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PERFORMANCE OF AN INDOOR WETLAND SYSTEM TRETATING BLACKWATER

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Abstract

Reclaimed water reuse is becoming an increasingly relevant topic in the context of urbanization, water scarcity risk and finite natural resources. Urban centres are by far the biggest consumers of energy, water, and raw materials and as such they produce a big amount of “urban metabolites”. With the help of nature-based technologies the latter can be recycled and reused, contributing to a cleaner environment and a viable economy.

The current study examines the performance of an indoor horizontal flow (HF) wetland, treating blackwater, installed in a single-family house in the city of Vienna. The system consists of three planted HF beds in series with a hydraulic loading rate 2.3 $cm.d^{-1}$ and an organic loading rate 67.5 g BOD_5 $m^{-2}.d^{-1}$. An UV/VIS spectrometer was calibrated for the specific blackwater matrix. Spectrometer measurements showed mean effluent values and removal rates for the HF wetland as follow: COD 45 mg/l and 70%, BOD_5 14 mg/l and 39%, TSS 16 mg/l and 70%, NO_3-N 24.3 mg/l, TOC 9.9 mg/l and 67%, DOC 8.0 mg/l and 55%, and TU 1.3 NTU and 71%. Effluent concentrations and the respective reduction rates of ammonium nitrogen, orthophosphates, and potassium were measured at 86.7 mg/l and 47%, 23.5 mg/l and 39%, 60.8 mg/l and 0%. Most of the organic matter and TSS removal took place in the first bed. COD:BOD_5 ratio was 4, one of the reasons the system is prone to clogging. Nitrogen was converted but not removed from the system. The pathogen indicators total coliforms*, E. coli and Enterococci* have been reduced by 1.3, 3.5 and 2.8 log_{10}, respectively. The effluent meets the EU regulation on water reuse for agriculture irrigation crop classes with lower risk for human health. When the pre-treatment is included, COD, BOD_5 and TSS meet the EU and Austrian concentration limits for surface water discharge and treatment efficiency.

Kurzfassung

Die Wiederverwendung von gereinigtem Abwasser wird im Zusammenhang mit der zunehmenden Urbanisierung, dem Risiko der Wasserknappheit und den endlichen natürlichen Ressourcen ein immer relevanteres Thema. Urbane Zentren sind die größten Verbraucher von Energie, Wasser und Rohstoffen und als solche produzieren sie eine große Menge an "urbanen Stoffwechselprodukten". Mit Hilfe von naturbasierten Technologien können letztere recycelt und wiederverwendet werden und so zu einer sauberen Umwelt und einer lebendigen Wirtschaft beitragen.

Die Masterarbeit untersucht die Leistung eines Indoor horizontal durchströmten (HF) bepflanzten Bodenfilters zur Behandlung von Schwarzwasser. Die Versuchsanlage wurde in einem Einfamilienhaus in Wien installiert. Das System besteht aus drei bepflanzten HF-Betten in Serie. Die hydraulische Belastung betrug 2,3 $cm.d^{-1}$ und die organische Belastung 67,5 g BSB_5 $m^{-2}.d^{-1}$. Ein UV/VIS-Spektrometer wurde für die spezifische Schwarzwassermatrix kalibriert. Die Spektrometermessungen ergaben folgende Ablaufwerte und Entfernungsraten für die Anlage: CSB 45 mg/l und 70%, BSB_5 14 mg/l und 39%, TSS 16 mg/l und 70%, NO3-N 24.3 mg/l, TOC 9,9 mg/l und 67%, DOC 8.0 mg/l und 55%, und TU 1,3 NTU und 71%. Die Ablaufkonzentrationen und Reduktionsraten von Ammonium-Stickstoff, Orthophosphaten und Kalium betrugen jeweils 86,7mg/l und 47%, 23,5 mg/l und 39%, 60,8 mg/l und 0%. Der größte Teil der Entfernung von organischen Stoffen und TSS fand im ersten Bett statt. Das CSB:BSB_5-Verhältnis beträgt 4, einer der Gründe, warum das System anfällig für Verstopfungen ist. Stickstoff wurde umgewandelt, aber nicht aus dem System entfernt. Die Indikatororganismen Gesamtcoliforme, *E. coli* und Enterokokken wurden um 1,3, 3,5 bzw. 2,8 log_{10} reduziert. Das gereinigte Schwarzwasser erfüllt die EU-Verordnung zur Wasserwiederverwendung für landwirtschaftliche Bewässerungsklassen mit geringerem Risiko für die menschliche Gesundheit. Unter Einbeziehung der Vorreinigung erfüllen CSB, BSB_5 und TSS die EU und österreichischen Konzentrationsgrenzwerte für die Einleitung in Oberflächengewässer und die Reinigungsleistung.

