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Retention and biological uptake of phosphorous in the Leikvollbekken constructed wetland

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Abstract

Phosphorous is the limiting nutrient for primary production in freshwater systems and excessively applied as inorganic fertilizer in agriculture. Elevated phosphorous concentrations in recipients can cause eutrophication which reduces the recipient water quality. The main focus of this study is to investigate the retention of phosphorous in a mature constructed wetland and the phosphorous uptake and removal in/with plant biomass. The constructed wetland studied is a two pond free water surface system treating agricultural runoff to improve the water quality of the adjacent recipient. The investigation of the phosphorous retention includes determination of retention of different phosphorous fractions, and how their retention is influenced by other parameters. Furthermore, the uptake of phosphorous by plants is studied and the amount of phosphorous that can be removed by harvesting vegetation is determined. The determination of phosphorous retention/removal by physiogeochemical and biological processes was achieved by performing weekly analyses of water and plant samples. Water samples were analyzed for total phosphorous, total dissolved phosphorous and phosphate. Additional analysis of total and dissolved iron, turbidity, total suspended solids, and color were performed to identify a possible impact on phosphorous retention. Plant samples were analyzed for total phosphorous content in root, stem and leave. The total phosphorous retention efficiency in the constructed wetland was 31 %, which accounts for 19.9 kg P/year. The phosphate retention was only 3 %, which accounts for 0.3 kg P/year. The total amount of phosphorous that could be removed by harvesting vegetation of one pond once a year was 2.42 kg P/year, which accounts for 4 % of the total phosphorous mass entering the wetland per year. The sedimentation zones in the constructed wetland are filled up with sediment, which causes particle wash-out at high hydraulic loadings and a low retention of phosphorus. Sediment conditions are not favorable for phosphate retention, as adsorption sites are saturated and precipitation with iron cannot occur. The latter may be due to anaerobic conditions during winter and a too high pH-value. The efficient phosphorous retention depends on the regular sediment and/or biomass withdrawal and the avoidance of peak flow sediment wash out by implementing a storm water by-passing.

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Stavanger, June 2017

Friederike Krahner

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Abbreviations

BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CW	Constructed wetland
DON	Dissolved organic nitrogen
DOP	Dissolved organic phosphorous
FWS	Free water surface
HSF	Horizontal subsurface flow
IPCC	Intergovernmental Panel in Climate Change
NIBIO	Norwegian Institute of Bioeconomy Research
PAO	Phosphorous accumulating organisms
TDP	Total dissolved phosphorous
TDS	Total dissolved solids
TFS	Total fixed solids
TP	Total phosphorous
TS	Total solids
TSS	Total suspended solids
TVS	Total volatile solids
VSF	Vertical subsurface flow

1. Introduction

Humans are continuously changing Earth to meet emerging challenges, where between one-third and one-half of the Earth's land surface has already been altered by human actions (Vitousek, Mooney, Lubchenco, & Melillo, 1997). Efficient agriculture is key to keep up with the worlds increasing demand for food. Use of fertilizers to increase growth is common practice in modern agriculture. Although use of fertilizers has a positive effect on the world's food production, excessive use of fertilizers has a negative effect on the ecological function and biodiversity of ecosystems. Agriculture disturbs natural, independent systems by changing their complex biological and physical structures and functions. (Moss, 2008).

Phosphorous is a major reason for eutrophication of waters as it is a common constituent of inorganic fertilizers applied in agriculture. P reaches water bodies either bound to soil particles which follow surface runoff due to erosion, or due to the washout of dissolved P (Søvik, 2007). The application and discharge of phosphorous has already been limited legally, and restrictions tend to continue, as a consequence of extensive eutrophication problems. However, the role of phosphorous in changing our environment globally continues for generations (Smil, 2000).

Experiments using wetland plants to treat different kinds of water were performed for the first time in the 1950s in Germany by Dr. Käthe Seidel. Her work was the start of a new era of natural water treatment and was followed by the first operation of a full-scale wetland system in the late 1960s (Vymazal, 2008). Since that time, constructed wetlands have gained a lot of interest due to their economic and ecological cost-benefits and research on constructed wetlands is expected to increase (Zhi & Ji, 2012). Constructed wetlands are considered as a low-cost alternative for treating different kinds of wastewater, including agricultural effluents (Kadlec & Reddy, 2001). They come in numerous designs – with horizontal or vertical flow, surface or subsurface flow, vegetated with different kinds of macrophytes or non-vegetated, and with a variety of different filter media.

The treatment of agricultural runoff to reduce phosphorous controlled eutrophication is of high interest to improve water quality in recipients. Despite the extensive use of constructed wetlands, they are often presented as a black-box and little knowledge on the retention mechanisms involved and long-term efficiency is present. Research to better understand the retention mechanisms so wetlands can be adjusted to treat water most efficiently is therefor of high interest. Furthermore, research on how treatment efficiency will develop gradually as constructed wetlands mature is important to ensure a good long-term treatment efficiency. This thesis will investigate the use of constructed wetlands to retain phosphorous – which mechanisms are involved and how will vegetation influence phosphorous retention.

2. Theoretical background

2.1 Water Quality Parameters

Access to water is essential for life. Although quantity is important, water quality is of equal importance. Poor quality has a negative influence on human and animal health, and can perturb natures ecological cycles. Reduced water quality can either be a result of natural processes or human pollution. Several parameters are of particular interest for qualitative estimations, which will potentially restrict the use of water (Ødegaard, Norheim, & Norsk Vann, 2012). This chapter describes and defines relevant water quality parameters regarding the theoretical background for this thesis.

2.1.1 Physical Characteristics

Particles

There are several ways of classifying particles. Depending on their size, particles are divided into three categories. (1) Settable solids are larger than 100 µm and will settle out of suspension within the period of hydraulic retention. (2) Suspended particles are in the size range from 1 µm to 100 µm and will settle out of suspension extremely slowly – they will not settle within the time determined by the hydraulic retention time and stay to some extent in suspension. (3) Colloidal particles vary from 0.01 µm and 1µm and will stay in suspension (Ødegaard et al., 2012).

Based on particles' physical characteristics, they can be divided into several groups. Total solids (TS) is defined as the residues left after evaporating a sample to dryness at 105 °C. TS consists of total volatile solids (TVS) and total fixed solids (TFS). Total volatile solids is defined as the solids that can be burned off when TS is ignited at 550 °C, while total fixed solids is the residue that remains after ignition (Tchobanoglous et al., 2003).

Total solids can also be defined as the sum of total suspended solids (TSS) and total dissolved solids (TDS). TSS is the residue left on a filter with a specified pore size, measured after being dried at 105°C. Total dissolved solids are simply the difference between TS and TSS, and consists of both colloidal and dissolved solids. TDS is the fraction that passes through a filter with specified pore size, measured after being dried at 105°C. Both, TSS and TDS, can be divided into their volatile and fixed fractions the same way as total solids (Tchobanoglous et al., 2003).

Particles occur naturally in water and can have many different origins. Erosion of land results in the transport of particles from one location to another, such as from land to water bodies. Particles that are being transported to different locations can carry other compounds with/within them, thereby spreading these into nature. A common example are particles that are being transported from agricultural land and that carry nutrients such as phosphorous and nitrogen. These nutrients can cause problems which will be explained later. Particles entering water bodies will either settle or stay in suspension, depending on the particles properties and flow characteristics. Particles that settle, become part of the sediments and, both particles and components bound to them, can participate in physical, chemical and biological processes. Particles that will stay in suspension will cause water to be more turbid (next section) and enables components bound to their surface to diffuse out into the open water phase.

Turbidity

Turbidity is a measure which indicates how cloudy, or turbid, water is (Ødegaard et al., 2012). Turbidity is primarily caused by colloidal particles and small suspended matter, such as clay, silt, finely divided organic and inorganic matter, nano-plankton, and other sub-micron organisms. Colloidal matter will scatter and absorb light, thus limiting its transmission. Turbidity is measured by comparing the intensity of light scattered by a sample to the intensity of light scattered by a reference suspension (Tchobanoglous et al., 2003). The measurements are given as FTU (Formazine Turbidity Units), referring to the use of formazine as the reference suspension. NTU (Nephelometric Turbidity Units) is another commonly used turbidity unit and is equal to FTU (Ødegaard et al., 2012).

Turbidity is not only a measure of colloidal and suspended particles, but also of the waters light transmittance. The more turbid the water is, the less light will reach to deeper parts, which could in turn reduce processes like photosynthesis. Turbidity is a common water quality parameter used to determine the waters suitability for human usage. For water to be considered safe for recreational activities, like swimming, turbidity should be less than 2 NTU, while turbidity > 5 NTU is not acceptable for this purpose (Andersen et al., 1997). For water to be used as drinking water, turbidity should be less than 1 NTU (Drikkevannsforskriften, 2016).

Color

Color is caused by organic matter (humus), which originates naturally or is a result of pollution from domestic homes, industry, or agriculture. Humus is the end product of biodegradation of plant and microbial tissue, and consists of large, slowly biodegradable organic compounds (Ødegaard et al., 2012). Color can also be caused by natural minerals such as iron and manganese. The true color of water is defined as color measured after removing suspended materials by centrifugation or filtration. It has the unit mg platina per liter (mg Pt/l), which refers to the standard color solution. The measurement is based on visually comparing a standard solution, consisting of potassium chloroplatinate tinted with cobalt chloride, with the sample. The color produced by 1 mg Pt/l and 0.5 mg/l cobalt equals 1 standard color unit. A spectrophotometer is usually used for color measurements (M. J. Hammer & Hammer, 2004). A high color unit caused by a high content of humus has no known effect on human health. However, if the water is to be used as drinking water, humus can reduce the effect of certain disinfection processes and cause bad taste, especially if the water is chlorinated (*Vannrapport 127. Vannforsyning og helse – veiledning i drikkevannshygiene*, 2016). For water to be used as drinking water, the color unit should be lower than 20 mg Pt/l (Drikkevannsforskriften, 2016). Color could also be used to determine the degree of degradation of organic matter. A high color unit represents a high content of humus and, hence, a high degree of degradation which again indicates a high content of organic matter.

2.1.2 Organic Constituents

Organic compounds consist mainly of carbon, hydrogen and oxygen, and possibly some minor components of nitrogen, phosphorous, sulfur, and certain metals. Organics containing inorganic substituent are called hetero-organic compounds. Organics serve as an energy source for animal consumers and microbial decomposers. Common characteristics of organics are low water solubility, high molecular weight, and they are combustible (M. J. Hammer & Hammer, 2004). In general, organic matter consists of a number of organic constituents that cannot be distinguished separately (Tchobanoglous et al., 2003). Specific individual organic compounds may be identified by advanced analytical methods like gas chromatography or mass spectrometry. Organic constituents are either present as, or adsorbed to, particles, or dissolved in water. They can be divided into biodegradable and nonbiodegradable components. Biodegradable organics can be degraded by microorganisms, either easily or slowly. Slowly biodegradable solids are only degraded partially within the time the compound is present within a given system, while nonbiodegradable organics cannot be degraded biologically within that period of time (Ødegaard et al., 2012).

Biodegradable organics are characterized by the biochemical oxygen demand (BOD) and may be present in either dissolved or suspended form (Crites, Reed, & Middlebrooks, 2006). BOD is the amount of oxygen required by microorganisms to oxidize organic matter. Degradation of organic matter by microorganisms follows equation 2.1. It is used to determine the organic content of water, which is determined based on the amount of oxygen used for degradation. BOD is either given as BOD_5 or BOD_7, which refers to the consumption of oxygen during decomposition after 5 or 7 days, respectively (Ødegaard et al., 2012).

$$\text{Organics} + O_2 + \text{microorganisms} \rightarrow CO_2 + H_2O + \text{new biomass} \qquad \text{Equation (2.1)}$$

A more rapid estimation of the organic matter content can be obtained by determining the COD (chemical oxygen demand) or the total organic carbon (TOC). COD is obtained after 2 hours digestion and represents the amount of oxygen equivalent to the organic material that can be oxidized chemically (Tchobanoglous et al., 2003). Organic matter is oxidized by potassium permanganate ($KMnO_4$) or potassium dichromate ($K_2Cr_2O_7$), according to equation 2.2 (Ødegaard et al., 2012), and Cr^{3+} is measured spectrometrically.

$$\text{Organics} + Cr_2O_7^{2-} + H^+ \rightarrow Cr^{3+} + CO_2 + H_2O \qquad \text{Equation (2.2)}$$

A high content of organic matter in water can result in oxygen-free zones due to a high oxygen consumption by microorganisms. Bottom layers are the first to become oxygen-free, as most organic matter will settle down and be degraded at the bottom. Shallow lakes can under extreme circumstances get almost completely oxygen-free. Oxygen depleted water is not suitable for most aquatic organisms. Absence of fish, or other aquatic organisms, can therefore be an early indication of oxygen depletion. When oxygen is absent, anaerobic processes will occur, and this can lead to the production of methane (CH_4) and hydrogensulfide (H_2S). Both are highly flammable gasses and H_2S has bad odor and is toxic.

2.1.3 Inorganic Constituents

Inorganic constituents in water can be present naturally, be a result from background levels in water supply, or be a result from additions in domestic, industrial and commercial use (Tchobanoglous et al., 2003). Most inorganic constituents are dissolved in water, while a minor part may be suspended in water such as clay particles. Common dissolved inorganics are cations like calcium (Ca^{2+}), sodium (Na^+) and magnesium (Mg^{2+}), and anions like chloride

(Cl^-) and sulfate (SO_4^{2-}). Water hardness is determined by calcium and magnesium concentrations in water. The higher concentration, the harder the water. In water treatment, calcium carbonate ($CaCO_3$) is often added to increase the pH. This reduces corrosion problems in the water distribution network, but too high Ca-concentrations result in the deposition of $CaCO_3$ in heating elements.

Inorganics also include metals like iron (Fe), cadmium (Cd), manganese (Mn) or zinc (Zn), which are found in trace quantities in water (Tchobanoglous et al., 2003). Trace quantities are amounts less than 0.1 percent by volume ("Trace element," 1998). Metals can either be dissolved or suspended in water. Dissolved metals are metals present in unacidified water that pass through a membrane filter with specified pore size, while those retained on the filter are classified as suspended metals. Many metals are essential for growth of biological life, however, when present in elevated quantities, metals can be toxic (Tchobanoglous et al., 2003). Iron and manganese occur often naturally in waters and can cause taste and color. Sufficient amounts of oxygen will cause the dissolved metals Fe^{2+} and Mn^{2+} to precipitate in water as $Fe(OH)_3$ and MnO_2, respectively. This will result in particles with a characteristic red color (Ødegaard et al., 2012). Elevated quantities of metals can also cause changes in flora and fauna, usually by reducing species diversity. Species able to tolerate or adjust to higher metal concentration will start to dominate, while those not being able to tolerate increased concentrations will be numerically reduced or will disappear.

Nutrients

Macronutrients, such as nitrogen (N) and phosphorous (P) are essential for the growth of biological life (Ødegaard et al., 2012). They are essential parts of plant components like proteins, and are important for energy transfer and enzyme functions. Common sources of nutrients in waters are release of nutrients from soil reserves, decomposing plant residues, discharge of domestic and industrial wastewater, fish farming, and agriculture (Lægreid, Kaarstad, & Bøckman, 1999).

Phosphorous is often the limiting nutrient for primary production in freshwater and can be present either as organic or inorganic P, be dissolved in water, or be associated with other compounds (Ødegaard et al., 2012). Inorganic phosphorous includes orthophosphates (PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$) and polyphosphates (two or more P atoms), where dissolved phosphates are the most reactive forms (Kadlec & Wallace, 2008). Orthophosphate is the only form which is available for biological uptake (Ødegaard et al., 2012). Phosphorous can be associated with suspended particles, and will have the designation particulate phosphorous in that form. Phosphorous that is combined with, or is part of, dissolved organic materials, is defined as dissolved organic phosphorous (DOP) (Kadlec & Wallace, 2008). Major sources of organic phosphorous include microbes, algae, vegetation, detritus, and soil organic matter. Although organic phosphorous accounts for the larger fraction of total P, only a small portion may be biologically available (Reddy & DeLaune, 2008).

Nitrogen is often the limiting factor for primary production in marine water and can be present as organically bound or inorganic N. Inorganic nitrogen includes dissolved forms such as ammonium (NH_4^+) and nitrate/nitrite (NO_3^-/NO_2^-) (Ødegaard et al., 2012). Ammonium will either be present as ammonium ion (NH_4^+) or ammonia gas (NH_3), depending on the pH. NH_4^+ will be dominant at pH levels below 7, while NH_3 will dominate at pH levels higher than 7 (Tchobanoglous et al., 2003). Nitrogen associated with suspended particles is classified as particulate nitrogen, while N that is combined with, or is part of, dissolved organic materials, is defined as dissolved organic nitrogen (DON).

2.2 Recipient pollution

Moss (2008) defines pollution as “any man-made impact that increases the risk of damage to a natural system” (Moss, 2008). Water pollution is a major problem across the globe and is often associated with the discharge of effluents from sewer systems, drains and factories. Such discharges, including accidental and illegal effluents, are also known as point-source pollution, as they arise from a single point. Pollutant concentrations are initially high and decrease with distance from the point of discharge. Pollution effects get more difficult to observe if pollutants enter the recipient from diffuse sources, which is also known as nonpoint-source, or diffuse pollution. This form of pollution is often less immediately obvious because there is no adjacent unpolluted area to compare with. Surface runoff and groundwater infiltration from agricultural areas is a nonpoint source discharge and a common source for pollution of adjacent water bodies (Abel, 1989). This chapter describes agricultural runoff and the environmental challenges associated with it.

2.2.1 Agricultural runoff

Agricultural land receives water either as precipitation, irrigation, or due to snowmelt. If the rate at which land receives water exceeds the soil’s infiltration rate, the water will leave the area as runoff and enter adjacent water bodies (Hudspeth & Reeve, 2009). In agriculture, fertilizers, pesticides, and other agrochemicals are applied to increase productivity, which increases the availability of these in terrestrial ecosystems (Hautier, Niklaus, & Hector, 2009). Thus, surface water draining off agricultural land can erode soil and transport washed out dissolved components and soil particles to surrounding waters. (Lægreid et al., 1999; Søvik, 2007). The presence of agrochemicals varies greatly with springtime thaw, tillage, and agricultural application (Droste, 1997). This nonpoint-source pollution is difficult to capture and treat before entering recipients (Hudspeth & Reeve, 2009). According to a report from the Norwegian Environment Agency, more than 400 lakes, 3000 rivers, 100 coastal areas, and more than 50 groundwater sources are influenced significantly by agricultural runoff (Miljødirektoratet, 2013).

Phosphorous applied as fertilizer in agriculture is of special concern when reaching surrounding waters, as explained in the next section. According to Braskerud, the majority of total phosphorous from agricultural runoff is in the form of particulate phosphorous (Braskerud, 2002). The fraction of particulate phosphorous that can be transformed to bioavailable P is often expressed as potentially bioavailable particulate P. Thus, the portion of the particulate P that is potentially or directly bioavailable is an indicator of the potential consequences in surrounding waters caused by agriculture (Maynard, O'Geen, & Dahlgren, 2009).

2.2.2 Eutrophication and Saprobiation

Primary production is predominantly limited by light, physical characteristics of the habitat, and the availability of nutrients, especially phosphate in freshwater environments (Lægreid et al., 1999). At concentrations below 0.1 mg/L phosphorous, plant biomass will increase linearly with P concentrations. At concentrations higher than 0.1 mg/L phosphorous, other factors become more important (Smil, 2000). An increased supply of nutrients can result in a higher primary production, which is referred to as eutrophication (Lægreid et al., 1999). Eutrophication can either be caused naturally or by anthropogenic sources, like agriculture (Abel, 1989). According to a NIVA report (1992), approximately 30 % of phosphorus in agricultural runoff is available for biological uptake, which is the key factor for eutrophication (Braaten, Johnsen, Källqvist, & Pedersen, 1992; Maynard et al., 2009). Smil (Smil, 2000) states that the mobilization of phosphorous has tripled compared to its natural flows by the year 2000, where the application of inorganic fertilizers is a major cause. Although crops utilize applied nutrients efficiently, there is still a large fraction that is lost to surrounding waters causing eutrophication. In general, concentrations above 0.01 mg/L dissolved P are most likely resulting in eutrophication, although it is the loading rather than the concentration that is the key factor for this process (Smil, 2000).

Unwanted consequences of eutrophication are changes in the species composition, higher production of organic matter, clogging of waterways with plants, or reduced suitability of water for human and animal use. Changes in the composition of species that are present in the water can appear as the arise of undesired species such as noxious algae. The production of toxins by noxious algae would in turn make the water unsuitable for animal and human consumption (Lægreid et al., 1999). Changes in the species composition can also appear as the loss of species diversity (Hautier et al., 2009; Yang et al., 2012), which in turn decreases population and community stability (Yang et al., 2012). The fact that a loss of diversity decreases population and community stability is a nevertheless a controversial issue (Yang et al., 2012; Yann et al., 2014).

High production of organic matter, of which the bulk part eventually settle out of the photic zone, will reduce the oxygen content in the deeper water during decomposition (Lægreid et al., 1999). The increased level of organic matter and the potential subsequent oxygen depletion is also referred to as saprobiation. This can in turn create conditions that are not suitable for fish to exist in and subsequent fish-kills (Ødegaard et al., 2012). Oxygen depletion can also result in the recycling of phosphorous due to reduced conditions, and furthermore, initially stimulated eutrophication (Lægreid et al., 1999).

2.3 Wetlands

With a great diversity of biogeochemical processes, wetlands are among the most important ecosystems on this planet. The fact that they are called “ecological supermarkets” is no coincidence (Mitsch & Gosselink, 2007). Wetlands embrace unique disciplines which are neither covered by aquatic nor terrestrial ecology. Wetlands are often defined as boundaries between terrestrial and aquatic ecosystems. They can be classified further, depending on their hydrology, physiochemical properties, and biota. An important characteristic of wetlands is the presence of standing water, either continuously or periodically. Soil conditions are unique and vegetation is adapted to the wet conditions. Wetlands protect fish and wildlife and provide unique habitats for a variety of flora and fauna. They act as treatment plants by purifying polluted water, and balance water levels by acting as a stabilizer (Mitsch & Gosselink, 2007). This chapter describes the most important processes which make wetlands to be one of the most unique and valuable ecosystems on Earth.

2.3.1 Hydrology

The circulation of water and interactions between water and wetland is described by the wetlands hydrology (Kadlec & Wallace, 2008). Many processes define the hydrology, such as direction and velocity of the moving water, water sources, depth, and duration and frequency of flooding (Reddy & DeLaune, 2008). The balance between water inflows and outflows is called the wetland water budget (Mitsch & Gosselink, 2007). Water enters wetlands by precipitation, surface runoff, and groundwater infiltration (Reddy & DeLaune, 2008). The water inflow contains nutrients, sediment, and toxins that are transported into the wetland. Precipitation can either enter the wetland directly, or be transported on the surface as runoff. During or immediately after a rainfall, the rate at which water reaches the ground may be higher than the soil's infiltration rate, and most of the precipitation will then be transported as overland flow. Also, seasonal spring thaw or episodic flooding from adjacent water bodies can serve as surface inflow. Times when the water table of land surrounding a wetland is higher than the surface water or groundwater level of the wetland, groundwater infiltration will be significant. In the opposite case, groundwater will leave the wetland. Water can also be lost through evapotranspiration. This term is composed of the term "evaporation", referring to water that vaporizes from the wetland, and "transpiration", referring to water that is lost through vascular plants to the atmosphere (Mitsch & Gosselink, 2007). The flow of water through a wetland may be differentiated into surface and subsurface flow, depending on if the water flows on the wetland surface or subsurface, respectively. The capacity of a wetland to store water, will depend on several factors such as inflow and outflow rate, physiochemical properties and vegetation density (D. A. Hammer, 1989).

The hydrology in a wetland will modify and determine the physiochemical environment such as sediments, nutrient availability, and soil and water chemistry. These abiotic processes will in turn create different habitats, allowing for the establishment of specific plants, animals, and microbes. Vice versa, the biota will also modify the wetland's physiochemistry and hydrology, thereby completing the cycle. On the basis of hydrology influencing nutrient cycling, it will also affect the primary productivity of a wetland (Mitsch & Gosselink, 2007).

2.3.2 Biogeochemistry

Wetlands act as sources, sinks and transformers of numerous materials. The transport and transformation of materials in wetlands is known as biogeochemical cycling, which includes many physical, chemical and biological processes. These processes cause the chemical form of materials to change and generate internal fluxes of materials through the wetland (Mitsch & Gosselink, 2007).

The permanent or periodic flooding of wetlands creates conditions which allow some processes to be more dominant than processes in terrestrial and aquatic systems (Mitsch & Gosselink, 2007). Wetland soils are often classified as hydric soils, which means that the saturated conditions have created anaerobic zones (USDA, 2016). When soils are saturated with water, the pore volume is filled, and the diffusion rate of oxygen is 10 000 times slower compared to gas porous materials (Mitsch & Gosselink, 2007). This often results in oxygen consumption exceeding supply, and anaerobic conditions will develop. Under these conditions, oxidized forms may be reduced, if reducing agents are available. The presence of reduced materials is therefore often used as an indicator for anaerobic conditions or hydric soil identification (Reddy & DeLaune, 2008). In submerged soils, redox potential ranges from -400 mV (reduced) to +700 mV (oxidized). At high redox potentials (oxidized), oxygen is the most favorable oxidant as it yields the most energy. As the redox potential decreases, other electron acceptors, successively yielding less energy, are being used. Figure 2. 1 shows the successive utilization of electron acceptors as redox potential is being reduced with soil depth (Kadlec & Wallace, 2008). In general, soils are not totally free for oxygen, because there will always be a thin oxidized top layer at the soil-atmosphere interface. In this thin layer, oxidized ions are present, which are otherwise reduced in deeper parts of the wetland. This is an important feature in the chemical transformation and nutrient cycling within a wetland (Mitsch & Gosselink, 2007). Changing the soil’s chemical state from oxidized to reduced will in general increase the electrical conductivity and ionic strength, while it decreases the soil redox potential (Reddy & DeLaune, 2008).

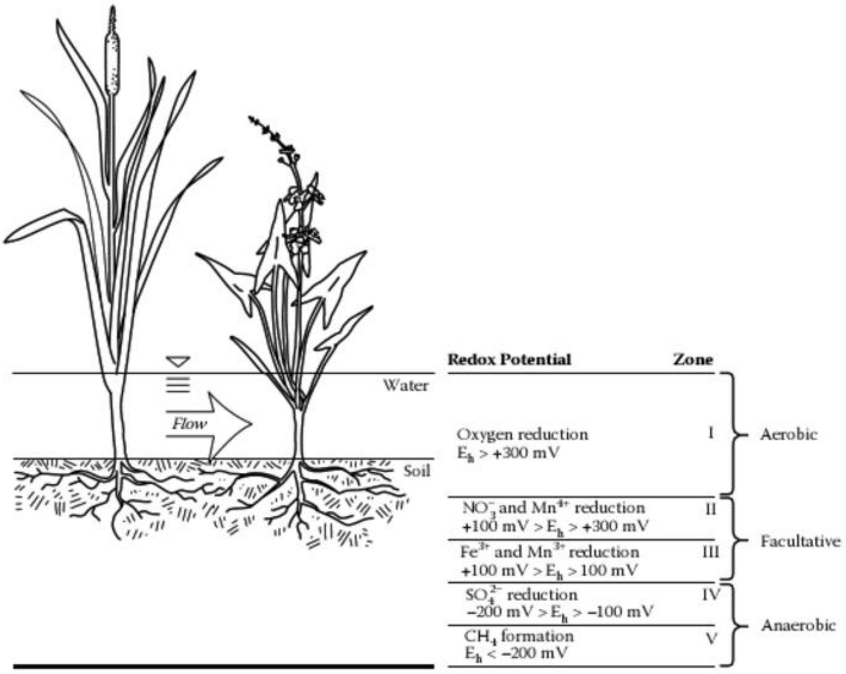

Figure 2. 1. Successive utilization of electron acceptors with vertical redox zonation in the soil.

