 2021). Reactive oxygen species such as superoxide radicals (O_2^-), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2) can cause phytotoxicity, affecting plant cell membranes and photosynthesis while also altering soil microbial populations crucial for nutrient cycling (da Silva et al., 2022; López et al., 2018; Zhang et al., 2022). Additionally, ROS generated during AOPs is linked to soil redox conditions, affecting the availability of nutrients like nitrogen and phosphorus for plant uptake (Usman et al., 2020). Persistent ozone-based AOP by-products, such as aldehydes and organic acids, may accumulate in soil or crop tissues, potentially affecting soil health and crop quality (Tiwari et al., 2020). Changes in soil pH due to AOP by-products are reported to influence nutrient availability and plant growth, while residual chemicals like chlorinated compounds pose risks of chemical contamination (An et al., 2022; Wang et al., 2021).

2.3.7 Potential human health concerns associated with AOP catalysts

The current study reports the use of photocatalysts such as TiO_2, iron (Fe^{2+} and Fe^{3+}), MnO_2, and Ag in wastewater treatment (**Table 2.3**, **Table 2.4**, and **Table 2.5**). While effective in treating contaminants in water, these photocatalysts can also introduce human health risks if not properly managed, especially in crop irrigation scenarios. Studies suggest that TiO_2 nanoparticles may cause adverse effects on human health upon inhalation or ingestion of significant quantities (Baranowska-Wójcik et al., 2020; Grande and Tucci, 2016). Similarly, iron (Fe^{2+} and Fe^{3+}) and silver (Ag) ions can pose health risks if present in irrigation water in elevated concentrations. Iron ions, for instance, are essential micronutrients for plants and humans, but excessive intake can lead to micronutrient toxicity and gastrointestinal issues, respectively (Gurzau et al., 2003; Mehri, 2020). Silver ions, known for their antimicrobial properties, can cause argyria in humans if consumed in excessive quantities (Lelievre et al., 2021; Medici et al., 2019). Therefore, supplying these photocatalysts in crop irrigation scenarios requires careful consideration to minimize potential human health effects (Zeinali et al., 2019).

2.4 Future Research Directions

While this state-of-the-art review assessed the extent and understanding of AOPs in reducing pathogens and potential regrowth, it also pinpoints potential gaps that need further investigation. Forming an assumption that a similar study with the same trends exists in all other languages apart from English, this study relied on peer-reviewed journals published in English. Subsequently, this may have excluded valuable insights from other sources, such as the grey literature (project reports, academic theses, and conference proceedings) and articles published in other languages. Even though peer-reviewed articles are regarded as an assurance of quality (Horbach and Halffman, 2018; Wicherts, 2016), future studies could consider and incorporate relevant information from a wider range of sources and databases, including wider selection criteria besides peer-reviewed journals in English only.

In the current study, only 3 out of 67 datasets have information on understanding the crucial aspect of pathogen regrowth dynamics (**Figure 2.4**). Future studies focused on the simultaneous reduction of organics alongside pathogens are needed to optimize the efficacy of AOPs. This could be achieved by adjusting AOP parameters or integrating them into complementary treatment processes like the decentralized wastewater treatment systems (DEWATS) with reduced organic levels as a post-treatment procedure (da Silva et al., 2021). Experimental studies focusing on these aspects can yield valuable insights into optimizing AOP performance, especially in scenarios with varying organic loads commonly found in wastewater effluents.

The present study has revealed the resistivity of *E.coli* in a wide range of the AOPs studied. In some studies, *E.coli* was reduced, although with incomplete elimination (Aguas et al., 2019; Ahmed et al., 2020b; da Silva et al., 2021). In future studies, *E.coli*, which is hard to remove, should be prioritized to achieve the required limits for agricultural reuse.

While the present study shows the varied potential reduction of pathogens by these AOPs, a clear trend pointing to the best AOP offering complete pathogen degradation and regrowth elimination is lacking. Therefore, a study is needed to simultaneously compare the efficacy of ozone-based AOPs, photolysis and photocatalytic-based AOPs, and hybrid AOPs in complete pathogen oxidation and eliminating the pathogen regrowth potential in the treated effluents.

Since there is a pressing need for well-treated effluents in agriculture (Sdg, 2019), a notable research gap exists regarding the potential bioaccumulation effects of catalysts used in AOP-treated effluents for crop production (Bracamontes-Ruelas et al., 2022). A comprehensive assessment of the impacts of AOP-treated effluents on agricultural systems is essential to determine potential risks such as bioaccumulation of catalysts in edible plant tissues, changes in soil biota composition, and alterations in nutrient mineralization processes. Investigating these aspects will address the immediate concerns of human health risks associated with bioaccumulation and provide a more holistic understanding of the implications of AOPs in wastewater treatment and their integration into sustainable agricultural practices.

2.5 Conclusions

Pathogen reduction and eliminating regrowth in wastewater are crucial for meeting stringent agricultural reuse standards. Only a few studies highlighted pathogen regrowth in AOP-treated effluents, underscoring the need for further investigation. High organic content hindered complete pathogen reduction and promoted regrowth by providing nutrients to surviving pathogens. By-products from AOP treatment, such as catalysts, reactive oxygen species (ROS), and aldehydes, have raised concerns about environmental and human health risks in wastewater reuse. These by-products can impact soil organisms, nutrient cycling, and plant metabolism in agriculture. Upon accumulation in crop produce, these by-products, such as catalysts, could pose risks to human health when ingested as food. Addressing these gaps (pathogen regrowth, by-product formation, and their impacts on crop, soil, and human health risks) can make AOP-treated effluents valuable for crop production. In a circular economy framework, recovering water and nutrients from wastewater for agricultural use is crucial for sustainable resource management.

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CHAPTER 3: MUNICIPAL ANAEROBIC FILTER EFFLUENT TREATMENT USING ADVANCED OXIDATION PROCESSES FOR POTENTIAL USE IN UNRESTRICTED CROP PRODUCTION

Abstract

Advanced treatment technologies have been considered to meet quality standards for unrestricted crop production. One promising avenue is the integration of advanced oxidation processes (AOPs) with Decentralized Wastewater Treatment Systems (DEWATS). This study sought to optimize the efficacy of selected AOPs (ozonolysis, UV photolysis, UV/TiO_2 photocatalysis) for polishing the anaerobic filter (AF) effluent from DEWATS for unrestricted agricultural use as an alternative to the constructed wetlands. Metrics evaluated included pathogen reduction efficiency, post-disinfection regrowth, and the treatment effects on the physical parameters (pH, EC, and turbidity), organic matter (soluble COD, BOD, DOC, humic), and nutrient concentration (ammonium, nitrates, and ortho-P). Ozonolysis and TiO_2 photocatalysis attained a 6.4-log pathogens reduction, whereas UV photolysis recorded a 6-log pathogen reduction. Ozonolysis samples showed no pathogen regrowth, whereas TiO_2 photocatalysis registered *E. coli* and Total coliforms regrowth of 2.5-log and 2.7-log, respectively. UV photolysis registered 0.5-log and 2.2-log regrowth for *E. coli* and Total coliforms, respectively. TiO_2 photocatalysis significantly reduced the BOD, soluble COD (sCOD), humic substances, ortho-P, turbidity, and nitrates while increasing the pH, EC, ammonium, and DOC. Ozonolysis significantly lowered BOD, sCOD, humics, and turbidity; however, it increased ortho-P, nitrates, pH, EC, ammonium, and DOC. UV photolysis resulted in marginal reductions in BOD, nitrates, and turbidity with increased EC, pH, ammonium, DOC, ortho-P, and humic levels. TiO_2 photocatalysis lowered the concentrations of Al, Fe, Mn, and Zn in the treated effluent. Overall, ozonolysis was the best AOP, demonstrating an efficient effluent treatment rate with no pathogen regrowth.

Keywords: Advanced oxidation processes; Anaerobic filter effluent; Ozonolysis; TiO_2 photocatalysis; UV photolysis

3.1 Introduction

With stringent pathogen standards to ensure safe water resources for sustainable agricultural practices and other uses, the advancement of wastewater treatment technologies is of utmost significance (Periasamy and Sundaram, 2013). While effective to a certain extent, conventional wastewater treatment methods often fall short of fully mitigating the diverse range of contaminants present in domestic wastewater (Lourenço and Nunes, 2020; Singh et al., 2019; Späth et al., 2021). As a result, inadequately treated wastewater can pose significant risks to human health and the environment, particularly when used to irrigate crops (Henze and Comeau, 2008; Okeyo et al., 2018; Pal et al., 2018). The pressing need to bridge this gap and establish a more comprehensive approach to wastewater treatment has prompted researchers to explore alternative methods (Periasamy and Sundaram, 2013). One promising avenue in this pursuit is the integration of Advanced Oxidation Processes (AOPs) with Decentralized Wastewater Treatment Systems (DEWATS). Merging these innovative techniques offers a potential solution to enhance the efficacy of treating domestic wastewater and achieving safety standards conducive to unrestricted crop production (Kokkinos et al., 2021).

DEWATS employs constructed wetlands to polish the effluent obtained from the anaerobic filter (AF) (Mladenov et al., 2018). These constructed wetlands have been reported to take longer to polish the effluent and do not achieve complete elimination of pathogens and emerging contaminants like pharmaceuticals (Gearheart, 1999; Späth et al., 2021; Wu et al., 2016). The constructed wetlands are land intensive and would not fit the overpopulated areas with limited land (Ilyas and Masih, 2017). Moreover, their treatment efficiency reduces over time (Kadlec, 2016) AOPs use strong oxidizing agents such as hydroxyls (OH-), hydrogen peroxides (H_2O_2), and superoxides (O_2^-) to degrade organic and inorganic compounds, including pathogens, into simple products such as water and carbon dioxide in a short period (Ariunbaatar et al., 2014; Santos et al., 2007; Tufail et al., 2021). The AOPs currently used, such as ozonolysis, UV light photolysis, and TiO_2 photocatalysis, have several advantages over treatment methods like adsorption, biological, and ultrafiltration, which would generally result in the production of sludge or necessitating further treatment processes (Santra et al., 2020; Srivastava et al., 2022). These advantages include (i) the ability to reduce turbidity, odor, taste, and color, (ii) improved decomposition of several pollutants at low temperatures and basic pH, (iii) reduction of waterborne pathogens, (iv) oxidation of organic and inorganic compounds, and (v) environmental friendliness in wastewater treatment which minimizes the effects on the downstream processes (Kumar and Shah, 2021; Sangave et al., 2007; Takashina et al., 2018). By incorporating these processes within the framework of DEWATS, there is a potential to improve the removal efficiency of pathogens and other contaminants and thereby enable the production of treated wastewater that adheres to safety standards suitable for irrigating crops intended for consumption (Sangave et al., 2007). Moreover, the use of AOPs can hasten the polishing step and eliminate the need for land-intensive wetlands.

In light of this context, this study aimed to investigate the extent to which AOPs can be used to enhance the performance of DEWATS technology as a polishing step in place of the constructed wetlands. The ultimate goal was to achieve a level of treated wastewater quality that complies with discharge standards and allows for unrestricted use in crop production. The study investigated the effect of ozonolysis, UV light photolysis, and TiO_2 photocatalysis on the reduction of pathogen load (*Escherichia coli* and Total coliforms) and wastewater quality parameters (soluble chemical oxygen demand, humic concentration, biochemical oxygen demand, dissolved organic carbon, pH, electrical conductivity and turbidity), on the DEWATS AF effluent. Also, pathogens regrowth was evaluated to investigate the potential challenges in maintaining microbes upon storing the AOPs-treated effluents for days before use.

3.2 Materials and instrumentation

3.2.1 Chemicals

Titanium dioxide (P-25, nanopowder, ≥99.5%, primary particle size: 21 nm) used as a photocatalyst, Sodium thiosulfate, starch, sodium hydroxide, potassium iodide (KI), and hydrochloric acid were purchased from Merck (Pty) Ltd. (South Africa). All chemicals used were of laboratory grade.

3.2.2 Wastewater source and the experimental site

Wastewater was collected after the anaerobic filter (AF) in DEWATS (a local plant in Newlands Mashu, Durban-South Africa). Domestic wastewater from about 84 households in Newlands Mashu was collected and directed to the DEWATS plant for treatment. The plant employs three treatment steps: (i) settling chambers, (ii) three parallel Anaerobic Baffled Reactor trains (ABR), (iii) two AF modules, and (iv) Vertical Flow Constructed Wetland (VFCW) and Horizontal Flow Constructed Wetland (HFCW) to polish the effluent. The wastewater was then transported and stored at 4^0C at the University of Kwazulu-Natal in Durban.

3.2.3 Experimental procedure

Ozonolysis was conducted in a fluidized reactor at a constant ozone dosage of 90 mg per minute. The reactor was 6.6 cm long and 4 cm in radius. Ozone gas was generated from the air (source of oxygen) using an ozone generator (AquaZO^{3}NE 5G), which was coupled to a JUN-AIR air compressor (Model OF302-25B; air displacement 3.81CFM). The air compressor’s flow meter was set at 5 LPM. The generated ozone was then bubbled through the reactor containing the substrate (AF effluent) from the bottom through a gas diffuser. The exhaust gas from the reactor was passed through a conical flask containing 100 ml of 0.2M potassium iodide (KI) solution to capture unreacted ozone. A schematic representation of the ozonolysis unit is given in **Figure 3.1**(**a)**. The ozone content was determined using a titrimetric method with potassium iodide (KI) solution, while the quantity of O_3 consumed was determined by subtracting the amount supplied from the amount that did not react (Otieno and Apollo, 2021; Venkatesh et al., 2015).

For photolysis and photocatalysis, a 2-liter UV-Vis photoreactor connected to a peristaltic pump for feeding and fluidization was used (Yusoff et al., 2018). During photolysis, 2 L of the AF effluent was pumped into the reactor and then subjected to photolysis. For TiO_2 photocatalysis, TiO_2 nanoparticles were first added to 2L of the AF effluent at a catalyst loading of 1 $g.L^{-1}$ and allowed to attain adsorption-desorption equilibrium for 30 minutes. Thereafter, the substrate was transferred to the UV reactor for photocatalytic treatment. A schematic representation of the photolysis and photocatalytic unit is given in **Figure 3.1(b)**. During the AOP treatment, samples were withdrawn and analyzed at predetermined time intervals.

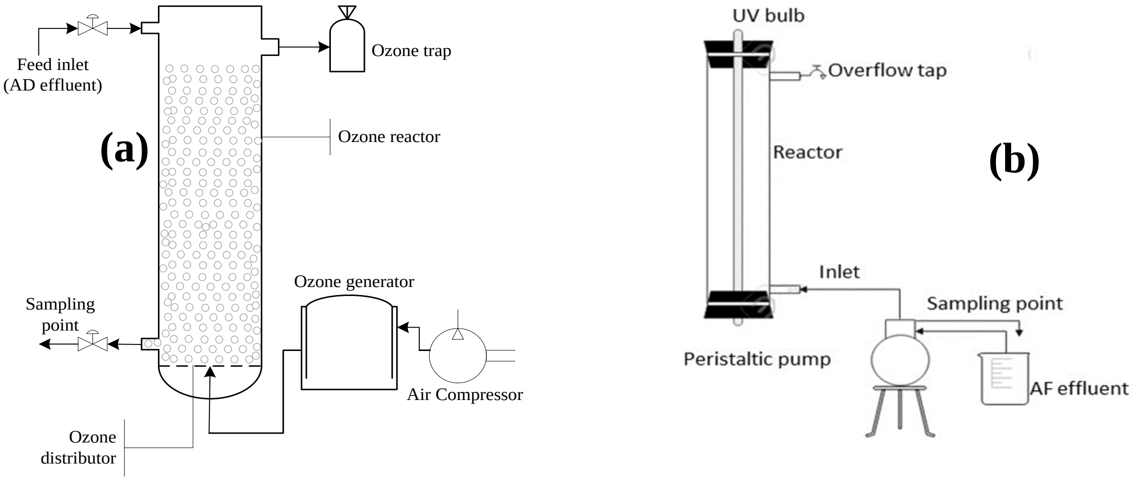

Figure 3.1: Schematic representation of the (a) ozonolysis and (b) UV photolysis and TiO_2 photocatalysis units.

3.2.4 Chemical analysis

Samples were analyzed for pH, electrical conductivity (EC), soluble chemical oxygen demand (sCOD), dissolved organic carbon (DOC), biochemical oxygen demand (BOD), and turbidity as per the standard methods of analysis (Baird et al., 2017). The culture-based microbiological analysis method with Most Probable Number (MPN) reporting was used to analyze the *E. coli* and total coliforms (Velkushanova et al., 2021). UV-Vis absorption spectroscopic analysis was done at a maximum absorption wavelength of 254 nm (Antonio da Silva et al., 2021; Otieno et al., 2019b). Samples with absorbance measurements above the range (>9.99) were serially diluted until the absorbance could be determined. Nutrient analysis was done to determine the concentrations of ammonium (NH_4^+), nitrates (NO_3^-), and orthophosphates (PO_4^{3-}) according to the standard methods (Beutler et al., 2014). The elemental analysis of the effluents was done using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) according to standard methods (APHA et al., 2017).

3.3 Results and discussion

The real DEWATS AF effluent was filtered through a 450 µm microfilter to remove large solid particles and then analyzed for physical and chemical properties, as presented in **Table 3.6**.

Table 3.6: Physicochemical properties of real DEWATS AF effluent.

	Parameter	Units	Value
Organics	Humic	$mg.L^{-1}$	0.337
	s.COD	$mg.L^{-1}$	129
	BOD	$mg.L^{-1}$	18
	DOC	$mg.L^{-1}$	3.4
Nutrients	Ammonium	$mg.L^{-1}$	0.47402
	Nitrate	$mg.L^{-1}$	-0.074
	Ortho-P	$mg.L^{-1}$	43
Trace Elements	Al	$mg.L^{-1}$	0.2895
	Fe	$mg.L^{-1}$	0.0692
	Mn	$mg.L^{-1}$	0.0434
	Zn	$mg.L^{-1}$	0.39
Pathogens	*E. coli*	$MPN.100mL^{-1}$	2419600
	Total coliforms	$MPN.100mL^{-1}$	3200400
Other parameters	Turbidity	ntu	14
	EC	uS/cm	1051
	pH	----	7.3

3.3.1 Reduction of organics

The effect of AOPs on the concentration of organics is given in **Figure 3.2.** High BOD and COD levels indicate the presence of organic pollutants that can reduce oxygen levels in water bodies (Venkatramanan et al., 2014). High DOC and humic content can lead to color, odor, and bad taste in water, affecting the effluents' aesthetic and reuse quality (Otieno et al., 2019b). In **Figure 3.2(a)**, DOC levels increased from 3.4 mg.L^{-1} to 12.8 mg.L^{-1}, 7.9 mg.L^{-1}, and 6.6 mg.L^{-1} after ozonolysis, UV photolysis, and TiO_2 photocatalysis treatments, respectively. Intense solubilization of suspended organic matter, such as pathogen cell walls, significantly increases DOC levels during ozonolysis (Ariunbaatar et al., 2014; Gomes et al., 2013). Humic compounds can also build complexes with other organic and inorganic molecules in the effluent or be adsorbed onto surfaces (Gautam et al., 2021). UV photolysis is known to decompose these binding bonds, causing previously sorbed organic molecules to be released, thus potentially increasing the concentration of DOC (Wang et al., 2017). The high affinity of the organic ions to be adsorbed on the TiO_2 active sites hindered the access of reactive oxygen species (ROS) on the TiO_2 surface by the suspended organic matter, leading to minimal mineralization, hence the slight increase in DOC during photocatalysis (Gusain et al., 2020).

