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Assessment and characterization of dissolved organic nitrogen removal in simulated soil aquifer treatment systems

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by

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ABSTRACT

Treated wastewater (reclaimed water) is becoming more critical as an alternative water source due to population growth and extreme precipitation patterns. The more we rely on water reuse schemes, the more we have to expand our knowledge about the efficiency of treatment processes. As part of the water reuse programs, soil aquifer treatment systems are widely applied to enhance groundwater resources and the quality of treated wastewater by providing environmental barriers, including soil and water. There have been decades of applied research on different types of soil/aquifer-based treatment systems focusing on their operation and maintenance as well as their capability to remove contaminants. However, considering the fact that the list of contaminants of concern are continually developing and these systems are highly site-specific, there is always a need to understand better the fate of compounds that can act as a contaminant themselves in specific doses or become pre-cursors of other contaminants.

Therefore, this research focused on dissolved organic nitrogen (DON) that has yet to be adequately investigated. Effluent DON could be problematic in the receiving waters as a nitrogen source causing eutrophication. It can also act as a precursor for nitrogenous disinfection by-products. In this study, we used unsaturated soil columns to simulate the vadose zone treatment systems in the lab. We simulated field characteristics by using the soil from the vadose of effluent rapid infiltration basins and reclaimed water from a full scale water reclamation rather than artificial wastewater. We observed steady DON removal in the columns for more than a year and a half, as well as the improvement of water quality through

the removal of other organic compounds. Since N-Nitroso-dimethylamine (NDMA), one of the nitrogenous disinfection by-products, concentrations were pretty low in our feed reclaimed water, and we did not reach conclusive results about the columns' capability of further removal of NDMA. However, our results showed that the columns could remove NDMA precursors by removing the reclaimed water organic matter.

In addition, we characterized the organic matter in the reclaimed water applied to the columns using fluorescence spectroscopy EEM spectra. EEM spectra analysis has been widely used for organic matter characterization in drinking or surface water. Here, we focused on its capability to monitor the performance of soil aquifer treatment systems. We were able to detect humic-like, fulvic-like, and tyrosine-like compounds in the influent and effluent of the columns using the fluorescence regional technique. We followed their change through the columns and found that most of the DON removal was attributed to humic-like compounds, even though the removal was observed in different defined regions. We fitted a PARAFAC model with four components to our data that was able to be compared with other models across different aquatic systems. The main component of the model (C1) was attributed to the aromatic molecules of terrestrial origin and associated with humic-like compounds. Another detected component (C3) corresponded with microbial humic-like and terrestrially delivered and was reported at drinking water treatment plants. The results confirmed the capability of EEM spectra as a monitoring tool for the organic material and that its potential should be further investigated in the lab and field-scale soil aquifer treatment systems under different hydrogeological conditions, operation and maintenance techniques, and source water quality.

Acknowledgments

When a ship started on a journey, it would rely on the guiding stars to show them the path and help them explore uncharted waters. I like to think of my journey in academia in the same way where I had the privilege of having a constellation of support and encouragement from my mentors and friends. I would like to thank my advisor, Dr. Krishna Pagilla, sincerely. Since day one, when he accepted me to his research group, his belief in me has been the main fuel that kept me going, and I would not have gotten the chance to write my dissertation's “Acknowledgment” section without the wisdom, patience, knowledge, and kindness he shared with me over the past years. I would also express my gratitude towards my committee members, Dr. Eric Marchand, Dr. David Hanigan, Dr. Sage Hiibel, Dr. Jackson Crews, and Dr. Rishi Parashar, who helped to shape my research with valuable insights and feedback and were always there to help.

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I talked about the journey, but every ship has to come home at the end. My husband, Milad, was the lighthouse that always showed me the way home. At the end of the hard days, he was there with a cup of tea or a hot meal and a few jokes on hand. He became familiar with all the aspects of my research by listening to my practice presentations or just being my sounding board. He always knew what to say to cheer me up. Milad and our pet, Milo, never stopped being a source of joy and stability for me.

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List of important abbreviations

AOPs	Advanced Oxidation Processes
ASR	Aquifer Storage and Recovery
BDOC	Biodegradable Organic Carbon
CDOM	Colored Dissolved Organic Matter
CNX	Cyanogen halides
CON	Colloidal Organic Nitrogen
DCAAs	Dissolved Combined Amino acids
DFAAs	Dissolved Free Amino acids
DOM	Dissolved Organic Matter
DON	Dissolved Organic Nitrogen
EDCs	Endocrine-Disrupting Compounds
EDTA	Ethylenediaminetetraacetic Acid
EfOM	Effluent Organic Matter
EOCs	Emerging Organic Contaminants
EON	Effluent Organic Nitrogen
FDOM	Fluorescent Dissolved Organic Matter
FRI	Fluorescence Regional Integration
GAC	Granular Activated Carbon
HAcAms	Haloacetamides

HANs	Haloacetonitriles
HLR	Hydraulic Loading Rate
HNMs	Halonitromethanes
HRT	Hydraulic Retention Time
IPR	Indirect Potable Reuse
MAR	Managed Aquifer Recharge
MBR	Membrane Bioreactor
N-DBPs	Nitrogenous Disinfection By-Products
NDMA	N-nitrosodimethylamine
NOM	Natural Organic Matter
NTA	Nitrilotriacetic Acid
OMPs	Organic Micropollutants
PAC	Powdered Activated Carbon
PARAFAC	Parallel Factor Analysis
PhACs	Pharmaceutically Active Compounds
PON	Particulate Organic Nitrogen
PPCPs	Pharmaceutical and Personal Care Products
RBF	Riverbank Filtration
SAT	Soil Aquifer Treatment
SMPs	Soluble Microbial Products

THM	Trihalomethane
TN	Total Nitrogen
TOC	Total Organic Carbon
TOrCs	Trace Organic Compounds
WWTPs	Wastewater Treatment Plants

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Chapter 1 Introduction

1.1 Background

The high demand for water resources versus the available supplies has made it critical to develop new ways to secure, manage, and treat water supplies for drinking water. Wastewater reclamation by advanced treatment processes to produce potable quality water has gained attention in recent years to build water resilience against ever-intensifying demand. Wastewater reclamation is usually defined with advanced technologies, including reverse osmosis membranes or ozonation coupled with biological filtration. However, there are other natural and low-cost treatment processes to improve water quality.

Managed aquifer recharge (MAR) systems that are based on the soil and aquifers, including soil aquifer treatment (SAT), are recognized and promising post-treatment techniques for further treatment of wastewater effluent (Figure 1.1). For instance, rapid infiltration of treated water into the ground has a long history and counts as a common disposal method in small community treatment systems. While such a method of treated effluent disposal could be considered a de facto approach to aquifer replenishment, there are many cases in which these systems are purposefully designed and engineered to address better our current issues, including the fate of contaminants of concern, microbial pathogens, and disinfection by-product (DBP) precursors. The better we understand the treatment efficacy of these systems in regard to different contaminants, the better we can attribute removal credits to them as part of the larger water reuse schemes. To this end, this study has focused mainly on effluent dissolved organic nitrogen (DON) removal through SAT systems.

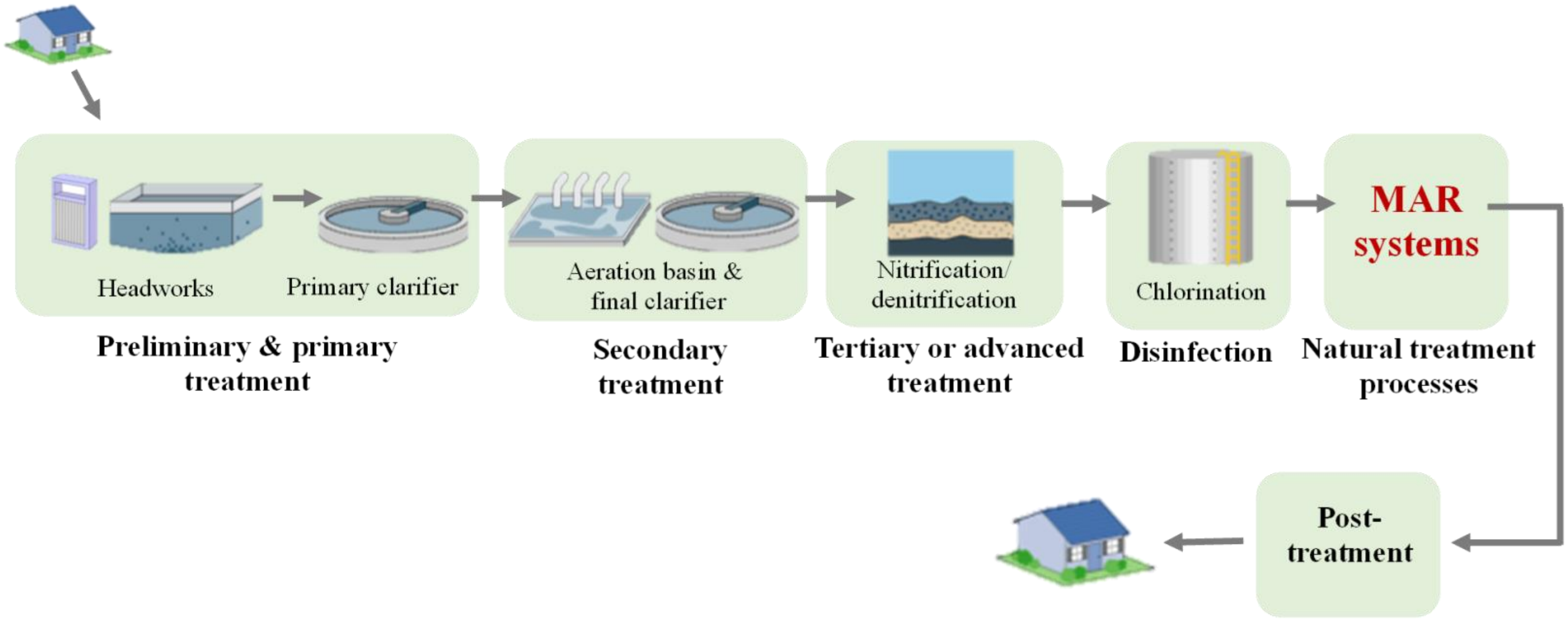

Figure 1.1 MAR treatment systems in water reuse schemes

1.2 Significance of research

Engineered SAT systems are great assets to recharge groundwater supplies as well as further treat the reclaimed water when they are used as part of the natural barrier in the indirect potable reuse systems. This research will provide essential information on the efficacy of SAT systems on two classes of water quality constituents that are of considerable interest from a public health perspective: DON and NDMA, which can be found in the reclaimed water that is applied to SAT. Further assessment will be conducted on the capability of fluorescence spectroscopy to detect and characterize the organic matter at the vadose zone.

1.3 Research hypothesis

The general hypothesis in this study is that SAT systems can further polish the treated water from wastewater treatment facilities and protect groundwater resources in the case of de facto reuse. Therefore, the following research questions are presented and will be investigated using lab-scale soil columns simulating large-scale SAT systems (vadose zone).

A. Are SAT systems reliable methods for further removal of organic matter with a focus on DON and low concentration NDMA from the treated wastewater via the subsurface environment when acting as the natural barrier in water reuse systems?

    A.1. In the case of organic matter removal, what are the major removal mechanisms?

    A.2. In the case of organic matter removal, will the potential for NDMA formation decrease following SAT?

B. Considering the unknown nature of organic nitrogen, is fluorescence spectroscopy, a non-intrusive and relatively fast method, capable of organic matter characterization in soil columns representing the vadose zone?

1.4 Chapters and contents

The following chapters construct the dissertation structure of this research.

Chapter 1: Introduction

In this chapter, a brief introduction of the whole dissertation is presented. This provides some key terms, including background, objectives, and the scope of the study. It introduces the chapters and contents of the dissertation.

Chapter 2: Literature review

This chapter gives a critical and detailed literature review of previous studies on the capability of soil/aquifer-based treatment methods in chemical compounds' removal. First, the chapter starts by defining key terms, such as distinguishing different managed aquifer recharge systems, including soil aquifer treatment systems. Then, the capability of soil/aquifer-based treatment processes with a focus on SAT and riverbank filtration (RBF) in removing organic matter, mainly nitrogen, will be researched, including the characterization of dissolved organic nitrogen, the importance of DON removal, its mechanisms, and critical evaluations of lab/field studies on DON removal. Based on this critical review, research needs and gaps are identified to take the SAT as a standard practice for DON removal from treated wastewater effluents for reuse as well as for environmental discharge.

In summary, the chapter emphasizes the importance of DON fate in SAT systems, which sets the objectives of the dissertation in later chapters.

Chapter 3: Dissolved organic nitrogen removal and its mechanisms during simulated soil aquifer treatment

This chapter focuses on the capability of soil columns representing the vadose zone in increasing the overall water quality with a focus on organic nitrogen. The hypothesis in this chapter is that 1) organic nitrogen can be removed effectively using biodegradation or sorption; 2) biodegradation plays an essential role in the removal because of the established biomass in the columns. 3) the soil columns can remove NDMA and NDMA precursors.

Chapter 4: Characterization of organic matter during simulated soil aquifer treatment (vadose zone)

The chapter will provide further analysis on the characterization of fluorescent organic matter fractions present in the influent and effluent of the columns and the identification of the patterns in the removed organic fractions. The chapter presents the EEM spectra of samples that were collected from the soil columns and different techniques applied to analyze and interpret the data. Furthermore, the chapter suggests that fluorescence spectroscopy can be a reliable way to monitor the organic matter changes in SAT systems and should be further investigated at larger scales as a promising technique.

Chapter 5: Conclusions

This chapter summarizes the conclusions of the research, compiles and discusses the results of both studies, and identifies the remaining questions for future research.

References

Dillon, P., Australia, National Water Commission, 2009. Managed aquifer recharge: an introduction. National Water Commission, Canberra, A.C.T.

Chapter 2 Literature review

This chapter has been published and can be found at: "Gharoon, N., & Pagilla, K. R. (2021). Critical review of effluent dissolved organic nitrogen removal by soil/aquifer-based treatment systems. Chemosphere"

2.0 Abstract

Soil/aquifer-based treatment systems improve wastewater effluent quality by removing trace contaminants in the soil and/or aquifer prior to groundwater recharge. This paper critically reviews these systems with a focus on removing nitrogen, particularly low levels of dissolved organic nitrogen (DON), from wastewater effluent. DON in wastewater effluent is a concern because of its contribution to nitrogen concentration in the surface or groundwater and its role as a precursor of nitrogenous disinfection by-products that harm human health. Biodegradation and sorption are the main DON removal mechanisms in the soil, where most of the removal happens in the vadose zone. Different factors such as temperature, redox conditions, retention time, indigenous microbial community, and soil type affect DON removal in soil/aquifer-based treatment systems. The lack of sufficient current knowledge underlines the need for designing lab/field scale systems for further determination of the relative contribution of biodegradation and sorption, optimal hydraulic loading rate, and the relationship between DON characteristics such as functional groups and physiochemical processes. Future research needs for DON removal in soil/aquifer-based treatment systems are identified.

Keywords: Dissolved Organic Nitrogen; Soil/Aquifer-based Treatment Systems; Wastewater Effluent Treatment; Disinfection By-products; Water Reuse.

2.1 Introduction

Water scarcity and uncertain groundwater sources have made it crucial to develop new ways to secure and treat water supplies for drinking water. Reclamation of wastewater by advanced treatment to produce potable reuse quality water has gained attention in recent years due to its proximity to end-users and as a secure, locally controlled water supply. Although engineered advanced water treatment methods, including reverse osmosis and biological treatment coupled with advanced oxidation, have been common, ground-based treatment systems upgrading rapid infiltration and other wastewater effluent ground disposal methods may be more desirable. They can remove trace contaminants and pathogens from treated effluent for potable reuse applications. For example, rapid infiltration of treated water into the ground is a common disposal method in small community treatment systems. Such a method of treated effluent disposal is considered a de facto recovery of treated water into the groundwater aquifer. The main constituents of concern in wastewater effluents to upgrade them to potable reuse water include pharmaceuticals, personal care products, synthetic chemicals, microbial pathogens, disinfection by-product (DBP) precursors, and nanoparticles. Managed aquifer recharge (MAR) is one of the methods to secure water for future use, improve groundwater quality, and prevent saltwater intrusion. MAR includes the intentional recharge of water to aquifers for environmental benefits or later recovery (Figure 2.1) (Dillon *et al.*, 2009). MAR systems such as riverbank filtration (RBF), soil aquifer treatment (SAT) by surface infiltration, and aquifer storage and recovery (ASR) are soil/aquifer-based treatment processes where removal mechanisms take place in soil and/or aquifer. Based on the input location of the water and treatment zone, media characteristics, removal mechanisms, and their potential application for water reuse purposes can be described more accurately and thoroughly, as on some occasions, multiple names are used for a given method. To use these systems as part of the wastewater treatment process, there is a need to meet certain factors such as sufficient infiltration rate, filtration, and residence time for natural attenuation processes in soil and aquifer sediments, and sufficient length of vadose zone (Maliva, 2020). In regions where vadose zone hydraulic conductivity and aquifer storage capacity are not limiting factors, soil/aquifer-based treatment of wastewater effluents to remove trace levels of organic, inorganic, and microbial contaminants could be explored.

During SAT in-situ treatment, water percolates through unsaturated and saturated zones of the subsurface environment, and the subsurface media removes contaminants mainly by sorption and biodegradation. Biodegradation in SAT is initially aerobic in the vadose zone and anoxic in the aquifer (Amy & Drewes, 2007). RBF is mostly a vadose zone treatment method where the unsaturated subsurface has the ability to improve water quality. It has the advantage of microbial activity and chemical reactions that usually occur more frequently in the colmation layer within the riverbank than on the surface. It counts as a natural and low-cost treatment that does not require any chemical additions, compared to advanced oxidation processes (AOPs) and membranes where AOP utilize the highly reactive hydroxyl radicals to mineralize the organic pollutants completely (Hiscock & Grischek, 2002; Bertelkamp *et al.*, 2014; Hamann *et al.*, 2016; Krishnan *et al.*, 2017).

Using soil in the subsurface environment or above-the-ground soil reactors has improved water quality in terms of inorganic and organic constituents' removal. Soil columns in laboratory-scale studies often are used to simulate SAT and RBF systems.

Column tests essentially consist of the percolation of feed water through a cylindrical column filled with soil to represent field conditions and determine its capability for water quality improvement. Soil column studies have the advantage of improved control and monitoring over the operation and sampling process (Gibert *et al.*, 2014) compared to in-situ studies.

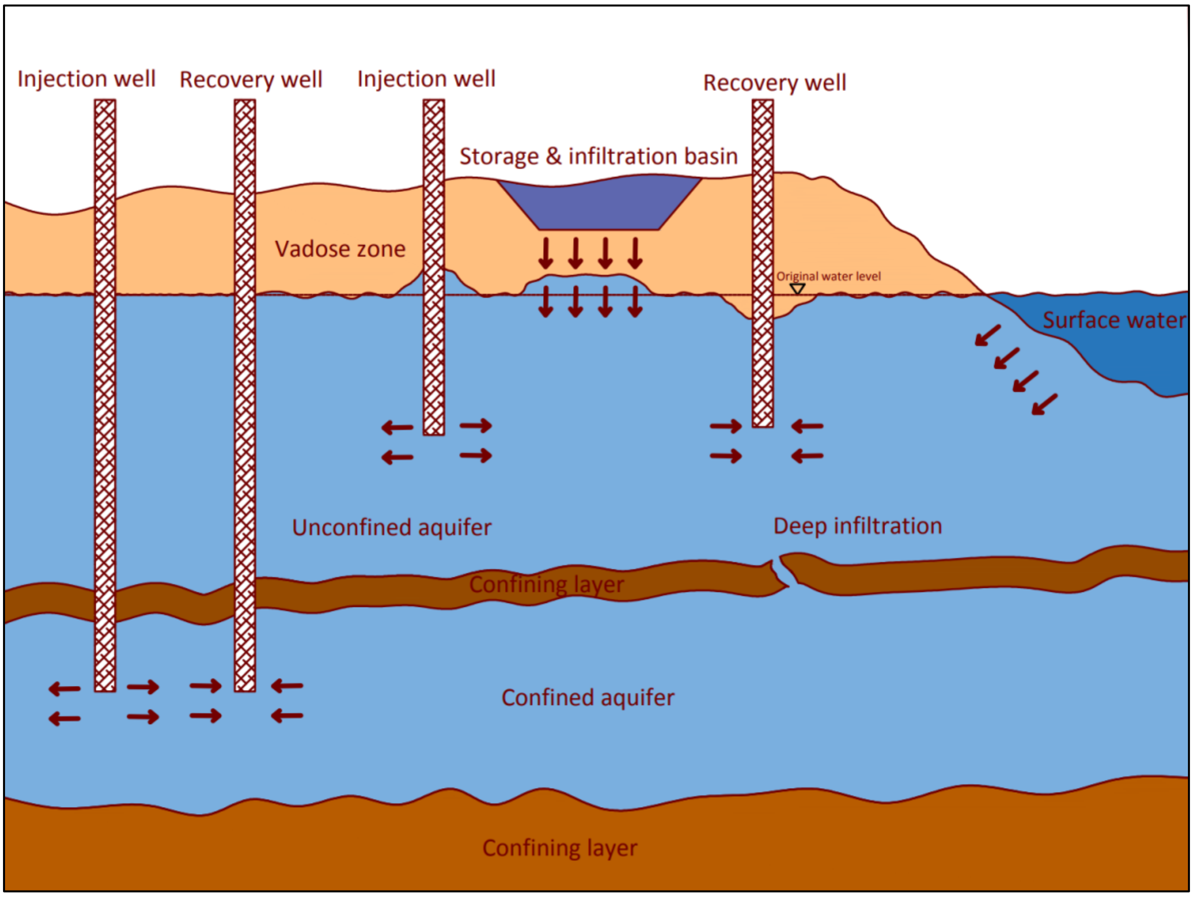

Figure 2.1 Managed aquifer recharge treatment of effluent for reuse

For water reclamation facilities that discharge effluents to SAT systems influencing surface water bodies, the occurrence of not only bulk constituents such as organic matter, N, and P, but also trace constituents are of concern. One particular concern is the occurrence of dissolved organic nitrogen (DON) in effluent both from a nitrogen input perspective as well as potential trace organic contaminants. DON in effluents has become a focused concern because DON is a precursor to N-nitrosodimethylamine (NDMA), a suspected human carcinogen, and other N-based DBPs when the groundwater is recovered for water supply (Mitch & Sedlak, 2002, 2004; Pehlivanoglu-Mantas & Sedlak, 2008).

