CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

Department of Ecology
Nature Conservation Master's Degree

The influence of wastewater with different microplastic loads on plant health in constructed wetlands

Diploma Thesis

Thesis Supervisor: doc. Zhongbing Chen
Author: Efrain Sandoval

Prague
2024

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

B.Sc. Efrain Sandoval

Nature Conservation

Thesis title

The influence of wastewater with different microplastic loads on plant health in constructed wetlands

---

Objectives of thesis

This research aims to determine the perspective of constructed wetlands as a sink of microplastics from wastewater. We will examine the effect of two different microplastic types with two different loads on the efficiency of vertical flow constructed wetlands in nutrient removal. It is to be determined how microplastic changes the nitrogen, carbon, and phosphorus cycle in the wetland, as well as its effect on plant growth. We will also examine the influence of microplastic on arbuscular mycorrhizal fungi in some of the given constructed wetland reactors.

Methodology

The experiment will be set up in a greenhouse at the Czech University of Life Sciences. Ten vertical flow-constructed wetland reactors will be designed. Five reactors will be inoculated with arbuscular mycorrhizal fungi, which help the system in nutrient removal. The other five reactors will be without fungi inoculation. There will be two different microplastic types in different loads added to the reactors. Two control reactors will be free of microplastic load. Laboratory analysis of water influent and effluent nutrient concentrations will be performed weekly. Plant health will be observed.

The proposed extent of the thesis

50

Keywords

constructed wetland, microplastics, plant health, nutrient removal

---

Recommended information sources

Azeem I, Adeel M, Ahmad MA, Shakoor N, Jiangcuo GD, Azeem K, Ishfaq M, Shakoor A, Ayaz M, Xu M, Rui Y. Uptake and Accumulation of Nano/Microplastics in Plants: A Critical Review. Nanomaterials (Basel). 2021 Nov 2;11(11):2935. doi: 10.3390/nano11112935.

Eerkes-Medrano D., Thompson R.C., Aldridge D.C. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs (2015) Water Research, 75, pp. 63 – 82 DOI: 10.1016/j.watres.2015.02.012

Chen Y., Li T., Hu H., Ao H., Xiong X., Shi H., Wu C. Transport and fate of microplastics in constructed wetlands: A microcosm study (2021) Journal of Hazardous Materials, 415, art. no. 125615 DOI: 10.1016/j.jhazmat.2021.125615

Lavrova, S., & Koumanova, B. (2013). Nutrients and Organic Matter Removal in a Vertical-Flow Constructed Wetland. InTech. doi: 10.5772/56245

Murphy F., Ewins C., Carbonnier F., Quinn B. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment (2016) Environmental Science and Technology, 50 (11), pp. 5800 – 5808 DOI: 10.1021/acs.est.5b05416

Saeed T., Sun G. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media (2012) Journal of Environmental Management, 112, pp. 429 – 448 DOI: 10.1016/j.jenvman.2012.08.011

Vymazal J. Removal of nutrients in various types of constructed wetlands (2007) Science of the Total Environment, 380 (1-3), pp. 48 – 65 DOI: 10.1016/j.scitotenv.2006.09.014

XU Z., BAN Y., JIANG Y., ZHANG X., LIU X. Arbuscular Mycorrhizal Fungi in Wetland Habitats and Their Application in Constructed Wetland: A Review (2016) Pedosphere, 26 (5), pp. 592 – 617 DOI: 10.1016/S1002-0160(15)60067-4

Expected date of thesis defence

2023/24 SS – FES

The Diploma Thesis Supervisor

doc. Zhongbing Chen

Supervising department

Department of Applied Ecology

Advisor of thesis

Ing. Kristina Kralj

Electronic approval: 17. 1. 2024

prof. Ing. Jan Vymazal, CSc.

Head of department

Electronic approval: 23. 1. 2024

prof. RNDr. Michael Komárek, Ph.D.

Dean

Prague on 28. 03. 2024

Acknowledgment:

I would like to thank my supervisor, doc Zhongbing Chen, for his trust in me and allow me to be part of his team for this period. To Kristina Kralj for being the best teacher and having the patience to work with me. For my family for supporting me in this incredible journey of going to academics after 8 years, Micol Genazzi for the emotional support through this period, my colleagues for the journey on this master program, and last but not least Frantiska for giving me the strength to be able to finish.

Author's declaration: I hereby declare that I have independently elaborated the diploma final thesis entitled "The influence of microplastics on carbon, nitrogen and phosphorus removal in vertical flow constructed wetlands.", and that I have cited all the information sources that I used in the present document as listed at the end of the thesis, in the list of used information sources.

I am aware that my diploma/final thesis is subject to Act No. 121/2000 Coll., on copyright, in rights related to copyright, and on amendment of some acts, as amended by later regulations, particularly the provisions of Section 35(3) of the act on the use of the thesis.

I am aware that by submitting the diploma/final thesis I agree with its publication under Act No. 111/1998 Coll., on universities and the change and amendments of some acts, as amended, regardless of the result of its defense.

With my signature, I also declare that the electronic version is identical to the printed version.

In on Signature:

Abstract

Constructed wetlands (CWs) have been studied and developed for being a cost-effective, efficient, sustainable, and low-maintenance wastewater treatment system for decades. CWs use the natural processes of different nutrient cycles, such as Carbon, Nitrogen, and Phosphorus to treat the effluents of small-density populated areas. The effectiveness of CWs in treating the water lies in the design that depends on the local necessities and characteristics of climate, effluent characteristics, plant species, and number of inhabitants. Several studies have investigated the removal efficiency (RE) of nutrients with the use of many combinations of different hydraulic loading rates, the efficiency of different CW designs, and Macrophytes. The symbiotic interaction Arbuscular mycorrhizal fungi (AMF) has been found in 80% of land plant families and has been widely reported to play an important role by providing many benefits to the host plants such as uptake of nutrients, enhanced resistance to soil-borne pests and diseases, improved resistance to drought, tolerance of heavy metals, and better soil structure. Since the discovery of microplastics in the sediments on the coast of the United Kingdom in 2004, microplastics have been presented as a threat to the health of humans and nowadays is known that microplastics can be found in terrestrial and aquatic ecosystems. In this study, we have merged the variables of AMF, and of two types of microplastics in different loads, to analyze the impact on the removal efficiency of CWs of Nitrogen, Carbon, and Phosphorus. The results conclude that the effect of microplastics in general does not generate an impact on the performance of the RE of TOC, TC, Phosphate, and Nitrates but it affects the RE of TN. In the case of the presence of AMF, it generated a significant influence on the RE of the Phosphate, TOC, TC, and TN.

Key Words: Nitrogen Cycle, Carbon Cycle, Phosphorus Cycle; wastewater treatment; constructed wetlands; Arbuscular mycorrhizal fungi, Microplastics

Umělé mokřady (CWs) jsou již po několik dekád studovány a vyvíjeny jako hospodárný, účinný, udržitelný a na údržbu nenáročný systém čištění odpadních vod. Využívají přirozené procesy koloběhů různých živin, jakými jsou uhlík, dusík a fosfor, k čištění odpadních vod v oblastech s nízkou hustotou osídlení. Účinnost umělých mokřadů při čištění vody spočívá v jejich návrhu, který závisí na místních potřebách a charakteristikách klimatu, vlastnostech odpadních vod, druzích rostlin a počtu obyvatel. Řada studií se zabývala účinností umělých mokřadů při odstraňování živin z vody prostřednictvím využitím kombinace různého hydraulického zatížení, různých designů umělých mokřadů a makrofyt. Symbiotická interakce arbuskulárních mykorhizních hub (AMF) byla nalezena u 80 % čeledí suchozemských rostlin a je všeobecně známo, že hraje důležitou roli, protože poskytuje hostitelským rostlinám mnoho výhod, jako je příjem živin, zvýšená odolnost vůči půdním škůdcům a chorobám, lepší odolnost vůči suchu, tolerance vůči těžkým kovům a lepší struktura půdy. Od objevu mikroplastů v sedimentech na pobřeží Spojeného království v roce 2004 jsou mikroplasty prezentovány jako hrozba pro lidské zdraví a dnes je již známo, že se mikroplasty vyskytují v suchozemských i vodních ekosystémech. V této studii jsme sloučili proměnné AMF a dvou typů mikroplastů v různých zátěžích s cílem analyzovat jejich vliv na účinnost odstraňování dusíku, uhlíku a fosforu umělými mokřady. Výsledky dospěly k závěru, že účinek mikroplastů obecně nemá vliv na výkonnost RE z TOC, TC, fosfátů a dusičnanů, ale ovlivňuje RE TN. V případě přítomnosti AMF generoval významný vliv na RE fosfátů, TOC, TC a TN.

Klíčová slova: koloběh dusíku, koloběh uhlíku, koloběh fosforu; čištění odpadních vod; umělé mokřady; arbuskulární mykorhizní houby, mikroplasty

List of Abbreviations:

AMF – Arbuscular mycorrhizal fungi
C – Carbon
CW – Constructed wetland
CWs – Constructed wetlands
DO – Dissolved Oxygen
FWS – Free water surface
HLR hydraulic loading rate
HM – Heavy metals
HRT - hydraulic retention time
HSSF – Horizontal subsurface flow
N – Nitrogen
NH_4^+ - Ammonium
NO – Nitric oxide
NO_2^- - Nitrite
NO_3^- - Nitrate
N_2O - Nitrous oxide
ORP - Oxidation-reduction potential
O_2 – Oxygen
P- Phosphorus
pH- Potential of hydrogen
PO_4^{3-} - Phosphate
RE – Removal efficiency
TN – Total Nitrogen
TC – Total Carbon
TOC – Total Organic Carbon
VFCWs – Vertical flow constructed wetlands
VSSF – Vertical sub-surface flow

.

Table of Content

1. Chapter 1: Introduction

The present research focuses on constructed wetlands (CW), their importance as an efficient solution for wastewater problems, the different processes involved in the cleansing of water, their removal efficiency under various conditions, arbuscular mycorrhizal fungi (AMF), and the influence that microplastics can generate in their capacity of removal efficiency.

As it is widely known, constructed wetlands (CWs) have been studied for their many benefits in comparison with traditional wastewater plant treatments, and their high capability of adaptation to the requirements. The simulation of natural processes is an important factor to take into consideration, due to its high capacity to remove carbon, nitrogen, and phosphorus compounds. There are various types of constructed wetland designs that can support various types of macrophytes and wetland vegetation. CWs can provide different efficiency results based on their design. Arbuscular mycorrhizal fungi (AMF) generate a symbiotic interaction between the plants and the fungi, and it is present in 80% of the plant families. This interaction generates many benefits in the plant host such as increasing the root network extensions and establishing a connection between the plant and the substrate (Zhu, 2022).

In this study, the removal efficiency of constructed wetlands was analyzed with the idea of evaluating the performance under the presence of two types of microplastic in different loads, and how AMF can influence it.

2. Chapter 2: Aim of diploma thesis

This research aims to determine the perspective of constructed wetlands as a sink of microplastics from wastewater. it examined the effects of two different microplastic types with two different loads on the efficiency of vertical flow constructed wetlands in nutrient removal. It is to be determined how microplastics change the nitrogen, carbon, and phosphorus cycle in the wetland, as well as its effect on plant growth. It will also examine the influence of microplastics on arbuscular mycorrhizal fungi in some constructed wetland reactors.

3. Chapter 3: Literature Review

3.1. Constructed wetlands

The use of wetlands or constructed wetlands for water treatment began around 1950, but the results were unpredictable. The use of natural ecosystems was replaced by artificial ones, which improved the outcome. These artificial wetlands had specific compositions in terms of substrates, vegetation types, flow patterns, and their associated microbial assemblage, depending on the needs of pollutants that they were focusing on removing. (Vymazal, 2022).

As far as is known, studies on wetlands have found that this type of ecosystem provides many ecological benefits and ecosystem services, including improved water quality, climate regulation, nutrient processing, carbon sequestration, recreation, and habitat improvement (Eller, F et al. 2021). The European Commission has defined Nature-based Solutions (NbS) as "actions inspired, supported or reproduced by nature" (EC 2015), meaning that some ecosystems have natural processes that can be replicated to solve problems of processes. Therefore, NbS clearly emphasizes the link between biodiversity conservation and the goals of sustainable development and climate adaptation (Balian et al. 2014; Eggermont et al. 2015) and demonstrates innovation and feasibility.

In this sense, constructed wetlands are seen as replicating the natural processes that take place in a wetland, but with the idea of using them for anthropocentric problems. Vymazal (2022) states that constructed wetlands designed for wastewater treatment are systems created to take advantage of natural wetland processes involved in the conversion and removal of pollutants but under more controlled conditions. Several processes are involved in the wastewater treatment in a constructed wetland such as sedimentation, filtration, precipitation, plant uptake, and microbial degradation (Kadlec et al., 2000). In conclusion, we can say that constructed wetlands are a nature-based solution for anthropogenic activities, like domestic wastewater but can also be used in other contexts as well, such as the treatment of groundwater, industrial wastewater, and sludge dewatering. (Haberl et al., 1994)

3.2. Types of Constructed Wetlands

Every constructed wetland is built to solve a specific problem, meaning that the designs of each CW are unique due to many factors that are involved in the construction such as vegetation, sediments, hydraulic system, area, weather, etc. However, it is important to note that there are three more relevant factors in relation to the others, such as hydrology, macrophyte growth, and flow path in sub-surface wetlands (Parde et al., 2021, Vymazal 2014)

After reviewing the literature, we can see that there are many types and subtypes of CWs and different ways of classifying them, depending on the different authors' perspectives on the field and how they want to approach it. For purposes of this research, we use as a reference the approach that is described by Vymazal (2010). In this Classification (Figure N°3.2.1) that is divided by the three consecutive main factors, in first place is the relevance of the vegetation that is used in the CW. Four types of plants are in this category which are:

a. Free Floating Plants
b. Floating leaved Plants
c. Emergent Plants
d. Submerged Plants

Vymazal (2010) also implies that this classification relies on the hydrology of the system, using the water level in relation to the surface, which can be above or below the surface (open water-surface flow and sub-surface flow). Additionally, he classified the direction of the flow of the water, which can be horizontal or vertical. Finally, some designs can be different types of constructed wetlands combined with each other to utilize the specific advantages of the different systems (Vymazal,2007). The combinations between all configurations should be defined according to the characteristics of the pollutants to be removed as shown in the samples in Figure N°3.2.2

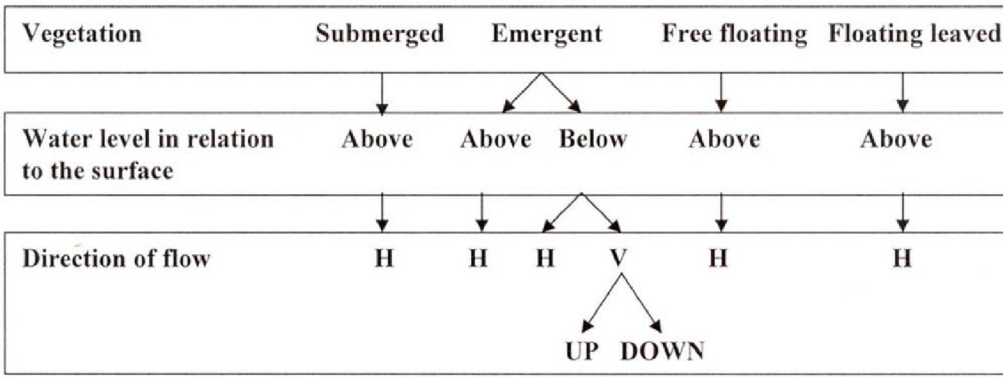

Figure N°3.2.1 The major characteristics of various types of constructed wetlands for wastewater treatment. H horizontal, V = vertical. (Vyzmal,2010)

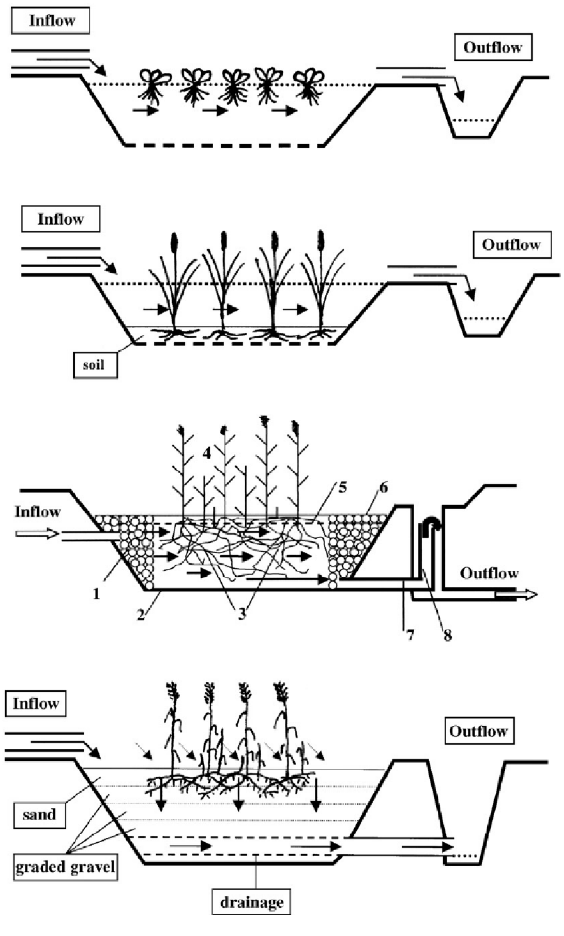

Figure N°3.2.2 -From Top to bottom - CW with free-floating plants (FFP), CW with free water surface and emergent macrophytes (FWS), CW with the horizontal sub-surface flow (HSSF, HF), CW with the vertical sub-surface flow (VSSF, VF). (Vymazal,2001)

In the Czech Republic, most of the CWS for wastewater treatment has been designed with horizontal subsurface water flow. Since the 2010s the design has changed into a combination of vertical and horizontal flow or a two-stage vertical flow CW. Nowadays there are about 210 CWs in operation, designed for small municipalities and a population estimated at 235 on average (Vymazal, 2023).

3.3. Hydrologic Regime of Constructed Wetlands

The retention time (RT) and wetland performance are directly related to water velocity and flow rate, so for a better performance of the wetland is important the time of interaction between the wastewater with the system (Kadlec and Knight 1996; Persson et al. 1999; Kadlec and Reddy 2001). As explained by Tanner (2013), hydrological regimes affect nutrient wetland plant nutrient intake. Also, it is considered that hydrology is a basic driver of mass and energy and that the water inflow in constructed wetlands differs from natural wetlands due to the specificity of the requirements of the systems (Jiang,2022).

The hydrology of wetlands also influences the sedimentation, aeration, biological transformations, soil adsorption processes, retention time, and water velocity (Kadlec and Knight 1996; Persson et al. 1999; Kadlec and Reddy 2001).

According to Jiang (2022), hydrological processes in CW are divided into three (3) categories: 1) Precipitation and Evapotranspiration, 2) Surface Hydrological processes, and 3) Subsurface flow. The importance of any of these processes varies due to environmental factors, for example, the evapotranspiration relevance is higher than the other processes in hot weather. If water balance calculations are not accurate, this can affect the formation and persistence of plant communities in wetlands, which means that the capacity for nutrient decomposition is also affected (Lott, 2001).

To summarize, the hydrological regime is important for the design of CW. It affects the whole system, the capacity of nutrient removal, plant communities, biomass production, sedimentation of pollutants, and the nutrient cycles. To achieve this, it's crucial that the environmental variables associated with the hydrology of the system are accurately managed to maintain a stable water level throughout the entire system.

3.4. Role of Plants in Constructed wetlands

Wetlands or Constructed wetlands are ecosystems that present a very specific type of vegetation that needs to be adapted to environments that are saturated by water. In the case of larger aquatic plants, are usually macrophytes, these plants are classified according to their biotype and their interaction with the environment as immersed, emergent, floating, submerged free, submerged rooted, submerged with floating leaves, or amphiphytes. (Leticia & Kochi et al. 2020).

As part of the role of plants in constructed wetlands, it has been found that the role of plants in wetlands are divided into the next seven following main roles 1)the Physical Effects of Root Structures, 2)Root as Base for Microorganisms, 3)Plant Uptake, 4)Evapotranspiration, 5)Microclimatic Conditions, 6)Other Functions of Plants in the CW and 7)Plant Production (Shelef et al.,2013).

In the case of the Physical Effects of Root Structure, we can see that the main effects on the system are filtering, flow velocity reduction, improved sedimentation, decreased resuspension, and even the distribution of water and prevention of clogging (Stottmeister et al., 2003). For the role of "Root as Base for Microorganisms", we found that the Rhizosphere is important for facilitating the right conditions for the microorganisms, which are key drivers in the treatment processes of the wetlands. (Brix, 1996, Vymazal, 2011).

Plant Uptake is considered by Vyzamal (2011) relatively important, according to the characteristics of the CW the uptake of nutrients may vary but in general, they are able to collect N and P of the wastewater and store them as biomass. It is also important to state that plants are able to gather heavy metals from the water and store them.

In the case of water balance in the CW, plants have a critical role in determining the dynamics of water loss, mainly by dictating the water loss through evaporation and plant transpiration. (Zhang, 2023).

Smith (1997) and Karczmarczyk (2013) stated that the microclimatic conditions provided by the physical structure of plants growing in the CW medium affect the environment in different ways, from generating a shade that reduces algal growth, reducing wind velocity by the upper parts of the plants to stabilize the sediment surface, to isolation from radiation in spring or frost in winter.

It is important to mention that CW plants can be used for the production of marketable goods depending on the species that is used in the CW, but we can find examples as fibers for construction purposes, bioenergy crops, and ornamental plants That can benefit the locals and generate an income to cover the actual costs of the CW. (Belmont et al., 2003, Aronsoson Et Perttu, 2001, Zhang. et al., 2011). Finally, Other functions of plants in the CW can include the improvement of aesthetic appearance, and the elimination of pathogens, insects, and odors. (Wood, 1995).

Summarizing and as also described by Brix (1997), CW plants, as macrophytes, provide the ecosystem with many assets that mainly contribute to the functioning of wetlands with the role of purifying the water. For the author, the macrophytes can also help to stabilize the surface of the beds, improve filtration, the prevention of clogging the system, insulate the surface against frost during winter, and help the microbial presence in the system. The interaction in wetlands between aquatic microbial communities, plant roots, and supporting mineral matrix generates an effective system for removing pollutants, such as suspended solids, dissolved and particulate organic matter, nitrogen, phosphorus, metals, and pathogenic organisms from effluent streams (Randerson, 2006)

The meta-analysis which included about 87 CWs from 21 countries, showed that the four most commonly flowering ornamental vegetation genera differ according to geographical location, *Canna spp* in Asia, Zantedeschia *spp*, North America, *Iris* in Asia, Europe, North America and *Heliconia* genus in Asia and parts of America (Sandoval L. et al., 2019)

As explained before, there are several authors with different approaches, in this case, we can see that the division of the macrophytes that is most commonly in constructed wetlands is the following.

a. Emergent aquatic macrophytes, are the most common type of plants in wetlands and marshes, general speaking, they produce aerial stems and leaves and an extensive root and rhizome system.
b. Floating-leaved aquatic macrophytes, include species that are rooted in the substrate and species that are freely floating on the water surface.
c. Submerged aquatic macrophytes, are plants that grow entirely underwater, rooted in the substrate of lakes, ponds, rivers, and other freshwater bodies

So according to the needs of CW the plant used can improve the process of denitrification in subsurface constructed wetlands (Gu X. et al., 2021). In terms of biomass, as explained by Xia Yu (2012) the correlation between the production and the nutrient removal efficiency is positive, but the production of biomass is mainly below the ground surface.