Abbreviations and acronyms

BOD_5	Biological Oxygen Demand in 5 days
CE	Circular Economy
CFU	Colony Forming Units
CI	Confidence Interval
COD	Chemical Oxygen Demand
DOC	Dissolved Organic Carbon
EC	European Commission
EEA	European Environmental Agency
EEC	European Economic Community
EGC	Effluent Global Calibration
Eq.	Equation
EU	European Union
GWGC	Groundwater Global Calibration
HDT	Hydraulic Detention Time
HF	Horizontal Subsurface Flow
HLR	Hydraulic Loading Rate
HRT	Hydraulic Retention Time
IGC	Influent Global Calibration
IPCC	Intergovernmental Panel on Climate Change
IQR	Interquartile Range
IWA	International Water Association
K	Potassium
LOQ	Limit of Quantification
N	Nitrogen
NBS	Nature-based Solutions
NH_4-N	Ammonium-nitrogen
NO_3-N	Nitrate-nitrogen
NTU	Nephelometric Turbidity Unit
OLR	Organic Loading Rate
P	Phosphorus
p.e.	People Equivalent
PI	Prediction Interval
PO_4-P	Orthophosphate
RO	Reverse Osmosis
RSE	Residual Standard Error
SIG	Siedlungswasserbau, Industriewasserwirtschaft und Gewässerschutz
SSF	Sub-surface Flow
TC	Total Coliforms
TOC	Total Organic Carbon
TSS	Total Suspended Solids
TU	Turbidity
TW	Treatment Wetlands

UDT	Urine-diverting Toiles
US EPA	United States Environmental Protection Agency
UV/VIS	Ultraviolet/Visual
VF	Vertical Flow
WFD	Water Framework Directive
WHO	World Health Organization
WWTP	Wastewater Treatment Plant

1. Introduction

Water in Europe is relatively abundant – only 13% of the available water resources are abstracted each year (EEA, 2009). However, they vary greatly in space and time. All Mediterranean countries are affected by water scarcity. Densely populated countries like Germany, Poland and England have the lowest water availability per capita in the EU (EC, 2007). Between 1976 and 2006 the number of areas affected by droughts in Europe went up by almost 20% and the total costs of droughts amounted to 100 billion € (EC, 2012a). Droughts are likely to intensify in southern and central Europe and the Mediterranean area (IPCC, 2012). Water exploitation index, as an indicator of the level of pressure that human activity exerts on the natural water resources shows high water scarcity risks in large parts of the Mediterranean area, but also in parts of Western and Eastern Europe (EEA, 2012).

After a public consultation in 2012, the EU recognized that treated wastewater reuse, having lower environmental impact than other alternative water supplies like desalination, should be encouraged (EC, 2012b). As a result, a regulation on water reuse that provides minimum water quality requirements for agricultural irrigation came out in 2020 (EC, 2020). Agriculture uses 69% of all consumed water, which makes it the most water demanding sector in Europe. For comparison, the public supply consumes only 13% (EC, 2007). In addition to alleviating water scarcity, treated water reuse could contribute to carbon emissions reduction thanks to the energy saved through less extraction and transport, less treatment for drinking water supply and less transport and treatment of the wastewater.

In 2015 EU adopted the circular economy concept as part of its new agenda for sustainable growth and one of the main components in the European Green Deal. It has been a response to other initiatives relating to waste management, recycling, reuse, and CO_2 emissions reduction, sharing the concept of closed loops. Based on different definitions Geissdoerfer et al. (2017) summarize the Circular Economy as a „*regenerative system in which resource input and waste, emission, and energy leakage are minimised by slowing, closing, and narrowing material and energy loops. This can be achieved through long-lasting design, maintenance, repair, reuse, remanufacturing, refurbishing, and recycling*“. The first EU CE Action plan prioritizes, among others, the reuse of treated wastewater as a means of increasing the water supply and alleviating pressure on water resources in EU, as well as a contribution to nutrients recycling (EC, 2015). Different sources of treated wastewater can be used for one or more purposes, e.g. aquatic systems restoration, aquifer recharge, process or cooling water for the industry, parks irrigation, streets cleaning, toilets flushing (EC, 2016). The CE aims to keep resources and products at their highest value for as long as possible, as well as to include the reuse and recycling already in the products/systems design. Decentralized technologies, like TWs fit well in the circular economy concept. They are preferred option in remote and rural areas where the centralized systems would be economically inefficient but can find certain applications also in the urban environment. The advantages of treating wastewater at the spot are lower dilution by storm water and groundwater intrusion and therefore reduced energy, chemicals and infrastructure needed to treat the water. One of the first to suggest a concept for avoiding centralized sewerage system of a settlement of 300 inhabitants was Otterpohl et al. (1997). The system suggested included domestic water separation and treatment in semi-centralized plants, nutrients recovery, storm water retention and infiltration.

