Universidad de Alcalá

Microbial Electrochemical Strategies for Recovering Nutrients after Treating and Re-using Urban Wastewater from a University Campus

Akram Gashtasebi 2024

Ph.D Thesis

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Ph.D in Hydrology and Water Resource Management

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TESIS DOCTORAL

Microbial Electrochemical Strategies for recovering nutrients after treating and re-using urban wastewater from a University Campus

Memoria presentada para optar al título de Doctor por la Universidad de Alcalá por:

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My dear family and colleagues

خانواده عزیزم و همکارانم

Wise is powerful as knowledge brings youth to old souls and bodies

Ferdowsi

You never know how strong you are, until being strong is your only choice.

BOB MARLEY

Water is the soul of the Earth

W. H. Auden

ACKNOWLEDGMENTS

Looking back on the past four years, my dissertation journey has been a rich blend of joyous milestones and significant challenges.

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Summary

Global water demand is rising due to urbanization, industrialization, and agricultural expansion, leading to increased wastewater production and environmental issues. Freshwater scarcity is a growing concern, with many regions facing water stress exacerbated by climate change. To address this, countries are implementing policies to reuse wastewater.

In this context, this doctoral thesis explores wastewater as a double resource, water itself and nutrients, through the use electroactive biofilters so-called METland®, for agricultural purposes. The METland® systems of the current thesis were made of a sustainable carbon-based matrial: electroconductive biochar as bed material. Although their efficiency in removing organic pollutants and nitrogen from urban and industrial wastewater was previously demonstrated, their performance for cleaning up wastewater from a university campus had not been thoroughly analyzed to re-use the water for irrigation of soil crops. Moreover, a strategy for treating and reusing treated wastewater from External Campus of Universidad de Alcalá, was established. Finally, this research evaluated a new circular economy concept, first to assess the capacity of the electroconductive bed (EC biochar) of METland® for adsorpting nutrients during wastewater treatment and, eventually, to re-use the material as soil fertilizer. Thus, the dissertation is organized into seven chapters, four of which are experimental base and summarized below.

Chapter 1 consisted an introductory section to review general aspects regarding nature-based solutions for wastewater treatment and water reuse. Moreover, it also covers the use of biochar as sustainable soil amendment. Finally, the chapter provides a comprehensive overview of METland® technology, discussing the state-of-the-art systems under various operational modes and electrochemical configurations

Chapter 2 details the materials and methodologies employed throughout the research, encompassing experimental setups, biofilters design and construction,

the cultivation of plants in soil and hydroponic systems, and the procedures for testing and analysis. Various biofilters and biochar types were utilized to assess their effectiveness in contaminant removal and plant growth support. Plants were grown in both soils amended with biochar and hydroponic systems to assess the impact on soil health, nutrient availability, water quality, and overall plant health. This detailed approach provided a robust framework for investigating the interactions between biochar, electroactive bacteria, and plant growth in different environments.

Chapter 3 presents an experimental study focused on the adsorption capabilities of biochar and the mechanisms through which nutrients are released into the soil. It assesses the efficiency of biochar in capturing nutrientes from wastewater and its subsequent benefits as a soil amendment. The transformative potential of biochar as a sustainable solution for converting nutrients from wastewater into valuable soil fertilizers was explored. Previous studies reported its capacity to improve plant growth, particularly when combined with organic matter that facilitates gradual nutrient release. Moreover, biochar plays a crucial role in waste management by efficiently absorbing and transforming pollutants in biofiltration systems, thereby contributing to water purification and pollution reduction. The chapter also explores innovations such as biochar-based slow-release fertilizers (SRFs). By elucidating biochar's mechanisms for nutrient storage and release, this chapter underscores its potential to advance sustainable agriculture and waste management, paving the way for future innovations in nutrient reclamation from wastewater.

Chapter 4 explores the impact of EC biochar as a soil amendment in the context of nutrient availability within wastewater treatment systems. Soil, recognized for its natural filtration capabilities, serves dual roles as an active participant in ecosystems and as a medium facilitating crucial interactions among soil, water, and crop systems. Nature-based solution for treating wastewater like green filters capitalize on soil's complexity, reactivity, fertility, and permeability to effectively filter water and facilitate biological, chemical, and physical processes. This chapter investigates the patterns of main cation and anion release from EC biochar. Soil-based biofilters were utilized to assess two types of biochar at diffrent doses, using biochar-free soil serving as the control.

Chapter 5 introduces the circular economy strategy for implementing METland® technology, focusing on two main aspects: reusing urban wastewater after treatment and using electroconductive EC-biochar from METland® beds as soil amendment. Sunflowers (Helianthus annuus L.), a significant global oilseed crop, were used to evaluate the impact of i) irrigating with treated water and ii) applying EC-biochar to soil. The study assesses the effectiveness of reusing water with different nitrate doses (15 and 35 ppm NO3-) versus tap water. Moreover, two types of electroconductive biochar: raw biochar and biochar previously used in METland® for wastewater treatment, were demonstrated to play a positive role regarding sustainable enhancement of soil fertility for sunflower cultivation.

Chapter 6 addresses the critical water scarcity challenge at the External Campus of the University of Alcalá (UAH) by evaluating the potential of METland® technology to treat wastewater and reuse the effluent for campus irrigation. The chapter begins by testing METfilter® biofilters' efficacy in treating real wastewater sourced from UAH's campus, focusing on the removal of COD and nitrogen contaminants using different bed materials. Additionally, this chapter evaluates the use of various materials, including humus, to enhance the efficiency of wastewater treatment.

The quality of the treated wastewater was subsequently assessed through comprehensive chemical analysis and by evaluating its suitability for hydroponic crop growth, including fluorescence emission analysis to discard any stress impact on photosynthesis. Moreover, the chapter proposes a design for implementing multiple METfilter® units aimed at reducing reliance on groundwater and promoting sustainable water use practices at UAH's External Campus

Finally, chapter 7 offers a comprehensive discussion, drawing conclusions and proposing future research directions based on the experimental findings. This section is structured in a question-and-answer format to enhance readability and facilitate understanding for the reader. The discussion synthesizes key results from the previous chapters, addressing the efficacy of biochar and METland® technology in wastewater treatment, nutrient recovery, and sustainable agriculture. Conclusions are drawn regarding the practical applications and potential benefits of these technologies. Additionally, future research directions are proposed, highlighting areas for further investigation to optimize and expand the use of biochar and METland® technology in various environmental and agricultural contexts.

Table of content

CHAPTER 1:
Introduction

Introduction

Introduction

1.1. Addressing the urgency of water reuse in the face of the water crisis

The escalating global demand for water stems from the burgeoning urban populations, rapid industrialization, and expansive agricultural activities. As this demand surges, the volume of wastewater generated also rises, posing significant environmental risks when not managed effectively. The strain on existing water resources intensifies as a result of these widespread trends, making access to fresh water increasingly constrained. Consequently, many countries worldwide are now adopting policies to recycle domestic wastewater as a strategic response to the mounting water demand (Pandey, Srivastava, and Singh 2014; Melo et al. 2020). The global issue of freshwater scarcity continues to escalate, driven by a profound imbalance between water supply and demand. Regions such as North Africa, the Middle East, Southern Europe, Australia, and the southern United States are particularly afflicted by this challenge. In many instances, the scarcity of freshwater can be attributed to regional climate-related factors exacerbating the situation (Norton-Brandao, Scherrenberg, and van Lier 2013). Water consumption is increasing as the world's urban population grows. Concurrently, climate change is increasing extreme weather occurrences in cities, such as droughts and floods, with serious social and economic effects. New needs for water security solutions are rising in a global context where about four billion people - 60 percent of the world's population - live in areas with near-permanent water stress. One in every four large cities is currently experiencing water stress, and demand is expected to rise by 55% by 2050. Pollution further exacerbates water stress; in underdeveloped nations, 80-90% of all wastewater is released directly into surface water bodies, posing serious health dangers (Corcoran et al. 2010). Although agricultural irrigation accounts for around 70% of total water usage, the rising use of energy obtained from biological sources increases the problem of water shortage (Tsoutsos et al. 2013).

1.1.1. Global freshwater withdrawals: understanding water usage on a global scale

According to FAO's AQUASTAT database, global freshwater withdrawals amount to 3,928 km³ annually. Approximately 44% of this water, equivalent to 1,716 km³ per year, is consumed, primarily in agriculture where it evaporates in irrigated cropland. The remaining 56%, accounting for 2,212 km³ per year, is discharged into the environment as wastewater in the form of municipal and industrial effluent and agricultural drainage water (UN 2017).

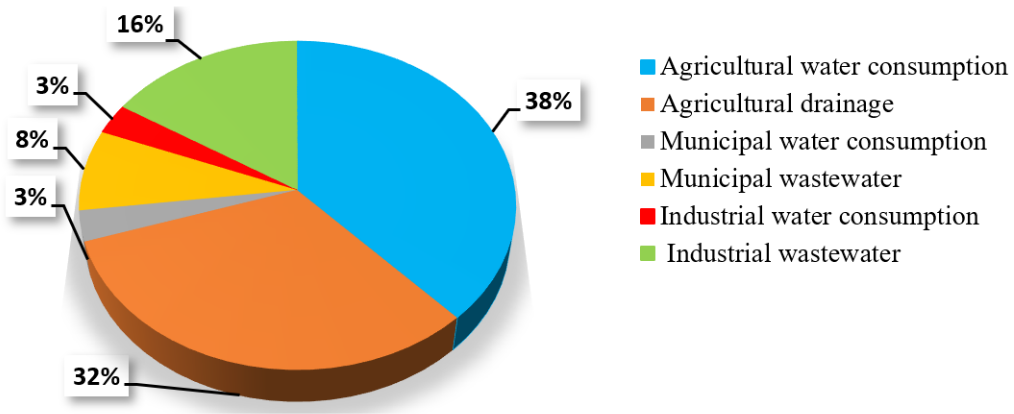


Figure 1. 1. The fate of worldwide freshwater withdrawals: consumption and wastewater generation by the largest water use industry (about 2010) (based on data from AQUASTAT)

Estimation indicate that worldwide water demand will rise significantly in the next decades. Although the agricultural sector now dominates, accounting for 70% of global water abstraction, there are expectations of significant increases in demand, particularly in industries and energy production (WWAP 2015; UN 2017).

Food processing in Europe utilizes approximately 5 m³ of water per person daily (UN 2017). Annually, an estimated 1.3 billion tonnes of food go to waste with a global loss of 250 km³ of water per year (FAO 2013a).

Introduction

The extent of wastewater treatment differs according to the income levels of countries, categorized into high-income, upper middle-income, and low middle-income countries. The rates of treating wastewater for these categories are 70%, 38%, and 28%, respectively. However, only 8% of industrial and municipal wastewater undergoes any form of treatment in lower middle-income countries. (Sato et al. 2013) .

This exacerbates the situation for the impoverished, particularly in slum areas, where individuals often encounter untreated wastewater owing to inadequate water and sanitation amenities. The provided statistics reinforce that over 80% of wastewater is discharged into the environment without adequate treatment (WWAP 2012; UN 2015a, 2017).

- AQUASTAT classifies water withdrawal into three specific categories:
- Agricultural water withdrawal: Encompasses irrigation, livestock, and aquaculture.
- Municipal water withdrawal: Involves domestic usage.
- Industrial water withdrawal: Relates to industrial water use.

Additionally, a fourth category of anthropogenic water use involves the evaporation of water from artificial lakes or reservoirs associated with dams.

On a global scale, the withdrawal ratios are distributed as follows: 70 percent for agriculture, 11 percent for municipal use, and 19 percent for industrial purposes. It's important to note that these percentages are significantly influenced by a small number of countries with exceptionally high-water withdrawals. When averaging the ratios for each country, the proportions become 59 percent for agriculture, 23 percent for municipal use, and 18 percent for industrial purposes (FAO 2016).

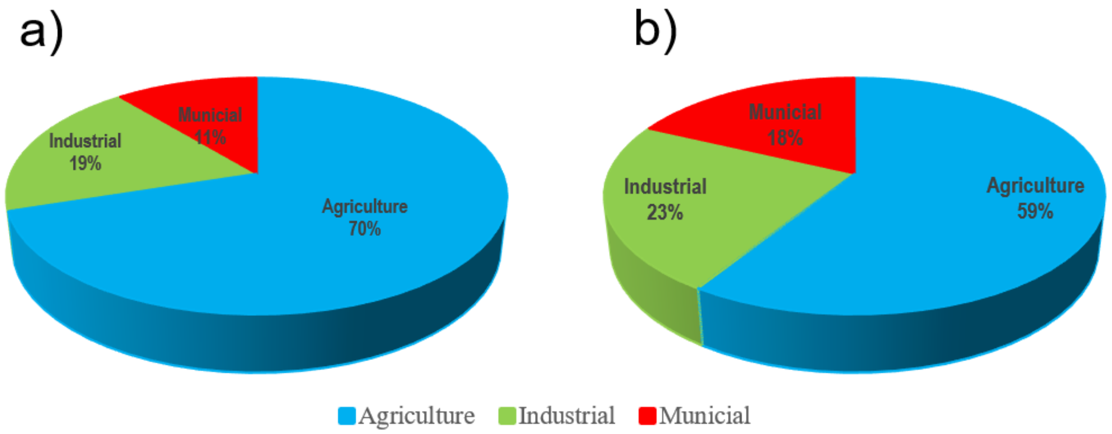


Figure 1. 2. (a) Global sum of all water withdrawals (b)average of country ratios (based on data from AQUASTAT FAO 2016)

Water withdrawal ratios display notable diversity across regions. In South Asia, for instance, agricultural water withdrawal accounts for 91%, while municipal and industrial withdrawal represent 7% and 2%, respectively. Conversely, in Western Europe, the proportions shift dramatically, with agricultural withdrawal decreasing to 5%, while municipal and industrial withdrawal rise to 23% and 73%, respectively. The importance of water withdrawal in agriculture is heavily determined depending on climate conditions and agriculture's economic function. The graphic below shows water loss proportions by region, with the agricultural component ranging from more than 80% in Africa and Asia to just over 20% in Europe (FAO 2016).

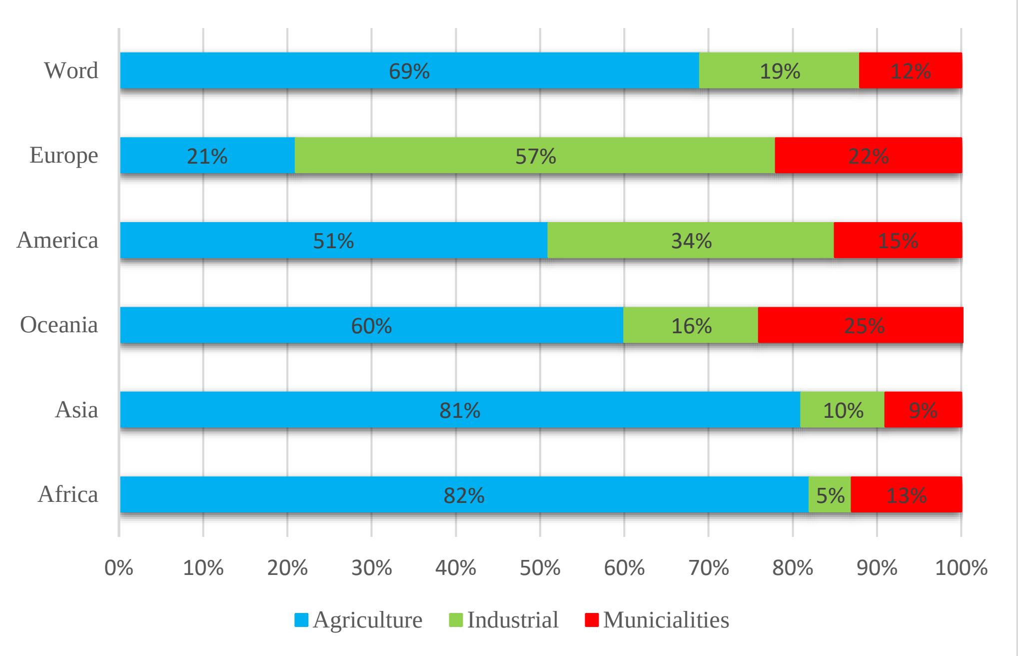


Figure 1. 3. Water withdrawal ratios by continent (based on data from AQUASTAT)

1.1.2. The impact of water scarcity on groundwater, a vital resource

Groundwater is one of the water resources that approximately 50% of the world's population relies on groundwater for drinking water, and it constitutes 43% of the water used in irrigation. However, groundwater resources are depleting, with approximately 20% of global aquifers facing overexploitation (EC 2019b). Water availability, environmental health, and human well-being are all significantly impacted by groundwater. Around the world, 2.5 billion people solely rely on groundwater resources to meet their basic daily water needs, and hundreds of millions of farmers depend on groundwater to support their livelihoods and help ensure the food security of many others (UNESCO 2012; EC 2019b). In areas facing water scarcity, there has been a significant rise in the extraction of well water or groundwater overdraft. Groundwater overdraft has gained significant attention due to its adverse environmental and economic repercussions, as well as its favorable socioeconomic outcomes. The detrimental effects of excessive groundwater withdrawal encompass economically unsustainable pumping conditions, deterioration in water quality due to the infiltration of brackish or low-quality groundwater, diminished flow in streams, wetlands, and springs, land sinking, disruption of pre-existing water use and rights, and gradual depletion of groundwater reservoirs. This issue is a global occurrence, leading to ecological challenges like water contamination, land subsidence, intrusion of seawater, reduction in streamflow, and degradation of ecological conditions (Zhao et al. 2020).

In water-scarce regions, effective management of water resources is of heightened importance for sustainable development. Ensuring the judicious development of water resources necessitates establishing a coherent relationship between available resources such as surface water and groundwater, considering factors like spatiotemporal demands, geo-climatic characteristics, and cultural values (Esmaeili, Habibi, and Esmaeili 2022).

There is an additional issue that relates to water quality in addition to the scarcity of water. Aquifers are frequently contaminated by nitrate pollution from excessive fertilizer use in agriculture, which affects the nitrogen cycle and lowers groundwater quality.

Drinking water safety is a global concern raised by this topic. Nitrate pollution can originate from sources other than agriculture, such as precipitation, animal dung, septic tanks, and runoff (Muñoz-Palazon et al. 2023). The European Union has identified Belgium, Denmark, Spain, and Cyprus as countries with notable nitrate pollution in groundwater. Additionally, some European nations, including Germany and Spain, exhibit high levels of pesticides in groundwater, surpassing 0.1 μg·L-1. Notably, these countries, along with France and Italy, collectively represent a significant portion of the total EU pesticide sales, accounting for about two-thirds between 2011 and 2020 (Eurostat 2020). It is noteworthy that studies have looked into the relationship between variations in

Introduction

groundwater levels attributed to precipitation and the occurrence of nitrate contamination. Nitrate concentrations have been found to rise when groundwater levels drop (Kawagoshi et al. 2016).

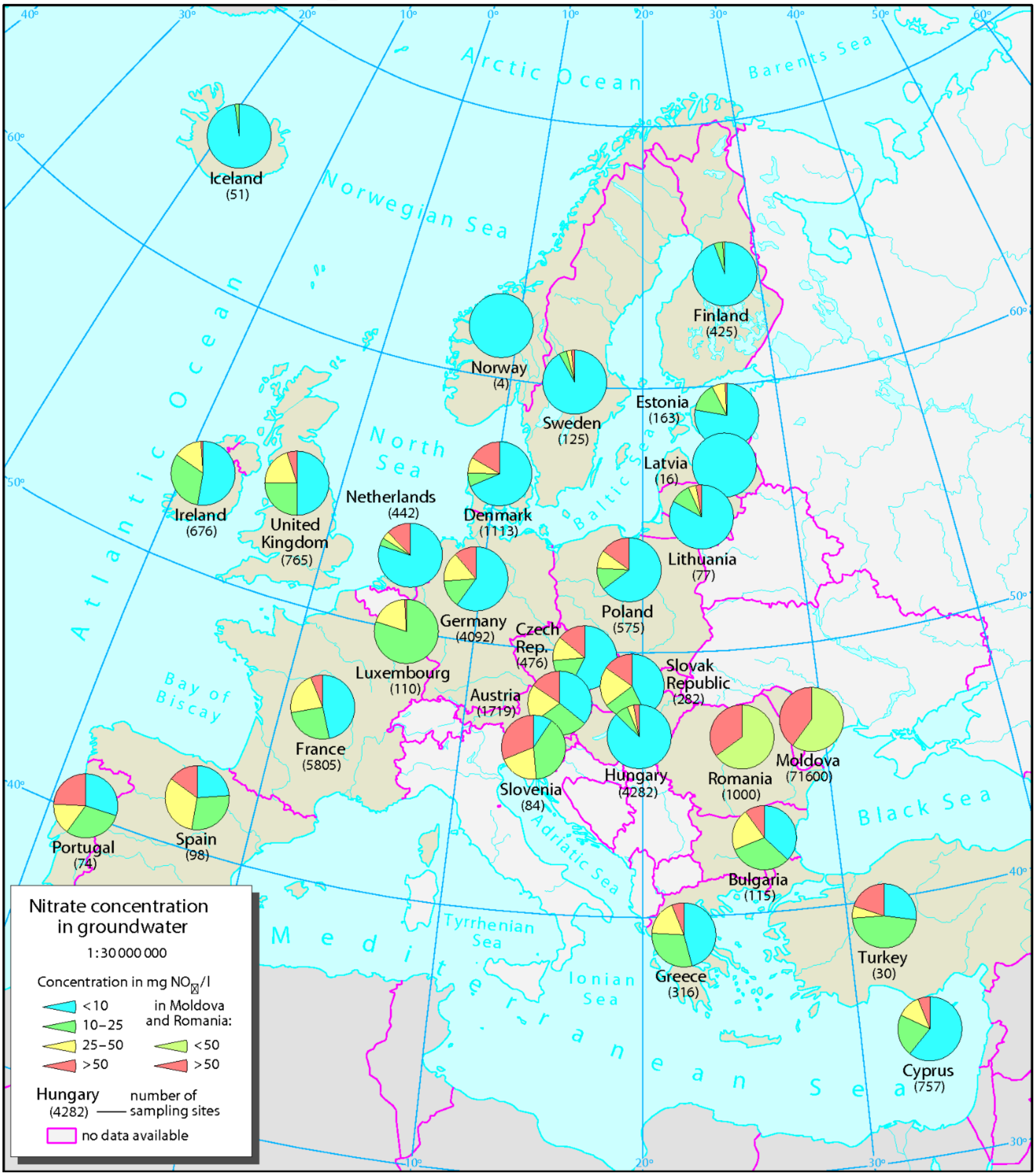


Figure 1. 4. Frequency distribution of nitrate concentration (NO3 mg/l) in groundwater at the country level (European Environment Agency, 2009, Modified 2012)

In Figure 1.4, the map presents nitrate concentrations in groundwater across European countries as provided by the European Environment Agency. The figure illustrates the number of sampling sites below the name of each country, with four classifications representing the nitrate concentrations in groundwater (NO_3 mg/l)

1.2. The framework for managing wastewater

Wastewater treatment (WWT) is a broad concept that applies to any location generating wastewater. The appropriate deployment process needs to be customized based on local parameters, with population size being a crucial factor. Population size can be determined by considering the number of inhabitants and/or the organic load. The common criterion used to categorize populations into small, medium, and large is the value of population equivalents (p.e.).

Population equivalents (p.e.) in WWT quantify the total pollution load generated in a 24-hour period by various sources such as households, industrial facilities, and services. It is expressed using the individual pollution load in household sewage produced by one person during the same timeframe as the unit of measure. Specifically, one p.e. corresponds to the organic biodegradable load with a five-day biochemical oxygen demand (BOD_5) of 60 grams of oxygen per day, as defined by Directive 91/271/EEC dated May 21, 1991. This directive establishes a standard for assessing and categorizing wastewater pollution based on the organic load's oxygen demand over a five-day period.

The Council Directive 91/271/EEC covering urban WWT, which was issued on May 21, 1991, is the fundamental rule governing WWT in Spain, as it is in the rest of the European Union. Its goal is to safeguard the environment from the negative impacts of urban wastewater discharges and discharges from specific industrial sectors (refer to Annex III of the Directive). To that end, the Directive establishes some minimum standards for wastewater collection and treatment based on the size of the urban agglomeration and the characteristics of the receiving waters. The Directive specifically specifies that:

- Collect and treat wastewater in agglomerations with more than 2,000 people p.e.

- Secondary treatment of all discharges from agglomerations of > 2,000 p.e., and more advanced treatment for agglomerations >10,000 population equivalents in designated sensitive areas and their catchments;
- In the case of agglomerations of less than 2,000 p.e., the urban wastewater entering collecting systems shall before discharge be subject to appropriate treatment.

In this context, "appropriate treatment" pertains to a wastewater treatment procedure designed to guarantee that, upon release, the receiving bodies of water adhere to the designated quality objectives and stipulations outlined in the Community Directives, particularly Article 2.9 of Directive 91/271/EEC dated May 21, 1991.

Regarding discharges from urban wastewater treatment plants (WWTP), the Directive mandates compliance with the specific criteria outlined in Annex I.b. This involves implementing secondary treatment in regular regions, as indicated in Table 1-1, and employing a more sophisticated treatment approach in identified sensitive areas and their associated catchments, as detailed in Table 1.2. In Spain, these sensitive areas encompass all intercommunity hydrographical basins.

It is noted that adopting primary treatment as the single and comprehensive treatment method is discouraged. García et al. (2001) proposed a secondary therapy for all communities. The Directive has been implemented in Spanish legislation by the Real Decreto 11/1995, which specifies the rules for urban wastewater treatment, and the RD 509/1996, which expands on it. The most recent changes to these regulations took place in February 2019.

According to the European Water Framework Directive (WFD) (Directive 2000/60/CEE), waters must be in good ecological and chemical condition to ensure human health, water supply, natural ecosystems, and biodiversity. The idea of excellent ecological state involves the maintenance of physicochemical and hydro morphological conditions that allow surface water's biological communities to coexist.

Table 1. 1. Requirements for discharges from urban WWTP of agglomerations of > 2,000 p.e. The values for concentration or the percentage of reduction shall apply

Parameter	**Concentration**	**Minimum reduction required (%)** (1)
Biochemical oxygen demand (BOD_5 at 20 °C) without nitrification (2)	25 mg O_2/L	70-90 40 under Article 4 (2)
Chemical oxygen demand (COD)	125 mg O_2/L	75
Total suspended solids	35 mg/L 35 under Article 4 (2) (more than 10,000 p.e.) 60 under Article 4 (2) (2,000-10,000 p.e.)	90 90 under Article 4 (2) (more than 10,000 p.e.) 70 under Article 4 (2) (2,000-10,000 p.e.)

(1) Reduction to the load of the influent.

(2) The parameter can be replaced by another parameter: total organic carbon (TOC) or total oxygen demand (TOD) if a relationship can be established between BOD_5 and the substitute parameter.

Table 1. 2. Requirements for discharges from urban WWTP to identified sensitive areas subject to eutrophication. The values for concentration or the percentage of reduction shall apply

Parameter	**Concentration**	**Minimum reduction required (%)** (1)
Total phosphorous	2 mg/l P (10,000 – 100,000 p.e.) 1 mg/l P (more than 100,000 p.e.)	80
Total nitrogen (2)	15 mg/l N (10,000 – 100,000 p.e.) 10 mg/l N (more than 100,000 p.e.)	70-80

(1) Reduction in relation to the load of the influent.

(2) Total nitrogen means: the sum of total Kjeldahl-nitrogen (organic N + NH3), nitrate (NO3)-nitrogen and nitrite (NO2)-nitrogen.

Hence, wastewater treatment should be sufficient to achieve the objectives set by the Water Framework Directive (WFD), along with meeting the quality standards outlined in various connected European Directives (such as the Bathing Water Directive, Drinking Water Directive, Shellfish Water Directive, and Fish Directive). Considerations also extend to additional factors like the reuse of treated wastewater and anticipating changes in landscapes due to climate change. To effectively implement this, it is crucial to thoroughly characterize the water body's quality in both current conditions and the intended objectives before choosing the wastewater treatment (WWT) method. In essence, the selected technology must enable compliance with the defined objectives.

Promoting environmental preservation for future generations necessitates a shift toward sustainability. The water cycle is important in this setting, with wastewater emerging as a potentially significant source of water, nutrients, organic matter, energy, and other things. The water sector holds significant potential to transition into a more resource-efficient and circular system (EEA 2022b).

Beyond water, energy, nutrients, and organic materials all demonstrate potential for reuse, recycling, and recovery. Existing EU water legislation has primarily focused on enhancing the water cycle, improving water quality, and striving to restore biodiversity. However, it has a limited impact on reducing water usage, whether in environmental abstraction or the efficiency of water networks and products (EEA 2022b).

The legislation governs what can be discharged into water and onto land, although the list of controlled substances is relatively small compared to the wide array currently used and produced, and it does not encompass greenhouse gases. Despite improvements brought about by the 1991 Urban Waste Water Treatment Directive (UWWTD) in Europe's water quality, urban wastewater treatment plants (UWWTPs) remain the primary point source of pollutants in European waters (EEA 2020; EC 2019b; EEA 2022b).

Sewage treatment plays a crucial role as a vital service, providing purified water, valuable nutrients, and organic fertilizers. It has the potential and should actively contribute to realizing the overarching objectives of the Green Deal, playing a significant role in advancing the goal of achieving zero pollution.

Numerous water flows are associated with water management, control, and reuse in this context (EEA 2022a).

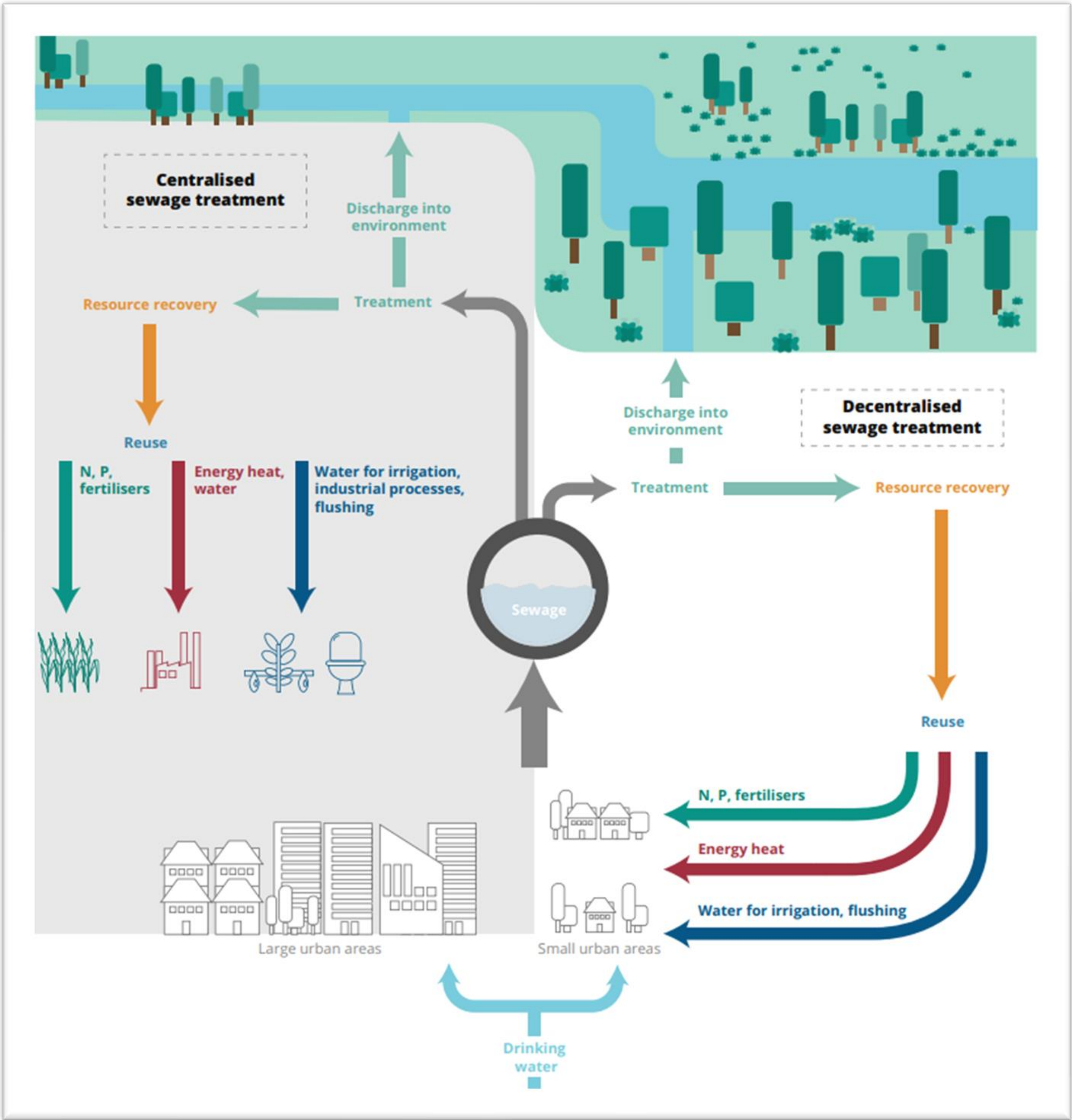


Figure 1. 5. Centralized and decentralized sewage management schemes (EEA 2022)

Introduction

Wastewater can be characterized in various ways, and one widely accepted definition is as follows: "Wastewater is considered a composite of one or more of the following components: domestic effluent comprising black water (consisting of excreta, urine, and fecal sludge) and greywater (utilized water from washing and bathing); effluent from commercial establishments and institutions, including hospitals; industrial wastewater; stormwater and other runoff from urban areas; and runoff from agricultural, horticultural, and aquaculture activities" (Raschid-Sally and Jayakody 2009).

1.2.1. Wastewater sources in urban and municipal systems

- Wastewater is produced by various origins, resulting in variations in its components and concentrations. According to the categorization and explanations outlined in the regulatory document (Council Directive 91/271/EEC, 1991), sources of wastewater can be classified as follows:
- Domestic Wastewater
- This type of wastewater originates in residential settings and is primarily generated by human activities and metabolism within the home environment. Additionally, it can be further divided into:
- Black Waters: These consist of urine and toilet wastewater.
- Gray Waters: Comprising soapy water potentially containing fats, originating from sources like sinks, showers, bathtubs, dishwashers, washing machines, and laundry facilities.

- Urban Wastewater: This type of wastewater encompasses either domestic wastewater or a combination of domestic wastewater with industrial wastewater and/or runoff rainwater. The components within urban wastewater can be further classified into:

❖ Domestic Wastewater: Consisting of typical components mentioned earlier, such as human metabolism by-products, nutrients, organic matter, and emerging pollutants. Contaminants such as heavy metals and other pollutants.

❖ Urban Runoff: This type of wastewater involves a broad spectrum of contaminants, including motor oil, microplastics, chemical fertilizers, pesticides, heavy metals, rubber, and various types of debris and waste.

1.2.2. Industrial wastewater:

Industrial wastewater is defined as any wastewater discharged from premises used for trade or industry, excluding domestic wastewater and runoff rainwater. The characteristics of industrial wastewater vary significantly depending on the products and processes employed by the respective industries. Here are some examples of industrial wastewater from different sectors:

- Mining Activities: The composition varies based on the particular mining activity, with typical compounds including suspended particles, dissolved salts, and the occurrence of heavy metals.

- Energy Generation: This category may contain nitrogen, thermal pollution, solids that dissolve, heavy metals, fossil fuels, and other related pollutants.

- Food Industry: In the food sector, wastewater generated from industrial processes often exhibits elevated concentrations of organic substances, emerging pollutants, suspended particles, acidic compounds, and oil, alongside other constituents

- Textiles Industry: Wastewater from textile industries may contain heat, hazardous substances, metals, increased acidity, solvents, salt, sulfide, suspended particles, emerging pollutants, and various other compounds.

- Agricultural Runoff: the runoff, originating from farmland, involves the flow of water and carries a varied range of substances resulting from agricultural activities. This runoff has the potential to include pesticides, insecticides, fertilizers, and nitrogen.

- Livestock Production: wastewater stemming from livestock production is characterized by very high organic loadings and elevated concentrations of nutrients and organic pollutants. Furthermore, it may contain veterinary residues, including pharmaceutical products.

1.3. Utilization of reclaimed water for irrigation

1.3.1. Minimum requirements for reclaimed water in agriculture

The European Commission's Communication "Closing the Loop – An EU Action Plan for the Circular Economy" (COM (2015) 614) paved the way for enhanced water reuse measures, including legislation defining minimum standards for water reuse in irrigation and groundwater recharge. This initiative materialized in Regulation (EU) 2020/741, approved by the European Parliament and the Council on May 25, 2020. The regulation outlines minimum requirements for reclaimed water in agricultural irrigation, specifying quality classes, permitted uses, and associated irrigation methods (Table 1.3). Emphasizing routine monitoring for parameters such as E. coli, BOD_5, TSS, and turbidity, the regulation underscores the importance of validation monitoring for reclaimed water used in agricultural irrigation (Table 1.4)(EC 2020).

Table 1. 3. Classes of reclaimed water quality and permitted agricultural use and irrigation method

Class	**Water Quality Criteria**	**Permitted Agricultural Use and Irrigation Method**
Class A	High-quality reclaimed water	All crops and irrigation methods
Class B	Good-quality reclaimed water	Most crops and irrigation methods
Class C	Medium-quality reclaimed water	Non-edible crops and restricted irrigation methods
Class D	Low-quality reclaimed water	Non-food crops and restricted irrigation methods

Table 1. 4. Reclaimed water quality requirements for agricultural irrigation

Reclaimed water quality class	**Indicative technology target**	**Quality requirements**				
		E. coli (nymber/100ml)	**BOD_5 (mg/l)**	**TSS (mg/l)**	**Turbidity (mg/l)**	**Other**
A	Secondary treatment, filtration, and disinfection	≤ 10	≤ 10	≤ 10	≤ 5	Legionella spp. : < 1000 cfu / l where there is a risk of aerosolisation Intestinal nematodes (helminth eggs): ≤ 1 egg / l for irrigation of pastures or forage
B	Secondary treatment, and disinfection	≤ 100	In accordance with Directive 91/271/EEC (Annex I, Table 1)		-	
C	Secondary treatment, and disinfection	≤ 1000			-	
D	Secondary treatment, and disinfection	≤ 10000			-	

1.3.2. Quality of water for irrigation

Soluble salts in water significantly impact water quality for various purposes, including drinking, livestock, and crop irrigation. Water quality is crucial for sustainable irrigated agriculture, particularly in addressing salinity issues. Evaluation of water quality for irrigation is guided by four criteria (Zaman, Shahid, and Heng 2018)

- Total soluble salts content, indicating salinity hazard
- Sodium adsorption ratio (SAR), assessing sodium proportion to calcium and magnesium, indicating sodium hazard
- Residual sodium carbonates (RSC), considering bicarbonate and carbonate anions about calcium and magnesium ions
- Monitoring elements to prevent ionic imbalances or plant toxicity

To assess the first three criteria, characteristics such as electrical conductivity (EC), soluble anions and cations must be determined in irrigation waters. Additionally, boron levels must be measured. The pH of irrigation water is not a reliable criterion for water quality since it tends to be buffered by the soil, and most crops can tolerate a wide pH range. Detailed descriptions of commonly employed techniques for analyzing irrigation water are available in the literature (USSL-Staff 1954; Bresler, McNeal, and Carter 1982).

1.3.2.1. Salinity hazard

Excessive salt in the soil elevates osmotic pressure, leading to physiological drought conditions. Despite apparent soil moisture, high osmotic potential prevents plant roots from absorbing water, causing wilting. Total soluble salts (TSS) in irrigation water are measured via electrical conductivity (EC) in micro-Siemens per centimeter (µS cm^-1) or salt content in parts per million (ppm). Table 1.5 provides guidelines for water use based on salt content.

Table 1. 5. Salinity hazard of irrigation water (Follett and Soltanpour 2002; Bauder et al. 2011)

Hazard	Dissolved salt content	
	ppm	EC (µS cm^{-1})
None-Water for which no detrimental effects will usually be noticed.	500	750
Some-Water that may have detrimental effects on sensitive crops.	500-100	750-1500
Moderate - Water that may have adverse effects on many crops thus requiring careful management practices.	1000-2000	1500-3000
Severe -Water that can be used for salt tolerant plants on permeable soils with careful management practices.	2000-5000	3000-7500

1.3.2.3. Sodium hazard

High sodium concentrations in irrigation water, measured by the Sodium Adsorption Ratio (SAR), can harm soil structure, causing compaction and reduced water penetration. It's recommended to avoid water with SAR exceeding 10 (moles/L) ^0.5 for prolonged exclusive irrigation, even with low total salt content. Salinity and SAR should both be considered in assessing the impact of water quality on soil water penetration. The USSL (United States Salinity Laboratory) diagram is a valuable tool in agricultural water management for evaluating irrigation water quality.

The USSL diagram is structured as a grid, with EC values plotted on the x-axis and SAR values on the y-axis. The different regions within the diagram represent distinct water quality classes, each holding significance for agricultural practices.

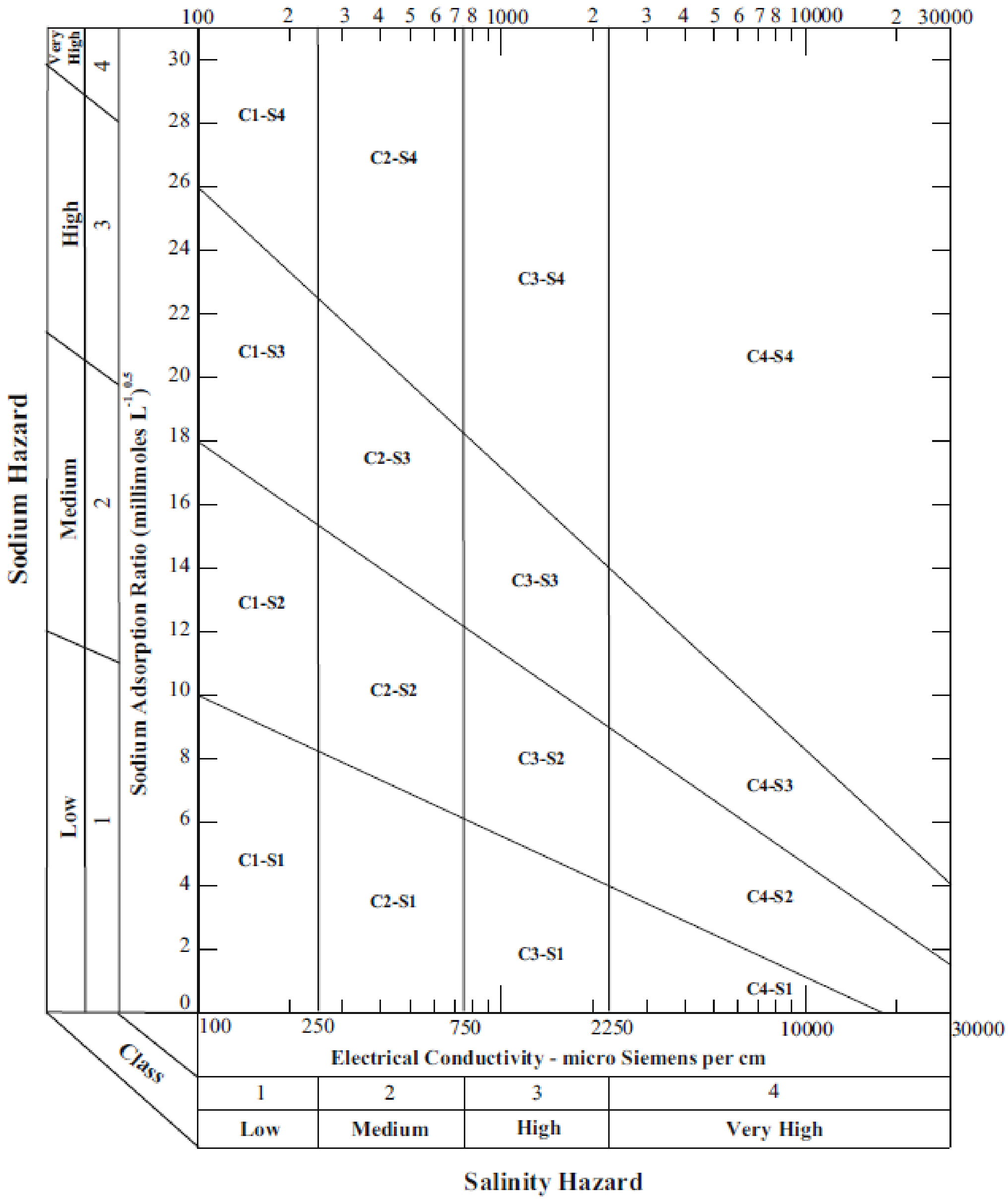


Figure 1. 6. Diagram for the classification of irrigation waters (USSL Staff 1954; modified by Shahid and Mahmoudi 2014)

1.3.2.4. Carbonates and bicarbonates concentration

High levels of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) in water can result in the precipitation of calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) as the soil solution concentrates through evapotranspiration. This process leads to an elevation in the Sodium Adsorption Ratio (SAR), with a higher relative concentration of sodium ions. As a result, the sodium hazard of the soil-water increases beyond the indication provided by the SAR value alone (Zaman, Shahid, and Heng 2018).

1.3.2.5. Impact of specific ions in agriculture

Certain crops are sensitive to particular ions in irrigation water and soil solution, including potential toxicity from trace elements like boron, chloride, and sodium. Conducting soil and water tests is crucial to identify potential toxins as their concentrations vary by crop. Elements introduced through irrigation may undergo reactions in the soil, either becoming inactive or accumulating to toxic levels. The time for an element to reach toxic levels can vary, with possible immediate toxicity or accumulation over several years (Zaman, Shahid, and Heng 2018).

- Chloride toxicity

Crop toxicity in irrigation water is often due to chlorides, which are soluble and easily leach into drainage water. Although essential for plant growth, high chloride concentrations can inhibit growth and be toxic to certain plants. Water quality assessments should include chloride concentration analysis. Chloride toxicity typically starts at leaf tips, progressing to edges, causing necrosis, early leaf drop, or total plant defoliation (Ayers and Westcot 1985).

Table 1. 6. Effects of Chloride (Cl^-) concentration in irrigation water on crops (Follett and Soltanpour 2002; Bauder et al. 2011; Ludwick et al. 1990; M Zaman et al. 2021)

Chloride concentration (ppm)	**Effect on crops**
< 70	Generally safe for all plants
70–140	Sensitive plants usually show slight to moderate injury
141–350	Moderately tolerant plants usually show slight to substantial injury
> 350	Can cause severe problems

1.4. Wastewater treatment (WWT)

1.4.1. Nature-based solutions (solutions for extensive water management)

Nature-based solutions (NBS) for water management strategically leverage ecosystem services to enhance water quantity, quality, and climate resilience, often integrated with conventional water infrastructure for sustainable outcomes. In a healthy ecosystem, natural processes, such as moderated rainfall and temperature events, slowed water flows, and natural storage and filtration, lead to the gradual release of clean water (UNEP 2020).

Global urban population growth and climate change lead to increased water demand and extreme events like droughts and floods, impacting societies and economies. About 60% of the world's population, approximately four billion people, reside in regions with near-permanent stress due to water. One out of every four major urban centers experiences water stress, and there is a forecasted 55% increase in water demand by the year 2050. Pollution exacerbates this stress, as 80-90% of wastewater in developing nations is directly released into surface water bodies, resulting in significant health hazards (Corcoran et al. 2010).

Advanced or unconventional wastewater treatment systems depend on natural purification mechanisms present in soils and aquatic environments. These systems offer an environmentally friendly approach to treating wastewater. However, their primary drawback is the substantial land area they require, resulting in a large footprint.

Utilizing Nature-based Solutions (NBS) and leveraging the water-related services provided by natural ecosystems like wetlands, floodplains, and forests, is instrumental in addressing the water crisis risks, especially amid impending climate challenges (DiFrancesco et al. 2015).

Several nations are presently integrating Nature-based Solutions (NBS) into their national climate strategies. The significance lies in guaranteeing the development and implementation of these actions based on the most effective criteria and practices. Nature-based solutions are described as "Actions to protect, sustainably use, manage, and restore natural or modified ecosystems, which address societal challenges effectively and adaptively, providing human well-being and biodiversity benefits" (IUCN 2020).

To be classified as Nature-based Solutions (NBS), an intervention should fulfill the following criteria: 1) tackle distinct climate change threats and their consequences, 2) play a role in conserving, restoring, or enhancing biodiversity and ecosystems, and 3) aim for socio-economic advantages by aiding vulnerable populations in adapting to the effects of climate change (Donatti et al. 2021).

1.4.2. Treatment wetlands as a wastewater treatment system

Wetlands are often acknowledged as nature-based solutions, providing diverse services with notable social, economic, and environmental value. Changes in land-use, water-use, and climate can impact wetland functions and services, extending beyond the local scale of individual wetlands (Thorslund et al. 2017). Wetlands possess distinctive qualities setting them apart from other Earth ecosystems. Their plant life is uniquely adapted to abundant water and the absence of certain essential chemical elements like oxygen. Consequently, wetlands are globally recognized as one of the most biologically diverse ecosystems, hosting a wide range of flora and fauna. This includes rare birds, mammals, reptiles, amphibians, and fish that are not commonly found elsewhere. Additionally, wetlands can convert many conventional wastewater pollutants into harmless byproducts or essential nutrients, contributing to further biological productivity (Kadlec 2009).

Several mechanisms play a role in improving water quality. These include adsorption, chemical transformations, and ion exchange on substrate, plant, or sediment surfaces. Filtration and chemical precipitation on the substrate, as well as the settling of suspended solids, are also essential factors. Furthermore, the breakdown, transformation, and absorption of nutrients and pollutants by microorganisms and plants, alongside predation and the natural death of pathogens, are crucial contributors to this process (Rahman et al. 2020).

The structure of the TW comprises the following elements:

- Water: Water serves as a habitat for a wide variety of organisms, encompassing both vertebrate and invertebrate animals, submerged and floating plants, algae, and microbial communities. Circulation within the ecosystem is facilitated by the presence of filter material and/or vegetation.

- The substrate, or bed material: The substrate serves a dual role: supporting plant growth and acting as a medium for microorganism fixation, while also functioning as a hydraulic conductor. Examples of substrate materials encompass construction waste, gravel, sand, zeolite, sludge, tire chips, lightweight expanded clay aggregate, or biochar (Yang, Lou, et al. 2018).

- Vegetation: The growth of bacterial biofilms is encouraged by aquatic macrophytes, particularly plants, as they absorb nutrients and release oxygen through their roots (Brix 1997).

- Microorganisms: Microorganisms are vital in decomposing pollutants, including nitrogen, iron, carbon, and sulfur (Zhou et al. 2020).

Treatment wetlands (TWs), also know as treatment wetlands (TW), mimic natural wetland treatment conditions and can be used for diverse wastewater types, such as domestic, storm-water, industrial, agricultural, and mine effluents. They are resilient, cost-effective, and technically feasible systems that blend into landscapes. Additional benefits include lower construction costs, reduced maintenance requirements, adaptability to changing flow and pollutant levels, provision of habitats for various organisms, improvement of water quality, and positive societal acceptance (Kadlec 2009).