3.5. Nutrient Cycle in Constructed wetlands

Constructed wetlands are a solution that uses less resources than conventional treatment systems, especially useful for small communities or remote localities due to their capacity to be adaptable to local requirements. For CWs, plants act to enhance a variety of removal processes of nitrogen, phosphorous, and other pollutants from the water. (Haiming W. et al. 2015). According to the system configuration, the capacity of the uptake of nutrients from the water may vary due to factors such as environmental conditions, loading rates, retention times, and wastewater composition. (Saeed and Sun, 2012). As explained by Vyzamal (2007), part of the removal of compounds like Nitrogen or Phosphorus is by replicating their cycles on wetlands ecosystems, which involves the interaction of the vegetation, soil, and their associated microbial assemblages.

3.5.1. Removal Mechanism

Constructed wetlands are engineered to mimic the natural activities in removing pollutants of wetlands by biological, chemical, and physical processes and mechanisms for removing pollutants. (Hassan et al.,2021). The main processes involving wastewater treatment are sedimentation, filtration, volatilization, chemical precipitation, and biological degradation. (Shukla A. et al., 2021).

3.5.2. Nitrogen Cycle

For living beings, the nitrogen cycle is considered the second most important cycle, after the carbon cycle, because nitrogen is a compound that is important for plant growth, photosynthesis, energy transfer, and fertilizer synthesis. (Zhou et al,. 2023). Meaning that, nitrogen is present in different forms in the most common wastewater, such as the ones that come from urban drainage, sewage, industrial, and agricultural activities. The different forms of Nitrogen are also known for the impact that it has on aquatic ecosystems as the toxicity to fish or causing oxygen depletion in water biota. (Lee et al., 2009). Li (2017), found that in common wastewater around 70%-82% is ammonia nitrogen ($NH4+-N$) is the main nitrogen form, of the TN concentration. Also, it can be found in the form of organic nitrogen, nitrite nitrogen ($NO2--N$), and nitrate nitrogen ($NO3--N$). Gaseous nitrogen may exist as dinitrogen ($N2$), nitrous oxide ($N2O$), nitric oxide ($NO2$ and $N2O4$), and ammonia ($NH3$) (Vymazal, 2006). In the next figure, Vymazal (2007) explains the process and transformation of Nitrogen on wetlands.

Process	Transformation
Volatilization	ammonia-N (aq)→ammonia-N (g)
Ammonification	organic-N→ammonia-N
Nitrification	ammonia-N→nitrite-N→nitrate-N
Nitrate-ammonification	nitrate-N→ammonia-N
Denitrification	nitrate-N→nitrite-N→gaseous N_2, N_2O
N_2 Fixation	gaseous N_2→ammonia-N (organic-N)
Plant/microbial uptake (assimilation)	ammonia-, nitrite-, nitrate-N→organic-N
Ammonia adsorption	
Organic nitrogen burial	
ANAMMOX (anaerobic ammonia oxidaton)	ammonia-N→gaseous N_2

Figure 3.5.1 *Nitrogen cycle in wetlands (Vymazal, 2007)*

- Volatilization

As is explained by Vymazal (2007), ammonia volatilization is a physicochemical process where ammonium-N is known to be in equilibrium between gaseous and hydroxyl forms.

- Ammonification

Reddy and Patrick (1984) explained that ammonification is defined as the biological conversion of organic N to ammonium, N. Ammonia is converted from organic form by different biochemical processes that release energy that is used by the microbes for development and the ammonia is incorporated into microbial biomass. (Vymazal, 2007). This process depends on many factors such as temperature, pH, Carbon/Nitrogen ratio, available nutrients, and soil conditions such as texture and structure. (Reddy and Patrick, 1984). Some important conditions are a pH is between 6.5 to 8.5 and the temperature in the range of 40°C to 60°C (Vymazal, 1995).

- Nitrification

Nitrification is defined as the biological oxidation of ammonium in two steps, first to nitrite and finally to nitrate. Also, it is the process where the composition of mineral N is regulated in soils. Biological Nitrification, which is done by nitrifiers is the first part of the process, in which the bacteria gain energy for their development and the production of nitrites. (Norton & Ouyang , 2023).

As explained by Vymazal (2007), Each transformation is performed by different bacterial genera that are facultative in the use of ammonia or nitrites as a source of energy, oxygen as an oxidizing agent, and carbon dioxide as a carbon source. In the case of ammonia oxidation (1), the genus of bacteria used and found is *Nitrosomonas*, and for the purpose of nitrite oxidation (2) is *Nitrobacter*. With the following overall equation for each reaction.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2)$$

On Constructed wetlands, the dissolved oxygen in the system limits the nitrification process, so artificial aeration is added to enhance the process (Ma et al., 2021).

- Nitrate-ammonification.

In general, nitrate reduction is commonly referred to as denitrification, but nitrate ammonification is another route for reducing the nitrates that differ from denitrification on the product. In denitrification, the products are related to gaseous end products such as N_2O and N_2, and in nitrate ammonification, the product is ammonium NH_4^+. (Balk et al., 2015). It is important to state that, the number of electrons used in the reduction of a molecule of nitrate, in the case of Denitrification is 5, while it is 8 in Nitrate Ammonification. So, Nitrate-Ammonifying bacteria can oxidize more organic matter per molecule of nitrate than in the regular process of Denitrification (Laanbroek ,1990).

- Denitrification

Denitrification is the biochemical process where the Nitrate (NO_3^-) is reduced to Dinitrogen (N^2) through a series of intermediates (NO_2^-, NO, N_2O) by denitrifying bacteria in their respiration process. (Vymazal ,2007).

This denitrifying process can be carried out by heterotrophic and autotrophic bacteria, the former is widely used in domestic wastewater treatment due to the presence of organic matter that is the main source of carbon and electro acceptor, meanwhile, the autotrophic presents better results in the treatment of groundwater due to no organic matter is required and the bacteria utilize carbon from inorganic compounds (e.g. carbonates) and, since the electron donor is inorganic such as sulfides H_2, Iron species. (Vymazal. ,2007, Cecconet et al., 2018).

As already mentioned, heterotrophic denitrification only occurs under the availability of organic matter and only under anaerobic or anoxic conditions. Denitrification process is illustrated by the following Equation (Vymazal ,2007).

$$6\,(CH_2O)+4\,NO_3^- \Rightarrow 6\,CO_2+2\,N_2+6\,H_2O$$

The rate of this process is influenced by several environmental factors, such as the absence of O2, redox potential, soil moisture, temperature, pH value, presence of denitrifiers, soil type, organic matter, nitrate concentration, and the presence of overlying water.

In the results of Lin et al. (2002), he found that 89% to 96% of the nitrogen removed in their research was due to denitrification, and due to biological uptake 4% to 11% of nitrogen was removed.

- Fixation

Nitrogen fixation is the process where the gaseous nitrogen (N2) is converted to ammonia (NH4+) with the aim of nitrogen being converted to nitrogen-containing organic compounds. In general, nitrogen fixation is realized via bacteria and requires nitrogenase, an oxygen-sensitive iron-, sulfur- and molybdenum-containing enzyme complex which also generate the reduction of other substrates containing triple covalent bonds (e.g., nitrous oxide, cyanides or acetylene) (Wang & Yu , 2023).

In wetland soils, biological N2 fixation may occur in the floodwater, on the soil surface, in aerobic and anaerobic flooded soils, in the root zone of plants, and on the leaf and stem surfaces of plants. The ability of fixation is distributed among aerobic, facultative, and strict anaerobic bacteria, but under anaerobic conditions, the fixation of N2 is greater. (Buresh et al., 1980, Vyzamal, 2007)

- Plant uptake

In plants, nutrients are very much needed for development, according to each species there are different preferences in the form of nitrogen absorbed. In the case of macrophytes are fundamentally taken up by roots. Ammonia and nitrate-nitrogen are the two forms of nitrogen generally used for assimilation due to ammonia nitrogen is more reduced energetically than nitrate. It is also important to consider that algae and other microorganisms benefit from the use of nitrogen that is located in the sediments. (Vymazal, 2007)

In the case of emergent and rooted floating-leaved macrophytes, the nutrients can be gathered from the sediments and in the case of free-floating macrophytes, it can be obtained from the water. (Wetzel, 2001)

Finally, Vymazal (2007) explained that for the design of constructed wetlands, some traits of the plants help to increase the capacity of nutrient uptake, these traits are related to high tissue nutrient content, rapid growth, and the capability of a high-standing crop. In the case of a constructed wetland treating municipal wastewater, potential nutrient uptake of about 1.9% of the influent nitrogen and phosphorus load can be expected.

- Ammonia adsorption

In the case of ionized ammonia, it can be adsorbed through a cation exchange reaction with detritus, inorganic sediments, or soils. This adsorption is bound loosely to the substrate and can be released easily when water chemistry conditions change. The fixed ammonia will react differently according to the water chemistry conditions. When the ammonia concentration in the water column is reduced, some ammonia will be desorbed to regain the equilibrium with the new concentration. And, when the ammonia concentration in the water column is increased, the adsorbed ammonia also will increase. The ammonium ion is generally adsorbed as an exchangeable ion on clays and adsorbed by humic substances. (Vymazal, 2007 & Lee et al., 2009)

- Organic nitrogen burial

For Vymazal (2007), organic nitrogen burial is when some fractions of the organic nitrogen incorporated in detritus in a wetland may be eventually become unavailable for additional nutrient cycling through the process of peat formation and burial. The values of organic nitrogen burial have been reported for various natural wetlands, however, in constructed wetlands there are practically no data available.

- ANAMMOX

In wetlands and constructed wetlands ANAMMOX pathway is significant and is responsible for the capability of removing nitrogen. (Negi et al, 2022). The anaerobic ammonium oxidation (ANAMMOX) is when the NO2- and NH4+ to N2 and is a process where autotrophic microorganisms obtain energy by oxidizing inorganic compounds defined in the next formula. (Amils, 2011).

$$\mathrm{NH_4^+} + \mathrm{NO_2^-} \rightarrow N_2 + H_2O$$

This process is used to reduce nitrogen in ammonium-rich wastewater or the remotion of ammonia from municipal and industrial wastewater. In this process, the anoxic oxidation of ammonia with nitrite as a preferred electron acceptor consumes 50% less oxygen and 100% less organic carbon. (Anjali and Sabumon, 2014)

3.5.3. Carbon Cycle

Wetland ecosystems are known for the capability of sequestration and long-term storage of carbon dioxide (CO2) from the atmosphere (William et al.,2012).

Wetlands are ecosystems that cover around 6–8% of the land surface but are estimated to account for one-third of the world's organic soil carbon pool. (Bernal & Mitsch, 2011).

The capacity of the storage and emission of a wetlands are connected to the hydrogeochemical characteristics of the ecosystem, which depend on the wetland vegetation communities. (Bernal & Mitsch, 2011). Also, as explained by Whitting and Chanton (2011) characteristics such as high productivity, high water table, and low decomposition rate related to a wetland lead to carbon storage in the soil, sediment, and detritus. In the next Figure, we have the representation of the inland wetland carbon cycle.

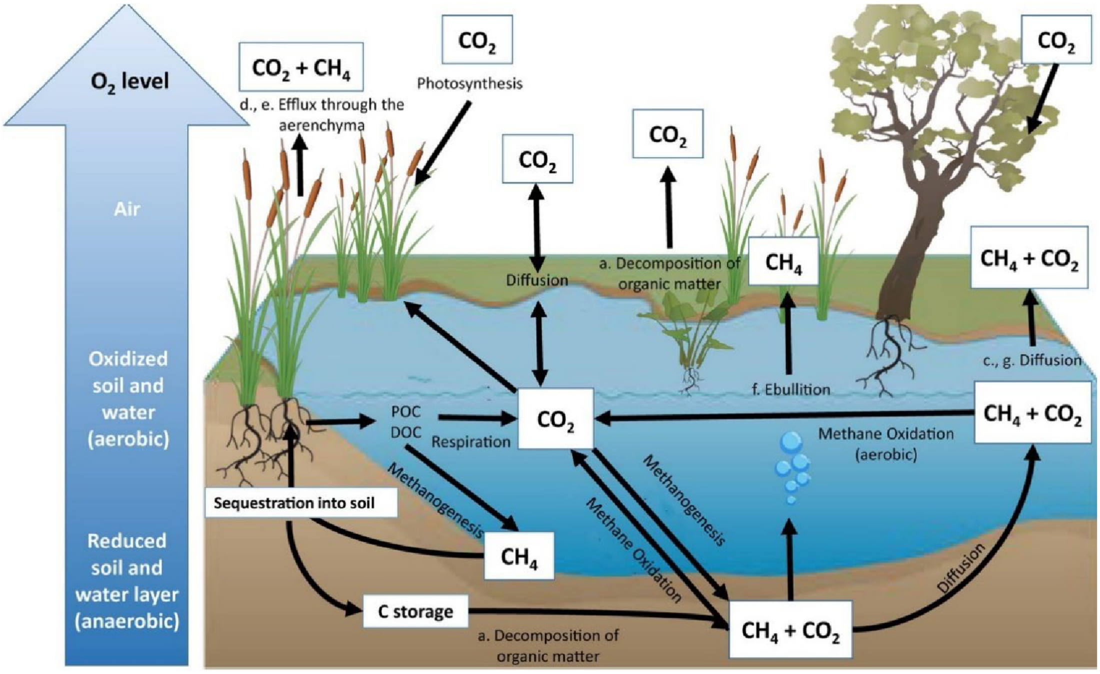

FIGURE 3.5.2. Representation of the inland wetland carbon cycle. Major pathways of carbon sequestration include photosynthesis and organic carbon accumulation (Bernal and Mitsch, 2012)

In the case of constructed wetlands, carbon cycle contributes to the global greenhouse gases balance through their carbon dioxide (CO2) and methane (CH4) emissions. (Barbera et al. 2014). The sources of carbon in the system are the inflowing wastewater, dead belowground plant biomass, and plant root exudates.

(Picek T. et al., 2007). Is important to state that, the carbon source is a controlling factor in the process of denitrification (Ragab et al., 1994).

In constructed wetlands, one of the main factors for nitrogen removal is the presence of carbon sources represent a factor to consider. In the experiment done by Ding et al., (2013) found that the DO and COD/N significantly affected the removal of ammonia nitrogen (NH4+-N) in horizontal subsurface-flow constructed wetlands.

3.5.4. Phosphate Cycle

Phosphorus in wetlands can be present as phosphate in organic and inorganic compounds, such forms include precipitated forms that can physically adsorbed onto mineral surfaces, biologically assimilated in cells, and in detritus (Di Luca G. et al.,2017). Algae and macrophytes are only able to utilize phosphorus in the form of free Orthophosphate, therefore represents a major link between organic and inorganic phosphorus cycling in wetlands. (Vymazal ,2007). Organic Phosphorus forms can be generally grouped into two categories 1) easily decomposable P (nucleic acids, phospholipids or sugar phosphates) and 2) slowly decomposable organic P (inositol phosphates or phytin) (Dunne and Reddy, 2005). In the next figure we can see the phosphorus transformation (Reddy and Delaune, 2005)

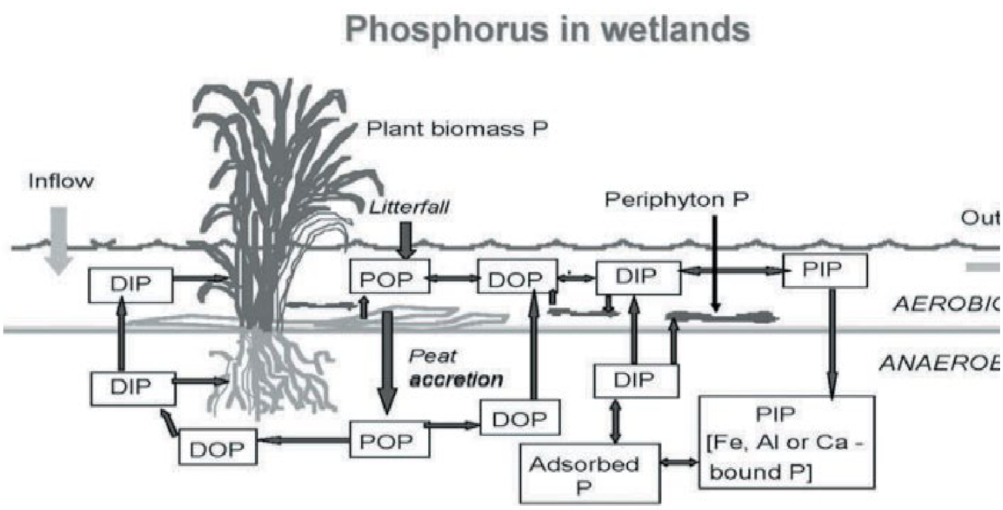

Figure N°3.5.3. Schematic showing phosphorus (P) cycle in wetlands. DIP = dissolved inorganic P; DOP = dissolved organic P; PIP = particulate inorganic P; POP = particulate organic P (Reddy and Delaune, 2005)

There are several ways of phosphorus removal in constructed wetlands, such as natural sedimentation, absorption and the assimilation of plants, excessive absorption of phosphorus by denitrifying phosphate accumulating organisms (DPAO), and the adsorption of phosphorus by substrate (Gao & Zhang , 2022), and according with the literature, the phosphorus removal of traditional constructed wetlands based on gravel is only approximately 15% (Gao & Zhang , 2022). The efficiency of sorption and precipitation processes relates to the pH, oxidation-reduction potential, presence of iron and aluminum ions, or the sorption capacity of the soil (Jakubaszek 2021).

Phosphorus uptake by microbial activity is very fast, but generally speaking, the amount it is very low, on the other hand is rapid because these organisms grow and multiply at high rates (Richardson et al., 1997). In the case of plant uptake, the phosphorus is taken up by plant roots, absorption through leaves and shoots is restricted to submerged species but this amount is usually very low. Phosphorus uptake by macrophytes is usually highest during the beginning of the growing season (in most regions during the early spring) before the maximum growth rate is attained (Vymazal ,2007).

3.6. Microbial Activity

Microbial communities in relation to constructed wetlands are divided into two groups: autochthonous (indigenous), and allochthonous (foreign) microorganisms. In the case of autochthonous microbes, this community exhibits adaptive features that allows them to participate in the treatment processes such as they are able to possess metabolic activity, survive, and grow in wetlands systems. On the other hand, allochthonous microbes usually do not survive or have any functional importance in the wetland environment (Truu M., et al., 2009). The following figure shows the external and internal factors that may affect microbial community structure and activity in constructed wetlands. (Truu M., et al., 2009).

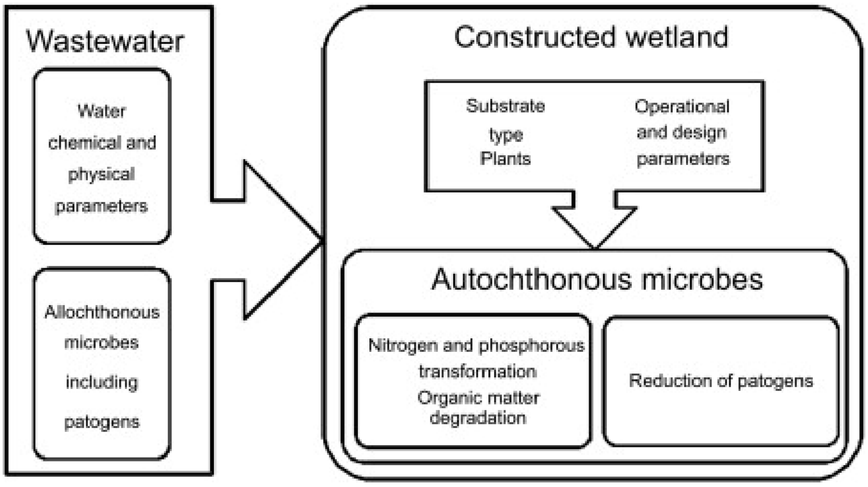

Figure N°3.6.1. External and internal factors which may affect microbial community structure and activity in constructed wetlands. (Truu et al., 2009).

Wang et al (2022) stated that microorganisms in CWs, as an important component, play a key role in processes such as pollutant degradation and nutrient transformation, mineralization of organic compounds. Also they play a key role in the nitrogen removal processes, specifically in the ammonification, nitrification, denitrification and ammamox that are carried out by microorganisms in anaerobic or aerobic conditions. (Meng et al., 2014), as well as physicochemical processes such as the fixation of phosphate by iron and aluminum in the soil filter. (Stottmeister, 2003).

Additionally, the microorganisms can help to remove heavy metal compounds, through biosorption, bioaccumulation and speciation transformation (Si et al., 2019) and can improve the tolerance and removal efficiency of CWs to pollutants by enhancing phytoremediation (Syranidou et al., 2018;Vassallo et al., 2020).

3.7. Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular Mycorrhiza is a mutualism between species in the fungal phylum Glomeromycotan and higher plant roots (Willis A, Rodrigues F & Harris P.J.C, 2012). So, AMF are the soil microbes that colonize the majority of the plant root and are able to make a connection between the plant and the substrate to enhance the uptake. (Kumari P. et al., 2021). Also, are known to form a symbiotic association with more than 80% of land plant families. (Newman and Reddell, 1987). These symbiotic associations are widespread in the natural environment, providing the host plant with many benefits such as improved nutrition, (Gosling et al., 2006).

The AMF consists of an internal phase inside the root and an external phase, or extraradical mycelium (ERM) phase, which can form an extensive network within the soil, facilitating the plant host in the uptake of relatively immobile phosphate ions, due to the ability of the fungal ERM to grow beyond the phosphate depletion zone that quickly develops around the root, in exchange of carbon from the host plant. (Gosling P. et al., 2006).

The presence and significance of AMF in wetland ecosystems have been demonstrated in recent studies, finding the existence of the association in various types of wetlands as mangroves, fen, marshlands, and urban wetlands. (Hu, B et al.,2021).

As it has been explained, AMF promotes the biodegradation of pollutants that are commonly found in domestic wastewater (Xu Z. et al., 2016). However, the application of AMF in constructed wetlands for contaminants of emerging concern or heavy metals in polluted wastewater is not vast. (Hu, B et al.,2021)

3.8. Microplastics

Plastics are made from natural gas, petroleum, and coal, which are processed resulting in the outcome of a long chain of polymers (Supid et al. 2018). Due to the high demand for production and the physical characteristics of plastics, it has brought a problem of pollution of plastic to different ecosystems due the improper waste disposal and management, especially, for the very slow capacity of biodegradation and chemical inertness, (Espinoza et al. 2016). For Merlin N Issac (2020), the mechanical and photochemical processes, that occur in natural environments, affect plastics, and cause a degradation of plastic into microplastics (MP).

