Biological Water Purification

The effect of growing aquatic plants as *Eichhornia crassipes, Pistia stratiotes, Stratiotes aloides, Salvinia natans* on the nutrient removal of treated domestic waste water

Bachelor Thesis

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Abstract

Industrial, agricultural, and domestic sectors have an impact on the pollution of surface waters. More specifically, discharges of wastewater treatment plants also have a major impact on the quality of surface waters. According to PBL Netherlands Environmental Assessment Agency, Dutch surface waters do not meet the limit values of the Water Framework Directive. This study aims to determine if a post-purification technique with different aquatic plant species can successfully purify treated effluent from a domestic wastewater treatment plant, in order to meet the nitrogen and phosphorus limit values.

To test the main hypothesis that plant treatments with the highest wet weight production also have the highest removal efficiency of nutrients (ammonium, nitrate, and phosphate), a field experiment with floating, aquatic plants is carried out. During the field experiment *Eichhornia crassipes* (water hyacinth), *Pistia stratiotes* (water lettuce), *Stratiotes aloides* (water soldier), and *Salvinia natans* (floating fern) were researched. Additionally to the four plant species, two control series called treatment CC (Control-Control) and treatment CU (Control-UVC source) were added, to make a total of six treatments. The six treatments were monitored for twice 14 days on water conditions as pH, dissolved oxygen concentration, thermal conditions, bicarbonate and carbon dioxide concentrations, elements, and nutrients (ammonium, nitrate, and phosphate).

Nonetheless, for this specific research report only the first period of 14 days is taken into account. The results of the gathered data showed that there is not one main conclusion to this experiment. The conclusion is dependent on different visions of the aspects as wet weight production, removal efficiency of nutrients, and the Water Framework Directive limit values. The results suggest that over a short period of 14 days *Salvinia natans*, also known as floating fern, ensured that the total nitrogen and phosphorus limit values were met in the best way.

Preface

This is a Bachelor thesis with the title: ‘The effect of growing aquatic plants as *Eichhornia crassipes, Pistia stratiotes, Stratiotes aloides, Salvinia natans* on the nutrient removal of treated domestic waste water. A study with applied research on the topic of biological water purification, wet weight production, and nutrient removal. Likewise, the end product of the study program Aquatic Ecotechnology at HZ University of Applied Sciences, Middelburg, the Netherlands.

A research study carried out in cooperation with Radboud University Nijmegen over a time period of February 2021 until June 2021. As part of the research study I consecutively monitored the water conditions of the experiment over a time span of four weeks.

First of all, I would like to thank my in-company supervisors Annelies Veraart and Lisanne Hendriks with whom I had a pleasant cooperation during my internship period. Besides that, I would like to the thank the staff members of the greenhouse and the department of Aquatic Ecology and Environmental Biology, people who were always there for you. Furthermore, I would like to thank my first examinator Anne Oele for the useful conversations and helpful feedback.

Enjoy your reading.

Willemijn Elzinga
Nijmegen, June 2021

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List of abbreviations

AA Samples
AutoAnalyzer samples

CBS
Centraal Bureau voor de Statistiek or Central Agency for Statistics

ICP Samples
Inductively Coupled Plasma samples

IUCN Red List
International Union for Conservation of Nature and Natural Resources

PBL
Planbureau voor de Leefomgeving or the Netherlands Environmental Assessment Agency

RIVM
Rijksinstituut voor Volksgezondheid en Milieu or the National Institute for Public Health and Environment

Treatment CC
Control – control, no plants and no UVC-lightning source

Treatment CU
Control – UVC-lightning source, no plant but with UVC-lightning source

Treatment EC
Eichhornia crassipes (water hyacinth) plant treatment with UVC-lightning source

Treatment PS
Pistia stratiotes (water lettuce) plant treatment with UVC-lightning source

Treatment SA
Stratiotes aloides (water soldier) plant treatment with UVC-lightning source

Treatment SN
Salvinia natans (floating fern) plant treatment with UVC-lightning source

WFD
Water Framework Directive or Kaderrichtlijn Water

WUR
Wageningen University and Research

1. Introduction

1.1 Context

Stagnant or flowing water, fresh, salt or brackish water, isolated water bodies or open water bodies. The world, but in this case more importantly the Netherlands, has a wide variety in surface waters (CBS, PBL, RIVM, WUR, 2009). Surface waters have a major function in society, therefore it is important that the quality of water bodies is maintained (RIVM, 2019).
In a report by the PBL Netherlands Environmental Assessment Agency (2014), Dutch surface waters have been assessed on chemical and ecological water quality. For both measures it appeared that the water quality does not meet the limit values of the Water Framework Directive. The Water Framework Directive consists of agreements, at European level, in order to ensure that surface waters will be chemically clean and ecologically balanced in 2027 (CBS, PBL, RIVM, WUR, 2020).

It is known that the industrial, agricultural, and domestic sector have an impact on the pollution of surface waters. However, the discharge of waste water treatment plant effluent also has a significant effect on the quality of surface waters (Klein, Rozemeijer, & Mul, 2016). In fact, they discharge their effluent directly into surface waters. In many cases, the concentration of nitrogen and phosphorus in the treated effluent is considerably high with eutrophication as an important negative result (Centraal Bureau voor de Statistiek, 2017).

Table 1- Overview of the difference between the limit values of the Water Framework Directive and the measured values of surface waters (European Water Framework Directive, 2020).

	Nitrogen concentration (mg/L)	Phosphorus concentration (mg/L)
Limit values Water Framework Directive	4-8 mg/L	< 0.42 mg/L
Values treated effluent Waterboard Rivierenland	15 mg/L	2.0 mg/L

1.2 Problem statement

According to the Dutch Ministry of Infrastructure and Water Management (2021), a sufficient water quality is vital for people, animals and plants. Ecologically and chemically clean water is needed for drinking water companies, agriculture, fisheries, industry, nature, and recreation. Therefore, the water quality of, in this case, Dutch surface waters needs to be improved so the limit values of the Water Framework Directive can be reached.
However, the measured values of the concentration of total phosphorus and total nitrogen in the treated effluent are still too high to be discharged into surface waters (Table 1) (Schuijt, Van Bergen, Verdonschot, Smolders, & Lamers, 2018).

In the past, several post-purification techniques were applied in the water technology sector, think of aeration or filtering. Over time, more innovative treatment techniques emerged, among which the self-purification principle. This principle is the process in which organic waste is broken down by plants or animals. (UNESCO, 1982). A good example of an innovative post-purification technique that already is applied in the Netherlands, is the Biomakerij. The Biomakerij consists of a small waste water treatment plant where they treat brewery waste water with plant roots and synthetic roots (Heijden, van der, 2018). However, an experiment with a successful post-purification technique for domestic waste water does not exist yet, because the technique is a compact treatment system. In the *theoretical framework* of this research study, alternatives regarding purification techniques will be discussed in more detail.

1.3 Research goal

Because of this, it matters that an innovative water purification technique is extensively tested.
The goal of the experiment is to successfully post-purify the excessive nitrogen and phosphorus concentrations out of the treated effluent from a domestic waste water treatment plant. This has been done by an experiment with four types of floating aquatic plant species, which have been monitored twice over a time period of 14 days.
During the field experiment different plant cultures are researched. More specifically:
Eichhornia crassipes
Pistia stratiotes
Stratiotes aloides
Salvinia natans

1.4 Research question and sub questions

The most important parameters during this experiment are the plant growth and the nutrient removal of the effluent. At the end of the experiment, there will be concluded if there are realistic possibilities regarding the use of floating, aquatic plants as a post-purification technique, with the use of the following research question:

'Which plant treatment removes the highest concentration of nitrogen and phosphorus from the treated domestic waste water over a period of 14 days?'

In order to answer the main research question, the following sub questions have to be answered first in the theoretical framework or during the field experiment. The sub questions are as follows:

Is it possible for a plant treatment to have a high vegetative growth, but a low removal efficiency of nutrients in the specific time limits and circumstances?
Which plant species has the highest wet weight production in grams?
Which plant species has overall the highest removal efficiency of nutrients?
Which plant treatment meets the limit values of the Water Framework Directive the best at the end of period 1?

1.5 Hypothesis

It is expected that the plant treatment with the highest wet weight production has the highest removal efficiency of nutrients and so the highest concentration of nitrogen and phosphorus removal. In addition, *Eichhornia crassipes*, also known as water hyacinth, is expected to perform well by thriving in polluted and nutrient rich waters.

1.6 Thesis structure

The research study begins with a theoretical framework where the background regarding water quality, choice of plant species, characteristics of plant species, and chemical factors will be examined. The research study will lead to the methodology where quantitative and qualitative research are explained and how they are connected to the appendices. Next on, in the observations, the findings of the conditions in the water are projected. After that, the data results of the wet weight production and nutrients are analyzed and explained in more detail. Followed by a conclusion, discussion, and suitable recommendations.

2. Theoretical framework

Within the chapter theoretical framework the findings of previous experiments are discussed. As well as factors that influence the water quality, substantiation for the choice of plants and their characteristics, the parameters that influence the conditions of the water and the growth of aquatic vegetation.

2.1 Factors that influence the water quality of Dutch surface water

As mentioned in the introduction of this research study, the water quality of surface waters in the Netherlands is not only influenced by discharges more upstream, but also by the influence of the industrial, agricultural, and/or domestic sector. The concentration of phosphorus and nitrogen is in several places high, with eutrophication as a result that should not be forgotten.
Globally, eutrophication is an increasing problem for a lot of water bodies in highly populated areas. The process of eutrophication arises when excessive nutrients are discharged or leached into water bodies, which can lead to a shortage of oxygen and excessive algal blooms. Since the Netherlands is a quite populated country, and also surrounded by other highly populated countries, the impact of a bad water quality can have disastrous effects on society. (Rozemeijer, 2016)

In case of this research study, effluent of a domestic waste water treatment plant in Rhenen (Gelderland), the Netherlands, is researched. According to Hoogheemraadschap Stichtse Rijnlanden (n.d.) the domestic waste water treatment plant discharges the effluent in the Nederrijn. The Nederrijn comes originally from Switzerland, but crosses more countries as Germany and France (Ministerie van Infrastructuur en Waterstaat, 2020b). That is why the chance is high that more upstream the water quality of the river is already influenced by leaching or discharges. Hence, it is important that the Water Framework Directive limit values are met in catchment areas.

In the Netherlands, the Nederrijn splits into two branches, namely the Lek and the Kromme Rijn. River Lek continues into the New Meuse, which flows into the North Sea (Ministry of Infrastructure and Water Management, 2020a). Because all water bodies are connected with each other, it is important to aim for a good ecological water quality status. It is relevant to monitor the quality of discharged water. In this case, they can come up with innovative solutions to improve the quality of discharged effluent from the domestic waste water treatment plant in Rhenen.

2.2 Conventional wastewater treatment and the Aquafarm principle

Most of the wastewater treatment plants in the Netherlands still run conventional water treatment, which mainly consists of primary and secondary treatment with possibly a tertiary and quaternary treatment step. However, the tertiary and quaternary treatment step are only applied when the quality of the effluent water is not sufficient. For this research study, a conventional wastewater treatment plant in Rhenen has a primary and secondary treatment process, figure 1.

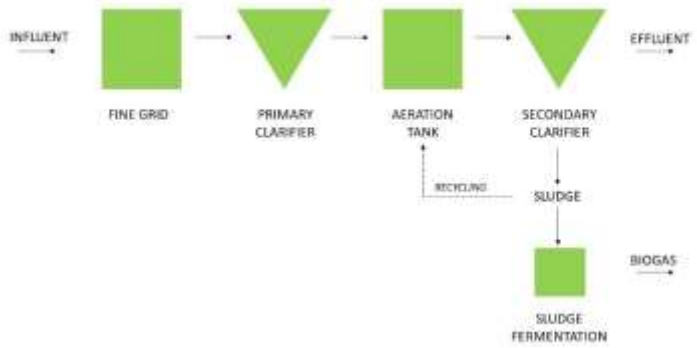

Figure 1- Treatment train of a conventional wastewater treatment plant.

Primary treatment consists of a fine grid system and a primary clarifier, where the rough and heavy particles such as paper and plastic are removed. In the secondary treatment process, an aeration tank is present where sludge removes residues, nutrients, and organic material. Sludge and water are separated in the secondary clarifier. The discharged water, now called effluent, leaves the wastewater treatment plant and is discharged into a surface water body, for example the Rhine.

Possible tertiary and quaternary treatment steps are a rapid sand filter, chemical additives as iron chloride or biological purification. A relevant example of an innovative post-purification technique is the Biomakerij that is applied in the Netherlands. The Biomakerij consists of a small waste water treatment plant where they treat waste water from brewery Koningshoeven. The treatment process is expressed by micro-organisms with plant roots and synthetic roots, which undergo metabolic processes with the wastewater from the brewery. After this treatment, the effluent is ready to be discharged in surface waters. In the means of biological purification, the Aquafarm principle is upcoming which is in some ways similar to the Biomakerij. (Heijden, van der, 2018)

The main idea of the Aquafarm principle is to realize complementary water treatment and biomass production by growing aquatic plants and animals on wastewater. Originally, the principle is based on plants and animals. However, in this research study only aquatic plants are researched. Nutrients are accumulated by aquatic plants (self-purification) and retained as biomass. By harvesting the plants later on, the nutrients can be removed from the water permanently. In the case of aquatic plants, nutrients are directly removed from the water by plant growth. Another way of removing nutrients, is indirectly through the interaction of plants with their surrounding environment. In general, the rhizosphere has a high oxygen concentration which is due to the loss of oxygen by roots. Thus, bacteria and archaea, whom are dependent on oxygen, grow in the rhizosphere, and take up-, and transform these nutrients. Whereas helophytes in general have a high growth rate, floating plants have a higher rate of accumulation of nutrients. In addition, floating plants decrease the amount of light available in the water column, which prevents the growth of algae and cyanobacteria in the toxic water layer. (Schuijt et al., 2018)

2.3 Substantiation for the choice of plants

According to a research paper about floating aquatic plants and nutrient removal from wastewaters, aquatic plants are widely used for nutrient and heavy metal removal from different types of waste waters (Muradov et al., 2014; Seo et al, 2010; Tel-Or and Forni, 2011).
In addition, different types of plant species have different nitrogen and phosphorus removal capacities (Iamchaturapatr et al., 2007). Furthermore, treatments of wastewater with floating aquatic plants are more desirable, because these plants have a high growth rate, are easy to maintain, and are easy to harvest after the treatment (Tel-Or and Forni, 2011).

Throughout the previous experiment, fast growing aquatic plants were used. Not all these aquatic plants belonged to the same classification. The selected plants were a combination of emerged, submerged, and floating aquatic plants. Plant species as *Lemna minor*, *Ceratophyllum demersum*, *Callitriche platycarpa*, *Azolla filiculoides*, were used. According to the previous experiment, which came to an end in January 2021, it can be concluded that *L. minor* and *C. demersum* are suitable plant species that can be used for future experiments. However, for the current research study, other aquatic plants were selected.

In this research study the following species have been chosen:
Eichhornia crassipes: water hyacinth
Pistia stratiotes: water lettuce
Stratiotes aloides: water soldiers
Salvinia natans: floating fern

All above stated plant species are non-rooted, floating, aquatic plants. In the upcoming paragraphs, the plant species will be discussed in more detail.

2.3.1 Defining characteristics for research

In table 2, the main characteristics for the researched plant species are shown by category.

Table 2- Characteristics for experimental research of the plant species

Plant species	Presence	Main accumulation	Vegetative growth	Climate	Other
Eichhornia crassipes	Eutrophic water bodies	Grows on high nitrate levels. Accumulation of total nitrogen: NH_4-N, NH_3-N	Number of plants can double within a week. Can occupy the entire water column with dense mats	Tropical climates	IUCN's list of 100 most invasive species
Pistia stratiotes	Rapid growth in water bodies with high nutrient levels	Accumulation of PO_4^{3-}, SO_4^{2-}, NO_3^-	Expand easily to horizontal coverage and forms dense mats	Tropical climates	
Stratiotes aloides	High CO_2 and inorganic nitrogen concentrations, but failure in growth with polluted waters	Accumulation of total nitrogen: NH_4-N, NH_3-N	Dominant and covers the water surface with dense mats of rosettes	Climates with a clear winter and summer season: continental and oceanic climate	
Salvinia natans	Stagnant water bodies	Accumulation of total nitrogen: NH_4-N, NH_3-N	Rapid dispersal rate, can result in complete coverage of stagnant water bodies	Optimal temperature of 25-30 degrees °C	

2.3.2 *Eichhornia crassipes*

The water hyacinth, see figure 2, is a floating invasive macrophyte, that is native of the Amazon basis. The plant is on the IUCN's list of the 100 most invasive species. Most of the problems are the result of rapid and aggressive growth, high rate of successful competition, and ease of propagation. (Téllez et al., 2008) These problems are quite negative in the means of a natural ecosystem. However, for the Aquafarm principle the growth of *E. crassipes* is beneficial.

Figure 2- Eichhornia crassipes: Water hyacinth illustration. (University of the Western Cape, 2017)

E. crassipes reproduces vegetatively through the formation of stolon's and propagates through seeds, which can survive in the water for many years. Under suitable conditions, the number of plants can double within a week. The water hyacinth is prevalent in eutrophic water bodies and is able to form dense mats, covering large areas of water bodies. (Su, Sun, Xia, Wen, & Yao, 2018)

The root length of the plant varies between 5 cm and 100 cm, which occupies the entire water column. High nitrate levels are mainly responsible for the growth of this plant. A research study by Wang et al. (2017) confirmed the plant species to purify water by removing excess nitrogen and phosphorus. In addition, it has been known that total nitrogen, COD, NH_3-N, NH_4-N are effectively removed from water bodies.

2.3.3 *Pistia stratiotes*

Water lettuce, see figure 3, is a free-floating macrophyte (Odjegba & Fasidi, 2003). The plant has developed roots that are extended into the bottom or altered on the mud surface. The roots of *P. stratiotes* increase oxygen transport in the water. The root age and the growth status determine the alternative effects of roots and the dissolved oxygen concentration. In comparison with the water hyacinth, water lettuce has stiffer roots and roots with a length to 20 cm. The plant can grow rapidly in water bodies with a tropical climate and expands easily to horizontal coverage on water bodies. As a result, dense mats of water lettuce lead to water stratification and oxygen deficiency, because they shade the water from sunlight (Wang et al., 2017).

Figure 3- Pistia stratiotes: Water lettuce illustration. (Pistia stratiotes (lechuga de agua), n.d.)

Water lettuce is capable of lowering parameters regarding the water quality. It is proven that there is a visible improvement of PO_4^{3-}, SO_4^{2-}, NO_3^-, COD, BOD, and DO by 70%, if water lettuce is growing on water nutrient rich water bodies.This is mainly because the rapid growth of *P. stratiotes* demands a high nutrient level. (Théophile, 2002)

2.3.4 *Stratiotes aloides*

The water soldier, see figure 4, is a free-floating macrophyte which floats during summer and is submerged during winter. The plant sinks to the bottom of the water body during fall (Smolders, Lamers, Den Hartog, & Roelofs, 2003). The availability of CO_2 influences its growth. *S. aloides* has the best growth in water bodies with high CO_2 concentrations (Abeli, Rossi, Smolders & Orsenigo, 2014). Furthermore, as the species has a high growth rate, they accumulate a significant biomass. According to a research study by Kufel, Strzalek, Konieczna, and Izdebska (2010), *S. aloides* mainly exploits nutrients from the water when it is in its submerged, rootless stage during spring. In summer, *S. aloides* obtains nutrients from the bottom sediments through roots, which are independent on the absorption of nutrients from surface water.

