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Management of fish farm sludge in vertical flow treatment wetlands planted with macrophytes: mesocosm experiment

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Abstract

Management of fish farm sludge in vertical flow treatment wetlands planted with macrophytes: mesocosm experiment

Inland fish farms are significant sources of water pollution. The main objectives of this work were to: a) develop on-site methods for fish farm sludge management and treatment with modified partly hydraulically saturated vertical flow treatment wetlands (TWs); b) to determine if effective TWs' plant species, *Phragmites australis* subsp. *australis* (invasive in North America), could be replaced with native *Phragmites australis* subsp. *americanus*. The performance of 12 on-site mesocosms (both species and unplanted mesocosms in 4 replicates) during two vegetation periods was assessed. The TWs were fed with a batch load of raw sludge, influent and effluent quality and plant development were monitored. Good sludge dewatering and percolate treatment was achieved in TWs (solids removal over 80%; COD 50-70%; TKN and TP 50-60%). As there were no significant differences in efficiency of the sub-species and unplanted mesocosms, the native *Phragmites* could replace the invasive in TWs. Longer lasting full-scale studies should be done to assess the influence of plants and species choice to the TW performance in order to validate the findings of this experiment.

Keywords: Vertical flow treatment wetland, fish farm sludge dewatering, *Phragmites australis*

CERCS: T270 Environmental technology, pollution control;

Annotatsioon

Kalakasvanduse reoveemuda käitlemine taimestatud vertikaalvooluliste märgalade katses

Sisemaa kalakasvandused on märkimisväärsed veereostuse allikad. Käesoleva töö põhieesmärk oli: a) arendada välja efektiivne meetod kalakasvanduste muda käitlemiseks kasutades modifitseeritud osaliselt hüdrauliliselt küllastunud vertikaalvoolulisi tehismärgalasid (TW-d); b) teha kindlaks, kas tõhusa TW-de taimeliigi *Phragmites australis* subsp. *australis* (Põhja-Ameerikas invasiivne), saaks asendada kohaliku *Phragmites a.* subsp. a*mericanus*'ega. Töös hinnati 12 katsekeha (mõlemad liigid ja taimestamata katsekehad kõik neljas korduses) efektiivsust kahe vegetatsiooniperioodi jooksul. TW-sid koormati perioodiliselt toormudaga ning määrati sisse- ja väljavoolude veekvaliteet ning jälgiti taimede arengut. TW-des saavutati hea muda veetustamise ja selle nõrgvee töötlemise efektiivsus (tahkete ainete eemaldamine üle 80%; KHT 50-70%; lämmastik ja fosfor 50-60%). Kuna alamliikide ja taimestamata katsekehade efektiivsuses ei olnud olulisi erinevusi, võiks kohalik *Phragmites* asendada invasiivset. Katse tulemuste kinnitamiseks tuleks teha pikaajalisemad täiemahulised uuringud, et hinnata taimede ja liikide valiku mõju TW-de efektiivsusele.

Võtmesõnad: Vertikaalvooluline tehismärgala; kalakasvanduse muda tahendamine, *Phragmites australis*

CERCS: T270 Keskkonnatehnoloogia, reostuskontroll

1. Introduction

There has been a large increase of inland fish farming over the last decades. The freshwater aquaculture is a prosperous and developing sector of industry and expected to contribute more than half of world production of fish products by the year 2030, explaining the considerable efforts of several countries to develop this sector. However, inland fish farms cause environmental problems as they discard in water bodies a significant load of pollutants. The high water content sludge collected at the bottom of conventional settling zones, filters, ponds and sedimentation units in fish farms is usually transported to storage tanks from where the settled sludge can be spread on agricultural land once per year. However, with these sludge treatment methods a vast amount of pollutants from the solids in fish farm sludge are dissolving into water (to the supernatant of the sludge) and increasing water pollution. Alternatively, the collected sludge and leachate/percolate from the dewatering process can be rapidly treated on-site in treatment wetlands (TWs) and biofilters. Methods based on the natural filtering capacity of wetlands have proven to be a prospective ecological alternative to traditional wastewater and sludge treatment.

Treatment wetlands (TWs) have been used to treat a diverse range of polluted effluents – domestic, agricultural, industrial, storm water, landfill leachate etc (Kadlec and Wallace, 2008) and sludge for dewatering and treatment (for fish farms: Comeau et al., 2001; Gagnon et al., 2013; Kõiv et al., 2016; Naylor et al., 2003; Summerfelt et al., 1999)

Vertical subsurface flow treatment wetlands have proven to provide rapid separation of solids from polluted water and sludge (e.g. from raw municipal wastewater and activated sludge), and to ensure effective mineralisation of the solids on top of the wetland substrate in aerobic conditions. Furthermore, TWs provide additional treatment of the polluted water percolating through the wetland substrate after solids separation.

There is already a vast amount of scientific studies conducted and TWs in usage for solids separation from wastewater (e.g. French systems = vertical subsurface flow treatment wetlands) and for sludge dewatering (e.g. with sludge dewatering reed beds). However, there is still a lack of information about the suitability and effectiveness of vertical flow TWs for fish farm sludge management and treatment.

Furthermore, an important part of TWs is the interactions between the chosen substrate, microbial community and the plants. The optimal selection of plant species provides many benefits and enhances the effectiveness of TWs in pollutants removal. Most efficient and commonly used TW plant species is macrophyte Phragmites australis subsp. australis. However, this species is highly invasive in North America and therefore, its usage in TWs is prohibited. To tackle this problem, there is a need in North America for alternative TW plant species that would ensure similar benefits and efficiency as invasive European common reed.

Objectives

The main objective of this work was to: a) develop on-site methods for fish farm sludge management and treatment that is cost-effective and environmentally-friendly by using modified partly hydraulically saturated vertical flow treatment wetland technology; b) to determine if effective TW plant species, invasive *Phragmites australis* subsp. *australis* (European common reed), could be replaced with North-American native *Phragmites australis* subsp. *americanus*.

Additionally, this work aimed to provide a short overview and examples of common Estonian and Canadian inland freshwater fish farm types, and comparison of composition of different sludges and most used treatment wetland technologies used for polluted water and sludge management.

2. Theoretical background

2.1 Water pollution and eutrophication

Eutrophication is a major global challenge for aquatic ecosystems due to man-made enrichment of these ecosystems with nutrients (Gooddy et al., 2016). Both phosphorus and nitrogen are the main inorganic nutrients that cause eutrophication of sea- and freshwater. Eutrophication leads to increased primary production and growth of algal biomass, creating unbalanced ecosystems, degrading water quality and ecosystem capacity, and reducing oxygen on the seabed (Letelier-Gordo and Fernandes, 2020). Abundant organic matter and nitrogen are associated with eutrophic freshwater that directly affect greenhouse gas emissions from the water column (Li et al., 2021). Sources of nutrient pollution that are responsible for eutrophication can be divided into two categories: point sources and nonpoint sources.

Point sources are usually relatively small and have high concentrations of nutrients in the waste stream, for example, effluents from municipal wastewater treatment plants and from livestock, and farmyards (Rodgers et al., 2005). On the other hand, non-point sources, such as run-off from pastures, farmlands and woodlands represent a flow of large amounts of low concentrations of nutrients (Ahmad and Hameed, 2010). Non-point sources are particularly difficult to control for a number of reasons, although the primary challenge is simply that the source of pollution has spread over such a large area, which makes it extremely difficult to implement effective drainage strategies (Callery and Healy, 2019). Therefore, from the perspective of reducing eutrophication it is easier to allocate first the nutrient pollution caused by point sources. One of the growing point sources of pollution are aquacultures (i.e. fish farms) (Sindilariu et al., 2009).

2.2 Freshwater aquacultures

The freshwater aquaculture is a prosperous and developing sector of industry and expected to contribute more than half of world production of fish products by the year 2030, explaining the considerable efforts of several countries to develop this sector. The socio-economic importance of aquaculture lies in the majority of jobs it has created, from 15 000 to 25 000 in Canada, including 8000 to 12 000 direct jobs associated with production, and concerns especially the rural and coastal communities. The fish production of the European Union in 2017, reached nearly 700 000 tons per year (FEAB, 2021). Strict legislations on discharges of pollutants from inland (freshwater) fish farms have been adopted in Canada and Europe, and are about to come into force (EU, 2000). Thus, the goal of the production of the industry of aquaculture in Quebec (in Canada) is to reduce emissions to 4.2 kg of phosphorus (P) released per ton of fish produced by year 2010 (Lefrançois et al., 2010). In Canada, total aquaculture production is 187 026 tonnes with a value of $1 230 146 000 in 2019 (Government of Canada, 2020). In 2019, Canada's farmed seafood exports were just over $1 billion. Trout farming is the second largest finfish farming after salmon in Canada. While Ontario is the largest trout producer in Canada, trout farms can be found in all ten Canadian provinces (CAIA, 2020). In 2019, Canadian trout farmers produced 8771 tonnes of trout valued at about $58 million. Quebec province has the smallest aquaculture production with 1295 tonnes (value $10 540 000), of which trout is produced 405 tonnes (value $2 451 000) (Government of Canada, 2020).

Despite being a small and young country, Estonia has a history of professional fish farming over 100 years (OECD, 2009). As the result of new funding's in aquaculture sector and an Aquaculture Development Strategy in 2012 (TU, 2020), the fish farming production has increased from less than 400 ton (including 333 ton of trout) in 2011 to more than 1000 ton of fish (incl. 927 ton of trout) in 2019 in Estonia (Statistics Estonia, 2020). According to the Veterinary and Food Board, in 2018 55 licensed companies were active in the aquaculture sector, of which 30 raised fish and 25 crayfish (Fisheries Information Center, 2019).

2.3 Fish farming as a water pollution source

The aquacultures are divided into marine and inland (i.e. land-based) farms. Controlling the pollution of open sea farms is more difficult as the effluent scutters into the surrounding seawater (Dean et al., 2007). It is easier to control the pollution of inland fish farms, especially when the fishes are kept in tanks. In inland aquacultures, mostly, different species of freshwater fish are grown, e.g. trout, cod and carp (Fisheries Information Center, 2015). In the context of the current thesis the main focus is set on inland trout farms.

There has been a large increase of inland fish farming over the last decades, representing the highest growth rate of all animal productions since the 80's (Chevassus and Lazard, 2009). According to the *State of World Fisheries and Aquaculture 2020* the total production of captured fish was 96.4 and total production from aquacultures was 82.1 million tonnes (live weight) in 2018 (FAO, 2020). During the last 30 years, the global captured fish industry has shown a slight increase from 87 to 96 million tonnes, at the same time global aquaculture production has grown more than five times from 15 to 82 million tonnes of live weight of fishes (FAO, 2020). The global aquaculture projection estimates to reach the point where aquaculture production equals global captured production by 2030 (The World Bank, 2013).

Fish farms cause an environmental problem as they discard in water bodies a significant load of organic matter and nutrients (Kliger, 2019; Sarà, 2007; Sindilariu et al., 2009). On one hand, to increase the economic benefit of fish farming, the goal is to intensify the production by keeping large amounts of fishes in the tanks. However, the big population of fish in small areas causes higher pollution in the water. The fish farm main effluents have still 20 to 25 times lower nutrient concentration than municipal wastewater, but the high flow rates result in pollutants (especially phosphorus and nitrogen) accumulation in ecosystems (Naylor et al., 2003), causing the pollution of natural areas and leading to eutrophication (Metcalf & Eddy et al., 2002). Furthermore, as the global aquaculture industry has grown exponentially in recent years, they are using a variety of veterinary drugs to control infections and diseases, including antibiotics, antifungals and antiparasitic agents. These drugs have different routes of emissions, causing side effects and environmental persistence to aquatic organisms (Alvarado et al., 2021). In this thesis context the veterinary drugs impact is not investigated, but must be considered to be analysed in further research in the field of treatment wetlands (He et al., 2021). The growth of the aquaculture industry has increased the need for improvement of wastewater treatment, and especially sludge management (Asche and Tveteras, 2005).

Inland fish farms are an important source of organic pollutants, estimated between 100-150 g/d per ton of fish produced for ammonium-nitrogen and between 20-60 g/d per ton of fish produced for P (Boaventura et al., 1997; Koçer et al., 2013). Two main goals to separate sludge from fish farm wastewater are: to reduce environmental impact of the wastewater released back to nature and to recirculate the water several times to decrease the need to supplement new fresh water into the water system. Several authors (Cripps and Bergheim, 2000; Gagnon et al., 2013; Kõiv et al., 2016; Naylor et al., 2003; Yeo, 2004) have shown that the best strategy to reduce pollutants from the effluent of fish farms was to separate the solids (fish faeces, uneaten food, etc.) from water as quickly as possible to prevent re-dissolution of phosphorus and nitrogen (sludge contains 7 to 30% nitrogen and 30 to 85% of phosphorus excreted by fish) to the receiving water. Therefore, the most relevant and common technology used regardless of the type of fish has been simple physical settling (removes particles up to 20 μm in diameter). However, once settled and collected, fish sludge still represents an environmental problem since areas for sludge application are getting smaller and smaller, and treatment and reuse of the sludge and sludge leachate (i.e. percolate) remains critical for many fish farms since few low-cost and high efficiency technical pathways exist.

2.4 Water pollution requirements for fish farms in Estonia and Canada

In Canada, fish farming is jointly managed among federal, provincial and territorial governments (DFO, 2018). How it's managed varies across provinces and territories. The effluent from any treatment plant must comply with the following standards: the 5-day carbonaceous biochemical oxygen demand ($CBOD_5$) must be less than or equal to 25 mg/L; the concentration of suspended solids (SS) must be less than or equal to 25 mg/L, the pH value must be between 6.0 and 9.5 (Gouvernement du Québec, 2019).

In Estonia, wastewater discharged from pond type fish farms, raceway farms and recirculating aquaculture systems into the waterways must comply with the following quality limit values: the 7-day biochemical oxygen demand (BOD_7) must be less than or equal to 15 mg/L; the concentration of suspended solids (SS) must be less than or equal to 25 mg/L; total P less than or equal to 0.5 mg/L and total N 15 mg/L. If the recirculating aquaculture systems has annual production less than 200 tons, then the effluent must comply with the following limits: the 7-day biochemical oxygen demand (BOD_7) must be less than or equal to 15 mg/L; the concentration of suspended solids (SS) must be less than or equal to 25 mg/L; total P less than or equal to 1.0 mg/L and total N 45 mg/L (Riigi Teataja, 2020a).