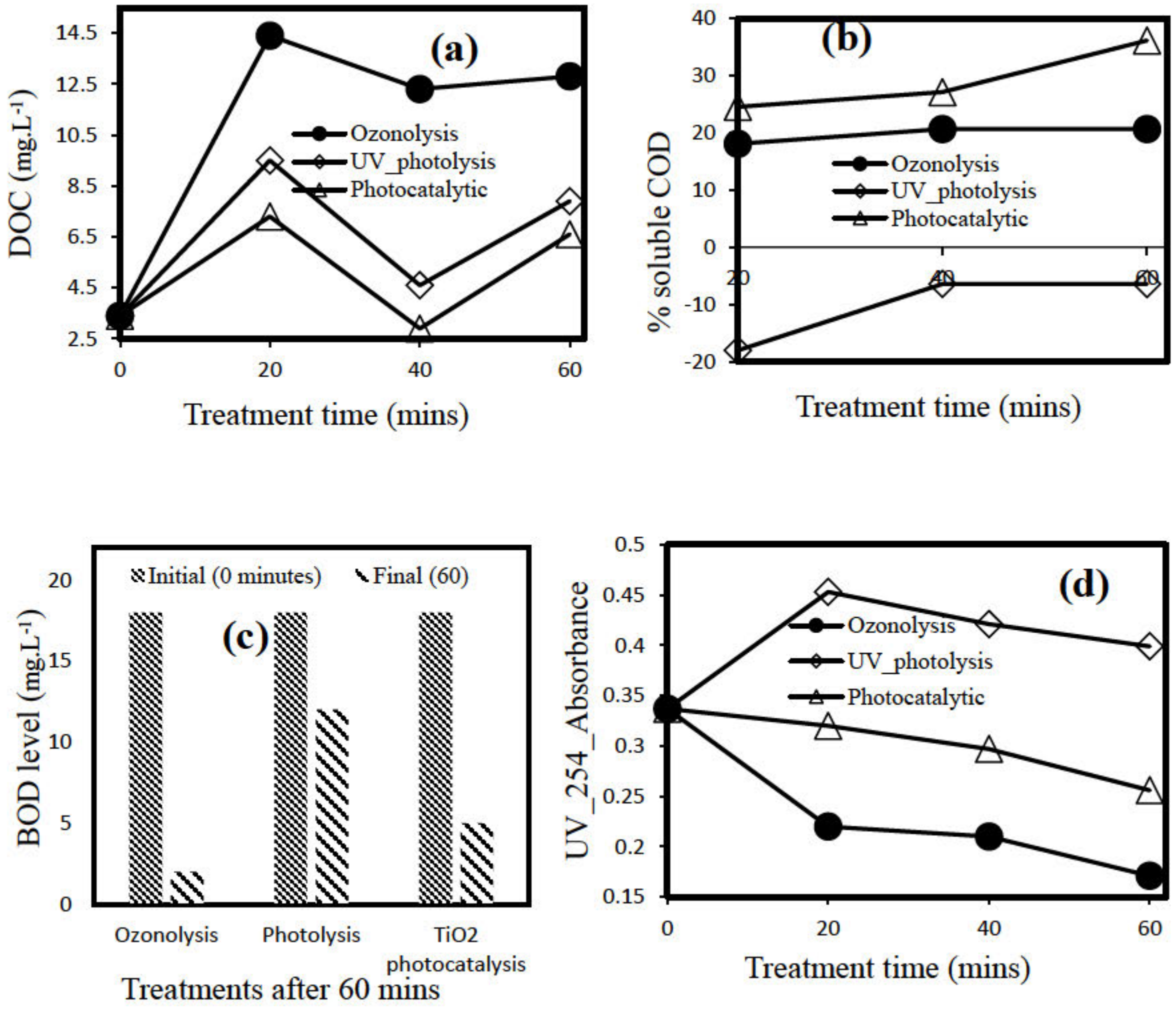

Figure 3.2: Reduction in organic matter: **(a)** Dissolved Organic Carbon (DOC), **(b)** soluble Chemical Oxygen Demand (sCOD), **(c)** Biochemical Oxygen Demand (BOD), and **(d)** Absorbance (UV-254) during DEWATS AF effluent treatment using different AOPs.

As observed in **Figure 3.2(b)**, sCOD levels after ozonolysis and TiO_2 photocatalysis decreased to 20.26% and 36.12% within 60 min, respectively, while UV photolysis recorded a 6.45% increase. The slight reduction of sCOD after ozonolysis and TiO_2 photocatalysis could be due to the concurrent solubilization and mineralization of the suspended organic matter (Gusain et al., 2020; Mehmood et al., 2022). On the other hand, a 6.45% increase in sCOD following UV photolysis can be attributed to the release of dissolved organic matter (DOM) with no subsequent mineralization (Zhu et al., 2010).

BOD levels were reduced to 2 mg.L^{-1} and 5 mg.L^{-1} from 18 mg.L^{-1} after 60 min when subjected to ozonolysis and TiO_2 photocatalysis, as observed in **Figure 3.2(c)**. Intense oxidation of the organic compounds present in the AF effluent by ozone and TiO_2 photocatalysis resulted in organic matter mineralization, significantly reducing the BOD levels (Al-Mamun et al., 2019; Otieno et al., 2019a). The slight reduction in the BOD level, from 18 mg.L^{-1} to 12 mg.L^{-1} after 60 minutes of UV photolysis, resulted from interferences of UV absorption by certain substances in the effluent. As shown in figure **Figure *3.3*:** Effect of AOPs on **(a)** turbidity, **(b)** EC, and **(c)** pH during DEWATS AF effluent treatment under different AOPs.**Figure 3.3**(**a)**, high turbidity content indicates the presence of dissolved solids that would interfere with the effectiveness of UV photolysis (Zhao et al., 2020). These substances may absorb or scatter UV light, reducing the efficiency of the process and, consequently, a minimal reduction of BOD (Sheng et al., 2013).

Humic concentration decreased significantly from 0.337 to; 0.171 and 0.256 during ozonolysis and TiO_2 photocatalysis, respectively, while increasing from 0.337 to 0.399 after 60 minutes of UV photolysis. The reduction in humic levels by ozonolysis is linked to the oxidation of the humic compounds into smaller molecules such as aldehydes and organic acids. These byproducts are more hydrophilic and cannot be detected as humic (Zhong et al., 2018). Increased humic values after UV photolysis would be associated with photo-fragmentation, a process by which UV light breaks down solid humic substances, such as lignin and tannins, into soluble products. This fragmentation may increase the functional groups of humic compounds, increasing humic values (George et al., 2014). Also, the transformation of labile organic compounds into more stable humic substances through the rearrangement of functional groups by UV photolysis could be responsible for the increase. The transformation results in more recalcitrant and complex structures, such as aromatic compounds and persistent organic pollutants (POPs), increasing the humic level (Liu et al., 2019). A slight reduction in humic level by TiO_2 photocatalysis is linked to the presence of inorganic ions (**Table 3.6**) in the AF effluent (Tang et al., 2018). These inorganic ions reduce the degradation of organic compounds such as humics by competing for the available Reactive Oxygen Species (ROS) (Gusain et al., 2020).

3.3.2 Change in Turbidity, EC, and pH conditions

The effect of AOPs on turbidity, EC, and pH is given in **Figure 3.3**. Reduction in turbidity, EC, and pH signifies increased quality of the polished effluent (Tahreen et al., 2020). Decreased turbidity from 14 NTU to 2 NTU (**Figure 3.3(a)**) indicates an effective removal of suspended particles from the effluent by ozonolysis (Otieno et al., 2019b). A slight reduction in turbidity after 60 minutes of UV photolysis indicates a slight removal of suspended particles from the wastewater. Due to the slow mineralization of the suspended particles, UV photolysis can only reduce a small percentage of turbidity (Ali et al., 2022). The efficient (from 14 to 3 NTU) turbidity reduction by TiO_2 photocatalysis could be attributed to the efficiency of the ROS produced during TiO_2 photocatalysis, which oxidized the suspended particles and the adsorption of the dissolved organic matter by the catalyst (Boroski et al., 2009; Gusain et al., 2020).

Figure 3.3(c) shows an increase in the pH of the DEWATS effluent when subjected to the AOPs, which is more pronounced in the ozonolysis process. Oxidation of the amino acids, proteins released from the lysed pathogen cells, produces basic amine oxides or other alkaline nitrogen-containing compounds, consequently increasing the pH of the effluent (Ma et al., 2012; Schumperli et al., 2012). By breaking chemical bonds (ionization process) in the AF effluent, hydroxyl ions (OH^-) are generated by the AOPs (Wang and Xu, 2012). This ionization process increased the number of charged particles in the effluent, raising the levels of electrical conductivity (**Figure 3.3(b)**) as well. The ionization increased with the abundance of contaminants in the effluent in the initial stages of treatment. After 20 min, most of the contaminants were already degraded, leading to decreased ionization, subsequently decreasing the EC.

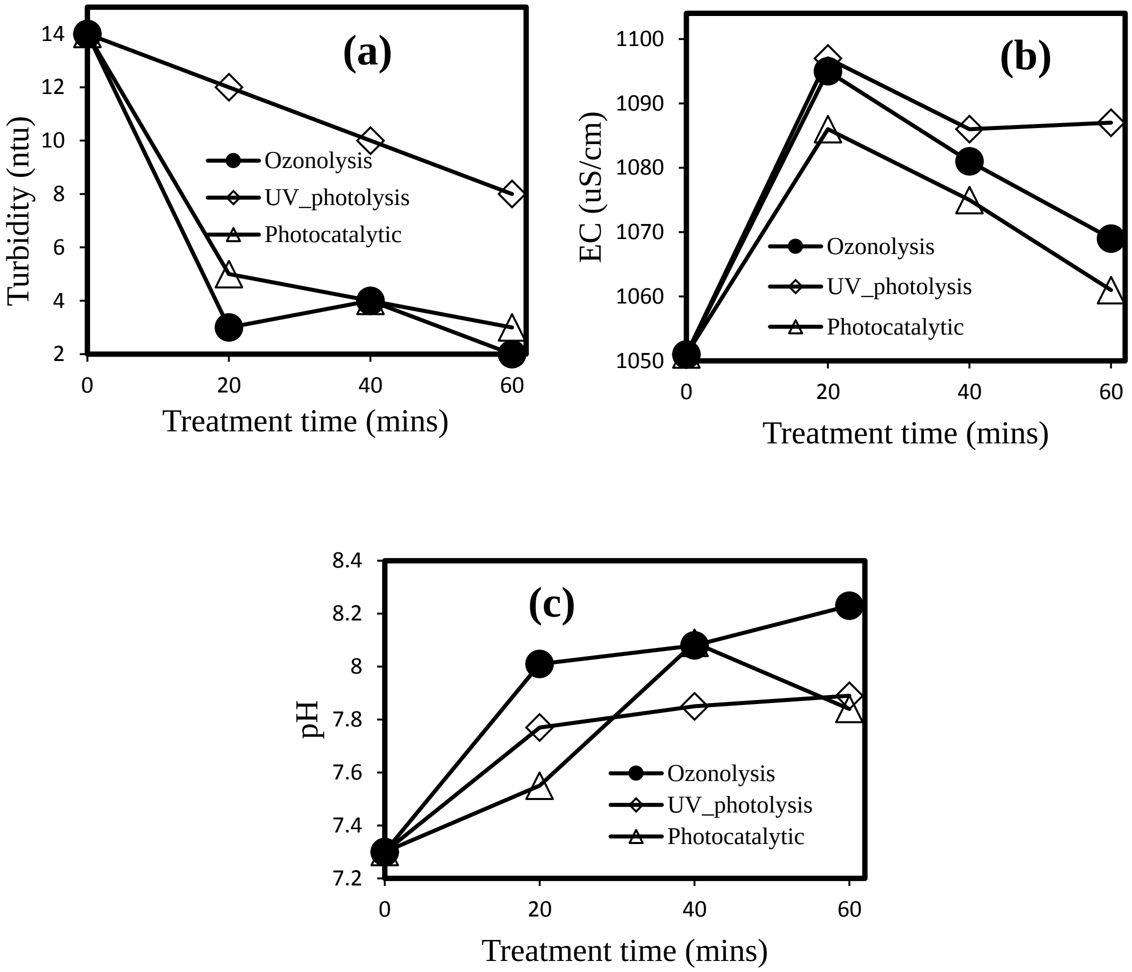

Figure 3.3: Effect of AOPs on **(a)** turbidity, **(b)** EC, and **(c)** pH during DEWATS AF effluent treatment under different AOPs.

3.3.3 Change in the nutrient content of the effluent

The effect of AOPs on the nutrient contents in the supernatant is given in **Figure 3.4**. An increase in ammonium concentration across the treatments was observed, though it was pronounced during ozonolysis (**Figure 3.4(a)**). A significant increase in nitrate concentration during ozonolysis was also observed, as shown in **Figure 3.4(c)**. Dissolved orthophosphates increased during ozonolysis and UV photolysis, while a significant decrease was observed during TiO_2 photocatalysis (**Figure 3.4(b)**). Ammonium accumulation in the effluent results from the solubilization of hard cell walls of the pathogens by the AOPs, releasing the cellular contents full of amino acids and carbohydrates into the effluent (Otieno et al., 2019c). With the continued breakdown of these amino acids (nitrogen-containing compounds) by ozonolysis, UV photolysis, and TiO_2 photocatalysis, ammonium ions are formed as a byproduct (Bamba et al., 2017; Jing et al., 2011; Kim and Tanaka, 2009; Schumperli et al., 2012). In the case of TiO_2 photocatalysis, the ammonium ions can be in the polished effluent or adsorbed onto the TiO_2 surface (Dong et al., 2007).

The decomposition of amino acids by ozonolysis in the effluent produces unstable primary ozonide intermediates (Schumperli et al., 2012). These intermediates decompose further to produce nitrogen-containing fragments that react with ozone or its decomposition products to generate nitrates (Zahardis et al., 2008). In the case of UV photolysis and TiO_2 photocatalysis, the insignificant change in nitrate levels is associated with nitrates potentially undergoing transformation reactions leading to intermediates or end products, such as nitrite (NO_2-) or nitrogen gas (N_2), which may not be measured as nitrates (Dai and Mitch, 2015).

Organic phosphorus compounds (Adenosine diphosphate (ADP), phospholipids, and nucleic acids) are released into the effluent when pathogen cells are solubilized (Mackey and Paytan, 2009). Further oxidation of these organic phosphorus compounds by ozonolysis and UV photolysis releases phosphate groups from the organic molecules, forming orthophosphates (Hu et al., 2021). Also, the mineralization of phosphate groups that link nucleotides together by UV photolysis would result in fragmentation and subsequent release of orthophosphates and nucleotides as byproducts (Malvestiti and Dantas, 2018). The decrease in orthophosphates after 60 minutes of TiO_2 photocatalysis is linked to the oxidation of orthophosphates into other phosphate species, such as polyphosphates or condensed phosphates, which are in less soluble form and have reduced biological availability (Volhard et al., 2020).

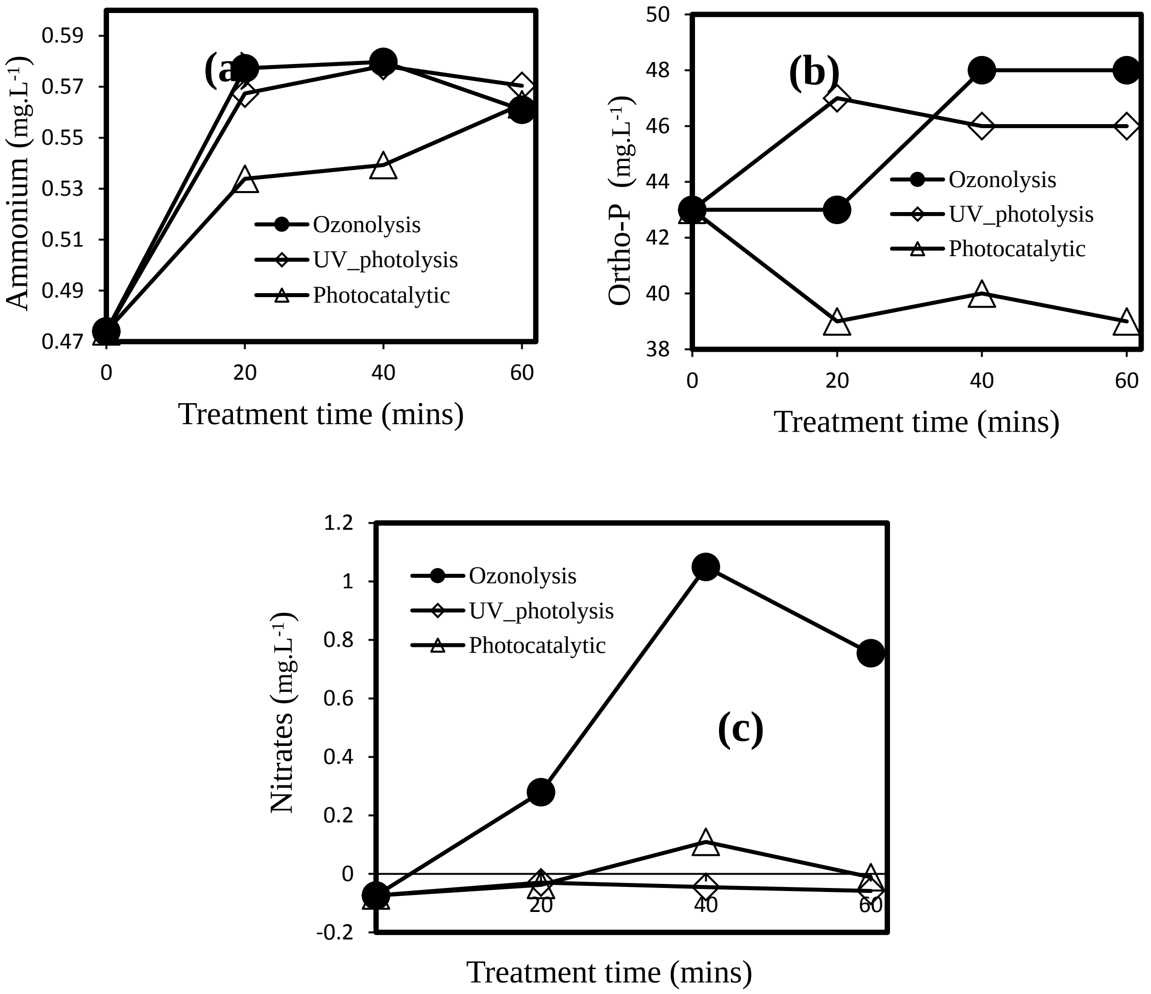

Figure 3.4: Effect of AOPs on the nutrient content: **(a)** ammonium-N, **(b)** ortho-P, and **(c)** nitrates during the treatment of DEWATS effluent under different AOPs.