1.4.2.1. Types of treatment wetlands

TWs can be classified based on plant type, substrate, or water circulation mode, with the latter being the most commonly used in literature (Vymazal 2010). The categorization considering water circulation mode is as follows:

- Free water surface treatment wetlands (FWS) emulate the features of natural wetlands, closely resembling lakes with their surface flow dynamics. Described as free water surface treatment wetlands, these natural wetlands involve the flow of wastewater over their surfaces. This approach brings about several advantages, including flood mitigation, control of shoreline erosion, and improvement of wastewater quality (Farooqi, Basheer, and Chaudhari 2008; Parde et al. 2021). Water flows freely in a sheet across the substrate, interacting with leaves, roots, and stems of plants, leading to the development of biofilm. Plants in these wetlands may take on different forms, this involves floating on the water surface, being submerged beneath the water sheet, or emerging with half of its structure underwater. including floating on the water's surface, submerged beneath the water sheet, or emerging with half of its structure underwater (Ingrao, Failla, and Arcidiacono 2020). It has average removal effectiveness for trace metals (Iron 53%, Copper 45%, Zinc 52%, and Lead 52%), BOD and COD (50%-60%), TSS (70%-80%), and nitrogen (50%-65%) (El-Sheikh et al. 2010).

- Horizontal subsurface flow treatment wetlands (HSSF) water flows horizontally through the spaces between the granular material and the roots, remaining below the substrate surface (Fig. 1.7b)(Huertas et al. 2013). This design reduces the risk of microorganisms being introduced into wastewater. However, these systems are more susceptible to clogging and require careful study throughout the planning step.

- This design minimizes the risk associated with exposing wastewater to pathogens. However, these systems are more prone to clogging and require careful consideration during the design phase (Ortega de Miguel et al. 2010). Anaerobic processes have an important role in water treatment in HSSF, especially in systems with water-saturated course material beds (Torrens et al. 2020). Because of the anaerobic circumstances, nitrification processes are limited. As a result, when utilized as a single-stage treatment, HSSF has limited nitrogen removal capacity (Mosquera-Romero et al. 2023).

- Vertical subsurface flow treatment wetland (VSSF) uniformly distribute wastewater over the substrate surface. The treatment occurs as the water percolates through the bed material and roots. VSSF is a system where wastewater is introduced from the top, percolates vertically through the bed, and exits from the bottom (Tilley et al. 2014; Parde et al. 2021). The vertical flow treatment wetland creates aerobic conditions, leading to high levels of nitrification, as well as removals of BOD, COD, and other pollutants. Vertical flow treatment wetlands require a land area of 1–3 m^2 per population equivalent (pe), which is less than horizontal flow treatment wetlands. However, they typically demand more maintenance compared to horizontal systems (Parde et al. 2021).

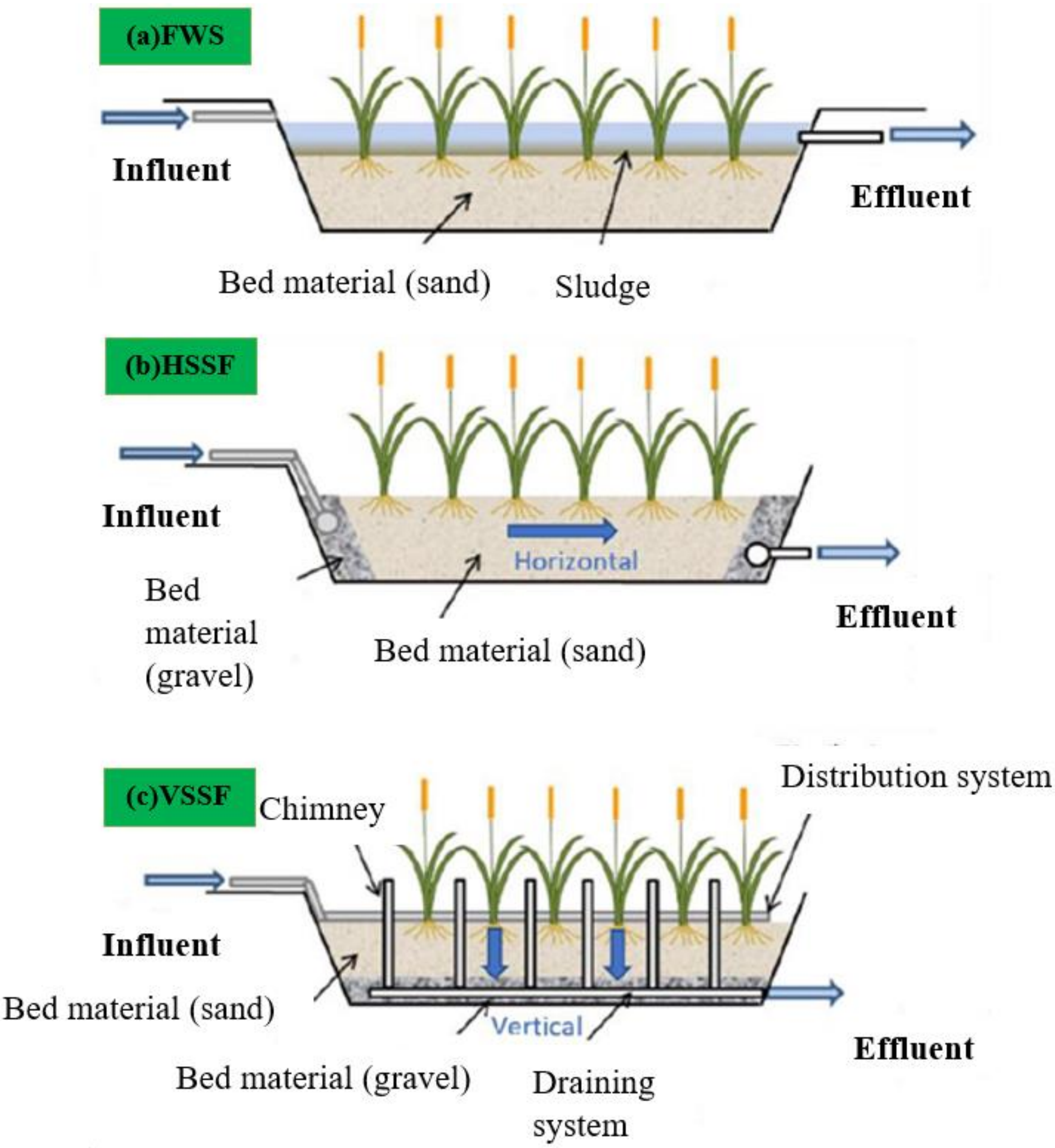


Figure 1. 7. Treatment Wetlands classification regarding water flow.a) Free water surface wetland (FWS); b) Horizontal subsurface flow wetland (HSSF) and c) Vertical subsurface flow wetland (VSSF) (Huertas et al., 2013)

Employing sequential filling and draining of wastewater can be utilized to enhance oxygen availability within Constructed Wetlands (TWs), aiming to improve treatment efficacy. This approach, commonly known as tidal flow, fill-and-drain, or reciprocating wetlands, enhances the removal of oxygen-demanding compounds (Ilyas and Masih 2017). In contrast to constructed wetlands with a constant level of water, those with varying water levels and utilizing a reciprocating operational mode have shown improved treatment efficacy (refer to Table 1.7). This improvement is attributed to the oxygen transfer rate, facilitating air ingress into the bed during the drainage phases.

Consequently, the alternating aerobic and anaerobic environment fosters a resilient and varied microbial population. To enhance pollutant elimination, active aeration can be implemented in both horizontal and vertical flow treatment wetlands. The intermittent application of aeration has demonstrated effectiveness in enhancing nitrogen and Rates of organic matter elimination, as outlined in Table 1.7. Despite the higher investment and operational expenses associated with intensive treatment wetland designs, which require pumps and components to ensure oxygen availability, they remain competitive with other wastewater treatment technologies (Dotro et al. 2017).

Table 1. 7. Comparing conventional treatment wetlands with intensified systems (Aeration and Reciprocating) - HSSF (Horizontal Flow) and VSSF (Vertical Flow)(Dotro et al. 2017)

	Mass percentage removal (%)			**Mass removal rate ($g.m^{-2}. d^{-1}$)**		
	BOD_5	**NH_4-N**	**TN**	**BOD_5**	**NH_4-N**	**TN**
HSSF	81.1	2.8	23.2	6.8	0.1	0.6
VSSF	99.5	87.2	27.6	21.4	4.3	1.9
VSSF + aeration	99.4	99.1	44.6	22	5.2	3.1
HSSF + aeration	99.9	99.3	40.6	31.1	7.3	3.9
Reciprocation	99.3	91.3	72.3	29.9	6.6	7.1

1.5. Electrobioremediation strategies for treating wastewater

Microbial electrochemistry delves into the interplay between microorganisms and electronic devices, emphasizing the distinctive electrical characteristics of microorganisms. Several microorganisms can naturally exchange electrons with electrodes, obviating the need for artificial electron shuttles. This field scrutinizes the correlation between microorganisms and electron-conducting materials such as electrodes (Schröder, Harnisch, and Angenent 2015a). The earliest experimental evidence in this domain, demonstrating the generation of current, dates back over a century, with both yeast and bacteria showcasing current production (Potter 1911). Electrobioremediation combines biological processes with electrochemical technologies to treat wastewater. It relies on the metabolic activity of electroactive bacteria, which use solid-state electrodes to oxidize various compounds. These processes lead to chemical synthesis, bioremediation of polluted matrices, and energy recovery. Researchers have explored merging electrobioremediation with treatment wetlands, creating intensified systems that maintain high performance with a smaller footprint (Ramírez-Vargas et al. 2018).

1.5.1. METland® technology: electromicrobiology integrated in treatment wetland

In conventional bioelectrochemical systems, electroactive bacteria (EAB) can metabolize organic compounds in oxygen-deprived settings, by transferring electrons to an electrode (anode). Then, electrons circulate to external circuit to reach the cathode, where they contribute to the reduction of O2, or any other electron acceptor available. Maintaining charge balance is accomplished through the introduction of an ion separator or by allowing the movement of ions within the fluid bulk (Ramírez-Vargas et al. 2018; Ramírez-Vargas et al. 2019).

Microbial electrochemistry is the core of METland® solutions, driven by electroactive microorganisms capable of transferring electrons to conductive materials. The METland® technology is developed simply and robustly, replacing inert materials like gravel in Treatment Wetlands (TW) with electroconductive materials that stimulate electroactive bacteria (Aguirre-Sierra et al. 2016). Microorganisms are in e particularly responsible for transporting electrons to and from conductive materials (Aguirre-Sierra et al. 2016). Electroactive bacteria (EAB) are utilized in METs to exchange electrons with conductive materials (Esteve-Núñez et al. 2011). METland®s enhance EAB growth by transporting electrons to an electroconductive material that serves as an unlimited acceptor, enhancing organic pollutant oxidation (Penacoba-Antona et al. 2022). In contrast to a TW-MFC, in a METland® system, the released electrons travel through the electroconductive media instead of an external circuit, effectively operating in short circuit mode so-called snorkel (Erable, Etcheverry, and Bergel 2011). Meanwhile, the ions travel with the bulk fluid to the anaerobic/anoxic zones of the system. Here, consortia of bacteria, comprising both heterotrophic and Electroactive Bacteria (EAB) communities, utilize these ions to reduce O2 or NO3 (Ramírez-Vargas et al. 2019). Electric potential (EP) profiles serve as an indicator of Electroactive Bacteria (EAB) development within the system. By utilizing EP electrodes, the flow of electrons can be measured, occurring from the anodic zones where EABs produce electrons to the cathodic zones where electrons are consumed. These measurements enable the quantification of electron flow and the identification of the direction of electrons, thereby determining the anodic and cathodic zones within the system (Prado et al. 2020; Prado, Berenguer, and Esteve-Núñez 2022). Due to the absence of electron movement in gravel systems or non-conductive carbonaceous materials, the EP profiles remain flat. This observation serves as a dependable indicator of the effectiveness of METland® systems (Penacoba-Antona et al. 2022).

Various granulometries and designs have undergone testing to optimize pollutant degradation rates in METland®. The bed incorporates different conductive materials, with conductive coke being a primary choice (Aguirre-Sierra et al. 2016; Ramírez-Vargas et al. 2019) and more sustainable options like conductive biochar obtained through high-temperature pyrolysis of biomass such as wood (Prado et al. 2020; Prado, Berenguer, and Esteve-Núñez 2019; Schievano et al. 2019). Biochar not only provides conductivity but also possesses substantial electron storage capacities due to its redox-active moieties (Prévoteau et al. 2016). This characteristic allows biochar to act as a redox buffer in cases of local limitations in electron donors or acceptors (Yuan et al. 2017). Recent research has explored the use of artificial elements for accepting electros so-called e-sink devices, enabling the regulation of electron flux within the METland®, thereby establishing new redox gradients (Prado et al. 2020). This device allows for the supply of unlimited electron acceptors without altering the composition of the wastewater. Implementation of the e-sink in METland® systems has shown to significantly enhance COD removal efficiency, reaching levels as high as 98% in urban wastewater treatment (Prado et al. 2020).

Different operational modes can be applied in the METland® technology. Initially designed for flooded conditions, it operated under either horizontal subsurface flow or upflow, promoting anoxic metabolism and favoring nitrate removal (Aguirre-Sierra et al. 2016; Prado, Berenguer, and Esteve-Núñez 2022).

In this operational mode, the natural redox gradient between the bottom and the naturally oxygenated surface was stimulated, intensifying microbial reactions (Aguirre-Sierra et al. 2016; Ramírez-Vargas et al. 2019). Consequently, the anaerobic zone promotes oxidation reactions, with electrons flowing through the conductive material along the increasing redox gradient to the upper zone, where reduction reactions occur such as oxygen reduction to produce water (Peñacoba-Antona 2021).

In a METland® system, electroactive bacteria (EAB) are stimulated to generate and transfer electrons to an electro-conductive material, acting as an unlimited electron acceptor. This maximizes substrate consumption, avoiding free electrons for methane generation and, consequently, leading to an increase in microbial metabolism rates, due to the unlimited nature of the electron acceptors (Ramírez-Vargas et al. 2019). Recently, various conductive materials have been employed, emphasizing sustainability and treatment efficiency. The studied materials include a combination of minerals and carbon, such as electroconductive coke (Aguirre-Sierra et al. 2016; Ramírez-Vargas et al. 2019).

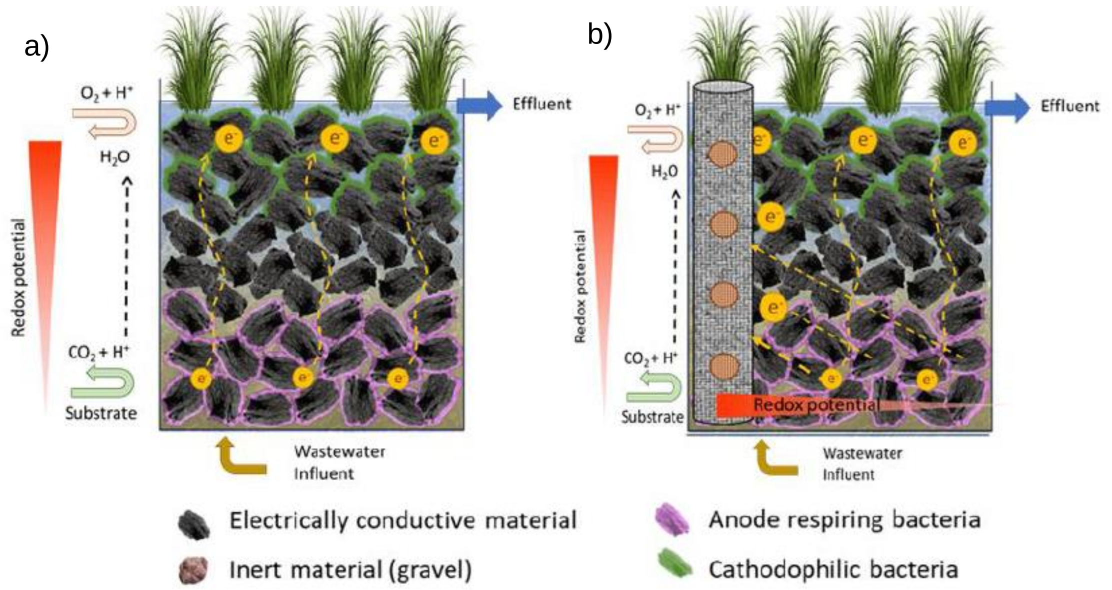


Figure 1. 8. METland® scheme operating as snorkel mode (single electrode-based configuration under short circuit) under (a) upflow mode , and (b) upflow mode in presence of an electron-sink device (Prado de Nicolás 2021)

To understand the internal dynamics of a flooded METland®, it was essential to explore the flow of electrons within the bed and microbial communities. Electric potential measurements along the material's depth demonstrated the flow of electrons from the anaerobic zones (bottom of the METland®) to the surface (oxygenated top). Providing an additional electron acceptor throughout the depth of the METland (the ec-sink concept) demonstrated that electrons migrate toward the nearest electron acceptor, aligning with the steepest redox potential gradient (Ramírez-Vargas et al. 2019; Prado de Nicolás 2021).

In non-flooded mode (down-flow operation), METland® has proven beneficial, supporting passive aeration without energy expenses. In this mode, oxygen serves as the electrochemical acceptor, consuming all generated electrons, favoring nitrification, and improving COD and nutrient removal (Aguirre-Sierra et al. 2020). Furthermore, flooded METland® configuration can remove over 95% of micropollutants (Pun et al. 2019).

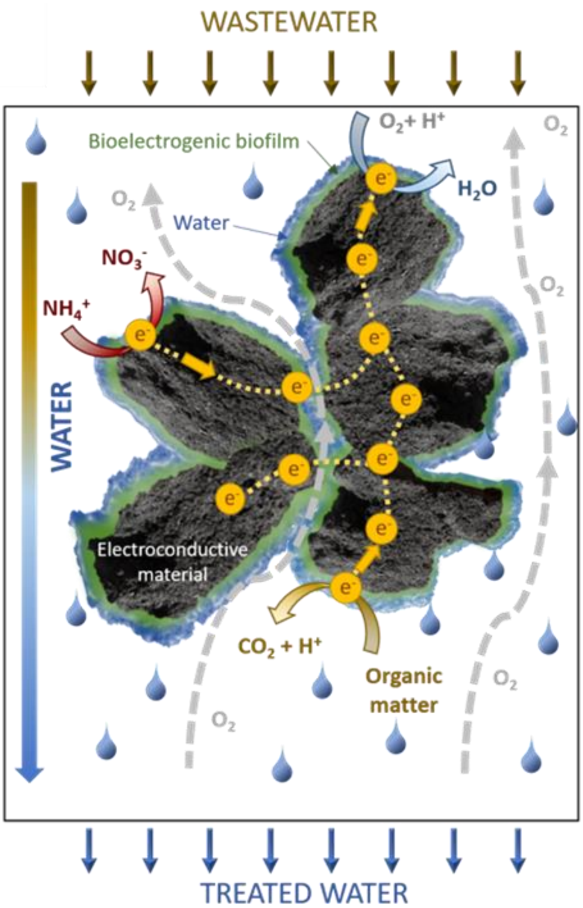


Figure 1. 9. Electrons flow through the conductive material METland® configurations (Peñacoba-Antona 2021)

The research, such as the ELECTRA project (www.electra.site), focuses on METland®'s performance in specifically degrading micropollutants in wastewater in Europe and China. Additionally, the latest generation of METland® operates in modular mode through a 3D lego configuration currently investigated in the project mobiMET (www.mobimet.es).

1.5.2. From laboratory trials to full-scale implementation

Over the past decade, METland® technology has transitioned from the laboratory to international large-scale implementation through the spinoff company METfilter , as shown in figure 1.10 (Esteve-Núñez 2022).

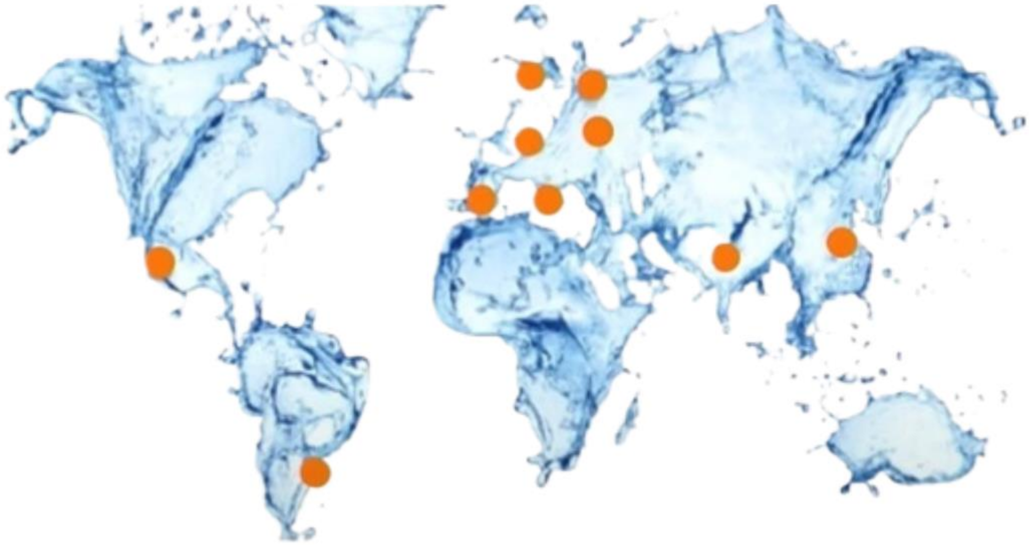


Figure 1. 10. A depiction of nations where there is at least one METland system either currently operational or in progress (Esteve-Núñez 2022)

Over the past decade, the METland® technology has achieved remarkable success across diverse geographic locations, consistently attaining COD removal efficiencies of about 90% (Penacoba-Antona et al. 2022). Furthermore, the solution was proved to be sustainable according to Life Cycle Analysis of full scale designs (Peñacoba-Antona 2021). This exceptional performance underscores its innovative approach to wastewater treatment, particularly in efficiently removing pollutants. For instance, in iMETland project the technology underwent validation in four diverse climatic sites, spanning the Mediterranean region (Spain), Northern Europe (Denmark), South America (Argentina), (Figure. 1.11).

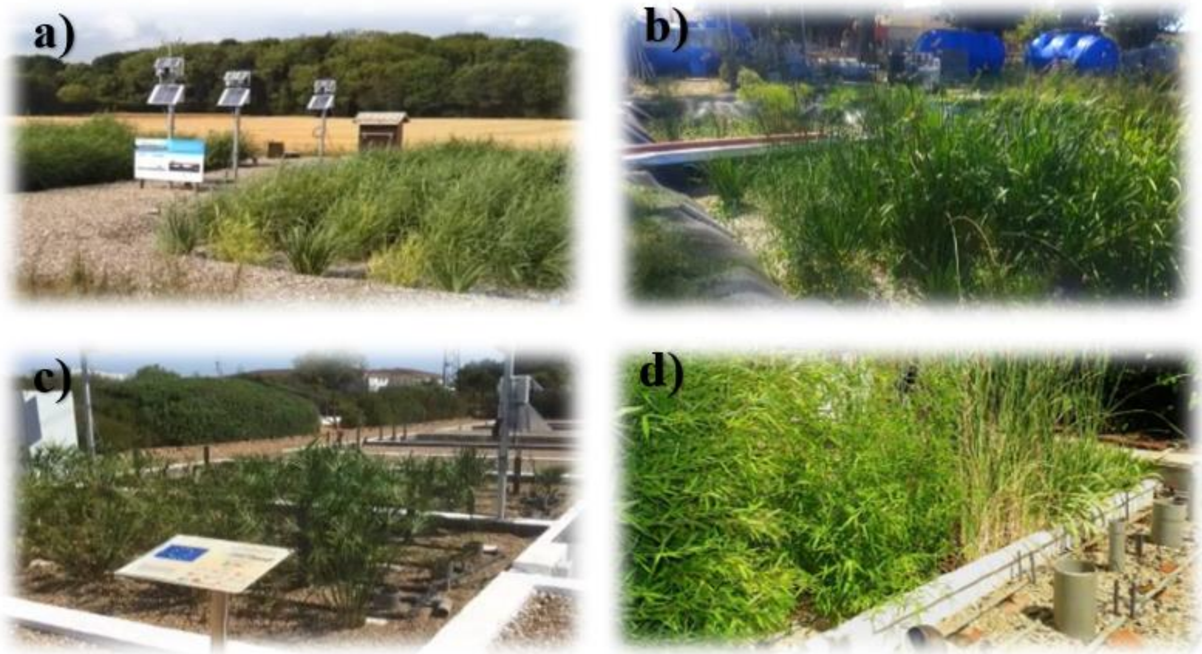


Figure 1. 11. Images of METland® solutions constructed during the iMETland project: a) Denmark, b) Argentina c) Sevilla, Spain, and d) Alcalá de Henares, Spain.

Figure 1. 12. METland® system for treating 100 m3/day of urban wastewater at Indian Institute of Technology Kharagpur

Introduction

It is worth highlighting the SARASWATI 2.0 project in India, which implemented a METland® solution for treating approximately 100 m^3/day of urban wastewater from the campus of the Indian Institute of Technology Kharagpur.

METlands® technology can be also designed under modular METfilter units that have demonstrated outstanding organic removal rates for treating industrial wastewater from different sectors, ranging from 2 kg COD/ m3bed day for treating wastewater from the oil and gas sector to 10 kg COD/ m3bedday for winery wastewater (Figures 1.13)

Figure 1. 13. Modular METfilter units for treating wastewater from a) winery, b) petrochemistry, c) urban, and c) oil&gas sector

1.6. Biochar: environmental applications

1.6.1 Biochar synthesis

Biochar is a porous solid material rich in carbon, resembling charcoal, and characterized by its black color. It is typically generated through the thermochemical conversion of biomass, often in environments with minimal or no oxygen present (Ahmad et al. 2014). Biochar is a porous carbonaceous material formed through the thermochemical breakdown of biomass feedstock in low-oxygen environments. This feedstock can comprise various organic waste materials, such as agricultural and forest waste, chipped wood, compost, algae, sewage sludge, and organic municipal solid wastes (Colantoni et al. 2016). The advancements in converting organic materials into valuable substances like biochar have garnered significant interest from various fields. Early investigations primarily examined the potential of biochar as a soil amendment to adsorb inorganic nutrients, enhance soil quality, or facilitate other environmental benefits (Sanroman MA et al. 2017). Biochar is derived from various thermochemical processes such as pyrolysis, hydrothermal carbonization, gasification, and torrefaction. The properties of biochar, including surface area, porosity, and elemental composition, are influenced by the pyrolysis temperature and the type of feedstock utilized (Wang et al. 2020). Biochar manufactured at lower temperatures tends to contain more oxygen-containing functional groups, enhancing its capacity to adsorb polar compounds, and it may have increased mechanical strength, rendering it suitable for utilization in treatment wetlands. Conversely, biochar produced at higher temperatures usually exhibits greater porosity, surface area, aromaticity, carbon content, and hydrophobicity (El Barkaoui et al. 2023).

1.6.2. Biochar application in agriculture (Biochar reactions in soil)

Recognized as an eco-friendly and plentiful energy source, biomass is subjected to thermochemical treatment to produce biochar. Originating from a range of biomass or organic waste materials, biochar is characterized by its high carbon content, significant specific surface area, ability to exchange cations, capacity to retain nutrients, and durable structure. The growing interest in biochar stems from its impressive properties, making it a valuable resource across multiple applications (Sakhiya, Anand, and Kaushal 2020).

Biochar is acknowledged for its capacity to enhance soil fertility and sequester carbon. Significant research has been dedicated to studying its chemical properties and its influence on plant and microbial growth. However, the underlying mechanisms driving these beneficial effects and potential environmental consequences, such as the release of organic contaminants or nutrients, remain uncertain, and require further investigation (Mukherjee and Zimmerman 2013). Given biochar's exceptional ability to adsorb and retain nutrients, its beneficial impact on the soil ecosystem, influencing both plants and microbes, arises from the nutrients within biochar or from its capacity to adsorb and retain nutrients (Hammes and Schmidt 2009; Lehmann et al. 2011; Mukherjee and Zimmerman 2013). One of the benefits of biochar is its effect on the rate of nitrification, achieved through its influence on soil nitrifier activity. This influence stems from modifications in various soil physicochemical properties resulting from the addition of biochar (Liao et al. 2022; Zhang et al. 2023). The capacity of biochar to improve soil aeration is due to its high porosity and structural properties, which provide optimal conditions for aerobic microbial activities like nitrification. This promotes nitrogen cycling and improves soil health overall (Wang et al. 2020).

1.6.2.2. Effects on seed germination and early seedling growth

Biochar has several applications, including its crucial role in seed germination and early seedling development. Additionally, it plays a vital role in removing the toxicity of heavy metals such as aluminum, particularly when biochar with alkaline properties is used to raise the pH in the soil. This increase in pH effectively mitigates potential adverse effects on root growth, especially in acidic soils. These chemical impacts of biochar on soils and soil water solutions are significant for agriculture and environmental management (Lauricella et al., 2021; Shetty et al., 2020; Van Zwieten, et al., 2015).

Biochar has the ability to provide sufficient oxygen and enhance soil aeration, leading to a reduction in soil bulk density. This characteristic significantly contributes to seed germination by creating an improved soil structure, enabling easier root penetration and facilitating seedling emergence. As a result, biochar promotes healthier seedling growth (Obia et al. 2018). Biochar, generated by pyrolyzing organic materials, offers the potential to enhance crop yields significantly (Dong et al., 2015; Hou et al., 2020). In the field of agricultural sciences, extensive research has been carried out to examine the contributions of biochar to enhancing soil fertility and its capability to sequester carbon in a stable form over extended periods. (Schievano et al., 2019).

1.6.2.3. Biochar and crucial oilseed crops

Sunflowers (Helianthus annuus L.), a prominent oilseed crop, have received significant research attention, ranking fourth on the FAO's 2017 list of crucial oilseed crops. Sunflowers possess several capabilities, including phytoremediation, resilience to saline irrigation, and economic benefits in both food and non-food sectors, particularly in biodiesel production. These attributes collectively make sunflowers a highly advantageous crop choice. Furthermore, sunflowers present an advantageous option for farmers practicing sustainable agriculture due to their short growth cycle, adaptability to diverse environmental conditions, and suitability for irrigation with reclaimed water (Souza, Oliveira, and Castiglioni 2004; Tsoutsos et al. 2013; Silva et al. 2013; da Costa Marques et al. 2015).

Moreover, alongside the acknowledged economic and agricultural significance of sunflowers, a recent field study carried out in the Mediterranean region explored the advantageous effects of biochar on sunflower production, especially during drought conditions. The study emphasized the compatibility of sunflowers with biochar and illustrated how biochar influenced sunflower development (Paneque et al. 2016).

1.6.3. Biochar application in water and wastewater treatment

1.6.3.1.A sustainable approach for contaminant removal

Researchers have explored alternative materials like charcoal, zeolite, and biochar, in addition to conventional choices such as gravel and sand, to optimize the efficiency of Treatment Wetlands (El Barkaoui et al. 2023). Biochar, specifically, has attracted considerable interest due to its stability, porous nature, carbon-rich composition, and the cost-effective production methods it offers through thermochemical conversion techniques like gasification, and pyrolysis (De Rozari, Greenway, and El Hanandeh 2016; Deng, Chen, and Chang 2021). Due to its remarkable sorption capacity for both organic and inorganic pollutants, biochar shows great promise as a substrate in Treatment Wetlands (TWs), offering the potential to substantially enhance system efficiency (Srivastava, Gupta, and Chandra 2008; Wang and Wang 2019). The ability of biochar to absorb substances varies based on factors such as the type of material it is made from and the conditions under which it is produced, such as the temperature at which pyrolysis occurs. This means that different types of biochar may have varying capacities to adsorb pollutants from water or soil, depending on their specific characteristics and how they were created (Tan et al. 2015; El Barkaoui et al. 2023).

1.6.3.2. Biochar's proficiency as a highly effective adsorbent for organic and inorganic contaminants

In water and wastewater treatment, biochar acts as a proficient adsorbent for eliminating diverse contaminants. Many research studies have showcased its effectiveness in eliminating pollutants, including heavy metals, organic compounds, and nitrogen and phosphorus. This highlights the versatile and valuable contribution of biochar to improving water treatment procedures.

-Heavy metal removal

Recent attention has been drawn to pollutants, especially those that resist biodegradation, revealing challenges in natural degradation processes. These pollutants are commonly found in rainwater, mining effluents, and industrial wastes, significantly contributing to heavy metal pollution. To address this issue, biochar, with its unique pore structure, high organic carbon content, and diverse functional groups, shows promise in effectively interacting with heavy metals through various pathways (Oliveira et al. 2017). The presence of heavy metals in wastewater poses a grave danger to human health, animals, and plants alike. Even when present in the aqueous phase at low concentrations, prolonged exposure to these metals can lead to serious health hazards (Ahmed et al. 2016). Functional groups present in biochar, such as COOH, OH, and R-OH, enable the absorption of heavy metals through mechanisms like complexation and ion exchange, as heavy metal ions interact with them (Lu et al. 2012).

Biochar has shown notable ability to adsorb various heavy metals. For instance, biochar derived from paper mill sludge exhibits a maximum adsorption capacity of 34.1 mg/g for As^{3+} (Cho et al. 2017). According to research findings, the adsorption capacity of As^{3+} has risen from 5.7 mg/g to 7.0 mg/g (Van Vinh et al. 2014). Another study illustrates the effectiveness of biochar in efficiently removing Cd^{2+} (Higashikawa et al. 2016). Biochars had Pb^{2+} removal efficiency of 359 mg/g and 193 mg/g, respectively (Zhou et al. 2017). Furthermore, biochars shown adsorption capacities for Cr^{3+}, Ni^{2+}, and Cu^{2+} (Agrafioti et al. 2013). The highest adsorption capacity of Ni^{2+} from water using biochar was recorded at 11 mg/g (Higashikawa et al. 2016). Magnetic biochars derived from marine macro-algae exhibited notable selectivity and adsorption capacity for Cu^{2+}, with values reaching 69.37 mg/g for kelp magnetic biochar and 63.52 mg/g for hijikia magnetic biochar (Son et al. 2018; Xiang et al. 2020).

Introduction

-Organic contaminant removal

The occurrence of common organic pollutants like herbicides, pesticides, and antibiotics, along with decreasing levels of dissolved oxygen in water, presents threats to both aquatic ecosystems and human health. It is essential to address these harmful compounds to prevent potential damage to the environment and ensure the well-being of the public (Ahmed et al. 2016). Utilizing techniques like adsorption, hydrolysis, chemical reduction, oxidation, filtration, and microbial degradation is widespread for eliminating organic matter during wastewater treatment processes (Vymazal and Brezinova 2015). By implementing Treatment Wetland (TW) systems, the efficient removal of conventional organic compounds like chemical oxygen demand (COD) and biological oxygen demand (BOD_5) is accomplished through the combined action of anaerobic and aerobic degradation processes (Saeed and Sun 2017; Zhao et al. 2020). Although there is a possibility of organic matter leaching from biochar (Zhou et al. 2019), its integration into Treatment Wetlands (TWs) notably enhances chemical oxygen demand (COD) removal. Several research studies have shown that the addition of biochar improves COD removal efficiency in TWs (Deng et al. 2019; Guo et al. 2020a; Guo et al. 2020b).

-Nitrogen and phosphorus removal

Biochar has the capability to uptake nutrients like nitrogen and phosphorus from the liquid phase (Zhang and Gao 2013; Zhang et al. 2014; Xue et al. 2016; Xiang et al. 2020). Ammonium, nitrate, and phosphate are typical forms of reactive nitrogen and phosphorus found in wastewater, which can lead to eutrophication (Yang, Zhao, et al. 2018; Xu et al. 2018). Enhanced adsorption capacities for nitrogen and phosphorus are observed in modified biochars compared to unmodified ones. This improvement is attributed to the increased specific surface area, heightened reactivity, and greater abundance of surface functional groups present in the modified biochar (Xiang et al. 2020).

1.6.4. Agronomic impact of filter biochar

Biochar exhibits the potential to serve as a slow-release fertilizer, supporting plant growth through the retention of nutrients within its pores during composting (Kammann et al. 2015).

Utilizing biochar as a filtration medium for water treatment offers a straightforward method for purifying wastewater to meet irrigation standards. The adoption of a slow-flow system aids in pathogen removal. Additionally, the residual biochar from the filtration process can be repurposed for agricultural applications. Regular replacement of exhausted biochar with fresh material is necessary over time, with the re-used biochar serving as a soil amendment. The quantity of residual biochar available for soil amendment depends on the composition of the treated wastewater. Therefore, employing biochar as a filter medium provides two benefits: effective water treatment and utilization of residual biochar for agricultural purposes (Werner et al. 2018). Research on the recycling of residual biochar used in treatment wetlands or biofilters for application in agricultural soils or degraded lands is limited, emphasizing the necessity for additional research and practical applications in these domains.

1.6.4.1. Recycling of spent biochar substrates

One of the challenges in managing treatment wetlands (TWs) is the disposal of depleted filter media, which can lead to environmental concerns. Exhausted substrates in TWs or biofilters may lose effectiveness or become obstructed after extended periods of operation, requiring the replacement of substrates with fresh or new ones to maintain pollutant removal efficiency.

It is important to highlight that using biochar as a filter media for TWs and biofilters carries minimal environmental concerns regarding its disposal. Additionally, it offers the benefit of being repurposed to improve soil structure, enriched with nutrients for use as a slow-release fertilizer, ultimately leading to enhanced crop yield and soil fertility (Deng, Chen, and Chang 2021). Considering the variety of agronomic and environmental advantages, including the utilization of nutrients derived from reused biochar for agriculture sourced from wastewater, the use of biochar offers a practical solution for organic farming while also it aids in alleviating non-point source (NPS) pollution runoff from agricultural activities. However, it is crucial to address concerns about potential toxic micropollutants and pathogens that may be present in reused biochar, as they could pose a risk of secondary pollution (Werner et al. 2018; Zheng et al. 2019). The combustion of used biochar for energy generation provides a versatile solution that tackles waste management, energy needs, and environmental sustainability (Kaetzl et al. 2019). In addition to the economic benefits of biochar compared to conventional filtration materials, it's worth highlighting that utilizing biochar in biofilters and treatment wetlands offers a viable solution for wastewater treatment in remote or rural areas. The ease of producing biochar using small-scale pyrolysis systems, like pyrolytic stoves and pits, which are increasingly prevalent, makes it especially suitable for these regions. Moreover, utilizing biowaste to produce biochar not only addresses wastewater treatment challenges in these regions but also provides a practical solution for managing biowaste. Biochar with sufficient mechanical strength can serve as the primary substrate layer in biofilters and TWs, allowing for biochar recycling. While current research often combines biochar with other materials in treatment wetlands or biofilters, using biochar alone has the potential to transform continuous and unlimited biomaterial flows into cyclic loops, fostering a circular economy (Gwenzi et al. 2017).

Figure 1. 14. Biochar Recycling: from wastewater treatment to soil enrichment

1.7. Objectives of the thesis:

According to the previous considerations, we proposed the following specific objectives to be aimed in this thesis:

- Evaluate the potential of METland® technology for treating urban wastewater generated in a University Campus.
- Design a preliminary plan to cope with water scarcity on campus by decentralized METland® solutions.
- Investigate how humus or other materials rich in humic acid affect METland® performance for treating wastewater.
- Assess the role of electroconductive biochar as bed material in METland® for nutrient recovery from urban wastewater.

- Determine the preferential adsorption of elements from ww in electroconductive biochar from METland®.
- Assess the impact of electroconductive biochar, either raw or used after treating ww, for agriculture use.
- Validate if urban wastewater treated by METland® technology meets current legal standards for reuse in irrigation.
- Assess the impact of using treated urban wastewater by METland® for irrigating soil crops.
- Verify that water treated by METland® technology meets toxicity standards using algal and chlorophyll as bioreporters.
- Address the dilemma between removing or retaining nutrients in re-use water.
- Explore the potential of using used biochar as a slow-release fertilizer.

CHAPTER 2:
Material and Methods

2. Material and Methods

In this chapter, all specific methodologies applied in the various experiments conducted in the current thesis are detailed across four experimental sections.

2.1 Analytical methods for wastewater analysis

2.1.1 Measuring PH and EC

The pH of the bulk solution in each experiment was determined using a Crison PH 25 pH meter. At the same time, electrical conductivity was measured using a Crison CM 35 instrument (Chapters 4 and 5).

2.1.2. Anions and cations determination

Anions and cations were identified using Ionic Chromatography (Metrohm 930 Compact Ion Chromatograph Flex). As part of the sample preparation process, the samples were filtered through nylon membranes with a pore size of 0.45 µm before being stored at -20 ºC in polypropylene Falcon tubes. This instrumental setup enabled the analysis of major anions (F^-, Cl^-, NO_3^-, NO_2^-, PO_4^{3-}, SO_4^{2-}) and major cations (NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+}).

2.1.3. Chemical oxygen demand

Chemical oxygen demand (COD) analysis was conducted using an optimized standard method (Noguerol-Arias et al. 2012). The sample compounds underwent oxidation using 1.5 mL of digestion reagent, comprising potassium dichromate (0.5 N), mercury sulfate, and sulfuric acid (95-97%), alongside 1.5 mL of catalyst containing silver sulfate (1%) and sulfuric acid (95-97%). Subsequently, the mixture was incubated at 150 ºC for 2 hours in a digester (Hach Lange LT 200). Dichromate concentration was then measured colorimetrically using a spectrophotometer (Hach Lange DR 2800). Additionally, three distinct ranges of commercial kits from HACH were employed for analysis in this study.

2.1.4 Total nitrogen

The determination of total nitrogen content in the samples was carried out using an element analyzer (Analytic Jena). This process involved subjecting the samples to either high-temperature combustion or chemical oxidation. These methods effectively transformed both organic and inorganic nitrogen compounds into nitrogen gas (N_2).

2.1.5. Total inorganic carbon

In our research, we utilized an element analyzer supplied by Analytic Jena to detect the presence of inorganic carbon in our samples. The detected inorganic carbon primarily includes carbon dioxide (CO_2), along with other species such as carbonic acid, bicarbonate anion, and carbonate. The methodology of the machine involves initially acidifying the sample to convert all inorganic carbon species to CO_2. Following this step, the sample is subjected to high-temperature treatment (800ºC) in an oven, where all carbon is oxidized to CO_2, which is then measured.

To determine the quantities of these species, we considered the pH of the water sample and applied a Bjerrum plot methodology. Specifically, for our calculations, we referenced data at 20˚C and an electrical conductivity of 250µS cm^{-1}, utilizing Gutz's approach (2012) with apparent $pK_1 = 6.532$ and $pK_2 = 10.329$ as derived by Schwarzenbach and Meier in 1958 (Schwarzenbach and Meier 1958).

$$CT= [CO_2] + [HCO_3^-] +[CO_3^{2-}]$$

In this equation,

C_T represents the total inorganic carbon concentration.

$[CO_2^*]$ denotes carbonic acid simultaneously, the combined concentration of carbon dioxide and carbonic acid

$([CO_2^*] = [CO_2] + [H_2CO_3])$

$[HCO^-_3]$ stands for the bicarbonate concentration

$[CO_3^{-2}]$ indicates concentration of carbonate.

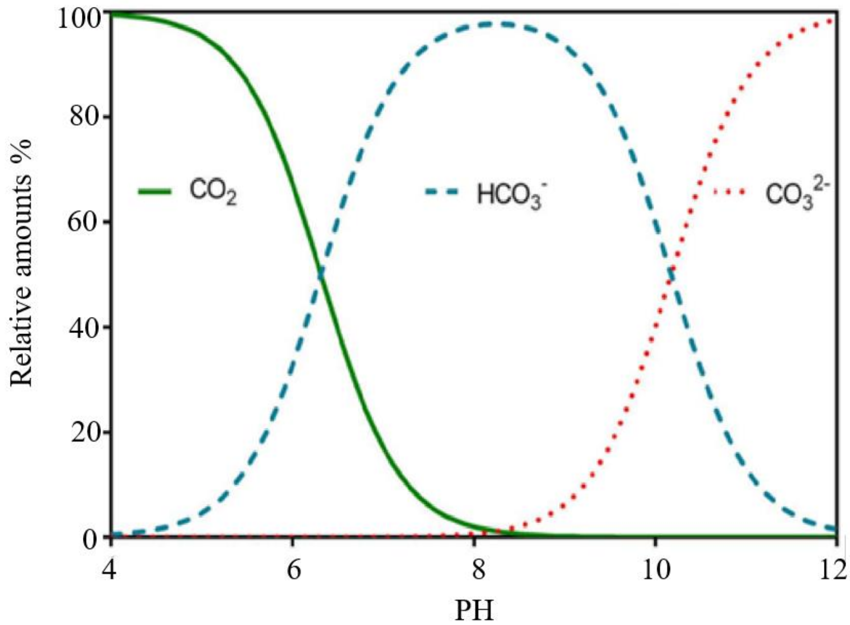


Figure 2. 1. Distribution of carbon dioxide (CO_2), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) in water at various pH values

2.2. Analytical methods for soil

2.2.1. PH and EC in soil

Soil pH was determined by preparing soil-water suspensions with a ratio of 1:2.5 (Bao et al. 2024). Electrical conductivity (EC) was measured at 25°C using an unfiltered 1:5 soil suspension in deionized water (Hardie and Doyle 2012).

2.2.2. Nutrient extraction from soil

To assess soil nutrient content, soil samples underwent sieving (2mm) to remove coarse particles. Subsequently, each sample was mixed with a 5% citric acid solution at a ratio of 1:20. The soil-solution blends were continuously agitated for 2 hours at a constant speed of 150 revolutions per minute (rpm) and maintained at an ambient temperature of 25°C. Following this, the mixture underwent filtration to separate the liquid extract from the soil particles. The identification of both cations and anions was conducted using ionic chromatography, as detailed in Chapter 4 and 5. Citric acid was found to be highly effective in extracting metal ions from soil, as demonstrated by the utilization of two different concentrations, 0.5 M and 0.3 M, for metal extraction (Bassi, Prasher, and Simpson 2004).

2.2.3. Microbial community assessment soil

The analysis of microbial diversity and composition in diverse soil samples involved collecting soil samples from plots growing sunflowers with and without biochar at the conclusion of the experiment, with repetition of sampling to ensure robustness. These samples were meticulously stored at -20ºC prior to DNA extraction. Subsequently, the DNA extraction, PCR amplification, and high-throughput sequencing processes were outsourced to an external service provider, specifically the Unit of Microbial Ecology at the Laboratory of IMDEA Agua.

-DNA extraction, library preparation and nanopore sequencing

The process of DNA extraction involves three main steps: sample preparation, library preparation, and statistical analysis.

-Sample Preparation

The samples underwent a 4-hour drying process in a laminar flow cabinet. For DNA purification, the Mag-Bind Environmental DNA kit from Omega Bio-Tek was employed, known for its efficiency in removing humic acid and other PCR inhibitors. Subsequently, the quality of the extracted DNA was evaluated using a nanofotometer (EPOCH, BioTek), while quantification was conducted using the Qubit x1 dsDNA High-Sensitivity Assay kit from Invitrogen.

-Library preparation

For library preparation, the 16S Barcoding Kit (SQK-RAB204) from Oxford Nanopore Technologies (ONT) was utilized, involving PCR procedures. In this process, a different master mix, specifically the repliQa HiFi ToughMix from Quanta bio, was used instead of the one recommended by the kit. This substitution aimed to ensure the efficiency of the PCR amplification process by leveraging its known tolerance to inhibitors.

Statistical analysis

Statistical analysis of the Fastq files obtained from sequencing was carried out using the Spaghetti pipeline (Latorre-Pérez et al. 2021) for data visualization and statistical analysis. Data analysis primarily relied on the phyloseq R package (McMurdie and Holmes 2013) within the R software version 4.2.1.

2.3. Analytical methods for biochar

2.3.1. PH and EC in biochar

The pH and electrical conductivity (EC) of the biochar were evaluated by mixing the biochar with deionized water in a ratio of 1:20. This mixture underwent shaking for 1.5 hours on a reciprocating shaker at 25°C. Afterward, the samples were allowed to settle for 30 minutes, following which pH and EC measurements were taken (Singh et al. 2017).

2.3.2. Biochar nutrient extraction

For biochar nutrient extraction, we use deionized water, which involves regularly changing the water and measuring the main cations and anions until no additional nutrients are released.

2.3.3. Elemental analysis technique (LECO CHNS-932)

The elemental analysis technique in the LECO CHNS-932 involves a destructive process, where a known amount of sample, typically between 1 or 2 milligrams, is weighed. The sample undergoes thermal oxidation at 1000°C in an oxygen atmosphere, leading to a complete and quantitative conversion of solid components into gaseous phase. These gases are then carried by a helium flow. Subsequently, they pass through heated granulated copper to eliminate excess oxygen and reduce nitrogen oxides to N_2. Another copper oxide is used to convert CO to CO_2. Finally, the obtained gases (CO_2, N_2, H_2O, and SO_2) pass through a series of traps for adsorption. This equipment utilizes independent infrared detectors to measure CO_2, SO_2, and H_2O, and a thermal conductivity detector to measure N_2. The results are provided in percentage values for %C, %H, %N, and %S.

2.3.4. Adsorption capacity analysis of biochar

The adsorption capacity (q_e) of biochar for nutrients was determined using Equation 1.

$$q_e = \frac{(C_0 - C_e)V}{W} \qquad \text{Eq1}$$

where C_0 and C_e (mg/L) are the initial and equilibrium concentrations, respectively; V is the volume (L) of the solution, and W is the weight of the biochar.

2.4. Biochar adsorption and release evaluation

2.4.1. Short-term adsorption

The research examines the short-term adsorption properties of electroconductive biochar sourced from diverse materials including Miscanthus Straw, Oil Seed Rape Straw, Soft Wood, and Wheat Straw. These biochar specimens were manufactured by the UK Biochar Research Centre (UKBRC) and underwent pyrolysis at two distinct temperatures: 550 and 700 degrees Celsius. Short-term adsorption offered valuable insights for identifying biochar that is well-suited for long-term adsorption purposes. To evaluate the adsorption capacity, one gram of each variety of biochar was mixed into 80 ml of nutrient-rich domestic wastewater. This method was carried out three times for each biochar variation. The biochar and solution mixes were agitated for 24 hours at a continuous speed of 150 rpm and controlled at a constant temperature of 25 degrees Celsius by placing the bottles inside a chamber. Following the 24-hour period, the samples were filtered through nylon membranes with a pore size of 0.45 µm. Subsequently, Ionic Chromatography was employed to identify both major anions and cations. The analysis entailed comparing the ion concentrations in the initial solution with those in the filtered solution to determine the degree of adsorption with each type of biochar, employing Equation 1 as specified in section 2.3.4.

2.4.2. Batch adsorption experiment setup (long-term adsorption)

For the analysis of maximum adsorption capacity, we conducted a batch adsorption experiment using two different biochar types produced at a pyrolysis temperature of 550°C: Oil Seed Rape Straw (OSR 550) and Soft Wood (SWP 550). Each type, totaling 40 grams, was placed in a batch container holding 0.5 liters of nutrient-rich wastewater. The powdered biochar was completely immersed in the nutrient-rich wastewater solution and subjected to experimental conditions for one month.

To ensure precise control, the experiment was conducted under controlled conditions, with a constant temperature of 25 degrees Celsius maintained by housing the containers in a chamber. To optimize the interaction between the biochar and wastewater, a horizontal shaker was employed at a speed of 150 revolutions per minute.

In order to comprehensively monitor the adsorption process, we implemented daily wastewater replacement and sampling procedures throughout the 33-day experiment duration. This approach allowed us to capture the dynamics of the adsorption process over time and accurately assess the maximum adsorption capacities of the respective biochar types. (Chapter 3).