Fish Farm pollution charges in Estonia. From 2020 in Estonia there are no more pollution fines if the pollutants (e.g. organic substances, phosphorus compounds, nitrogen compounds) are released into the environment as a result of aquaculture activities and the permitted amounts of pollutants released into the environment per year are not exceeded (Riigi Teataja, 2020b). As the Estonian aquaculture sector is small, the environmental impact of agricultural wastes is also considered low. Estonia's neighbouring countries do not apply such pollution charges to aquacultures, which puts Estonian aquaculture companies in an unequal competitive position, especially if world market sales prices are lower than the cost price. The aquaculture sector is a food production, and in a situation where agriculture is exempt from the fertilization tax, the difference between the two primary food production sectors creates an unequal situation. Aquaculture is a sector where setting up new businesses involves high investment and risk. The development of the sector is facilitated by the avoidance of different tax policies of neighbouring countries. (Savisaar and Keskkonnakomisjon, 2019)

2.5 The wastewater and sludge management in aquacultures

The first step in fish farm wastewater treatment is to separate sludge (i.e. all solids) from water as soon as possible to prevent dissolution of pollutants (discussed in chapters, 2.2). The different raw sludge separation methods in fish farms are the following:

1. Physical passive settling in the bottom of fish ponds and ditches - sludge removal periodically (e.g. once per year in spring);
2. Sludge separation units in the end of the raceways, e.g. a cone-shaped, sloped floor as gravity separation tank in the end of the raceways;
3. Vacuum cleaning in the end of the raceways from solids separation section (Figure 1);
4. Mechanical filtering units - e.g. sieve filters, drum filters, microscreens, rotating belt filters (ACE, 2021; Bergheim and Kelly, 1993; Bregnballe et al., 2015; Salsnes Filter Ltd., 2017).

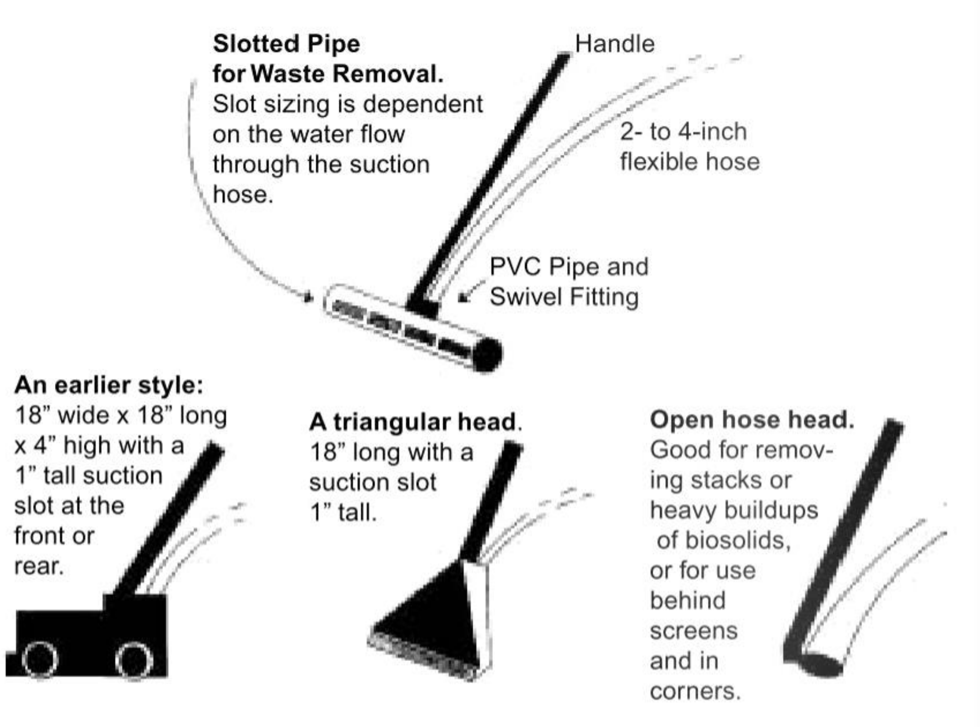

Figure 1. Vacuum removal of solids: the solids that are collected are pumped or gravity-flowed to the off-line settling ponds or tanks (IDEQ, 1997).

The result of the sludge separation is cleaner main effluent that is recirculated to or discharged from the system and separated raw sludge with high water content.

Two main goals of treating raw sludge before disposal are to reduce the volume and to stabilize and separate the solids, organic matter and nutrients. The suitable methods for raw sludge dewatering or further management in fish farms are the followings:

1. Raw sludge used directly in agriculture as soil fertilizer (e.g. (IDEQ, 1997));
2. Settling of raw sludge in tanks (i.e. silos), resulting with settled sludge and the supernatant (i.e. remaining water on top of the settled sludge), and afterwards use of settled sludge on agricultural fields;
3. Sludge drying and composting;
4. Humification fields;
5. Sludge fermentation for biogas production;
6. Rapid separation of sludge with treatment wetlands (TWs).

The treatment of the raw sludge in a conventional manner may involve a combination of thickening (i.e. dewatering), digestion and dewatering processes. Thickening is usually the first step in sludge treatment, as it is impractical to handle thin sludge, a slurry of solids, suspended in water. Thickening usually takes place in tanks called gravity thickeners. The thickener can reduce the total volume of the sludge by less than half the original volume. Sludge digestion is a biological process in which organic solids decompose into stable substances. Digestion reduces the total weight of solids, destroys pathogens and makes it easier to dewater or dry the sediment. Sludge drying beds offer the simplest method of dewatering. The digested sludge is spread on an open layer of filter bed and allowed to dry. Drying takes place through a combination of evaporation and gravity drainage through the substrate. The final destination of treated sewage sludge is usually land. Dehydrated sludge consisting of high levels of harmful pollutants can be buried underground in sanitary landfills. If proven safe, it can also be applied to agricultural land to use its value as a soil improver and fertilizer (Encyclopedia Britannica, 2021).

Many authors (Balkema et al., 2002; Boguniewicz-Zabłocka and Capodaglio, 2017; Istenič et al., 2014) point out that conventional treatment methods of wastewater are not cost effective in rural areas. In these areas individual onsite sewage disposal systems are commonly used, like septic tanks with infiltration beds and treatment wetlands. Most of the inland fish farms are away from cities in rural areas and an effective approach for raw sludge treatment is needed. A medium to large size inland fish farm (1000 tonnes of feed per year) can produce up to 15 tonnes of sludge (dry matter) per month, corresponding to 150 m^3 of wet sediment (10% total solids in wet sediment) - i.e. about 200 g of suspended solids (SS) per kilogram of fish feed (DHI Group, 2021). Proper sludge management is needed to make sludge from waste into new resources.

Some of the methods, like usage of raw sludge and settling it in tanks over a long period of time can have high negative environmental impacts. Direct use of raw sludge in agriculture can have a negative influence on plant growth and may even be harmful to some plants (Brod et al., 2017) when compared to the usage of digested sludge. For example, the roots of the plants treated with raw fish sludge were severely damaged, probably due to high levels of organic matter in the anoxic zone, as well as potential competition for nutrients from roots and soil microbial biomass (Brod et al., 2017).

Settling of raw sludge in tanks (i.e. silos) over a long period of time causes dissolution of pollutants to the supernatant that needs further treatment (usually done with bioponds, -filters) before discharge to nature. Furthermore, with anaerobic conditions present in tanks there is high production of methane and other harmful gases (Hobson, 1999).

Therefore, there is a need for alternative environmentally friendly technology for rapid fish farm sludge dewatering and treatment of the remaining water. Constructed treatment wetlands have proven to be efficient in sewage sludge dewatering (Nielsen and Stefanakis, 2020) and in raw wastewater treatment (Paing et al., 2015). Furthermore, sludge supernatant or leachate treatment can be solved also with treatment wetland technologies, e.g. as shown by Kõiv et al., (2016).

2.6 Comparison of fish farm sludge to other common sludge types

The fish farms usually have large volumes of effluent water and low levels of solids (van Rijn, 2013, 1996) compared to other rural and urban wastewaters like municipal sewage, surface runoff, storm sewer effluent etc. Municipal sewage has approx. 10 times higher TSS, 20 times higher BOD, 50 times higher TN and more than 10 times higher TP concentrations than fish farm effluents (Yeo, 2004). On the other hand, the primary treatment (solids separation) of fish farm wastewater (i.e. effluent of fish tanks) results with raw sludge characteristics (TSS, COD, BOD, TN, TP) that can be compared to most of the raw municipal sludges (Table 1).

Table 1. Comparison of average fish farm sludge composition with municipal sludge and raw wastewater.

Sludge origin	**Water content (%)**	**TSS** (mg/L)	**COD** (mgO_2/L)	**BOD** (mgO_2/L)	**TN** (mg/L)	**TP** (mg/L)	**References**
Fish farm sludge	95	7860 ±1849	6855 ±1251	N/A	234 ±20	238 ±19	(Summerfelt et al., 1999)
Fish farm sludge	N/A	32500 ±14000	N/A	N/A	2000 ±800	750 ±200	(Gagnon et al., 2013)
Fish farm sludge	N/A	28500 ±16400	68000 ±26500	N/A	1800 ±600	820 ±300	(Gagnon et al., 2013)
Municipal primary sludge	96	34000	N/A	N/A	7300		(Al-Malack et al., 2008)
Raw municipal sludge	N/A	23000	42000	N/A	1423	517	(Vincent et al., 2011)
Raw municipal wastewater	N/A	720	1300	520	97	14	(Budych-Gorzna et al., 2021)
Raw municipal wastewater	N/A	353 ±207	841 ±340	360 ±159	94 ±27	12 ±4	(Paing et al., 2015)
Industrial sludge	99.8	1262	N/A	N/A	2300	6960	(Nielsen and Stefanakis, 2020)

2.7 The different types of fish farms and common pollution control methods used

Choosing the best available wastewater and sludge management and treatment solution for inland aquacultures depends on the type of the fish farms. Most common fish farm types are ditch and pond systems, flow-through systems and recirculating aquaculture systems (Wikipedia, 2020; Yeo, 2004).

Flow-Through Systems

Most common type of fish farms in Europe are flow-through systems. Flow-through aquaculture systems have a continuous water inflow and outflow, which help to maintain suitable water quality for fish culture by the high oxygen level in the incoming water and removal of waste products in the outflow (Tucker et al., 2008).

The high water exchange rate dilutes dissolved waste and allows fish to be reared at high densities in raceways, tanks and ponds. These systems usually operate with a very short water retention time, often less than one hour. Because of high fish density, strict formulated diets are needed. Fish farms have different sizes and shapes, including circular units, but the most common are the linear raceways. Concrete and fiberglass are popular building materials used in larger commercial hatcheries (Yeo, 2004). Earthen raceways can be found in many smaller private fish farms. To achieve higher production potential, pure oxygen injectors, mechanical aeration or gravity aeration are used between raceways to maintain high dissolved oxygen concentration. Flow-through facilities carry large amounts of highly diluted effluent, making nutrient recovery difficult. Very often the raceways are followed by a pond or several ponds, in purpose to settle the sediments before release (Yeo, 2004). From time to time these ponds need to be cleaned from settled sludge. After cleaning, the gathered sludge must have proper management. Furthermore, the use of resources must be handled in such a way that aquaculture does not reduce the value of the used water for some other uses (Tucker et al., 2008).

Ponds

Ponds differ from flow-through systems in that they are essentially static and do not rely on water replacement to maintain water quality. Ponds rely mainly on internal natural processes to purify water. The biological community acts on dissolved waste and helps to stabilize and recycle waste. Sediment solids accumulate and decompose in ponds by microbes, much like in a municipal wastewater treatment plant (Yeo, 2004).

Ponds are more similar to a recirculating aquaculture system, although water is treated in separate processes in recirculating systems, waste treatment and aeration are inherent in the pond ecosystem (Tucker et al., 2008). Ponds' water treatment needs a long water retention time and makes the fish farm production much smaller than in intensive systems. The production capacity of the pond is directly related to the maximum daily addition of fish feed while maintaining adequate water quality. For example, catfish ponds, a daily feeding rate of 30–50 kg/ha limits annual production to 2000–3000 kg/ha (Yeo, 2004).

Recirculating Aquaculture System (RAS)

Recirculating aquaculture systems (RASs) consist of a culture unit connected to a set of water treatment units, which allows part of the water leaving the culture unit to be recovered and reused again in the same culture unit (Figure 2). The RAS demands at least a primary water treatment process to remove solids, to reduce nitrogenous wastes, and to add oxygen to the water (Tucker et al., 2008). Most RASs are simplified systems where water treatment is limited to solids, sometimes also preliminary nutrients removal. In many fish farms only a small part of the used water is recirculated and the main volume of water is discharged from the farm. In more complicated systems temperature control, pH adjustment, gas removal, and disinfection are used (Tucker et al., 2008).

Although the construction, maintenance and operation of RASs is often much more expensive and more mechanically complex than other fish farming methods, the fish can be raised intensively in more ideal water conditions all year round. One of the main advantages of RAS is the possibility to reuse all or a significant portion of their rearing water multiple times (Yeo, 2004).

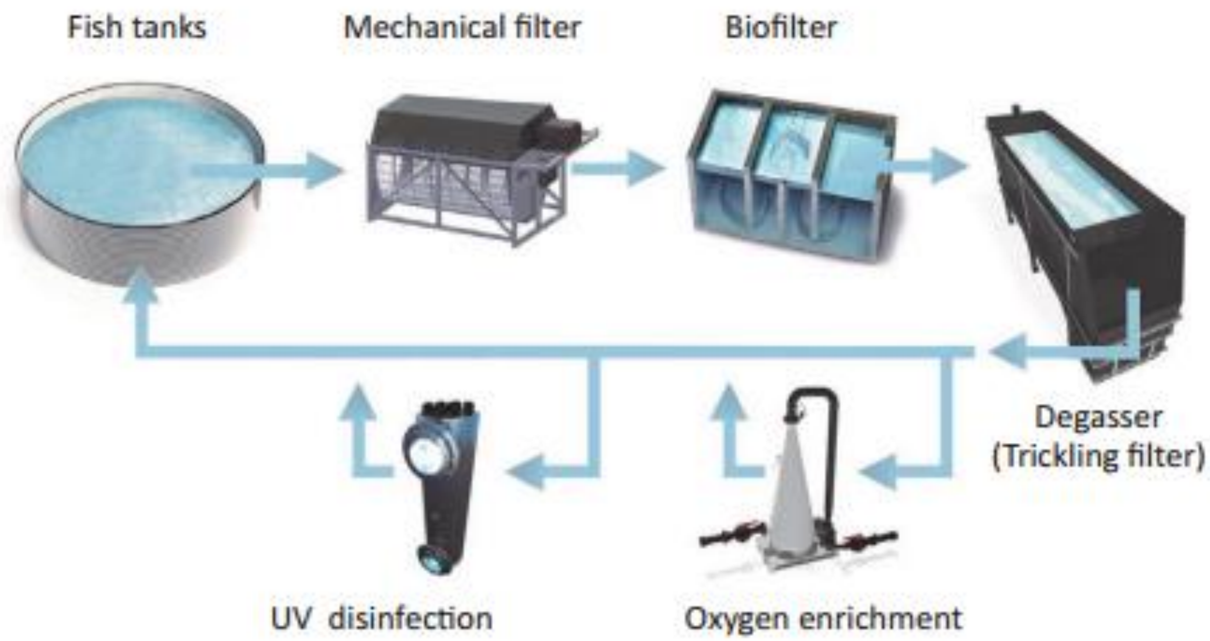

Figure 2. A schematic of a typical recirculating aquaculture system (RAS) with high treatment efficiency (Bregnballe et al., 2015).