Soil/Aquifer-based treatment systems can act as an environmental buffer and provide multiple natural barriers to remove the concerning contaminants, such as indirect potable reuse (IPR) (Regnery *et al.*, 2017). Although these systems have been applied widely to further improve the wastewater effluent quality under different conditions, there are not many studies investigating DON removal from effluents by SAT. However, there are studies that investigated nitrogen species removal in MAR systems which can provide insights into DON removal (Table 2.1). A limited number of studies investigated organic nitrogen removal during SAT and, more recently, the trace organic compounds (TOrCs) that may contain nitrogen (Table 2.1).

Table 2.1 Compilation of studies focused on nitrogen removal in MAR systems

Key Focus of the Study	Type of Study	Reference
Ammonia and/or Nitrogen Removal in MAR Systems		
Factors affecting nitrate removal during RBF	Lab & field	Grischek *et al.*, 1998
Nitrogen removal from heavily polluted rivers by RBF	Field	Wu *et al.*, 2007
Nitrogen removal by using a saturated soil column representing SAT	Lab	Essandoh *et al.*, 2011
Nutrients (N and P) removal under different temperatures and redox conditions by SAT	Lab	Abel *et al.*, 2012
Fate of nitrogen in batch experiments simulating SAT using primary effluent	Lab	Abel *et al.*, 2013b
Nitrogen removal by both saturated and unsaturated soil columns representing SAT	Lab	Essandoh *et al.*, 2013
Nitrogen removal by four successive ASR cycles	Field	Vanderzalm *et al.*, 2013
Nitrogen removal by using soil columns simulating MAR system with varying drying cycles	Lab	Abel *et al.*, 2014
Ammonia removal by surface water infiltration	Field	Hu *et al.*, 2016

Ammonia, nitrite, and TOrC removal by short-term SAT system with and without prior ozonation	Lab	Lakretz *et al.*, 2017
Nitrogen removal by long-term SAT using infiltration basins	Field	Mienis & Arye, 2018
DON and Nitrogenous TOrC Removal in MAR Systems		
Ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) removal by long-term SAT	Field	Drewes *et al.*, 2003
DON, effluent organic matter (EfOM), pharmaceutically active compounds (PhACs), and endocrine-disrupting compounds (EDCs) removal by short-term and long-term SAT	Field	Amy & Drewes, 2007
Organic nitrogen removal by saturated soil column study simulating SAT under different hydraulic rates	Lab	Essandoh *et al.*, 2011
Organic nitrogen removal by both saturated and unsaturated soil columns representing SAT	Lab	Essandoh *et al.*, 2013
Pharmaceutical and personal care products (PPCPs) removal by SAT	Lab	Onesios & Bouwer, 2012
Organic micropollutants (OMPs) removal by RBF	Lab	Bertelkamp *et al.*, 2014
Total organic carbon (TOC), NDMA, and CECs removal by soil columns	Lab	Trussell *et al.*, 2018
OMPs removal by batch studies representing RBF	Lab	Abdelrady *et al.*, 2019
Model-based analysis of TOrC removal during bank filtration	Field	Sanz-Prat *et al.*, 2020

The main nitrogen transformation mechanisms in all these studies (Table 2.1) are redox reactions, including nitrification/denitrification, where nitrification is the main reaction at first, and denitrification becomes dominant by soil depth due to lower dissolved oxygen. Specifically, organic nitrogen removal happens mostly through ammonification (organic nitrogen to ammonium nitrogen), while the main removal mostly occurs in the vadose zone. According to Amy and Drewes (2007), DON was reduced by 50% in shorter-term SAT and 90% in longer-term SAT when the tertiary treated wastewater effluent contained 2mg N/L as DON. Organic nitrogen removal of 71% to 76% was reported by Essandoh *et al*. (2011) in a saturated soil column study simulating SAT for different hydraulic rates. Later, Essandoh *et al*. (2013) showed that more than 80% organic nitrogen removal could be obtained in an unsaturated soil column where different thicknesses of unsaturated soil were investigated to assess the effect of the wastewater travel length through the unsaturated zone on removal. Drewes *et al.* (2003) documented EDTA and NTA removal by long-term SAT, 89% and 100%, respectively.

Meanwhile, different studies on soil/aquifer-based treatment capabilities in removing nitrogenous TOrCs and OMPs can shed more light on DON removal (Table 2.1). For instance, the presence of biodegradable organic carbon (BDOC) increased the TOrC degradation in the initial zone by enhancing soil biomass growth, but high TOrC removal was also reported in the presence of low BDOC concentrations (Rauch *et al.*, 2010). Some OMPs exhibited lower persistence with the increase in temperature. The temperature has the most influence on OMP removal with hydrophilic and low-hydrophobic character compared to highly hydrophobic compounds (Abdelrady *et al.*, 2019). In some studies, numerical modeling is used to predict the fate of emerging organic contaminants (EOCs) by applying linear degradation (Strauss *et al.*, 2011), first-order kinetics (Strauss *et al.*, 2011; Arye *et al.*, 2011; Koroša *et al.*, 2020), or sorption models (Strauss *et al.*, 2011; Arye *et al.*, 2011). Modeling studies are conducted to describe contaminant behavior and main removal mechanisms and to obtain the major controlling parameters (Gibert *et al.*, 2014). Bertelkamp (2014) obtained a model (first-order kinetics in combination with linear and reversible sorption) to predict the biodegradation rate of complex mixtures of OMPs, based on the functional groups present (Bertelkamp *et al.*, 2014; Gibert *et al.*, 2014). The influence of environmental conditions such as redox reactions, pH, temperature, travel times, the microbial community, amount, and type of BDOC, and their effect on the microbial community on TOrCs' and OMP's removal have been analyzed more thoroughly compared to DON removal. The results can be used as a guideline for designing future bulk DON removal experiments.

In this paper, the capability of soil/aquifer-based treatment processes with a focus on SAT and RBF in removing organic nitrogen has been critically reviewed. First, the characterization of DON and, following that, the importance of DON removal, its mechanisms, and lab/field studies on DON removal are critically reviewed. Based on this critical review, research needs and gaps are identified to make SAT systems applicable for DON removal from treated wastewater effluents for potable reuse as well as for environmental discharge applications.

2.2 Dissolved organic nitrogen

An understanding of wastewater effluent organic nitrogen (EON) characteristics is needed to determine what specific fractions and chemical types are amenable for treatment in the soil systems. EON consists of particulate (PON), colloidal (CON) organic nitrogen, and DON. The PON is removed during the settling and filtration processes in wastewater treatment, while CON and DON still could remain in the final effluent (Sattayatewa *et al.*, 2010; Pagilla *et al.*, 2011) and would potentially need to be treated in the soil aquifer systems. The CON could presumably be solubilized to DON in the environment. Most DON compounds are released by living organisms or from the degradation of dead organisms (Jørgensen, 2009). A compilation of DON in various waters is shown in Table 2.2. Dissolved free amino acids (DFAA) refer to protein amino acids such as alanine (Ala), glycine (Gly), and serine (Ser), and non-protein amino acids such as ornithine (catalyst in urine cycle, Barmore & Stone, 2019, theanine (found in tea and mushrooms, Kardashev, Ratner & Ritsner, 2018), and tranexamic acid (medication, Dunn & Goa, 1999). In secondary treated wastewater effluents discharged into rivers and lakes, DFAA and dissolved combined amino acids (DCAA) have been found to make up 0.05–3% and 0.6–21% of DON (Table 2.2) (Pehlivanoglu-Mantas & Sedlak, 2008; Huo *et al.*, 2013). As it can be seen from Table 2.2, the remaining DON in treated wastewater effluent consists of low molecular weight (LMW, <1kDa) amines (e.g., dimethylamines), chelating agents from detergents such as EDTA and NTA, pharmaceuticals, and a broad spectrum of organic nitrogen compounds at low concentrations (Pehlivanoglu-Mantas & Sedlak, 2008). The LMW DON pool is quantitatively essential, comprising 70–80% of marine DON (Worsfold *et al.*, 2008). Moreover, dissolved NDMA precursors are mostly of LMW DON (Pehlivanoglu-Mantas & Sedlak, 2008), and they will be discussed more in the next section. LMW and HMW characteristics of compounds can be found in Table 2.2 (Berman & Bronk, 2003; Pehlivanoglu-Mantas & Sedlak, 2008). There is a considerable portion of EON that is not characterized by its chemical nature. Mentioned compounds usually account for less than 10% of wastewater effluent DON

(Pehlivanoglu-Mantas & Sedlak, 2008), and only less than 14% of DON in oceanic environments is identifiable at the molecular level (Worsfold *et al.*, 2008; Jørgensen, 2009). Hence, there is a need to identify the remaining DON compounds for a comprehensive understanding of their distribution and removal from wastewater effluents.

Table 2.2 Characterization of DON compounds in various sources

DON compounds	**Conc (μM N)**	**% Of DON fraction**	**Water sample**	**References**	**Molecular weight**
DON		60-69 of TDN	Lakes, rivers, surface ocean waters	Curtis-Jackson *et al.*, 2009; Worsfold *et al.*, 2008	NA
		77-99 of TDN	Soil leachates	Yu *et al.*, 2002	
DFAA: Protein and non-protein amino acids	0.04-2	0.1-3	Raw WW	Pehlivanoglu-Mantas & Sedlak, 2008	LMW
		0.05-3	Effluent	Pehlivanoglu-Mantas and Sedlak, 2008; Huo *et al.*, 2013; Pehlivanoglu-Mantas and Sedlak, 2006b	
		1.5-10.6	Soil leachates	Yu *et al.*, 2002	
		1	Marine Environments	Sommerville & Preston, 2001	

DCAA: Peptide and protein-bound amino acids, amino acids	1-19 0.2-15	4 0.6-21 48-74 15	Raw WW Effluent Soil leachates Oceans	Pehlivanoglu-Mantas and Sedlak, 2008 Pehlivanoglu-Mantas and Sedlak, 2008; Huo *et al.*, 2013; Pehlivanoglu-Mantas and Sedlak, 2006b Yu *et al.*, 2002 Keil & Kirchman, 1991	HMW *Peptides: LMW
Methylamines include mono-, di-, and trimethylamine	0.3-5.1 (Dimethylamine)		Effluent	Mitch & Sedlak, 2004 Pehlivanoglu-Mantas and Sedlak, 2008	LMW
Humic-like substances	4-20		Effluent	Pehlivanoglu-Mantas and Sedlak, 2006b	HMW for those with relatively low content

EDTA	0.1-1	<5	Effluent	Pehlivanoglu-Mantas and Sedlak, 2006b
NTA	0.1-0.5		Effluent	Pehlivanoglu-Mantas and Sedlak, 2006b Bucheli-Witschel & Egli, 2001
N-containing pharmaceuticals	<0.001		Effluent	Pehlivanoglu-Mantas and Sedlak, 2008

2.2.1 Why is dissolved organic nitrogen a concern?

DON is of concern mostly due to its role as a precursor in forming DBPs, causing incomplete chlorination in wastewater treatment systems, as well as its contribution to N load in receiving waters. When chlorine or chloramine disinfection is practiced during wastewater treatment, DBPs will be produced (Krasner *et al.*, 2009a). One of the DBP categories that occur in the chlorination process as the result of the chemical reactions between natural organic matter (NOM) and disinfectants, especially chlorine, is nitrogenous disinfection by-products (N-DBPs). Although N-DBPs such as haloacetonitriles (HANs), halonitromethanes (HNMs), and haloacetamides (HAcAms) generally have lower concentrations compared to regulated carbon-based DBPs (Chu *et al.*, 2011), they are more toxic and subsequently pose a higher public health risk (Muellner *et al.*, 2007; Bond *et al.*, 2011; Hou *et al.*, 2012). In N-DBP formation, the disinfectant type is vital as, depending on the compound and reaction, the nitrogen can also be derived from the organic precursors, i.e., DON (Krasner *et al.*, 2009b). While DON includes urea, amino acids, peptides, proteins and viruses, and small bacteria (Jørgensen, 2009), amino acids are known to act as precursors of HANs, HAcAms, and cyanogen halides (CNX) (Bond *et al.*, 2011). The wastewater-derived DON can also act as a precursor for NDMA formation (Mitch & Sedlak, 2002, 2004; Pehlivanoglu-Mantas & Sedlak, 2008). This involves a reaction between organic nitrogen-containing compounds and inorganic chloramines (Pehlivanoglu-Mantas & Sedlak, 2006a). Although, as mentioned earlier, N-DBPs are present at lower concentrations, industrial wastewater effluent and fertilizer runoff can feed algal blooms, which can cause a high increase in DON (Westerhoff & Mash, 2002; Chu *et al.*, 2011). Following this, N-DBPs formation may occur in eutrophic waters if there is any remaining chlorine.

The presence of DON in wastewater effluents can also interfere with the removal of residual chlorine-based disinfectants prior to discharge. Dechlorination is mainly achieved by adding dechlorinating agents such as sulfite salts (sodium metabisulfite, sodium bisulfite, and sodium sulfite) to chlorinated waters (Basu & De Souza, 2011). When sulfur dioxide or sulfite salts are dissolved in the water, the generated S(IV) species, such as the sulfite ion (SO_3^{-2}), react with both free and combined forms of chlorine (US EPA, 2000). However, some N-chloro compounds, such as DFAA and DCAA-based chlorinated products, react sluggishly with sulfite dechlorinating agents and lead to incomplete dechlorination during wastewater treatment, which eventually threatens the aquatic life (Pehlivanoglu-Mantas & Sedlak, 2006b. These findings suggest that treated effluents containing DON can also form N-DBPs in drinking water systems that source effluent-dominated surface or groundwater.

Additionally, the discharge of wastewater effluents into surface waters provides a rich source of organic nitrogen, which may disturb the delicate balance of estuarine and coastal ecosystems (Heaton, 1986). Although the removal of inorganic nitrogen by nitrification and denitrification processes in WWTPs can be more than 95%, the removal of organic nitrogen is usually less efficient (Pehlivanoglu-Mantas & Sedlak, 2006b). Additionally, WWTPs that can remove nitrogen to less than 3mg TN per liter are faced with the challenge of residual effluent DON, which constitutes about one-third to one-half of the effluent TN (Pagilla *et al.*, 2006). This can become more problematic as regulations become stricter and WWTPs try to reduce their effluent nitrogen to very low levels. Therefore, there is a clear need to investigate DON transport behavior in the soil to remove DON residues from wastewater effluent by SAT and RBF systems to eliminate their input into groundwater and surface waters.

2.3 DON removal by SAT/soil-based treatment systems

2.3.1 DON transport

In general, DON occurrence in surface waters is mostly caused by wastewater discharges and agricultural fertilizers (Westerhoff & Mash, 2002). During wastewater treatment, proteinaceous materials are converted to soluble (nitrate, ammonia) and gaseous (nitrogen gas) inorganic forms, but a certain portion remains as DON. Secondary and tertiary treated wastewater contains 5–25 and <4 mg DON/l, respectively, while higher DON concentrations can be present in industrial discharges (Westerhoff & Mash, 2002).

Usually, it is accepted that nitrogen in aquifers occurs almost exclusively as nitrate, as ammonification and nitrification occur in unsaturated zones above aquifers and that nitrate is mobile enough in soils to be transported to aquifers in quantity (Bouwman, Brecht & Van der Hoek, 2005). Ammonium-nitrogen is seldom present more than 1 mg/L unless ammonia fertilizer or wastewater is added to the water supply (Ağca *et al*., 2014). Even under anaerobic conditions, anaerobic ammonium oxidation (anammox) is mediated by autotrophic bacteria that can oxidize ammonium with nitrite to N_2 without the need for an organic electron donor (Wang *et al*., 2020). But in addition to nitrate, it was seen that DON concentrations in some cases are quite high in groundwater since DON proportion to total dissolved nitrogen (TDN) varies significantly (<1-99%), which indicates the importance of monitoring DON presence in groundwater supplies (Valiela *et al.*, 2000; Corbet *et al.*, 2002; Kroeger *et al*., 2006; Lorite-Herrera *et al*., 2008). However, it was seen that 76% of shallow groundwater and 81% of deep groundwater aquifers had less than the detection limit DON. The lower DON concentration in groundwater compared to surface water can be due to DON ammonification (mineralization) to ammonia and high adsorption of DON to aquifer materials, where there were some exceptions near wastewater sources (Westerhoff & Mash, 2002; Viers *et al*., 2012). Based on these numbers and the capability of subsurface soil and aquifers, an adequately designed soil/aquifer-based treatment system such as SAT could mitigate DON input into groundwater.

2.3.2 Nitrogen removal mechanisms and efficiency

Biodegradation, adsorption, filtration, chemical precipitation, and ion exchange are the main removal mechanisms of different contaminants during soil passage (Mansell *et al.*, 2004; Abel, 2014; Sharma & Kennedy, 2017). Abiotic processes such as sorption and complexation, and biotic processes such as mineralization of DON (Pabich *et al*., 2001), and sorption and nitrification of NH_4^+ (DeSimone & Howes, 1998) in vadose zones and aquifers minimize DON and N/NH_4^+ levels in groundwater (Kroeger *et al*., 2006). The adsorption of ammonia can be a result of cation exchange, which will then be further reduced by nitrification (Fox, 2011; Abel, 2014).

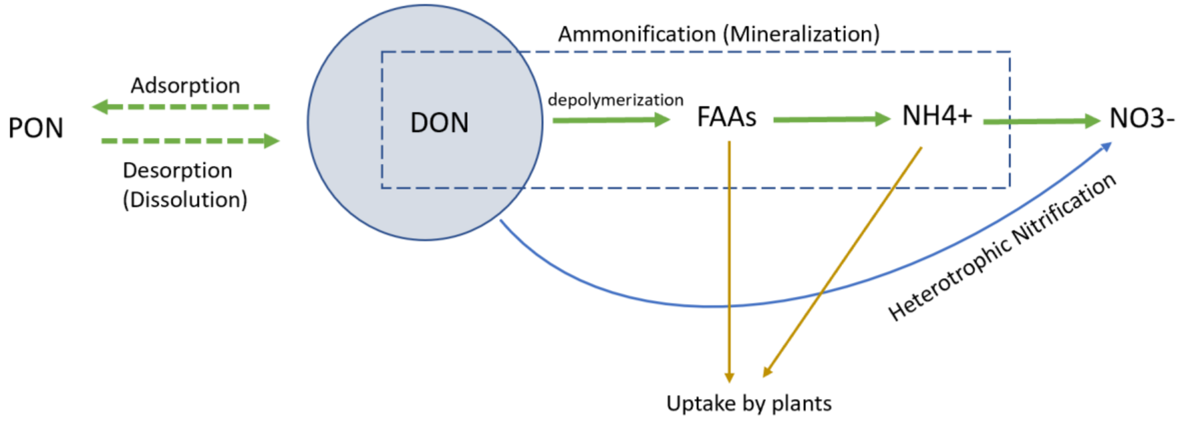

Figure 2.2 Overview of soil DON cycle

The main attenuation mechanisms for trace organic chemicals in soil-aquifer systems are biotransformation (through ammonification in the case of organic nitrogen) and sorptive processes (within the first few meters of infiltration), which determine the fate of organic contaminants in groundwater during artificial recharge (Figure 2.2) (Massmann *et al.*, 2006; Díaz-Cruz & Barceló, 2008; Regnery *et al.*, 2017). Heterotrophic nitrification could also occur on rare occasions where DON is converted to nitrate (Hood-Nowotny, 2018). DON removal by sorption in the long term should be further examined in future research as sorption may enhance removal by providing more time for biodegradation as well as decrease it by reducing the bioavailability of the compound (Storck *et al*., 2012). Both sorption and biodegradation processes depend on a chemical compound's physicochemical properties, including functional groups, distribution coefficients, Henry's law constants, polarity, and site-dependent conditions such as site hydraulic, hydrogeochemical, and microbiological properties (Regnery *et al.*, 2017). In recharge methods such as infiltration basins or vadose zone injection, hydraulic loading rate (HLR) plays an important role. HLR is typically 15 to 100 m/year, depending mostly on soil, influent quality, and wetting/drying cycles. As HLR decreases with time, it can be restored by drying cycles during the process (Sharma & Kennedy, 2017). Although a higher loading rate is desirable from the groundwater recharge perspective, there must be enough retention time for removal mechanisms. Essandoh (2011) reported higher ammonium removal with lower HLR under saturated conditions, which can be attributed to biosorption onto the biofilms coating the sand grains. Increasing the loading rate resulted in higher organic nitrogen removal; however, the removal efficiency was lowered (Essandoh *et al.*, 2011). Similarly, the soil column with an HLR of 0.625 m/d exhibited higher overall removal of ammonium compared to the soil column with an HLR of 1.25 m/d, suggesting ammonium reduction through adsorption to the media due to longer hydraulic retention time (HRT) at lower HLR (Abel *et al*., 2013a). Thus, the effect of HLR on overall DON removal, especially in the first few meters of infiltration, should be further investigated.