Figure 4- Stratiotes aloides: Water soldier illustration. (Sowerby, 1869)

The species is known to be dominant and cover the water surface with a dense mat of rosettes up to 50 cm in height. The surface water where water soldiers disappear, are characterized by high inorganic nitrogen concentrations. However, the main reason of failure in growth is the pollution of surface waters. (Abeli et al., 2014)

Increased sulphate- and iron-mediated mechanisms seem to lead to increased phosphate mobilization from the sediment, which is known as internal eutrophication. Internal eutrophication can strongly affect the *S. aloides* vegetation (Smolders et al., 2003). However, in the current research study no substrate is used during the experiment. If no phosphate mobilization takes place total nitrogen, COD, NH_3-N, NH_4-N can be effectively removed from water bodies.

2.3.5 *Salvinia natans*

Salvinia natans, see figure 5, is a free-floating heterosporous fern that reproduces vegetatively. The fern has no true roots and is anisophyllous, which means that they produce two types of leaves. Each individual has a light floating leaf with hairs and brown rootlike submerged leaves. Since the fern has no real roots, the rootlike submerged leaves act the same way as a true root, as they absorb nutrients from the water. The roots are part of the sporocarps (reproductive organs) and desorb oxygen in the water. (Jampeetong & Brix, 2009)

A cluster of a maximum of 10 sporocarps is produced by each plant during summer when the water temperature is optimal, around 25 to 30 degrees Celsius. In winter, the sporocarps are detached and float on the surface of the water body. High temperatures and sufficient lightning during summer support a maximal biomass production of the plant. (Jampeetong & Brix, 2008)

The floating fern has a rapid dispersal rate. The vigorous growth and floating habits have resulted in complete coverage of stagnant water bodies with negative impacts on the ecosystem. *S. natans* has shown to prefer ammonium as nitrogen source for the uptake, because of the higher energetic costs associated with NO_3- uptake and the assimilation. (Zutshi & Vass, 1971)

Figure 5- Salvinia natans: Floating fern illustration. (Thomé, 1885)

2.4 Parameters that influence the conditions in the water and aquatic vegetation

Several water measurements were carried out throughout the experimental period. The most important measurements for this research study are: potential of hydrogen (pH), dissolved oxygen concentration, thermal conditions, bicarbonate and carbon dioxide concentration, nutrients as nitrogen and phosphorus. These parameters are indicative for the ecological state of the water.

2.4.1 Potential of Hydrogen

pH is a scale used to specify the acidity or basicity of an aqueous solution. The quantity of hydrogen or hydroxyl ions in a solution determines whether the solution is acid or alkaline. pH measures the relative alkalinity or acidity of a solution. Natural and human processes determine the pH of water bodies. (Water Quality Plus, n.d.)

The acidity has an important influence on the physiology of water organisms. A high pH, as a cause of an increasing buffer capacity, means that the decomposition of organic material accelerates with eutrophication and cloudiness as a result. The composition of macroflora species has a direct relation with the acidity, because species disappear when eutrophication takes place. In addition, a high pH can decrease the growth and development of plants. (Evers, 2007)

Acidification may occur as a result of leaching acidifying substances, for example ammonium. Acidification has, together with eutrophication, a significant impact on the water environment. By the change in pH, and so the change in nutrient balance, the biodiversity in water environments can increase. Depending on the optimum pH level of the water body, the pH increases or decreases. (Ecopedia, n.d.)

2.4.2 Bicarbonate and carbon dioxide concentrations

Furthermore, pH largely determines the form in which inorganic carbon is present in water. A pH lower than 6.4 ensures that all inorganic carbon is present in the form of CO_2. A pH higher than 6.4 ensures that all inorganic carbon is present in the form of HCO_3.

The dispersion of aquatic vegetation is mainly determined by the availability of alkalinity or, in other words, the acid binding capacity. Alkalinity of water is most of the time the result of bicarbonate alkalinity. Buffers are used to balance the pH of a solution, for this study the effluent water. Bicarbonate alkalinity occurs as a reaction of the presence of bicarbonate, which is formed in water when CO_2 molecules are brought into contact with carbonates. (Janssen & Freeman, 2017)
The acid binding capacity is mainly expressed by the (bi)carbonate concentration and plays a major role in the counteracting pH fluctuations. Besides that, alkalinity determines the rate of availability of inorganic carbon (CO and HCO_3), an important nutrient. Therefore, it can be said that alkalinity determines the distribution of aquatic plants. (Arts et al., 2007)

2.4.3 Dissolved oxygen concentration

Dissolved oxygen is an indicator for how much oxygen is dissolved in water. Oxygen dissolves in water as a result of diffusion of surrounding air, flow acceleration, and photosynthesis in water. The concentration of dissolved oxygen is influenced by thermal conditions, but also by phosphates and nitrates as they have an indirect positive effect on bacterial growth. The bacterial growth has a negative effect on the dissolved oxygen concentration of the water. Oxygen is a major indicator for the livability of water environments and so the water quality. (Lenntech, n.d.)

A low oxygen concentration in the water can lead to the death of aquatic organisms. Whereas, a high oxygen concentration can be an indicator for the growth of algae. (Vonk et al., 2008)
However, according to a research study by Miranda and Hodges (1999), macroflora affects physical and chemical conditions of the water. Respiration by macroflora can reduce dissolved oxygen concentrations, especially at night and warmer months. In addition, dissolved oxygen concentrations were inversely related with vegetation coverage. Dissolved oxygen concentrations dropped rapidly as vegetation coverage increased.

2.4.4 Thermal conditions

Thermal conditions express themselves occasionally in high temperatures (Evers, 2007). Water temperature is measured in degrees Celsius. The rate of plant growth and development is dependent upon the temperature surrounding the plant. Each plant species has a specific temperature range with an optimal temperature. How a plant species responds to temperature, differs among various species throughout their life cycle. In general, the vegetative development increases as temperature rises to the species optimum level. (Hatfield & Prueger, 2015)

2.4.5 Nitrogen

Aquatic plants can absorb nitrogen in two forms: ammonium and nitrate. Some aquatic plants prefer either one or two forms, but some plants even take up both nitrogen forms. Next to the different nitrogen forms, there are also two different methods which plants absorb their nitrogen, namely: by soil and/or by water. In the case of this research study, floating aquatic plants were selected which do not root in substrate. All researched plants absorb their nitrogen from the water column.
Low levels of ammonium are an important source for vegetation. However, high concentrations of ammonium can have a negative effect on vegetation development. The toxicity of ammonium depends on thermal conditions, pH, and sensitivity of the plant itself. Thermal conditions influence the speed at which plants can absorb ammonium. Besides that, temperature influences the dissociation equilibrium of ammonium and ammonia gas. At high values of ammonium, the amount of undissociated ammonium (ammonia gas) increases. In the end, ammonia can be very toxic for aquatic plants. (Arts et al. 2007)

High nitrate concentrations occur in water bodies that are influenced by leaching groundwater of agricultural land. Surface waters with a too high nitrogen concentration can have a negative effect on the vegetation development (Arts et al. 2007). Nonetheless, fast growing plants often need to accumulate a high nutrient concentration, such as total nitrogen, in order to survive.

According to several studies, one of which is written by UKTAG (2008), it became clear that the role of nitrogen, and specifically in the form of nitrate, is not well understood. It has been said that the effects of nitrogen are not distinguishable enough with phosphorus to set a standard for nitrate in fresh water systems for the Water Framework Directive (Lambert and Davy, 2011). However, excess nitrate and also phosphate can result in eutrophication of surface waters, which can lead to oxygen depletion and death of organisms. (Suresh and Choi, 2011)

2.4.6 Phosphorus

Substrates and soil have a major function on the growth of aquatic plants in shallow surface waters. If the phosphorus concentration is too high, due to the supply of nutrient rich water for example, the role of the bottom of a water body is limited. Most of the time, algal blooms dominate in water bodies with a high nutrient concentration, such as phosphorus. If the bottom layer of a water body can contain high phosphorus concentrations, submerged aquatic plants can dominate. However, if the phosphorus concentration is lower than the optimum concentration, emerged aquatic plants proliferate of the better light availability.

Warming of shallow waters, as a result of heating of the earth, will lead to high phosphate concentrations in the bottom layer and fast growing algal blooms with cyano bacteria. Especially when the oxygen consumption is high and the solubility of oxygen is low due to a high temperature. With less extreme, but only high phosphorus concentrations, the proliferation of fast growing aquatic plant species will increase. Especially exotic species with a high temperature optimum. (Lamers et al., 2012)

2.5 Aquatic plant species and vegetative growth

Biomass production determines the rate of vegetative growth. The ultimate biomass production of a particular plant species is influenced by the efficiency of the process of photosynthesis, which supplies raw materials for vegetative growth. Photosynthetic efficiency is dependent on their ability to concentrate carbon dioxide compounds in their leaves and their efficiency in water use. An increased leaf area, for light energy capture, has been shown to be positively correlated with biomass production. (Demura & Ye, 2010)

In order to determine the biomass, it is needed to quantify the potential biomass production of floating plants grown on the effluent. Moreover, sustainability of nutrient removal systems is also determined by further biomass utilization (Sudiarto et al., 2019). In addition, a high biomass production of the used plant species is needed, so the plants can be harvested continuously (Schuijt et al., 2018).

Biomass and weight

Biomass is a quantitative term that is used to address the total weight of biological material of an organism. Biomass can be expressed in wet weight, dry weight, and ash-free dry weight.

Wet weight: Gross weight of a living or dead organism
Dry weight: Weight after drying in an oven to evaporate water
Ash-free dry weight: Weight after incineration in an oven to remove hydrocarbons

In the case of this experiment the dry weight of the total plant species is only weighed at the beginning and end of the experiment. Also, the wet weight for each plant species and replicate is measured before, during, and at the end of the experiment. However, in the results only the wet weight production is discussed. (Soortenbank, n.d.)

3. Methodology

In order to answer the main research question of this study, qualitative and quantitative research has been carried out. Qualitative research has been performed by the means of online and offline literature research. Quantitative research has been performed by an experiment in the greenhouse of Campus Huygens, Nijmegen.

3.1 Qualitative research

The purpose of literature research was to gather information about topics as hydroponic culture, nutrient uptake and vegetative growth of certain plants, characteristics of floating plants as *Eichhornia crassipes*, *Pistia stratiotes*, *Stratiotes aloides*, *Salvinia natans*, the Water Framework Directive, and the water quality of Dutch surface waters.

3.2 Quantitative research

Extended literature research was followed by field research, by the means of a 28-day long lasting experiment. During this experimental period, the following plants species were monitored:

Eichhornia crassipes
Pistia stratiotes
Stratiotes aloides
Salvinia natans

3.2.1 Experimental set-up

In total, four plants were monitored during the experimental period. Each plant species had a quadruplicate, which means that there was a total of 16 tanks with plant cultures. In addition, two different control tanks were added. Each control tank had a quadruplicate as well. The experiment had a total of 24 tanks. In figure 6 below, a top view of the experimental set-up is shown. Each tank, except for control series CCx, had an UVC lightning source. The UVC source acted as a disinfection source, in order to prevent algae growth in the tanks. In addition, every tank had a water pump as well. The pump absorbed the water in the tank and transported it through a tube into the UVC device, see figure 6. In table 3 below, an overview of the treatments, their applications, and explanations are shown.

Table 3- Overview of the treatments with their abbreviation and explanation.

Treatment abbreviation	Treatment	Explanation
CC	Control – control	No plants and no UVC-lightning source
CU	Control – UVC lightning source	No plants but with UVC-lightning source
EC	*Eichhornia crassipes* plant treatment	*Eichhornia crassipes* plant species and UVC- lightning source
PS	*Pistia stratiotes* plant treatment	*Pistia stratiotes* plant species and UVC- lightning source
SA	*Stratiotes aloides* plant treatment	*Stratiotes aloides* plant species and UVC-lightning source
SN	*Salvinia natans* plant treatment	*Salvinia natans* plant species and UVC-lightning source

Each tank has the following dimensions; 60 cm * 40 cm * 30 cm and a total surface of 2400 cm^3. For each tank, 10% of the surface area is used to assign the suitable amount of plants at the start of the experiment. For the experiment, a percentage of 10 is approximately 240 cm^2, which is equal to 0.024 m^2.

The effluent domestic wastewater that was added to all the tanks came from the domestic wastewater treatment plant in Rhenen (Gelderland), the Netherlands. In each tank an amount of 60 liters effluent domestic wastewater was added.

In figure 6 a top view of a single tank is shown. The UVC lightning source is placed at the short side of the tank. On the left side of the UVC device, a hose is connected with the device to a water pump. The water pump is located at the left bottom of the tank, underwater. On the other side of the UVC device is another hose connected with a PVC hose. The PVC hose has multiple holes so water can flow out gradually. In addition, in figure 7 a side view of the experimental set-up in the greenhouse is shown.

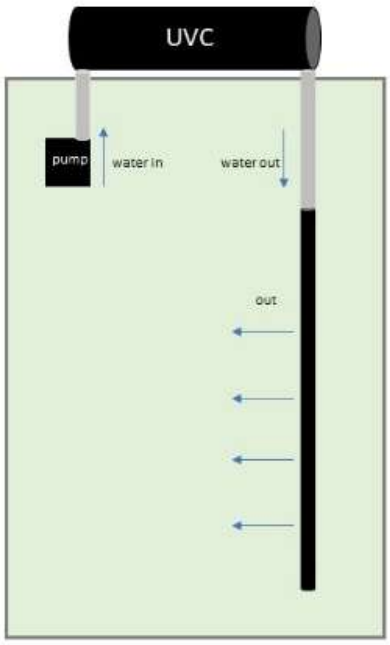

Figure 6- Top view of one treatment tank with the UVC device and the hoses. (Elzinga, 2021).

Figure 7- Overview of one side of the greenhouse. (Elzinga, 2021).

3.2.2 Planning of the experiment

The experiment has been carried out for seven days in a row, three days for every other day, followed by another seven day in a row experiment, and again three days for every other day. See table 4 for a weekly overview.

Table 4- Overview of the planning of the experiment with the corresponding dates.

Sunday	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**
		Day 1 (16/03/21) **Start**	Day 2 (17/03/21)	Day 3 (18/03/21)	Day 4 (19/03/21)	Day 5 (20/03/21)
Day 6 (21/03/21)	Day 7 (22/03/21)		Day 9 (24/03/21)		Day 11 (26/03/21)	
	Day 14 (29/03/21)	Day 15 (30/03/21) **Refreshment**	Day 16 (31/03/21)	Day 17 (01/04/21)	Day 18 (02/04/21)	Day 19 (03/03/21)
Day 20 (04/04/21)	Day 21 (05/04/21)		Day 23 (07/04/21)		Day 25 (09/04/21)	
	Day 28 (12/04/21) **End**			**Cleaning experiment**		

3.2.3 Sampling and measurements

During the experiment, daily measurements and intermediate measurements were carried out. See Appendix II for the detailed protocols of the daily measurements. In addition, table 5 shows an overview and explanation of the daily measurements.

Table 5- Overview and explanation of daily measurements.

Device of measurement	**Measurement**	**How**
HACH Lange Multimeter Kit HQ40d	pH, temperature (°C), dissolved oxygen (mg/L and percentage)	Use of the HACH kit for every individual tank.
TIC Analyzer Infrared Spectroscopy	HCO_3, CO_2.	Sampling with 10 ml syringe in the greenhouse and analysis with the TIC Analyzer in the laboratory.
Inductively Coupled Plasma (ICP) filtered samples	Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Sr, Zn.	Sampling with rhizon samplers and 60 ml syringes. Analysis in RU Laboratory.
Inductively Coupled Plasma (ICP) unfiltered samples	Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Sr, Zn.	Sampling with rhizon samplers and 60 ml syringes. Analysis in RU Laboratory.
AutoAnalyzer Continous Flow Analyzer (AA)	PO_4, NO_3, NH_4, Na, K, Cl.	Sampling with rhizon samplers and 60 ml syringes. Analysis in RU Laboratory.

Figure 8- Side view of HACH HQ40d while measuring pH, dissolved oxygen, and temperature. (Elzinga, 2021)

Figure 9- TIC Analyzer Infrared spectroscopy machine. (Elzinga, 2021)

See table 6, for an overview and explanation of intermediate measurements. Also, see appendix III for the protocols of the intermediate measurements of the experiment.

Table 6- Overview and explanation of intermediate measurements.

Measurement	**How**	**Why**
Measuring, weighing, and counting of the plants.	With the use of an analytical balance and a measuring tape.	To determine an increase or decrease in wet weight of the plant treatments.
Roots	The length of the tallest root was measured with a measuring tape.	
Leaves	The amount of leaves and the length of the tallest leaf of the plants.	
Color	Observations during measuring.	Important factor and determinative for the health state of the plant.

3.2.4 One-time measurements

On day 28 (April 15, 2021), sludge samples were taken to determine the amount of Total Suspended Solids in the effluent domestic wastewater after the experiment took place. See Appendix IV for the detailed protocol of the Total Suspended Solids measurements.

3.2.5 Data-analysis

After the literature research and field research were carried out, ICP and AA samples were analyzed by professionals in the RU laboratory. As mentioned before in table 5, ICP samples were analyzed on elements and AA samples were analyzed on nutrients. Since the main focus of this research study is on the removal of nutrients, the data of the AA samples are the most important.

When all ICP and AA data was available by the RU laboratory, the data was converted to the right unit and ratio in Microsoft Excel. After which the average values, standard deviation, removal rate for every treatment were calculated. In the case of the analyzed nutrients, scatter charts of each treatment and for each nutrient were made in Microsoft Excel. After that, each scatter chart has to contain a linear trendline and a linear formula (y = ax+b). Within this formula *a* is the removal rate and *b* is the start value of the data. Next on, all 24 *a*- values were put in a new Microsoft Excel document and uploaded in a statistical computer program called Jasp. In Jasp a boxplot and descriptive statistics can be made. In addition, a statistical test called Anova is used as well in Jasp. Anova is used to determine if there are significant differences between the treatments. If the answer is yes, a post hoc test can be carried out. A post hoc test is a possible function to determine if a significant difference is present between the treatments.

4. Observations

Within this chapter, the findings of certain measurements over period 1 are shown in scatter charts. For each treatment, the average measured value is first calculated. This way, every treatment has one measured value for each measuring day. In table 7, the treatments and their abbreviations that were used during the experiment are shown.

Table 7- Overview of treatments and their abbreviations.

Treatment abbreviation	**Treatment**
CC	Control without UVC
CU	Control with UVC
EC	*Eichhornia crassipes*
PS	*Pistia stratiotes*
SA	*Stratiotes aloides*
SN	*Salvinia natans*

4.1 Potential of Hydrogen

Figure 10 is a scatter chart with on the x-axis time in days and on the y-axis the average pH value. The average pH value is shown over a time period of 14 days for six different treatments. During the first days of the research period there is a slight decrease in pH value. On day 5, all treatments reach their lowest ph. All treatments reach their highest pH at the end of the first research period, which is day 14. There are little to no outliers in pH values over time. However, the data has shown that treatment CC has a higher pH in comparison to the other treatments.

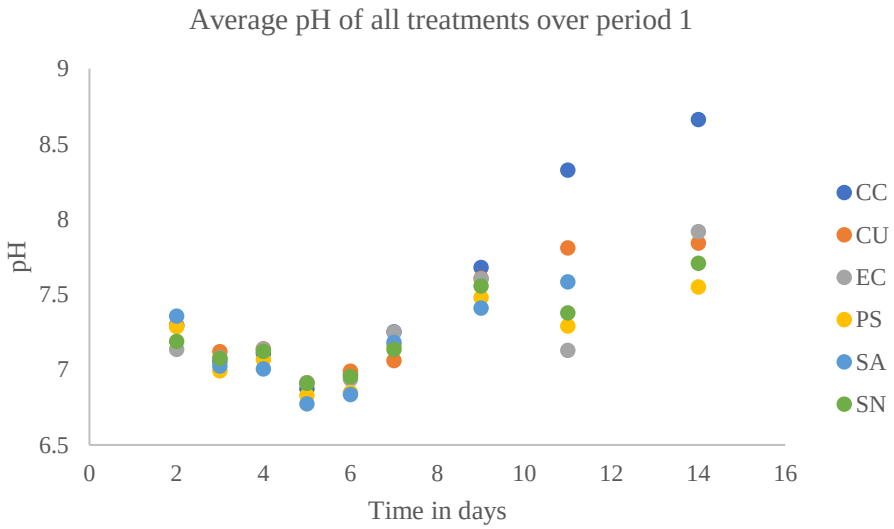

Figure 10- Scatter chart of average pH for each treatment over a time period of 14 days.