3.3.4 Pathogen reduction by AOPs

The effect of AOPs on pathogen reduction is given in **Figure 3.5**. Ozonolysis and TiO_2 photocatalysis registered 6.4-log for both *E. coli* and Total coliforms, whereas UV photolysis, in both cases, attained 6-log. Ozonolysis and TiO_2 photocatalysis in employing ROS could have led to attaining the log1 pathogen reduction mark through the following oxidation processes. The cell membrane is initially attacked, followed by glycoproteins, glycolipids, certain amino acids, and finally, sulfhydryl groups of certain enzymes. The bacterial cell begins to break down, followed by perforation of the cell membrane, and eventually, the cell disintegrates, a condition known as lysis (Kaur et al., 2022).

The inability of UV photolysis to reduce pathogens entirely after 60 minutes resulted from other effluent components that lowered the UV intensity through absorption, shielding, or shadowing of the UV light (Yasar and Tabinda, 2010). Increased humic compounds likely reduced the amount of UV radiation available for pathogen disinfection. Moreover, extracellular substances or biofilm matrices released by some microorganisms when exposed to UV radiation can absorb or scatter UV light, shielding the pathogens present in the biofilm from effective disinfection (de Carvalho, 2017; Raza et al., 2021).

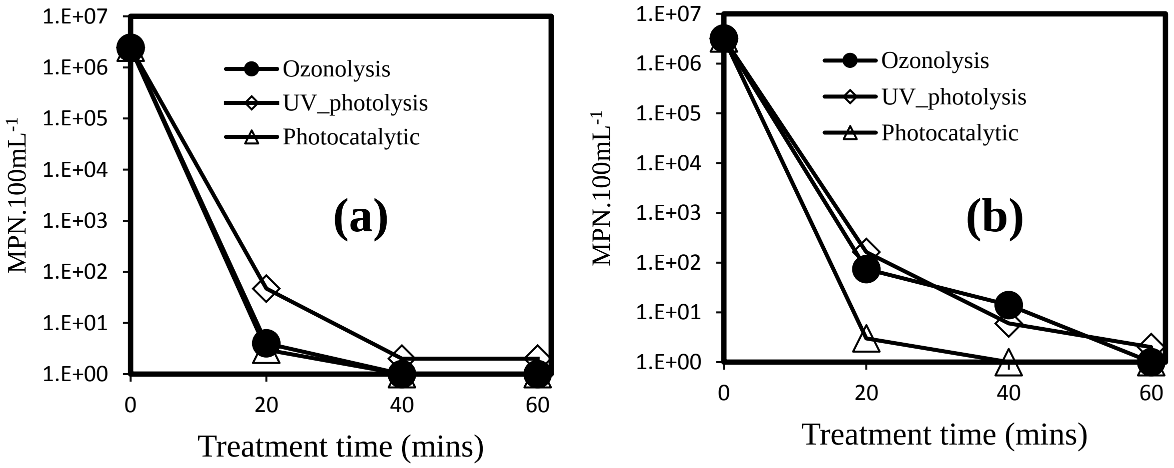

Figure 3.5: Effect of AOPs on pathogen reduction: **(a)** *E. coli* and **(b)** Total coliform during the treatment of DEWATS AF effluent.

3.3.5 Pathogen regrowth after polishing with AOPs

The effect of AOPs on pathogen regrowth is given in **Figure 3.6**. Notable regrowth logs occurred in samples polished for 20 and 40 minutes across all the AOP treatments, probably because complete lysis of pathogen cells was not achieved. The remaining viable pathogens, such as *E. coli* (gram-negative bacteria), are fed on the nutritious cellular remains of other dead pathogens, resulting in exponential regrowths (Das et al., 2020). The highest *E. coli* and Total coliform log regrowth on samples treated for 60 minutes were observed on TiO_2 photocatalysis (2.5-log and 2.7-log, respectively), followed by UV photolysis (0.5-log and 2.2-log). Conversely, Ozonolysis recorded a 0-log, suggesting no pathogen regrowth. When ozone molecules combine with nucleic acids, adducts, strand breakage and cross-linking form in the DNA and RNA, thus impairing the capacity of bacteria to regrow their genetic material. A gradual regrowth with an increase in time and temperature during storage (from 20°C to 35°C) was observed after pathogens were 99% eliminated by ozone at a rate of 300 mg/h and 1000 mg/hr, respectively, for 20 minutes (Magdeburg et al., 2014; Rizvi et al., 2013; Yasar et al., 2007). According to these reports, achieving complete oxidation of the pathogen cells demands a considerable extension of the exposure duration to attain a 0-log regrowth level, as the case in this study.

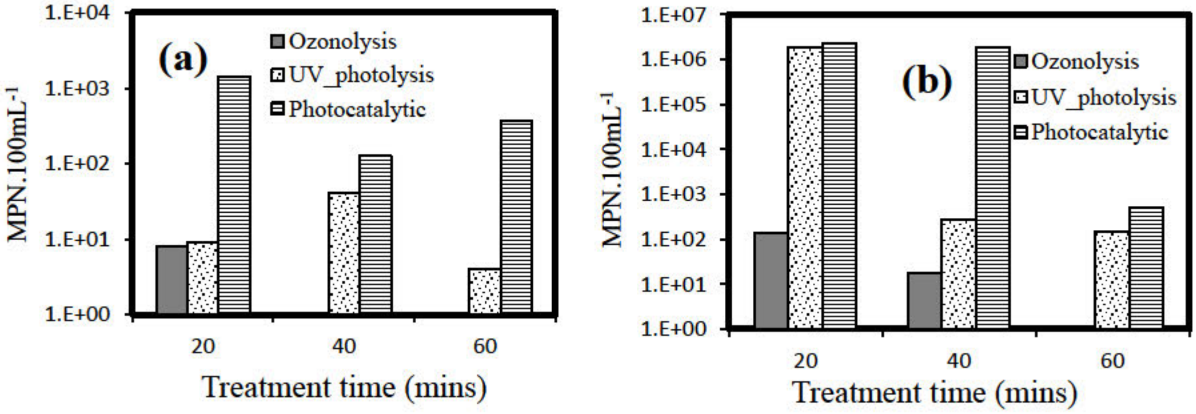

Figure 3.6: Effect of AOPs on pathogen regrowth; **(a)** *E. coli* and **(b)** Total coliform concentrations after four days of storage after different AOP treatments.

When the DNA or RNA molecules of pathogen cells are subjected to ultraviolet (UV) radiation, pyrimidine dimers form between them. These dimers cause disruptions in the structure of the nucleic acids, leading to mutations or even cell death if left unrepaired (Delorme et al., 2020). Bacterial cells can counter the formation of pyrimidine dimers through various mechanisms to avoid cell death or mutations that may affect the ability of bacteria to grow and reproduce, thereby contributing to the exponential regrowth of the pathogens in the UV photolysis experiment (Barlev and Sen, 2018; Cordero and Casadevall, 2020; Pavan et al., 2020; Raza et al., 2021). He et al. (2021) observed that TiO_2 photocatalysis can only perforate the cell membranes and not the nucleic acids (RNA and DNA) of most gram-negative pathogens like *E. coli*. Joost et al. (2015) reported the presence of cellular debris indicating incomplete pathogen cell destruction after 60 minutes of TiO_2 photocatalysis. Also, complete oxidation of the genomic DNA of pathogens under TiO_2 photocatalysis is very slow and can take longer for the impact to be seen (Joost et al., 2015; Kim et al., 2013). Thus, TiO_2 photocatalysis has a high probability of producing under-oxidized pathogen cells that can quickly repair and regrow in the effluent.

3.3.6 Elemental composition of the raw and polished effluent

Both ozonolysis and UV photolysis minimally mineralized the trace elements (**Table 3.7**). However, the observed concentrations are within Word Health Organisation (WHO) and the United States Environmental Protection Agency (USEPA) agricultural reuse standards (Gabr, 2020). A significant reduction in the trace elements during TiO_2 photocatalysis was observed (**Table 3.7**). Due to the favorable chemical and physical properties of the TiO_2 catalyst, such as pH and surface functional groups, metals like Fe, Zn, Al, and Mn can be adsorbed onto the TiO_2 surface (Pan et al., 2021; Zhang et al., 2021). In the current study, the reduced treatment time of pathogens in the effluent by TiO_2 photocatalysis was a result of the adsorption of Fe, Zn, Al, and Mn from the effluent, which are metal dopants that counter the recombination of the photo-generated electrons (**Figure 3.5**) (Al-Mamun et al., 2019).

Table 3.7: Different concentrations of trace elements and heavy metals before and after polishing.

	Concentration (mg.L^{-1})			
Sample ID	**Al**	**Fe**	**Mn**	**Zn**
A	0.2814	0.0402	0.0344	1.04
B	0.282	0.0453	0.0358	0.369
C	0.288	0.1795	0.0431	0.2845
Q	0.2846	0.0615	0.0427	0.339
R	0.2835	0.0316	0.03	0.32
S	0.2838	0.0158	0.0199	0.2785
X	0.273	-0.0297	0.0034	0.244
Y	0.2736	-0.0328	0.0003	0.231
Z	0.2718	-0.0333	0.0005	0.233
RAW	0.2895	0.0692	0.0434	0.39

(A) Ozonolysis (20 mins); **(B)** Ozonolysis (40 mins); **(C)** Ozonolysis (60 mins); **(Q)** UV photolysis (20 mins); **(R)** UV photolysis (40 mins); **(S)** UV photolysis (60 mins); **(Y)** Photocatalytic (40 mins); **(X)** Photocatalytic (20 mins); **(Z)** Photocatalytic (60 mins).

3.4 Conclusion

The current study investigated ozonolysis, UV photolysis, and TiO_2 photocatalysis as treatment methods to polish up DEWATS effluent for compliance with unrestricted agricultural reuse standards for pathogens. The results showed significant trends in the influence of these treatment methods on the parameters studied. TiO_2 photocatalysis significantly reduced BOD, soluble COD, humic levels, ortho-P, turbidity, nitrates, and pathogens while increasing the pH, EC, ammonium, and DOC. Also, significant pathogen regrowth was observed after four days of storage, suggesting potential challenges in maintaining microbial control under TiO_2 photocatalysis. On the other hand, ozonolysis treatment significantly reduced BOD, soluble COD, humic, and turbidity and eliminated the pathogens while increasing the ortho-P, nitrates, pH, EC, ammonium, and DOC. Notably, the absence of pathogen regrowth demonstrated the ability of ozonolysis to sustain improved microbial control. Lastly, UV photolysis resulted in marginal reductions in BOD, nitrates, turbidity, and pathogens, with increased EC, pH, ammonium, DOC, humic, and ortho-P levels, though a slight pathogen regrowth was observed. Based on the current findings, ozonolysis proved to be the best AOP for the treatment of the

AF effluent to meet the WHO (Organization, 2006), USEPA (Tzanakakis et al., 2023), FAO (Shoushtarian and Negahban-Azar, 2020), and EU (Angelakis and Gikas, 2014) pathogens standards for unrestricted agricultural use, making it a suitable alternative over the constructed wetlands. Therefore, cost analysis for ozonolysis should be further investigated to inform relevant stakeholders willing to out scale this method for intensive crop production in wastewater-irrigated scenarios.

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CHAPTER 4: NITROGEN AND PHOSPHOROUS RELEASE PATTERNS IN SOIL IRRIGATION SCENARIOS WITH ADVANCED OXIDATION RECLAIMED WASTEWATER

Abstract

Using municipal effluents treated through advanced oxidation processes (AOPs) for crop production can reduce agricultural water demand. However, there is little information on how AOP-treated effluents can affect nutrient mineralization in the soil. An incubation study was conducted in the laboratory to determine the effects of ozone-treated effluent (O-TE), UV-treated effluent (UV-TE), and TiO_2-photocatalysis-treated effluents (Ti-TE) on N and P mineralization in the soil with both positive (municipal tap water; MTW) and a negative (Raw Anaerobic Filter Effluent; RW) used as controls. The experiment followed a single factorial, completely randomized design with three replications, resulting in 285 experimental units. Variables observed included pH, ortho-P, ammonium-N, and nitrate-N levels. Notably, O-TE, UV-TE, and Ti-TE significantly increased soil pH from 5.68 to 6.0, while RW significantly decreased soil pH to 5.5 after 70 days of incubation. Furthermore, all treatments—RW, O-TE, UV-TE, and Ti-TE—showed significant levels ($P < 0.05$) of ammonium-N mineralization and nitrate-N release, contrary to MTW. Notably, RW, O-TE, and UV-TE exhibited a significant increase in nitrate-N within 14 days of incubation. A mismatch in ammonium-N reduction and nitrate-N release was observed, suggesting ammonification possibilities. Ortho-P was significantly released in the soil across all treatments except for Ti-TE. However, in O-TE and UV-TE treatments, the ortho-P release slowed after day 35 of incubation. While O-TE and UV-TE treatments did not interfere with P and N mineralization but showed a slower ortho-P release over time, Ti-TE treatment effectively prevented significant ortho-P release into the soil.

Keywords: Advanced oxidation processes; Ammonium-N; Mineralization; Nitrate-N; Nutrient release; ortho-P; Reclaimed wastewater

4.1 Introduction

The escalating global demand for water resources and mounting concerns over environmental degradation have resulted in an increased emphasis on wastewater reclamation and reuse (Botai et al., 2018). Reclaimed water, mainly sourced from urban domestic wastewater, contains various nutrients, including phosphorus and nitrogen, which can reduce the use of chemical synthetic fertilizer while improving soil fertility upon irrigation (Poustie et al., 2020; Qadir et al., 2020). Nitrogen in the soil mainly exists in organic form (Gao et al., 2019). However, plants directly absorb and utilize inorganic nitrogen and a small amount of water-soluble organic nitrogen only in the presence of soil animals (beetles, ants, and worms) and microorganisms (Dubey et al., 2021; Zhang et al., 2019). On the other hand, phosphorus in soil primarily exists in organic compounds and is crucial for various biochemical processes within plants (Malhotra et al., 2018; Tian et al., 2021). Plants primarily take in phosphorus in the form of inorganic phosphate, which is soluble in water and readily available for plant uptake (Kunwar et al., 2018). The mineralization of soil nitrogen and phosphorus is crucial to their respective cycles within the soil (Wang et al., 2022). Both nitrogen and phosphorus mineralization processes are fundamental for understanding and managing soil fertility and nutrient availability for plant growth (Arenberg and Arai, 2019; Shrivastav et al., 2020; Zeng et al., 2022).

The reclamation of domestic wastewater through advanced oxidation processes (AOPs) has emerged as a promising solution to address water scarcity and pollution concerns (Garrido-Cardenas et al., 2020). However, the specific impacts of effluents from these processes on nitrogen and phosphorus, essential nutrients that play important functions in plant growth and ecosystem health (Guignard et al., 2017), remain inadequately understood. The nitrogen and phosphorus release patterns, particularly in soil-irrigation scenarios, are lacking in the literature. While the existing literature predominantly focuses on contaminants removal and the improvement of water quality by AOPs (Feng et al., 2013), a comprehensive assessment of the subsequent effects on nutrient dynamics in soil and water is important to ensure the sustainability of wastewater reclamation practices and reuse (Corominas et al., 2020).

Furthermore, the divergent outcomes observed in the previous study (**3.2.3**) - where ozonolysis increased nitrate-N, ammonium-N, and ortho-phosphates levels, UV photolysis had marginal effects on the three nutrients, and TiO_2 photocatalysis exhibited significant reductions in ortho-phosphates (ortho-P) and nitrate-N - demonstrate the need for a more refined investigation. Addressing these gaps is crucial to optimizing the design and operation of AOPs for wastewater reclamation, ensuring that the reclaimed water meets regulatory standards and facilitates sustainable agriculture by supporting nutrient availability for plant growth while mitigating adverse environmental impacts (Chartres and Noble, 2015; Helmecke et al., 2020).

This study aims to contribute to the understanding of nitrogen and phosphorus release patterns in soil irrigation scenarios with domestic wastewater reclaimed through AOPs. The focus on these nutrients is justified by their important roles in agricultural productivity and their significant potential for transformation and mobility within soil-water systems (Kehler et al., 2021; Zhu et al., 2018b). By investigating the effects of nutrients present in the ozonolysis, UV photolysis, and TiO_2-photocatalysis treated effluents, alongside the chemical properties of these AOPs on the soil nitrogen and phosphorous release patterns, this study seeks to shed light on the efficacy of these AOP-treated effluents in managing nutrient loads and improving the quality and safety of agricultural soils upon irrigation.

4.2 Materials and Methods

4.2.1: Soil collection and preparation

The soil was sourced from Bishopstowe, Pietermaritzburg-South Africa (29° 35.6398' S and 30° 25.3089' E). The topsoil was collected from the upper 300mm layer using a spade, placed into plastic containers, and transported to UKZN Pietermaritzburg. The soil was sieved past a

2mm mesh to remove the debris and stones. Three soil subsamples were randomly collected and bulked to form a representative sample of every replicate. The soils were then submitted to the Fertility and Advisory Service (FAS) at the KwaZulu-Natal Department of Agriculture Land Reform and Rural Development (DALRRD) in Cedara, Pietermaritzburg, for the analysis of soil chemical properties according to standard methods (Manson et al., 2020). Soil samples were also digested using aqua regia (3:1 HNO_3 and HCl) and filtered. The filtrates were taken for elemental analysis using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) at the University of KwaZulu-Natal Chemistry Department (APHA et al., 2017). The soil nutrient characteristics used in the study are given in **Table 4.8**.

Table 4.8: The measured characteristics of the soil used in the incubation study.