2.4.3. Determination of nutrient release patterns

To assess the nutrient release potential of biochar, a sequential methodology was employed. Five grams of both raw and used biochar were placed in 50 mL of deionized water in 100 mL serum bottles, and this procedure was repeated three times. The bottles were incubated in an orbital shaker a150 rpm) at 25 degrees Celsius,

The process was conducted daily for a week, by adding fresh 50 mL of deionized water and samples collected each day. This procedure was repeated with fresh deionized water added on day 10, and subsequently, weekly until 2 months to monitor the release of nutrients over time.

To quantify the released nutrients, samples were collected at each time point, and the concentrations of potassium (K^+), phosphate (PO_4^{3-}), and nitrate (NO_3^-) were measured using ion chromatography.

2.5. Biofilter configuration

2.5.1. Design and construction

Ten laboratory-scale vertical biofilters were established to investigate the impact of bed materials on wastewater treatment and to simulate the release pattern of nutrients from soil and biochar. These biofilters consisted of glass columns measuring 20 cm in height and 4 cm in diameter, with a total volume of 250 ml each. Each biofilter contained 180 grams of soil and biochar. The soil used in this research was sourced from Alcalá University's campus with a particle size smaller than 2mm. The biochars utilized were produced by the UK Biochar Research Center (UKBRC) from Oil Seed Rape (OSR). Two variants of biochar, labeled OSR 550 and OSR 700, were employed, derived at distinct pyrolysis temperatures of 550 and 700 degrees Celsius, respectively. We use a peristaltic pump to maintain wet media and water at a flow rate of 2 ml per hour.

This research also involved constructing five laboratory-scale vertical biofilters (Fig. 2.3). Each biofilter was a 2.5 L polyvinyl chloride (PVC) cylinder with a diameter of 90 mm and a height of 40 cm, featuring a downflow feed. The biofilter assembly began with 0.2 L of 2-3 mm dimension gravel at the bottom, followed by 1.5 liters of EC-biochar (powder sawdust biochar (0.1-0.075 mm)) for one pair of biofilters. Another pair of biofilters included 0.2 liters of gravel at the bottom and 1.5 liters of EC-biochar mixed with 10% humus, with a total volume of 1.7 L for all biofilters. Additionally, an identical biofilter comprised of inert gravel served as the control. The bottom of the biofilters was reinforced with a mesh with 0.1 cm holes to retain materials and enhance airflow. The mesh facilitated water drainage into the bio tank and allowed air circulation through the media. The rate was then increased to 3 liters per day, and this continued until the experiment ended after 170 days.

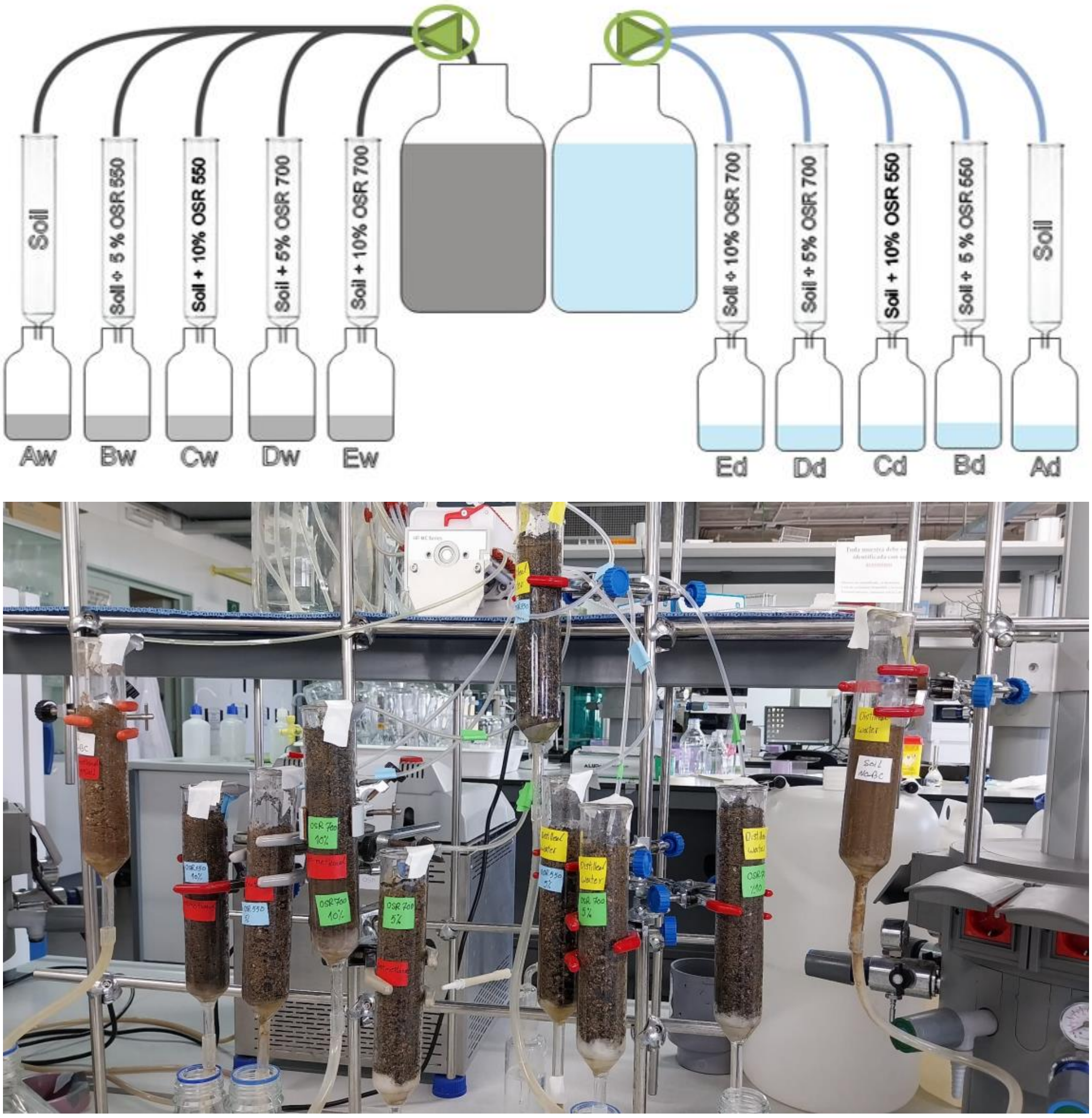


Figure 2. 2. Schematic Representation of the vertical biofilter (VF Soil Filter)

Figure 2. 3. METland® Downflow Biofilters (VF MET)

2.5.2. Operation of the biofilters

Two separate reactor configurations were used to carry out a wide range of experimental setups. Chapter 4 focuses on vertical biofilters (VF Soil Filter) which employ soil and biochar for their operation. We initiated the experiments by meticulously saturating the soil and biochar with 120 ml volume of deionized (DI) water. The water was added until it reached the level of the top of the soil surface, after which any excess water was immediately drained.

In our experimental setup, we employed a low-flow pump to ensure a gradual passage of water through the soil at a slow rate. This method was designed to mimic the leaching of nutrients, maintaining a consistent and evenly wet environment for the soil throughout the simulation.

This study employed two different types of wastewaters. Initially, brewery wastewater with high chemical demand (COD) was used; however, after one month, low-concentration domestic wastewater was used. During the 16-week study period, major ion analysis was performed weekly for each of the ten VF Soil Filter. To evaluate the nutrient release patterns and interactions in soil-biochar mixes, both main cations and anions were assessed. The influent water for the wastewater-fed columns was measured weekly to determine the initial nutrient levels prior to interaction with the soil-biochar mixture.

To assess nutrient release patterns, we utilized ten columns labeled as A, B, C, D, and E, which were irrigated with deionized water and denoted as Ad, Bd, Cd, Dd, and Ed, respectively. Additionally, five columns were supplied with wastewater and labeled as Aw, Bw, Cw, Dw, and Ew (Figure 2.2).

The vertical METland® biofilter (VF MET) was utilized in this research to evaluate how different materials mixed with biochar can impact wastewater treatment, as elaborated in Chapter 6. It operated using two types of wastewaters: brewery wastewater for up to 140 days and wastewater from Alcala University's campus for up to 170 days. In these biofilter experiments, the removal efficiency of ammonium and COD was assessed by computing the ratio of the different concentrations in the bio tank. Measurements were taken at four intervals: 70 days, 120 days, 140 days, and 170 days. Continuous measurements were conducted and recorded 24 hours a day, with readings taken every two hours throughout a 12-hour period. The final measurement was recorded after 24 hours.

2.6. Monitoring water quality for reuse

Evaluating the quality of reused water requires a thorough examination of numerous critical factors, including the Sodium Adsorption Ratio (SAR), Soluble Sodium Percentage (SSP), Sodium Percentage (Na^{+}%), Magnesium Hazard (MH), Kelly's Ratio (KR), and Residual Sodium Carbonates. These measurements take into account ion concentrations (meq/L), Potential salinity (PS) (mmol/L), and as well as total hardness (TH) and total dissolved solids (TDS) concentrations (mg/L). This assessment includes both the effluent from biofilters mentioned in Chapters

4 and 6, as well as treated wastewater from METland®, which are used in sunflower growing as detailed in Chapter 5.

-Sodium adsorption ratio (SAR)

The Sodium Adsorption Ratio (SAR), as defined by Ayers and Westcot (1985), has a significant impact on water infiltration rates into crops and soil permeability (Ayers and Westcot 1985). Our study estimated SAR using Equation (2):

$$SAR = Na^{+}/\sqrt{(Ca^{2+} + Mg^{2+})/2} \qquad \text{Eq2}$$

-Soluble sodium percentage (SSP)

The presence of sodium in irrigation water can hinder the rate at which water penetrates the root zones, consequently influencing plant growth. The Soluble Sodium Percentage (SSP) is of considerable importance as it is closely linked to the quality of irrigation water and its effect on soil permeability (Nagaraju et al. 2006). In our study, we computed SSP using Equation (3):

$$SSP = ((Na^{+}) / (Ca^{2+} + Mg^{2+} + Na^{+})) \times 100 \qquad \text{Eq 3}$$

-Sodium percentage (Na^{+}%)

Another vital factor in assessing water suitability for irrigation purposes is the sodium percentage (SP). The Na% (Wilcox 1955) is calculated using Equation (4):

$$Na\% = ((Na^{+} + K^{+}) / (Ca^{2+} + Mg^{2+} + Na^{+} + K^{+})) \times 100 \qquad \text{Eq 4}$$

Material and Methods

-Magnesium hazard (MH)

Increased levels of magnesium in irrigation water can result in heightened soil alkalinity and diminished crop yields. To gauge the possible consequences of magnesium presence in irrigation water, the magnesium hazard (MH) is computed using Eq. (5) (Szabolcs and Darab 1964). Paliwal (1972) introduced the magnesium hazard (MH) index to assess whether magnesium levels in irrigation water might negatively impact soil and crops (Paliwal 1972). This index aids in evaluating the potential risks linked to magnesium content in irrigation water.

$$MH = (Mg^{2+} / (Ca^{2+} + Mg^{2+})) \times 100 \qquad \text{Eq 5}$$

-Kelly's ratio (KR)

Kelly's ratio, introduced by Kelly in 1963, acts as a measure for assessing the suitability of groundwater for irrigation (Kelley 1963). It is determined using Eq. (6), where the concentration of Na^+ is compared to Ca^{2+} and Mg^{2+}. Water samples with Kelly's ratio exceeding 1 are typically considered marginal for irrigation. Hence, the comparison between sodium concentration and the combined concentrations of magnesium and calcium ions in irrigation water is of crucial significance.

$$KR = Na^+ / (Ca^{2+} + Mg^{2+}) \qquad \text{Eq 6}$$

-Residual Sodium Carbonates (RSC)

To predict the supplemental sodium hazard associated with $CaCO_3$ and MgCO3, the widely used empirical approach proposed by Eaton in 1950 is used (Eaton 1950).This method computes residual sodium carbonates (RSC). The equation utilized for this computation is denoted as Eq. (7):

$$RSC = (CO_3^{2-} + HCO_3^{-}) - (Ca^{2+} + Mg^{2+}) \qquad \text{Eq 7}$$

-Total hardness (TH)

Total hardness (TH) in water is categorized into temporary and permanent forms. Temporary hardness arises from dissolved bicarbonates such as $CaHCO_3$ and $MgHCO_3$, which can be eliminated through boiling. On the contrary, permanent hardness results from substances like $CaSO_4$, $MgSO_4$, $CaCl_2$, and $MgCl_2$, which can be removed through ion exchange processes (Nag 2014). The calculation of total hardness (TH) in parts per million (ppm) was determined using Equation 8 (Todd and Mays 1980; Hem 1985).

$$TH = 2.5 \times Ca^{2+} + 4.1 \times Mg^{2+} \qquad \text{Eq 8}$$

-Potential salinity (PS)

PS evaluates the danger posed by high salt levels stemming from Cl^- and SO_4^{2-}, which can amplify the osmotic potential of the soil solution when soil moisture content dips below 50%. According to this parameter, water is divided into three categories: beneficial (<3 mmol/L), moderate (3–15 mmol/L), and not advisable (>15 mmol/L) (Delgado et al. 2010).

$$PS = Cl^- + \tfrac{1}{2} SO_4^{2-} \qquad \text{Eq 9}$$

-Total dissolved solids (TDS)

The salinity level in water is commonly assessed through its total dissolved solids (TDS), comprising both anions (negatively charged ions) and cations (positively charged ions). These dissolved solids contribute to changes in the water's color and properties. The connection between total dissolved solids and electrical conductivity (EC) holds significance. This relationship is expressed by the equation:

$$\text{TDS (mg/L)} = \text{EC (dS/m)} \times K \qquad \text{Eq 10}$$

2.7. Ecotoxicity assays

2.7.1. Green alga

Ecotoxicity assessments were conducted using the green alga Raphidocelis subcapitata, and the growth inhibition test followed a modified Organization for Economic Co-operation and Development (OECD) Test Guideline 201 open system (OECD, 2011), consistent with prior research (González-Pleiter et al. 2013). Each tested sample underwent four replicates, with randomized placement in each run, including blanks and controls devoid of pollutants. Nutrient solutions and organisms for the ecotoxicity tests were sourced from MicroBio Test Inc. (Belgium). This methodology was applied to assess the outflow of biofilter with soil and biochar in Chapter 4.

2.7.2. Photosynthetic efficiency (Chlorophyll fluorescence test)

Fluorescence parameters were evaluated using a portable modulated fluorimeter, the FMS-2 from Hansatech Instruments Ltd., UK, which encompassed both dark-adapted and light-adapted fluorescence assessments. This procedure involved acquiring fluorescence induction curves, commonly referred to as Kautsky's curves. Among the parameters determined were F0, representing minimal fluorescence with a modulated light pulse, and Fm, denoting maximal fluorescence following a saturation pulse. Additionally, the Fv/Fm ratio, serving as an indicator of the maximal quantum efficiency of Photosystem II (PSII), was computed, where Fv denotes the disparity between Fm and F0.

During steady-state photosynthesis under actinic light, the steady-state fluorescence yield (Fs) was gauged. Furthermore, following a subsequent saturation pulse when the plant was light-adapted, supplementary parameters were established. These included F0′ (light-adapted minimal fluorescence), Fm′ (light-adapted maximal fluorescence), and Fv′/Fm′, which reflects the efficacy of excitation capture by open PSII centers, with Fv′ representing the contrast between F0′ and Fm′. The comprehensive list of parameters measured in this study is presented in Table 2.1.

Table 2. 1. Common parameters of chlorophyll fluorescence and their interpretations

`	**Illustration/formula**
Fo	Minimal fluorescence (dark): Fluorescence intensity with all PSII reaction centers open in the non-energized state.
Fm	Maximal fluorescence (dark): This indicates the classical maximum fluorescence level observed in the dark-adapted state. It signifies the fluorescence intensity when all PSII reaction centers are closed.
Fo'	Minimal fluorescence (light): The fluorescence intensity observed when all PSII reaction centers are open in any light-adapted state.
Fm'	maximal fluorescence (light): Fluorescence intensity with all PSII reaction centers closed in any light-adapted state
Fs	Steady-state fluorescence: The fluorescence level immediately before the flash.
Fv	Variable fluorescence (dark)Maximum variable fluorescence in the state at which all non-photochemical processes are at their minima (Fm–Fo)
Fv'	Maximum variable fluorescence in any light-adapted state (Fm'–Fo')
QP	Photochemical quenching coefficient (Fm'–Fs)/(Fm'–Fo')
NPQ	Non-photochemical quenching of chlorophyll fluorescence (Fm –Fm')/Fm'
Fv/Fm	The maximal photochemical quantum efficiency of PSII (Fm–Fo)/Fm
φPSII	Quantum yield of PSII electron transport (Fm'–Fs)/Fm'

2.8. Validation of re-use applications and practical solutions

The practical applications of METland® outflow discussed in Chapter 5 primarily revolve around utilizing this water source for sunflower cultivation. This utilization extends into Chapter 6, where the method is implemented to address the irrigation needs and water shortages of the University of Alcala campus.

Material and Methods

2.8.1. Reuse water for crop irrigation in the presence/absence of electroconductive biochars

2.8.1.1. Case study area

The study, which lasted from June to September 2022, was carried out in a **12**× **6** m^2 area at the Scientific Research and Technological Institute (IMDEA) in Alcala, Madrid. Positioned at coordinates 40°30'49.12"N, 3°20'15.91"W.

It comprised twelve experimental plots, each measuring 3 × 2 m^2. These plots, labeled A through H, were selected to represent various experimental conditions for evaluating the effect of using raw and used biochar, as well as different treated water from the METland® system and tap water. The aim was to assess the multitude of factors influencing sunflower growth, development, and productivity.

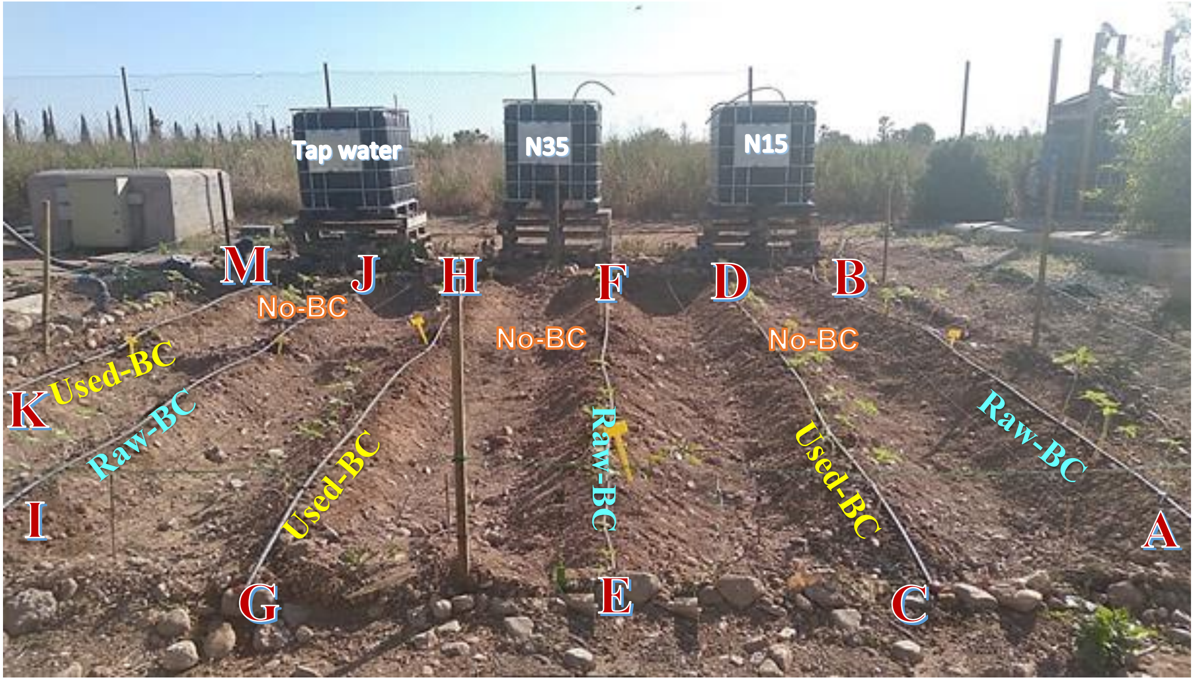


Figure 2. 4. Design and arrangement of lanes

2.8.1.2. Understanding the climate

The research region has a Mediterranean climate with hot summers (Csa). Alcala de Henares, Spain, receives an average annual precipitation of 267mm (10.51 inches), with roughly 91.7 days of rainfall reported throughout the year.

2.8.1.3. Seedling production and plant transplantation

Sunflower seedlings were cultivated in expanded polystyrene trays using coconut fiber and compost as substrate. During the early phases of development, the seedlings were simply irrigated with tap water. After 15 days of development, six sunflower seedlings were put into each plot. The initial irrigation was completed on the following day.

Material and Methods

2.8.1.4. Irrigation

Drip irrigation was employed for watering the sunflowers, utilizing driplines provided by Driplines-Caudal. These driplines are engineered to deliver water to the plants with precision and uniformity, ensuring optimal irrigation.

2.8.1.5. Reuse-water

For irrigation purposes, we utilized effluent from full-scale METland® systems situated at the facilities of IMDEA Water in Alcalá de Henares, Spain.

We designated twelve lanes for irrigation using three different types of water: treated water with two nitrate concentrations (N35 with 35 ppm NO_3^- and N15 with 15 ppm NO_3^-) and tap water. To assess the irrigation water quality, we collected samples on five separate occasions during the reload process for water tanks. These samples were analyzed for concentrations of main cations and anions.

2.8.1.6. Measuring dried cluster

After three months, the sunflower harvest was carried out. To assess the dried biomass, the sunflower clusters were arranged for drying in ambient room conditions with suitable airflow. Once fully dried, their weights were measured and recorded to determine the dried biomass of the sunflowers for subsequent analysis and comparison between different cultivation conditions.

2.8.2. Water scarcity on the external campus of the University of Alcala

2.8.2.1. Study area

This research was carried out at the University of Alcalá in Alcalá de Henares, a city 35 kilometers (22 miles) northeast of Madrid, Spain.

2.8.2.2 Water resources and demand

2.8.2.2.1. Availability of groundwater on campus

The water resources on the university campus, including the total water yield from wells and the allowable amount of water for each well, are detailed in Table 2.2.

Table 2. 2. Overview of Campus Water Resources: Insights from Botanic Garden Report

Well Number/Name	**m^3/year**			**Authorized C.H.T. (m^3/year)**
	2014	**2015**	**2016**	
Number 1 (P1. pozo del vivero)	52	757	490	3400
Number 2 (P2. pozo del lago-1 &lago-2)	9775 +20	3957+30	5450	4000
Number 3 (P3. pozo de francisco)	4617	9304	10421	-------
Number 5 (P5. pozo de ciencias)	384	2967	3423	1080
S5. sondeo de ciencia	609	8390	6331	600
Qanat (ciencias)	12794	2618	2251	-------
S2. sondeo de la capilla	0	0	879	2800
Total annual	18456	27996	29245	11880

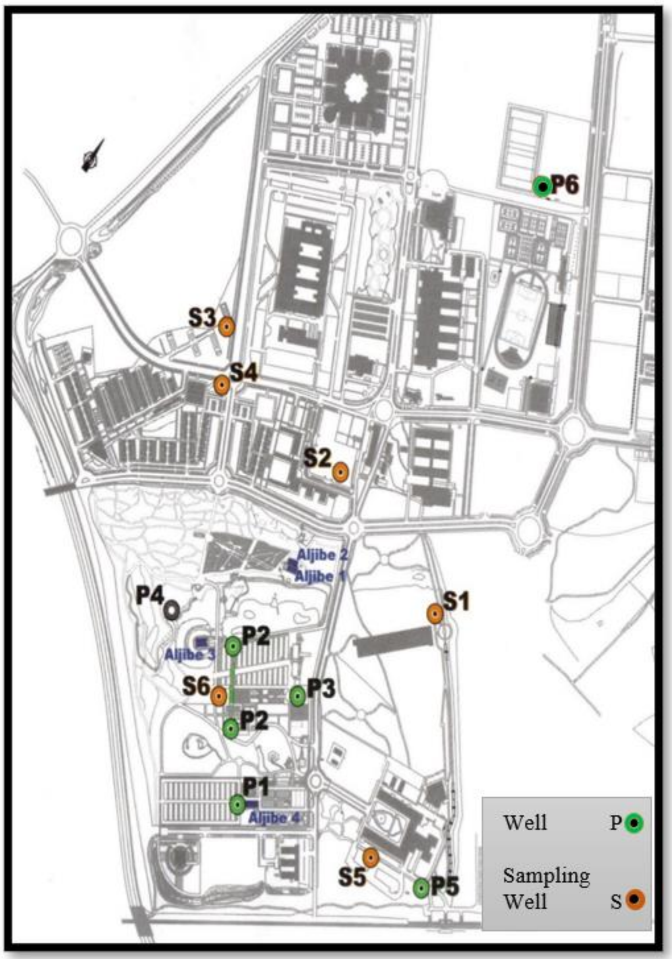


Figure 2. 5. Map indicating the locations of wells on the campus of University pf Alcalá

2.8.2.2.2. Water demand

The various water usage categories corresponding to distinct green spaces are depicted in Table 2.3. The table showcases the total amount of each consumption category, area covered, and annual consumption. On campus, there are several consumption rates including high, medium-high, medium-low, low, and no watering. The specific requirements and regions for each category are outlined in Table 2.3. The total area of green space under irrigation spans 36 hectares. Additionally, some green spaces receive tap water irrigation for which there are no records available.

Table 2. 3. Consumption of water for irrigation purposes on campus (based on Botanic Garden Report)

Consumption Type	**Demand(m^3/ha/year)**	**Area (ha)**	**Consumption (m^3/year)**
High consumption	2000	5.360	10719.60
Medium-high consumption	1500	4.004	6005.70
Medium-low consumption	1000	8.085	8085.40
Low consumption	500	18.113	9056.45
Replacement of ponds	250	0.053	13.33
Null or occasional watering	0	0.426	0
Total		36.041	33880.48

2.8.2.3. Existing irrigation reservoirs

Reservoirs located on the Alcala University campus were shown in Figure 2.6. along with their specifications outlined in Table 2.4. The collective capacity of these reservoirs is approximately 2100 m^3. It's worth noting that there is a lack of available information regarding the dimensions and capacity of reservoir 5. Furthermore, due to damage to the wall of reservoir 4, its capacity has been diminished to 150-200 m^3. Consequently, the estimated total storage capacity for irrigation water stands at 1800 m^3.

Figure 2. 6. Reservoirs located on the external campus at University of Alcalá

Table 2. 4. Characteristics of Reservoirs Located on the Alcala University Campus

Name	Construction	Dimensions	Capacity	Description
Lago	buried	15×5×4	300	Irrigation of the campus and the Botanical Garden
Higueras	buried	25×10×3.2	800	Irrigation of the campus and the Botanical Garden
Auditorio	buried	18×9×3	500	Irrigation of the campus and the Botanical Garden.
Vivero	buried	25×10×2	500	Nursery irrigation.
Crusa	buried	-------	-------	Exclusive for irrigation of the residential development. Compartmented with a fire-fighting tank

2.8.2.4. Assessing water distribution system capacity with EPANET 2.2

In this study, EPANET 2.2 software was employed to evaluate the capacity of the existing water distribution pipes to meet current demand. The physical properties of the network, including pipe lengths, diameters, roughness coefficients, and node elevations, were input into the model. Junctions and reservoirs were accurately represented, and the total water demand was considered to assess the transfer capability of the existing pipes (chapter 6).

2.9. Hydroponically growing lettuce with biofilter outflow and chlorophyll fluorescence testing

2.9.1. Hydroponic cultivation

Lettuce seeds were carefully planted in a controlled environment within a culture chamber, maintaining a steady temperature of 18°C. A 16-hour exposure to daylight was established each day, with watering conducted using tap water. After a two-week germination period, the young seedlings were transferred into plastic containers with a volume of 700 ml. Each container housed five hydroponic glasses, with each glass containing two lettuce seedlings. Pebbles were used as a supportive medium for the plant bed within each glass (Chapter 4). In another hydroponic experiment, three containers were employed, each containing four hydroponic glasses. Within each glass, two lettuce plants were cultivated and supported using sponges. The nutrient solution utilized originated from the effluent of the VF MET biofilter. Among these three containers, one served as a positive control containing fertilizer, one acted as a negative control containing nutrient-free water, and one utilized the effluent from the METland® system, as outlined in Chapter 6.

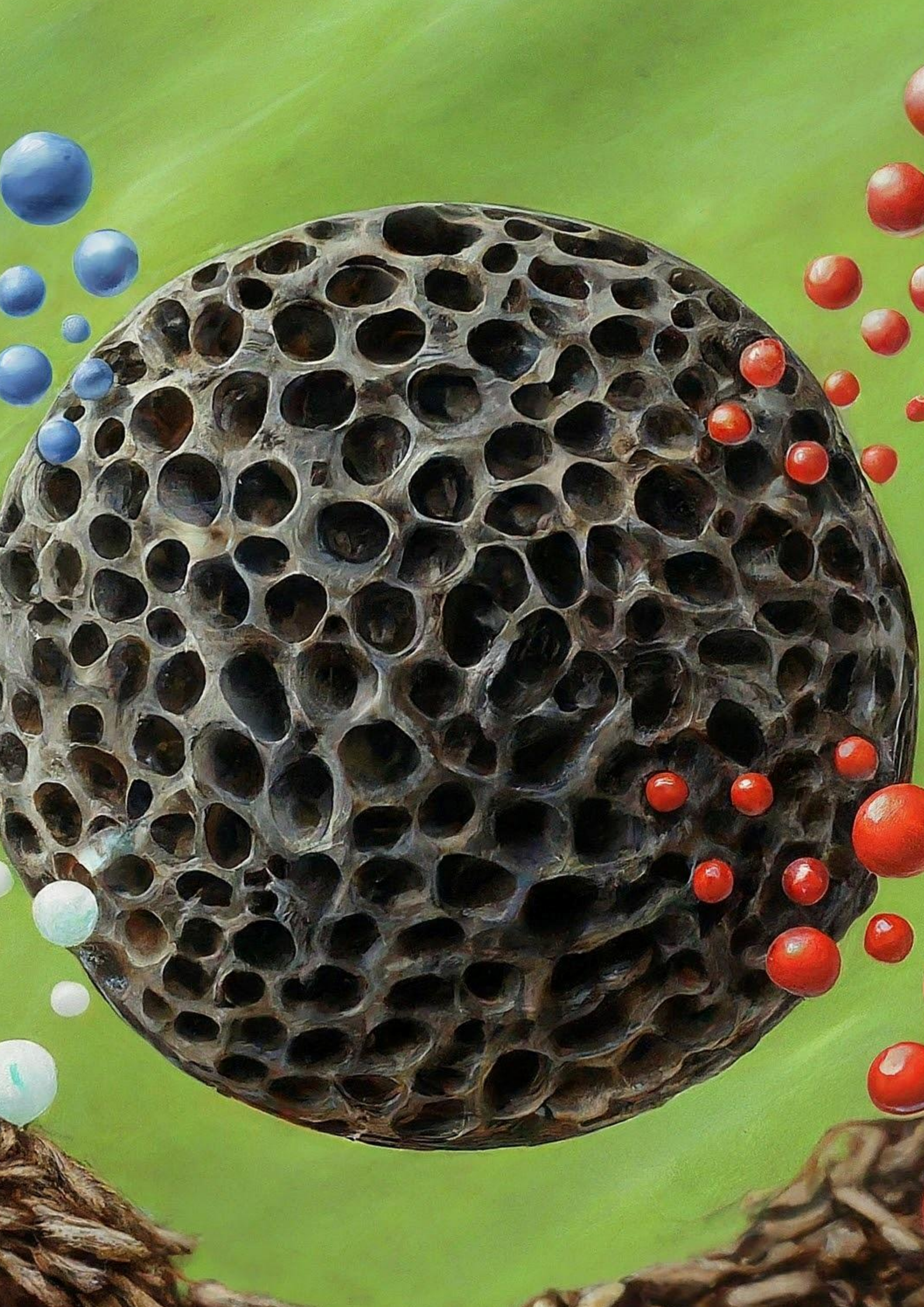
2.9.2. Measurement of fresh and dry plant weights

Two weeks after the lettuce development cycle began, we collected plant biomass from the pots. The harvested plants were then weighed to determine their fresh biomass weights, as discussed in Chapters 4 and 6. Subsequently, we employed a two-day air-drying method at room temperature to determine the dried biomass, as detailed in Chapter 4.

2.9.3. Measuring dimension of leaf and root

For assessing leaf and root dimensions, plant photography was utilized, and ImageJ software was employed for analysis to measure the dimensions of leaves and root lengths (Chapters 4 and 6).

Chapter 3:
Turning Wastewater into Fertilizer: Biochar for Nutrient Reclamation



3. Turning Wastewater into Fertilizer: Biochar for Nutrient Reclamation

Converting nutrients from wastewater into soil fertilizers is a major challenge in promoting the circular economy. This challenge is further limited by factors such as unregulated wastewater release, inadequate access to fertilizers in underdeveloped regions, and the high costs of fertilizers (Saliu and Oladoja 2021). Soil amendment with biochar is recommended as a global method to counteract climate change and soil degradation by sequestering carbon, lowering soil greenhouse gas emissions, and improving nutrient retention. According to studies, biochar enhances plant development, especially when paired with nutrient-rich organic matter. This growth enhancement is linked to the gradual release of nutrients, while the processes of nutrient storage in biochar need to be further investigated (Hagemann et al. 2017).

In agriculture, chemical fertilizers are commonly used to address soil deficiencies in nitrogen (N), phosphorus (P), and potassium (K). However, a significant portion of these fertilizers is lost through runoff or volatilization. According to estimates, approximately 40–70% of nitrogen, 80–90% of phosphorus, and 50–70% of potassium applied as fertilizers are lost to the environment. This loss not only results in economic losses for the farmer but also contributes to environmental pollution (Duhan et al. 2017).

A natural strategy to improve the availability of fertilizers in soil is the use of biochar. This material is a porous solid material rich in carbon, resembling charcoal, and characterized by its black color. It is typically generated through the thermochemical conversion of biomass, often in environments with minimal or no oxygen present (Ahmad et al. 2014). Biochar shows some natural content in nutrients so it can can perform slow-release fertilizers (SRFs) can supply nutrients for crop development for the entire growing season following a single application. Moreover, they mitigate the stress and potential toxicity resulting from an overabundance of nutrients in the root zones (Kim et al. 2018). Interestingly, some authors have considered the possibility of artificially enriching biochar in nutrients for enhancing plant growth. Thus, applying organic substances to biochar surfaces improved microporosity and improved the biochar's ability to retain nutrients and water in the soil (Hagemann et al. 2017). Applying organic matter to biochar looks to be a potential method for increasing its effectiveness in low-fertility soils. Organic molecules on the top of biochar operate as an adhesive, trapping dissolved nutrients in the soil (Conte and Laudicina 2017).

In this context of using nutrient-enriched biochar for agriculture purpose, biochar may play a critical role in extracting nutrients from wastewater while boosting microbial biodegradation in biofiltration systems so-called METland®. Thus, such strategy would, provide a dual solution for waste management and agricultural sustainability.

This chapter aims to explore the capacity of different types of electroconductive biochar for adsorption of nutrients from synthetic and real urban wastewater. A complete series of analysis assessed the different adsorption and release properties while discussing the potential use of the material.

3.1. Short-term adsorption test

A batch adsorption experiment, often referred to as an immersion experiment, is a widely used method for evaluating adsorption equilibrium and kinetics from liquid solutions (Brandani 2020) from both synthetic and real urban wastewater (Table 3.1).

Table 3. 1. Main cations and anions in wastewater & Synthetic Solution (ppm)

Description	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**NO_3^-**	**PO_4^{3-}**	**SO_4^{2-}**
Wastewater	112.00	177.00	23.02	10.61	226.03	191.11	56.15
Synthetic Solution	1.18	154.03	19.82	6.98	156.24	190.18	8.42

The adsorption capacities of the four tested biochar samples, namely Miscanthus Straw (MSP), Oil Seed Rape Straw (OSR), Soft Wood (SWP), and

Wheat Straw (WSP), were found to vary significantly. The OSR biochar demonstrated a phosphate adsorption capacity of 4.90 mg/g. Conversely, the WSP biochar exhibited the highest adsorption capacity for potassium removal at 2.48 mg/g, and both OSR 550 and SWP 550 biochar showed slight nitrate adsorption at just 0.02 mg/g under wastewater conditions. In a synthetic solution, the OSR 550 biochar showed a phosphate adsorption capacity of 1.93 mg/g and nitrate adsorption of 0.55 mg/g, with no potassium adsorption. Comparing these four biochar samples provides valuable insights into selecting the most suitable biochar for further long-term adsorption.

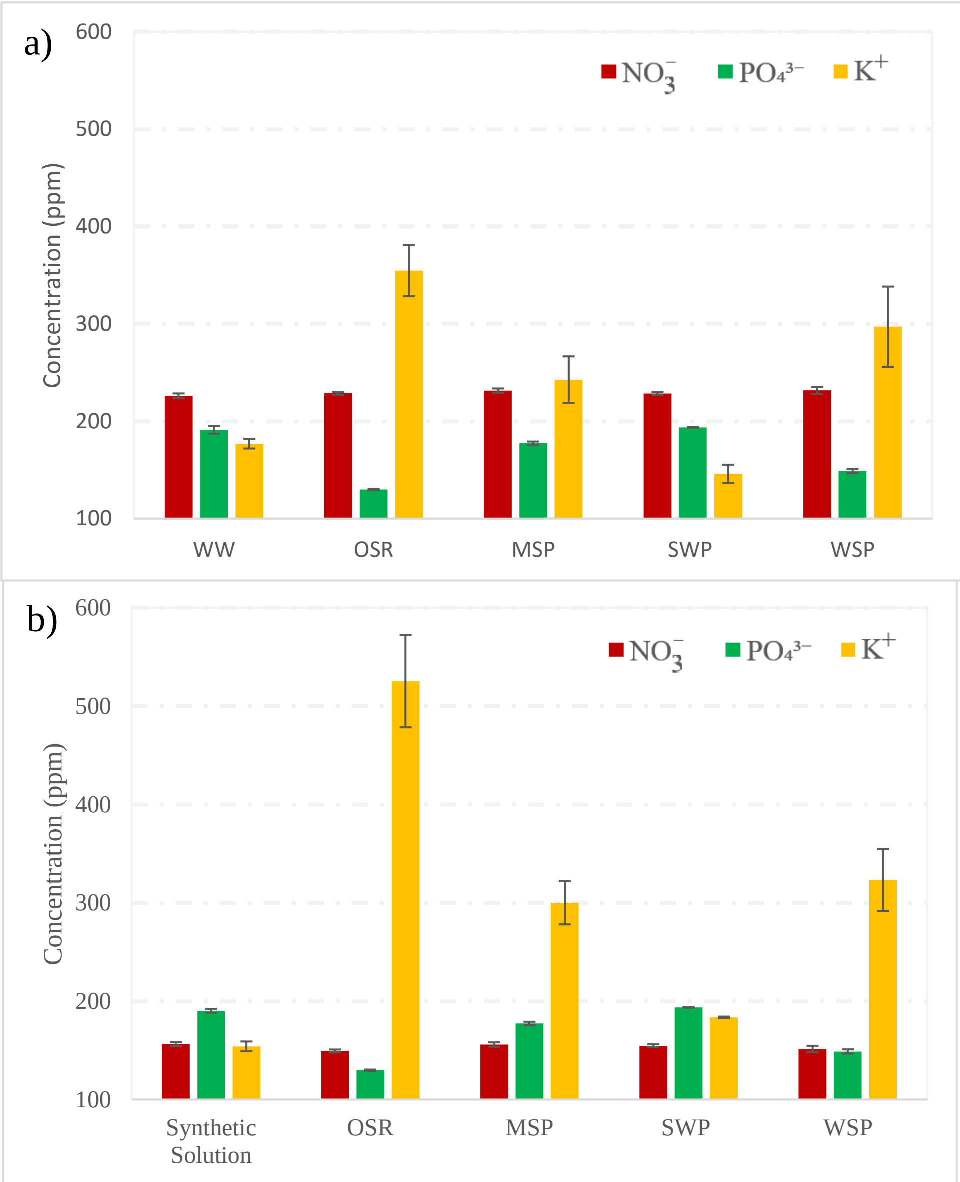


Figure 3. 1. Amount of nitrate, phosphate, and potassium adsorbed by biochar after 24 hours incubation with (a) urban wastewater and (b) synthetic solution

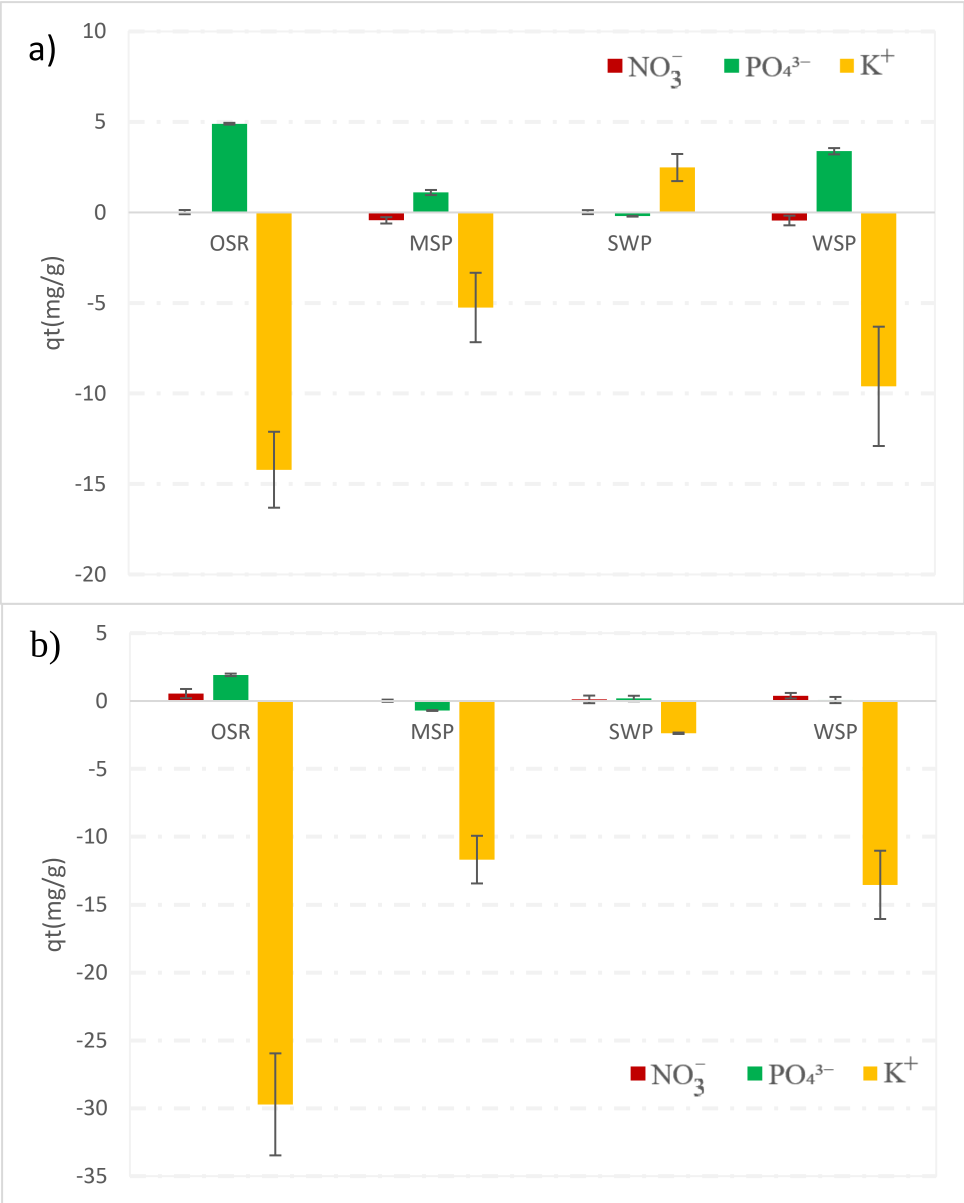


Figure 3. 2. The amount of adsorption and release of nitrate, phosphate, and potassium in the batch adsorption experiment after 24 hours of influent wastewater (a) and synthetic solution (b) (mg/g)

3.2. Long-term Adsorption

Building upon the short-term adsorption findings, the research delved deeper into the long-term adsorption properties of two distinct biochar types: OSR 550 and SWP 550. Over a month period, both materials were evaluated regarding adsorption efficiency for nitrate, phosphate, and potassium.

3.2.1. Adsorption of nitrate

The findings on nitrate adsorption using two types of biochar, OSR 550 and SWP 550. Nitrate levels in batch bottles were monitored over a span of 33 days to observe the daily absorption patterns of these biochars. Figure 3.3 presents the adsorption trends over this period. The overall nitrate adsorption capacities of the two biochar types were very similar: OSR biochar absorbed a total of 5.74 mg/g, while SWP 550 biochar had a slightly lower total absorption of 5.64 mg/g.

Additionally, it's important to note that the quantities mentioned above account for both adsorption and release over the course of a month, considering the daily fluctuations in nitrate concentrations that the biochar encounters in the batch. This scenario simulates biochar's role as a medium in biofilters for treating wastewater.

While Soft Wood biochar SWP 550 initially demonstrates superior nitrate adsorption according to short-term adsorption results, OSR 550 exhibits better long-term adsorption compared to SWP 550. This indicates that the adsorption behavior of biochar differs between short-term (24 hours) and long-term periods.

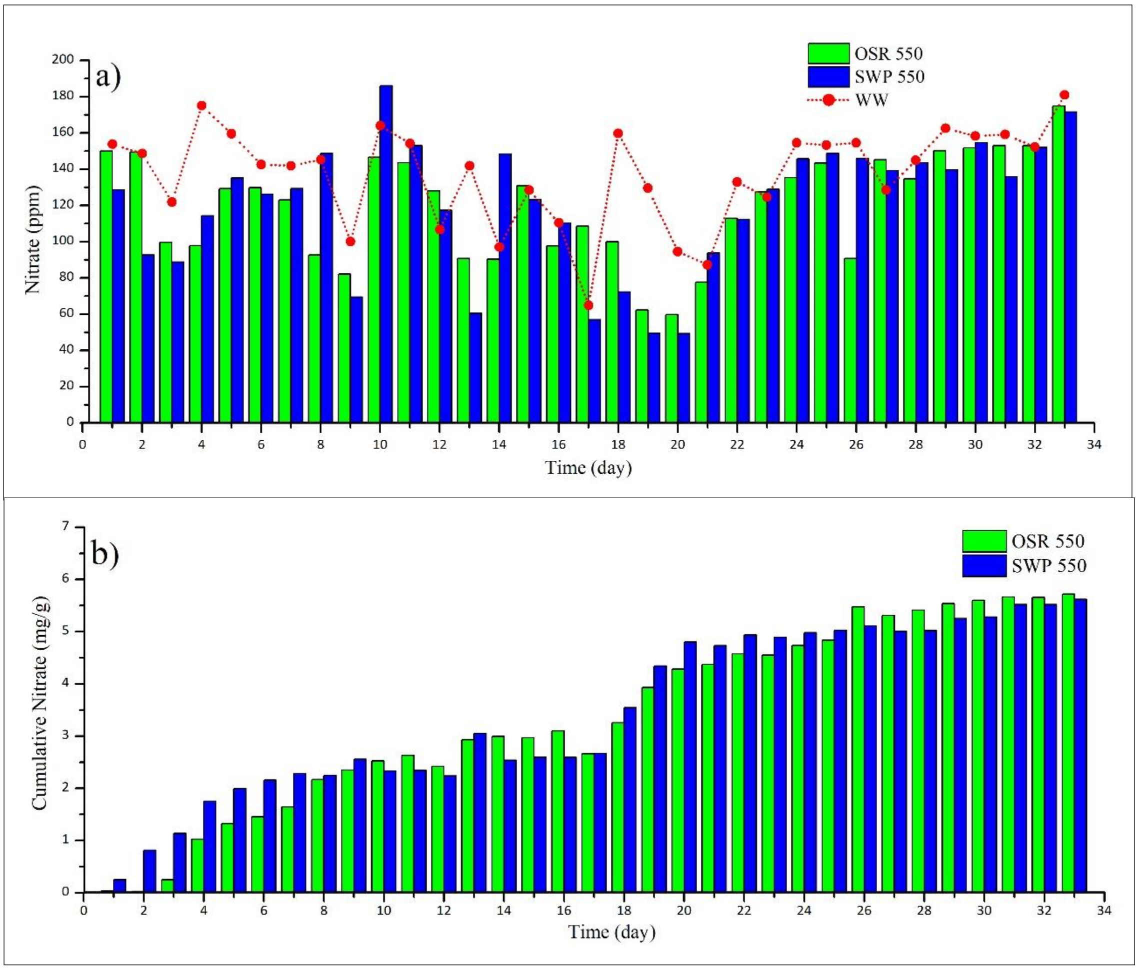


Figure 3. 3. Levels of nitrate concentration (ppm) observed in wastewater (WW) in comparison to concentrations found in batch bottles containing OSR and SWP biochar (a) and cumulative adsorption of nitrate (mg/g) (b)

3.2.2. Adsorption of phosphate

The study revealed that OSR 550 had a total phosphate adsorption capacity of 5.2 mg/g. Graph 3.2b showed that SWP biochar exhibited variable phosphate adsorption and release patterns across different days. Furthermore, the cumulative phosphate graph indicated that the specific SWP biochar used did not contribute to any adsorption.

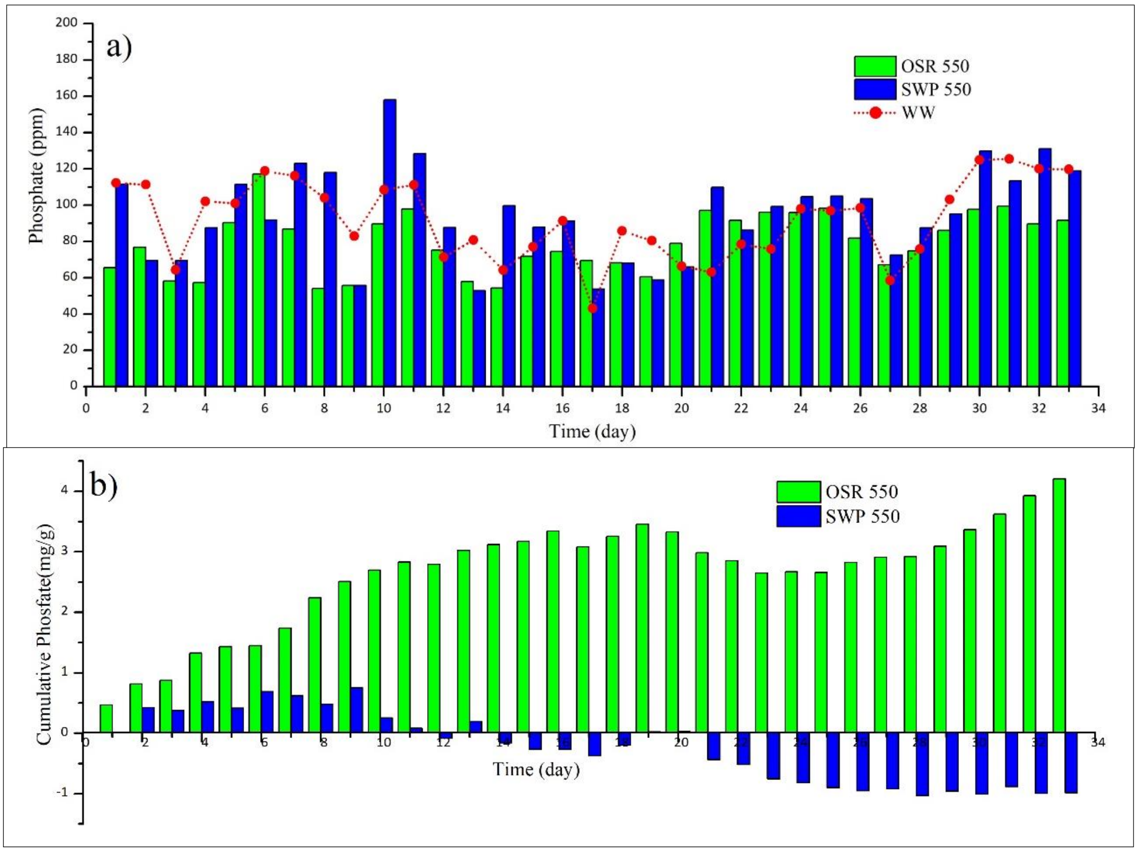


Figure 3. 4. levels of phosphate concentration (ppm) observed in wastewater (WW) in comparison to concentrations found in batch bottles containing OSR and SWP biochar (a)) and cumulative adsorption of phosphate (mg/g) (b)

3.2.3. Adsorption of potassium

The experiment on adsorption of potassium using two different biochar types, OSR 500 and SWP 500, was conducted, and the results are presented in graph 3.3. Biochar OSR 500, derived from oilseed, contains a significant amount of potassium and initially releases a large quantity of potassium within the first few days, as shown in graph 3.3a. During the experiment, potassium is continuously released, with minor adsorption observed only on the 5th day, totaling 1.7 mg/g, followed by further release. On the other hand, biochar SWP 550, obtained from soft wood, exhibits an initial release of potassium within the first four days, followed by adsorption on day 5, and then another release. During the 33-day adsorption period, significant adsorption events are observed on 11 days, totaling 4.52 mg/g, while the remaining days show biochar release. It is noteworthy that the total adsorption capacities for both biochar types are zero.