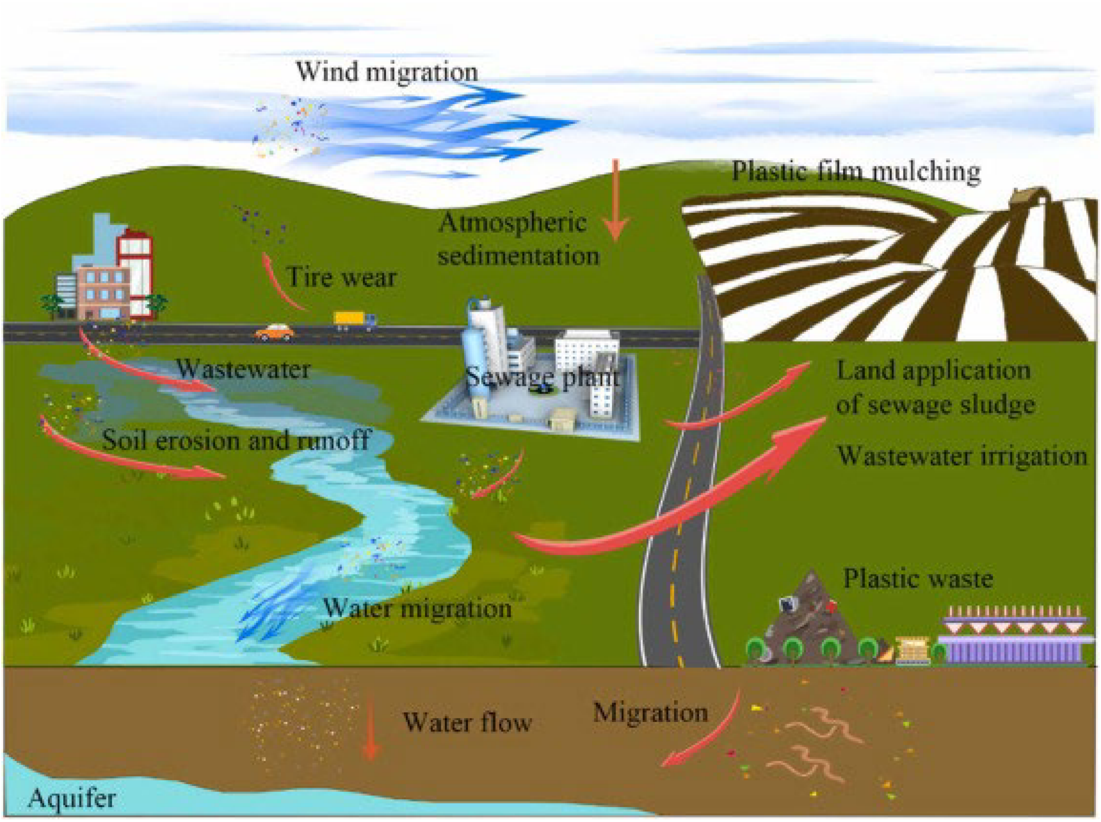

Fig. 3.8.1. *Potential sources and migration of MPs in the soil. (Wang F. et al,.2022)*

Microplastics are fragments of any plastic less than 5 mm long with an anthropogenic origin. According to the National Oceanic and Atmospheric Administration (NOAA, 2023). Microplastics come from a variety of sources, including from larger plastic debris that degrades into smaller and smaller pieces. In addition, microbeads, a type of microplastic, are very tiny pieces of manufactured polyethylene plastic that are added as exfoliants to health and beauty products, such as some cleansers and toothpastes. These tiny particles easily pass through water filtration systems and end up in the ocean and Great Lakes, posing a potential threat to aquatic life".

The migration of anthropogenic pollutants into water bodies has caused floating microplastics to become the most widespread pollutant in the aquatic environment, acting as a contaminant for all aquatic organisms due to their small size. Microplastics are ingested by aquatic organisms, causing physiological problems such as, the development of disorders and behavioral changes (Homin et al. 2023).

Especially since it is known that 10% of plastic annual production ends up in oceans, having as primary inputs as beaches and land-based sources such as rivers, stormwater runoff, wastewater discharges, or transport of land litter by wind.

3.8.1. Problematic of Microplastics

The microplastics issue has been in the sight of the scientific community for not for long, as Carlo Giacomo (2017) explains the first scientific report about the presence of microplastics that was found in beach sediments in the United Kingdom in 2004 (Thompson et al., 2004). In other words, since 20 years ago started the concept of microplastic as emerging pollutant, and nowadays has changed to as an emergent threat. Also, it is imperative to say that microplastics are considered diverse and almost "omnipresent" contaminants, a global change driver with the potential to modify any ecosystem properties and processes. (Lehmann et al., 2021)

As previously mentioned, microplastics are present in both land and water environments. For instance, they can swiftly enter the soil and alter its structure and texture through external influences. In highly polluted soils, the impact that microplastics can generate is in visual changes in soil structure and composition, largely because commercial polymers tend to be less dense than common soil particles. (Lehmann et al., 2021; Wang et al., 2021).

The effect of microplastics in plants is still under study, and every plant has particular cases. In the next studies it was found by Taylor et al., (2020) that microplastics and even nanoplastics tend to accumulate at the root surfaces but are not able to get into the internal root system of Arabidopsis thaliana. Also, it has been proven that, depending on the physical characteristics of the microplastic, especially in the limit of the size that some authors can consider as nanoplastics or the electric charge, it can be absorbed and bioaccumulated in the plant in Arabidopsis thaliana (Sun X. et al., 2020). And lastly, it has been discovered that Submicron (0.2 μm) or even micron-sized (2.0 μm) plastic particles were able to penetrate the root surface of Triticum aestivum L., and Lactuca sativa (Li et al., 2019, 2020d). These studies indicate that microplastics can be accumulated and transported to different parts of the plants, emphasizing the last two cases where the plants are used as crops on farmlands for human consumption.

4. Chapter 4: Methodology

4.1. Location

This experiment was placed in two (2) areas of the Czech University of Life Sciences Prague (Česká zemědělská univerzita v Praze – "CZU") in the City of Prague – Czech Republic, at the laboratories of applied ecology of the Faculty of Environmental Sciences (Fakulta životního prostředí) where the samples were tested, and at the greenhouse of the university where the reactors that simulated the constructed wetlands were located and where samples were gathered.

4.2. Materials

4.2.1. Reactors

For this experiment, 15 reactors were prepared to simulate the vertical flow CWs, dimensions of 15 x 55 cm each (diameter x height). Each reactor was filled with 15 cm of gravel with particles sized 4 - 5 cm and 25 cm of sand, representing the substrate of the CW. The substrate from bottom to top was made in the following order: 15 cm of gravel, 10 cm sand, 10 cm sand mixed with 300 g of arbuscular mycorrhizal fungi (AMF), then the plant was planted, and lastly, 5 cm of sand was added.

The AMF inoculum was Rhizophagus irregularis, the most studied AMF and easy to propagate in pot culture with different plant hosts (Malbreil et al., 2014). They are also pioneer AMF strains in the constructed wetlands (Fester T., 2013).

The reactors that simulated the constructed wetlands were divided into three (3) groups of five (5) reactors per group. In the first group called "A AMF+", we had the presence of AMF, at the second group we had the presence of AMF called "B AMF+" that worked as double, and the third group had the absence of AMF called "A AMF-".

The Plant used in this experiment was Iris pseudacorus, a common wetland plant usually found at sites with permanently high soil-water content, but the soil doesn't necessarily need to be submerged and the plant is also capable of growing in dry sandy soil. However, it is mostly found on peats, as well as on permanently submerged organic and inorganic soils on the edges of ponds, lakes and rivers (Dykes, 1974). Iris pseudacorus is an erect perennial, 40 - 150 cm tall. Rhizome is 1 - 4 cm in diameter, with roots usually 10 - 20 cm long. Leaves are long (50 - 100 cm) with a diameter of 10 - 30 mm, with raised midrib coming to a fine point. Flowers are 8 - 10 cm in diameter, yellow, varying from pale shade to almost orange (Sutherland, 1990)

4.2.2. Chemical Compounds

For this experiment, we needed to recreate the effluent, which simulated domestic wastewater. So for that we used the synthetic municipal water (Nopens, Capalozza, Vanrolleghem, 2001), that involves next lists of compounds that were divided by Macronutrients and Micronutrients as is shown in the Tables N°4.1 and N°4.2, this formula was.

Table N°4.1 – Macronutrients		
Compound Name	Chemical Composition	Concentration (mg/5L)
Urea		0.52
Ammonium chloride	NH4CL	0.08
Sodium Acetate Trihydrate	CH3COONA*3H20	1.275
Peptone		0.1
Skim Milk		0.295
Yeast		0.66
Sodium Bicarbonate	NaHCO3	0.125
Magnesium Chloride	MgCl2*6H2O	0.205
Monopotassium phosphate	KH2PO4	0.205

Table N°4.2 – Microelements		
Compound Name	Chemical Composition	Concentration (ml/5L)
Copper sulfate pentahydrate	CuSO4*5H2O	0.25
Ferrous sulfate Heptahydrate	FeSO4*7H2O	0.25
Boric acid	H3BO3	0.25
Sodium molybdate dihydrate	Na2MoO4*2H2O	0.25
Chromic potassium sulfate	MnSO4*H20	0.25

4.2.3. Microplastics

For this experiment, it was required to simulate the introduction of microplastics to the systems of the CW in controlled concentrations. So, for that, it was used two types of microplastics: Polyethylene (PE) and Polyester (PES) in different concentrations according to the designated reactor in the following order: 1) Blank, 2) PE 0.25mg 3) PE.2.5mg, 4)PES 0.25, 5) PES 2.5

4.2.4. Laboratory Equipment

A few machines were utilized during this test to measure the concentration of different parameters of the water samples that were taken from the reactors.

The first machine that was used after getting the samples was the HQD Field Case (HACH) which is a portable meter with detachable probes. The probes are able to measure the pH and oxidation-reduction potential (ORP) in the water samples.

The Cary 60 UV-VIS spectrophotometer (Agilent Technologies) was used to calculate the concentration of ammonium in the sample. The spectrophotometer measures the number of photons (the intensity of light) absorbed after it passes through sample solution (chem.libretexts.org)

The FormacsSERIES TOC/TN analyzer (SKALAR) analysis of Total Nitrogen (TN), Total Carbon (TC), Total Inorganic Carbon (TIC), and Total Organic Carbon (TOC). The machine uses an injection to collect the sample and uses a high-temperature catalytic combustion to analyze the sample (skalar.com).

The 883 Basic IC plus machine was used to find the nitrate (NO3-), nitrite (NO2-), sulfate (SO42-) and phosphate (PO43-) in the samples.This machine is used to separate and identify different chemical compounds by passing a gas or solution through columns containing beads that can selectively retain or control the rate of movement of different chemical species based on molecular size (Simon, 2012).

4.3. Sample Analysis

Once a week, we needed to simulate the wastewater that was going to be used for the reactors, so for that, we used the macronutrients and microelements and mixed them to have uniformity in buckets of 5 liters, and to each reactor it was poured 2.5 liters.

After the first week, before pouring the water we needed to collect the outflow of each reactor of approximately 50 ml for sampling. So, for each week, we had 15 samples of outflow, their doubles, and 8 samples for inflow. At the same time, we were measuring the total volume of the outflow to be able to calculate the hydric balance.

The 15 samples were taken to the laboratory and were first analyzed for pH, temperature (C°), and oxidation-reduction potential (ORP) using the multiparameter HQD Field Case (HACH).

Then the samples were prepared for the ammonium (NH4+) analysis by first diluting 50 times the concentrations. So first in a beaker, we put 50 ml of water and then 1 ml of sample and mixed it. After that, we took 1 mL of the dilution and added 7.4 mL of distilled water, .8 mL of reagent A, and .8 mL of reagent B (color additive). The samples were then placed in the dark for approximately 30 minutes and were then analyzed using the Cary 60 UV-VIS spectrophotometer (Agilent Technologies).

For the parameters of nitrate (NO3-), nitrite (NO2-), and phosphate (PO43-) we use filters of 45 mm on 12ml of samples to clean them of any type of solid suspended or algae that could be on the sample. The filtered samples were then placed on the 883 Basic IC plus to determine their concentrations. The machine would take approximately 15-20 minutes per sample and the results were processed the next day.

In the case of the analysis of Total Nitrogen (TN), Total Carbon (TC), Total Inorganic Carbon (TIC), and Total Organic Carbon (TOC) we took approximately 3 mL of the outflow sample and diluted with deionized 6 mL water. Once the samples were diluted, they were placed on the FormacsSERIES TOC/TN analyzer (SKALAR). The machine would take approximately 20-30 minutes per sample and the results were taken the next day.

4.4. Statistical Analysis

This experiment has two important statistical analyses to perform. In the first one it was needed to analyzed the effect of the microplastic treatment over the reactors. And in the second one, it was required to analyzed the effect of AMF on the 3 groups. For reaching both goals, it was required to measure the concentrations of the compounds target and analyze the inflow versus the outflows, obtaining the removal efficiency.

Once the removal efficiency was retrieved, we needed to run the statistical basic analysis to be able to choose the proper statistical analysis. Afterwards, a statistical analysis was done the RE values using a non-parametric test Kruskal-Wallis test with Dunn´s multiple comparisons test as post analysis get their P-Value.

This statistical analysis is a statistical test that is used to compare the means of more than two groups. It is often used in hypothesis testing to determine whether a process or treatment actually has an effect on the subject of interest, or whether more than two groups are different from one another in this case, the four treatment of the microplastic and the control.

5. Chapter 5: Results

We grouped the 15 reactors in groups of 5, with the purpose of analyzing the change over time of the Removal Efficiency Percentage (RE) of the reactor under the conditions that were set up in the methodology. Also, as it has been described in the methodology, every week we needed to collect the samples of the outflow and the inflow to run the laboratory analysis. In the case of the inflow, eight samples were prepared with concentrations described in Tables N° and Table N°, and for the outflow, 15 samples were collected from the reactors.

5.1. Field Parameters

5.1.1. Water Volume Balance

Measuring the volume of the outflow was the first task of the experiment which consisted in collecting the water from the reactors. In this part, it was needed to measure the exact volume of water that was going out of the reactors to observe the water loss percentage due to evapotranspiration or linkage to any type of water loss that could happen in the system.

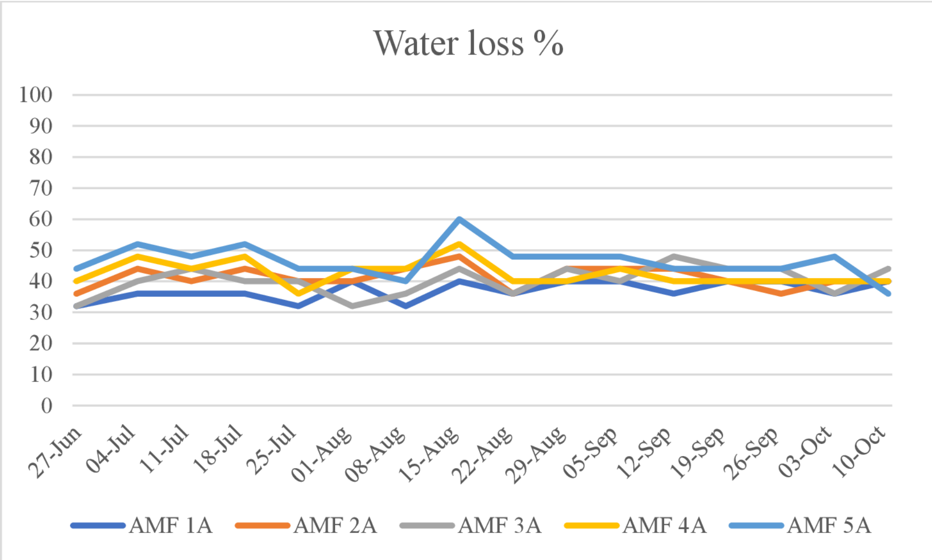

Figure 5.1.1. Water loss percentage in the first group of treatment

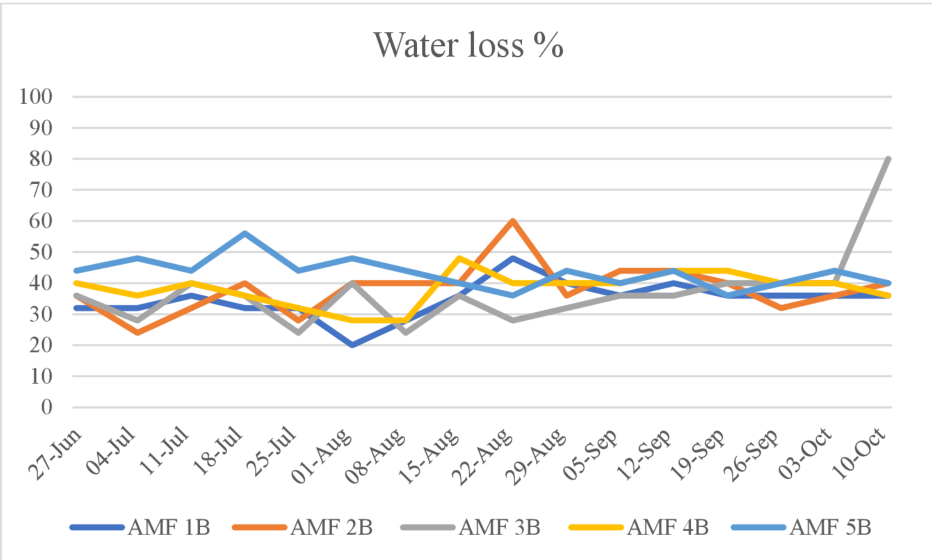

Figure 5.1.2. Water loss percentage in the second group of treatment

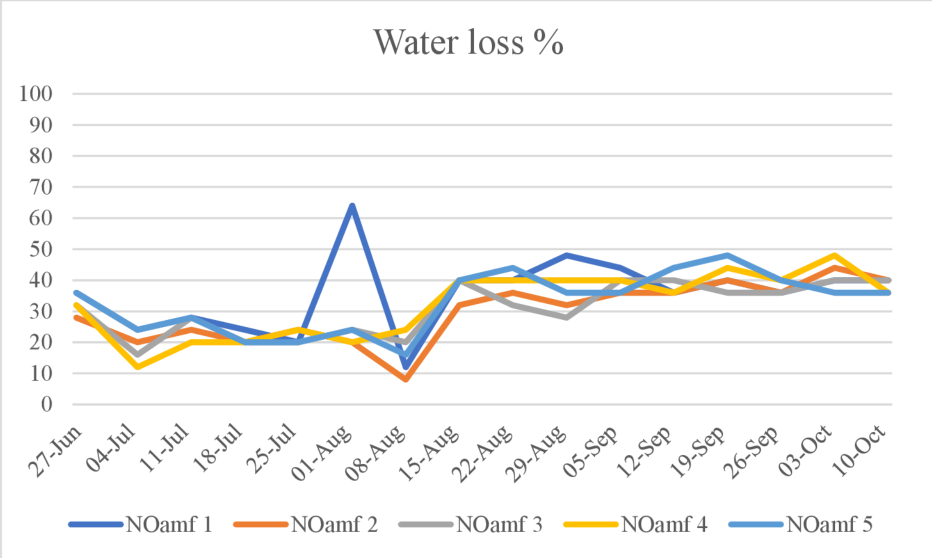

Figure 5.1.3 Water loss percentage in the third group of treatment

From the figures 5.1.1., 5.1.2., 5.1.3 that we have presented, we can observed that the first group (A-AMF +) had a mean of 41.5% water loss, in the second group (B-AMF +) it is 38.5% water loss, and in the last group (A-AMF -) 32.5% water loss, it is important to state that this experiment was carried out in the months of summer, so the percentage of loss water is mostly related to the evapotranspiration of the reactors. In the case of the outliners, happened due technical problems in the reactors such as leakages.

5.1.2. pH

In the case of the potential of hydrogen (pH), it was needed to measure the 8 samples of inflow in addition to the outflows. For the first group (A-AMF+), the min value is 6.93pH, the max pH is 8 and the mean is 7.58 pH, for the second group (B-AMF+), the min value is 6.93pH, the max pH is 8.2 and the mean is 7.61 pH, for the third group (A-AMF-), the min value is 6.91pH, the max pH is 8.1 and the mean is 7.69 pH, and finally for the inflow, the min value is 6.89 pH, the max pH is 7.46 and the mean is 7.10.