4.2 Dissolved oxygen concentration

The dissolved oxygen concentration is shown in figure 11. The scatter chart has on the x-axis time in days and on the y-axis the dissolved oxygen concentration in mg/L. It is noticeable that treatment CC has a higher dissolved oxygen concentration at the end of the research period. The obvious difference can be seen around since day 7 until the end of the research period. Treatment EC has the lowest start value, but has together with the other treatments a value of approximately 8 mg/L on day 14. Treatment PS shows the smallest increase in dissolved oxygen concentration over the specific period.

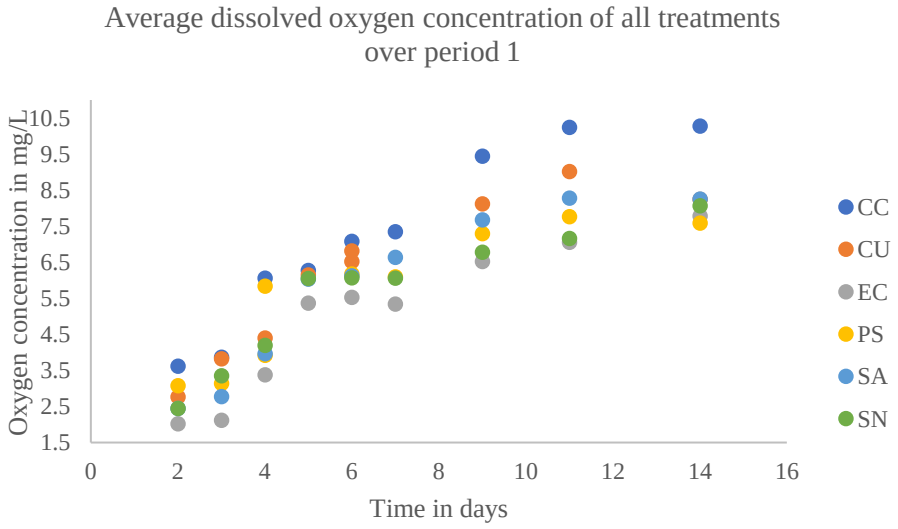

Figure 11- Scatter chart of average dissolved oxygen concentration (mg/L) over a time period of 14 days.

4.3 Bicarbonate concentration

Figure 12 shows a scatter chart with on the x-axis time in days and on the y-axis the bicarbonate concentration in mg/L. It is noticeable that the data for the bicarbonate concentration follows the remarkable parabola shape. Almost all values decrease until day 6, after which the values slightly increase. The start and end values of the bicarbonate measures are at the same level. The minimum value of bicarbonate is found for treatment CU, namely 411.63 mg/L. After which SA has a value of 482.83 mg/L. The highest bicarbonate value is found on day 3 with treatment SA, namely 2579 mg/L.

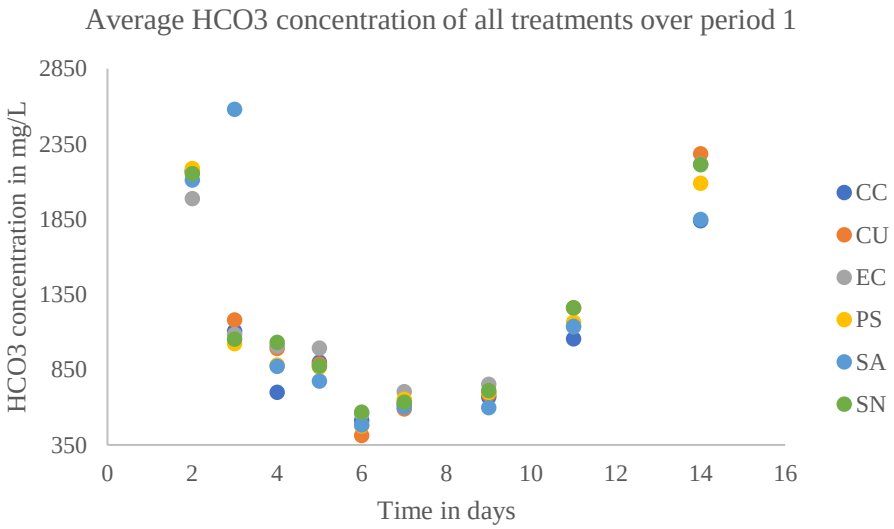

Figure 12- Scatter chart of average bicarbonate concentration (mg/L) over a time period of 14 days.

Lastly, the carbon dioxide concentration in the water. In figure 13, on the x-axis time in days is shown and on the y-axis the carbon dioxide concentration in mg/L is shown. The dispersion of values for the treatments is quite small. The data of the different treatments follow in general the same pattern. It is remarkable that on day 3 treatment SA has an outlier of 1512.6 mg/L. A similar peak is signaled at the same day for treatment SA.

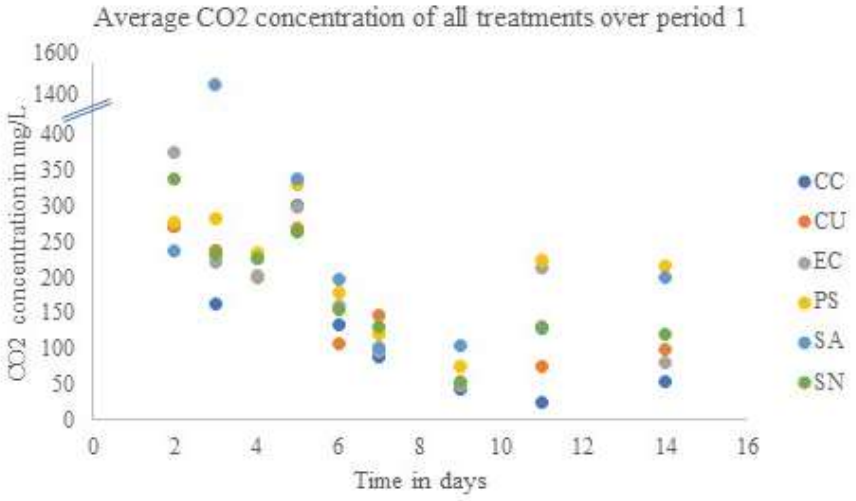

Figure 13- Scatter chart of average carbon dioxide concentration (mg/L) over a time period of 14 days.

5. Results

In order to determine the removal efficiencies of the nutrients (ammonium, nitrate, and phosphorus), first scatter charts of each treatment and for each nutrient were made in Microsoft Excel. After that, each scatter chart had to contain a trend line and a linear formula (y = ax+b). Where *a* is the removal rate and *b* is the start value of the data. Next on, all 24 *a*- values are put in a Microsoft Excel document and are uploaded to Jasp. Jasp is a program to do statistical analysis. In Jasp a boxplot and descriptive statistics are made. In addition, a statistical test called Anova is used as well, in order to determine if there is a significant difference between the treatments. If the answer is yes, then a post hoc test can be carried out in Anova as well.

The following abbreviations still apply, see table 8.

Table 8- Overview of treatment abbreviations.

Treatment abbreviation	**Treatment**
CC	Control without UVC
CU	Control with UVC
EC	*Eichhornia crassipes*
PS	*Pistia stratiotes*
SA	*Stratiotes aloides*
SN	*Salvinia natans*

5.1 Wet weight production

The box plot shown in figure 14 shows the average wet weight production for four different plants over the first research period. Treatment SN has the highest average increase in wet weight with a maximum weight of 200 grams. Treatment SA has the lowest average increase in wet weight of almost 70 grams. Treatment EC and PS are in the same location and range of the box plot. Treatment PS has a high dispersion of the minimal and maximal value of weight.

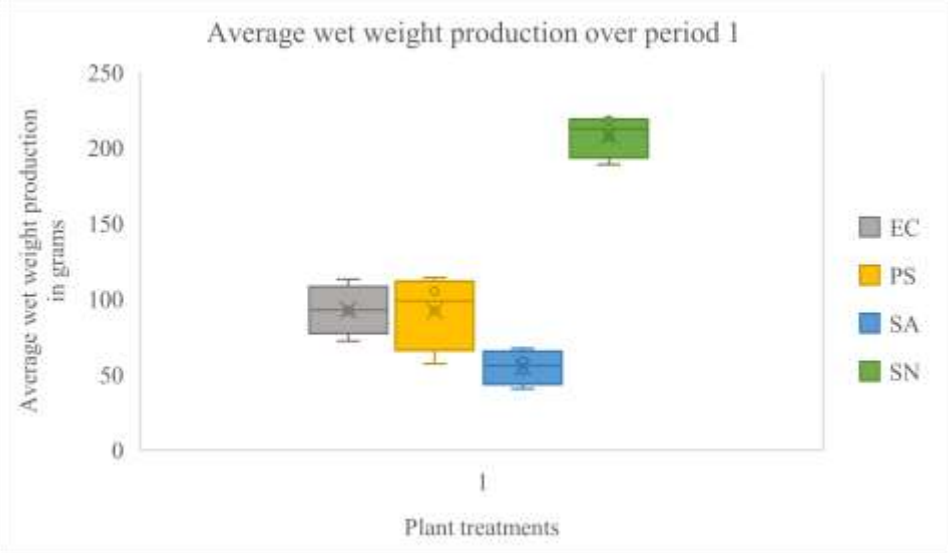

Figure 14- Box plot of average wet weight production of the four plant treatments over period 1.

The box plot above, figure 15, is made in Anova/Jasp to prove that there is a significant difference in wet weight between the different plant treatments.

$F(3, 12) = 57.742$, $P < .001$, $\eta^2_p = .935$

In this case there is a significant difference between treatment EC and SN of < .001, between treatment PS and SN of < .001, and treatment SA and SN of < .001.
According to the Anova post hoc test of the wet weight production, it can be proved that SN has a significantly higher production than the other plant treatment during the first 14 days of the experiment.

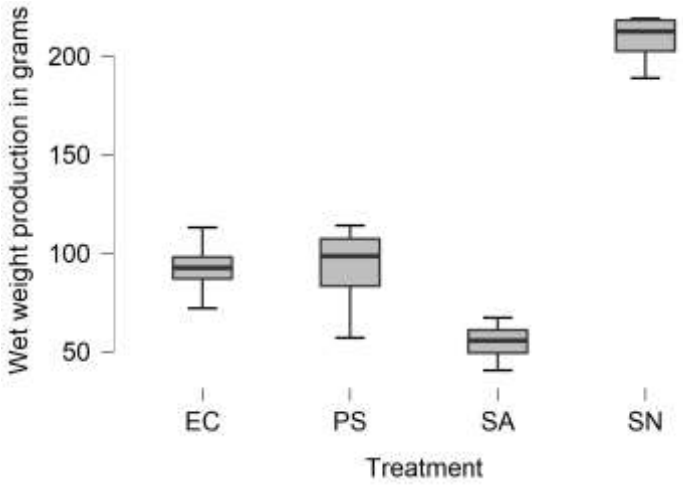

Figure 15- Box plot of the wet weight production over period 1.

5.2 Ammonium

Nutrients over time

Figure 16 shows the average ammonium concentration with standard deviation over period 1. The standard deviation in the line chart shows the degree of dispersion in the ammonium data, it indicates how far the observed values deviate from the mean value. On the x-axis the time in days is shown and on the y-axis the ammonium concentration in mg/L is shown.

In figure 16, it can be seen that there is a clear decrease in ammonium concentration for all treatments. On day 3, the values are almost constant, after which the values stay constant for the whole research period. It is noticeable that treatment CU has the highest ammonium concentration on day 3, but after that the concentration decreases fast and follows the same pattern as the other treatments.

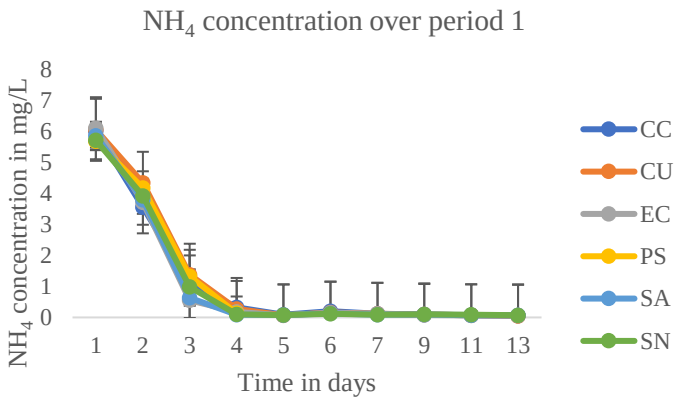

Figure 16- Line chart of the average ammonium concentration over period with standard deviation.

Removal efficiency

For the decrease of ammonium a significant difference of $p < .001$ is found, see figure 17. Therefore, a post hoc test is carried out in Anova to determine which treatments differ significantly with each other. $F(5, 18) = 11.581$, $P < .001$, $\eta^2_p = .763$

The post hoc test shows that there is a significant difference of <.001 between treatment CC and SA and between treatment CC and SN. Treatment CC (M = -0.031 and sd = 0.006) has a higher removal rate in comparison with treatment SA (M = -0.012 and sd = 0.002). Also, treatment CC (M = -0.031 and sd = 0.006) has a higher removal rate in comparison with treatment SN (M = -0.009 and sd = 0.003). According to the descriptive statistics, treatment SA and SN have the lowest removal rate of ammonium over period 1.

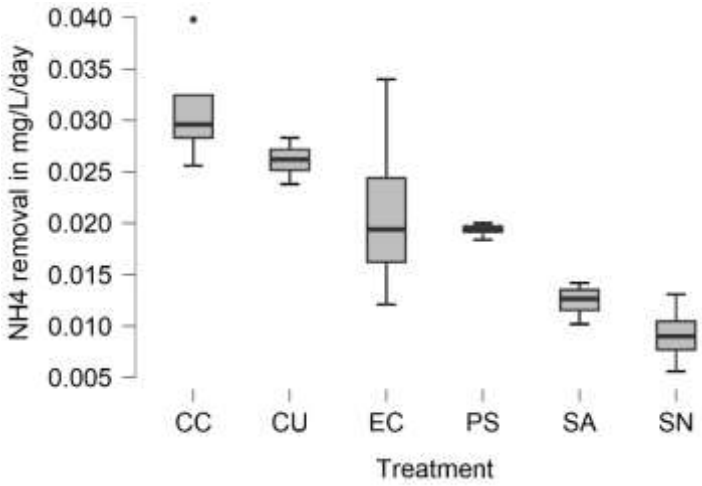

Figure 17- Box plot of ammonium removal efficiency.

Ammonium concentration after day 5

In figure 18, a box plot of the ammonium concentration after day 5 is shown. The values are taken from day 6 until the end of period 1, because on day 5 treatment CU has reached the lowest ammonium concentration of 0.04344 mg/L. In the days after, treatment CU has reached rounded 0.0 mg/L. There is no significant difference found between the other treatments.

$F(5, 13) = 0.914$, $P = 0.475$, $\eta^2_p = 0.039$.

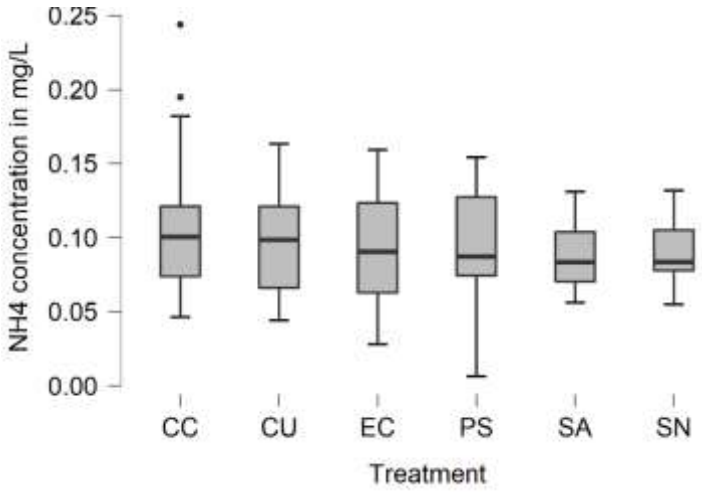

Figure 18- Box plot of ammonium concentrations after 0 mg/L is reached at day 5.

5.3 Nitrate

Nutrients over time

In the line chart, figure 19, the average nitrate concentration over period 1 is shown. The line chart contains the standard deviation of the nitrate data as well. The standard deviation shows the degree of dispersion in the nitrate data. It indicates how far the observed values deviate from the mean value. On the x-axis time in days is shown and on the y-axis the nitrate concentration in mg/L is shown.

It is clear that there is an increase right after the beginning of the experiment. Most peaks are noticed around day 2-3. Treatment PS has an early peak in comparison with the other treatments, because they have a peak at day 3. After the outlier, the values decrease on day 4, which after the values are kept constant until day 9. Treatment PS has a late peak on day 11 instead of day 9. After all treatments have gotten their outliers, the nitrate concentration decreases. The nitrate values of the beginning and end of the experiment are at a similar level.

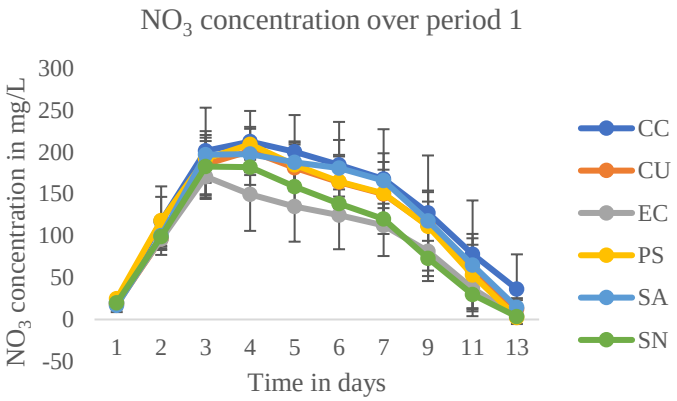

Figure 19- Line chart of the average nitrate concentration over period 1 with standard deviation.

Removal efficiency

In figure 20, the removal efficiency for each treatment is shown. For the decrease of nitrate no significant difference is found between the different treatments.
Treatment SA (M = -34.043 and sd = 10.442) has the highest removal rate over period 1.
Treatment EC (M = -21.012 and sd = 5.508) has the lowest removal rate over period 1.

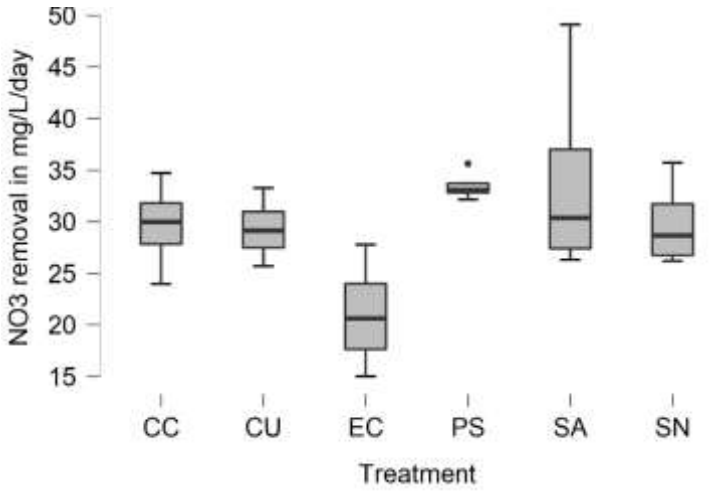

Figure 20- Box plot of nitrate removal efficiency.