These experiments demonstrate the differences in adsorption of potassium by two distinct biochar types, with biochar derived from oilseed OSR 550, which is rich in potassium, showing a higher initial release of potassium, and the soft wood biochar exhibiting higher adsorption capacity over time. The results of high adsorption capacity with soft wood biochar in this period rely on the results of short-term adsorption.

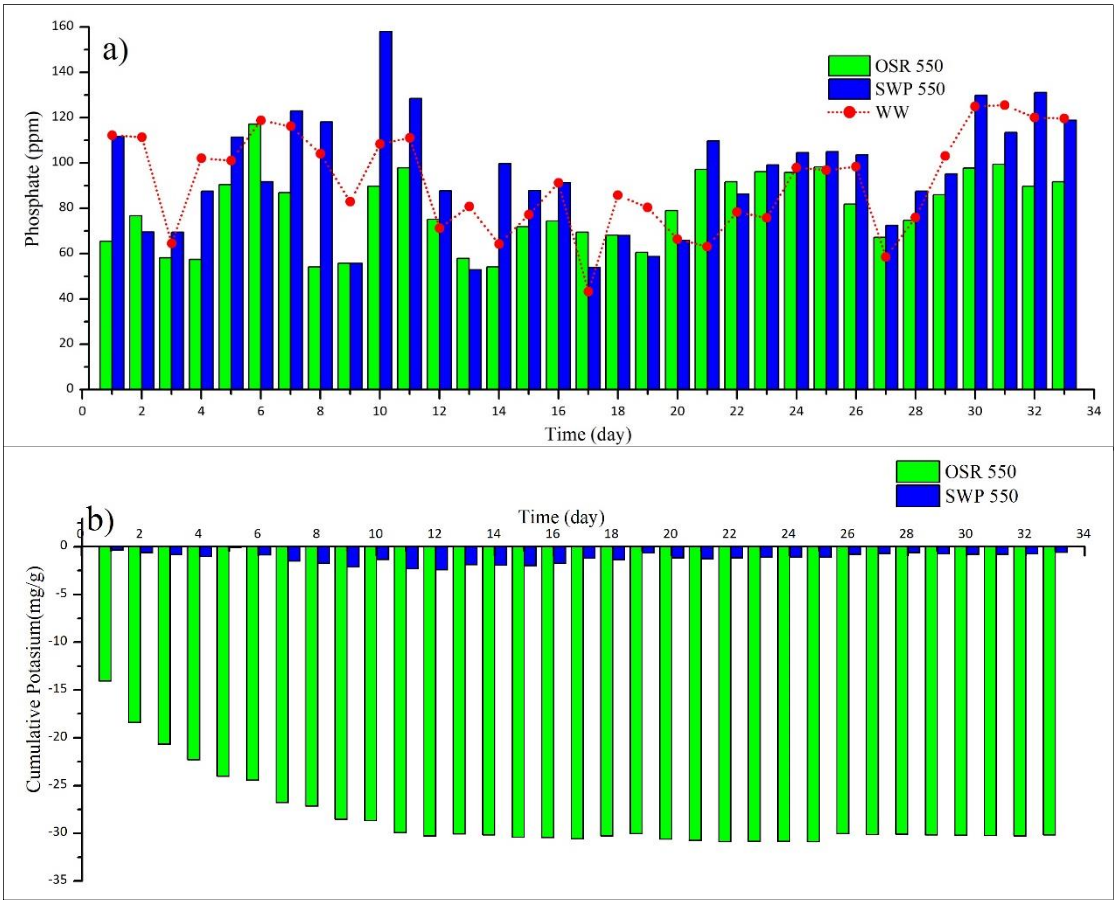


Figure 3. 5. Levels of potassium (ppm) observed in wastewater (WW) in comparison to levels measured in batch bottles containing OSR 550 and SWP 550 biochar (a) and (b) cumulative adsorption of potassium (mg/g)

3.3. Nutrient release

The investigation focused on the release of nitrate, phosphate, and potassium from biochar subjected to a 33-day wastewater incubation batch. A 60-day water-incubation batch experiment was conducted to simulate the release behavior of these nutrients from both used and raw biochar. Daily measurements of nutrient release were recorded during the first week, on day 10, and then weekly for two months.

3.3.1. Release of nitrate

Figures 3.6a and 3.6b depict the nitrate release from raw and used biochar. for OSR and SWP. It was observed that raw biochar did not release any nitrate, while the total nitrate released from used biochar was 1.84 mg/g and 1.64 mg/g for used-OSR and used-SWP, respectively. Upon comparing this to the nitrate adsorption with OSR 550 at 5.74 mg/g and with SWP 550 biochar at 5.64 mg/g, it was found that only 32% and 29% of the total nitrate in used biochar was released. This indicates the slow-release biochar characteristic.

The nutrient release pattern from nutrient-enriched biochar varies over time, forming a slow-release pattern. In a previous study, after 90 days of leaching, the amount of total release of NO_3^- was found to be 55.47–50.84% (Das and Ghosh 2021).

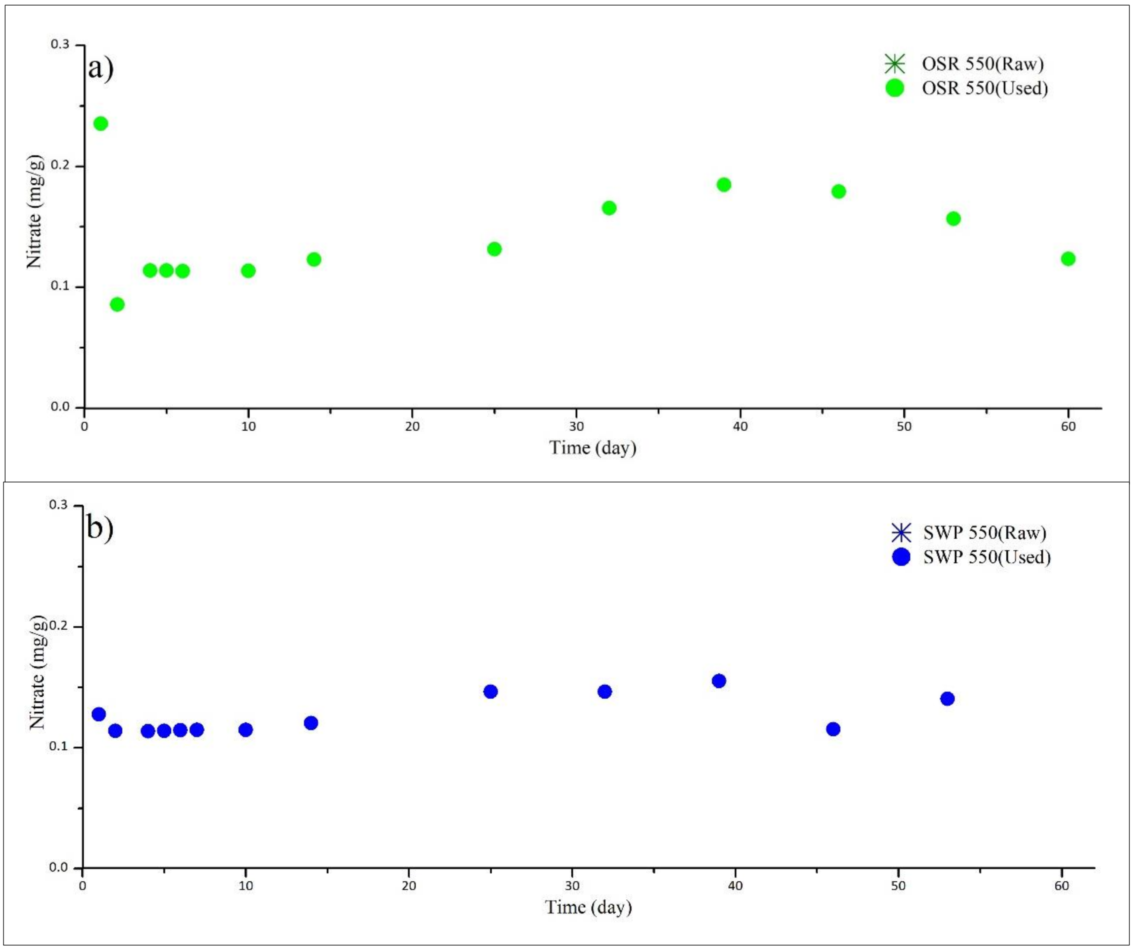


Figure 3. 6. Nitrate release of used and raw OSR 550 biochar(a) and SWP biochar (b) (mg/g)

3.3.2. Release phosphate

Phosphorus is crucial for life, but many soils lack available P, causing overuse of water-soluble P fertilizers. Most applied P is either lost to runoff or becomes unavailable in the soil. To improve efficiency and reduce environmental harm, P release from fertilizers should match crop needs, which can be achieved with slow-release fertilizers (Hart, Quin, and Nguyen 2004; Weeks and Hettiarachchi 2019).

The objective of the 60-day water-incubation batch experiment was to investigate the phosphate release from raw and used biochar samples. The purpose of this study was to simulate the release patterns of potassium from OSR 550 and SWP 550 biochar. The results of the experiment showed that the total phosphate release from OSR 550 raw biochar was 1.24 mg/g, whereas it was 0.98 mg/g for OSR 550 used biochar. It was observed that during the 60-day release period, 21% of the phosphate was released from used-biochar. It is important to note that the OSR used-biochar had a phosphate adsorption capacity of 4.5 mg/g over a 33-day period of adsorption test. These findings suggest that the biochar exhibits a slow-release behavior. Furthermore, release kinetic seem not be affected by the amount of phosphate adsorption in the material, either raw or used.

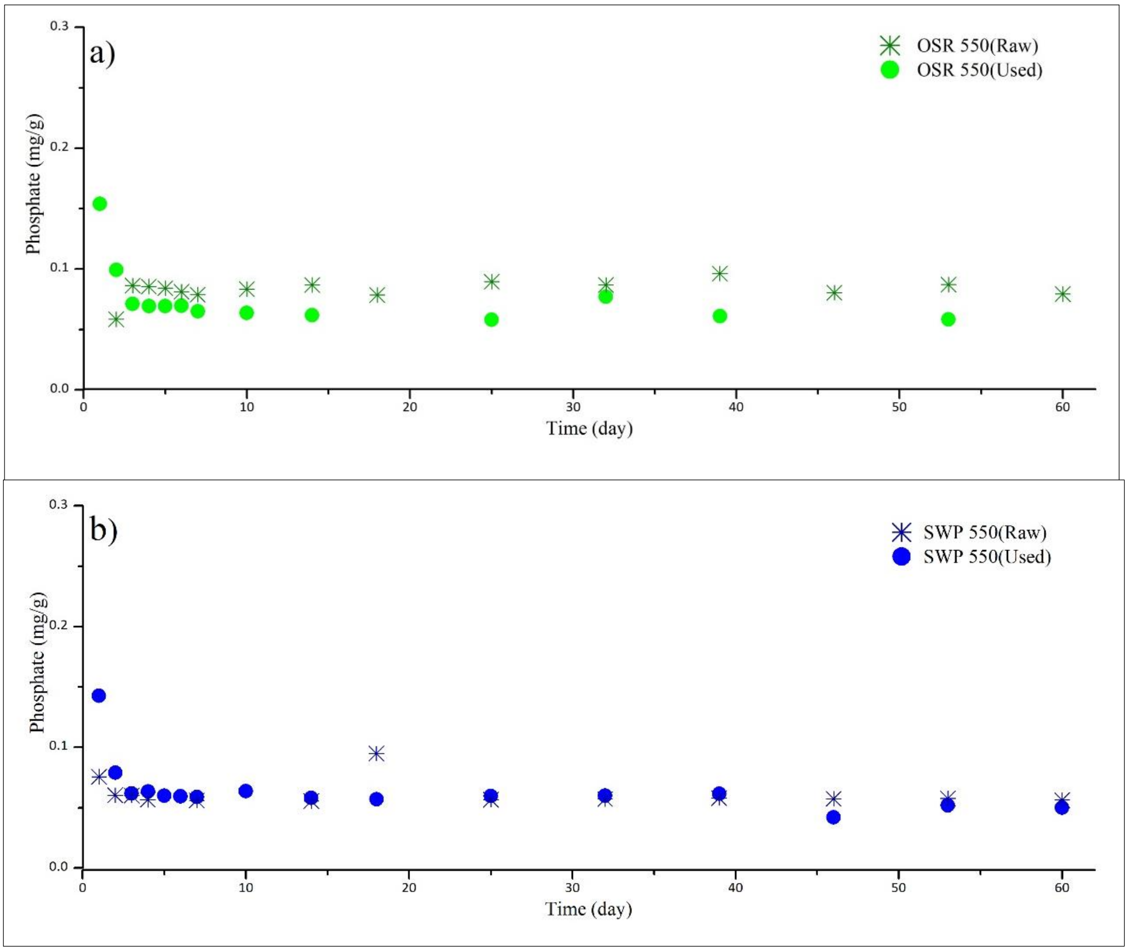


Figure 3. 7. Phosphate release of used and raw OSR biochar(a) and SWP biochar (b) (mg/g)

Regarding SWP 550 biochar, no detectable adsorption was detected during previous assays (Figure 3.4b). Then, overall release of approximately 1 mg/g observed after 60-day period should be primarily originated from phosphate within the original biochar matrix. Values for phosphate release from raw from used SWP biochar, was slightly higher than the one from raw SWP biochar (0.91 mg/g). It is worth noting that since the biochar had no adsorption, the release of phosphate from SWP 550 used-biochar.

3.3.3. Release potassium

To explore the release of potassium from raw and used biochar, a 60-day water-incubation batch experiment was conducted. Interestingly, there was no detectable adsorption of potassium during the previous incubation period for both OSR 550 and SWP 550 biochars. However, some potassium release was observed over the 60-day period, and it was directly related to the potassium content within the original biochar.

Analyzing the release pattern of potassium in OSR biochar, known for its high potassium content, revealed that potassium release from raw biochar occurred predominantly on the first day, accounting for approximately 60% of the total release that was about 42.75 mg/g. In contrast, potassium release from used biochar exhibited a different pattern, with the initial release on the first day constituting approximately 30% of the total release (2.82 g/mg). This release from used biochar primarily originated from potassium within the biochar matrix.

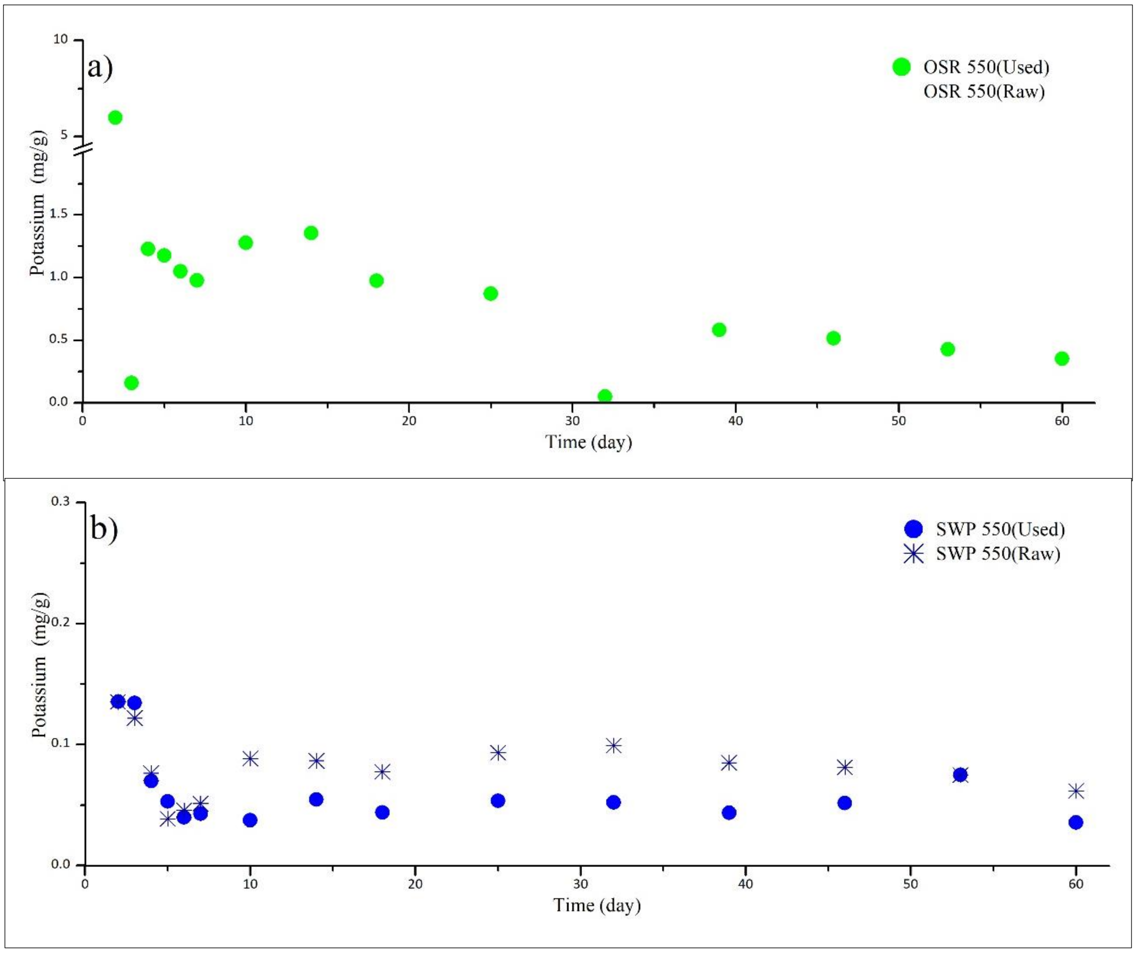


Figure 3. 8. Potassium release of used and raw OSR biochar(a) and SWP biochar (b) (mg/g)

3.4. Electroconductive biochar used in long-term real treatment of wastewater

In this section, we have characterized the release of nitrate, phosphate, and potassium from electroconductive sawdust biochar used in a real METland® for treating urban wastewater from Carrion de los Céspedes municipality for more than 3 years. Furthermore, this very same material was used as nutrient-amendment for growing sunflower crops as part of the research developed in Chapter 5 of the current thesis. As reference, raw electroconductive from same material was also evaluated through identical methodology.

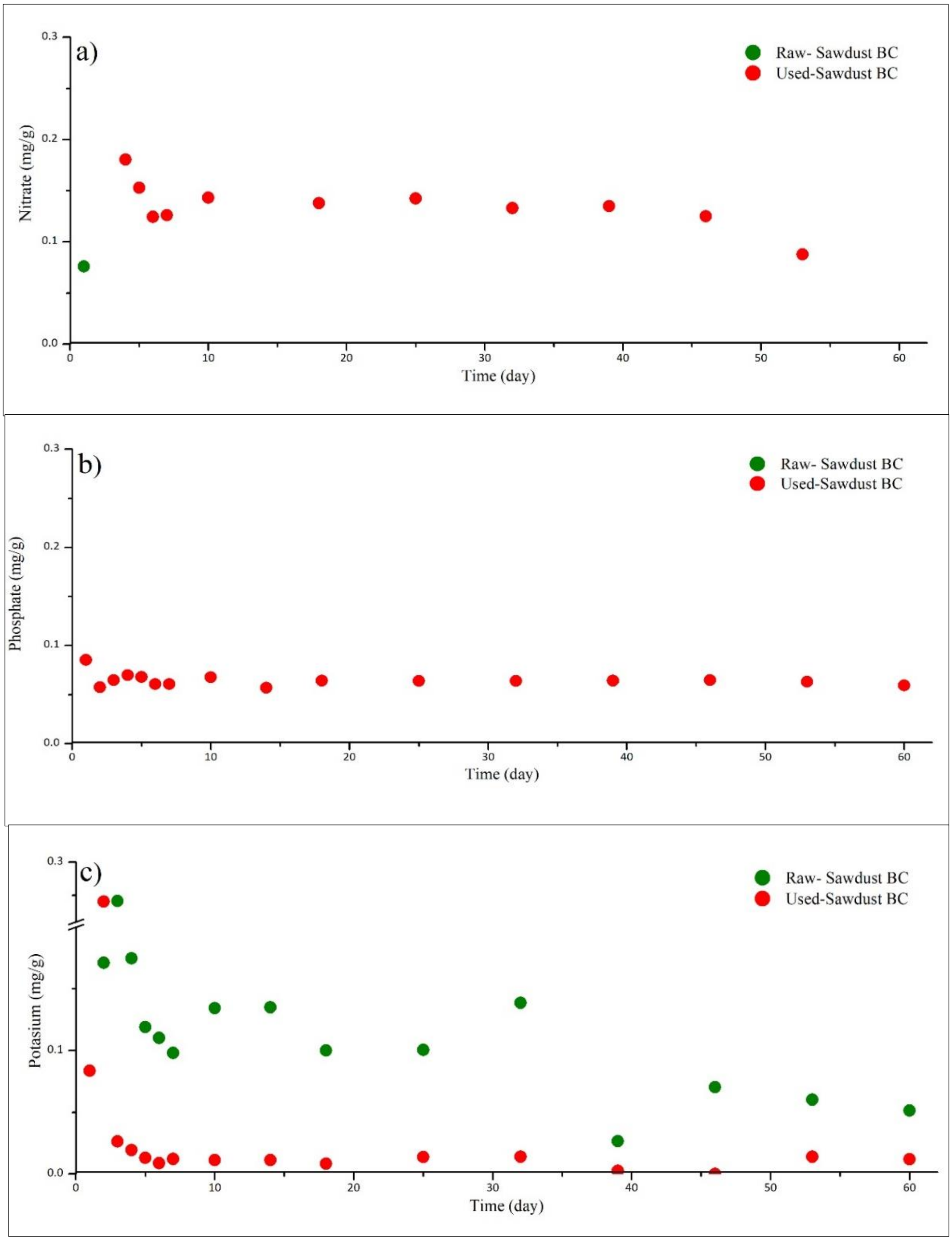


Figure 3. 9. Nitrate (a), phosphate (b), and potassium (c) release of used and raw sawdust biochar (mg/g)

3.5. Results of total nitrogen, total organic carbon, inorganic carbon, and total carbon

The following presents the findings regarding TN, TOC, IC, and total carbon from one day of released used and raw biochar. The results show that the total nitrogen content from 24-hour release in used-OSR is 3.5 times higher than in raw biochar, while in SWP biochar, it is twice as high as in raw biochar.

The discrepancy in Total Nitrogen (TN) content between used and raw biochar can be attributed to the biochar's exposure history and subsequent alterations in chemical composition. Biochar when applied in environmental settings such as wastewater treatment, undergoes complex interactions with organic and inorganic compounds present in the environment. These interactions can lead to the sorption and accumulation of nitrogen-containing compounds within the biochar matrix.

The higher TN content observed in used biochar samples compared to raw biochar can be explained by the prolonged exposure of the former to organic matter and microbial activity in the application environment. Microbial degradation of organic matter and the transformation of nitrogen-containing compounds contribute to the enrichment of TN in used biochar. Conversely, raw biochar, having undergone minimal exposure, exhibits lower TN levels due to limited interaction with environmental nitrogen sources. The higher TN content observed in used biochar compared to raw biochar suggests the accumulation of nitrogen-containing compounds during its previous application. These compounds may include organic nitrogen from organic matter decomposition and inorganic nitrogen from fertilizer residues or microbial activity.

Additionally, the disparity in Total Carbon (TC) and inorganic Carbon (IC) content and total carbon between used and raw biochar samples may indicate carbon loss through microbial degradation or chemical transformations during previous applications. Total carbon is 6 times and inorganic Carbon is 5 times higher in raw biochar of OSR and SWP compared to used biochar.

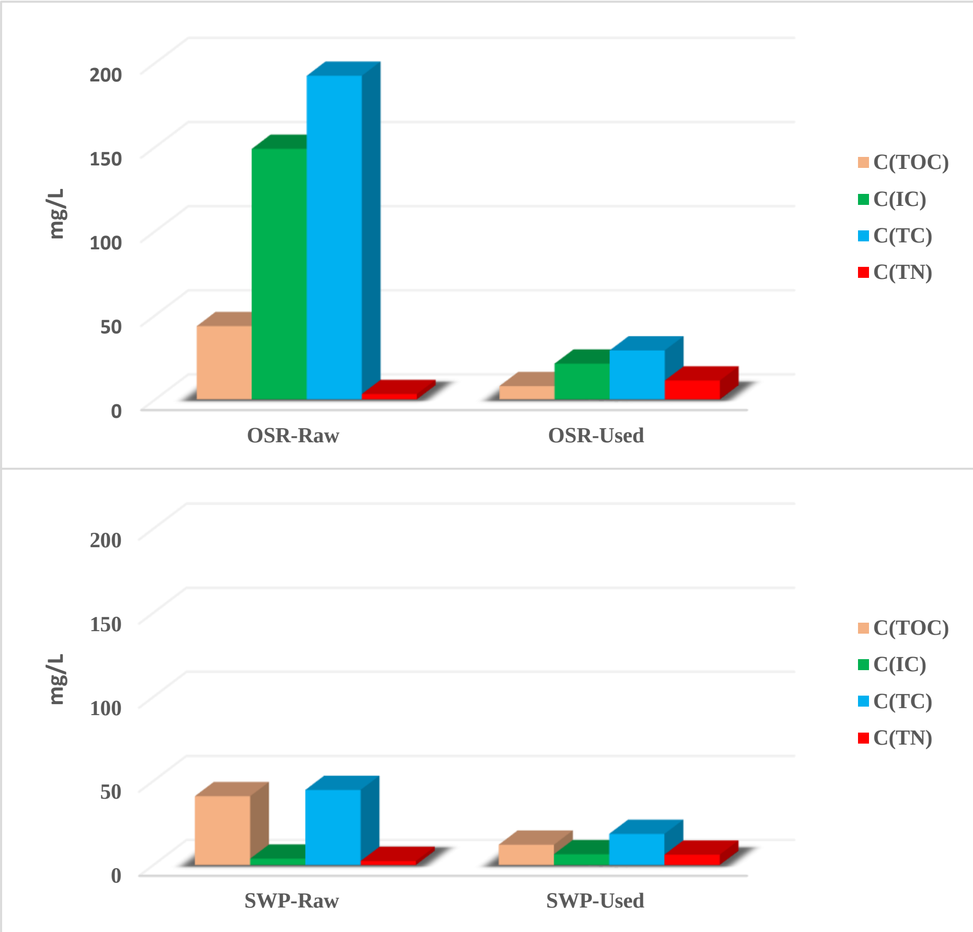


Figure 3. 10. Total nitrogen, total organic carbon, inorganic carbon, and total carbon over a two-day release period

3.6. Elemental analyzer (CHNS)

An elemental analyzer was employed to determine the composition of both raw and used Soft Wood biochar (SWP) and Oil Seed Rape Straw (OSR). This analysis assessed the presence of carbon, hydrogen, nitrogen, and sulfur (CHNS) in the biochar samples, with the results displayed in Figure 3.11.

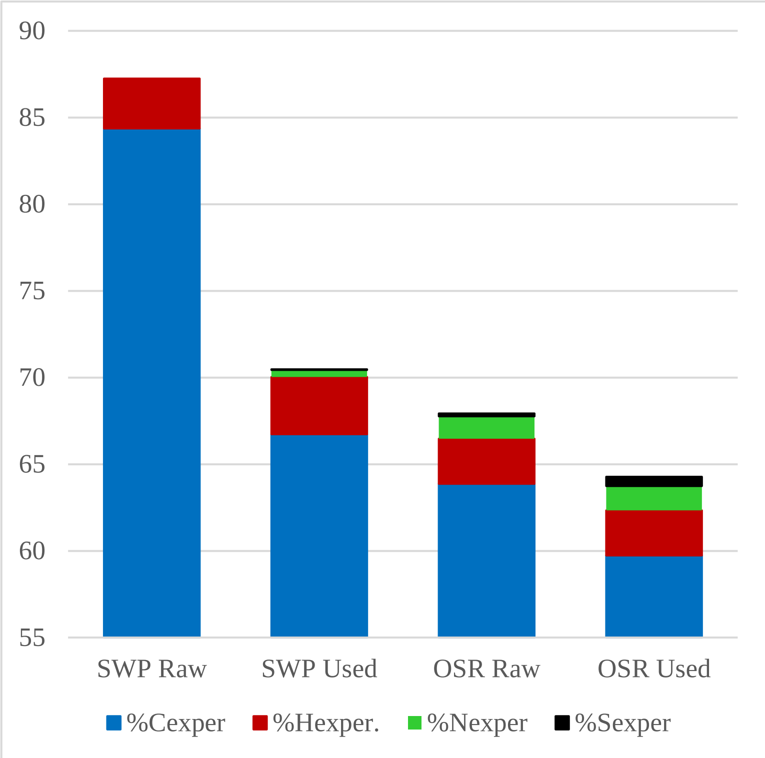


Figure 3. 11. Result of elemental analysis CHNS-932 of raw and used biochar

Chapter 4:
Nutrient Release Dynamics in Vertical Soil Filter (Biochar, Soil, and Wastewater Interactions)

4. Nutrient Release Dynamics in Vertical Soil Filter (Biochar, Soil, and Wastewater Interactions)

Soil functions as a natural filter, removing contaminants from urban wastewater. It also has two important functions: as an active participant in ecosystems when receiving wastewater, and as a medium that facilitates biological, chemical, and physical interactions within the soil, water, and crop systems (Carballo et al. 2019). Wastewater treatment systems come in various forms, including an innovative method called green filters, which use soil. Soil, a complex, reactive, fertile, and permeable medium, serves as the initial filter for water. This approach provides two main environmental benefits: acting as a buffer to store water, carbon, and nutrients, and enabling filtration by allowing the passage of water and carbon and converting chemical compounds (Kadam et al. 2009; Deurer et al. 2019).

This chapter investigates the impact of biochar as a soil amendment on nutrient availability. Biochar may also have an additional role if the soil is acting as a green filter for treating wastewater. In this context, a number of soil tubular reactors were setup in order to i) identify nutrients released by biochar and i) investigate how biochar stimulates microbial transformation of nutrients from wastewater. Two different kinds of biochar at different doses were assayed, using biochar-free soil as control.

During a 16-week experiment, weekly ion analyses were conducted on each of the 10 vertical flow soil reactors (VF Soil Filter) with the main objective of assessing nutrient release patterns and interactions within the soil-biochar mixture. This was achieved by quantifying essential cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) and anions (NO_3^-, PO_4^{3-}, and SO_4^{2-}). Initial nutrient levels in the influent water for columns receiving wastewater were determined weekly to establish baseline values before interacting with the soil-biochar mixture. It is important to note that the data for the first week represent cumulative values obtained from both the initial washing and the first week of operation.

Nutrient Release Dynamics in Vertical Soil Filter
(Biochar, Soil, and Wastewater Interactions)

For evaluating nutrient release patterns, ten soil reactors were used. Biochar-free soil reactors were labeled as A. Reactors fed with deionized water were designated as Ad, Bd, Cd, Dd, and Ed, while Reactors fed with wastewater were designated as Aw, Bw, Cw, Dw, and Ew (as shown in Figure 4.1).

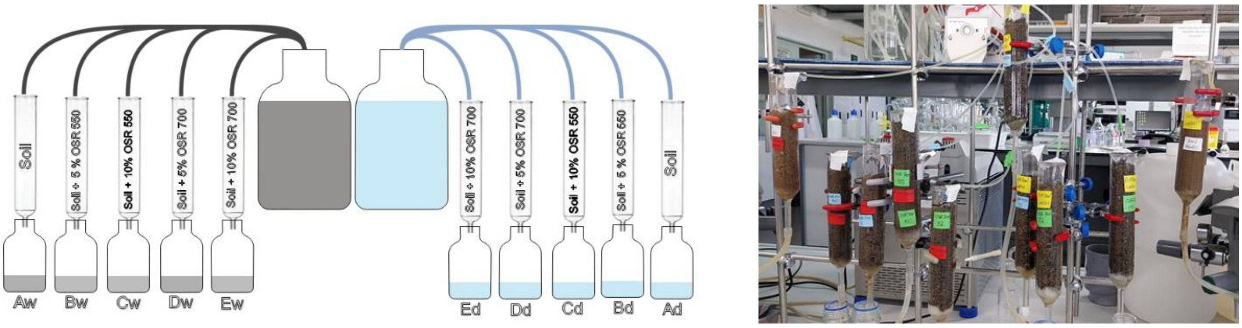


Figure 4. 1. Vertical Biofilters (VF Soil Filter) for Nutrient Release

4.2. Soil and biochar characteristics in VF Soil Filter

The nutrient composition of soil from Alcala University campus used in the Vertical soil reactor (VF Soil Filter) and two kinds of oil seed biochar OSR 550 and OSR 700 are presented in Table 4.1.

Table 4. 1. Main cations and anions in soil and biochar (mg/g)

Description	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**NO_3^-**	**PO_4^{3-}**	**SO_4^{2-}**
Soil	0.04	0.20	52.16	1.33	0.49	0.47	-----
OSR 550	1.11	43.45	4.55	0.86	------	1.20	9.09
OSR 700	1.08	44.16	3.69	0.66	0.21	1.53	8.11

4.3. Main cation release: Na^+, K^+, Ca^{2+}, Mg^{2+}

After a 16-week operational phase involving deionized water and wastewater, we conducted a comprehensive analysis to investigate the main cation release patterns within the Vertical soil reactor (VF Soil Filter). Our study was primarily aimed at discerning the dynamic behavior of Na^+, K^+, Ca^{2+}, and Mg^{2+} ions.

- Sodium (Na^+)

Soil reactors fed by deionized water revealed that biochar-supplemented soil released ca. 11-fold higher Na^+ than soil control. However, such difference was not sustained in time more than two weeks and eventually all soil reactors stop releasing Na^+ after 6 weeks of operation. (Figure 4.2).

In contrast, soil reactors fed by wastewater revealed a different profile determined by the variability in the sodium content of the wastewater. During the first month Na^+ content in WW was high (350-100ppm) so high concentration of Na^+ was detected in effluent, suggesting adsorption and desorption mechanisms. After 5 weeks of operation, Na^+ content in WW became stable and influent and effluent showed identical values (Figure 4.3).

- Potassium (K+)

The release of potassium from control soil during deionized water feeding consistently generated a <5ppm K^+ effluent. However, the biochar supplement increased the K^+ released values as high as 10-fold soil was amended with 10% OSR 700. Subsequent washings kept removing a significant portion of potassium in the subsequent weeks. Soil reactors Dd and Ed released 75% and 85%, respectively, during the first washing period, while the remaining columns exhibited a more gradual decrease in potassium release over the entire 16-week period.

The low concentration of K^+ in wastewater did not significantly contribute to values in the effluent, except after 10 weeks of operation where increasing levels of K^+ in wastewater were also observed in effluent.

Potassium leaching is a commonly encountered issue in agricultural practices, particularly in soils with limited cation exchange capacity or those with a sandy texture (Rashmi et al. 2017; Jalali and Jalali 2020). Farmers may need to modify their irrigation practices, use potassium-rich fertilizers, or employ soil amendments to improve potassium retention (Goulding et al. 2020). The rapid loss of potassium caused by irrigation water has the potential to harm both soil fertility and plant nutrition.

Potassium release from soils happens in two clear phases: a fast initial release followed by a slower, extended release. Magnesium ions (Mg^{2+}) significantly aid in this process. Moreover, soils lacking potassium fertilizers showed reduced rates of potassium release, and prolonged cultivation without potassium fertilization resulted in a significant decrease in soil potassium fertility (Ruan et al. 2014).

- Calcium (Ca^{2+})

Figure 4.2 illustrates the calcium release trends from columns throughout the 16-week study period. Notably, a consistent and uniform calcium release pattern is observed across the control soil reactor with ca. 20% of the total calcium content released during the initial washing and first week, indicating that calcium release persists beyond this initial experimental phase. The presence of biochar increased the Ca^{2+} released in such an initial period, but then it became a relatively stable process during the whole period.

In soil reactors operating with wastewater, the calcium content (ca. 50 ppm) in the influent did not significantly have an impact on the cation released. However, after 10 weeks of operation, Calcium released significantly increased regardless of its stable value wastewater. We did not observe such behavior using deionized water in the absence or presence of biochar, so we hypothesized that some element present in wastewater beyond 10 weeks of operation may participate in such unexpected Calcium release.

Moreover, upon analyzing nutrient dynamics in the soil, it becomes evident that the soil serves as the primary source of calcium release.

- Magnesium (Mg^{2+})

The magnesium release patterns observed within the soil reactors demonstrate that roughly 30% of the total magnesium content was released within the first week. However, magnesium release continued during the 16-week period, showing similar concentration regardless the presence of biochar after the first of operation. Thus, we can conclude that all magnesium stored in biochar was released during the initial period of feeding.

Regarding the operation with wastewater, after 10 weeks of operation, magnesium released significantly increased regardless its stable value wastewater. An identical behavior was also found for calcium and the reason is currently under investigation.

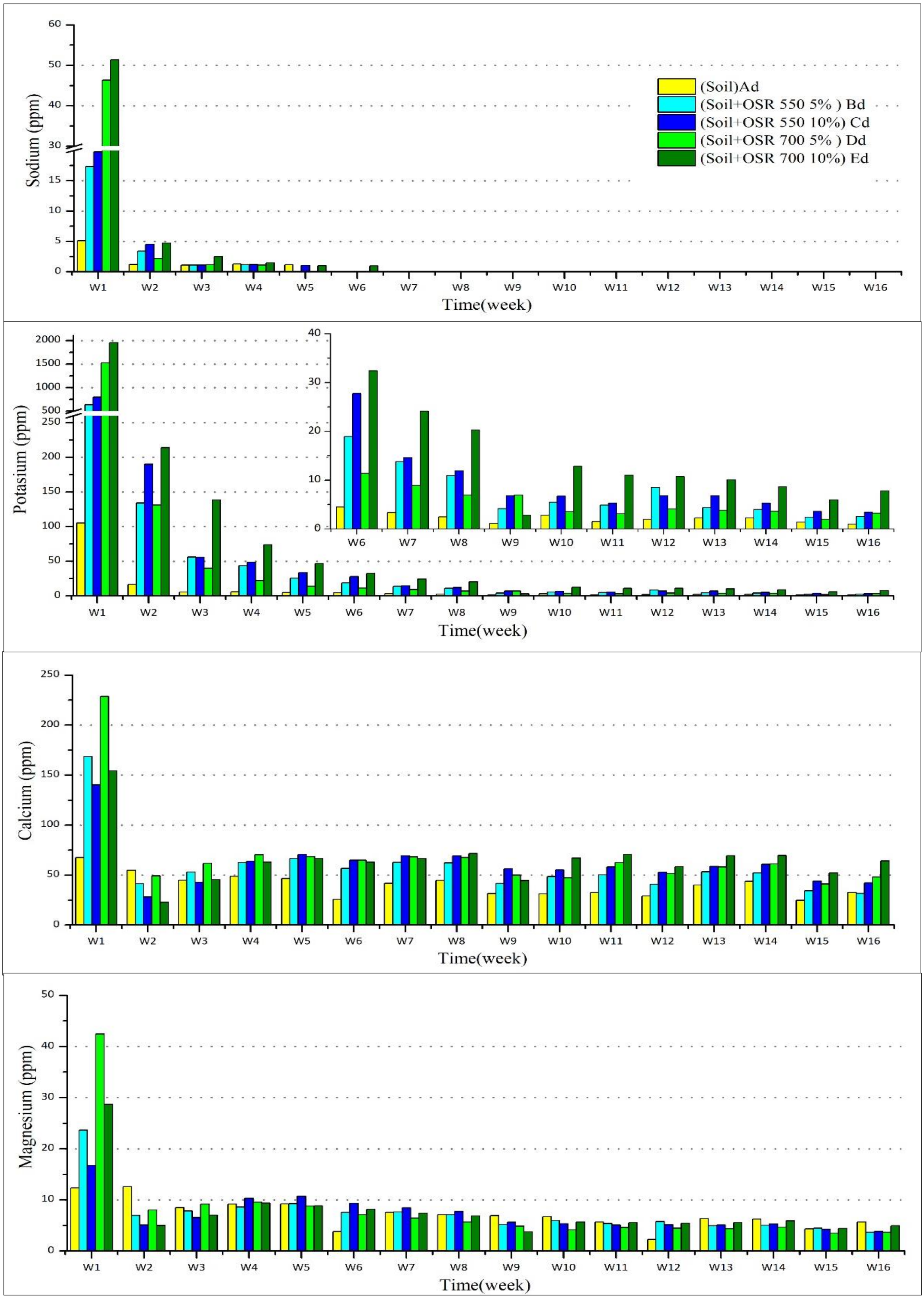


Figure 4. 2. The concentration of Main Cation (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}) in the effluent (VF Soil Filter Operated with deionized water)

Nutrient Release Dynamics in Vertical Soil Filter (Biochar, Soil, and Wastewater Interactions)

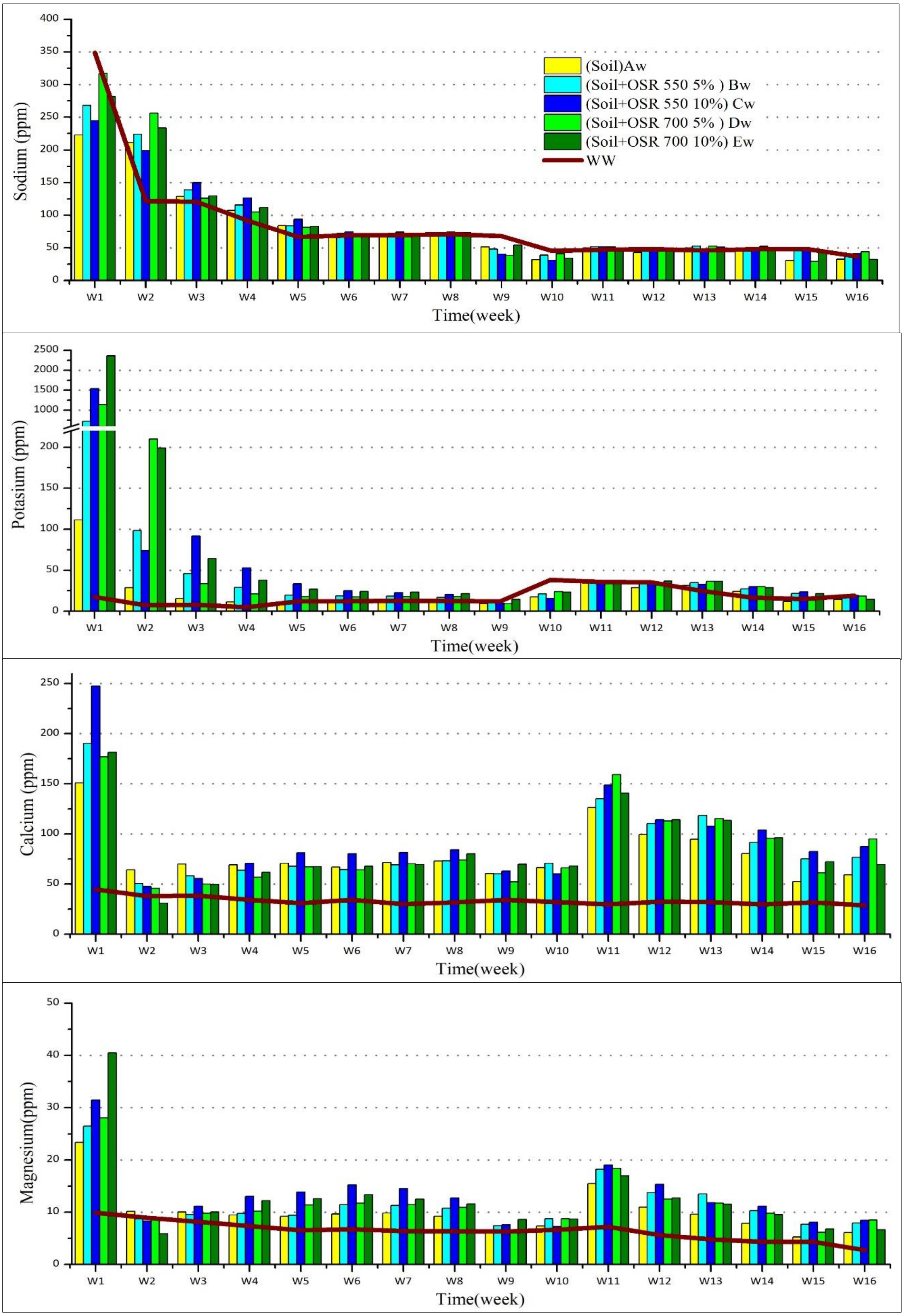


Figure 4. 3. The concentration of main cation (Na^+, K^+, Ca^{2+}, Mg^{2+}) in the effluent (VF Soil Filter Operated with wastewater)

The levels of calcium and magnesium in water are pivotal as they act as indicators of water hardness. According to recommendations from the Center of Agriculture, Food, and the Environment at Massachusetts University, optimal levels for irrigation water typically range from 40 to 100 ppm of calcium and from 30 to 50 ppm of magnesium.

Previous research has demonstrated the beneficial role of calcium in preventing the disturbance of the equilibrium of potassium ions (K^+) in plants and mitigating the effects of salt stress. Elevated sodium ions (Na^+) can disrupt the balance of potassium ions (K^+) in plants. However, the presence of calcium ions (Ca^{2+}) can significantly reduce these deleterious effects, highlighting calcium's critical function in protecting plants from the harmful consequences of salt stress (Cramer et al. 1985; Rengel and Elliott 1992; Marschner 1995). The consistent and substantial release of calcium throughout the experiment highlights its continuous availability. The importance of calcium at the soil-plant interface is emphasized, as it significantly contributes to crop tolerance under salinity stress conditions by preventing the absorption of sodium ions (Na^+) from the soil throughout the year (Jaramillo and Restrepo 2017).

Figure 4.4 provides a comprehensive analysis of the concentration ratio of soil+biochar/soil of main cations released from the VF Soil Filters during the 16-week research study, including both deionized water and wastewater operation. This indicates a slower release of main cations in VF Soil Filters irrigated with wastewater, highlighting a slow-release situation. Graph (a) illustrates the cation release comparison during deionized water operation, while graph (b) highlights the comparison during wastewater operation.

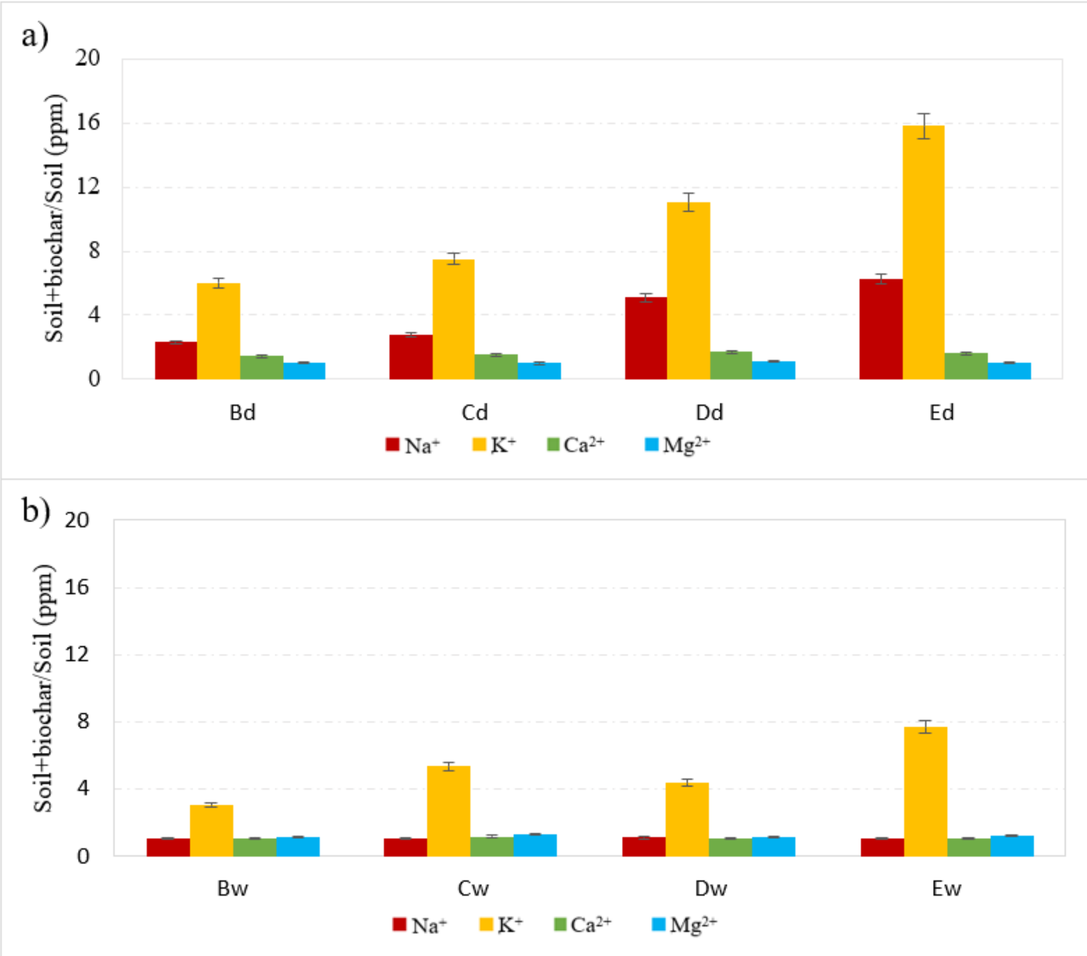


Figure 4. 4. Concentration ratio of main cation in the VF Soil Filter effluent (soil+biochar/soil) Deionized Water (a) and Wastewater (b) Operation

4.4. The release of main anion (NO^{3-}, PO_4^{3-}, SO_4^{2-})

The principal anion release patterns of nitrate, phosphate, and sulfate from VF Soil Filter during the 16-week experiment, operating with deionized water and wastewater, are illustrated in Figures 4.5 and 4.6, respectively.