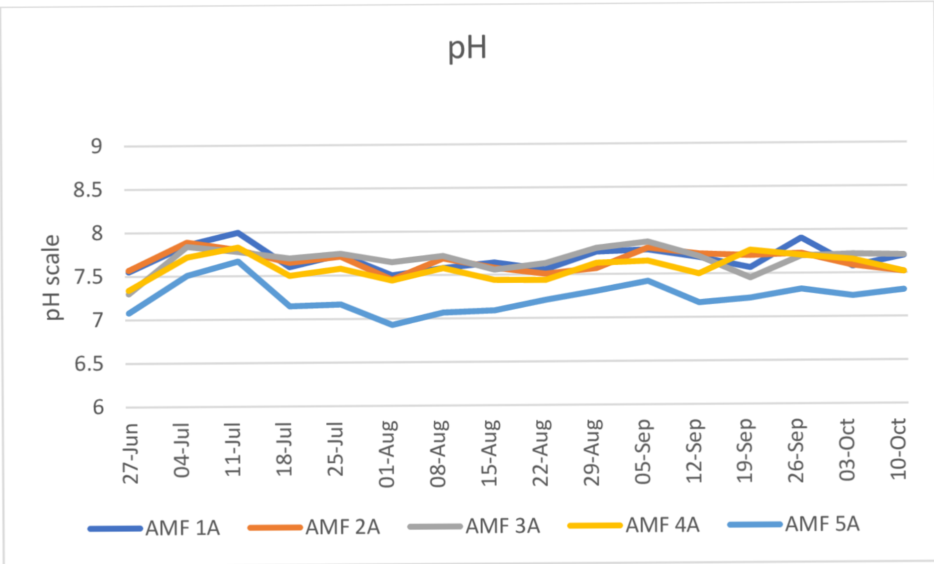

Figure 5.1.4. *pH in the first group of treatment over time*

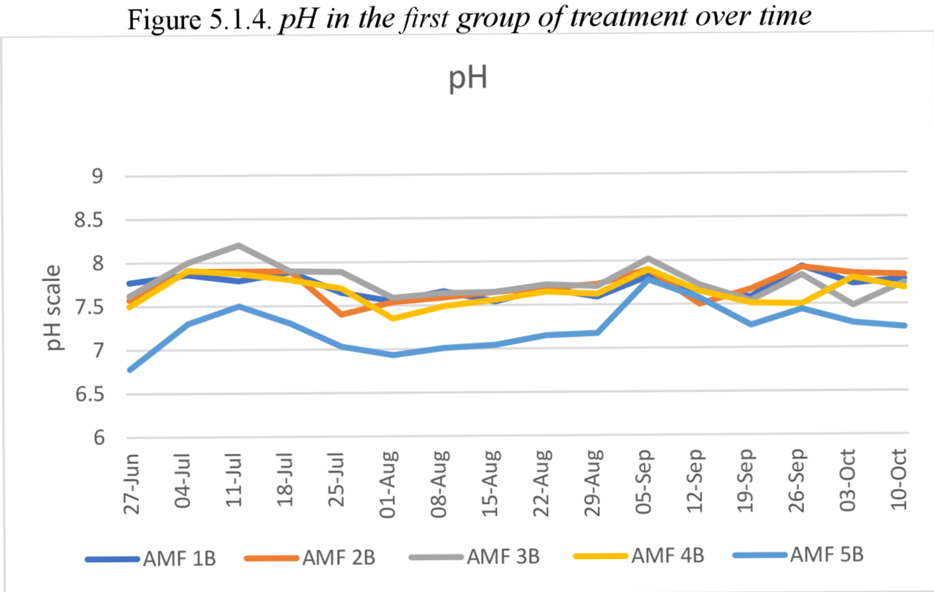

Figure 5.1.5. *pH in the second group of treatment over time*

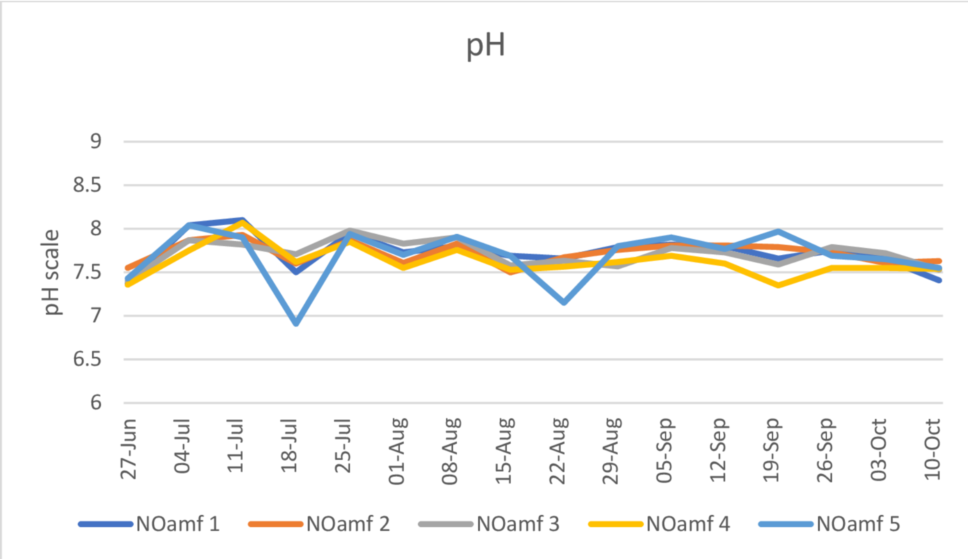

Figure 5.1.6. *pH in the third group of treatment over time*

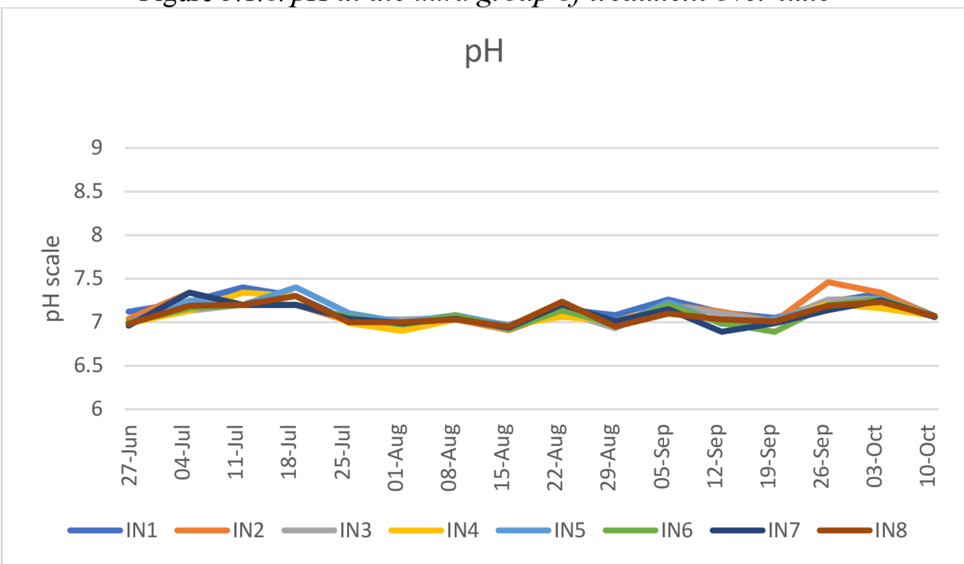

Figure 5.1.7. *pH in the inflow group of treatment over time*

5.1.3. ORP

The oxidation-reduction potential (ORP) or redox potential is used to describe a system's overall reducing or oxidizing capacity. ORP can be divided into three levels (Gui, 2007). An ORP > 200 mV represents a totally aerobic status; an ORP in the 100 to 200 mV range is indicative of a mix of anaerobic and aerobic status; and an ORP < -100 mV represents a totally anaerobic status. So this indicates that in the reactors, at the very beginning there was a mixture of anaerobic and aerobic conditions, but at the end of the experiment we could observe the transition to a more anaerobic status.

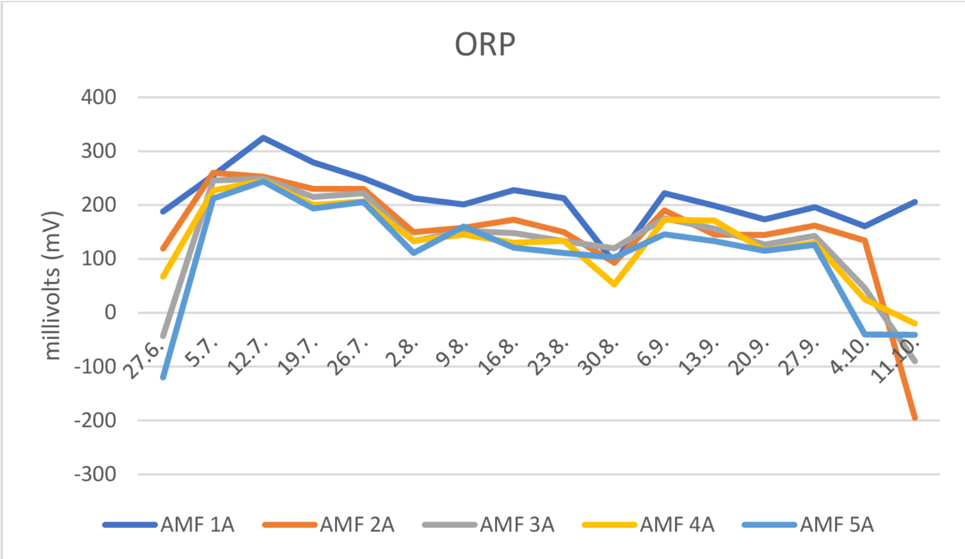

Figure 5.1.8. *ORP in the first group of treatment over time*

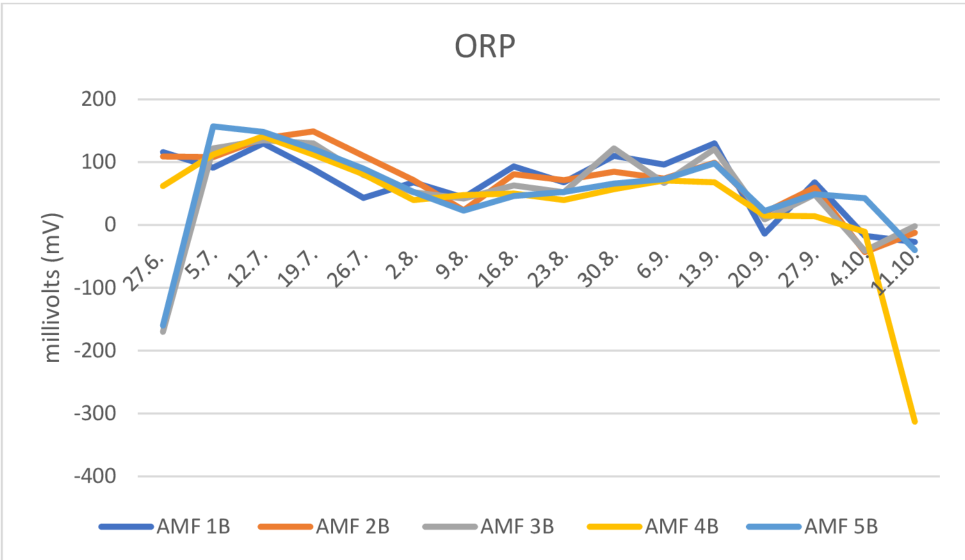

Figure 5.1.9. *ORP in the second group of treatment over time*

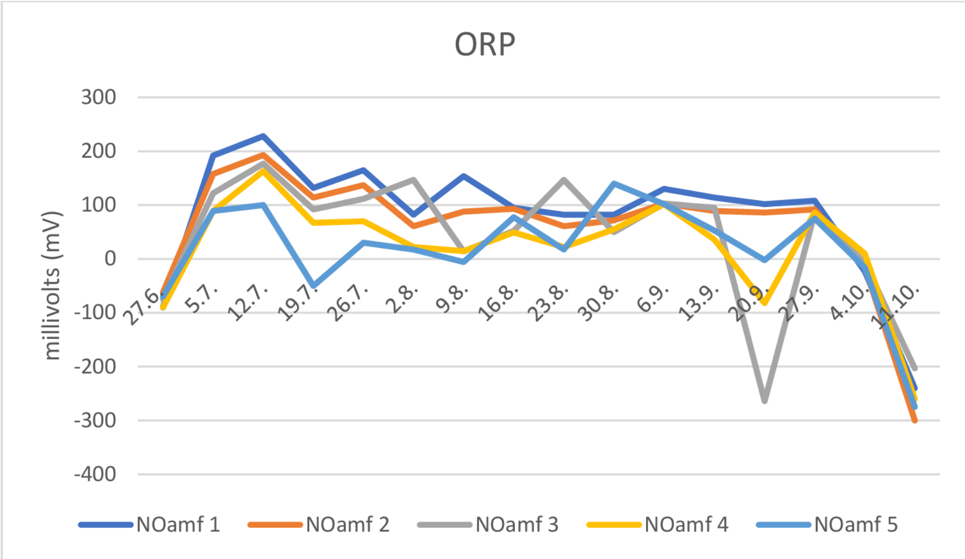

Figure 5.1.10. *ORP in the third group of treatment over time*

5.2. Chemical Compounds

5.2.1. Ammonium

For the analysis of ammonium, the concentrations were diluted 50 times and then analyzed in the spectrophotometer. In this case we could see that in the inflows presented in the figure N°5.2.1. were lower than all the outflows, implying that in the reactor a process of ammonification was occurring, so we cannot indicate a process of removal efficiency.

The average inflow concentration in the initial group (A-AMF+) was 5.54 mg/L, while the outflow had an average of 11.51 mg/L. Additionally, it was observed that the levels of ammonia were gradually rising over the weeks. In group B-AMF+, the average inflow concentration was 5.10 mg/L, while the outflow had an average of 18.53 mg/L. Additionally, it was observed that the ammonia concentration was gradually rising over the weeks. The average inflow concentration for the third group (A-AMF-) was 5.07 mg/L, whereas the outflow had an average of 27.7 mg/L.

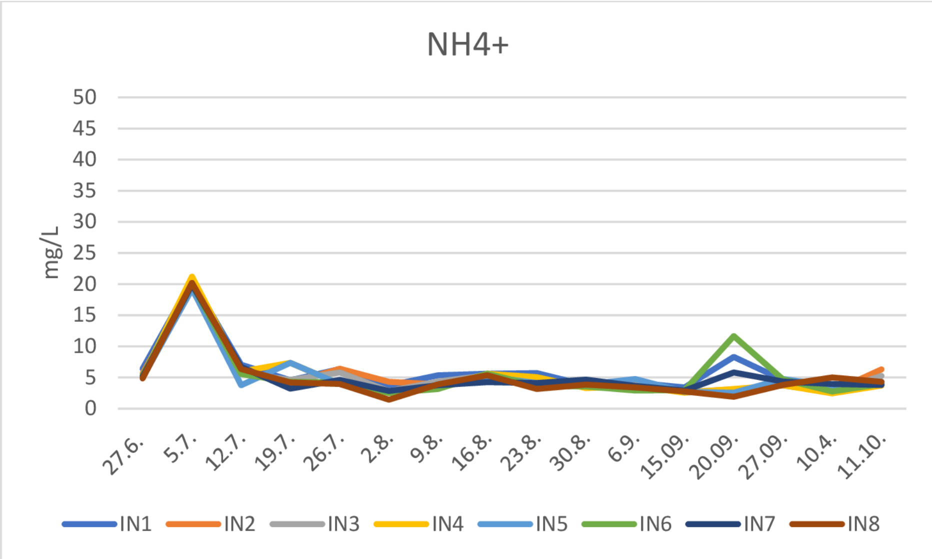

Figure 5.2.1. *Ammonium in the inflow group over time*

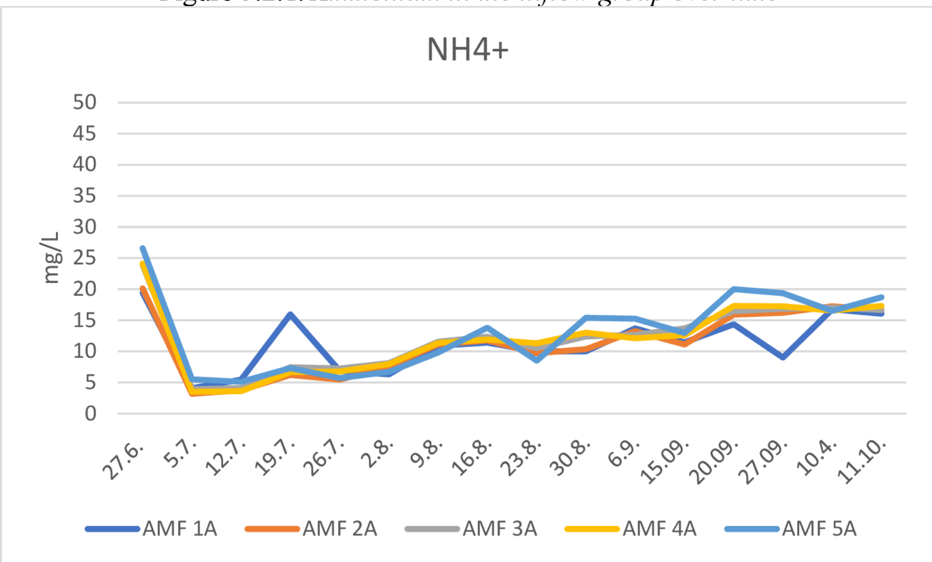

Figure 5.2.2. *Ammonium in the second group of treatment over time*

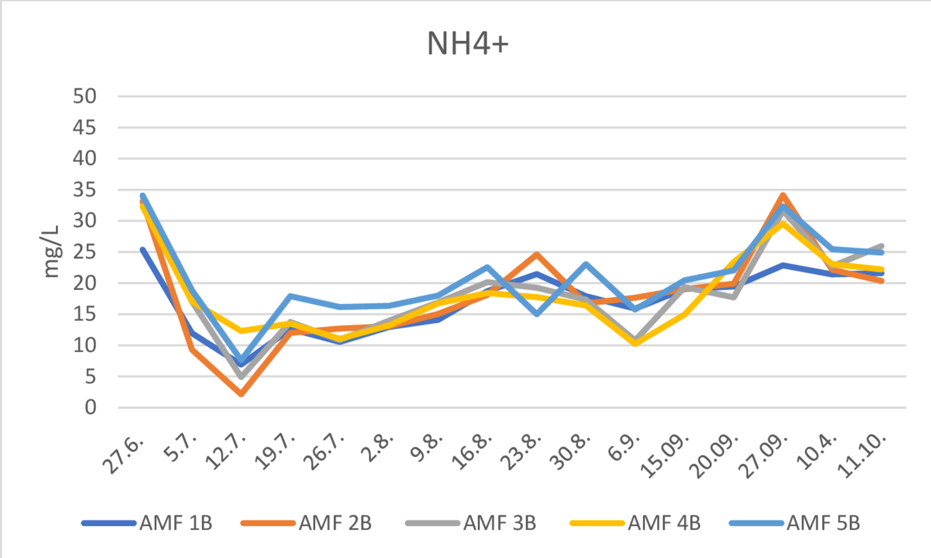

Figure 5.2.3. *Ammonium in the second group of treatment over time*

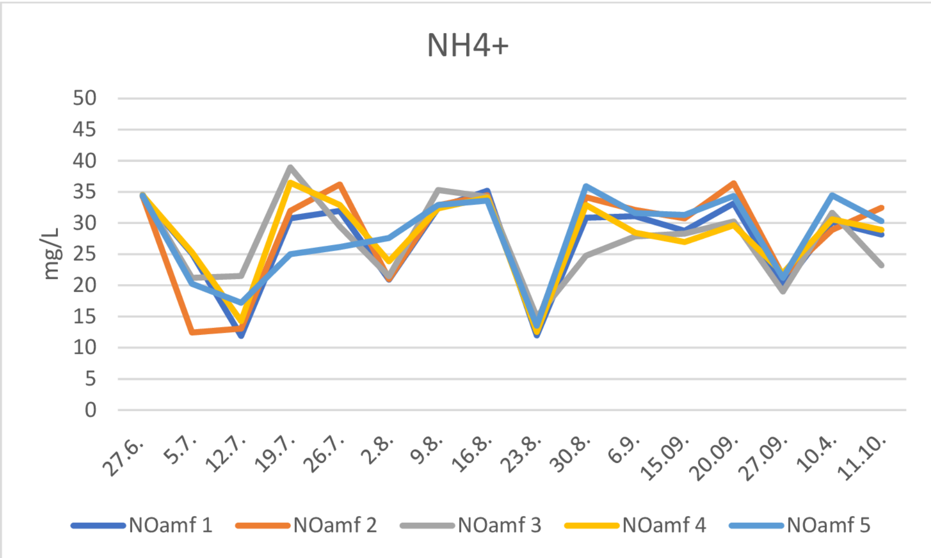

Figure 5.2.4. *Ammonium in the third group of treatment over time*

5.2.2. Nitrate

In group A-AMF+, the average inflow concentration was 4.95 mg/L, while the outflow had an average of 0.06 mg/L. Concurrently, there was a noticeable rise in nitrate concentrations throughout the weeks. In the B-AMF+ group, the average inflow concentration was 4.96 mg/L, while the outflow had an average of 0.07 mg/L. Additionally, it was observed that the nitrate concentration was gradually rising over the weeks. The average inflow concentration for the third group (A-AMF-) was 5.05 mg/L, whereas the outflow had an average of 0.07 mg/L.

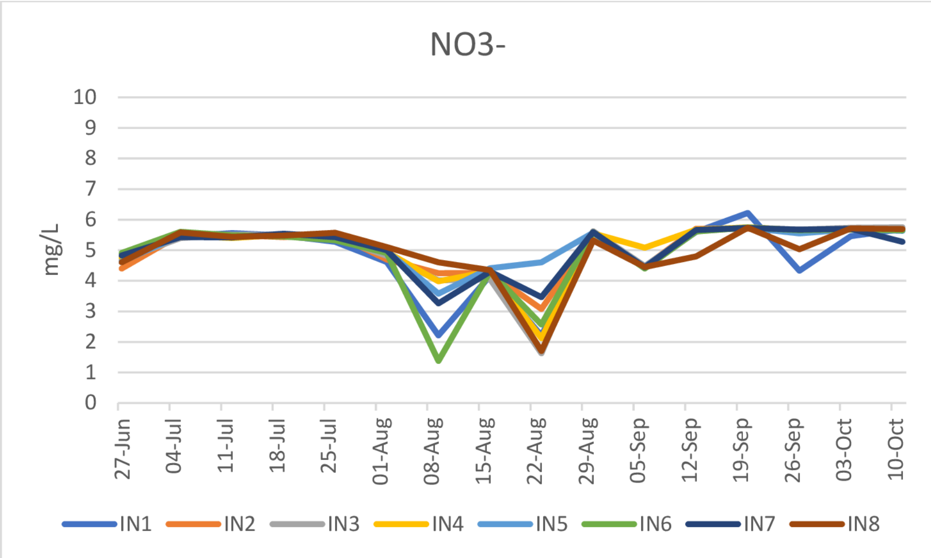

Figure 5.2.4 *Nitrates in the inflow group over time*

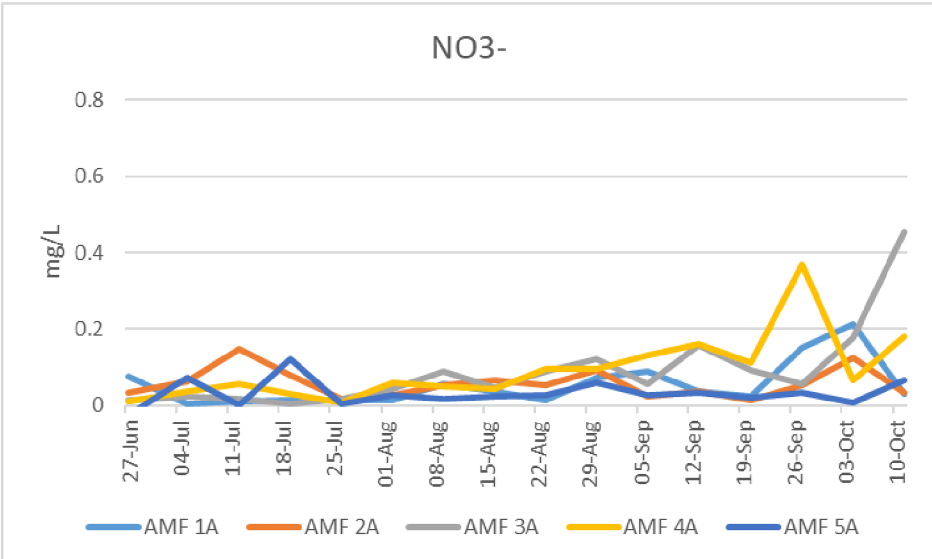

Figure 5.2.5 *Nitrates in the first treatment group over time*

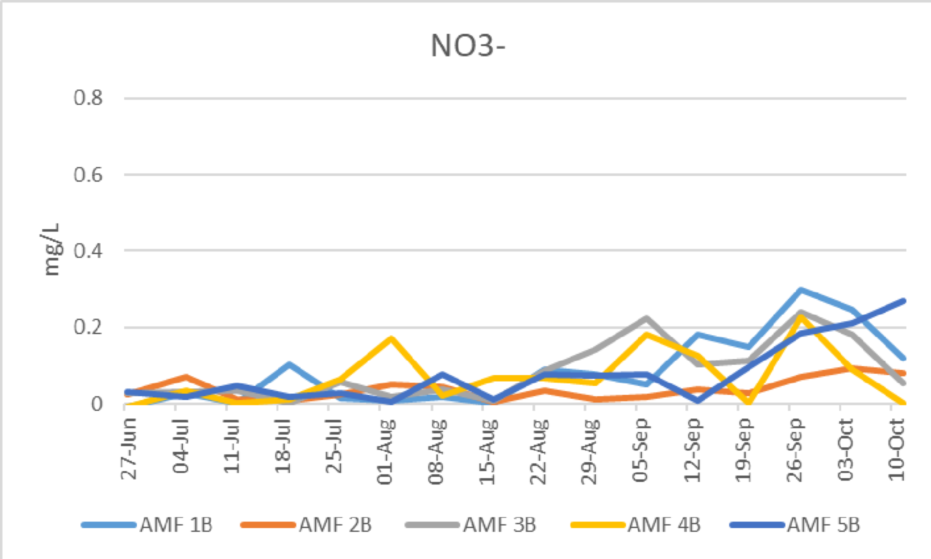

Figure 5.2.6 *Nitrates in the second treatment group over time*

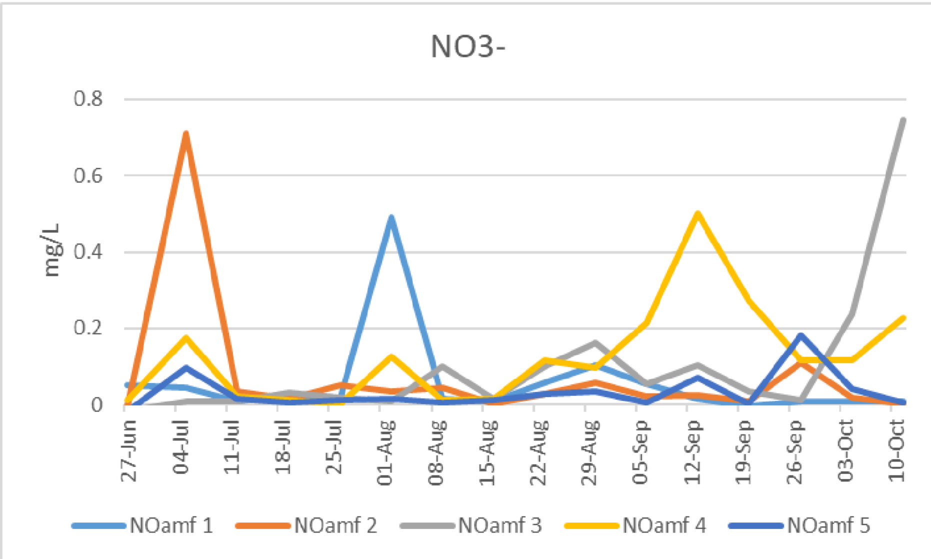

Figure 5.2.7 *Nitrates in the third treatment group over time*

In the case of nitrates, the concentration that was going out it was lower than the inflow, indicating a removal process of nitrates as part of denitrification. It can be seen that the removal efficiency is high in all three groups.