The full water treatment system of RAS (Figure 2) consists of mechanical filtration, biological treatment, aeration/stripping to remove CO_2 and N_2 before the water is oxygenated and returned to the fish tanks (Bregnballe et al., 2015). Depending on the requirements, oxygen enrichment or UV disinfection may be added (Bregnballe et al., 2015). This method produces a large amount of sludge to be operated outside the treatment system.

According to a report assembled by Aqua Consult Baltic Ltd. (2012) conducted the Life Cycle Assessment of RAS and flow-through system analysed trout production systems at the global level. Their environmental impact assessment revealed that the main differences between the two systems were between water use, eutrophication potential and energy use. Regardless of the system used, the main determinant of environmental impact is fish feed. In a recirculation system, the feed coefficient (the amount of feed required to produce 1 kg of fish) is 0.8, and in a flow-through system without recirculation, it is 1.1. Therefore, a recirculation system is more economically beneficial at both global and regional level, except for energy use. The dependence of the recirculation system on water is 93%, the eutrophication potential is 26-38% lower than in the flow-through system. In contrast, a recirculation system consumes 24-40% more energy for aeration and water treatment. By improving aeration systems and biofilters, energy consumption can be reduced to the level of energy demand of farms with a flow-through system, which is common in Europe (Aqua Consult Baltic Ltd., 2012). The Figure 3 shows the main differences between flow-through and recirculating aquaculture systems.

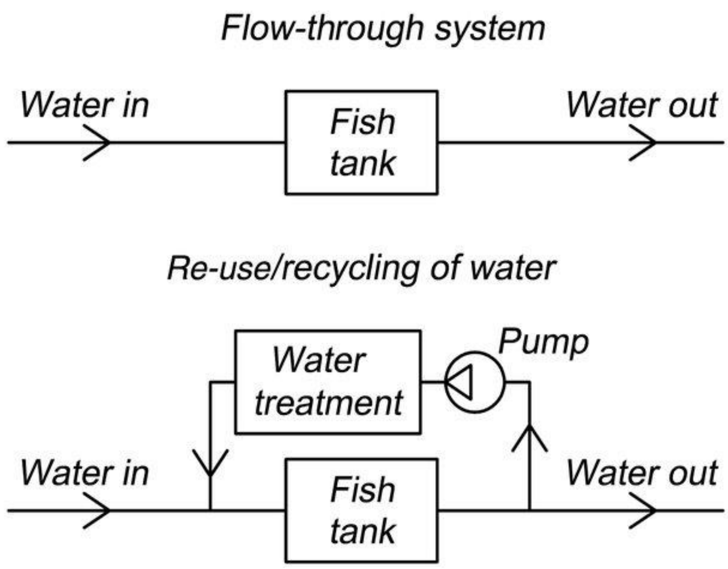

Figure 3. Comparison of a flow-through system (top) and a recirculated system (bottom). Figure from (Lekang, 2013).

Examples of different types of inland trout farms in Estonia and Canada

In this chapter a short overview of trout producing fish farms in Estonia (Table 2) and some examples from Canada are provided.

In Estonia, most common fish farm types for trout production are pond systems and raceways (Table 2.; (PRIA, 2021)). The typical effluent water and sludge treatment is done with simple passive settling ponds or in case of water recirculation in the raceway tanks the more complex sludge separation and settling techniques are used (Table 2).

For example, Pähkla fish farm (Table 2) settling pond is an example of a basically closed simple passive pond system. According to the representative of the farm, Taneli Leivat, the pond's ecosystem, including the help of carps, is purifying the water quite well. More intensive fish production is done in Aquamy Ltd fish farm where RAS and deep cleaning system is used (Table 2).

Trout farming is a small but growing aquaculture industry in Canada (Seafood Watch, 2018). In some provinces like, Ontario and Saskatchewan (and British Columbia), the trout production is mainly done in freshwater open net pens in lakes. In Quebec, Canada, the trout production is done via flow-through and semi-closed raceways and tank systems, that are also used to some degree in other provinces. According to Seafood Watch Consulting Researcher (Seafood Watch, 2018): "Land-based and net pen systems all have potential to discharge nutrients and wastes that can cause harmful farm-level and cumulative effects to receiving waterbodies. Canada has implemented waste and nutrient release reduction strategies, including improved feeds, regulations on feeding, and improved water retention and filtration approaches (particularly for semi-closed systems)".

St-Alexis-des-Monts fish farm, where the experimental work of current thesis was performed, has trout production in raceway tanks and sludge removal system from the raceways with vacuum cleaning and settling of solids in settling tanks and subsequent polishing of the supernatant and treatment of the farm main effluent in large ponds (more information provided in Materials and Methods; see also pictures in Annex: Figures A1, A2, A3).

Another example of intensive raceway trout production farm in Quebec, Canada is Les Bobines fish farm (FPB, 2021).In this farm the raw sludge separation is done with micro-sieves and the collected sludge is stored end settled in the settling tanks. The supernatant from the settling tanks is treated with lime for phosphorus removal. The phosphorus-rich solids from treated supernatant are sent back to the settling tank and the cleaned supernatant is discharges together with the main effluent of the fish farm (information provided by M. Kõiv-Vainik).

Table 2. Overview of Estonian fish farms for trout production (PRIA, 2021).

Business name - Name of the fish farm	**Location**	**Species**	**Type of farm**	**Wastewater-sludge treatment system type**
ARAVUSE KALAKASVANDUS	Vinni parish	Rainbow trout	Freshwater raceways and ponds.	Settling ponds after raceways
KALATALU HÄRJANURMES	Jõgeva parish	Carp, Whitefish, Whitefish, Siberian sturgeon, Rainbow trout, Norway pout	Freshwater raceways and ponds, incubator building.	Sludge removal systems, settling ponds and aeration systems. Fish faeces is made into compost.
SIMUNA IVAX Ltd. - ÄNTU	Väike-Maarja parish	Rainbow trout	Freshwater raceways (recirculation), ponds, incubator building.	Settling - cones, biofilter, settling tanks, settling ponds. Year-around recirculation with aeration.
PÄHKLA VÄHI- JA KALAKASVATUS Ltd.	Saaremaa parish	River trout, Carp, Rainbow trout	Freshwater raceways (recirculation), ponds	Mechanical filter, Settling - cones, recirculation, settling bioponds (with craps cleaning the water).
SIMUNA IVAX Ltd. - NÕMMEVESKI	Väike-Maarja parish	Rainbow trout	Freshwater raceways (recirculation)	Settling-cones, large settling pond, recirculation aeration.
SIMUNA IVAX Ltd. - KÄRUVESKI	Väike-Maarja parish	Rainbow trout	Flow-through freshwater ponds (with gravel bottom), sediment-rich inflow sedimentary water.	Yearly removal of sediments from ponds and from inflow and outflow channels.
AVIISO Ltd.	Kadrina parish	Rainbow trout, Sturgeon, Arctic bale, Russian sturgeon, Siberian sturgeon	Freshwater raceways (recirculation), ponds (a "copy" of fish farm Äntu).	Settling - cones, biofilter, settling tanks, settling ponds. Year-around recirculation with aeration.
SIMUNA IVAX Ltd. - MÕDRIKU	Vinni parish	Rainbow trout, Arctic bale	Indoor fish tank, Freshwater ponds	Settle removing, recirculation during feeding.

Business name - Name of the fish farm	Location	Species	Type of farm	Wastewater-sludge treatment system type
KARILATSI KALAMAJAND Ltd.	Põlva parish	Rainbow trout, Siberian sturgeon	Freshwater raceway channels (recirculation), ponds	Mechanical-biological treatment
AQUAMYK Ltd.	Saaremaa parish	Rainbow trout, Arctic bale	Indoor freshwater RAS	Deep cleaning
RAHO Ltd.	Tori parish	Rainbow trout	Freshwater raceway channels	Unknown. (The environmental permit is invalid 26.04.21)
SK TRADE Ltd.	Rapla parish	Rainbow trout	Freshwater raceway channels, ponds, net cages	Unknown. (The environmental permit is invalid 26.04.21)
NELI ELEMENTI Ltd.	Paide town	Rainbow trout	Freshwater ponds	Mechanical-biological treatment
LAPAVIRA Ltd.	Järva parish	Rainbow trout	Freshwater Raceway channels (recirculation)	Mechanical-biological treatment, settling-cones, settling ponds.
ÖSEL HARVEST Ltd.	Saaremaa parish	Rainbow trout	Freshwater raceway channels (recirculation)	Mechanical-biological treatment

Some fish farms have mixed production systems and these farms can be called a hybrid or mixed type of fish farms. A good example of a mixed fish farm is Käru-Veski fish farm in Estonia which has ponds, flow-through systems and a simple RAS (Figure 4). This farm has several ponds which are situated like raceways and each pond has dams for inflow and outflow to change the water retention time separately in each pond. It has a mobile RAS and aeration system that could be used intensively for one pond, according to the urgent need for improvement of water quality (e.g., low level of oxygen or decrease of inflow water supply due to dry period). The mobile RAS may be used also to recirculate and aerate the water between outflow and two inflow channels to have an overall quality improving effect on all or half of the ponds.

Figure 4. Käruveski fish farm ponds (Photo: Rene Mets)

2.8 Treatment wetlands for sludge management

Growth of the aquaculture industry and its potential to negatively impact freshwater and estuarine ecosystems have garnered TWs as potentially cost-effective treatment systems (van Rijn, 2013, 1996). It is essential to understand the advantage of fish farm sludge management with treatment wetland.

Many terms are used for naming treatment wetlands, such as constructed wetlands, reed beds, soil infiltration beds, engineered wetlands, man-made or artificial wetlands (Wikipedia, 2021). In the book Treatment Wetlands (2008) authors Kadlec and Wallace describe treatment wetlands (TWs) as designed systems that optimize processes in the natural environment to treat different types of polluted water. Compared to other wastewater treatment solutions, TWs are environmentally friendly and sustainable options for wastewater treatment. These systems have low operation and maintenance requirements and are durable because performance is less susceptible to input variations. Treatment wetlands

(TWs) have been used to treat a diverse range of polluted effluents – domestic, agricultural, industrial, storm water, landfill leachate etc (Kadlec & Wallace, 2009) and sludge for dewatering and treatment (for fish farms: Summerfelt et al., 1999; Comeau et al., 2001; Naylor et al., 2003; Roque d'Orbcastel & Blancheton, 2007).

The main advantage of TWs over conventional treatment methods is based on the favourable adsorbing and filtering characteristics of the media, its large surface area, long retention time, flexibility in alternating aerobic-anaerobic zones, diverse microbiological populations and removal of the pollutants by plant uptake (Brisson and Chazarenc, 2009; Gagnon et al., 2007) and microbial degradation. A variety of contaminants are removed from water through various chemical, physical and biological processes (Kadlec and Wallace, 2008).

There are several different types of treatment wetlands (Kadlec and Wallace, 2008). At first, TW systems can be divided according to the hydrology to water position (to surface flow and subsurface flow); and after that based on flow direction (to horizontal and vertical flow); saturation of media and influent loading type (Fonder and Headley, 2013). TW systems can also be divided according to vegetation: the vegetation sessility refers if the plants are anchored to the environment or the plants are floating around (last one is relevant only to surface flow TW). By vegetation can SF TWs differentiate by dominant vegetation as defined emergent, submerged, floating leaved and free-floating plants (Fonder and Headley, 2013).

The overview of main types of TWs are illustrated on the following Figure 5. Sub-surface (SF) TWs are densely planted units that have water flow above the filter bed. SF TW is generally used for tertiary wastewater and stormwater, and agricultural runoff treatment (Fonder and Headley, 2013; Kadlec and Wallace, 2008). Horizontal flow (HF) and Vertical flow (VF) TWs are generally used to treat already mechanically pre-treated wastewater to avoid clogging of filter materials. In HF TWs mostly anaerobic conditions and in VF TWs aerobic conditions and according to that different treatment processes are favoured (Kadlec and Wallace, 2008).

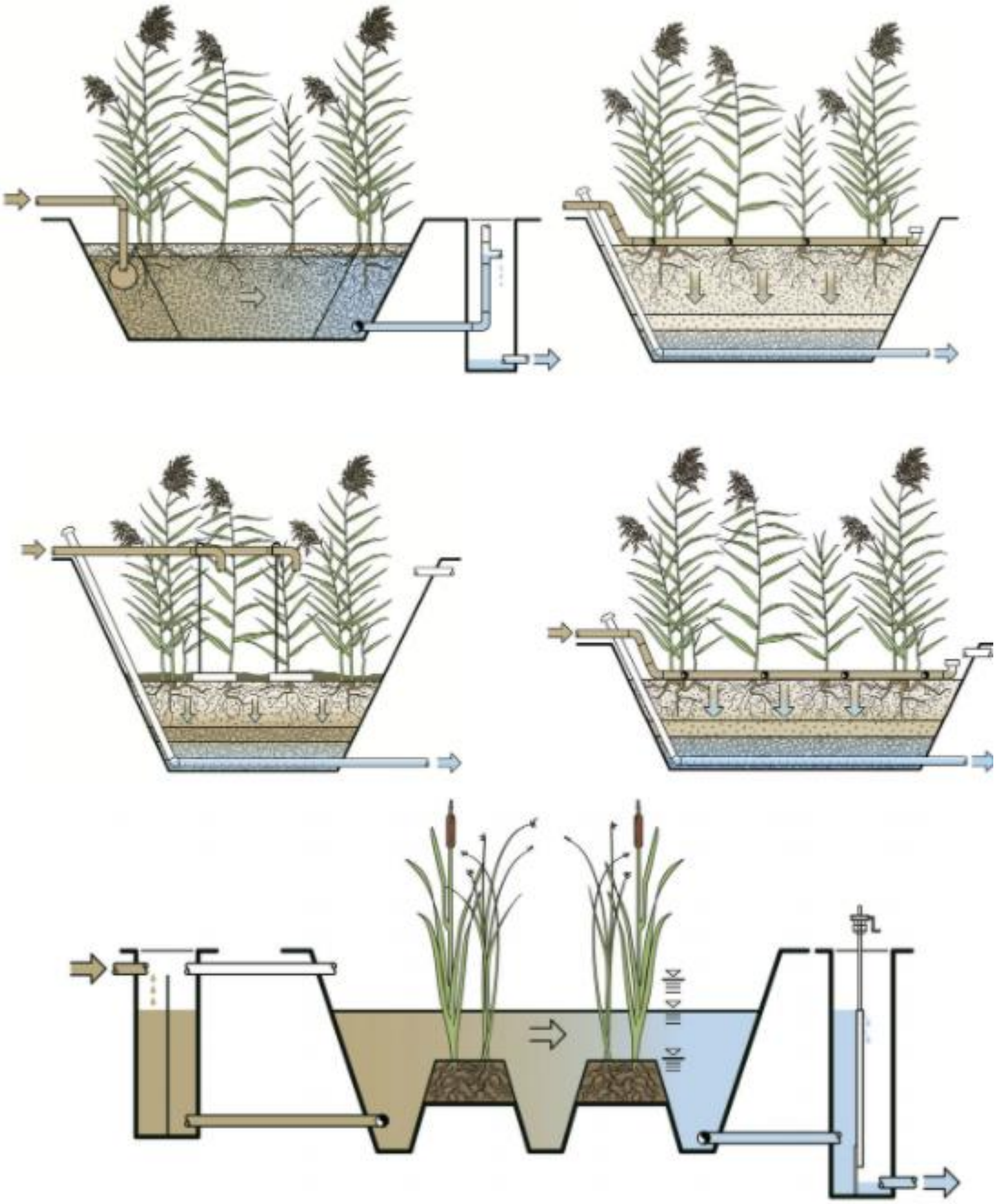

Figure 5. Overview schematics of treatment wetlands addressed in this volume. Top left: horizontal flow TW; top right: vertical flow TW; middle left: French vertical flow system first stage; middle right: French vertical flow system second stage; bottom: sub-surface TW (Kadlec and Wallace, 2008).