- Nitrate (NO_3^-)

The pattern of nitrate release shows variability in soil reactors operating with deionized water, as illustrated in Figure 4.5, with instances of zero release followed by subsequent release periods. Soil supplemented with both type of biochar did no released significantly higher doses of nitrate during the first two weeks of operation suggesting their nitrate content was low. In contrast, soil filters operating with wastewater display a more uniform pattern of nitrate release. Additionally, the incorporation of biochar into the soil filter led to an increase in nitrate release. This increase was consistent across all soil filters, regardless of the type of biochar used or the percentage mixed with soil. In spite of the low presence of nitrate in WW from our assays (ca- 40-90 ppm), nitrate was released from WW-supplemented soil in values reaching ca. 400 ppm. The role of nitrification to explain such results will be properly discussed in section 4.5 of this chapter.

- Phosphate (PO_4^{3-})

The use of biochar as soil amendment led to an increase in the presence of phosphate in effluent. However, these concentrations were dependent on the type and proportion of biochar applied. Phosphate was released uniformly until the experiment was completed, except for the soil control which did not exhibit any release after week 14). These show the availability of phosphate throughout the experiment, which typically ranges between 25 and 7 ppm.

- Sulfate (SO_4^{2-})

Regardless of the percentage of biochar added to soil, both type of biochar produced significant amounts of sulfate (SO_4^{2-}) in the effluent. These values were 4.5 to 5.5 times higher than in soil filters containing only soil, indicating the presence of sulfate in the biochar. Notably, roughly half of this sulfate emission occurred in the first week. The sulfate concentration of the wastewater used in this investigation varied between 50 and 20 ppm.

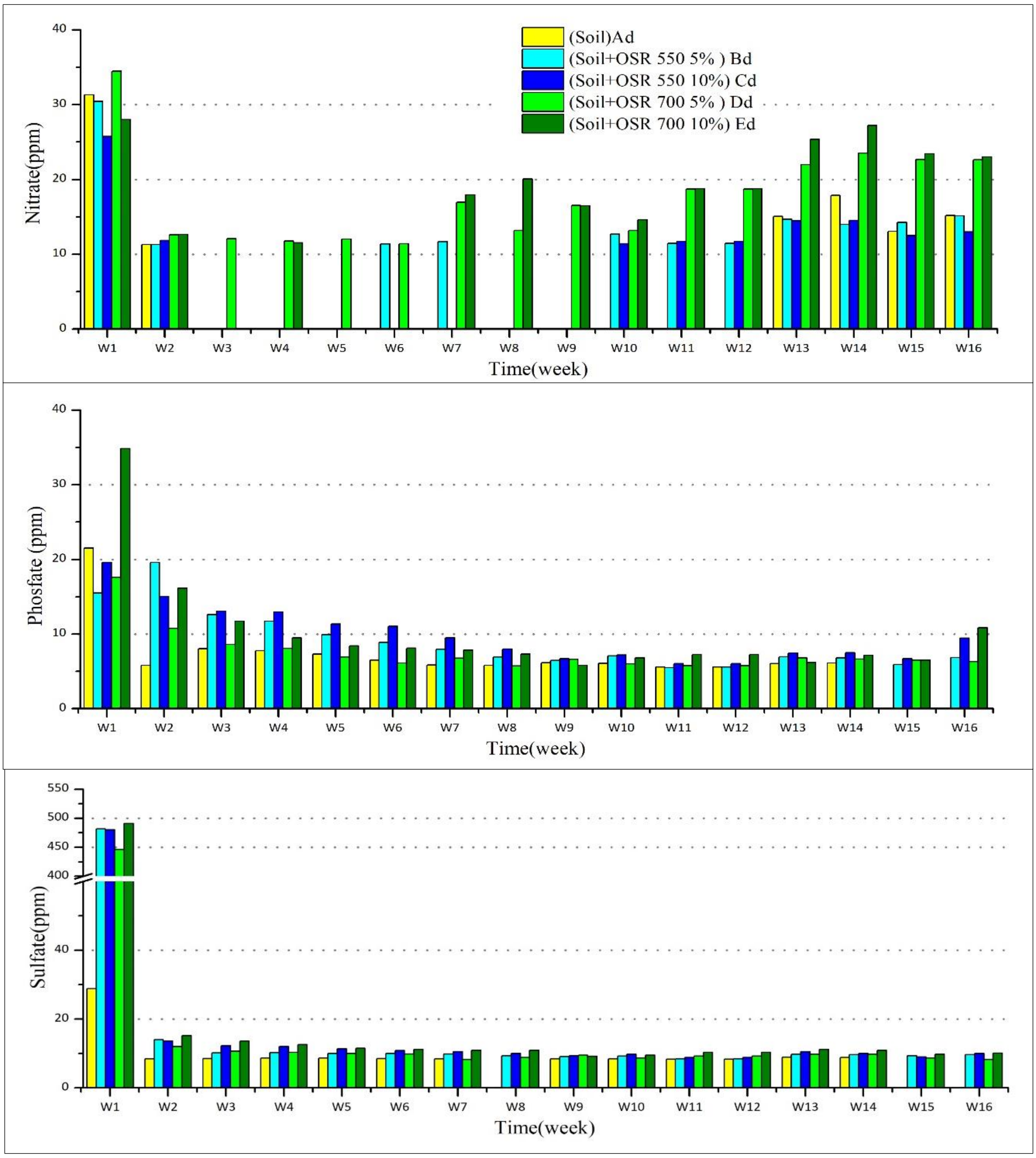


Figure 4. 5. Main anion concentrations ((NO^{3-}, PO_4^{3-}, SO_4^{2-}) in effluent (VF Soil Filter Operated with deionized water)

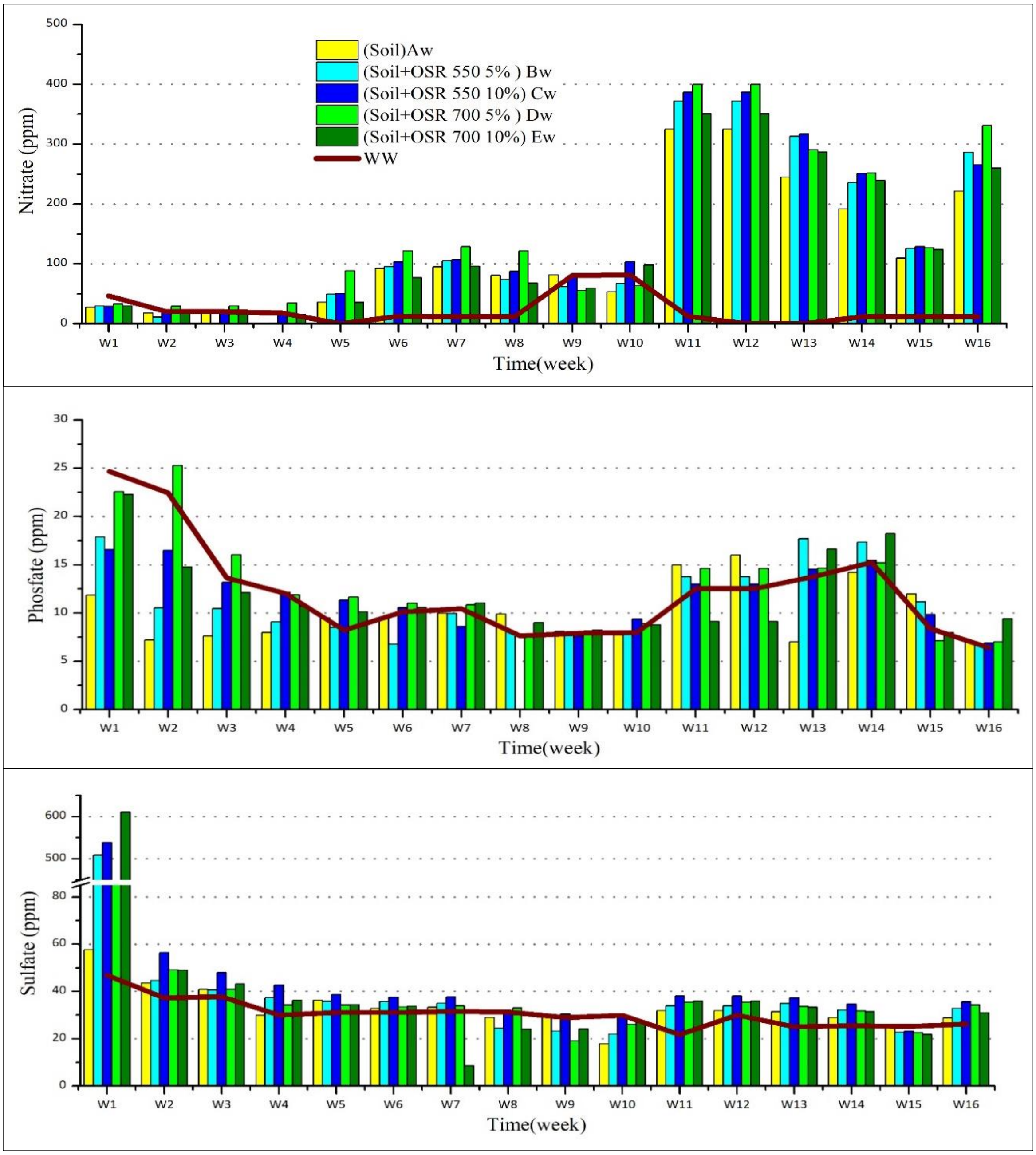


Figure 4. 6. Main anion concentrations ((NO_3^-, PO_4^{3-}, SO_4^{2-}) in effluent (VF Soil Filter Operated with wastewater)

Figure 4.7 provides an in-depth analysis of the concentration ratio of soil+biochar/soil for main anions released by VF Soil Filter over a 16-week study, which included both deionized water and wastewater feeding. Results suggested a slower release of key anions in the soils fed with wastewater, suggesting a slow-release condition. Graph (a) illustrates the comparison of anion release during deionized water operation, whereas graph (b) focuses on the comparison during wastewater operation.

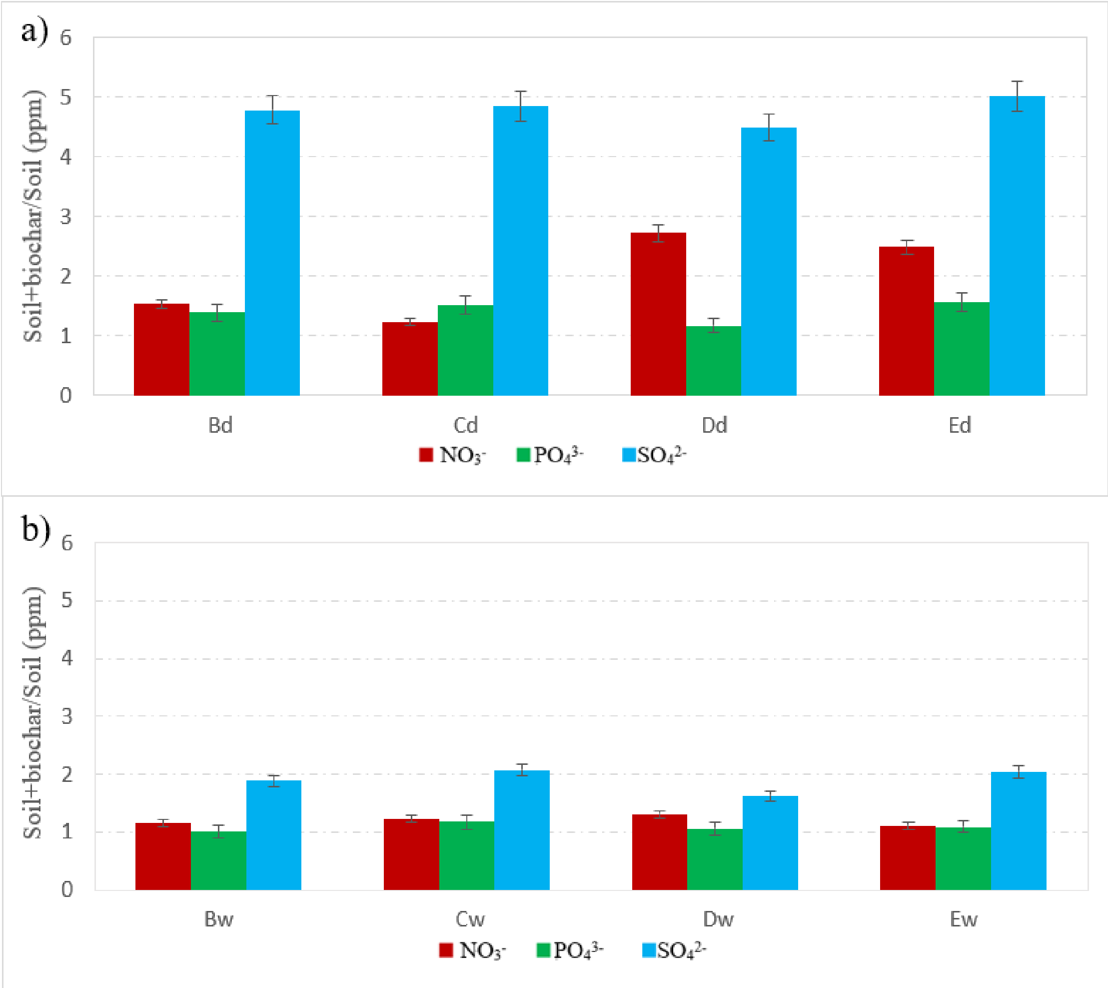


Figure 4. 7. Concentration ratio of main anion in the VF Soil Filter effluent (soil+biochar/ soil) Deionized Water (a) and Wastewater (b) Operation

Angst and Sohi's study indicates (Angst and Sohi 2013) that potassium (K) demonstrates a rapid release compared to phosphorus (P) and magnesium (Mg). Their findings suggest that while K release is initially substantial, it decreases swiftly from the first extraction to the last (six extractions in total), with only 6–18% of the initial extraction being recovered in subsequent extractions. This high rate of K release and its short-term availability in the soil align with findings from other studies (Gaskin et al. 2010; Silber, Levkovitch, and Graber 2010; Yao et al. 2010).

Our findings unveil distinctive release patterns for various main cations and anions. The introduction of biochar into the soil significantly alters the concentrations of these ions in the effluent. Notably, our study elucidates intricate dynamics in their release, suggesting complex interactions between biochar and the soil, mimicking nutrient release patterns observed in agricultural fields.

4.5. The Impact of biochar on nitrification and denitrification

Biochar application in soil affects nitrification processes by changing nitrogen concentrations, soil pH, and nitrifier populations, with effects influenced by factors like soil moisture and biochar type. However, its impact on nitrification is variable and poses challenges for managing nitrogen losses in agriculture (Hale et al. 2023). According to previous studies, biochar enhanced (ca. 56%) the soil nitrification rate (Qi Liu et al. 2024). In spite of the low presence of nitrate in WW from our assays (ca- 40-90 ppm), nitrate was released from WW-supplemented soil in values reaching ca. 400 ppm. The presence of ammonium in WW after week 4 suggest that microbial nitrification is after such nitrate released from soil columns (Figure 4.8), indeed detected values of ca. 400 ppm for nitrate were consistent with a full nitrification process of 100 ppm ammonium present in WW.

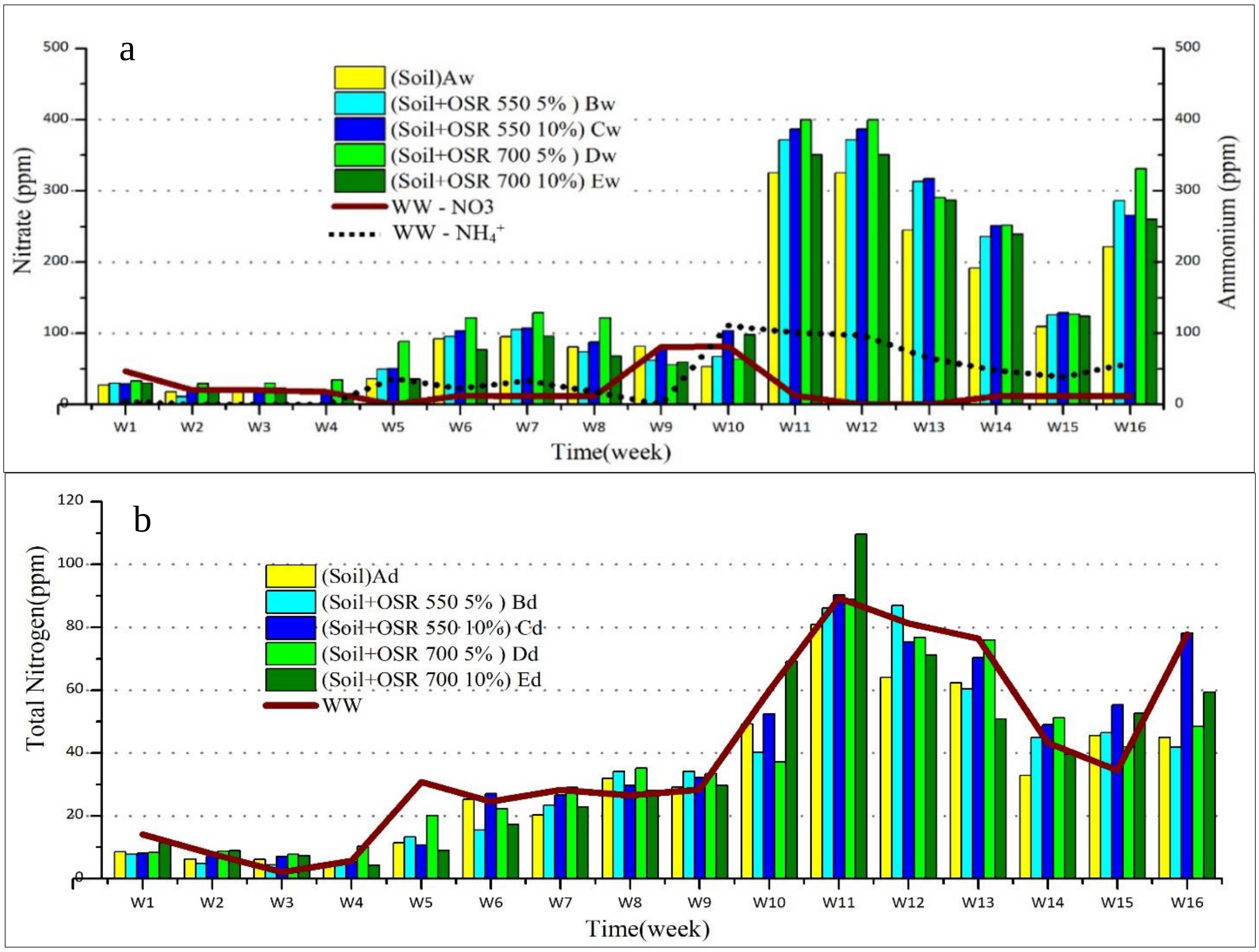


Figure 4. 8. Release of nitrate (a) and total nitrogen (b) under wastewater operation

4.6. Evaluating water quality for irrigation

We specifically explored the impact of adding biochar regarding soil mobility of biochar-associated elements, in a green filter-like system for treating wastewater.

Our results encompass calculating a series of parameters including Electrical conductivity (EC), Sodium Adsorption Ratio (SAR), Soluble Sodium Percentage (SSP), Sodium Percentage (Na^{+}%), Magnesium Hazard (MH), Kelly's Ratio (KR), MH, TDS and Residual Sodium Carbonates (RSC) with all ion concentrations expressed in milliequivalents per liter (meq/L). Additionally, the impact of nutrient transfer between influent and effluent in soil filters was examined.

- Electrical conductivity (EC)

Assessing Electrical Conductivity (EC) is crucial for determining health of those soil devoted to agriculture purpose. In our study, we investigated the impact of adding electroconductive biochar to soil regarding conductivity and according to FAO classifications (Figure 4.9). Our findings indicate that wastewater and its nutrients significantly influence EC levels. Initially, we observed a substantial increase in EC in the effluent from the EW biofilter during the first week, due to the high nutrient release from the biochar, resulting in EC values exceeding 3000 µS/cm. However, over time, EC levels gradually declined, with all water samples eventually falling within the permissible range, approximately below 1250 µS/cm.

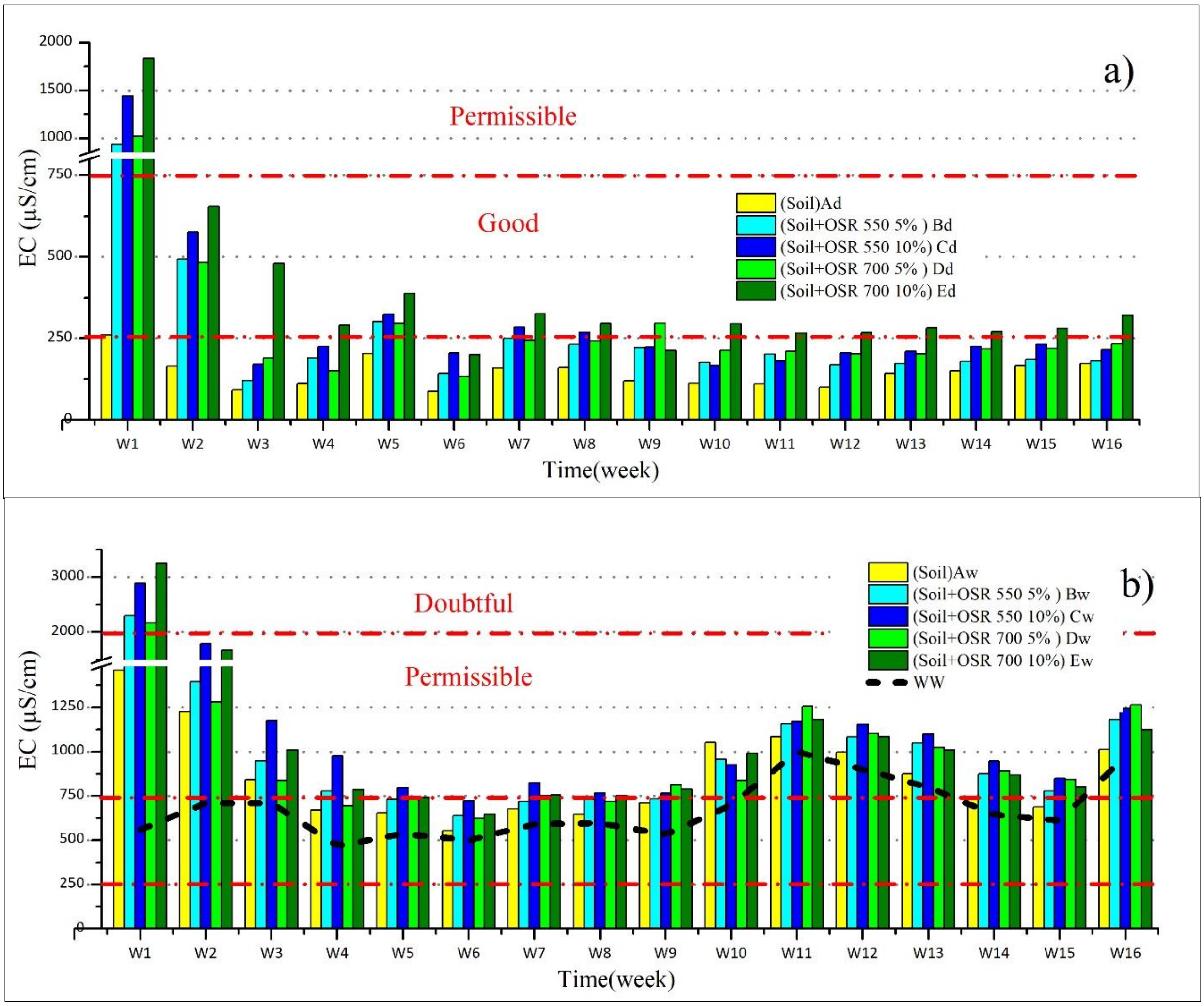


Figure 4. 9. A comparison of electrical conductivity (EC) in effluent water from VF Soil Filter operated with deionized water (a) and wastewater (b), categorized based on FAO's classification

- Sodium adsorption ratio (SAR)

The findings regarding SAR indicate its utility in assessing the sodium hazard in irrigation water. SAR values ranging from 0 to 10 are regarded as indicative of excellent water quality for irrigation in terms of sodium adsorption ratio (Richards 1954).

Prolonged utilization of water with SAR values surpassing 10 can result in the degradation of the soil's physical integrity. If the sodium concentration compared to calcium and magnesium becomes disproportionately high, the soil is classified as sodic (Zaman, Shahid, and Heng 2018). The heightened salinity and sodium ratio in the soil hinders water infiltration and could potentially result in the proliferation of weeds, seed decay, and hindered downward water movement to the roots (Ayers and Westcot 1985; Suarez and Lebron 1993).

All effluent water from the soil filters in our study exhibited SAR values below 10, indicating excellent quality in terms of sodium adsorption ratio.

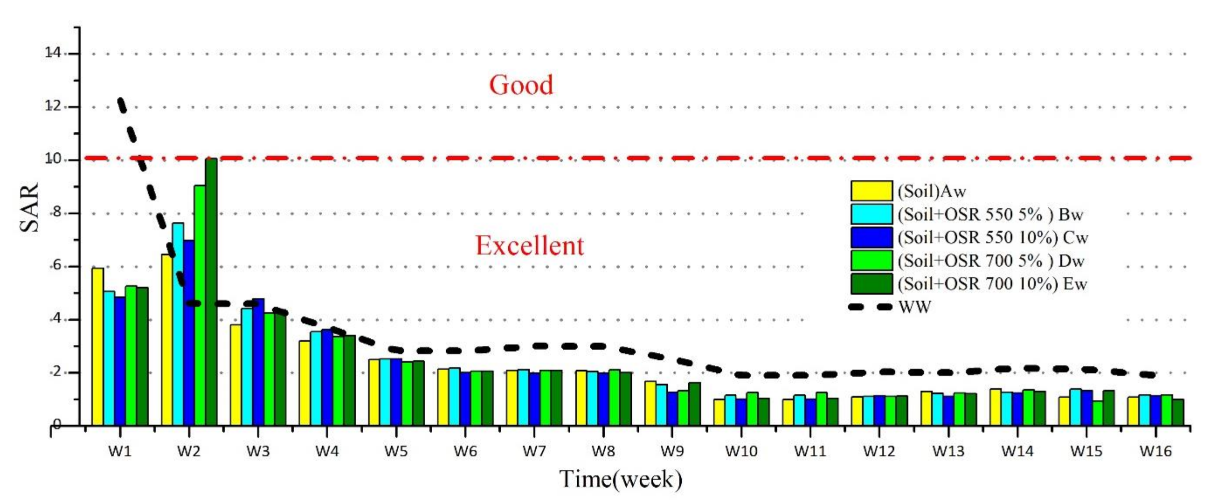


Figure 4. 10. Evaluation of SAR in effluent water from VF Soil Filter operated with wastewater

-Soluble sodium percentage (SSP)

Soluble sodium percentage is an important parameter to determine the irrigation water quality in terms of soil permeability (Nagaraju et al. 2006). To maintain soil permeability, it's important for irrigation water to possess lower sodium ion levels and higher calcium and magnesium ion levels. This helps prevent a decrease in permeability caused by elevated sodium content (Subramanian and Baskar 2022).

In our study, the values of Soluble Sodium Percentage (SSP) in VF Soil Filter effluents ranged from 69.38 to 19, as shown in Figure 4.11. Notably, starting from week 6, all SSP values in the wastewater gradually decreased, reaching below 40 by week 8. Consequently, all effluent was classified as having "good" water quality based on criteria for agricultural use (Todd 1960). The trend of decreasing SSP values can be attributed to the continuous release of calcium ions from the soil in VF Soil Filter.

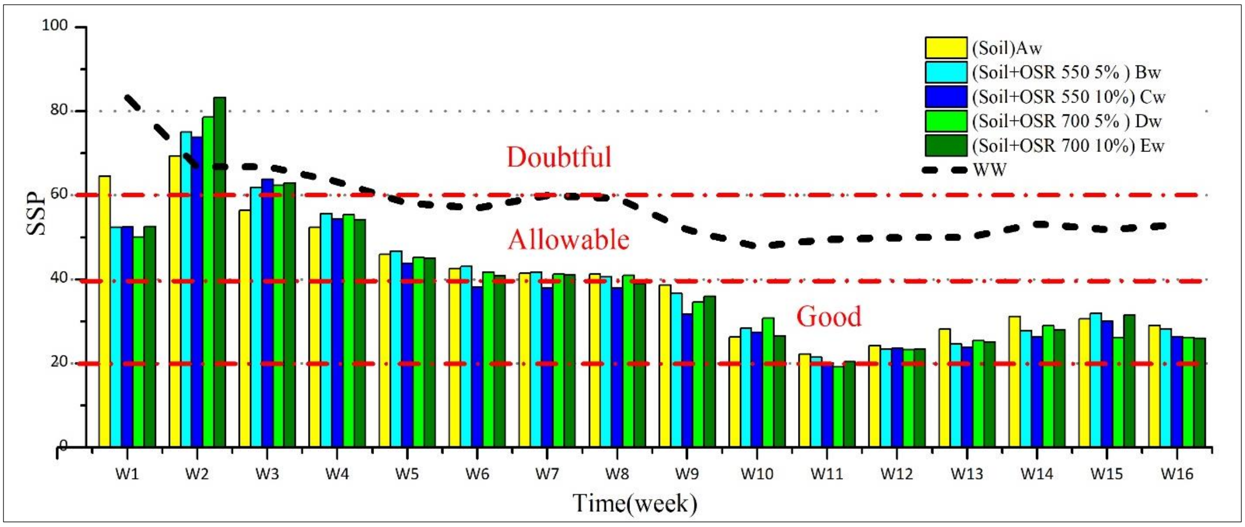


Figure 4. 11. Evaluation of SSP in effluent water from VF Soil Filter operated with wastewater

-Sodium Percentage (Na^+ %)

Another vital aspect in evaluating irrigation water quality is the sodium percentage. Our samples fall in classification good (20-40) (Mahammad, Islam, and Shit 2023).

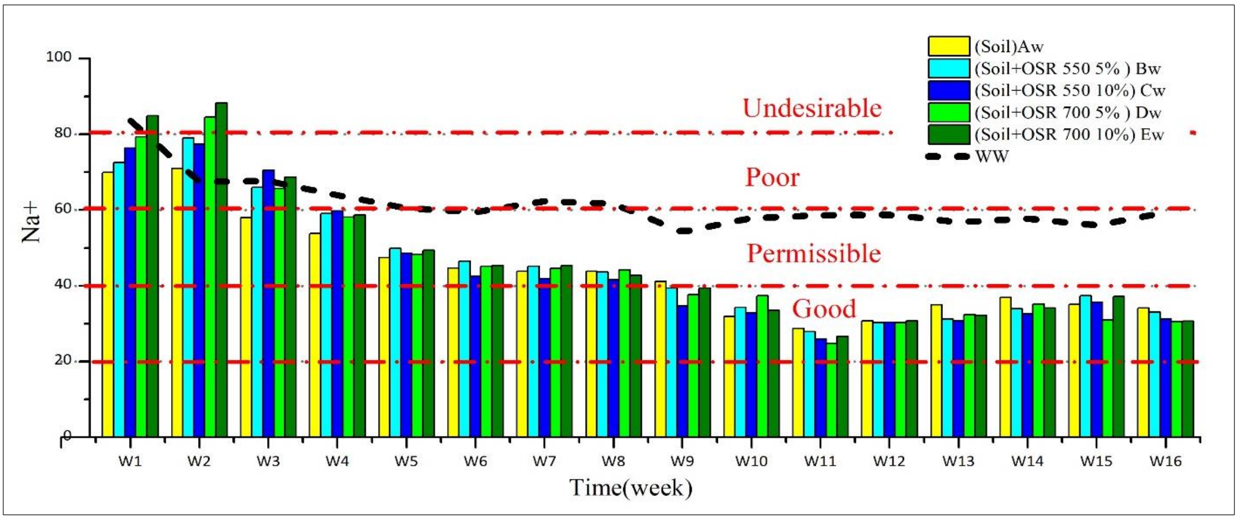


Figure 4. 12. Evaluation of Na+ in effluent water from VF Soil Filter operated with wastewater

-Magnesium hazard (MH)

The high level of magnesium in water will deteriorate the soil structure especially the soil with high exchangeable sodium content. The magnesium hazard (MH) index is for determining the effects of magnesium in irrigation water

(Paliwal 1972).Thus, Magnesium Hazard (MH) represent a problem for values higher than 50, so the water is considered unsuitable for irrigation due to a lower agricultural productivity (Anonna et al. 2021). Based on the data presented in Graph 4.13, it can be observed that all samples had a Magnesium Hazard (MH) index of less than 50. This indicates that all the water samples were suitable for irrigation according to the MH index.

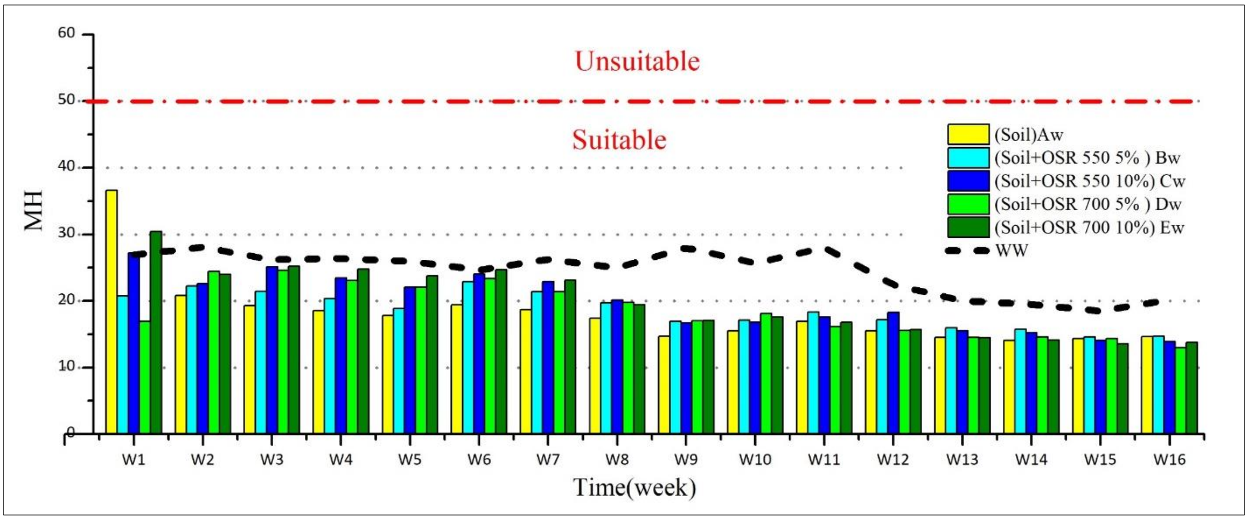


Figure 4. 13. Evaluation of MH in effluent water from VF Soil Filter operated with wastewater

-Kelly's ratio (KR)

Kelly's ratio plays a pivotal role in assessing irrigation water quality, serving as an indicator of excess sodium presence. It is calculated by comparing sodium levels to the combined concentrations of calcium and magnesium ions (Kelly 1963). A KR <1 is deemed suitable for irrigation, while a KR >1 indicates an excess of sodium in water (Kelly 1940). Throughout our 16-week study, we observed variations in Kelly's ratio ranging from 0.24 to 5. However, a significant shift occurred after the fourth week, as the ratio dropped to less than 1 across all effluents from the VF Soil Filter, except wastewater, which served as the influent. This decline in Kelly's ratio signifies a noteworthy improvement in water quality, falling within the desired range for irrigation purposes, where values below 1 are considered optimal. Figure 4.14 visually illustrates this trend, demonstrating the dynamic interaction between nutrient release from soil and biochar, leading to the favorable adjustment of Kelly's ratio over time and its alignment with the optimal range for irrigation.

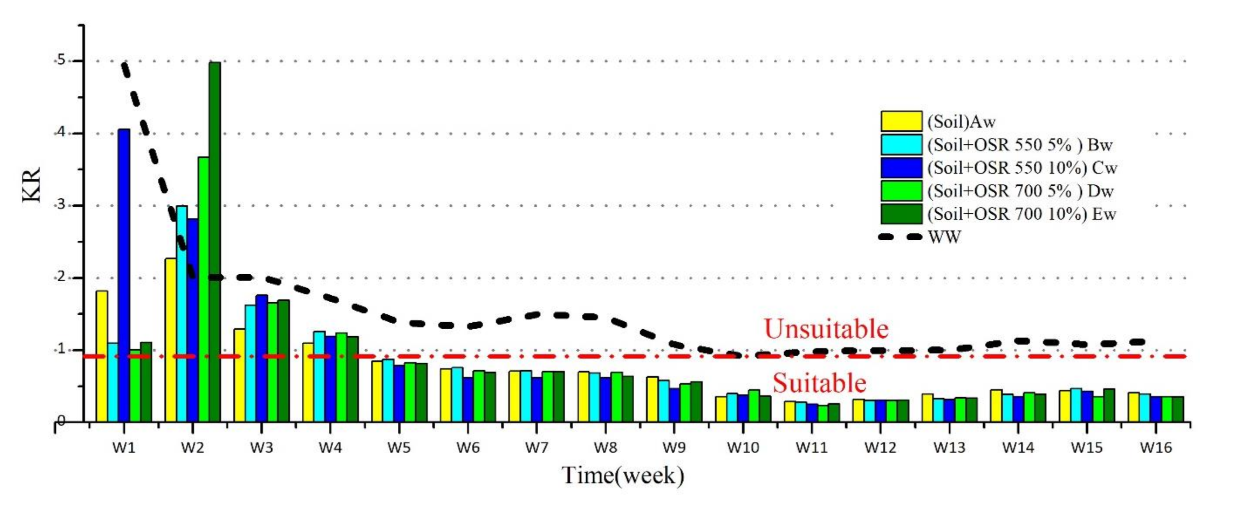


Figure 4. 14. Kelly's ratio throughout the 16-week experiment

-Total hardness (TH)

Total hardness was measured in milligrams per liter (mg/L). Our samples exhibited very hard water during the initial week and notably in week 12, due to the release of high amounts of calcium and magnesium. During these periods, we observed a significant release of these minerals, indicating elevated levels of calcium and magnesium. In other weeks, the samples were categorized as hard water.

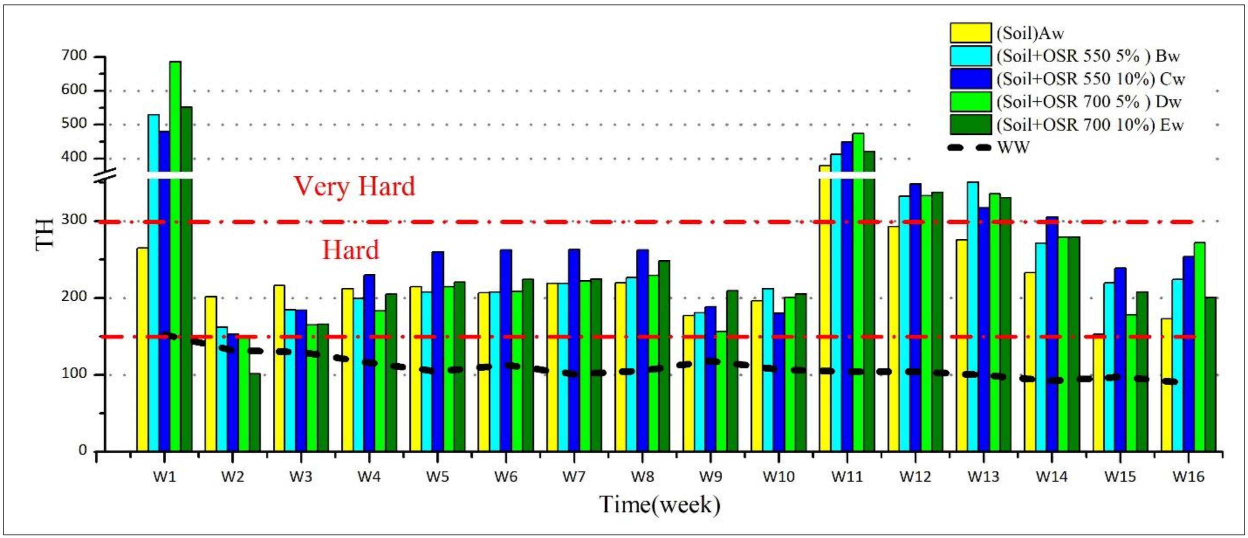


Figure 4. 15. Total hardness over the 16-week experiment

Total dissolved solids (TDS)

The level of salinity in water is often indicated by its total dissolved solids (TDS), which encompass both anions (negatively charged ions) and cations (positively charged ions). These dissolved solids alter the color and characteristics of the water (Arshad and Shakoor 2017). In our research, all samples demonstrated an electrical conductivity (EC) below 5 decisiemens per meter (dS/m). The Total Dissolved Solids (TDS) were computed using a coefficient (k) value of 640. Our samples exhibited a range from 450 to 2000, suggesting a slight to moderate degree of restriction on use (Ayers and Westcot 1985).

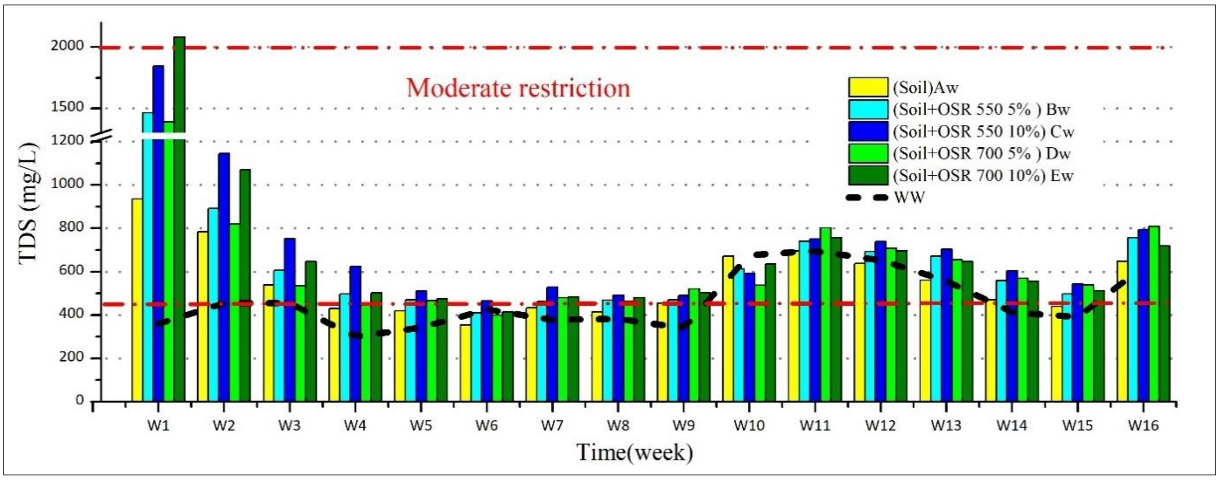


Figure 4. 16. Total Dissolved Solids (TDS) over the 16-week experiment

Residual sodium carbonate (RSC)

A commonly used method for evaluating irrigation water quality involves measuring Residual Sodium Carbonate (RSC), which indicates the detrimental effects of carbonate and bicarbonate on water quality (Acharya, Sharma, and Khandegar 2018). The presence of carbonate and bicarbonate in water affects its appropriateness for irrigation (Shil, Singh, and Mehta 2019). All of our samples have a pH ranging from 7.5 to 8.5, and according to the Bjerrum plots, the inorganic carbon in these samples is primarily derived from bicarbonate ions (HCO3-). Considering this, all samples fell below the threshold of 1.25 on the residual sodium carbonate (RSC) scale, indicating a good water category and being safe for agriculture in terms of residual sodium carbonate levels.

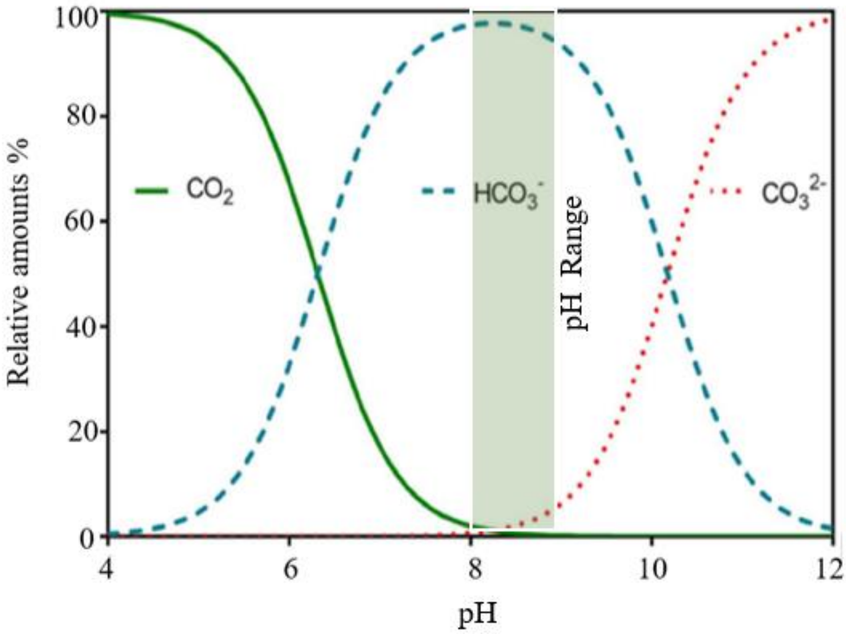


Figure 4. 17. pH range of our samples on Bjerrum plots

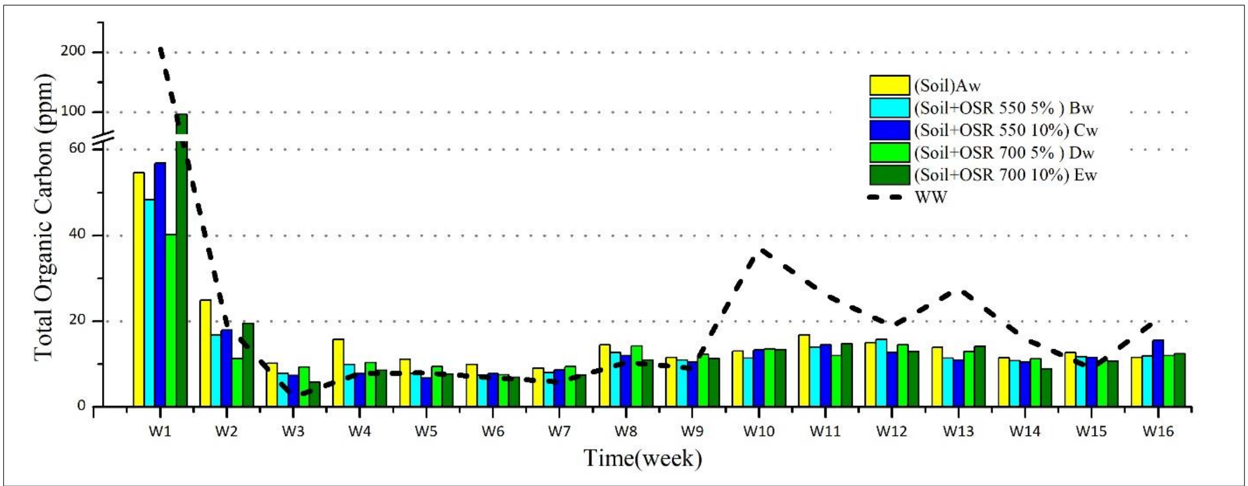


Figure 4. 18. Evaluation of total organic carbon concentrations in the effluent water released from a VF Soil Filter system across a 16-week operational span using wastewater

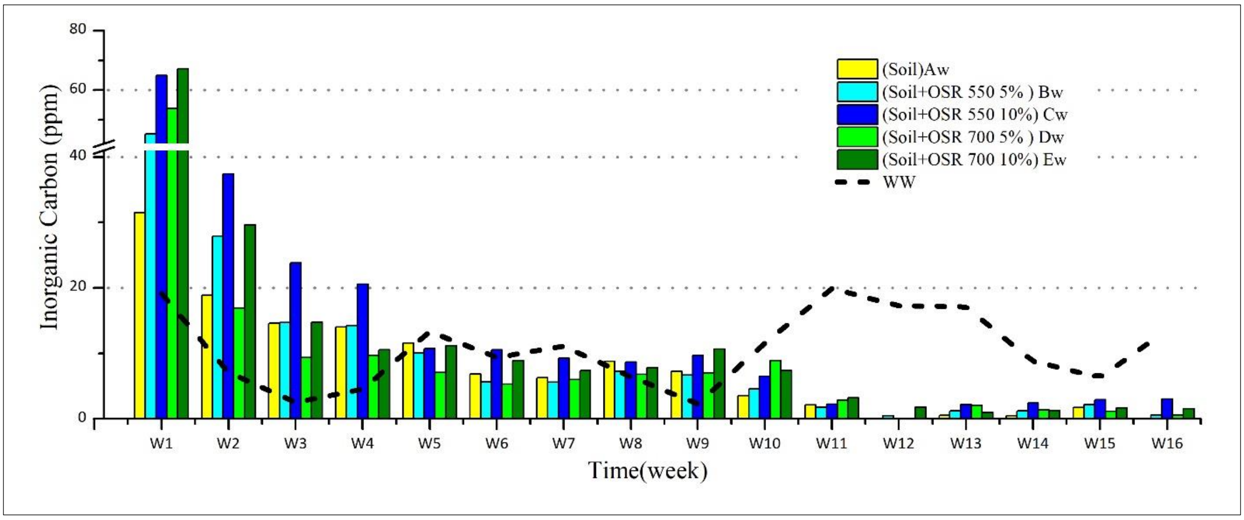


Figure 4. 19. Evaluation of Inorganic Carbon Concentration in Effluent Water from VF Soil Filter System Operated with Wastewater Over 16 Weeks

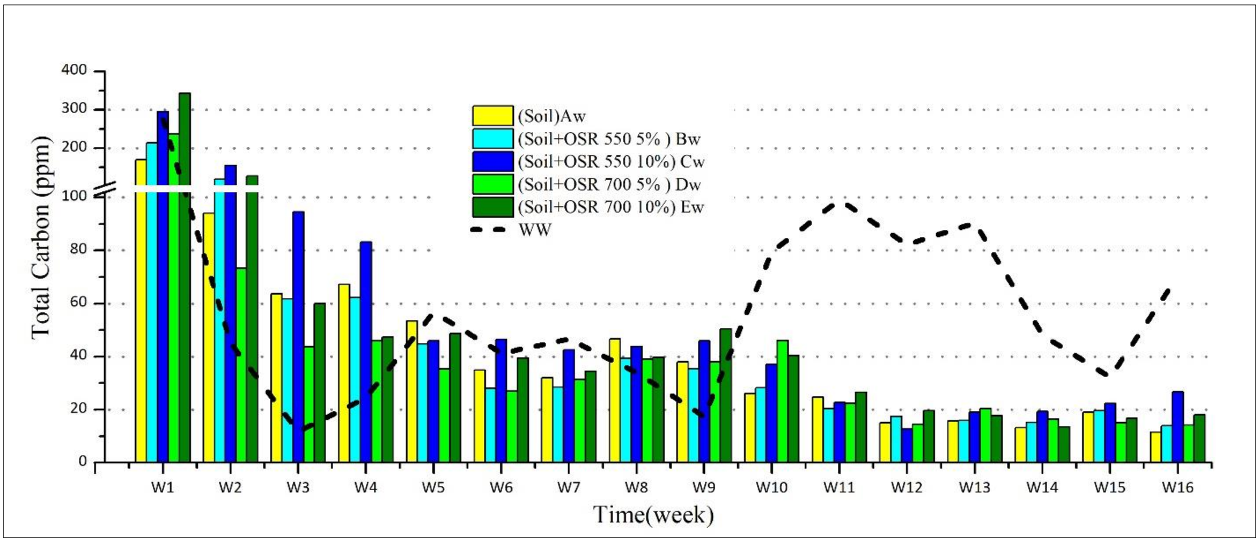


Figure 4. 20. Assessment of the total carbon levels in the effluent water discharged from a VF Soil Filter system over a 16-week operational period utilizing wastewater

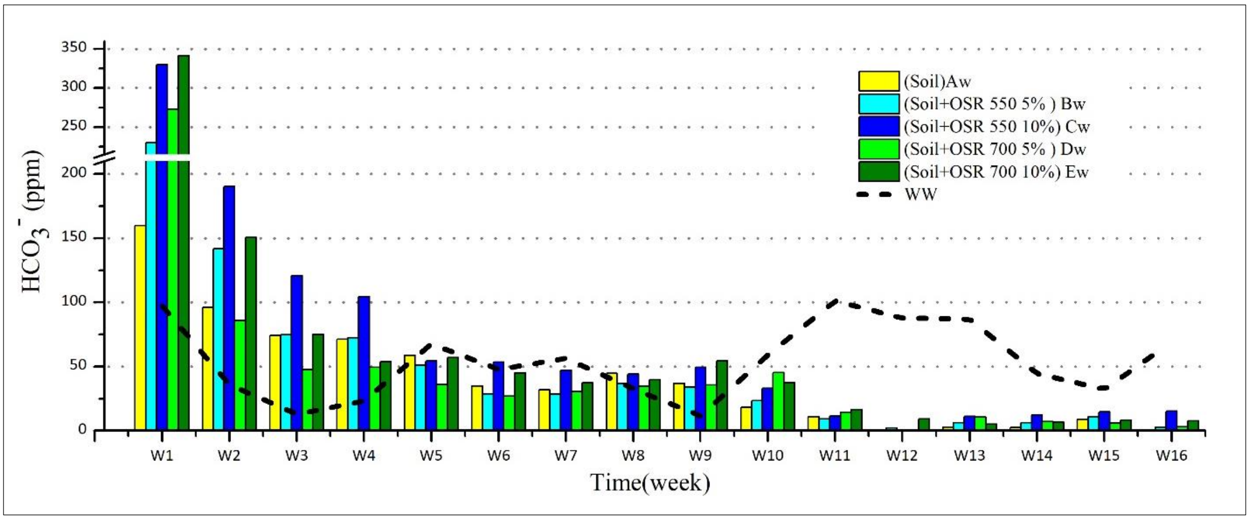


Figure 4. 21. Assessment of bicarbonate ion (HCO3-) levels in the effluent water discharged from a VF Soil Filter system during a 16-week period of operation with wastewater

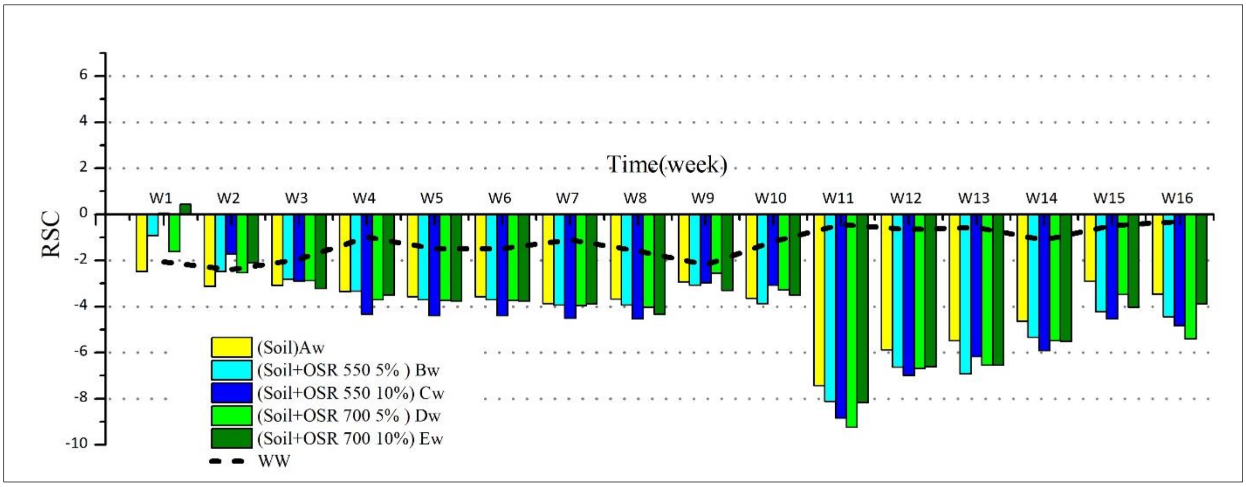


Figure 4. 22. Evaluation of Residual Sodium Carbonate (RSC) levels in the effluent water discharged from a VF Soil Filter system during a 16-week operational period utilizing wastewater

4.7. Results of lettuce growth on different nutrient solutions

4.7.1. Fresh and dry plant weights from hydroponic (treated water with biofilter)

Figure 4.23 illustrates the combined fresh and dried weights of lettuce for plants that are two weeks old. In this dataset of lettuce observations exposed to various treatments, the positive control test (PCT) condition is represented by the use of hydroponic fertilizer, while the negative control test (NCT) condition is indicated by the use of nutrient-free Water.