Nitrate concentration in mg/L at day 14

In figure 21, all treatments are shown. The values of the treatments are taken at day 14, because on this day treatment PS has the lowest value compared to the other treatments. Treatment PS has a concentration of 2.3859 mg/L at day 14. It seems like treatment PS has the lowest nitrate concentration at the end of this period. However, according to Jasp/Anova, there is no significant difference found between the other 6 treatments.

$F(5, 18) = 1.965$, $P = 0.133$, $\eta^2_p = 0.353$

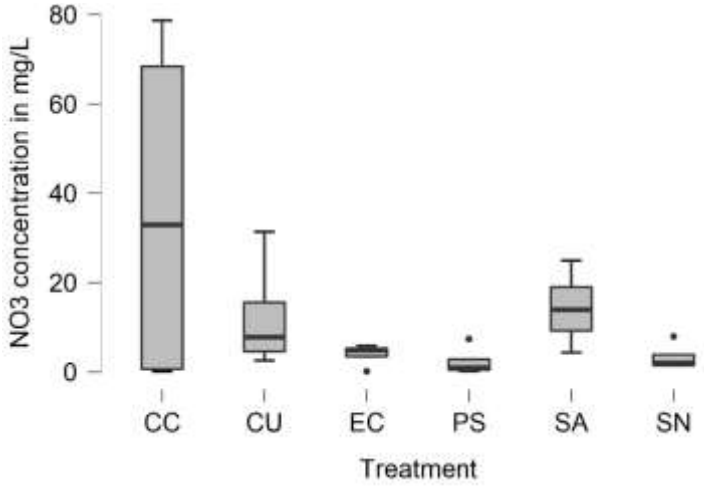

Figure 21- Box plot of nitrate concentrations in mg/L when the lowest value is reached at day 14.

5.4 Phosphate

Nutrients over time

In the line chart above, the average phosphate concentration with their standard deviation is shown over period 1. The standard deviation in this line chart shows the degree of dispersion in the phosphate data. It indicates how far the observed values deviate from the mean value. On the x-axis time in days is shown and on the y-axis the phosphate concentration in mg/L is shown.

After day 1, the value of phosphate decreases significantly to a point where there is no more phosphate available. On day 3, the phosphate concentration increases again. Treatment PS increases on day 5 linearly, but the treatment does not reach the highest phosphate concentration at the end of the research period. Treatment PS has a concentration of 4.13 mg/L and is, in comparison to treatment SA, quite low. Treatment SA succeeds to reach the highest phosphate concentration, namely: 5.85 mg/L.

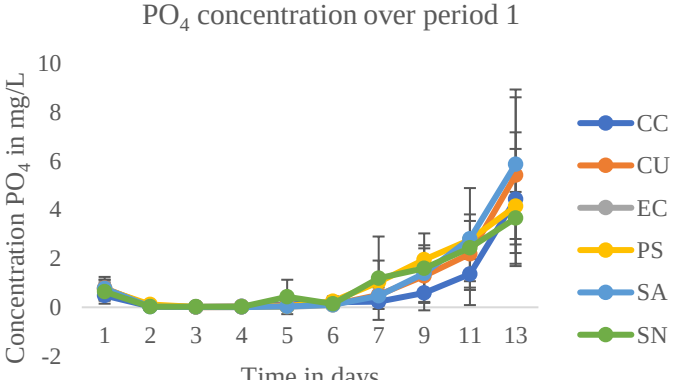

Figure 22- Line chart of the average phosphate concentration over period 1.

Release speed

For the increase of phosphate no significant difference is found between the different treatments. Treatment EC (M = 0.907 and sd = 0.140) has the highest rate of increase in phosphate concentration. Treatment CC (M = 0.852 and sd = 0.039) has the lowest rate of increase in phosphate concentration.

$F(5, 18) = 0.150$, p 0.977, $\eta^2_p = 0.040$.

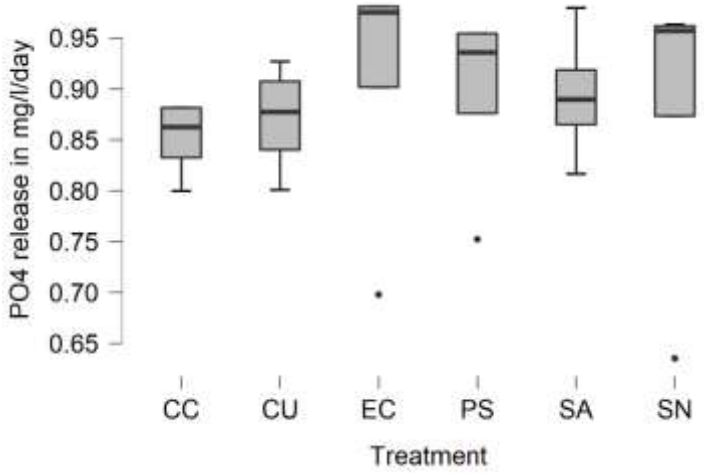

Figure 23- Box plot of phosphate removal efficiency.

6. Conclusion

In this study, research has been executed in order to answer the following research question:
'Which plant treatment removes the highest concentration of nitrogen and phosphorus from the treated domestic wastewater over a period of 14 days?'

The answer to the research question can be approached in different ways.
In case of the wet weight production, it was possible to achieve the highest wet weight in grams at the end of period 1 as well as the overall highest wet weight production in grams over period 1. Focused on the research question, only the wet weight production is taken into account. Small and fast growing plants were able to prove that they also can achieve a high wet weight production. *Salvinia natans* achieved a significantly higher wet weight production than the other three plant treatments.

Furthermore, on the topic of nutrient removal, it is visible that every researched nutrients prefers a certain treatment and thus has the best removal efficiency. It has been proved that there is a significant difference in ammonium removal between all treatments. Treatment CC has the highest ammonium removal efficiency. Nonetheless, there is no significant difference present in the nitrate and phosphate removal. Since there is no prove for a significant difference, it is not possible to draw a conclusion about nitrate and phosphate removal.

Besides that, *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia natans* meet the limit values of the Water Framework Directive the best. *Stratiotes aloides* overtops the range of 4-8 mg/L of total nitrogen, set-up by the WFD. Moreover, the phosphate concentration of all treatment overtops the phosphorus limit value of < 0.42 mg/L P. At the end of the experiment, *Salvinia natans* has the lowest phosphate concentration of 3.64 mg/L, but still overtops the phosphorus limit value.

Not to mention, it was expected that the plant treatment with the highest wet weight production would also have the highest removal efficiency of nutrients. Thus, the highest concentration of total nitrogen and phosphorus removal. As well as it was expected that *Eichhornia crassipes* would perform the best of all treatments. Though, this is not the case. In fact, *Eichhornia crassipes* even has the highest release of phosphate during the experiment. The hypothesis can be disproved.

To conclude, the main research question does not legitimately have one answer. The conclusion to the research question is dependent on different visions as wet weight production, removal efficiency, and the Water Framework Directive limit values. Looking at the achievement of the Water Framework Directive limit values, *Salvinia natans*, also called floating fern, ensured that the limit values of the WFD were met in the best way. The total nitrogen and phosphate concentrations were the closest to the limit value of 4-8 mg/L. Although, the dispersion in phosphate concentration is remarkable. Within the chapter *Discussion*, the phosphate concentration and the possible causes are considered.

7. Discussion

In order to gather data for this research study, qualitative research is carried out in the form of a field experiment. The experiment consisted of six different treatments, whereof four plant treatments growing on wastewater in a mesocosm environment in greenhouse Huygens. After gathering the data with use of the experiment, data is analyzed. Based on this, it can be stated that the results of the experiment are valid but arguable. The mesocosm environment has a temperature in a solid range of 20 to 25 degrees Celsius. Therefore, the climate in the greenhouse should feel somewhat natural to the plant species.

With help of the data analysis, it was found that the ammonium concentration significantly decreased for treatment CC over period 1. However, for nitrate removal there is no prove that the concentration significantly decreased. Additionally, the phosphate concentration actually increased over period 1. The high phosphate concentration may be explained by the lack of substrate in the treatment tanks. In this experiment floating, aquatic plants were used, which do not need substrate to root. The pH of substrate has an influence on the uptake of phosphate by plants (Verheggen, 2016).

Nutrient-rich wastewater in combination with the presence of algae and a low flow velocity, resulted eutrophic water on day 7 of the first period. In addition to this explanation, the algal blooms found were micro cystins. Micro cystins are cyanobacterial toxins mainly produced by *M. aeruginosa* in fresh water systems (Wu et al., 2019). In theory, algal blooms ensure that oxygen concentrations are decreasing, they need oxygen to survive and ammonium and nitrate get a chance to rule. Nonetheless, the dissolved oxygen concentration actually increased and the ammonium and nitrate concentration decreased to a fair 0 mg/L at the end of period 1.

As mentioned previously, six treatments were researched, whereof two treatments without plants acted as a control. It was predicted that treatment CC (Control-Control) and treatment CU (Control-UVC source) would have a difference in their data. The UVC-lightning source was mainly placed to ensure that algal blooms were removed from the treatment tanks and would not influence the water conditions and vegetative growth of the plants. It is noticeable that the expected difference between treatments did not take place. Both control treatments followed the dispersion patterns of the other plant treatments. A possible explanation for this pattern is the presence of microbial populations in the wastewater. More specifically, microbial populations attached to the small roots of the plants. Rapid growth and an extensive root zone ensure a large area for micro-organisms. Therefore, they stimulate the biodegradation of organic matters and nutrients in the wastewater, such as ammonium and nitrate removal. However, this reasoning does not answer the question mark regarding the pattern in phosphate concentration. Namely, the phosphate concentration follows a dispersion pattern similar to the ammonium and nitrate removal, but exactly in the opposite way. When the phosphate concentration increases, the ammonium and nitrate concentration decreases, and the other way around. Helmer and Kunst (1998), reported a reduction in phosphorus release when the operating temperature was lowered from 20 degrees Celsius to 5 degrees Celsius. Additionally, Boswell et al. (1999) indicated that when the temperature increases between 4 and 37 degrees Celsius, the phosphorus release also increases. These observations seam similar to the findings of the current research study.

The fact that there is not a remarkable difference between the treatments and their data of water conditions and nutrients, does not mean there is no difference in vegetative development over time. As shown in the results, see page 20, it is known that there is a significant difference in wet weight production between the treatments. *Salvinia natans* has the highest wet weight production and a significant difference of <.001 between the other three plant treatments. A high wet weight production means a high rate of vegetative growth. The high rate of growth may be due to optimal conditions of water temperature. The water temperature was always at least 20 degrees Celsius and warming during the day. In addition, sufficient light was available and adjusted if necessary.

It is noticeable that ammonium and nitrate concentrations are decreasing for all treatments over time. However, these parameters are decreasing as wet weigh production increases over time. According to Zuthsi and Vass (1971), it is shown that *Salvinia natans* prefers ammonium and nitrate for uptake and assimilation. Additionally, a correlation observed by Reddy and Tucker (1983), about nitrogen and phosphorus uptake rates by the *Eichhornia crassipes* showed that the nitrogen recovery of both shoots and roots is way higher than phosphorus recovery. It suggests that more nitrogen can be translocated to the shoots instead of phosphorus. Next to *Eichhornia crassipes*, this is also possible for the other three treatments.

This research study is an addition to the previous study of research project Aquafarm. In the previous study, a combination of emerged and submerged plant species were researched, but in the current research study non-rooted, floating, aquatic plants were researched. Within the experiment, this specific research study is a separate direction of the entire experiment. During the experiment also measurements regarding nitrogen and nitrous oxide emissions, methane, and elements were executed. Anyhow, these additional measurements do not have any effect on the current results.

As mentioned in the previous chapter, there is not one answer to the main question of this study. Since all points of discussion are handled within this chapter, it is important to explain and substantiate suggestions for future research in the *Recommendations*.

8. Recommendations

Based on the results, the main suggestion for this research study is to execute an additional research study with slight changes in measuring and field experiment. It would be interesting to gather results regarding the influence of substrate and no substrate on the nutrient removal, especially on the removal efficiency of phosphorus.

Approach
The current method and approach of measurements applied to this research study are sufficient and can still be applied to the suggested experiment.

Experimental period
The length of 28 days of monitoring, with in total 8 days of no monitoring, is sufficient.
For this research study only the first period, consisting of 14 days, is considered. Though, it would be beneficial for the validity of the data to have at least a period of 28 days of consecutively monitoring. 28 Days of consecutively monitoring will ensure a large dataset and in this way will allow small errors in the data. Right now, it is not possible to afford small errors in the dataset of period 1.

Research design
As mentioned previously, it is suggested to research the difference between rooted and non-rooted aquatic plants, since substrate can have an influence on the nutrient uptake and pH. Therefore, an experiment with a combination of rooted and non-rooted plants can be set-up with practically the alike research design. The only difference is the addition of two more control tanks: One control with substrate and no UVC-lightning source and one control with substrate but with a UVC-lightning source. The current control tanks will still exist and are more valid in the research. In addition, the treatment tanks can be a combination of, for example, three rooted plants with substrate and three non-rooted plants without substrate. Thus, the experimental set-up in the greenhouse can still be used.

Algal blooms
In response to the current experiment, it is an useful test to sieve the algal threads out of the treatment tanks in order to observe if the algal blooms are returning or stay absent during the remaining research period. In general, it is recommended to clean the tanks more often, especially when monitoring over a longer period than 14 days. The sides and the bottom of the tank should be cleaned more often.

In addition, the filter of the water pump and the outside of the hoses should be cleaned and cleared of algae and sludge.

Duplicate measurements
All along the experiment, duplicate measurements of among other things pH, dissolved oxygen, thermal conditions, Total Inorganic Carbon samples, nutrient and element samples would be favorable for the validity of the dataset to prevent mistakes or confusion.

Biomass production
In this research study, only the wet weight production of the vegetative growth is monitored. However, it is beneficial for the validity of the experiment to monitor the real biomass production instead of the wet weight production. Biomass sampling can be done by destructive methods, where plant material is actually collected from the site and weighed, or by a non-destructive method, in which an alternative measure related to weight has been carried by using subsampling of destructive plant samples measuring weight.

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Appendices:

I) List of treatment abbreviations

Treatment abbreviation	Treatment	Explanation
CC	Control – control	No plants and no UVC-lightning source
CU	Control – UVC lightning source	No plants but with UVC-lightning source
EC	*Eichhornia crassipes* plant treatment	*Eichhornia crassipes* plant species and UVC- lightning source
PS	*Pistia stratiotes* plant treatment	*Pistia stratiotes* plant species and UVC- lightning source
SA	*Stratiotes aloides* plant treatment	*Stratiotes aloides* plant species and UVC-lightning source
SN	*Salvinia natans* plant treatment	*Salvinia natans* plant species and UVC-lightning source

II) Protocol daily measurements

HACH Lange Multimeter Kit: pH, temperature, and dissolved oxygen measurements.

Materials:

- HACH Lange Multimeter Kit: HQ40d;
- Demi water;
- 3 Buckets;
- Tissue paper;
- Tripod;
- pH and oxygen probes;
- 24 Sampling tanks

Method:

- Fill 1-2 buckets with demineralized water. Keep one bucket full with water and the other bucket empty.

- Unscrew the protective covers of both pH and oxygen probes and rinse them with demi water, by pouring water over the probes. Take care of the protective covers, especially the cover of the pH probe. The protective cover of the pH probe contains a chemical liquid that protects the vulnerable pH probe. Put both protective covers back in the kit.

- Connect both probes to the main device and put the probes in the tripod. Adjust the tripod to the correct height by adjusting the screws and clamp. When both probes are 1 centimeter underwater, the tripod is at the right height. During the measurements, the tripod can be kept at the same height.

- When both probes are at the right height, press the green button on the right to read the values of the water. After a while, depending on the stability of the values, the values associated with the measuring tank are given.

- Write the values in your lab journal and repeat the method above for all tanks. In between each measurement, both probes have to be rinsed with demi water and cleaned with a tissue if needed.

- At the end of the measurements, the probes have to be rinsed with demi water again and also cleaned with a tissue.

- Screw the protective covers on the pH and oxygen probe. Take into account that the protective cover is filled sufficient, to prevent the probe from drying out.

- After that, remove both probes from the main device and tripod. Put both probes, together with the main device, away in the HACH kit.

Total Inorganic Carbon samples

Materials:

- 1 ml syringes (24 times);
- Vacuum pillow;
- 24 Sampling tanks

Method:

- Pull the syringe until it is filled with 0.5 ml of sample. Check if there are any air bubbles present in the syringe and tap to the middle of the syringe and the transition from the needle to the syringe, in order to get rid of the air bubbles.

- When the air bubbles are almost out of the needle, push the syringe so the air bubbles can escape from the needle.

- Continue to take the sample if there are no more air bubbles present in the needle and syringe. If not, continue the method above.

- After taking all 24 samples, the syringes can be pricked into a vacuum pillow. The samples can be taken to the cooling for a maximum of four days or can be measured immediately after taking the samples with the TIC machine in the laboratory. See appendix IV, to follow the protocol of the TIC Analyzer Infrared Spectroscopy.

Inductively Coupled Plasma: unfiltered samples

Materials:

- 10 ml ICP tubes (24 times);
- ICP caps (24 times);
- 10 ml syringe;
- Demi water;
- Tissues;
- 65% nitric acid;
- 24 Sampling tanks

Method:

- Rinse the syringe first one time for each tank. Refill the syringe with sample and fill the ICP tube that is connected to the sample. The tube has to be filled up to the edge of the tube, which is approximately 10 ml.

- Rinse the syringe with demi water and clean the outside with a tissue, before you continue with the next tanks.

- Repeat these method for all the 24 tanks.

- Acid all 24 samples with 1 ml of nitric acid. Twirl the samples and press the caps before the samples are stored in the cooling.

Inductively Coupled Plasma: filtered samples and AutoAnalyzer samples

Materials:

- 10 ml ICP tubes (24 times);
- ICP caps (24 times);
- 10 ml syringe;
- Demi water;
- Tissues;
- AutoAnalyzer jars (24 times);
- AutoAnalyzer Caps (24 times);
- Rhizon samplers (24 times);
- 100 ml syringe;
- 65% nitric acid;
- 24 Sampling tanks

Method:

- Take the sample by pulling the 100 ml syringe and put a screw into the syringe. Wait until the syringe is filled to approximately 50 ml and disconnect it from the rhizon samplers.

- The step above is the same for rinsing the syringe and taking the real sample.

- Taking the sample; fill an ICP tube with 10 ml of sample and continue to fill the AA jar with the rest of the sample from the syringe. Repeat this step for all 24 tanks.

- When all samples are done, connect the syringe again to the rhizon sampler.

- Acid all 24 ICP samples with 1 ml of nitric acid. Twirl the samples and press the caps before the samples are stored in the cooling.

- Put all 24 AA sampling jars in the fridge and put all 24 ICP tubes in the cooling.

III) Protocol Intermediate Measurements

Roots and leaves

Materials:

- Tapeline;
- Tweezers;
- Gloves;
- Analytical balance;
- Net baskets;
- Buckets;
- Demi water;
- Sieve;
- Plants: Stratiotes aloides, Eichhornia crassipes, Salvinia natans, Pistia stratiotes.

In all cases, put on gloves in order to prevent skin contact with blue-green algae and microcystins.

Method: Measuring the length

- Measure the tallest root by putting the plant straight on the counter.

- Measure the tallest leaf.

- Measure the total length of the plant, which is the bottom of the root to the top of the leaf.

Method: Counting the amount of plants and leaves

- Count every plant for each tank, except for the two control series.

- Count every leaf for each plant, in the case of Salvinia natans and Stratiotes aloides count and measure only 5 random plants.Method: Weighing the plants (wet weight and dry weight)

- Weigh the plants of each tank on the analytical balance. If the plants are weighed with a net basket, subtract the weight of the net baskets then. Keep in mind that the weight of the plants is probably influenced by the weight of residual water, it is the wet weight.

