

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences



Effect of agricultural runoff treatment with different C/N  
ratios in continuous flow floating treatment wetlands

## Diploma Thesis

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# CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

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## DIPLOMA THESIS ASSIGNMENT

Bc. Filip Bokros

Landscape Planning

Thesis title

**Effect of agricultural runoff treatment with different C/N ratios in continuous flow floating treatment wetlands**

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### Objectives of thesis

1. Evaluate the removal performance of continuous flow FTWs for treating agricultural runoff under different C/N ratio.
2. Investigate the changes in plants under the stress of different C/N ratios and operation regimes.

### Methodology

#### Experimental setup

This study will be conducted using 6 FTWs at the Czech University of Life Sciences Prague. The hydroponic systems will have internal recirculation units, then planted *Iris pseudacorus*. The FTWs will be protected from rain throughout the experiment.

#### Operation of experimental systems

This experiment will simulate the intake of FTWs by peristaltic pumps. The hydraulic retention time (HRT) is 7 days. The influent C/N ratio is set at 1:5, 1:1, 5:1. Experiments were conducted using simulated wastewater.

#### Water sample analysis

Inflow and outflow water samples in the 6 FTWs will be taken every 7 days; pH, ORP, and DO in inflow and outflow will be monitored by using HQD Field Case (HACH), TOC and TN in outflow will be monitored by FormacsSERIES Total Organic Carbon (TOC)/Total Nitrogen analyzers,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  concentrations will be determined by 883 Basic IC plus. Biomass in shoots and roots will be measured by the gravimetric method. All photochemical efficiency measurements will be conducted every week by PAM 2500. Wetland plant samples and substrates in FTWs will be analyzed after the experiment. Plant shoots and roots will be harvested separately; the height and fresh weight will be measured afterward, and then washed carefully with deionized water for further analysis.



## The proposed extent of the thesis

50

### Keywords

Agricultural runoff; C/N ratios; floating treatment wetlands; flow regime

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1906

## **Recognition:**

I'd want to express my gratitude to the doc. Dr. Ing. Zhongbing Chen for his remarks on the completed work once it was submitted. My gratitude also goes to Ph.D. applicant Yingrun Chen for his valuable guidance, willingness, and patience during the preparation of this thesis.

## **Declaration:**

*I hereby declare that I have independently elaborated the bachelor/final thesis with the topic of: The effect of nitrogen source on phytoremediation of heavy metals in plant-sand systems and that I have cited all of the information sources that I used in the thesis as listed at the end of the thesis in the list of used information sources.*

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## **Abstract:**

Floating mats are a widespread phenomenon in both temperate and (sub)tropical wetlands across the world. The most sophisticated and model versions of constructed wetlands (CWs) are floating treatment wetlands (FTWs). Other names for FTWs include planted floating system beds, artificial or vegetated floating beds, and constructed floating wetlands (CFWs).

The roots, rhizomes, leaves, and shoots of hydrophytes that develop as a floating mat in FTWs serve a critical role in wastewater treatment. They can accumulate nutrients (such as N and P) and insecticides, as well as create conditions for biological decomposition of organic waste. Although the use of phytoremediation to treat agricultural runoff is becoming increasingly popular, it has rarely been explored if the manner of water intake impacts the efficacy of pollutant removal.

From the experiment outcome we found out that pots with concentration of C/N 1:5 have the highest removal efficiency of TN, TOC, IC and TC. Consequently, following the C/N 1:1 and 5:1 respectively. Continuous flow wetland systems show higher ability to increase removal performance of the system.

The results of this study help to clarify how well FTWs work to reduce pollution from agricultural runoff at different nutrient levels. Moreover, the knowledge acquired from this study might help optimize FTW operation and design to improve their efficacy in managing agricultural runoff and safeguarding aquatic environments. This knowledge is essential for creating long-term plans for managing agricultural water resources and enhancing the quality of the water in affected areas.

## **Abbreviations:**

ANPSP - nonpoint source pollution

ARBs – Antibiotics resistant to bacteria

BOD – Biochemical oxygen demand

CMs – carbamates

CWs – Constructed Wetlands

DDT - dichloro diphenyl trichloroethane

ETR – Electron transport rate

FAO - Food and Agriculture Organization of the United Nations

FTWs – Floating Treatment Wetlands

HFBs – Horizontal flow beds

HRMs - Hydroponic root mats

HSSFCW - horizontal sub-surface flow CW

OPs – Organophosphates

POPs – Persistent Organic pollutants

USDA - U.S. Department of Agriculture

VFBs – Vertical flow beds

WWTPs – Waste water treatment plants



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# 1. Introduction

Various pollutants have been causing environmental pollution for a number of decades, dating back to the periods of industrialization, urbanization, raw material consumption, mining, metal production, and fabric manufacturing. Although all of these processes have raised our quality of life, they have also somewhat degraded the environment in which we live. Every interaction they have with living things has negative toxicological effects and places some strain on the ecosystem. They spread gradually to people through industry, food, water, and medications. Studies indicate a rise in the concentration of heavy metals in human bodies over the past fifty years, attributed to a greater utilization of products containing pollutants. (Kizeková et al. 2017) The growing amount of foreign and toxic substances in the environment is making people reevaluate their behavior in an effort to reduce risk and avoid the threat that their careless choices posed. In recent decades, an increasing number of dangerous substances have been discharged into the environment, impacting all terrestrial life forms, including humans. (Auvinen, Du, Meers, 2016)

Excessive levels of many contaminants, including nutrients, pathogens, pesticides, veterinary medications, sediments, and metals, are present in agricultural runoff. Agrochemicals, which offer a higher yield in a shorter amount of time, such pesticides, herbicides, and hormones, are frequently utilized in modern agriculture. The intensity of agricultural practices and the production of feed for livestock has grown along with the demand for food. Consequently, the use of pesticides and inorganic fertilizers in agriculture has increased in recent years. Herbicide usage was 43.3% of the time for North American farmers in 1993, compared to 26.3% for European farmers, according to Carvalho and colleagues (1997).

Most kinds of green plant have the ability to absorb harmful (contaminating) and toxic compounds from the air, water, soil, and wetlands. They eventually engage in the phytoremediation process, which involves the removal of potentially harmful substances from the environment through fixation, accumulation, and eventual breakdown, with the aid of specific bacteria. The upper green plants are referred to as the "green liver" of nature for a reason. This title is based not only upon their capacity to break down and metabolize a wide range of contaminants, but also upon the ability to accumulate harmful matter in significant amounts within their tissues (Pauková et al., 2020).

We currently have a number of methods for addressing the environmental problem of pollutant removal, but in order to get the best results, the procedure must be either highly

expensive or very complex. Contrarily, phytoremediation is less expensive and environmentally more approachable than other technological approaches and methods for removing harmful elements and pollutants from contaminated soil, which allows for its application in a far wider range of conditions (Tangahu et al., 2011)

Popular sustainable management technique for managing different wastewaters are constructed wetlands. A thorough and critical review on the removal of pollutants other than nutrients that occur in agricultural field runoff and wastewater from animal facilities is currently lacking, despite a number of articles on the subject. A study was conducted on the presence of contaminants in agricultural runoff water, how different wetlands remove pollutants (intermittent flow, continuous flow), how the removal processes work, and what influences the removal process.

These compounds are ideal for raising production, but when they get out of agricultural ecosystems in storm-related runoff water, they pose a threat to the environment. According to studies, only 1% of pesticides used on crops are really effective; the remaining 99% contaminate soils, waterways, and the environment through non-targeted pollution. Animal manure used in livestock production can serve as an ideal environment for bacteria and genes resistant to antibiotics (ARBs). According to another study, there is a greater likelihood of medicinal products for animals finding their way to water resources through agricultural runoff from soil. These compounds have the potential to have negative long-term impacts on agricultural sustainability and food security. (Tank, Wood, Smith, 2021)

A number of advantageous characteristics, including wastewater remediation under various circumstances, ecosystem quality preservation, landscape protection, and aesthetic advantages, are contributing to the growing popularity of floating treatment wetlands (FTWs). FTW is a phyto-technology in which macrophytes use a variety of physicochemical and biological processes to eliminate pollutants from the environment while growing on a floating raft with their roots always in touch with water.

Pallis originally described the floating wetlands, also known as "plavs," from the Romanian Danube delta in 1915. In 1986, Arcata, California utilized the floating wetlands for the first time to perform tertiary treatment of wastewater. Van Oostrom and Russell (1992) provided information about FTW use in New Zealand during the early 1990s. The more advanced and model types of constructed wetlands (CWs) are called floating treatment wetlands (FTWs). Other names for FTWs include artificial or vegetated floating beds, constructed floating wetlands, and planting floating system beds. The fundamental principle of FTWs is the ability

of plants to quickly and efficiently remove pollutants from water bodies by means of their self-cleaning capabilities. Since FTWs also use interactions between water, microorganisms, plant parts, algae, and pollutants to remove such contaminants from water, they function similarly to CWs. The roots, rhizomes, leaves, and shoots of hydrophytes, which grow as a floating mat in FTWs, are essential to the treatment of wastewater because they accumulate nutrients (such as N and P) and heavy metals and create the conditions necessary for the biological degradation of organic wastes. (Sharma, Vymazal, Malaviya, 2021)

## **1.1 Agricultural Pollution**

Agricultural production processes that release livestock and poultry manure, use pesticides and fertilizers, and discharge agricultural waste are the main causes of agricultural tridimensional pollution. These pollutants can be transferred and transformed in the hydrosphere, soil sphere, biosphere, and atmosphere, resulting in cross-pollution between these four spheres. (Hang, Yang, 2024) A major environmental fear worldwide is agricultural runoff, or the flow of polluted water from agricultural fields into neighboring waterbodies. Despite the negative impact on the environment, agricultural production methods are the main means of obtaining food security and feeding the world's population of around 7.8 billion people with food and fibre. Approximately 37% of the earth's surface is utilized for agricultural activities. Less arable land forces countries to increase their usage of pesticides and fertilizers in their agriculture practices. During storms and runoff, the residues of these pesticides and fertilizers may be carried as NPS (Non-Point Sources) in the form of dissolved or particulate matter. The rise of hypoxic zones in recent years has drawn a lot of attention to this kind of pollution. The resulting water pollution continues to be a major worldwide issue, contributing to issues with human health, eutrophication of fresh and marine waters, and increased treatment costs for the supply of water. Water pollution is largely caused by the use of agriculture, which requires almost 70% of the world's water resources (Pericherla, Karnena, Vara, 2020).

The complex composition of pollutants found in agricultural runoff includes heavy metals, nitrates, ammonium, phosphorus compounds, and persistent organic pollutants. As the primary affecting nutrient during eutrophication, N and P, which are necessary components of amino acids and genetic material, respectively, are critical to the growth of aquatic plants. Since anthropogenic eutrophication is widely acknowledged to have significant potential to affect the



safety and well-being of aquatic ecosystems worldwide, it has emerged as the main issue. Millions of people could be impacted by a problem if the water supply plant closes due of the huge "cyanobacteria mat." Simultaneously, ongoing agricultural runoffs containing heavy metals and persistent organic pollutants (POPs) can simply build up in organisms to cause a variety of health hazards (such as contaminated drinking water). Hence, it is essential to significantly reduce non-point source pollution from agriculture in order to manage eutrophication in rivers and lakes, protect the aquatic ecosystem, and ensure the quality of drinking water. The fertilizers that are most commonly used worldwide are N and P. The Food and Agriculture Organization of the United Nations (FAO) released statistics showing that, as of 2015, the average global usage of N fertilizer per farmland area was 68.6 kg/ha, while the average use of P fertilizer was 30.1 kg/ha. The United States of America continues to use more fertilizer. Furthermore, China is the world's biggest manufacturer and user of fertilizers (Xia, Zhang, Tsang, 2020).

Ultimately, less than 15% of pesticides that are distributed actually reach their intended targets, which causes these toxins to unintentionally spread to other environmental segments. These substances, however, are categorized as persistent organic pollutants (POPs), which have an inclination to build up in sediments and/or biota over time while remaining in the aqueous phase. Additionally, the agrochemicals have a tendency to move across large areas, which harms both the environment and ecosystems. The United Nations Sustainable Development Goal No. 2, which emphasizes "zero hunger" and sustainable agricultural practices in the current context, is closely associated with the right use of agrochemicals. The potential acute and chronic toxicity of certain POPs, such as herbicides, insecticides, fungicides, and their metabolites, has been extensively proven in literature, even at small amounts (ng/L to mg/L), found in aquatic bodies. Prior research has demonstrated that exposure to pesticides can cause a variety of health problems, including Parkinson's disease, non-Hodgkin lymphoma, and Hodgkin's disease. Previous research has shown that a number of substances found in nature are stable and do not simply degrade under either biotic or abiotic conditions. Because of these factors, traditional wastewater treatment facilities (WWTPs) are not able to efficiently treat these pollutants to the required levels.

Globally, several research investigations are being carried out to create economical, environmentally sustainable, and effective techniques for the quick and thorough breakdown of pesticides. The majority of technology experiments conducted to date have focused on advanced oxidation processes (AOPs), adsorption, electrochemistry, biological degradation,

and nano-based methods. Every technology has pros and cons. For example, biological processes are limited by temperature, pH, oxygen content, and nutrition quantity. Adsorption processes, for instance, have disadvantages including high sorbent costs, capital expenditure, and secondary pollutants. The use of chemical treatments is also limited by issues including expensive treatment, drawn-out procedures, and partial deterioration. As a result, choosing the best treatment technology is still a difficult undertaking. The treatment of agricultural runoff that is contaminated with pesticides and herbicides is the focus of emerging technologies including cyanobacteria, raceway pond reactor photocatalyst, and biofilters made of sand, pine bark, and peat soil. (Singh, Sanghvi, Yadav, 2023)

Meat was seen as a luxury for the majority of human history. However, during the past century, our desire for and consumption of meat, dairy, and eggs has increased along with our disposable income. Global meat consumption doubled to 43 kilograms per person annually between 1961 and 2014. The average individual consumed 19 kilograms of meat annually. According to the U.S. Department of Agriculture (USDA), Americans would consume an estimated 102 kilograms of red meat and poultry per person annually in 2022.