Carbon

The primary driver for all biogeochemical processes in wetlands is carbon (Reddy & DeLaune, 2008). The cycle starts when inorganic carbon as CO_2 is transformed into organic matter by photosynthesis, which is further transported throughout the food web (Taksdal & Hågvar, 2001). If oxygen is present, aerobic respiration is the dominant process for decomposition. Aerobic respiration is nature's most efficient way to decompose carbon based on energy transfer. Low oxygen levels in wetlands will however prohibit aerobic respiration and fermentation and methanogenesis will often take place. The fermentation of organic matter carried out by facultative or obligate anaerobe microorganisms, will result in the formation of low-molecular-weight acids and alcohols, and CO_2, which can serve as substrate for other microorganisms. Methanogens can utilize carbon dioxide or low-molecular-weight organic compounds to produce methane (Mitsch & Gosselink, 2007). Methane is a greenhouse gas and released to the atmosphere either due to disruption of soil sediments, or through internal gas lacunas of wetland plants (Laanbroek, 2010). According to the IPCC (Intergovernmental Panel in Climate Change), natural wetlands are the largest natural source of methane emission to the atmosphere (Mackay, 2008).

Phosphorous

Phosphorous may be released into the environment by decomposition reactions, anthropogenic sources, or weathering of minerals, (Reddy & DeLaune, 2008). Phosphorous is usually transported from uplands to wetlands with surface or subsurface flow, and further to aquatic ecosystems. The biogeochemical cycling of phosphorous, as shown in figure 2. 2 (Mitsch & Gosselink, 2007), takes place in the sediments, water column and biotic communities, as there is no stable gaseous P. Hence, a major proportion of P will be bound to organic matter, tied up in inorganic sediments, or be dissolved in water (Mitsch & Gosselink, 2007).

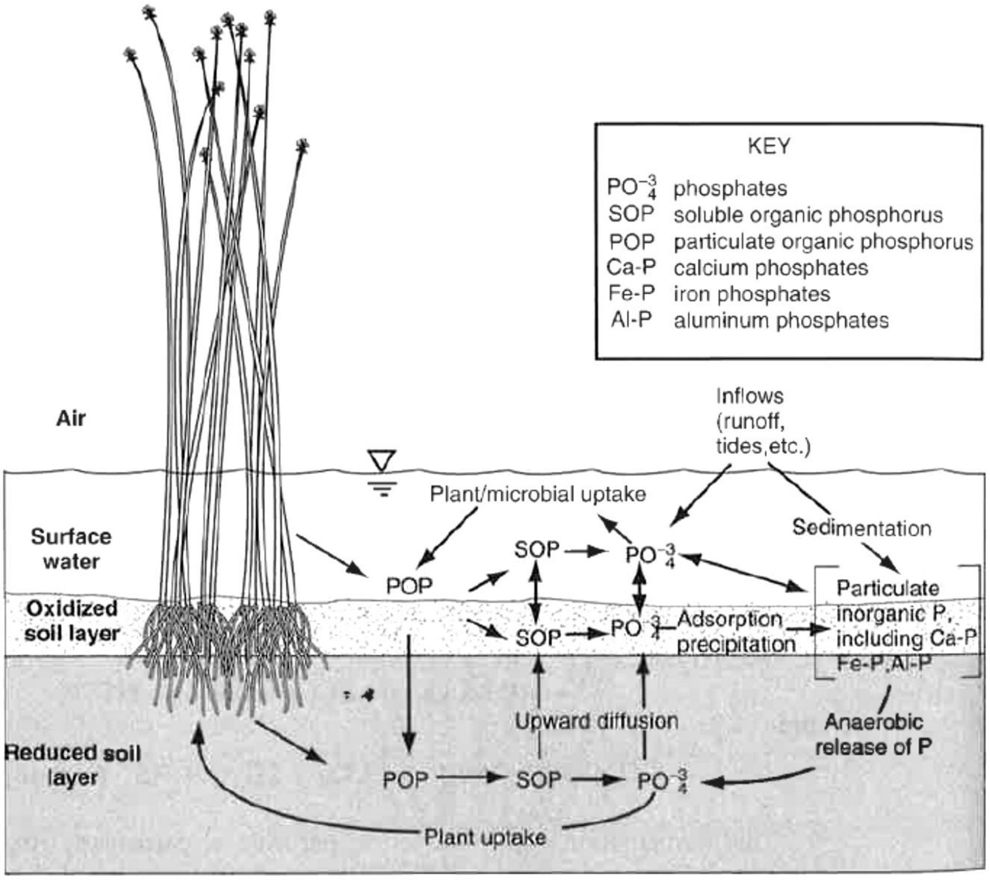

Figure 2. 2. Biogeochemical cycle of phosphorous in wetlands.

Phosphate ions may either be present as H_3PO_4 (phosphoric acid), $H_2PO_4^-$, HPO_4^{2-}, or PO_4^{3-}, depending on the pH:

$$H_3PO_4 \leftrightarrow H_2PO_4^- + H^+ \qquad \text{Equation (2.3)}$$

$$H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+ \qquad \text{Equation (2.4)}$$

$$HPO_4^{2-} \leftrightarrow + PO_4^{3-} + H^+ \qquad \text{Equation (2.5)}$$

Under acid soil conditions, phosphoric acid will be the dominant species, while as under alkaline conditions, PO_4^{3-} will dominate. $H_2PO_4^-$ and HPO_4^{2-} are the dominant phosphate species under the most natural conditions. The relative distribution of phosphate with changes in pH is shown in figure 2. 3. Under highly reduced, acidic conditions, phosphate may be reduced to phosphine gas (PH_3). However, phosphine is unstable under aerobic conditions and an aerobic top soil layer can act as a phosphine sink through oxidation (Reddy & DeLaune, 2008).

Phosphates under aerobic conditions have a great tendency to form complexes with other elements like calcium, iron, and aluminum, a process that will contribute to the retention of P in the sediments. However, under anaerobic conditions, oxidized elements will be reduced to more soluble forms, such as iron(III) to iron(II), thereby releasing the bound phosphate (Mitsch & Gosselink, 2007). An exception is Ca-bound phosphate, which will not be released under anaerobic conditions because it is not affected by changes in the redox potential (Bruland & Richardson, 2006).

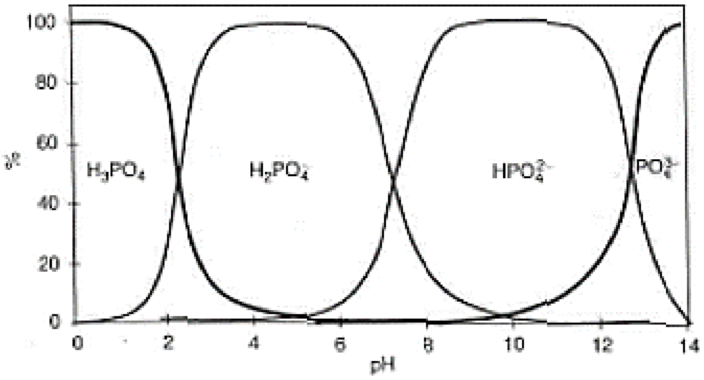

Figure 2. 3. Distribution of phosphate species as a function of pH (Reddy & DeLaune, 2008).

Organic phosphorous entering the wetland can either become part of the sediment, or be oxidized by microorganisms to inorganic forms (Mitsch & Gosselink, 2007). The bioavailable phosphorous can be utilized biologically and become part of biological tissues, which either becomes part of the sediments through burial, or may be released again through decomposition (Vymazal, 2007). In general, phosphorous is bioavailable at slightly acidic to neutral pH, given that it precipitates with aluminum and iron at acidic pH, and with calcium and magnesium at alkaline pH. (Mitsch & Gosselink, 2007). The assimilation of phosphorous in biotic communities is a form of short term storage as plants eventually will die and decompose, thereby releasing the phosphorous again (Reddy & DeLaune, 2008).

As the pH increases significantly, for example due to the removal of CO_2 by algal photosynthesis, a phenomenon called co-precipitation may occur (Mitsch & Gosselink, 2007). Under these conditions phosphorous will co-precipitate with calcium carbonate ($CaCO_3$) to form calcium phosphate, where dissolved Ca^{2+} is available (Reddy & DeLaune, 2008).

Iron

The biogeochemical cycling of iron predominantly occurs in the geosphere and to a lesser extent in the biosphere and hydrosphere. Depending on the redox condition in soils, iron will be present as reduced ferrous (Fe^{2+}) or oxidized ferric (Fe^{3+}) (Reddy & DeLaune, 2008). Iron is present in its reduced form under anaerobic conditions, which is also the most available form to organisms. In aerobe soils, Fe^{2+} will be oxidized to insoluble Fe^{3+} (Mitsch & Gosselink, 2007). Microorganisms accelerate this process in the presence of oxygen by using iron as an electron donor. Iron can also be released as a result of microbial activity under anaerobic conditions, or dissolution or weathering of minerals. Bacteria able to use iron(III) as electron acceptor link the iron reduction to the decomposition of organic or inorganic substrates (Reddy & DeLaune, 2008). As a matter of fact, iron plays a key role in the decomposition of organic matter (Vodyanitskii & Shoba, 2015). Dissolved iron can also form stable complexes with dissolved organic matter and be present as particulate iron. In the presence of sulfides, the precipitation of iron sulfide can be significant (Reddy & DeLaune, 2008). Reduced iron may also form minerals such as magnetite (Fe_3O_4) and siderite ($FeCO_3$), although high levels of phosphorous create unfavorable conditions for the formation of magnetite (Vodyanitskii & Shoba, 2015). The oxidized ferric iron will cause the characteristic red-brown color of sediments due to the formation of ferric hydroxide ($Fe(OH)_3$), while ferrous hydroxide ($Fe(OH)_2$) will cause a grey-green color of the sediment. Due to the leakage of oxygen from plant roots, iron(II) can be oxidized to iron(III), which can cover the roots with an iron oxide coat. However, too high concentrations of iron can be toxic for plants, due to the prevention of nutrient uptake (Mitsch & Gosselink, 2007).

2.3.3 Biota

The stress caused by standing water, water fluctuations, and partly anaerobic soil conditions in wetlands demand vegetation and organisms that are adapted to that harsh environment. Typical adaptations are the development of pore space by plants to increase the oxygen diffusion capacity, or physical and structural adaptations by animals. In general, there are two types of organisms – tolerators and regulators. Tolerators are capable of tolerating stress and thrive despite of stressful conditions. Regulators are more sensitive and avoid stress. (Mitsch & Gosselink, 2007).

Organisms with restricted mobility adapt to wetland conditions metabolically. Most organisms in wetland environments are facultative anaerobes. These can switch between aerobic and anaerobic respiration, in tune with changing wetland conditions. (Mitsch & Gosselink, 2007). Wetland plants, also called helophytes or macrophytes, either adapt to wetland conditions morphologically or physiologically. A typical structural adaptation is the development of aerenchyma, which are air spaces in roots and stems that allow oxygen to diffuse from aerial parts of the plant into the roots. This allows for increased oxygen supply throughout the plant, which is no longer dependent on diffusion of oxygen from the surroundings. The development of adventitious roots on the stem above the anaerobic zone also helps the plant to increase the transport of oxygen to the roots beneath the water line. The capacity of oxygen supply is also increased by a swelling of the lower stem of vascular plants due to larger cells, which is called stem hypertrophy. Another morphological adaptation of wetland plants is the development of shallow root systems to avoid anaerobic conditions. A typical physiological adaptation in order to increase oxygen supply to the roots is by pressurizing the gas flow through the plant (Mitsch & Gosselink, 2007; Stottmeister et al., 2003). The driving force of the pressurization can either be a gradient in temperature or water vapor between internal gas spaces of the plant and the atmosphere (Brix, Sorrell, & Orr, 1992). Oxygen can diffuse out into the rhizosphere, if the supply to the roots is high enough, thereby oxidizing the surrounding soil. Iron and manganese can reach toxic levels in anoxic soils since they are only needed in low quantities. Plants can tolerate the excessive availability of these elements by creating an oxidized rhizosphere where iron can precipitate and reduce the concentration around the roots, by sequestering minerals that are taken up in tissues where they do not affect the metabolism, or by having a higher tolerance for these elements. When oxygen is depleted, plants can adjust by respiring anaerobically, as many organisms do. However, anaerobic respiration results in the formation of products which are toxic to root tissues. Plants have therefore developed adaptations that minimize the toxicity of these compounds. In addition to physiological and morphological adaptations, there are several strategies that plants use to either avoid or escape certain situations. These include the development of buoyant seeds, the germination of seeds while the fruit is still attached to the tree, and the development of tubers, roots and seeds able to survive submergence for a longer period of time (Mitsch & Gosselink, 2007). Some plant species adapt by changing their time of reproduction, either by delaying or accelerating the flowering and seed production (Blom et al., 1990).

2.3.4 Mass balance

The mass balance of a wetland is a specification of inputs, outputs and cycling of materials in the wetland and is illustrated in figure 2. 4. Hydrological inputs, geological input such as the weathering of rock, or biological inputs such as photosynthesis or biotic transport by animals are the dominant inputs for wetlands. The main losses occur through outflow of groundwater or surface water, unless the wetland is isolated and has no outflow. Materials can also be removed biologically by respiration, denitrification, and in small amounts as methane, sulfide, or ammonia. The long-term storage of chemicals within the sediments may also be considered as an outflow. The internal cycling of materials includes processes like litter production, remineralization, and a number of transformation processes. The uptake of chemicals by plant roots is also part of internal cycling, if the roots are not being removed (Mitsch & Gosselink, 2007). It is difficult to identify one mass balance only due to great variations among wetlands. It is therefore more accurate to adjust mass balances individually to each wetland.

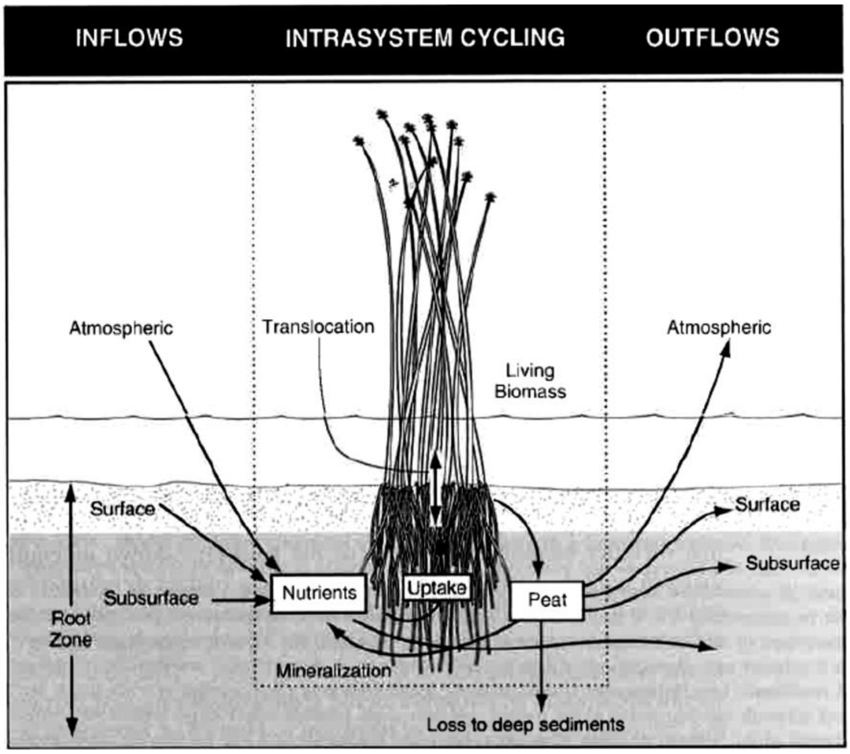

Figure 2. 4. Massbalance in a wetland.

2.4 Constructed wetlands

The great diversity of complex processes together with the high productivity of wetlands can be used for human use and benefit in constructed wetlands (CW). These are man-made wetlands that simulate processes and interactions of natural wetlands within a more controlled environment (D. A. Hammer, 1989). Wetlands have a high potential to accumulate and transform organic matter and other substances and are thus considered to act as water purification systems and nutrient sinks. However, the great variety of functions in natural wetlands make it difficult to adopt these into other geographical areas due to differences in hydrology, physiochemistry, biology and in some cases climate (Moshiri, 1993). Wetlands, either natural or constructed, will respond to new supplies of water and nutrients by adjusting storages, pathways, and structure, where newly constructed wetlands will need a successional period to adapt to these inputs (Kadlec & Wallace, 2008). For most purposes, constructed wetlands consist of five essential components: (1) A filter medium with varying hydraulic conductivity, (2) vegetation adapted to anaerobic, water-logged conditions, (3) water flowing through the wetland on the surface or subsurface, (4) invertebrates and vertebrates, and (5) aerobic and anaerobic microorganisms (D. A. Hammer, 1989). This chapter describes constructed wetlands and their most important features.

2.4.1 Classification and design

In general, constructed wetlands can be divided into three different types based on the flow of water through the wetland, as shown in figure 2. 5: (1) Horizontal surface flow systems, where wastewater flows above the soil surface, (2) Horizontal subsurface flow systems, where wastewater flows below the soil surface, and (3) Vertical flow systems, where wastewater is loaded continuously or intermittently and flows through the root zone in a upstream or downstream manner (Stottmeister et al., 2003). Another commonly used classification is calling horizontal surface flow systems for free water surface systems (or open pond systems), while systems with subsurface flow are often called reed-bed filters (Ødegaard et al., 2012).

There are many different ways of designing a wetland, depending on the treatment criteria, wastewater characteristics, local conditions, climate and the availability of land. One of the oldest concepts of constructing wetlands is with a free water surface (FWS) flow. The design usually consists of several connected ditches which may be vegetated. The ditches are typically 3 to 5 m wide and more than 100 m long (Moshiri, 1993). Systems with horizontal subsurface flow (HSF) consist of a bed, usually planted with helophytes, and an impermeable membrane underneath to prevent leakage (Moshiri, 1993). Systems with vertical subsurface flow (VSF) are constructed in a similar way as HSF systems, but the water is spread over the whole wetland surface via a distribution system. The water flows through the filter in a vertical manner and is collected at the bottom in a drainage system (Ødegaard et al., 2012). A permeable barrier is often used as distribution system in CW. It is usually a pile of larger rocks and/or gravel which is placed across the wetland at inlet zones. Water is forced through and gets spread across the whole pond. This is an important factor to achieve good treatment efficiency, as the whole area is utilized. This also reduces the nature of water to create small paths/streams where water masses gather and increase forces to wash out sediment in open pond systems. Permeable barriers can also be placed at outlet zones when water flows from one pond to another, where they function in regulating the water level and water flow-through. The designs mentioned above can also be combined with each other or other treatment technologies (Moshiri, 1993). However, the choice of design or possible design combinations has to be adapted to each individual situation in order to ensure optimal treatment efficiency.

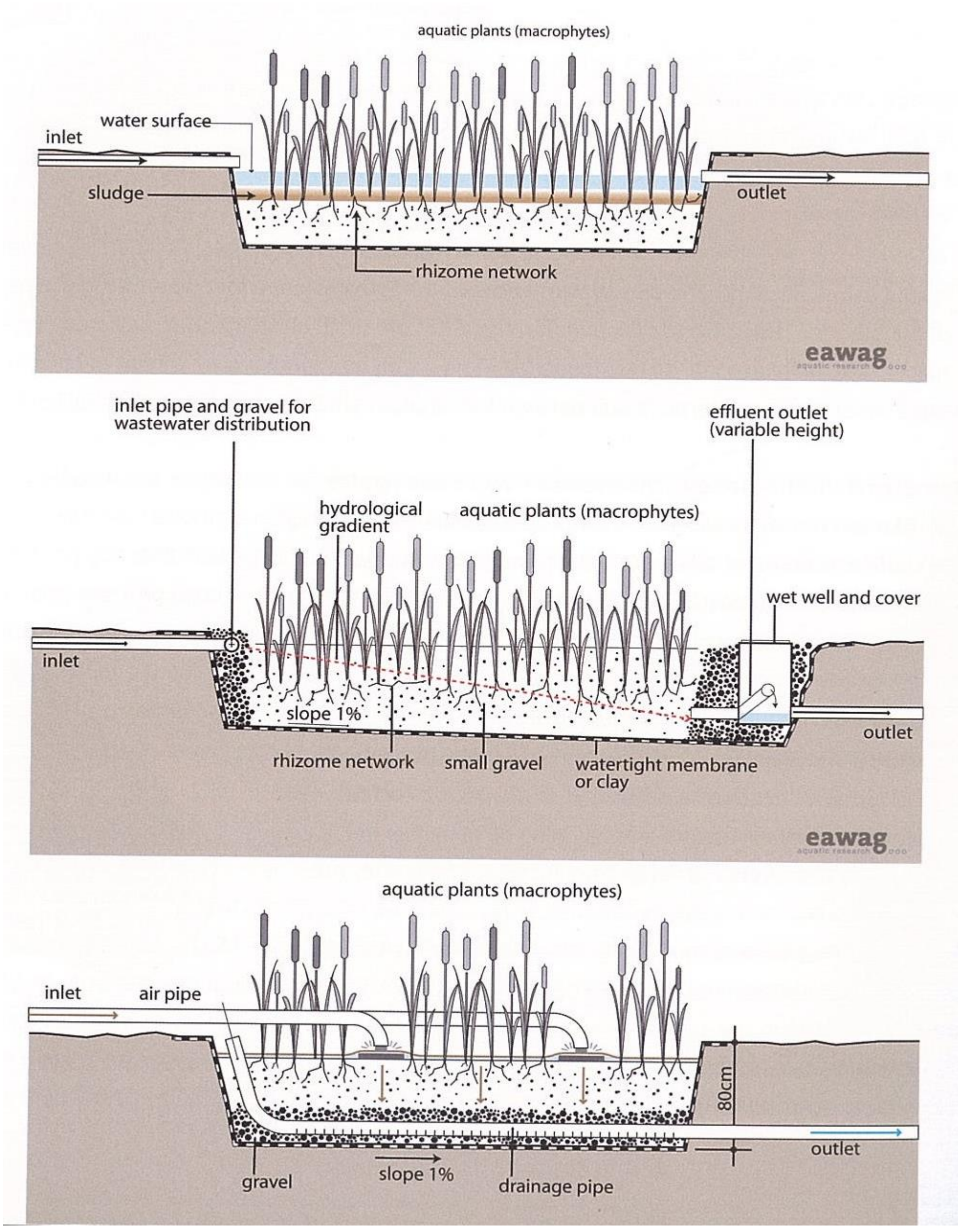

Figure 2. 5. Horizontal surface flow system on the top, followed by a horizontal subsurface flow system, and a vertical flow system at the bottom (Ødegaard et al., 2012).

In systems with VSF and intermittent loading, oxygen is forced out of the soil during loading periods, while air is drawn back into the pore spaces of the soil during drying periods. This increases the oxygenation of the soil during drying periods compared to HSF systems, and creates alternating oxidized and reduced conditions (Moshiri, 1993). The question whether horizontal or vertical flow is the best choice, should be answered based on present conditions.

2.4.2 Sediment/soil type

The root zone, or rhizosphere, of constructed wetlands is also called the active reaction zone. Here, microorganisms, plants, soil and compounds interact to initiate a number of physiochemical and biological processes (Stottmeister et al., 2003).

There are many different types of soil, but not every type will be suitable as a filter medium in constructed wetlands. The soil plays a crucial role for many hydraulic processes, and should support plant growth and growth of microbial communities (Stottmeister et al., 2003). Hence, soil with high surface area is usually a good choice for constructed wetlands, because it provides important attachment areas for microbial activity (Moshiri, 1993). Physical properties like grain size and pore space will influence the flow through the wetland greatly and effect its removal mechanisms by influencing the contact time between water and the rhizosphere, and thereby the time treatment processes can occur (Stottmeister et al., 2003). In general, high hydraulic retention time, and hence maximum contact between wastewater and soil, is a key factor for good removal mechanisms (Vohla, Alas, Nurk, Baatz, & Mander, 2007). A study by Hunter et al. (2001) showed a higher nutrient removal after increasing the hydraulic retention time from 3 days to 6 days (Hunter, Combs, & George, 2001). Also, Braskerud (2015) obtained higher retention of P at higher hydraulic retention times (Braskerud, 2002). Hydraulic retention times are generally long in systems with horizontal subsurface flow, while they are shorter in intermittently loaded vertical flow systems. (Stottmeister et al., 2003). Hydraulic retention times can also be influenced by hydraulic loadings (Braskerud, 2002; Dzakpasu, Scholz, McCarthy, & Jordan, 2015; Vohla et al., 2007; Yates & Prasher, 2009; Zhimiao et al., 2016). Increased hydraulic loadings will decrease the hydraulic retention time, and with it, the removal of nutrients (Braskerud, 2002; Yu, Tan, Chong, & Long, 2015).

Filter substrate clogging can present a risk for constructed wetlands by decreasing its treatment functionality (Hua, Li, Zhao, Zhu, & Shen, 2013). Adjustment of operational factors such as loading rates and bed size, and the right selection of filter medium are important in preventing clogging. The main factors contributing to pore clogging are development of biofilm, solids retained in the wetland, and plant detritus. The reduction of pore spaces by filter substrate clogging can lead to changes of flow conditions. This could in turn lead to short-circuiting, decrease of hydraulic retention times, odors, and presence of undesired insects (Hua et al., 2013)

2.4.3 Vegetation

Constructed wetlands can either be vegetated or not. Typical wetland plants are reeds, rushes, and cattails, together with fast growing trees like willow (Stottmeister et al., 2003). In general, there are three different types of wetland plants: (1) Free floating helophytes, (2) Emergent helophytes, and (3) Submerged helophytes. Free floating helophytes are not fixed in the soil, but float on the water surface. They range from large plants with well-developed large roots, to surface floating plants with no or few roots. The most dominant wetland plants are rooted, emergent helophytes, with root depths ranging from 50 cm below the soil surface to as deep as 150 cm or more. Common reed (*Phragmites australis)*, cattail *(Typha latifolia)*, and bulrush *(Scirpus lacustris)* all belong to emergent wetland plants. Rooted, submerged helophytes are entirely submerged in water, in contrast to emergent helophytes which have their photosynthetic tissue above the water surface. Submerged wetland plants only grow well in oxygenated water and are therefore not suitable for the treatment of water with high content of easily biodegradable organic matter (Moshiri, 1993).

Vegetation can bring about several advantages. First of all, plants increase the surface area for microbial attachment and hence, enhance microbial activity which in turn increases microbial transformation processes. Secondly, transport of oxygen to the roots by rooted helophytes enables them to survive under anaerobic conditions (D. A. Hammer, 1989), and is important in the development of a thin oxygenated layer around the roots (Mitsch & Gosselink, 2007). Another important function of vegetation is to slow down incoming water, which will increase the hydraulic retention time (Yates & Prasher, 2009). Finally, vegetated constructed wetlands provide both an esthetic pleasing system and enhance biodiversity by creating a green environment with different appearances throughout the year (Calheiros et al., 2015). A concern about vegetated CW is the clogging of the filter bed in subsurface-flow CW. Several studies showed that vegetation helps to prevent clogging of the filter substrate, most certainly by improving porosity (Brix & Arias, 2005; Hua et al., 2013; Wang et al., 2010). In temperate regions with frost during winter, dead plant material can also serve as isolation layer on top of a reed-bed filter (Ødegaard et al., 2012) However, in open pond systems, dead plant material can cover the open surface and disturb the flow of water through the wetland. For systems where this is significant, removal of vegetation might be necessary (Moshiri, 1993). The removal of wetland plants would also prevent the release of nutrients by decomposition of dead plant material. The question if wetland plants should be removed needs to be answered for each individual CW.