- After the experiment is done, the dry weight of the starting plants and the dry weight of the ending plants is measured on the analytical balance. This way, the wet weigh production over time can be determined.

IV) Protocol One-time Measurements

Sludge samples for TSS analysis

Materials:

- 500 ml glass bottles (24 times);
- Vacuum lids (24 times);
- Dishwashing brush;
- Gloves;
- 24 Sampling tanks;

Method:

- At the end of the experiment, when all plants were removed from the tanks, sludge samples were taken.

- The bottom and sides of the tank, the water pump, and the hoses are all brushed with the dishwashing brush to ensure that sludge is released from surfaces.

- Put the glass bottle underwater and make sure the water is bubbling. When the air bubbles are gone, put the vacuum lid on the glass bottle, the bottle is still underwater. The bottle can be removed from the water, cleaned from the outside and stored in the cooling. After the TSS samples were taken, the samples were stored in the cooling for one night. The next morning, the samples were analyzed in the lab.

Total Suspended Solids analysis

Materials:

- Weighing box (24 times);
- Glass microfiber filter (24 times);
- Filtration apparatus: filter pump, 1 liter receiving flask, filter funnel, vacuum tubing.
- Drying oven at 100°C;
- Analytical balance (reading to 0.1 mg);
- Tweezers;
- Measuring cylinder;
- Demi water;
- 24 Sludge samples

Method:

- First of all, weigh all 24 weighing boxes on the analytical balance and write down the value in the lab journal.

- After that, connect the filter apparatus and rinse the 24 filters with demi water. Use 200 ml demi water for each filter, to rinse the filter. When all filters are rinsed, weigh the filters with the weighing box on the analytical balance and write down the values again.

- Put the filters and there weighing boxes in the drying oven at 100 degrees Celsius for one hour. This is to dry the filter after rinsing it. After one hour, the filters and their weighing boxes can be weighed again on the analytical balance.

- Use 210 ml of sample to filter the sludge out. After sampling, weigh each filter in their weighing box again on the analytical balance. When all samples are filtered and the filters are weighed, put the filters

and their weighing boxes again in the drying oven at 100 degrees for one hour. After one hour, the samples can be put in an desiccator for at least 30 minutes. In this case, the samples stayed in a desiccator for at least 48 hours.

- The samples were weighed after the desiccator again on the analytical balance. With these data, the amount of Total Suspended Solids (mg/L) for each tank can be measured.

V) Protocol Total Inorganic Carbon Measurements

Daily task
210 mg of NaHCO3 (F177) dissolved in 100 ml of MillliQ, which is 25 mM NaHCO3.
This is the stock solution.

Put 1 ml of the stock solution in a 100 ml volumetric flask and fill up with MilliQ.
> This dilution depends on the concentration. If you expect a high concentration, then you start with a smaller dilution.

Weekly task
Make an acid solution of 2.5 ml phosphoric acid (E5) in 100 ml MilliQ.
After these tasks are done, turn on the nitrogen tap. Check if the glass tube of the IRGA device is dry and if necessary, replace the magnesium perchlorate if the tube is moist or wet.

Computer program settings
- Turn on the computer: Gas Analysis.
- Put on Action Triggering/Calculate Integral and Beep.
- Check if integral functions is set at CO2 and trigger start and end is set at 1.
- Go to Trend Logging and Integral Logging and put on Log Data for both functions.

Calibration
Add 1 ml of acid in the glass tube and calibrate the device.
Check on the IRGA device the screen. Push menu and click enter until the calibration is set. Push the green button meas(ure). Ignore the Error on the screen.

Calibration line
- Pour the standard solution in the NaHCO3 beaker. Flush the syringe for NaHCO3 and inject 0.1 ml of the standard solution in the injection chamber. Prick the needle of the syringe as deep as possible in the septum of the injection chamber. Watch out that there are no air bubbles present in the syringe.
- When as solution is added to the injection chamber, the red button on the window lights up. In the bottom left area of the screen the value will increase. The measurement is finished when the red button is not lighting up anymore. After the value is written down according to the table 5 below, a new sample can be injected to the injection chamber.

Table 9- Scheme used for setting up calibration line

Number	ML standard solution	0.25 nmol	1.25 nmol	Integral values
1	0.1	25	125	
2	0.2	50	250	
3	0.4	100	500	
4	0.8	200	1000	
5	1.0	250	1250	

During this experiment, only the 0.25 and 1.25 nano mol for different standard solutions is used. With the use of the ml standard solution, the nano mol for different standards solutions, and the integral values, a calibration line can be made in Microsoft Excel.

Measuring of the samples

- Before starting to measure your own samples, inject 1 ml of acid solution to the injection chamber. Prick the needle of the syringe as deep as possible n the septum of the injection chamber. Watch out that there are no air bubbles present in the syringe.
- Inject 0.1 ml of your sample to the injection chamber. The red button on the window lights up and an increasing value is visible. After the value is written down, a new 0.1 sample can be injected into the chamber. Continue this processes with all samples.

Shutting down the device

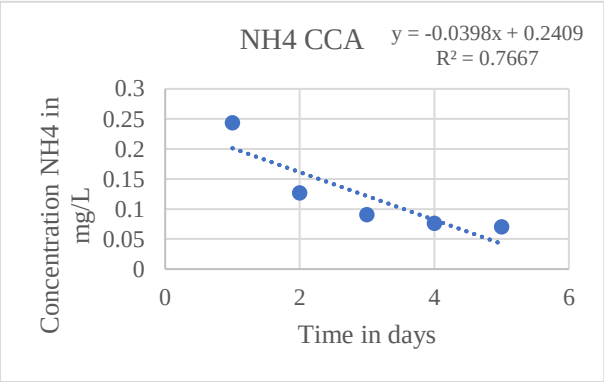
- Close the windows on the computer, turn off the computer.
- Close the nitrogen gas tap, but TIC device should be left on.
- Empty the injection chamber rinse and place back the empty chamber
- Replace the magnesium perchlorate and glass wool after use

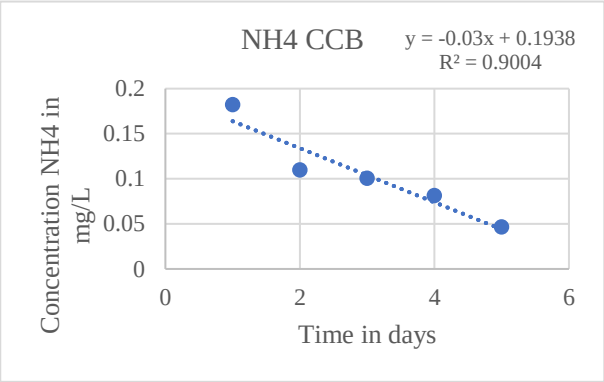
Take into account

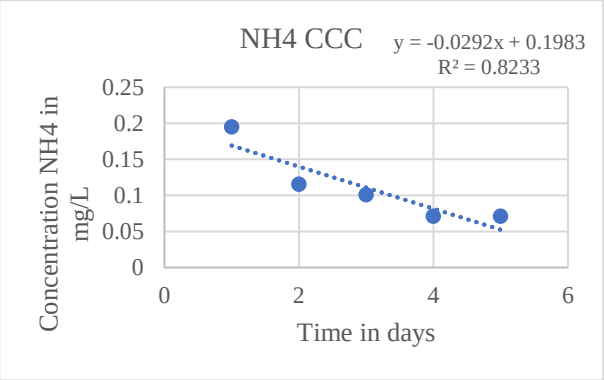
- If the chamber gets too full easily:
Empty the chamber and fill again with 1 ml acid solution or check if the septum is not leaching sample. If necessary, replace the septum in the injection chamber.
- If the magnesium perchlorate in the glass tubes is wet:
Remove the glass tubes from the device. Remove the glass wool and rinse the tubes with MilliQ. Dry the inside of the tubes with an air gun. Place new glass wool at the end of the tube and put new magnesium perchlorate inside the tub. Place on the other side of the glass tube also new glass wool. Place the tube back at the same position on the TIC device.

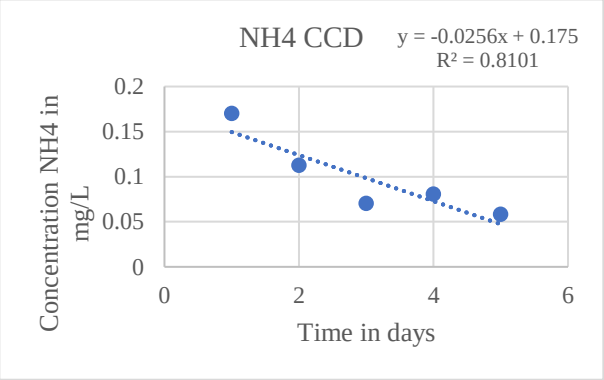
VI) Scatter charts nutrients over time

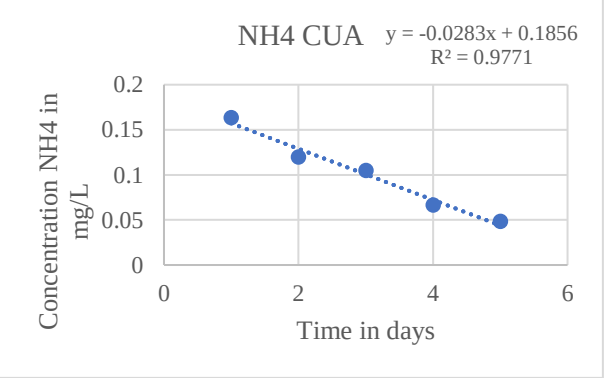
NH_4 scatter over time for each treatment

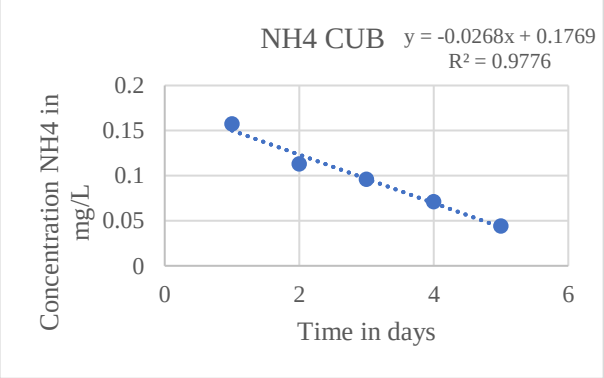


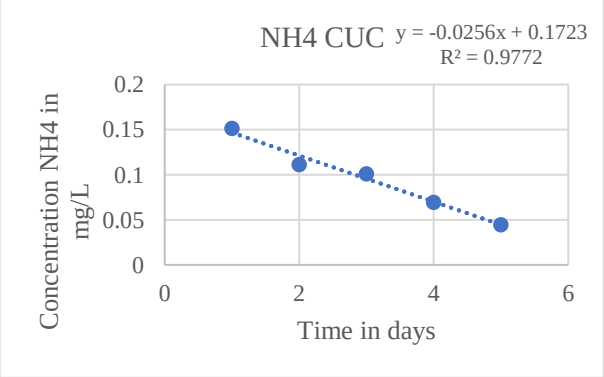


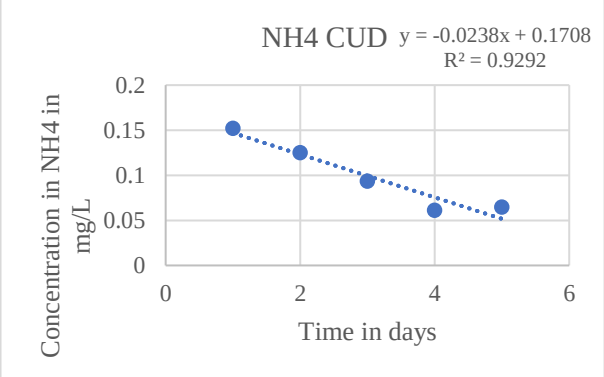


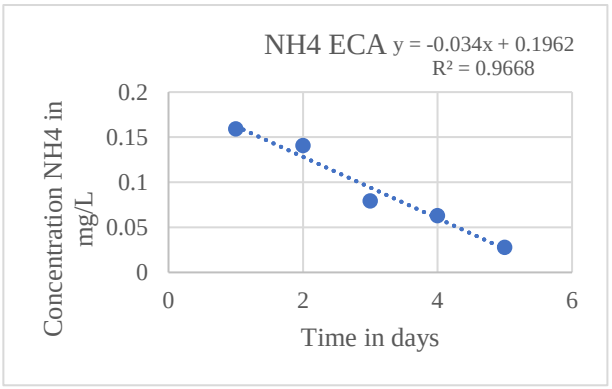


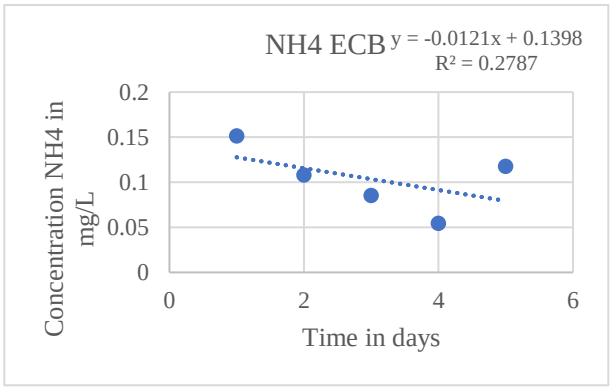









NH4 ECA
y = -0.034x + 0.1962
R² = 0.9668
Concentration NH4 in mg/L
0.2
0.15
0.1
0.05
0
0
2
4
6
Time in days


NH4 ECB
y = -0.0121x + 0.1398
R² = 0.2787
Concentration NH4 in mg/L
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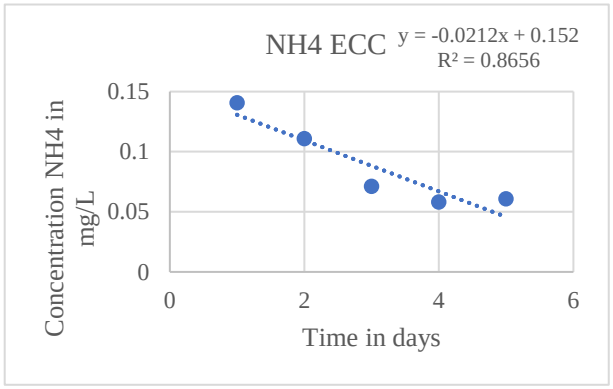
0.1
0.05
0
0
2
4
6
Time in days


NH4 ECC
y = -0.0212x + 0.152
R² = 0.8656
Concentration NH4 in mg/L
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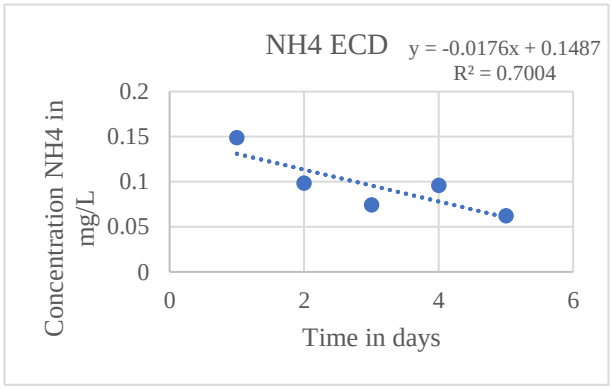
0.1
0.05
0
0
2
4
6
Time in days


NH4 ECD
y = -0.0176x + 0.1487
R² = 0.7004
Concentration NH4 in mg/L
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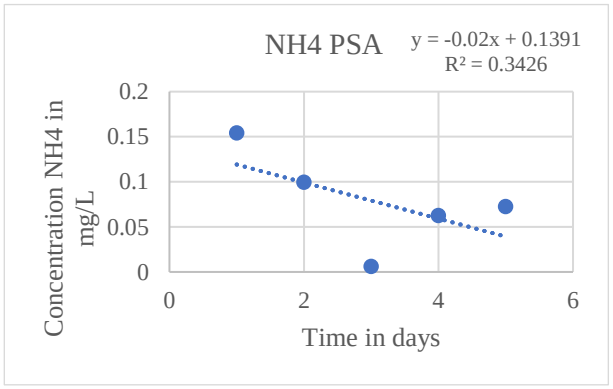
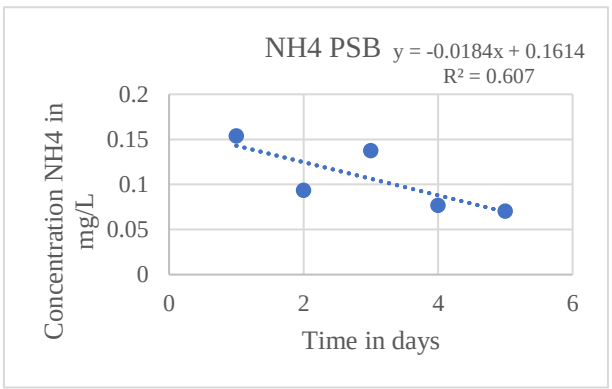
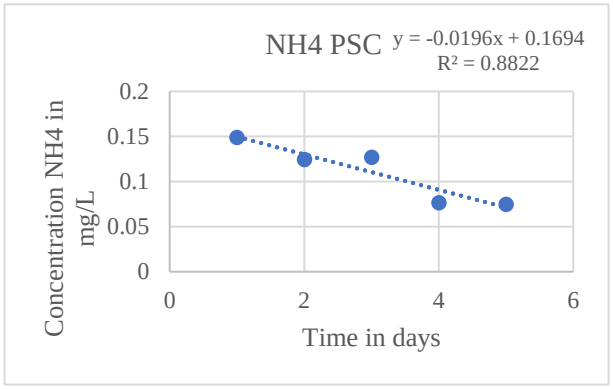
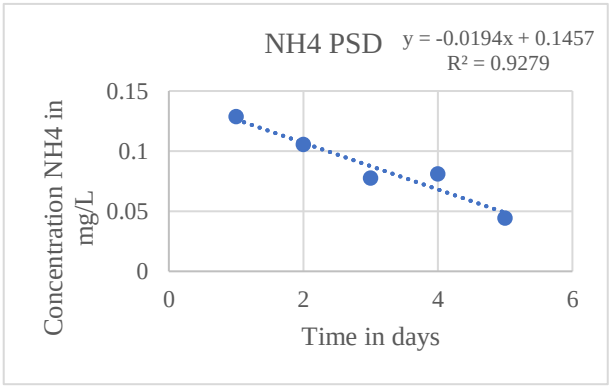
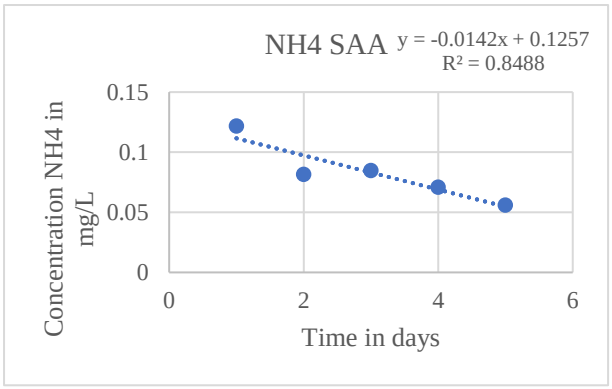
0.15
0.1
0.05
0
0
2
4
6
Time in days

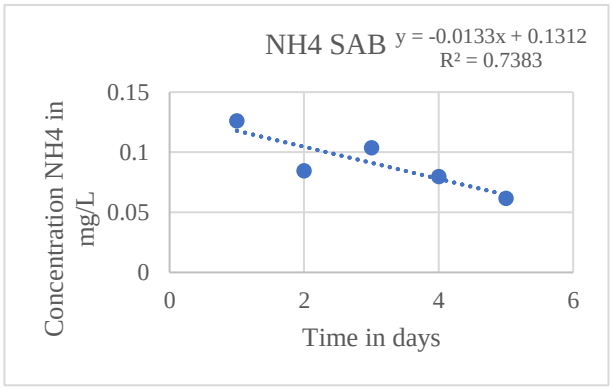

NH4 PSA
y = -0.02x + 0.1391
R² = 0.3426
Concentration NH4 in mg/L
0.2
0.15
0.1
0.05
0
0
2
4
6
Time in days