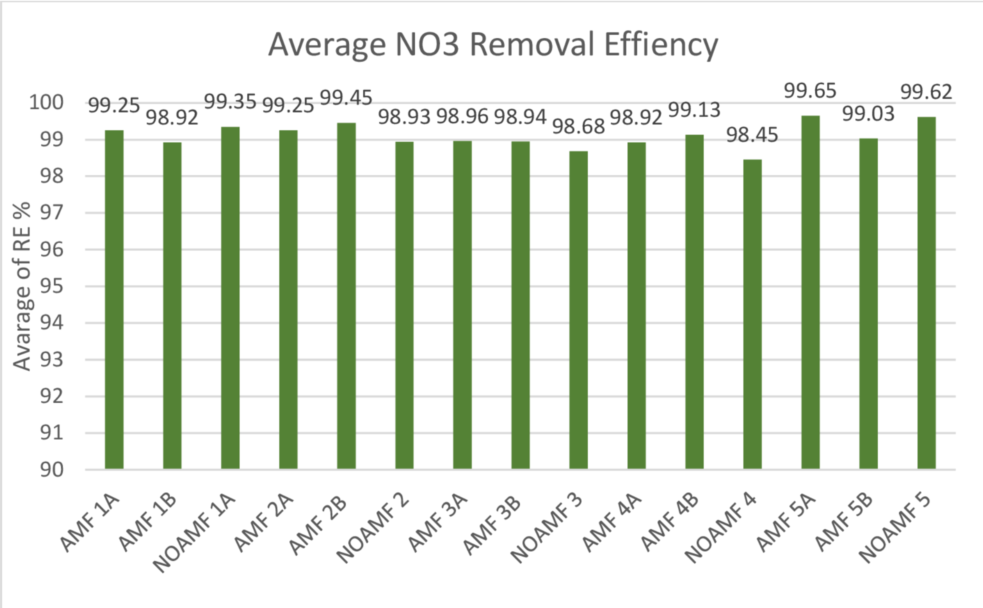

Figure 5.2.8 Avarage of Nitrates Removal Efficency

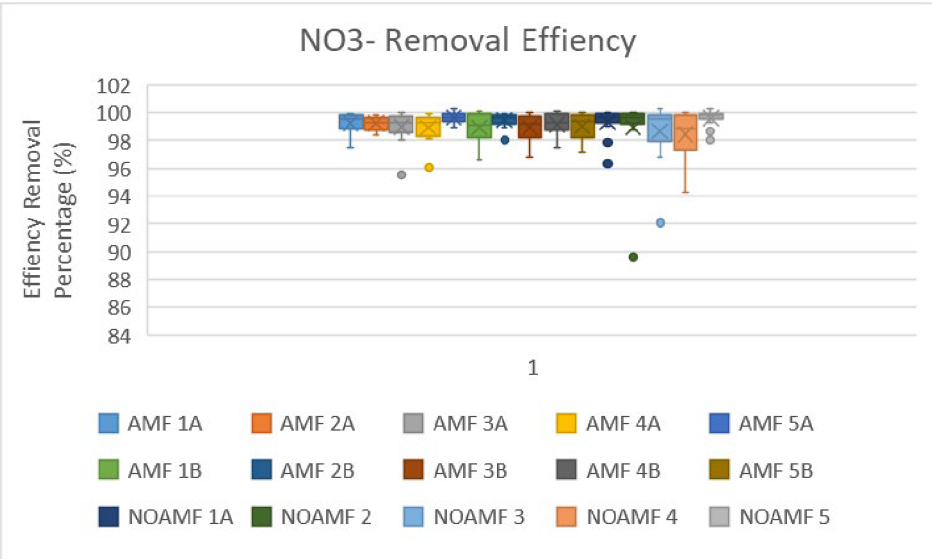

Figure 5.2.9 Variation of Nitrates Removal Effiency

All reactors displayed consistent findings, with each one achieving approximately 90% to 100% removal efficiency. The P-Values show that there is no significant impact of the treatments on the nitrate removal efficiency when comparing their effects within the group..

Table 5.2.1 p-values between the effect of the different treatments on the three groups	
Group	P-values
A - AMF +	0.0540
B – AMF +	0.6852
A – AMF -	0.0736

When considering the impact of AMF, it can be concluded that the presence of the fungi does not impact the nitrate removal efficiency.

Table 5.2.2 p-values between the effect of the different treatments on the three groups	
Group	P-value
Control	0.4279
PE0.25	0.2304
PE2.5	0.8605
PES 0.25	0.3444
PES 2.5	0.1048

5.2.3. Nitrites

In the first group (A-AMF+), the mean concentration of inflow was 0.12 mg/L, while the outflow presented a mean of 0.49 mg/L. In the second group (B-AMF+), the mean concentration of the inflow was 0.13 mg/L, while the outflow was presenting a mean of 0.48 mg/L. For the third group (A-AMF-) the mean concentration of the inflow was 0.11 mg/L, meanwhile the outflow was presenting a mean of 0.49 mg/L.

The process of denitrification converts the ammonium in nitrites and then nitrates, in this case the production of nitrites can be related to this process, especially when we can also observe production of ammonium in the reactors as it was seen in the results 5.2.1.

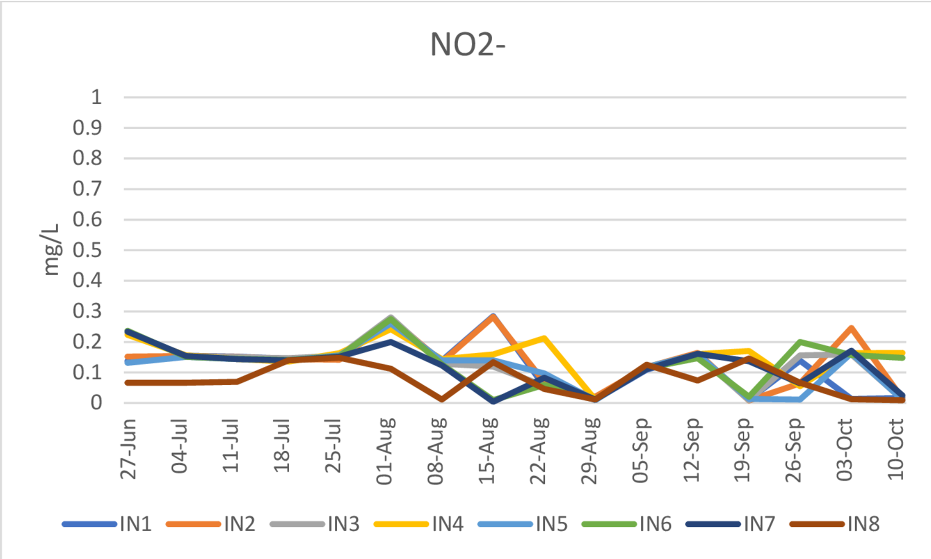

Figure 5.2.10 *Nitrites in the inflow group over time*

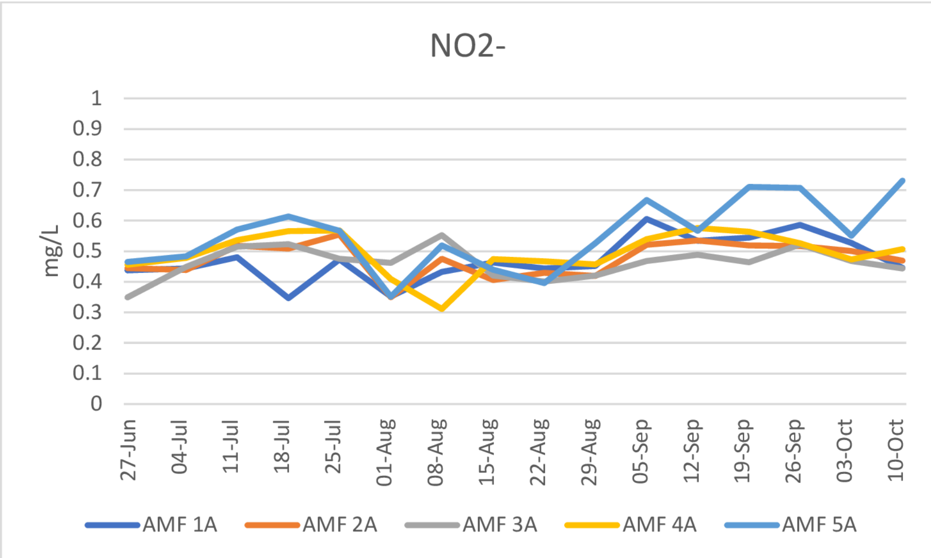

Figure 5.2.11 *Nitrites in the inflow group over time*

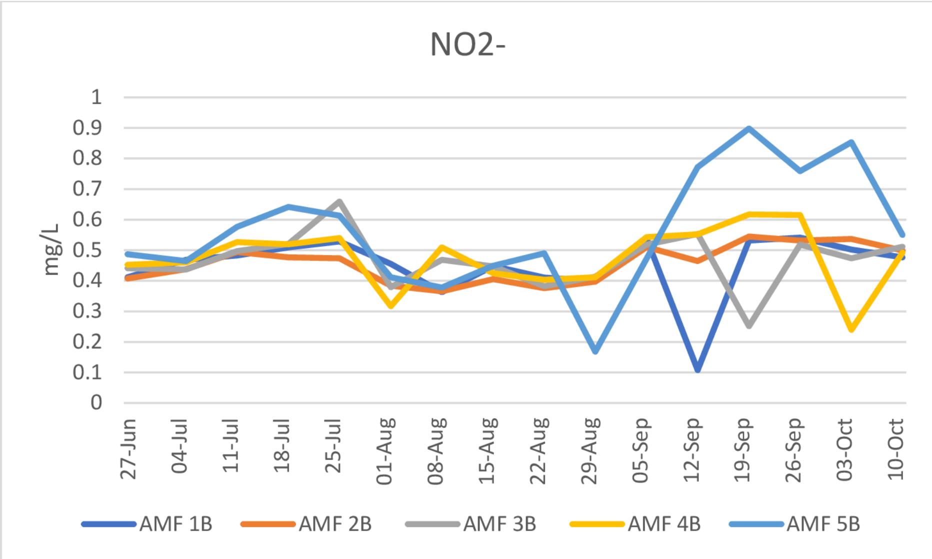

Figure 5.2.12 *Nitrites in the second group over time*

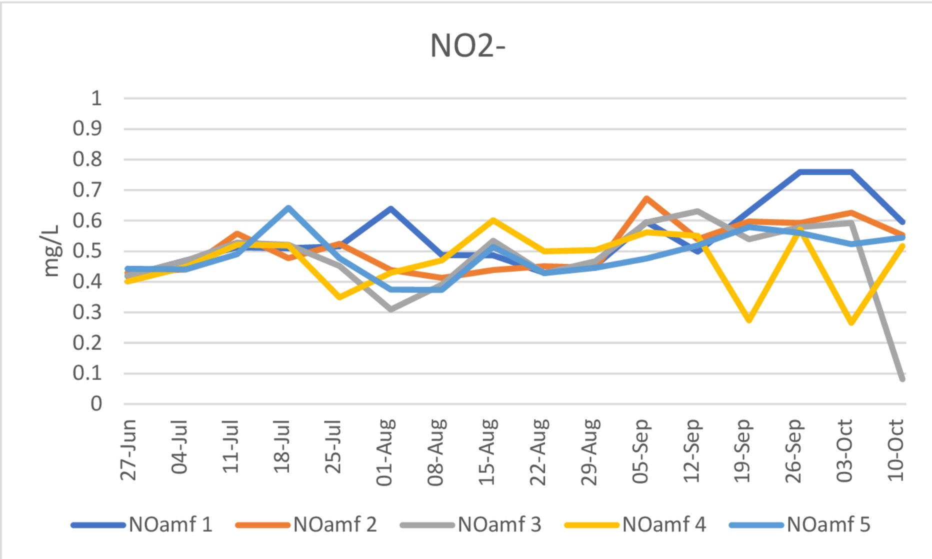

Figure 5.2.13 *Nitrites in the third group over time*

5.2.4. Phosphate

In the first group (A-AMF+), the mean concentration of the inflow was 10.14 mg/L, while the outflow was presenting a mean of 5.89 mg/L. In the second group (B-AMF+), the mean concentration of the inflow was 10.04 mg/L, while the outflow was presenting a mean of 7.16 mg/L. For the third group (A-AMF-) the mean concentration of the inflow was 0.11 mg/L, while the outflow was presenting a mean of 9.73 mg/L.

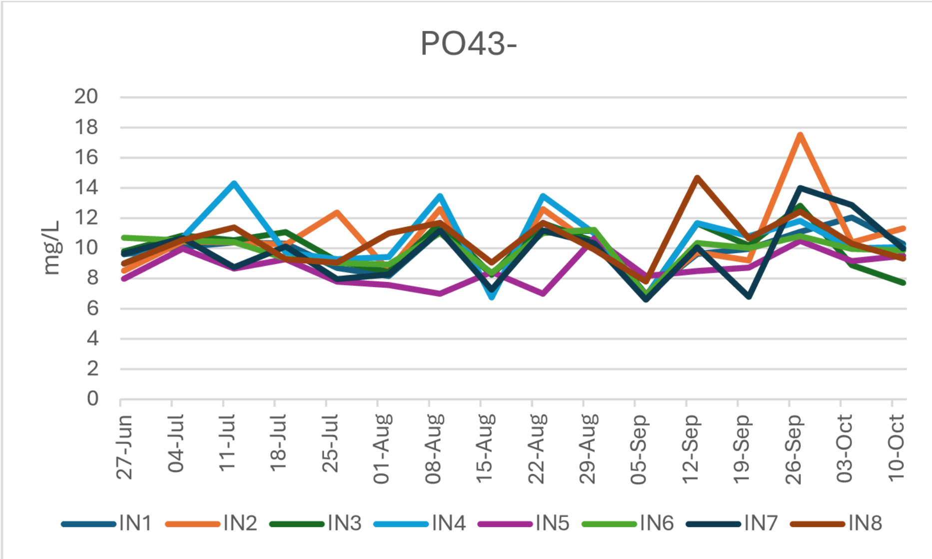

Figure 5.2.14 *Phosphates in the inflow group over time*

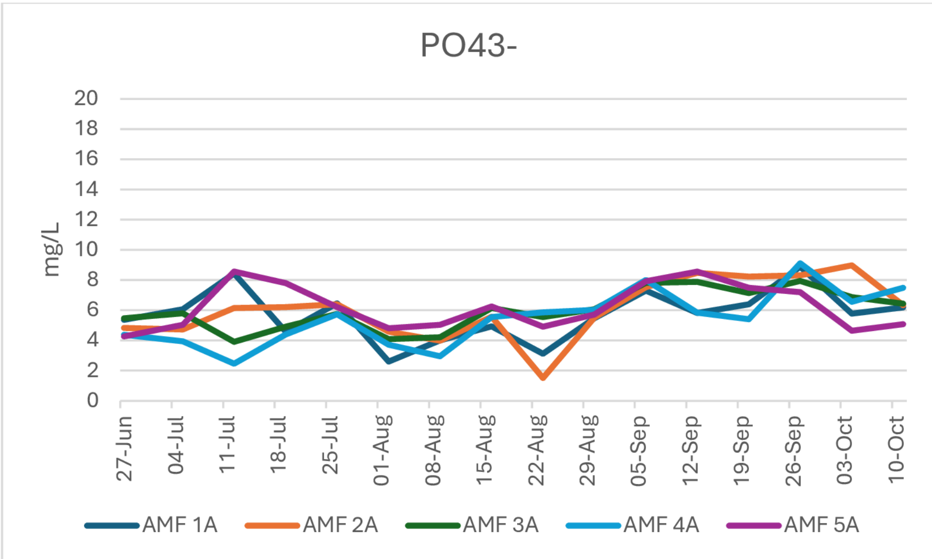

Figure 5.2.15 *Phosphats in the first group over time*

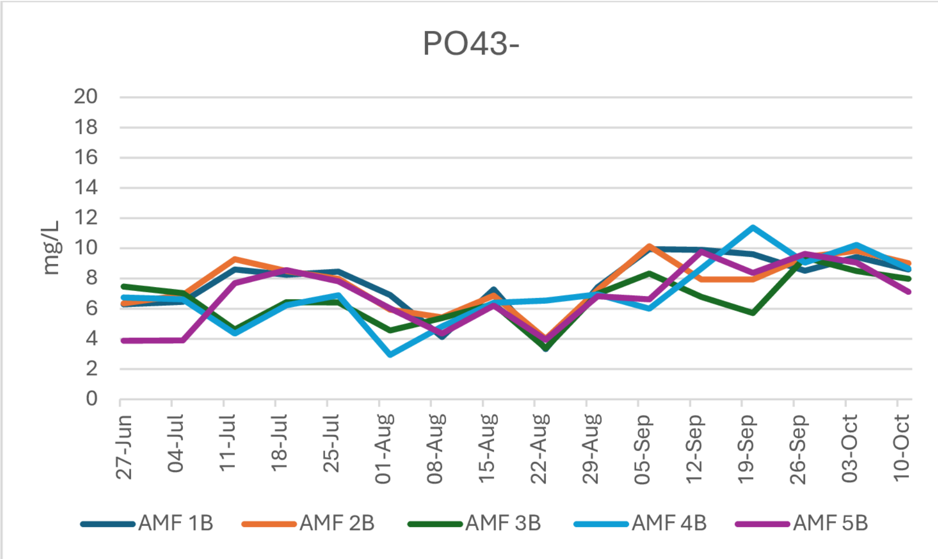

Figure 5.2.16 *Nitrites in the second group over time*

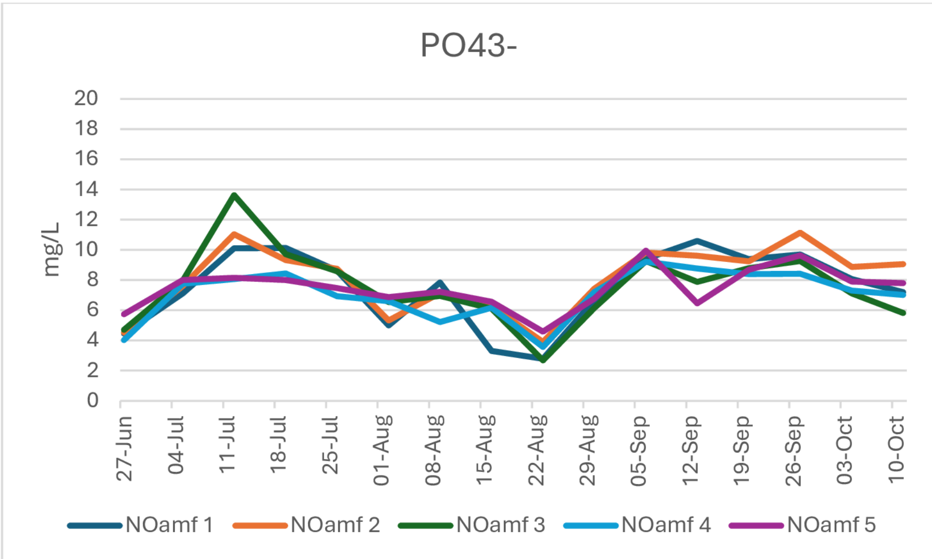

Figure 5.2.17 *Phosphates in the third group over time*

For the case of phosphates, the concentration that was going out was lower than the inflow, so it can be stated that there is a process of removing phosphates from the system as part of the microbial activity process and plant uptake mechanism. In the three groups, it can be observed that removal efficiency is high.