2.4.4 Advantages and Disadvantages

The biggest advantage of constructed wetlands compared to conventional treatment systems is the low costs (Gachango, Pedersen, & Kjaergaard, 2015; Kadlec & Reddy, 2001). Costs associated with CW include establishment costs, operational and maintenance costs, and relevant land costs, like putting productive land out of production (Gachango et al., 2015). No need for high educated staff will also keep operational costs low. Energy input is low, if it is needed at all, which will benefit the environment and costs associated with operation (Moshiri, 1993). Finally, constructed wetlands are more flexible and susceptible to variations in loading rate compared to conventional treatment systems (Calheiros et al., 2015; Moshiri, 1993). However, a larger land area is required for constructed wetlands to obtain the same treatment effect as for conventional treatment facilities (Moshiri, 1993; Tyler, Moore, & Locke, 2012).

2.4.5 Cold climate and seasonal variations

The applicability of constructed wetlands in cold climates, compared to warm climates, has raised several questions concerning the treatment efficiency. The main questions are: Would CW freeze in winter?; How good would removal efficiency be in cold climate?; Is it possible to remove significant amounts of phosphorous? Biological processes are sensitive to low temperatures, but play an important role in CW. Microbial activity will decrease with decreasing temperature, and plants will be dormant during colder months (Beutel et al., 2014; Dierberg et al., 2002). In order to avoid reduced treatment efficiency and the risk of freezing during colder months, appropriate design is crucial. Cold climates require larger and deeper systems than warmer climates do. A larger and deeper design can be achieved by CW with horizontal subsurface flow. By using a HSF design, water will flow beneath the soil surface, which will give some degree of isolation. Additional isolation can be obtained by constructing deeper wetlands, and with artificial or natural isolation layers, such as snow, rock wool, straw, or polystyrene (Mæhlum & Jenssen, 2003). Physical processes such as sorption reactions are not significantly affected by temperature. However, hydraulic retention time and dilution factors may vary with seasonal changes in precipitation quantities, which could affect treatment efficiency (Kadlec & Reddy, 2001). As plants are dormant during colder months, oxygen transport to the rhizosphere will be reduced (Ouellet-Plamondon, Chazarenc, Comeau, & Brisson, 2006). Although the solubility of oxygen increases with decreasing temperature, diffusion processes are reduced (Kadlec & Reddy, 2001). Artificial aeration during colder months improves removal efficiency of constructed wetlands by compensating for reduced transport of oxygen by plants (Ouellet-Plamondon et al., 2006).

The seasonal variation of treatment performance has become of greater interest in recent years. Mæhlum and Stålnacke (1999) studied three CW with horizontal subsurface flow located in Norway with a cool temperate continental climate. Temperature did not affect treatment efficiency significantly, with differences in efficiency between cold and warm periods of less than 10 percentage points for all parameters (organic matter and nutrients) (Mæhlum & Stålnacke, 1999). Seasonal variations for plant uptake, however, have varied for some studies. For example, Beutel et al. (2014) found some seasonal variations for phosphorous uptake for a constructed wetland planted with cattail (*Typha* sp.*)* and soft stem bulrush (*Scirpus* sp.) in Washington, USA. Warmer temperatures and increased sunlight in spring stimulated plant growth and enhanced P removal. In summer, plants continued to take up phosphorous, but leaf litter was also produced. Decay of leaf litter, which was enhanced by increased temperatures, increased the release of P. In fall, root storage and low litter decay resulted in improved P removal, before removal rates were reduced in winter with temperatures down to 0°C (Beutel, Morgan, Erlenmeyer, & Brouillard, 2014).

2.5 Phosphorous retention in constructed wetlands

Excessive use of fertilizers in agriculture causes high nutrient concentrations in agricultural runoff (Lægreid et al., 1999). To reduce phosphorous controlled eutrophication, the treatment of agricultural runoff before entering the recipient is of high interest. Constructed wetlands can be used to treat the runoff by being a transition zone between agricultural land and recipient, thereby acting as a buffer for the retention of phosphorous. Constructed wetlands are considered to be effective in removing P from agricultural runoff at low costs (Beutel et al., 2014; Gachango et al., 2015), with removal rates ranging from 20 % up to 94 % (Beutel et al., 2014; Braskerud, 2002; Dzakpasu et al., 2015; Liu, Huang, Tang, Liu, & Scholz, 2012; Lu et al., 2009; Yates & Prasher, 2009). The major processes for P retention are sedimentation, sorption, precipitation, ion exchange, and uptake in biological tissues. Sorption and biomass-building are saturable mechanisms, because they reach a limited capacity where they can retain no more phosphorous. Storage of refractory residuals (burial), such as particulate settling, are sustainable mechanisms, because they can continue without limit (Kadlec & Wallace, 2008). As phosphorous is the main contributor to poor recipient quality, retention mechanisms described in this chapter is restricted to phosphorous only. However, it should be noted that unlike carbon or nitrogen, phosphorous will only be retained within the wetland, but not removed from it naturally or degraded into harmless forms (Kim et al., 2016).

2.5.1 Sedimentation

Sedimentation is defined as the separation of suspended particles that are heavier than water, by gravitational settling. Based on particle interactions and concentration, gravitational settling can be divided into four groups: (1) Discrete particle, (2) Flocculent, (3) Hindered, and (4) Compression settling (Tchobanoglous et al., 2003).

The laws of sedimentation formed by Newton and Stokes can be used to describe the settling of discrete, nonflocculating particles. This kind of settling applies to suspensions where particle concentrations are so low, that particles settle as individual entities without interacting significantly with neighboring particles. The discrete particle will settle due to the gravitational force acting upon it, while the frictional drag force will restrain settling. Equating the gravitational force to the frictional drag force yields the terminal settling velocity of a spherical, discrete particle, as shown in equation 2.6 (Tchobanoglous et al., 2003).

$$v_{p(t)} = \sqrt{\frac{4g}{3C_d}\left(\frac{\rho_p - \rho_w}{\rho_w}\right) d_p} \qquad \text{Equation (2.6)}$$

where, $v_{p(t)}$ is terminal settling velocity of the particle (m/s)

C_d is the drag coefficient (unitless)

ρ_p is the density of the particle (kg/m^3)

ρ_w is the density of water (kg/m^3)

d_p is the diameter of the particle (m)

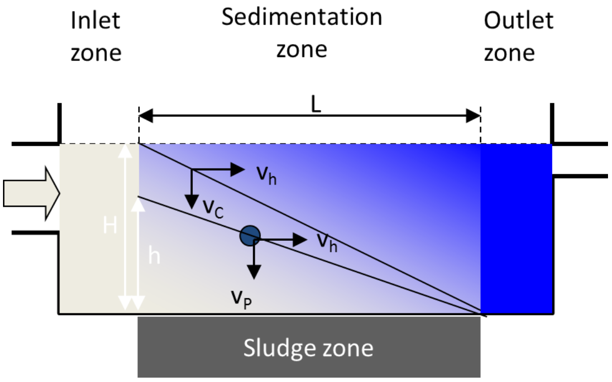

Figure 2. 6. Sketch of flow path for particles with critical settling velocity (Tchobanoglous, Burton, Stensel, Metcalf, & Eddy, 2003).

Depending on whether the flow regime surrounding the particle is laminar or turbulent, the drag force will vary. The design of sedimentation processes, usually start with the selection of a minimum particle size with a terminal settling velocity, v_c. The settling path of a particle is illustrated in figure 2. 6 and shows that the sinking velocity, v_c, has to be greater than the upward water velocity, v_h, for particles to be removed by sedimentation. With other words, the particle retention time, τ_p, should be smaller than the hydraulic retention time, τ_h. However, there will be a size dependent fraction of particles with sinking velocity less than v_c, that will be removed as well due to a larger size (Tchobanoglous et al., 2003).

Particle and hydraulic retention times are given as follows:

$$\tau_p = \frac{h}{v_p} \text{ and } \tau_h = \frac{V}{Q} \qquad \text{Equation (2.7)}$$

where, h is the inlet height of the particle (m)
V is the volume of the water (m^3)
Q is the flowrate (m^3/s)
τ_p is the particle retention time (s)
τ_h is the hydraulic retention time (s)

Inserting retention times from equation 2.7 into the requirement for removal by sedimentation:

$$\tau_p \leq \tau_h$$

$$\frac{h}{v_p} \leq \frac{V}{Q} = \frac{L * B * H}{Q}$$

where, H is the water height
L is the length of the settler
B is the width of the settler

To ensure all particles with a settling velocity v_c are removed, inlet height of the particle and the height of the water (as shown in figure 2. 6) should be equal. Inserting the latter, h = H, yields:

$$\frac{H}{v_c} \leq \frac{L * B * H}{Q}$$

$$Q \leq v_c * L * B = v_c * A$$

$$v_c = \frac{Q}{A} \qquad \text{Equation (2.8)}$$

Hence, the particle settling velocity (equation 2.8) equals the surface loading rate, and is independent of depth. This is important as several reports claim water depth to be important for sedimentation removal (for example Braskerud et al.). This is a misinterpretation in the literature and is probably linked to the risk of resuspension (which would depend on water depth).

Flocculation is the process whereby particles aggregate and increase in mass. Flocculent particle settling occurs as particle concentrations slightly increase. Particles will not settle as independent, discrete particles anymore, but will flocculate during sedimentation, increase in mass, and settle faster. The degree to which flocculation occurs depends on contact time, settler depth, advective velocity gradients, particle concentrations, particle size distribution, and particle chemical surface properties. Macroflocculation refers to the aggregation of particles greater than 1 or 2 μm due to an induced velocity gradient or differential settling, illustrated in figure 2. 7. Faster moving particles can overtake slower moving particles in a velocity field or during gravity settling. Microflocculation refers to the aggregation of particles due to the constant thermal bombardment by small water molecules that surround the particles, also called Brownian motion. After collision, the particles will stick together and sink at a faster rate. However, for flocculation to occur, particles have to coagulate first. Colloidal particles found in wastewaters are too small to settle by gravitational settling. They have a negative surface charge and are kept in suspension by Brownian motion, which takes place under stable conditions when attractive forces between particles are less than repelling forces of the electrical charge. The coagulation process is the destabilizing of colloidal particles which enables them to collide and flocculate. The coagulation theory is very complex and explained by the DLVO theory. The basis for the DLVO theory are two principle forces. The forces of repulsion due to the electrical charge and Van der Waals forces of attraction which are significant only if the two particles are brought together close enough. In order for two particles to collide, the total potential energy of the particles must be so that the attractive forces are high enough, and repulsive charge forces low enough, for Brownian collisions to occur.

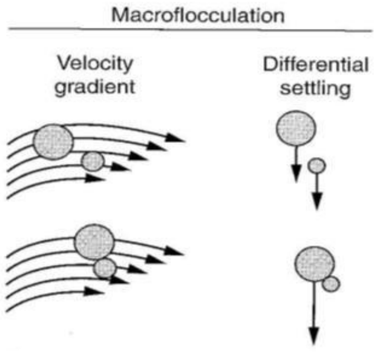

Figure 2. 7. Macroflocculation by a velocity gradient and differential settling (Tchobanoglous et al., 2003).

In addition to discrete and flocculent settling, hindered and compression settling will occur as particle concentrations increase into the sediments. During hindered settling, or zone settling, interparticle forces are strong enough to hinder the settling of neighboring particles. Water will move upwards through the space between the contacting particles. This results in the formation of a "blanket" where particles stay in a fixed position relative to each other, and the mass of particles settles as a unit. On top of the settling mass, a solid-liquid interface will develop and a clear layer of water is produced above the particles. The rate of hindered settling will depend on the particle concentration and their characteristics, and is probably not very important for constructed wetlands performance. The exception might be sedimentation severe mixing during plant and sediment removal. As settling continues, particle concentrations increase and a compressed layer of particles will form. Particles are brought in close physical contact and the net sinking velocity decreases with increasing solids concentration (Tchobanoglous et al., 2003). This is most likely to happen inside sediments and limited compression settling may lead to "fluffy" sediments readily re-suspended during elevated water velocities.

Sedimentation in free water surface systems

The sedimentation of suspended particles plays a major role in the removal of phosphorous systems with a free water surface. A large fraction of phosphorous entering wetlands is bound to particles or organic matter (particulate phosphorous), or will be transformed in the wetland to precipitates of low solubility or detrital particles (Braskerud, 2002; Reddy & DeLaune, 2008). These phosphorous containing compounds can be retained in the wetland by sedimentation, as constructed wetlands act as effective sediment traps (Kadlec & Reddy, 2001). The retention of potentially bioavailable P depends therefor often on the particle settling potential of particulate phosphorous. A study by Maynard et al. (2009) showed that wetlands are less effective in removing bioavailable particulate phosphorous which is associated with clay particles, since these will remain in suspension (Maynard et al., 2009). Braskerud (2002) also mentions the difficulty of clay particle settling, which can reduce phosphorous retention in the wetland (Braskerud, 2002). However, it should be noted that sedimentation is relevant for surface-flow constructed wetlands, rather than subsurface-flow constructed wetlands. In subsurface-flow CW, sedimentation occurs in spaces between particles, which could rather be described as filtration by the filter medium.

In wastewater treatment, particle settling may be enhanced by using incline plates and tube settlers. These consist of stacked offset trays or bundles of small plastic tubes. Sedimentation is enhanced based on the theory that settling depends on the area rather than detention time. Hence, increased area will increase settling efficiency (Tchobanoglous et al., 2003). For constructed wetlands, vegetation could be used to function the same way as plates or tubes. Plants will increase the settling area the same way as artificial surfaces and enhance settling of particles.

In storm runoff situations, when hydraulic loadings are very high, resuspension of particles can be an issue (Braskerud, 2002). As water flows past a surface, velocities will decrease with proximity to the surface and equal zero at the surface. Triggering mechanisms, such as the interaction of unsteady flow structures that develop naturally within the fluid or small disturbances will cause the flow to change from being laminar near the surface to become turbulent in the bulk, where inertia forces become sufficient to amplify the triggering mechanisms (Incropera, DeWitt, Bergman, & Lavine, 2007). Whether a flow is laminar or turbulent is defined by the Reynolds number (N_R), shown in equation 2.9 (Tchobanoglous et al., 2003). Laminar flow is classified by $N_R < 1$ and turbulent flow by $N_R > 2000$ (Tchobanoglous et al., 2003).

$$N_R = \frac{v_p \cdot d_p \cdot \rho_w}{\mu} \qquad \text{Equation (2.9)}$$

where, v_p is the terminal velocity of a particle (m/s)
d_p is the diameter of a particle (m)
ρ_w is the density of water (kg/m^3)
μ is the dynamic viscosity (m^2/s)

As Reynolds number increases, particle settling will be counteracted by higher particle lifting forces. During turbulent flow conditions, eddies will keep particles in suspension and prevent particle settling. As hydraulic loadings increase, velocities will increase and, hence, the laminar region is reduced. More turbulent flow conditions will be established near the surface and particles will be detached. The slow-down of incoming water, for example by vegetation, is therefor of benefit to reduce particle resuspension.

2.5.2 Sorption and Precipitation

Phosphorous sorption is an abiotic retention mechanism of P, which includes both, adsorption and absorption. Soluble inorganic P can move from the water to soil particle surfaces and accumulate without penetrating the particle by a process called adsorption (Reddy & DeLaune, 2008). The adsorption process starts with the movement of P, by advection and dispersion, through the bulk fluid to the boundary layer of a fixed film of liquid surrounding the particle. By diffusion, P is transported through the fixed film and finally adsorbed at the particle surface (Tchobanoglous et al., 2003). The adsorbed inorganic P can be released again from the particle surface into the water by desorption. Adsorption and desorption processes will maintain an equilibrium between phosphorous attached on a solid surface and phosphorous dissolved in the water. At equilibrium, adsorption equals desorption. Particles adsorption capacity is limited and soil particles surfaces will eventually get saturated, which will increase phosphorous concentrations in water. With time, some of the adsorbed P will be absorbed into the solid phase and form phosphate minerals. However, the latter is a very slowly process which occurs over days to months or years (Reddy & DeLaune, 2008). The soils phosphorous adsorption capacity will vary greatly among different soil types, whereas a high amount of positively charged clay particles or minerals will increase adsorption capacity (Reddy & DeLaune, 2008). The pH of the soil will also influence sorption processes greatly (Kadlec & Reddy, 2001; Reddy & DeLaune, 2008). Under acidic conditions, excess H^+ will cause positively charged particle surfaces, where negatively charged phosphate ions can be adsorbed. Under alkaline conditions, the loss of H^+ will result in negatively charged surfaces and no adsorption of phosphate will occur (Reddy & DeLaune, 2008). This agrees with results by Kim et al. (2016), where P release was higher under alkaline conditions compared to acidic conditions (Kim et al., 2016). Besides, new constructed wetlands have a higher adsorption capacity due to little saturation of particle surfaces (Yu et al., 2015). However, soil will only adsorb phosphorous when added concentrations are higher than P concentration in water. Otherwise, phosphorous will be released (desorption) from the soil until a new equilibrium is reached (Reddy & DeLaune, 2008).

Phosphorous sorption occurs at low concentrations. As concentrations increase, precipitation will occur. The occurrence of precipitation reactions is determined by the reactant concentrations, which again is determined by the solubility product of the precipitate. Precipitation is the formation of insoluble precipitates when inorganic phosphate reacts with metallic cations such as iron (Fe), aluminum (Al), calcium (Ca), and magnesium (Mg). In aerobic soils, metallic cations such as Fe^{3+} and Al^{3+} often exceed phosphate concentrations, which will result in the precipitation of iron and aluminum phosphate (Reddy & DeLaune, 2008), according to equation 2.10 and 2.11, respectively (Tchobanoglous et al., 2003).

$$Fe^{3+} + H_nPO_4^{3-n} \leftrightarrow AlPO_4 + nH^+$$ Equation 2.10

$$Al^{3+} + H_nPO_4^{3-n} \leftrightarrow FePO_4 + nH^+$$ Equation 2.11

However, these minerals are sensitive to changes in pH (figure 2. 8), where solubility will increase with increasing pH, as the equilibrium shifts to the left when H^+ concentrations increase (Tchobanoglous et al., 2003). Solubility products for $FePO_4$ and $AlPO_4$ are $9.9 \cdot 10^{-16}$ and $9.0 \cdot 10^{-21}$, respectively (Steen, 2009). Phosphorous minerals associated with iron are also sensitive for changing redox conditions, as iron is reduced under anaerobic conditions (Reddy & DeLaune, 2008). This coincides with results by Vohla et al. (2007), where phosphorous concentrations increased in the outlet of the horizontal subsurface flow CW after anaerobic conditions were established (Vohla et al., 2007).

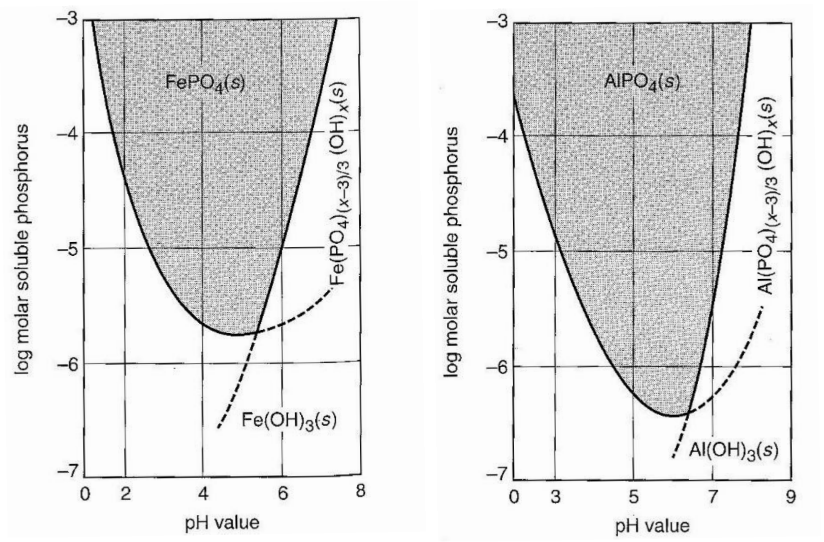

Figure 2. 8. pH influencing precipitation of iron and aluminium phosphate.

As the pH increases beyond 10, phosphate will precipitate with calcium to form hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$), according to equation 2.12 (Tchobanoglous et al., 2003). The presence of humic substances can nevertheless inhibit the precipitation of calcium and phosphate (Kim et al., 2016).

$$10\ Ca^{2+} + 6\ PO_4^{3-} + 2\ OH^- \leftrightarrow Ca_{10}(PO_4)_6(OH)_2 \qquad \text{Equation 2.12}$$

However, for precipitation reactions, if the concentration of any of the two reactants decreases below the solubility product of the precipitate, the compound will dissolve. (Reddy & DeLaune, 2008).

2.5.3 Ion and ligand exchange

Ions of a given species can be replaced by ions of a different species from an insoluble exchange material by a process called ion exchange (Tchobanoglous et al., 2003). In acidic soils, the positive charge on the surface of minerals, clay particles and organic matter results in the adsorption of anions such as OH^-, NO_3^-, SO_4^{2-}, and Cl^-. These can be replaced by other anions such as phosphate. This exchange results from electrostatic attraction of phosphate ions to the positively charged mineral surface (Reddy & DeLaune, 2008).

Hydroxide ions are often coordinated with a metal cation such as iron or aluminum as a complex. The hydroxide ions can be replaced by phosphate ions in a process called ligand exchange, as shown in figure 2.9. However, the phosphate associated with metal ions may be replaced again by OH^- or organic acids (produced by microorganisms) at higher pH (Reddy & DeLaune, 2008; Stumm & Morgan, 2013).

Hence, low pH and high redox potential will favor processes like adsorption, precipitation with Fe and Al, ion exchange, and ligand exchange, and thus, increase the retention of phosphorous in the soil. High pH will favor the precipitation with Ca and, thus, increase the retention of P in calcium rich soils. The accumulation of P in soil is the major long-term retention mechanism in constructed wetlands.

Figure 2. 9. Ligand exchange of ironhydroxide with phosphate (Stumm & Morgan, 2013).

2.5.4 Plant and microbial uptake

Although physiochemical processes are the major retention mechanisms of TP, plants can assimilate a smaller fraction too (Lægreid et al., 1999). A study by Dzakpasu et al. (2015) showed that plants accounted for up to 16 % of the total retained phosphorous, while sediment storage contributed with nearly 60 % (Dzakpasu et al., 2015). An improvement of phosphorous retention by vegetation was achieved in several studies. The improvement could be a result of biological uptake of dissolved P, an oxidized layer around the roots supporting aerobic soil conditions, and an increased microbial activity due to a larger attachment area for microorganisms (Hunter et al., 2001; Liu et al., 2012; Tyler et al., 2012; Yates & Prasher, 2009).

Phosphorous is available for plant uptake in its soluble inorganic form, and will be transformed by plants to organic P (Mitsch & Gosselink, 2007). The storage of the nutrient in plants is primarily in the form of phytic acid, or inositol phosphate, which constitutes the major portion of total phosphorous in plants (Hidvégi & Lásztity, 2003; Turner, Richardson, & Mullaney, 2006). Phytic acid consists of an inositol ring where each hydroxyl group is esterified as phosphates, as shown in figure 2. 10 (Turner et al., 2006).

Plants have two direct effects on phosphorous retention in wetlands: (1) Storage and release, as they store and release P during their seasonal growth cycle; and (2) Sediment accretion, where new stable residuals are created which accrete in the wetland and contribute to the long-term storage (Kadlec & Wallace, 2008). Phosphorous concentrations in plant tissue will vary greatly depending on the plant species, the availability of phosphorous, and growth stage of the plant. Phosphorous concentrations typically range from less than 1 to 7 g P per kg dry weight (Reddy & DeLaune, 2008).

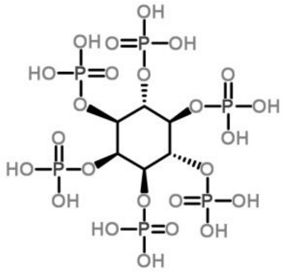

Figure 2. 10. Phytic acid ("Phytic acid [Figure],").

Some examples of phosphorous concentration in various wetland plants are given in table 2.1. All data in table 2. 1 are from low P wetlands, with exception of *Carex fascicularis*, which grew under high P-concentrations. The variety of P uptake by plants is clearly seen by *Typha latifolia* and *Phragmitis australis*, where P uptake also varies among the same species grown in different wetlands. Table 2. 1 also shows that dead aboveground biomass contains less phosphorous than their live counterparts (Kadlec & Wallace, 2008).

Table 2. 1. Phosphorous concentration of various wetland plants in live and dead aboveground biomass and belowground biomass. (Kadlec & Wallace, 2008).

Plant	**Above live [mg P/kg dry weight]**	**Above dead [mg P/kg dry weight]**	**Below [mg P/kg dry weight]**
Typha latifolia	895	-	-
Typha latifolia	1460	-	-
Typha angustifolia	2000	-	-
Typha spp.	1343	709	1945
Phragmitis australis	1870	520	-
Phragmitis australis	2800	500	1000
Carex fascicularis	2700	-	1900

In general, emergent macrophytes have a higher potential for long-term phosphorous storage compared to submerged and floating plants, due to a larger network of roots. The storage of P in the aboveground biomass is usually of short-term. However, a high ratio of belowground to aboveground biomass create good anatomical structures for long-term storage, where approximately 50 % of the phosphorous is transported to the roots for storage. Floating and submerged plants have a higher turnover and phosphorous will be released back rapidly during decomposition. In general, plants in early growth stages or plants grown in nutrient rich environments, will have higher tissue phosphorous concentrations. As mentioned in chapter “2.4.5 Cold climate and seasonal variations”, the uptake rate of P is highest during early spring growth before the maximum growth rate is achieved. During fall, nutrients will be transported to the roots for storage and will be utilized again during early spring growth. Phosphorous will eventually be released again, either after their death due to decomposition or as older tissues leach (Reddy & DeLaune, 2008).

Microorganisms in wetlands are important for the decomposition of organic material and the remobilization and cycling of nutrients. One of the first interactions of phosphorous when entering a wetland is with microorganisms rather than vegetation. Microbes can store significant amounts of P and place it in the sediment-soil system, where other processes can transform it to recalcitrant forms. Turnover is quick as the lifecycle is short for microorganisms. Hence, most of the uptake is returned as dissolved organic phosphorous and particulate phosphorous, leaving only a small fraction as permanently buried (Kadlec & Wallace, 2008). Phosphorous can also be incorporated into cellular constituents by microorganisms beyond their needs and stored as polyphosphate, a phenomenon called "luxury uptake". The formation of polyphosphates enables microorganisms to survive under altering redox conditions. (Reddy & DeLaune, 2008). Microorganisms able to accumulate phosphorous beyond their needs in their cellular tissues are called phosphorous accumulating organisms (PAO) (Tchobanoglous et al., 2003), and are abundant in biologically enhancing wastewater treatment plants.

2.6 Objectives

The main objective of this thesis is to investigate the retention of phosphorous in a mature constructed wetland designed to treat agricultural runoff. The hypothesis is that phosphorous is being retained by biogeochemical conversions and physical retention, and will accumulate in the constructed wetland. Furthermore, the influence of vegetation on phosphorous retention was studied. The main interest with studying vegetation was to investigate the phosphorous content in plants during different seasons. This could be used to determine seasonal variations and to quantitively estimate annual phosphorous retention by harvesting plant biomass. To achieve these objectives, weekly water- and plant samples were analyzed for phosphorous concentrations. In addition, water flow entering and leaving the wetland, iron concentrations, color, and suspended solids concentrations were analyzed in order to investigate their possible connections to phosphorous retention.