Several factors that can affect the biotransformation of trace organic chemicals during SAT processes, in general, are redox conditions, temperature, retention time, and metabolic capability of the indigenous soil microbial community (Heberer *et al.*, 2008; Grützmacher & Reuleaux, 2011; Li *et al.*, 2013; Alidina *et al.*, 2014). Other factors, such as volatilization, photolysis, or hydrolysis, have less importance in the attenuation of organic contaminants of concern during MAR (Regnery *et al.*, 2017). There can also be a direct uptake of DON, specifically amino acids, by plant roots which need a significant amount of low molecular weight DON and lack of competition by other microorganisms for DON as they can utilize it as a source of nitrogen (Streeter, Bol & Bardgett, 2000; Owen & Jones, 2001; Jones *et al.*, 2004). It was seen that plant roots captured only 6% of amino acids, and the rest was captured by microbial biomass (Owen & Jones, 2001).

Sorption may not be a sustainable DON removal mechanism due to its finite capacity and the possibility of desorption. Organic carbon removal in SAT systems is sustainable as it occurs through biodegradation processes rather than sorption (Fox *et al*., 2005). However, the sorption capacity of soil/aquifer-based treatment systems may be so large relative to the DON loading rates expected from wastewater effluents and may not be exhausted over the operational life of a system (Maliva, 2020). Although sorption and biodegradation have been found to be the main attenuation mechanisms of DON in SAT systems, no studies differentiate how much each of these two mechanisms contributes to DON removal. There are more studies done on RBF systems and how to differentiate between sorption and biodegradation in OMP removal. In one study, soil column studies were conducted to differentiate these two processes where some of the columns were designed not to have any biodegradation as the biomass was destroyed intentionally by adding sodium azide. Statistically significant differences in OMP removal between the biologically active columns and the biologically inactive columns were reported (Bertelkamp *et al.*, 2014). This result is also compatible with DON removal in most aquatic systems, as degradation by bacterial activity probably accounts for the major removal in those systems (Berman & Bronk, 2003). There was no statistically significant difference in OMP removal between columns with “inactive sorption biomass” and “sorption on sand grains with no biomass present” that the latter showed a water matrix effect. This shows that OMP sorption was not affected by the inactivated biomass or the water matrix in specified circumstances (Bertelkamp *et al.*, 2014). Although these results are reported for OMPs (ng/l - µg/l range), which are in lower concentrations compared to EON (about 1mg N/L), they might be able to give an idea regarding the contribution of each mechanism. Hence, similar investigations are needed in order to determine the contribution of biodegradation and sorption to DON removal in soil columns. The results can also be useful in numerical modeling, where most of them do not differentiate between sorption and biodegradation roles in EOCs removal and report the result as a whole (Gibert *et al.*, 2014).

In the same study (Bertelkamp *et al.*, 2014), the relationship between OMP biodegradation and physiochemical properties, such as charge, hydrophobicity, and molecular weight, or their functional groups, was investigated. There were trends observed between charge or hydrophobicity and biodegradation rate for charged compounds, but there was no significant linear relationship for all OMPs mixture. On the other hand, there was a statistically significant relationship between biological degradation rates and functional groups of OMPs. It was seen that the availability of ethers and carbonyl groups would increase biodegradation; however, the presence of amines, ring structures, aliphatic ethers, and sulfur will decrease it (Bertelkamp *et al.*, 2014). Hiscock and Grischek (2002) also reported that the biodegradation of some aromatic amines is relatively slow in RBF (Hiscock & Grischek, 2002).

Some of the common DON compounds are amino acids, amino sugars from cell walls, nucleotides from RNA and DNA, metabolites, and urea (Jørgensen, 2009). If the functional groups of these compounds influence their removal through SAT by biodegradation, it would be beneficial to know. The main functional groups that can be seen in DON compounds are amine, carboxyl, and a carbonyl group. The results can also be compared with Bertelkamp's (2014) study and see if the effect of functional groups in DON is the same as the ones in the OMPs. This would help with determining the fate of new compounds in the future by their functional groups. It should also be investigated when both amine and carbonyl groups are present, which one would be the leading and determinative functional group because the previous study showed that they have opposite effects on the biodegradation rate.

In another study by Schaffer *et al*. (2015), the attenuation of OMPs was investigated by increasing solid organic matter in a large-scale column study. It was seen that for neutral and anionic compounds, with solid organic matter and compound hydrophobicity, sorption increases in the system. Also, biodegradation was improved for the majority of compounds with higher portions of biodegradable dissolved organic carbon (Schaffer *et al.*, 2015). Although most of these studies are investigating OMPs, their results can also lead to more investigations in the organic nitrogen area. For instance, most of the amino acids are neutral and anionic, except for lysine and histidine, which are cationic. They are not the majority of DON as most of its constituents are unknown, but they are a large known part of it.

2.3.3 Hydrophobic and hydrophilic DON fraction removal

Hydrophilic DON typically accounts for approximately 80% (Liu *et al.*, 2012) and 64% to 72% (Qin *et al.*, 2015) of the total DON, as opposed to hydrophobic DON, which accounts for the rest of the effluent DON. This can be justified as most of the hydrophobic DON will be removed during the treatment process, and mostly the hydrophilic portion will remain in the effluent. Hydrophobic trace organic contaminants can sorb onto organic matter and metal oxides, leading to their attenuation in subsurface systems. This sorption slows them down, which leads to more time for their biotransformation (Tolls, 2001; Higgins & Luthy, 2006; Regnery *et al.*, 2013). Following this, sorption may be a central mechanism for the removal of approximately 20% of DON. According to another study in Oa (topsoil layer with a high amount of organic matter) horizontal leachates, most of the DON was found in the hydrophobic dissolved organic matter (DOM) (60% to 90%) rather than the hydrophilic portion, where hydrogen bonding between proteins and polyphenols tends to make the compounds more hydrophobic as it increases the complexation (Yu *et al.*, 2002). Hydrophobic compounds such as protein-tannin complexes and amino acids complexed with humic substances will be sorbed in mineral soil horizons selectively, which results in more mobile hydrophilic substances such as amino acids, free peptides, and free proteins in solutions with soil depth increase (Yu *et al.*, 2002). Although this may seem contradictory to the previous study, it can be implied that before adsorption happens, most of the DON fraction is hydrophobic, and after adsorption, mostly hydrophilic fraction remains, which can be biodegraded. Also, there is a higher chance that it reaches groundwater or surface water as it is more mobile (Yu *et al.*, 2002).

2.4 Lab- and field-scale findings on DON removal in SAT systems

DON removal using different methods of soil/aquifer-based treatment systems has been investigated by both lab- and field-scale studies. While reviewing the results of previous studies, it must be noted that the removal efficiency of different laboratory- and field-scale setups cannot be compared effectively only by means of the final percent removal. Although biotransformation due to microbiological activities in the subsurface happens naturally, microbial processes depend on geochemical and hydrological subsurface conditions (Haack & Bekins, 2000; Regnery *et al.*, 2013). Most of the studies are conducted under different conditions such as HRT, HLR, depth of vadose zone (in field studies), saturated or unsaturated conditions, and their depth and varying wetting and drying cycles. Hence, understanding the effect of each variable can be more reliable for predicting future systems results rather than solely depending on the removal.

As it was mentioned before, although there are many SAT experiments investigating nitrogen removal, few addressed DON removal specifically. However, a significant DON removal was achieved through the vadose zone, where tertiary-treated wastewater containing 2mg/L of DON was reduced to 1mg/L by shorter retention time (6-20 months) SAT and to 0.2 mg/L by longer retention time (12-96 months) SAT (Amy & Drewes, 2007). The SAT process included a mostly anoxic vadose zone with only 1.5m out of 15m thickness being aerobic. They also reported DON reduction from 9 mg/l to below the detection limit after only 11 days on-site. This result was attributed to the deep vadose zone of 30m. Due to the high concentrations of wastewater EfOM and ammonia, the vadose zone becomes anoxic at a typical depth of about 1.5 m (Amy & Drewes, 2007). Infiltration of tertiary treated wastewater decreased DON from 0.7 to 0.3–0.6 mg N/l over 10 m of unsaturated soil but did not show any further decline in the saturated zone, and the results were similar to other studies (DeSimone & Howes, 1998;

Westerhoff & Mash, 2002). Kroeger *et al*. (2006) showed that percent of total dissolved N (TDN) as DON in groundwater is negatively related to the path length of groundwater through aquifers (Kroeger *et al*., 2006). The long-term behavior of nitrogen and its components were investigated in an SAT system of the Dan Region Reclamation Project (Shafdan), Tel-Aviv, Israel (Mienis & Arye, 2018). TN removal in the upper part of the aquifer was 47-63% by denitrification and absorption, and overall removal across the aquifer was 49-83%. Soil/aquifer-based treatment systems can also occur on a smaller scale, unmanaged and unintentionally, such as discharging to ephemeral streams. Retention basins that were primarily designed only for effluent discharge or stormwater infiltration rather than groundwater augmentation and contaminant removal are unmanaged MAR systems. Due to the high number of these basins, it becomes crucial to conduct purposefully designed systems to monitor DON removal and groundwater quality at the field scales. Lysimeters and monitoring wells should all be located in the same aquifer to monitor the removal, as the results could be misleading if, for example, some of the wells are in the second untouched aquifer; those samples could overestimate the total removal. At the same time, the contaminants are still present in the first aquifer.

Meanwhile, based on a saturated lab-scaled soil columns study, high organic nitrogen removal, 71% to 76%, for different loading rates was seen in less than 100 mm of the column depth, where it was transformed to ammonium (Essandoh *et al.*, 2011). Ammonia removal was seen at the lower loading rates, which can be attributed to biosorption onto the biofilms coating the sand grains. As nitrification can hardly occur in soil columns due to a lack of oxygen, the difference in the amount of ammonia can be a result of adsorption, which is a removal mechanism during the SAT (Essandoh *et al.*, 2013). As this study was only in a saturated column, Essandoh (2013) also performed the column studies under saturated and unsaturated conditions. In the unsaturated column, three different water tables were investigated in order to represent the maximum and minimum length of unsaturated zones and in between. Greater than 80% organic nitrogen removal was achieved after 850 mm travel through the soil at all three water table lengths. Some increases in organic nitrogen were seen at some points, which can be a result of soluble microbial products (SMPs) released into the liquid phase of the soil column (Essandoh *et al.*, 2013). However, when the soil particles are saturated, and effluent with lower DON is applied, desorption might have occurred in the soil column, which can lead to a higher DON concentration at the end compared to the influent amount. Desorption occurs when the concentration gradient in the adsorber reverses, and adsorbed compounds are driven into the liquid phase by back diffusion (Corwin & Summers, 2011) and resulting in a delayed breakthrough curve (Banzhaf & Hebig, 2016). The desorption process has been seen in PPCPs, and how both sorption onto and desorption from sediments will affect their transport and fate (Martinez-Hernandez *et al.*, 2013), and CECs such as sulfonamides where adsorption and desorption influence the transport, transformation, and bioavailability (Doretto *et al.*, 2014). In another study, Krasner *et al*. (2009b) showed that SAT removed more trihalomethane (THM) precursors than other processes, such as membrane bioreactors (MBRs) and granular/powdered activated carbon (GAC/PAC) processes. It was also shown that SAT removed a substantial amount of all types of NOM (Krasner *et al.*, 2009b). Although only a few studies have investigated DON removal by SAT, DON removal has been reported, which makes it crucial for further investigations.

2.5 Improving DON removal efficiency with pre-treatment

Pre-treatment refers to processes that are applied to the source water to enhance the performance of subsequent soil/aquifer-based treatment systems. Lack of or inadequate pre-treatment processes may cause clogging in the SAT system, and consequently, its removal capabilities will be lowered. Additional post-treatment may become necessary, which leads to a costlier system (Sharma *et al*., 2016; Sharma & Kennedy, 2017). Different pre-treatment methods can be used in order to enhance water quality, depending mostly on the source water quality (Table 2.3). One of the pre-treatment processes that can be used for further removal of DON is adsorption on activated carbon, which can remove DON up to 80% from secondary effluent. Another option is using coagulation with metal salts that allow for DON removal from secondary effluent by up to 55% (Czerwionka & Makinia, 2014). Moreover, using denitrification filters, deep bed, and membrane filtration can be effective in the removal of colloidal fractions of organic N from effluents (Pagilla *et al.*, 2006). Enhanced coagulation using alum followed by microfiltration was also seen to be able to remove DON up to 69% (Arnaldos & Pagilla, 2010).

Table 2.3 Pre-treatment methods to improve DON removal during SAT

Pre-treatment method	**Benefits**	**Reference**
Coagulation using alum + microfiltration	Up to 69% DON removal at secondary effluent	Arnaldos & Pagilla (2010)
Adsorption on activated carbon	Up to 80% DON removal at secondary effluent	Czerwionka & Makinia (2014)
Coagulation using metal salts	Up to 55% DON removal at secondary effluent	Czerwionka & Makinia (2014)
Ozonation	Improving TOrCs removal	Hübner et al. (2012)
Biofiltration (coagulation/flocculation, hydrogen peroxide addition, and filtration) + Ozonation	Decreasing oxygen demand during SAT by reducing DOC & ammonium, improving TOrCs removal, increase in OM biodegradability	Zucker et al. (2015)
Ozonation	Improving TOrCs removal	Kim et al. (2017)
Ozonation	DOC reduction, decreasing biological stability, improving TOrCs removal	Kim et al. (2019)
UV/H_2O_2 AOP	Higher biodegradability of DOM, Improving micropollutants removal	Wünsch et al. (2019)

The reduction of DOC and ammonium during biofiltration as a pre-treatment system significantly decreases the oxygen demand during SAT infiltration by removing organic matter and ammonium. Ozonation prior to SAT allows efficient TOrC removal, an increase in organic matter biodegradability, and enough DO to establish and maintain oxic conditions throughout the SAT system (Hübner *et al.*, 2012; Zucker *et al.*, 2015). Although ammonification takes place in both aerobic and anaerobic conditions, it proceeds rapidly in the oxygen-rich layers (Stefanakis *et al.*, 2014). The use of pre-treatment systems in improving DON removal should be investigated more thoroughly in the future to increase the performance of the soil/aquifer-based treatment systems in general.

2.6 Conclusions

SAT has been proven to be an efficient natural barrier in removing contaminants and polishing the water quality further for recharging groundwater supplies and water reuse purposes.

One of the wastewater effluent constituents of concern is DON which can act as a precursor for the formation of N-DBPs and increase the nitrogen load in receiving waters. As it is also responsible for a significant portion of total effluent nitrogen, it becomes more important to investigate its removal by post-treatment systems. Hence, the application of the SAT process to further remove the DON from effluent wastewater has become noteworthy. Nevertheless, there are not many studies investigating DON removal efficiency by SAT systems either in lab- or field-scales. In the few available related studies, it has been shown that DON can be removed successfully (more than 80% removal). Disadvantages of DON removal by SAT systems can be perceived as SAT systems' limitations in general and in DON removal specifically. In general, the SAT's performance is site-dependent, and it needs specific hydrogeological conditions. Potential clogging in the system due to the accumulation of suspended solids or chemical precipitation is another concern that should be addressed by proper design, operation, and maintenance of the system, such as adequate drying cycles or pretreatment. Limitations regarding DON removal may be caused due to inadequate understanding of DON characteristics itself and the removal mechanisms happening under the ground that make it difficult to predict the performance. Based on a review of the nitrogen removal mechanism and previous studies, the following needs and gaps are suggested for future studies:

- More field-/lab-scale studies should be done to evaluate DON removal by SAT systems under different conditions, such as shorter/longer SAT regarding time and distance, different hydraulic rates, and applying various pre-and/or post-treatments for SAT. In order to optimize the process, investigations should be done to determine the contribution of both biodegradation and sorption mechanisms in DON removal in SAT systems.
- In the case of biodegradation being the major removal mechanism, the effects of different functional groups in DON and biodegradation rates should be investigated to see whether any dominant functional groups can affect the rate of biodegradation in the SAT. Amino acids are the main known group of DON that contain amines and carboxylic functional groups. As it was reported in the Bertelkamp (2014) study, amines will decrease the biodegradation rate for OMPs in RBF systems. It should be investigated if the carboxylic group has the same effect as the amine group on the biodegradation rate. Any relation between

physicochemical properties of the known DON content, such as hydrophobicity, charge, molecular weight, and biodegradation rate in the SAT system, can be further investigated by using soil columns in future research.

- Increases in DON concentration have been seen at some points within the soil column that was portrayed by a drop in the soil profile (Essandoh *et al.*, 2013). This increase could be attributed to the release of SMP (containing protein) into the liquid phase. However, other processes, such as desorption, should also be considered in soil column removal experiments. The probability of desorption occurrence during soil column run time and the effect of desorption on sustainable DON removal rates should be further investigated in future studies.
- Most of the DON removal happens in SAT systems occurs in the first few meters of the vadose zone in field sites. This could be partly attributed to uptake by the plant roots. The effect of vegetation on DON uptake in field sites could lead to improved infiltration systems with or without combined effects of Phyto-uptake.

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Chapter 3 Dissolved organic nitrogen removal and its mechanisms during simulated soil aquifer treatment

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3.0 Abstract

Soil aquifer treatment systems are known to further remove contaminants in wastewater effluent when applied through infiltration into the ground. Dissolved organic nitrogen (DON) in the effluent, a precursor for nitrogenous disinfection by-products (DBPs) such as *N*-nitrosodimethylamine (NDMA), is of great concern upon subsequent use of the groundwater infiltered into the aquifer. In this study, a soil aquifer treatment system was simulated using 1 m laboratory soil columns under unsaturated conditions representing the vadose zone. The final effluent of a water reclamation facility (WRF) was applied to these columns to investigate the removal of N species with a focus on DON, as well as NDMA precursors. DON removal achieved was up to 99% with an average of 68% and was accompanied by a 52% nitrate increase suggesting the occurrence of ammonification and nitrification through the soil columns. Around 62% of total DON removal was seen at less than 10 cm travel distance, which was in accordance with higher adenosine triphosphate (ATP) concentrations at the top of the column due to more oxygen and organic matter availability. Total Dissolved N removal was drastically lowered to 4.5% in the same column without microbial growth, which highlights the importance of biodegradation. Soil columns could remove NDMA precursors up to 92% through the column with the initial concentration of 89.5 ng/L, possibly due to the

removal of DON fractions. The results demonstrate the capability of the vadose zone in further treatment of DON and other organic matter before reaching the groundwater through infiltration or indirect discharge to surface water.

3.1 Introduction

Water scarcity and less predictable water resources are the main consequences of extreme precipitation and drought events caused by climate change (UNICEF, 2021). The growing need for sustainable water management has made wastewater reclamation and reuse a promising technique to secure water supplies. Managed aquifer recharge (MAR) systems, including soil aquifer treatment (SAT), are recognized and capable practices for further treatment of wastewater effluent for water reuse. In these methods, the treated effluent percolates the unsaturated (vadose) zone or is directly injected into the aquifer when the hydrogeological conditions are not suitable for infiltration. De facto disposal of treated effluent through land-based systems such as rapid infiltration systems can also achieve additional constituent removal prior to the treated effluent reaching the groundwater or nearby surface waters. In these systems, the removal mechanisms consist of physical, chemical, and biological processes that mainly include the sorption and biodegradation of nutrients by the subsurface media (Sharma and Kennedy, 2017).

Multiple studies have shown that lab and field-scale SAT systems are capable of removing nutrients (Pan et al., 2017), heavy metals (Wei et al., 2016), contaminants of emerging concern, including pharmaceutical and personal care products (PPCPs) (Onesios and Bouwer, 2012; Qin et al., 2020; Sallwey et al., 2020) and endocrine-disruptive compounds (EDCs) (Mansell et al., 2004); and pathogens such as viruses (Morrison et al., 2020). The removal differs based on multiple factors, such as the compounds' characteristics, source water quality, travel time or distance, and hydrogeological conditions (Sharma and Kennedy, 2017; Sidhu et al., 2015). Compared to studies that investigated the removal of nitrogen species such as nitrate and ammonia (Miller et al., 2006), fewer studies are addressing dissolved organic nitrogen (DON) removal in SAT systems (Amy and Drewes, 2007; Essandoh et al., 2013, 2011; Gharoon and Pagilla, 2021) despite the presence of DON in the wastewater effluent discharged into the subsurface.