Fonder and Headley (2013) pointed out seven “standard types” to clarify and generalize the types of TWs among many different applications around the world.

The three standard types of surface flow TWs are:

1. Surface flow TW, dominated by emergent herbaceous macrophytes;
2. Free-floating macrophyte (FFM) TW contains free-floating vascular aquatic plants growing on the water surface;
3. Floating emergent macrophyte (FEM) TW with emergent macrophytes growing on a buoyant structure.

The four standard types with sub-surface flow TWs are:

1. Horizontal sub-surface flow (HSSF) TW, with subsurface loading (without intentional surface flooding);
2. Vertical down flow (VDF) TW, with free-draining substrate and subsurface loading (without surface flooding);
3. Vertical up flow (VUF) TW with a flooded surface for outflow;
4. Fill and drain (FaD) TW in which the flow direction is mixed, often periodically alternating between up and down flow.

According to review by Messer et al., (2021) there are some examples of usage of TWs for treatment of fish farm effluents (Buřič et al., 2015; Konnerup et al., 2011; Schwartz and Boyd, 1995; Zachritz et al., 2008; Zhang et al., 2010). However, there are few pilot studies (Gagnon et al., 2013, 2012; Summerfelt et al., 1999) and no full-scale examples of usage of TW technology for management of fish farm sludge.

Some TW systems have an extra sludge layer above the surface, e.g. Sludge Treatment Reed Beds and French VF TWs (ARM, 2021; Paing et al., 2015). Specially designed VF TWs are capable of treating screened raw wastewater (containing high concentration of solids). So-called French VF TWs with downflow have integrated both raw wastewater and sludge treatment in single two step systems and no primary treatment is needed (Kadlec and Wallace, 2008); Figure 6). To enhance the performance of French systems one novel method is the use of a hydraulically saturated bottom layer in the filter bed (Morvannou et al., 2017). This enhancement increases the hydraulic retention time in the TW, provides anaerobic conditions in the bottom layer and improves denitrification potential (Morvannou et al., 2017; Prigent et al., 2013).

Especially for wastewater sludge treatment, sludge treatment reed beds (i.e. VF TWs for sludge treatment) have been developed. Sludge treatment reed beds (STRB) have been used to treat sewage and drinking water sludges. Developed from the planting of common reeds (*Phragmites australis*) into a pre-existing sludge drying beds, they differ from the conventionally constructed reed bed, designed for the purpose of wastewater treatment, as the STRB's basic function is to hold sludge on top of the filter bed, allowing it to dewater and mineralize leaving behind a sludge cake (ARM, 2021). STRB have many design similarities with VF TWs for wastewater treatment (ARM, 2021), however, they have extra freeboard for the accumulation of sludge sufficient for an operating cycle of approximately 10 years.

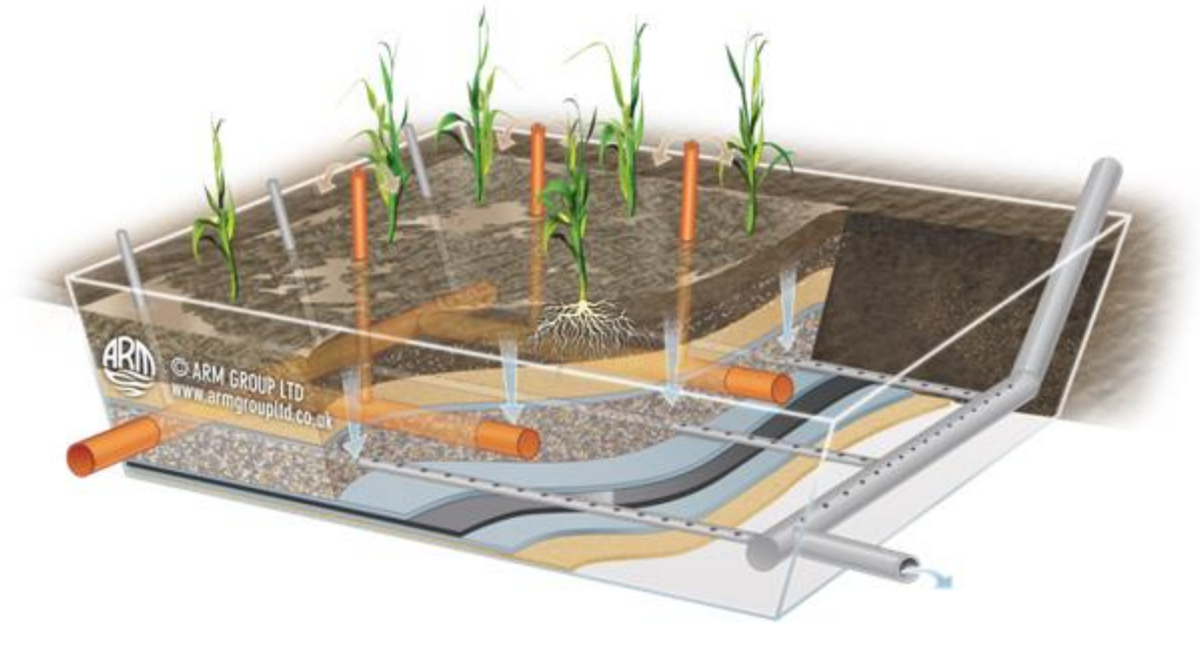

Figure 6. Typical design example of sludge treatment reed bed (ARM, 2021).

Activated sludge has a higher solid content than raw wastewater. The fish farm raw sludge TSS is roughly comparable with activated raw sludge (see Table 1).

STRB's main goal is to stabilize and mineralize activated sludge with already high TSS content and have less frequent sludge loading and longer resting periods for the sludge layer to try (Brix, 2017). On the other hand, French system treats constant loads of wastewater with low TSS. The French system is not optimized for total nitrogen (TN) removal (i.e. it nitrifies but does not denitrify; (Paing and Voisin, 2005). The process of nitrification needs an aerobic environment (Noorvee et al., 2007). Denitrification, on the other hand, is the first anoxic process that takes place after consumption of oxygen and it is the main nitrogen removal process in most types of TWs (Noorvee et al., 2007).

Several authors (Morvannou et al., 2017; Panuvatvanich et al., 2009; Prigent et al., 2013; Silveira et al., 2015) have combined the advantages of STRB and French systems by adding the saturated layer to single-staged French VF TW and treating raw wastewater. Gagnon (2012) used a new system design for fish farm sludge treatment, in which the wetland was not completely drained and a saturated layer was created using an overflow. The result showed that such wetlands were highly efficient: removal rates were between 94-99% (Table A 1) that is comparable with STRB results.

2.9 Role of plants in treatment wetlands used for sludge dewatering

Plants are an important component of wetland systems. Still, the role of plants in the treatment of polluted water by TWs is under debate. Shelef (2013) points out several ways how plants can affect TWs performance:

1. Physical effect of root structure;
2. Roots as a base for microorganisms;
3. Plant uptake;
4. Evapotranspiration (ET);
5. Microclimatic conditions.

The most important mechanism by plants is not the uptake but the physical effect of root structure combined with enhanced aeration (Brisson and Chazarenc, 2009) and other processes, including filtering, flow velocity reduction, improved sedimentation, decreased resuspension and even the distribution of water (Vymazal, 2011). Gagnon et al. (2013) found that plants created tunnels in the sludge cake and favoured sludge drainage and TW aeration.

The rhizosphere of plants improves the conditions for various microorganisms, which are key drivers in the biological treatment processes (Brix, 2017; Vymazal, 2011). Another key component of plants' effects in TWs is the roots release of liquid and gas (Armstrong and Armstrong, 1990), e.g. additional oxygen from roots improves degradation, supports heavy-metal sedimentation and increases nitrification.

The plant uptake importance has been under debate. Some authors (Brisson and Chazarenc, 2009; Vymazal, 2011) agree that the role of plant uptake in TWs is negligible, but other authors (Langergraber and Simunek, 2005; Tanner, 2001) found plant uptake to be significant as plants provide storage and uptake of nutrients - advantage of phytoremediation. Evapotranspiration, transfer of water to atmosphere, is generally an advantage in TWs and in humid environments plants help to accelerate the process of dewatering (Gagnon et al., 2013; Gregersen and Brix, 2001). On the other hand, in warm areas water loss in TWs may be fateful (Masi and Martinuzzi, 2007). The plants create the microenvironmental conditions in TWs, e.g. prevent algal growth, protect sediments from wind, insulate from radiation in the spring and from frost in the winter (Shelef et al., 2013).

2.10 The choice of plant species

Although, the logic by which plant populations affect treatment efficiency of TWs is still to be realized completely, there are suggestions to choose the suitable plant species for TWs. The author Tanner (1996) gives general requirements of plants suitable for use in TW wastewater treatment:

1. Ecological acceptability; i.e., no significant weed or disease risks or danger to the ecological or genetic integrity of surrounding natural ecosystems;
2. Tolerance of local climatic conditions, pests and diseases;
3. Tolerance of pollutants and hypertrophic waterlogged conditions;
4. Ready propagation, and rapid establishment, spread and growth;
5. High pollutant removal capacity, either through direct assimilation and storage, or indirectly by enhancement of microbial transformations such as nitrification (via root-zone oxygen release) and denitrification (via production of carbon substrates).

Tanner (1996) tested 8 different species (*Schoenoplectus talidus, Phragmites australis, Glyceria maxima, Baumea articulata, Bolboschoenus fluviatilis, Cyperus involucratus, Juncus effusus* and *Zizania latifolia*) suitability for TWs. Three species had the best result: *Zizania, Glyceria* and *Phragmites*. The first two species were suitable in warm temperate climates for year around vegetation. However, *Phragmites* remain standing over winter providing continues root-zone aeration. Many authors confirm the appropriateness of *Phragmites australis* for cold climate (Mander and Mauring, 1997; Noorvee et al., 2007; Pell and Wörman, 2011).

High potential growth, deep rhizome and root system, ready propagation and wide distribution of *Phragmites* have made it the most common plants in TW (Tanner, 1996), especially in Europe where *Phragmites australis* subsp. *australis* (referred to hereafter as "exotic Phragmites") is native species and wildly spread. However there are many regions where this subspecies of *Phragmites australis* is not native, e.g. North-America (Rodríguez and Brisson, 2015; Tanner, 1996).

Ecological acceptability is important to consider, because invasive species may represent a treat to local biodiversity (Rodríguez and Brisson, 2015). Basically the same matter, why exotic *Phragmites* is popularly used in TWs all over the world (high growth and intensive spread rate, dense stems, well developed rhizomes and roots system, an amazing survivor) cause the reason of European subspecies to be forbidden in Canada by law (MDDEP Québec, 2009). Since introduced in North-America in the early 1800s, the exotic *Phragmites* has expanded rapidly, causing the tolerate regime in wide range of hydrologic conditions by displacing the native vegetation and reducing animals diversity (Bohling, 2013; CABI, 2019). In addition of becoming monoculture, the plant obstructs roadside and agricultural ditches, block shoreline views and create a flammable situation due to its dry shoots (Rodríguez and Brisson, 2015). Therefore, it is important to find alternative TW plant species that would ensure similar benefits and efficiency as invasive European common reed.

One possible alternative to exotic *Phragmites* is the native common reed in North America - *Phragmites australis* subspecies *americanus* (referred to hereafter as "native Phragmites"). There have been few tests with native *Phragmites* used in TWs (Rodríguez and Brisson, 2015), mostly because this subspecies is introduce quite lately (Saltonstall et al., 2003). In overall native *Phragmites* seems to be "weaker" competitor to exotic subspecies in pollutant removal: the native produce less total biomass, has shorter shoots, smaller shoots density and less efficient nutrient uptake (League et al., 2006; Mozdzer et al., 2013; Price et al., 2013). Accordingly, it is essential to understand the suitability of using native instead of exotic *Phragmites* in TW in Canada.

According to different studies (Allen et al., 2002; Mander and Mauring, 1997; Noorvee et al., 2007; Rodríguez and Brisson, 2015) another commonly used species in TWs is *Typha latifolia*. For example, *Typha* had good results in nitrogen removal in winter (Riley et al., 2005).

As discussed in chapter 2.5, sludge treatment processes have two main purposes: dewatering and stabilization of the organic matter through microbial decomposition. The TW macrophytes help sludge treatment by filtering, aerating, transferring of nutrients and by creating improved microenvironmental condition for microbic processes (considered in chapter 2.9). By combining with the previous knowledge to advanced solution of adding saturated layer to single-staged VF TW system (talked in chapter 2.8), it is possible to develop cost-effective and environmentally friendly on-site methods for fish farm sludge management.

3. Material and Methods

3.1. Experimental set-up

The mesocosm experiment was conducted in the fish farm St-Alexis-des-Monts (Pisciculture St-Alexis-des-Monts inc. 46°27'29.7"N 73°08'32.5"W, in Saint-Alexis-des-Monts, Québec, Canada; Figure 7). This inland fish farm had a production capacity of 150 tons of fresh water trout per year (in 2011) and relies on raceway production (Figure A 1; Figure A 2). This fish farm is equipped with a hydraulic vacuum system (Figure A 3) for sludge removal and raceway cleaning, and three silos (i.e. settlers) for sludge storage and settling (i.e. solid/liquid separation from raw sludge).