3. Methods

This chapter explains the design of the constructed wetland which was studied for this thesis, as well as sample procedures and analytical methods used in this study. The latter includes analysis procedures for phosphorous, iron, color, and suspended solids in water samples, as well as phosphorous analysis of plant samples.

3.1 Site description

The constructed wetland "Leikvollbekken" is located northwest of Stavanger between the municipalities of Stavanger and Randaberg. Leikvollbekken is part of the Store Stokkavatn water shed, as illustrated in

Figure 3. 1. The area surrounding the CW is dominated by agricultural activities, including mainly grassland and some greenhouses. According to figure 3. 2, mineral soil with a top layer rich in humus is dominating around the wetland. This soil composition is characteristic for podzol, which is the most common soil type in Norway. Beneath the humus top layer, there is a grey, nutrient poor soil layer, which is followed by a precipitation layer at the bottom. The bottom layer is often characterized by iron rich precipitates and causes a red/brown color (Låg, 1979). A typical red/brown color can be observed at the wetland location, indicating iron rich bedrock and/or soil.

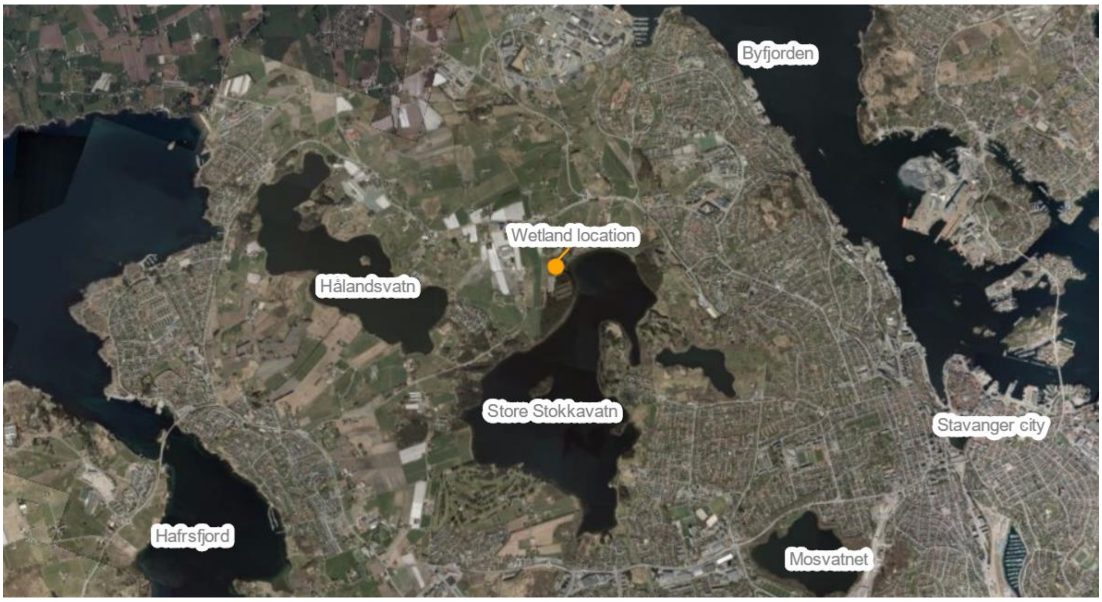

Figure 3. 1. Location of the constructed wetland "Leikvollbekken" marked with an orange dot and text "wetland location", together with other place descriptions.

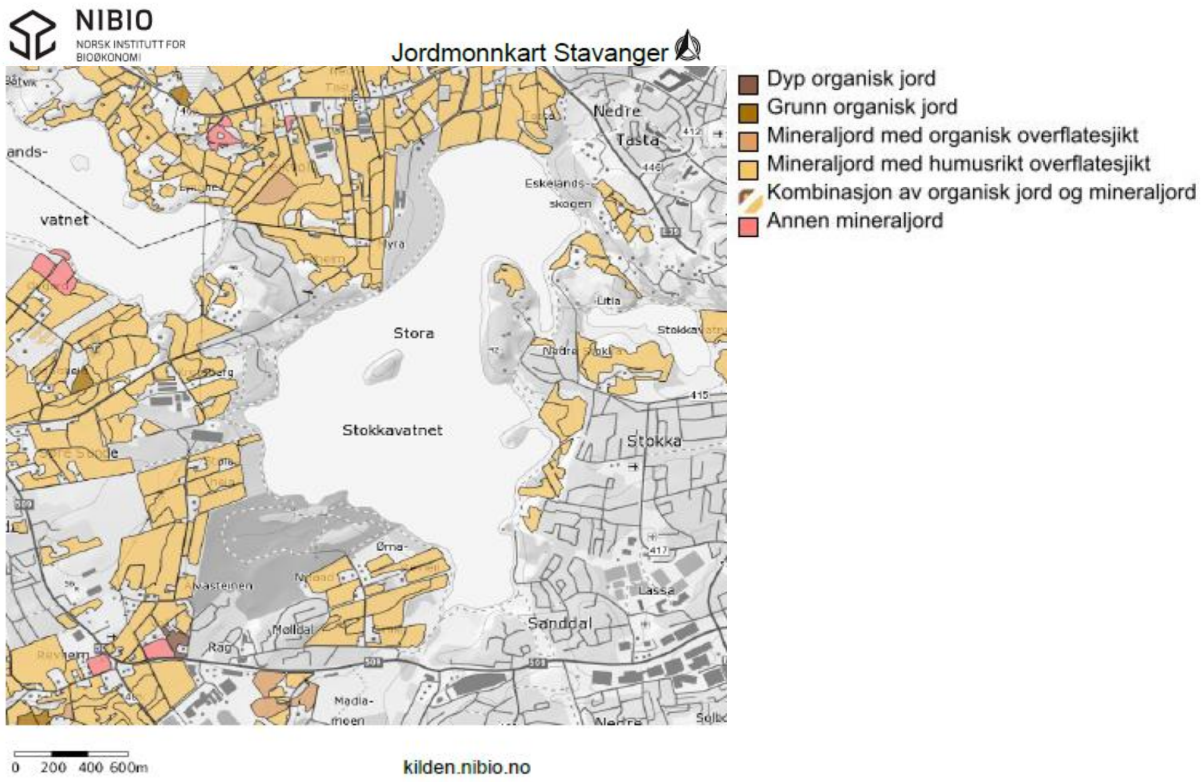

Figure 3. 2. Map showing dominating soil types near the constructed wetland "Leikvollbekken" and its adjacent areas.

The wetland was constructed in 1993/94 in order to remove nutrients, especially phosphorous, from agricultural runoff before it enters lake "Store Stokkavatnet". Leikvollbekken is a two-pond system with free water surface flow. The total wetland area is approximately 1410 m^2, where pond 1 is slightly larger than pond 2 with approximately 754 m^2 and 656 m^2, respectively (see figure 3. 3). The wetland was planted with more than 25 different macrophyte species in the mid-90, but is now dominated by Reed Sweet-grass (*Glyceria maxima*), Yellow Iris (*Iris pseudacorus*), Common Cattail (*Typha latifolia*) and Willow (*Salix*). The effluent zones were originally designed with a depth of 1.0 – 1.5 m, but these are now totally filled and the average water depth is approximately 30 cm (Kommedal, R.). The water enters pond 1 at the "inlet" marked in figure 3. 3 and is spread by a permeable barrier consisting of cobblestone. At the outlet of pond 1, another barrier, consisting of fine and coarse gravel, functions in regulating the water level and flow through. The water is transported beneath the path in a pipe and enters pond 2, where it is spread by a permeable barrier. Before entering Store Stokkavatnet, the water flows in a loop around a mound where trees will shade the ditch and create good spawning conditions. A monitoring station is placed at the outlet channel.

Figure 3. 3. Illustration of pond 1 and 2, which constitute the constructed wetland together with areal dimensions and important terms.

3.1.1 Weather data

Monthly weather data for the master thesis period (September 2016-May 2017) is presented in table 3. 1 (Yr, 2017). The table contains average, highest and lowest temperature, as well as total and highest precipitation for each month. According to average temperatures, September 2016 was the warmest month, while February 2017 was the coldest. Most precipitation was received in September 2016, while October 2016 was the driest month. Snow was observed in January and February. The weather observation station is located in Stavanger, Våland at 72 m. a. s. l. The change of the mean temperature over time (figure 3. 4) shows that temperature decreases from September on and starts to increase again in March. Temperature below 0 occurred in November, January, February and March (Yr, 2017). The precipitation variation (figure 3. 5) shows that October and February were the driest months, while November had the highest daily precipitation peak.

Table 3. 1. Monthly weather data of temperature and precipitation from September 2016 until May 2017 (Yr, 2017).

Month	**Temperature**			**Precipitation**	
	Average	Highest	Lowest	Total	Highest
September 2016	15.7 °C	27.3 °C (sep. 13)	8.5 °C (sep. 30)	152.4 mm	27.2 mm (sep. 11)
October 2016	8.8 °C	16.9 °C (oct. 07)	2.0 °C (oct. 14)	22.1 mm	11.1 mm (oct. 01)
November 2016	4.6 °C	11.5 °C (nov. 22)	-5.0 °C (nov. 08)	146.3 mm	31.8 mm (nov. 15)
December 2016	6.1 °C	10.2 °C (dec. 08)	-0.9 °C (dec. 03)	152.2 mm	27.0 mm (dec. 01)
January 2017	3.5 °C	9.2 °C (jan. 27)	-5.6 °C (jan. 05)	144.3 mm	22.9 mm (jan. 10)
February 2017	2.8 °C	8.8 °C (feb. 27)	-5.7 °C (feb. 08)	100.6 mm	23.1 mm (feb. 22)
March 2017	4.6 °C	14.6 °C (mar. 31)	-4.2 °C (mar. 07)	120.8 mm	19.4 mm (mar. 22)
April 2017	5.9 °C	13.6 °C (apr. 09)	-1.2 °C (apr. 25)	116.8 mm	21.1 mm (apr. 07)
May 2017	12.1 °C	26.2 °C (may 08)	4.1 ° C (may 01)	107.9 mm	32.1 mm (may 31)

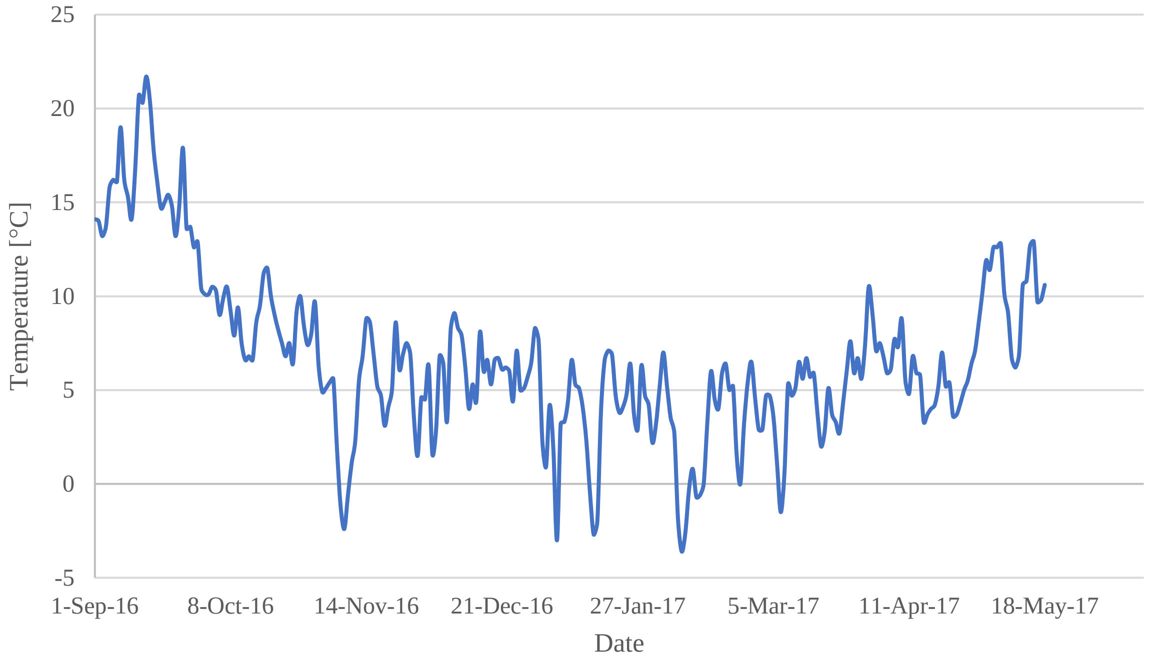

Figure 3. 4. Temperature change from September 2016 until May 2017 (Yr, 2017).

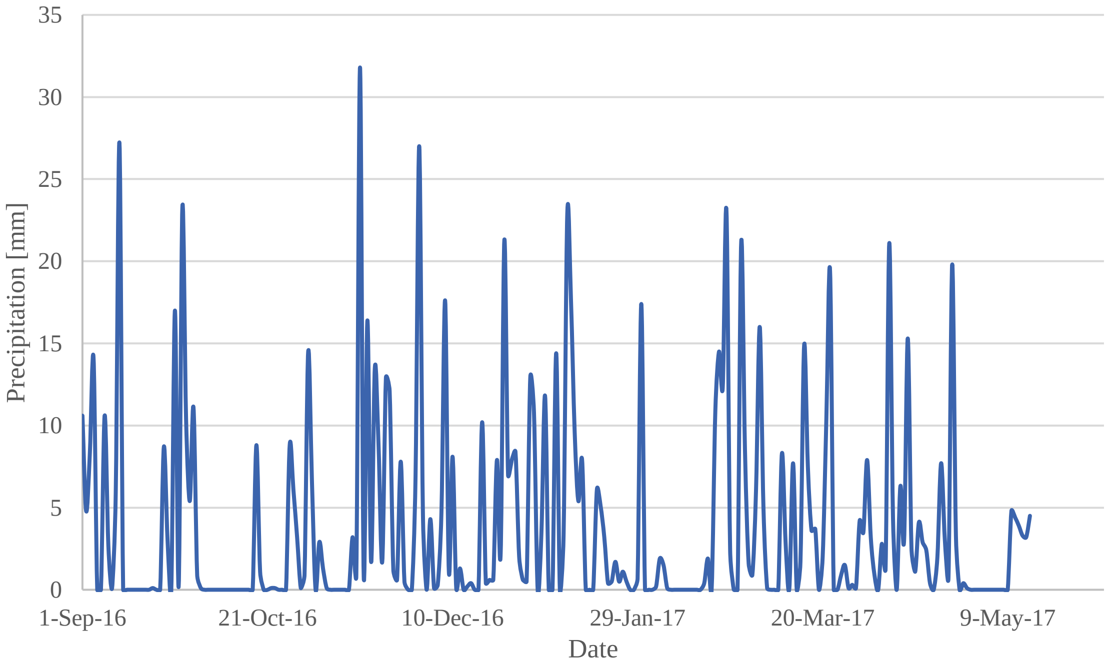

Figure 3. 5. Precipitation from September 2016 until May 2017 (Yr, 2017).

3.2 Sampling procedures

3.2.1 Water samples and flow measurement

Inlet samples were taken from the discharge of the pipe located beneath the ground to connect pond 1 and 2 (at the inlet of pond 2). Outlet samples were taken at the monitoring station located at the outlet channel. Automatic composite sampling for the inlet and outlet was performed weekly by two ISCO 6712 Full Sized Portable Samplers (auto samplers). This kind of sampling provides a representative sample for average concentrations (weekly average). Samples were pumped into 11 liter bottles (one for inlet and one for outlet samples), which were kept dark and cool in refrigerators, for storage. From September to the end of March, automatic samples were taken every 2 hours. From 30 March 2017, samples were taken with a flow-based sampling as the auto-samplers were connected to a flow-meter. Inlet and outlet flow rates were measured by using an ISCO Signature Flow Meter for open channel flow monitoring. The signature flow meter can measure flow rates by using standard open channel level-to-flow conversions. To measure flowrates, a bubbler level sensor technique was used. Bubbles are forced out at the end of a submerged bubble line. The amount of pressure required to force bubbles from the end of the line depends directly on the hydrostatic pressure of the flow stream over the end of the bubble line. A pressure transducer senses this pressure and converts it into a level signal that the flow meter can use to calculate flow rate and total flow.

3.2.2 Plant samples

Plant samples of *Glyceria maxima* and *Iris pseudacorus* were taken weekly. Whole plants were harvested and divided into root, stem and leave. When there was no clear, visual distinction between stem and leave, the plant was rather divided into root, lower leave and upper leave. Several plants of *Glyceria maxima* were taken, while one to two plants of *Iris pseudacorus* were sufficient for analysis. The plant parts were rinsed with distilled water and either stored at -70°C in plastic bags, or analyzed directly.

3.3 Analytical methods

This chapter describes the analytical methods used for this thesis. These include analysis of total phosphorous (TP), total dissolved phosphorous (TDP), phosphate (PO_4^{3-}), total iron (tot. Fe), total dissolved iron (tot. diss. Fe), color, turbidity, and total suspended solids (TSS). Total phosphorous analyses are described for both water and plant samples, as well as water content measurements of plant samples.

3.3.1 Water samples

Inlet and outlet water samples were taken from the 11 liter bottles weekly and analyzed. The analytical procedures, summarized graphically in figure 3. 6 , were carried out the same way for inlet and outlet samples. Analytical methods for phosphorous, turbidity, and total suspended solids are given with a standard method number. The number abbreviation and the corresponding method name are given in table 3. 2 (Clesceri et al., 1998).

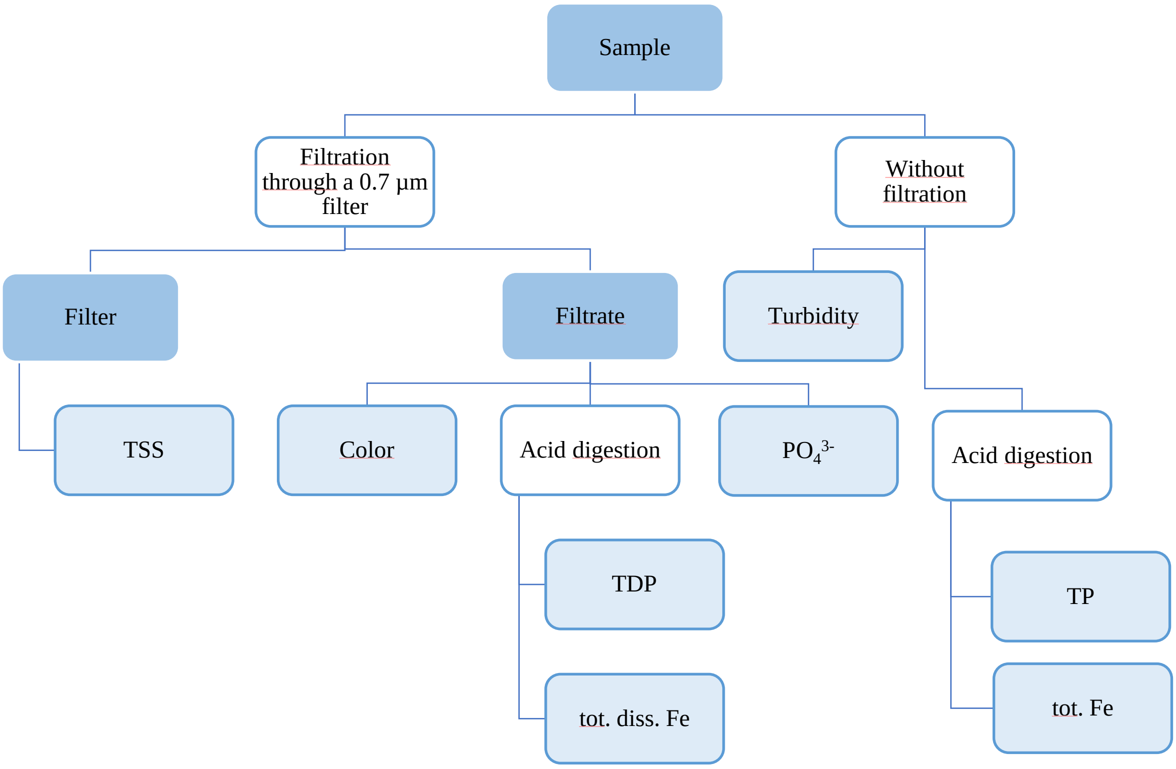

Figure 3. 6. Graphical illustration of all analytical methods performed with water samples.

Table 3. 2. Standard method abbreviation and corresponding method name.

Abbreviation	Method name
2540 D	Total suspended solids dried at 103-105°C
4500-P E	Ascorbic Acid Method
4500-P B.1	Preliminary Filtration
4500-P B.5	Persulfate Digestion Method
4500-P.C.3.e	Stock Phosphate solution
4500-P.E.3.e	Combined reagent
2130 B	Nephelometric Method

A summary of all chemicals used to analyze water samples is given in table 3. 3. The table includes chemical formula and name of the chemicals, as well as which analytical procedures the chemical was used for. For more expletive information on standard method abbreviations, see table 3. 2.

Sample Pre-treatment

Filtration was performed according to Standard method 4500-P B.1 (Clesceri et al., 1998) for analysis of TSS, color and dissolved fractions of phosphorous and iron. A standard glass microfiber filter with pore size 0.7 µm of type Whatman (GF/F) was used. TP and TDP samples as well as a distilled water blank and an independent control sample (385 µg/l P) were digested in the autoclave according to "Standard method 4500-P B.5"(Clesceri et al., 1998). For the analysis of total phosphorous, raw sample was used, while the filtrate was used for dissolved fractions. For iron analysis, a volume of the digested samples was used.

Table 3. 3. Chemicals used for water sample analysis.

Name	**Chemical formula**	**Analytical method**	
Sulfuric acid	H_2SO_4	Standard method (Clesceri et al., 1998)	4500-P B.5 4500-P E
Ammonium Persulfate	$(NH_4)_2S_2O_8$		4500-P B.5
Sodium Hydroxide	NaOH		4500-P B.5
Potassium antimonyl tartrate	$K(SbO)C_4H_4O_6 * ½H_2O$		4500-P E
Ammonium molybdate	$(NH_4)_6Mo_7O_{24} * 4H_2O$		4500-P E
Ascorbic acid	$C_6H_8O_6$		4500-P E
Ceric ammonium sulfate	$Ce(NH_4)_4(SO_4)_4$	Modified Thiocyanate method (Goswami & Kalita, 2013)	
Potassium thiocyanate	KSCN		

Total suspended solids

Total suspended solids were analyzed according to “Standard method 2540-D” (Clesceri et al., 1998). A rinsed standard glass microfiber filter with 0.7 µm pore size was used for this analysis. Total suspended solids is the residue retained on the filter after filtration, and is calculated according to equation (2.13) (Clesceri et al., 1998).

$$TSS\left[\frac{mg}{l}\right]=\frac{(weight\ of\ filter\ and\ dried\ residue\ [mg]-weight\ of\ filter[mg])*1000}{sample\ volume\ [ml]} \qquad \text{Equation (2.13)}$$

Phosphorous analysis

Total phosphorous, total dissolved phosphorous, and phosphate were analyzed according to Standard method 4500-P E (Clesceri et al., 1998), with some modifications. Two parallels were performed for each sample for error analysis. Pre-treated samples were used for TP and TDP analysis, while the filtrate without digestion was used for the analysis of PO_4^{3-} . In addition, a distilled water blank and calibration curve standards with concentrations from 50 µg/l P to 400 µg/l P were prepared. For the latter, a stock phosphate solution, prepared according to 4500-P.C.3.e (Clesceri et al., 1998), was used. Combined reagent was prepared as described in 4500-P.E.3.e (Clesceri et al., 1998), and added to all volumetric flasks. The samples were analyzed in the spectrophotometer at 880 nm. A linear relationship (calibration curve) was obtained by plotting absorbance against the standard phosphorous concentrations. The calibration curve was determined and used to calculate unknown phosphorous concentrations of the sample.

The ascorbic acid method measures the orthophosphate content in a sample. Phosphorous may be present in combination with organic matter or other forms than orthophosphate. Polyphosphate is converted to reactive orthophosphate by sulfuric acid hydrolysis and organophosphorous is converted to reactive orthophosphate by persulfate digestion. The preliminary filtration separates dissolved from suspended phosphorous forms. The ascorbic acid method is based on the formation of a heteropoly acid-phosphomolybdic acid by reacting orthophosphate with an acid solution containing ammonium molybdate and potassium antimonyl tartrate. The heteropoly acid-phosphomolybdic acid is reduced by using ascorbic acid, which results in the formation of a blue colored molybdate complex. The absorbance of the blue-colored complex is measured with a spectrophotometer (Clesceri et al., 1998).

Iron

Total iron and total dissolved iron were analyzed according to the Modified Thiocyante Method (Goswami & Kalita, 2013). For total and total dissolved iron analysis, a volume of the digested total and total dissolved phosphorous samples, respectively, were used. In order to calculate iron concentrations, a calibration curve with standard concentrations ranging from 0.1 to 4.0 was prepared. A linear relationship was obtained by plotting absorbance against standard iron concentrations and a slope of 7.7 mg*L^{-1}*abs^{-1} was found. It was not necessary to prepare a calibration graph for iron each time samples were analyzed, since it did not deviate significantly from other calibration curve results. Unknown iron concentration were found by multiplying the absorbance with the slope constant and the dilution factor (Goswami & Kalita, 2013).

When analyzing iron, ceric ammonium sulfate in sulfuric acid is used as an oxidizing agent. Complete oxidation of iron(II) to iron(III) is indicated with a remaining weak yellow color in the solution. A red color results after addition of potassium thiocyanate, due to the formation of a ferric thiocyanate complex. Absorbance of the complex is measured with a spectrometer at 480 nm (Goswami & Kalita, 2013).

Turbidity

Turbidity was measured according to Standard method 2130 B. Sample cells were filled with raw sample water and measured as NTU in a turbidimeter (Clesceri et al., 1998).

The analysis is based on measuring the intensity of light scattered by the sample compared to the intensity of light scattered by a standard reference suspension. The sample is placed 90° to the incident light. The higher the intensity of the scattered light, the higher the turbidity of the sample (Clesceri et al., 1998).

Color

Color was measured according to Norwegian standard NS-4787. Filtered sample was measured in the spectrophotometer at 410 nm. In contrast to the standard, where a pore size of 0.45 µm to filter the sample is specified, filtered sample from the pretreatment step was used (filtered through a 0.7 µm pore size filter) (*Vannundersøkelse : bestemmelse av farge : metode for spektrofotometrisk måling av absorbans ved 410 nm*, 2002).

Color is analyzed as true color, meaning the color of the water after suspended matter has been removed by filtration. To calculate color as mg Platina per liter, the absorbance at 410 nm is multiplied with 1900 mg*l^{-1}*abs^{-1}, which is a constant found from a prepared calibration curve. As color analysis is a stable analysis and the calibration curve does not vary significantly, it is not necessary to prepare a calibration graph for color each time samples are analyzed (*Vannundersøkelse : bestemmelse av farge : metode for spektrofotometrisk måling av absorbans ved 410 nm*, 2002).

3.3.2 Plant samples

Plant samples of *Glyceria maxima* and *Iris pseudacorus* were analyzed weekly for total phosphorous and water content. Analysis were performed for each plant part (root, stem, leave) and phosphorous analysis were also performed with two parallels for error analysis. Chemicals used for the analysis of plant samples are given in table 3. 4.

Table 3. 4. Chemicals used for plant analyses.