Following more stringent discharge limits for nutrients, tertiary or advanced treatment processes have been implemented in water reclamation facilities (WRFs) (Zheng et al., 2021). Even though these processes are successful in total nitrogen (TN) removal, it mostly relies on ammonia and nitrate decrease rather than DON. The residual effluent DON can be responsible for one-third to one-half of the total effluent nitrogen when the TN has been decreased to less than 3 mg/L (Pagilla et al., 2006). It was seen that DON to TDN ratio in the effluent was increased from 10 to 23% when the CAS was upgraded to a pre-denitrification BNR process at lab-scale with the same solids retention time (SRT) (Eom et al., 2017). At the field scale, Eom et al. (2017) reported that following the upgrade of CAS to BNR with the pre-denitrification process in a WRF, total organic nitrogen in the effluent was significantly increased despite the removal of total dissolved nitrogen (TDN).

Effluent DON is of concern since it contributes to the N load in receiving waters and simulates algal growth (Pehlivanoglu and Sedlak, 2004), causing hypoxia and threatening aquatic life (Eom et al., 2017). It can also act as a precursor in the formation of *N*-nitrosodimethylamine (NDMA), a carcinogen nitrogenous disinfection by-product (DBP) (Lee et al., 2007; Mitch and Sedlak, 2002). NDMA has notification and response levels of 10 and 300 ng/L, respectively (*California State Water Resources Control Board*, 2022) and is currently on the Draft Fifth Contaminant Candidate List (CCL 5) published by EPA ("Drinking Water Contaminant Candidate List 5-Draft," 2021). US EPA Integrated Risk Information System (IRIS) has estimated the carcinogenicity assessment of NDMA for lifetime exposure in drinking water to be 7 ng/L at the risk level of 10^{-5} (US EPA). Meanwhile, NDMA has been reported to occur in wastewater effluents ranging from below the limit of quantification to 73 ng/L (Yoon et al., 2011). Even though the use of chloramination has been shown to decrease the regulated DBPs formation during effluent disinfection, it favors the formation of N-nitrosamines, including NDMA (Le Roux et al., 2011). There is also the possibility of NDMA concentrations rebounding in the effluent while traveling through the distribution systems when there are NDMA precursors and chloramine residuals (Sgroi et al., 2015). Therefore, there is a need to monitor the formation and fate of NDMA when disinfectants such as chloramine are applied, particularly when the effluent water is contributing to the indirect potable reuse (IPR) SAT systems. By applying the dechlorinated tertiary effluent to lab-scale sand columns for treatment, the median NDMA concentration was decreased from 901 ng/L to 3 ng/L and less than 2 ng/L after 30 and 150 days, respectively (Trussell et al., 2018). However, Reny et al. (2021) reported a much lower initial NDMA concentration in the influent of infiltration basins, only up to 9.0 ng/L, when they were fed by advanced purified reclaimed water (Reny et al., 2021). Considering the fact that NDMA concentration coming to infiltration basins could be low or below detection limits, a further step would be to investigate the vadose zone soil's capability in decreasing the NDMA formation by removing its precursors.

This paper presents the results of the experimental study using four laboratory-scale unsaturated soil columns fed by wastewater effluent representing SAT systems with a focus on their capability in DON and NDMA precursors removal in the vadose zone. To better simulate the field-scale SAT systems in the lab, the soil was taken from the actual SAT site, and actual wastewater effluent of the region with naturally occurring nutrients was used as opposed to artificial wastewater. Following the observed DON and DOC decrease during this research, further investigation was conducted to 1) detect the presence of microbial activity through the columns, 2) determine the relative contribution of biodegradation and/or sorption mechanisms towards overall DON removal, and 3) characterize the removed organic matter using fluorescence spectroscopy. The use of fluorescence spectroscopy as a surrogate for micropollutants or determination of treatment processes efficacy has been investigated in multiple studies (Gerrity et al., 2018; Park and Snyder, 2018). The fluorescent organic matter compositional changes in SAT systems could be used to monitor the different management strategies of infiltration basins (Hägg et al., 2021). This study aimed to investigate this method further to characterize the wastewater effluent organic matter in the vadose zone where it is hard to obtain enough volume of samples for multiple analytical tests. Moreover, the NDMA formation potential test was also conducted as a surrogate test to determine the removal of NDMA precursors through the columns.

3.2 Materials and methods

3.2.1 Soil columns setup and operation

Four structurally similar soil columns were used for the purpose of this study to run simultaneous experiments. Figure 3.1 shows the schematic of the soil column. Soil columns were set up in a controlled temperature room set to 22℃ ± 2℃. The soil columns were made of acrylic with an inner diameter of 7 cm (2.75 in) and an outer diameter of 7.6 cm (3 in). The height of the column was 1.2 m (4 ft). A layer of glass beads was used on top of the column to ensure even distribution of the flow and on the bottom of the column to prevent the soil from washing out. A layer of glass wool was also placed at the bottom of the column beneath the glass beads. Five sampling ports were created in each column at 10 cm, 20 cm, 40 cm, and 65 cm depth from the top of the soil surface. The ports were closed by means of flexible tubing and a clip. The tubing length was minimized to prevent potential sorption in them. Cole-Parmer Masterflex L/S® Digital Drive (Vernon Hills, Illinois, USA) with variable speed was used to deliver the influent to the column top through Masterflex L/S 13 Tygon lab tubing. The tubing was replaced regularly to prevent biofilm formation. The flow through the columns was by gravity.

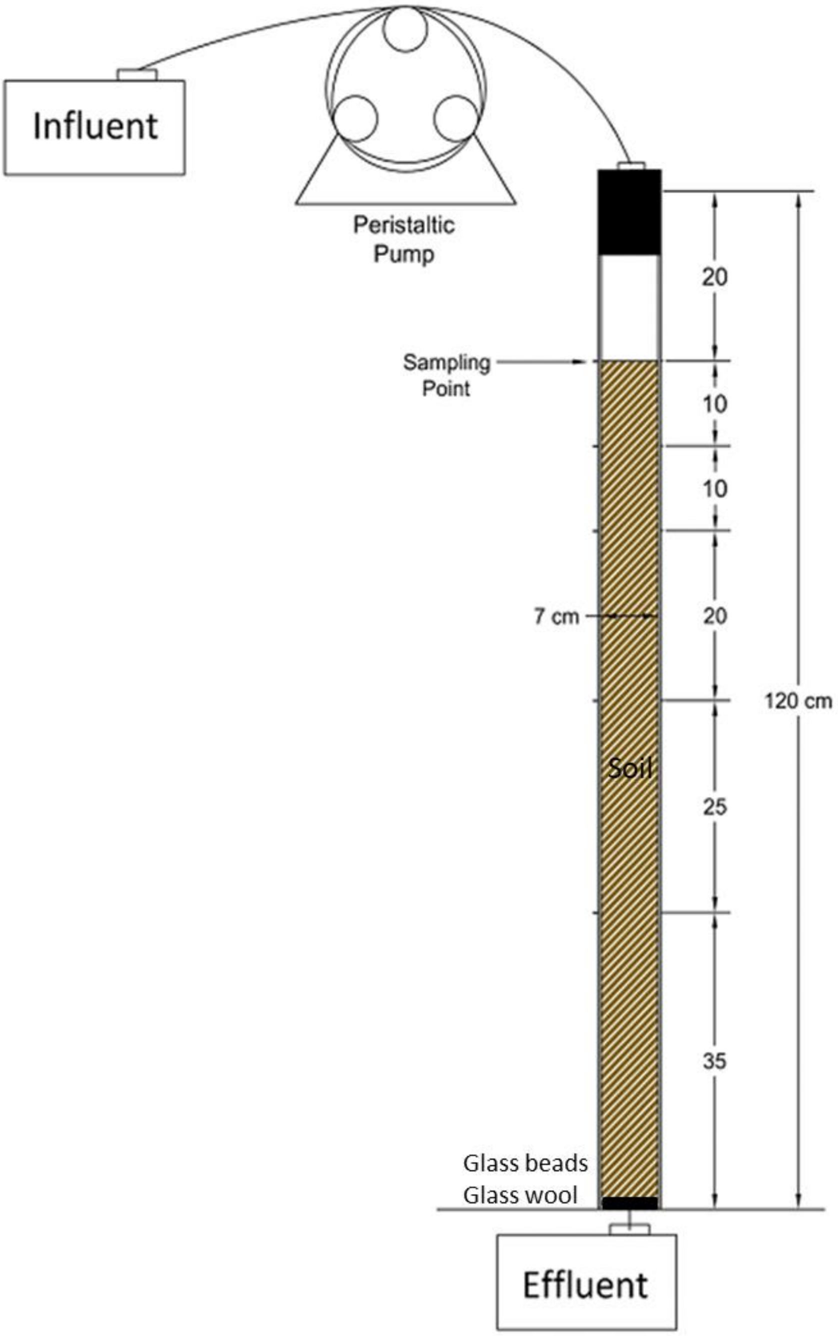

Figure 3.1 Lab-scale soil columns setu**p**

Columns were dry packed to a density of 1.9 g/cm^3 with the soil gathered from the vadose zone at Cold Springs Water Reclamation Facility, Washoe County, Nevada, USA, where rapid infiltration basins are used for secondary treated wastewater effluent disposal. The soil samples were taken from various depths of the ground by drilling a soil core. The representative soil of this study includes a mixture of soil samples from different layers (48.4 kg) from the vadose zone to represent the field conditions better. A sieve analysis was performed to get the grading curve for the soil. In this analysis, to characterize the soil properties, 10 kg out of a total mixed 48.4 kg soil was chosen at first. To select an appropriate sample of full soil size, the soil was shaped into a cone. Then it was divided into four equal parts. 5 kg of soil was chosen by taking equal amounts of all four parts. It was then dried in the oven at a constant temperature of 110 ± 5 °C for four hours to remove any water content from the soil sample. Following that, the soil was sieved and characterized. The results of sieve analysis are generally expressed in terms of the percentage of the total weight of soil that passed through different sieves (supplementary material, Table S3.1). Based on those results, the soil was found to be a combination of 86% sand, 13% silt and clay, and <1% gravel. The uniformity coefficient (C_u) and coefficient of gradation (C_c) of the soil were 5.7 and 0.7, respectively, representing well-graded soil (For more information, see supplementary material).

The influent to all soil columns was the final effluent from a WRF in Washoe County, Nevada, USA, with an average operating flow rate of 114×10^3 m^3/day, which was applied to the columns regularly. The WRF effluent used for experiments was treated by secondary treatment with enhanced biological phosphorous removal (EBPR), anaerobic-oxic (A/O) aeration basins, tertiary nitrification and denitrification, filtration, and final chlorination/dechlorination (Lacroix et al., 2020). The influent and effluent of the columns were regularly tested, and the sampling began after nine weeks of applying continuous wastewater effluent through the soil columns. During this acclimation period, biological activity was established in the columns, and the effluent characteristics were almost constant after the acclimation period.

3.2.2 Experiments design and analytical methods

3.2.2.1 Nitrogen, carbon, and other water quality parameters

After the acclimation period, the influent and effluent samples were obtained from the inlet and outlet of the columns, and samples were drawn from sampling ports along the columns as needed. The captured samples were divided into aliquots and analyzed for TN, DON, nitrate, nitrite, ammonia, DOC, UV absorbance measurements at 254 nm (UVA_{254}), and dissolved oxygen (DO).

TN and TKN were measured by HACH (Loveland, CO, USA) simplified TKN TNT 880 kit, which measures the TKN by subtracting the inorganic nitrogen (nitrate + nitrite) from TN. Nitrate, nitrite, and ammonia were measured by TNT835, TNT839, and TNT830, respectively. The DON concentration was calculated by the difference between measured TKN and ammonia. Furthermore, the DOC concentration was measured using a total organic carbon (TOC) analyzer (Shimadzu, Japan), and UVA_{254} and DO were measured using the HACH DR6000 and HACH HQ40d portable meter, respectively. If the values were below the range, half of the lower range value was assigned to them.

To indicate the presence and abundance of microbial activity through the columns, adenosine triphosphate (ATP) in the soil was determined using the Deposit and Surface Analysis (DSA™) test Kit (Luminultra Technologies Ltd., Fredericton, Canada) based on the manufacturer's manual and a luminometer (PhotonMaster™ Luminometer, LuminUltra Technologies Ltd., Fredericton, Canada). Sterile swabs were applied to collect microbial particles of a measured surface area using sampling ports along the depth of the columns. Further in the experiments, one of the columns was fed with wastewater effluent with 400 mg/L sodium azide (NaN_3) to inactivate the established biomass (Bertelkamp et al., 2014). At that point, ATP measurements were compared in the influent and through the soil column before and after NaN_3 addition to confirm biomass inactivation. The Quench-Gone Aqueous (QGA) test kit (Luminultra Technologies Ltd., Fredericton, Canada) was used to measure the abundance of microbial activity in the liquid sample.

3.2.2.2 NDMA formation potential sample preparation and analysis

An NDMA formation potential (FP) test was used to measure the columns' abilities to remove the NDMA precursors load. The true value of NDMA FP was calculated as the difference between the results of the NDMA FP test and the initial NDMA in the sample. To measure the initial NDMA concentrations, 500 mL samples were collected from the influent and effluent of the columns in baked amber bottles containing sodium thiosulfate as a preservative. Samples were kept at 4°C until they were delivered to Eurofins Eaton Analytical laboratory (Monrovia, CA) for NDMA measurement according to EPA 521 method. The non-detect samples (less than 2 ng/L) were assigned a value of half a detection limit for calculation purposes. In addition, as described by Zhang et al. (2016), the FP method included providing the ideal nitrosamine-formation conditions by adding high concentrations of chloramine. Monochloramine was made by adding 10 mL of a 5.65-6% sodium hypochlorite stock solution in 240 mL of MilliQ water. The free chlorine of the solution was measured and used to calculate the required amount of NH_4Cl to dissolve in 245 mL of high-purity water and 5 ml of the 1 M borate buffer. The sodium hypochlorite solution was added slowly to the NH_4Cl solution using a burette to cause monochloramine formation. After an hour of equilibration in the dark, chloramine was measured to determine the required volume to add 500 mL samples. After completing the chloramination step, the samples were kept at room temperature in the dark for 72 hours to provide the conditions for nitrosamine formation (McKenna, 2020; Zhang et al., 2016). Samples were sent to Eurofins Eaton Analytical laboratory (Monrovia, CA) for NDMA measurements, as described earlier. Samples were shipped in amber bottles containing sodium thiosulfate, which was used as a chloramine quenching agent to prevent further NDMA formation during transport. All NDMA or NDMA FP samples were taken in duplicates.

3.2.3 Statistical analyses

To assess whether the concentrations of each parameter were significantly different between the influent and effluent of the columns, paired t-test analysis was performed.

3.3 Results and discussion

3.3.1. Nitrogen transformation and removal

During the soil column treatment, samples were collected twice or three times a week for more than a year (n=164). The results for nitrogen transformations and removal in the soil columns are shown in Figure 3.2, where the box plots represent the minimum, maximum, and median values. Average concentrations of TDN, Nitrate + Nitrite, and DON were 1.5 ± 0.5, 0.2 ± 0.1, and 1.4 ± 0.5 mg N/L, respectively, in the soil column influent, and they were 0.8 ± 0.3, 0.4 ± 0.2, and 0.4 ± 0.3 mg N/L, respectively in the soil column effluent. In most cases, ammonia concentrations were below the detection limit (< 0.015 mg N/L) before and after the soil column treatment. It is clearly evident that the DON concentrations decreased through the 1 m soil columns. The DON removal ranged from 34 to 99%, with an average of 68% (SD=23%). Even though the DON removal varied due to variations of influent DON and its low concentration, this result was confirmed to be significant at $p\text{-value} = 0.0007 < 0.05$ (paired t-test). In addition, TDN decreased significantly (paired t-test; $p\text{-value} = 0.0037 < 0.05$) with an average of 42% removal. Similarly, Essandoh et al. (2013) showed more than 80% organic nitrogen removal after a 0.85 m travel distance in an unsaturated soil column (Essandoh et al., 2013). Meanwhile, an up to 52% increase in nitrate concentration was seen in the soil column effluent. The removal of DON, accompanied by an increase in nitrate and no detectable nitrite, shows the nitrogen transformation processes occurring, including ammonification (organic nitrogen to ammonium nitrogen) and full nitrification (ammonium nitrogen to nitrate). Due to aerobic conditions in columns, denitrification processes were not prominent, and nitrate concentrations were present in the effluent.

Wastewater effluent DON has been reported to vary greatly from 0.01 to 10.9 mg N/L (Mallick et al., 2022). In this study, while the soil column's influent DON (wastewater effluent) always remained below 2.5 mg/L, the DON concentration was less than 1 mg N/L in all effluent samples. During the sampling period, the seasonal variation did not cause any noticeable difference in DON removal. Even though the ratio of DON to TDN has been observed to be from 0.07 to 99.7% in wastewater effluent samples

(Mallick et al., 2022), due to the WRF efficiency in ammonia and nitrate removal, the ratio of DON to TDN of WRF effluent always remained above 85% throughout this study.

The average concentrations of other water quality parameters, including DOC, UVA_{254}, $SUVA_{254}$, and chemical oxygen demand (COD), have been presented in Table S3.2. The DOC content was removed by more than 34% on average, from an average of 8.4 mg C/L to 5.6 mg C/L. In addition, UVA_{254} and COD decreased by 25% and 60%, respectively, improving the water quality. The observed increase in $SUVA_{254}$ within one meter of the soil column indicates the preferential removal of non-aromatic compounds. Previous studies on soil aquifer treatment reported higher DOC removal, including more than 90% removal on average in a field-scale study that included a 37 m percolation zone (Quanrud et al., 2003), where the decrease was mainly occurring at the uppermost 3 meters with only a few DOC concentrations higher than 5 mg C/L. Given that Rauch & Drewes (2005) reported that higher initial DOC concentrations lead to higher DOC removal (Rauch and Drewes, 2005), two of the columns of this study were spiked to increase the initial concentration from 8.4 to 19.1 mg C/L on average. Consequently, the DOC removal increased to 70 ± 2. What stands out here is that despite the approximately doubled increase in the initial DOC concentration, the concentration stays in the same range (5-6 mg C/L) in the columns' effluent. This could imply that the remaining fraction is recalcitrant under the column's operating conditions.

Further in the experiment, to better understand the changes in nitrogen concentration across the depth of the soil column, a set of samples taken from sampling ports along the depth were tested for total nitrogen, nitrate, and DON. As can be seen in Figure 3.3, even though the nitrate concentration slightly fluctuates across the column, it increases up to 62% at 100 mm depth of the soil column. In addition, considering that 62% of the total DON removal occurred at the same depth, the occurrence of complete nitrification within the short distance through unsaturated columns can be implied. Similarly, it was reported that most organic nitrogen and ammonium were converted to nitrate when the infiltration basins were operated under aerobic conditions (Mienis and Arye, 2018). Further inspection of Figure 3.3 shows an increase in DON concentrations at two sampling points through the column. Likewise, Essandoh et al. (2011) reported drops in organic nitrogen removal efficiencies through the column at some points (Essandoh et al., 2011), which could be due to the release of soluble microbial products (SMPs) back into the liquid phase of the soil column.