N (mg.L^{-1})	P (mg.L^{-1})	Fe (mg.L^{-1})	Mn (mg.L^{-1})	Zn (mg.L^{-1})	pH
0.19	27	18.7	18	19.6	5.68

4.2.2 Experimental material

Anaerobic Filter Effluent (AF) was collected from the Decentralized Wastewater Treatment System (DEWATS), a local treatment plant in Newlands-KwaMashu, Durban-South Africa. The AF effluent was reclaimed through advanced oxidation processes: Ozonolysis, UV-photolysis, and TiO_2 photocatalysis (**3.2.3**). The content traits of the reclaimed effluents are given in **Table 4.9**.

Table 4.9: Measured characteristics of the water sources used in the study.

W.S	Nitrate-N (mg.L^{-1})	Ammonium-N (mg.L^{-1})	Ortho-P (mg.L^{-1})	Fe (mg.L^{-1})	Mn (mg.L^{-1})	Zn (mg.L^{-1})	pH	DOC (mg.L^{-1})
RW	-0.074	0.474	43	0.069	0.043	0.39	7.30	3.4
MTW	-0.002	-0.008	9	-0.001	-0.004	-0.001	7.20	0.21
O-TE	0.754	0.563	48	0.180	0.043	0.285	8.23	12.8
UV-TE	-0.012	0.570	46	0.016	0.020	0.279	7.89	7.49
Ti-TE	-0.012	0.563	39	-0.033	0.001	0.233	7.84	6.6

4.2.3 Study site

The incubation study was carried out in an incubation room (a darkroom at 25 °C) at the University of KwaZulu-Natal's Soil Science Laboratories Department in Pietermaritzburg, South Africa (29°37′37.5″ S and 30°24′10.4″ E).

4.2.4 Experimental design

The study followed a completely randomized design (CRD) with a single factor, comprising five treatments ((controls, raw Anaerobic Filter Effluent (RW), municipal tap water (MTW)), ozone-treated effluent (O-TE), UV-treated effluent (UV-TE), and TiO_2-photocatalysis treated effluent (Ti-TE)), each replicated three times. Destructive sampling was carried out 19 times on 0, 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49, 56, 63,70,77 and 84 days of incubation, giving a total of 285 experimental units.

4.2.5 Incubation set up

One hundred grams of soil was placed in each ventilated plastic incubation pot. Following the approach of Haney and Haney (2010), 40 grams of soil were used to assess the soil's water retention capacity. Moisture was first set at 100% WHC and then maintained at 80% WHC, adjusted based on regular weight loss measurements during the study.

4.2.6 Analyses

4.2.6.1 Determination of phosphorus

The AMBIC extraction technique was used to extract orthophosphates (Schmidt and du Preez, 2004; Venter, 2018; White et al., 2020). The extracted solution was discolored to blue after mixing with a color reagent. A calibration curve was drawn based on the readings of the prepared phosphorus standards (0, 10, 20, 40, and 60 mg/L) on the UV spectrophotometer. The discolored blue liquid was read on a UV-Visible spectrophotometer at 670 nm absorbance to determine the extractable P. The calibration curve equation was used to determine the concentration of the extracts.

4.2.6.2 Determination of Nitrogen (ammonium and nitrates)

Each soil sample of about 5 g was weighed into the conical flasks. 50 ml potassium chloride (2 M KCl) was added, followed by shaking at 180 cycles per minute for 30 mins on an overhead shaker (Okalebo et al., 2002). Through Bowmen 250 nm filter papers, samples were filtered, and the filtrates were analyzed on a HI801-02 iris visible spectrophotometer, 230 VAC using HI93728-01 Nitrate, cadmium reduction method, Reagent kit (NO_3^--N), HI93700-01 Ammonia LR and HI93733-01 Ammonia HR, Nessler method, Reagent kit (NH_4^+ LR) and (NH_4^+ HR).

4.2.7 Data analysis

Analysis of variance (ANOVA) was used to evaluate changes in pH and nutrient N and P release in the incubated soil using GenStat 23rd (VSN International 2023). Means were separated using Fisher's protected least significant difference (LSD) at a 5% significance level.

4.3 Results and Discussions

4.3.1 Effects of water sources on soil pH change

No significant differences ($P < 0.05$) were observed among the treatments with different water sources regarding pH level. However, soil pH decreased with incubation time (**Figure 4.1**). At day 0, the pH ranged from 6.4 to 6.7, decreasing to 6.1 to 5.9 after 63 days across MTW, O-TE, Ti-TE, and UV-TE treatments. Notably, RW showed the lowest pH decreases to 5.5 after 77 days of incubation. During advanced oxidation processes, protein-containing organics are broken down (Schumperli et al., 2012), resulting in the production of basic amine oxides or other alkaline nitrogen-containing compounds (Ma et al., 2012), significantly raising the pH of the supernatants (**Table 4.9**). The increased effluent pH, therefore, can neutralize the soil acidity, in this case, from 5.68 to 6.0 across the AOP treatments after 84 days of incubation (**Figure 4.1**).

Conversely, continuous irrigation using RW after 70 days can lead to increased soil acidity with a pH of 5.5 or lower (**Figure 4.1**), which makes nutrients less available to soil microorganisms (Havlin, 2020; Xiao et al., 2020). These low-pH contents could also lead to increased nitrifying bacteria's aluminum toxicity (Zhao and Shen, 2018), resulting in low nitrate-N release (Havlin, 2020). Additionally, research indicates that nitrogen and phosphorus levels tend to be lower when the pH is 5.5 or below (Havlin, 2020; Silva-Sánchez et al., 2019).

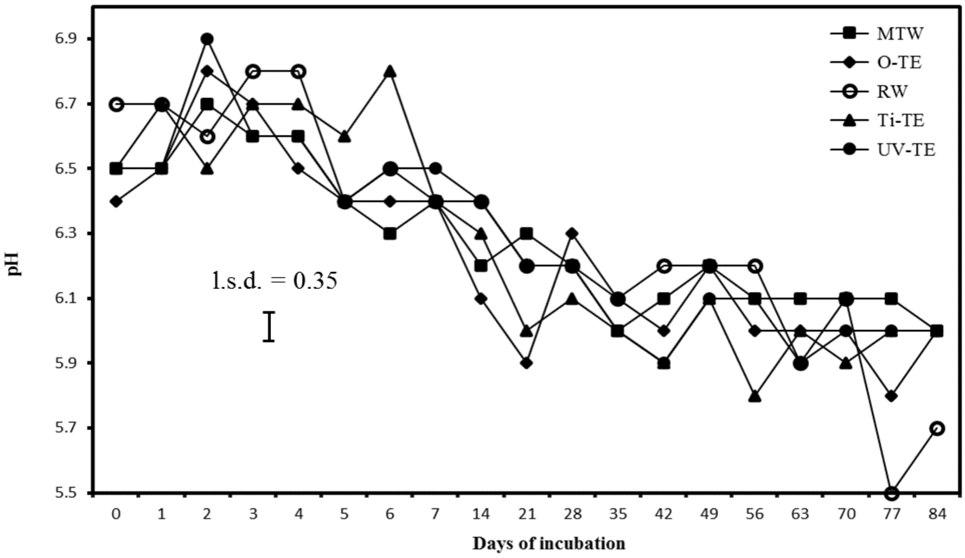

Figure 4.1: Changes in soil pH at different incubation time intervals.

4.3.2 Effects of water sources on soil nutrient mineralization

4.3.2.1 Ammonium-N mineralization

Treatments where MTW was used resulted in significantly lower ammonium-N release ($P < 0.05$) compared to all other treatments (**Figure 4.2**). Conversely, the remaining treatments did not show significant differences ($P > 0.05$) in ammonium-N release, although they exhibited higher levels of ammonium-N compared to the control (MTW) up to the 56th day of incubation time (**Figure 4.2**). RW showed the highest ammonium-N release among all treatments (**Figure 4.2**). The high levels of ammonium-N seen at the initial stages of incubation in RW, O-TE, Ti-

TE, and UV-TE could be attributed to the introduction of ammonium-N into the soils from the effluents upon moisture correction (**Table 4.9**). Based on the amount of ammonium-N supplied by the AOP-treated effluents (O-TE, Ti-TE, and UV-TE), there was low ammonium mineralization compared to RW. When pathogens are oxidized during AOPs, non-targeted microorganisms such as nitrifying bacteria are also eliminated (Shahid et al., 2020; Xiong et al., 2018). Subsequently, this reduces the abundance of these beneficial microorganisms in the soil for proper soil functioning (Xiong et al., 2018). In contrast, RW is reported to have high microbial densities (Adebayo and Obiekezie, 2018), providing plenty of beneficial microorganisms to the soil.

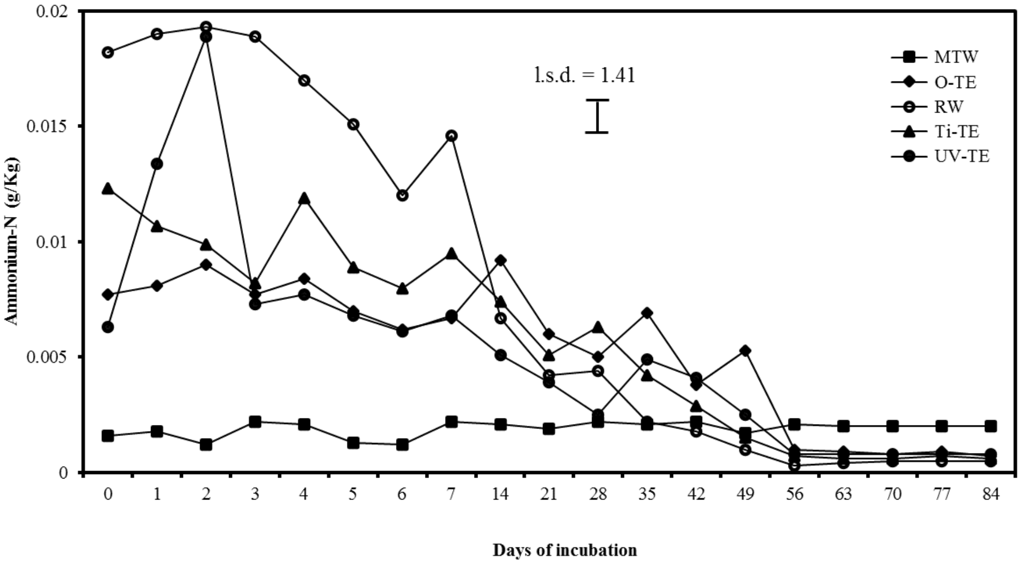

Figure 4.2: Release pattern of Ammonium-N at different incubation time intervals.

4.3.2.2 Nitrate-N release

Nitrate-N levels showed a significant ($P < 0.05$) increase over time across all treatments during the incubation period, except for MTW ($P > 0.05$) (**Figure 4.3**). However, significant differences ($P < 0.05$) were observed between treatments, with RW notably releasing more nitrate-N than any other treatment. Evidently, RW, O-TE, and UV-TE were the earliest (within 14 days) to show a significant increase in nitrate-N levels (**Figure 4.3**). In the present study, increased nitrate-N (**Figure 4.3**) is associated with the reduction of ammonium-N (**Figure 4.3**), which signifies the nitrification process (a process by which nitrifying bacteria oxidizes ammonium-N, increasing the nitrate-N contents) (Cáceres et al., 2018). The mismatch in ammonium supply from effluents (**Table 4.9**), ammonium-N reduction in the soil (**Figure 4.2**), and low nitrate-N yield (**Figure 4.3**) suggest a likelihood of ammonification, where ammonium-N turns into ammonia gas (Wang et al., 2018). Research also notes that liquid sources of ammonium-N, in this case, effluents, are prone to losing ammonia to the air (Liu et al., 2019). The increased ammonium-N at the start and nitrate-N later in the incubation study in O-TE, Ti-TE, and UV-TE, compared to the control (MTW), indicate that AOP-treated effluents can significantly supplement N supply for crop growth.

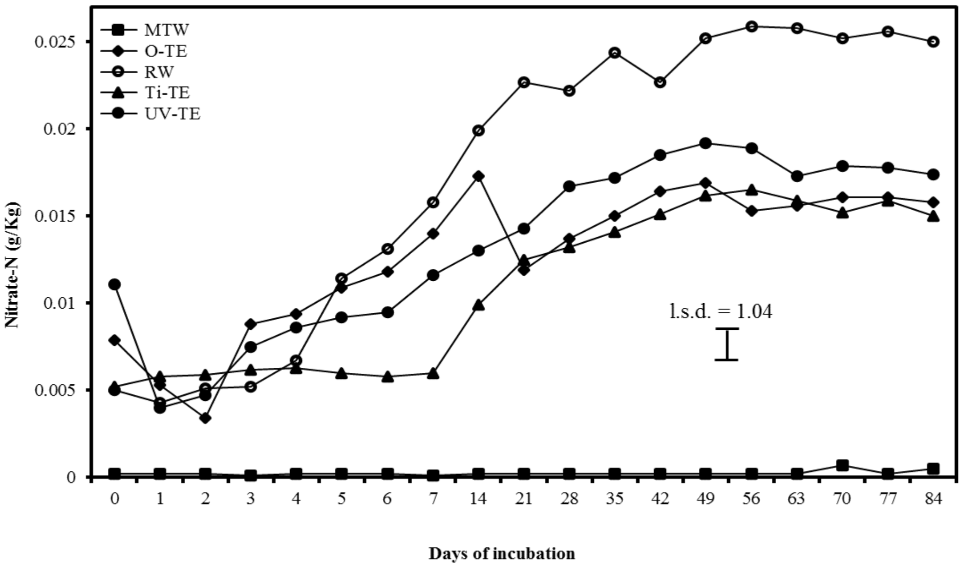

Figure 4.3: Release pattern of Nitrate-N at different incubation time intervals.

4.3.2.3 Effects of water sources on soil phosphorus mineralization

Ortho-P levels increased significantly ($P < 0.05$) over time in all treatments except for Ti-TE ($P > 0.05$) (**Figure 4.4**). Significant differences ($P < 0.05$) were observed between treatments, with RW notably releasing more ortho-P. By day 35 of incubation, RW, O-TE, MTW, and UV-TE had significant ortho-P available for crops. However, there was a noticeable decrease in the rate of ortho-P release after the 35th day of incubation in O-TE and UV-TE treatments. These findings are supported by Li et al. (2022), who reported that dissolved organic matter (**Table 4.9**) can bind to phosphorus, reducing its leaching potential and facilitating its slow release through microbial degradation (Kulikova and Perminova, 2021; Mosa et al., 2020; Zhu et al., 2018a). On the other hand, ammonium-N (**Table 4.9 and Figure 4.2**) can interact with soil phosphorus, affecting its transformation and availability (Chen et al., 2019). Moreover, nitrate-N concentrations (**Figure 4.4**) can lead to increased microbial activity that promotes phosphorus mineralization but may also lead to leaching under certain conditions (Cui et al., 2021; O'Neill et al., 2021).

No significant release of ortho-P was noted in Ti-TE treatments, even after 77 days of incubation (**Figure 4.4**). Trace elements are crucial nutrients for soil microbes, aiding in effective enzymatic phosphorus mineralization (Hussain et al., 2018; Rajkumar and Kurinjimalar, 2021). Studies report that TiO_2, present in Ti-TE, binds trace elements like Mn, Fe, and Zn (Pan et al., 2021; Zhang et al., 2021). This binding capacity might reduce or inhibit enzymatic phosphorus mineralization in the soil (Mosa et al., 2018). Moreover, TiO_2 is reported to have the ability to adsorb and immobilize phosphorus, resulting in a significantly lower release of ortho-P in Ti-TE treatments (Kaur et al., 2022; Yupeng et al., 2023; Zhang et al., 2024).

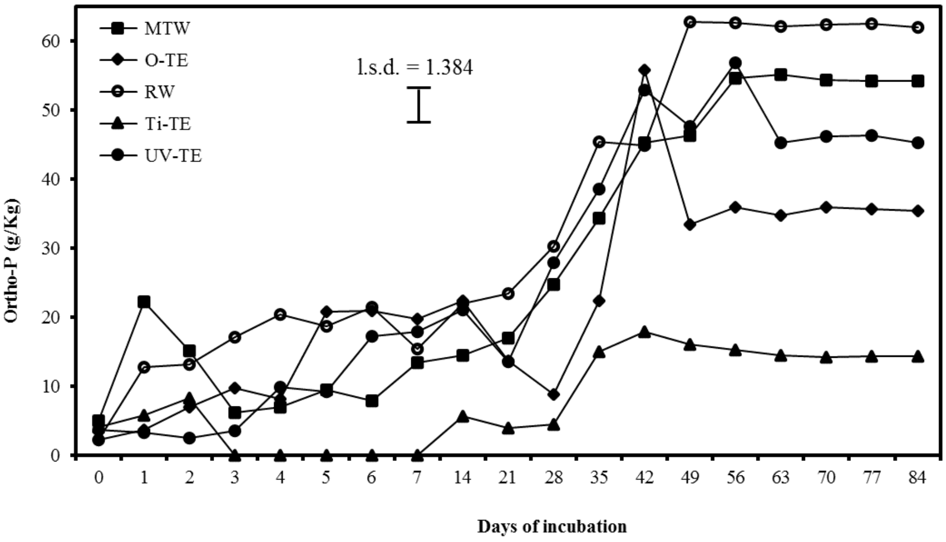

Figure 4.4: Release pattern of ortho-P at different incubation time intervals.

4.4 Conclusion

The elevated pH levels in O-TE, U-TE, and Ti-TE can also contribute to amending acidic soils through irrigation. Irrigation with O-TE and UV-TE increases ammonium-N and nitrate-N availability in the soil. Ortho-P is slowly released in O-TE and UV-TE treatments in the early stages of incubation, making it abundant for crops after 42 days. Furthermore, Ti-TE treatment effectively prevents significant ortho-P release into the soil. Future studies should investigate the potential effects of O-TE, U-TE, and Ti-TE on soil microbial diversity and trace element availability upon irrigation.