The weight differences among cultivars exposed to various nutrient solutions were distinct, particularly evident in the notable contrast between the PCT and NCT conditions. The nutrient-enriched solution (PCT) demonstrated an average weight increase approximately six times greater than that observed with the nutrient-free water (NCT). This variance was also reflected in the area of leaves and length of roots, underscoring the significant influence of nutrient concentration on plant growth (see Figure 4.24). The average biomass weight appears to correlate closely with the nutrient content, aligning with findings from previous studies emphasizing the pivotal role of nutrient composition in lettuce growth within hydroponic solutions. Research by (Sapkota et al. 2019) highlights the multifaceted influences on growth dynamics, encompassing factors such as temperature, water availability, and nutrient levels. Significantly disparate outcomes were evident between containers supplied with nutrient-free water and fertilizer, indicative of substantial differences. However, among the remaining containers, variations in nutrient compositions were relatively minor, likely accounting for the observed disparities.

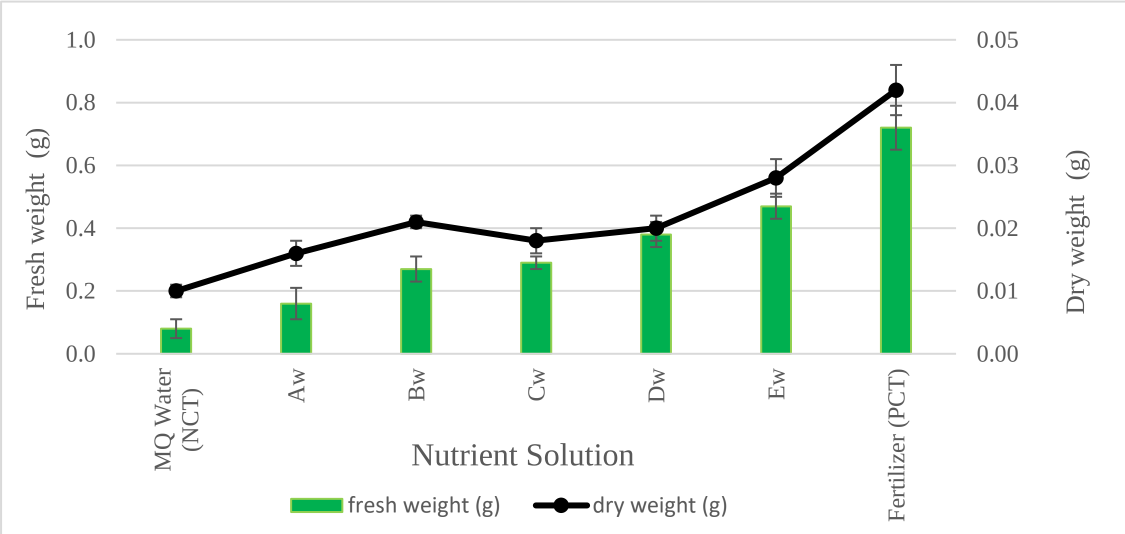


Figure 4. 23. The average weight of fresh and Dried biomass

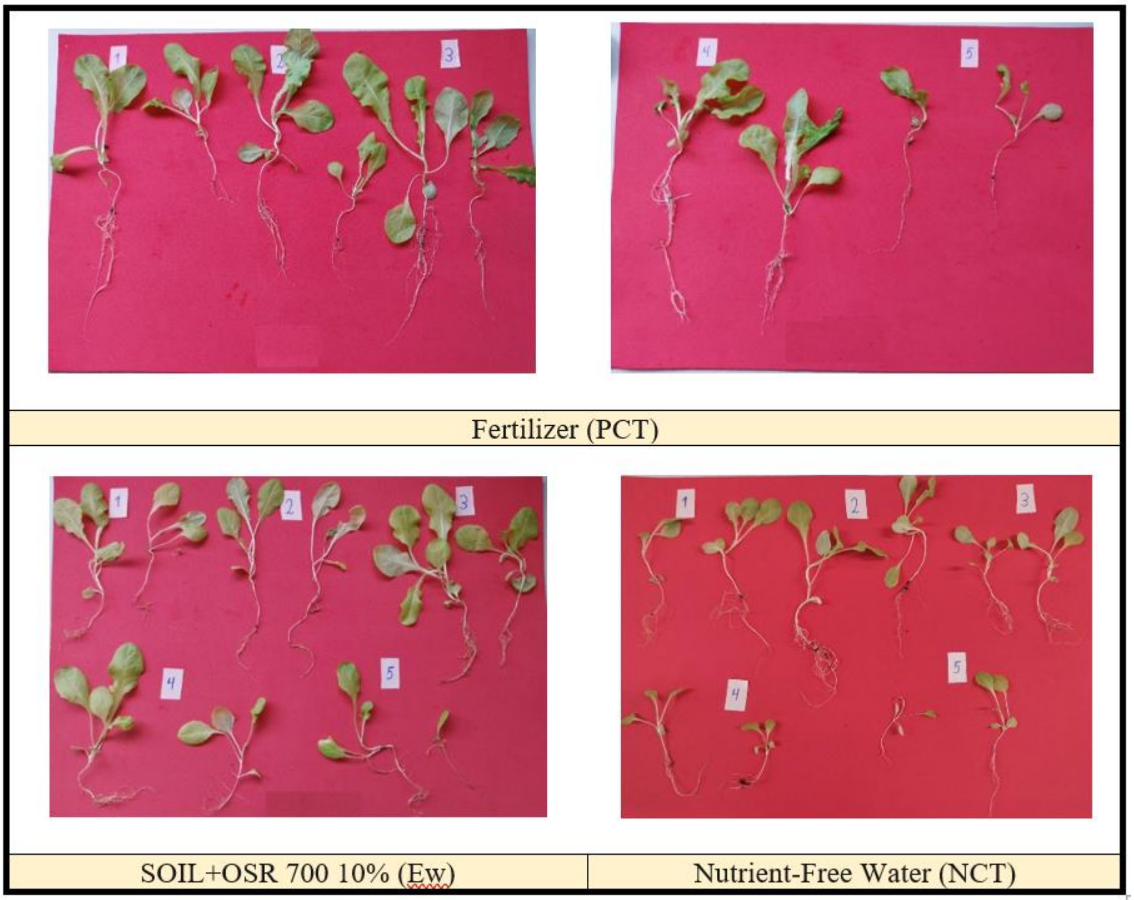


Figure 4. 24. Visual Comparison of Two-Week-Old Lettuce Cultivated in PCT, NCT, and Ew

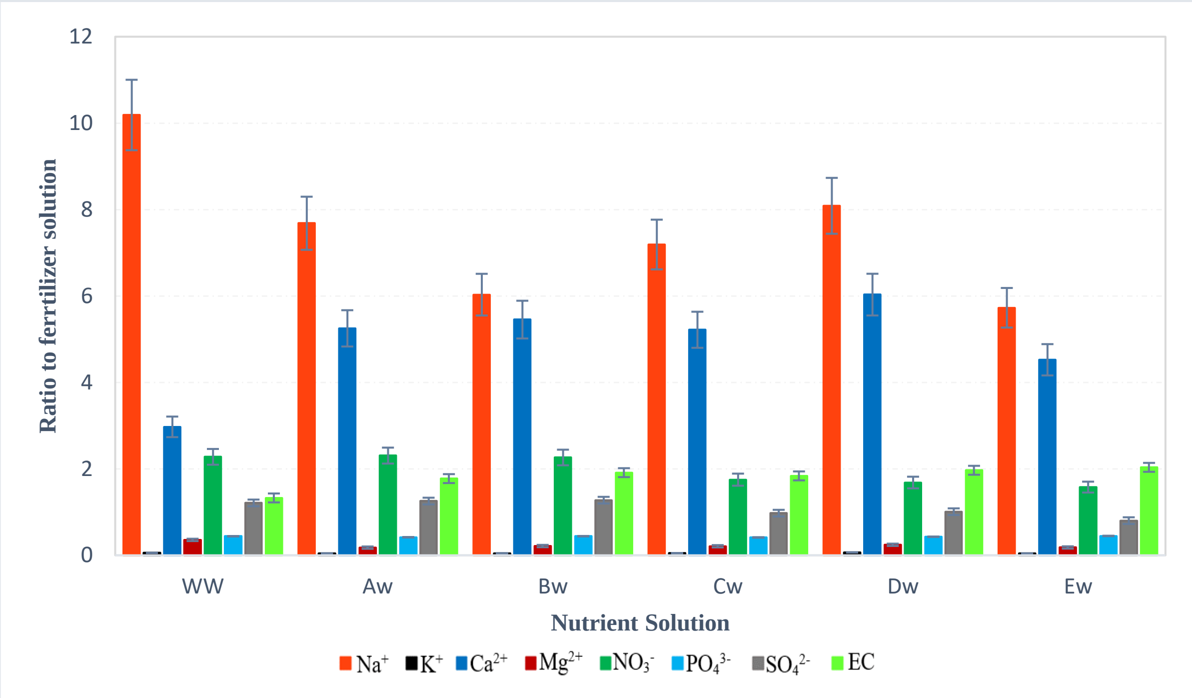


Figure 4. 25. The ratio of main cations and anions, and electrical conductivity of the effluent from VF Soil Filters compared to fertilizer solution

The ratio of main cations and anions, as well as the electrical conductivity of the effluent from VF Soil Filter during week nine, is compared to the fertilizer solution in Figure 4.25. This effluent is utilized for hydroponic cultivation. The graph serves as an index, comparing each parameter in the soil filter effluent to the positive control (fertilizer solution). Our investigation revealed that lettuce demonstrated optimal growth when utilizing the Ew effluent. This favorable outcome could be attributed to the decreased sodium (Na) concentration in the solution. However, it's essential to acknowledge that a balanced proportion of all nutrients may have contributed to this success.

A notable impact on the biomass yield of the aerial parts of lettuce was observed at higher sodium concentrations, resulting in a decrease of over two-fold compared to the control. This decrease was particularly pronounced at the highest NaCl concentration (Breś et al. 2022). Salinity stress affects plants through ion toxicity, such as Na, surpassing the plant's salt tolerance threshold and leading to decreased water uptake (Brès, Pérot, and Freed 2009).

4.8. Fluorescence parameters results

Both theoretically and empirically, Fv/Fm has been established as a robust indicator of the maximum quantum yield of PSII chemistry (Butler 1978; Genty and Meyer 1995). Under normal conditions, unstressed leaves consistently exhibit a value of approximately 0.83 for Fv/Fm, correlating with the maximum quantum yield of photosynthesis (Demmig and Björkman 1987). However, the presence of stress factors such as inactivation damage of PSII (photoinhibition) or sustained quenching leads to a reduction in Fv/Fm (Long, Humphries, and Falkowski 1994; Demmig-Adams and Adams 2006). In our research the amount of Fv/Fm all of samples from biofilter with biochar have the amount of 0.7 to 0.85.the soil filter with soil has lower amount of Fv/Fm is 0.7.

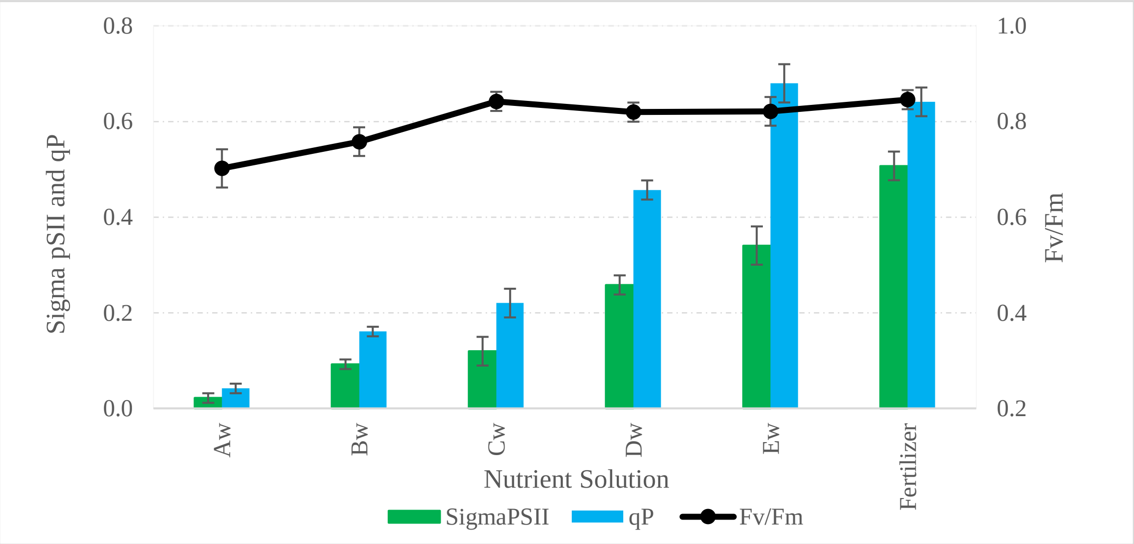


Figure 4. 26. Average qP, Sigma pSII, and Fv/Fm Measurements

Additionally, ΦPSII and qP are also significant photochemistry ratios. These measurements exhibit various trends closely related to pollutant concentrations, with both values decreasing as pollutant concentrations increase (González-Naranjo et al. 2014). Furthermore, the values of ΦPSII and qP are influenced by the range of variations in the presence of contaminants, as indicated by results from studies conducted by other researchers using various photosynthetic organisms

(Wu et al. 2012; Qiu, Wang, and Zhou 2013). The conversion of absorbed light energy into chemical energy (photosynthesis) is associated with qP. Osmotic stress dramatically decreases qP and Fv/Fm, impacting photochemical processes within PS II and influencing overall photosynthetic performance in plants (Lu, Zhang, and Vonshak 1998).

A biofilter that contain only soil does not seem to be able to remove some contaminants; however, a biofilter that uses soil in addition to biochar can remove certain contaminants. When you combine soil with biochar, they may remove contaminants from wastewater more effectively and economically. A recent study indicates that the efficacy of biofilters that use soil as a substrate to remove toxins is typically limited. To significantly increase the pollutant removal capacity of biofilters, biochar can be added to the biofilter media (Boehm et al. 2020).

4.9. Results of algae growth inhibition test

Results from the algae growth inhibition test revealed that the wastewater exhibited a notable level of toxicity, with a significant 53% inhibition observed in the growth rate of the algae. Similarly, the effluent from the biofilter containing soil displayed some degree of toxicity, albeit less pronounced, with a 22% inhibition observed in the algae growth rate. In Figure 4.27, it's important to note that the control test, represented by 0%, corresponds to the algae culture media. Points above the control test values indicate inhibition, while points below signify stimulation of algae growth. The high stimulation observed in biofilter Ew can be attributed to the abundance of nutrients present in the effluent. This nutrient-rich environment fosters increased algal growth compared to the control condition. The surplus nutrients serve as nourishment for the algae, encouraging their rapid growth and proliferation.

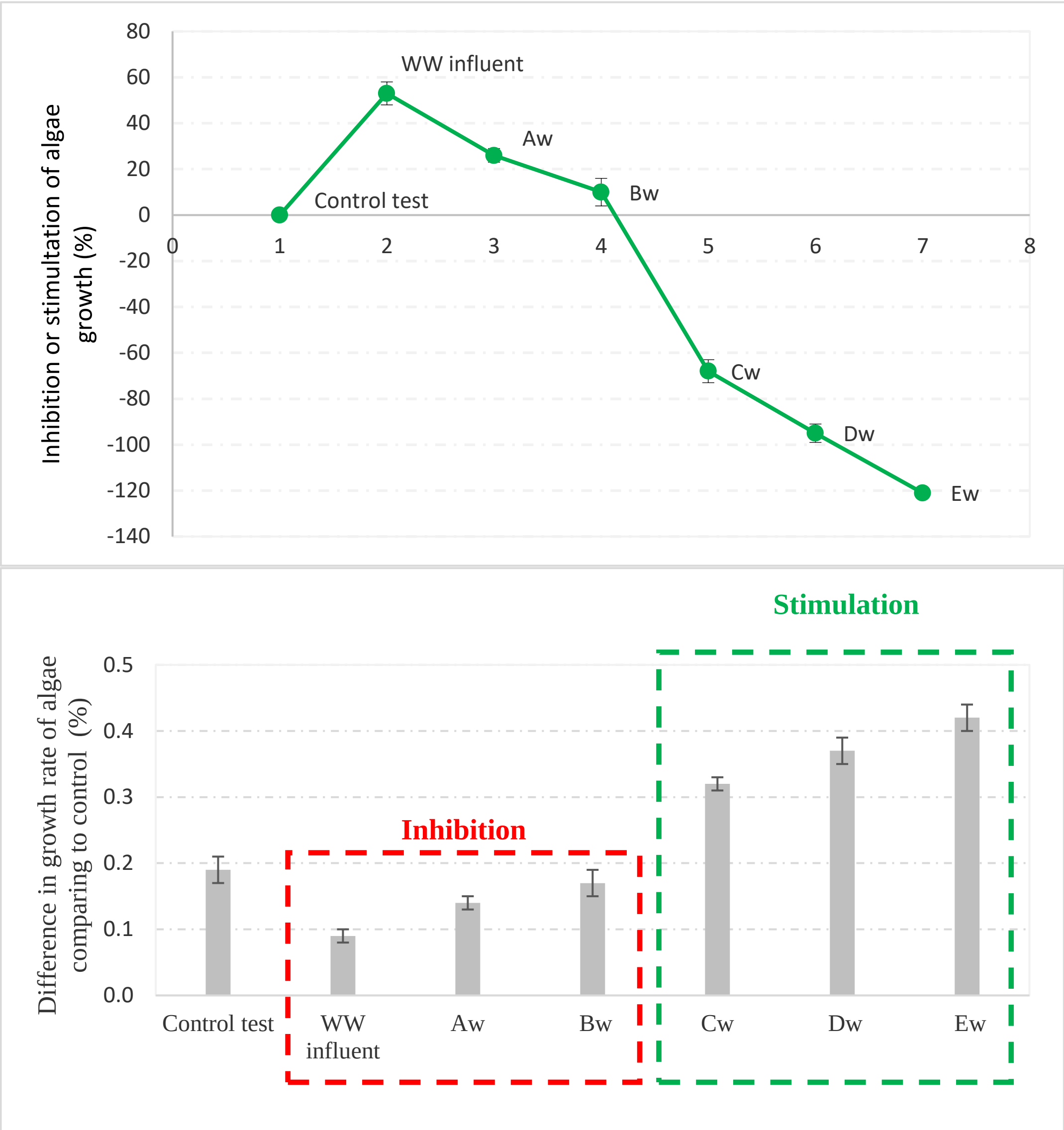


Figure 4. 27. Inhibition or stimulation of algae growth (%) Difference in the growth rate of algae compared to control (%)

However, despite the stimulation of algae growth, it's important to note that the biofilter with biochar remains effective in treating wastewater. Moreover, it serves an additional beneficial purpose by releasing nutrients suitable for irrigation. This dual functionality underscores the significance of biochar, as it not only contributes to water purification but also provides a natural source of nutrients for irrigation needs. By reducing the dependency on additional fertilizers, the biochar promotes sustainable agricultural practices, aligning with environmentally friendly approaches to farming.

Chapter 5:
Assessment of Sunflower Crop Production Using Electroconductive Biochar and treated Wastewater for Irrigation

5. Assessment of sunflower crop production using electroconductive biochar and treated wastewater for irrigation

Due to the current water scarcity, the concept of reusing wastewater in agriculture, particularly for irrigation, is gaining popularity. This strategy not only alleviates water pressure but also addresses water pollution concerns. Historically, the improper use of wastewater in agriculture has posed significant threats to public health and the environment. However, when properly regulated, wastewater reuse emerges as an effective solution, especially in mitigating water scarcity caused by seasonal variations or inconsistent water supply for crop irrigation throughout the year (Jaramillo and Restrepo 2017).

The current chapter aims to explore for the first time circular economy aspects of METland® technology at two independent but complementary levels: i) reuse of urban wastewater after METland® treatment, and ii) reuse of EC-biochar from METland® bed as a soil amendment.

Given the substantial agricultural interest of sunflowers (Helianthus annuus L.), as a major oilseed crop (ranked fourth on the FAO's 2017 list of essential oilseed crops) we decided to use it as a target to validate the impact of irrigating with re-used water and using EC-biochar as soil amendment. Specifically, we examine reused water derived from the urban wastewater from Campus after METland® treatment. To evaluate the impact of nutrients (nitrate) present in treated water we used two sets of water with different nitrate levels: N35 (35 ppm NO_3^-) and N15 (15 ppm NO_3^-). Additionally, we investigated the application of two types of electroconductive biochar for sunflower cultivation: raw biochar (EC-biochar not used before for treating ww) and used biochar (EC-biochar after wastewater treatment). Our experiments were conducted on twelve lanes: four irrigated with N30 water, four with N15 water, and four with tap water. Additionally, six lanes were treated with 0.5% EC-biochar (three with raw biochar and three with used biochar).

Figure 5. 1. Farming assay to explore the impact of i) irrigating with tap water or treated wastewater (N35 and N15) and ii) supplementing soil with (raw and used) for growing sunflowers. N35 and N15 corresponded to treated wastewater containing 35 ppm and 15 ppm Nitrate, respectively

5.1. Quality of treated water from campus

5.1.1. Nutrient composition of treated water

The main cations and anions were analyzed in 5 independent sampling actions along the whole experimental season (Figures 5.2 and 5.3). All ions showed similar level regardless sampling except for nitrate (35 and 15 ppm) so we concluded to name water batch as N35 and N15.

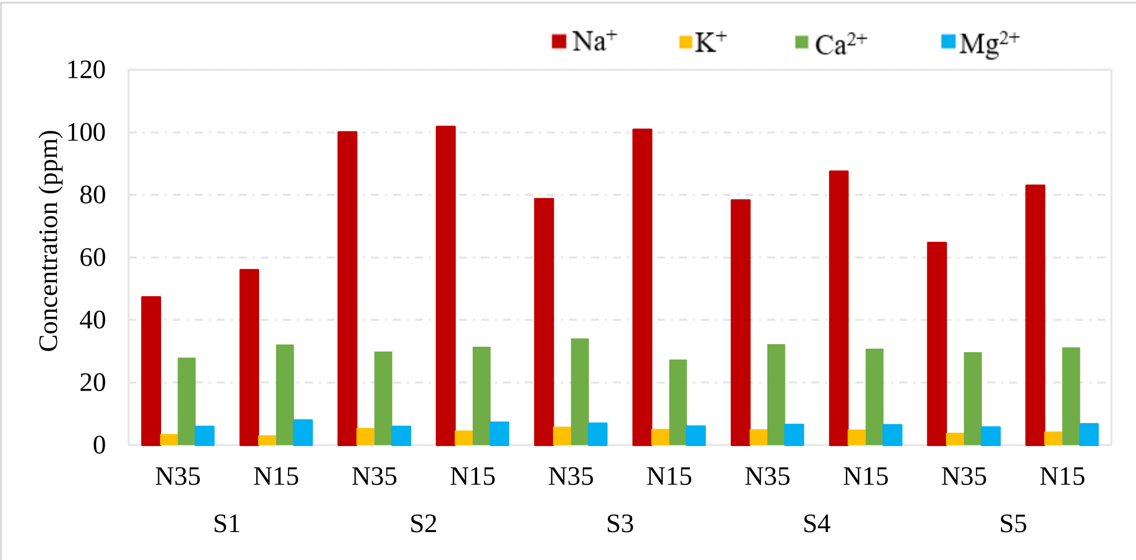


Figure 5. 2. Main cation concentrations in five independent samples of treated water

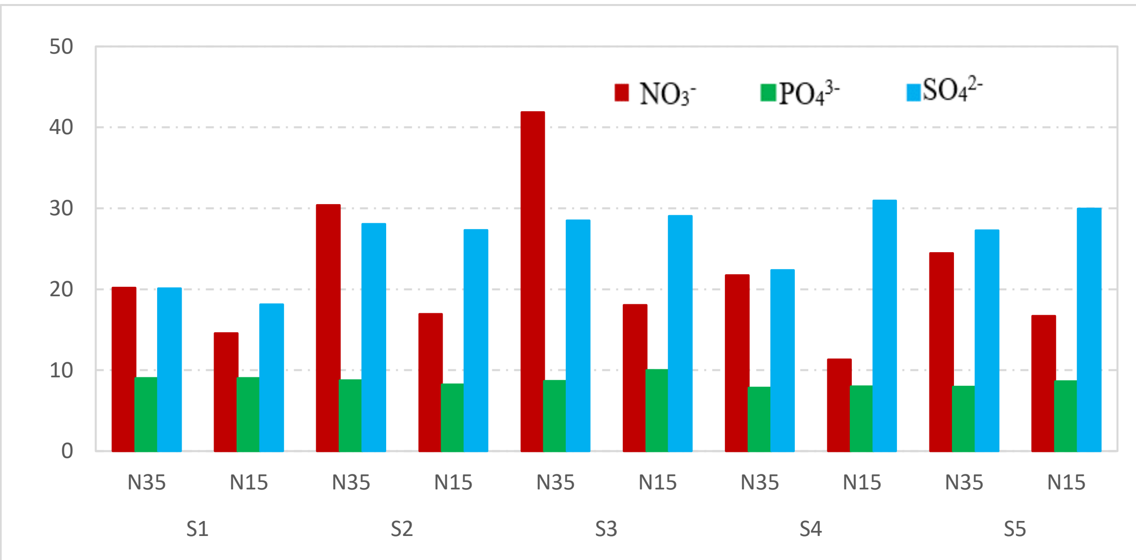


Figure 5. 3. Main anion concentrations in five independent samples of treated water

5.2. Treated water quality and criteria of use for irrigation

The quality of treated water can be influenced by multiple factors, including the concentration and types of total soluble salts not removed during the treatment. To evaluate the suitability of METland®-based treated water for irrigation, several essential criteria were considered, such as electrical conductivity (EC), total dissolved solids (TDS), sodium adsorption ratio (SAR), soluble sodium percentage (SSP), magnesium hazard (MH), and Kelly's ratio (KR). total hardness (TH) and potential salinity (PS).

5.2.1. Electrical conductivity

Through the use of electrical conductivity (EC) measurements, which are commonly utilized to assess irrigation water quality, the FAO has established different classifications for water. For EC values below 250 µS/cm, the water is considered excellent, while values ranging from 250 to 750 µS/cm are deemed good. Permissible water falls within the range of 750 to 2000 µS/cm, doubtful between 2000 and 3000 µS/cm, and unsuitable if EC exceeds 3000 µS/cm.

Our study revealed that all samples after METland® did not reach the threshold of 750 µS/cm, which is generally regarded as suitable for irrigation without significant issues.

5.2.2. Total dissolved solids (TDS)

The salinity level in water, reflected by total dissolved solids (TDS), includes both anions and cations, which influence the water's color and characteristics (Arshad and Shakoor 2017). In our study, all samples had an electrical conductivity (EC) below 5 dS/m. With a coefficient (k) of 640, TDS values below 450 ppm indicate no usage restriction (Ayers and Westcot 1985).

5.2.3. Sodium adsorption ratio (SAR)

The sodium adsorption ratio (SAR) serves as a critical parameter for assessing the sodium content in irrigation water. SAR value ranging from 0 to 10 indicates excellent water quality (Richards 1954). However, prolonged use of water with a SAR exceeding 10 can lead to soil structure degradation. If the concentration of sodium relative to calcium and magnesium becomes high, the soil is characterized as sodic (Zaman, Shahid, and Heng 2018). In our research, the water samples collected from the effluents of treated water N35 and N15 were classified under the medium salinity and low sodium hazard category, as indicated by the USSL diagram. One of the N15 samples falls within the C1-S1 category, suggesting low salinity and low sodium hazard, while the remaining N15 samples were categorized as C2-S1. These findings imply that water from both N35 and N15 is generally suitable for irrigation purposes.

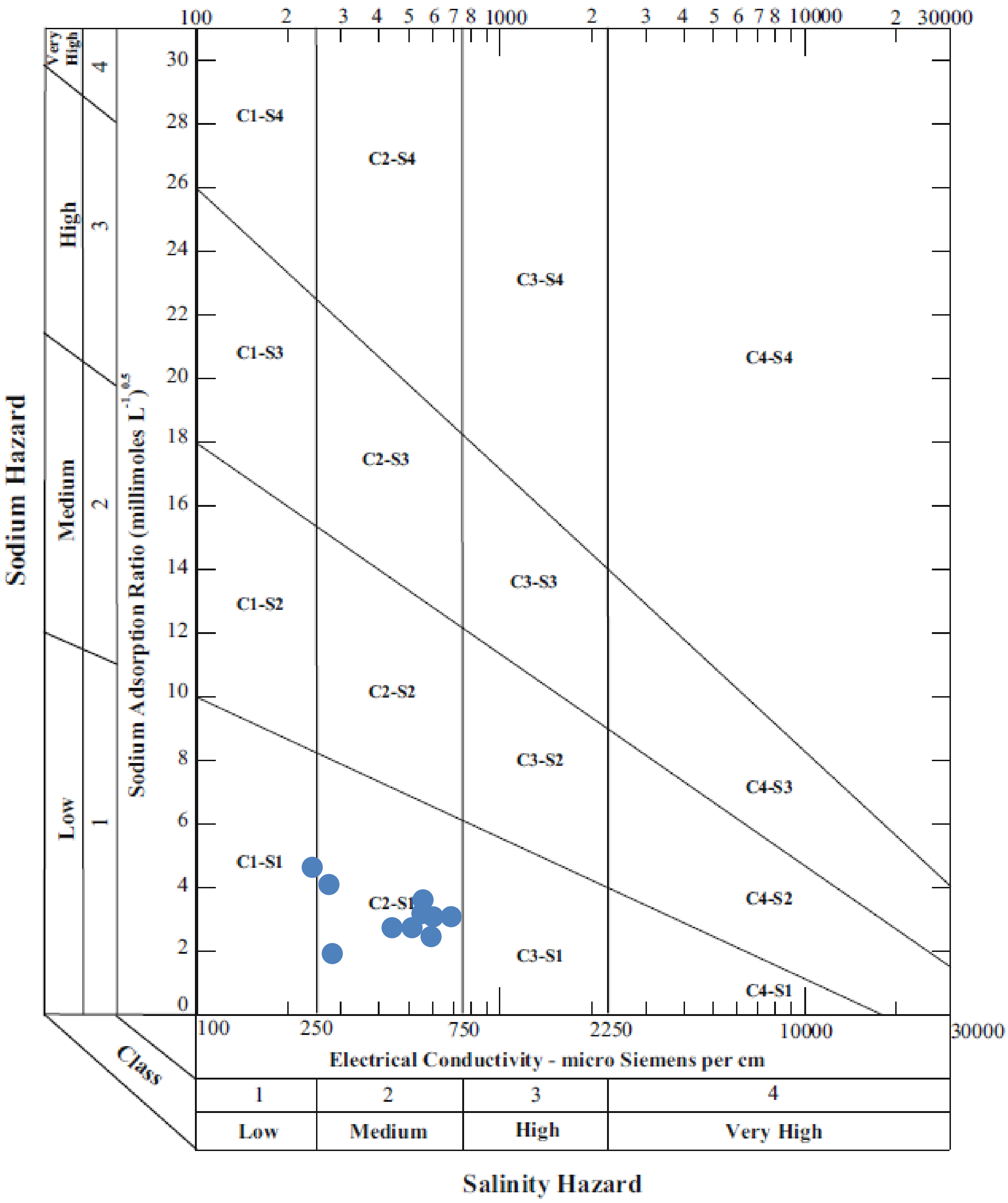


Figure 5. 4. USSL diagram for classifying irrigation waters based on SAR and EC as described by Richards (1954)

5.2.4. Percentage sodium (Na^{+}%)

The suitability of water for irrigation hinges on its mineralization and its impact on plants and soil. Sodium percentage is one method that has been utilized to classify and comprehend the fundamental nature of the chemical composition of water (Wilcox 1955; Richards 1954). In our study, 30% of our samples fall within the permissible category, while the remainder are classified as doubtful in terms of percentage sodium.

5.2.5. Soluble sodium percent (SSP)

Soluble Sodium Percent (SSP) with values lower than 60 correspond to water safe for irrigation, whereas values exceeding this threshold indicate unsafe conditions (Todd and Mays 2004). In our study, SSP values ranged from 55.82 to 72.35 (Table 5.2). Notably, only two samples exhibited SSP values falling within the lower range (<60 - considered safe), while the rest exceeded this threshold (>60 - considered unsafe).

3.2.6. Magnesium hazard (MH)

High magnesium levels in water can detrimentally impact soil structure, particularly in soils with elevated exchangeable sodium content. The magnesium hazard (MH) index serves as a tool to evaluate the influence of magnesium in irrigation water (Paliwal 1972). Magnesium ratio surpasses 50% can lead to adverse effects on crop yields. In our study, the magnesium hazard (MH) of our samples ranged from 16.02% to 19.88%, all below the critical 50% threshold. This shows treated water as suitable for irrigation purposes in term of magnesium hazard.

5.2.7. Kelly’s ratio (KR)

The Kelly's ratio (KR) serves as a pivotal parameter in evaluating irrigation water quality. Ratios below 1 signify suitability, while those falling between 1 and

2 are deemed marginal. Any KR exceeding 2 is considered unsuitable for irrigation. In our study, 70 percent of the samples exhibited KR values ranging from 1.40 to 1.92, indicating marginal suitability. Conversely, 30 percent displayed KR values higher than 2, signifying an excessive sodium content that renders them unsuitable for irrigation based on Kelly's index.

These parameters were outlined (Table 5.1) to provide a comprehensive assessment of water quality concerning irrigation suitability.

5.2.8. Potential salinity (PS)

PS assesses the risk posed by elevated salt levels from Cl^- and SO_4^{2-}, which can increase the osmotic potential of the soil solution when soil moisture drops below 50%. According to this criterion, our samples fall into the beneficial category.

5.2.9. Total hardness (TH)

Water is considered soft when total hardness (TH) is below 75 ppm and moderate when TH is between 75 and 150 ppm. Our samples had water hardness ranging from 92 to 113 ppm, placing them in the moderate hardness category.

Table 5. 1. Key parameters to assess the irrigation water quality

Type of water	Samples	pH	EC	TDS	SAR	SSP	MH	KR	TH	PS
Treated water N35	N35-1	9.18	279	178.56	2.11	53.17	26.13	1.09	94.16	0.57
	N35-2	8.85	598	382.72	2.29	52.59	29.26	1.07	112.98	0.55
	N35-3	8.85	511	327.04	4.37	69.37	24.53	2.19	98.79	2.06
	N35-4	8.97	262	167.68	4.24	67.61	27.76	2.03	108.36	2.44
	N35-5	8.87	357	228.48	3.20	61.02	25.23	1.50	113.61	1.55
Treated water N15	N15-1	9.13	250	118.40	4.54	70.75	26.92	2.35	93.02	1.58
	N15-2	9.18	614	392.96	3.28	62.16	24.98	1.58	107.16	0.54
	N15-3	8.86	565	361.60	3.74	65.47	25.68	1.83	103.25	0.73
	N15-4	8.82	508	325.12	2.84	59.77	24.12	1.43	97.57	0.62
	N15-5	9.02	420	268.80	3.50	63.62	26.43	1.70	105.88	1.16

Table 5. 2. Irrigation parameters and their classification

Parameters	Range	Water Class	No. of Samples	% Of samples
Sodium absorption ratio (SAR) (Richards 1954)	0-10	Excellent	10	100
	10-18	Good	----------	----------
	18-26	Doubtful	----------	----------
	>26	Unfit	----------	----------
Soluble sodium percentage (SSP) (Nagaraju et al. 2006)	<20	Excellent	----------	----------
	20-40	Good	----------	----------
	40-60	Permissible	2	20
	60-80	Doubtful	8	80
	>80	Unfit	----------	----------
Percentage sodium (Na%) (Wilcox 1955)	<20	Excellent	----------	----------
	20–40	Good	----------	----------
	40–60	Permissible	3	30
	60–80	Doubtful	7	70
	>80	Unsuitable	----------	----------
Magnesium Hazards (MH) (Szabolcs and Darab 1964)	<50	Suitable	10	100
	>50	Unsuitable	----------	----------
Kelly Ratio (KR) (Kelly 1963)	<1	Suitable	----------	----------
	1-2	Marginal.	7	70
	>2	Unsuitable	3	30
Total hardness (TH) (Todd and Mays 2004)	<75	Soft	----------	----------
	75-150	Moderate	10	100
	150-300	Hard	----------	----------
	>300	Very hard	----------	----------
Potential salinity (PS) (Delgado et al. 2010)	<3	Beneficial	10	100
	3-15	Moderate	----------	----------
	>15	Not advisable	----------	----------

In Table 5.3, the Pearson correlation matrix of water parameters was used to identify and quantify the linear relationships between irrigation parameters.

Table 5. 3. Pearson correlation matrix of water parameters

	pH	EC	TDS	SAR	SSP	MH	KR	TH	PS
pH	1								
EC	-0.38657	1							
TDS	-0.41098	0.992828	1						
SAR	-0.02339	-0.24852	-0.295	1					
SSP	-0.00749	-0.23011	-0.27671	0.990022	1				
MH	0.07723	-0.19209	-0.20343	-0.12113	-0.23933	1			
KR	0.025501	-0.28264	-0.3368	0.99091	0.987614	-0.1491	1		
TH	-0.35487	0.329997	0.374309	-0.17771	-0.24016	0.348838	-0.30315	1	
PS	-0.14444	-0.53617	-0.52644	0.763829	0.708484	0.075671	0.728483	0.049179	1

3. Soil analysis and microbial community assessment results

5.3.1. Soil nutrient analyses

Following the sunflower growth cycle, the main cations and anions were identified using a 5% citric acid extraction, as shown in Figure 5.5.

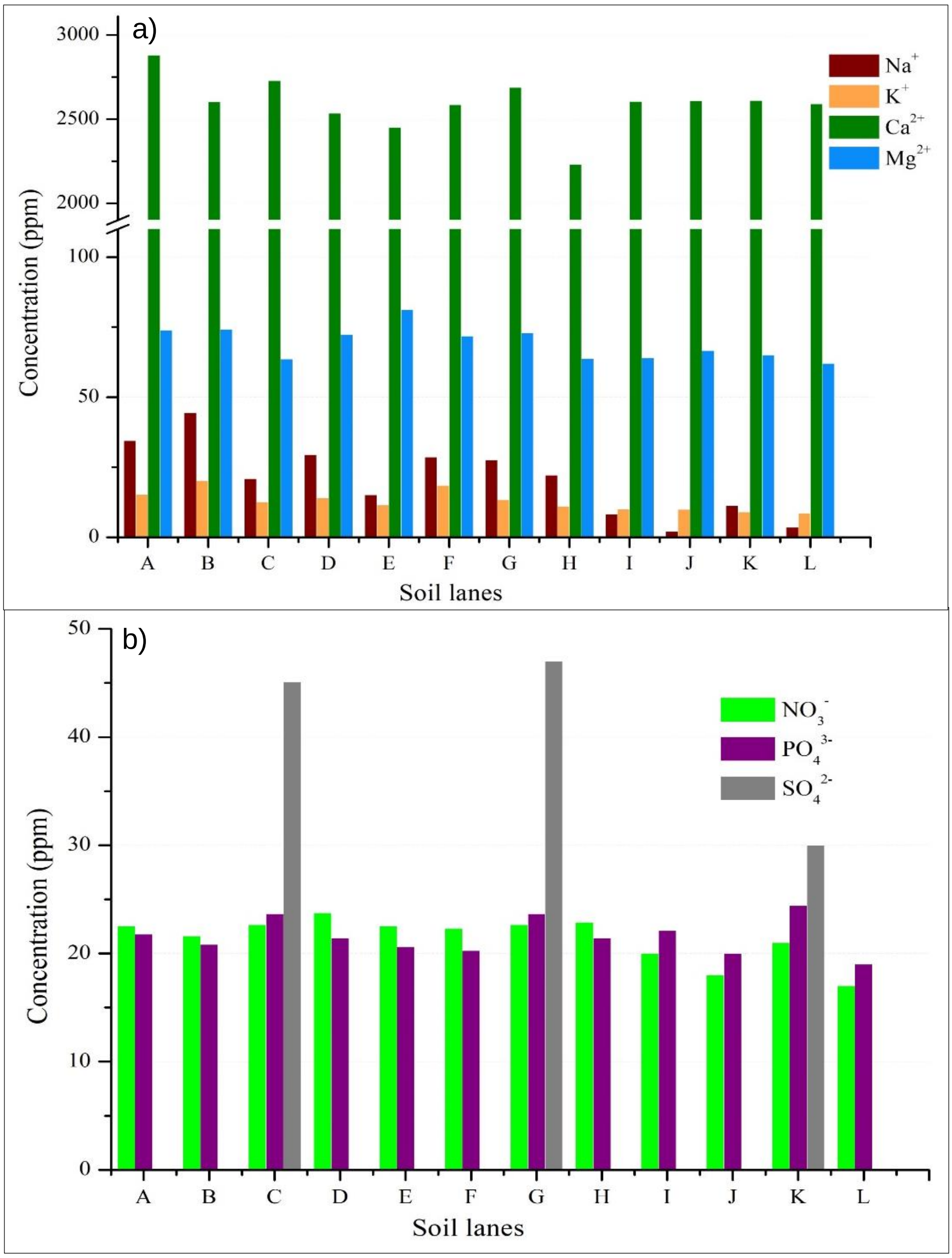


Figure 5. 5. Main Cation (a) and Anion (b) concentrations in the different lanes of soil. Lanes A, B, C, and D were irrigated with treated N15 water; lanes E, F, G, and H with treated N35 water; and lanes I, J, K, and L with tap water. Specifically, lanes A, E, and I were treated with raw biochar; lanes C, G, and K used biochar; while lanes B, D, F, H, J, and L without biochar.

5.3.2. Supplementing EC-biochar to soil: impact on microbial community

Amendment of biochar to soil for agriculture purposes has been demonstrated to be successful due to several reasons like water and nutrient retention and consequently stimulation of microbial activity (Joseph et al. 2021; Oliveira et al. 2017). Together with activity it is reasonable to expect also some kind of change in the profile of microbial community so we proceed to sequence 16s RNA using Nanopore technology in order to analyze the soil biodiversity in presence or absence of biochar.

The taxonomic analysis of soil bacterial communities revealed significant shifts not just due to the presence of biochar but also regarding the nature of the biochar , either raw or used. (Figure 5.6 and 5.7). Thus, the composition of bacterial communities of control soil showed a decrease in the relative abundance of Acidobacteria after addition of raw biochar till non-detected level, while Actinobacteria increased 2-fold. This shift was likely due to synergistic interactions such as co-metabolism or syntrophy, or because these bacteria respond similarly to various biological, chemical, or physical factors, thus occupying similar ecological niches (Nielsen et al. 2014). However, soils treated with used biochar, so with an additional load of nutrients, exhibited opposite response than raw biochar, thus Acidobacteria population increased and Actinobacteria decreased. These findings suggest that raw biochar provides conditions that favor Actinobacteria, likely due to its rich carbon content and impact on soil structure, while used biochar loaded with nutrients rather select for Acidobacteria.