Figure 7. The fish farm St-Alexis-des-Monts on aero photo with raceways inside the buildings and outside; sludge settling silos (round structures); and subsequent effluent treatment ponds before discharge to the river (Google Maps, 2010).

The experimental set-up (Table 3) consisted of twelve pilot units made of PVC columns (Figure 8). Each mesocosm was filled with four layers of granite gravel according to the typical particle size distribution (Table 3) that is employed in vertical flow constructed wetlands treating raw domestic wastewater in French systems (Gagnon et al., 2012; Paing et al., 2015).

Table 3. Experimental design parameters of the mesocosms and choice of plant species.

Type of experimental units	**Vertical flow mesocosms**		
Number of mesocosms	12		
Dimensions of mesocosms (diameter*height, m*m)	0.6*1.0		
Empty volume of the mesocosms (m^3)	0.28		
Height of filter material (m)	0.7		
Volume of filter material per mesocosm (m^3)	0.2		
Type of filter material	Granitic gravel		
Thickness of filter material layers (from top to bottom, m)	0.3; 0.1; 0.1; 0.2		
Particle size of filter material (from top to bottom, mm)	2.5 - 5.0; 10 – 12; 14 – 20; 20 - 40		
Water flow conditions	Vertical down-flow		
Water level in mesocosms (saturated layer, m)	0.45		
Void volume of saturated layer (m^3)	0.045		
Experimental periods (years)	May - Sept. 2012		May -Sept. 2013
Plant species	North-American (native) *Phragmites australis*	European (exotic) *Phragmites australis*	Unplanted control (originally planted with *Typha latifolia*)
Nb of mesocosms per plant species	4	4	4

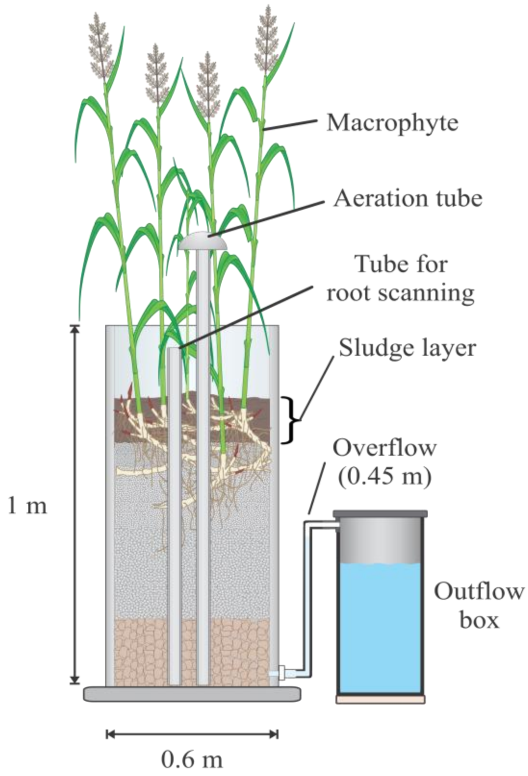

Figure 8. Schematic representation of an experimental mesocosm *(drawing: V.Gagnon).*

Three plant species (Table 3) were chosen and planted as monocultures in four replicates of TW mesocosms for each species. Plant's rhizomes were harvested from natural wetlands and planted in May 2011 with the same density, representing about 50-100 stems per mesocosm. The vegetation period of year 2011 was used as the establishment period for acclimatization and growth of the plants in the experimental conditions. The North-American native common reed (*Phragmites australis* subsp. *americanus*) will be referred to as 'Native' throughout this paper; the exotic and invasive European common reed (*Phragmites australis* subsp. *australis*) will be referred to as 'Exotic'. The mesocosms initially planted with NorthAmerican native broadleaf cattail (*Typha latifolia*) will be referred to as 'Unplanted' as there was no normal plant development of this species from spring 2012 when the experimental period started (with no above- or below-ground plant parts visible). After the plantation of mesocosms in spring and acclimatisation summer of year 2011 cattail showed initial growth (not comparable with common reeds), however, this species did not survive the winter 2011/2012.

The twelve columns were placed according to a randomized disposition to avoid any potential effect of the space placement (Figure A 4). During vegetation period of years 2012 and 2013 the mesocosms were insulated with thick layer of insulation wool covered with layer of weatherproof plastic film. This insulation prevented extreme temperature fluctuations during cold and warm periods (also overheating) of the aboveground mesocosms.

3.2 Feeding strategy

The raw sludge separated from effluent of raceways was produced twice a week during raceway cleaning, by harvesting all the solids with vacuum cleaners from the end of each raceway (Figure A 5). The raw sludge (Figure A 6) consisted of settled fish-feces and uneaten fish-food. It was characterized by high content in ammonium-nitrogen (NH_4-N), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD_{Cr}) and low content of nitrate (NO_3-N).

During the acclimatisation period from March to October 2011 the system was gradually inoculated with raw sludge to enable a good plant establishment. During this first vegetation period, the water level was kept high (0.6 m from the bottom of the mesocosms) in the filter material for the first three months. The raw sludge application concentration was gradually increased during the acclimatisation period.

In the first experimentation period, from May to September 2012, each unit was manually fed twice a week (on Mondays and Thursdays) by adding a batch of 45 L of effluent (hydraulic loading rate 380 L/m^2 per week) to each mesocosm (Table 4).

Table 4. Feeding strategies and loading rates of the mesocosms.

Year	**Unit**	**2012**	**2013**
Feeding	L per batch	45	90
Number of feeding events	times per week	2	1
Hydraulic loading rate	L m^{-2} $week^{-1}$	318	318
Mass loading rate per vegetation period	kg COD/m^2	7.89	10.29
	kg TSS/m^2	7.50	6.10
	kg VSS/m^2	6.42	4.68
	kg TP/m^2	0.26	0.25
	kg TKN/m^2	0.52	0.41

During the second vegetation period, from May to September 2013, the pilots were fed once a week with a batch of 90 L of influent raw sludge (hydraulic loading rate 380 L/m^2 per week) to test the full capacity of mesocosms to treat an increased load per batch. The feeding strategy of the vegetation period of 2012 also fits better the operating reality of most of the fish farms with vacuum cleaning systems.

At each feeding event (on Mondays and Thursdays), raw sludge was collected in one m^3 plastic tank, mixed and manually applied to the top of the mesocosms by using pump, hose and 20 L bucket. Influent sludge percolated vertically through the mesocosms, mixed with water of the hydraulically saturated layer in the bottom half of the mesocosms and through drainage pipeline and overflow system, the effluent was collected into 60 L tanks.

In the end of each feeding the effluent of the mesocosms were sampled and then the collection tanks were flushed and cleaned.

During winter period of years 2011-2012 and 2012-2013 the mesocosms were fed with settled sludge from the fish farm settling tanks. These experimental periods gave information about the performance of these systems in cold winter conditions and the effect of frozen sludge layer on top of the TW mesocosms on the system overall efficiency. However, in current thesis the results of these winter periods are not presented.

3.3. Sampling and analysis

Combined sample of the influent raw sludge was collected to separate tank during the feeding of the mesocosms. One litre of influent sludge was taken before feeding of each mesocosm. Total 12 L of influent sludge was collected and a sample was taken from this volume.

Homogenized water samples were taken from the outlet collection tanks of each mesocosm. In addition to influent sample, one litre of sample per mesocosm was stored in cooler, transported to the laboratory and analysed according to the standard methods (APHA, AWWA WEF et al., 2012) for parameters: TSS, VSS, COD, total phosphorus (TP), total Kjeldahl nitrogen (TKN), NH_4-N, and NO_3-N.

Plant development parameters were followed every week during the feeding periods, and height of shoots, stem density, plant colour, and presence of flowers were monitored. Furthermore, plant health was visually monitored for potential diseases, fungi and aphids' attacks.

For observation and comparison of the plant rhizosphere development of two common reed species, the root scanner (CI-600 In-Situ Root Imager; CID Bio-Science to take photographs 360°, total of 70 cm depth from the substrate surface to the bottom) was used inside the rhizotrons during the vegetation period (from April until August 2012). Scanning provided us with the 360 degrees pictures of below-ground plant parts. At the end of the vegetation periods the above-ground plant parts were harvested and dried. Total dry biomass weight per each mesocosm was measured. Thereafter, the above-ground biomass of each mesocosm was separated into three categories of organs: stems, leaves and flowers, then again weighed.

Data analysis and visualization was done with R (ver. 4.0.2) software and Microsoft Excel.

4. Results and discussion

4.1 Plant monitoring results

The plants' density was the same between exotic and native *Phragmites* in vegetation period of year 2012 (Table 5). In the second vegetation period (year 2013), native *Phragmites* stem density declined to half of its first-year density, whereas exotic *Phragmites* increased considerably. Native and exotic *Phragmites* both were shorter in length in 2013. The native remained taller than exotic during both two vegetation periods of experiment (Table 5), what is not usually to be expect from invasive nature of exotic *Phragmites* (Bohling, 2013; League et al., 2006; Mozdzer et al., 2013). Rodríguez and Brisson (2015) reached the same result were both exotic and native *Phragmites* stem length was significantly shorter (approx. 1 meter) and density about 20 times higher in the mesocosms feed also by concentrated sludge. An interesting similarity between current and Rodríguez and Brisson's study was the fact that native *Phragmites* stem length was longer in the experiments than in wild compared to exotic subspecies.

Despite the density increase, the dry biomass of exotic *Phragmites* decreased in 2013, what could be explained by the noticeable decrease in size and the absence of flower production in 2013 as reported in Table 5. The native *Phragmites* produced four times more flowers than exotic in 2012, but the flowering process seems inhibited for both reeds in the second year (Table 5). The increased density of exotic compared to native in 2013 confirms the invasive nature of exotic *Phragmites* (discussed in chapter 2.10). The rapid growth despite of the absence of flowering should be explained by the fact of efficient expansion of exotic *Phragmites* through the production and fragmentation of underground rhizomes (League et al., 2006; NOAA, 2021). The growth differences of two vegetation period could be also affected by variations between the seasonal conditions (e.g. temperature, amount of precipitation, length of the growing season) and also by the winter experimentation. However, winter period results are not discussed in detail in current work.

Table 5. Plant parameters (standard deviation in parenthesis; n=4 per species).

Year	**2012**		**2013**	
Subspecies of *Phragmites australis*	**Native**	**Exotic**	**Native**	**Exotic**
Stem density (stems.m^{-2})	673 (204)	688 (115)	365 (380)	950 (358)
Length (m)	1.83 (0.10)	1.60 (0.04)	1.33 (0.36)	1.20 (0.07)
Dry aboveground biomass (g.m^{-2})	3215 (1333)	2746 (479)	995 (996)	2320 (850)
Stems dry mass (g.m^{-2})	1907 (797)	1571 (261)	589 (562)	1272 (410)
Leaves dry mass (g.m^{-2})	1164 (421)	1159 (240)	392 (441)	1048 (440)
Number of flowers (total)	88	19	2	0

4.2 Changes in the physicochemical parameters of the sludge and mesocosm effluent water

The overall temperature inside the planted mesocosms (Table 6) dropped between 2012 and 2013 approx. 2 degrees. The inside temperature of unplanted mesocosms remained the same, what does reflect the official climate data tendency of the area for these periods (Figure A 8). This is explained by the fact that plants create a microenvironment and isolate TW system from outer influence, in this case the direct sunlight (discussed in chapter 2.9). Furthermore, the insulation layer around the mesocosms kept the inside temperature more stable and prevented overheating.

The dissolved oxygen (DO) presented a significant drop for the unplanted units in 2013. The correlation between DO and temperature should be negative (Rajwa-Kuligiewicz et al., 2015), which does not explain the drop of DO in unplanted mesocosms. It may be explained that an unknown factor reduced the overall drop of DO, but plants helped to keep the DO on the same level in both years.

Redox potential (Eh) could not be measured during the first year due to technical difficulties. Eh acquired in 2013 displayed high negative values (Table 6). Evapotranspiration (EvT) rate was higher in 2013, which may be caused by the change of sludge loading strategy to mesocosms in 2013 (described in chapter 3.1) from 45 L two times per week to 90 L once per week. This change gave the mesocosms a longer resting periods and the water in saturated layer a longer hydraulic retention time.

Table 6. Average *In situ* measurements of effluent in 2012 and 2013 periods (standard deviation in parenthesis).

	2012			2013		
Species	**Native**	**Exotic**	**Unplanted**	**Native**	**Exotic**	**Unplanted**
Temperature (°C)	19.7 (3.6)	19.5 (3.5)	19.1 (3.6)	17.5 (4.2)	17.8 (4.0)	18.8 (3.5)
pH	6.24 (0.26)	6.29 (0.18)	6.31 (0.18)	7.04 (0.82)	7.05 (0.78)	7.12 (0.90)
Dissolved Oxygen (mg L^{-1})	1.78 (0.29)	1.76 (0.23)	1.76 (0.24)	1.75 (0.65)	1.87 (0.73)	1.58 (0.51)
Redox potential (mV)	N/A	N/A	N/A	-136.7 (106)	-170.0 (125)	-196.0 (92)
Evapo-transpiration rate (L m^{-2} d^{-1})	19.62 (9.18)	16.33 (6.06)	11.71 (7.05)	24.68 (12)	22.14 (10)	21.65 (11)

The development of native and exotic *Phragmites*' roots throughout the vegetation period of 2012 is shown on Figure A 7. On average, exotic *Phragmites* rhizosphere was denser and grew deeper compared to native. The differences are even more considerable in the beginning of vegetation period (Figure A 7, see "May"), which refers to exotic *Phragmites* invasive nature (discussed in chapter 2.10). League (2006) found that exotic *Phragmites* starts putting up new shoots earlier than native. This fact coupled with noted flowering times suggests that exotic *Phragmites* benefits from longer vegetation season, allowing more time for growing (League et al., 2006). It would have been interested to study with root scanner the differences in roots development in 2013, as the exotic *Phragmites*' number of stems were increased, while native stems decreased and both had an absence of flowering (Table 5).