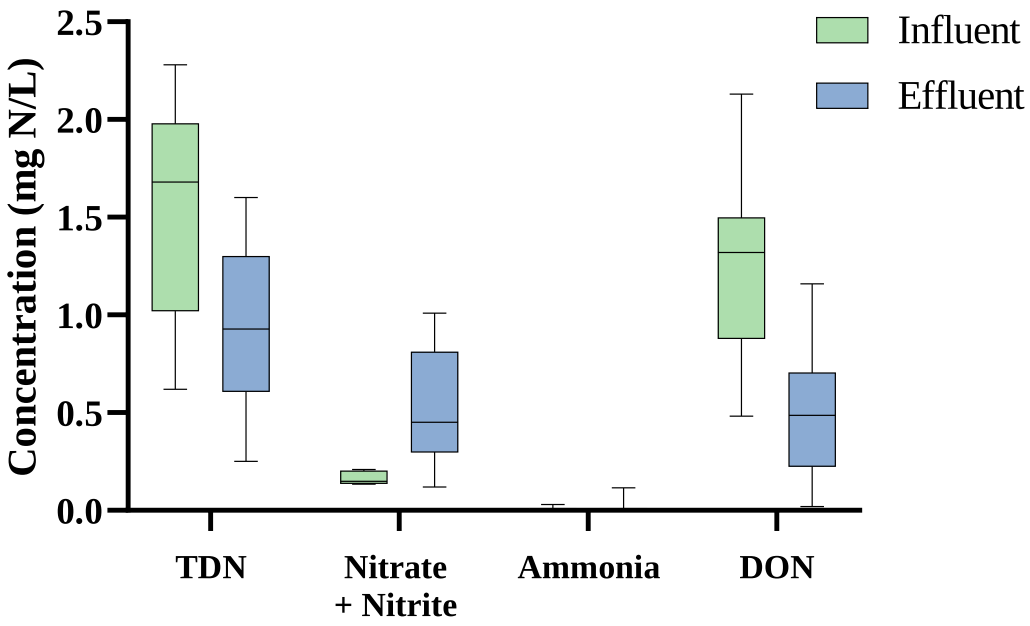

Figure 3.2 Nitrogen species concentrations boxplot before and after the soil column treatment, representing minimum, maximum, and median values (n = 164)

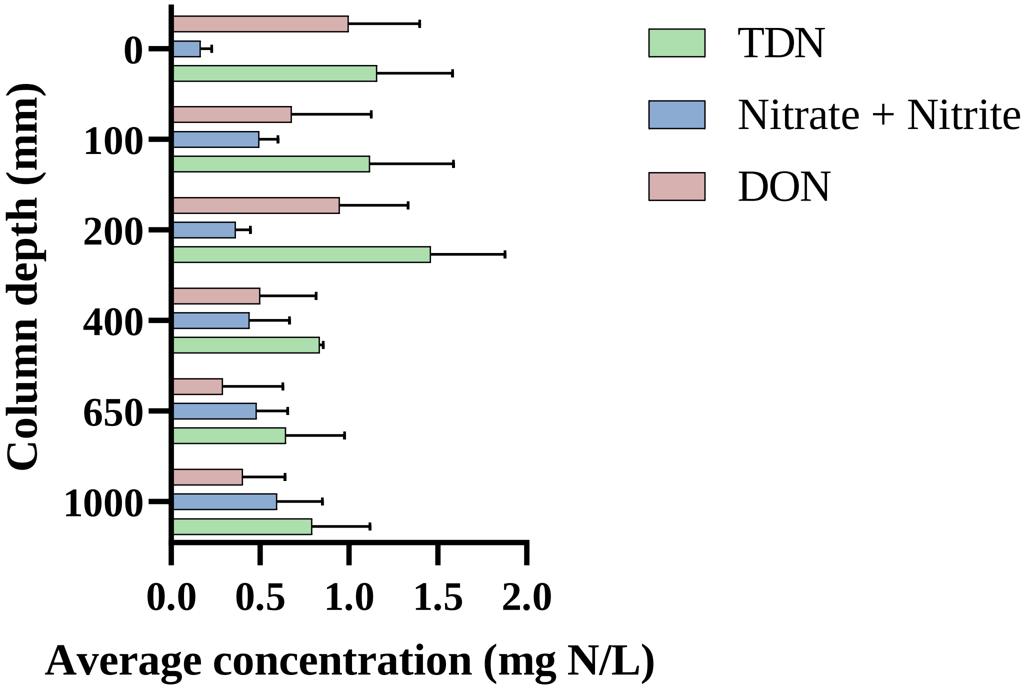

Figure 3.3 Total nitrogen, nitrate + nitrite, and DON changes through the column depth

3.3.2 Microbial community presence and biodegradation role

To confirm the presence of microbial activity through the columns, soil ATP values were measured alongside the DON concentration. As shown in Figure 3.4, ATP concentration was measured through the column, indicating the presence of microorganisms in the columns and biodegradation, as was mentioned earlier. This result also indicates the abundance of microbial activity, which is highest at a depth of 100 mm from the top, where a 32% decrease in the DON can be seen. A high concentration of ATP on top of the column could be due to the higher availability of dissolved oxygen and organic matter that causes rapid removal in the active layer.

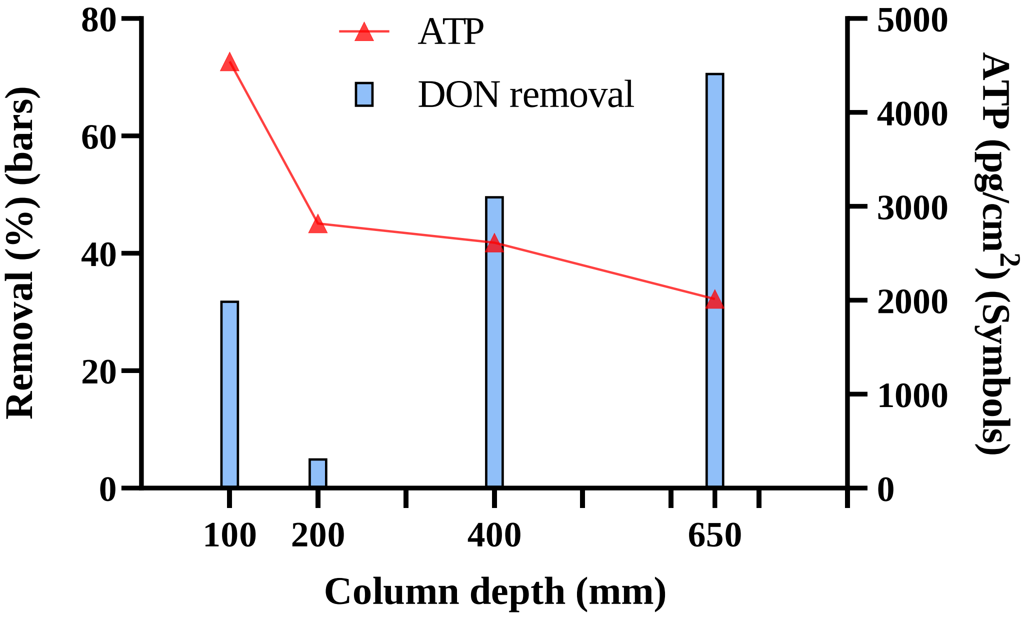

Figure 3.4 DON removal and ATP concentrations through the column depth

To gain a better insight into the removal mechanisms through the columns, one of the columns was fed with the wastewater effluent supplemented with 400 mg/L NaN_3 to inactivate the biomass present in the feed water and the biomass that had already been established in the soil column (Bertelkamp et al., 2014). Following this step, the biomass in the feed water was decreased from 340 to 0.0 pg ATP/mL by NaN_3 addition. Similarly, as shown in Figure 3.5, ATP values were measured along the depth of the column to confirm the inactivation of the biomass that was already established in the soil column. Even though a smaller amount of biomass is still present in the column after NaN_3 addition, more than 82% reduction of biomass was seen at all sampling ports in the column.

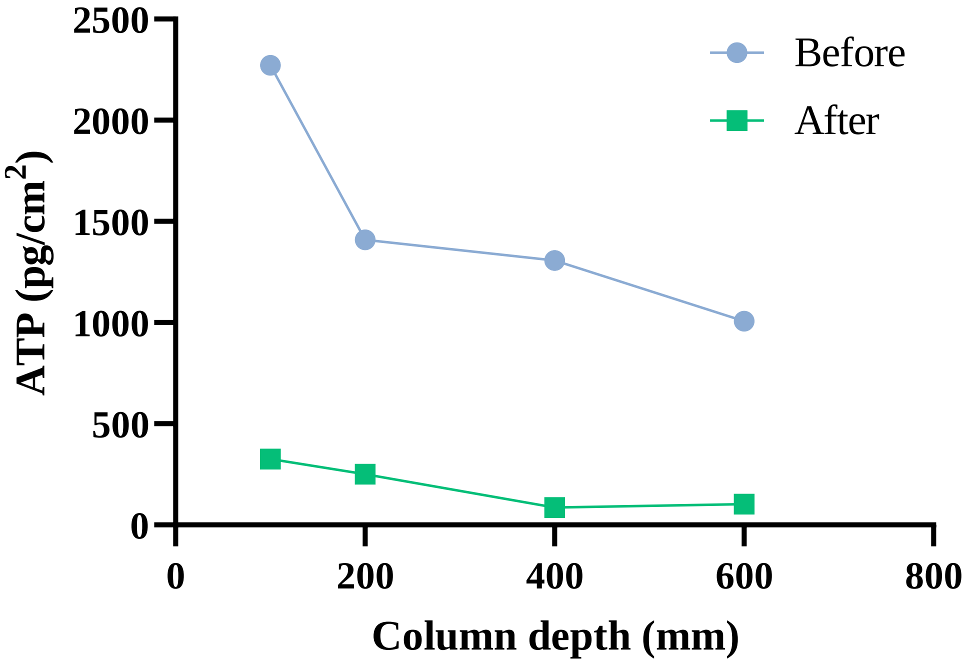

Figure 3.5 ATP concentrations through the column depth before and after sodium azide (NaN3) addition

After the confirmation of biomass inactivation, the water samples were collected and tested at the influent and effluent of the column (n=16). The average TDN removal was only 4.5% (SD=3.2) in the absence of microbial community, which is considerably lower than the average TDN removal (68%) in the columns with biomass present. Moreover, nitrate concentration only slightly increased (3.7%), which could imply the absence of biodegradation as opposed to before azide addition. Observing the decrease in the TDN removal through the column, it can be implied that biodegradation is an important factor that contributes to the total removal under the operating conditions pointing to the fact that a well-developed and established biomass is essential for water quality improvement through SAT. Moreover, soil sorption capacity could get exhausted after a while; however, during the run of the columns for over a year, their DON removal efficiency did not change significantly. This further strengthens the point since the soil in the columns included less than 13 percent silt and clay and more than 86 percent sand. However, since this study used unsaturated soil columns in order to focus on the vadose zone, the removal mechanisms' contributions could change in the saturated soil zone, where the microbial community would be different due to different oxic conditions.

3.3.3 NDMA precursors' removal in the columns

An NDMA FP test was conducted to measure the NDMA precursors' removal in the columns. The results showed the columns’ capability to remove the NDMA precursors by more than 91 percent when the average initial NDMA FP was reported to be 89.5±7 ng/L, which is in accordance with the organic matter removal that was reported in Section 4.1 (Figure 3.6). These results highlight the capability of soil columns to remove the NDMA precursors' which leads to the decreased potential for the formation of

NDMA in the soil subsurface. The removal of NDMA precursors becomes more important as NDMA is photolyzed to dimethylamine (DMA), a precursor, through the photolysis that happens in the recharge basins (Reny et al., 2021). Therefore, even though natural photolysis has shown to be capable of NDMA removal before infiltration, the capability of the soil to remove the generated precursors would be beneficial in preventing future NDMA formation.

It is noteworthy to mention that the initial NDMA concentration was only detected in one out of three influent samples at low levels (2.2 ng/L). NDMA was shown to be below the detection limit (< 2 ng/L) in all effluent samples. Since NDMA was only detected at low levels in the influent sample, it is hard to attribute the non-detect NDMA to the soil contaminant removal capability and assess the treatment capacity of the vadose zone in regard to NDMA removal in general. Still, future studies on the removal of NDMA through SAT systems with a lower detection limit could be beneficial when the initial NDMA concentration is low. As reported by Reny et al. (2021), the natural photolysis could remove the NDMA to less than 1.5 ng/L in infiltration basins (Reny et al., 2021), which would be the initial concentration that further enters the vadose zone.

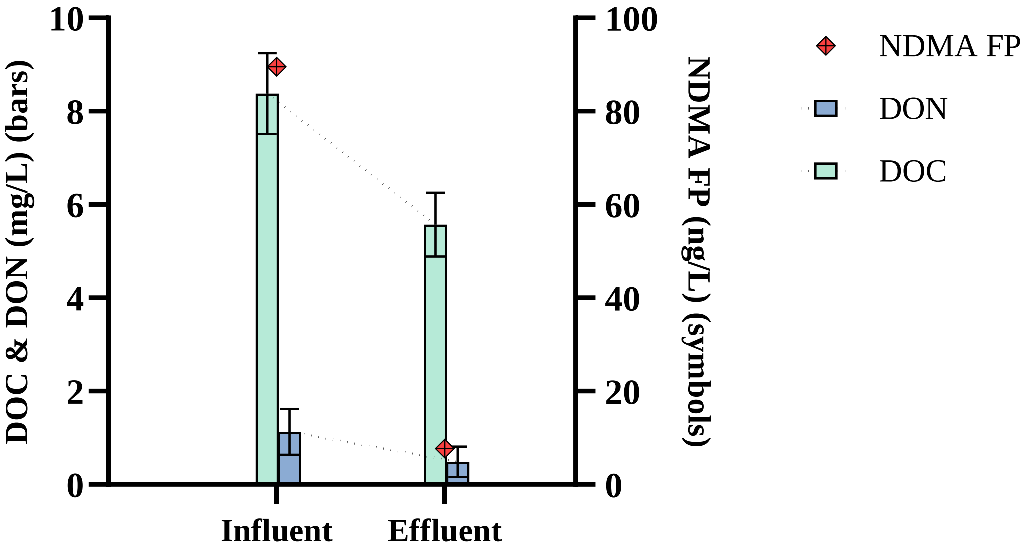

Figure 3.6 NDMA FP, DON, and DOC changes before and after the soil column treatment

3.4 Conclusions

Using lab-scale unsaturated soil columns to simulate SAT systems, this study showed the capability of the vadose zone in further treatment of remaining organic matter in the wastewater effluent, in particular, DON, as evidenced by samples collected from the influent and effluent as well as through the sampling ports along the depth of the columns.

- The removal of DON, accompanied by an increase in nitrate concentration, pointed to the occurrence of ammonification and nitrification. ATP analysis showed that the highest biomass concentration is in less than 100 mm of soil depth, which resulted in the occurrence of the majority of the total DON removal at a short distance or time traveled by the effluent through the column.
- The slight increase in nitrate and low TDN removal following the inactivation of the biomass in the control column showed the important role of biodegradation for ammonification and nitrification reactions under aerobic conditions.
- The NDMA precursors were significantly removed by the soil columns. However, these results were measured at a limited time or distance frame. Longer travel distances through the soil and changes in the oxic conditions could cause further reactions in the nitrogen cycle or the possibility of NDMA rebounding. Hence, to comment on the treatment capabilities of SAT systems, it is important to identify when the snapshot of the soil profile was taken.

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Supplementary materials and methods

Table S3.1 Sieve analysis of the soil

Sieve #	Diameter (mm)	sieve weight (Kg)	sieve & soil (Kg)	mass of soil retained (g)	percent retained on each sieve (g)	cumulative percent %	percent finer
4	4.75	0.51	0.51	3.5	0.7	0.7	99.3
10	2	0.49	0.53	36	7.2	7.9	92.1
20	0.85	0.35	0.39	41	8.2	16.1	83.9
40	0.425	0.42	0.48	58.5	11.7	27.8	72.2
60	0.25	0.41	0.47	57.5	11.5	39.3	60.7
100	0.15	0.35	0.41	65	13.0	52.4	47.6
200	0.075	0.35	0.52	173.5	34.7	87.1	12.9
pan		0.50	0.57	64.5	12.9	100.0	0.0

Continued from Table S3.2:

D(10)= effective particle size (10% percent of the particles are finer than D10) = 0.05

D(30)= (30% percent of the particles are finer than D30) = 0.11

D(60)= (60% percent of the particles are finer than D60) = 0.3

The uniformity coefficient (Cu)= D(60)/D(10) = 5.85

Coefficient of curvature (Cc) = (D(60)^2)/(D(10)*D(30))= 0.7

Table S3.2 Average of water quality parameters for the influent and effluent samples of the column

Sampling location	DOC (mg C/L)	$UVA_{254}(cm^{-1})$	SUVA (L/mg-m)	COD (mg/L)
Influent	8.38 ± 0.87	0.16 ± 0.008	1.9	34.00 ± 3.4
Effluent	5.57 ± 0.68	0.12 ± 0.01	2.15	13.75 ± 4.2

Chapter 4 Characterization of organic matter during simulated soil aquifer treatment (vadose zone)

4.0 Abstract

Optical characterization, including the application of UV absorbance measurements at 254 nm for colored dissolved organic matter (CDOM) and fluorescence spectroscopy for fluorescent organic matter (FDOM), has been employed for a long time for water quality assessments. Multiple analyses, including fluorescence regional integration (FRI), PARAFAC, and peak picking, were applied to the fluorescence spectroscopy dataset to characterize and model the fluorescence properties of dissolved organic matter in samples acquired from soil columns simulating the soil aquifer treatment (SAT). FRI technique nfollowed by fulvic-acid-like compounds and tyrosine-like compounds (28% and 15%), respectively. The columns were capable of removing 56% of the fluorescent dissolved organic matter (FDOM), where the main removal was associated with humic-like compounds. A four-component EEM-PARAFAC modeling indicated the predominance of terrestrial humic-like component (C1), followed by tyrosine-like (C2) and microbial-humic-like (C3), and humic-acid-like compounds (C4). Since fluorescent spectroscopy is a fast, highly sensitive, and non-invasive technique, there is a need to explore its monitoring potential in water reuse schemes further when managed aquifer recharge techniques, including SATs, are being employed.

Keywords: Fluorescence spectroscopy; Multivariate analysis; Fluorescence regional integration; PARAFAC modeling

4.1 Introduction

Dissolved organic matter is a heterogeneous mixture of molecules containing carbon that is present in aquatic environments, including wastewater systems. DOM’s role as a substrate for microbial growth and nutrient cycling makes it an important water quality parameter (Heibati et al., 2017). DOM has a notable effect on the ecosystem’s health, including contaminant transport, as well as being a challenge in engineered systems, such as its role in coagulant efficiency, DBPs formation, biomass growth, and contaminant mobility (Ishii and Boyer, 2012). Therefore, it becomes important to track DOM changes and concentration fluctuation spatially and temporally from its source. DOM mixtures include aliphatic and aromatic polymers whose compositions depend on the source and the degrading processes (Stedmon et al., 2003). Since the chemical nature of the DOM can be seen in its optical properties, many researchers have used optical measurements to follow the DOM changes across the surface waters (Coble, 2007).

The chromophoric/colored DOM (CDOM) is the optically active fraction of the organic matter. Fluorescent dissolved organic matter (FDOM) is a fraction of CDOM that emits fluorescence after light excitation. In this process, when a molecule absorbs light, an electron moves to the excited state with a higher energy level from the ground state. Eventually, the energy will be released, and the electron will return to the lower energy level. One way of energy release is in the form of a photon which is called fluorescence. The amount of energy released, or the wavelength of the photon, is always the same in the fluorescent molecule (Figure 4.1). Besides emitting a photon (fluorescence), internal conversion and vibrational relaxation are other ways to dissipate the energy in the excited molecule (Jaffe and Miller, 1966). Fluorescence data can be presented in three-dimensional excitation-emission matrices to be interpreted.

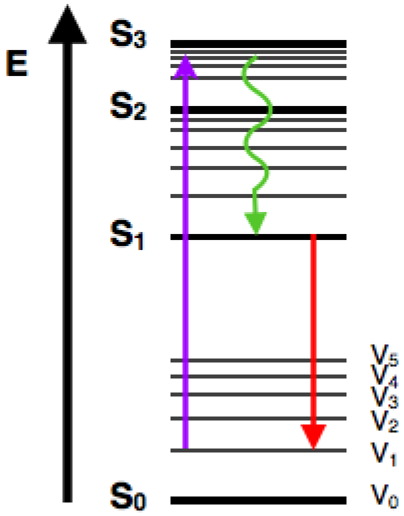

Figure 4.1 Jablonski diagram showing possible scenarios including absorption (purple arrow), internal conversion and vibrational relaxation (green arrow), and fluorescence (red arrow) processes.

Fluorescence spectroscopy analysis, based on the presence of the fluorophores associated with humic or protein-like compounds, is a highly sensitive, relatively fast, and non-invasive technique for organic matter characterization (Sanchez et al., 2013; Shi et al., 2021). Due to minimal sample preparation, it has the potential for online implementation in full scale natural or engineered treatment systems.

Many studies have used this technique to characterize organic matter in engineered systems, such as treatment plants and non-engineered systems, including rivers and lakes (Table 4.1). As mentioned, soil aquifer treatment systems' performances are highly site-specific and dependent on multiple variables. Hence, there is a constant need to monitor their treatment efficacies. Multiple studies have applied fluorescence spectroscopy to characterize the organic matter fractions in SAT systems (Abel et al., 2012; Cohen et al., 2014; Drewes et al., 2006; Stahlschmidt et al., 2016; Xue et al., 2013; Xue et al., 2003). In this study, organic matter in the lab scale soil columns was characterized using fluorescence spectroscopy. This technique was chosen in this study to assess the columns' performance since it is 1) highly sensitive and is able to handle the low organic matter-containing reclaimed water samples that are usually the case in SAT systems. 2) relatively fast and easy to operate, which gives it the potential to be used for online monitoring. 3) capable of identifying the treatment performance efficiencies with varying degrees of data processing, and 4) an efficient way to compare the organic matter characteristics across other studies if the appropriate steps of data processing have been taken.