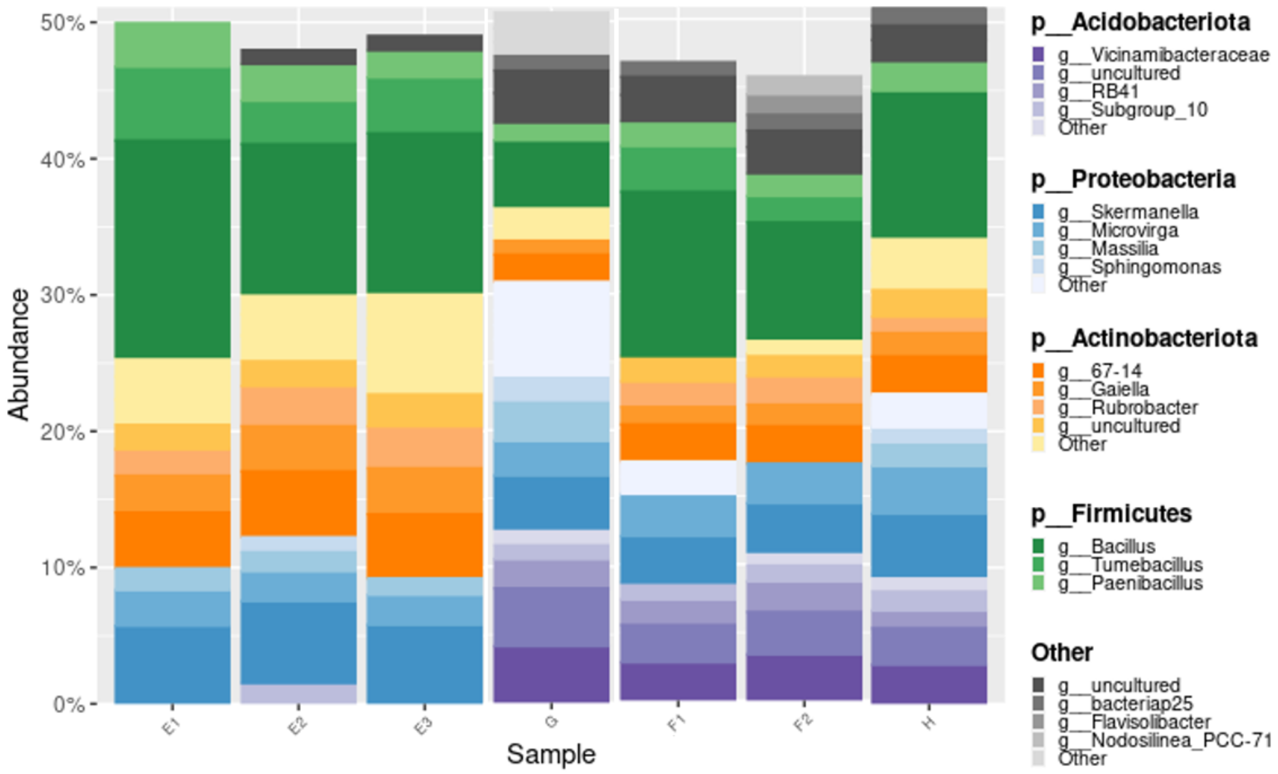


Figure 5. 6. Taxonomic analysis of soil bacterial communities' relative abundances of major taxonomic groups at the phylum and genus levels for bacteria (Soil with raw Biochar (E1, E2, E3), Used Biochar (G), and Biochar-free soil (F1, F2, H))

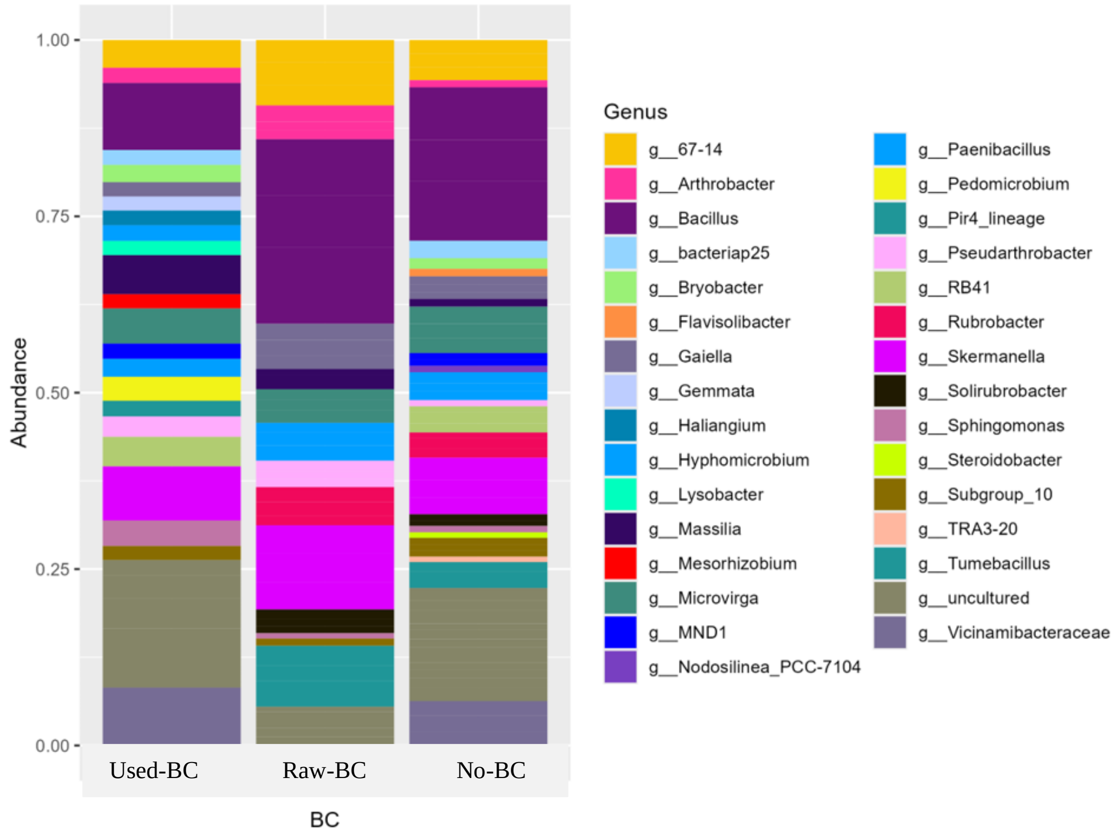


Figure 5. 7. Taxonomic Analysis of Soil Bacterial Communities (Genus >1% of Abundance) in the absence (No BC) and in the presence of used biochar (Used-BC) and raw biochar (Raw BC)

-Alpha diversity

The alpha diversity analysis of soil bacterial communities revealed distinct impacts of raw and used biochar treatments. Specifically, the Observed species count and ACE (Abundance-based Coverage Estimator) index were highest in soils treated with raw biochar, indicating a notable increase in species richness. This suggests that raw biochar enhances the habitat, promoting a greater variety of bacterial species. In contrast, the Shannon diversity index and Inverse Simpson (InvSimpson) index were highest in soils treated with used biochar. These indices, which account for species abundance and evenness, suggest that used biochar creates a more balanced and diverse bacterial community. This balanced microbial environment might result from the soil's longer-term stabilization and adaptation processes. These findings highlighted the differential effects of biochar type on soil microbial diversity, with raw biochar boosting species richness and used biochar enhancing overall diversity and evenness.

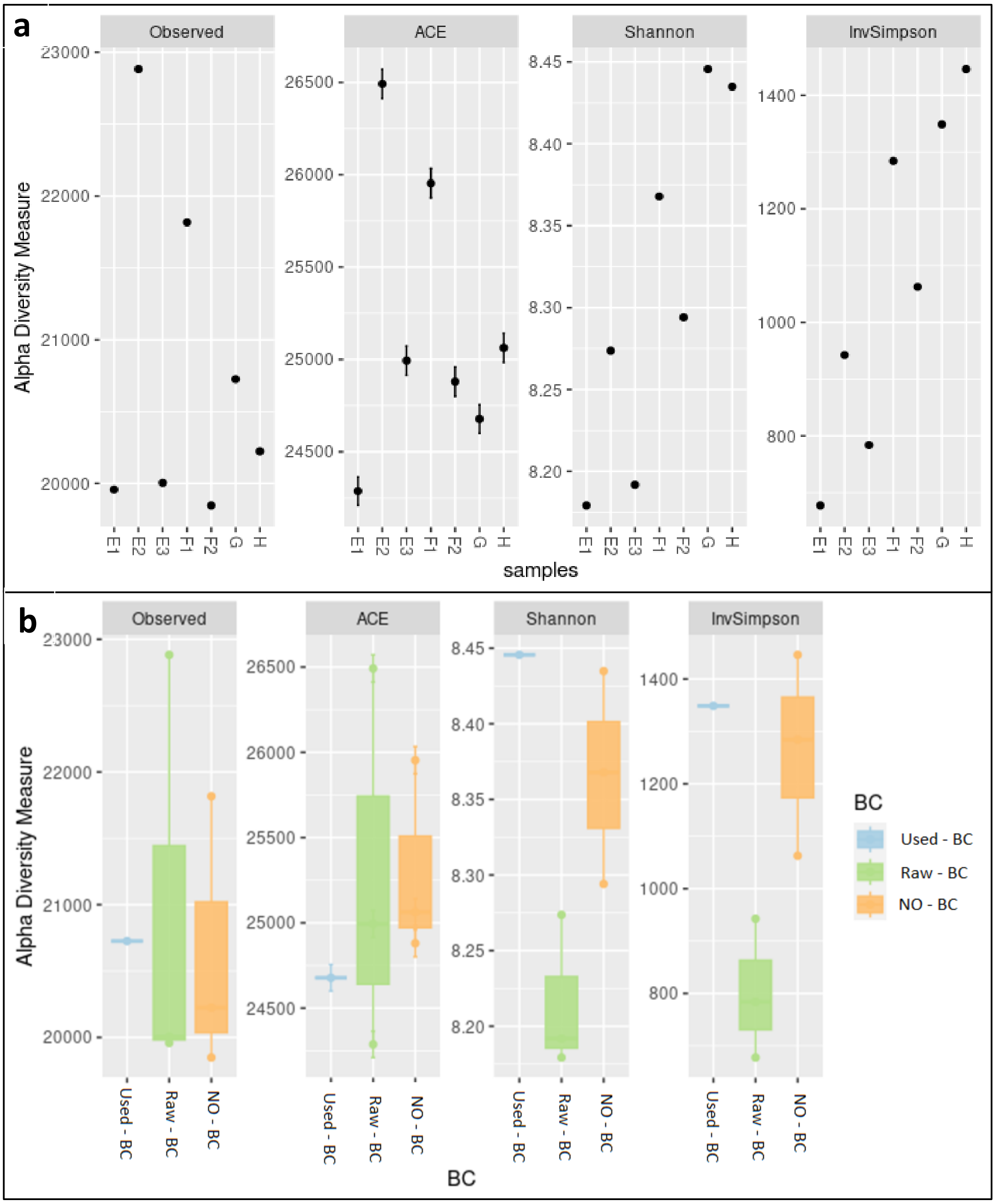


Figure 5. 8. Biodiversity parameters shown as a) Alpha Diversity Measurement for the following soil Samples: Soil with raw Biochar (E1, E2, E3), Used Biochar (G), and biochar-free soil (F1, F2, H). (b) Average values for alpha diversity in different samples (used-BC, raw-BC, and biochar-free soil)

5.4. Fertilizer impact of electroconductive biochar: raw versus used

After treated wastewater, the second circular economy element we validated using sunflower crops was electroconductive biochar. Typically biochar has been used as soil amendment for centuries (reference); however, conventional biochar is produced at moderate pyrolysis temperatures which does not graphitize carbon so the final product is far from being electrically conductive. In contrast, technological solutions like METland® requires the use of electroconductive material for the construction of the biofiltering bed. Thus, the EC-biochar we used for wastewater treatment was highly conductive and we aim to explore the impact of this material as soil amendment.

5.4.1. Chemical adsorption in electroconductive Biochar

Similar to other types of biochar, raw EC-biochar contains various elements integral to its structure. The functional groups on its surface, such as hydroxyl, carboxyl, and phenolic groups, play a significant role in its adsorption capabilities (Ambaye et al. 2020), EC-biochar used for METland® is expected to trap a number of elements originally present in wastewater. The nutrient analysis revealed that the used biochar contains levels of nitrate were ca. 2-fold higher than raw biochar. The results was even more remarkable for phosphate since levels were 3-fold higher if biochar was previously used for treating wastewater. The last revealed used biochar as a more promising soil amendment for soil.

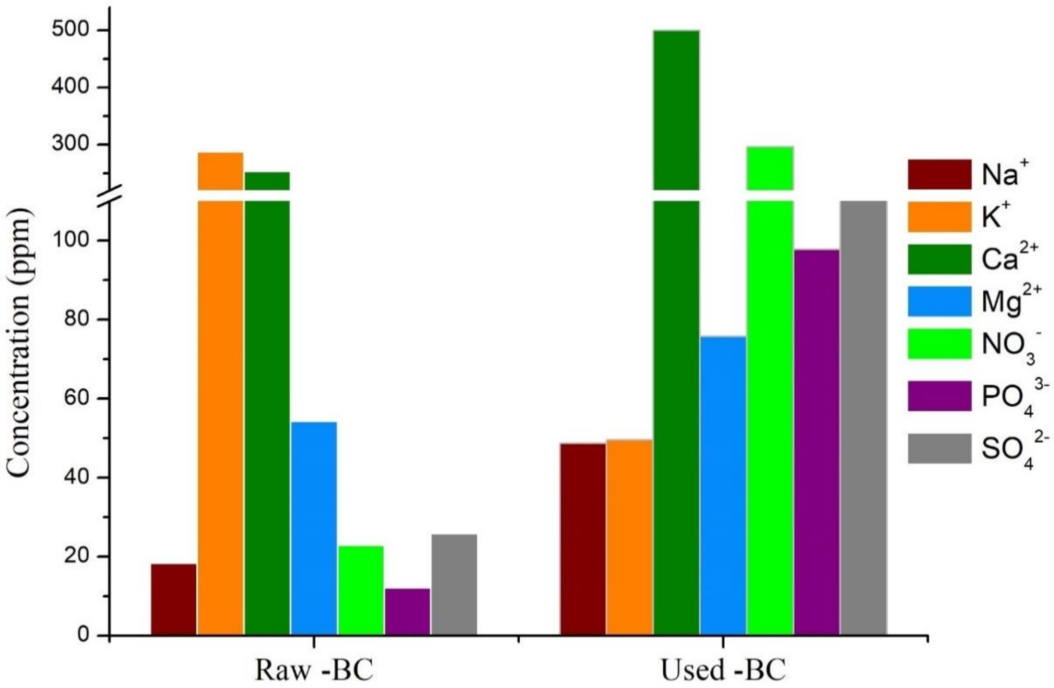


Figure 5. 9. Main ion Concentrations in raw and used sawdust biochar

5.4.2. Elemental analyzer (CHNS)

An elemental analyzer was used to determine the elemental composition of both raw and used sawdust biochar, which were applied for soil treatment during sunflower cultivation. The analysis focused on evaluating the presence of carbon, hydrogen, nitrogen, and sulfur (CHNS) in the biochar samples. The results of the elemental analysis for both raw and used biochar are shown in Figure 5.10.

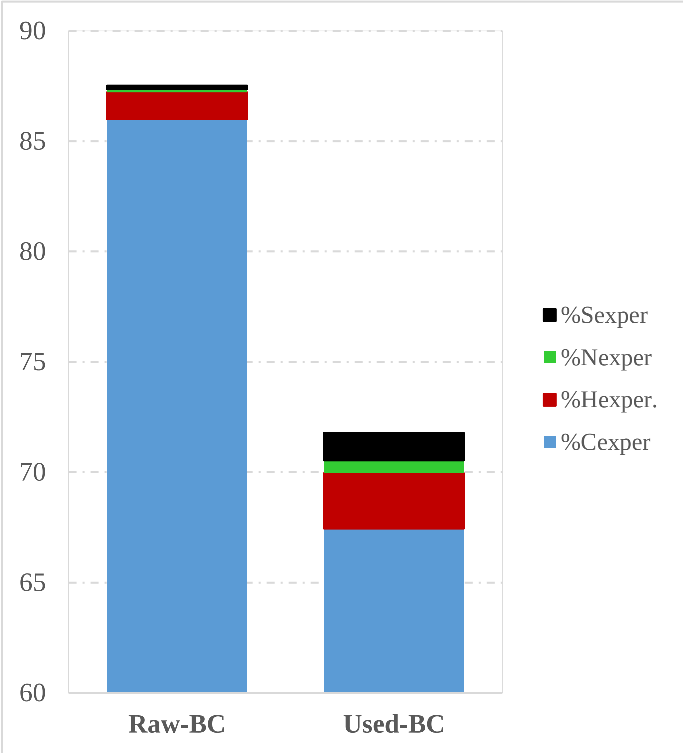


Figure 5. 10. Result of elemental analysis CHNS-932 of raw-BC and used-BC

5.4.3. Results of total nitrogen, total organic carbon, inorganic carbon, and total carbon

The results showed a significant difference in total nitrogen between raw biochar and biochar previously used in wastewater treatment. Raw biochar contains more inorganic carbon, whereas the amounts of total nitrogen and total organic carbon were significantly higher in used biochar.

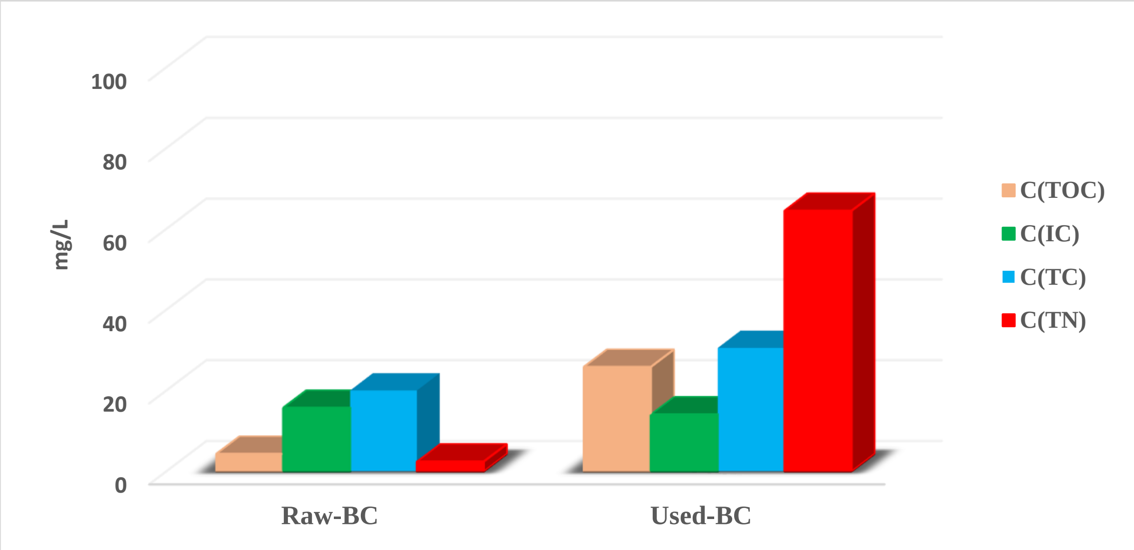


Figure 5. 11. Total nitrogen, total organic carbon, inorganic carbon, and total carbon release from raw and used sawdust biochar over 24 hours of incubation

5.5. Impact of irrigating with treated wastewater and Ec-biochar for sunflower cultivation.

After three months of growing Sunflower using tap water and two types of treated water (N35 and N15) together with raw and used EC-biochar, all crops were harvested and evaluated regarding a number of variables: the average flower cluster diameter (in centimeters), surface area (in square centimeters), volume (in cubic centimeters), average dry weight of flowers (in grams), and overall product weight (in grams).

Regardless the type of water used for irrigation, sunflower grew healthy and following expected standard parameters, confirming then that treated wastewater did not exhibit any toxicity and revealing itself as suitable for agriculture purposes. Actually, in absence of biochar, the sole use of treated water yield larger flower cluster (ca. 300 cm^3) than tap water (ca. 150 cm^3) did. This remarkable result demonstrate the advantage of using re-using treated wastewater for crop irrigation. More conventional nutrients like nitrate and phosphate were also present in tap water so we cannot conclude what element present in treated wastewater is responsible for the higher yield. Furthermore, lanes irrigated with tap water could be also more productive (ca. 250 cm^3 flower) when electroconductive biochar, either raw or used, was added.

A significant contrast was observed between lanes with and without biochar biochar amendment. Clusters grown in biochar-treated lanes were 17% heavier than those grown in natural soil. Additionally, early yield was observed in those lanes where biochar was present. The first flowers appeared earlier in biochar-treated lanes, and it was found that using biochar can save water. Biochar not only enhanced the strength of sunflower stalks during the growing period but also led to an increased yield and water savings due to earlier blooming.

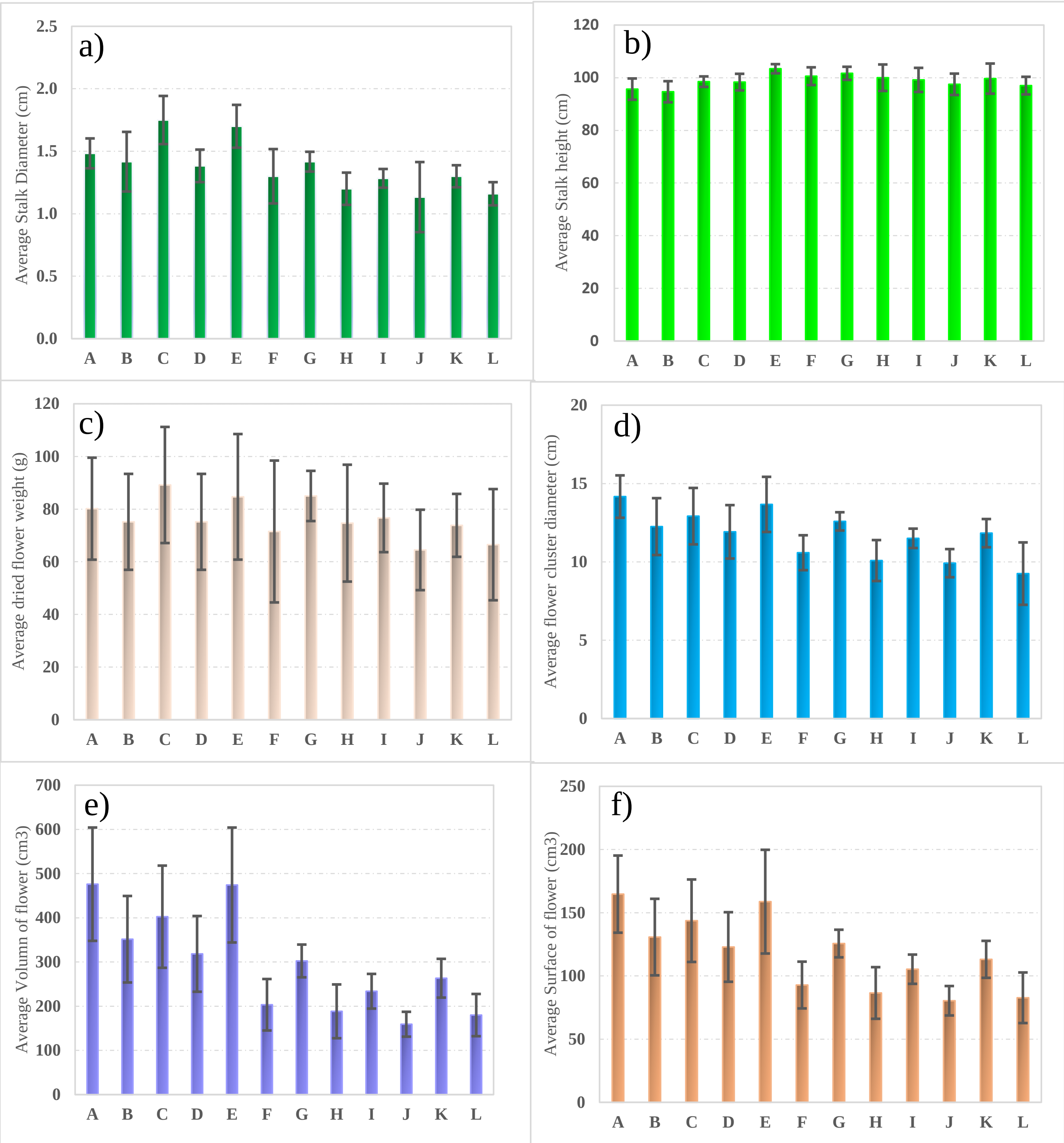


Figure 5. 12. Comparison of average stalk diameters (cm) (a), average stalk heights (cm) (b), average dry cluster weights (g) (c), average flower cluster diameters (cm) (d) average surface of flower (cm2) (e) and average volume of flower (cm3) across eight experimental lanes. (Lanes A, B, C, and D were irrigated with treated N15 water; lanes E, F, G, and H with treated N35 water; and lanes I, J, K, and L with tap water. Specifically, lanes A, E, and I were treated with raw biochar; lanes C, G, and K used biochar; while lanes B, D, F, H, J, and L without biochar).

Flower production was by far the variable showing a higher impact due to the addition of biochar, regardless its nature (raw or used) in comparison with biochar-free soil.

Figure 5. 13. Early yield of sunflowers in lanes with biochar

However, the use of nutrient enriched biochar (used biochar) did not show a significant improvement regarding flower productivity in respect to raw biochar. The advantage of using such nutrient-rich biochar could be shaded by the fact that irrigation water was also loaded with nutrients (15 ppm or 35 ppm Nitrate) so raw wastewater may adsorb nutrients from water and perform like used biochar . Beyond this first preliminary result, further research is required to verify this hypothesis by irrigating with different amount of nutrients or even with no nutrients at all. Unfortunately, the nitrate and phosphate present in the tap water we use did not allow us to verify the last hypothesis.

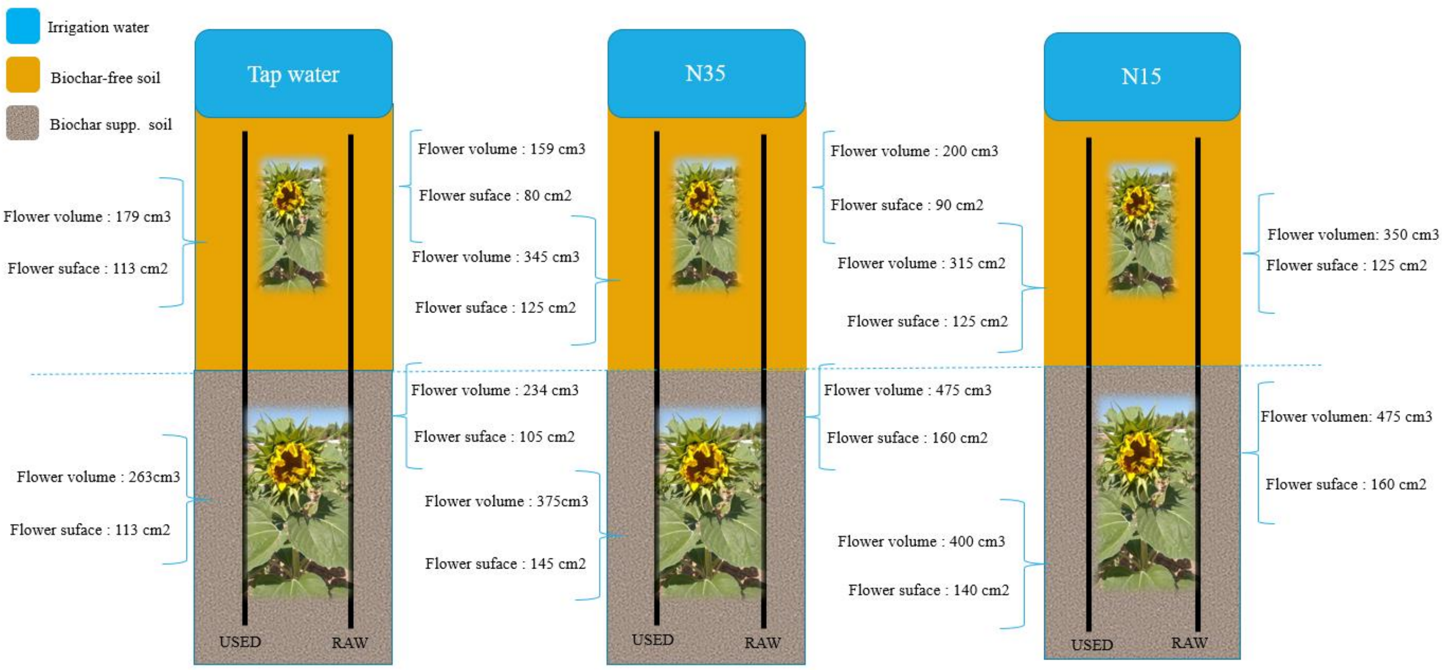


Figure 5. 14. Impact of i) irrigation water (tap water and treated wastewater with 15 and 35 ppm in nitrate) and ii) biochar amendment (biochar free, raw biochar and used biochar) on the production of sunflower

Chapter 6:
Validating METland® Technology for Re-using Wastewater in University Campus

6. Validating METland® Technology for re-using wastewater in University Campus

External Campus of University of Alcalá (UAH) shows heavy reliance on well water for irrigation, especially during dry months like July and August, when rainfall is minimal so addressing this water scarcity challenge is crucial. In this context, the current chapter focuses on evaluating if METland® technology may treat wastewater produced on campus, with the ultimate aim of re-using the effluent to irrigate the campus's vegetation. Firstly, we tested the effectiveness of METland® biofilters for treating real wastewater harvested in UAH´s Campus. Thus, the impact of bed material, particularly concerning the removal of COD and nitrogen, was evaluated. Secondly, the quality of the treated wastewater was assessed by i) chemical analysis, and ii) growing hydroponic crops including analysis of fluorescence parameters in order to discard the potential toxicity for plants. Finally, according to irrigation needs on Campus, together with wastewater flow rate, a design proposal was elaborated to implement a number of METland® units to replace groundwater use.

6.1. The performance of vertical downflow METland® biofilters (VF MET)

METland® biofilters made of three different materials (gravel, EC-biochar and EC-biochar supplemented with 10% humus) were evaluated regarding their efficiency for removing COD and nitrogen from real wastewater harvested on Campus and industrial wastewater from the brewery sector. The biofilters were operated downflow under recirculation mode for 170 days along 4 different phases associated with wastewater of different compositions.

6.1.1. Removal of organic pollutants (COD)

Real wastewater from Campus suffered of high variability according to academic season, due student attendance and rainfall. Thus, in order to operate biofilters with a more representative COD, campus wastewater (ca. 200 ppm COD) was also tested after a supplement of brewery wastewater till reached constant

COD values of ca. 1000 and 1300-1400 ppm. Furthermore, the impact of the recirculation rate was also monitored at 2L/d and 3 L/d.

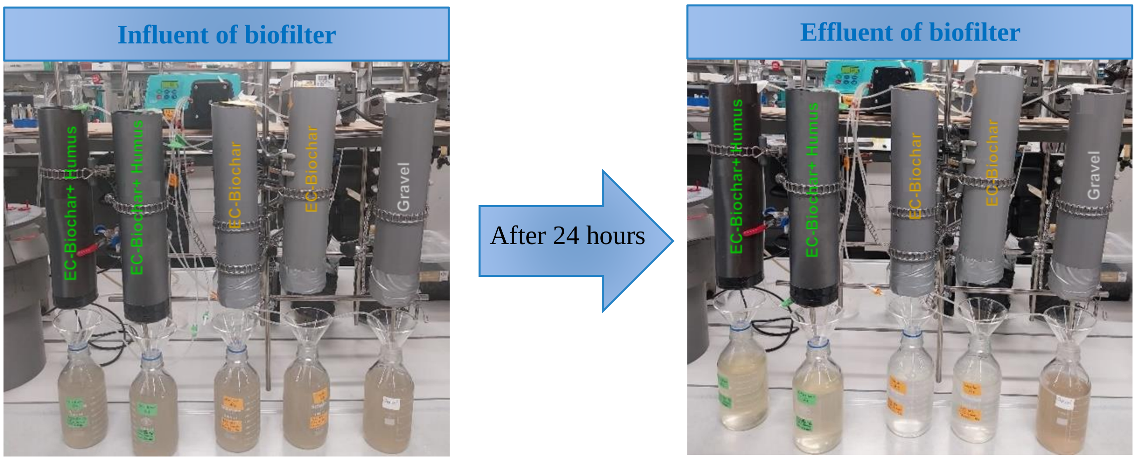


Figure 6. 1. Biofilter performance for treating wastewater during 24 hours. Biofilters made of EC-biochar and EC-biochar supplemented with humus were operated in two replicas. Gravel Biofilter was operated as control for conventional wetland made of inert bed material.

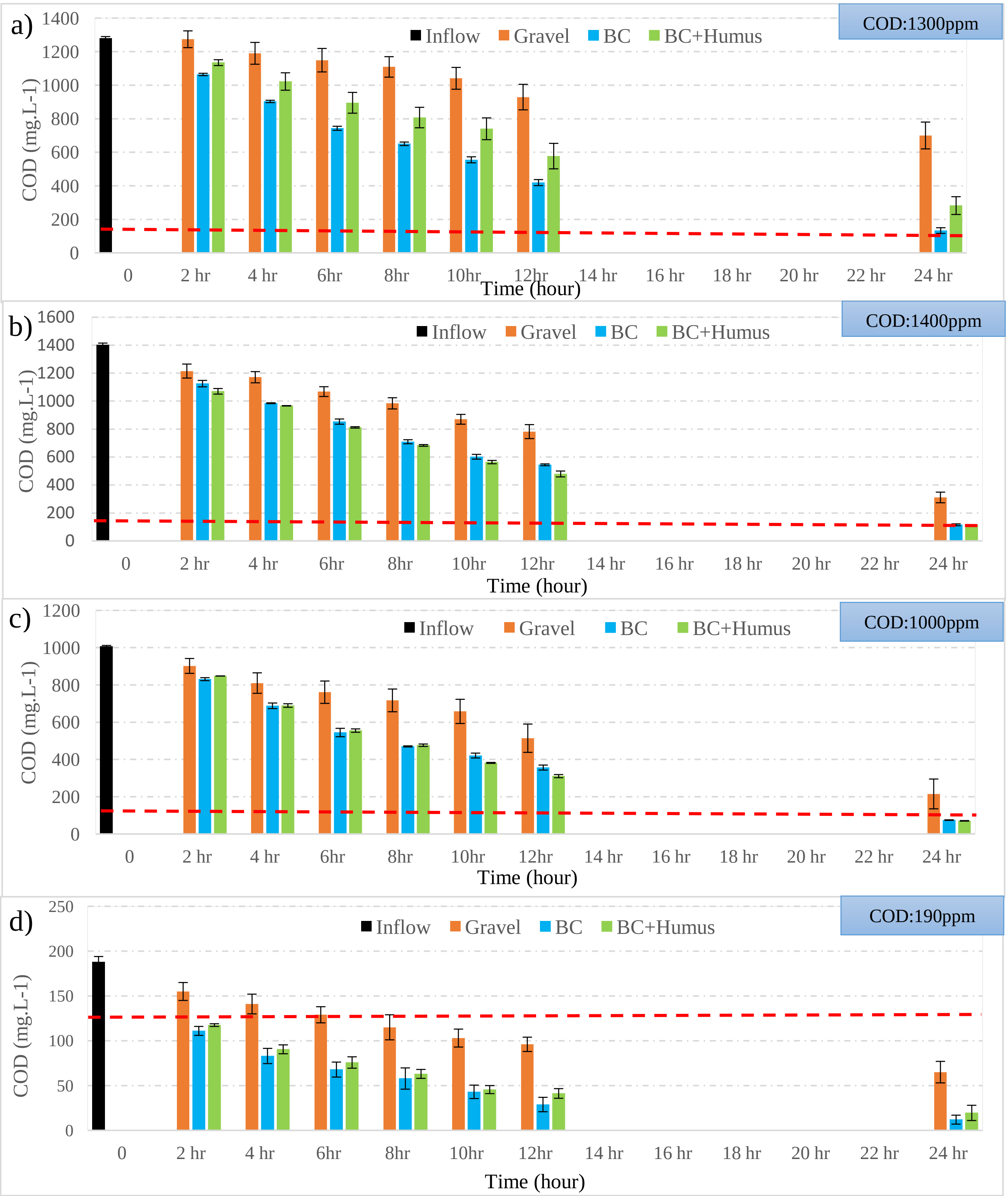


Figure 6. 2. Biofilter performance regarding COD removal from ww fed at different COD load (black column): a) 1300 ppm at 2L/day, b) 1400 ppm at 3L/day, c)1000 ppm at 3L/day, d) 190 ppm COD at 3L/day. The following biofilters were operated for 24 h: gravel (orange), ec biochar (blue), and ec-biochar mixed with humus (green). The red line represents the European discharge limit of 125 mg/L, as outlined in Council Directive 2000/60/EC of 23 October 2000

Our results showed how biochar-based biofilters outperformed gravel biofilter under all tested conditions. Indeed, biochar-based biofilters generated an effluent with residual COD value lower than 25ppm when sole wastewater from campus was used. This is remarkable considering difficulties to reach values lower than 50 ppm conventional biofilters. Indeed, gravel biofilter reached just 70 ppm COD in effluent (Figure 6A). Rest of assays using wastewater in a higher COD range (1000-1400 ppm) showed identical trend when EC-biochar biofilters in comparison to gravel ones. Furthermore, just EC-biochar biofilters fulfill discharge limit (<125 ppm COD) after 24 hours operation. Indeed, systems were capable of further biodegradation so operating systems 4 additional hours improved water quality till reaching remarkable COD values below 50 ppm. During the first 12 hours of operation, efficient behavior of electroconductive material resulted in removal rates for EC-biofilter as high as 44 g COD/m^3_{bed} h in contrast with 31 g COD/m^3_{bed} h observed in gravel biofilter (Table 6.1).

Table 6. 1. Impact of recirculation on COD removal rate

Flow rate*	**2L/day**	**2L/day**	**3L/day**	**3L/day**
Biofilter	gravel	biochar	gravel	biochar
Removal rate (g COD/m^3_{bed} h)	19	34	31	44

* One liter of wastewater was recirculated for 12 hours at 2 or 3 L/h

The efficiency analysis revealed that biofilter made of EC-biochar removed ca. 95% of COD from campus wastewater (inlet of 190 ppm COD) in contrast with 64% for gravel biofilter. In case wastewater from campus is supplemented till reaching 1400 ppm, then EC-biofilter removed 92% of COD while gravel biofilter just removed 74%. Regarding the use of humus as a supplement, this quinone-rich material did not improve the performance of biofilters regarding COD removal. However, we cannot exclude alternative impact in metal and nutrient adsorption since this material has previously shown this role (Lipczynska-Kochany 2018).

In conclusion, we can estimate that the removal rate for wastewater for campus was roughly 200 g COD/m^3_{bed} day so a building producing 1 m^3/day would require a METland® solution of 1m^3 of electroconductive biofiltering bed.

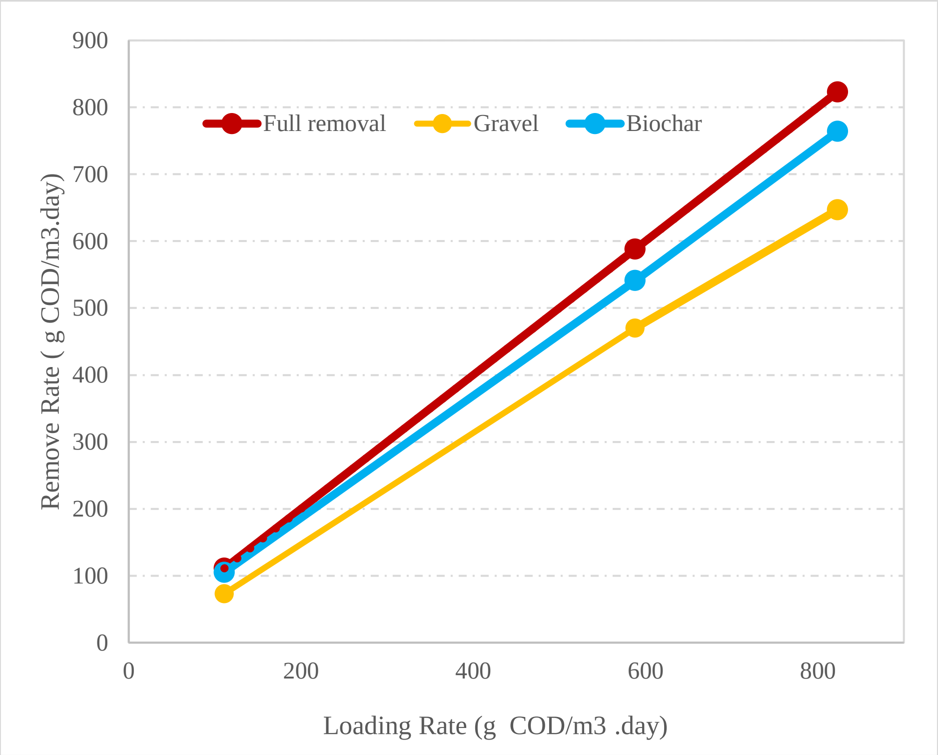


Figure 6. 3. Loading Rates and Removal Rates for COD (g COD/m³bed. day) for biofilter made of Gravel and Biochar. Operation for full removal also included

6.1.2. Removal of nitrogen

The removal of ammonium from effluents occurs through microbial assimilation or nitrification processes. Nitrification, which relies on oxygen, proceeds in a two-step sequence involving two types of bacteria: ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Initially, AOB converts ammonia into nitrite. Subsequently, NOB transfers nitrite into nitrate. Previous research reported enhanced efficiency, particularly in nitrogen compound oxidation, during the performance of biofilters using electroconductive biochar as a bed material (Prado et al. 2020). Such a study highlighted an efficiency range of 90% removal of total nitrogen. However, operation of these biofilters occurred under flooded conditions where anoxia and nitrate conversion to nitrogen gas were favored.

Similar results we also reported in downflow electroactive biofilters by(Aguirre-Sierra et al. 2020). Interestingly, they also reported a 95% removal of ammonium at a rate of 280 mm/d, which resulted in an average concentration as low as 1.6 ± 0.2 mg NH_4-N/L, which implies denitrification regardless of the presence of oxygen typical of downflow operation. This fact was unexpected considering that oxygen typically inhibits nitrate reduction by competing for accepting electrons from COD oxidation. The fact that our wastewater on campus showed a low ratio of COD/N (ca. 2) allowed us to test our system under conditions where nitrogen removal could be limited. The nitrification performance of biofilters revealed that gravel material just removed 51% of ammonium after 24 hours of operation, in contrast with 90% removed by EC-biochar. Ammonium was indeed converted into nitrate that eventually accumulated in the recirculation tank from gravel biofilter till reaching values as high as 100 ppm. This is consistent with the low COD level detected in such a tank after 24 hours (60 ppm) so electron donors were not available for denitrification. Consequently, just 19% of total nitrogen was removed in the gravel biofilter. However, regardless of such low COD, the EC-biochar performance revealed a very different scenario where nitrate was not accumulated (<10 ppm) suggesting an efficient denitrification, that indeed was confirmed by a ca.90% of total nitrogen removed. We hypothesized that electroactive bacteria are using those electrons either stored in the functional groups (eg. Hydroquinones) from biochar surface (Schievano et al. 2019; Prado et al. 2020; Prado, Berenguer, and Esteve-Núñez 2022) or stored as part of organic pollutants adsorbed to the material.

The biochar used for our assays was previously used for supporting wastewater treatment for long periods so it is reasonable to admit that it may store enough electrons to support denitrification. Such a result requires further analysis out of the scope of this thesis.

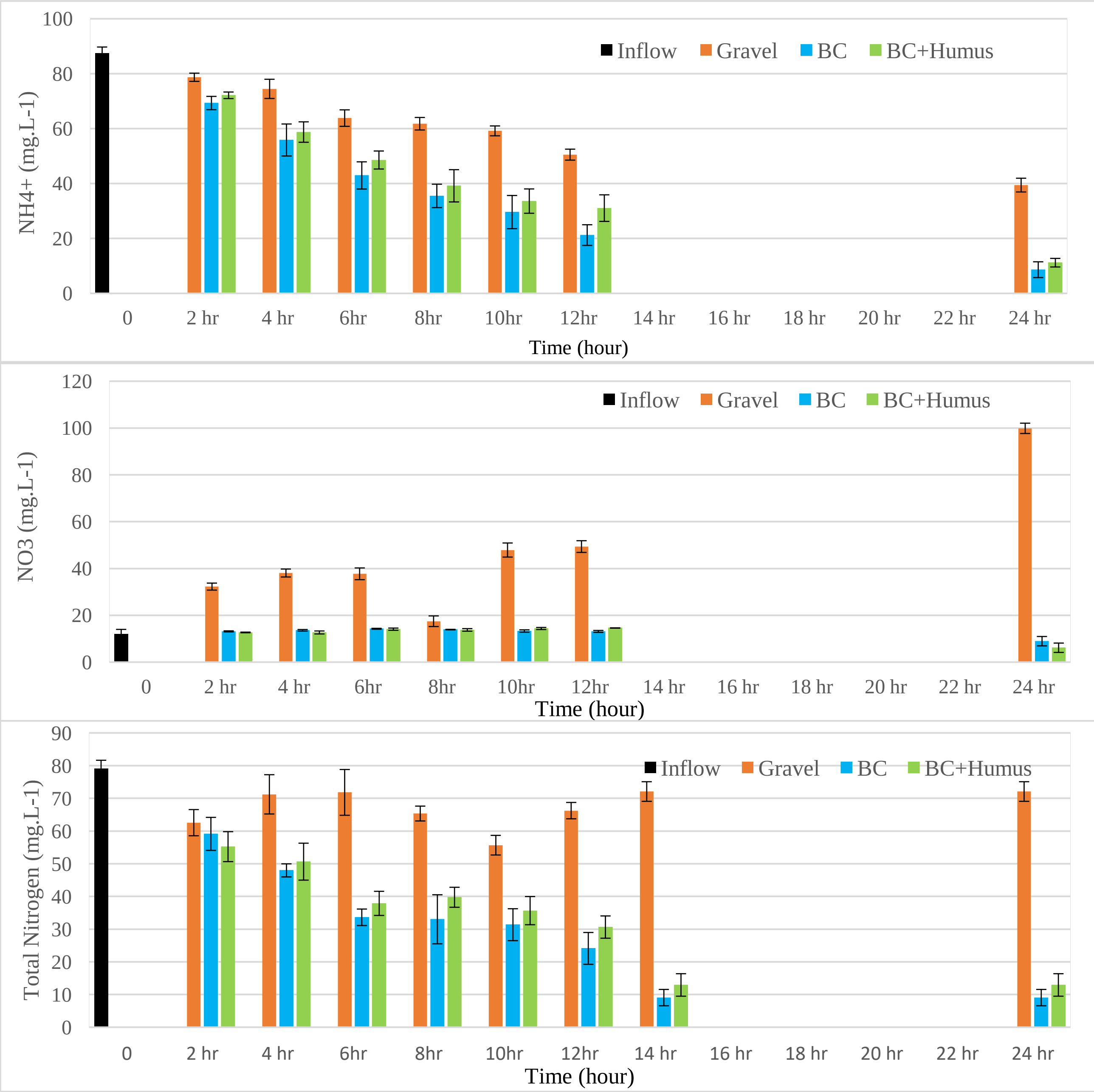


Figure 6. 4. Biofilter performance regarding ammonium (A), Nitrate (B), and total nitrogen (C) from WW from campus (black column). The following biofilters were operated in batch at 3L/h for 24 h: gravel (orange), EC-biochar (blue), and EC-biochar mixed with humus (green).

Another condition that typically inhibits the nitrification of ammonium in wastewater is the presence of COD. Thus, we analyzed the impact of COD (1000 and 1400 ppm) in such ammonium oxidation (Figure 6.4) after supplementing our campus wastewater with brewery wastewater.

The analysis measurement of ammonium removal revealed significant disparities in efficiency based on the nature of bed material. Thus, despite the high COD load (1000-1400 ppm), Biochar-based biofilters exhibited ammonium removal efficiency higher than 90-93%, while gravel-based biofilters showed values in the 60-65 % range. Furthermore, biochar biofilters supplemented with humus revealed a slight enhancement in ammonium removal. Similar role of humus was previously reported by (Jiménez Conde 2024) in electroconductive biofilters acting as tertiary treatments of WWTP effluents. However, they observed a higher impact probably due to the very low COD (< 50 ppm) present in such effluents. Additional studies reported also how the presence of humic acid represent a beneficial impact on electron transfer efficiency, enhancing the activity of enzymes responsible for nitrogen removal and altering the composition of functional bacteria within the system (Li et al. 2016).

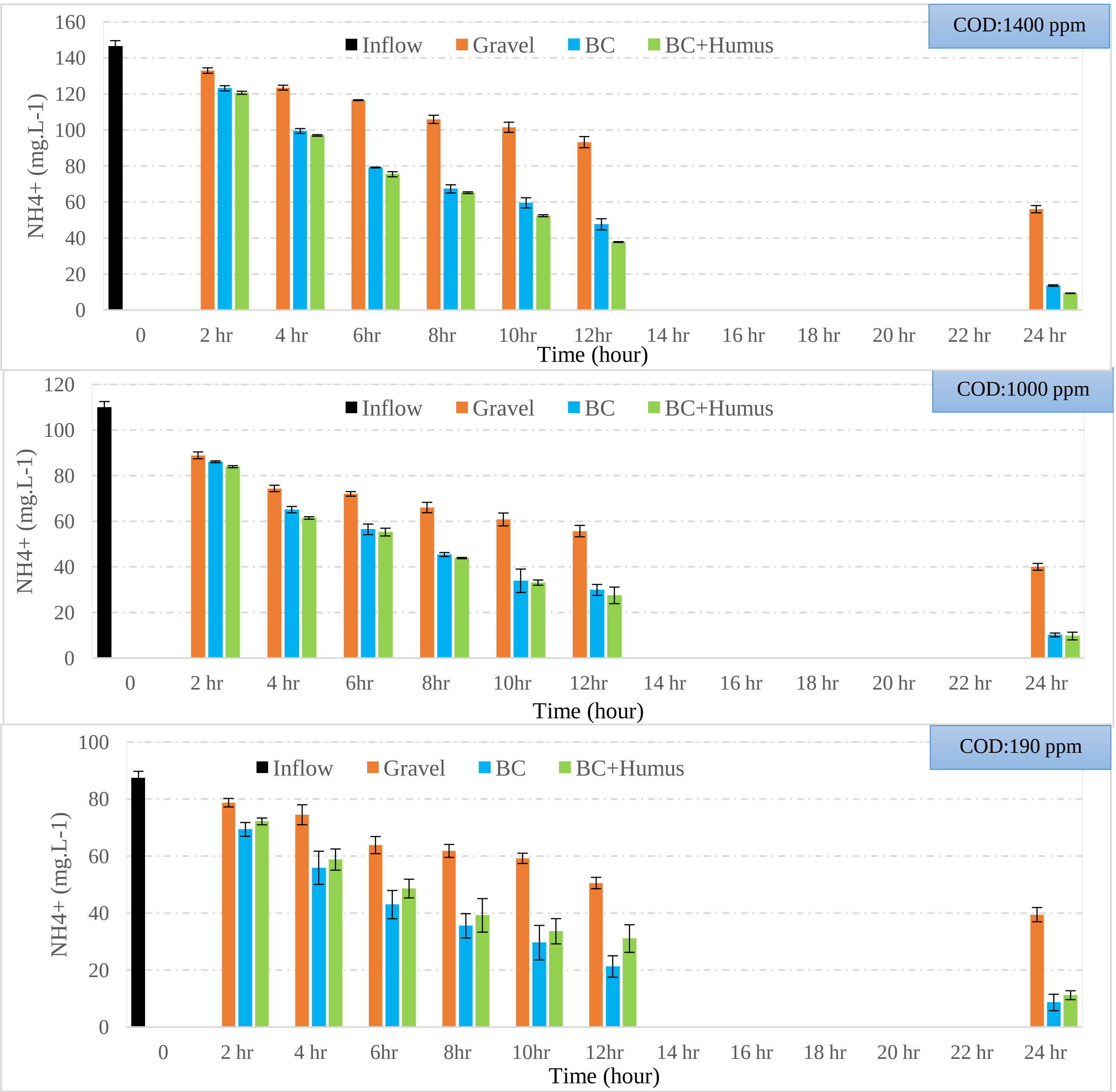


Figure 6. 5. NH4+ concentration at the influent (black) and effluent of each biofilter, including gravel (orange), EC-biochar (blue), and EC-biochar mixed with humus (green), operating operating operated at COD levels of 1400 ppm, 1000 ppm, and 190 ppm

So finally, we have demonstrated that an EC-biochar biofilter can efficiently remove ca. 93-95% of both COD and total nitrogen from urban wastewater at campus even after supplementing the pollutant load to values as high as 1400 ppm COD and ca. 150 ppm ammonium. The water quality of the effluent suggested a proper re-use for irrigation in agriculture.

6.2. Validating the water quality of effluent for hydroponic agriculture: lettuce growth and toxicity assessment

In order to test the potential use of treated water by EC biofilters for hydroponic agriculture, a number of assays using lettuce as a vegetal species were performed. Three sets of hydroponic growth were independently irrigated by: i) effluent from the EC biofilter after treating campus wastewater, ii) A nutrient-free solution as negative control, and iii) a commercial fertilizer solution as positive control, respectively (Table 6.2). Furthermore, to evaluate the potential toxicity of effluent in plants, we conducted a chlorophyll fluorescence assay using the hydroponic plants.

Table 6. 2. Physical-Chemical Characteristics of Water Used in Hydroponic Systems

	Concentration (ppm)										
	CL^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Na^+	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	pH	EC*
EC biofilter	89	17	5	69	78	9	21	91	31	8	600
Fertilizer	-----	105	18	24	7	-----	215	12	35	7	650

*micromhos per centimeter (µmhos/cm)

In the process of biomass production through photosynthesis, leaves are indispensable plant components crucial for this metabolic activity. Their significance extends to plant productivity, with leaf area serving as a critical parameter (Tondjo et al. 2015). After two weeks of cultivation, the leaf area of the largest leaf from each plant and the average total leaf area for each set of plants were measured. Specifically, the leaf area for plants treated with commercial fertilizer was 17-fold higher than setups germinated with nutrient-free water. Moreover, commercial fertilizer promoted plants with leaf areas 3-fold larger than those grown with treated wastewater from campus.