Name	**Chemical formula**
Sulfuric acid	H_2SO_4
Sodium Hydroxide	$NaOH$
Potassium antimonyl tartrate	$K(SbO)C_4H_4O_6 * \frac{1}{2}H_2O$
Ammonium molybdate	$(NH_4)_6Mo_7O_{24} * 4H_2O$
Ascorbic acid	$C_6H_8O_6$
Nitric acid	HNO_3

Water content

Rinsed plant samples of root, stem and leave were weighed in a porcelain dish, dried at 105°C to constant weight, and weighed again.

After drying plant samples at 105°C, water has evaporated and plant dry mass will remain in the porcelain dish. The water content in percent can be calculated according to equation 2.14:

$$Water\ content\ [\%] = \frac{(weight\ before\ drying\ [g] - weight\ after\ drying\ [g])}{weight\ before\ drying\ [g]} * 100 \quad \text{Equation (2.14)}$$

Phosphorous analysis

Root, stem and leave samples were prepared according to Norsk standard NS-EN 14672 (Standard, 2005). The dried plant parts were mixed and grinded in a coffee grinder. After boiling dry plant samples in an acidic solution, the mixture was transferred to volumetric flasks, and used for phosphorous analysis by Standard method 4500-P E (Clesceri et al., 1998). In addition, a distilled water blank and calibration curve standards with concentrations ranging from 50 µg/l P to 600 µg/l P were prepared (Clesceri et al., 1998). The analysis was performed the same way as described earlier for phosphorous analysis of water samples.

The oxidation of organic matter by a boiling mixture of sulfuric and nitric acid, releases any phosphorous present and converts it to orthophosphate. Nitric acid is lost during the boiling process due to oxidation and distillation. The boiling temperature increases as the nitric acid content decreases and remaining organic matter can react with the sulfuric acid, which causes the mixture to char. Small additions of nitric acid are used to complete the oxidation. When the oxidation is complete, no charring occurs resulting in white fumes and a clear solution (Standard, 2005). The mixture is neutralized in order to analyze it according to the ascorbic acid method. The principle for the ascorbic acid method is described above for phosphorous analysis of water samples.

3.4 Error analysis

The standard deviation was calculated for phosphorous analysis of water and plant samples, according to Standard method 1030 C (Eaton et al., 1995). The method is based on two parallel samples which were taken for total P, total dissolved P, and phosphate analyses of water samples, as well as for total P analysis of plant samples. Two parallels were taken each time water and plant samples were analyzed. The calculated standard deviation includes the random errors involved in sampling as well as errors in sample preparation and analysis.

4. Results

Results obtained from the analysis of water- and plant samples are presented in this chapter. Samples were taken from September 1st 2016 until May 18th 2017. Exceptions are iron and color measurements, which were started September 15th and October 13th, respectively. The presented data and figures summarize the collected raw data. The latter is presented in the Appendixes. The chapter is divided into three main sections. First, inlet and outlet hydraulic loadings are presented for the whole sampling period. The next section presents results obtained from water samples, which includes phosphorous, iron, total suspended solids, turbidity and color. The last section presents results obtained from plant samples, which includes phosphorous content in different plants and plant parts.

4.1 Hydraulic loading

Hydraulic loadings for incoming and outgoing water were measured every 15 minutes. Inlet and outlet hydraulic loadings were measured at locations with close proximity and no significant differences for inlet and outlet values were found. Hence, inlet and outlet hydraulic loadings are assumed to be equal, and outlet values are used for plotting and further calculations. For the master thesis period, hydraulic loadings [l/s] are presented in figure 4. 1 , showing the variation of hydraulic loading with time. Hydraulic loadings increase from dry water levels of 6 l/s to > 150 l/s during heavy rainfall. Heavy rainfall resulted in several peaks (figure 4. 1), and high hydraulic loading fluctuations.

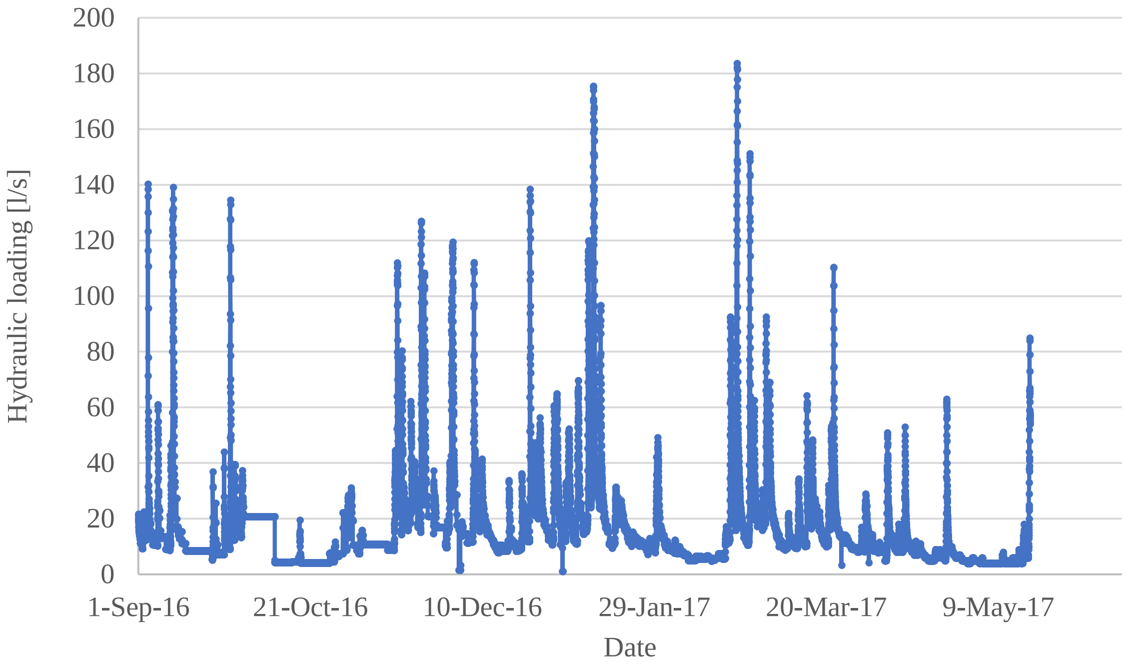

Figure 4. 1. Hydraulic loading variation with time.

4.2 Water samples

All results obtained from the analysis of water samples are summarized in this chapter. Results are plotted against time in order to show time dependent variations. In addition, the weekly average hydraulic loading (flow) is plotted as a grey stippled line in each figure in order to show how the different parameters vary together with hydraulic loadings. Raw data used for figures and tables can be found in appendix A and B When examining the results, it should be noted that there were some technical issues in the period February 9th 2017 – February 16th 2017 due to low temperatures. During large parts of this period, the inlet sampling tube was frozen. This resulted in the suction of air and particles, rather than water, and water inlet samples contained a lot of particulate matter, but only very little water. The high amount of particulate matter resulted in a high peak of inlet samples for total and particulate phosphorous, total and particulate iron, total suspended solids, and turbidity. These peaks are plotted, but can be considered negligible.

4.2.1 Phosphorous

Total phosphorus consists of particulate and dissolved phosphorous fractions, whereas the latter can be roughly divided into phosphates and polymeric phosphorous. Total phosphorous concentrations as well as concentrations of TP fractions are plotted against time for inlet and outlet samples in figure 4. 2 and figure 4. 3, respectively.

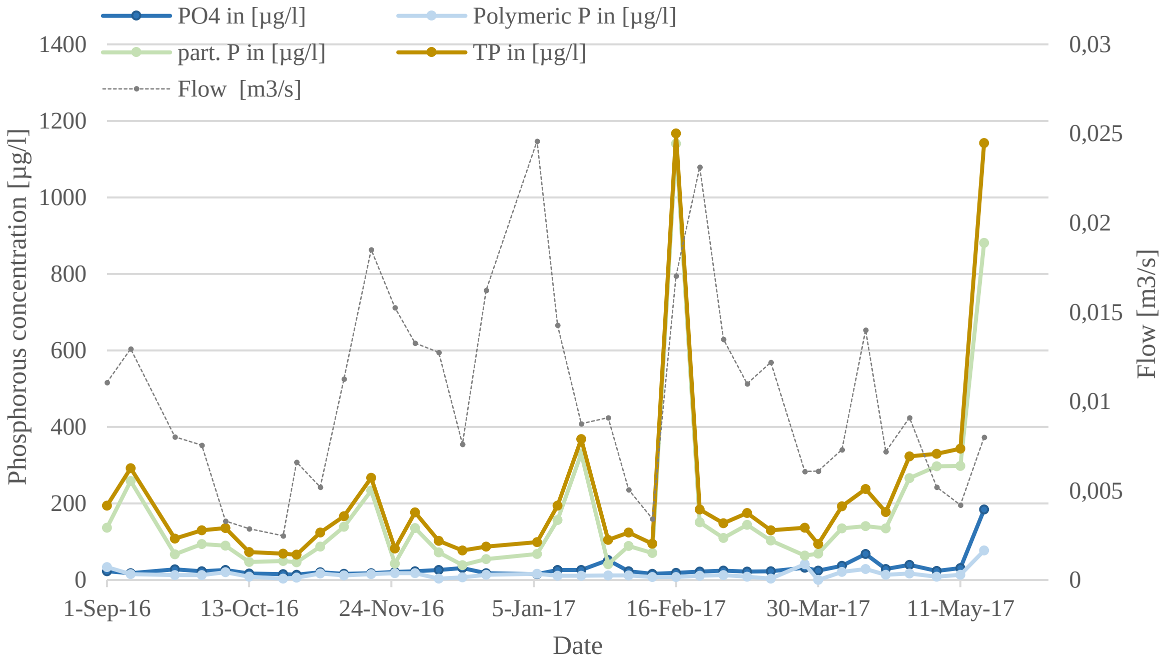

Figure 4. 2. Inlet concentrations of total P, particulate P, polymeric P and phosphate varying with time.

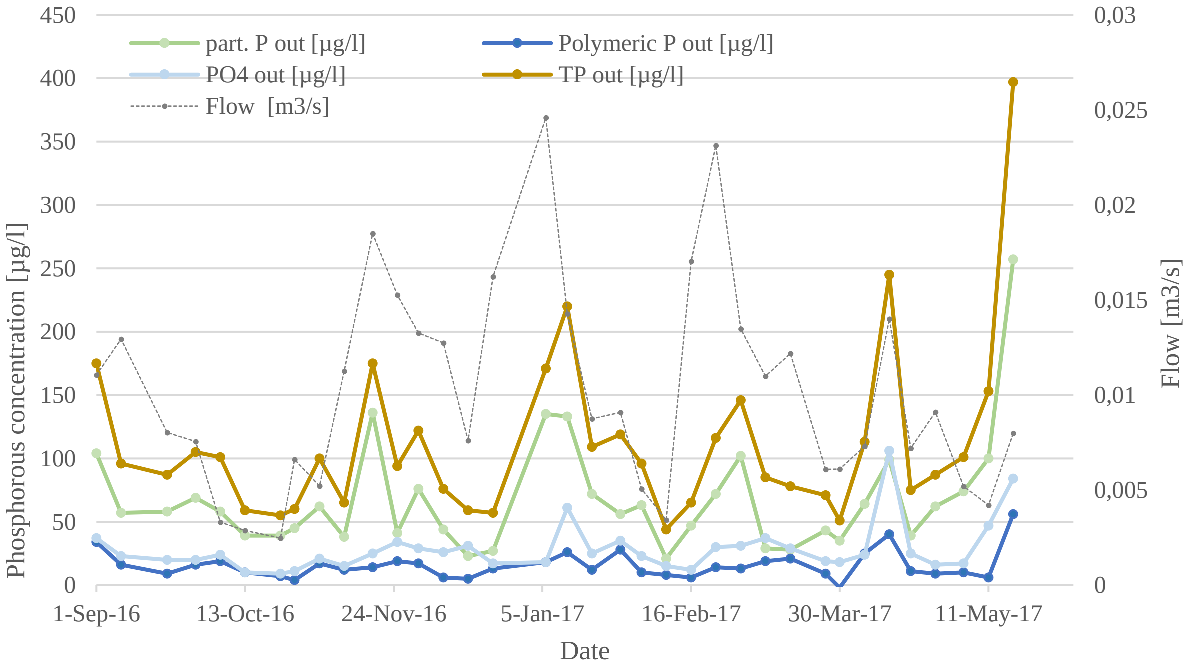

Figure 4. 3. Outlet concentrations of total P, particulate P, polymeric P and phosphate varying with time.

Phosphorous mass flux

Multiplying the concentration with the hydraulic loading, yields the mass flux. The mass flux also takes hydraulic loadings into consideration, in contrast to concentration vs. time plots above. The total phosphorous mass flux can also be divided into its particulate and dissolved fractions. The mass flux of total phosphorous and its fractions is given for inlet and outlet samples in figure 4. 4 and figure 4. 5, respectively.

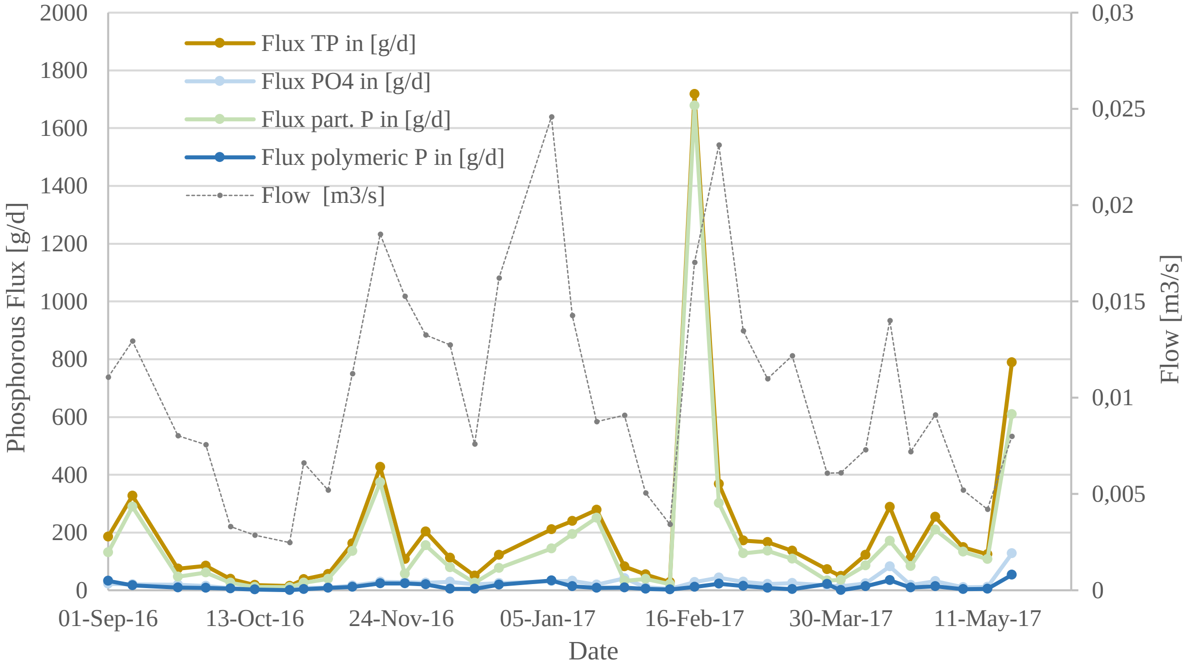

Figure 4. 4. Inlet mass flux of total P, particulate P, polymeric P and phosphate plotted against time.

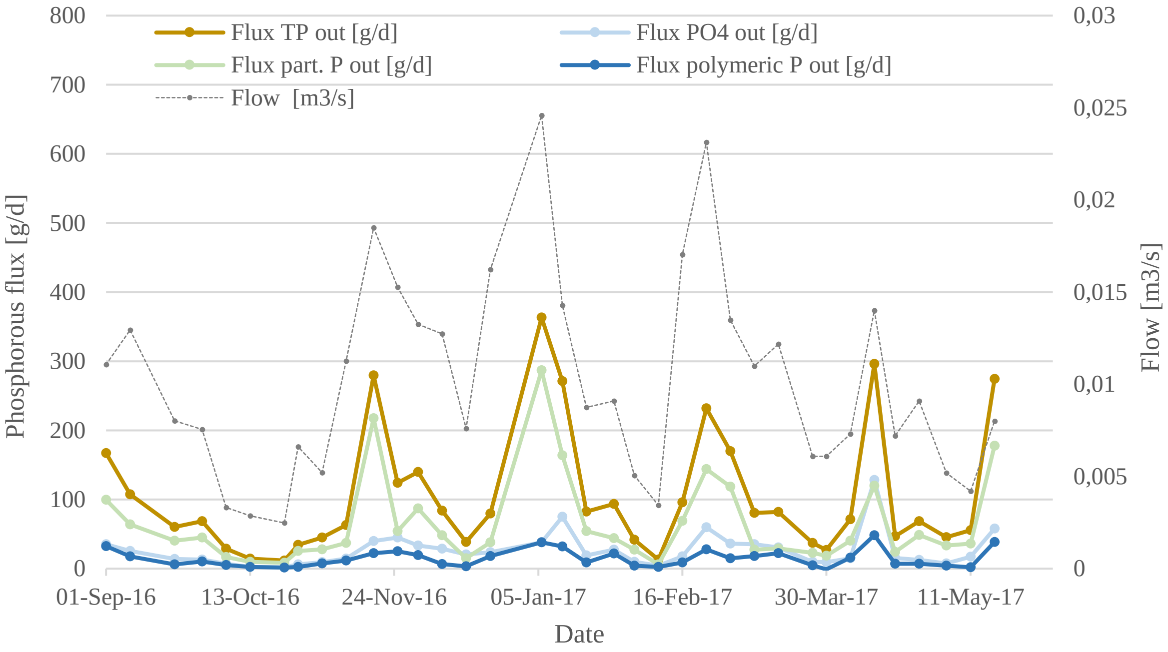

Figure 4. 5. Outlet mass flux of total P, particulate P, polymeric P and phosphate plotted against time.

Phosphorous retention

The phosphorous retention can be calculated as the difference between inlet and outlet values of the different phosphorous fractions, as well as of total phosphorous values. The total phosphorous retention may be positive or negative, indicated with yellow marks at the top or bottom of figure 4. 6, respectively. The bars in figure 4. 6 are divided into the fractions of total phosphorous – particulate P, polymeric P and phosphate. A negative retention indicates a higher amount of phosphorous leaving the wetland compared to the amount entering the wetland. By dividing the bars into the different phosphorous fractions, the contribution of each fraction to the, either negative or positive retention, is indicated (figure 4. 6). The retention of particulate phosphorous constitutes the largest part of the total phosphorous retention. During the master thesis period, retention was negative 5 times.

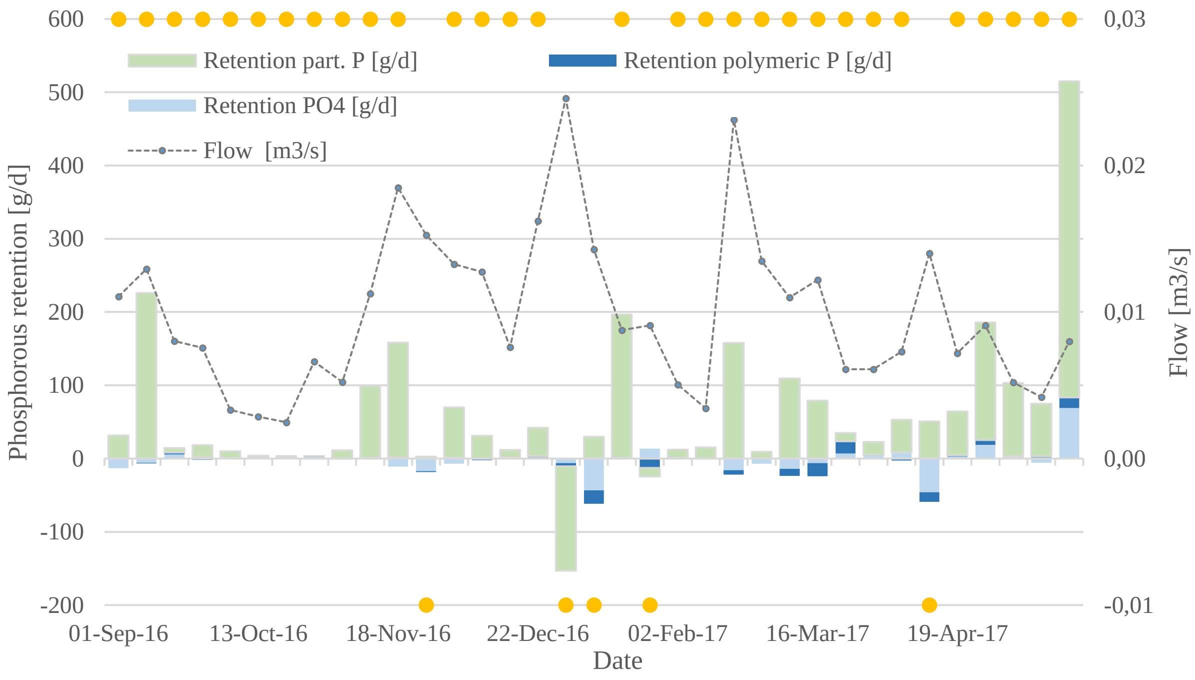

Figure 4. 6. Phosphorous retention with contributing phosphorous fractions variating with time.

The annual retention of total phosphorous, total dissolved phosphorous, particulate phosphorous and phosphate (table 4. 1) was calculated based on the retention of the mass of phosphorous retained during this master thesis period. Furthermore, based on the mass of phosphorous retained, the overall retention efficiency was calculated. It should be noted that the retention of the individual phosphorous fractions is calculated based on their incoming mass, not on the mass of total phosphorous entering the wetland. The corresponding standard deviations are given in table 4. 2, where the standard deviation in percent is based on average phosphorous concentrations.

Table 4. 1. Annual retention and retention efficiency of total P, total dissolved P, particulate P and phosphate.

	TP	**sTP**	**Part. P**	**PO_4^{3-}**
Phosphorous retention [kg/year]	19.89	-1.07	20.96	0.25
Phosphorous retention [g/(m^2*year)]	14.11	-0.76	14.87	0.18
Retention efficiency [%]	31	-7	43	3

Table 4. 2. Standard deviation for total P, total dissolved P, particulate P and phosphate.

	TP	**sTP**	**Part. P**	**PO_4^{3-}**
Standard deviation [µg/l]	6.97	2.11	9.19	1.69
Standard deviation [%]	4	5	6	6

Phosphorous calibration curve

For the analysis of phosphorous, a calibration curve was determined each time with a standard phosphorous solution. An example of a calibration curve is given in appendix E. The R^2-value for the calibration curve varied between 0.9596 and 1, with an average R^2 of 0.9976. The control sample ranged from 379 to 415 µg/l, with an average of 390 µg/l.

4.2.2 Iron

Total iron (tot. Fe) and dissolved iron (diss. Fe) were measured and particulate iron (part. Fe) is calculated as the difference between total and dissolved iron. The variation of total iron concentrations in the inlet are plotted against time in figure 4. 7. The total iron fractions – dissolved and particulate iron – are also plotted in figure 4. 7 to indicate their quantitative contribution to the total iron inlet concentration. Total iron concentrations in the outlet are plotted against time in figure 4. 8. Iron fractions are plotted as well in order to indicate their individual contribution to the total iron outlet concentration. No significant peaks can be observed for outlet concentrations (figure 4. 8).

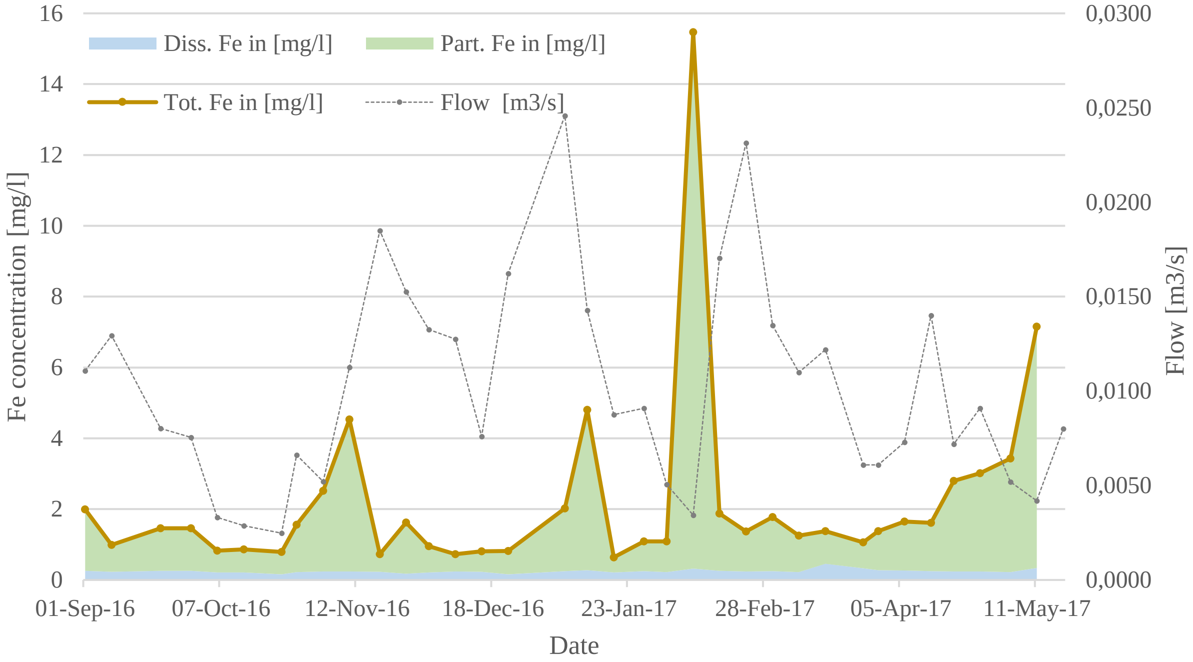

Figure 4. 7. Total iron concentration in the inlet with iron fractionation plotted against time.

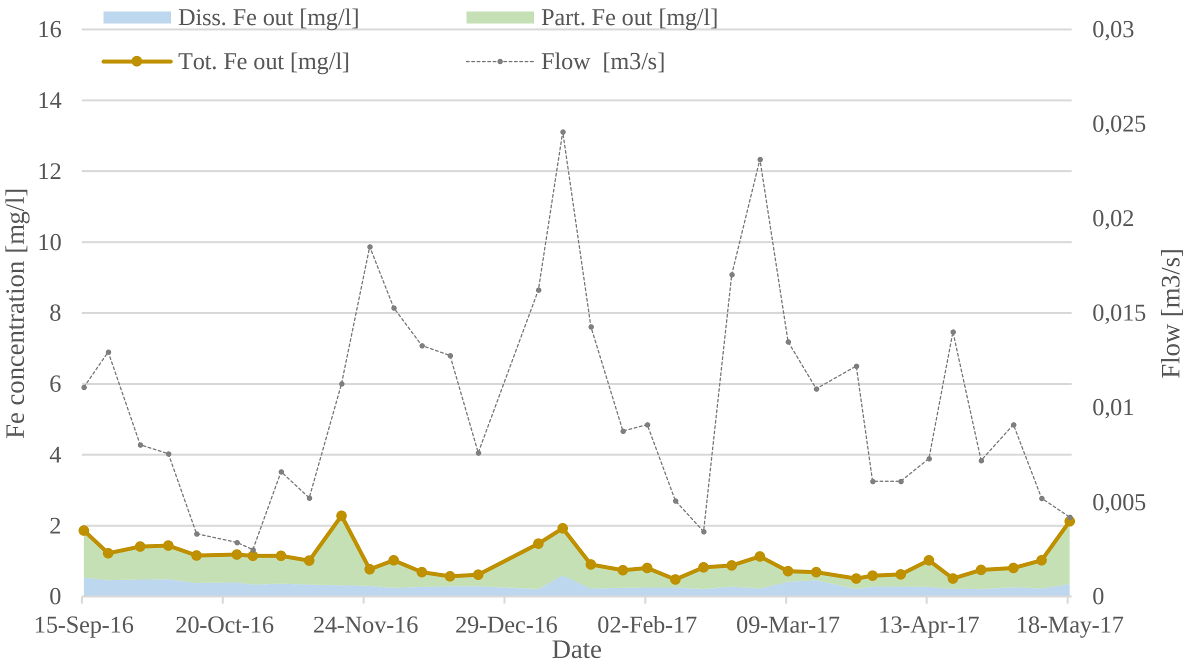

Figure 4. 8. Total iron concentration in the outlet with iron fractionation plotted against time.