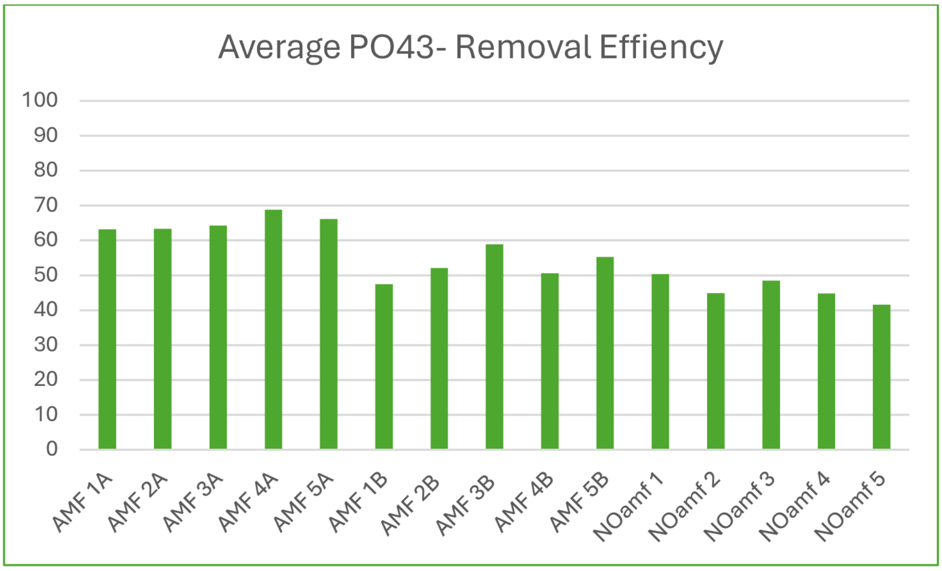

Figure 5.2.18 *Average of Phosphates Removal Efficiency*

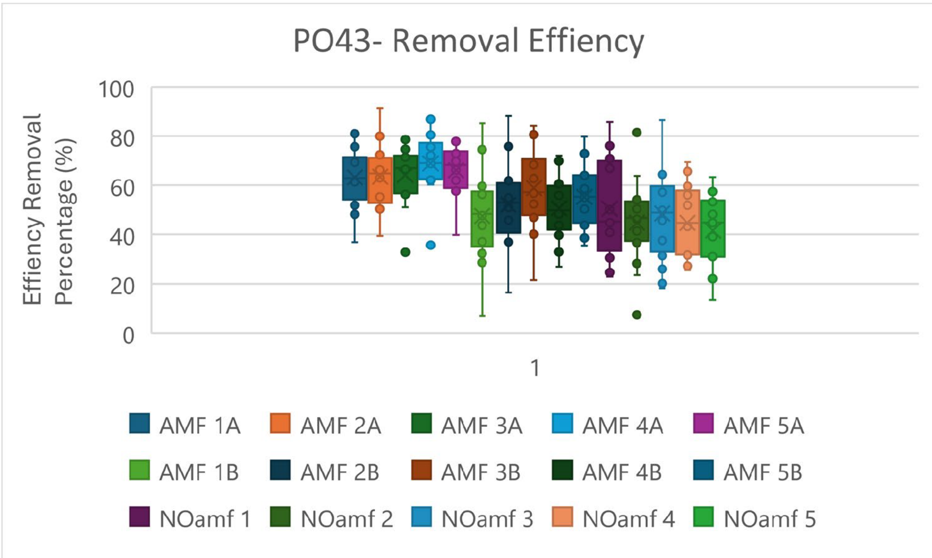

Figure 5.2.19 *Variation of Phosphate Removal Efficiency*

The reactors within the groups show consistent results, however there is observed variation across the AMF groups. The 5A AMF- has a minimum removal efficiency of 41.55%, the reactor 4A AMF+ has a maximum removal efficiency of 68.80%, and the average removal efficiency is 55.66%. The P-Values indicate that the various treatments do not have a significant effect on the efficiency of phosphate removal when comparing the groups

Table 5.2.3 p-values between the effect of the different treatments on the three groups	
Group	P-values
A - AMF +	0.5363
B – AMF +	0.2937
A – AMF -	0.8061

In the case of the effect of the effect of the AMF it can be state that in the case of the presence of the AMF fungi does affect the removal efficiency of the phosphates.

Table 5.2.4 p-values between the effect of the AMF treatments on the three groups	
Group	P-value
Control	0.0205
PE0.25	0.0026
PE2.5	0.0251
PES 0.25	<0.0001
PES 2.5	<0.0001

5.3. Total Nitrogen

For the analysis of Total Nitrogen, the concentrations were diluted 3 times and then analyzed in the TOC. In this case we could see that inflows presented in the figure N°5.3.1 were higher than all the outflows.

In the first group (A-AMF+), the mean concentration of inflow was 67.55 mg/L, meanwhile the outflow was presenting a mean of 18.42 mg/L. At the same time, it can observed that over the weeks the concentration of Total Nitrogen was increasing. In the second group (B-AMF+), the mean concentration of inflow was 60.36 mg/L, meanwhile the outflow was presenting a mean of 25.38 mg/L. At the same time. Also, it can be witness that over the weeks the concentration of Total Nitrogen was increasing. For the third group (A-AMF-) the mean concentration of inflow was 62.72 mg/L, meanwhile the outflow was presenting a mean of 47.62mg/L.

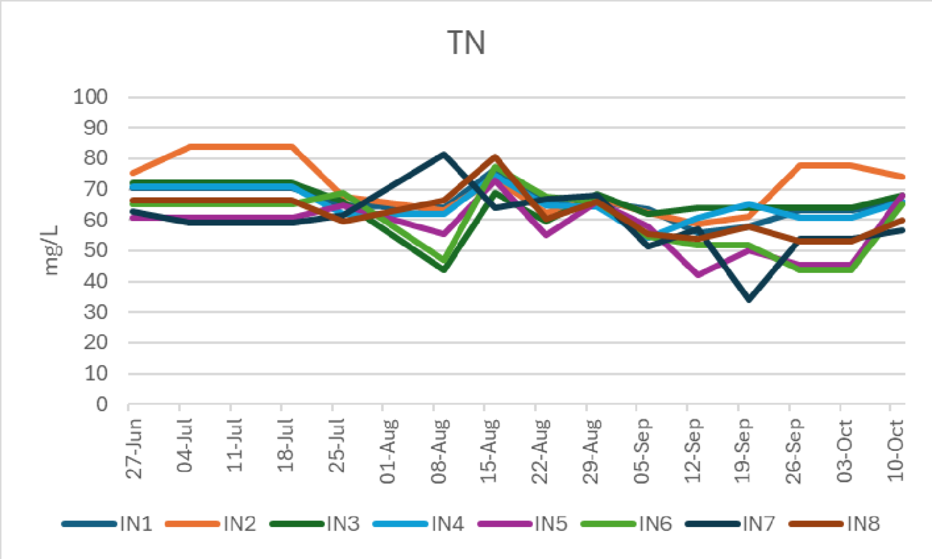

Figure 5.3.1. *Total Nitrogen in the inflow group over time*

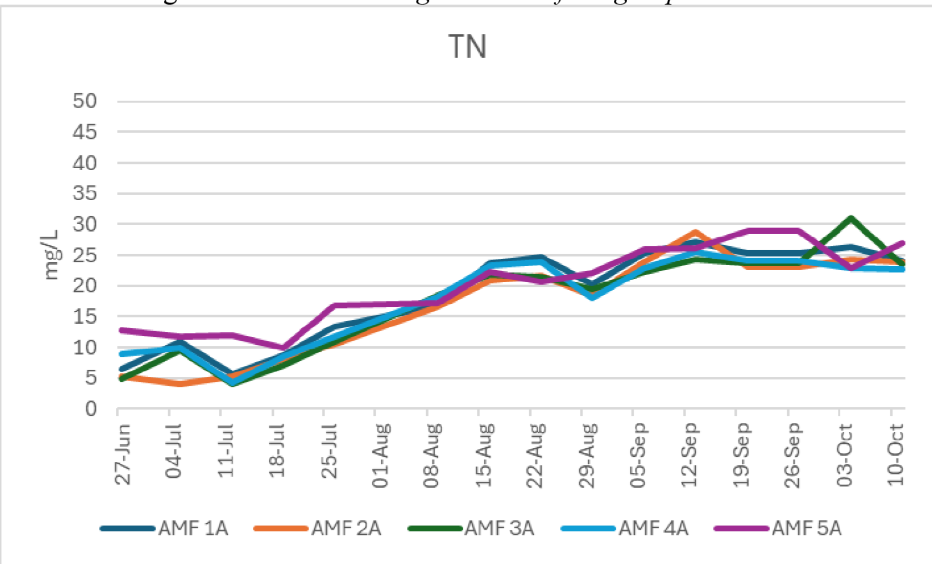

Figure 5.3.2. *Total Nitrogen in the first treatment group over time*

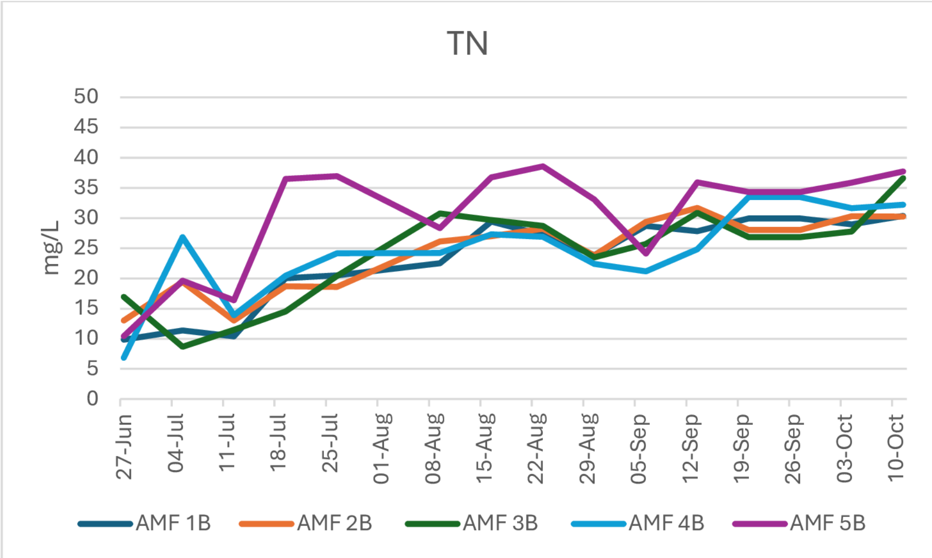

Figure 5.3.3. *Total Nitrogen in the second treatment group over time*

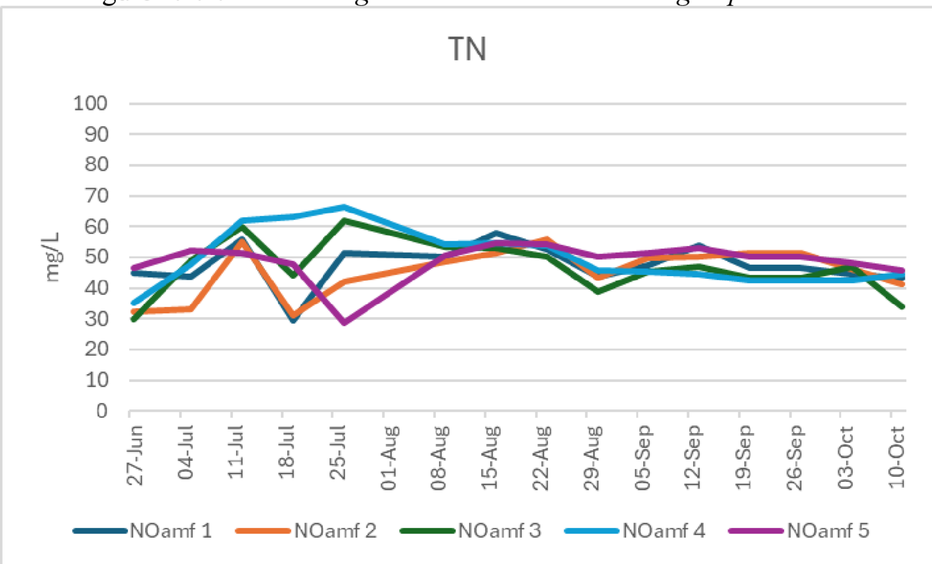

Figure 5.3.4. *Total Nitrogen in the third treatment group over time*

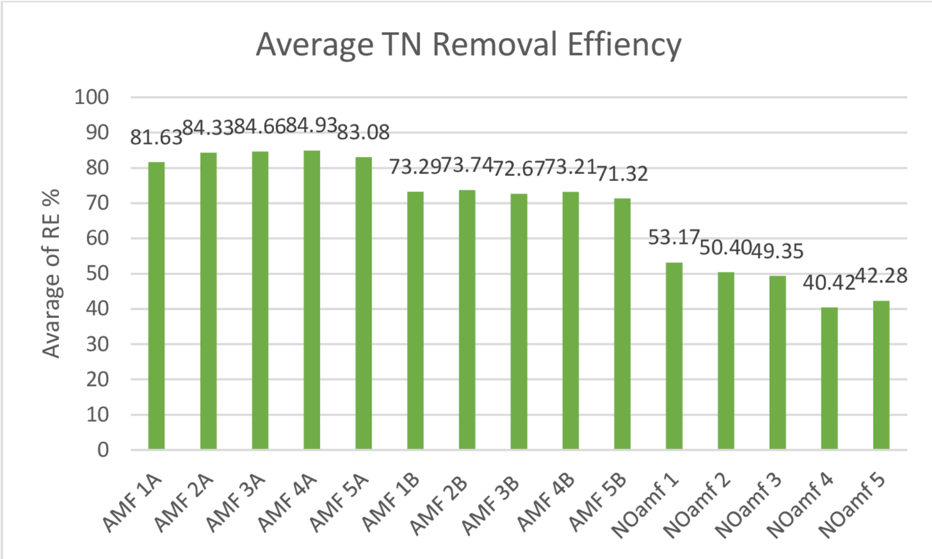

Figure 5.3.5 *Average of Total Nitrogen Removal Efficiency*

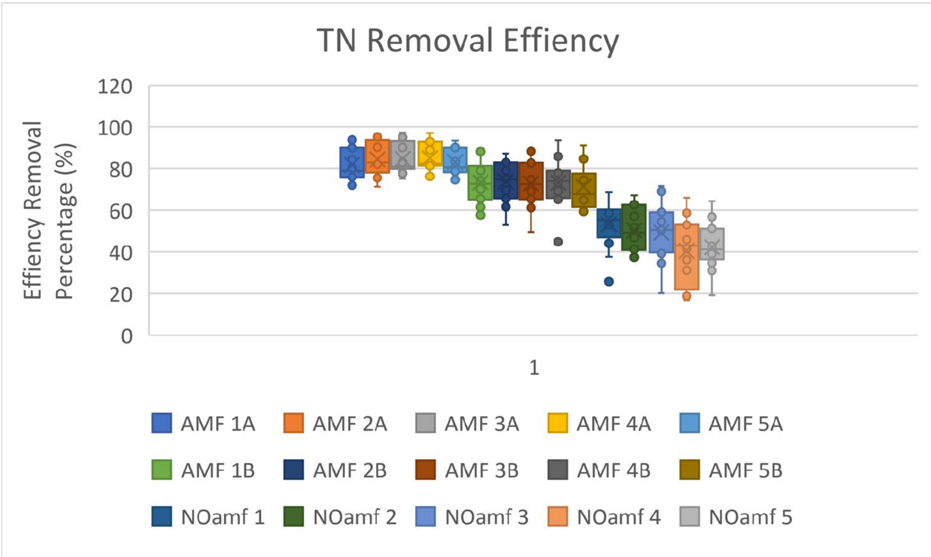

Figure 5.3.6 *Variation of Total Nitrogen Removal Efficiency*

The reactors present consistent results within the groups but it can be observed variation over the AMF groups. The minimum percentage of removal efficiency is 16.6% from the 4A AMF-, the maximum percentage of removal efficiency is 97.28% from the reactor 3A AMF+, and a mean percentage of removal efficiency is 67.89%. Comparing the effect of the different treatments in the groups, the P-Values prove that might be a significant impact of the treatments in relation to the Total Nitrogen removal efficiency in the third group (A- AMF -).

Table 5.3.1 p-values between the effect of the different treatments on the three groups	
A - AMF +	0.5346
B – AMF +	0.9178
A – AMF -	0.0373

In the case of the effect of the effect of the AMF it can be state that in the case of the presence of the AMF fungi does affect the removal efficiency of the Total Nitrogen.

Table 5.3.2 p-values between the effect of the AMF treatments on the three groups	
Group	Pvalue
Control	<0.0001
PE0.25	<0.0001
PE2.5	<0.0001
PES 0.25	<0.0001
PES 2.5	<0.0001

5.4. Total Carbon

For the analysis of Total Carbon, the concentrations were diluted 3 times and then analyzed in the TOC. In this case we could see that inflows presented in the figure N°5.4.1 were slightly higher than all the outflows.

In the first group (A-AMF+), the mean concentration of inflow was 144.05 mg/L, meanwhile the outflow was presenting a mean of 106.31 mg/L. In the second group (B-AMF+), the mean concentration of inflow was 140.34 mg/L, meanwhile the outflow was presenting a mean of 139.28 mg/L. For the third group (A-AMF-) the mean concentration of inflow was 140.34 mg/L, meanwhile the outflow was presenting a mean of 109.37 mg/L.