Figure 6. 7. Image of hydroponic growth of Lettuce after two weeks of cultivation under different nutrient solutions

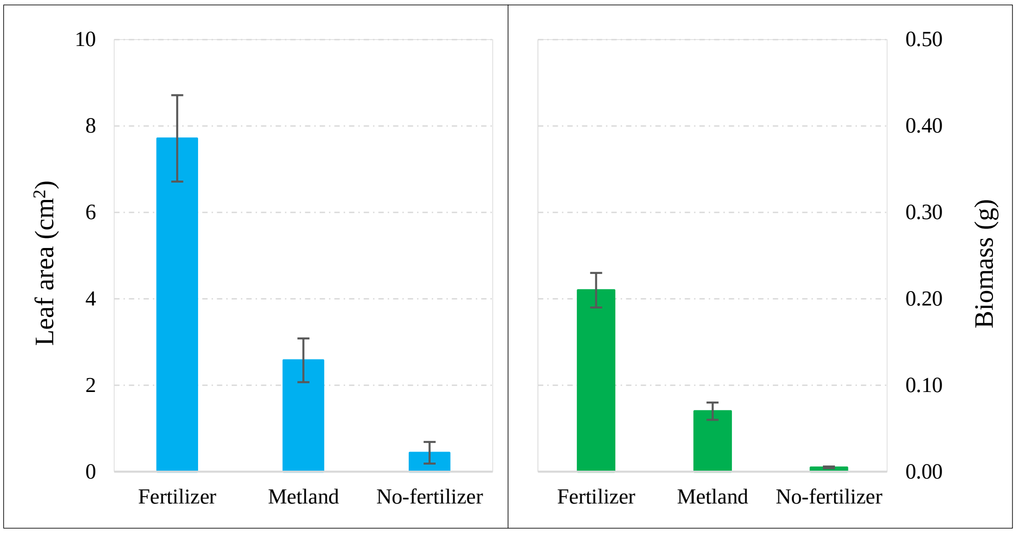


Figure 6. 6. Average leaf area (cm²) and biomass weight (g)

The nutrient composition of biofilter effluent could be variable depending on activity of campus over time. The effluent includes nutrients (nitrate and phosphate) capable of promoting plant growth although at a 10-fold lower presence than commercial fertilizers. These findings align with prior research highlighting the importance of nutrient composition in hydroponic solutions for lettuce growth (Fraile-Robayo et al. 2017; Sapkota et al. 2019). The findings indicate that lettuce grows in these conditions, implying that effluent water from our biochar biofilter system is suitable for irrigation however the nutrient composition was not optimal, at least for germination.

6.2.2. Evaluation of fluorescence emission by plants

In our study, we focused on measuring fluorescence parameters to assess the potential toxicity of water sources affecting plant stress. We conducted measurements on plants grown with i) effluents from EC- biochar biofilter, and ii) a commercial fertilizer, serving as a positive control. Due to the small leaf size of plants treated with nutrient-free solution, measurements were not feasible.

Fv/Fm is a commonly used indicator of the maximum quantum efficiency of Photosystem II (PSII) in plants. It represents the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) and is used to assess the health and stress level of photosynthetic organisms (Butler 1978; Genty and Meyer 1995). Our findings revealed that plants exposed to effluent from EC-biochar exhibited an average Fv/Fm ratio of approximately 0.80, whereas plants treated with hydroponic commercial fertilizer showed a slightly higher value of 0.83. These results are consistent with established knowledge indicating that under optimal conditions, unstressed leaves typically maintain an Fv/Fm ratio close to 0.83, representing the maximum quantum yield of photosynthesis (Butler 1978; Genty and Meyer 1995; Demmig and Björkman 1987). Furthermore, ΦPSII and qP are vital photochemistry ratios that deserve attention, as they reflect the state of photosynthetic efficiency. These metrics exhibit diverse trends closely associated with pollutant concentrations, showing a decrease as pollutant levels increase (González-Naranjo et al. 2014). Moreover, their values are subject to variation influenced by the presence of contaminants, as evidenced by prior studies involving different photosynthetic organisms (Qiu, Wang, and Zhou 2013; Zezulka et al. 2013; González-Naranjo et al. 2014).

In our investigation, we noted that the qP value for plants irrigated with effluent from EC-biochar biofilter was marginally lower compared to those receiving fertilizer, measuring 0.11 and 0.13, respectively. Additionally, the average ΦPSII value for plants treated with commercial fertilizer was 0.84, whereas, for those exposed to effluent from EC-biochar it notably increased to 0.91. These results suggest that there was no discernible stress on plants grown after irrigation with effluent from biochar biofilter, especially considering the higher ΦPSII value, highlighting its potential suitability for promoting plant growth.

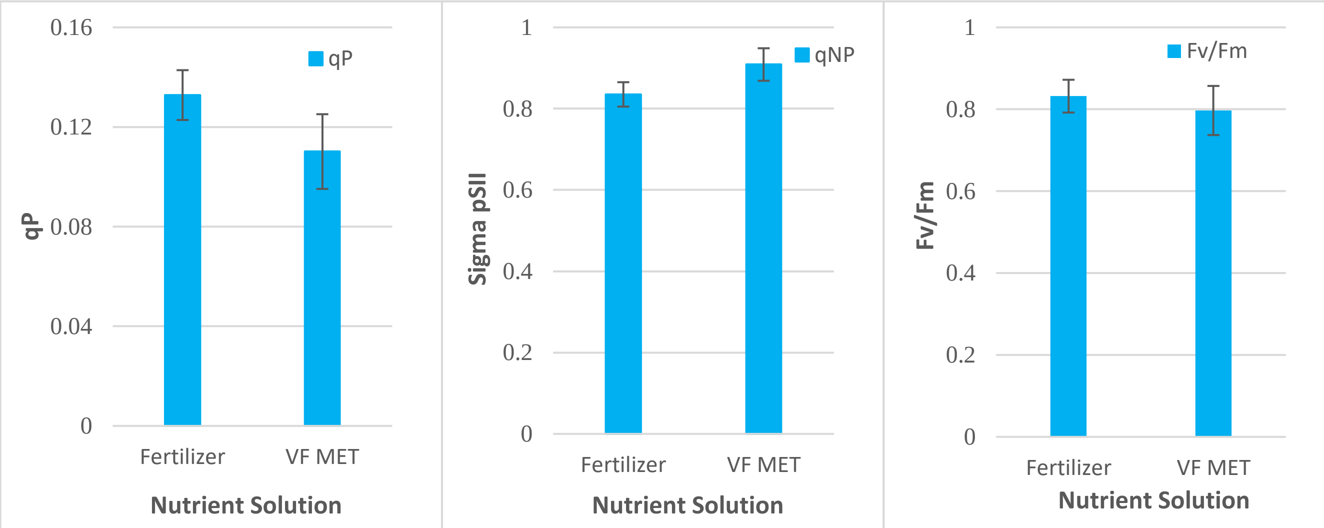


Figure 6. 8. Photosynthesis activity based on average values for qP, Sigma pSII, and Fv/Fm

6.2.3. Assessment of water quality for irrigation purposes

In this section, we detail the physical and chemical properties of the water effluent from EC-biochar biofilter, alongside the characteristics of water resources utilized across the campus. The campus water sources included well number one from Jardín Botánico (Shallow aquifer), well number two from Facultad de Biología (150 m Aquifer), and a blend of water from both wells known as Balsa Botánico. Water sampling was carried out at three distinct locations, and the specific attributes of these water samples are outlined in Table 6.3.

Table 6. 3. Physical and Chemical Characteristics of Water Used for Campus Irrigation and ec-biofilter Effluent

	Concentration (ppm)											
	F^-	CL^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Na^+	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	pH	EC*
ec biofilter	-----	89	17	5	69	78	9	21	91	31	8.20	600
Well 1	0.6	29	16	6	81	61	4	-----	63	57	8.86	834
Well 2	0.7	26	28	161	-----	91	-----	7	66	45	8.56	844
Well 1&2	0.5	9	13	247	-----	136	-----	-----	36	17	8.28	929

*micromhos per centimeter (µmhos/cm)

The suitability of irrigation water quality is determined by numerous factors. To assess its appropriateness for irrigation purposes, various essential criteria are considered, including electrical conductivity (EC), Sodium Adsorption Ratio (SAR), Soluble Sodium Percentage (SSP), Magnesium Hazard (MH), and Kelly's Ratio (KR). These parameters are outlined in Table 6.3 to provide a comprehensive evaluation of water quality in terms of its suitability for irrigation. Additionally, the results were compared with the standard range for each criterion, presented in Table 6.4.

Table 6. 4. Irrigation Water Quality Assessment Parameters

		SAR	Na^+%	SSP	MH	KR	EC
1	**ec biofilter**	1.80	35.67	32.37	35.96	0.48	600
2	**Well 1**	1.34	25.28	25.28	59.75	0.34	834
3	**Well 2**	2.12	37.12	36.11	53.29	0.57	844
4	**Balsa Botánico**	4.66	64.68	64.68	44.26	1.83	929

[1] effluent of EC-biochar biofilter
[2] water from well number 1
[3] water from well number 2
[4] Balsa Botánico

As depicted in Table 6.4, all samples exhibit an excellent water class in terms of the Sodium Adsorption Ratio (SAR). Regarding the Sodium Percentage, samples 1, 2, and 3, were classified as suitable, whereas water from Balsa Botánico (sample 4) is deemed unsuitable. Additionally, concerning Soluble Sodium Percentage (SSP), the effluent of the EC biofilter, as well as samples 2 and 3, demonstrate a good water class, while water from Balsa Botánico falls within the permissible class. In terms of Magnesium Hazard, water from well 1 and well 2 classified as unsuitable, while the remaining samples exhibit a suitable classification. Regarding the Kelly Ratio, water from Balsa Botánico falls within the marginal water class, whereas the other samples are classified as having a suitable water class.

Table 6. 5. Comparison of Irrigation Water Quality Parameters with Standard Ranges

Parameters	Range	Water Class	Samples Number
Sodium absorption ratio (SAR)	0-10	Excellent	1-2-3-4
	10-18	Good	----------
	18-26	Doubtful	----------
	>26	Unfit	----------
Soluble sodium percentage (SSP)	<20	Excellent	----------
	20-40	Good	1-2-3
	40-60	Permissible	4
	60-80	Doubtful	----------
	>80	Unfit	----------
Sodium percentage (Na^+%)	<50	Suitable	1-2-3
	>50	Unsuitable	4
Magnesium Hazards (MH)	<50	Suitable	1-4
	>50	Unsuitable	2-3
Kelly Ratio (KR)	<1	Suitable	1-2-3
	1-2	Marginal	4
	>2	Unsuitable	----------

In our study, water processed through the EC biofilter was classified as having medium salinity and a low sodium hazard (C2-S1). In contrast, water from various wells was categorized as C3-S1, signifying high salinity and a low sodium hazard, as illustrated in Figure 6.9. Based on these findings and the additional parameters listed in Table 6.4, it is evident that water treated by the EC biofilter is more suitable for irrigation than the existing water sources from the natural wells on campus.

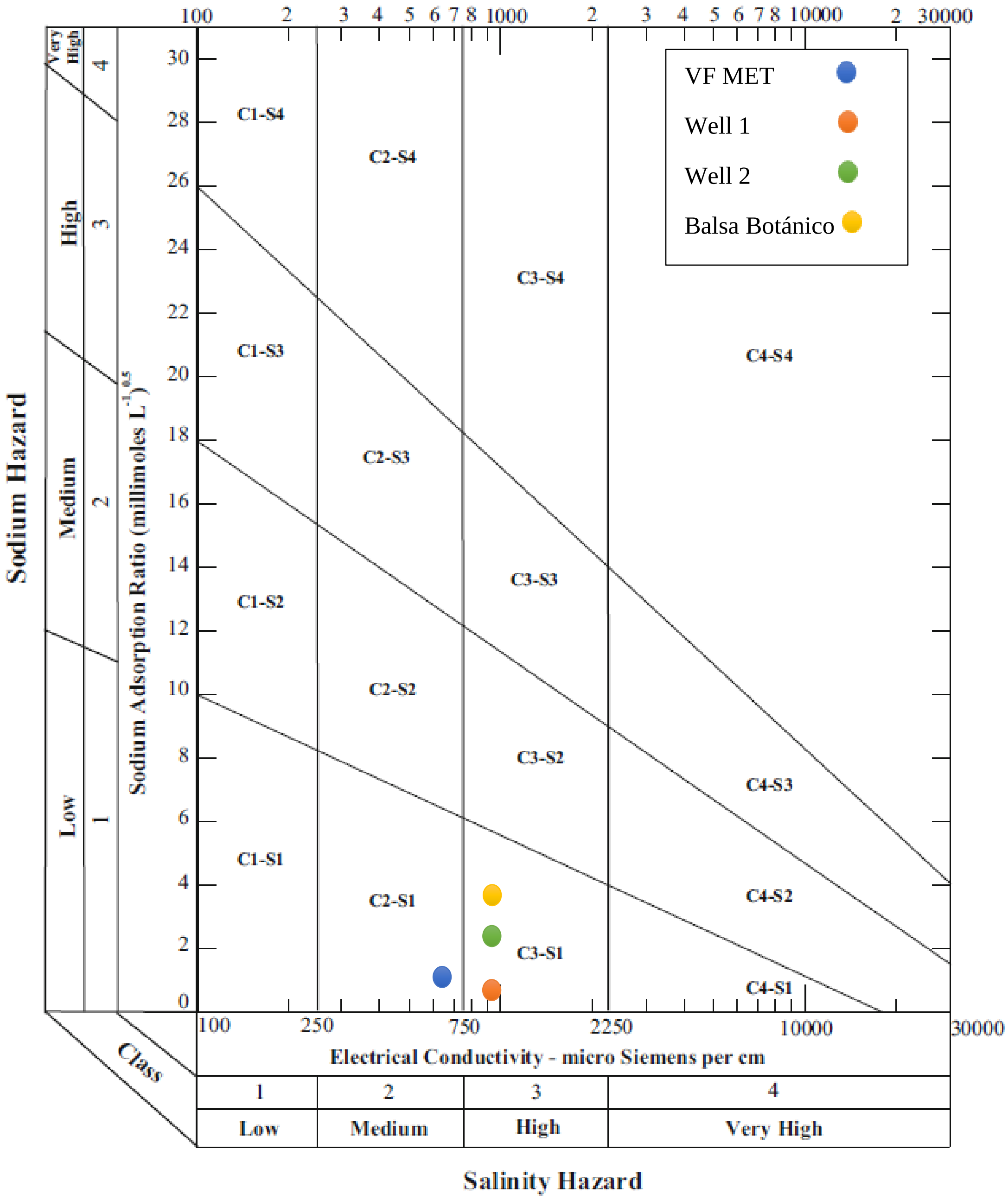


Figure 6. 9. Salinity (EC) and Sodium (SAR) Hazard Plots for Classifying Irrigation Water Quality

6.3. Assessment of EC- biochar from biofilters for agricultural purposes

We utilized electroconductive biochar as the bed material for our biofilter. Indeed, we used a fine powder size (0.1-0.075 mm) to enhance adsorption capacity. After a 170-day experimental period, we performed a nutrient analysis on this biochar to evaluate its potential for agricultural use. The nutrient content in the biochar, as shown in Figure 6.10, was determined through CHNS testing.

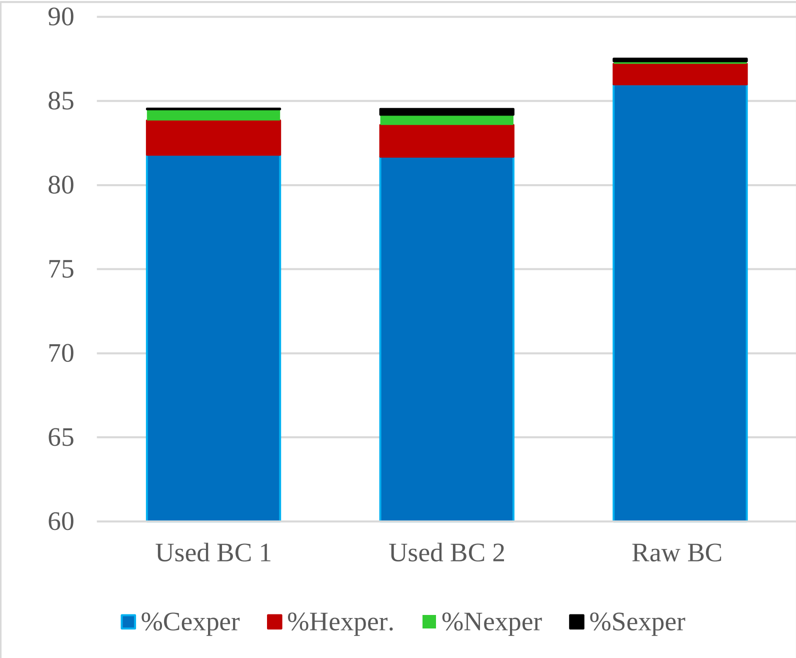


Figure 6. 10. Elemental analysis results (CHNS-932) for raw and used biochar from biofilter replicas

As depicted in Figure 6.10, the data showcases the percentage composition of carbon (%C), hydrogen (%H), nitrogen (%N), and sulfur (%S) in three distinct samples. It's notable that there's a higher percentage of nitrogen observed in the used biochar samples, likely attributed to the adsorption of wastewater during the treatment process. The nutritional content of biochar is precisely determined by both the intrinsic properties of the biochar and the composition of the wastewater used for treatment.

6.4. Managing water resources on the campus of Alcalá University

To address the challenge of irrigation water scarcity at the University of Alcalá campus, our goal is to employ METland® treatment and wastewater treatment for irrigation purposes.

6.4.1. Disparity between authorized extraction and irrigation demand

Consumption of water associated with irrigation on campus (including Botanical Garden) reached 33880 m^3/year while just 11880 m^3/year was authorized to extract from campus wells. Such disparity revealed a substantial water deficit, with a shortage of 22000 m^3/year that could be provided by re-used water after treating urban wastewater from campus.

Table 6. 6. Irrigation needs on the university campus and Botanical Garden (theoretical estimate)

Month	April	May	June	July	August	September	October	Annual total
Demmand(m3/month)	1694.02	3388.05	5082.07	8470.12	8470.12	5082.07	1694.02	33880.47
Demmand (L/S)	0.65	1.26	1.96	3.16	3.16	1.96	0.63	13.07

As detailed in the 'Report on Irrigation Resources on the Campus and Botanical Garden ' the highest irrigation demand on the campus was 8470.12 m^3 per month (equivalent to 3.16 L/s), during July and August. Such peak demand underscores the importance of effective water resource management and infrastructure planning during this critical period. A significant disparity in irrigation needs is evident, with April representing the minimum demand and July and August exhibiting a demand five times greater.

6.4.2. Wastewater production in university campus

To estimate the amount of wastewater generated on campus, we utilized consumption data from four consecutive years. By averaging this data, we obtained the annual average consumption. Assuming that 80% of this water can be converted into wastewater, we then calculated the daily consumption and daily wastewater production (Table 6.7).

Table 6. 7. Estimation of Wastewater Generation Based on Average Water Consumption Data on Campus

External Campus	(m^3/year)		(L/day)	
	Annual Average Consumption	**Annual wastewater***	**Daily Average Consumption****	**Daily wastewater***
Almacén de Gases	4	3	10	8
Edificio de Biología Celular y Genética	5248	4198	15711	12569
Edificio de Ciencias	483	387	1447	1157
Edificio de Ciencias Ambientales	218	174	652	521
Edificio de Enfermería y Fisioterapia	13914	11131	41658	33326
Edificio de Farmacia	672	537	2010	1608
Edificio de Medicina	971	777	2907	2326
Edificio Politécnico	9538	7630	28557	22846
Edificio Polivalente	15731	12585	47099	37679
Jardín Botánico	22753	18202	68123	54498
Jardín Botánico - Riego	936	749	2803	2243
Planta de Tratamiento de Isótopos	152	122	455	364
Riego Farmacia	190	152	568	454
Servicio de Deportes	3592	2873	10754	8603
Servicios Informáticos	137	110	411	329
Total	**74537**	**59630**	**223165**	**178532**

*Approximately 80% of water consumption is allocated to wastewater
**Daily without considering August

Based on the generated wastewater volume, we propose the implementation of METland® solution for the decentralized treatment of wastewater produced at different campus buildings. as outlined in Table 6.8. Additionally, the table presents the corresponding capacities of these METland®s and the pipe demand required for transferring treated water from each building. The total combined capacity of the METland®s across campus amounts to 178 m^3 and also in figure 6.11 was presented the proposed METland® system in the campus of the university of Alcala.

Table 6. 8. Daily Wastewater production per building

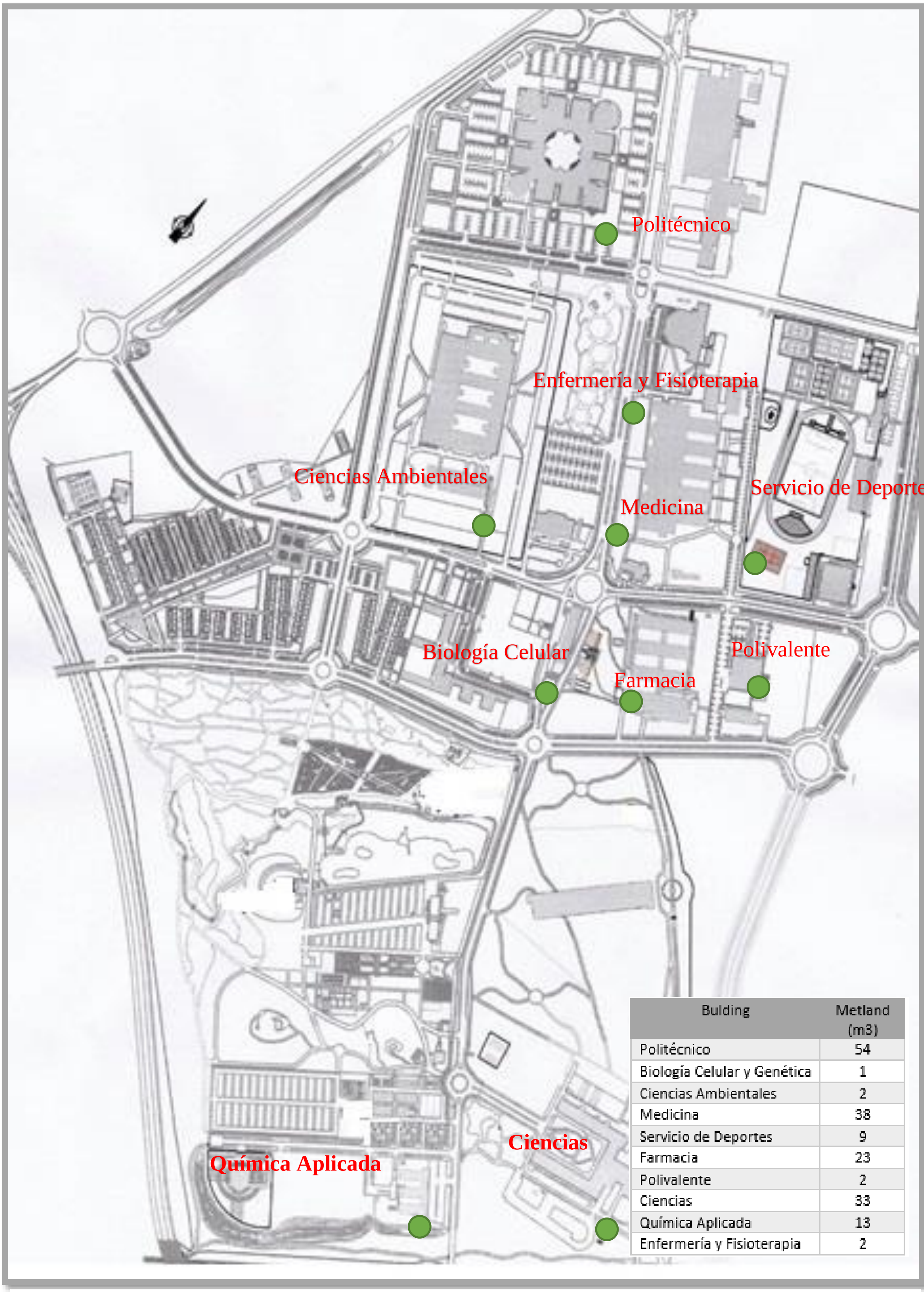
External Campus	Capacity treatment (m3/day)	Pipe Demmand (L/S)
Centro de Química Aplicada y Biotecnología	12.6	0.145
Edificio de Biología Celular y Genética	1.2	0.013
Edificio de Ciencias	33.3	0.386
Edificio de Ciencias Ambientales	1.6	0.019
Edificio de Enfermería y Fisioterapia	2.3	0.027
Edificio de Farmacia	22.8	0.264
Edificio de Medicina	37.7	0.436
Edificio Politécnico	54.5	0.631
Edificio Polivalente	2.2	0.026
Jardín Botánico	0.4	0.004
Planta de Tratamiento de Isótopos	0.5	0.005
Servicio de Deportes	8.6	0.100
Servicios Informáticos	0.3	0.004
Total	178.0	2.066

In order to treat wastewater from campus we should design METland® systems to operate in a decentralized manner per building. According to the research developed in section 6.1 of this chapter we estimated that EC biofilter can treat campus pollutants at removal rate of 200 gCOD/m^3bed day. Thus, a building producing 1 m3/day would require a METland® solution of 1 m^3 of an electroconductive biofiltering bed.

The highest demand for irrigation was calculated to be 3.16 L/s, which can be supported by 110 and 125 PVC pipes implemented in the main loop of the irrigation network. Utilizing Epanet software, the head loss and velocity in pipes were calculated to ensure the effective transportation of water within our existing pipe network.

Despite treating wastewater with METland®, we are only capable of covering 2 liters per second of the total demand. However, the peak demand requires 3.16 liters per second. The permissible volume of water extraction from the wells was set at 11,880 cubic meters per year, equivalent to approximately 0.37 liters per second. Overall, we can secure 2.37 liters per second, falling short by about 0.7 liter per second compared to the peak demand in August and July, and for storage this amount of demand the capacity of the reservoir is calculated 2000 m^3.

To manage this peak demand, especially during months like August when there is no wastewater production, it was essential to construct storage tanks to store and reuse water accumulated from previous months. However, according to Table 6.5, the need for irrigation only occurs for 6 months, and we can reserve water for months with high peaks. Currently, there was a total of 1800 m^3 reservoir for storing water, which is insufficient for irrigation needs and additional storage capacity is required. A potential element for storing water would be the artificial pond from the Botanical Garden with capacity for 15000 m^3 of water storage.



Bulding	Metland (m3)
Politécnico	54
Biología Celular y Genética	1
Ciencias Ambientales	2
Medicina	38
Servicio de Deportes	9
Farmacia	23
Polivalente	2
Ciencias	33
Química Aplicada	13
Enfermería y Fisioterapia	2

Figure 6. 11. Plan for METland® implementation to decentralized treatment of wastewater on campus

Using METland® units, we will cover 2 liters per second of the total demand, but the peak demand requires 3.16 liters per second. The permissible volume of water extraction from the wells was set at 11,880 cubic meters per year, which translates to approximately 0.7 liters per second. Altogether, we can guarantee 2.7 liters per second, which falls short by about one liter compared to the peak demand in August and July. To manage this peak demand, especially during months like August when water, it's imperative to construct storage tanks to store and reuse water accumulated from previous months.

6.5. Evaluation of existing pipes and hydraulic performance of the main pipeline

Figure 6.12 illustrates the layout and profile of the main pipeline, showing a slight incline in the campus. However, relying solely on gravity for water transport presents challenges, such as head loss within the pipes and the need to maintain adequate pressure to ensure water reaches all areas along the route. Therefore, the option of using pumps for water transfer should be considered. This profile was created using Google Earth to provide an overall view of the campus site. For future work, a precise map will be required.

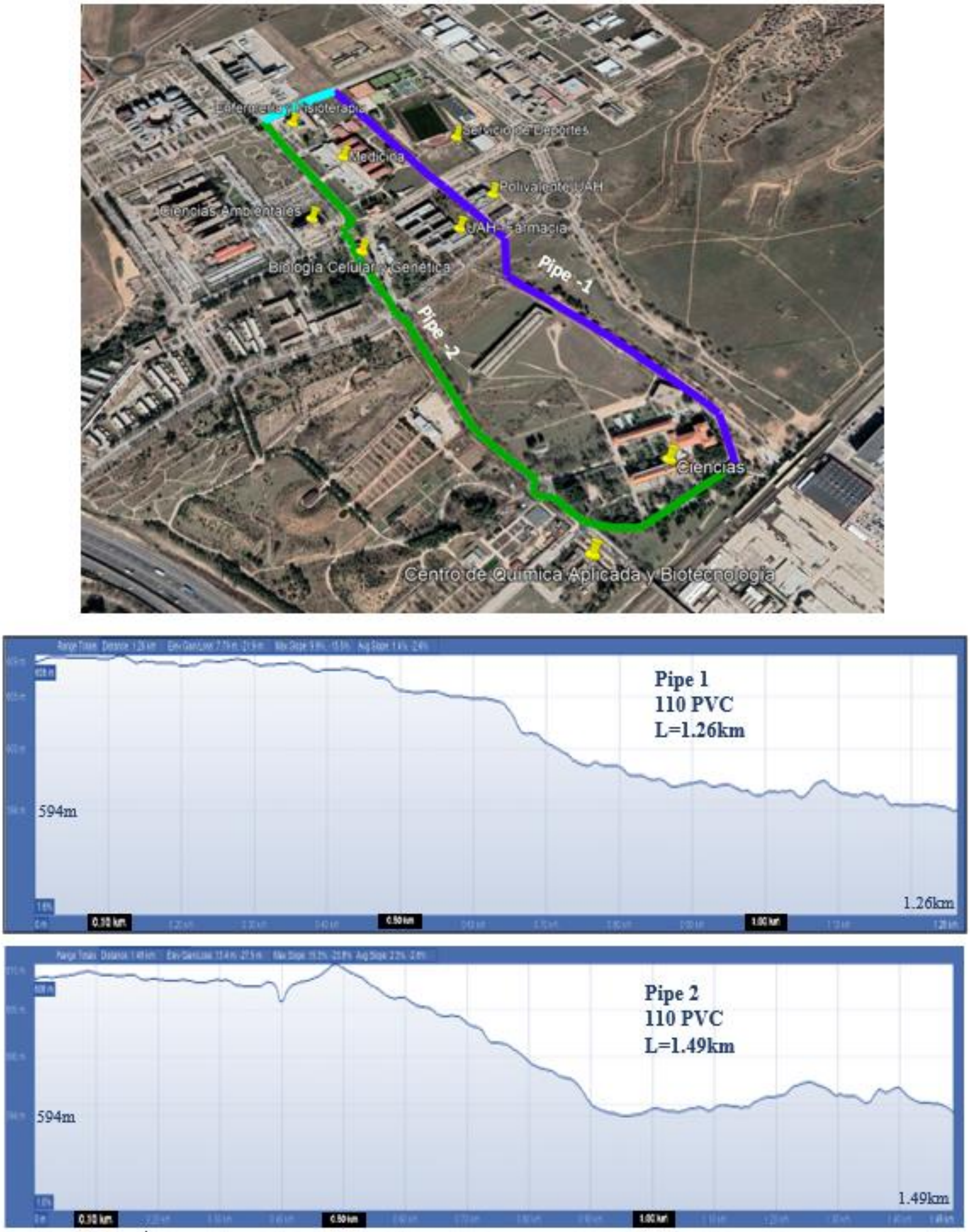


Figure 6. 12. Profile of the main pipeline pathway (Google Earth)

We used EPANET 2.2 software to calculate head loss and velocity in pipes, ensuring efficient water transportation within our pipe network. The analysis in EPANET uses the Hazen-Williams equation, with two PVC pipelines of sizes 110 mm and 125 mm, each with respective inlet diameters of 105.6 mm and 120 mm. The Hazen-Williams coefficient is set to 100. The main pipe can transfer a maximum flow rate of approximately 3.16 L/s, required during July and August. Figure 6.12 shows a contour plot map and the elevation of a junction close to some of the campus buildings, depicted in terms of elevation.

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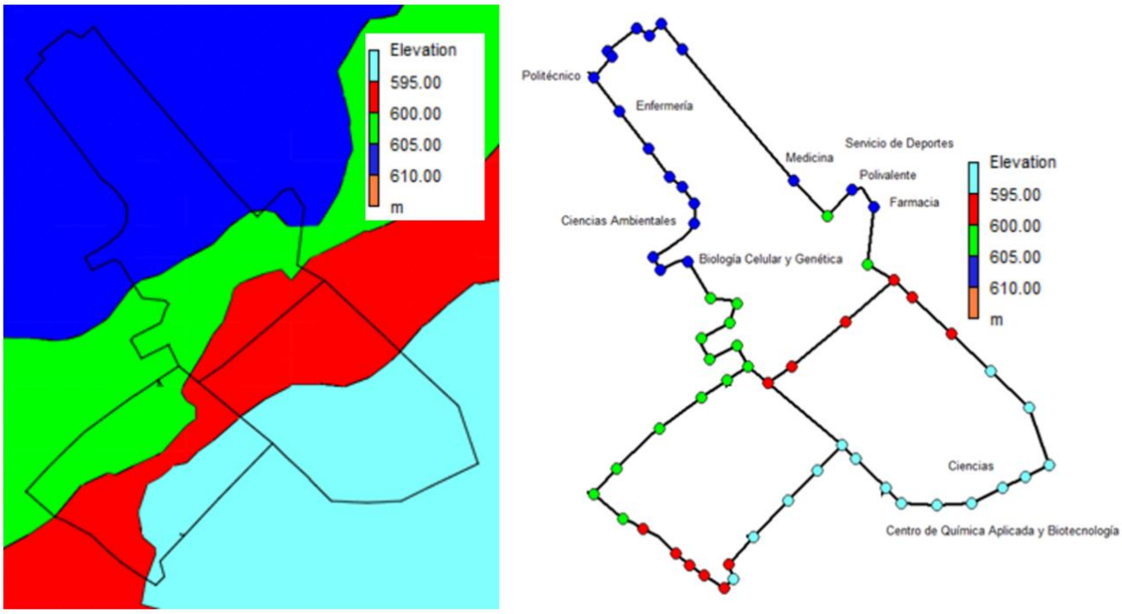

Figure 6. 13. Contour plot and map elevation in EPANET2.2

Chapter 7:
General Discussion, Conclusion and Future Work

7. General Discussion, Conclusions, and Future Work

The main objective of this thesis has been to study the potential of METland as tertiary and quaternary treatment systems. The focus has been on two locations where these systems have been developed on a demo scale. METland is proposed for the removal of nitrogen and emerging pollutants from the treated wastewater from the Carpio and Otos WWTPs. The relationship between the electroactive bacteria in METland and nitrogen compounds, as well as emerging pollutants, has also been explored. In the general discussion below, the most important findings are presented in a question-and-answer format.

7.1. General discussion

What is METland® technology and how it works?

The METland® system has emerged as a pioneering solution in wastewater treatment, distinguished by its effectiveness in removing organic pollutants, significantly reducing COD (Chemical Oxygen Demand), and lowering ammonia levels from wastewater. Unlike traditional approaches that typically aim to eliminate nutrients such as nitrogen and phosphorus, METland® systems are uniquely designed to retain these essential nutrients. This capability is particularly beneficial in agricultural settings where nutrient-rich water can be directly used for irrigation in soil or hydroponic systems. By preserving nutrients crucial for plant growth, METland® not only improves water quality and mitigates downstream environmental impacts but also promotes sustainable agricultural practices by potentially decreasing the need for synthetic fertilizers. This integrated approach, highlighted in chapters 4, 5, and 6 of relevant studies, underscores METland®'s capacity to support efficient wastewater treatment while fostering environmental stewardship and sustainable agriculture.

The potential of METland® technology for treating various types of wastewaters is significant. As part of this thesis (chapter 6), our analysis showed how a biofilter made of EC biochar removed approximately 95% of COD from campus wastewater (190 ppm COD), compared to just 64% removal by a gravel biofilter. When the COD concentration of the campus wastewater was increased to 1400 ppm, the EC biochar biofilter still achieved a high removal rate of 92%, while the gravel biofilter only managed to remove 74% of the COD.

Nutrients in wastewater: removing them or keeping them?

Urban Wastewater contain vast amounts of nutrients, mainly nitrogenous and phosphorous compounds that may represent a hazard for the environment, leading to eutrophization events if they reach water bodies at certain concentration. Thus, wastewater treatment is typically designed to include the removal of nitrogenous compounds through a series of steps including the mineralisation of organic nitrogen to ammonium, followed by the nitrification of ammonium to nitrate and, eventually, the reduction of nitrate to nitrogen gas (Paredes et al., 2007). Regarding phosphorus removal, treatments are not so efficient and are mainly based on PAO or precipitation by physicochemical process in presence of Al and Fe salts.

In parallel with the nutrient removal strategy, farmers suffer the cost of adding chemical fertilizers commonly used to address soil deficiencies in nitrogen (N), phosphorus (P), and potassium (K). However, a significant portion of these fertilizers is lost through runoff or volatilization. According to estimates, approximately 40–70% of nitrogen, 80–90% of phosphorus, and 50–70% of potassium applied as fertilizers are lost to the environment. This loss not only results in economic losses for the farmer but also contributes to environmental pollution (Duhan et al. 2017).

In this context of agriculture and recent water scarcity, the percentage of treated wastewater that may be available for irrigation purposes is increasing. Thus, due to the growing interest of reusing treated wastewater after proper disinfection it probably makes sense to keep the original nutrients so treated wastewater would become a double resource: water by itself and soluble nutrients as fertilizers. In this sense, the mere oxidation of ammonium to nitrate through biological nitrification processes would guarantee the generation of an ammonia-free liquid fertilizer. Thus, converting nutrients from wastewater into soil fertilizers is a major challenge in promoting the circular economy. This challenge is further limited by factors such as unregulated wastewater release, inadequate access to fertilizers in underdeveloped regions, and the high costs of fertilizers (Saliu and Oladoja 2021).

However, an alternative more sophisticated strategy would be to remove but not destroy nutrients from wastewater. A number of applications like the production of struvite has been developed in the last two decades, although its commercialization is limited by the national legislation regarding its nature: fertilizer or waste.

In the context of this thesis, we hypothesized the use of the electroconductive biochar bed could be used not just for boosting electrobioremediation of organic pollutants but also for retaining nutrients so such material could have a second life by using it as nutrient-enriched biochar. Actually, all experimental chapters were following this circular economy strategy, either for assessing nutrient adsorption from wastewater or assessing nutrient releasing for soil and hydroponic crops.

What is the real potential of biochar as nutrient-recovery role apart from boosting bioremediation of pollutants?

Biochar has gained significant attention due to its stability, porous structure, carbon-rich composition, and cost-effective production through thermochemical methods like gasification and pyrolysis (De Rozari, Greenway, and El Hanandeh 2016; Deng, Chen, and Chang 2021). Its remarkable ability to adsorb both organic and inorganic pollutants makes biochar a promising substrate for Treatment wetlands (TWs), potentially enhancing their efficiency (Srivastava, Gupta, and Chandra 2008; Wang and Wang 2019). The adsorption capacity of biochar varies based on the source material and production conditions, such as pyrolysis temperature, which influences its effectiveness in pollutant removal (Tan et al. 2015; El Barkaoui et al. 2023).

In this context, METland® technology appeared as novel strategy to treat wastewater in a way as sustainable as nature-based solutions like treatment wetland, but with a significant lower footprint (Mosquera-Romero et al. 2023). Among the electroconductive materials used for constructing METlands®, EC biochar offers an additional role to the mere stimulation of electroactive bacteria for biodegrading pollutants (Prado et al. 2020; Prado, Berenguer, and Esteve-Núñez 2022): the adsorption of nutrients presents in urban wastewater. Thus, incorporating electroconductive biochar into TWs could synergistically combine its adsorption capabilities with enhanced microbial activity, potentially leading to more efficient wastewater treatment.

What elements were preferentially adsorbed in EC-biochar?

Considering the scope of our research we focused on those elements present in wastewater that are already key for agricultural activity: nitrate, phosphate and potassium. Thus, in chapter 3 we carefully evaluated the adsorption capacity of different biochar with either synthetic solution containing the nutrients o real urban wastewater to test the retention under a more real scenario. Our results revealed that nitrate was adsorpted by all biochar tested and in a similar range (ca. 5.7 mg/g). In contrast phosphate was retained (ca. 5.5 m/g) by OSR550 biochar from oil seed but adsorption was found negligible for SWP biochar from soft wood. The differences in chemical surface of such biochars is currently under investigation to give insights into the adsorption processes. Regarding potassium, OSR biochar revealed a low adsorption capacity (ca. 1.7mg/g) while SWP showed none. Indeed, the pattern was the opposite and biochar were releasing K^+ to the water medium suggesting a value as high as 30mg/g in the original biochar composition. Our adsorption assays were followed by a series of experiments to measure the release of nitrate, phosphate and potassium. Thus, only 32% (OSR) and 29% (SWP) of the total nitrate previously adsorpted in biochar was released. This indicates the slow-release biochar characteristic, consistent with previous studies showing how after 90 days of leaching, the amount of total release of NO_3^- was in the range of 50-55% (Das and Ghosh 2021).

Regarding Phosphate, during the 60-day release period, 21% of the phosphate was released from used-biochar. It is important to note that the OSR used-biochar had a phosphate adsorption capacity of 4.5 mg/g over a 33-day period of adsorption test. These findings suggest that the biochar exhibits a slow-release behavior. Furthermore, release kinetic seem not be affected by the amount of phosphate adsorption in the material, either raw or used. Phosphorus is crucial for life, but many soils lack available P, causing overuse of water-soluble P fertilizers. Most applied P is either lost to runoff or becomes unavailable in the soil. To improve efficiency and reduce environmental harm, P release from fertilizers should match crop needs, which can be achieved with slow-release fertilizers (Hart, Quin, and Nguyen 2004; Weeks and Hettiarachchi 2019).

Potassium is another key element as crop fertilizer and all previous adsorption biochar test showed that such raw material was already saturated in potassium so not additional amounts present in water are further retained. Indeed, Analyzing the release pattern of potassium in OSR biochar, revealed that potassium release from raw biochar occurred predominantly on the first day, accounting for approximately 60% of the total release that was about 42.75 mg/g. In contrast, potassium release from used biochar exhibited a different pattern, with the initial release on the first day constituting approximately 30% of the total release (2.82 g/mg). This release from used biochar primarily originated from potassium within the biochar matrix.

In spite of such lab scale analys to explore the adsorption capacity of this material, our final goal was to evaluate the potential of using biochar material after long periods of treating wastewater under real conditions. In this context, we harvested some sawdust biochar material from a real METland facilities already operative for 3 years and test the release of nutrients storage in the granular bed. Thus, our analysis revealed a slow and continuous release of 0.1mg nitrate/g biochar per day, 0.05 mg phosphate/gbiochar per day and 0.1mg potassium/g biochar per day. Such sawdust biochar was evaluated as fertilizer for growing sunflower in chapter 5.

What are the effects of EC biochar on sunflower crops, and how do these effects differ between raw biochar and biochar used for treating wastewater?

The measurement of sunflower stalks' height and diameter across different plots revealed that while height differences were not significant, stalks in biochar-treated plots were 18% thicker. Throughout the observation period, sunflowers in biochar-treated soil exhibited thicker and sturdier stalks. On average, the diameter of sunflower clusters in biochar-treated soil was 16% greater than those grown without biochar. A notable contrast was observed between lanes with and without biochar amendment. Clusters in biochar-treated lanes were 17% heavier than those in natural soil. Additionally, an early yield was noted in biochar-treated lanes, with the first flowers appearing sooner. The use of biochar was also found to conserve water. Overall, biochar not only enhanced the strength of sunflower stalks during the growing period but also increased yield and promoted water savings due to earlier blooming.

Is water treated by METland® technology suitable for irrigation regarding current legal standards?

In chapter 5 discuss about our research, the water samples collected from the effluents of re-use water N35 and N15 were classified under the medium salinity and low sodium hazard category, as indicated by the USSL diagram. One of the N15 samples falls within the C1-S1 category, suggesting low salinity and low sodium hazard, while the remaining N15 samples were categorized as C2-S1. These findings imply that water from both N35 and N15 is generally suitable for irrigation purposes.

In chapter 6, water treated by EC-biofilter was categorized as medium salinity and low sodium hazard (C2-S1), while water from different wells corresponded to the C3-S1 category, indicating high salinity and low sodium hazard. These results indicate that water treated by ec biofilter would be more suitable for irrigation purposes compared to the current water sources from natural wells on campus.

Does the water treated by the METland® meet toxicity standards when assessed using algal and chlorophyll assays?

In treating wastewater, addressing contaminants such as Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) is crucial. However, this approach often overlooks the subtler toxicities produced by certain recalcitrant chemicals present at low levels. Ecotoxicology enables a comprehensive analysis by using bioindicators to assess these toxic effects, providing a more thorough understanding of environmental impacts.

In Chapter 4, the results showed that despite the stimulation of algae growth, the biofilter with biochar remains effective in treating wastewater. Moreover, it serves an additional beneficial purpose by releasing nutrients suitable for irrigation. This dual functionality underscores the significance of biochar, as it not only contributes to water purification but also provides a natural source of nutrients for irrigation needs. By reducing the dependency on additional fertilizers, the biochar promotes sustainable agricultural practices, aligning with environmentally friendly approaches to farming.

Previous research indicates that the Quantum yield of PSII electron transport (ΦPSII) and the Photochemical quenching coefficient (qP) are significant photochemistry ratios, both decreasing as pollutant concentrations increase. These measurements, influenced by contaminants, exhibit trends related to pollutant levels. The conversion of absorbed light energy into chemical energy (photosynthesis) is associated with qP. Osmotic stress significantly reduces qP and

the Maximal photochemical quantum efficiency of PSII (Fv/Fm), impacting photochemical processes within PS II and overall photosynthetic performance in plants (Lu, Zhang, and Vonshak 1998; Wu et al. 2012; Qiu, Wang, and Zhou 2013; González-Naranjo et al. 2014).

In Chapter 4, the results show that plants irrigated with effluents from a biofilter containing 10% biochar have high amounts of ΦPSII and qP. Additionally, in Chapter 6, our investigation revealed that the qP value for plants irrigated with effluent from an electroconductive biochar-supplemented soil biofilter was marginally lower compared to those receiving fertilizer. Moreover, the average ΦPSII value for plants treated with commercial fertilizer was 0.84, whereas for those exposed to effluent from EC-biochar, it notably increased. These results suggest that there was no discernible stress on plants irrigated with effluent from the biochar-supplemented soil biofilter, especially considering the higher ΦPSII value, highlighting its potential suitability for promoting plant growth.

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Building on the potential of biochar in pollution mitigation, understanding METland® technology requires examining the electron flow through its bed. Full-scale METland® systems have utilized various electroconductive granular materials, including electroconductive coke (Aguirre-Sierra et al. 2016) and more sustainable options like electroconductive biochar (EC-biochar) derived from high-temperature wood pyrolysis (Prado, Berenguer, and Esteve-Núñez 2019). Previous research has shown that electroactive biochar outperforms other highly conductive carbon materials in biodegrading pollutants by enhancing microbial extracellular electron transfer (EET). Thus, incorporating electroconductive biochar into CWs could synergistically combine its adsorption capabilities with enhanced microbial activity, potentially leading to more efficient wastewater treatment.

What elements were preferentially adsorbed in EC-biochar from METland®?

In a previous study, biofilters were employed to evaluate the efficacy of various electrically conductive bed materials electroconductive coke, electroconductive biochar, non-electroconductive biochar, and gravel in enhancing wastewater treatment efficiency, specifically focusing on COD and nitrogen removal. The findings indicated that electrically conductive materials outperformed non-conductive ones, achieving impressive COD removal rates of up to 175–180 g COD/bed×m^3/day, thereby supporting a compact footprint as small as 0.4 m^2 per person equivalent (PE). Notably, the highest nitrogen removal rates (80%) were observed with non-conductive biochar when plants were present, irrespective of the assay's anoxic conditions (Prado, Berenguer, and Esteve-Núñez 2022).

What are the effects of EC biochar on cultivated plants, and how do these effects differ between raw biochar and biochar used for treating wastewater?

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How does the humus or other materials rich in humic acid affect METland® performance in treating wastewater?

(Jiménez Conde 2024) reported that addition of earthworm humus to electroconductive coke bed of METfilter significantly improved nitrification efficiency (Jiménez Conde 2024). Thus, a more complete nitrification process was achieved, reducing the occurrence of highly toxic intermediate compounds such as nitrites. The author hypothesized the ability of the humus to act as an insoluble redox mediator, facilitating the exchange of electrons between bacteria, and the electroconductive material. Actually, (Jiménez Conde 2024) suggested that humic compounds could play a role similar to the biochar (Prado, Berenguer, and Esteve-Núñez 2019). In this context, we design a number of experiments using use quinone-rich humus as supplement for electroconductive bed made of biochar. Our results revealed no positive impact regarding COD removal after adding such substance. Considering the large presence of quinones in biochar surface, then the external addition of quinone-rich humus may be non-significant for microorganisms that are already growing on a quinone rich environment. This would explain the different observation from (Jiménez Conde 2024) who reported such effect using a quinone-free material like electroconductive coke. Anyway, we cannot discard potential benefits from humus in metal and nutrient adsorption, as reported elsewhere (Lipczynska-Kochany 2018).

7.2. Future Work

METland® solutions have undergone extensive study over the past decade, yet significant opportunities for optimization and development remain, positioning them as a promising new nature-based solution for wastewater treatment. The success of this versatile technology depends on achieving widespread social acceptance and a thorough understanding of its performance. Future work should focus on the following recommendations to further enhance METland® systems:

- Exploring New Material Mixes with EC-Biochar: Investigate novel combinations of materials incorporating electroconductive (EC) biochar to maximize the efficiency and effectiveness of METland® systems.
- Enhancing Sodium Removal Capabilities: Develop new materials or modify existing METland® configurations specifically designed to enhance the removal of sodium from wastewater. This is critical for improving water quality suitable for irrigation purposes.
- Performance Evaluation and Optimization: Conduct comprehensive evaluations of METland® systems under various environmental conditions to optimize their performance, ensuring efficiency and reliability.

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ANNEX

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Abbreviations

ACE	Abundance-based Coverage Estimator
AOB	Ammonium oxidizing bacteria
BOD	Biological oxygen demand
BOD_5	Five-day biochemical oxygen demand
COD	Chemical oxygen demand
COM	Circular Economy Chemical oxygen demand
CW	Constructed wetland
CW-MFC	Constructed wetland - microbial fuel cell
dS/m	Direct extracellular electron transfer
DF	Down-flow
DO	Dissolved oxygen
EAB	Electroactive bacteria
EC-biochar	electrically conductive biochar
EC	electrical conductivity
FWS	Free water surface
HRT	Hydraulic retention time
HSSF	Horizontal subsurface flow
KR	Kelly’s ratio
MET	Microbial electrochemical technology
MFC	Microbial fuel cell
MH	Magnesium hazard
N	Nitrogen
NBS	Nature-based solutions
NOB	Nitrite oxidizing bacteria
P	Phosphorous
p.e.	Population equivalent
PS	Potential salinity
RSC	Residual sodium carbonates
SAR	Sodium adsorption ratio
SRFs	Slow-release fertilizers
SSF	Subsurface flow
SSP	Soluble sodium percentage
TH	Total Hardness
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TSS	Total suspended solids
TW	Treatment wetland
UF	Up-flow
UKBRC	UK Biochar Research Centre
UWWTD	Urban Waste Water Treatment Directive
UWWTPs	Urban waste water treatment plants
USSL	United States Salinity Laboratory
WFD	Water framework directive
WWT	Wastewater treatment
WWTP	Wastewater treatment plant