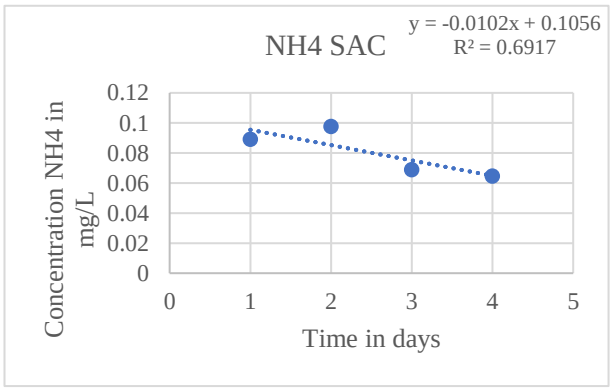

NH4 PSB
y = -0.0184x + 0.1614
R² = 0.607
Concentration NH4 in mg/L
0.2
0.15
0.1
0.05
0
0
2
4
6
Time in days

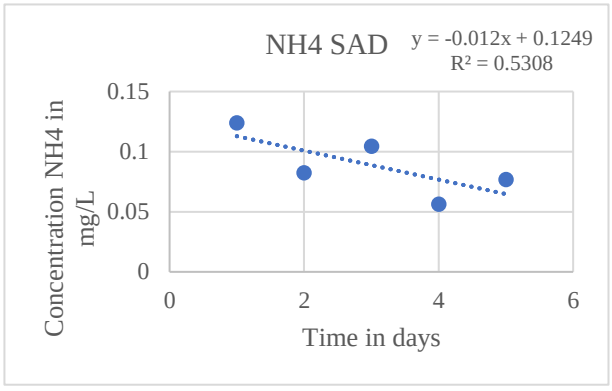

NH4 PSC
y = -0.0196x + 0.1694
R² = 0.8822
Concentration NH4 in mg/L
0.2
0.15
0.1
0.05
0
0
2
4
6
Time in days

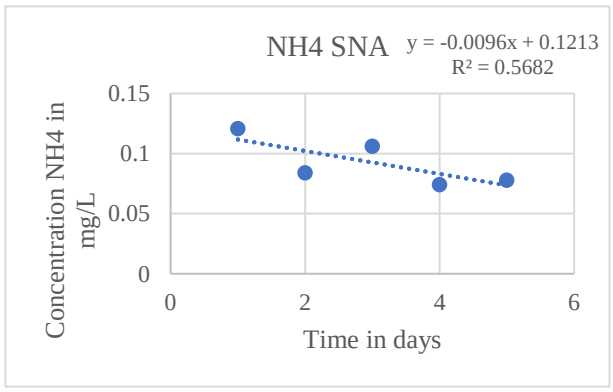

NH4 PSD
y = -0.0194x + 0.1457
R² = 0.9279
Concentration NH4 in mg/L
0.15
0.1
0.05
0
0
2
4
6
Time in days

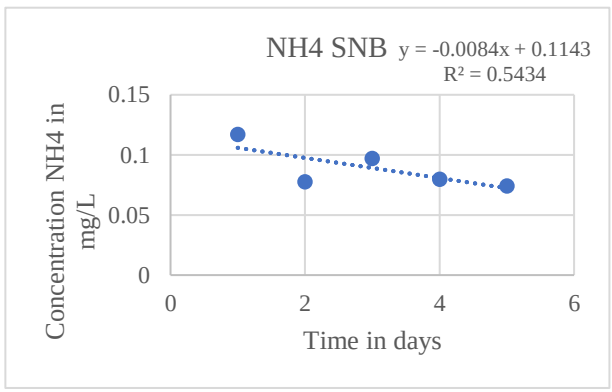

NH4 SAA
y = -0.0142x + 0.1257
R² = 0.8488
Concentration NH4 in mg/L
0.15
0.1
0.05
0
0
2
4
6
Time in days

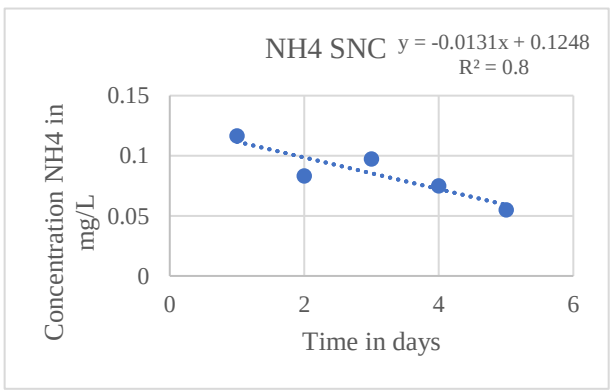

NH4 SAB
y = -0.0133x + 0.1312
R² = 0.7383
Concentration NH4 in mg/L
0.15
0.1
0.05
0
0
2
4
6
Time in days

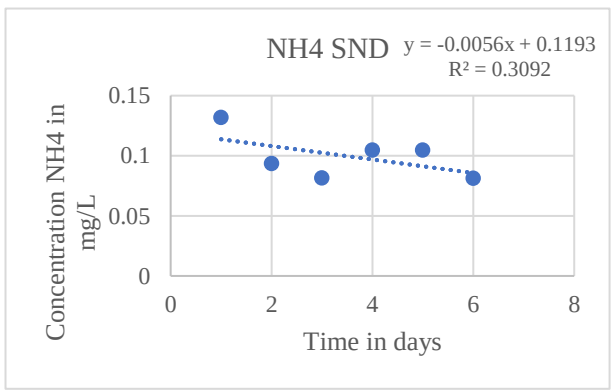

NH4 SAC
y = -0.0102x + 0.1056
R² = 0.6917
Concentration NH4 in mg/L
0.12
0.1
0.08
0.06
0.04
0.02
0
0
1
2
3
4
5
Time in days


NH4 SAD
y = -0.012x + 0.1249
R² = 0.5308
Concentration NH4 in mg/L
0.15
0.1
0.05
0
0
2
4
6
Time in days

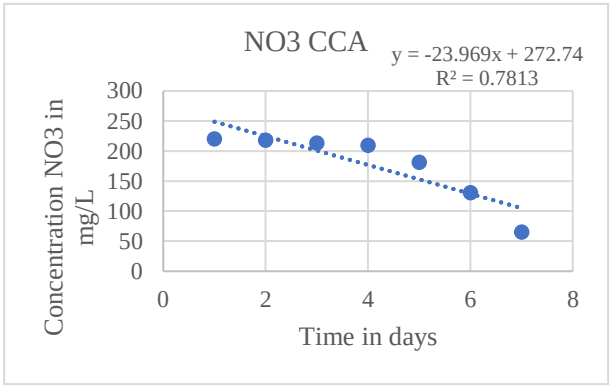

NH4 SNA
y = -0.0096x + 0.1213
R² = 0.5682
Concentration NH4 in mg/L
0.15
0.1
0.05
0
0
2
4
6
Time in days

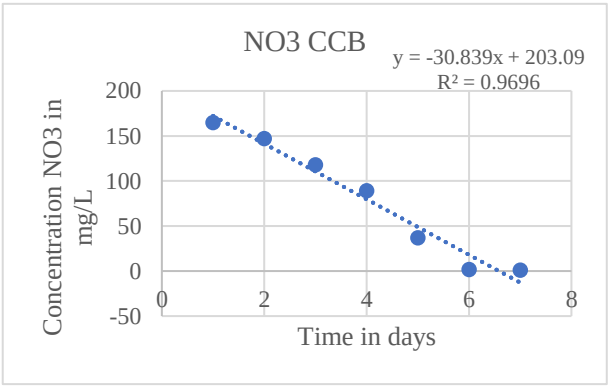

NH4 SNB
y = -0.0084x + 0.1143
R² = 0.5434
Concentration NH4 in mg/L
0.15
0.1
0.05
0
0
2
4
6
Time in days

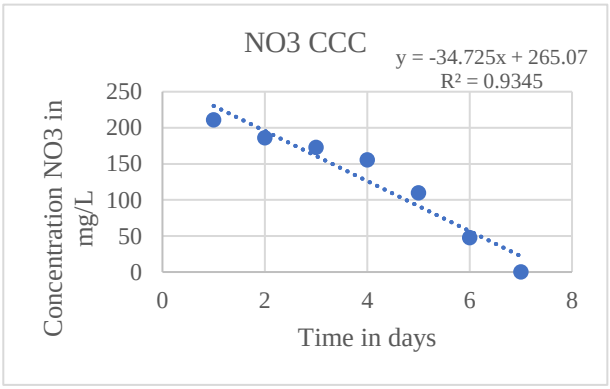

NH4 SNC
y = -0.0131x + 0.1248
R² = 0.8
Concentration NH4 in mg/L
0.15
0.1
0.05
0
0
2
4
6
Time in days

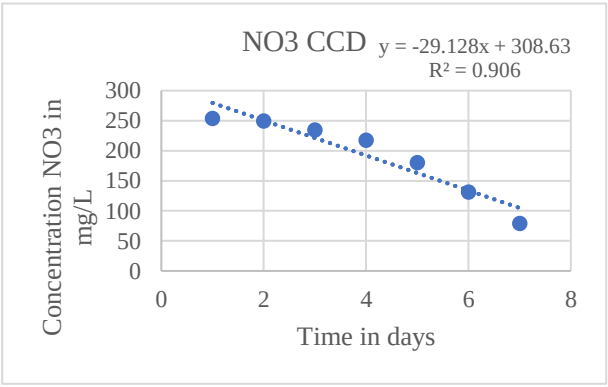

NH4 SND
y = -0.0056x + 0.1193
R² = 0.3092
Concentration NH4 in mg/L
0.15
0.1
0.05
0
0
2
4
6
8
Time in days

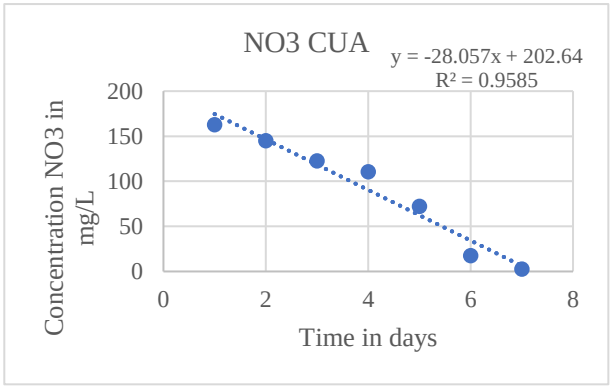
NO_3 scatter chart over time for each treatment