4.3 Removal efficiency of water pollutants

In Tables 7 and 8 the influent and effluent water parameters are presented for vegetation period 2012 and 2013, respectively. In Table A 2, the average pollutants removal efficiencies and sludge volume reduction are shown. Furthermore, the changes in the concentration of main water pollutants in time are presented in Annex Figure A 9. Overall, the raw sludge has relatively similar pollutants concentrations on both periods and the average effluent values of different types of the mesocosms are quite similar also. In general, the removal efficiencies were high and the hybrid VF TW mesocosms showed good performance (Table A 2). There seems to be no significant effect on the overall performance by the difference in the loading frequency and rate per week between the two vegetation periods. Previous studies have shown that the sludge loading rate (kg ds/m^2/year) represents a key parameter in STRB design, and mostly depends on the sludge quality and the climate (Nielsen and Stefanakis, 2020). When comparing with previous studies, current study is in accordance with its design and operation with others. According to Nielsen and Stefanakis (Nielsen and Stefanakis, 2020): "A typical pilot study would consist of 3–12 beds, each usually with an area of up to 2 m^2. A typical testing period lasts 4–12 months, although there are pilot experiments operated for up to 3 years". Similarly, to ours, such experiments usually aim to identifying the suitability of the feed sludge for its treatment in an STRB, the optimum SLR and length of the feeding and resting periods, the dewatering efficiency, the drained water (i.e. percolate) quality, and the plant growth (Nielsen and Stefanakis, 2020). Furthermore, the required number of parallel beds, the residual dewatered sludge quality and also the seasonal effects on performance (especially the effect of freezing and melting periods) can be studied (Nielsen and Stefanakis, 2020).

Table 7. Inlet and outlet pollutants characteristics and changes in sludge volume of the mesocosms according to the species and unplanted, after vegetation period in 2012.

Sampling location		**Volume (L/week)***	**TSS (mg/L)**	**VSS (mg/L)**	**COD (mg/L)**	**TKN (mg/L)**	**TP (mg/L)**	**NH_4-N (mg/L)**	**NO_3-N (mg/L)**
Inlet:	Sludge	90	1228	1051	1292	125	62	15	0.47
Outlet:	Native	64	235	170	644	53	18	28	0.54
	Exotic	67	241	175	677	58	19	34	0.44
	Unplanted	77	317	226	797	65	19	51	0.48

Note: *Sampling performed on Mondays from batch of 45L feeding per mesocosm.

Table 8. Inlet and outlet pollutants characteristics and changes in sludge volume of the mesocosms according to the species and unplanted, after vegetation period in 2013.

Sampling location		**Volume (L/week)**	**TSS** (mg/L)	**VSS** (mg/L)	**COD** (mg/L)	**TKN** (mg/L)	**TP** (mg/L)	**NH_4-N (mg/L)**	**NO_3-N (mg/L)**
Inlet	Sludge	90	1224	956	2240	111	67	16	5.05
Outlet	Native	56	270	201	937	47	28	21	9.00
	Exotic	57	294	213	905	53	28	26	9.54
	Unplanted	61	326	242	1188	56	27	34	6.52

Total suspended solids and volatile solids removal

The removal of solids was highly efficient, with a reduction of 80-84% of TSS and VSS at the outlet of both *Phragmites* mesocosms, while efficiency of unplanted mesocosms was a slightly lower 74-79% (Figure 9). There were no considerable differences between vegetation periods of 2012 and 2013 in solids removal (Table A 2, Figure A 9). The relevantly high content of VSS (Tables 7 and 8) from total solids indicates that the total suspended solids of fish farm sludge are mostly made of volatile (i.e. organic, degradable) solids (80% average) regardless of the year.

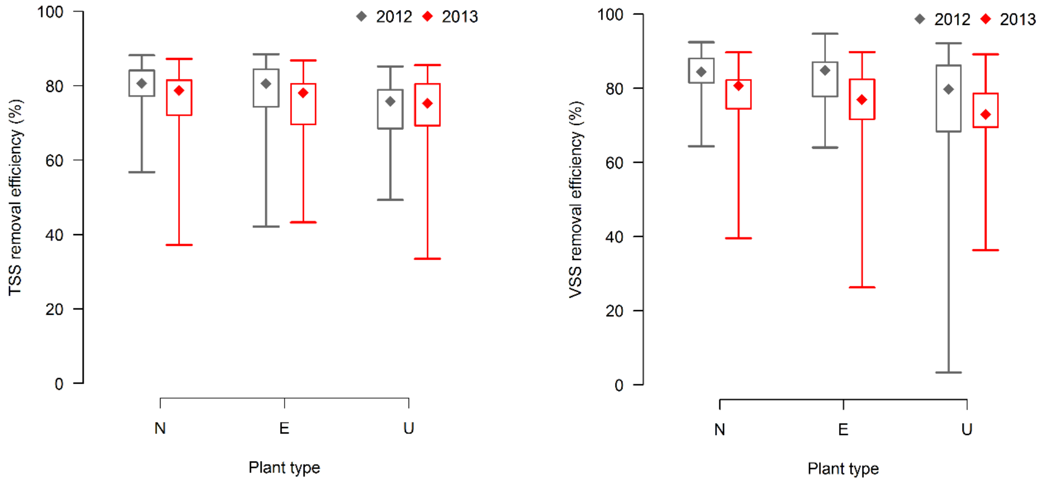

Figure 9. TSS and VSS removal efficiency for Native (N), Exotic (E) and Unplanted (U) mesocosms compared in vegetation periods of years 2012 and 2013.

The remarkable efficiency of solids removal can be explained by the effective filtration capacity of the mesocosms in the experiment. The solids in raw fish farm sludge (Tables 7 and 8, Figure A 9) are mainly in particulate form, and therefore remained efficiently on the surface of the mesocosm filter media. In addition, the plants create an extra dense network in between the filter media seen on root scanner photos (Figure A 7). The created saturated layer in experimental mesocosms favoured the high evapotranspiration efficiency by not letting all the influent sludge water (i.e. percolate) to discharged from the system (Gagnon et al., 2012).

Removal of organic matter

The raw sludge treatment efficiency on removal of COD (average influent and effluent values in Tables 7 and 8) was considerably good, with reduction of about 50% in 2012 and this increased to over 60% in 2013 (Figure 10). In 2013 there were increase in raw sludge COD values with high variability (Figure A 9). There is no markable difference between efficiency of exotic and native *Phragmites*, only a slightly higher removal by exotic reed in second year. The dissolved oxygen presents a significant drop for the unplanted units in 2013 (Table 6). By contrast, there is a bit more dissolved oxygen in the exotic *Phragmites* in the second year of the experiment and that could also affect organics removal. In comparison, the unplanted control unit's efficiency was lower: increased from 40% in 2012 to 50% in 2013 (Figure 10). The better efficiency of the planted treatment result could be linked to plant oxygen transfer to rhizomes (discussed in chapter 2.9). The overall efficiency increase of COD removal can be explained by thicker layer of sludge cake on top on the mesocosms substrate on the second year of the experiment, which probably also increased the mechanical filtering capability. Another relation between the increase in the quantity of waste in the inflow and the treatment efficiency increase refers to knowledge that the more waste the system receives, the more the system is capable to treat (Mozdzer et al., 2013; Rodríguez and Brisson, 2015).

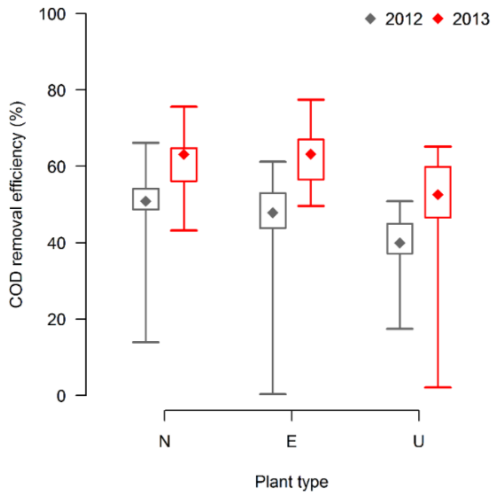

Figure 10. COD removal efficiency for Native (N), Exotic (E) and Unplanted (U) mesocosms compared in vegetation periods of years 2012 and 2013.

Removal of nitrogen

The removal efficiency of TKN (consisting of organic and ammonium-nitrogen) is 50-60% without significant differences in both vegetation periods of the experiment (Figure 11). The raw sludge inflow TKN was with high concentration variability (Figure A 9). There were no markable differences neither between *Phragmites* subspecies nor between planted and unplanted mesocosms, which makes this result interesting as the overall nitrogen removal should have been more successful in planted wetlands, especially with the additional saturated layer. Overall, in hydraulically unsaturated vertical downflow treatment wetlands the conditions are aerobic and favour nitrification of ammonium-nitrogen to nitrate (Kadlec and Wallace, 2008). However, in current setup with application of raw fish farm sludge, it brings nitrogen to the systems mostly in organic solid form (and partly as dissolved ammonium-nitrogen; Tables 7 and 8). It could be, that in our aerobic TW upper part the ammonification (transformation of organic nitrogen to ammonium-nitrogen; (Nature, 2021)) was primary process (Tables 7 and 8; Table A 2) and nitrification (ammonium-nitrogen transformation to nitrate;(Noorvee et al., 2007)) was taking place only partly(Tables 7 and 8; Table A 2). Secondly, in the lower hydraulically saturated bottom layer with anaerobic or anoxic conditions the produced nitrate is usually denitrified by microorganisms to gaseous nitrogen and removed from the treatment system (Noorvee et al., 2007). However, in these mesocosms there appeared to be no changes in nitrate concentration on 2012 and even though the concentrations are relatively low, there was significant NO_3-N increase when comparing influent and effluent values on 2013 (Table 8). Ammonium-nitrogen, in anaerobic/anoxic conditions could be going through anaerobic oxidation process (called ANNAMOX; (Jetten et al., 1998)), however, half of the TKN effluent concentration is only related to NH4-N and other half is still in form of organic nitrogen (Tables 7 and 8). Furthermore, the gained removal of TKN shows a complexity of the removal processes contributing to this parameter.

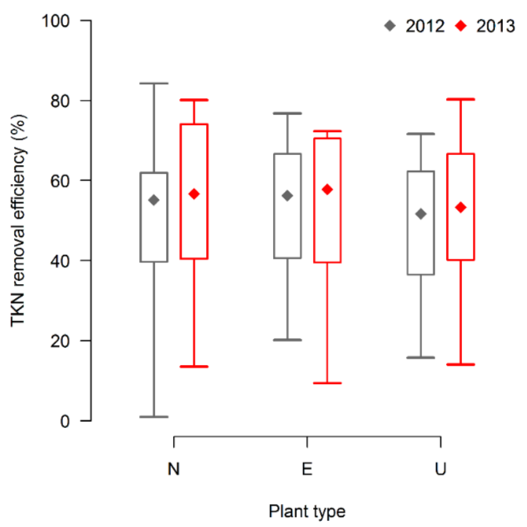

Figure 11. TKN removal efficiency for Native (N), Exotic (E) and Unplanted (U) mesocosms compared in 2012 and 2013.

Removal of phosphorus

Interestingly, relatively high TP removal rate over 70% was observed in planted and unplanted mesocosms in the first vegetation period (Table 7). In 2013, there was a decrease in efficiency below 60% of all mesocosms (Figure 12). The raw sludge inflow TP was with high concentration variability (Figure A 9). The overall good removal rate of TP could be explained by effect of saturated layer, which favoured evapotranspiration and lengthened the hydraulic retention time, allowing more time for pollutant removal by physical, chemical and biological processes (Gagnon et al., 2012), even in unplanted units. Furthermore, as most of the TP in raw sludge is in solid form, the sludge cake on top of the mesocosms can enhance the physical removal of phosphorus from the sludge. TP removal efficiency was similar in unplanted and planted mesocosms, which indicates that the removal process of TP was independent from plants. In treatment wetlands, the biological phosphorus removal by microbial processes and plants is usually quite low (Kadlec and Wallace, 2008) and therefore, our results with no significant differences between the planted and unplanted mesocosms were expected.

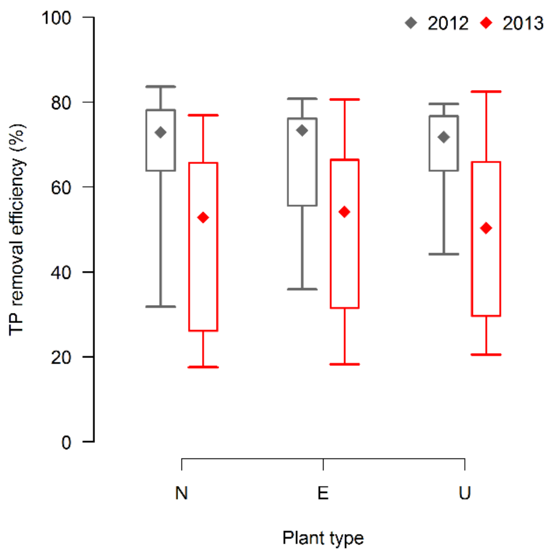

Figure 12. TP removal efficiency for Native (N), Exotic (E) and Unplanted (U) mesocosms compared in 2012 and 2013.

4.4 The role of plant species

Overall, previous research shows that plant species choice has significant importance and effect on treatment wetlands' performance (Brisson and Chazarenc, 2009).

Furthermore, previous studies have shown the importance of plants in sludge drying beds as they bring several benefits, e.g. better oxygen transfer to the drying beds, by movement of the stems the plants make cracks in the sludge cake and therefore, improve also hydraulic performance and prevent clogging of the top layer (Brix, 2017).

The invasive nature of exotic *Phragmites* outmatches the native subspecies in wild. The same scenario seemed to be logic in pollutants removal. On the contrary, the results of the experiment showed the opposite – *Phragmites australis* subsp. *americanus* seems to be appropriate alternative to *Phragmites australis* subsp. a*ustralis*. The level of pollutant treatment outcome is comparable in all tested parameters. Native *Phragmites* mesocosms were slightly more efficient at TSS and VSS removal, equal in TKN and TP treatment, and in the removal of COD exotic *Phragmites* performed a bit better. Only morphological differences in shoot density and development of roots the exotic reed had significantly better result. However, despite having shorter shoots in wild, native *Phragmites* had longer shoots in both years of the experiment. Rodríguez and Brisson (2015) highlighted also the unexpectedly good performance of the native reed in their study. They found similar or slightly higher performance of native *Phragmites* in pollutants removal. An idea for further research could be to test native and exotic *Phragmites* hybridized form's suitability and efficiency in TWs (Meyerson et al., 2010).

The overall performance of both *Phragmites* subspecies were the same. The mesocosms planted with native and exotic *Phragmites* outperformed the unplanted mesocosms in pollutants removal, which approves the fact that native *Phragmites* is suitable alternative macrophyte for TWs.

4.5 The correlation of conditions and ideas to be investigated

However, there are still a number of processes and relationships in sludge management TWs that are not fully understood: e.g., the role of plants in TWs and their effect on the removal efficiency; the correlation between the height of saturated layer and the efficiency; the effect of sludge loading rate or frequency and inflow concentrations on the removal efficiency.