There must be a place for all of that animal manure from farms. However, animal waste from CAFOs is not sent to a wastewater treatment plant via the municipal sewer system, as is the case with human waste. Rather, such waste is dumped by spreading it over land without any treatment. Operators are expected to apply no more manure than what crops can utilize, but in practice, manure is frequently applied in excess, exceeding the ground's natural rate of absorption and causing runoff into water sources. To make matters worse, the manure typically rests in enormous manure lagoons on-site, some of which can reach the size of a football field, prior to being spread to land. The antibiotic residue, chemicals, and bacteria that break down the waste combine to form a hazardous stew in the lagoons that can eventually take on an unsettling color. They frequently have no lining, which makes them vulnerable to spills, leaks, and overflows that let the contents seep into the groundwater and soil. Due to its ability to produce large yields even on overflowed soil, nitrogen-based fertilizer has played a significant role in the modernization of agriculture throughout the past century. However, fertilizer has detrimental effects on our climate and water resources. In soil water, nitrogen is comparatively mobile. Soil ammonia can be leached below the root zones of crops and pastures, where it can accumulate in groundwater or flow into streams, after being converted by microorganisms into nitrates ( $\text{NO}_3$ , a soluble salt form of nitrogen) (Hatfield and Follett, 2008).<sup>4</sup> Moreover, surface

runoff from treated pastures and crop fields, as well as roadside ditches and drains, are ways that nitrogen enters waterways. (Chai, Pannel, Pardey, 2022)

Chemical fertilizer and pesticide application, as well as sewage discharge, can contaminate soil; on the other hand, hazardous materials can contaminate river water environments through surface runoff and other channels. When plants and animals burn, toxic gases including CO<sub>2</sub>, N<sub>2</sub>O, and NH<sub>3</sub> are released into the atmosphere and can contaminate surface water when rains carry them into it. Plants and animals can absorb harmful compounds from the soil and subsequently consume them, posing a risk to human health. The three dimensions of compound cross-pollution, spatial and temporal extension, and circular pollution have become more prevalent in agricultural pollution. Traditional point and nonpoint source pollution prevention and control techniques are no longer able to handle the intricate agricultural tridimensional pollution problem because of the highly interwoven and complicated nature of agricultural pollution. Through multidisciplinary cross-sectional study, Chinese scientists and technologists have pioneered the new idea of agricultural tridimensional pollution. This idea substantially resolves the issue of agricultural pollution by innovating the traditional research of individual pollutants and establishing a tridimensional ecosystem management and pollution control system with a water-soil-biology-atmosphere cycle based on system science theory. (Hang, Yang, 2024)

### **1.1.1 Effects of Agricultural Pollution on the Environment**

Risks to ecosystems and hence critical ecosystem services might arise from agricultural contamination. For instance, a variety of biologically and commercially significant species, such as pollinators, birds, natural predators, and microbial ecosystems, might be harmed by some pesticides. For instance, pollinators are essential to 35% of food production and can be adversely affected by pesticide residues left on plants or direct contact with the chemicals. A variety of agricultural inputs, including pesticides, fertilizers, and antibiotics from animal agriculture, find their way into drinking water due to the introduction of harmful chemicals, minerals, and infections. This can have an effect on human health. Removing these contaminants from tap water is a notoriously tough task, as many of them are recognized

carcinogens. Agricultural pollution has rendered drinking water unfit for human consumption in numerous parts of the world, spanning from China to the United States.

Agricultural methods like drainage tiling have the potential to worsen water contamination. While tiling a field improves yield, fields with faster drainage decrease filtering, which makes it possible for more fertilizer and pesticide to reach rivers and exacerbates issues like eutrophication. Climate change encompasses a multitude of intricate issues, including increased frequency of extreme weather events, altered disease and insect dynamics, and global warming. These factors have a significant impact on agriculture. A significant portion of greenhouse gas emissions from agriculture are released into the atmosphere, where they trap heat and exacerbate climate change. (Gordon, 2022)

### **1.1.2 Pollutants**

Pesticides nowadays are not properly managed, their concentrations in the air, water, and soil are steadily rising year by year. Specifically, substantial harm to human health and the ecosystem is caused by agricultural runoff, leaching, erosion, spray-drift and deposition. (Singh, Sanghvi, Yadav, 2023) Pesticides are substances used to manage a range of pests, either naturally occurring or chemically created. Insecticides, herbicides, fungicides, nematicides, rodenticides, molluscicides, plant growth regulators, and other substances are among them. Numerous industries, including food, forestry, agriculture, and aquaculture, use these chemical compounds. (Rajmohan, Chandrasekaran, Varjani, 2020)

The toxicity of pesticides is evident in living systems. The World Health Organization (WHO) emphasizes the importance of public health by classifying them according to their harmful consequences. By utilizing them sparingly and fully understanding their classification, the utilization can be reduced to the lowest possible level, which is advantageous for the environment and human health. Pest management has involved the application of numerous chemical substances since earlier times. One well-known example of a pesticide for controlling insects and mites is Sulphur compounds. For more than 2000 years, pyrethrum, a pesticide produced from the plant *Chrysanthemum cinerariaefolium*, has been in use. Before Paul Herman Muller introduced dichloro diphenyl trichloroethane (DDT) as a powerful pesticide in 1939, salty water and chemical compounds, both organic and inorganic, were frequently utilized to reduce insect populations. Nonetheless, DDT use contributes to longer food product shelf lives and higher food yield. As a result, the need for DDT grew daily on a global scale,

which made it possible to create other chemicals with pesticidal properties. Organophosphates (OPs) and carbamates (CMs) took the role of DDT.

In 2019, the total amount of pesticides used worldwide was estimated to be 4.19 million metric tons. China accounted for the majority of this consumption, with 1.76 million metric tons, followed by the US (408 thousand tons), Brazil (377 thousand tons), and Argentina (204 thousand tons). The largest portion of synthetic pesticides consisting of organic compounds, which were divided into four categories: pyrethroids, carbamates, organochlorines, and organophosphates. Biopesticides, or naturally occurring pesticides, are made by living things like fungi, bacteria, and plants. (Pathak, Verma, Rawat, 2022)

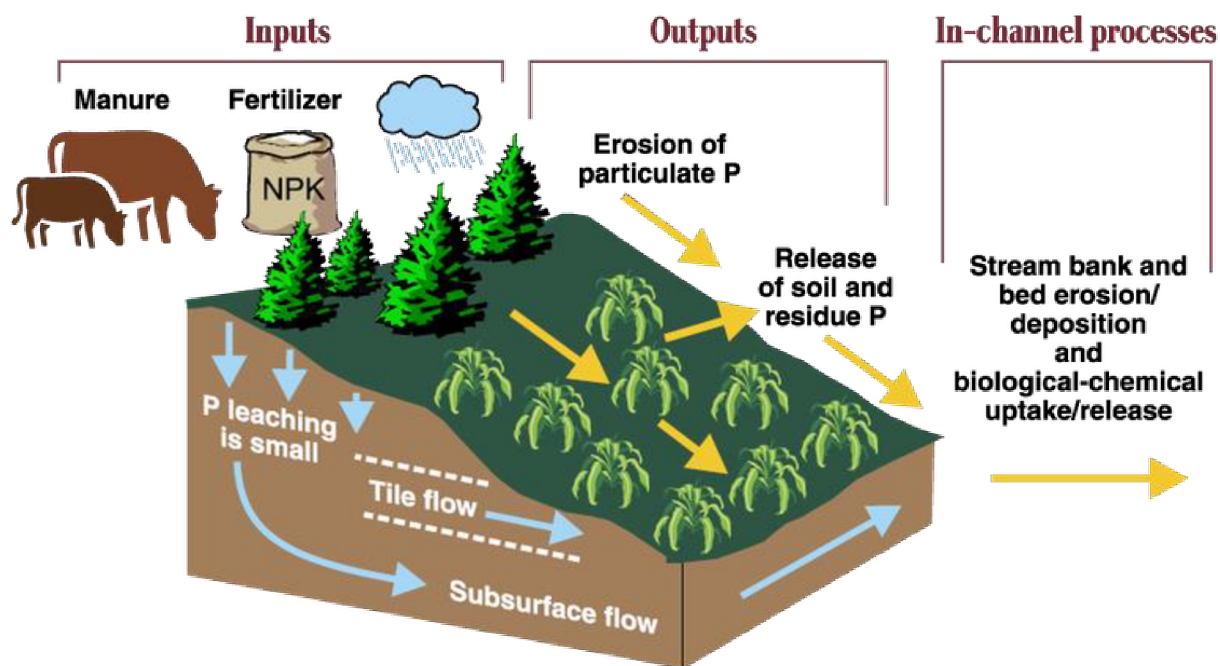
According to research on pesticides, pesticide buildup in plants stunts their growth and results in a range of metabolic diseases. For example, chlortoluron interferes with the plant's photosynthetic electron transport chain mechanism, causing disruption of the PS II reaction center. Uracil-type herbicides stop the Hill reaction and photosystem II during the photosynthetic process. The more fungicide doses that are applied to plant leaves, the more total chlorophyll, as well as chlorophyll a, b, and carotenoid content, are reduced. According to some study, using herbicides on plants can have harmful effects such as burns, necrosis, stunting, chlorosis, and leaf bending. Nonetheless, Donald (2004) found in his experiment that using too many pesticides can significantly lower the diversity of structural vegetation. The majority of scientists have documented that using pesticides has a negative impact on the growth and development of plants. (Pathak, Verma, Rawat, 2022)

Farmers and environmental scientists are aware of the deadly chemicals that are released into water bodies and the long-term impacts of pesticides. Notably, over the past 50–60 years, there has been a rise in the incidence of cancer and other chronic diseases. This trend may be related to both the growing population and the pesticidal chemicals that function as a catalyst to carcinogens. Water bodies contaminated by pesticidal detritus pose a serious hazard to the aquatic ecosystem and drinking water supplies. Water bodies become contaminated when large amounts of foreign substances are introduced, negatively affecting the bodies of water. The Environmental Protection Agency (EPA) reported in 1990 that the release and mixing of pesticides used in agricultural soils is to blame for half of the water pollution from rivers and streams. Through soil erosion, aerial sprinkling, drifting sprinkling, and negligent chemical discharge during machinery washing, pesticides are indirectly drained into water bodies. (Rajmohan, Chandrasekaran, Varjani, 2020)



A naturally occurring element, phosphorus (P) can be found in water, minerals, soil, and living organisms and is the least transportable of the main plant nutrients. Like nitrogen, high concentrations of phosphorus are necessary for plant growth and development. The elements speed up plant maturity and is necessary for early root formation. Phosphorus can be found in soil in organic, soluble, or "bound" forms. To comprehend how plants use phosphorus and how much phosphorus can travel through the environment, one needs to understand the relationships between different kinds of the element. (Dvořáková, Vymazal, 2016)

All living things, including microbial tissues and plant waste, contain organic phosphorus. It is the main kind of phosphorus found in most animal manures. Fresh manure contains around two thirds of its phosphorus in organic form. Available inorganic phosphorus, also known as soluble phosphorus. It may contain trace levels of both the form that plants absorb, orthophosphate, and organic phosphorus. It is also a substance that is lost due to leaching and, to a lesser extent, dissolving in runoff. Soluble phosphorus content of manure and commercial fertilizers is significantly higher than that of soil. The concentration of soluble phosphorus in runoff from rainstorm events that transpire shortly after the application of commercial fertilizer or manure on the surface can surpass 100 times that of other runoff events. High amounts of phosphorus in a form that aquatic organisms can easily access are present in flash losses of soluble phosphorus.



*Figure n. 1 - Phosphorous movement in the environment. (Keena, Meehan, Fronzen, 2023)*

The majority of phosphorus is bonded to soil particles. P travels with the wind and water when soil particles move. Phosphorus can be held in the soil by finer particles than by coarser ones. Regrettably, increased fine particle mobility brought about by soil erosion results in "enriched" phosphorus-containing eroded silt. (Keena, Meehan, Fronzen, 2023)

Wetlands offer a setting where all types of phosphorus can interconvert. Plants absorb soluble reactive phosphorus, which is then transformed into tissue phosphorus or may seep into the sediments and soils of marshlands. If the organic matrix is oxidized, organic structural phosphorus may be liberated as soluble phosphorus. In certain situations, insoluble precipitates can form, but they can also re-dissolve in different situations. Peat and soil accretion, adsorption and desorption, precipitation and dissolution, plant and microbial uptake, fragmentation and leaching, mineralization, and burial are the phosphorus transformations that occur in wetlands. Thus, each of these elements needs to be measured while assessing a wetland ecosystem in order to preserve P. It was discovered that the long-term phosphorus sequestration in wetlands is regulated by soil adsorption and peat accretion. However, because biomass storage and sorption are saturable processes with limited capacity, they are unable to support long-term sustainable removal. (Vymazal, 2007)

Adsorption and precipitation are two methods used in CWs to remove phosphorus, and some is also absorbed by plant growth.