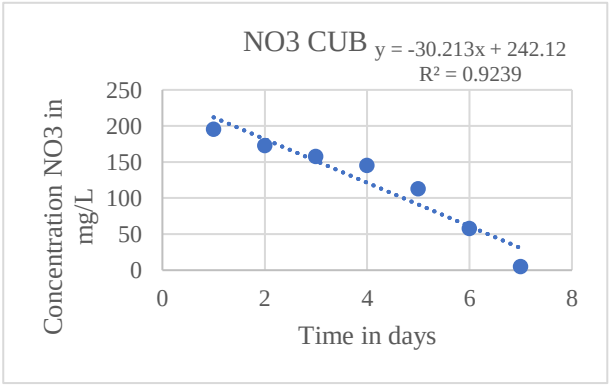


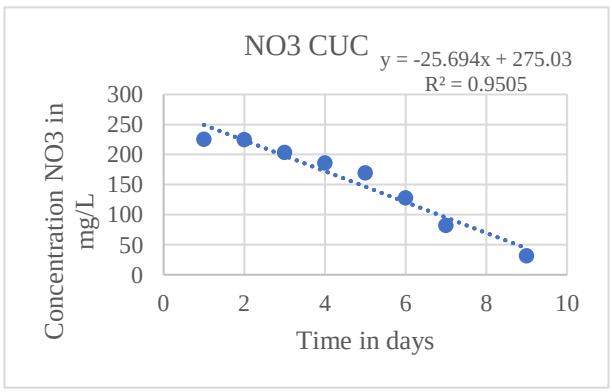


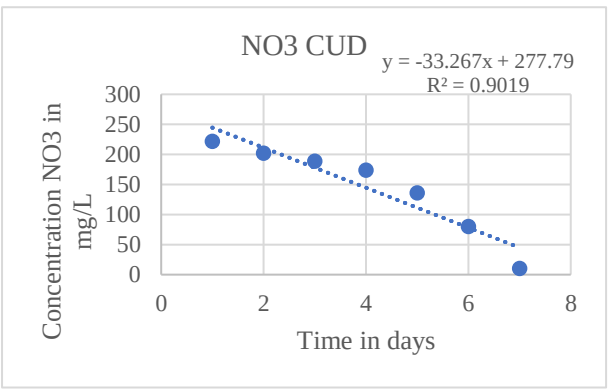


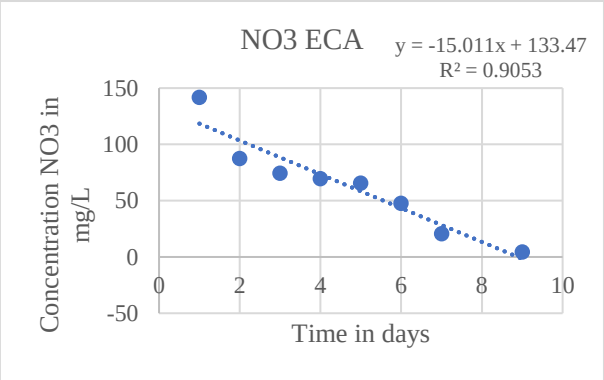


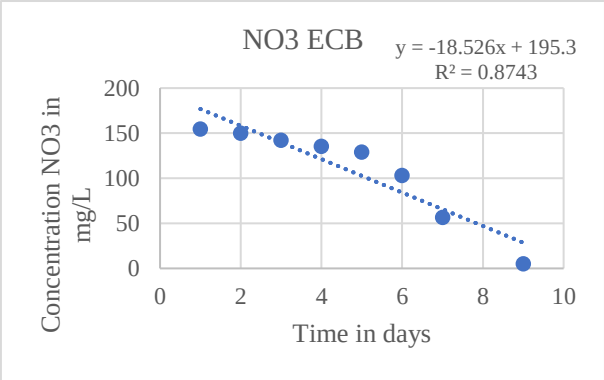


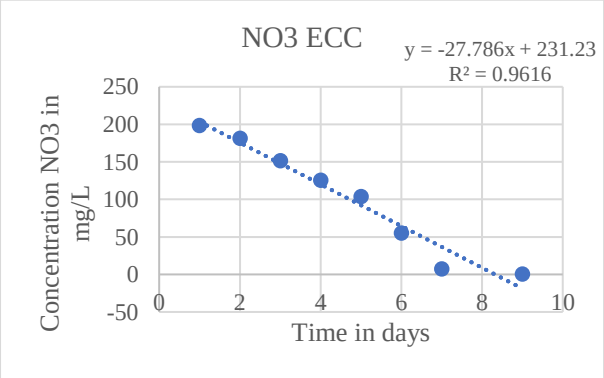


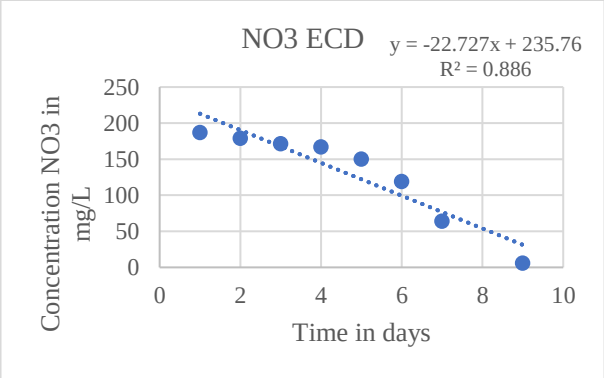


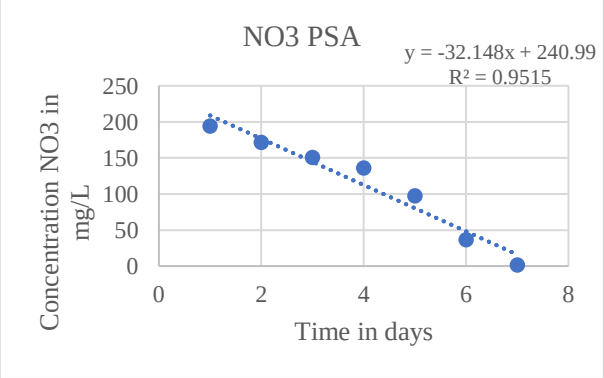


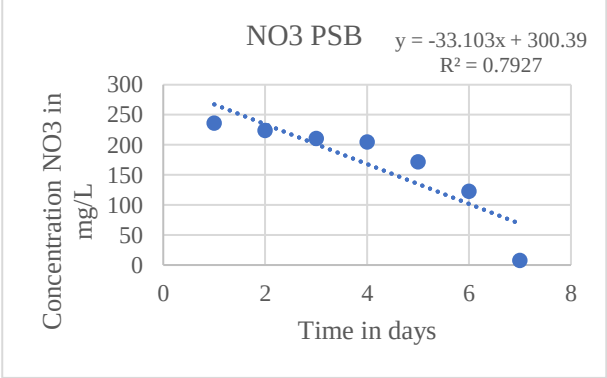

NO3 ECA
y = -15.011x + 133.47
R² = 0.9053
Concentration NO3 in mg/L
Time in days

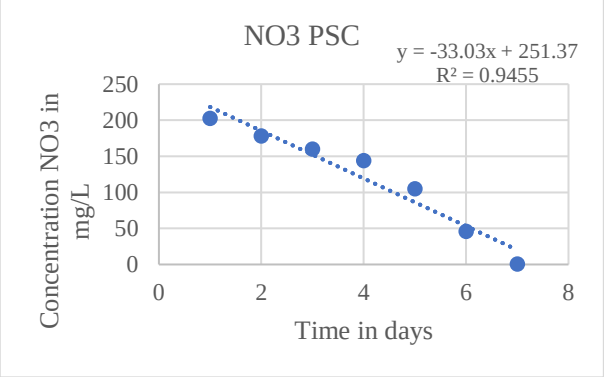

NO3 ECB
y = -18.526x + 195.3
R² = 0.8743
Concentration NO3 in mg/L
Time in days

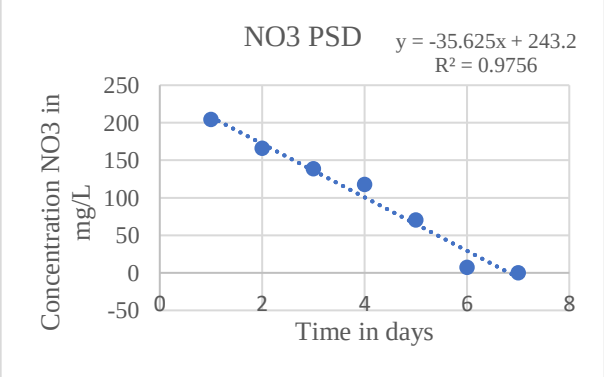

NO3 ECC
y = -27.786x + 231.23
R² = 0.9616
Concentration NO3 in mg/L
Time in days

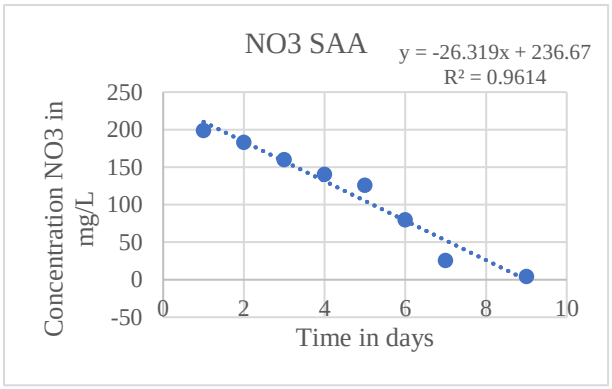

NO3 ECD
y = -22.727x + 235.76
R² = 0.886
Concentration NO3 in mg/L
Time in days

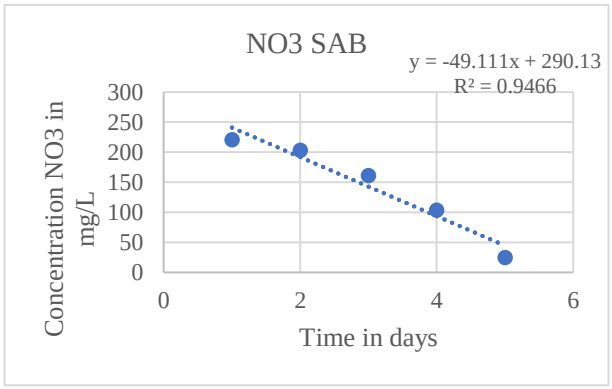

NO3 PSA
y = -32.148x + 240.99
R² = 0.9515
Concentration NO3 in mg/L
Time in days

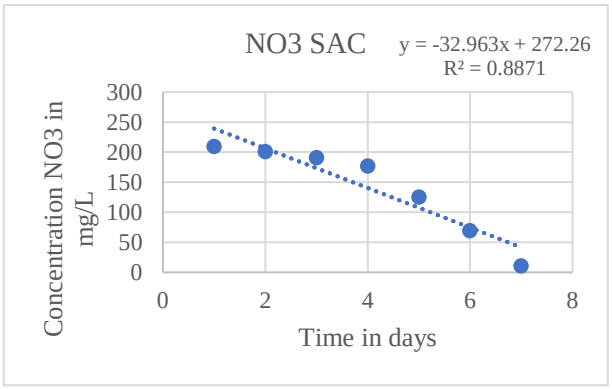

NO3 PSB
y = -33.103x + 300.39
R² = 0.7927
Concentration NO3 in mg/L
Time in days

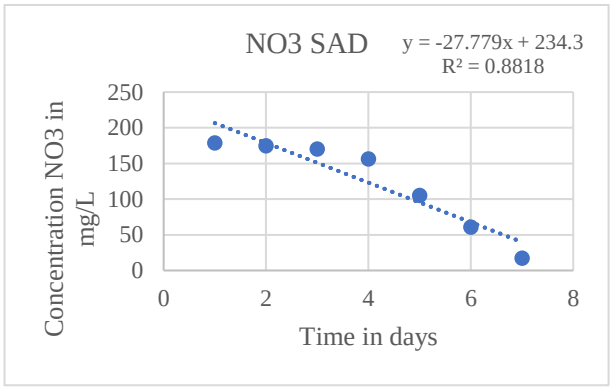

NO3 PSC
y = -33.03x + 251.37
R² = 0.9455
Concentration NO3 in mg/L
Time in days

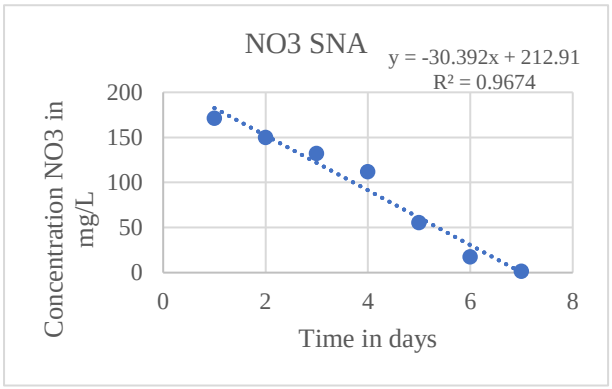

NO3 PSD
y = -35.625x + 243.2
R² = 0.9756
Concentration NO3 in mg/L
Time in days

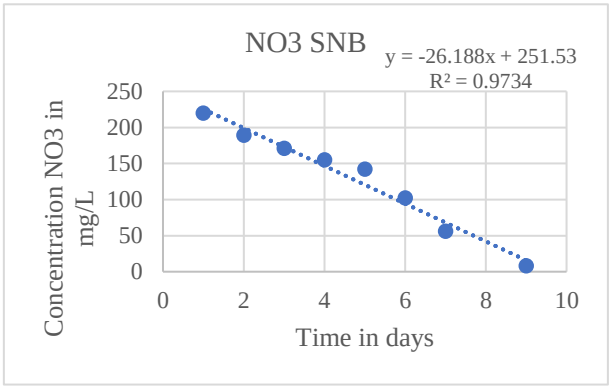

NO3 SAA
y = -26.319x + 236.67
R² = 0.9614
Concentration NO3 in mg/L
Time in days

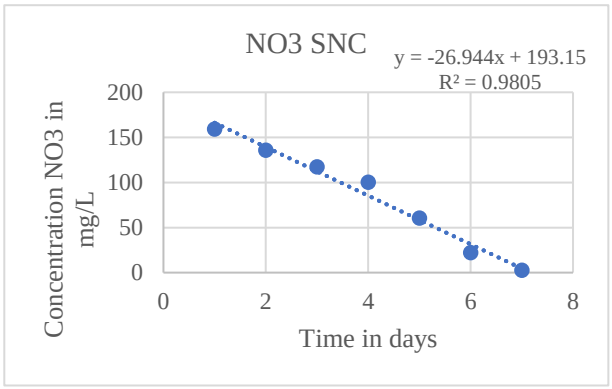

NO3 SAB
y = -49.111x + 290.13
R² = 0.9466
Concentration NO3 in mg/L
Time in days

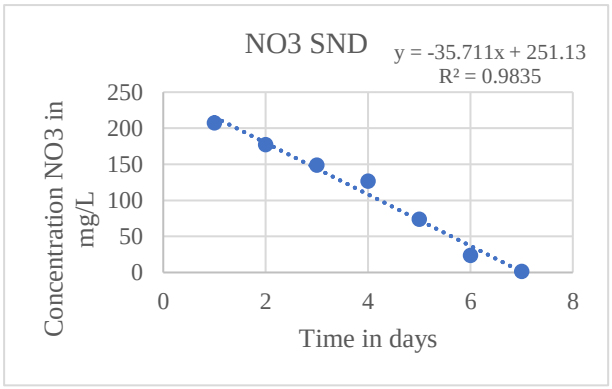

NO3 SAC
y = -32.963x + 272.26
R² = 0.8871
Concentration NO3 in mg/L
Time in days


NO3 SAD
y = -27.779x + 234.3
R² = 0.8818
Concentration NO3 in mg/L
Time in days

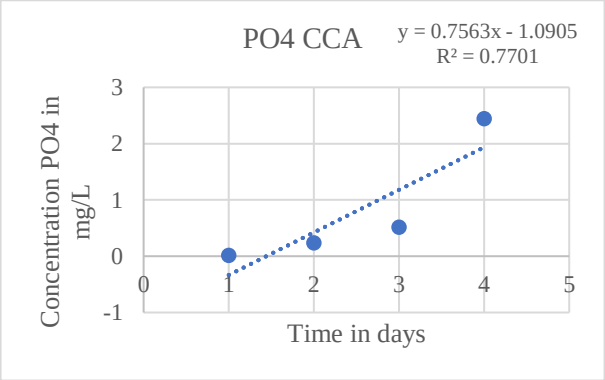

NO3 SNA
y = -30.392x + 212.91
R² = 0.9674
Concentration NO3 in mg/L
Time in days

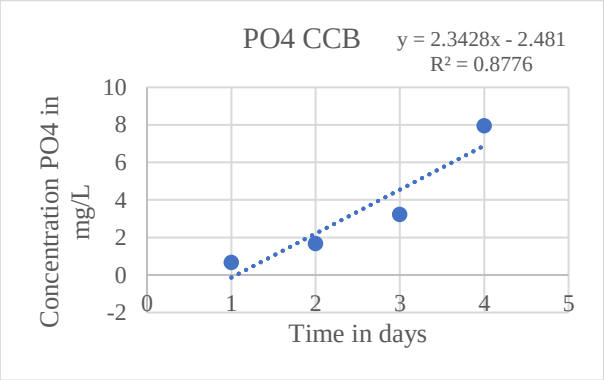

NO3 SNB
y = -26.188x + 251.53
R² = 0.9734
Concentration NO3 in mg/L
Time in days

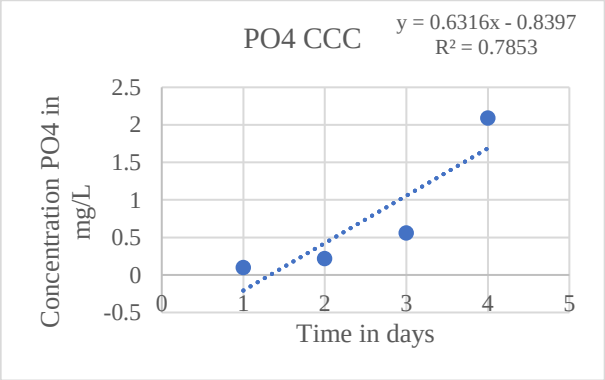

NO3 SNC
y = -26.944x + 193.15
R² = 0.9805
Concentration NO3 in mg/L
Time in days

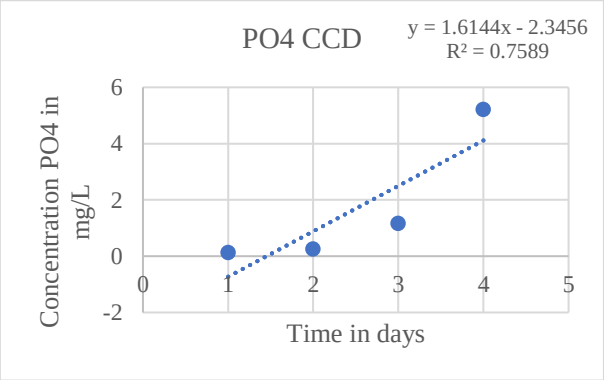

NO3 SND
y = -35.711x + 251.13
R² = 0.9835
Concentration NO3 in mg/L
Time in days

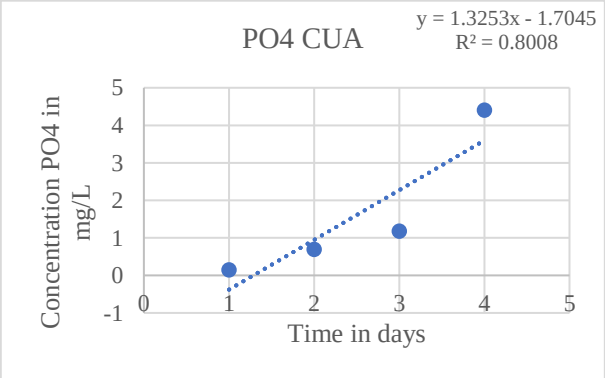
PO_4 scatter chart over time for each treatment