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CHAPTER 5: USING ADVANCED OXIDATION-POLISHED DECENTRALIZED WASTEWATER TREATMENT SYSTEM EFFLUENTS FOR IRRIGATION; EFFECTS ON CROP PHYSIOLOGICAL FUNCTIONS BIOACCUMULATION AND RISKS TO CONSUMER HEALTH AND SAFETY

Abstract

Advanced oxidation processes (AOPs) like ozonolysis, UV-photolysis, and TiO_2-photocatalysis have proven effective in treating secondary municipal wastewater effluent to meet unrestricted agricultural reuse standards. However, concerns arise due to residual byproducts such as catalysts and reactive oxygen species (ROS) in the treated effluents, potentially impacting crop and human health. The current work explored the impacts of AOPs-treated effluents on crop physiological traits and the potential microbial and chemical health risks associated with the consumption of such crops. Lettuce and Swiss chard were irrigated using municipal tap water (MTW) (control) and Anaerobic Filter effluent treated with AOPs (TiO_2-photocatalysis (Ti-TE), UV (UV-TE) and ozone (O-TE) -treated effluents) and evaluated the effects on crop growth. The experiment followed a 4 x 2 factorial design, which was completely randomized, with each treatment combination replicated three times, resulting in a total of 24 experimental units. The results revealed significant phytotoxic stress on the examined crops, with Ti-TE showing more pronounced effects than UV-TE and O-TE. Notably, TiO_2 deposition in stomata and leaf coverage led to significant chlorophyll fluorescence and gas exchange stresses on the crops. Ti-TE caused the highest percentage of electrolyte leakage in Swiss chard (34.22%) and lettuce (25.29%), followed by O-TE (19.07% in Swiss chard and 20.20% in lettuce), and UV-TE (12.90% in Swiss chard) and 19.57% in lettuce). Interestingly, Swiss chard exhibited higher tolerance to phytotoxicity stress resulting from Ti-TE, UV-TE, and O-TE than lettuce. No pathogens were detected on the crop leaves, with E. coli reading < 1 MPN and no fecal coliform reaction observed. However, the levels of titanium found on the edible leaves (Swiss chard: 2.7, lettuce: 1.46, g kg-1 dry-leaf weights) indicated potential health risks associated with consuming Ti-TE-irrigated crops. Conversely, O-TE and UV-TE demonstrated a promising approach to maintaining environmental integrity and food quality standards, highlighting their potential in safe agricultural wastewater treatment practices.

Keywords: Advanced oxidation processes; Crop irrigation effects; Reclaimed wastewater; TiO_2 bioaccumulation; TiO_2 exposure health risks

5.1 Introduction

Clean and reliable water resources are critical to sustainable agriculture (Xiang et al., 2021), particularly in regions with no centralized wastewater treatment facilities. Decentralised Wastewater Treatment Systems (DEWATS) have emerged as a promising solution for local wastewater treatment (Lourenço and Nunes, 2020). Musazura and Odindo (2021) highlight the possibility of reusing the DEWATS effluent for crop production. However, there is a need to develop additional post-treatment approaches for the DEWATS effluent to ensure compliance with microbial standards for unrestricted crop cultivation (Periasamy and Sundaram, 2013). Combining advanced oxidation processes (AOPs) with DEWATS can reduce pathogens in the effluent to acceptable levels (Di Cesare et al., 2020), thus making it suitable for irrigation without risks of pathogenic contamination to humans. Moreover, the effluent could present a valuable resource of nutrients for crop production. Applying DEWATS effluents for crop production is particularly beneficial in areas facing water scarcity and limited access to traditional irrigation sources (Musazura and Odindo, 2021).

Ozonolysis, UV-photolysis, and TiO_2 photocatalysis, among other AOPs, have been tested as potential polishing steps for DEWATS effluents and found to meet the chemical, physical, and biological standards for unrestricted crop production (**Figure 3.2**, **Figure 3.3**, **Figure 3.4**, and **Figure 3.5**). However, effluents treated by AOPs have been found to have harmful byproducts, such as catalysts and reactive oxygen species (ROS) (Fernández-Castro et al., 2015; Ike et al., 2019). Some studies have linked ROS to crop phytotoxic stress, which may affect root development and damage cellular components such as chloroplasts (Emberson et al., 2018). Subsequently, it could affect nutrient absorption, water uptake, and reduced CO_2 assimilation in plants (Dağhan, 2018; Dağhan et al., 2020; Emberson et al., 2018; Khodakovskaya, 2011; Ma et al., 2016; Wang et al., 2007). Additionally, titanium, a catalyst in TiO_2 photocatalysis, has been identified to compete with essential elements for absorption sites on the root surface or in the plant’s transport system (Dağhan et al., 2020). This competition could prevent crops’ absorption of essential elements (iron, manganese, and zinc), leading to nutritional deficiencies, poor crop quality, increased susceptibility to diseases, and reduced yield (Khodakovskaya, 2011; Ma et al., 2016).

Despite the potential for using AOP-treated effluents for irrigation, there is little information about crop-specific responses to the AOP-treated effluents (Babu et al., 2019). Understanding the diverse responses of various crops to these effluents is crucial due to potential variations in physiological and biochemical processes from crop to crop (Maiti et al., 2000; Mukami et al., 2020; Sarabi et al., 2017). Moreover, further investigations are needed to uncover the uncertainties surrounding the persistent pathogens and AOP-toxic byproducts in treated effluents and their potential transfer to crops and humans (Adegoke et al., 2018; Bernstein, 2011). This research gap is critical for developing evidence-based guidelines and recommendations for the safe and sustainable use of AOP-treated effluents in agriculture.

The current study aimed to explore how AOPs-treated effluents affect crop growth and the associated health risks from consuming the subsequent irrigated crops. The objectives of this study were (i) to investigate the effects of DEWATS anaerobic filter effluent treated with ozone, UV photolysis, and TiO_2 photocatalysis on crop (lettuce and Swiss chard) physiology and (ii) to quantify the accumulation of TiO_2 and pathogens traces on crop edible parts that would provide scientific evidence to inform policy on potential human health risks linked to consuming crops irrigated with AOP-treated effluents.

5.2 Materials and methods

5.2.1 Irrigation water sources

Irrigational water sources were obtained following advanced oxidation treatment of secondary municipal effluent. The raw Anaerobic Filter Effluent was subjected to three different oxidation processes, ozonolysis (O-TE), UV-photolysis (UV-TE), and TiO_2-photocatalysis (Ti-TE), for pathogen elimination (**3.2.3**).

5.2.2 Experimental site and materials

Pot experiments were carried out in a glasshouse at the University of KwaZulu-Natal's Controlled Research Facility (CRF) in Pietermaritzburg, South Africa (29°37′37.5″ S and 30°24′10.4″ E). Two-week-old seedlings of the lettuce (*Lactuca sativa*) butterhead variety and Swiss chard (*Beta vulgaris)* Ford hook giant variety were obtained from a local seedlings supplier. Lettuce and Swiss chard were chosen for the study due to their widespread consumption and vulnerability to contamination, especially since they are often eaten raw and can be contaminated at various production stages (Rahman et al., 2021).

5.2.3 Experimental design

The treatments comprised a 4 x 2 factorial experiment with the following factors: water sources (4 levels – Anaerobic Filter effluent (RW), Municipal tap water (MTW), Ozone treated effluent (O-TE), UV treated effluent (UV-TE), and TiO_2 photocatalysis treated effluent (Ti-TE)) and crop species (2 levels - lettuce and Swiss chard), giving a total of 8 treatment combinations. The experiment was laid out using a completely randomized design, with each treatment combination replicated three times, resulting in a total of 24 experimental units.

5.2.3 Soil collection, preparation, and analysis

The soil was collected from Bishopstowe, Pietermaritzburg, South Africa (29° 35.6398' S and 30° 25.3089' E). The topsoil was obtained from the top 300 mm depth using a spade, packed in plastic sacks, and transported to UKZN Pietermaritzburg. The soil was sieved past a 2 mm mesh and sterilized for 12 hrs in a Biobase autoclave (Model: BKQ-B50II) at a pressure of 15 Pa and 121°C. Five soil subsamples were collected randomly and bulked to make a representative sample of each replicate. The soils were analyzed for chemical properties and enteric pathogens according to standard methods (Manson et al., 2020; Velkushanova et al., 2021). Soil samples were also digested using aqua regia (3:1 HNO_3 and HCl) and then filtered. The filtrates were subjected to elemental analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) according to standard methods (APHA et al., 2017).

5.2.4 Cultural practices

At planting, the sieved sterile soils were mixed with a basal fertilizer (N: P: K, 2:3:4) with the following application rates. The nutrient concentrations of water sources were measured: MTW contained 9 mg L^{-1} ortho-P and -0.008 mg L^{-1} ammonium, O-TE contained 48 mg L^{-1} ortho-P and 0.563 mg L^{-1} ammonium, UV-TE contained 64 mgL^{-1} ortho-P and 0.57 mg L^{-1} ammonium, and Ti-TE contained 39 mg L^{-1} ortho-P and 0.563 mg L^{-1} ammonium. The nutrient contributions per hectare were calculated with a 10 000 L ha^{-1} target application rate for each effluent (water requirement for the fixed experimental period) (Zaman et al., 2018). The contributions from O-TE were 480 g ha^{-1} of ortho-P and 5.63 g ha^{-1} of ammonium, from UV-TE were 640 g ha^{-1} of ortho-P and 5.7 g ha^{-1} of ammonium, and from Ti-TE were 390 g ha^{-1} of ortho-P and 5.63 g ha^{-1} of ammonium. The remaining nutrient requirements for lettuce and Swiss chard were then determined based on hypothetical nutrient requirements: 138.74 kg ha^{-1} of N and 100 kg ha^{-1} of K. No additional P was required, apart from MTW treatment, due to excess P in the effluent. The nitrate-N and K discrepancy between the hypothetical nutrient requirements and soil nutrient analysis was addressed by applying 30 g pot^{-1} (200 kg/ha) of N: P: K (2:3:4).

The overhead irrigation method was chosen to mimic unrestricted crop production practices (Drysdale and Hendricks, 2018). Moreover, the direct crop-effluent interaction facilitates easy stress monitoring and persisting pathogen/contaminant tracing on crop leaves (Al-Karablieh et al., 2024). Soil moisture content was maintained at 70% field capacity based on daily irrometer readings (Irrometer Co., Riverside, California) (Kashyap and Kumar, 2021). Automated wetwall humidifiers and forced ventilation systems were used in the tunnel to maintain stable conditions, with temperatures consistently at 28 ± 2 °C and humidity levels at 63 ± 2%. This controlled environment aimed to prevent heat stress and ensure optimal conditions for the duration of the experiment.

5.2.5 Crop sampling and analyses

5.2.5.1 Determination of electrolyte leakage

Electrolyte leakage was determined following the methods used by Hampton and Tekrony (1995) and improved by Hussain et al. (2023). Fresh leaf samples were washed with deionized water and cut into uniform pieces. Each test tube containing 1 g of the prepared leaf samples was filled with 20 ml of deionized water and soaked for 24 hrs in a thermostatic water bath shaker (FWS-30, China) maintained at 25 °C in an incubator and set to 80 rpm. Based on Hampton and Tekrony (1995), the electrolyte leakage corresponds to the initial and final electrical conductivity difference. The water’s initial electrical conductivity (EC1) was measured using an EC meter (Model: HI98129). The soaked tissue samples were extracted and dried through gentle blotting, and the final EC (EC2) was recorded. The percentage of electrolyte leakage was determined using **Equation 1**:

$$Electrolyte\ leakage(\%) = \frac{EC2 - EC1}{EC1} \times 100 \qquad \textbf{Equation 1}$$

5.2.5.2 Identification and quantification of TiO_2

Ten grams of fresh leaves were sampled using sterilized scissors. The samples were placed in fresh plastic bags, sealed, and labeled with the plant species, the sampling date, and the applied treatment (Ti-TE). The samples were cut into tiny pieces (about 10 mm × 10 mm) and fixed for 2 hrs in a solution of 3% glutaraldehyde, followed by washing twice in sodium cacodylate buffer each 5 min. The samples were dehydrated by submerging them for 10 mins each in ethanol solutions with ethanol concentrations (10%, 30%, 50%, 70%, 90%, and 100%). The samples were subjected to Critical Point Drying for 1 hr to remove ethanol. The titanium traces were mapped using a scanning electron microscope (SEM) coupled to energy-dispersive X-ray Spectroscopy (EDX), according to Sawidis et al. (2011). Additionally, image J software (https://imagej.nih.gov/ij/download.html) was used to determine the size (diameter) of agglomerates identified by SEM scans (Radu et al., 2021; Schroeder et al., 2021).

Further elemental analysis was conducted using spectroscopy. Samples were collected into different brown bags; each crop species planted, from each treatment, and replicates. The harvested samples were oven-dried at 40 °C for 72 hrs. Dried samples were crushed using a motor and pestle into fine powder. About 0.5 g of the crushed leaf samples were digested using aqua regia (3:1 HNO_3 and HCl) on a hot sand digestion system, according to Kalra (1997). The mixture underwent filtration using 2.5 µm Whatman filter papers, followed by filtration through 0.45 µm filters into 100 ml volumetric flasks. The solutions were then diluted to the 100 ml mark using deionised water and subjected to elemental analysis using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) at the University of KwaZulu-Natal Chemistry Department (APHA et al., 2017).

5.2.5.3 Pathogen tracing and quantification

According to European Union 2020/741 regulations, public health assurance is key in wastewater reuse of any kind (Berbel et al., 2023). The Environmental Protection Agency (EPA) regulations also mandate monitoring of pathogens upon wastewater reuse in agriculture to inform the public of potential underlying health risks (Verbyla et al., 2023). In this study, *Escherichia coli*, a fecal contamination indicator in water and agricultural products, was chosen as an ideal organism for the study alongside fecal coliform (Adams et al., 2023; Luna-Guevara et al., 2019). The Terrific Broth (TB) growth medium was prepared according to methods adopted and adjusted from Song et al. (2021). In this study, 12 $g.L^{-1}$ tryptone, 24 $g.L^{-1}$ yeast extract, and 4 $mL.L^{-1}$ glycerol were thoroughly mixed and then autoclaved, after which 2.31 $g.L^{-1}$ KH_2PO_4 and 12.54 $g.L^{-1}$ K_2HPO_4 were added and mixed. About 10 g of leaves were washed in 100 ml sterile plastic containers and immediately filled with sterile TB to remove *E. coli* from edible parts of the crop. The containers were centrifuged using Eppendorf 5804 R Centrifuge for 10 mins at 60 rpm. The leaves were washed by decanting from the plant material into sterile containers and incubated at 37 °C for 12 hrs using Infitex Heating Incubator, ICB (Jinan, Shandong-China). The culture-based microbiological analysis method with Most Probable Number (MPN) reporting was used to analyse the resulting solution (Velkushanova et al., 2021).

5.2.6 Data collection

The crop physiological parameters **(Table 5.10)** were measured by clamping each leaf per pot into the sensor head of the Licor (LI-6400 XT) (Evans, 2009; Mashilo et al., 2017).

Table 5.10: The measured physiological parameters.

Gas exchange parameters	**SI unit**
gs (stomatal conductance)	mol/m^2/s
Ci (intercellular CO_2 concentration)	ppm
VpdL (vapour pressure deficit of the leaf)	kPa
Trans (transpiration rate)	mol/m^2/s
R(W/m^2) (amount of radiant energy absorbed per unit area)	W/m^2
SVTleaf (specific leaf area per unit leaf area)	mol/m^2/s
H_2O_i (water vapor concentration)	--------
CndTotal (total conductance)	mol/m^2/s
CndCO$_2$ (conductance of CO_2)	mol/m^2/s
Ci_Pa (canopy index plant analysis)	ppm
Ci/Ca (ratio of intercellular and atmospheric CO_2)	--------
PARabs (photosynthetically active radiation absorbed)	µmol electrons/m^2/s
Chlorophyll fluorescence parameters	**SI unit**
*Fv’ (*variable fluorescence)	--------
*Fv’/Fm’ (*maximum quantum yield of PSII in photosynthesis)	--------
*PhiPS2 (*the effective quantum efficiency of PSII photochemistry)	--------
LeafAbs (amount of light absorbed by the leaf)	--------
PhiCO$_2$ (partial pressure of CO_2)	--------
*qP (*photochemical quenching)	--------
*qN (*nonphotochemical quenching)	--------
*ETR (*electron transport rate)	µmol electrons/m^2/s

5.2.7 Statistical analysis

Analysis of variance (ANOVA) was done using GenStat 23rd Edition (VSN International, Hempstead, United Kingdom). To show differences between treatments on measured parameters at a 5% significance level, Fisher’s protected least significant difference (LSD) test was done on the means. Principal component analysis (PCA) was conducted using XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France 2023), and biplot diagrams were generated to select parameters influenced by each water source. Additionally, Pearson correlation heatmaps were drawn based on mean values using Origin Pro 2023 (OriginLab Corporation) to visually identify and analyze the strength and direction of linear relationships between physiological parameters related to lettuce and Swiss chard (Perlo et al., 2020).

5.3 Results

5.3.1 Characteristics of the AOP-treated effluents and other water sources

The AOP-treated effluents and the municipal tap water were analyzed for biological, physical, and chemical properties, and data presented in **Table 5.11**.

Table 5.11: Summary of the nutrient ($mg.L^{-1}$) and biological ($MPN.100mL^{-1}$) properties of the study water sources (W.S).

W.S	Nitrate-N	Ammonium	Ortho-P	Fe	Mn	Zn	pH	*E.coli*	Total coliforms
R.W	-0.074	0.474	43	0.069	0.043	0.39	7.30	< 1	< 1
MTW	-0.002	-0.008	9	-0.001	-0.004	-0.001	7.20	< 1	< 1
O-TE	0.754	0.563	48	0.180	0.043	0.285	8.23	< 1	< 1
UV-TE	-0.012	0.570	46	0.016	0.020	0.279	7.89	1	1
Ti-TE	-0.012	0.563	39	-0.033	0.001	0.233	7.84	< 1	< 1

5.3.2 Crop physiological response to irrigation with AOP-treated effluents

The ANOVA for evaluated gas exchange and chlorophyll fluorescence parameters indicated that the effects of water sources (W.S), crop species (C.S), and their interaction were significantly different for most traits ($P < 0.01$) (**Table 5.12**).

Among the assessed leaf gas exchange parameters, significant differences were observed across *VpdL, Trans, SVTleaf,* H_2O_i*, CndTotal,* $CndCO_2$*,* CO_2*, Ci_Pa, Ci/Ca* ($P < 0.01$) (**Table 5.12**). O-TE recorded the highest means in $CndCO_2$ (0.38 $mol/m^2/s$) and *gs* (0.6372 $mol/m^2/s$) as compared to other W.S in Swiss chard. In lettuce, Ti-TE recorded the lowest mean *gs* (0.3561 $mol/m^2/s$) while recording the highest means in *Ci* (336.2 ppm) and *CndTotal* (0.51 $mol/m^2/s$). The same trend was observed in Swiss chard, with Ti-TE recording the lowest mean *gs* (0.2792 $mol/m^2/s$) while also recording the highest in *Ci* (342.9 ppm) and *CndTotal* (0.71 $mol/m^2/s$) (**Figure 5.1**).