The authors calculate that, depending on the plants, wastewater type, climate, and other factors, a phosphorus removal ratio by plant growth of up to 10% may be achievable. As a subsurface flow CW ages, its ability to bind phosphorus chemically diminishes, lowering the efficiency of phosphorus removal. This is because the sand's adsorption sites are limited. If phosphorus removal is really necessary, the substrate can be changed whenever its phosphorus adsorption capacity is achieved in a separate unplanted soil filter that is employed downstream of the subsurface flow CW. (Hoffman, Platzer, Winker, 2011)

Another biogenic element that is essential to the survival and expansion of life on Earth is nitrogen (N). On the other hand, a large amount of nutrients supplied to the Earth's system can cause a variety of environmental problems. The rapid expansion of the social economy has led to an increase in both the population and the level of urbanization in recent times. Human activity disrupts the N cycle at a far higher intensity than the classic natural cycle, and each link in the N cycle has a longer metabolic pathway. (Pericherla, Karnena, Vara, 2020) Under normal circumstances, a number of biogeochemical processes allow N to enter the terrestrial ecosystem and surrounding environment. N enters the soil, is taken up by plants, and then finds its way into products from agriculture. The food chain has a dynamic, compact cycle that is less likely to cause pollution, has a greater recycling efficiency for nutrients, and has less nutrient loss and build-up. Since the start of the 21st century, humankind's capacity to apply nitrogen to agricultural activities has risen dramatically, allowing agriculture to produce more food to meet the growing need for food due to population expansion. The original nutrient cycle has undergone major alteration due to the massive usage of nutrients in agriculture and other human activities. (Deng, Zhang, Song, 2024)

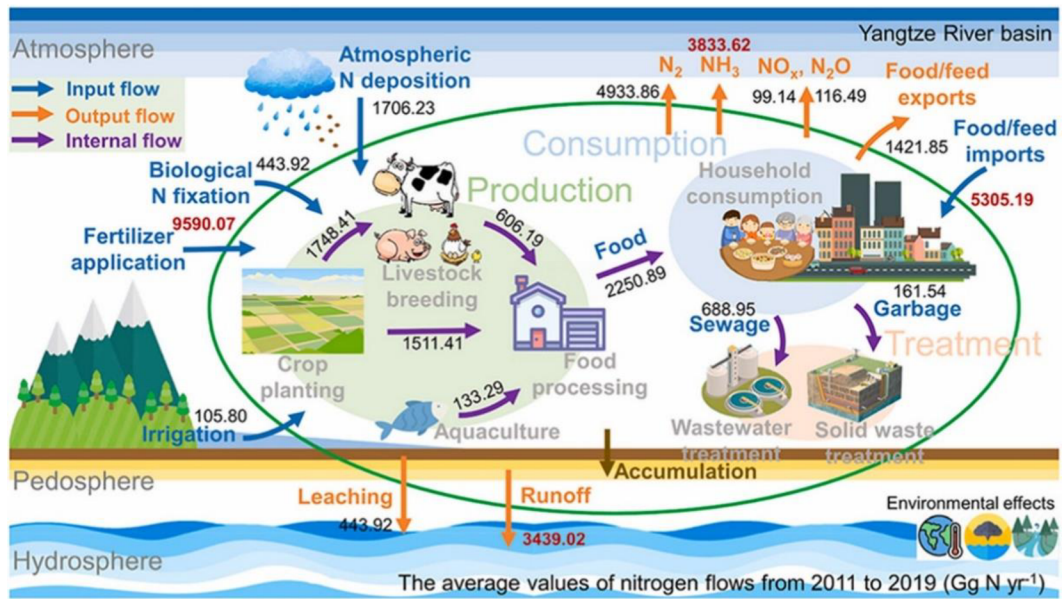


Figure n. 2- The average values of nitrogen flow from 2011 to 2019 (Gg.N.yr<sup>-1</sup>) (Deng, Zhang, Song, 2024)

Overabundance of nitrogen and phosphorus in the air and water can harm our land and water resources, have a negative impact on human health, and negatively impact the economy. An algal bloom, or huge proliferation of algae, can be caused by nutrients. Eating shellfish that has been contaminated by toxins released by some types of algae can cause short-term memory loss and gastrointestinal ailments. Absorption of toxins from algal blooms by drinking or touch can result in more serious issues such as rashes and stomach discomfort. In agricultural areas, excess nitrogen is a typical pollutant of drinking water that can be especially dangerous for new-born under six months of age. Chemicals used for purifying drinking water contaminated with nutrients may also be harmful to people's health. Chlorine and other chemicals have the ability to react with water algae to produce disinfection by-products that have been linked to issues with reproductive and developmental health. Water quality is negatively impacted by nutrient contamination, which also hurts the environment. The oxygen that fish, shellfish, and other animals require to exist is greatly consumed by algal blooms. Algal blooms have the potential to clog fish gills, cloud the water, and hinder aquatic life's ability to obtain food.

Approximately 24%–71% of the pollution load in surface water bodies in EU member states comes from agricultural discharge of P, and the issue of excessive ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) in water bodies due to nutrient loss in agricultural ecosystems is also quite problematic. The primary source of N and P pollution in China is ANPSP. The ANPSP (nonpoint source pollution) was responsible for about 1.4 million tons of N and 0.2 million tons

of P pollution in 2017. The longest river in China, the Yangtze, directly affects the Pacific Ocean's water quality. Consequently, limiting the amount of N and P that cropland loses is essential to lowering ANPSP. The growing N and P difficulties that most river basins experience have prompted a number of actions. Among these, the Yangtze River Basin, the third-largest river in the world by volume, features numerous restoring farmlands to wetlands (RFWs) programs that have received backing from the Chinese government. RFWs convert riparian zones that were previously utilized as farming but are now disorderly inhabited back to wetlands, hence restoring water bodies and wetland ecosystems. (Page, Badiou, Steele, 2023)

Many nations have expressed concern over the eutrophication of surface waters, and it is generally acknowledged that anthropogenic nutrient inputs to surface aquatic ecosystems must be reduced. But since nutrient control comes at a high cost, it is imperative to identify the source of nutrient contamination as soon as possible in order to create a suitable nutrient management plan. Dissolved organic matter's (DOM) optical characteristics are a sensitive indication of water quality as well as the trajectory and effects of water pollution. (Shen, Huang, Zhang, 2021)

High NO<sub>x</sub> concentrations have negative effects on human health in addition to negatively affecting agricultural crop development and (semi)natural vegetation growth. These effects could result in significant yield decreases in high NO<sub>x</sub> concentration areas. Due to an absence of research, distinct critical limits for NO<sub>x</sub> have not been established for various vegetation types, yet it is believed that the sensitivity decreases as one moves from (semi)natural vegetation to agricultural crops and woods. A lower uncertainty bound of 15  $\mu\text{g m}^{-3}$  NO<sub>x</sub> represented as NO<sub>2</sub> has been proposed for the most sensitive vegetation. The long-term critical level for NO<sub>x</sub> is defined at 30  $\mu\text{g m}^{-3}$  NO<sub>x</sub>, expressed as NO<sub>2</sub>.

The damage of the epicuticular wax layer, increased susceptibility to drought stress due to increased stomatal opening causing higher transpiration rates, and an increased risk of fungal infection and pest attacks due to increased nitrogen levels in plant tissue are some of the adverse effects of elevated NH<sub>3</sub> concentrations on higher plants resulting from direct exposure and uptake. When the rate of NH<sub>3</sub> uptake by the leaves is greater than the rate at which the leaves detoxify, there are direct negative impacts on the plants.

Terrestrial ecosystems can be impacted by soil-mediated effects in addition to the direct effects of N gases. It is crucial to distinguish between forests and other ecosystems in this context. Through increased N availability, N deposition in forests may initially boost growth and productivity. However, over time, it may also result in eutrophication and acidification,



which disrupt nutrient balances and make trees more vulnerable to drought, disease, and pests. Growth augmentation only poses a problem in other environments since it reduces the diversity of plant species, which is a compromise in forest ecosystems. (Vries, 2021)

In horizontal flow beds it is not possible to anticipate improved nitrification due to the restricted oxygen movement within HFBs. However, even at very low carbon to nitrogen ratios, denitrification can be highly effective. Denitrification is the process by which heterotrophic bacteria convert the generated nitrate to nitrogen ( $N_2$ ) in an anoxic environment.

In vertical flow beds autotrophic bacteria can oxidize ammonia to nitrate in VFBs with an adequate oxygen supply; this process is known as nitrification. For VFBs, reports of nearly total nitrification and 90% ammonia oxidation are typical. Nonetheless, the availability of oxygen is critical to nitrification. The oxygen consumption in the VFB must be calculated for dimensioning. However, because VFBs do not produce much denitrification, the nitrogen in the wastewater remains as nitrate, resulting in a total nitrogen removal ratio of only about 30%. When nitrogen removal is necessary, a VFB, an HFB, and flow recirculation are frequently utilized in combination. (Hoffman, Platzer, Winker, 2011)

In agricultural soils, soil organic matter, or SOM, performs a variety of crucial tasks, including as enhancing the soil's ability to retain water, enhancing its structure, and supplying plants with nutrients. However, long-term monitoring has shown a diminishing tendency in the amount of organic carbon (OC) in Finnish agricultural soils. The amount of dissolved organic carbon (DOC) in farmed soil discharges may be detrimental to surface waters. Depending on the characteristics of the soil or agricultural management techniques, the load's magnitude may change. The quality of inland or seawater as well as boreal agriculture may be affected by the removal of organic carbon from the soil. The risk of eutrophication in the receiving waters may rise as a result of DOC leaching with runoff water. (Manninen, Soenne, Lemola, 2018)

## 1.2 Agricultural pollution solutions

An important environmental problem that can harm ecosystems, public health, and water quality is agricultural pollution. It takes a diverse strategy including many players, technology, and legislation to address agricultural pollution. Storm water that drains off of farms can carry nutrients like nitrogen and phosphorus from commercial fertilizers and manure, as well as pesticides and soil. These contaminants travel upstream through streams and eventually reach bigger bodies of water. By encouraging algal blooms, which can cause aquatic animals to become oxygen-starved, these pollutants deteriorate the quality of the water. They also produce substances and poisons that can be dangerous to human health when they enter surface and groundwater systems. Pollutants are not the only things that riparian buffers help filter out. They improve aquatic habitat and give birds and other animals food, cover, and places to nest by creating woody debris and shading streams. They absorb carbon as well. (Marinelli, 2022)

Sustainable farming techniques include crop diversification, contour farming, cover crops, and organic farming. Crop variety lowers the risk of water contamination by enhancing soil health and lowering the requirement for chemical pesticides and fertilizers. Cover crops and contour farming aid in reducing soil erosion and runoff, which can introduce contaminants into waterways. In organic farming, natural methods are utilized to improve soil quality and manage pests, rather than using artificial fertilizers and pesticides. NBS (nature-based solutions) have an equally vital role in protecting water quality by addressing socio-environmental concerns through natural processes. In order to improve water quality and availability, NBS for water involves managing wetlands, soils, and vegetation. These methods improve ecosystem resilience and health by imitating nature and fostering a symbiotic interaction between human activity and the environment.

One of the most effective NBS for enhancing water quality is constructed wetlands. They act as organic biofilters, capturing silt, phosphates, and other contaminants from agricultural runoff and keeping them out of waterways. Additionally, these wetlands support a variety of plant and animal species, which helps to conserve biodiversity. Combining trees with cattle or crops on the same piece of land is known as agroforestry, and it provides another integrated NBS. Trees serve as filters, drawing in pollutants and excess nutrients while preventing soil erosion. In addition, agroforestry systems enhance soil fertility, boost biodiversity, store carbon, and give farmers access to alternate sources of income.

To sum up, NBS and sustainable agriculture offer viable ways to balance environmental health and agricultural productivity. They show that farming and water management don't have to be zero-sum games where environmental degradation occurs in order to increase productivity. Rather, by employing comprehensive, unified, and inventive methods, we can establish mutual benefits between societal well-being and ecological conservation. By doing this, we make sure that our water and food systems are resilient, sustainable, and able to sustain future generations. (Brears, 2023)

### **1.3 Constructed Wetlands**

Our ancestors have long since noted that water exits from the wetlands area cleaner and purified. Wetlands have been recognized for hundreds of years as a highly advantageous method of treating wastewater because of such. In the past 20 years, a number of artificial wetland types have dramatically expanded, giving us the opportunity to see the treatment of heavily loaded wastewater or sludge from a variety of municipal and industrial sources. At the same time, people's understanding of how humans affect the environment globally has increased, particularly in relation to global sustainability and the threat posed by climate change. Due to their ease of use, low energy consumption, low carbon footprint, robustness, ability to be naturally incorporated into the existing landscape, consistency, and impressive performance, constructed wetland treatment systems are now valued as highly relevant water treatment solutions for the modern era rather than just being curious. (Treatment Wetlands - Constructed Wetlands, 2022) Wetlands have great potential for utilization in developing countries due to their low cost. Their potential can serve as a substitute for global water treatment facilities. (Almuktar et al., 2018)

The ongoing reduction in water supply has prompted a re-evaluation of human impact on the environment and society, as well as resource management. As a result, a lot of researchers are looking into more environmentally friendly tactics and inventions that combine the ideas of clean technology with a circular economy. Regarding the utilization and conservation of natural resources, water is essential to social, environmental, and economic endeavors. Due to the importance of wastewater treatment and water reuse for a more sustainable approach to water management—particularly in developing nations with inadequate or non-existent wastewater

treatment infrastructure—many technologies have been developed in these areas. (Tara, Arslain, Hussain, 2019)

In this context, low-cost treatment solutions with minimal environmental impact, such as constructed wetlands (CWs), have been designed and studied. According to Dotro et al. (2017), these are engineered systems that are meant to be used and maintained to purify various kinds of contaminated water. They maximize natural processes that occur in the environment and require minimal maintenance. In floating treatment wetland (FTW) systems, naturally occurring plants are applied to the water's surface as hydroponic floating mats. The root system of these floating macrophytes acts as a natural filter, facilitating the hydraulic passage of water through the plants and below them. (Chen, Curevo, Muller, 2016) The crowns and shoots of emergent plants in these systems grow above the water line, while the root system declines deeper into the water column. The plants are placed in a buoyant mat suspended in a floating structure. The primary purpose of its design and operation is to eliminate various pollutants and nutrients, such as phosphorus and nitrogen, from water. Nowadays, rainwater, agricultural runoff, wastewater from secondary effluents, and even the cleaning of contaminated rivers is all treated with FTWs. Numerous researchers have acknowledged the effectiveness of FTWs in enhancing wastewater quality by eliminating contaminants through an integrated system of physical, biological, and chemical processes, due to their simple establishment. (Colares, Dell'Osbel, Wiesel, 2020)

The majority of contaminants that are found in waters can be treated by using wetlands. Because of their "green" reputation and the passive treatment technology's cheap operational costs, these treatment wetlands have grown in popularity. They have been widely used for a variety of purposes, including stormwater management, mining water reclamation, industrial sources, residential wastewater, and agricultural and natural water contamination. Wetlands fall into two main categories: subsurface flow and free water surface flow. Regardless of design, wetlands remove substances through a variety of methods. For example, solids are removed through filtration and some settling; BOD is reduced as organic matter is taken in by microbes; and ammonia is oxidized by microbes close to the water's surface, with the resulting nitrate being removed through anoxic metabolism further into the wetland. Wetlands do not biologically remove phosphorus; however certain sediment components have the ability to sorb phosphate. Surprisingly enough, plants don't really affect pollutant removal all that much. Because they provide surfaces for microbial attachment, shade and insulate the water's surface,

and alter the redox potential of the wetland substrate, plants do have a shown effect on treatment.