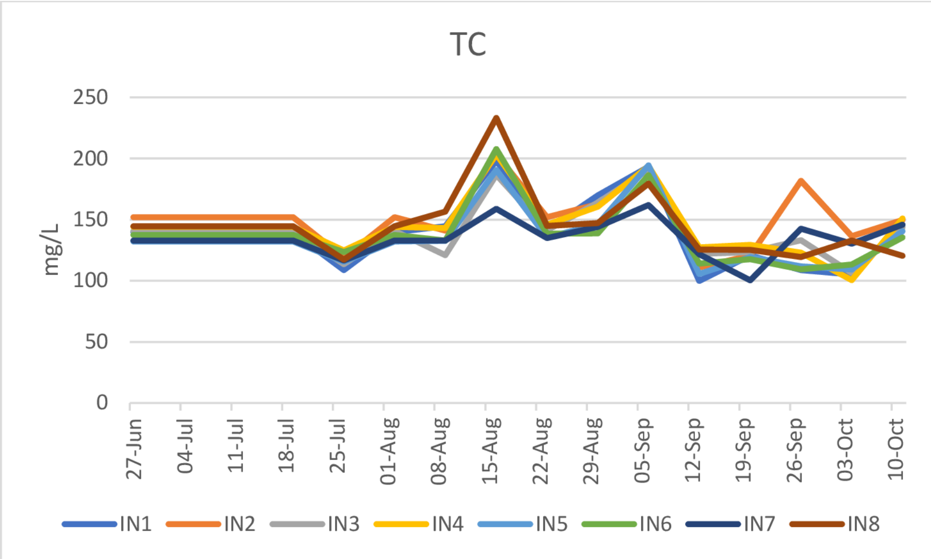

Figure 5.4.1. *Total Carbon in the inflow group over time*

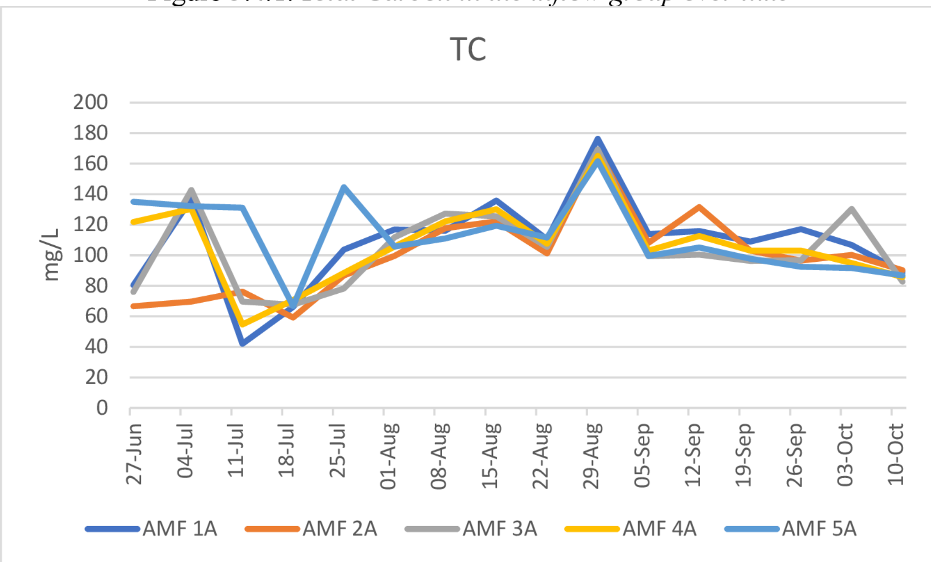

Figure 5.4.2. *Total Carbon in the first treatment group over time*

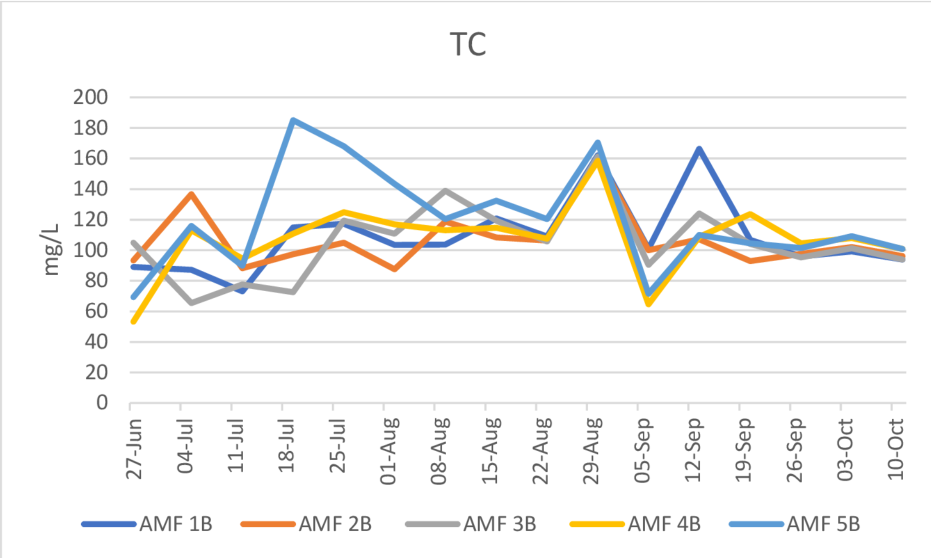

Figure 5.4.3. Total Carbon in the second treatment group over time

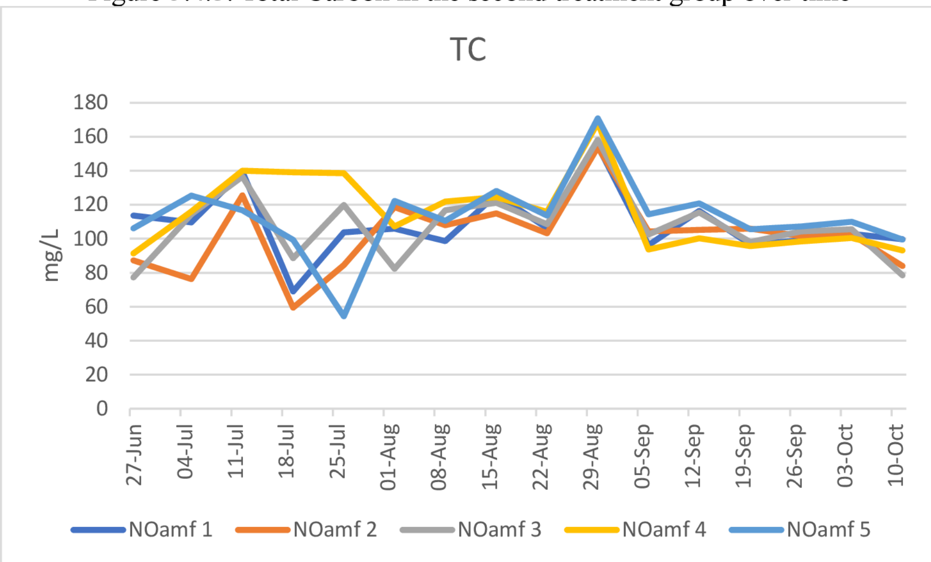

Figure 5.4.4. Total Carbon in the third treatment group over time

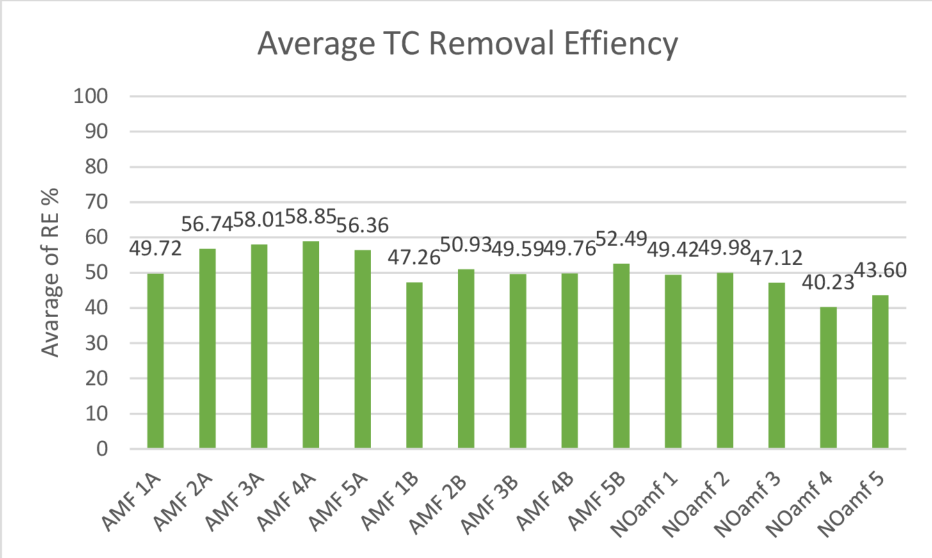

Figure 5.4.5 *Average of Total Carbon Removal Efficiency*

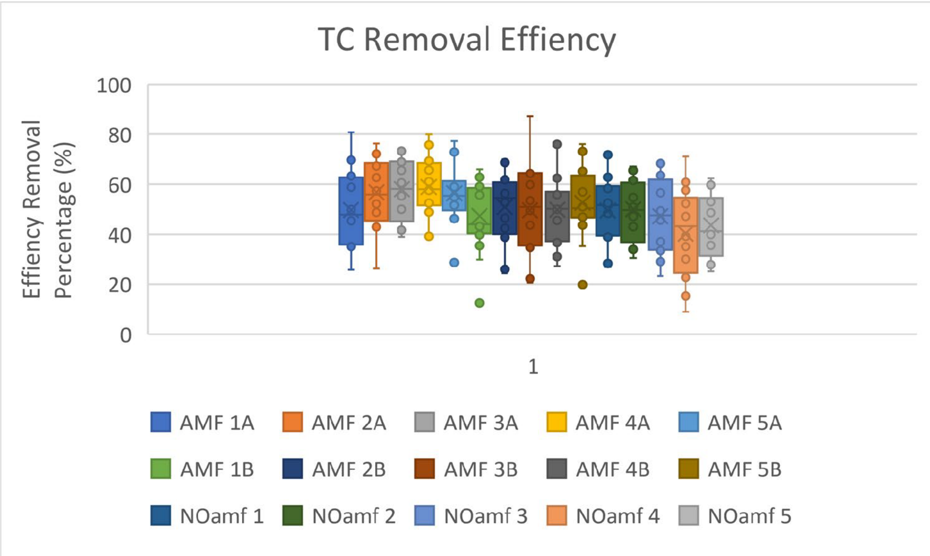

Figure 5.4.6 *Variation of Total Nitrogen Removal Efficiency*

The reactors present consistent results disregarding the groups, but with some reactors having outliners. The minimum percentage of removal efficiency is 8.91% from the 4A AMF-, the maximum percentage of removal efficiency is 87.13% from the reactor 3B AMF+, and a mean percentage of removal efficiency is 50.67%. Comparing the effect of the different treatments in the groups, the P-Values prove that might be a significant impact of the treatments in relation to the Total Carbon removal efficiency in the third group (A- AMF -).

Table 5.4.1 p-values between the effect of the different treatments on the three groups	
A - AMF +	0.3474
B - AMF +	0.8891
A – AMF -	0.4814

In the case of the effect of the effect of the AMF it can be state that in the case of the presence of the AMF fungi does affect the removal efficiency of the Total Carbon when is related to the type of microplastic PES in both concentrations.

Table 5.4.2 p-values between the effect of the AMF treatments on the three groups	
Group	Pvalue
Control	0.9751
PE 0.25	0.2966
PE 2.5	0.0701
PES 0.25	0.0106
PES 2.5	0.0461

5.5. Total Organic Carbon

For the analysis of Total Carbon, the concentrations were diluted 3 times and then analyzed in the TOC. In this case we could see that inflows presented in the figure N°5.5.1 were slightly higher than all the outflows.

In the first group (A-AMF+), the mean concentration of inflow was 105.61 mg/L, meanwhile the outflow was presenting a mean of 20.74mg/L. In the second group (B-AMF+), the mean concentration of inflow was 94.16 mg/L, meanwhile the outflow was presenting a mean of 21.85 mg/L. For the third group (A-AMF-) the mean concentration of inflow was 100.4mg/L, meanwhile the outflow was presenting a mean of 25.90 mg/L.

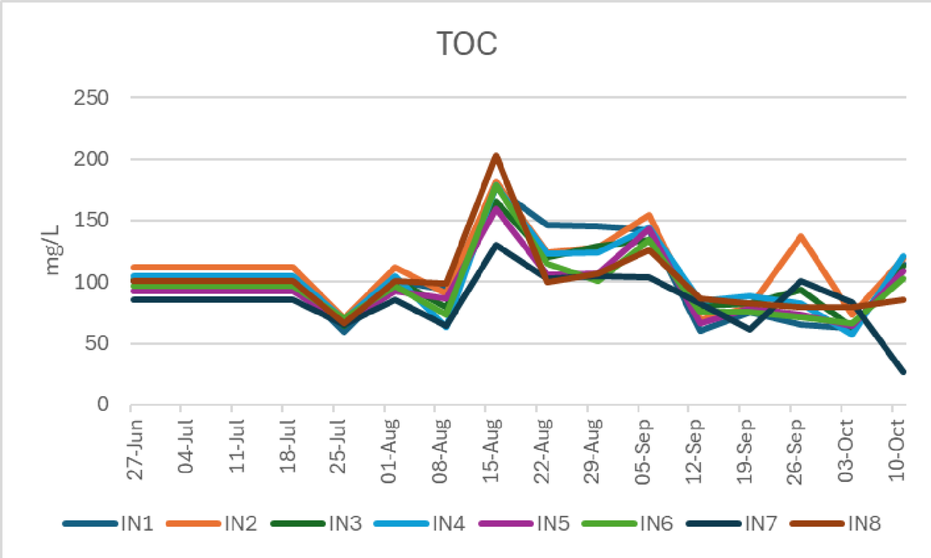

Figure 5.5.1. *Total Organic Carbon in the inflow group over time*

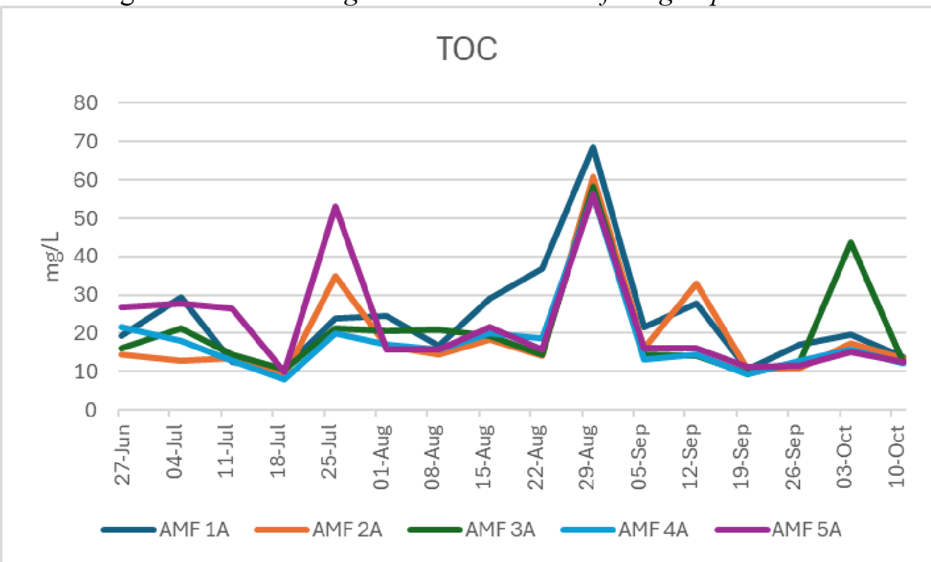

Figure 5.5.2. *Total Organic Carbon in the first treatment group over time*

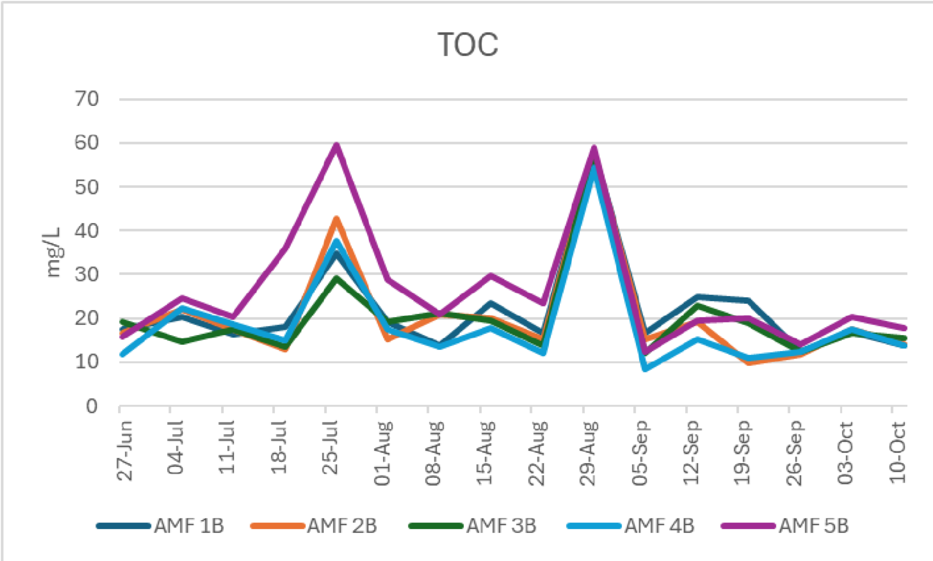

Figure 5.5.3. *Total Organic Carbon in the second treatment group over time*

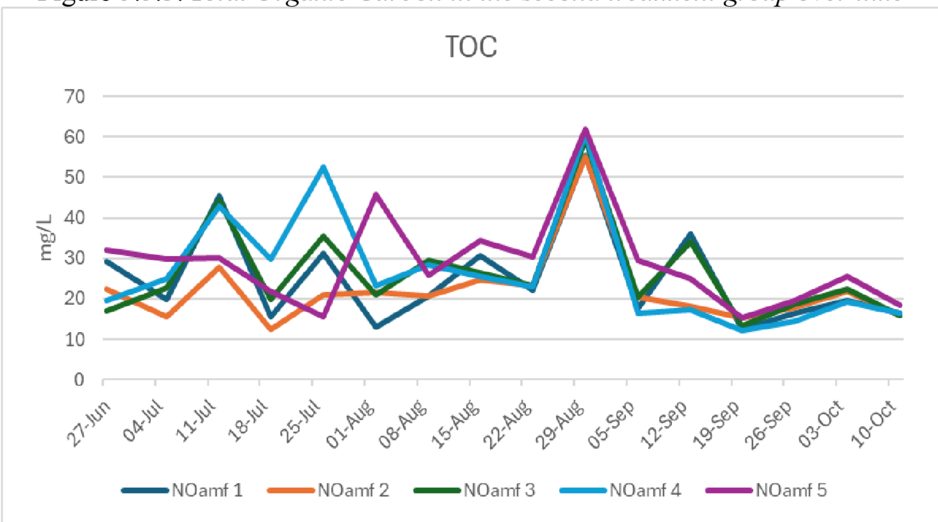

Figure 5.5.4. *Total Organic Carbon in the third treatment group over time*

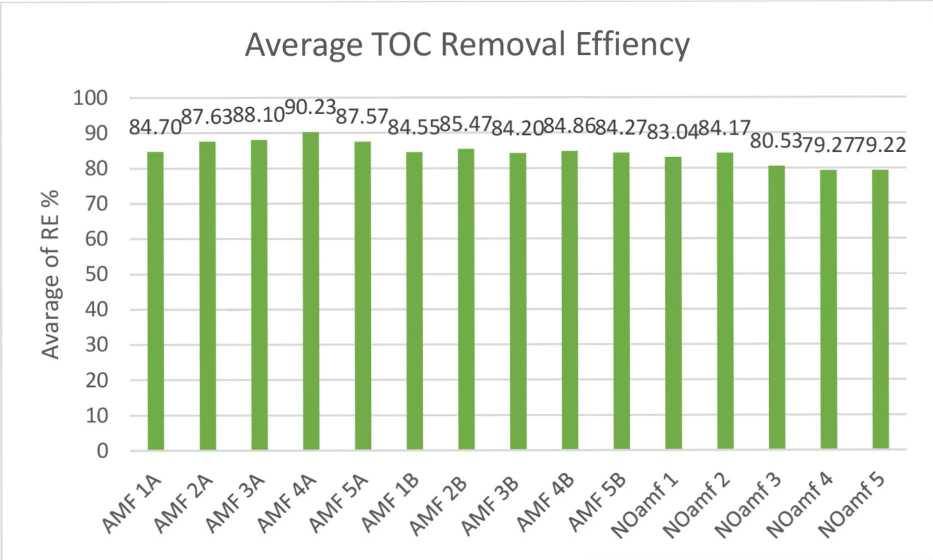

Figure 5.5.5 *Average of Total Organic Carbon Removal Efficiency*

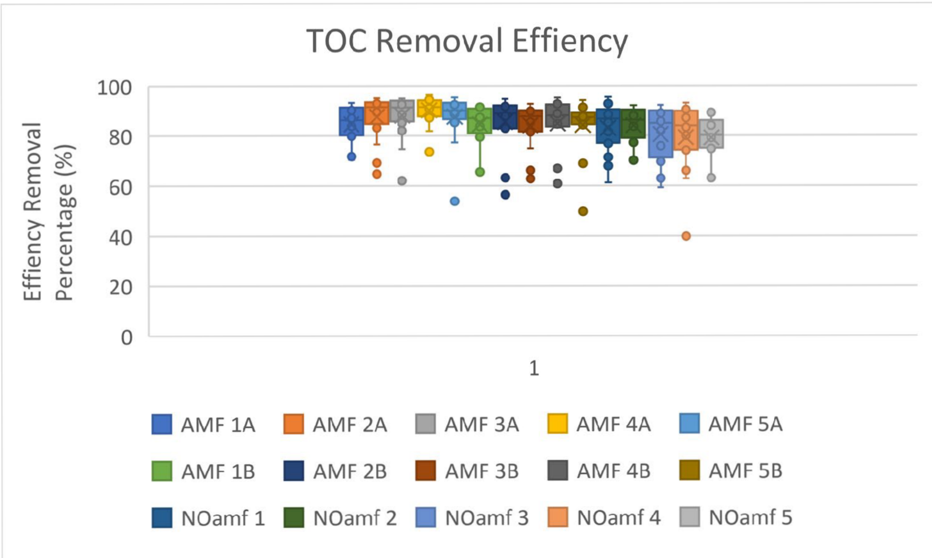

Figure 5.5.6 *Variation of Total Organic Carbon Removal Efficiency*

The reactors present consistent results disregarding the groups, but with some reactors having outliners. The minimum percentage of removal efficiency is 39.79% from the 4A AMF-, the maximum percentage of removal efficiency is 96.29% from the reactor 4A AMF+, and a mean percentage of removal efficiency is 84.52%. Comparing the effect of the different treatments in the groups, the P-Values prove that there is not a significant impact of the treatments in relation to the Total Organic Carbon removal efficiency (.

Table 5.5.1 p-values between the effect of the different treatments on the three groups	
A - AMF +	0.1399
B - AMF +	0.7943
A – AMF -	0.4163

In the case of the effect of the effect of the AMF it can be state that in the case of the presence of the AMF fungi does affect the removal efficiency of the Total Carbon when is related to the type of microplastic PE 2,5, and PES in both concentrations.

Table 5.4.2 p-values between the effect of the AMF treatments on the three groups

Group	Pvalue
Control	0.9155
PE0.25	0.0869
PE2.5	0.0253
PES 0.25	0.0058
PES 2.5	0.0014

5.6. Physical Observations

The descriptions below are for the physical observations that exalted from the reactors and were place during the experiment.

- The reactor 1A no AMF was presenting problems and needed to be replanted the 25.07.
- The reactor 4A no AMF and 5 no AMF started to present signs of algae in the top of the reactors from the week of 26.07 and started a process of stress.
- The reactor 5B AMF+ started to present signs of white fungus from the week of 12.09 and started a process of stress.
- The reactor 4B AMF+ started to present signs of white fungus from the week of 19.09 and started a process of stress.

6. Discussion

The use of constructed wetlands has been proven effective in the treatment of wastewater; we could see that from the results, the removal efficiency from some components were as expected but others not. In this chapter, is going to be discuss the results and try to give an explanation to them. It is important to state that in the first analysis we wanted to have the approach of the effect of microplastics in the reactors, and in the second step of this research the analysis was focus on the effect of the AMF Rhizophagus irregularis in relation to the treatments.

6.1. Ammonium (NH4+), Nitrite (NO2-), and Nitrate (NO3-)

As we can see in Figures N°5.2.1 , N°5.2.2 , N°5.2.3 and N°5.2.4 , we can appreciate that the inflow concentration of ammonium were lower than the outflows in all the treatments, so we cannot analyze the removal efficiency of ammonium in the system, instead, there is evidence of ammonium production. The production of ammonium is related to the process of ammonification and is a process that proceeds more rapidly than nitrification, and the rates depend on temperature, pH, C/N ratio, availability of nutrients, and soil conditions (Kadlec and Knight, 1996; Reddy and Patrick, 1984), also this can be related to the process of nitrate ammonification that transform the nitrates into ammonium. According to Vymazal (1995), the conditions of the reactors in terms of pH (6.5 – 8.5), as is visible in the results 5.12 pH, and Temperature (40° - 60°)were close to optimal.

In the case of nitrates, the figures N°5.2.4, N°5.2.5, N°5.2.6, and N°5.2.7 demonstrated that the concentrations of nitrates in the inflow were higher than the concentration of nitrates in the outflow. In this case, it can be stated that there is a process of nitrate removal from the system and the percentage removal efficiency is close to 100% in all the treatments, as is show in the figure N°5.2.8. This is particularly happening through the nitrogen removal process in wetlands as nitrate-ammonification and denitrification (Vymazal, 2007).

In the case of nitrites, the figures N°5.2.10, N°5.2.11, N°5.2.12, and N°5.12.13, put in evidence the production or the accumulation of nitrites in the system. The concentrations of nitrites in the inflows that were used for the wastewater simulation were lower than the concentrations of the outflows, and this can be explained by the process of nitrification and denitrification, where the ammonium is oxidized first in nitrites and then to nitrates, and in the case of denitrification, the processes involved the initial NO3 −-N reduction to NO2 −-N (Norton J. & Ouyang B., 2023, Xiong et al. 2011), specially due the high presence of ammonium on the systems. In the results shown by Zhang et al.(2011), in the process of nitrogen removal, there is an accumulation of nitrites in the system, especially in the spring and summer seasons, the time that this experiment was done.

For the Total Nitrogen Removal, it is important to state that the concentrations of TN in the reactors tended to increase over the experiment period in the groups A AMF+ and B AMF+, but not in the A AMF-, as is shown in the figures N°5.3.2, N°5.3.3 and N°5.3.4. This can be explained as the maturity of the plants of the reactor AMF+ reached earlier than AMF- which was corroborated by the size of the plant. Additionally the presence of the carbon sources can help for the process of denitrification enabling and enhancing the nitrogen removal process (Mateus & Pinho, 2020).

In the first analysis, the results of the P values demonstrated that the concentration of microplastics or either the type does not affect the capacity of nitrogen removal in the reactors, but this does not follow some literature indicating that microplastics can affect the removal mechanism because microplastics tend to affect the Plant nitrogen uptake, microbial growth community composition, and nitrogen-related enzyme (Zhang et al.,2024). But also depends on the load of microplastics, the size of the microplastic, the type of constructed wetland, and the type of sediment that has been use in the conformation of the wetland (Zhang et al.,2024).

6.2. Total Organic Carbon (TOC) and Total Carbon (TC)

As it has been stated before, the presence of carbon is important for the functioning of the constructed wetlands, especially in relation of the process that the Carbon is involve as the electron donor (Mateus & Pinho, 2020). In addition, Total carbon plays a multifaceted role in constructed wetlands, influencing processes such as organic matter decomposition, nutrient cycling, redox conditions, pH buffering, and carbon sequestration

Based on the results of Total Organic Carbon and Total Carbon, the amount of carbon change over time, but in different ratios. It could be observed that in the case of TC, the concentration of carbon in the outflow were much higher than the concentration of TOC, disregarding the treatments of microplastic or the treatment of AMF. This means that the constructed wetlands could process the organic carbon and using it as in the other process such as denitrification , as expected (Vymazal, 2007 & Ragab et al., 1994). and also in the process carbon dioxide or methane are produced, increasing the amount of inorganic carbon in the system that it can liberate in the form of gases. The figures of the section 5.4 and 5.5, showed that concentration levels of TC were much higher over the whole period and the RE percentage was around the 50%, meanwhile in TOC it was observed that the RE was around 84%.

6.3. Phosphates

In the case of the phosphates, it can be seen that inflows in the figure 5.2.14 were higher than the outflows Figures 5.2.15, 5.2.16, 5.2.17 in that sense there is a phosphorus removal process in which and according to the results presented in the figure 5.2.18 the mean of the removal efficiency is 54%. These results match the some bibliographic information where vertical constructed wetlands have a similar percentage of phosphorus removal 59% mostly through the mechanism of adsorption (Vymazal, 2007).

6.4. Microplastic

As it was explained before, the analysis that was divided into one control group (no microplastic), two types of microplastic and different loads. So, in this section we will analyze the results that showed removal efficiency as nitrates, TN, TOC, TC and phosphate.

- Nitrates

In this case we did the analysis of the removal efficiency of nitrates in the 15 reactors, where we could observed in the graphics, and after with the statistical analysis that the influence of microplastic PE and in the loads of 0.25 and 2.5 mg did not affect the capacity of the reactors on the removal process.