Significant differences were observed across *Fv', Fv'/Fm',* $PhiCO_2$*, qP, qN, and ETR* in the assessed chlorophyll fluorescence parameters at ($P < 0.01$) (**Table 5.12**). O-TE recorded the highest means in *qP* (0.12), *ETR* (18564 um electrons/m^2/s), *PhPS2* (0.0333), and *LeafAbs* (795) as compared to other W.S in Swiss chard. In lettuce, O-TE registered a distinct lowest mean *Fv'* (246.18) and the highest in *LeafAbs* (812.3). In lettuce, Ti-TE recorded the lowest mean *Fv'/Fm'* (0.0914), *PhPS2* (0.0019), *qP* (0.01), and *ETR* (127.00 um electrons/m^2/s). The same trend was observed in Swiss chard, with Ti-TE recording the lowest means in *Fv'/Fm'* (0.1087), *PhPS2* (0.0103), and *qP* (0.05). UV-TE recorded the least mean *PhPS2* (0.034), *qP* (0.01), and *ETR* (917.00 um electrons/m^2/s) in lettuce and *PhPS2* (0.0105), *qP* (0.03), and *ETR* (744.00 um electrons/m^2/s) in s Swiss chard (**Figure 5.1**).

Table 5.12: Analysis of variance showing mean squares and significant tests for leaf gas exchange and chlorophyll fluorescence parameters of lettuce and Swiss chard evaluated upon irrigation with different AOP-treated effluents.

		Leaf Gas Exchange Parameters											
Source of variation	**d.f**	**Gs**	**Ci**	**VpdL**	**Trans**	**R(W/m2)**	**SVTleaf**	**H_2O_i**	**CndTotal**	**CndCO_2**	**Ci_Pa**	**Ci/Ca**	**PARabs**
W.S	3	0.076**	2924.51**	0.252**	3.99E05**	0.263×	0.202**	22.299**	0.051**	0.009**	10.928**	0.007**	2.97E+08**
C.S	1	0.004^{ns}	63.47^{ns}	0.005×	1.71E05**	0.257^{ns}	0.009×	0.347*	0×	0.001**	19.329**	0.002**	9.78E+06^{ns}
W.S × C.S	3	0.009×	54.46^{ns}	0.108**	2.31E05**	0.055^{ns}	0.012**	0.619**	0.05**	0.011**	5.355**	0.001**	2.39E+07×

		Chlorophyll fluorescence parameters							
Source of variation	**d.f**	**Fv’**	**Fv’/Fm’**	**PhiPS2**	**LeafAbs**	**PhiCO_2**	**qP**	**qN**	**ETR**
W.S	3	4401.894**	0.023**	0×	620.47**	1.327E10**	0.009**	0.017**	1.69E+08**
C.S	1	222730.463**	0.004×	0×	44.54^{ns}	9.067E10**	0.011**	0.025**	3.00E+08**
W.S × C.S	3	87553.697**	0^{ns}	0^{ns}	161.32^{ns}	3.77E-10**	0.001**	0.002**	7.96E+07**

d.f; degrees of freedom, ***gs***; stomatal conductance, ***Ci;*** intercellular CO_2 concentration, ***VpdL;*** vapor pressure deficit of the leaf, ***Trans;*** transpiration rate, ***R(W/m^2);*** amount of radiant energy absorbed per unit area, ***SVTleaf;*** specific leaf area (SLA) per unit leaf area, ***H_2O_i;*** water vapor concentration, ***CndTotal;*** total conductance, ***CndCO_2;*** conductance of CO_2, ***Ci_Pa;*** canopy index plant analysis, ***Ci/Ca;*** ratio of intercellular and atmospheric CO_2, ***PARabs;*** photosynthetically active radiation absorbed, ***Fv’;*** variable fluorescence, ***Fv’/Fm’;*** maximum quantum yield of photosystem II (PSII) in photosynthesis, ***PhiPS2;*** the effective quantum efficiency of PSII photochemistry, ***LeafAbs;*** amount of light absorbed by the leaf, ***PhiCO_2;*** partial pressure of CO_2, ***qP;*** photochemical quenching, ***qN;*** nonphotochemical quenching, ***ETR;*** electron transport rate, ***ns***; non-significant, ***W.S;*** water source, ***C.S***; crop species, ×and **; significance at 5 and 1% probability levels, respectively.

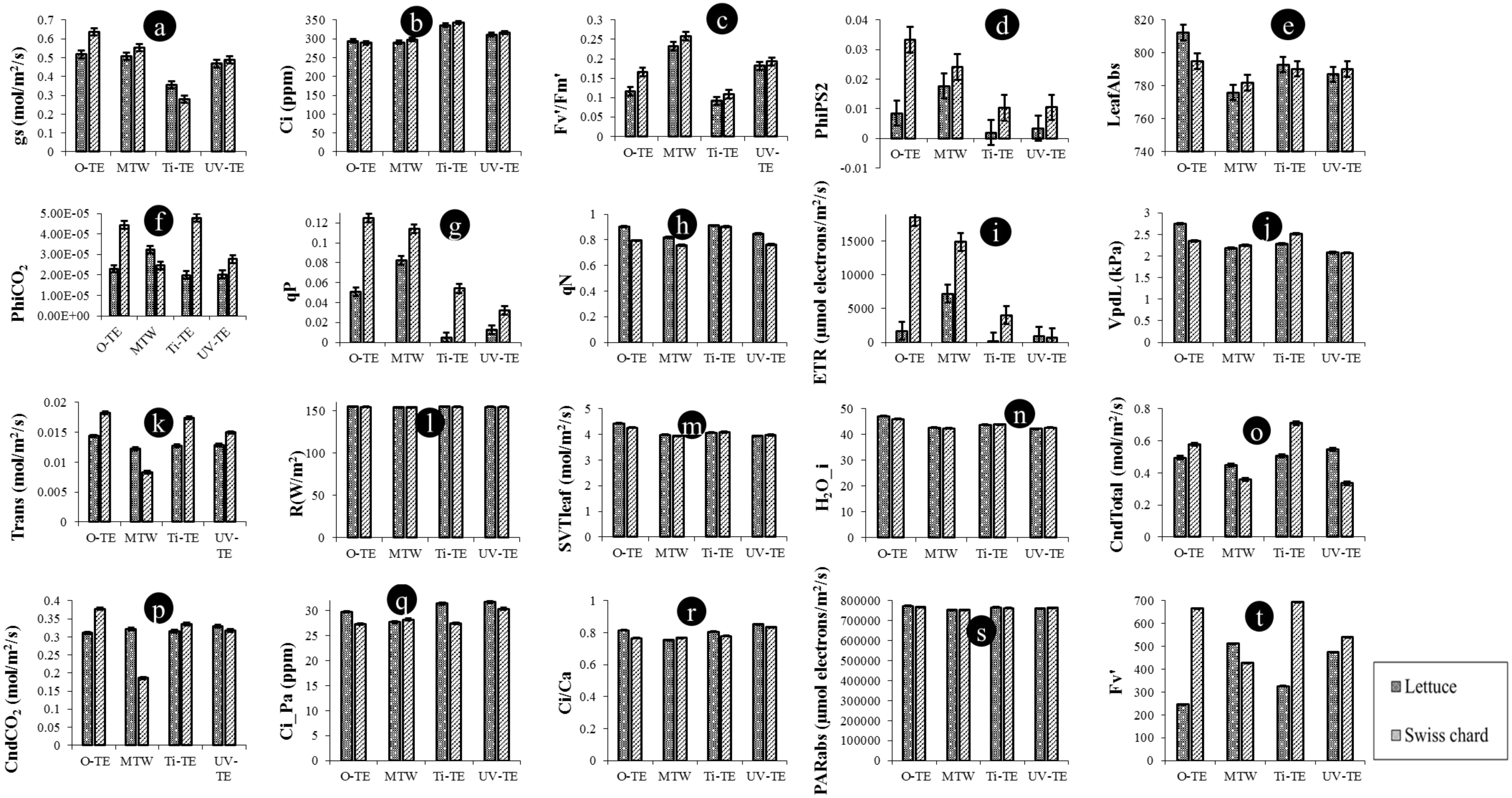

Figure 5.1: Effect of AOP-treated effluents on lettuce and Swiss chard leaves:***(a) gs;*** stomatal conductance, ***(b) Ci;*** intercellular CO_2 concentration, ***(c) Fv'/Fm';*** maximum quantum yield of photosystem II (PSII) in photosynthesis, ***(d) PhiPS2;*** the effective quantum efficiency of PSII photochemistry, ***(e) LeafAbs;*** amount of light absorbed by the leaf, ***(f) PhiCO₂;*** partial pressure of CO_2, ***(g) qP;*** photochemical quenching, ***(h) qN;*** nonphotochemical quenching, ***(i) ETR;*** electron transport rate, ***(j) VpdL;*** vapor pressure deficit of the leaf, ***(k) Trans;*** transpiration rate, ***(l) R(W/m²);*** amount of radiant energy absorbed per unit area, ***(m) SVTleaf;*** specific leaf area (SLA) per unit leaf area, ***(n) H₂O_i;*** water vapor concentration, ***(o) CndTotal;*** total conductance, ***(p) CndCO₂;*** conductance of CO_2, ***(q) Ci_Pa;*** canopy index plant analysis, ***(r) Ci/Ca;*** ratio of intercellular and atmospheric CO_2, ***(s) PARabs;*** photosynthetically active radiation absorbed, ***(t) Fv';*** variable fluorescence.

5.3.3 Principal component analysis (PCA) for evaluated parameters

Table 5.13 presents the PCA results with the assessed parameters' factor loadings, eigenvalues, and percent variance. For Swiss chard, PC1 accounted for 51.91% of the total variation and was positively correlated with *LeafAbs, $PhiCO_2$, qN, Trans, R(W/m^2), SVTleaf, H_2O_i, CndTotal, $CndCO_2$, PARabs,* and *Fv'*. PC2 positively correlated with *gs, PhiPS2, qP, ETR,* and *H_2O_i,* contributing 30.61% of the total variation. PC3 accounted for 17.47% of the total variation and was positively correlated with *Ci, qN, VpdL,* and *CndTotal*. On lettuce, PC1 accounted for 56.12% of the total variation and was positively correlated to *LeafAbs, qN, VpdL, Trans, R(W/m^2), SVTleaf, H_2O_i, CndTotal, Ci_Pa, Ci/Ca, and PARabs. gs, PhiPS2, qP, VpdL, Trans, SVTleaf,* and *H_2O_i* were positively correlated to PC2, accounting for 34.00% of the total variation. PC3 accounted for 9.86% of the total variation and positively correlated to *gs, Fv'/Fm', Trans, CndTotal, $CndCO_2$,* and *Ci/Ca*.

Based on evaluated physiological parameters, PC biplots derived from PCA analysis were used to picture the effects of AOP-treated effluents on Swiss chard **(Figure 5.2a)** and lettuce **(Figure 5.2b)**. Parallel or closely positioned vectors in the biplots indicated a strong positive association between the parameters represented, while vectors located nearly opposite (180°) exhibited a highly negative association (Mashilo et al., 2017). Vectors towards the sides of the biplots indicated a weaker relationship between the parameters.

For Swiss chard (**Figure 5.2a**), *Ci_Pa* and *Ci/Ca* were grouped based on the strong positive effects of U-TE, while *qP*, *ETR, gs*, and *Fv'/Fm'* are grouped as positively affected by MTW. O-TE showed strong positive effects on *PARabs, $PhiCO_2$, CndTotal, gs, PhiPS2, qP, VpdL, Trans, SVTleaf,* and *H_2O_i* with Ti-TE positively affecting *Ci, Fv', qN, Trans, R(W/m^2),* and *$CndCO_2$*. On lettuce (**Figure 5.2b**), U-TE showed a strong positive effect on *$CndCO_2$* and *Fv'*, whereas MTW positively affected *qP, ETR, gs, PhiPS2, $PhiCO_2$,* and *Fv'/Fm'*. On the other hand, O-TE showed strong positive effects on *PARabs*, *R*(W/m^2), *qN*, *VpdL*, *Trans*, *SVTleaf*, and H_2O_i, while Ti-TE positively affected *Ci*, *CndTotal*, *Ci_Pa*, and *Ci/Ca*.

These positive plant responses can collectively refer to (i) MTW quality supports efficient photosynthesis, stomatal conductance, and electron transport rates, (ii) UV-TE enhances photosynthetic efficiency, water use efficiency, and stomatal regulation, and lastly, (iii) Ti-TE contains TiO_2 nanoparticles stimulating plant physiological processes by altering gas exchange dynamics.

Table 5.13: Summary of factor loadings, eigenvalue, Kaiser-Meyer-Olkin measure of sampling adequacy, percent and cumulative variation for physiological parameters assessed in Swiss chard and lettuce upon irrigation with different AOP-treated effluents.

	Swiss chard				Lettuce			
Parameters	PC1	PC2	PC3	KMO	PC1	PC2	PC3	KMO
Gs	-0.321	0.777	-0.541	0.411	-0.373	0.651	0.661	0.373
Ci	0.341	-0.841	0.421	0.449	0.483	-0.723	-0.494	0.451
Fv'/Fm'	-0.929	0.355	-0.101	0.658	-0.946	0.019	0.323	0.583
PhiPS2	0.028	0.991	-0.133	0.501	-0.764	0.644	-0.032	0.640
LeafAbs	0.848	0.062	-0.526	0.495	0.877	0.443	0.188	0.591
PhiCO$_2$	0.976	0.075	0.204	0.655	-0.841	0.523	-0.141	0.604
qP	-0.062	0.984	0.169	0.512	-0.645	0.764	0.029	0.619
qN	0.753	-0.336	0.566	0.489	0.942	0.097	-0.320	0.579
ETR	-0.019	0.995	0.093	0.520	-0.885	0.458	-0.087	0.647
VpdL	0.688	0.160	0.708	0.446	0.616	0.787	0.042	0.579
Trans	0.940	-0.116	-0.322	0.567	0.709	0.562	0.425	0.498
R(W/m^2)	0.857	-0.275	-0.436	0.526	0.981	0.174	-0.089	0.681
SVTleaf	0.819	0.528	-0.224	0.575	0.647	0.756	0.101	0.575
H$_2$O_i	0.816	0.544	-0.197	0.585	0.679	0.734	0.038	0.592
CndTotal	0.900	0.030	0.435	0.562	0.548	-0.690	0.472	0.444
CndCO$_2$	0.888	-0.060	-0.456	0.520	-0.555	-0.708	0.437	0.457
Ci_Pa	-0.551	-0.597	-0.583	0.461	0.664	-0.733	0.147	0.562
Ci/Ca	-0.220	-0.752	-0.621	0.398	0.610	-0.522	0.596	0.425
PARabs	0.829	0.023	-0.559	0.484	0.928	0.368	0.063	0.657
Fv'	0.996	-0.082	-0.022	0.683	-0.898	-0.411	0.156	0.611
Eigenvalue	10.382	6.123	3.495	-	11.226	6.801	1.973	-
Variability (%)	51.910	30.616	17.474	-	56.129	34.005	9.866	-
Cumulative %	51.910	82.526	100.000	-	56.129	90.134	100.000	-

gs; stomatal conductance, ***Ci;*** intercellular CO$_2$ concentration, ***Fv'/Fm';*** maximum quantum yield of photosystem II (PSII) in photosynthesis, ***PhiPS2;*** the effective quantum efficiency of PSII photochemistry, ***LeafAbs;*** amount of light absorbed by the leaf, ***PhiCO$_2$;*** partial pressure of CO$_2$, ***qP;*** photochemical quenching, ***qN;*** nonphotochemical quenching, ***ETR;*** electron transport rate, ***VpdL;*** vapor pressure deficit of the leaf, ***Trans;*** transpiration rate, ***R(W/m^2);*** amount of radiant energy absorbed per unit area, ***SVTleaf;*** specific leaf area (SLA) per unit leaf area, ***H$_2$O_i;*** water vapor concentration, ***CndTotal;*** total conductance, ***CndCO$_2$;*** conductance of CO$_2$, ***Ci_Pa;*** canopy index plant analysis, ***Ci/Ca;*** ratio of intercellular and atmospheric CO$_2$, ***PARabs;*** photosynthetically active radiation absorbed, ***Fv';*** variable fluorescence.

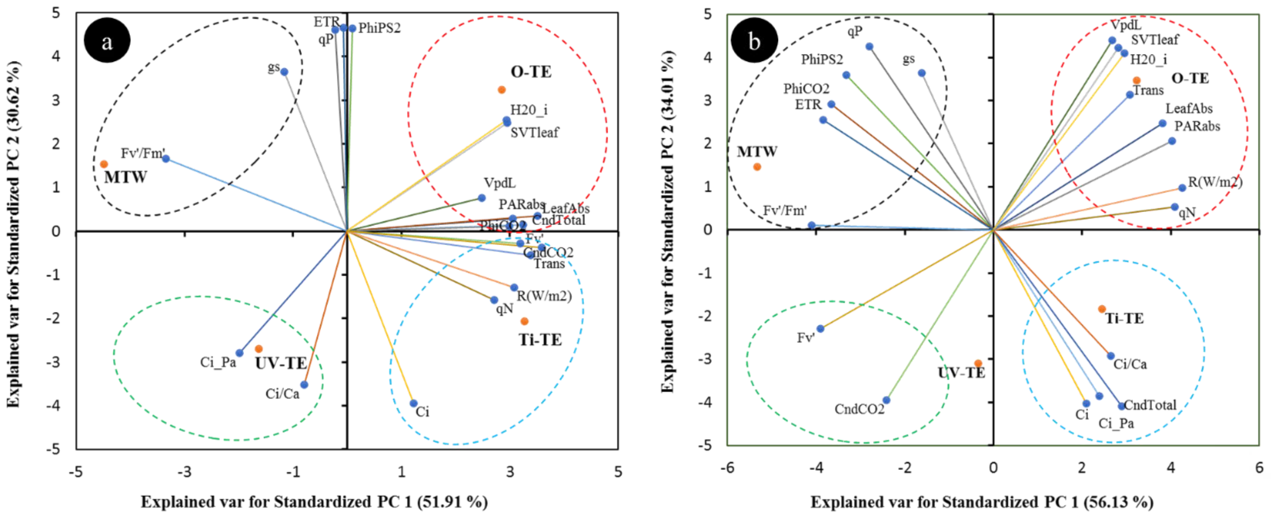

Figure 5.2: Principal component (PC) biplot of PC 1 vs PC 2 demonstrating the relationships among physiological traits evaluated under **(a)** Swiss chard and **(b)** lettuce upon irrigation with different AOP-treated effluents.