Wetlands are far more variable in their design and operation than other treatment approaches. Wetlands are heavily influenced by local factors due to their relatively modest volumetric activity. Because of this, the very complex system's daily performance is "noisy," and design equations are unable to adequately account for this unpredictability. This does not imply bad performance because water from well-sized wetlands has low levels of pathogens, BOD, sediments, and nitrogen. Instead, wetlands are often designed with extremely simple, zero-order area loading values. (Fitch, 2014)

The aquatic ecology is seriously endangered by current agricultural methods. The most deadly and persistent toxins that threaten human health and the planet's environment are synthetic pesticides originating from agricultural activities; conventional waste-water treatment systems that are not very effective enhance the problem. Because of this, it is necessary to update these old technologies, and built wetlands have gained significance as environmentally and user-friendly technologies. Comprising diverse biotic elements such as microorganisms and plants, as well as abiotic elements like sand, gravel, and other materials, CWs serve to eliminate a range of organic contaminants and facilitate their gradual breakdown into more basic forms. However, the selection of aquatic macrophytes is the element limiting the successful application of created wetlands.

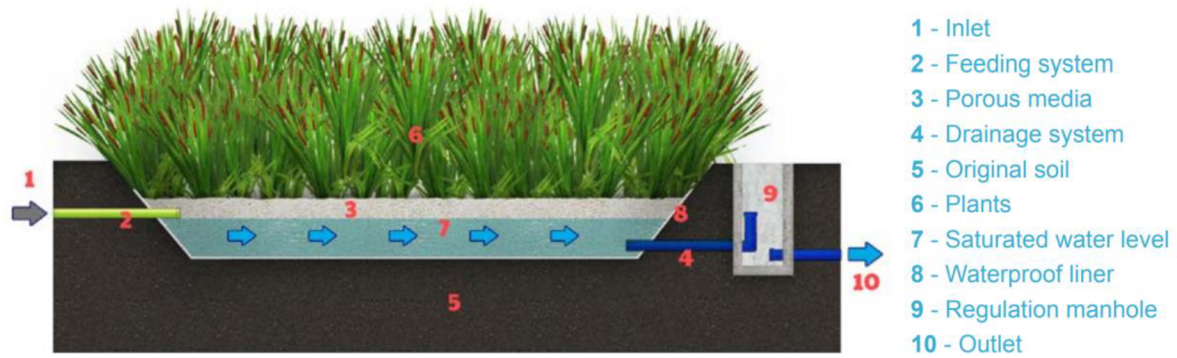
In addition to lowering the need for pesticides, macrophytes also efficiently support the bed surface, lessen clogging, enhance filtration, and encourage microbial growth. Subsequently, microorganisms have the ability to eliminate pollutants by expediting chemical reactions, facilitating biodegradation and biosorption, and stimulating plant growth. The overall goal of this chapter is to emphasize how crucial it is to investigate the macrophyte-microorganism interactions in their whole in order to maximize removal efficiency. (Sachadeva, Chowdari, Patro, 2023)

## BIOHAVEN FLOATING WETLAND



*Figure n. 3- Design of floating treatment wetland (FTW) (Midwest Floating Island LLC, 2014)*

Gravel beds covered in emergent wetland vegetation that encourages horizontal flow through the filter media are known as horizontal flow (HF) wetlands. Since the media are completely saturated with water, an anoxic environment may be created, sustaining a subsurface flow. Filtration or straining is used to hold onto particles, while biotic or abiotic partial absorption occurs for solubles. Subsequent chemical and primarily biological activities in the filter media cause additional transformation and degradation of the compounds that have been retained. In addition to supporting hydraulic flow, the root zone offers a very active environment for oxygen exchange and biofilm adhesion. As seen in figure n.6, horizontal flow wetlands have a number of benefits. Not particularly dangerous for mosquito breeding, robust; capable of withstanding hydraulic changes minimal energy consumption (gravity-based feeding), It is feasible for separate and combined sewer systems to operate. Possibility of reuse at the building scale (irrigation, toilet flushing). (Rizzo, Tondera, 2021)



*Figure n. 4- Horizontal flow wetland. (Rizzo, Tondera, 2021)*

One of the most popular forms of wetland is horizontal sub-surface flow CW (HSSFCW), which has been effectively applied for the past few decades to treat a variety of contaminants from wastewater. These wetlands are more efficient as a system than an unplanted wetland because of the utilization of macrophytes. Overall, the components of CWs, including as growth media, plants, microorganisms, and the pattern of water flow in the wetland system, determine their effectiveness and efficiency in terms of removing pollutants from wastewater. In order to improve the effectiveness of CWs, various media types are also used, including charcoal, zeolite, vermiculite, lime, etc. (Shukla, Gupta, Singh, 2021) Since no additional equipment is needed, constructed wetlands and ponds both receive good marks for process simplicity and reliability. The following are the key justifications for selecting subsurface flow CWs over ponds:

Subsurface flow CWs produce clear water, whereas ponds have a high algal production that affects the effluent quality. Ponds are much harder to integrate into a neighborhood, especially an urban one, because of their open water surface, mosquitoes, and odor. Subsurface flow CWs do not have open water bodies, so they do not encourage mosquito breeding.

When space is limited, VFBS (vertical flow beds) work better than HFBs (horizontal flow beds) because they require less room due to their higher treatment efficiency. Wastewater from VFBS is periodically pushed to the surface, where it descends vertically through the filter layer and onto a drainage system at the bottom. The wastewater percolates through the unsaturated substrate and the surface dries up during the lengthy resting periods that follow the irregular short-term loading intervals (4 to 12 dosages per day) that define the treatment process. High aerobic degradation activities are produced by the intermittent batch loading, which also improves oxygen transmission. Therefore, although HFBs can function without pumps, VFBS



require pumps constantly or at the very least siphon pulse loading. (Hoffman, Platzer, Winker, 2011)

## **1.4 Floating treatment wetlands: history and classification**

When it comes to water treatment, FTWs were most likely initially implemented in Germany and Japan to treat eutrophic lakes and rivers s conventional CW systems are usually not suitable for receiving stormwater peaks. They are still utilized in China for the same purpose. Since then, their unique usefulness for treating stormwater has been recognized and taken advantage of. They have been applied to the treatment of sewage, domestic wastewater, wastewater from poultry processing, and acid mine drainage. The commercial crop production business employed the "nutrient film technique" in hydroponic plant growing systems during the 1970s. The latter method has a strong resemblance to our definition of HRMs (Hydroponic root mats). Human resource management practices are currently used worldwide, particularly in developing nations (Chen, Curevo, Muller, 2016).

Fig. 5 displays a potential classification for natural treatment technologies, and it shows that FTWs fall halfway between ponds and standard artificial wetland systems. In order to remove pollutants from water, CWs and ponds—both of which are considered artificial systems—make use of the natural aquatic ecosystems that exist between soil, water, bacteria, photosynthetic organisms (plants and algae), and the atmosphere. But while CWs are typically depicted by a system where macrophytes develop in wet conditions, ponds are essentially open water bodies with a predominant phytoplankton population. (Colares, Dell’Osbel, Wiesel, 2020)



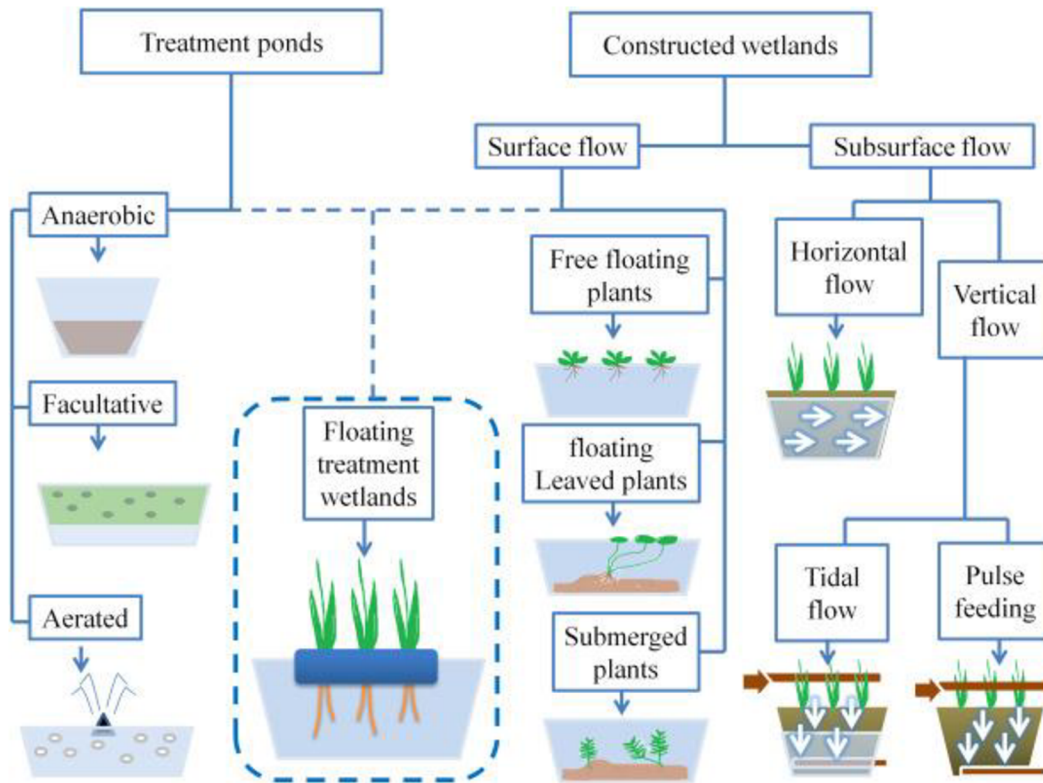


Figure n. 5- Classification of FTWs. (Colares, Dell’Osbel, Wiesel, 2020)

To take advantage of the unique benefits of each system, various artificial wetland types can be mixed together to create hybrid systems. Sand and gravel are the two different kinds of filter material. In Asia, Australia, New Zealand, North Africa, and South Africa, gravel bed systems are extensively utilized. Sand bed systems originated in Europe are being utilized globally. (Hoffman, Platzer, Winker, 2011)

## 1.5 Removal mechanism of N

The two main methods for removing nitrogen are physicochemical and biological treatment approaches. The conventional method of biological nitrogen removal from water and wastewater involves a combination of anaerobic denitrification and aerobic nitrification, and is often regarded as the most efficient and cost-effective form of nitrogen treatment. (Vymazal, 2007) Many problems remain unsolved, nevertheless, including the need for a secondary carbon source in wastewater with a low C/N ratio, the need for extensive treatment areas, and the high expense of upkeep. The majority of wastewaters lack an external organic source and sufficient

biodegradable carbon to support heterotrophic denitrification. Biological techniques in nitrogen removal treatment often face many challenges due to their lower energy consumption and higher costs in the wastewater treatment plant.

Ammonification, nitrification-denitrification, plant uptake, and physical chemical methods such as sedimentation, ammonia stripping, breakpoint chlorination, and ion exchange are recognized as the nitrogen removal processes in artificial wetlands.

The biological conversion of organic N to ammonia is known as ammonification. In both the aerobic and anaerobic zones of reed beds, nitrogen-containing pollutants are easily broken down, yielding inorganic ammoniacal nitrogen ( $\text{NH}_4\text{-N}$ ). In artificial wetlands, nitrification-denitrification reactions mostly eliminate inorganic  $\text{NH}_4\text{-N}$ . Ammonification happens more quickly than nitrification, in a kinetic sense. Ammonification rates peak in the oxygenated zone and subsequently fall when the mineralization circuit transitions from facultative anaerobic to obligatory anaerobic and aerobic anaerobes. Temperature, pH, C/N ratio, available nutrients, and soil structure all affect the rates.

It is thought that wetlands decomposition processes produce ammonia from a sizable portion of the organic nitrogen. The biological nitrogen removal in conventional nitrogen treatments necessitates a two-step process: nitrification and denitrification. The nitrification process requires a lot of oxygen. Compared to the heterotrophs, which remove BOD, the nitrifying bacteria of the autotroph group respire at substantially lower rates. Because of this, major nitrification in SSF systems often occurs after significant BOD reduction. Temperature, pH, alkalinity, inorganic carbon supply, moisture, microbial population, and concentrations of dissolved oxygen and ammonium-N all affect how quickly nitrification occurs. The reactor layout, substrate type, and influent ammonium concentration all affect the ammonia absorption rate (AUR). In low-oxygen locations nitrate serves as the terminal electron acceptor in the biological denitrification pathway. Denitrifying bacteria convert inorganic nitrogen, such as nitrate and nitrite, into harmless fundamental nitrogen gas during this process. Heterotrophs and autotrophs are the two main kinds of denitrifying bacteria, or denitrifiers. Microbes classified as heterotrophs derive their energy from organic materials and require organic substrates to receive their carbon supply for growth and evolution. Autotrophs, on the other hand, use carbon dioxide as a source of carbon and inorganic materials as a source of energy. A heterotrophic microbe carries out the second stage, denitrification. Even if there are some concerns about external organic carbon sources in heterotrophic denitrification, several studies have demonstrated that the denitrification rate in organic carbon-restricted water and wastewater may be continuously

enhanced by adding any carbon sources [60]. Right now, a lot of focus is on biological nitrogen removal, but denitrification takes a while, especially for industrial wastewaters with high nitrate content. (Lee, Fletcher, Sun, 2009)

The degree of the elimination operations varies between systems. Because single-stage manmade wetlands cannot simultaneously supply anaerobic and aerobic conditions, they are unable to remove a high percentage of total nitrogen from the environment. Although ammonia-N is effectively removed from vertical flow-built wetlands, very little denitrification occurs in these systems. Conversely, while horizontal-flow built wetlands offer favorable denitrification conditions, their capacity to nitrify ammonia is severely restricted. As a result, several kinds of artificial wetlands may be coupled with one another to take use of the unique benefits of each system. The primary route of removal in artificial wetlands including free-floating macrophytes is plant absorption. Emergent plants have very little potential, particularly in artificial wetlands used to clean home or municipal sewage. However, it appears that harvesting emergent plants might play a key removal pathway, especially for weakly loaded systems, in tropical settings where seasonal translocations are modest and many harvests are possible. (Vymazal, 2007)

In artificial wetlands, the elimination rate of N is only around 50%, which is not a very high rate. According to certain research, adding straw and methanol to raise the BOD may significantly increase the elimination rate of N from 30% to 90%. (Qin, Chen, 2016)

## **1.6 Removal mechanism of C**

In the anaerobic zone, organic matter is decomposed into carbon dioxide and methane by anaerobic bacteria by process of fermentation. Constructed wetland has a strong ability to purify organic matter. The soil has a large surface area, and when sewage flows through the surface of the particles, a large amount of SS blocks the filler and plant roots from interfering. The insoluble organic matter is also quickly retained through wetland matrix sedimentation, filtration, and adsorption. Thereafter, the organic matter will be used by small organisms, and the dissolved organic matter was removed by the adsorption of plant roots biofilm, absorption, and metabolism of microorganisms.