- Total Nitrogen

In the Total Nitrogen analysis, it was important to acknowledge that the groups A AMF+ and B-AMF+, they represented the same conditions. In this two groups, the removal efficiency performance didn't show any significant variability in the sense of the microplastic treatment. This can be supported by the fact that the presence of AMF was enhancing the performance of system and the ability to remove this compound from it. But in the case of the group A AMF- the statistical analysis shown in the Table 5.3.1, demonstrate that there are significant variations in the performance of removal efficiency in the relation to the type of microplastic and the load.

- Total Carbon and Total Organic Carbon

Total Carbon and Total Organic Carbon analysis showed us that the performance of the removal efficiency due the concentration of microplastic and type of microplastic didn't have any significant effect. Meaning that the process that are related to removal process are not connected to these types of treatment or has insignificant impact in the concentration. The literature indicate that this also can be related to low concentration of microplastics that it was added over the period of time, because the microplastic indeed can affect the capacity of removal of the constructed wetland due the capacity of microplastics can serve as surfaces for the adsorption of dissolved organic carbon (DOC) and other carbon-containing compounds. (Muñoz et al., 2021).

- Phosphates

In the analysis of the phosphates of the outflows, we could observe that neither the loads nor the type of microplastic have a significant impact on the removal process of the phosphates in the three groups. The literature indicates that mostly the effects of microplastic over the phosphorus removal process are related to adsorption and transport, where the microplastic sequesters the phosphate ions and then transports them into the effluents. In our case, the vertical systems mostly retain the microplastics in the sediment that was used in the reactor.

6.5. AMF

During this experiment, it was noted the differences between the groups that had the presence of AMF and the group that had the absence of AMF, especially when it was related to the concentrations of ammonium and the concentration and removal efficiency of Total Nitrogen.

- Ammonium

Due to the incapacity of analyzing ammonium removal efficiency, this analysis will be focused only on the difference in concentrations of ammonium in the three groups. In this case particular case, we can observe in Figures 5.2.2, 5.2.3, and 5.2.4 where the concentrations of ammonium in the systems are shown. We can state that in the groups that had the presence of AMF (A-AMF+ and B-AMF+), the concentration of ammonium was considerably less than the one that didn't have AMF (A-AMF-). This can explained as part of the processes that are related to ammonium removal being connected to the ability of the AMF to enhance the capacity of the plant to uptake nutrients, increase root exudates, and indirect effects of microbial communities. In the experiment carry by Zheng et al. (2023), the results in ammonium removal had the same outcome regarding the presence and absence of AMF.

- Total Nitrogen

In the analysis of removal efficiency done in Total Nitrogen, we could observe differences between the three groups, but between the groups of AMF + and AMF- the differences were statistically significant as was reflected in the P values of the treatments in Table 5.3.2, this can be explained in the way that the effect that has the AMF in influencing the capacity of the whole system is significant, AMF does not give a direct benefit but is known to extend the root networks of plant and this also beneficial to the uptake of NH4 +-N, as well as for the assimilation activities of plants and microorganisms (Xiong et al, 2011).

7. Conclusion

Constructed wetlands are a sustainable, efficient, and low-cost systems that can solve the problem of wastewater treatment in low-density communities, with the ability to regulate nutrients from the wastewater by including the cycles of carbon, nitrogen, and phosphorus to clean the effluents. Nowadays the abundance of microplastic in almost all ecosystems presents a potential hazard to the well-being of humans and all ecosystems. In this research, we studied the effect of microplastic in constructed wetlands to evaluate their capacity and resilience under this threat and how it could affect their capacity for water treatment. Also, it was important to investigate the influence of the AMF over the microplastic treatment.

After several analyses, this experiment concluded that the impact of the microplastic treatments on TOC, TC, nitrate, and phosphate does not influence the nutrient removal capacity of the constructed wetlands. In the particular case of TN, we can conclude that the removal efficiency can be affected when the condition of no presence of AMF is presented so for that generating possible health problem. The literature indicates that microplastics can affect all the nutrient cycles in constructed wetlands that were analyzed in this research. The results of our experiments could establish a baseline to analyze the loads and time to demonstrate the impact of microplastic in this particular type of constructed wetlands.

In the analysis realized to determine the influence of AMF in the microplastic treatments, it could be concluded that for the Phosphate, TOC, TC and TN the removal efficiency is significantly influenced by the presence of the AMF.

8. References

Rodríguez & P. J.C Harris. (2013). The Ecology of Arbuscular Mycorrhizal Fungi, Critical Reviews in Plant Sciences, 32:1, 1-20, DOI: 10.1080/07352689.2012.683375

Agnieszka, Karczmarczyk & Anna, Baryła. (2013). Cold season temperature pattern over a Horizontal subsurface flow constructed wetland - Application of thermography in environmental research. Fresenius Environmental Bulletin. 22. 3694-3699.

Amils, R. (2011). Chemolithoautotroph. In: Gargaud, M., et al. Encyclopedia of Astrobiology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-11274-4_272

Anjali, G., & Sabumon, P. (2014). Unprecedented development of anammox in presence of organic carbon using seed biomass from a tannery Common Effluent Treatment Plant (CETP). Bioresource Technology, 153, 30-38. https://doi.org/10.1016/j.biortech.2013.11.061

Balk M, Laverman AM, Keuskamp JA and Laanbroek HJ (2015) Nitrate ammonification in mangrove soils: a hidden source of nitrite? Front. Microbiol. 6:166. doi: 10.3389/fmicb.2015.00166

Barbera, A. C., Borin, M., Ioppolo, A., Cirelli, G. L., & Maucieri, C. (2014). Carbon dioxide emissions from horizontal sub-surface constructed wetlands in the Mediterranean Basin. Ecological Engineering, 64, 57-61. https://doi.org/10.1016/j.ecoleng.2013.12.032

Beebe, D. A., Castle, J. W., Molz, F., & Rodgers, J. H. (2014). Effects of evapotranspiration on treatment performance in constructed wetlands: Experimental studies and modeling. Ecological Engineering, 71, 394-400. https://doi.org/10.1016/j.ecoleng.2014.07.052

Belmont, Marco & Metcalfe, Chris. (2003). Feasibility of using ornamental plants (Zantedeschia aethiopica) in subsurface flow treatment wetlands to remove nitrogen, chemical oxygen demand and nonylphenol ethoxylate surfactants - A laboratory-scale study. Ecological Engineering. 21. 233-247. 10.1016/j.ecoleng.2003.10.003.

Bernal, B., & Mitsch, W. J. (2012). Comparing carbon sequestration in temperate freshwater wetland communities. Global Change Biology, 18(5), 1636-1647. https://doi.org/10.1111/j.1365-2486.2011.02619.x

Brix, H. (1996). Do macrophytes play a role in constructed treatment wetlands. Water Science and Technology, 35(5),11-17.https://doi.org/10.1016/S0273-1223(97)00047-4

Carlo Giacomo Avio, Stefania Gorbi, Francesco Regoli, Plastics and microplastics in the oceans: From emerging pollutants to emerged threat, Marine Environmental Research, Volume 128, 2017, Pages 2-11, ISSN 0141-1136, https://doi.org/10.1016/j.marenvres.2016.05.012.

Di Luca, G., Maine, M., Mufarrege, M., Hadad, H., Pedro, M., Sánchez, G., & Caffaratti, S. (2017). Phosphorus distribution pattern in sediments of natural and constructed wetlands. Ecological Engineering, 108, 227-233. https://doi.org/10.1016/j.ecoleng.2017.08.038

Ding, X., Xue, Y., Zhao, Y., Xiao, W., Liu, Y., & Liu, J. (2018). Effects of different covering systems and carbon nitrogen ratios on nitrogen removal in surface flow constructed wetlands. Journal of Cleaner Production, 172, 541-551. https://doi.org/10.1016/j.jclepro.2017.10.170

Espinosa C, Esteban MÁ, Cuesta A (2016) Microplastics in aquatic environments and their toxicological implications for fish. https://www.intechopen.com/chapter/pdf-download/52031/7357120

Fletcher, T. D., & Sun, G. (2009). Nitrogen removal in constructed wetland systems. Engineering in Life Sciences, 9(1), 11-22. https://doi.org/10.1002/elsc.200800049

Gan, Q., Cui, J., & Jin, B. (2023). Environmental microplastics: Classification, sources, fates, and effects on plants. Chemosphere, 313, 137559. https://doi.org/10.1016/j.chemosphere.2022.137559

Gosling, P., Hodge, A., Goodlass, G., & Bending, G. (2006). Arbuscular mycorrhizal fungi and organic farming. Agriculture, Ecosystems & Environment, 113(1-4), 17-35. https://doi.org/10.1016/j.agee.2005.09.009

Gu, X., He, S., & Huang, J. (2021). Efficient utilization of Iris pseudacorus biomass for nitrogen removal in constructed wetlands: Combining alkali treatment. Environmental Pollution, 291, 118170. https://doi.org/10.1016/j.envpol.2021.118170

Guo, J., Huang, X., Xiang, L., Wang, Y., Li, Y., Li, H., Cai, Q., Mo, C., & Wong, M. (2020). Source, migration and toxicology of microplastics in soil. Environment International, 137, 105263. https://doi.org/10.1016/j.envint.2019.105263

Haberl, R., Perfler, R., & Mayer, H. (1994). Constructed wetlands in Europe. Water Science and Technology, 32(3), 305-315. https://doi.org/10.1016/0273-1223(95)00631-1

Hassan, I., Chowdhury, S. R., Prihartato, P. K., & Razzak, S. A. (2021). Wastewater Treatment Using Constructed Wetland: Current Trends and Future Potential. Processes, 9(11), 1917. https://doi.org/10.3390/pr9111917

"Homin Kye, Jiyoon Kim, Seonghyeon Ju, Junho Lee, Chaehwi Lim, Yeojoon Yoon, Microplastics in water systems: A review of their impacts on the environment and their potential hazards, Heliyon, Volume 9, Issue 3, 2023, e14359, ISSN 2405-8440, https://doi.org/10.1016/j.heliyon.2023.e14359."

Hu, B., Hu, S., Vymazal, J., & Chen, Z. (2021). Arbuscular mycorrhizal symbiosis in constructed wetlands with different substrates: Effects on the phytoremediation of ibuprofen and diclofenac. Journal of Environmental Management, 296, 113217. https://doi.org/10.1016/j.jenvman.2021.113217

Issac, M.N., Kandasubramanian, B.(2021) Effect of microplastics in water and aquatic systems. Environ Sci Pollut Res 28, 19544–19562 . https://doi.org/10.1007/s11356-021-13184-2

Jakubaszek, A. (2021). Nitrogen and Phosphorus Accumulation in Horizontal Subsurface Flow Constructed Wetland. Agronomy, 11(7), 1317. https://doi.org/10.3390/agronomy11071317

Kalengo, L., Ge, H., Liu, N., Wang, Z. (2021). The Efficiency of Aquatic Macrophytes on the Nitrogen and Phosphorous Uptake from Pond Effluents in Different Seasons. Journal of Ecological Engineering, 22(8), 75-85. https://doi.org/10.12911/22998993/140308

Kochi, L.Y.; Freitas, P.L.; Maranho, L.T.; Juneau, P.; Gomes, M.P. Aquatic Macrophytes in Constructed Wetlands: A Fight against Water Pollution. Sustainability 2020, 12, 9202. https://doi.org/10.3390/su12219202

Kumari, P., Singh, A., & Kharwar, R. N. (2020). Phytostimulation and ISR responses of fungi. Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-Technology, 459-473. https://doi.org/10.1016/B978-0-12-821394-0.00018-4

Laanbroek, H. (1990). Bacterial cycling of minerals that affect plant growth in waterlogged soils: A review. Aquatic Botany, 38(1), 109-125. https://doi.org/10.1016/0304-3770(90)90101-P

Lee, C.-g., Fletcher, T.D. and Sun, G. (2009), Nitrogen removal in constructed wetland systems. Eng. Life Sci., 9: 11-22. https://doi.org/10.1002/elsc.200800049

Lehmann, A., Leifheit, E. F., Gerdawischke, M., & Rillig, M. C. (2021). Microplastics have shape- and polymer-dependent effects on soil aggregation and organic matter loss – an experimental and meta-analytical approach. Microplastics and Nanoplastics, 1(1), 1-14. https://doi.org/10.1186/s43591-021-00007-x

Li, L., Zhou, Q., Yin, N., Tu, C., Luo, Y., (2019). Uptake and accumulation of microplastics in an edible plant. Chin. Sci. Bull. 64, 928–934. https://doi.org/10.1360/n972018-00845.

Li, R., Zhou, Q., Li, Y., Luo, Y., Yang, J., Tu, C., et al., 2020d. Uptake and accumulation of microplastics in a cereal plant wheat. Chin. Sci. Bull. 65, 2120–2127. https://doi.org/10.1360/tb-2020-0030.

Li, Y., Li, H., Xu, X., Xiao, S., Wang, S., & Xu, S. (2017). Fate of nitrogen in subsurface infiltration system for treating secondary effluent. Water Science and Engineering, 10(3), 217-224. https://doi.org/10.1016/j.wse.2017.10.002

Meng, P., Pei, H., Hu, W., Shao, Y., & Li, Z. (2014). How to increase microbial degradation in constructed wetlands: Influencing factors and improvement measures. Bioresource Technology, 157, 316-326. https://doi.org/10.1016/j.biortech.2014.01.095

Munoz, M., Ortiz, D., Nieto-Sandoval, J., De Pedro, Z. M., & Casas, J. A. (2021). Adsorption of micropollutants onto realistic microplastics: Role of microplastic nature, size, age, and NOM fouling. Chemosphere, 283, 131085. https://doi.org/10.1016/j.chemosphere.2021.131085

National Oceanic and Atmospheric Administration (2023) -

Negi, D., Verma, S., Singh, S., Daverey, A., & Lin, J. (2022). Nitrogen removal via anammox process in constructed wetland – A comprehensive review. Chemical Engineering Journal, 437, 135434. https://doi.org/10.1016/j.cej.2022.135434

Nopens, I., Capalozza, C., & Vanrolleghem, P. A. (2001). Stability analysis of a synthetic municipal wastewater. *Department of Applied Mathematics Biometrics and Process Control, University of Gent, Belgium*.

Norton, J. M., & Ouyang, Y. (2022). Nitrification: Process, organisms and management. Encyclopedia of Soils in the Environment (Second Edition), 253-260. https://doi.org/10.1016/B978-0-12-822974-3.00252-4

P. Aronsson et K. Perttu. (2001). Willow vegetation filters for wastewater treatment and soil remediation combined with biomass production, FOREST CHRO, 77(2), pp. 293-299

P. Gui, R. Inamori, M. Matsumura, Y. Inamori (2007). Evaluation of constructed wetlands by wastewater purification ability and greenhouse gas emissions. Water Sci Technol 1; 56 (3): 49–55. doi: https://doi.org/10.2166/wst.2007.517

Parde, D., Patwa, A., Shukla, A., Vijay, R., Killedar, D. J., & Kumar, R. (2021). A review of constructed wetland on type, treatment and technology of wastewater. Environmental Technology & Innovation, 21, 101261. https://doi.org/10.1016/j.eti.2020.101261

Picek, T., Čížková, H., & Dušek, J. (2007). Greenhouse gas emissions from a constructed wetland—Plants as important sources of carbon. Ecological Engineering, 31(2), 98-106. https://doi.org/10.1016/j.ecoleng.2007.06.008

Ragab, M., Mohamed, S., Mehana, T. et al. Denitrification and nitrogen immobilization as affected by organic matter and different forms of nitrogen added to an anaerobic water-sediment system. Biol Fertil Soils 17, 219–224 (1994). https://doi.org/10.1007/BF0033632

Richard C. Thompson , Ylva Olsen, Richard P. Mitchell, Anthony Davis, Steven J. Rowland, Anthony W. G. John, Daniel Mcgonigle, and Andrea E. Russell (2004). Lost at Sea: Where Is All the Plastic?

Richardson, C.J., Qian, S., Craft, C.B. et al. (1996). Predictive models for phosphorus retention in wetlands. Wetlands Ecol Manage 4, 159–175. https://doi.org/10.1007/BF01879235

"Ryan Peter G., Moore Charles J., van Franeker Jan A. and Moloney Coleen L. (2009) Monitoring the abundance of plastic debris in the marine environmentPhil. Trans. R. Soc. B3641999–2012

http://doi.org/10.1098/rstb.2008.0207"

Sandenbergh, E., Petruzzella, A., & Coetzee, J. A. (2024). Distribution and sexual reproductive potential of Iris pseudacorus L. (Iridaceae) in South Africa. South African Journal of Botany, 167, 31-39. https://doi.org/10.1016/j.sajb.2024.01.067

Shelef, O., Gross, A., & Rachmilevitch, S. (2013). Role of Plants in a Constructed Wetland: Current and New Perspectives. Water, 5(2), 405-419. https://doi.org/10.3390/w5020405

Shukla, A., Parde, D., Gupta, V. et al.(2022). A review on effective design processes of constructed wetlands. Int. J. Environ. Sci. Technol. 19, 12749–12774 . https://doi.org/10.1007/s13762-021-03549-y

Stottmeister, U., Wießner, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O., Müller, R., & Moormann, H. (2003). Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnology Advances, 22(1-2), 93-117. https://doi.org/10.1016/j.biotechadv.2003.08.010

Stottmeister, U., Wießner, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O., Müller, R., & Moormann, H. (2003). Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnology Advances, 22(1-2), 93-117. https://doi.org/10.1016/j.biotechadv.2003.08.010

Sudip Ray, Ralph P. Cooney (2018). Chapter 9 - Thermal Degradation of Polymer and Polymer Compositeshttps://doi.org/10.1016/B978-0-323-52472-8.00009-5

Sun, X., Yuan, X., Jia, Y., Feng, L., Zhu, F., Dong, S., Liu, J., Kong, X., Tian, H., Duan, J., Ding, Z., Wang, S., & Xing, B. (2020). Differentially charged nanoplastics demonstrate distinct accumulation in Arabidopsis thaliana. Nature Nanotechnology, 15(9), 755-760. https://doi.org/10.1038/s41565-020-0707-4

Sutherland, W. J. (1990). Iris Pseudacorus L. Journal of Ecology, 78(3), 833–848. https://doi.org/10.2307/2260902

Tanner, C. C., & Kadlec, R. H. (2013). Influence of hydrological regime on wetland attenuation of diffuse agricultural nitrate losses. Ecological Engineering, 56, 79-88. https://doi.org/10.1016/j.ecoleng.2012.08.043

Taylor, S.E., Pearce, C.I., Sanguinet, K.A., Hu, D., Chrisler, W.B., Kim, Y.-M., et al., (2020). Polystyrene nano- and microplastic accumulation at Arabidopsis and wheat root cap cells, but no evidence for uptake into roots. Environ. Sci-Nano. 7, 1942–1953. https://doi.org/10.1039/d0en00309c.

Truu, M., Juhanson, J., & Truu, J. (2009). Microbial biomass, activity and community composition in constructed wetlands. Science of The Total Environment, 407(13), 3958-3971. https://doi.org/10.1016/j.scitotenv.2008.11.036

Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. Science of The Total Environment, 380(1-3), 48-65. https://doi.org/10.1016/j.scitotenv.2006.09.014

Vymazal, J. (2010). Constructed Wetlands for Wastewater Treatment. Water, 2(3), 530-549. https://doi.org/10.3390/w2030530

Vymazal, J. (2011). Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia 674, 133–156. https://doi.org/10.1007/s10750-011-0738-9

Vymazal, J. (2023). Thirty years of constructed wetlands for municipal wastewater treatment in the Czech Republic. Ecological Engineering, 194, 107054. https://doi.org/10.1016/j.ecoleng.2023.107054

Vymazal, J.(2022) The Historical Development of Constructed Wetlands for Wastewater Treatment. Land 2022, 11, 174. https://doi.org/10.3390/land11020174

Wang, F., Wang, Q., Adams, C. A., Sun, Y., & Zhang, S. (2022). Effects of microplastics on soil properties: Current knowledge and future perspectives. Journal of Hazardous Materials, 424, 127531. https://doi.org/10.1016/j.jhazmat.2021.127531

Wood, A. (1994). Constructed wetlands in water pollution control: Fundamentals to their understanding. Water Science and Technology, 32(3), 21-29. https://doi.org/10.1016/0273-1223(95)00601-X

Wu, H., Zhang, J., Ngo, H. H., Guo, W., Hu, Z., Liang, S., Fan, J., & Liu, H. (2014). A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation. Bioresource Technology, 175, 594-601. https://doi.org/10.1016/j.biortech.2014.10.068

XU, Z., BAN, Y., JIANG, Y., ZHANG, X., & LIU, X. (2016). Arbuscular Mycorrhizal Fungi in Wetland Habitats and Their Application in Constructed Wetland: A Review. Pedosphere, 26(5), 592-617. https://doi.org/10.1016/S1002-0160(15)60067-4

Yousefi, Z., & Mohseni-Bandpei, A. (2010). Nitrogen and phosphorus removal from wastewater by subsurface wetlands planted with Iris pseudacorus. Ecological Engineering, 36(6), 777-782. https://doi.org/10.1016/j.ecoleng.2010.02.002

Zhang, L., Marshall, M., Vrieling, A., & Nelson, A. (2023). The divergence of energy- and water-balance evapotranspiration estimates in humid regions. Journal of Hydrology, 624, 129971. https://doi.org/10.1016/j.jhydrol.2023.129971

Zhang, L., Xia, X., Zhao, Y., Xi, B., Yan, Y., Guo, X., Xiong, Y., & Zhan, J. (2011). The ammonium nitrogen oxidation process in horizontal subsurface flow constructed wetlands. Ecological Engineering, 37(11), 1614-1619. https://doi.org/10.1016/j.ecoleng.2011.06.020

Zhang, S., Shen, C., Zhang, F., Wei, K., Shan, S., Zhao, Y., Man, Y. B., Wong, M. H., & Zhang, J. (2024). Microplastics removal mechanisms in constructed wetlands and their impacts on nutrient (nitrogen, phosphorus and carbon) removal: A critical review. Science of The Total Environment, 918, 170654. https://doi.org/10.1016/j.scitotenv.2024.170654

Zhang, Yang & Chen, Z.-H & Zhang, Zhenhua & Zhou, X.-Y & Tu, Y.. (2011). Ornamental plants in constructed wetlands. Ornamental Plants: Types, Cultivation and Nutrition. 81-96.

Zheng, X., Liu, Q., Chen, X., Cao, M., Wu, F., Li, W., Zhang, L., Liu, S., & Jiang, J. (2023). Arbuscular mycorrhizal fungi decrease soil ammonium availability and nitrous oxide emissions under nitrogen input. Agricultural and Forest Meteorology, 333, 109385. https://doi.org/10.1016/j.agrformet.2023.109385

Zhou, Y., Zhu, Y., Zhu, J., Li, C., & Chen, G. (2023). A Comprehensive Review on Wastewater Nitrogen Removal and Its Recovery Processes. International Journal of Environmental Research and Public Health, 20(4). https://doi.org/10.3390/ijerph20043429