The water reuse has been only 2.4 % of the treated effluent in Europe, most of it in Spain and Italy (MED-EUWI, 2007). Despite the existence of numerous successful applications, water reuse practice is still not very common mainly because of increased capital and operational costs, potential risk of lower water quality, public perception, regulatory framework and engineering issues (Voulvoulis, 2018; West et al., 2016).

Introduction

Domestic wastewater is a resource rich in water, energy, and plant nutrients. The largest portion of the domestic flow is the greywater stream. While greywater is seen as an option for retrieving water, the blackwater is usually perceived as a source of energy and nutrients. Its share in the domestic water flow ranges from 40 to 9.6% (DWA, 2014) depending on the sanitation technology, but can be as low as 5,5% when vacuum toilets are used (Graaff, 2010; Todt et al., 2015). Energy can be produced from the solid part and the liquid part contains most of the nutrient. Before the liquid part can be used, however, it needs to be treated to meet certain hygienic standards.

Urban areas are the biggest consumer of energy and resources and therefore the biggest producer of secondary materials. Currently half of the human population lives in cities, producing 75% of the carbon emissions and consuming more than 75% of the world’s natural resources (UN, 2018). In the current thesis, the blackwater treatment efficiency of a 3-bed indoor wetland treatment system was tested. The system is in the city of Vienna in the laboratory of alchemia-nova Institute for innovative phytochemistry and closed loop processes GmbH. The experiment was part of their project HOUSEFUL whose main goal was to develop and demonstrate circular solutions for the housing sector. The project is expected, among others, to recycle more than 90% of the rainwater, greywater and blackwater for production of reclaimed water and biogas. The HOUSEFUL project is funded within the EU’s Horizon 2020 research and Innovation programme. The experiment lasted from February to October 2020.

2. Objectives

On-site water treatment is common for remote areas and for smaller communities that lack the infrastructure connecting them to centralized treatment system. In the light of resources use efficiency, closed loops concept and water scarcity, decentralization and (re-)use of local resources have been gaining popularity. On-site solutions are not seen any more only as a necessary alternative but rather as an opportunity to contribute to cities' sustainability.

An indoor treatment system designed by alchemia-nova GmbH was installed at a single-family household to study the possibilities for different domestic wastewater streams treatment. In the current work the liquid part of source separated blackwater stream of the domestic water was tested. The objective of the thesis was to:

- Investigate the performance of the horizontal flow indoor treatment wetland in its efficiency to treat blackwater and evaluate the potential uses of the treated blackwater.

For achieving the objectives, the following steps were taken:

- Analysis of the organic matter elimination along the system described by Biological oxygen demand (BOD_5), Chemical oxygen demand (COD), Total organic caron (TOC), Dissolved organic carbon (DOC)
- Analysis of reduction of Total suspended solids (TSS) and Turbidity (TU)
- Analysis of removal and conversion efficiencies of nutrients – nitrogen (N), phosphorus (P) and potassium (K)
- Analysis of the reduction of microbial contamination described by pathogen indicators and culturable microorganisms.

For measuring the BOD_5, COD, TOC, DOC, Nitrate-nitrogen (NO_3-N), TSS and TU an Ultraviolet/Visual (UV/VIS) spectrometer was used. A calibration of the spectrometer was done for the specific blackwater matrix. Three different factory integrated global calibrations were refined using multipoint linear models' local calibrations. For the latter 18 reference samples were tested in parallel in the SIG laboratory. Additionally, nutrients in the form of ammonium nitrogen, orthophosphates and potassium were measured with photospectrometer.

For examining hygienic standards of the treated water, pathogen indicators total coliforms, *E. coli* and *Enterococci* were analysed. The general microbial removal efficiency was tested with culturable microorganisms at 22°C and 37°C.

Structure of the thesis

Chapter three describes the characteristics of the blackwater as part of the domestic wastewater flow and its organic matter concentrations and nutrients content. It presents the treatment wetland systems in the context of NBS and their functioning in terms of organics and nutrients removal mechanisms. The chapter ends with an overview of existing documents regulating the wastewater reuse, treatment trains for enhancing water quality, possible applications of the treated water.