Aerobic bacteria break down organic materials in the aerobic zone to produce carbon dioxide and water. Anaerobic bacteria ferment organic materials in the anaerobic zone to produce carbon dioxide and methane. The majority of the organic stuff in sewage is eventually

transformed by heterotrophic microbes into microorganisms, carbon dioxide, methane, and water, as well as inorganic nitrogen and inorganic phosphorus. Constructed wetlands offer greater rates of COD and BOD removal; in fact, much of the processing system can remove material at rates as high as 90%. (Qin, Chen, 2016)

In constructed wetlands, organic carbon is obtained from wastewater that is treated there as well as from fixation by photosynthetic hydrophytes. The latter quickly recycle the carbon through respiration, but a large portion of the fixation process ends up as organic carbon in the soil. Carbon dioxide (CO<sub>2</sub>) is produced by soil organic C going through the biogeochemical mechanisms that control soil C accretion and microbial respiration. Methane (CH<sub>4</sub>) can be produced by anaerobic mineralization of organic C by methanogenic archaea and exchanged with the atmosphere. The effectiveness of C and N removal in CWs is often restricted and varies greatly between CW types, plant kinds, seasons, climatic zones, and management techniques. Although the removal rates are highly variable, it appears that 50 and 56% of the influent TN and TOC, respectively, may be eliminated. Since mixed species enhance microbial biomass and diversity, they outperform monocultures in the removal of C and N contaminants. (Jahangir, Fenton, Gill, 2020)

## **1.7 Nitrogen Transformation**

Wetlands contribute to a number of transformation processes and are essential to the nitrogen cycle. In wetland ecosystems, nitrogen goes through a number of changes. The first stage of the nitrogen attenuation process is called mineralization, also known as ammonification. Various bacteria that get their energy from organic carbon mineralize organic nitrogen to ammonia. (Wardrop, Fennessy, Moon, 2016) There are three types of bacteria that can aid in the ammonification process: facultatively anaerobic, obligately anaerobic, or aerobic. Larger organic nitrogenous molecules, including proteins and amino acids, are converted into smaller organic molecules that dissolve and particulate before becoming ammonium, which is either dissolved in soil or water or taken up by plants or soil microbes.

A critical chemoautotrophic process in the nitrogen cycle and for improving the water quality of the aquatic ecosystem is the biological oxidation of ammonia to nitrite (nitrification) by ammonium oxidizers or nitrate (nitrification) by nitrite oxidizers under aerobic

circumstances. Research has demonstrated that ammonia oxidizers may oxidize ammonia in anoxic conditions, despite the fact that nitrification is an anoxic process. Nitrite and nitrate are reduced to molecular or gaseous nitrogen by chemoorganotrophic, lithoautotrophic, or phototrophic bacteria during denitrification, an enzymatic anoxic reduction process.

When the pH in wastewater treatment wetlands is below 7.5, volatilization loses less ammonia than nitrification and denitrification.  $\text{NH}_3$  is released into the environment when the pH rises over 7.5, which also results in a relatively high concentration of the unionized form of ammonia. In many wetland ecosystems, the volatilization process is not thought to be a significant factor in the nitrogen cycle; however, in inadequately buffered waters, it can result in substantial nitrogen losses due to the rise in pH caused by the increased photosynthetic activity of different algae and submerged and free-floating macrophytes.

In many artificial wetlands, sedimentation attenuates the majority of the particulate organic nitrogen that may stick to plant stems or settle on the wetlands' bottom. The degraded matter, which includes TN, TP, and low molecular weight organic molecules, is used by a variety of microorganisms and plants. Recently, an enhanced sedimentation approach that employs magnesium-ammonium-phosphate as a precipitation reagent has been devised for wastewater treatment, with the potential to be used in artificial wetlands for the amelioration of nitrogen and phosphorous. (Yousaf, Khalid, Aqueel, 2021)

## **1.8 Carbon transformation**

The various ways that natural systems take in and release carbon are together referred to as the "carbon cycle." Carbon is used by both plants and animals to construct their cell walls. Soils may also accumulate carbon. This process of absorption, or "sequestration," of carbon. When plants burn, when cell structures break down, when soils are disturbed, or during natural respiration, stored carbon can be released or expelled. The processes of sequestering and releasing carbon were typically balanced before the Industrial Revolution, with large amounts of carbon being stored for millennia in highly condensed forms like coal, oil, and natural gas—collectively, fossil fuels. (Gilman, Ellison, Duke, 2008)

Wetlands' carbon transformation mechanisms are crucial to the ecosystems' ability to cycle carbon. Wetlands affect global carbon balances and climate control because of their important function in both storing and releasing carbon.

In constructed wetlands, organic carbon is obtained from wastewater that undergoes treatment there as well as from fixation by photosynthetic hydrophytes. These organisms swiftly recycle the carbon through respiration, but a large portion of the fixation process ends up as organic carbon in the soil. Carbon dioxide (CO<sub>2</sub>) is produced by soil organic C going through the biogeochemical mechanisms that control soil C accretion and microbial respiration. Methane (CH<sub>4</sub>) can be produced by anaerobic mineralization of organic C by methanogenic archaea and exchanged with the atmosphere. (Laanbroek, 2010) The dissolved organic carbon (DOC) load transfer to ground and surface waters, which may generate and exchange significant volumes of CO<sub>2</sub> and CH<sub>4</sub> with the atmosphere, can be facilitated by constructed wetlands. (Elberling, Louise, Hans, 2011)

## 1.9 Objectives

This thesis aims to evaluate the effectiveness of continuous flow Floating Treatment Wetlands (FTWs) in reducing the effects of agricultural runoff in a larger context. As the overflow may be inclusive of contaminants and nutrients, agricultural runoff seriously jeopardizes the health of ecosystems and water quality. This project attempts to further knowledge of sustainable water management techniques in agricultural landscapes by assessing the removal performance of FTWs, which are ecologically designed systems. The study specifically looks into how different Carbon-to-Nitrogen (C/N) ratios affect how effective FTWs are at treating runoff from agriculture. The C/N ratio influences microbial activity, nutrient cycling, and treatment efficiency overall, all of which are vital biological activities in wetland habitats. This research aims to give insights for improving FTW design and operation for increased pollutant removal by a thorough assessment under various C/N circumstances. By providing helpful advice for the application of FTWs as a sustainable method of treating agricultural runoff, the thesis seeks to further knowledge in the field of water quality management. The results of this study also have consequences for environmental practitioners, land managers, and politicians that work to preserve and restore aquatic ecosystems that are damaged by agriculture. The main objective is to aid in the creation of practical plans for protecting water resources and fostering ecological resilience in agricultural environments.

Another goal of this thesis is to investigate how plant communities respond dynamically to different operating regimes and carbon-to-nitrogen (C/N) ratios. In order to mitigate the effects of human activity on natural systems and to guide sustainable ecosystem management techniques, it is essential to comprehend how plants adapt and respond to various environmental stresses. In order to fill in fundamental knowledge gaps in plant ecology and ecosystem dynamics, the research aims to provide insight into how resilient and adaptable plant communities are to environmental stresses brought on by human disturbances. The thesis advances knowledge of ecosystem resilience to global environmental change by clarifying the complex relationships between C/N ratios, operational regimes, and plant performance.

## 2 Methods and Materials

Within the campus of the Prague-based Czech University of Life Sciences, *Iris pseudocorus* was planted in the hydroponic systems. These systems were protected by greenhouse from rain, snow and wind. This experiment simulates intake of FTWs by peristaltic pumps. The hydraulic retention time (HRT) was set for 7 days. The influent C/N ratio was set in proportion 1:5, 1:1, and 5:1 to perform simulation of wastewater as shown in the Table n.1. Table 1 displays the exact water distribution plan.



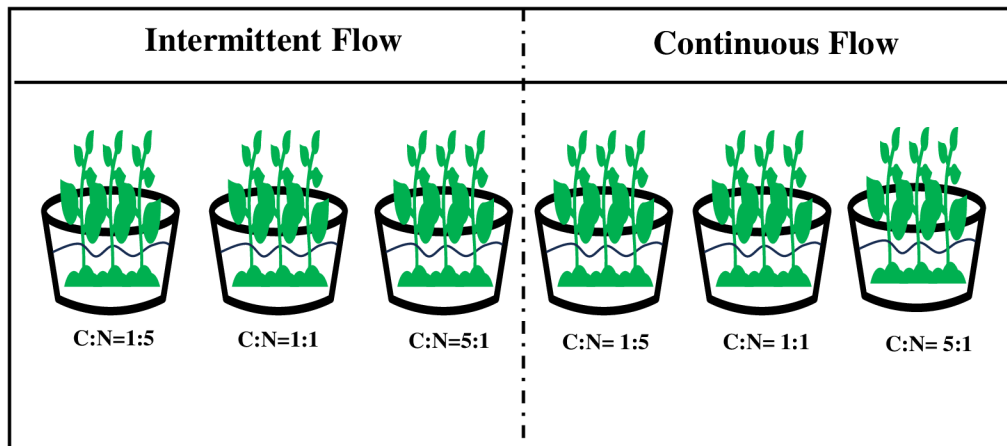
*Figure n. 6 - Experiment setup with peristaltic pumps.*

Reagents	C/N ratio	1:5 (mg L <sup>-1</sup> )	1:1 (mg L <sup>-1</sup> )	5:1 (mg L <sup>-1</sup> )
		FTW 1, FTW 4	FTW2, FTW5	FTW3, FTW6
	<b>CH<sub>3</sub>COONa.3H<sub>2</sub>O</b>	22.68	113.4	567
	<b>NH<sub>4</sub>Cl</b>	18.93	18.93	18.93
	<b>KNO<sub>3</sub></b>	108.32	108.32	108.32
	<b>KH<sub>2</sub>PO<sub>4</sub></b>	17.58	17.58	17.58
	<b>MgSO<sub>4</sub>.7H<sub>2</sub>O</b>	30.75	30.75	30.75

*Table 1- The characteristics of simulated wastewater.*



The FTW systems were carried out in PVC-U materials pots with a volume of 50l. Figure n. shows the experimental setup.



*Figure n. 7- Experimental setup*



*Figure n. 8- Experimental setup simulation in the Czech University of Life Sciences, Prague*

Inflow and outflow samples in the 6 FTWs were taken every 7 days, and were analyzed for pH, oxidation-reduction potential (ORP), total nitrogen (TN), total organic carbon (TOC), ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), sulphate ( $\text{SO}_4^{2-}$ ), and sulphide ( $\text{S}^{2-}$ ) concentrations. Wetland plant samples and substrates in FTWs were analyzed after experiment. Plant shoots and roots were harvested separately, the height and fresh weight will be measured afterwards, then washed carefully with deionized water for further analysis.

Dry weights of shoots and roots were determined after oven drying at 70 °C for 48 h. The PrimacsSN analyzer was used to measure the proportion of total carbon (TC) and nitrogen (TN) in dry plant samples. The dried samples were also used to measure the heavy metal concentrations, biomass, total carbon (TC), total nitrogen (TN), and total phosphorus (TP). Photochemical efficiency of plants in each treatment were analyzed by a pulse-amplitude modulation fluorometer (PAM). Measurements of pH and ORP were taken using the multi-parameter device Multi 3620 IDS SET C for field measurements, with two channel inputs. Set with IDS electrodes: digital pH electrode SenTix® 940, digital conductivity cell TetraCon® 925 displayed in **Figure 9**.



*Figure n. 9-multi-parameter device Multi 3620 IDS SET C / Determination of pH and conductivity of water samples.*

TOC and TN in outflow will be monitored by Formacs<sup>SERIES</sup> Total Organic Carbon (TOC)/Total Nitrogen analyzers where every sample is put into a high-temperature reactor (750–950 °C) and then it is auto-sampled by rotating on a carousel.,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  concentrations will be determined by 883 Basic IC plus. Biomass in shoots and roots will be measured by gravimetric method. All photochemical efficiency measurements will be

conducted every week by PAM 2500. The effective mass balance of the pollutants in the influent (1500 ml) and effluent was used to compute the mass removal efficiencies of the pollutants using the following equation.

$$\text{Mass removal efficiency (\%)} = \frac{V_{in} \times C_{in} - V_{out} \times C_{out}}{V_{in} \times C_{in}} \times 100$$

### 3 Results and discussion

#### 3.1 pH and ORP

Variations in pH and ORP (Oxidation-Reduction Potential) can be caused by a range of biological, chemical, and environmental variables in constructed wetlands (CWs). These variations may have a major impact on how well CWs function overall in treating wastewater. As shown on the graph of pH, there were small fluctuations over the experiment period with downward trend possibly altered by the decreasing temperature over the experiment period.

pH variations may be influenced by microbial activity, organic matter breakdown, and influent properties. Because of this, the relationship between pH and temperature in these kinds of systems can be more complicated and change based on the particular circumstances and activities taking place in the system. The highest differences between Inflow and Cycle n. 6 were observed in FTW-1, FTW-4 and FTW-5. Highest pH values are within the systems with the C:N=1:5 ratios i.e., FTW-3 and FTW-6.

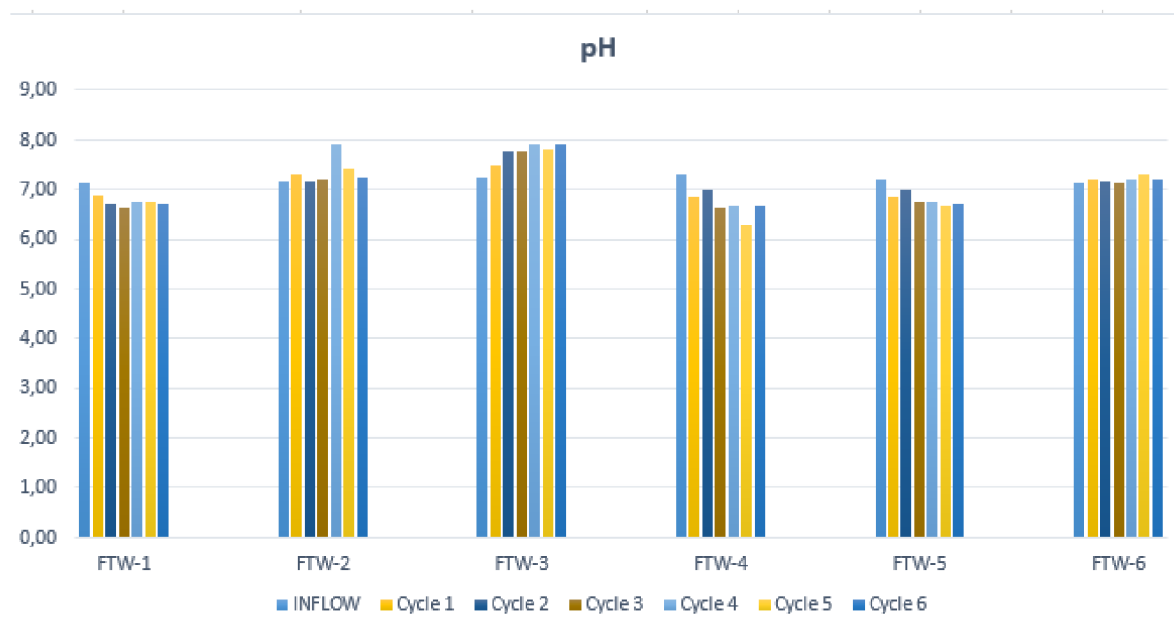


Figure n. 10– pH values of experiment pot during cycles.