Iron calibration curve

A calibration curve for the analysis of iron was controlled without detecting any deviation from the constant 7.7 mg Fe/l*Abs, and had a R^2-value of 0.9990. The calibration curve is shown in appendix E.

4.2.3 Total suspended solids, turbidity and color

Total suspended solids

Total suspended solids were measured for inlet and outlet samples, and plotted against time in figure 4. 9. Both, inlet and outlet concentrations follow the average hydraulic loading well, where TSS concentrations increase with increasing hydraulic loading.

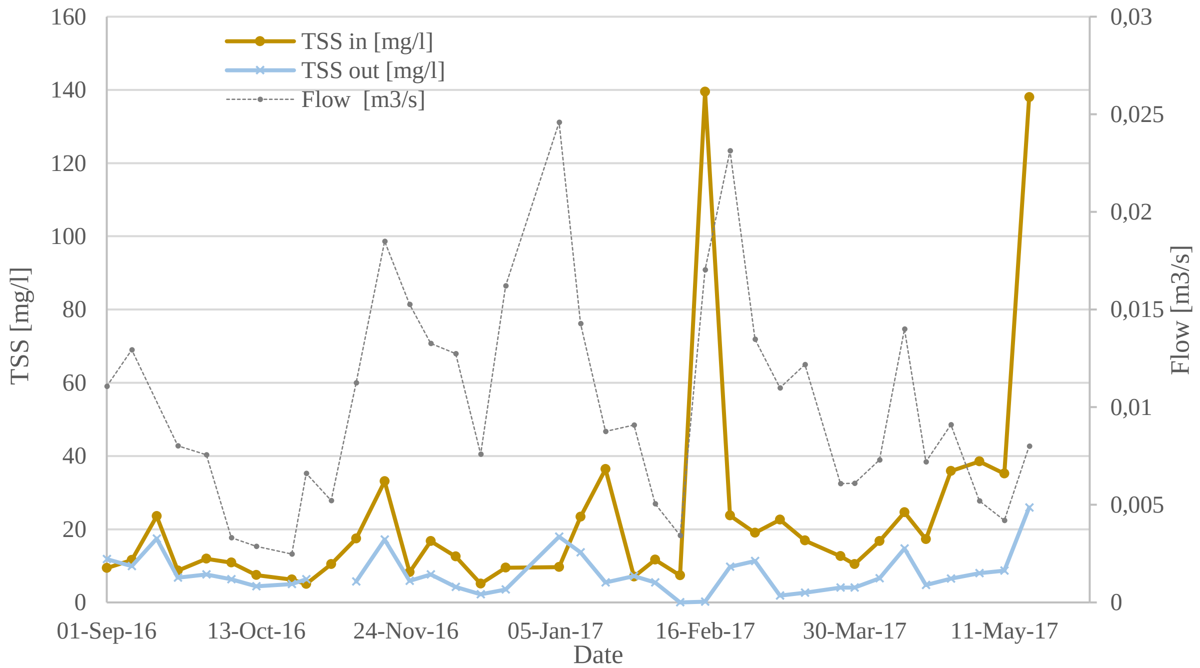

Figure 4. 9. Total suspended solids for inlet and outlet samples plotted against time.

Turbidity

Turbidity was measured for inlet and outlet samples and plotted against time in figure 4. 10. The figure shows inlet and outlet turbidity values, which increase as hydraulic loadings increase.

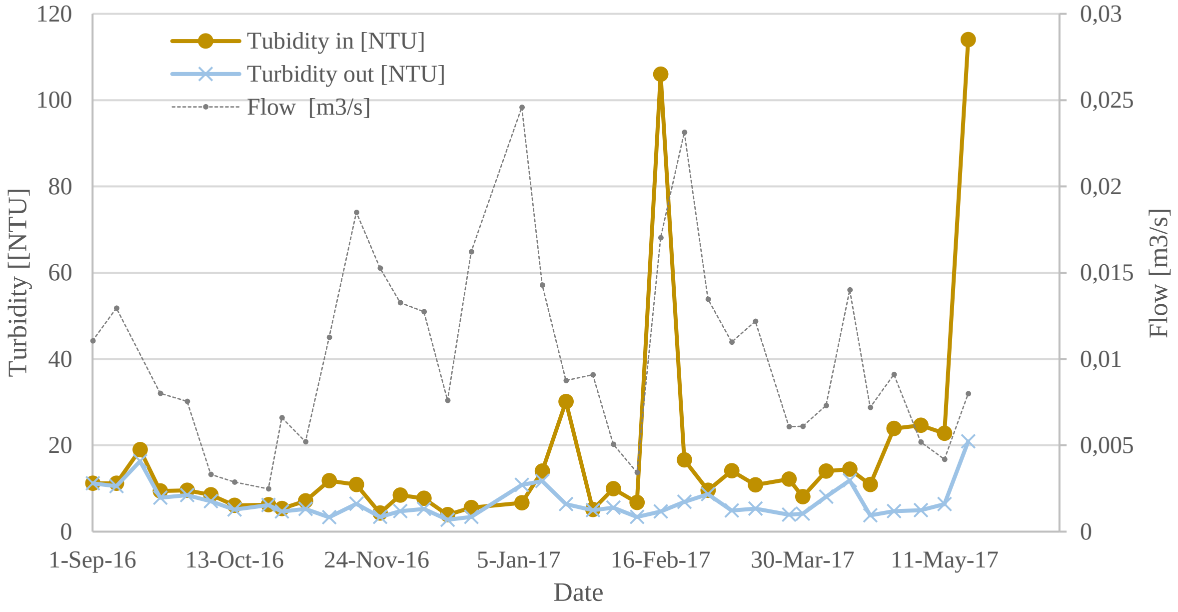

Figure 4. 10. Turbidity for inlet and outlet samples plotted against time.

Color

Color measurements did not vary significantly during the master thesis period and there is no need for a graphical illustration. However, average color values were calculated to be 48.53 mg Pt/l and 49.83 mg Pt/l for inlet and outlet water samples, respectively.

4.3 Plant samples

Results obtained from the analysis of plant samples are summarized in this chapter. Plant samples were taken in the period September 29th 2016 until May 18th 2017. This includes weekly results of *Glyseria maxima* and *Iris pseudacorus*, as well as singular analysis of other plants. Other plants than *Glyseria maxima* and *Iris pseudacorus* were obtained from NIBIO (Norwegian Institute of Bioeconomy Research) from different wetlands in the Jæren region and were analyzed for total phosphorous content. Results for *Glyseria maxima* and *Iris pseudacorus* are given as “grams P per kiolgrams plant wet weight” (g P/ kg ww). However, it should be noted that plants can differ significantly in size and large plants have consequently a larger mass per square meter than small plants. Hence, the amount of phosphorous taken up by large plants can be higher per square meter compared to small plants, although small plants may contain a larger amount P per kilogram plant weight. Results for other plants are given in “grams P per kilograms plant dry weight” (g P/ kg dw). Thus, when comparing *Glyseria maxima* and *Iris pseudacorus* with other plants, results are given per plant dry weight. Raw data used in the graphs and table can be found in appendix D.

For the phosphorous analysis, a calibration curve was determined each time with a standard phosphorous solution. The R^2-value for the calibration curve varied between 0.9923 and 1, with an average R^2 of 0.9978. The control sample varied between 385 and 420 µg/l, with an average of 403 µg/l.

4.3.1 *Glyseria maxima*

The total phosphorous content in root, stem and leave for *Glyceria maxima* is given in figure 4. 11. From March until May, the amount of phosphorous in the leaves increases, while it decreases in the root. The TP-content in the stem increases from March too, but decreases again in May. The average TP-content is given for the seasons fall, winter and spring in root, stem, leave and the whole plant for *Glyceria maxima* (table 4. 3). The standard deviation for the total phosphorous is 125.17 mg P/kg dw or 13.58 mg P/kg ww, which accounts for 3 % of TP. The standard deviation in percent is based on average phosphorous concentrations. TP-content in the root is highest in winter, but does not vary dramatically among the three seasons. For stem and leave, phosphorous increases from fall to winter and further in spring and is at its highest in spring.

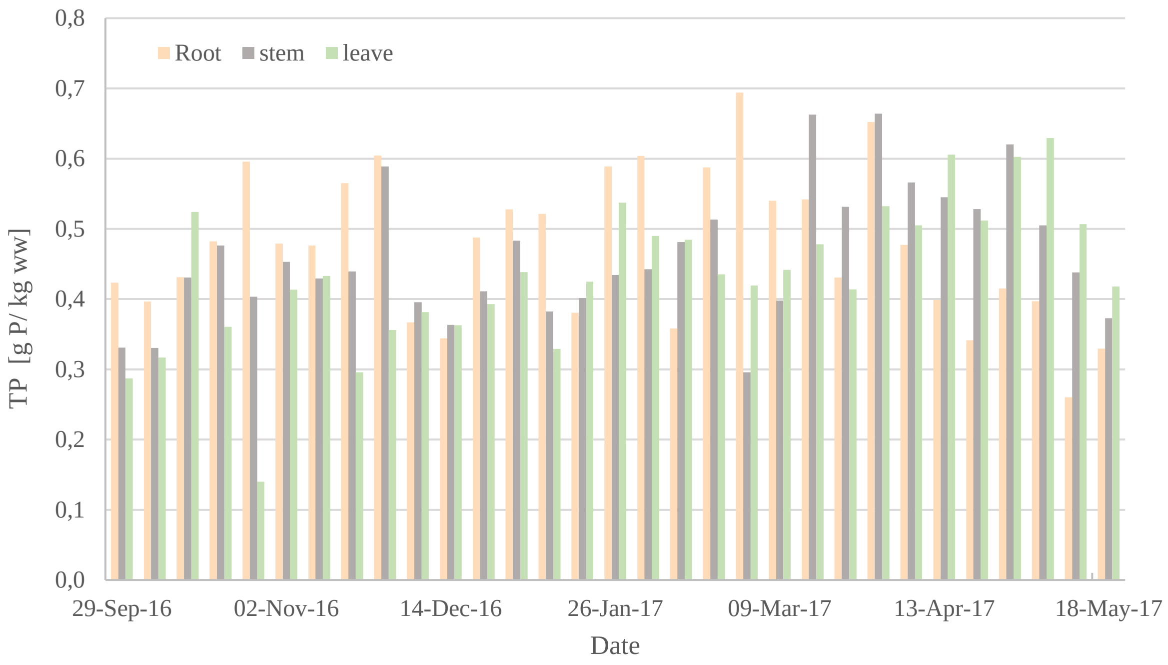

Figure 4. 11. Total phosphorous content in root, stem and leave for *Glyceria maxima* changing over time.

Table 4. 3. Average seasonal phosphorous content in plant parts of *Glyceria maxima*.

Season	**Average TP content [g P/ kg ww]**			
	Root	Stem	Leave	Whole plant
Fall (Sep, Oct, Nov)	0.47	0.41	0.35	1.24
Winter (Dec, Jan, Feb)	0.49	0.45	0.42	1.35
Spring (Mar, Apr, May)	0.46	0.51	0.51	1.47

4.3.2 *Iris pseudacorus*

Total phosphorous content in root, stem and leave is given for *Iris pseudacorus* in figure 4.12. The standard deviation for the total phosphorous is 110.91 mg P/kg dw or 13.80 mg P/kg ww, which accounts for 3 % of TP. Phosphorous content increases in November in the root and is high throughout the remaining sampling period. Phosphorous increases the most from March on in leaves, while it varies throughout the whole sampling period in the stem.

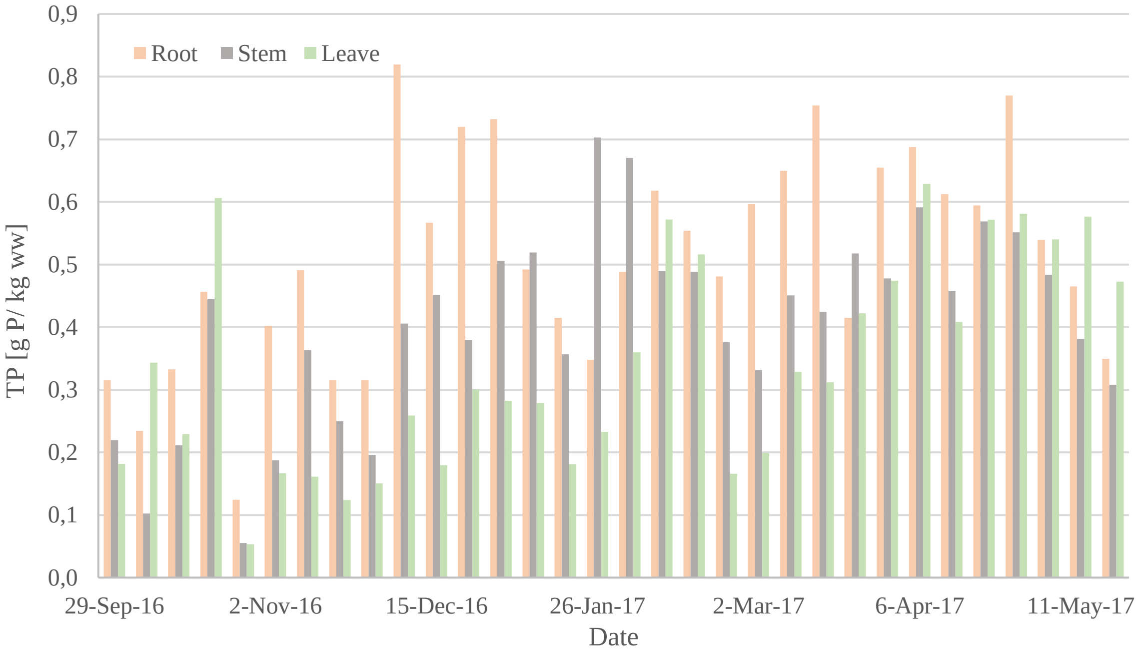

Figure 4. 12. Total phosphorous content in root, stem and leave for *Iris pseudacorus* changing over time.

The average TP-content is given for the seasons fall, winter and spring in root, stem, leave and the whole plant for *Iris pseudacorus* (table 4. 4). TP is low in fall in all three plant parts. However, it increases a lot in the root and stem in winter and is highest in spring. Phosphorous in the leaves is still low in winter, but increase in spring where it is highest.

Table 4. 4. Average seasonal phosphorous content in plant parts of *Iris pseudacorus.*

Season	**Average P content [g P/ kg ww]**			
	Root	Stem	Leave	Whole plant
Fall (Sep, Oct, Nov)	0.33	0.23	0.23	0.80
Winter (Dec, Jan, Feb)	0.52	0.46	0.29	1.30
Spring (Mar, Apr, May)	0.59	0.46	0.46	1.51

4.3.3 Additional plant analysis

Additional analysis of total phosphorous content in dead plant material and sprouts of both *Glyceria maxima* and *Iris pseudacorus* were performed. Also, total phosphorous was analyzed in the following plants obtained from NIBIO: *Phalaris arundinacea* (Reed Canary Grass), *Typha latifolia* (Common Cattail), *Juncus effuses* (Common Rush), *Poaceae* (Meadow Grass), *Phragmatis australis* (Common Reed), *Equisetum palustre* (Marsh Horsetail), and *Myosotis* (Forget-Me-Nots). The plants obtained from NIBIO were taken June 25th 2015 and were already dried when they were received for further analysis for this master thesis.

The total phosphorous content in the live plant material of the above-mentioned plants is illustrated in figure 4. 13. Most of these plants were divided into stem and leave, or upper and lower part. However, details on plant parts are given in the figure. In addition, average TP-values in root, stem and leave of *Glyceria maxima* and *Iris pseudacorus* from the Leikvollbekken CW are shown. Average TP values were calculated based on the months September, April and May. These months were chosen as samples of the other plants were taken in summer, and September, April and May are the months of the sampling period closest to summer. TP-content in sprouts of *Glyceria maxima* and *Iris pseudacorus* are also given in figure 4. 13. Due to limited text space in the figure, the first part of the scientific plant name is given only, together with the plant part. Plant parts of the same plants, are given with the same color. Sprouts of *Glyceria maxima* and *Iris pseudacorus* show the highest phosphorous content, together with the whole plant of *Equisetum palustre*.

Plant samples obtained from NIBIO contained dead plant material of *Typha latifolia* and *Phragmatis australis* in addition. In March, withered samples of *Glyceria maxima* and *Iris pseudacorus* were taken. All these dead/withered samples were analyzed for total phosphorous too and results are shown in figure 4. 14. Total phosphorous is lower in withered samples compared to live plant samples of *Glyceria maxima* and *Iris pseudacorus*, and extremely lower in dead plant materials of *Typha latifolia* and *Phragmitis australis*.

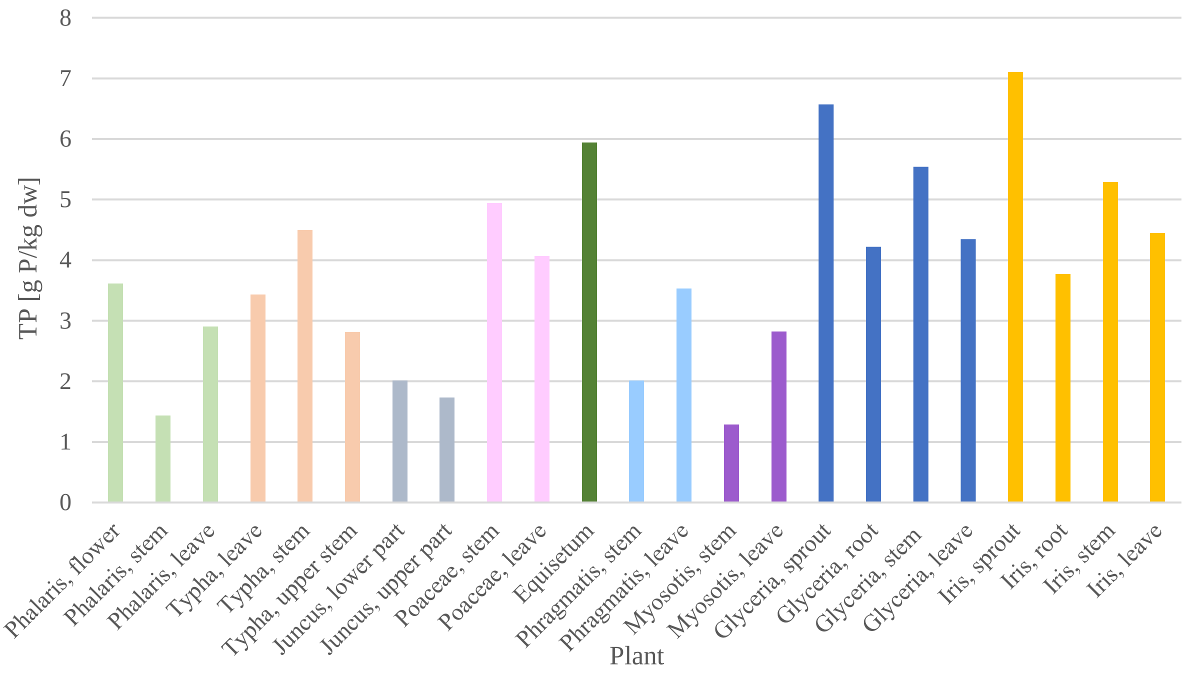

Figure 4. 13. Total P content in additional plants samples together with *Glyceria maxima* and *Iris pseudacorus*.

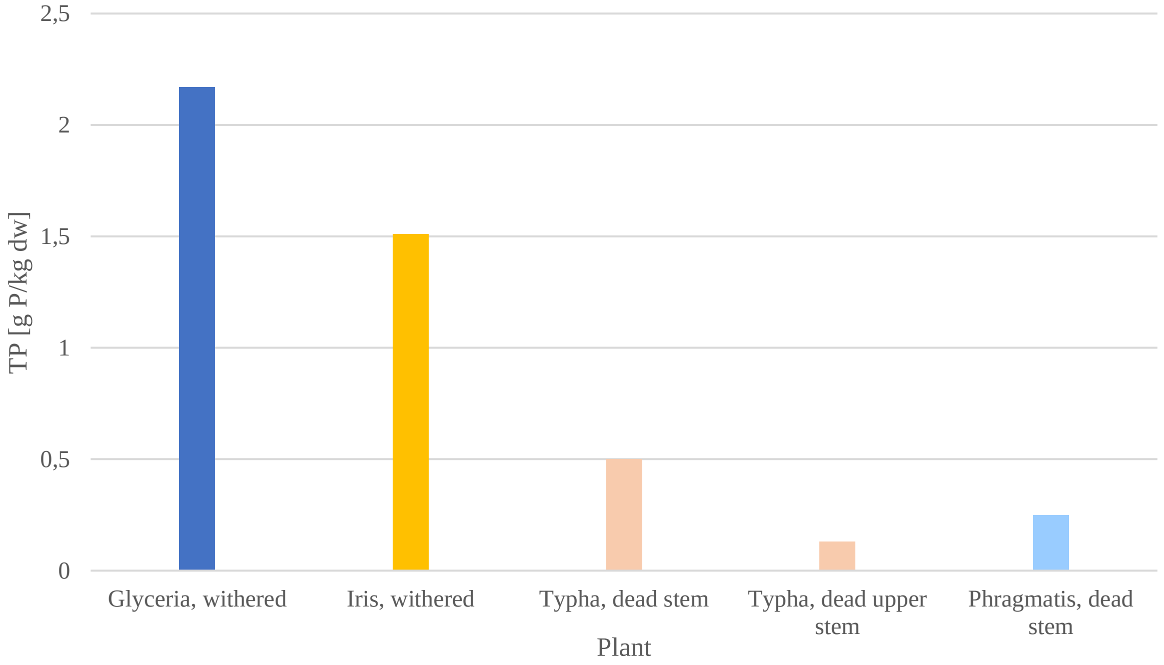

Figure 4. 14. Total phosphorous content in additional dead plant samples, *Glyceria maxima* and *Iris pseudacorus*.

4.3.4 Phosphorous removal by vegetation

In order to be able to calculate the amount of phosphorous that can be removed from the wetland in plant biomass, several wetland characteristics have to be identified. First of all, the plant biomass per square meter in the wetland needs to be estimated. In September 2015, the plant biomass per square meter was measured to be 4.3 kg plant/m^2 during an exercise (UIS). The measurement included only *Glyceria maxima,* but not *Iris pseudacorus* (which is larger in size). These measurements were done in September, when plants have grown all summer long and are probably at the highest. Therefore, a value of 3.7 and 3.9 kg plant/m^2 was estimated for *Glyceria maxima* and *Iris pseudacorus,* respectively. Secondly, the amount of phosphorous in the plant biomass needs to be determined. The only reasonable way to remove vegetation is by harvesting the aboveground biomass. Thus, phosphorous content in the roots are not being included in these measurements. As there is very little biomass to harvest in winter and biomass starts to wither fast in fall, results from spring are considered only. Based on these indications, the amount of phosphorous in plants was determined to be 1.02 g P/kg biomass and 0.92 g P/kg biomass for *Glyceria maxima* and *Iris pseudacorus*, respectively. Finally, the area of the wetland was presented earlier to be 1410 m^2, where pond 1 constitutes 754 m^2 and pond 2 constitutes 656 m^2 (figure 3. 3). Based on these numbers, the phosphorous removal in plant biomass was calculated for harvesting aboveground biomass once a year (table 4. 5). Calculations were done on harvesting pond 2 only, and harvesting both ponds. The standard deviation was calculated to be 3 %.

Table 4. 5. Phosphorous removal by *Glyceria maxima* and *Iris pseudacorus.*

	***Glyceria maxima* [kg P /year]**	***Iris pseudacorus* [kg P/year]**
Harvest pond 2	2.48	2.35
Harvest pond 1+2	5.32	5.06

4.4 Correlations

Correlations between different parameters were found by performing a regression analysis using the regression data tool in Excel and are presented in this chapter. Correlations were found to be significant if the p-value was smaller than 0.05.

4.4.1 Water samples

Different parameters measured for this master thesis, showed a significant correlation. For significant correlations, a plot with R^2 is included (figure 4. 15). All main correlations are presented in table 4. 6, showing R^2, p-value and the number of measurements (n).

Table 4. 6. Correlations for different water sample parameters.

Correlation parameters	R^2	p-value	n	Significant
Flow [m^3/s] vs. Retention TP [g/d]	0.0067	0.66	34	No
Flow [m^3/s] vs. Retention part. P [g/d]	0.0000	0.97	34	No
Flow [m^3/s] vs. Retention PO_4^{3-} [g/d]	0.1412	0.03	34	Yes
Flow [m^3/s] vs. Flux TP out [g/d]	0.6129	$3.54*10^{-8}$	34	Yes
Retention TSS [kg/d] vs. Retention TP [g/d]	0.7906	$2.14*10^{-12}$	34	Yes
Retention diss. Fe [g/d] vs. Retention PO_4^{3-} [g/d]	0.0019	0.80	34	No
Flux diss. Fe in [g/d] vs. Flux PO_4^{3-} in [g/d]	0.4912	$2.16*10^{-5}$	33	Yes

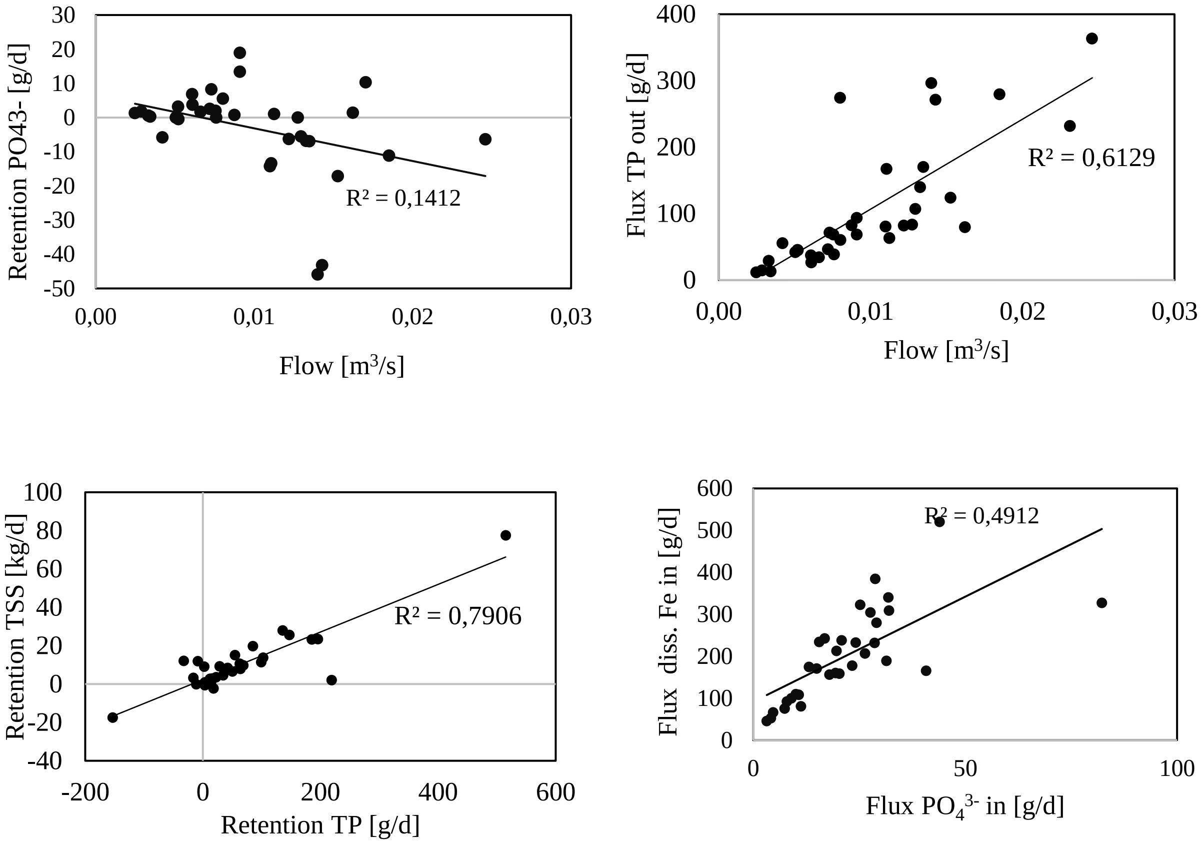

Figure 4. 15. Plot of significant correlations including R^2-value.