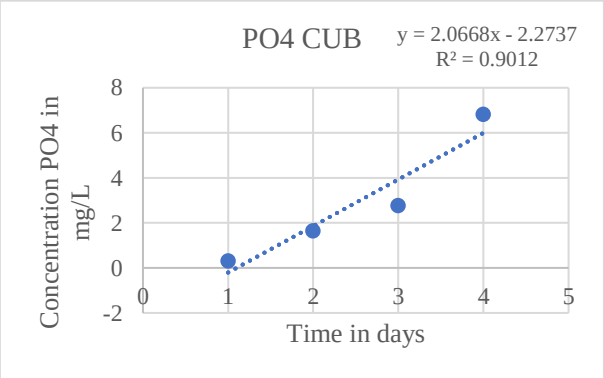


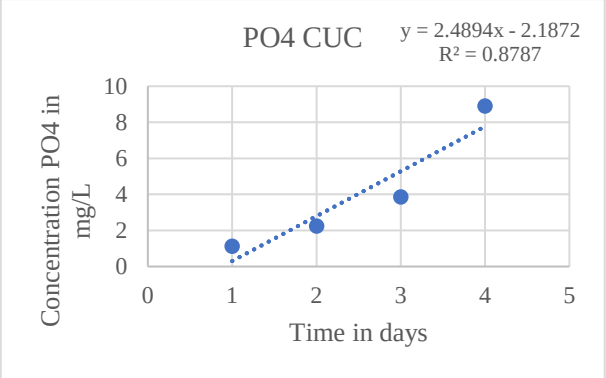


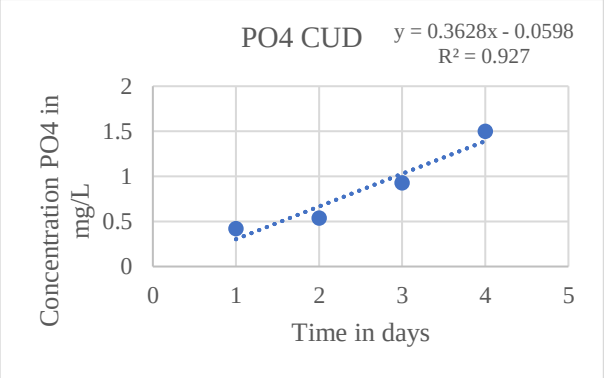


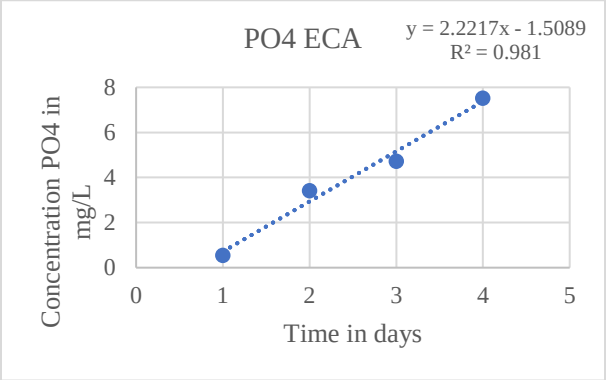


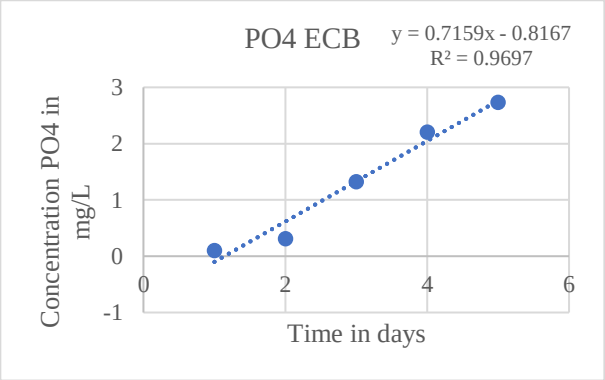


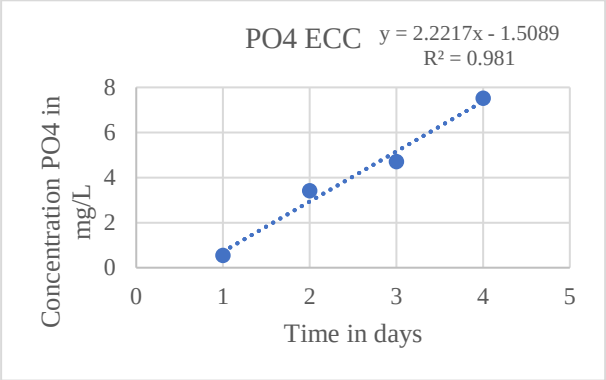


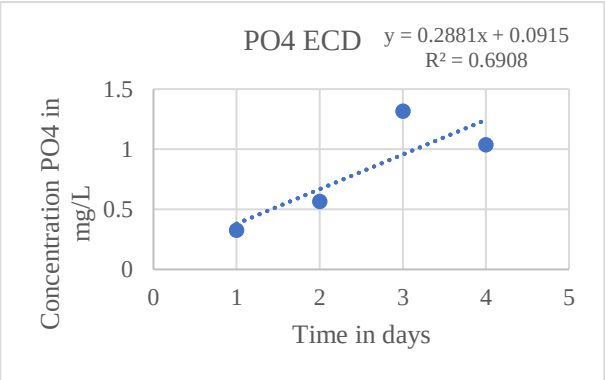


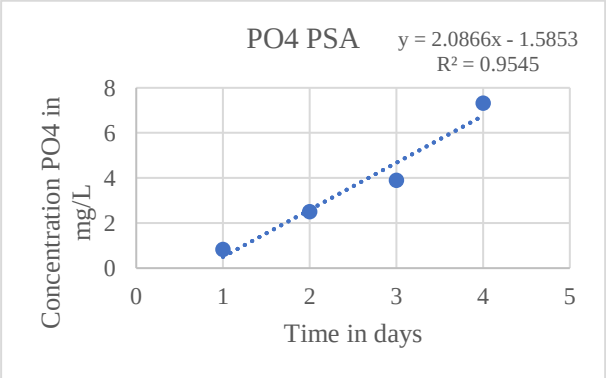


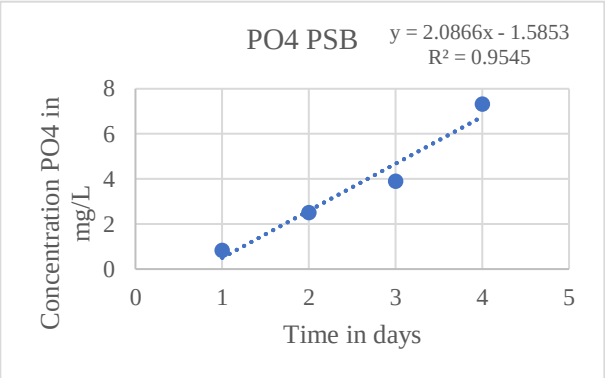

PO4 ECA
y = 2.2217x - 1.5089
R² = 0.981
Concentration PO4 in mg/L
Time in days

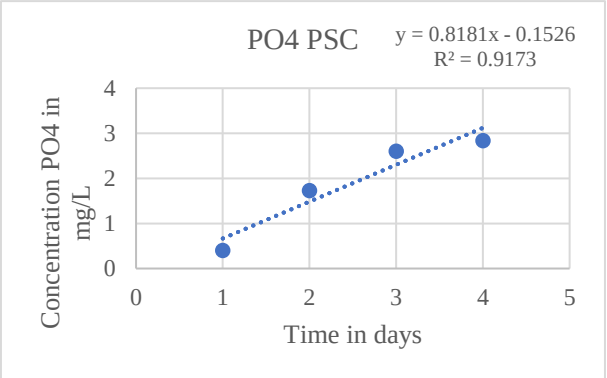

PO4 ECB
y = 0.7159x - 0.8167
R² = 0.9697
Concentration PO4 in mg/L
Time in days

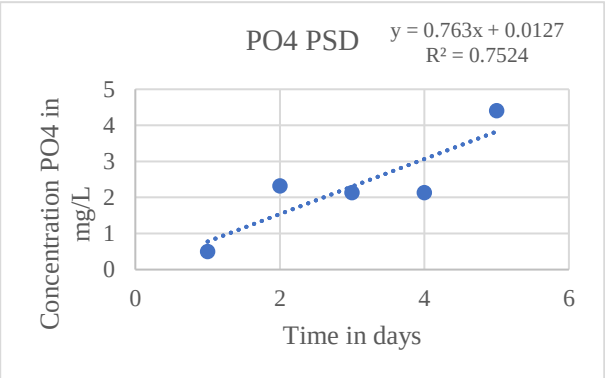

PO4 ECC
y = 2.2217x - 1.5089
R² = 0.981
Concentration PO4 in mg/L
Time in days

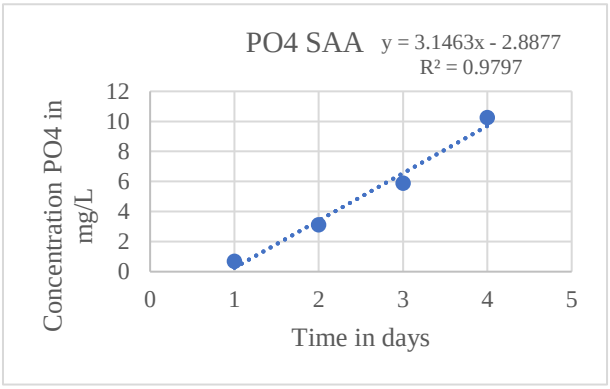

PO4 ECD
y = 0.2881x + 0.0915
R² = 0.6908
Concentration PO4 in mg/L
Time in days

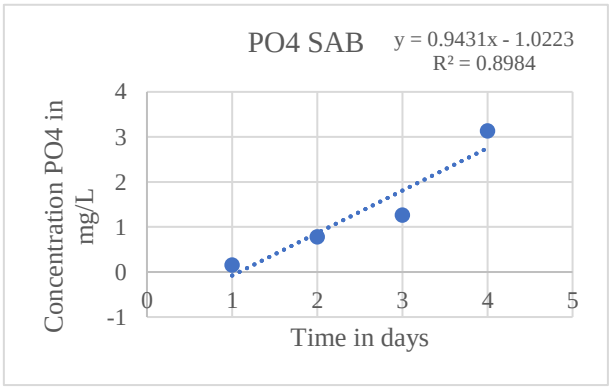

PO4 PSA
y = 2.0866x - 1.5853
R² = 0.9545
Concentration PO4 in mg/L
Time in days

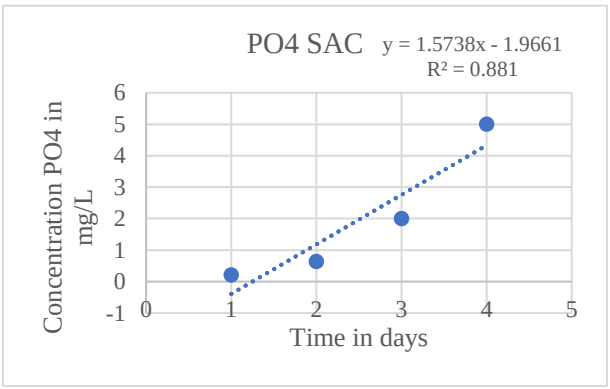

PO4 PSB
y = 2.0866x - 1.5853
R² = 0.9545
Concentration PO4 in mg/L
Time in days

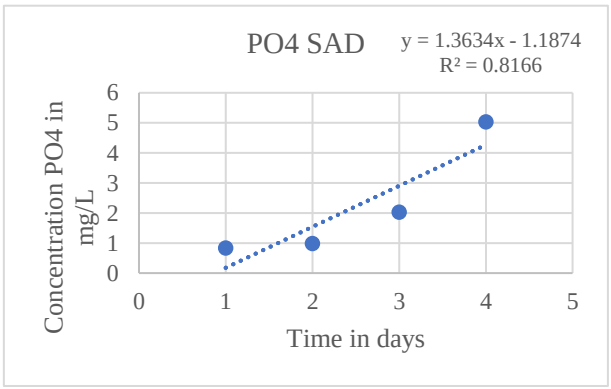

PO4 PSC
y = 0.8181x - 0.1526
R² = 0.9173
Concentration PO4 in mg/L
Time in days

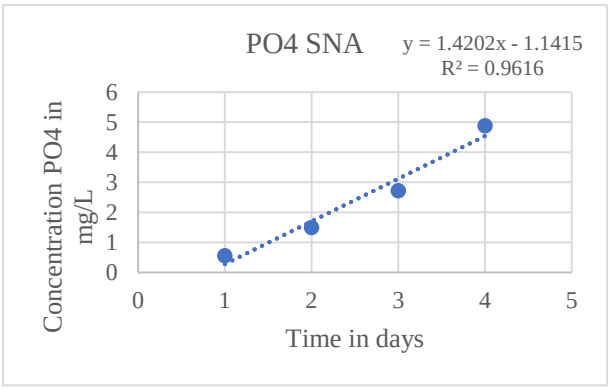

PO4 PSD
y = 0.763x + 0.0127
R² = 0.7524
Concentration PO4 in mg/L
Time in days

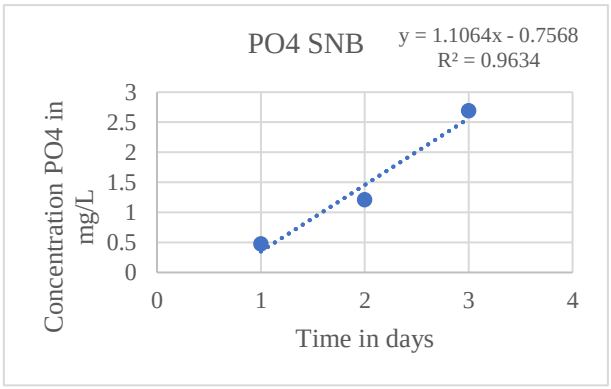

PO4 SAA
y = 3.1463x - 2.8877
R² = 0.9797
Concentration PO4 in mg/L
Time in days

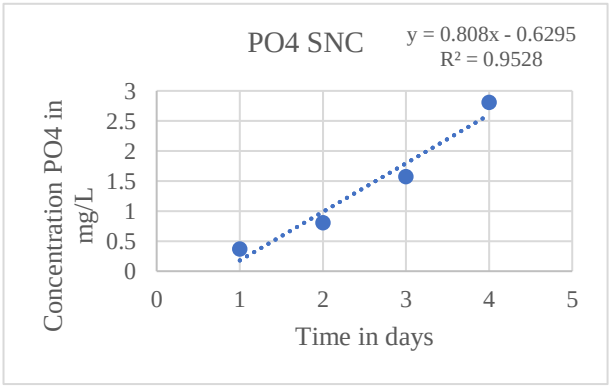

PO4 SAB
y = 0.9431x - 1.0223
R² = 0.8984
Concentration PO4 in mg/L
Time in days

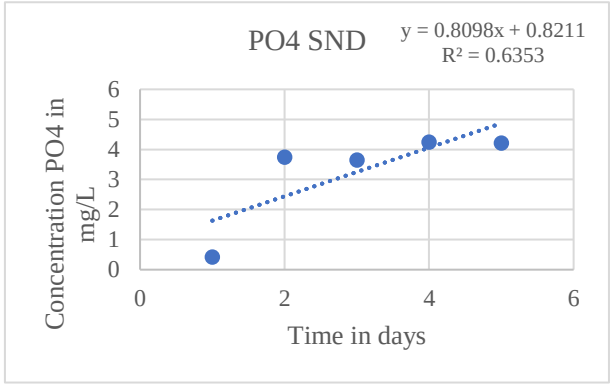

PO4 SAC
y = 1.5738x - 1.9661
R² = 0.881
Concentration PO4 in mg/L
Time in days


PO4 SAD
y = 1.3634x - 1.1874
R² = 0.8166
Concentration PO4 in mg/L
Time in days


PO4 SNA
y = 1.4202x - 1.1415
R² = 0.9616
Concentration PO4 in mg/L
Time in days


PO4 SNB
y = 1.1064x - 0.7568
R² = 0.9634
Concentration PO4 in mg/L
Time in days


PO4 SNC
y = 0.808x - 0.6295
R² = 0.9528
Concentration PO4 in mg/L
Time in days


PO4 SND
y = 0.8098x + 0.8211
R² = 0.6353
Concentration PO4 in mg/L
Time in days

VII) Descriptive statistics and post-hoc results

Wet weight production

Descriptive Statistics

	Biomassa in grams			
	EC	**PS**	**SA**	**SN**
Valid	4	4	4	4
Missing	0	0	0	0
Mean	92.500	92.000	54.725	208.185
Std. Deviation	16.743	25.020	11.274	14.005
Minimum	72.000	57.000	40.500	188.800
Maximum	113.000	114.000	67.200	219.000

ANOVA - Biomassa in grams

Cases	Sum of Squares	df	Mean Square	F	p	η^2	η^2_p	ω^2
Treatment	53248.571	3	17749.524	57.742	< .001	0.935	0.935	0.914
Residuals	3688.722	12	307.394					

Note. Type III Sum of Squares

Post Hoc Tests

Standard

Post Hoc Comparisons - Treatment

			95% CI for Mean Difference					
		Mean Difference	**Lower**	**Upper**	**SE**	**t**	p_{tukey}	
EC	PS	0.500	-36.307	37.307	12.397	0.040	1.000	
	SA	37.775	0.968	74.582	12.397	3.047	0.044	*
	SN	-115.685	-152.492	-78.878	12.397	-9.331	< .001	***
PS	SA	37.275	0.468	74.082	12.397	3.007	0.047	*
	SN	-116.185	-152.992	-79.378	12.397	-9.372	< .001	***
SA	SN	-153.460	-190.267	-116.653	12.397	-12.378	< .001	***

* p < .05, *** p < .001

Note. P-value and confidence intervals adjusted for comparing a family of 4 estimates (confidence intervals corrected using the tukey method).

NH_4 removal efficiency

Descriptive Statistics

	NH4 removal in mg/L/day					
	CC	**CU**	**EC**	**PS**	**SA**	**SN**
Valid	4	4	4	4	4	4
Missing	0	0	0	0	0	0
Mean	0.031	0.026	0.021	0.019	0.012	0.009
Std. Deviation	0.006	0.002	0.009	6.807e -4	0.002	0.003
Minimum	0.026	0.024	0.012	0.018	0.010	0.006
Maximum	0.040	0.028	0.034	0.020	0.014	0.013

ANOVA - NH4 removal in mg/L/day

Cases	Sum of Squares	df	Mean Square	F	p	η^2	η^2_p	ω^2
Treatment	0.001	5	2.706e -4	11.581	< .001	0.763	0.763	0.688
Residuals	4.206e -4	18	2.337e -5					

Note. Type III Sum of Squares

Post Hoc Tests

Standard

Post Hoc Comparisons - Treatment

			95% CI for Mean Difference					
		Mean Difference	**Lower**	**Upper**	**SE**	**t**	**p_{tukey}**	
CC	CU	0.005	-0.006	0.016	0.003	1.470	0.686	
	EC	0.010	-9.382e -4	0.021	0.003	2.904	0.085	
	PS	0.012	9.368e -4	0.023	0.003	3.452	0.029	*
	SA	0.019	0.008	0.030	0.003	5.478	< .001	***
	SN	0.022	0.011	0.033	0.003	6.429	< .001	***
CU	EC	0.005	-0.006	0.016	0.003	1.433	0.707	
	PS	0.007	-0.004	0.018	0.003	1.982	0.389	
	SA	0.014	0.003	0.025	0.003	4.008	0.009	**
	SN	0.017	0.006	0.028	0.003	4.959	0.001	**
EC	PS	0.002	-0.009	0.013	0.003	0.549	0.993	
	SA	0.009	-0.002	0.020	0.003	2.574	0.155	
	SN	0.012	0.001	0.023	0.003	3.525	0.025	*

Post Hoc Comparisons - Treatment

			95% CI for Mean Difference				
		Mean Difference	Lower	Upper	SE	t	p_{tukey}
PS	SA	0.007	-0.004	0.018	0.003	2.026	0.367
	SN	0.010	-6.882e -4	0.021	0.003	2.977	0.074
SA	SN	0.003	-0.008	0.014	0.003	0.951	0.927

Note. P-value and confidence intervals adjusted for comparing a family of 6 estimates (confidence intervals corrected using the tukey method).

* p < .05, ** p < .01, *** p < .001

NH_4 concentration in mg/Lafter day 5

Descriptive Statistics

	NH4 concentration in mg/L					
	CC	CU	EC	PS	SA	SN
Valid	19	20	20	20	20	20
Missing	0	0	0	0	0	0
Mean	0.111	0.098	0.095	0.096	0.088	0.091
Std. Deviation	0.052	0.039	0.038	0.039	0.024	0.020
Minimum	0.046	0.044	0.028	0.006	0.056	0.055
Maximum	0.244	0.163	0.159	0.154	0.131	0.132

ANOVA - NH4 concentration in mg/L

Cases	Sum of Squares	df	Mean Square	F	p	η^2	η^2_p	ω^2
Treatment	0.006	5	0.001	0.914	0.475	0.039	0.039	0.000
Residuals	0.151	113	0.001					

Note. Type III Sum of Squares

Standard

Post Hoc Comparisons - Treatment

		Mean Difference	SE	t	p_{tukey}
CC	CU	0.013	0.012	1.140	0.863
	EC	0.016	0.012	1.361	0.750
	PS	0.015	0.012	1.321	0.773
	SA	0.023	0.012	1.964	0.369
	SN	0.020	0.012	1.700	0.534

Post Hoc Comparisons - Treatment

		Mean Difference	SE	t	p_{tukey}
CU	EC	0.003	0.012	0.224	1.000
	PS	0.002	0.012	0.184	1.000
	SA	0.010	0.012	0.835	0.960
	SN	0.007	0.012	0.567	0.993
EC	PS	-4.618e -4	0.012	-0.040	1.000
	SA	0.007	0.012	0.611	0.990
	SN	0.004	0.012	0.344	0.999
PS	SA	0.008	0.012	0.651	0.987
	SN	0.004	0.012	0.384	0.999
SA	SN	-0.003	0.012	-0.267	1.000

Note. P-value adjusted for comparing a family of 6

NO_3 removal efficiency

Descriptive Statistics

	NO3 removal in mg/L/day					
	CC	CU	EC	PS	SA	SN
Valid	4	4	4	4	4	4
Missing	0	0	0	0	0	0
Mean	29.665	29.308	21.012	33.477	34.043	29.809
Std. Deviation	4.461	3.221	5.508	1.497	10.442	4.339
Minimum	23.969	25.694	15.011	32.148	26.319	26.188
Maximum	34.725	33.267	27.786	35.625	49.111	35.711

ANOVA

ANOVA - NO3 removal in mg/L/day

Cases	Sum of Squares	df	Mean Square	F	p	η^2	η^2_p	ω^2
Treatment	434.529	5	86.906	2.734	0.052	0.432	0.432	0.265
Residuals	572.176	18	31.788					

Note. Type III Sum of Squares

Post Hoc Tests

Standard

Post Hoc Comparisons - Treatment

			95% CI for Mean Difference					
		Mean Difference	**Lower**	**Upper**	**SE**	**t**	**p_{tukey}**	
CC	CU	0.357	-12.312	13.027	3.987	0.090	1.000	
	EC	8.653	-4.017	21.323	3.987	2.170	0.298	
	PS	-3.811	-16.481	8.859	3.987	-0.956	0.926	
	SA	-4.378	-17.048	8.292	3.987	-1.098	0.876	
	SN	-0.144	-12.813	12.526	3.987	-0.036	1.000	
CU	EC	8.295	-4.375	20.965	3.987	2.081	0.339	
	PS	-4.169	-16.839	8.501	3.987	-1.046	0.896	
	SA	-4.735	-17.405	7.935	3.987	-1.188	0.837	
	SN	-0.501	-13.171	12.169	3.987	-0.126	1.000	
EC	PS	-12.464	-25.134	0.206	3.987	-3.126	0.055	
	SA	-13.030	-25.700	-0.361	3.987	-3.268	0.042	*
	SN	-8.796	-21.466	3.874	3.987	-2.206	0.282	
PS	SA	-0.566	-13.236	12.103	3.987	-0.142	1.000	
	SN	3.668	-9.002	16.338	3.987	0.920	0.936	
SA	SN	4.234	-8.436	16.904	3.987	1.062	0.890	

* $p < .05$

Note. P-value and confidence intervals adjusted for comparing a family of 6 estimates (confidence intervals corrected using the tukey method).

NO_3 concentration in mg/L at day 14

Descriptive Statistics

	NO3 concentration in mg/L					
	CC	**CU**	**EC**	**PS**	**SA**	**SN**
Valid	4	4	4	4	4	4
Missing	0	0	0	0	0	0
Mean	36.163	12.401	3.946	2.386	14.317	3.374
Std. Deviation	41.582	13.070	2.563	3.387	8.801	3.115
Minimum	0.110	2.581	0.184	0.184	4.359	1.444
Maximum	78.660	31.402	5.893	7.415	24.991	7.983

ANOVA - NO3 concentration in mg/L

Cases	Sum of Squares	df	Mean Square	F	p	η^2	η^2_p	ω^2
Treatment	3284.119	5	656.824	1.965	0.133	0.353	0.353	0.167
Residuals	6015.279	18	334.182					

Note. Type III Sum of Squares

Standard

Post Hoc Comparisons - Treatment

		Mean Difference	SE	t	p_{tukey}
CC	CU	23.762	12.926	1.838	0.468
	EC	32.217	12.926	2.492	0.178
	PS	33.777	12.926	2.613	0.145
	SA	21.846	12.926	1.690	0.555
	SN	32.789	12.926	2.537	0.165
CU	EC	8.455	12.926	0.654	0.985
	PS	10.016	12.926	0.775	0.968
	SA	-1.916	12.926	-0.148	1.000
	SN	9.027	12.926	0.698	0.980
EC	PS	1.560	12.926	0.121	1.000
	SA	-10.371	12.926	-0.802	0.963
	SN	0.572	12.926	0.044	1.000
PS	SA	-11.931	12.926	-0.923	0.935
	SN	-0.988	12.926	-0.076	1.000
SA	SN	10.943	12.926	0.847	0.954

Note. P-value adjusted for comparing a family of 6

PO_4 removal efficiency

Descriptive Statistics

	PO4 removal in mg/l/day					
	CC	**CU**	**EC**	**PS**	**SA**	**SN**
Valid	4	4	4	4	4	4
Missing	0	0	0	0	0	0
Mean	0.852	0.871	0.907	0.895	0.894	0.878
Std. Deviation	0.039	0.056	0.140	0.096	0.067	0.162
Minimum	0.800	0.801	0.698	0.752	0.817	0.635
Maximum	0.882	0.927	0.981	0.955	0.980	0.963

ANOVA - PO4 removal in mg/l/day

Cases	Sum of Squares	df	Mean Square	F	p	η^2	η^2_p	ω^2
Treatment	0.008	5	0.002	0.150	0.977	0.040	0.040	0.000
Residuals	0.193	18	0.011					

Note. Type III Sum of Squares

Post Hoc Tests

Standard

Post Hoc Comparisons - Treatment

			95% CI for Mean Difference				
		Mean Difference	**Lower**	**Upper**	**SE**	**t**	**p_{tukey}**
CC	CU	-0.019	-0.251	0.213	0.073	-0.260	1.000
	EC	-0.056	-0.288	0.177	0.073	-0.762	0.970
	PS	-0.043	-0.275	0.189	0.073	-0.588	0.991
	SA	-0.042	-0.275	0.190	0.073	-0.578	0.991
	SN	-0.027	-0.259	0.206	0.073	-0.364	0.999
CU	EC	-0.037	-0.269	0.196	0.073	-0.503	0.995
	PS	-0.024	-0.256	0.208	0.073	-0.328	0.999

Post Hoc Comparisons - Treatment

			95% CI for Mean Difference				
		Mean Difference	**Lower**	**Upper**	**SE**	**t**	**p_{tukey}**
	SA	-0.023	-0.256	0.209	0.073	-0.318	0.999
	SN	-0.008	-0.240	0.225	0.073	-0.104	1.000
EC	PS	0.013	-0.220	0.245	0.073	0.174	1.000
	SA	0.013	-0.219	0.246	0.073	0.185	1.000
	SN	0.029	-0.203	0.262	0.073	0.398	0.998
PS	SA	7.500e -4	-0.232	0.233	0.073	0.010	1.000
	SN	0.016	-0.216	0.249	0.073	0.224	1.000
SA	SN	0.016	-0.217	0.248	0.073	0.214	1.000

Note. P-value and confidence intervals adjusted for comparing a family of 6 estimates (confidence intervals corrected using the tukey method).