The C/N ratio affects nitrogen cycling activities in FTWs. Increased C/N ratios could encourage denitrification, which lowers ORP by converting nitrate to nitrogen gas in anaerobic environments. On the other hand, lower C/N ratios can encourage nitrification, in which ammonia oxidizes to nitrate, using up oxygen and perhaps raising ORP. In continuous system, FTW-1 with C/N 1:5 was observed highest ORP values throughout the experiment.

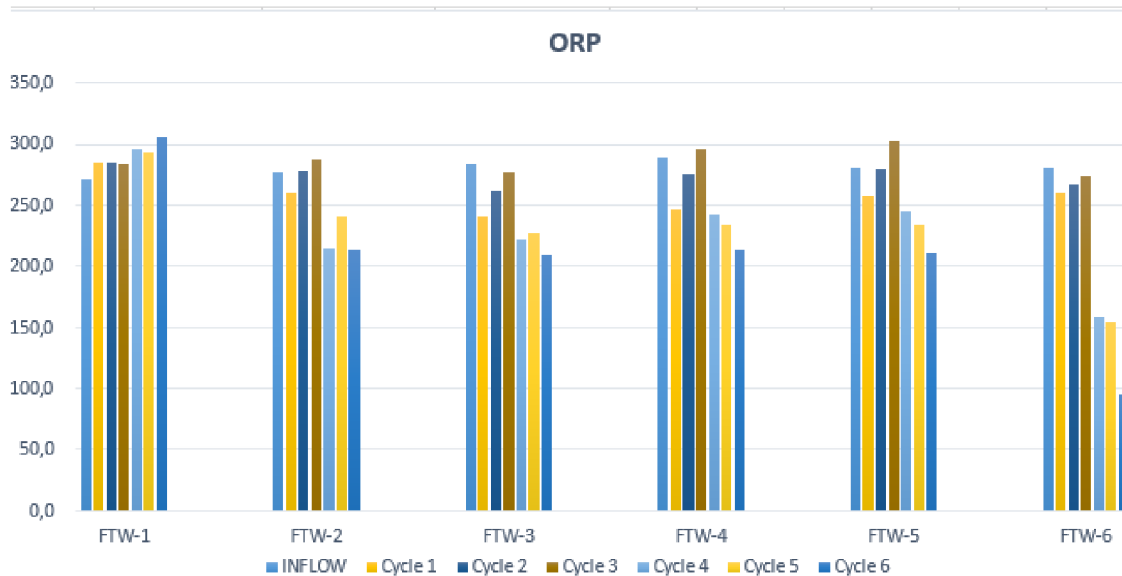


Figure n. 11– ORP values of experiment pot during cycles.

### 3.2 DO – Dissolved Oxygen

The effectiveness of the several biological and chemical processes involved in water treatment is strongly impacted by dissolved oxygen (DO), making it a crucial parameter in floating treatment wetlands (FTWs). Like other built wetlands, FTWs are dependent on aerobic processes for the breakdown of organic debris, the removal of nutrients, and the maintenance of microbial and plant populations. Nitrification is a crucial mechanism for removing nitrogen from FTWs. It involves the biological conversion of ammonia ( $\text{NH}_4^+$ ) to nitrate ( $\text{NO}_3^-$ ). Aerobic bacteria, which need oxygen to oxidize ammonia, carry out this activity. As a result, sufficient dissolved oxygen concentrations are required to promote nitrification and ease the removal of nitrogen. DO was gradually consumed as the influent was deeply purified in the later units, thus continuously expanding the anoxic environment and facilitating the denitrification reaction.

The DO value in our setup is with decreasing tendency even in continuous flow systems, with possible explanation of increased fertilizer concentrations in FTWs, especially those of nitrogen and phosphorus as they can promote the growth of algae. During the day, algae perform photosynthesis, which produces oxygen. However, algae need respiration to take in

oxygen throughout the night or during algal blooms, which might result in oxygen depletion if generation of oxygen does not outweigh consumption.

Pots simulating continuous flow treatment wetland have higher concentration values of dissolved oxygen that pots with intermittent flow. Water flows through continuous flow treatment wetlands continually, supplying a steady supply of oxygen from the atmosphere. This continuous flow contributes to the system's overall greater dissolved oxygen levels.

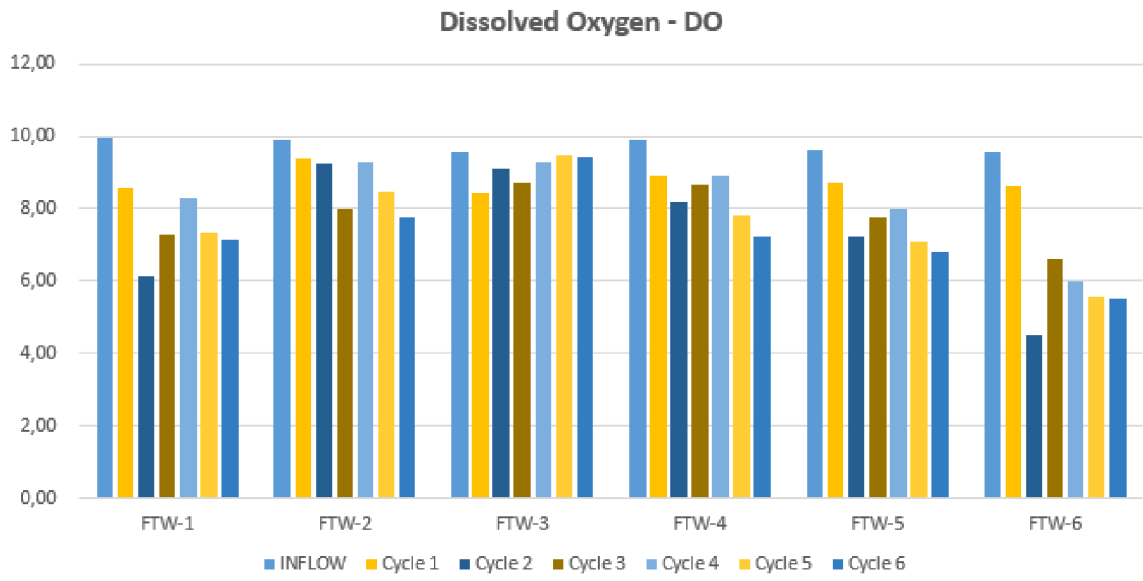
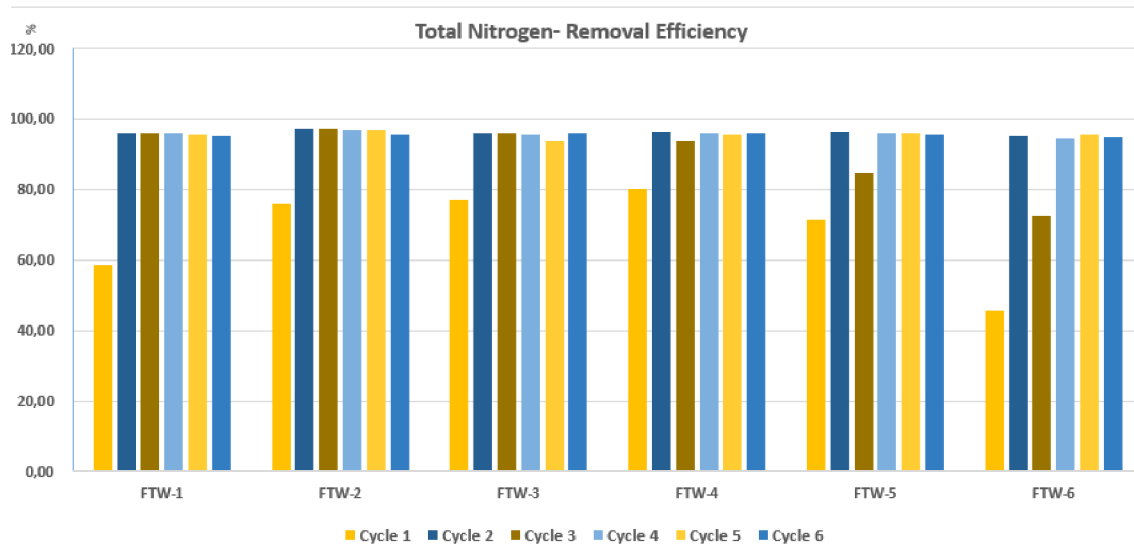


Figure n. 12– DO values of experiment pot during cycles.

### 3.3 Total Nitrogen (TN)

By utilizing a variety of techniques, such as plant absorption, microbiological processes, and sedimentation, floating constructed wetlands (FCWs) can be successful in eliminating nitrogen. However, a number of variables can affect how much nitrogen is removed precisely from FCWs. Although studies have shown removal efficiencies ranging from 30% to 90% depending on the particular design and operational factors, the effectiveness of nitrogen removal in FCWs can vary greatly. The following resulting graph of our experiment shows us Total Nitrogen (TN) removal efficiency in both intermittent flow and continuous flow after Cycle 2 exceeding 95%. The data shows, that the removal efficiency of both IFFWS and CFFWS may participate in a very efficient removal of TN in the ecosystem for all types of different C/N concentrations.

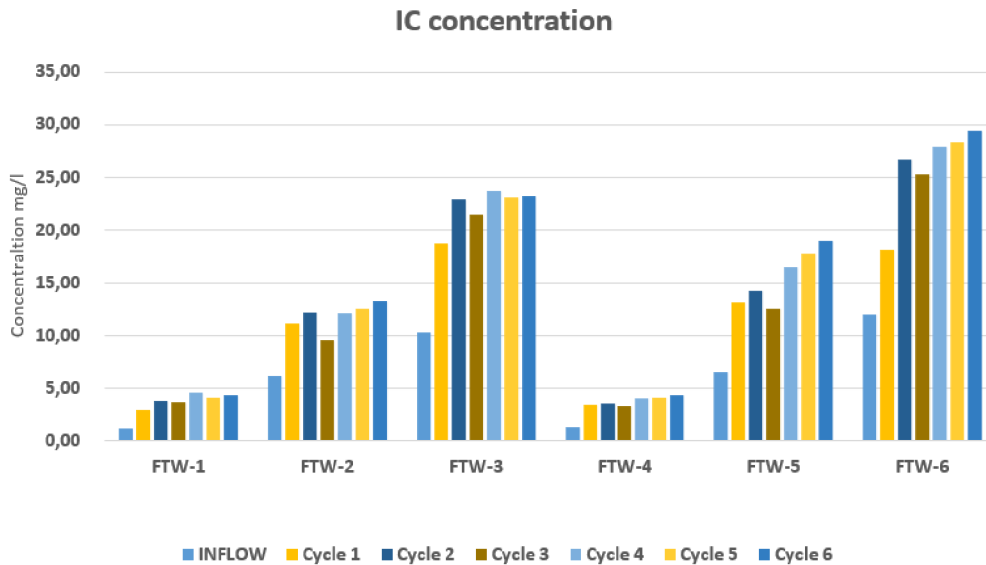




*Figure n. 13– Total Nitrogen removal efficiency.*

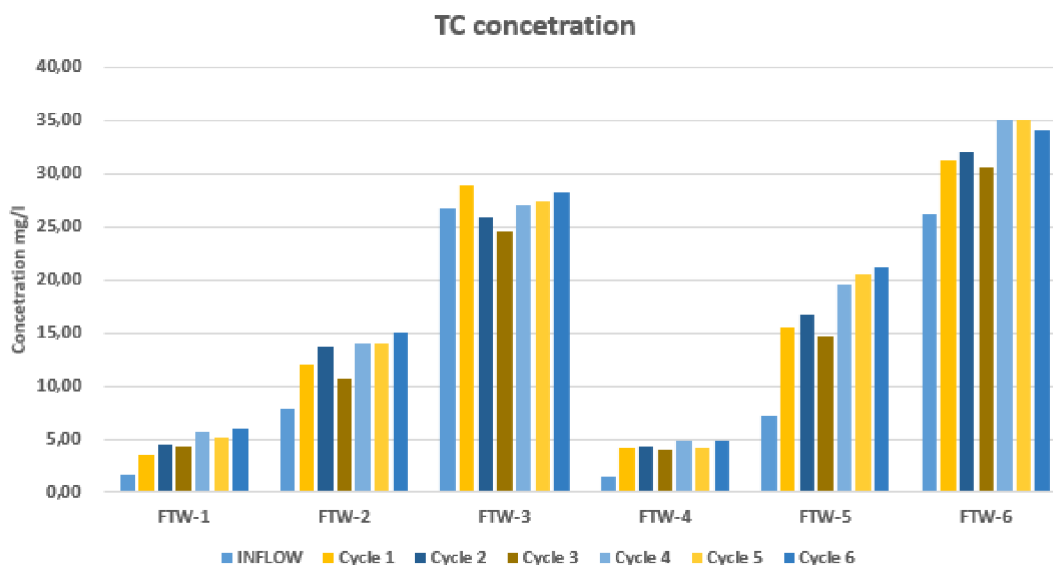
### 3.4 TC, IC and TOC

It is crucial to find and handle the underlying reasons of the poor mass removal efficiencies of TC and IC in CWs by using appropriate design, operating, and maintenance procedures. In order to do this, it could be necessary to balance nutrient inputs, ensure appropriate substrate conditions, improve flow dispersion, optimize hydraulic loading rates, and take seasonal fluctuations in treatment efficacy into consideration. It is essential to regularly monitor influent and effluent TC concentrations in addition to other pertinent water quality indicators in order to evaluate the efficacy of treatment and make the required modifications to improve pollutant removal. Both TC and IC concentrations were increased indicating that the effluent is higher than in the influent, indicating that the wetland is not effectively reducing the TC and IC load.