4.4.2 Plant samples

No significant correlations between phosphorous uptake in plant tissues and P-retention in the wetland was found, as regression analyses resulted in p-values higher than 0.05. However, correlations between phosphorous uptake in the different plant parts in *Glyceria maxima* and *Iris pseudacorus* were found (table 4. 7):

Table 4. 7. Correlations between TP content in plant samples.

Correlation parameters	R^2	p-value	n	Significant
TP root *Glyceria* [g P/kg ww] vs. TP root *Iris* [g P/kg ww]	0.011	0.58	31	No
TP stem *Glyceria* [g P/kg ww] vs. TP stem *Iris* [g P/kg ww]	0.1379	0.04	31	Yes
TP leave *Glyceria* [g P/kg ww] vs. TP leave *Iris* [g P/kg ww]	0.3357	$6.00*10^{-4}$	31	Yes

5. Discussion

Results obtained from the analysis of water and plant samples are discussed in this chapter. This chapter is divided into 6 sub-sections: (1) Hydraulic loading and temperature; (2) Phosphorous retention; (3) Phosphorous uptake in plant tissues; (4) The long-term retention efficiency; (5) Error analysis; and (6) Suggestions for improvement and further research.

5.1 Hydraulic loading and temperature

Hydraulic loadings varied from 1 l/s (February 1st 2017) to 182 l/s (February 22nd 2017) with a normal loading rate of approximately 6-7 l/s. Figure 4. 1 shows the hydraulic loading fluctuations with several peaks distributed throughout the master thesis period. Every month (September 2016-May 2017) had hydraulic loading peaks > 40 l/s, except October. The months September and November – March also had peaks with more than 100 l/s. If a flooding event is set to happen at a hydraulic loading > 40 l/s, the months with the most flooding events are November, December and January, while months with the least flooding events are October, April and May. These data fit well with the average precipitation data (table 3. 1), where November, December and January are months with the most precipitation, while October, April and May are months with the least precipitation. The high hydraulic loading fluctuations make it difficult to design a wetland with appropriate dimension. Appropriate dimensions means a wetland large enough to deal with high hydraulic loadings, without being too large. A too large wetland may be able to deal with peak flows, but designing a wetland larger will also cause higher costs (mainly in the building process) and a larger area. Although there are some high hydraulic loadings, these will not happen every day and the wetland will not have to be able to deal with peaks daily. Thus, there will be a limit at which costs (and area requirements) will rise much faster than the achieved treatment efficiency of the wetland. Another problem with high hydraulic loadings is that these will create storm runoff situations, meaning that the amount of water is so high that it will erode land surfaces. These runoff situations will not only transport a high amount of particles into the wetland, but also out of the wetland as the sedimentation sections are filled up. This phenomenon will be explicated further in the next chapter.

The average daily temperature during the master thesis period (figure 3. 4) varies from 21.7 °C (September 15th 2016) to -3.6 °C (February 8th 2017). There were only a few days with temperatures below 0°C, and an overall warm winter was observed. A thin ice cover over some parst of the wetland was observed January 6th, but the ice was completely melted a week later. The wetland was completely frozen February 9th which did last approximately 1.5 weeks. Warm temperatures are beneficial for bacterial decomposition of organic matter and, hence, may have contributed to a good decomposition efficiency throughout the year (although it was most likely reduced during colder months). Clear signs of a high bacterial decompositions were firstly observed in April when plants had started to grow, but were still low. These signs occurred as blue, oily-like depositions spread over the whole wetland which appear to be degradation products.

5.2 Phosphorous

In order to determine the phosphorous retention in the constructed wetlands, inlet and outlet concentrations were measured. Furthermore, to specify which phosphorous species is dominating, total dissolved phosphorous and phosphate concentrations were measured, while the particulate fraction was calculated.

5.2.1 Phosphorous mass flux and hydraulic loadings

Figure 4. 4 and figure 4. 5 show the phosphorous mass fluxes in the inlet and outlet, respectively, together with the average hydraulic loading. For the inlet, particulate P mass flux follows the total P mass flux well and is higher than the mass flux for the dissolved fractions. This indicates that the majority of the phosphorous entering the wetland is as particulate phosphorous. The dissolved fraction constitutes only a small portion of the TP, but follows the TP mass flux as well to some degree. The dissolved P fractions slightly increase frequently in March, April and May in the inlet, which may be due to the fertilization of agricultural land. The outlet mass fluxes in figure 4. 5 show approximately the same distribution of the phosphorous fractions, where particulate P is the main fraction, but the difference between particulate and dissolved P is smaller compared to the inlet. The smaller difference between the particulate and the dissolved fractions is due to a higher reduction of particulate P in the outlet compared to dissolved fractions as well lower concentrations in general.

The hydraulic loading in figure 4. 4 and figure 4. 5 (same hydraulic loading for inlet and outlet) shows some peaks. These peaks are followed by phosphorous mass flux peaks well, both for inlet and outlet. A significant positive correlation between the flow and the mass flux of total P in the outlet (R^2=0.6129, p-value < 0.05, n=34) was found, which can be explained by the reason given in the previous chapter: At times with a lot of precipitation, the hydraulic loading will increase which can result in storm runoff situations. During the runoff situations, land surfaces will get eroded and the water will transport the eroded materials. As particle concentrations increase, the concentration (and mass flux) of phosphorus bound to these particles will increase as well. Thus, at hydraulic loading peaks, the particulate P mass flux will increase (together with the TP flux). The increase of particles in the water samples due to storm runoff can also be seen in figure 4. 9, where total suspended solids concentrations have a peak at the same time hydraulic loadings have a peak. Comparing figure 4. 9 and figure 4. 10, shows that turbidity increases as TSS concentrations increase, as the latter will contribute to the turbidity.

5.2.2 Phosphorous retention

The difference between the two figures discussed above yields the phosphorous retention, shown in figure 4. 6. This figure shows also clearly that most of the total phosphorous is in its particulate form. The total phosphorus retention was 30.32 g/m^2* year, and the retention of particulate P was slightly higher with 31.95 g/m^2* year, while phosphate retention was low with 0.39 g/m^2* year. This yields a retention efficiency of 31 % for TP, 43 % for particulate P and only 3 % for phosphate. These results fit well with a TP retention efficiency of 37-43 % obtained by Beutel et. al (Beutel et al., 2014) and a TP retention of 26-71 g/m^2* year (21-44 %) obtained by Braskerud (Braskerud, 2002). The reason why more particulate P than total P is retained is due to the total dissolved P retention (-1.63 g/m^2*year, -7 %), which contributed to the TP retention is a negative way.

There were 5 situations with negative retention of total phosphorus (yellow marks) during the master thesis period (figure 4. 6), but there were several negative retentions of dissolved fractions. The negative phosphate retention indicates that retention mechanisms for dissolved fractions do not work well. Particulate P is mainly retained by sedimentation, while dissolved fractions are retained due to biological uptake, adsorption, precipitation, and exchange reactions. Sedimentation can continue without limit, and hence, so can the sedimentation of particulate P. On the other hand, biological uptake, precipitation and adsorption are capacity limited, and biological uptake is also season dependent. Hence, the main mechanisms for dissolved P retention are limited, while those for particulate P retention are not. However, as sedimentation is supposed to happen within a pond with given dimensions, (and the sedimentation regions are completely filled up) sedimentation could also be identified as a limited process for the Leikvollbekken CW. As soil and bedrock surrounding the CW are rich in iron, it is assumed that phosphate can be retained by the precipitation with iron. Figure 4. 7

shows the inlet concentrations of iron fractions together with the flow. As for particulate P, particulate iron follows the hydraulic loading pattern well as particulate iron will be transported bound to eroded particles as well, while dissolved iron concentrations are low. However, when looking at figure 4. 8, the particulate iron concentration is reduced in the outlet (sedimentation of particles in the wetland), while dissolved iron concentrations slightly increase. This indicates that the CW is a source of dissolved iron, which can be an indication that there are processes occurring in the wetland which transform iron(III) to iron(II). The wetland is vegetated, and hence, plant litter is produced. The Litter is degraded, which consumes oxygen, and as the wetland is constantly covered with water, anaerobic conditions may have been established which would cause iron(III) to be reduced to iron(II). Under these conditions, phosphate will not precipitate and possible precipitates are being dissolved. Another explanation could be a too high pH-value. As the pH increases the solubility of $FePO_4$ -precipitates will increase. As shown in figure 2. 8, $FePO_4$ will not precipitate at pH higher than approximately 7.5 at concentration of 10^{-3} M. In the Leikvollbekken CW, concentrations are considerably lower than 10^{-3}M, which would require an even lower pH than 6 for precipitation to happen. According to Espen Enge (UIS), the pH in the CW is between 6.3 and 6.5, and hence, precipitation will most certainly not occur due to too low phosphate concentration in relation to a too high pH. A significant positive correlation between the flux of dissolved iron and phosphate into the wetland ($R^2 = 0.4912$, p-value < 0.05, n= 33) may indicate that a lot of phosphate enters the wetland in connection with iron. Netherless, there was no significant correlation found between the retention of dissolved iron and phosphate, which strengthens the assumption that phosphate is not precipitating with iron in the wetland, as well as the assumption that Fe(III) is reduced to Fe(II) in the wetland. Adsorption processes will also depend on an acidic pH in order to occur, as phosphate can only adsorb to positively charged surfaces. As the pH is slightly acidic, adsorption might occur. However, the adsorption capacity of the sediment is probably reached as the sediment has never been removed, and no more phosphate will be adsorbed efficiently. Phosphate will enter and leave the wetland dissolved in water, unless it is being retained. As neither, precipitation nor adsorption processes seem to function efficiently, phosphate retention is low or negative. The negative retention can also be associated with high hydraulic loadings, as table 4. 6 shows that there is a significant positive correlation between hydraulic loading and the retention of phosphate (R^2=0.1412, p-value < 0.05, n=34). This can be explained by the fact that phosphate will fluctuate with the hydraulic loading. This means that if hydraulic loadings are high, a large amount of phosphate is being washed out as it is not being retained in the wetland, while a minor amount is being washed out at low hydraulic loadings, giving a better phosphate retention. This is also illustrated in figure 4. 6, where phosphate retention is low/negative at high hydraulic loadings, while it is positive at low hydraulic loadings.

Phosphate retention starts to increase in spring (except April 13th), it seems like plants play a role for phosphate retention as they will store it in their tissue and use it for growth. In fall (September and October), the phosphate retention was either positive or just slightly negative. This could be due to storage of phosphate in plant roots, as explained by Beutel et al. (Beutel et al., 2014). During winter, plant litter will decay and increase phosphate concentrations in the wetland. It should be noted that this phosphate will not enter the wetland, but will be produced in the wetland and only contribute to outlet concentrations. Plants will also be dormant during winter and no phosphorous will be taken up, hence, phosphorous retention is negative. In spring plants will start to grow and the uptake of phosphorous will increase, thus increasing its retention. As plants utilize nutrients which were stored in the root during the first growing stage, phosphate retention started to increase as plants were better developed and started to take up phosphate from the wetland. This may be the reason why phosphate retention still is negative in March and April, although the retention slightly increases (except April 13th, where high hydraulic loadings washed out phosphate). Also, fertilization of agricultural land increases the inlet loading of phosphate and contributes to a lower retention during spring.

The particulate phosphorus retention is mostly positive, except the period from December 22nd to January 6th, which had the highest negative TP retention and particulate P was the main contributor. During this period, there was also the highest hydraulic loading peak. Hence, the negative retention of particulate P was caused by the wash out of particles during the storm runoff. A problem for the retention of particulate P due to high hydraulic loadings arises as both ponds of the CW are completely filled up by now and no sedimentation regions are present anymore. If hydraulic loadings get too high, resuspension of particles will become a problem, and particulate phosphorus is being washed out. A factor enhancing this phenomenon is that water flows across the wetland in small streams, rather than being spread over the whole wetland surface. This gathers water masses and applies larger water forces to resuspend particles. This is mainly due to overgrown permeable barriers which are clogged and do not function as water spreading systems anymore. The fact that concentrations and mass fluxes of particulate and total P follow the hydraulic loading pattern and a positive correlation was found between flow and total P in the outlet, leads to the assumption that retention will do so too. However, there is no significant correlation between the hydraulic loading and the TP and particulate P retention, meaning that particulate and total phosphorous are retained independently of the flow. Figure 4. 6 shows a positive retention of particulate and total P even though the hydraulic loading is high (except January 6th). This weakens the assumption that particles are being washed out at storm runoff situations. There are several possible reasons for this: First of all, the automatic samplers were set on a time-paced composite sampling during a large part of the master thesis period (samples were taken every 2 hours). Thus, flooding events, when particles are washed out, were not captured effectively. This could have resulted in underestimated inlet/outlet concentrations of particulate P (and TP) and a consequently better retention at storm runoff events. Secondly, although particles were washed out during storm events, inlet concentrations could have been so high that the retention still was positive. The automatic samplers were set on a flow-paced sampling on March 30th, which means that samples were taken according to the amount of water passing the sampling station. Hence, several samples were taken at high hydraulic loadings and storm runoff situations were captured. However, two high peaks in April did not result in a negative retention, which indicates that a possible washout of particles was not significant. The positive retention in April may also be a result of the presence of plants. These will slow down incoming water and reduce turbulences, and consequent particle resuspension. Netherless, the positive correlation between the retention of TSS and the retention of TP (R^2 = 0.7906, p-value < 0.05, n= 34) indicates that the latter is strongly dependent on the retention of particles. With other words, as particles are being washed out, so will phosphorous.

5.3 Phosphorous uptake in plant tissues

The amount of total phosphorous in root, stem and leaves of *Glyceria maxima* and *Iris pseudacorus* was determined in order to estimate how much phosphorous that can be removed with plant biomass and to determine possible seasonal variations of phosphorous uptake by plants.

The TP concentration in root, stem and leave in *Glyceria maxima* is given in figure 4. 11. TP in the root is high during large parts of fall and winter, with an average of 0.47 and 0.49 g P/kg ww for fall and winter respectively. The high TP content in the root could be explained by storage of nutrients which are going to be used when plants will start to grow. At the beginning of March, the TP content in the root decreases (0.46 g p/kg ww average for spring) and continues to decrease as the storage is being consumed. The total phosphorous content in the leaves and stem is low during fall and winter, but increases slightly from fall with an average of 0.41 and 0.35 g P/kg ww in stem and leave, respectively, to 0.45 and 0.42 g P/kg ww in stem and leave, respectively, during winter. The increase may be due to less litter in the winter samples which would function as a dilution. Plants already start to decay in summer, and hence fall will be the period with the most litter. Figure 4. 14 and Figure 4. 13 show that withered plant parts contain less phosphorous compared to live plant mass, thus, it will add to the weight of the sample, but will only contribute little to the phosphorous content and dilute the sample. The TP content increases in March as plants starts to grow and sprouts will dominate which contain the most phosphorous (figure 4. 13). In May, the TP content decreases again, as most plants have grown high and no sprouts will add to the phosphorous content. The measurement fit well with the growing cycle of a plant, where nutrients are stored during fall and transported to stem and leave during the growing season in Spring.

The TP content in root, stem and leave in *Iris pseudacorus* is given in figure 4. 12. The weekly average TP content varies a lot during the master thesis period, whereas the TP content in the root usually is the highest during fall, winter and early spring. The TP content in the root increases a lot from fall to winter with an average of 0.33 to 0.55 g P/kg ww, respectively. This indicates that *Iris pseudacorus* continues the process of storing nutrients in the root until mid-December, in contrast to *Glyceria maxima* which had a quite constant TP content in the root during fall and winter. After December, the TP content stays high and increases only slightly from an average of 0.55 to 0.59 g P/kg ww from winter to spring, respectively. The TP content in the stem is low during fall and starts to increase in December, but decreases again in February. In March, total phosphorous increases again in the stem, and decreases in May. The increase from fall to winter may be due to the same reasons explained for *Glyceria maxima*, as there is less litter in winter samples which would cause a dilution. The reason why TP decreased in February is unclear, but it might be a result of the ice cover during larger parts in February. The Spring pattern is similar to *Glyceria maxima*, where the phosphorous content increases in the stem as plants start to grow and sprouts will dominate. In May, the TP content decreases in the stem, as most plants have grown high and no sprouts will add to the phosphorous content. The phosphorous content in the leaves is low in fall with an average of 0.23 g P/kg ww and increases only slightly in winter with an average of 0.29 g/kg ww, which may be due to the reduced amount of litter in the winter samples. In March, TP increases as plants start to grow and sprouts dominate, but it will stay high throughout spring. This indicates that *Iris pseudacorus* has a longer early growing stage compared to

Glyceria maxima and will consequently take up more phosphate for a longer period of time during spring. This is also represented in the average phosphorous content of the whole plant, where *Glyceria maxima* contained more phosphorous than *Iris pseudacorus* in fall and winter, but in spring, *Iris pseudacorus* contained more phosphorous than *Glyceria maxima* (table 4. 3 and table 4. 4).

To better understand correlations between the growing cycle of *Glyceria maxima* and *Iris pseudacorus*, regression analyses were performed. No significant correlation between the TP content in the root of the two plants was found, indicating that the nutrient storing/consumption process differs for the two plants. However, there is a significant positive correlation between the TP content in stem ($R^2 = 0.1379$, p-value < 0.05, $n=31$) and the TP content in leaves ($R^2 = 0.3357$, p-value < 0.05, $n=31$), indicating that both plants may transport phosphate to/in the aboveground biomass quite similar. *Iris pseudacorus* had a higher P-content in the root compared to *Glyceria maxima*, especially during winter and continued phosphate uptake during winter. As the root is the only plant part contributing to the long-term storage of phosphate, *Iris pseudacorus* is more suitable in retaining the phosphate which was taken up during months were plants are dormant. Sprouts of *Iris* contain more P than sprouts of *Glyceria*, which indicates a higher uptake of phosphate during the early growing stage of *Iris pseudacorus*. However, the TP content in the whole plant is higher in *Glyceria maxima* than *Iris pseudacorus* on an average basis during fall and winter. It should also be kept in mind that *Iris pseudacorus* is larger in size and will produce more litter per square meter during fall compared to *Glyceria maxima*, which would cause a larger release of P per square meter. Hence, *Iris pseudacorus* has a more efficient phosphate storage in the root and phosphate uptake in the early growing stage compare to *Glyceria maxima*, but might release more P during fall per square meter.

The plants obtained from NIBIO were also analyzed for phosphorous and compared to average TP values from September, April and May from *Glyceria maxima* and *Iris pseudacorus*. It should be noted that plants from NIBIO were taken June 25th, while no summer measurements are available for *Glyceria maxima* and *Iris pseudacorus.* Furthermore, wetland characteristics and phosphorous concentrations in the water are unknown for plant samples obtained from NIBIO, which makes it difficult to directly compare TP-values. Figure 4. 13 shows the phosphorous content in all plants analyzed. Sprouts of *Glyceria maxima* and *Iris pseudacorus* contain the most phosphorous and are followed by the whole plant of *Equisetum palustre.* The phosphorous content in *Glyceria maxima* and *Iris pseudacorus* is similar with the phosphorous content in *Poaceae*, while other plants like *Typha latifolia, Phragmitis australis* and *Phalaris arundinacea* have a slightly lower TP content. *Juncus effuses* and *Myosotis* contained the least phosphorous. Dead plant material of *Typha latifolia* and *Phragmitis australis* contained extremely low phosphorous with less than 0.5 g P/kg dw (figure 4. 14). TP content in dead plant material fits also well with values in table 2. 1, which were between 0.5 to 0.7 g P/kg dw. However, values for live plant mass is significantly higher for *Phragmitis australis* and *Typha latifolia* compared to values in table 2. 1. It should again be noted that no wetland or plant characteristics are known for TP-values in table 2. 1.

The annual phosphorous removal was also calculated for *Glyceria maxima* and *Iris pseudacorus* based on one harvest per year. The phosphorous that can be removed by harvesting pond 2 only is 2.48 kg/year and 2.35 kg/year for *Glyceria maxima* and *Iris pseudacorus*, respectively, which would account for 4 % of the total phosphorus entering the wetland. As the distribution between the two plants is approximately the same based on mass, an average of 2.42 kg P/year (4 %) is a good estimate for pond 2. If both ponds are harvested, the phosphorous removed by *Glyceria maxima* is slightly higher with 5.32 kg P/year (8 %) than 5.06 kg P/year (8 %) for *Iris pseudacorus*. Other plants than *Glyceria maxima* and *Iris pseudacorus* are present in pond 1, however, by estimating an equal distribution based on mass for the two plants as well, an average of 5.19 kg P/year (8 %) would be obtained. A removal of 8 % of the total phosphorous by harvesting both ponds once a year is a good result and could be even higher with a second harvest. As plants contain most nutrients in the early growing stage, and P-content actually started to decrease again in May, a first harvest in early June would be recommended. The second harvest should be done before withering starts to take over as phosphate is recycled to the wetland fast when decomposition has started. It should be noted that numbers in this section are based how much phosphorous that can be removed in the plant biomass compared to the total phosphorous entering the wetland. It is not an estimation of how much of the incoming phosphate is taken up by plants, as there is not enough information to calculate the latter. Hence, it is not possible to give a quantitative estimate of the phosphate retention as a result of biological uptake and the phosphate retention based on physiochemical mechanisms. However, as discussed earlier in section 5.2.2, vegetation has a positive effect on the phosphate retention as they will take up phosphate, and well developed plants will slow down incoming water, increase the hydraulic detention time and reduce wash-out events.

5.4 Long-term phosphorous retention

The determination of the phosphorous retention in the Leikvollbekken CW has been going on for approximately 2.5 year. Data from February 2015 to August 2016 were obtained from Roald Kommedal (supervisor) and plotted in figure 5. 1. During the first months in 2015, phosphorous retention is mainly positive, most likely due to few flooding events. During the first months in 2016, the auto-sampler was set on taking flow-paced samples, which resulted in a better capturing of particle wash-out events at high hydraulic loadings. These events are indicated by negative particulate phosphorous retention. As there were several flooding events during this period, particulate P retention was often negative. The annual phosphorous retention and retention efficiency is given in table 5.1. Retention efficiencies for TP and particulate P are lower compared to values obtained during this master thesis period, while the retention efficiency for phosphate is much higher with 7 % compared to 3 % obtained during this master thesis period. The same applies for the annual phosphorous retention, were the overall phosphate retention over the last 2.5 years is higher compared to the last 9 months. Based on these values, dissolved P retention mechanisms are affected negative the most as the CW matured, while retention mechanisms for particulate P are not affected as much. The higher phosphate retention based on the last 2.5 years may be due to a less saturated filter substrate, which had a higher capacity to precipitate and adsorb phosphate. As the years go by, the filter substrate gets more saturated and the capacity to retain more phosphate decreases, hence, phosphate retention decreases as well. Another factor is the hydraulic loading, which was high more frequently the last year compared to the previous sampling periods (February 2015 – August 2016). Although there were two high average peaks in the period November 2015 – February 2016, hydraulic loadings were relatively low elsewise. In contrast to this master thesis period, average hydraulic loadings were often above 15 l/s and washed out a higher amount of phosphate more often. The lower particulate and total phosphorous retention based on the 2.5 years could also be explained with the hydraulic loading variation. The peaks in the period November 2015 – August 2016 were higher compared to the average peaks during the last year and consequently washed out a higher amount of particulate phosphorous then the more frequent, but lower, peaks during the last year. The difference between the particulate and total phosphorous retention based on the 2.5 year period and this master thesis period is, however, small.

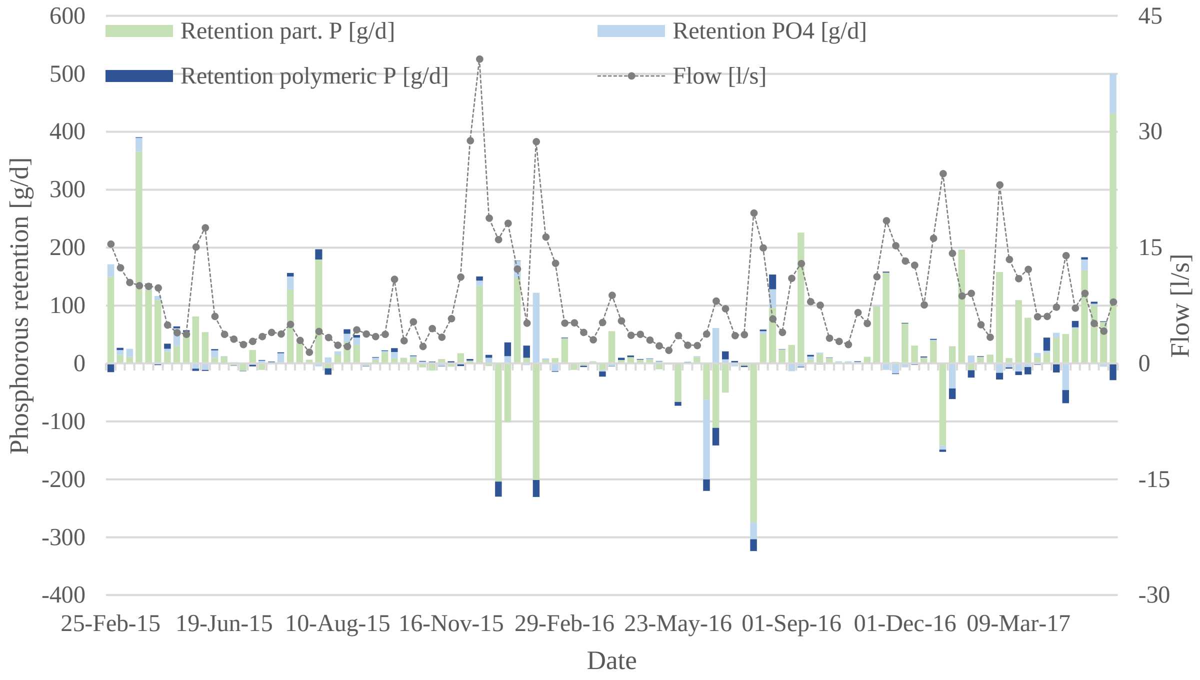

Figure 5. 1. Long-term phosphorous retention.

Table 5. 1. Annual phosphorous retention and retention efficiency for the last 2.5 years.

	TP	**PO_4^{3-}**	**Part. P**
Retention efficiency [%]	18	7	23
Phosphorous retention [kg/year]	11.43	0.83	10.78
Phosphorous retention [g/m^2*year]	17.42	1.26	16.43

A TP retention of 18 % is lower than results obtained by Beutel et al. with 37-43 % (Beutel et al., 2014) and 21-44 % obtained by Braskerud (Braskerud, 2002). The low phosphorous retention is mainly caused by the wash out of the wetland when the hydraulic loading is high and the wetland conditions which are not favorable for phosphate and particle retention. It should be noted that retention efficiencies are calculated based on the individual incoming mass of the P-fractions, and not on the total P mass.