Chapter four describes the design of the experiment, measurement instruments, calibration process, methods for analysing the chemical and microbiological data.

In chapter five results of the calibration and in-situ measurements are presented with their analysis. An attempt is made to explain the results through the physical, chemical and biological processes taking place in the system, taking as a guidance the results from other research in this field.

The thesis concluding statements and general recommendations for improvement of the treatment process as well as further investigation needs are presented in chapter 6.

3. Fundamentals

3.1 Blackwater characteristics

Blackwater is the mixture of urine, faeces, and flush water along with anal cleansing water and/or dry cleansing materials. Blackwater can be further separated to yellow water - urine with or without flush water and brown water - faeces with water (Tilley et al., 2014). The amount of blackwater is very different depending on the flush system used and ranges from 8 to 50 L/p.d, or between 9.6% and 40% of the domestic wastewater stream (DWA, 2014). In a compilation of sources Kujawa-Roeleveld and Zeeman (2006) report volume of urine and faeces (without flush water) 1.32 – 1.67 L/p.d compared to the grey water 91.3 L/p.d, or 1.4 -1.8% of the domestic wastewater flow. Conventional flush toilets use up to 12 litres of drinking water per flush, although modern houses are equipped with low-flush toilets only using 4-6 litres per flush (Kujawa-Roeleveld and Zeeman, 2006). Vacuum toilets, already applied in planes and trains, only use 1 litre of water per flush, so the water is almost as concentrated as UDT (Graaff, 2010). The advantage of vacuum toilets is that there is no change of in-house practice, which could increase their acceptance by the users, in contrast to dry sanitation solutions (Nordin et al., 2018).

The highest proportion of nutrients and TSS and about half of the organic materials are found in the blackwater (Table 3-1).

Table 3-1: Examples for absolute amounts and share of the domestic wastewater flow of BOD_5, COD, TSS, total P, total N and potassium in blackwater

	BOD_5 g O_2/p.d %	**COD g O_2/p.d %**	**P_{tot} g P/p.d %**	**N_{tot} g N/p.d %**	**K g K/p.d %**	**TS g/p.d %**
DWA (2014)	37 67%	50 52%	1.5 75%	12 92%		61 82%
Kujawa-Roeleveld and Zeeman (2006)	19-9.5 52%	55.7-66.5 36%	0.9-1.7 68.2%	8.5-13 79%	3.0-4.3 79%	
Daigger (2009)	25 42%		2 77%	12.1 86%	3.6 82%	
Todt et al. (2015)		69%	87%	83%		

A large fraction of the main components of domestic wastewater, including organics, nutrients (N, P and K), pathogens, pharmaceuticals residues and hormones are present in a very small volume of faeces and urine (Fig. 3.1). Urea, ammonia, and creatine contain respectively 80%, 7% and 6% of the N. Phosphorus is mostly present as inorganic phosphates (>95%) and the main proportion of the phosphorus in the faeces is found as undigested mineral calcium phosphates. Potassium is mainly found in its ionic form (Kujawa-Roeleveld and Zeeman, 2006).

The separation of different streams of household wastewater gives opportunities for better water reuse and nutrients recovery. By diverting blackwater from grey water, 80– 95% of the nutrients from households can be recovered (Kujawa-Roeleveld and Zeeman, 2006). Nutrients and energy are demonstrated to be recoverable to a big extent using different treatment processes. E.g. anaerobically treated concentrated blackwater contains 69 and 48% of the theoretically produced N and P in the household. Ninety five percent of the retained P was shown to be recoverable via struvite precipitation (Zeeman and Kujawa-Roeleveld, 2011).

Otterpohl et al. (1999) suggested several concepts for blackwater treatment with nutrient and energy recovery. The following one resembles closely the one used during the current study: solids are separated from the blackwater and the liquid part is treated aerobically with nitrification but without denitrification, which produces effluent containing most of the nutrients. This flow can be mixed with effluent of the digestor and reused as fertilizer.