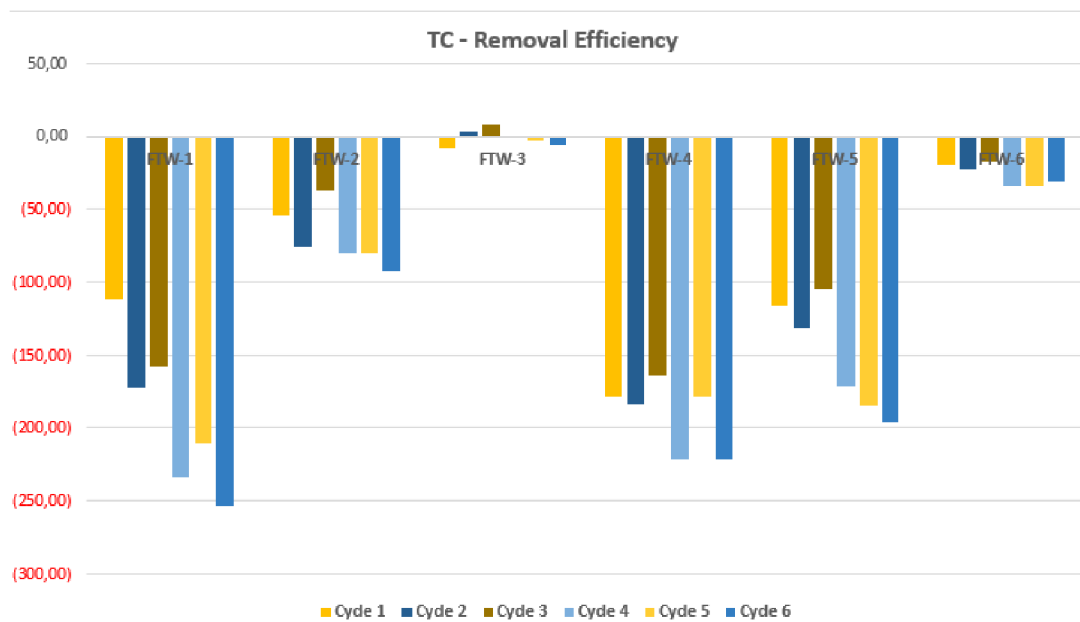
*Figure n. 14– IC concentration mg/l*

As shown in Figure n.13 and Figure n.14, the availability of carbon in FTWs in relation to nitrogen is influenced by different pollutant ratios. Greater carbon availability relative to nitrogen is indicated by greater C/N proportion, whereas lower ratios suggest the reverse. Higher C/N concentration in FTWs may have more carbon accessible for microbial activity, which results in a rise in the synthesis or release of inorganic carbon molecules. As the results show, IC and TC of pots with ratio 5:1 (FTW-3, FTW-6) have the highest concentration of Inorganic Carbon available. Wetlands with continuous flow system have higher ability to decrease the amount of IC and TC in comparison to the systems with intermittent flow.



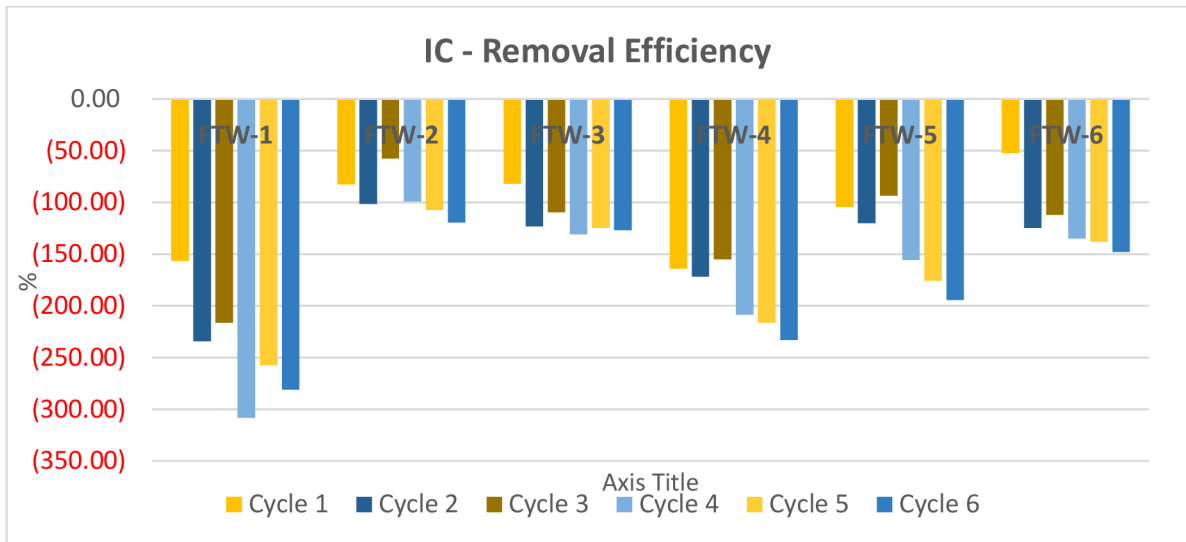
*Figure n. 15– Total Carbon concentration mg/l*





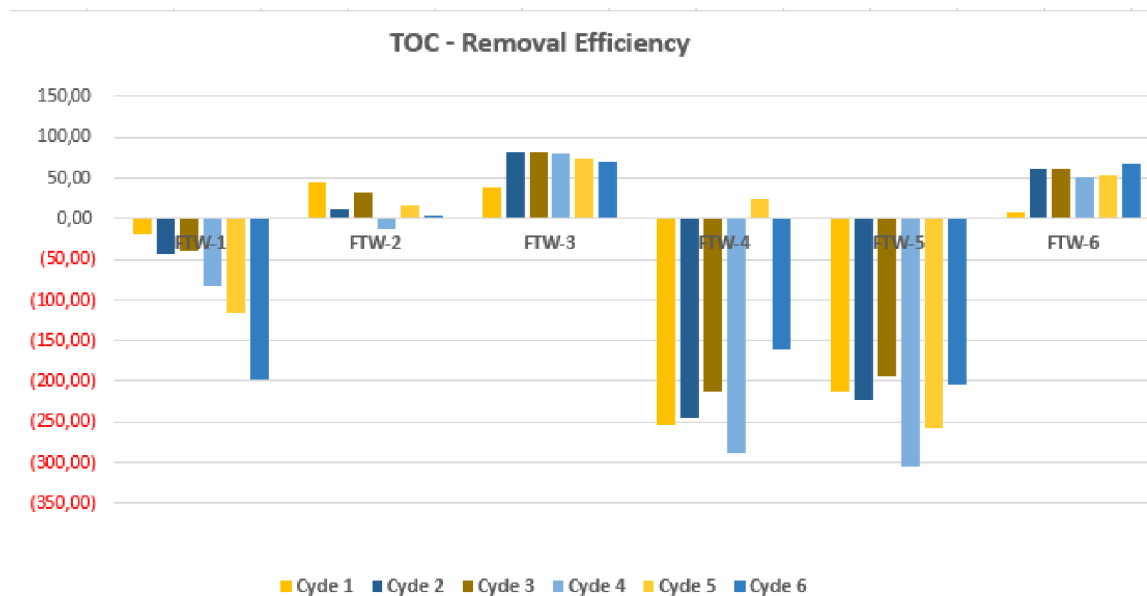
*Figure n. 16– Total Carbon removal efficiency.*

The high carbon concentration of the pollutants may have completely saturated the water-soil matrix, preventing it from extracting carbon as effectively, which might be one reason for this situation. The TC removal efficiency was found to be lower in the FTWs with a C/N ratio of 1:5, suggesting that the system was less successful in eliminating carbonaceous substances from the water. Pots with the ration of C/N 5:1 (FTW-3, FTW-6) have the highest removal efficiency rate of TC. In this case, the ratio of carbon to nitrogen may have been more advantageous for encouraging the TC elimination processes of denitrification or microbial carbon absorption. Continuous flow system with C/N 1:1 and 5:1 show better ability of TC removal in comparison to system with intermittent flow. Overall, while intermittent flow systems are often associated with enhanced TC removal due to their dynamic conditions, the specific characteristics of the continuous flow systems in this study, such as optimized nutrient availability, oxygenation, hydraulic residence time, and microbial community adaptation, may have collectively contributed to their superior TC removal ability.



*Figure n. 17– Inorganic Carbon removal efficiency.*

For both TC and IC removal efficiency, CFFWs resulted as more efficient with lower effluent concentration after Cycle 6. This may be contributed to continuous oxygenation of water, consistent hydraulic conditions and increased oxygen availability as shown in table of dissolved oxygen.



*Figure n. 18– Total Organic Carbon (TOC) removal efficiency.*

TOC includes dissolved, particulate, and colloidal forms of organic carbon compounds of the system. The TOC removal efficiency of was achieved by the CFFWs in a higher removal rate that the IFWs. Higher oxygen transfer rate and mixing of the water column may be one of the explanations of the higher removal rate of the continuous flow systems which

were also under constant inflow of pollutants into the system. Pots with the ration of C/N 5:1 (FTW-3, FTW-6) have the highest removal efficiency rate of TOC. The observation that in a continuous flow system, FTW-2 exhibits much higher removal efficiency of total organic carbon (TOC) compared to FTW-5, despite having the same concentration of C/N ratio. Differences in oxygen availability within the wetland substrate between FTW-2 and FTW-5 could affect microbial processes involved in TOC removal. FTW-2 has better oxygenation (as shown in Fig. n.12), promoting aerobic degradation of organic carbon compounds.

### 3.5 Phosphate removal efficiency

Wetlands can effectively remove phosphate ( $PO_4^{3-}$ ) through various physical, chemical, and biological processes such as already mentioned adsorption, precipitation, plant uptake, microbial processes, sorption by organic matter and hydraulic retention time. In both, CFFWs and IFWs we can spot a very high rate of phosphate removal efficiency.

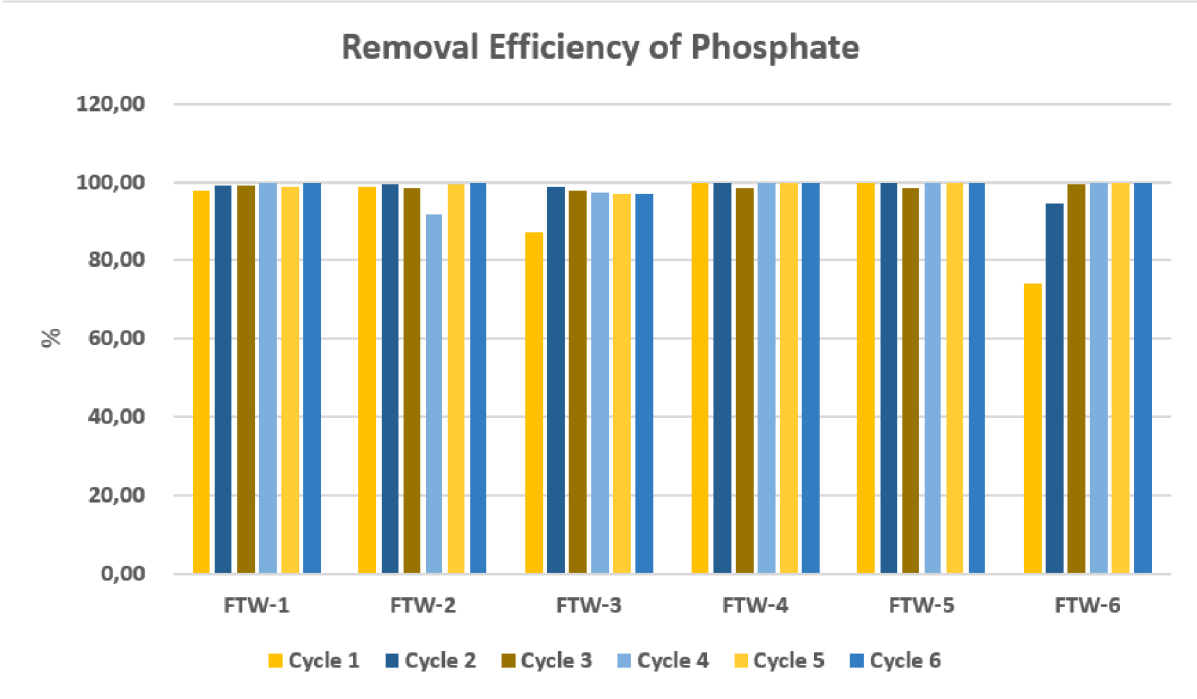


Figure n. 19- Phosphate removal efficiency

### 3.6 Photochemical analysis

In general, photochemical analysis is essential to comprehending how wetland ecosystems in treatment systems work, are healthy, and are resilient. Photochemical analysis helps to manage and conserve wetland habitats and its important ecosystem functions by offering insightful information about photosynthetic activity and plant responses to environmental factors and stressors. After the sample has been dark-adapted for a while, dark F0 ( $F_{0,d}$ ) is measured. Alterations in the photosynthetic system may be indicated by changes in  $F_{0,d}$  over time or in response to stressors. In our results, we can observe only smaller changes in the  $F_{0,d}$  values. The biggest alteration is visible in pots with intermittent flow FTW-4 and FTW-6 as shown in Fig. n. 18.