Table 4.1 Fluorescence spectroscopy potential applications

Reference	**System**	**Fluorescence spectroscopy analysis**	**Further aspects of the study**
Sanchez et al., 2013	raw and treated water after coagulation– filtration – drinking water plant	PARAFAC	Use of differential fluorescence dataset
Moradi et al., 2018	Nitrified and non-nitrified waters – drinking water plant	PARAFAC	
Retelletti Brogi et al., 2022	River – surface water	PARAFAC	Regulations of anthropogenic emissions
Zhang et al., 2022	Lake – surface water	PARAFAC	Coupled with self-organizing mapping neural network
Gou et al., 2017	Pharmaceutical wastewater effluent applied to coagulation process – industrial wastewater	PARAFAC	Coupled with PCA to analyze the correlations between physiochemical parameters and fluorescence component characteristics

Baghoth et al., 2011	Different points along two water treatment plants – drinking water plant	PARAFAC	One plant includes infiltration in sand dunes
Lavonen et al., 2015	Different points along four water treatment plants – drinking water plant	Indices including HIX, FI, and freshness index	Use of differential fluorescence dataset – Coupled with ESI-FT-ICR-MS analysis
Sgroi et al., 2015	Primary influent and final effluent of 10 wastewater treatment plants – wastewater plant	Peak picking, FRI, and PARAFAC	Identifying surrogates for emerging trace organic compounds
Vera et al., 2017	UV membrane samples in a drinking water treatment plant	PARAFAC	Identifying the correlation between PARAFAC components and membrane fouling indices
Maqbool et al., 2020	Influent and effluent of 10 drinking water treatment plants	Indices including HIX, BIX, and PARAFAC	Identifying the correlation between PARAFAC components and N-nitrosamines

Roccaro et al., 2020	Pilot-scale membrane biological reactor followed by nanofiltration (MBR-NF)	Peak picking	Identifying the correlation between NDMA precursors and NDMA FP with EEM peaks.
This study	Reclaimed water applied to lab-scale soil columns	Peak picking, FRI, and PARAFAC	Identifying the organic matter changes though soil columns simulating the vadose zone

4.2 Materials and Methods

4.2.1 Sampling plan

During the sampling event of over four months under steady state soil column operation with reclaimed water feed, samples were obtained from the influent and effluent of the columns as well as sampling ports across the columns along the depth. At each sampling location, four to five replicate samples were taken. Samples were collected in Teflon-lined cap glass bottles and stored at 4°C for a maximum of 4 days until analysis. All samples were filtered using 0.45 µm cellulose acetate membrane filters prior to analysis. A total of 54 samples were used in the characterization and data analysis.

4.2.2 3D excitation-emission matrices (EEMs) fluorescence spectroscopy analysis

In this study, excitation-emission matrices (EEMs) of filtered samples were determined using a spectrofluorometer (RF-6000, Shimadzu Corporation, Japan) with a built-in spectral correction function to characterize the organic matter identified in the influent and effluent of the columns and the removed fractions. The excitation wavelengths varied from 200 to 500 nm in 1 nm steps, and the emission wavelengths varied from 220 to 600 nm in 2 nm steps to obtain detailed and comprehensive information on organic matter. The excitation and emission bandwidths were 5 nm, and the scanning speed was set at 6000 nm/min (further details on instrument setup in Table S4.1).

There are multiple ways to interpret or quantify the fluorescence signals that require different data processing times, including excitation-emission pairs (peak picking; Coble, 2007), fluorescence regional integration (FRI, Chen et al., 2003; Jacquin et al., 2017) and parallel factor (PARAFAC) analysis (Murphy et al., 2013; Stedmon et al., 2003). In this study, the results of all the methods are reported. However, the raw EEM data was processed before any further analysis (Figure 4.2). For the FRI analysis, the EEM samples were corrected for Raman scattering and other possible background noises or systematic errors by subtracting the DI water EEM that was obtained under the same conditions as the samples from all sample spectra (Peiris et al., 2010). Additional steps in data preprocessing were conducted to apply 1) inner filter effect (IFE) correction (Lakowicz, 2006) and 2) fluorescence intensity normalization, including the conversion of arbitrary units (AU) to Raman units (RU) (Lawaetz and Stedmon, 2009). For PARAFAC and indices, the preprocessing of the data was conducted according to the staRdom package in R (Pucher et al., 2019). The reasoning behind each data correction has been discussed in the next sections.

Figure 4.2 Process of EEM data handling

4.2.2.1. Raman and Rayleigh light scattering

The raw EEM data shows the presence of diagonal scatter peaks that are not caused by the fluorescence. Rayleigh and Tyndall can be seen at the same wavelength as the excitation beam, where excitation and emission wavelengths are equal, but they usually are magnitudes higher than the fluorescence. Tyndall includes reflection from particulate matter in the sample. Primary Raman peaks occur at longer wavelengths. The secondary Rayleigh and Raman peaks can also be seen at twice the emission wavelength of the primary peaks (Murphy et al., 2013; Zepp et al., 2004). These regions of the data are non-linear and need to be eliminated before applying the PARAFAC model. There are multiple ways that can be used for spectra correction. One way that can partially account for the scattering is to subtract the fluorescence response of a distilled water (<0.2 mg/L) sample from the spectra sample. Furthermore, these sections of the spectra could be eliminated and replaced by the interpolated values from the surrounding area (Bahram et al., 2006; Gerrity et al., 2018; Murphy et al., 2013; Zepp et al., 2004) if deemed appropriate.

4.2.2.2. Inner filter effect

The raw data acquired from the fluorescence spectrometer should be corrected for IFE prior to any data analysis. IFE occurs when radiation gets absorbed by the sample when it goes in and out of the cuvette (primary and secondary IFE) and loses some of its energy. Therefore, the chromophores that are in the center of the cuvette absorb less amount of excited light, which eventually leads to a lower amount of emitted light picked up by the detector (Murphy et al., 2013). To correct for IFE, the observed EEM matrixes are multiplied elementwise by the correction matrix based on the following equation.

$$F_C = F_O * 10^{\left(\frac{Abs_{exitation}+Abs_{emission}}{2}\right)} \tag{1}$$

In equation 1, F_C and F_O refer to the corrected and observed fluorescence intensities, respectively. $Abs_{exitation}$ and $Abs_{emission}$ represent the absorbance at the excitation and emission wavelength, respectively where the F_O was observed (Lakowicz, 2006; Park and Snyder, 2018).

This correction method is accurate within 5% when the absorbance is below 2.0 cm^{-1} (Murphy et al., 2013). Otherwise, the samples need to be diluted first, and a dilution factor should be applied later. It has been mentioned that when absorbance is below 0.05 cm^{-1} across the wavelength range, there is no need for IFE correction. Even though that is the case with the raw data here, the correction was still applied since it could still be troublesome at shorter wavelengths due to the noises' presence.

4.2.2.3. Data normalization

The data obtained from the fluorescence spectrometer is challenging to use for comparison across multiple instruments since the fluorescence intensity is reported in arbitrary units (AUs). Therefore, there is a need to normalize the data prior to any data interpretation. One way of data normalization that is used in this study is by dividing the corrected values (AU) obtained in Section 4.2.2.2 by the Raman peak of DI water (Lawaetz and Stedmon, 2009; Murphy et al., 2010; Park and Snyder, 2018; Stedmon et al., 2003) and get the fluorescence intensity in Raman units (RU) based on the following equation:

$$F\ (R.U.) = \frac{F_C}{\int_{\lambda_{emission,l}}^{\lambda_{emission,u}} F_{DI} * (\lambda_{emission})} = \frac{F_C}{\int_{427.9\ nm}^{370.8\ nm} F_{DI\ (exitation=350\ nm)} * (\lambda_{emission})} \quad (2)$$

Here,

F = Final corrected fluorescence intensity in Raman unit;

F_{DI} = Fluorescence intensity of the DI water where the excitation wavelength is 350 nm;

$\lambda_{emission,l}$= Lower limit of the emission wavelength (427.9 nm);

$\lambda_{emission,u}$ = Upper limit of the emission wavelength (370.8 nm).

The emission wavelength boundaries are selected in a way that can capture the Raman peak position.

The overall steps of data preprocessing in this study are shown in Figures 4.3 to 4.6, using one of the study samples. At first, a blank sample EEM spectra were subtracted from the samples EEM spectra, followed by IFE correction. Then, the EEM spectra were normalized using the Raman peak of DI water. Finally, Raman and Rayleigh’s scatter were removed, and they were replaced by values obtained from interpolation based on the surrounding area.

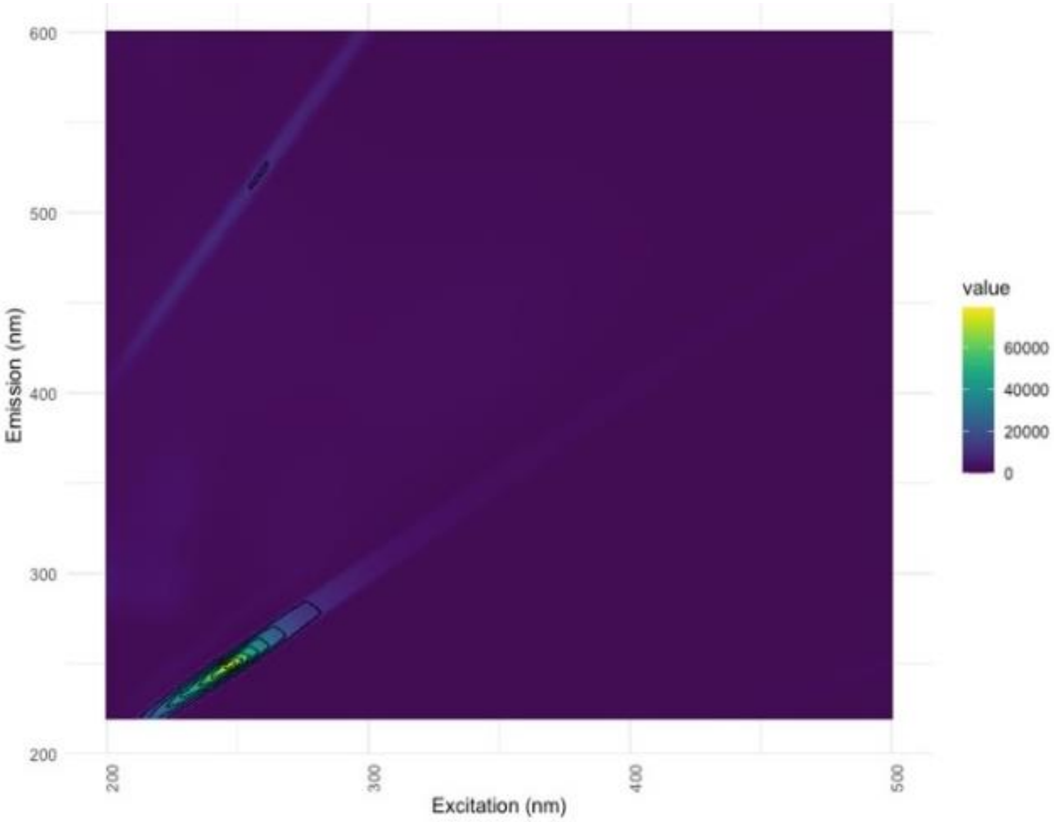

Figure 4.3 Data preprocessing steps, raw data

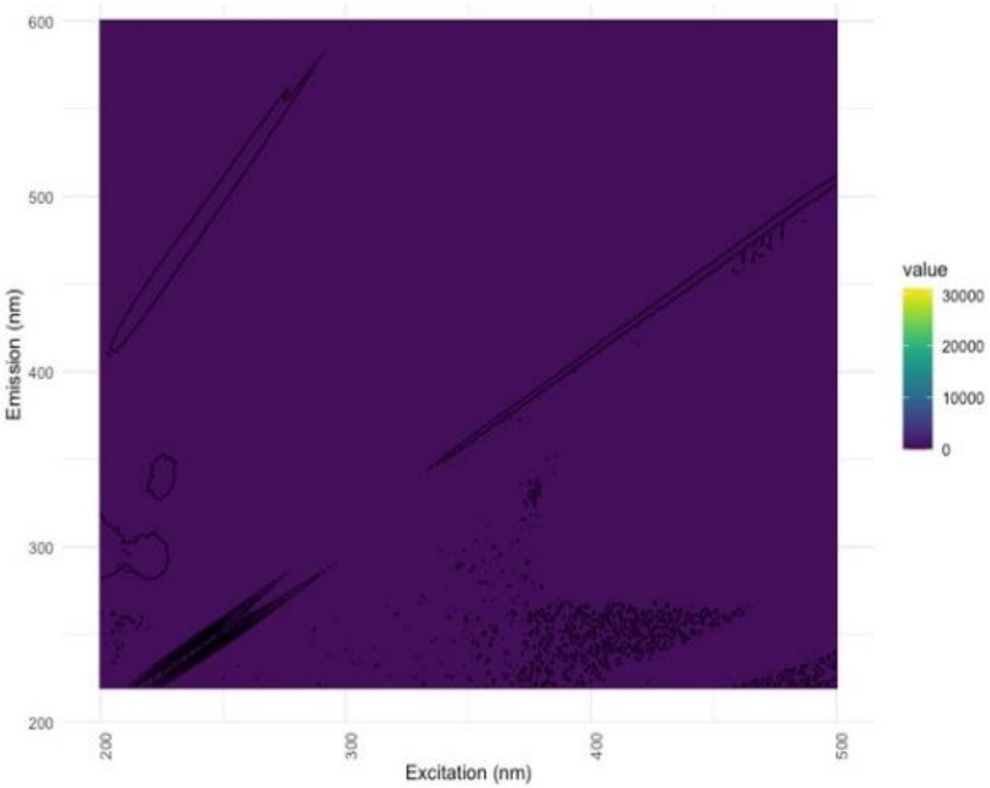

Figure 4.4 Data preprocessing steps, blank subtraction, inner filter effect correction, Raman normalization

Figure 4.5 Data preprocessing steps, scatter removal

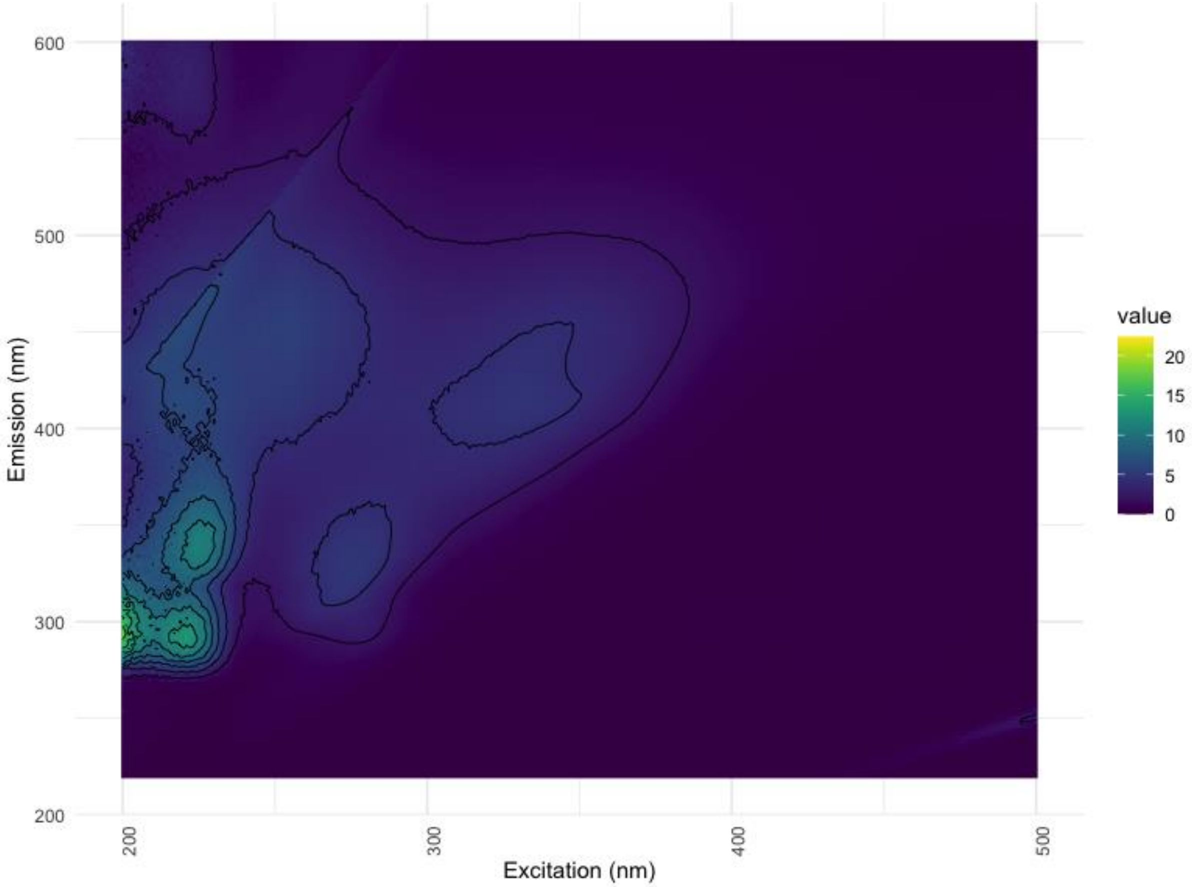

Figure 4.6 Data preprocessing steps, interpolation

4.2.3 Data processing

4.2.3.1 FRI technique

The FRI technique is a quantifying method for EEM analysis that integrates the volume beneath defined EEM regions that correspond to certain chemical compounds. Here, the EEM has been classified into five regions, as shown in Table 4.2 (Chen et al., 2003; Park and Snyder, 2018). However, there are other proposed boundaries for EEM categories that vary slightly among the literature (Gerrity et al., 2018). A MatLab code was used to preprocess, integrate, and report the results.

Table 4.2 Fluorescence regional definitions for excitation - emission boundaries

Region	**Description**	**Specifications**
I	Tyrosine-like aromatic protein	Ex: 200 - 250 nm; Em: 280 - 330 nm
II	Tryptophan-like aromatic protein	Ex: 200 - 250 nm; Em: 330 - 380 nm
III	Fulvic acid-like matter	Ex: 200 - 250 nm; Em: 380 - 550 nm
IV	Soluble microbial byproduct-like matter	Ex: 250 - 400 nm; Em: 280 - 380 nm
V	Humic acid-like matter	Ex: 250 - 400 nm; Em: 380 - 550 nm

4.2.3.1 PARAFAC modeling

PARAFAC analysis is used in chemical science for the decomposition of three or higher-order arrays. One example of the three-order arrays is the fluorescence EEMs. Since, EEMs = Samples * excitation wavelength * emission wavelength. The decomposition will help to detect and quantify each underlying signal since every analyte would have its own distinct excitation and emission spectrum. In layperson's terms, each mixture can be described as underlying pure excitation and emission wavelengths and their concentrations (Figure 4.7). One advantage of this method is that this decomposition happens mathematically as opposed to physically, which is the case in chromatography. Hence, this is a non-invasive way that the samples will not be ruined during the process for other analyses.

A PARAFAC model of a three-way array can be shown with the following equation:

$$X_{ijk} = \sum_{f=1}^{F} a_{ij} b_{jk} c_{kf} + e_{ijk} \qquad (i = 1, \dots, I; j = 1, \dots, J; k = 1, \dots, K) \tag{3}$$

Where:

X_{ijk} = Data point corresponding to i^{th} sample (Sample) at the j^{th} variable on dimension 2 (emission mode) and at the k^{th} variable on dimension 3 (excitation mode);

e_{ijk} = residual to represent the variability or interference that the model cannot handle;

f = Corresponds to a PARAFAC component.

When the model has been validated, each component has chemical interpretations. The parameter a_{if} is directly proportional to the concentration of the f^{th} analyte of sample i; the vector $\mathbf{b}_f$ with elements b_{jf} is a scaled estimate of the emission spectrum of the f^{th} analyte.

Likewise, the vector $\mathbf{c}_f$ with elements c_{kf} is linearly proportional to the specific absorption coefficient of the f^{th} analyte (Murphy et al., 2013).

There are two important assumptions to using PARAFAC. 1) No two chemical components can have identical spectra. 2) The number of components underlies the chemical variation in each dimension. 3) The total fluorescence signal includes the linear superposition of a fixed number of components. (Bro, 1997; Lakowicz, 2006; Murphy et al., 2013). Multiple constraints can be applied to the model, such as non-negative values since the fluorescence intensity is always positive (Pucher et al., 2019). In this study, the staRdom package was applied in R (Pucher et al., 2019) using the multiway package for model fitting compared to the drEEM, which uses the N-way toolbox in Matlab (Murphy et al., 2013), where the logic behind most of the steps remain the same. A summary of various steps of modeling by staRdom can be seen in Figure 4.8. The model was verified according to Murphy et al. 2013 by assessing the following criteria: core consistency, split-half analysis, residual analysis, and visualization of the spectral loading, which will be explained in detail in Section 4.3.2.