Previous study done by Morvannou et al. (2017) took advantage of the fact that nitrification efficiency is influenced by the height of the unsaturated zone (also influenced by recirculation rate) and turned the traditional French two-staged wetland into single-staged wetland with hydraulically saturated bottom layer with high removal efficiency. One step extra to the previous enhancement is the automatic height regulation of saturated layer according to the collected data from inflow and outflow or according to the on-site measurements (e.g. temperature, precipitation, humidity, evapotranspiration, redox potential, dissolved oxygen, pH etc.) in fish farm RAS. It must be taken into consideration that this kind of futuristic approach needs much more awareness of the processes in the saturated and unsaturated layers but is certainly worth of further investigations.

There are more questions raised, for example what are the reasons behind the success in pollutants removal with native *Phragmites*, despite being outmatched in wild by the invasive *Phragmites*. Furthermore, it is unknown if native *Phragmites* would survive in full-scale TWs. Another interesting laboratory study proved that native and exotic *Phragmites* hybridized form is possible candidate as TW plant species (Meyerson et al., 2010). Exotic *Phragmites* expands rapidly in North America and comes more and more into contact with novel populations of native Phragmites. The potential for interbreeding exists in the wild as the flowering period of both subspecies' overlap. Thus, if hybridized form may outstand the invasion of exotic *Phragmites* and could have even higher removal rates of pollutants, "new star" could be born. On the other hand, interbreeding may have opposite result what could mean even more aggressive form of exotic *Phragmites*, which is fortunately uncommon according to Mayarson et al. (2010).

Conclusion

The main objectives of this work were to: a) develop cost-effective and environmental-friendly methods for on-site fish farm sludge management by treatment wetland technology; b) determine if already proven but invasive TW species –*Phragmites australis* subsp. *australis* (European common reed) – could be replaced with North American native *Phragmites australis* subsp. *americanus*.

The technical solution investigated in this project consists in the use of vertical flow treatment wetlands (TWs) – sustainable and environmentally-friendly systems that require minimal energy and maintenance for the treatment of a variety of wastewaters and sludges.

Overall, the hybrid vertical flow TWs showed high efficiency in raw fish farm sludge dewatering and quite high performance in removal of organic matter and nutrients. The addition of hydraulically saturated layer in TWs supported absorption of pollutants by longer retention time. The role and the choice of plant species found to be important factor to affect sludge dewatering and mineralisation and the general fate of water treatment in TWs. The presence of plants in the mesocosms created tunnels in the sludge cake and favoured sludge drainage and aeration in planted mesocosm. However, the absence of considerable differences between performance of planted and unplanted mesocosms needs further investigation.

There were no clear differences determined between the efficiency of tested common reed (*Phragmites australis*) sub-species and unplanted mesocosms in sludge dewatering and effluent quality improvement. However, the results confirm that the North American native common reed – *Phragmites australis* subspecies *americanus* – was proven to be suitable alternative to invasive Eurasian *Phragmites* for using in treatment wetlands.

As the experimental study of this work presents the results of a mesocosm, we have to be conservative about interpretation of the results. Further studies should be done in a full-scale TW to validate the findings in this mesocosm experiment: the native Phragmites successful performance in mesocosm experiment should be tested in full-scale study; the reliability of this method should be tested in larger scale in cold conditions throughout the winter months.

In addition, the sub-aim of this work was to provide a brief overview of the types of freshwater inland fish farms in Estonia and Canada (with some examples) and to compare the most widely used treatment wetland technologies developed for high solids content wastewaters and for sludge treatment. Surveys show that trout farms in both countries use similar farming technologies as well as methods to reduce water pollution from farms. A comparison of sludges of different origins showed that the raw material sludge from fish farms is similar in composition to the raw sludge from activated sludge treatment plants. The efficiency of sludge dewatering and its residual water treatment achieved in the modified vertical flow treatment wetland tested in this work was also comparable to the efficiency of other similar treatment wetland technologies, such as activated sludge dewatering treatment wetlands and so-called French systems.

Summary in Estonian

Viimastel aastakümnetel on sisemaa kalakasvanduste arv oluliselt kasvanud üle kogu maailma. Sisemaal asuvad kalakasvandused põhjustavad keskkonnaprobleeme, kuna eraldavad veekogudesse arvestatava koguse saasteaineid. Käesolevas töös uuritud tehniline lahendus seisneb vertikaalvooluliste tehismärgalade kasutamises kalakasvanduste muda tahendamiseks ja puhastamiseks.. Tehismärgalad on efektiivsed, jätkusuutlikud ja keskkonnasõbralikud süsteemides, mis nõuavad minimaalselt energiat ja hooldust.

Magistritöö põhieesmärk oli: a) arendada välja kohapealne meetod kalakasvanduse reoveemuda käitlemiseks ja töötlemiseks modifitseeritud osaliselt hüdrauliliselt küllastunud vertikaalvooluliste tehismärgalade abil; b) teha kindlaks, kas tõhusa tehismärgaladel laialdaselt kasutuses oleva taimeliigi *Phragmites australis* alamliik *australis* (Põhja-Ameerikas invasiivne liik) saaks asendada Põhja-Ameerika kohaliku *Phragmites australis* alamliik a*mericanus*'ega.

Töös hinnati hüdrauliliselt küllastunud vertikaalvooluliste tehismärgala katsekehade efektiivsust kalakasvatuse reoveemuda käitlemisel ja selle nõrgvee puhastamisel kahel järjestikusel vegetatsiooniperioodil. Uurimus viidi läbi Kanadas, Quebeci provintsi St-Alexis-des-Monts forellikasvatuses kokku kaheteiskümne katsekeha abil, milledest neli olid taimestatud invasiivse pillirooga, neli kohaliku pillirooga ja neli katsekeha jäeti taimestamata. Katsekehasid koormati kohalikust kalakasvandusest kogutud toormudaga. Analüüsiti katsekehade sisse- ja väljavoolu veekvaliteeti (põhiparameetrid: hõljuvaine, keemiline hapniku tarve, Kjeldahli üldlämmastik ja üldfosfor) ja jälgiti taimede arengut.

Kokkuvõttes saavutasid tehismärgala katsekehad vegetatsiooniperioodil väga hea hõljuvaine (üle 80%) ning heaorgaanilise aine eemaldamise efektiivsuse (KHT 50–70%), mis aja jooksul paranes, sest muda hulk katsekehade pinnal kasvas ja seega ka nende filtreerimisvõime paranes. Kjeldahli üldlämmastiku ja üldfosfori eemaldamine mudast ja selle nõrgveest oli vahemikus 50-60%.

Hariliku pilliroo (*Phragmites australis*) alamliikide ja taimestamata katsekehade reoveemuda veetustamise ehk tahendamise ja katsekehade väljavoolude veekvaliteedi parandamise efektiivsuses ei olnud selgeid erinevusi. Uuringu tulemustest järeldati, et

Kanada kohalik hariliku pilliroo alamliik võib asendada tehismärgalades populaarse Põhja-Ameerikas invasiivse alamliigi, kuna nende reoveemuda käitlemise efektiivsus oli sarnane. Antud katses saadud tulemuste kinnitamiseks tuleks teha täiendavaid ulatuslikke uuringuid, et hinnata taimede mõju ja rolli tehismärgalades pikema aja jooksul, sh külmal perioodil.

Lisaks oli käesoleva töö alam eesmärk anda lühiülevaated Eesti ja Kanada siseveekogude magevee kalakasvanduste tüüpidest (koos mõnede näidetega) ning võrrelda enim kasutatud tehismärgala tehnoloogiaid, mis on välja arendatud hõljuvainerikka reovee ja muda käitlemiseks. Ülevaated näitavad, et mõlemas riigis kasutatakse forellikasvandustes nii sarnaseid kasvatustehnoloogiaid kui ka kasvandustest tuleneva veereostuse vähendamise meetodeid. Erinevat päritolu mudade võrdlus näitas, et kalakasvanduste toormuda on oma koostiselt sarnane aktiivmudapuhastite toormudale. Samuti oli töös katsetatud modifitseeritud vertikaalvoolulistes tehismärgala katsekehades saavutatud muda tahendamise ja selle jääkvee puhastamise efektiivsus võrreldav teiste sarnaste tehismärgala tehnoloogiate, nagu näiteks aktiivmuda tahendamise tehismärgalade ja olme toor-reovett puhastavate vertikaalvooluliste tehismärgalade (nn. *French systems*), efektiivsusega.

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Annex

Figure A 1. The outdoor raceways of fish farm Pisciculture St-Alexis-des-Monts.

Figure A 2. The outdoor raceways of fish farm Pisciculture St-Alexis-des-Monts.

Figure A 3. The hydraulic vacuum system of fish farm Pisciculture St-Alexis-des-Monts.

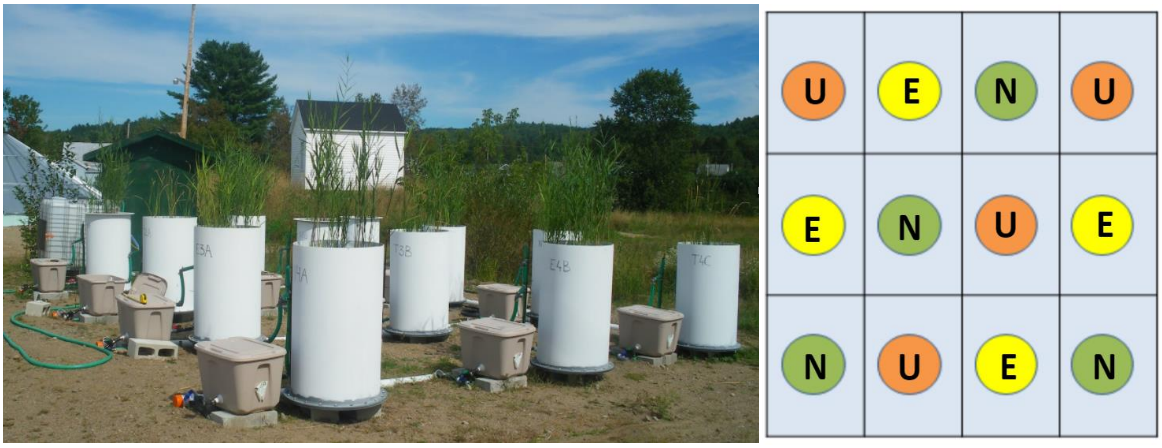

Figure A 4. Picture of the experimental setup in the fish farm and randomized location of each mesocosm in the experimental setup: E - Exotic; N - Native; U – Unplanted.

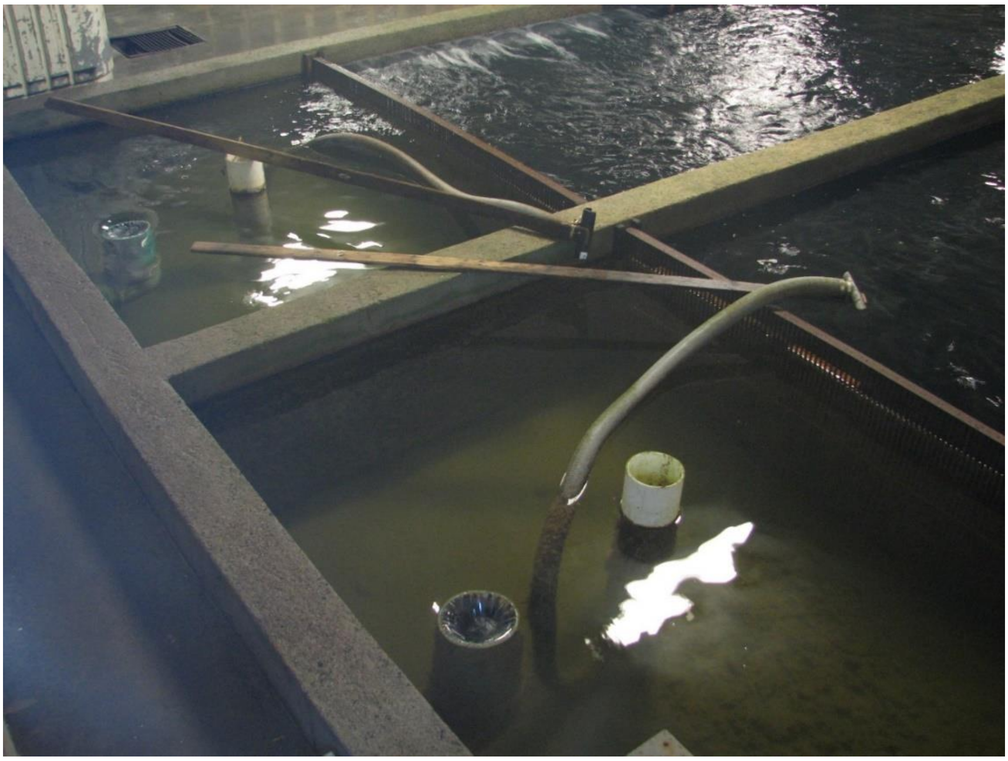

Figure A 5. Cleaning the end of raceway with vacuum system in the fish farm Pisciculture St-Alexis-des-Monts.

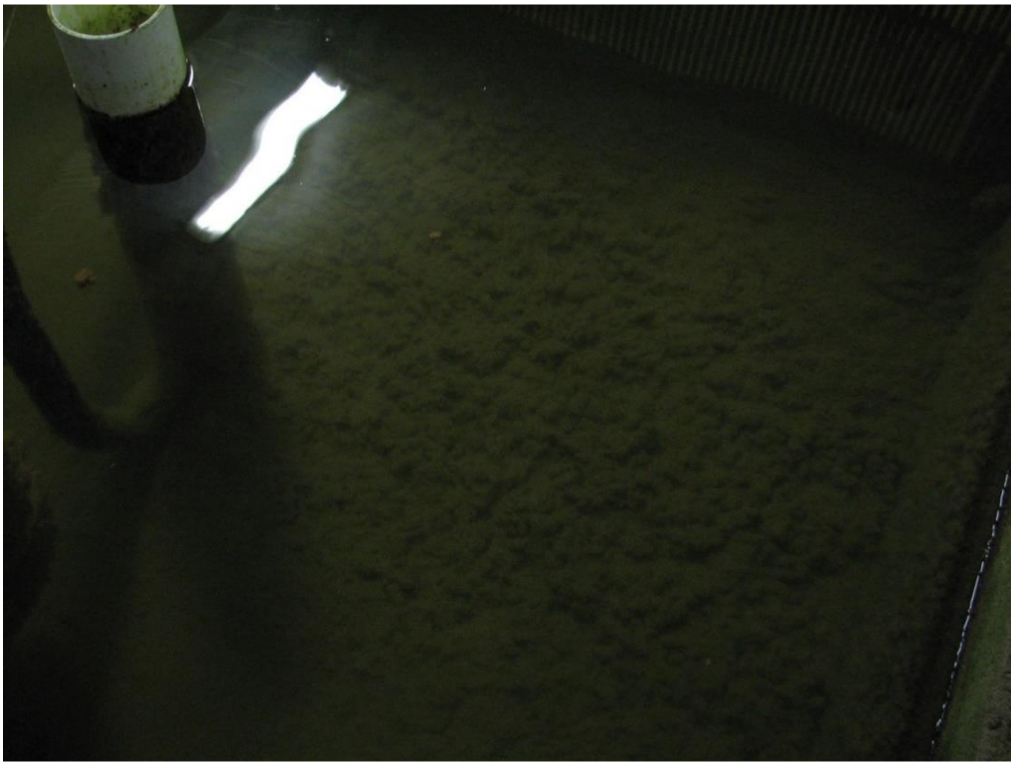

Figure A 6. The raw sludge in the end of the raceway system in the fish farm Pisciculture St-Alexis-des-Monts.