5.3.4 Pearson correlation

Pearson correlation was conducted to evaluate the negative relationship between the physiological parameters studied and TiO_2 cover on Swiss chard (**Figure 5.3a**) and lettuce leaves (**Figure 5.3b**). In Swiss chard, significant negative correlations were observed in several relationships. These include *CndTotal* with $PhiCO_2$ (r=-1: p= 0.00) and $R(W/m^2)$ (r=-1: p= 0.00), *ETR* with *PARabs* (r= -1: p= 0.00) and *Ci/Ca* (r=-1: p= 0.00), and lastly, *Fv'* with *LeafAbs* (r= -1: p= 0.00). Additionally, significant positive correlations were observed in the following relationships: *PhiPS2* with *gs* (r=1: p= 0.00), *VpdL* with *Ci* (r=1: p= 0.00), $R(W/m^2)$ with $PhiCO_2$ (r= 1: p= 0.00), H_2O_i with *qN* (r= 1: p= 0.00) and *VpdL* (r=1: p= 0.00), *Ci_Pa* with (r=1: p= 0.00), and lastly between $CondCO_2$ with *PARabs* (r=1: p= 0.00) and *Ci/Ca* (r=1: p= 0.00).

In lettuce, significant negative correlations were also observed, which included $PhiCO_2$ and *Ci* (r=-1: p= 0.00), $R(W/m^2)$ with *LeafAbs* (r= -1: p= 0.00) and *qN* (r= -1: p= 0.00), *Trans with qP* (r= -1: p= 0.00), *PARabs* (r= -1: p= 0.00) and *CndTotal* (r=-1: p= 0.00). Conversely, a few positive relationships were observed between *LeafAbs* with H_2O_i (r= 1: p= 0.00) and *qN* (r=1: p= 0.00), as well as *qP* with *CndTotal* (r=1: p= 0.00) and *PARabs* (r=1: p= 0.00).

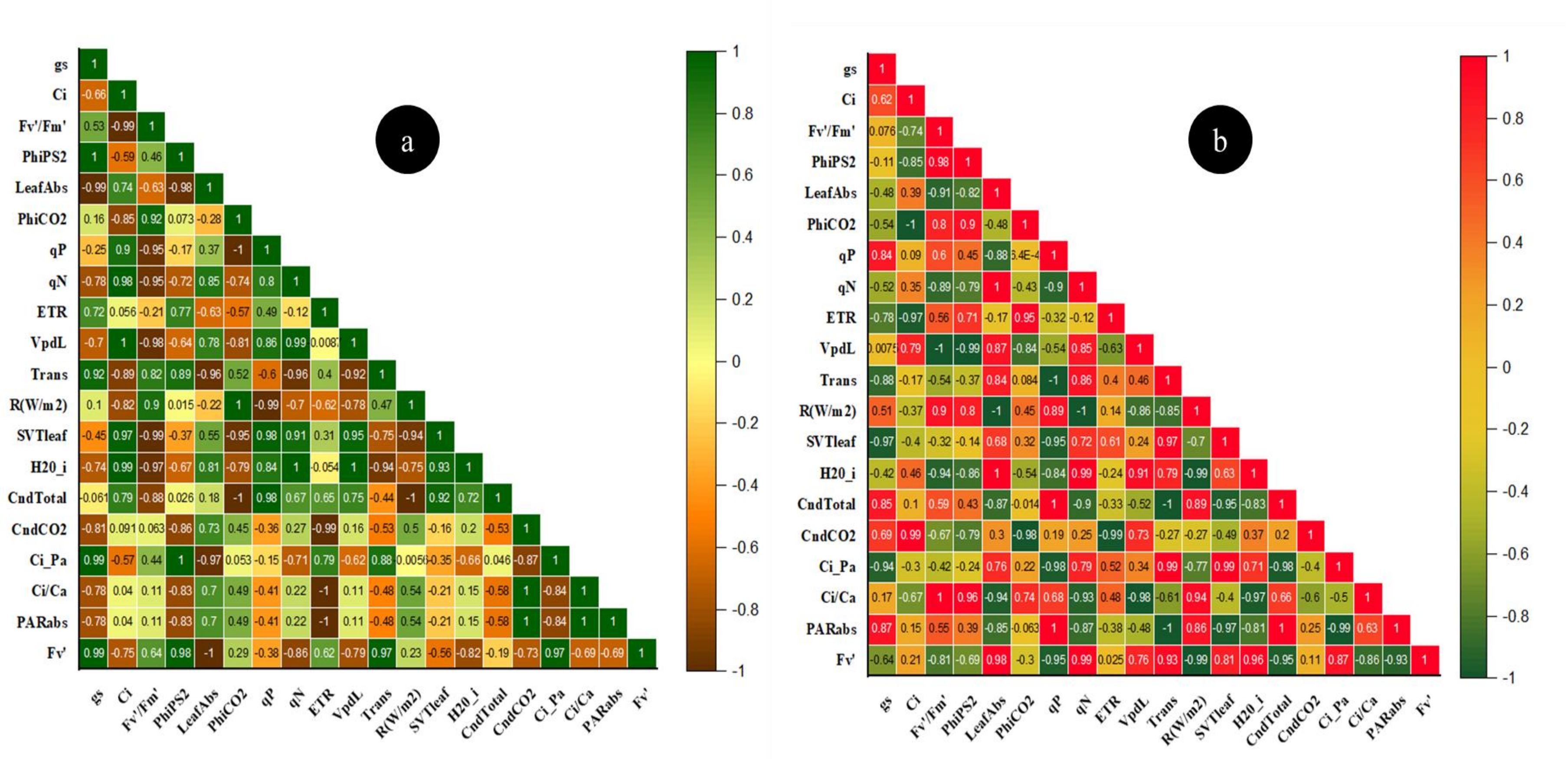

Figure 5.3: Pearson correlation coefficients showing a relationship among physiological parameters and TiO_2 cover on **(a)** Swiss chard **(b)** lettuce leaves upon irrigation with **Ti-TE**: **gs**; stomatal conductance, **Ci**; intercellular CO_2 concentration, **Fv'/Fm'**; maximum quantum yield of photosystem II (PSII) in photosynthesis, **PhiPS2**; the effective quantum efficiency of PSII photochemistry, **LeafAbs**; amount of light absorbed by the leaf, **PhiCO$_2$**; partial pressure of CO_2, **qP**; photochemical quenching, **qN**; nonphotochemical quenching, **ETR**; electron transport rate, **VpdL**; vapor pressure deficit of the leaf, **Trans**; transpiration rate, **R(W/m^2)**; amount of radiant energy absorbed per unit area, **SVTleaf**; specific leaf area (SLA) per unit leaf area, **H$_2$O_i**; water vapor concentration, **CndTotal**; total conductance, **CndCO$_2$**; conductance of CO_2, **Ci_Pa**; canopy index plant analysis, **Ci/Ca**; ratio of intercellular and atmospheric CO_2, **PARabs**; photosynthetically active radiation absorbed, **Fv'**; variable fluorescence.

5.3.5 Plasma membrane function and crop cell death

A high % of electrolyte leakage in Swiss chard was observed in Ti-TE (34.22%), followed by O-TE (19.07%) and UV-TE (12.90%), respectively. In lettuce, a high % electrolyte leakage was observed in Ti-TE (25.29%), followed by O-TE (20.20%), then UV-TE (19.57%). MTW showed a low % Electrolyte leakage in Swiss chard and lettuce (**Figure 5.4**).

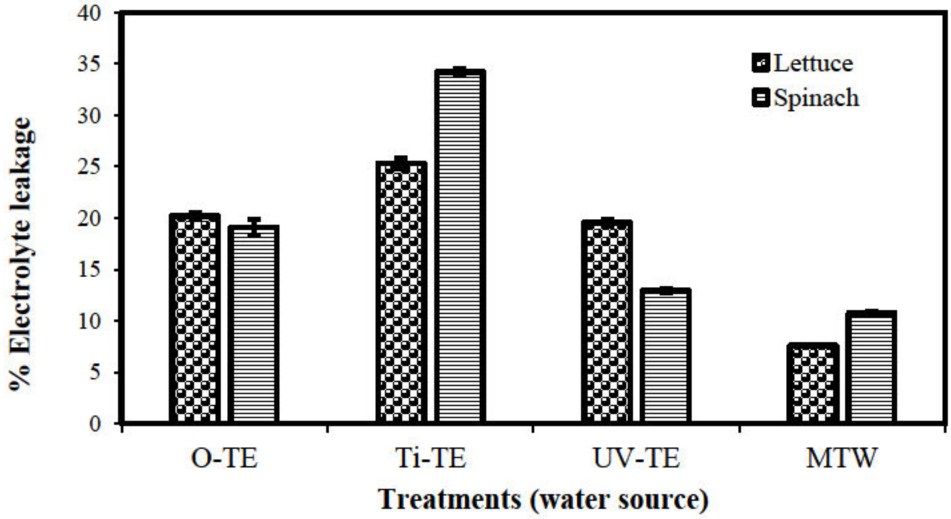

Figure 5.4: Percentage of electrolyte leakage in Swiss chard and lettuce leaves.

5.3.6 Effects of TiO_2 deposition in the crop's stomata

Crops irrigated with Ti-TE exhibited a unique thin white layer covering their surface. Upon SEM scanning, TiO_2 particle deposition within the stromal openings was revealed **(Figure 5.5)**. The deposition of TiO_2 nanoparticles on stomatal surfaces showed a significant effect on *CndTotal*, the rate of gas exchange through stomata, consequently reducing *gs*. Changes in *gs* consequently influence gas exchange for photosynthesis (Guan et al., 2015), lowering the mean *Ci* in lettuce and Swiss chard upon irrigation with Ti-TE (**Figure 5.1**).

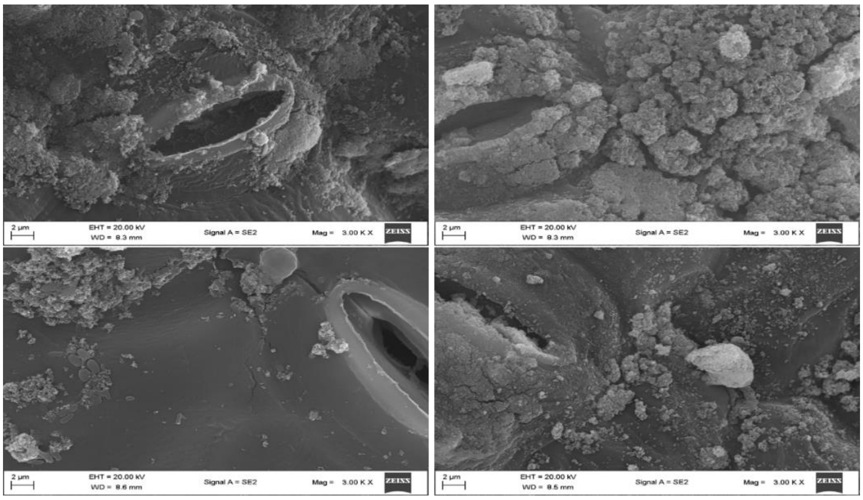

Figure 5.5: SEM images of TiO_2 deposition in stomatal openings.

5.3.7 Food safety and consumer risk

5.3.7.1 Pathogen tracing on the crop edible parts

In this study, *E. coli* levels were below detectable limits (<1 MPN.g^{-1} wet weight) on the edible leaves across all the treatments (**Table 5.14**). Additionally, no chemical reaction (NR) was observed upon detecting fecal coliforms, indicating their absence in the leaf samples.

Table 5.14: Pathogen analysis results of the leaf samples.

Treatment	Sample ID	*E. coli*	Fecal coliforms	Dilution factor
O-TE	L	<1	NR	1.00E+02
	L	<1	NR	1.00E+02
	S	<1	NR	1.00E+02
	S	<1	NR	1.00E+02
	L	<1	NR	1.00E+02
	S	<1	NR	1.00E+02
Ti-TE	L	<1	NR	1.00E+02
	S	<1	NR	1.00E+02
	S	<1	NR	1.00E+02
	L	<1	NR	1.00E+02
	S	<1	NR	1.00E+02
	L	<1	NR	1.00E+02
UV-TE	S	<1	NR	1.00E+02
	S	<1	NR	1.00E+02
	L	<1	NR	1.00E+02
	S	<1	NR	0.00E+00
	L	<1	NR	0.00E+00
	L	<1	NR	0.00E+00
MTW	S	<1	NR	1.00E+02
	S	<1	NR	1.00E+02
	L	<1	NR	0.00E+00
	L	<1	NR	0.00E+00
	S	<1	NR	0.00E+00
	L	<1	NR	0.00E+00

S; Swiss chard, ***L***; Lettuce, ***NR***; no reaction.

5.3.7.2 Accumulation of the nanomaterials on the plant's edible parts

SEM scans revealed an aggregation of irregular particles primarily made of inorganic crystalline components on crops irrigated with Ti-TE (**Figure 5.6 1a** and **2a**). Aggregates ranged between 3.27 µm and 18.19 µm in diameter (**Figure 5.7)**. The spread of the irregular inorganic crystals varied depending on the inclination of the leaves, with the crystals being more pronounced on the back of the lettuce leaves, contrary to the Swiss chard leaves. Fordhook Giant, the chosen Swiss chard cultivar, generally has a large leaf area (Motseki, 2008), allowing more TiO_2 nanoparticles to adhere to them, resulting in a higher Ti concentration on Swiss chard leaves than lettuce **(Figure 5.8)**. Elemental mapping revealed that the Ti particles appeared bluish in coloration, indicating their distinct presence on the leaf surface (**Figure 5.6 1b** and **2b**). The amount of Ti tapped on the leaves was quantified in dry-leaf weight (**Figure 5.8)**. On average, 1.46 g and 2.7 g of Ti were quantified per kilogram of lettuce and Swiss chard dry-leaf weights, respectively.

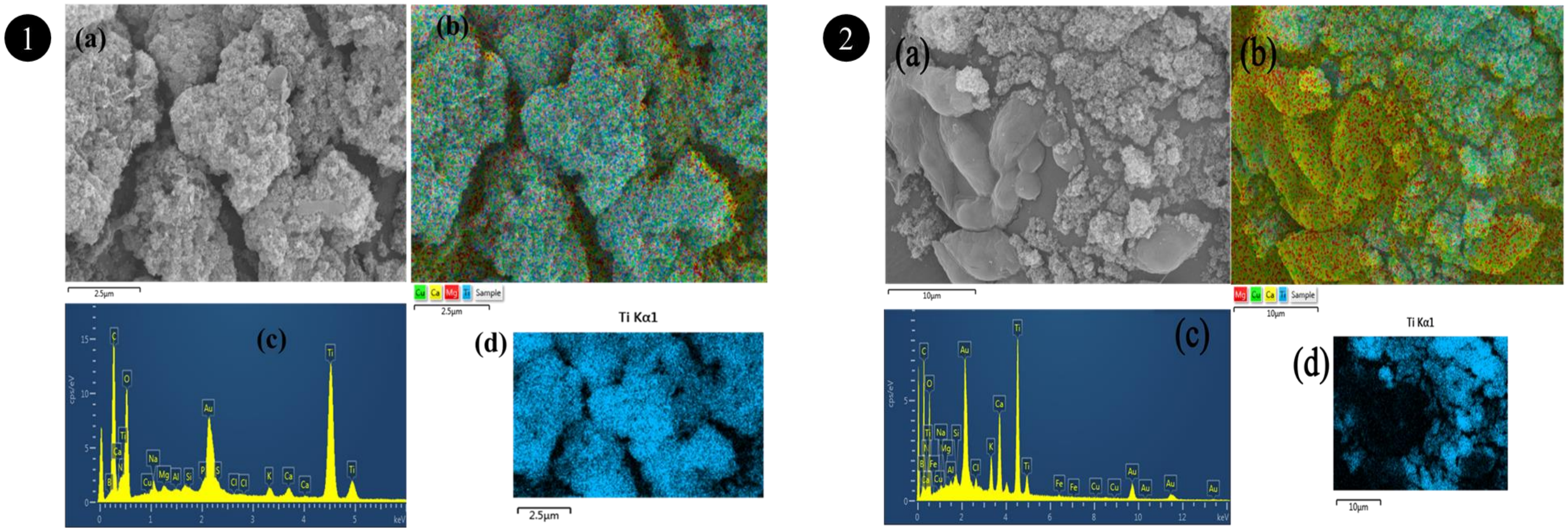

Figure 5.6: Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDX) mapped catalyst traces used in effluent treatment on the crop leaves upon irrigation: **(a)** Identified agglomerates by SEM scans, (**b)** Mapped elements by EDX scans, **(c)** EDX spectra, **(d)** Mapped Ti on the leaf, **(1)** Swiss chard leaf, **(2)** lettuce leaf.

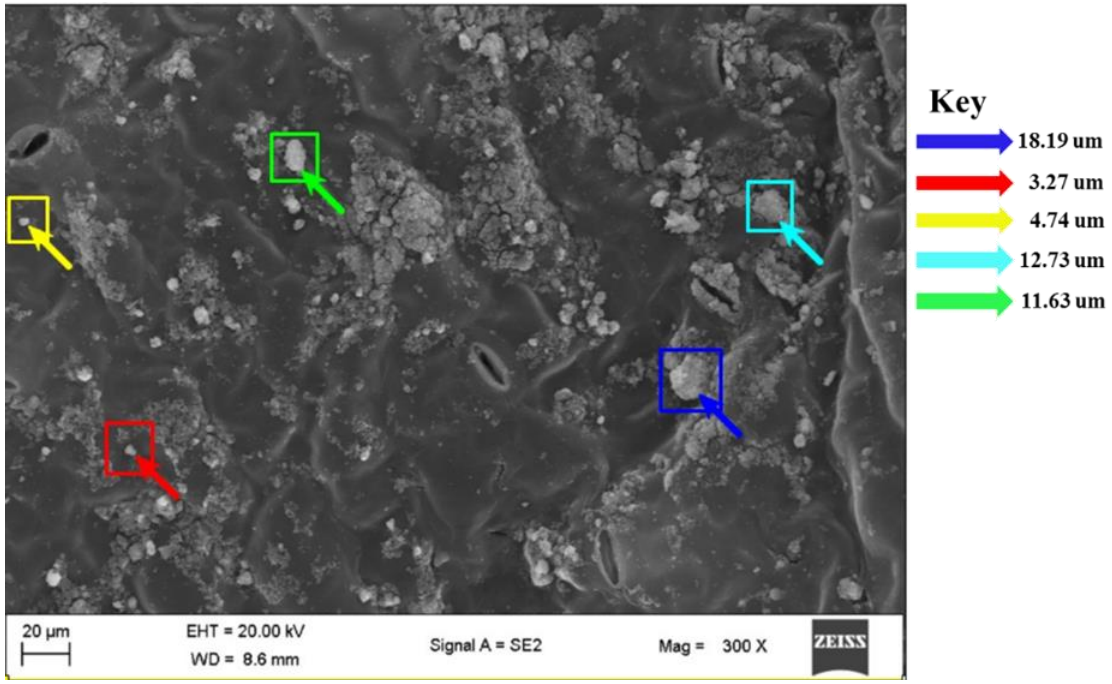

Figure 5.7: Image J diameter measurements of the SEM-identified agglomerates

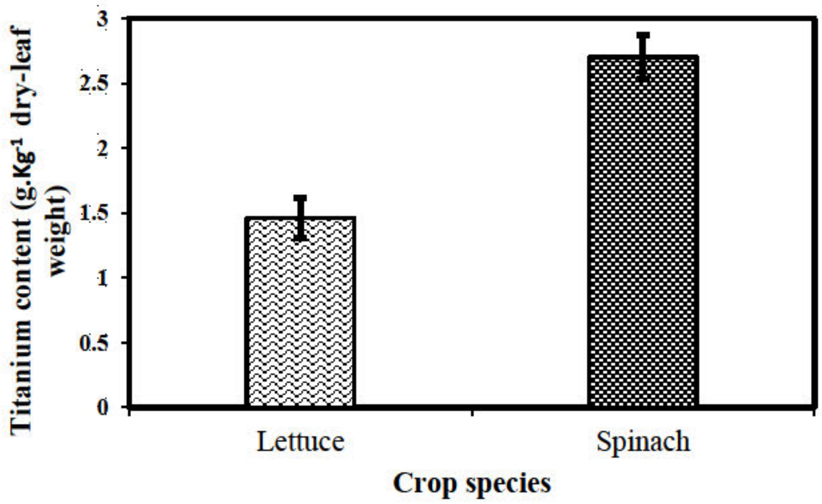

Figure 5.8: ICP/OES quantified Ti levels in the harvested dried leaves.