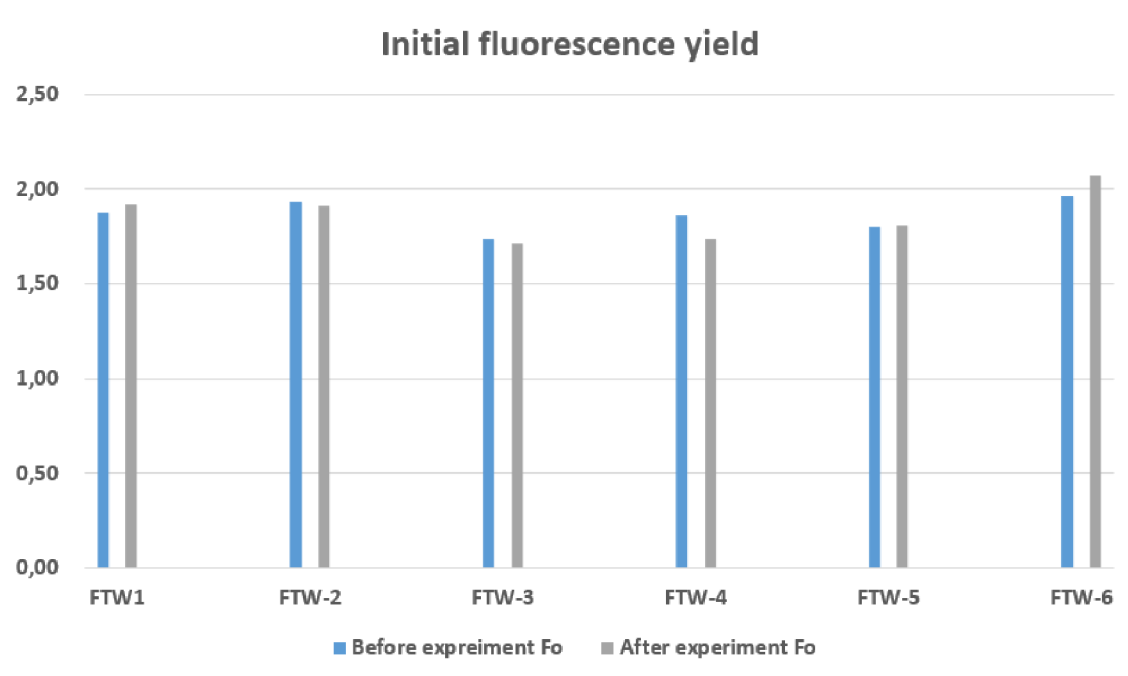
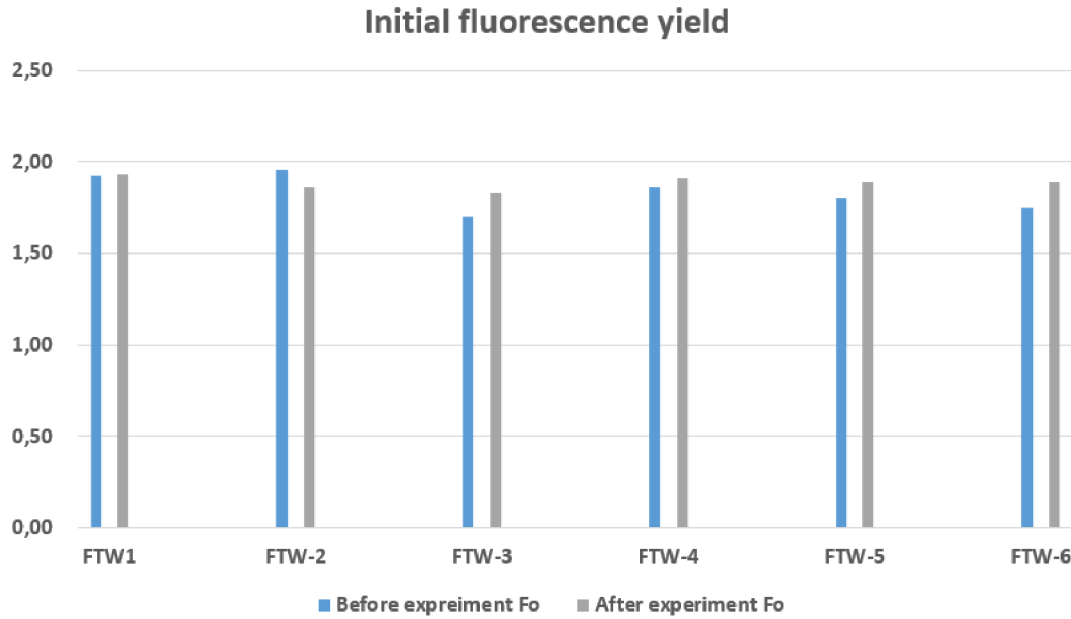
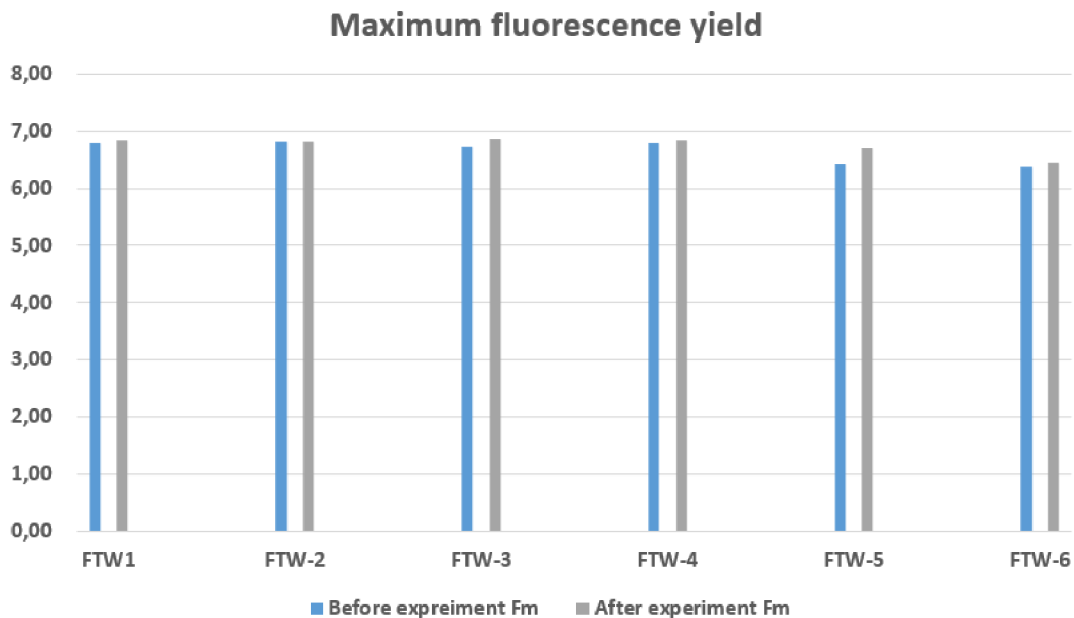


Figure n. 20- Initial fluorescence yield  $F_{0,d}$ – dark light



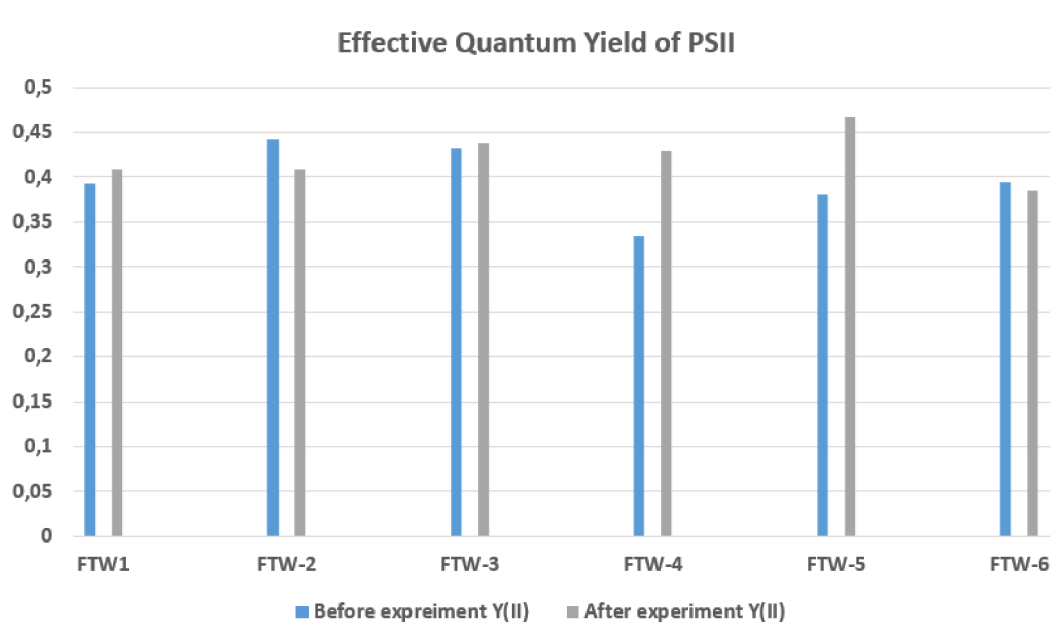
*Figure n. 21-Initial fluorescence yield  $F_0$  – light*

In figure n. 19 we can observe initial fluorescence yield under light condition when sample has been exposed to actinic light. The biggest differences before and after the experiment were observed in pots with C/N ratios 1:5.



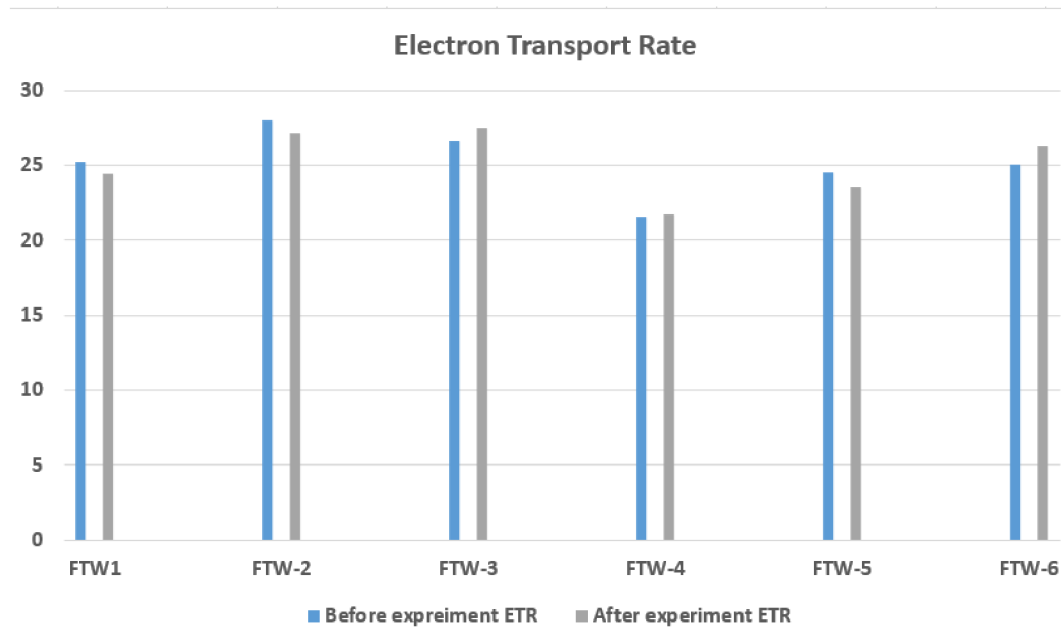
*Figure n. 22– Maximum fluorescence yield – light*

A vital metric in the measurement of chlorophyll fluorescence,  $F_m$  gives important details regarding the highest fluorescence yield that can be obtained under dark-adapted settings. In the Figure n. 20 we can see the before and after experiment  $F_m$  values.



*Figure n. 23- Effective Quantum Yield of PSII ( Y(II)) – light*

When evaluating PSII real photosynthetic activity and efficiency in different environmental settings, Y(II) is a useful indicator which provides light on PSII (photosystem II) photochemical energy conversion efficiency. As shown in the Fig n. 21, after experiment result of FTW1, FTW-3, FTW-4 and FTW-5 values are increased which may be caused due to light intensity or higher nutrient amount available in the systems. In the systems of intermittent flow, Y(II) values are often greater at higher CO<sub>2</sub> concentrations because PSII is better able to use absorbed photons for carbon fixation.



*Figure n. 24– Electron transport rate (ETR) – light*

To determine the rate of linear electron transport through PSII, a computed parameter called ETR is used. It offers details on PSII's total electron transport and photosynthetic process driving capability. Higher CO<sub>2</sub> concentrations may cause higher values of ETR as shown in the Fig. n. 22, intermittent flow pots (FTW-4, FTW-6). Lower CO<sub>2</sub> concentration may cause lower ETR values as shown in continuous flow pots (FTW-1, FTW-2). For maximum photosynthetic activity and electron transport, there must be an adequate supply of vital nutrients, especially nitrogen and phosphorus. Reduced ETR can result from nutrient deficits that affect electron transport and enzyme activity. (Baker, 2008)

## 4. Conclusion

In conclusion, the utilization of continuous flow floating treatment wetlands (CFFTWs) to treat agricultural runoff with different carbon-to-nitrogen (C/N) ratios highlights the intricate relationship between nutrient dynamics, plant-microbe interactions, and the resulting water quality. It is clear from our research that C/N ratios affect the effectiveness of nutrient removal as well as the overall treatment performance in FTWs. The variation in removal efficiency across various parameters highlights the complex interactions between biological, chemical, and physical processes in FTWs. The limited removal of TC, IC, and TOC indicates potential difficulties in addressing microbial contaminants and organic carbon in agricultural runoff. On the other hand, the observed high removal efficiencies for TN and phosphate can be attributed to the efficient utilization of nitrogen and phosphorus by microbial and plant communities.

The differences in removal efficiencies that have been reported might be caused by a number of factors. Removal mechanisms for nitrogen and phosphorus, including as denitrification, plant absorption, and precipitation, may be more dominant than those for microbial contaminants and organic carbon, which might obscure their effects. Furthermore, differing substrate properties, hydraulic loading rates, and plant compositions may have distinct effects on the dynamics of nutrients and contaminants, resulting in diverse treatment outcomes. Our results highlight the necessity of a thorough comprehension and optimization of FTWs design and operation in order to efficiently handle a wider range of water quality factors. Enhancing hydraulic residence time, maximizing substrate composition, and introducing a variety of plant species are some strategies that may be used to increase microbial activity, improve the breakdown of organic carbon, and improve the removal efficiency of microbial pollutants. Our research also emphasizes how crucial it is to take site-specific variables into account and modify treatment strategies in order to successfully achieve water quality objectives. Mitigating nutrient pollution requires TN and phosphate removal, but organic carbon and microbiological pollutants still need to be addressed to protect human health and ecological integrity.

In conclusion, even though the removal of phosphate and TN from agricultural runoff in FTWs with varying C/N ratios showed encouraging results, more management and research



work is required to maximize treatment efficiency and address a wider range of water quality issues in agricultural landscapes, such as microbial contaminants and organic carbon.

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