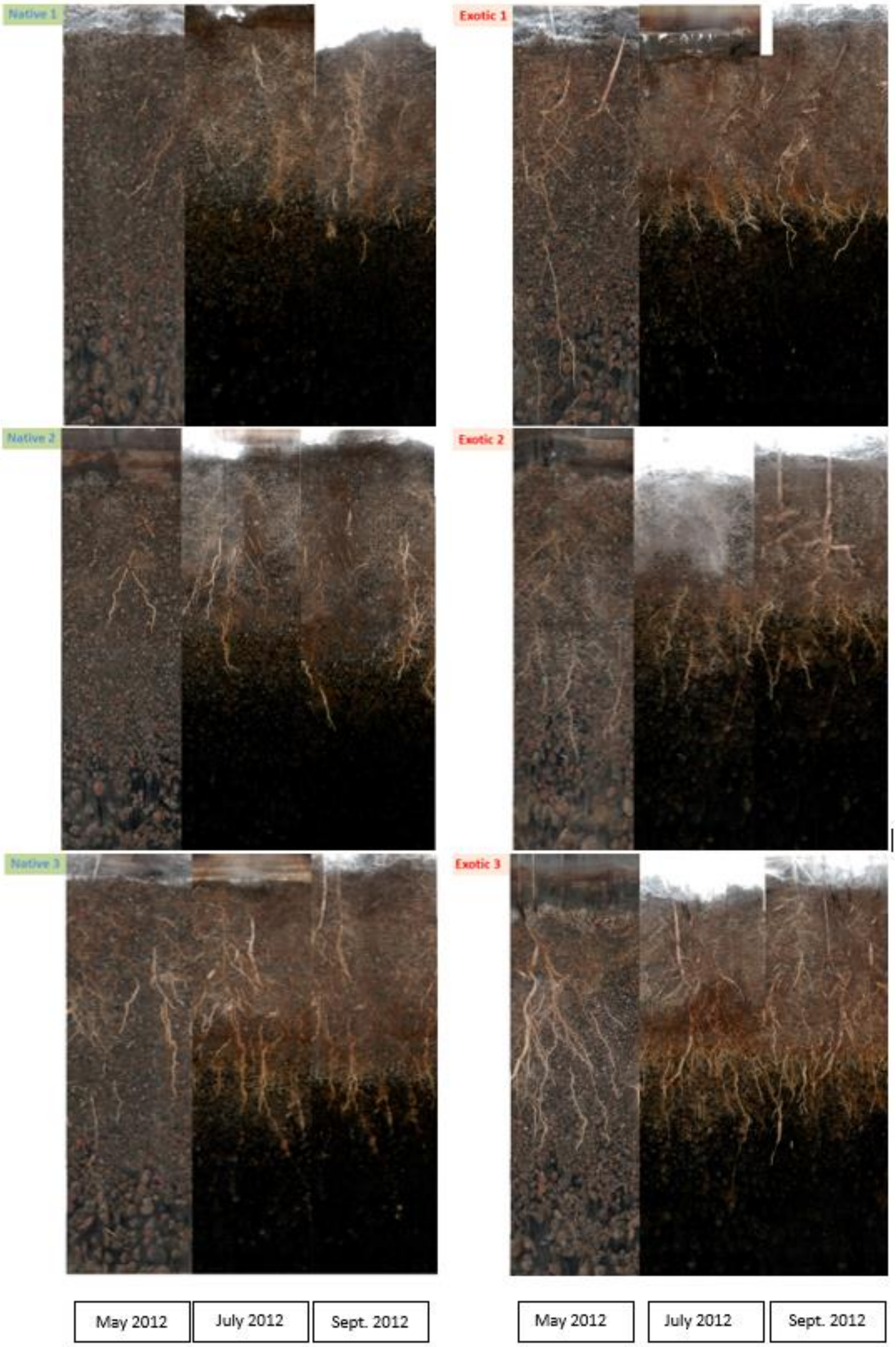

Figure A 7. Rhizospheres of Exotic and Native *Phragmites australis* subspecies along the growing period (May to Sept. 2012).

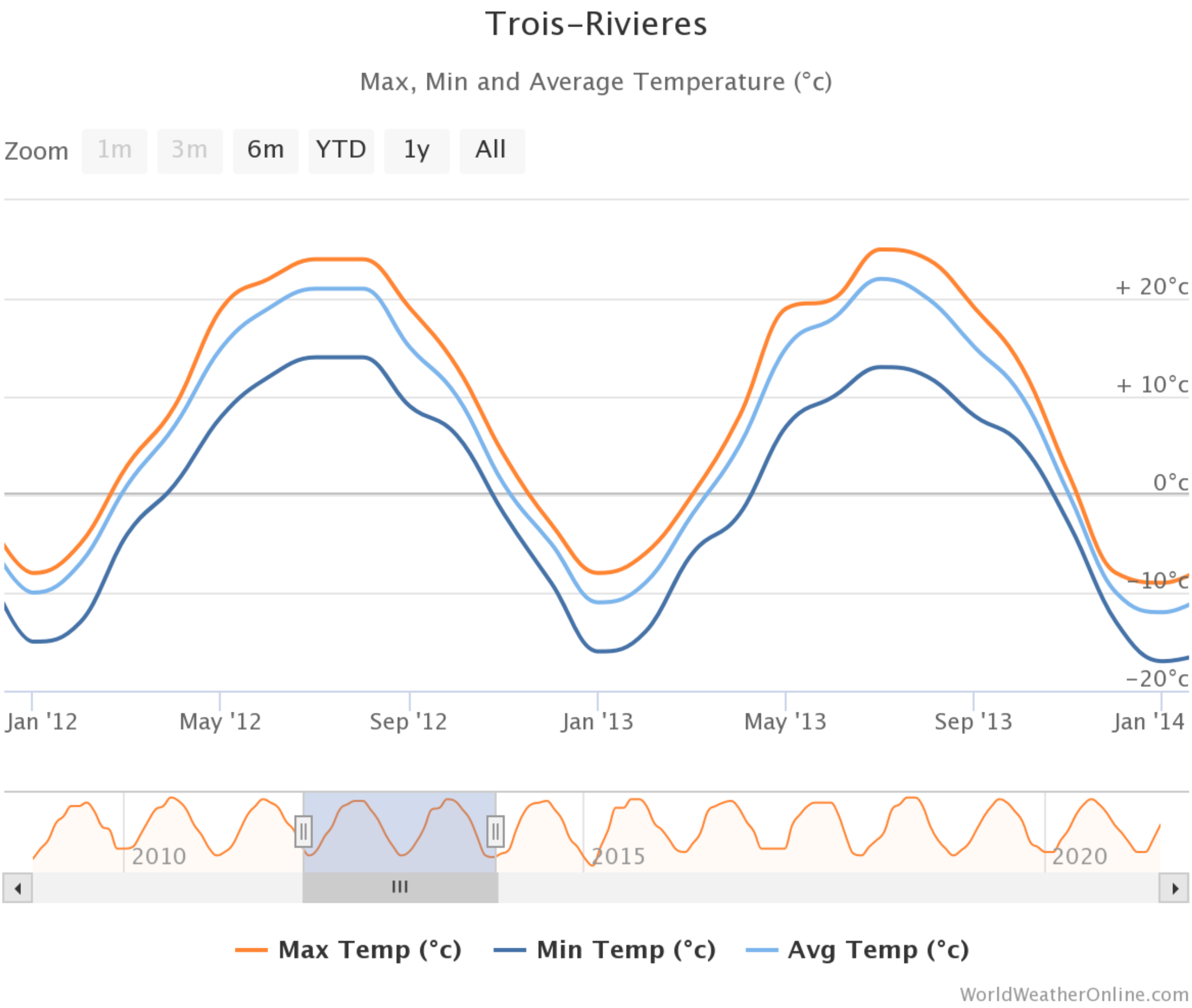

Figure A 8. Maximum, minimum and average temperatures in Trois-Rivieres (60 km from Saint-Alexis-des-Monts), Quebec 2012-2013 (WWO, 2021).

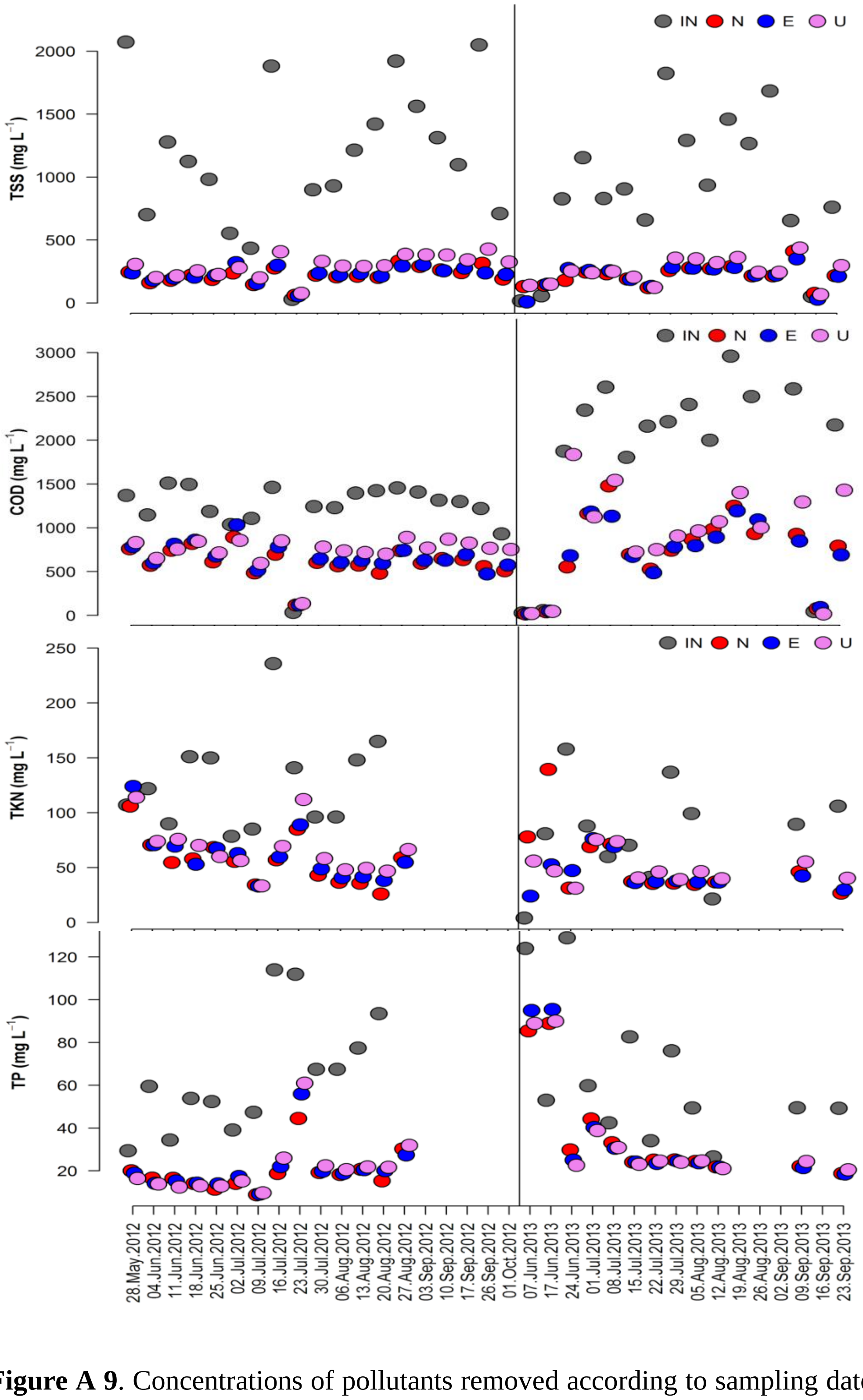

Figure A 9. Concentrations of pollutants removed according to sampling dates for the two vegetation periods. Vertical line indicates the change in years. Abbreviations: IN – influent; N – native

Table A 1. Examples of different treatment wetland systems for sludge management.

Treatment type	Study type	Size (m^2)	Sludge/ Wastewater type	Location	Water content (%)	TSS (%)	COD (%)	TN (%)	TP (%)	Reference
STRB (VF)	Full-scale	495	Municipal	USA	93-97	98	99**	90***	80	(Begg et al., 2001)
STRB (VF)	Mesocosm	1	Food industry activated sludge	France	N/A	99	98-99	89-99	99	(Korboulewsky et al., 2012)
French system	Full-scale	1030	Municipal wastewater	France	N/A	96	94	57	96	(Merlin et al., 2002)
STRB with Phragmites	Mesocosms	(4x) 0.3	Aquaculture sludge	Canada	98	99	99	99*	N/A	(Gagnon et al., 2012)
STRB with Typha	Mesocosms	(4x) 0.3	Aquaculture sludge	Canada	83	99	99	99*	N/A	(Gagnon et al., 2012)
STRB unplanted	Mesocosms	(4x) 0.3	Aquaculture sludge	Canada	52	99	99	94*	N/A	(Gagnon et al., 2012)
VF TW	Mesocosms	(3x) 4.4	Aquaculture sludge	USA	N/A	97	91	89	90	(Summerfelt et al., 1999)
HF TW	Mesocosms	(3x) 4.4	Aquaculture sludge	USA	N/A	86	72	86	82	(Summerfelt et al., 1999)

Note: *Measured as TKN; **Measured BOD_5;***nitrate-nitrogen

Table A 2. Pollutants' removal efficiency and volume reduction (%) differences between years 2012 and 2013.

Species	Volume reduction		TSS		VSS		COD		TKN		TP		NH_4-N		NO_3-N	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Native	29	37	81	78	84	79	50	58	57	58	71	58	-87	-31	-14	-78
Exotic	25	37	80	76	83	78	48	60	53	52	70	59	-127	-59	6	-89
Unplanted	15	32	74	73	79	75	38	47	48	49	69	59	-243	-108	-1	-29

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01.06.2021