5.4 Discussion

Reclaimed wastewater through AOPs provides effluents with reduced pathogen contamination risks for crop production. However, the properties of the resulting effluents (**Table 5.11**) could affect crop growth and, in some cases, cause human health risks upon consumption. Hence, the current research delved into the effects of AOP-treated effluents on the physiological functions of crops, specifically examining parameters such as gas exchange and chlorophyll fluorescence. Furthermore, the study aimed to elucidate the potential health hazards posed to consumers by these treated effluents.

The study uncovered varied responses for measured physiological parameters among the assessed Swiss chard and lettuce upon irrigation with AOP-treated effluents. Generally, increased *gs* facilitates the entry of CO_2 for photosynthesis, which is responsible for the dissipation of excess heat (Walker et al., 2021). In this study, changes in *ETR* and *qP* showed crop adaptations to maintain photosynthetic efficiency under such oxidative conditions caused by O-TE (Dusenge et al., 2019; Liu et al., 2019; Walker et al., 2021). On the other hand, the reduction in the mean *PhPS2, qP,* and *ETR* under UV-TE signified a decreased efficiency of PSII photochemistry, disrupting the electron transport chain and reducing the efficiency of energy conversion and electron transport in PSII (Sperdouli et al., 2021; Sperdouli et al., 2022; Zavafer and Mancilla, 2021). These responses are potential compensatory mechanisms to counteract the oxidative stress caused by ROS exposure from O-TE and UV-TE (Sperdouli et al., 2022; Ueda et al., 2013). Compared to other W.S, Ti-TE generally showed a pronounced stress effect on the crops, with lettuce being more affected (**Figure 5.1**).

As crops undergo transpiration, moisture is released onto the leaf surface (Tian et al., 2019). In the presence of TiO_2 (**Figure 5.6**) and UV rays from the sun, TiO_2 photocatalysis is triggered, breaking down the moisture on the leaf and generating reactive oxygen species (ROS) (Brooms et al., 2018). These ROS can subsequently interact with organic compounds in the transpired water or leaf surface, initiating oxidation reactions (Fernandes et al., 2019). ROS can induce stomatal closure through various signaling pathways (Sperdouli et al., 2022), damage the photosynthetic apparatus (Zavafer and Mancilla, 2021), and impair electron transport rates and photosynthetic activity (Bhattacharjee and Bhattacharjee, 2019), resulting in reduced *gs*, *Fv'/Fm', PhPS2, qP,* and *ETR* (**Figure 5.1**). Based on these adverse effects, it is evident that TiO_2 photocatalysis persisted on the leaves after irrigation with Ti-TE.

Plant tolerance to stress could vary from crop to crop based on several adaptation mechanisms (Raza et al., 2020). In this study, the observed consistent low means in *gs, Fv'/Fm', PhPS2,* $PhiCO_2$*, qP, ETR, Trans, CndTotal,* and *Fv'* on lettuce across all the treatments (**Figure 5.1**) indicate its low tolerance to oxidative stress caused by AOP-treated effluents than Swiss chard (Li et al., 2021). It is noteworthy that Swiss chard, as noted by Jabeen et al. (2019), possesses high levels of phenolics, proline, and glycine betaine, which confer the potential to counteract the detrimental effects of ROS on leaf tissues.

In Swiss chard, the negative correlations between *CndTotal* with $PhiCO_2$ and $R(W/m^2)$ suggested reduced chlorophyll levels and impaired photosynthetic activity (Ahammed et al., 2018). Similarly, the negative correlations between *ETR* with *PARabs* and *Ci/Ca* indicated decreased photosynthetic efficiency and metabolic activity (Rodrigues et al., 2018). Additionally, the observed negative correlations between *Fv'* and *LeafAbs* suggested reduced energy transfer efficiency, leading to alterations in fluorescence and absorption characteristics within the Swiss chard leaves (Kataria and Jain, 2019). In lettuce, the relationship between $PhiCO_2$ and *Ci* (r=-1: p= 0.00) signified a reduction in the CO_2 available for photosynthesis (Wang et al., 2023). Additionally, $R(W/m^2)$ with *LeafAbs* (r= -1: p= 0.00) and *qN* (r= -1: p= 0.00) showed a reduced capacity for light absorption and dissipation of excess light energy (Moradi et al., 2023). Moreover, the relationship between *Trans* with *qP* (r= -1: p= 0.00) indicated responses to regulate water loss and optimize photosynthetic efficiency (Rivero et al., 2022), *Trans* with *PARabs* (r= -1: p= 0.00) and *CndTotal* (r=-1: p= 0.00) indicated the crop adjustments in photosynthetic and stomatal responses to changing light conditions (Devireddy et al., 2018).

These negative correlations collectively point to the thin layer of TiO_2 covering the leaves irrigated with Ti-TE (**Figure 5.6**), potentially blocking stomatal openings (**Figure 5.5**) and physically preventing or deflecting sunlight from reaching the leaves (Hong et al., 2021). When stomata are blocked, CO_2 intake decreases, reducing photosynthesis (Brodribb et al., 2020; Wall et al., 2022). Moreover, deflection or blockage of photosynthetic photon flux density (PPFD) from the leaves can reduce photosynthetic activity (Kataria et al., 2019; Liu et al., 2020). Some deposited aggregates hindered complete stomatal closure (**Figure 5.5**), leaving them fully open, thus impacting CO_2 uptake, O_2 release, and water loss regulation (Brodribb et al., 2020; Xiong and Flexas, 2020).

High percentage of electrolyte leakage indicates a compromised plasma membrane function and heightened cell death within crop leaves (Zhang et al., 2018a). Generally, MTW achieved a low % electrolyte leakage range (Swiss chard:10.7%, lettuce:7.6%), indicating less stressed crops (Salgado et al., 2014; Zhang and Yang, 2017). In an earlier study, Zhang et al. (2021) confirmed a complete adsorption of Fe, Mn, and Zn by TiO_2. Irrigation with Ti-TE effluent leads to an undersupply of these adsorbed trace elements, resulting in nutrient imbalance. Potentially, this interferes with plant metabolism, affecting the membrane integrity and contributing to the high % of electrolyte leakage (Gautam and Dubey, 2018; Hussain et al., 2023). Moreover, increased % electrolyte leakage in the O-TE, Ti-TE, and UV-TE would also be linked to the ROS presence in the effluents, as confirmed by Alyemeni et al. (2018), higher ROS concentrations result in increased electrolyte leakage in crop tissues.

Food quality has attracted a lot of concern in recent years due to increased environmental contamination (Gupta and Singh, 2011; Lu et al., 2015). Stringent food quality standards have since been developed to ensure consumers' safety (Trienekens and Zuurbier, 2008; Webb, 2015). The absence of *E. coli* and *Faecal coliforms* on the grown lettuce and Swiss chard underscored the safety of the crops for human consumption (Amoah et al., 2006). The efficacy of these studied AOPs in eliminating pathogens underscores their reliability in treating domestic wastewater for agricultural use (Di Cesare et al., 2020). These results align with food safety standards, demonstrating compliance and risk mitigation measures that ensure the vegetables meet stringent criteria for pathogen-free status (Gemmell and Schmidt, 2012; Mdluli et al., 2013; Richter et al., 2021). Moreover, these results assure public health regarding raw vegetable consumption, such as lettuce, and signify a potential for innovative domestic wastewater treatment technologies to contribute to sustainable agricultural practices (du Plessis et al., 2021; Gemmell and Schmidt, 2012; Ijabadeniyi and Buys, 2012; Moses et al., 2016; Richter et al., 2021).

5.5 Risk assessment on TiO_2 accumulation

Studies have shown that the absorption of TiO_2 nanoparticles from the gut into the body tissues is influenced by particle size (Guillard et al., 2020; Jones et al., 2015). Guillard et al. (2020)

confirmed that the smaller the size of TiO_2 nanoparticles, the higher the chances of absorption into the body tissues. Additionally, aggregation of TiO_2 in the gut increases the particle sizes, reducing the absorption percentage into body tissues (Pujalté et al., 2017).

Recent findings challenge the notion of TiO_2 inertness in either form, nanoparticles, or aggregates, with several studies confirming the possible health risks associated with ingesting TiO_2. Health studies on rats suggest that exposure to TiO_2 at doses ranging from 0.005 mg/kg to 1 g/kg bw/day can lead to slight liver and heart injury (Bu et al., 2010), cause preneoplastic lesions (Chen et al., 2019), and colon cancer (Bischoff et al., 2020). Studies done with humans show that TiO_2 accumulation beyond 0.008 mg/kg and 0.14 mg/kg in the liver and spleen could pose health risks to elderly individuals (Heringa et al., 2018). Additionally, intestinal tumors, allergic responses, and gastrointestinal issues have been confirmed in human health studies (Antonio da Silva et al., 2021; Bettini et al., 2017; Bischoff et al., 2020; Urrutia-Ortega et al., 2016). Moreover, placental dysfunction or transplacental passage, which could harm fetal development, has been reported in maternal health studies (Umezawa et al., 2012; Yamashita et al., 2011; Zhang et al., 2018b). Furthermore, TiO_2 exhibits toxicity to multiple bodily systems, including the neurological, cardiovascular, and respiratory systems, raising further concerns about their safety for human consumption (Jin et al., 2022; Warheit and Donner, 2015).

While studies have investigated titanium's potential health effects and exposure pathways, there is a lack of comprehensive understanding regarding the rate at which ingested Ti compounds are absorbed into various body tissues. Bischoff et al. (2020) confirm that the effect of TiO_2 on microbiotic health and intestinal barrier integrity has made it impossible to validate whether its absorption increases with increased ingestion. Consumption of crops grown using Ti-TE (**Figure 5.8**) significantly increases the levels of TiO_2 in the gut. Whether absorbed into various body tissues or not, this TiO_2 accumulation could pose health risks based on findings from animal studies. Therefore, a dose-response survey is needed to accurately evaluate the potential risks associated with TiO_2 ingestion in humans. Addressing these gaps through studies will be essential for developing informed regulatory guidelines and risk assessment frameworks to safeguard public health.

5.6 Conclusion

The study investigated the effects of using AOP-treated municipal effluents for irrigation, their effects on crop growth, and the potential health risks of consuming crops irrigated with these effluents. The results showed significant phytotoxicity effects on the examined crops (Swiss chard and lettuce), with Ti-TE exhibiting more pronounced effects than other water sources. Interestingly, Ti-TE deposited TiO_2 traces in stomata and covered the leaves from sunlight, causing significant chlorophyll fluorescence and gas exchange stresses. Moreover, Swiss chard showed a more significant tolerance to water sources than lettuce. Notably, no pathogens were detected on the crop leaves, with *E. coli* reading < 1 MPN and no reaction from Fecal coliforms. However, the levels of TiO_2 found on the edible leaves, in reference to the literature, showed potential health risks associated with consuming Ti-TE-irrigated crops. Therefore, until a dose-response survey on TiO_2 is adequately studied, its use in the treatment of effluents meant for reuse in unrestricted agricultural production should not be recomended. In contrast, utilising O-TE and UV-TE demonstrated a promising approach to maintaining the environment’s integrity and food quality standards.

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CHAPTER 6: GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 General conclusions

While effective to a certain extent, conventional wastewater treatment methods often fall short of fully mitigating the diverse range of contaminants present in domestic wastewater (Lourenço and Nunes, 2020; Singh et al., 2019; Späth et al., 2021). For instance, the slow and land-intensive conventional wetlands have been identified as non-complying in relation to discharge and agricultural reuse standards of municipal effluents from decentralized wastewater treatment systems (DEWATS) (Gearheart, 1999; Späth et al., 2021; Wu et al., 2016). Therefore, establishing a systematic and reliable approach that optimizes wastewater resources for sustainable crop production while ensuring minimal environmental impact, public health, and overreliance on freshwater resources is important. The present study sought to understand the efficiency of advanced oxidation processes (AOPs) in pathogen control of DEWATS effluent to achieve unrestricted crop production standards with produce that has no health risks upon consumption. To date, little is known about the pathogen regrowth potential of effluents treated through AOPs, the potential effects the AOP-treated effluent has on soil health and crop growth, and possible health risks associated with consuming crops irrigated with AOP-treated effluent, which formed the respective objectives of the present study.

The first objective reviewed current AOPs and their potential use in crop-irrigated settings. Recent data highlighted ozone-based, photolysis-based, and hybrid AOPs as key focuses for pathogen reduction in wastewater. While AOPs showed promise against pathogens like *E.coli* and Total coliforms, their potential decreased with high organic levels in the effluent. Ozone-based and hybrid AOPs notably reduced *E.coli* and Total coliforms, while photolysis-based AOPs targeted Total coliforms without addressing *E.coli*. Notably, only 3 out of 66 studies explored pathogen regrowth in AOP-treated effluent, indicating a potential research gap.

The second objective determined the potential of selected AOPs (ozonolysis, UV photolysis, and TiO_2 photocatalysis) to eliminate pathogens in domestic wastewater and prevent regrowth. Ozonolysis and TiO_2-photocatalysis achieved a 6.4-log reduction in *E.coli* and Total coliforms, while UV-photolysis achieved a 6-log reduction. After four days of storage, ozonolysis-treated (O-TE) samples showed no pathogen regrowth, while TiO_2-photocatalysis treated effluent (Ti-TE) had regrowth of *E. coli* and *Total coliforms* at 2.5-log and 2.7-log, respectively. UV-photolysis treated effluent (UV-TE) had a regrowth rate of 0.5-log for *E. coli* and 2.2-log for *Total coliforms*. Following the exponential pathogen regrowth during storage, potential challenges in maintaining microbial control under TiO_2 photocatalysis were revealed. Notably, the absence of pathogen regrowth in O-TE demonstrated the ability of ozonolysis to sustain improved microbial control. In addition to pathogen elimination, ozonolysis treatment significantly reduced BOD, soluble COD, humic, and turbidity while increasing the ortho-P, nitrates, pH, EC, ammonium, and DOC. On the other hand, UV photolysis and TiO_2 photocatalysis resulted in marginal reductions in BOD, nitrates, and turbidity with increased EC, pH, ammonium, DOC, humic, and ortho-P levels. Based on the current findings, ozonolysis proved to be the best AOP for the treatment of the DEWATS effluent to meet the WHO (Organization, 2006), USEPA (Tzanakakis et al., 2023), FAO (Shoushtarian and Negahban-Azar, 2020), and EU (Angelakis and Gikas, 2014) pathogens standards for unrestricted agricultural use.

The possible effects of the AOP-treated effluents on soil nutrient mineralization dynamics was investigated using an incubation study (objective 3). A significant reduction in the ammonium levels across UV-TE, O-TE, and Ti-TE treatments was observed, which did not translate to significant changes in the nitrate levels. As reported by Wang et al. (2018), this incongruency suggested a likelihood of ammonification. Additionally, phosphorus mineralization was relatively slower across UV-TE and O-TE than in the controls (DEWAT Anaerobic Filter effluent (RW) and municipal tap water; (MTW)); however, total mineralization of P was achieved after day 70 of the incubation. The study confirmed a consistent and significant reduction of ortho-P in Ti-TE irrigated soils, indicating effective phosphorus binding by TiO_2 (Kaur et al., 2022; Yupeng et al., 2023; Zhang et al., 2024).

The fourth objective investigated the impact of AOP-treated effluents on crop physiological functions with respect to photosynthetic rates and capacity and potential health risks for consumers. Significant phytotoxicity effects in Swiss chard and lettuce irrigated with AOP-treated effluents were revealed, with Ti-TE showing the highest stress levels, followed by O-TE and UV-TE. While no pathogens were detected on the leaves across all the treatments, TiO_2 agglomerates were identified using SEM-EDX on crop leaves irrigated with Ti-TE. High TiO_2 levels were recorded upon ICP/OES quantification, suggesting potential TiO_2 health risks associated with consuming crops irrigated with Ti-TE (Umezawa et al., 2012; Yamashita et al., 2011; Zhang et al., 2018).

Overall, the study has shown that AOPs can be effectively used to polish secondary municipal effluents for unrestricted crop growth. Of the AOPs investigated, ozonolysis was the most promising approach, meeting the standards for unrestricted crop production. Moreover, it showed insignificant effects on soil nutrient mineralization as well as mild phytotoxicity effects on crops upon irrigation. Furthermore, upon irrigation, the O-TE did not present any potential health risks to consumers, as no traces of pathogens were detected on the crop leaves.

6.2 Recommendations for further research

The research suggests that farmers consider using ozonolysis-treated effluent (O-TE) for crop cultivation without restrictions due to its compliance with WHO, USEPA, FAO, and EU pathogen standards for agricultural use. However, it also proposes conducting a thorough cost analysis of ozonolysis to provide insights to stakeholders interested in implementing this method for intensive crop production in wastewater-irrigated environments. It is necessary to explore the impacts of O-TE, UV-TE, and Ti-TE on soil microbial diversity and trace element levels on post-irrigation. Additionally, given the concerns highlighted from animal studies regarding TiO_2 accumulation and potential health risks (Jin et al., 2022; Warheit and Donner, 2015), a dose-response survey is advised to accurately assess the dangers associated with TiO_2 ingestion in humans.

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