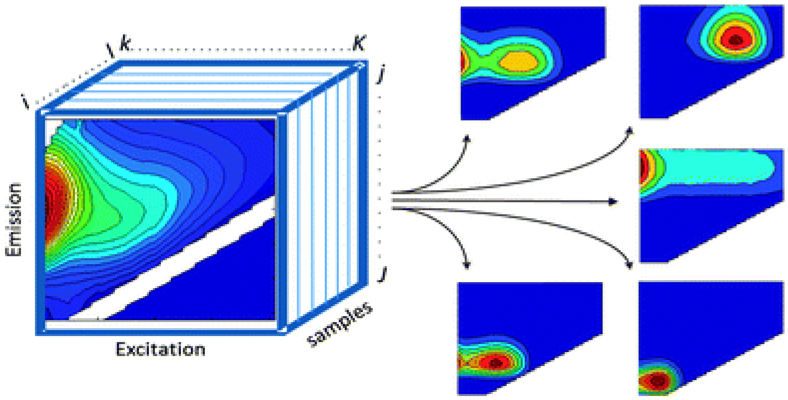

Figure 4.7 Three-way structure of EEM samples that are decomposed into five components (Murphy et al., 2013)

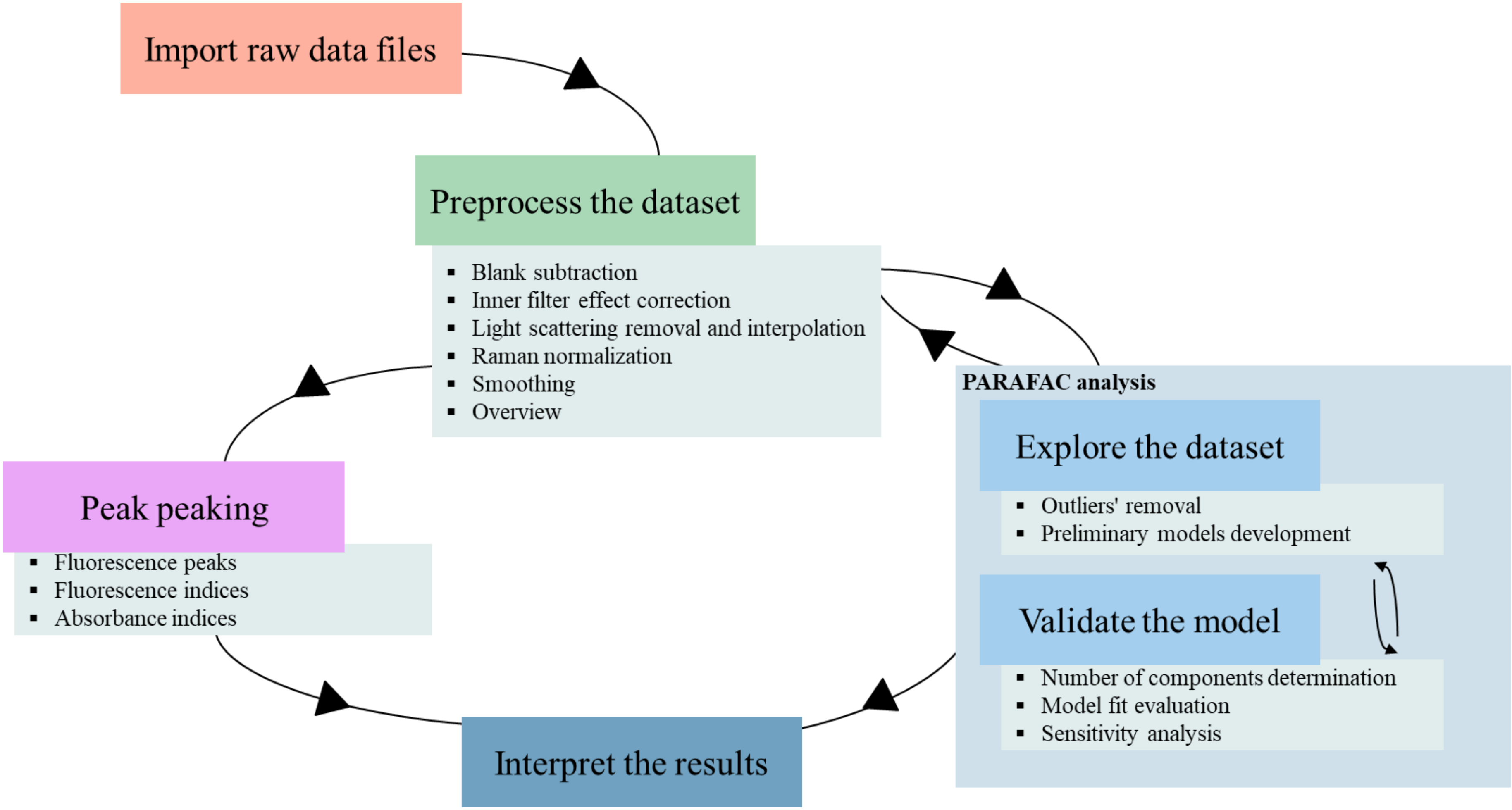

Figure 4.8 PARAFAC modeling structure with staRdom; adopted from Pucher et al. (2019)

4.2.3.2 Peak picking and indices

The modification and evolution of fluorescent DOM can be monitored by measurements of fluorescent intensities and calculations of certain intensity ratios (Huguet et al., 2009), which is a simpler way to interpret the EEM data and the organic matter composition with less data processing compared to FYI and PARAFAC modeling. These indices include fluorescence index (FI), humification index (HIX), and autotrophic productivity (BIX) (Table 4.3). McKnight et al. (2001) showed that FI, the ratio of emission intensity at a wavelength of 450 nm to 500 nm (f450/f500) when the excitation wavelength is 370 nm, can be used to distinguish sources of isolated aquatic fulvic acids (McKnight et al., 2001). Humification indices are calculated by diving the fluorescence intensity at higher wavelengths where higher values are associated with higher molecular weight aromaticity and humification (Chen et al., 2018). Ohno (2002) suggested another form of calculation for the humification index as opposed to the prior formula calculated by Zsolnay et al. (1999), where the new formula reduces the HIX coefficient of variation for most of the highly humified samples (Ohno, 2002; Zsolnay et al., 1999). Finally, it was observed that high values of BIX (>1) correspond to a predominately autochthonous origin of organic matter (Huguet et al., 2009).

Even though there could be multiple ways to define some of these indices, the staRdom package (Pucher et al., 2019) used in this study reported the following features of the EEM data:

- Fluorescence index (McKnight et al., 2001)

- Fluorescence peaks and indices (Massicotte, 2019)
- Autochthonous productivity index/freshness index (BIX) (Fellman et al., 2010; Huguet et al., 2009)
- Classical peaks based on manual peak picking [B, T, A, M, C; (Coble, 1996)]
- Humification index (Ohno, 2002)

Table 4. 3 Descriptions of fluorescence indices

Indices	**Description**	**Observations**
Fluorescence index (FI)	Ratio of emission intensity (450 nm/500 nm) at 370 nm excitation (McKnight et al., 2001)	1.9-2 in lakes representing microbially derived fulvic acids and ~1.4 for terrestrially derived fulvic acids (McKnight et al., 2001) 1.15-1.22 for estuary (Huguet et al., 2009)
Humification index (HIX)	Ratio of H/L in the emission spectrum for excitation at 254 nm. H represents the upper region (435-480nm) area, and L represents the lower region (330-345 nm) area (Zsolnay et al., 1999)	10-16 for strongly humified organic material mainly of terrestrial origin, <4 with autochthonous organic matter (Huguet et al., 2009)
	Ratio of H/(H+L)(Ohno, 2002)	0.83-0.84 for soil DOM extract (Ohno, 2002)
BIX	The β/α index was calculated as the ratio of emission intensity at 380 nm (β) to the maximum emission intensity observed between 420 and 435 nm (α) for an excitation wavelength of 310 nm (Wilson and Xenopoulos, 2009)	0.6-0.7 for low autochthonous component and >1 biological or aquatic bacterial origin

Table 4.4 Descriptions of fluorescence peaks by Coble (1996)

Peaks	**Ex (nm) /Em (nm)**
Tyrosine-like, protein-like (B)	275/310
Tryptophan-like, protein-like (T)	275/340
Humic-like (A)	260/380-460
Marine humic-like (M)	312/380-420
Humic-like (C)	350/420-480

4.3. Results and Discussion

4.3.1 FRI technique

Considering the established removal of organic matter through the columns, EEM spectra of the column influent and effluent, as well as samples from sampling ports along the depth, were analyzed to characterize the organic matter based on Table 4.2 boundaries. As demonstrated in Figure 4.9 and Table 4.5, humic-like substances were the largest fraction in the influent (35%), followed by fulvic-acid-like compounds and tyrosine-like compounds (28% and 15%), respectively. This order stayed the same in the effluent samples as well. 53-61% removal occurred through all regions (R1-R5,) indicating the capability of soil columns in removing SMP, fulvic and humic-like substances (Table 4.5). Meanwhile, the fulvic acid portion (R3) of the organic matter had the highest removal (61%). In comparison, humic-like substances (R3) had a lower removal (56%) which further confirms the earlier results (Section 3.3) when the increase of $SUVA_{254}$ suggested the preferential removal of non-aromatic organic matter. The reported SMP removal is in accordance with Trussell et al. (2018), where the SMP peak was removed through a soil column treatment (Trussell et al., 2018). As shown in Figure 4.6, there was an increase in fluorescence intensity during the first 200 mm of the column that decreased later in the soil profile. As mentioned earlier, the release of SMPs in the soil columns from biomass in the upper layers has been reported in the literature. However, this increase can be seen in all defined regions of organic matter, and there is a need for further investigation of the organic matter compositional change in the vadose zone that can affect the removal mechanisms in SAT systems.

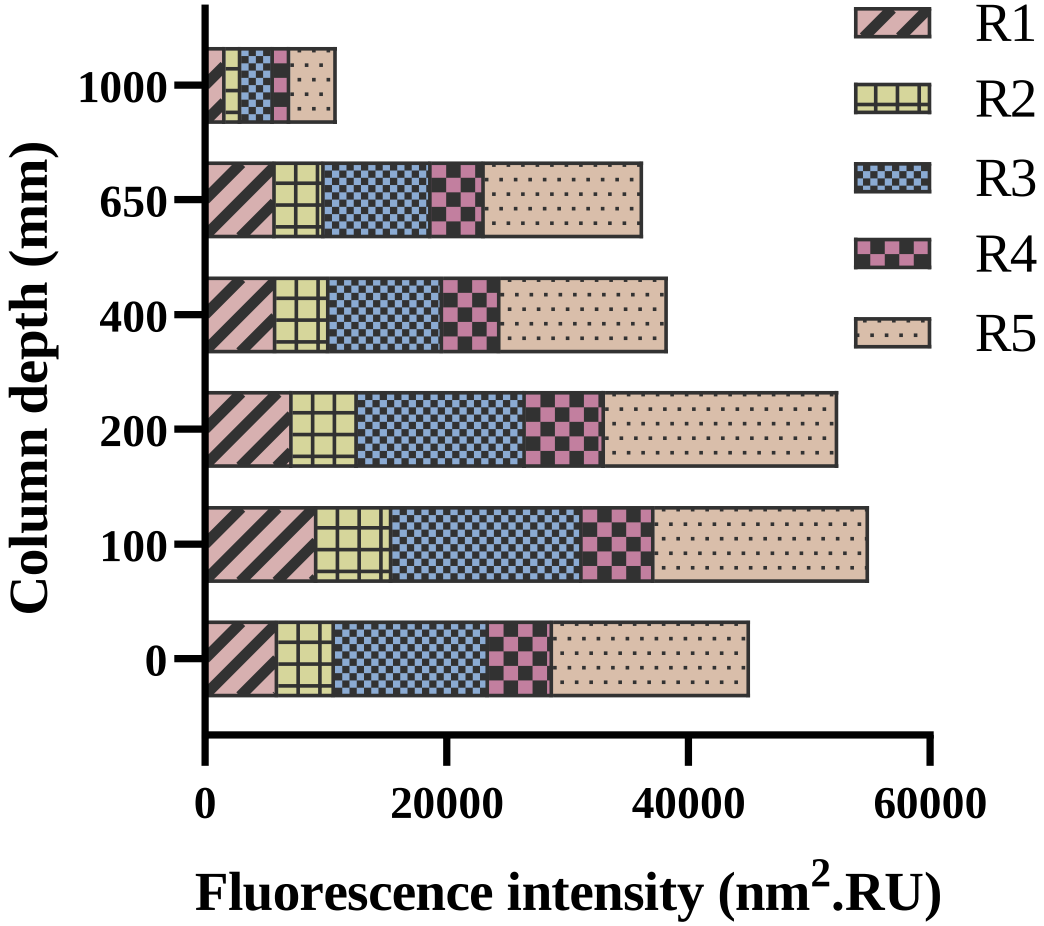

Figure 4.9 Comparison of EEM-defined region changes with respect to soil column depth

Table 4.5 Total and regional average fluorescence intensities (RU*nm^2) for defined EEM regions in soil column samples (SP = sampling port; 1 to 4 from the top to bottom order)

Region description	**Influent (nm^2.RU)**	**SP 1 (nm^2.RU)**	**SP2 (nm^2.RU)**	**SP3 (nm^2.RU)**	**SP4 (nm^2.RU)**	**Effluent (nm^2.RU)**	**Total Removal (%)**
Region I	3667.33	9309	7286	5937	5865	1712	53
Region II	2755.29	6204	5392	4372	4064	1289	53
Region III	7031.32	15742	13891	9434	8826	2747	61
Region IV	2865.61	5949	6550	4709	4403	1330	54
Region V	8709.49	17775	19305	13875	13136	3872	56
Total Fluorescence (TF)	25029.05	54979	52424	38327	36294	10949	56

4.3.2 PARAFAC modeling

Since it is crucial to find the appropriate number of components, at first, a series of PARAFAC models were built fitting 3 to 7 components (Figure S4.2 and S4.3) to be compared with each other. In another step, samples were normalized to make sure that there was no correlation between PARAFAC components. The samples that were identified as outliers were removed from the model during the exploratory phase using the leverage number. The leverage number of each sample varies between zero and one and shows the deviation of the sample from the average data distribution. Explained variance and core consistency values of the model were calculated when the number of components was changed from 3 to 7. Explained variance changed between 0.988 (3 components) to more than 0.997 (7 components, Figure S4.4). Core consistency diagnostic (CORCONDIA) is a tool to determine the proper number of components for multiway models that evaluates the degree of tri-linearity of PARAFAC loadings by comparison of least square Tucker3 (Bro and Kiers, 2003; Tucker, 1966). CORCONDIA evaluates the "appropriateness of the model" where it gradually decreases by increasing the number of components. At some point, by adding a new component, there will be an abrupt decline in the core consistency that determines the adequate number of components. However, the application of this metric for real-world data may not always be a reliable diagnostic tool and could lead to oversimplification of the FDOM composition in the samples (Murphy et al., 2013). There are some studies that, despite having low core consistencies, the models capture the real chemical composition (Bosco et al., 2006; Murphy et al., 2013, 2011, 2008) judged by the other assessment tools. Therefore, in this study, 4 components were selected to describe the FDOM in favor of protecting the model against under-fitting even though core consistency could be higher with a smaller number of components. Besides core consistency, other factors such as the model convergence to a unique solution, explained variance percentage, and comparison of the sum of square error (SSE) across the models were used to determine the number of components that are adequate for the dataset description.

The model was further validated using the split-half analysis, where the dataset will be divided in half in different ways, and the models are compared to check if they overlap using Tucker's congruence coefficients (TCC). The data is recombined in six different ways, and if the results from each sub-group are similar, it shows the stability of the model (Pucher et al., 2019). TCC values of 0.95 or greater are considered significant enough to be "equal", with values between 0.85 and 0.95 considered "fair" (Lorenzo-Seva and ten Berge, 2006). Here, TCCs for excitation and emission ranged from 0.87 to 0.98. Lastly, the residual dataset that is not captured by the model was checked to make sure that it only contains random errors, not systematic ones.

Based on the mentioned validation tools of the model, it was seen that the model with 4 components best fits the dataset (Figure 4.10). The four identified components and their characteristics are shown in Table 4.6. Component 1 (C1) was characterized by peaks at 344 nm excitation with 448 nm emission wavelengths, which was previously associated with high molecular weight and aromatic molecules of terrestrial origin. It is also similar to humic-like fluorescent peak C (Coble, 1996). Application of PARAFAC modeling enables us to compare our organic matter composition with global studies and identify the patterns using the OpenFluor database. The OpenFluor database has been set up for almost a decade and consists of plenty of models with sample entries ranging from wastewater to drinking water, as well as surface water to groundwater. Based on the OpenFluor database, C1 This component has been previously reported at eutrophic lakes during algal bloom season in China (Chen et al., 2018), the Congo river in Africa (Lambert et al., 2016), a wide range of lakes, rivers, and wetlands in Canada (Lapierre and del Giorgio, 2014), autochthonous DOM from the plankton community under different nutrient doses in a Norwegian bay (Stedmon and Markager, 2005). Vines and Terry (2020) reported C1 when they applied wastewater effluent to a bench biofiltration system (Vines and Terry, 2020). It has also been reported that a component with highly similar characterization (350/428) was removed effectively by chlorination, and it is associated with high nutrient and wastewater-impacted environments (Murphy et al., 2011). Further matches have been reported in Table 4.6. Component 2 (C2) peaks were at 271 nm excitation and 324 nm emission. The only similar signature was reported by Murphy et al. (2011) and is associated with protein-tyrosine-like compounds with a shift in excitation maxima compared to pure tyrosine (Murphy et al., 2011). Component 3 (C3), with peaks at 297 nm excitation and 382 nm emission, has the highest number of matches in the OpenFluor database (more than 45 matches that are shown in Table 4.6). C3 is associated with microbial humic-like and terrestrially-delivered and was reported at drinking water treatment plants (Shutova et al., 2014), at lakes (Osburn et al., 2017, 2011), rivers (Lambert et al., 2016; Yang et al., 2019). C3 present in the river water showed poor removal by biofiltration (Peleato et al., 2016). Finally, component 4 (C4) showed a primary excitation maximum at 269 nm, followed by a low-intensity secondary peak at 394 nm and an emission maximum at 524 nm. C4 was reported earlier in surface water samples collected from the Florida Keys and is considered to be of terrestrial origin (Yamashita et al., 2013). The overall components variations across the columns have been reported in Figure 4.11, demonstrating the removal of all 4 detected components (>92%), which is in agreement with Section 4.3.1 results.

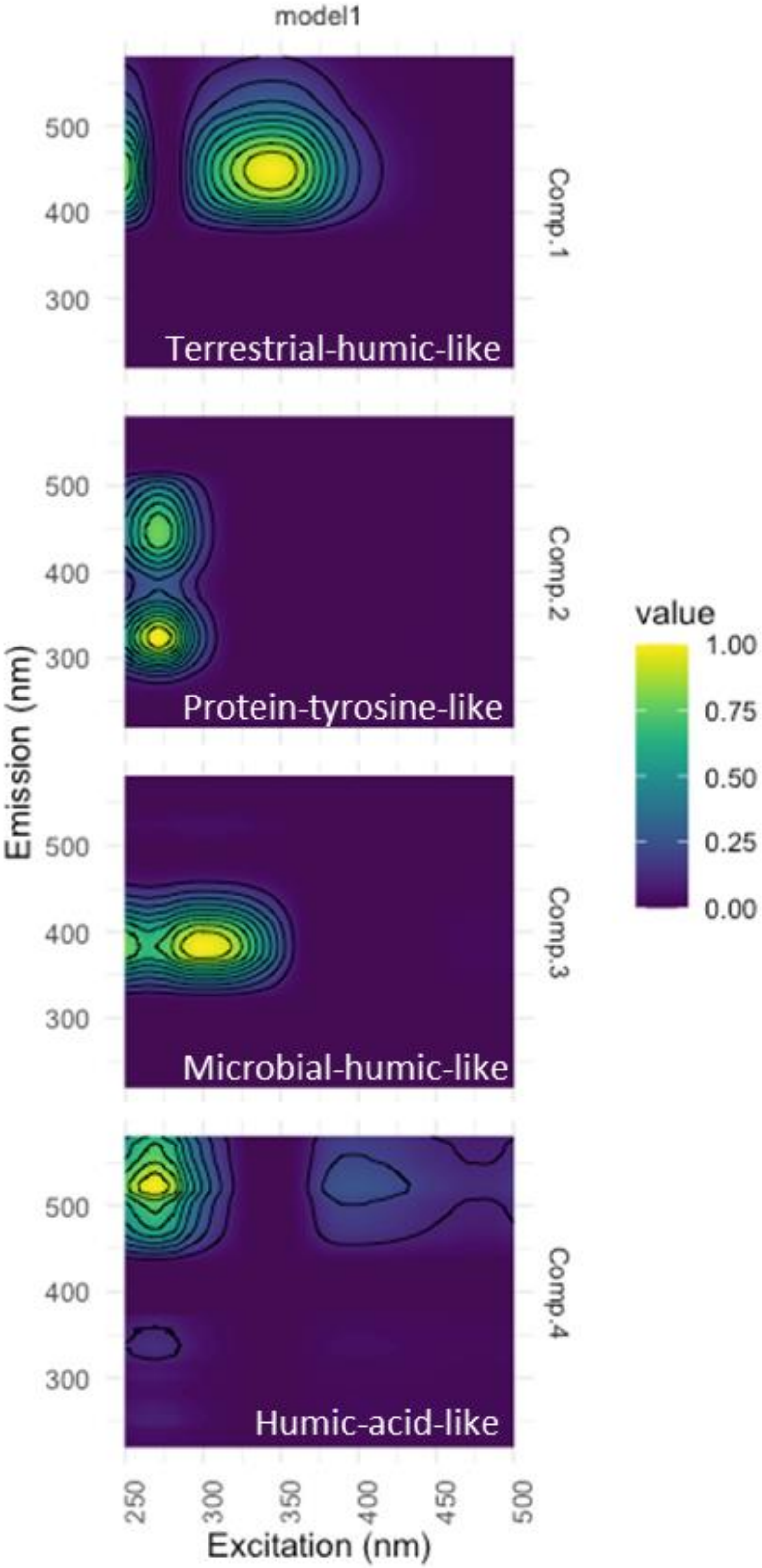

Figure 4.10 EEM spectra of four PARAFAC components identified in the model

Table 4.6 Description and wavelength positions of peak excitation and emission of PARAFAC components