5.5 Error analysis

One of the most important potential error sources for the analysis of water samples is the sampling procedure. As mentioned above, the automatic sampler was set on time-paced sampling rather than flow-paced sampling which did not capture storm runoff situations effectively. This affected the measured retention efficiency, which is probably lower during high hydraulic loadings than what was actually calculated. The water was stored in plastic bottles and phosphorous could start to stick to the walls during storage. As phosphorous concentrations are considerably low, this could result in measured P-concentrations which are lower than the actual value. During the phosphorous analysis in the lab, several contamination sources may contribute to potential errors. First of all, plants are prepared for analysis on the same bench as water samples. The grinding of dry plant samples results in the development of fine dust which is difficult to control. As plants contain more phosphorous than water samples, a contamination by plant residuals could result in the measurement of elevated phosphorous concentrations. Furthermore, during the fall, students were performing phosphorous analysis for a laboratory exercise and were using the same equipment and chemicals. This may have contributed to contamination of chemicals/equipment. Finally, inobservance during analytical procedures could cause the contamination of samples. This includes for example inadequate washing procedures of the equipment (especially volumetric flasks and glass jars), the contact of equipment with P-rich surfaces (hands without gloves) or the contamination of pipettes.

A standard deviation of 6.97 µg/l (4 %) for total phosphorous, 2.11 µg/l (5 %) for total dissolved phosphorous, 9.19 µg/l (6 %) for particulate phosphorous and 1.69 µg/l (6 %) for phosphate was calculated (table 4. 2). Standard deviations are low and indicate a relatively stable analysis. The R^2 value for the calibration curve for water analyses varies between 0.9596 and 1, with an average R^2 of 0.9976. This indicates a low variance with an uncertainty of less than 0.24 %. The control sample ranged from 379 to 415 µg/l, with an average of 390 µg/l. The average value indicates a systematic deviation from the actual concentration (385 µg/l) as measured values were often slightly higher than the actual value. This deviation may be a result of contamination and/or repeated analytical errors. The latter may include wrong pipetting and/or dilution. However, the average value does not deviate a lot and can be considered insignificantly.

One of the most important potential error sources for the analysis of plant samples is the rinsing of plant material. A lot of sediment sticks to the plants as they are taken from the wetland (especially to the roots). The sediment and other particles have to be removed from the plant samples properly as these can be an additional source of phosphorous. Also, the 4-digit balance that was being used to weigh plant dry weight is unstable on the two last digits and could cause wrong mass measurements. During boiling, charring of the solution causes some plant material to stick to the glass wall. The walls are flushed with distilled water, but some residuals may still stick to the walls and will not be part of the further analysis. Furthermore, inobservance during analytical procedures could result in the contamination of samples, as mentioned above. However, it should be noted that P-concentrations in plants are considerably higher compared to water samples and are not as sensitive to contaminations as water analyses are.

The standard deviation for TP for *Glyceria maxima* and *Iris pseudacorus* is 13.58 mg P/kg ww and 13.80 mg P/kg ww, respectively (3 %). These are low and indicate a stable analysis. The R^2-value for the calibration curve varied between 0.9923 and 1, with an average R^2 of 0.9978. This indicates a low variance with an uncertainty of less than 0.22 %. The control sample varied between 385 and 420 µg/l, with an average of 403 µg/l. The concentration of the control sample was higher than the actual 385 µg/l most of the time and the average is significantly higher. This may be due to contamination as the dust caused by the grinding was difficult to control and may have spread over the equipment. Also, repeated analytical errors, as mentioned for water samples, may have contributed to higher values as well.

5.6 Suggestion for improvement and further research

Leikvolbekken constructed wetland has a relatively low retention efficiency compared to results obtained by other CW. This is mainly caused due to nonexisting sedimentation regions (as these are filled up) and the consequent wash out of particles at high hydraulic loadings. The latter is enhanced by the development of water streams rather than water distribution across the wetland area. Phosphate is also washed out as it is not retained properly due to non-favorable wetland conditions and saturated sediment capacity.

The problems high hydraulic loading causes can be reduced by the implementation of storm water by-passing, for example with an overflow. Here, the most important question to answer is: How high hydraulic loadings cause a particle wash out event? The overflow should be designed based on that answer. With other words, it has to be decided when water should by-pass the wetland as it would wash out more particles than it would transport into the wetland. It should be kept in mind that high hydraulic loadings also will erode a lot of particulate matter which would be beneficial to be treated before entering recipients. Another important improvement would involve the sediment removal. This would create good sedimentation conditions and reduce sediment surface wash out. Vegetation clogging permeable barriers should be removed so water can pass through and is spread across the wetland surface. This would avoid the water to flow in streams. The replacement of the original filter substrate would also improve adsorption processes, as new particle surfaces are available. To improve precipitation processes, sediment condition should be clarified. However, as phosphate concentrations are probably too low in relation to a too high natural pH of incoming water, precipitation might still not happen even under aerobe conditions. Hence, other mechanisms such as adsorption and biological uptake should be promoted. The removal of plants would improve the retention of phosphorus, especially phosphate. Plants play also an important role in the oxygen transfer to the sediments, hence plants with deep roots (such as *Iris pseudacorous* and *Glyceria maxima*) are beneficial in supporting precipitation reactions. The slow-down of water by vegetation is also favorable to avoid particle wash out.

Further research on the Leikvollbekken constructed wetland should include the investigation of the sediment to examine the cause of the leaching of dissolved iron, as well as the low phosphate retention. Furthermore, the phosphorous uptake by plants during summer and a better estimation of the plant species distribution should be included.

In general, there were a lot of studies on the phosphorous retention in constructed wetlands, but only little information on the mechanisms involved. Further research on which mechanisms retain P, especially phosphate, and how these function is crucial to create favorable conditions in the wetland. Another research gap exists on the long-term retention efficiency of phosphorous. There are studies conducted over a few years, but little information on the retention efficiency of mature CWs, which are more than 15 to 20 years old. Especially interesting is which mechanisms turn out to dominate in mature constructed wetlands and how much maintenance is required to keep the retention efficiency high even after many years. Whether plants are beneficial or not is an often discussed question, but further research should be conducted on which mechanisms they are involved in and how plants interact with the physiogeochemistry of the wetland.

In addition to research how to reduce the consequences of an excessive fertilization of agricultural fields, it is also important to look at the cause. Further research on the fertilization reduction, for example by fertilizing more effectively, should be supported.

6. Conclusion

Leikvollbekken CW had a total phosphorous retention efficiency of 31 % during this master thesis period, which is good compared to other studies. The main phosphorous fraction was particulate phosphorous which contributed the most to the good TP retention with a 43 % retention efficiency. Total phosphorous and particulate phosphorus are primarily retained by physical sedimentation processes, while geochemical processes, especially adsorption, together with plant uptake are important processes for the retention of phosphate. The retention of phosphate was low with 3 % during this master thesis period, which is mainly caused by non-favorable conditions for physiochemical mechanisms such as precipitation and adsorption. Non-favorable conditions are assumed to be anaerobic conditions and a too high pH-value in the sediments. However, plants grow well in the wetland and can contribute to the uptake of phosphate, thereby retaining some of it in live biomass. The total phosphorous retention efficiency based on the last 2.5 years was lower with 18 %. This is mainly due to higher peak flows occurring in the period November 2015-February 2016 which resulted in sediment wash out. As the autosampler was set on a composite time-paced sampling for a large part of this master thesis period, storm runoff events were not captured properly and the resulting particle wash out was underestimated. This could have resulted in a higher retention efficiency than what was actually true. The phosphate retention efficiency based on the last 2.5 years was higher with 7 % compared to results obtained the last 9 months. This may be due to a better adsorption capacity of the sediment 2.5 years ago. Although peak flows were lower compared to previous periods, hydraulic loadings were relatively high more frequently during this master thesis period. This may not have been significant for the wash out of particulate P, but for the wash out of phosphate which gets washed out more easily unless it is being retained in the sediments. Dissolved P retention mechanisms are affected negative the most as the CW matured, while retention mechanisms for particulate P are not affected as much.

Plants are very important for the uptake of phosphate as it is an important plant nutrient. Phosphate retention was mostly negative in the wetland during this master thesis period when plants were dormant (winter). As plants started to grow, phosphate retention increased probably due to biological uptake and the slow-down of incoming water which reduces wash out events. TP analysis of root, stem and leave of *Glyceria maxima* and *Iris pseudacorus* showed that TP content was highest in the root during fall and highest in stem and leave during spring, indicating a storage of P in the root during fall and winter, and a transport of P to the growing stem and leave in Spring. In general, *Iris pseudacorus* had a more efficient phosphate storage in the root and uptake during the early growing stage compared to *Glyceria maxima.* The annual phosphorous removal by harvesting pond 2 once a year was 2.42 kg P/year, which would account for 4 % of the total phosphorous entering the wetland. Harvesting both ponds once a year would increase the P removal to 5.19 kg P/year, which would account for 8 % of the total P. As phosphate stored in plant tissues is returned to the wetland during decomposition, harvesting plant biomass could improve the removal of phosphate from the wetland.

Problems caused by high hydraulic loadings can be avoided by the by-passing of storm water, for example by the implementation of an overflow. Sediment should be removed to improve sedimentation processes and avoid the wash out of the sediment surface. However, as phosphate is the bioavailable form of P, phosphate retention processes should be promoted. This would include further investigation of the sediment to determine why physiochemical processes like adsorption and precipitation are not occurring sufficiently. The replacement of the original filter substrate would also increase the adsorption capacity and, hence, increase phosphate retention.

7. References

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Appendixes

Appendix A: Phosphorous and iron concentrations in water samples

Appendix B: Turbidity, TSS and color in water samples with average hydraulic loading

Appendix C: Total phosphorous concentrations and water content in plant samples

Appendix D: Iron mass flux

Appendix E: Calibration curve for phosphorous and iron

Appendix A: Phosphorous and iron concentrations in water samples

The measured total phosphorous, total dissolved phosphorous, phosphate, total iron and total dissolved iron concentrations are given in table A.1.

Table A. 1. Concentration of total P, total dissolved P, PO_4^{3-}, total Fe and total dissolved Fe.

Dato	**TP [µg/l]**		**sTP [µg/l]**		**PO_4^{3-} [µg/l]**		**Tot. Fe [mg/l]**		**Diss. Fe [mg/l]**	
	In	Out	In	Out	In	Out	In	Out	In	Out
09.01.16	194	175	57	71	23	37				
09.08.16	292	96	33	39	18	23				
09.15.16							1,99	1,86	0,26	0,53
09.21.16	108	87	41	29	28	20	0,99	1,22	0,23	0,46
09.29.16	130	105	36	36	23	20	1,46	1,41	0,26	0,48
10.06.16	136	101	46	43	26	24	1,46	1,44	0,26	0,49
10.13.16	73	59	26	20	17	10	0,83	1,16	0,21	0,38
10.23.16	69	55	19	16	15	9	0,86	1,18	0,21	0,4
10.27.16	66	60	20	15	14	11	0,79	1,15	0,16	0,33
11.03.16	124	100	37	38	20	21	1,56	1,15	0,22	0,36
11.10.16	167	65	28	27	16	15	2,52	1,01	0,24	0,33
11.18.16	267	175	33	39	18	25	4,53	2,28	0,24	0,32
11.25.16	82	94	39	53	21	34	0,73	0,77	0,23	0,3
12.01.16	177	122	41	46	23	29	1,62	1,02	0,18	0,24
12.08.16	102	76	30	32	26	26	0,95	0,69	0,21	0,27
12.15.16	77	59	38	36	31	31	0,73	0,57	0,24	0,29
12.22.16	87	57	32	30	18	17	0,81	0,61	0,23	0,29
01.06.17	99	171	31	36	15	18	0,82	1,49	0,16	0,21
01.12.17	194	220	37	87	26	61	2,02	1,92	0,25	0,6
01.19.17	368	109	37	37	26	25	4,8	0,9	0,28	0,23
01.27.17	105	119	64	63	52	35	0,64	0,74	0,21	0,24
02.02.17	124	96	35	33	23	23	1,09	0,8	0,25	0,25
02.09.17	95	44	24	23	16	15	1,09	0,48	0,22	0,25
02.16.17	1167	65	27	18	19	12	15,47	0,82	0,32	0,21
02.23.17	184	116	33	44	22	30	1,88	0,88	0,26	0,28
03.02.17	148	146	38	44	25	31	1,37	1,13	0,24	0,23
03.09.17	175	85	31	56	22	37	1,78	0,71	0,25	0,42
03.16.17	130	78	27	50	23	29	1,25	0,69	0,22	0,46
03.26.17	137	71	73	28	32	19	1,38	0,51	0,46	0,21
03.30.17	94	51	25	16	25	18	1,06	0,59	0,33	0,28
04.06.17	193	113	58	49	37	24	1,38	0,62	0,28	0,28
04.13.17	238	245	97	146	68	106	1,65	1,02	0,27	0,27
04.19.17	178	75	43	36	29	25	1,61	0,51	0,25	0,22
04.26.17	323	87	57	25	40	16	2,8	0,75	0,24	0,21
05.04.17	330	101	33	27	24	17	3,01	0,8	0,24	0,26
05.11.17	343	153	45	53	31	47	3,43	1,02	0,22	0,23
05.18.17	1142	397	261	140	184	84	7,15	2,12	0,34	0,35

Appendix B: Turbidity, TSS and color with average hydraulic loading

The measured turbidity, total suspended solids and color for water samples, as well as the average flow data is given in table A.2.

Table A. 2. Turbidity, TSS, color and average flow.

Dato	Tubidity [NTU]		TSS [mg/l]		Color [mg Pt/l]		Flow [m3/s]
	In	Out	In	Out	In	Out	
09.01.16	11,2	11,2	9,35	11,79			0,0111
09.08.16	11,2	10,5	11,61	9,92			0,0129
09.15.16	19	16,3	23,56	17,46			0,0129
09.21.16	9,45	7,88	8,65	6,77			0,0080
09.29.16	9,57	8,43	11,90	7,66			0,0076
10.06.16	8,5	7,05	10,83	6,33			0,0033
10.13.16	6,09	5,14	7,46	4,41	57,57	58,71	0,0029
10.23.16	6,22	6,18	6,21	5,01			0,0025
10.27.16	5,37	4,68	5,03	6,30	25,27	29,64	0,0066
11.03.16	7,15	5,26	10,41		50,54	55,67	0,0052
11.10.16	11,8	3,34	17,41	5,73	53,58	53,96	0,0113
11.18.16	10,9	6,46	33,05	17,15	49,78	52,82	0,0185
11.25.16	4,27	3,42	8,28	5,91	64,41	64,41	0,0153
12.01.16	8,47	4,74	16,73	7,59	57,00	55,86	0,0133
12.08.16	7,68	5,27	12,55	4,24	51,68	51,11	0,0127
12.15.16	3,9	2,76	5,13	2,22	51,49	67,07	0,0076
12.22.16	5,59	3,41	9,50	3,55	42,75	46,17	0,0162
01.06.17	6,65	10,8	9,63	17,92	42,75	42,75	0,0246
01.12.17	14	11,7	23,34	13,66	49,78	67,45	0,0143
01.19.17	30,1	6,38	36,36	5,46	44,46	38,76	0,0088
01.27.17	5,1	4,96	6,99	7,17	41,80	36,48	0,0091
02.02.17	9,9	5,54	11,67	5,49	41,99	41,99	0,0051
02.09.17	6,76	3,4	7,41	0,00	36,67	36,67	0,0034
02.16.17	106	4,68	139,46	0,19	31,16	20,52	0,0170
02.23.17	16,6	6,89	23,71	9,75	42,18	42,18	0,0231
03.02.17	9,57	8,67	19,01	11,30	47,31	47,31	0,0135
03.09.17	14,1	4,87	22,60	1,89	47,50	58,71	0,0110
03.16.17	10,8	5,32	16,93	2,66	45,41	63,27	0,0122
03.26.17	12,2	3,95	12,64	4,01	60,80	48,64	0,0061
03.30.17	8,11	4,16	10,47	4,05	45,41	39,33	0,0061
04.06.17	14	8,06	16,76	6,59	57,19	57,49	0,0073
04.13.17	14,5	11,9	24,55	14,74	64,22	82,46	0,014
04.19.17	10,9	3,75	17,25	4,73	51,11	45,41	0,0072
04.26.17	23,9	4,77	35,9	6,53	57,76	40,09	0,0091
05.04.17	24,6	5	38,5	7,99	42,18	47,69	0,0052
05.11.17	22,8	6,35	35,15	8,7	31,35	31,35	0,0042
05.18.17	114	20,9	138	25,93	70,87	70,87	0,008

Appendix C: Total phosphorous concentrations and water content in plant samples

The measured total phosphorous concentrations in root, stem and leave of *Glyceria maxima* and *Iris pseudacorus* is given in table A.3 and table A.4, respectively.

Table A. 3. Total P and water content in *Glyceria maxima.*

Date	**TP [g P/kg dw]**			**Water content [%]**		
	Root	Stem	Leave	Root	Stem	Leave
09.15.16	5,93		8,5	90	92	90
09.20.16		12,42		84	90	83
09.29.16	3,78	3,85	3,74	89	91	92
10.06.16	3,69	3,48	3,56	89	91	91
10.13.16	4,34	4,1	4,51	90	90	88
10.23.16	4,57	4,37	3,58	89	89	90
10.27.16	5,23	2,87	1,19	89	86	88
11.02.16	4,86	5,09	4,95	90	91	92
11.18.16	5,01	4,34	4,21	90	90	90
11.25.16	5,51	3,28	2,57	90	87	88
12.01.16	4,65	4,07	3,39	87	86	90
12.08.16	4,07	4,07	2,82	91	90	86
12.14.16	3,47	3,29	4,19	90	89	91
12.22.16	4,08	4,13	3,27	88	90	88
01.05.17	4,93	4,33	3,82	89	89	89
01.12.17	4,92	2,77	2,75	89	86	88
01.19.17	3,53	3,31	3,53	89	88	88
01.26.17	4,97	3,67	4,87	88	88	89
02.02.17	4,92	3,4	4,05	88	87	88
02.16.17	5,45	4,68	4,7	93	90	90
02.23.17	5,38	5,02	4,08	89	90	89
03.02.17	5,8	2,64	3,54	88	89	89
03.09.17	5,19	3,02	4,08	90	87	89
03.16.17	5,57	5,87	4,35	90	89	89
03.26.17	5,1	5,19	4,13	92	90	90
03.30.17	4,96	6,43	4,22	87	90	87
04.06.17	5,02	6,74	4,53	90	92	89
04.13.17	4,42	6,07	4,67	91	91	87
04.19.17	3,68	5,93	4,77	91	91	89
04.26.17	4,47	6,73	4,96	91	91	88
05.04.17	3,78	5,2	4,49	90	90	86
05.11.17	2,76	4,66	3,57	91	91	86
05.18.17	4,12	5,13	4,06	92	93	90

Table A. 4. Total P and water content in *Iris pseudacorus.*

Date	**TP [g P/kg dw]**			**Water content [%]**		
	Root	Stem	Leave	Root	Stem	Leave
09.15.16	7,32	2,83	3,68	92	92	82
09.20.16	7,42	2,33	2,96	89	90	84
09.29.16	2,27	1,29	1,42	86	83	87
10.06.16	1,75	0,96	1,85	87	89	81
10.13.16	2,14	1,85	1,55	84	89	85
10.23.16	3,41	4,28	4,22	87	90	86
10.27.16	0,65	0,58	0,45	81	90	88
11.02.16	1,90	1,89	1,20	79	90	86
11.18.16	2,47	3,08	1,05	80	88	85
11.25.16	1,61	2,29	1,10	80	89	89
12.01.16	1,79	2,12	1,41	82	91	89
12.08.16	5,13	4,10	2,09	84	90	88
12.14.16	3,29	3,18	1,51	83	86	88
12.22.16	3,86	3,60	1,86	81	89	84
01.05.17	5,47	5,05	2,47	87	90	89
01.12.17	3,97	5,16	2,57	88	90	89
01.19.17	2,03	3,19	1,74	80	89	90
01.26.17	2,17	5,09	1,90	84	86	88
02.02.17	2,74	5,55	3,17	82	88	89
02.09.17	3,37	4,68	3,53	82	90	84
02.16.17	3,34	4,16	2,80	83	88	82
02.23.17	2,86	3,86	1,30	83	90	87
03.02.17	4,39	3,92	1,60	86	92	88
03.09.17	3,90	4,50	2,85	83	90	88
03.16.17	4,53	4,73	2,71	83	91	88
03.26.17	2,77	5,65	3,13	85	91	87
03.30.17	3,46	5,43	3,22	81	91	85
04.06.17	4,19	6,52	4,55	84	91	86
04.13.17	3,91	5,76	3,67	84	92	89
04.19.17	3,71	7,97	5,75	84	93	90
04.26.17	5,96	7,36	6,31	87	93	91
05.04.17	3,92	7,30	5,73	86	93	91
05.11.17	3,28	6,09	4,86	86	94	88
05.18.17	2,90	5,42	4,72	88	94	90

Appendix D: Iron mass flux

The iron mass flux was obtained by multiplying the iron concentration with the hydraulic loading (figure A.1) As the difference between inlet and outlet flux for dissolved iron (blue lines) is small and therefore difficult to see, blue marks at the top and bottom in figure A.1 indicate differences. A blue mark at the bottom represents a higher flux of dissolved iron in the outlet compared to the inlet, while a blue mark at the top indicates a higher flux of dissolved iron in the inlet compared to the outlet.

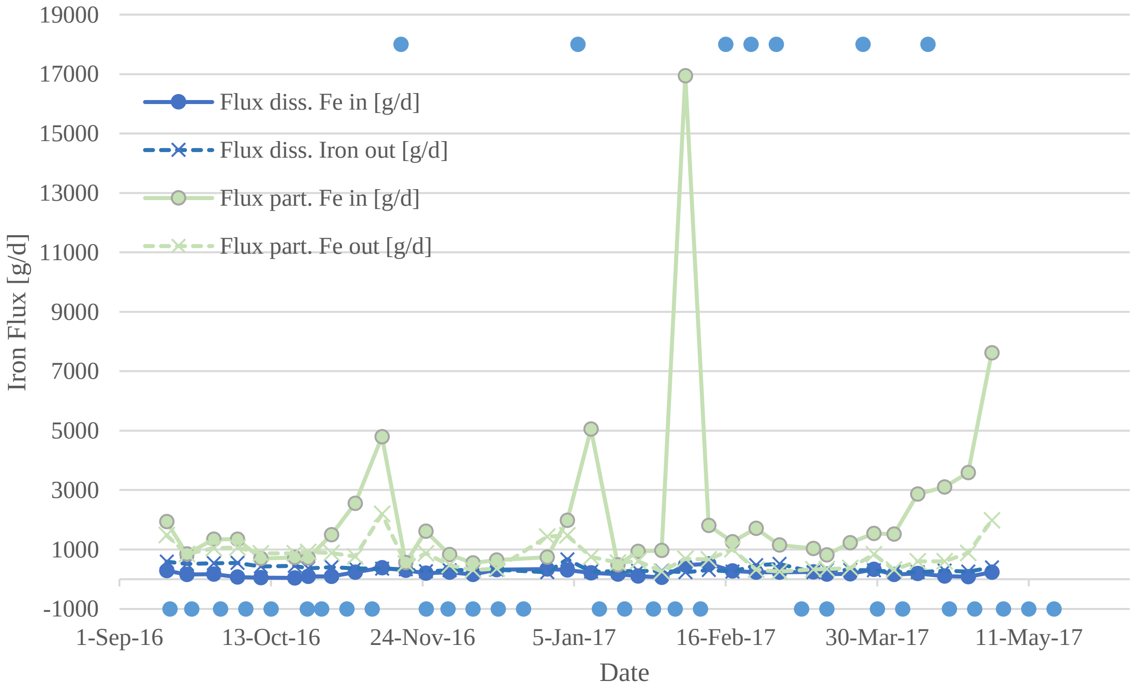

Figure A. 1. Iron mass flux plotted against time.

Appendix E: Calibration curve for phosphorous and iron

An example of a calibration curve obtained from standard phosphorous solutions of 50, 100, 200 and 400 µg/l is given in figure A.2. The curve with a slope of 1343.5 and a constant of 1.9 was used to calculate unknown phosphorous concentrations on April 26th 2017.

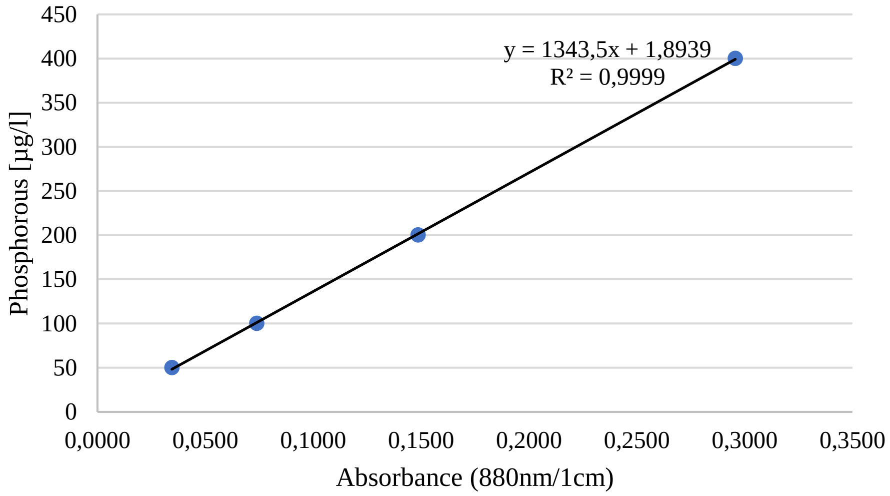

Figure A. 2. Calibration curve for phosphorous analysis.

An example of a calibration curve obtained from standard iron solutions of 0.1, 0.2, 0.4, 0.5, 0.8, 1.0, 2.0 and 4.0 mg/l is given in figure A.3. The curve with a slope of 7.7 was used to calculate unknown iron concentrations during the mater thesis period.

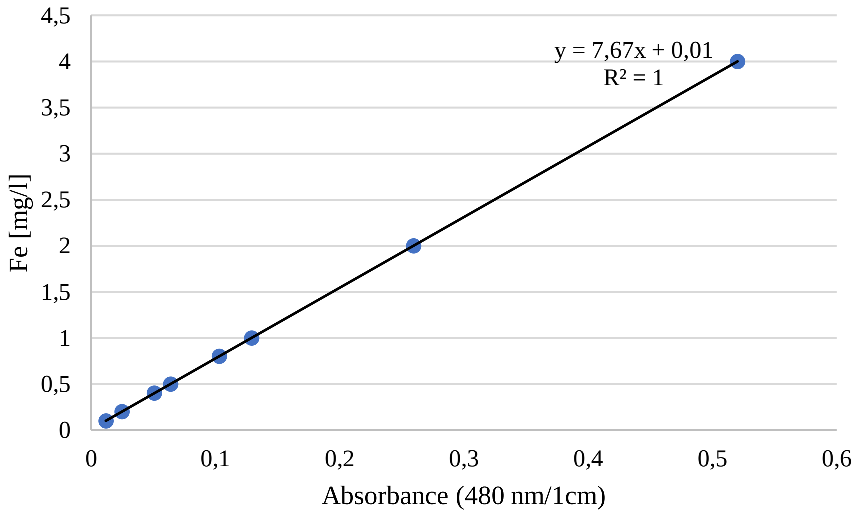

Figure A. 3. Calibration curve for iron analysis.