Component	Wavelengths of the maxima (nm)		Descriptions	Previously reported matches from OpenFluor (Min similarity score of 0.95 for Ex and Em)
	Excitation	Emission		
C1	344 (<250)	448	Terrestrial-humic-like, peak C	Hong et al., 2021; Kothawala et al., 2012; Murphy et al., 2011; Wang et al., 2022
C2	271	324	Protein-tyrosine-like, peak B	Murphy et al., 2011
C3	297(<250)	382	Microbial-humic-like	Groeneveld et al., 2020; Osburn et al., 2017, 2011; Peleato et al., 2016; Wauthy et al., 2018; Wünsch et al., 2017; Yamashita et al., 2010; Yang et al., 2019; Zhuang et al., 2021
C4	269	524	Humic-acid-like, peak A and C	Yamashita et al., 2013, 2011

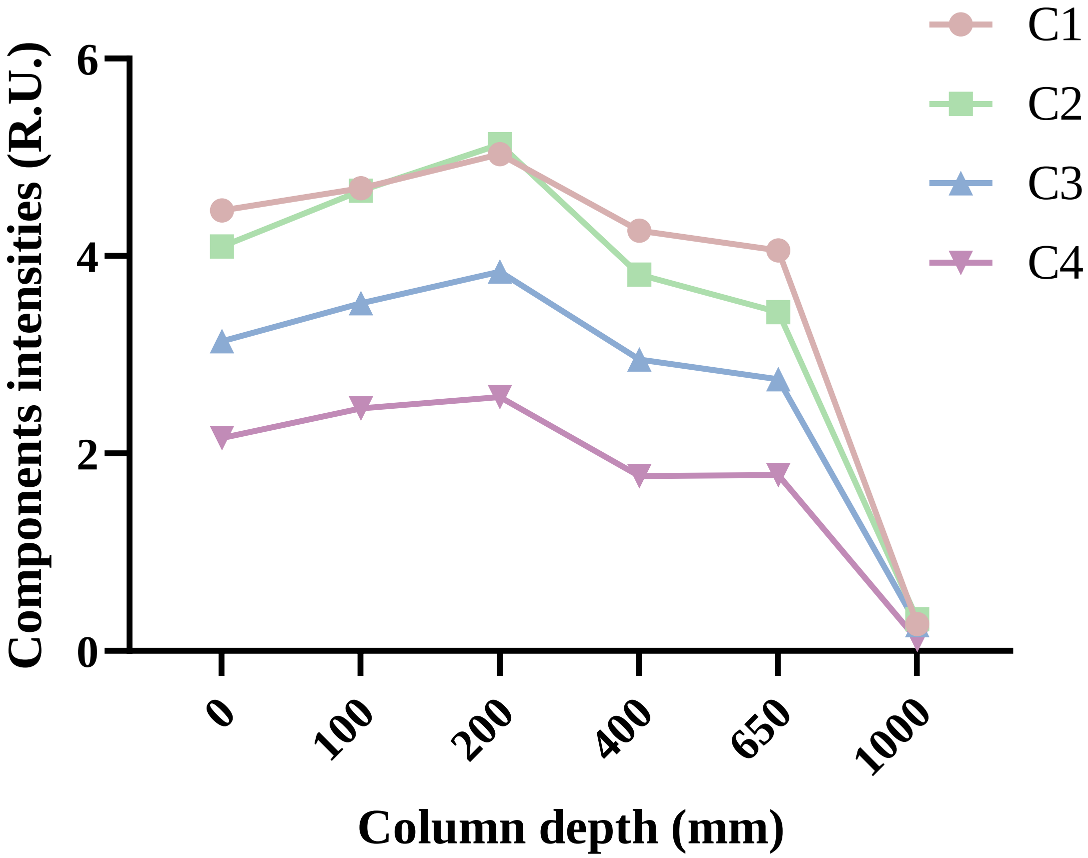

Figure 4. 11 Components changes along the column depth

4.3.3 Peak picking and indices

Based on the descriptions provided in Table 4.4, the intensities of humic-like peaks A, C, and M, and protein-like peaks B and T of influent samples decreased 93, 93, 92, 93, and 95% on average, respectively, upon passage through the columns (Table 4.7). Furthermore, it is shown that humic-like peak A has the biggest contribution to the influent samples. These results are in accordance with Section 4.3.1, when the FRI technique was applied to the data, it was shown that the removal occurred in all defined regions (R1-R5), with the R1 (humic-like) having the highest removal. These results are also in agreement with the findings of Abel et al. (2012) study, where the reduction of humic, fulvic, and protein-like peaks under aerobic conditions in lab-scale soil columns was reported. It is noteworthy to mention that DON concentration increased in the first sampling port of the columns and went down afterward (Chapter 3), and the same effect could be seen in all of the peaks' intensities. The values for HIX indices (all less than <4) indicate the autochthonous origin of the organic matter. The BIX is said to be higher than 1 for biological microbial origin in samples, and here, the values ranged from 0.95 to 1.01 across the columns.

Table 4.7 Peaks and indices of EEM samples

Column depths	**Indices**			**Peaks**				
	FI	**HIX**	**BIX**	**B (R.U.)**	**T (R.U.)**	**A (R.U.)**	**M (R.U.)**	**C (R.U.)**
0	1.67	0.66	0.95	3.98	4.68	5.48	4.33	3.95
100	1.55	0.66	0.98	4.69	5.45	6.15	4.67	4.05
200	1.44	0.65	0.96	5.16	5.70	6.65	5.14	4.11
400	1.61	0.64	1.00	3.69	4.36	4.77	4.20	3.71
650	1.60	0.67	1.01	3.25	3.75	4.54	3.95	3.61
1000	1.61	0.65	1.01	0.26	0.33	0.42	0.29	0.21

4.4 Conclusions

Overall, three EEM fluorescence data processing techniques have been applied in this study, and all of them report the same conclusions with different levels of detail. Peak picking and indices have the advantage of being fast and requiring minimum calculations. FRI technique can encompass more of the data information and be used as a monitoring tool for treatment processes capabilities. Peak picking technique showed that the columns were capable of removing humic-like and protein-like peaks in the recycled water where the protein-like peak (T) had the highest removal. PARAFAC detected terrestrial-humic (C1), protein-tyrosine (C2), microbial humic (C3), and humic acid-like (C4) components in the recycled water. The columns were capable of high removal of all four predominant components. Furthermore, PARAFAC application made it possible to find that the fluorescent components have spectral features similar to other components previously extracted from EEMs of organic matter in engineered or non-engineered systems. These results confirm the potential of fluorescence spectroscopy coupled with PARAFAC technique to monitor and track the organic matter from the source to the end-use in indirect potable reuse treatment units, including SATs.

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Supplementary materials and methods

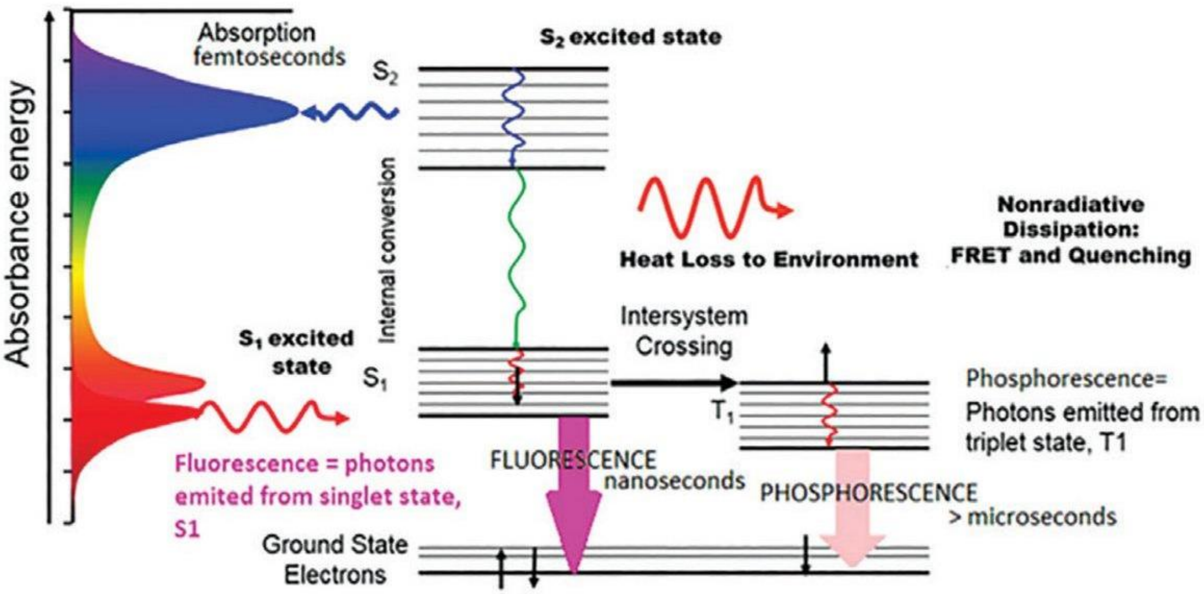

Figure S4.1 Jablonski diagram for Fluorescence and Phosphorescence (HORIBA Scientific)

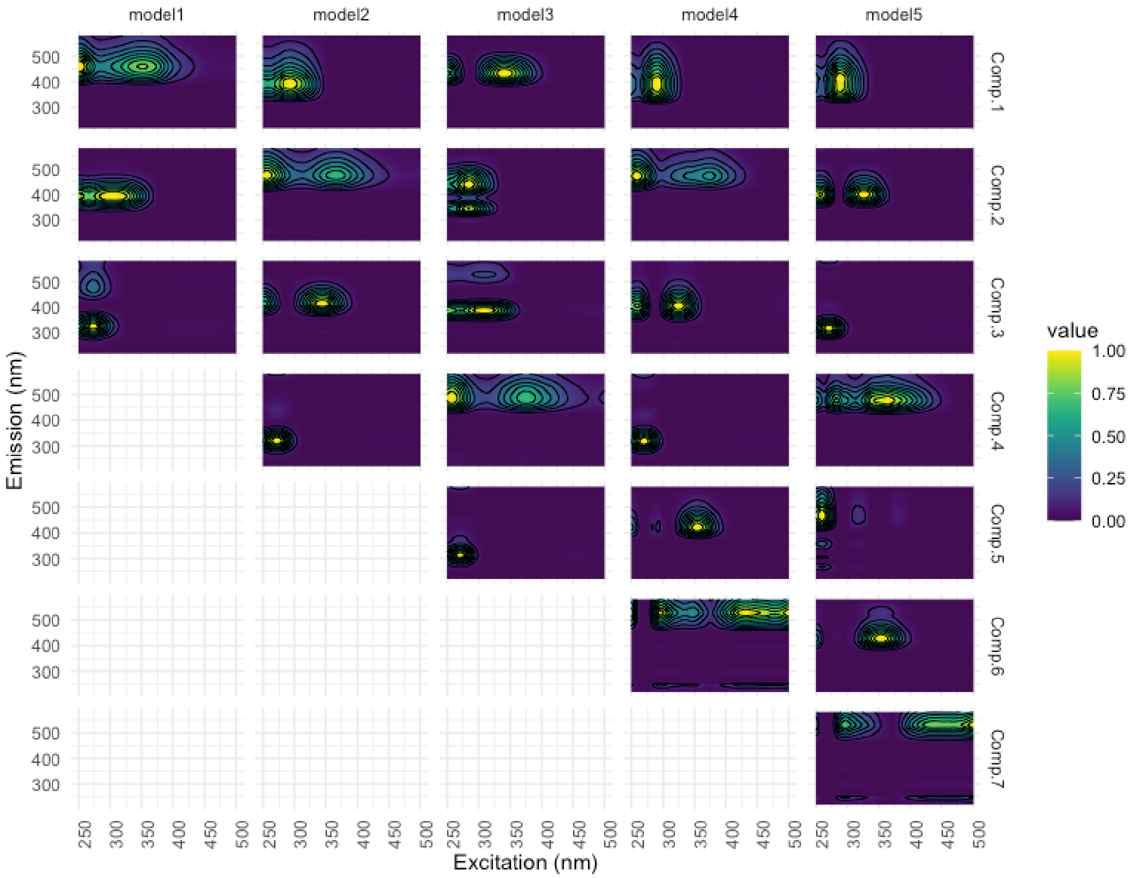

Figure S4.2 PARAFAC models with a number of components varying from 3 to 7

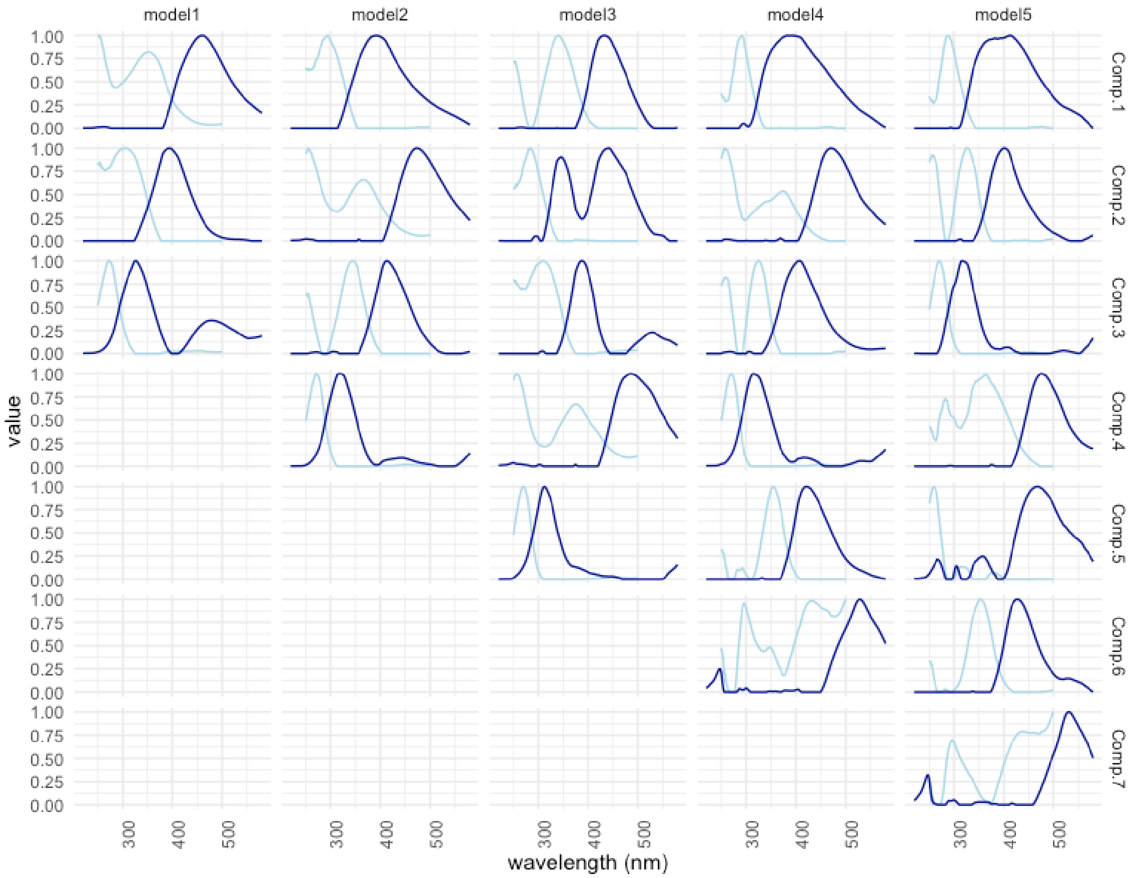

Figure S4.3 PARAFAC models: excitation and emission spectra of 3 to 7 components

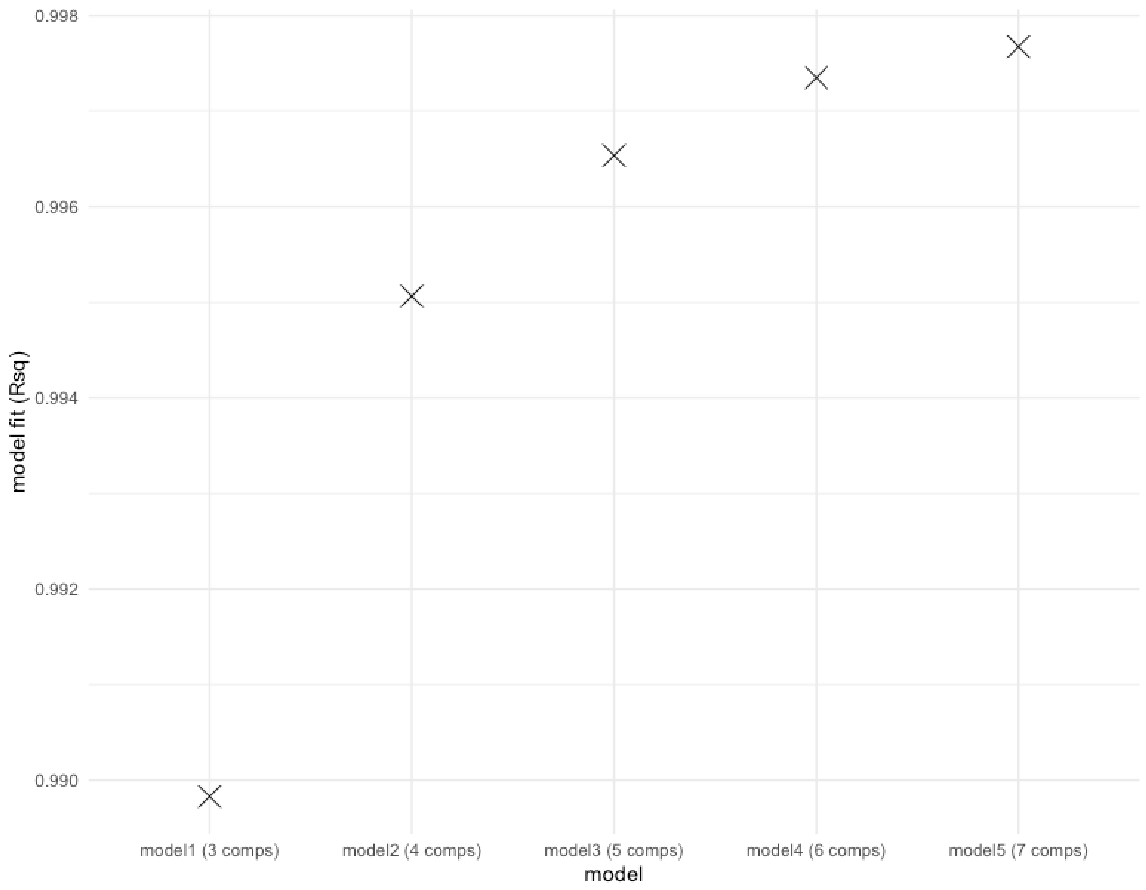

Figure S4.4 Model fits (R^2) for PARAFAC models with 3 to 7 components

Table S4.5 Fluorescence spectroscopy (RF 6000) settings during the study period

Item	Specifications
Lamp	150 W xenon arc lamp
Excitation wavelength range	200 – 500 nm
Emission wavelength range	220 – 600 nm
Excitation wavelength step	1 nm
Emission wavelength step	2 nm
Number of replicates for each sample	4 - 6

Chapter 5 Conclusions

The present research investigated the capability of the vadose zone of soil aquifer treatment systems in the removal of DON through lab-scale soil columns. This study further investigated the removal mechanisms of organic matter in the vadose zone. To our knowledge, this is the first study to use fluorescence spectroscopy EEM spectra to characterize organic matter through the soil columns, which could be a potential monitoring tool in SAT systems to follow the trends and changes in organic matter composition. Fluorescence spectroscopy presents the advantage of requiring a relatively small sample volume, which is particularly beneficial when the focus is on the unsaturated zone.

The main findings of this research include the following:

1) SAT systems are capable of DON removal in the vadose zone. The nitrate increase and presence of biomass in the columns pointed to the occurrence of ammonification and nitrification. Also, biodegradation had a major role in ammonification and nitrification reactions under aerobic conditions.
2) Vadose zone can be reliable for NDMA precursors' removal. However, there is a need for investigation of NDMA removal in low concentrations and the possibility of NDMA rebound in deeper soil profiles.
3) Fluorescence spectroscopy is sensitive enough to analyze samples with low organic matter concentrations, which is mostly the case in SAT systems and vadose zone.
4) Pick peaking could be a relatively fast method to follow the changing pattern of organic matter composition that requires minimum data processing and can have the

potential for online monitoring. However, FRI can give a more comprehensive picture of the organic matter removal by calculating the volume of defined regions and their changes. PARAFAC could be the best technique for data analysis if the focus is on identifying the previously reported compounds or detecting the new compounds. Even though it is time-consuming, it is crucial to follow all the data preprocessing steps to build a model capable of being compared with a global database that is growing. Also, there is a need to add more models to the OpenFluor database where the studies are focused on MAR systems, and the samples are collected from unsaturated zones or groundwater.

5) Fluorescence spectroscopy showed that the soil columns were capable of removing organic matter in all the defined regions, including humic-like, fulvic-like, and protein-like compounds. However, humic-like compounds had the highest removal. The compounds that PARAFAC detected have been previously reported in other aquatic environments, including rivers and lakes, as well as wastewater and drinking water treatment plants.

Overall, water scarcity has made us dependent on water reuse systems that include SAT techniques for not only increasing our groundwater resources but also treating the water further by adding environmental barriers. Field-scale SAT systems are highly site-dependent and require monitoring. Due to the concerns that imbalances of organic matter can cause in the environment, we suggest taking advantage of fluorescence spectroscopy as an optical technique to monitor better the variations in treatment efficiencies that are caused by seasonal variations, different drying and wetting cycles, and hydrogeological characteristics.