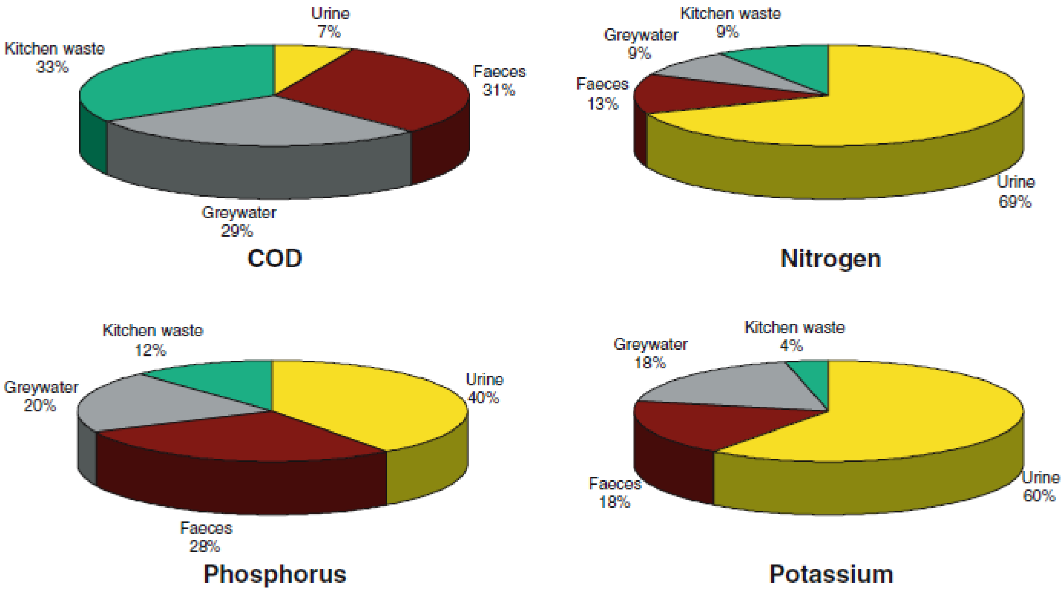


Fig. 3.1: Distribution of organic matter and nutrients in domestic wastewater streams (Kujawa-Roeleveld and Zeeman, 2006)

3.2 Nature-based solution and treatment wetlands

The European Commission has been promoting Nature-based solutions (NBS) since 2013 and sees their implementation as a contribution to policies such as climate change, biodiversity, circular economy and disaster risk reduction (Faivre et al., 2017). NBS are defined as "*solutions that are inspired and supported by nature, which are cost-effective, simultaneously provide environmental, social and economic benefits and help build resilience. Such solutions bring more, and more diverse, nature and natural features and processes into cities, landscapes and seascapes, through locally adapted, resource-efficient and systemic interventions*" (EC, 2016). (Re-)integrating nature and natural processes into built areas is increasingly considered as providing multiple services to the urban population. In line with the CE principles, the NBS also can contribute to solving drinking water shortages, enable resource recovery and improve the cities resilience to climate change (Kabisch et al., 2017). In an urban areas NBS can purify different water sources – greywater, rainwater, sewer overflow etc. enabling urban green growth with local water resources (Masi et al., 2020). Nature-based technologies like treatment wetlands (TWs) are energy extensive, cost-effective, using only natural approach solutions that closely imitate the treatment functions of natural wetland system (Sundaravadivel and Vigneswaran, 2001).

Treatment wetlands, or constructed wetlands for wastewater treatment, are currently recognized as an effective environmental biotechnology for wastewater treatment with high removal rate of organics and suspended solids. Hybrid TWs and well-designed COD/N ratio, hydraulic retention time, and high sorption capacity media can reach also high treatment efficiency of nitrogen and phosphorus with low energy consumption and low construction costs (Almuktar et al., 2018; Valipour and Ahn, 2016; Vymazal, 2010). TWs also demonstrate tolerance against fluctuations of flow, provision of habitat for wetland species and more aesthetic appearance compared to conventional WWTPs (Langergraber, 2001). A main advantage of TWs compared to natural wetlands is the greater degree of control, allowing well-defined composition of substrate, type of vegetation, and flow pattern. TWs offer additional flexibility including site selection, sizing, and not least, control over the hydraulic pathways and retention time (Brix, 1993).

The following advantages and disadvantage of TWs are listed by Sundaravadivel and Vigneswaran (2001):

Advantages:

- Low external energy input
- High level of treatment with little maintenance
- Relatively tolerant to shock hydraulic and pollutant loads
- Simplicity of design
- Lower cost of installation
- Provision of green space and wildlife habitat.

Disadvantages:

- Large areas requirement makes them unsuitable for centralized treatment of densely populated cities
- A few years needed before the vegetation develops and the treatment becomes optimal
- Their performance depends on environmental conditions – storm, flooding, etc.
- Mosquitos and other undesirable animals may find good ground for development
- Steep topography and high-water table may pose limitations.

3.3 Horizontal flow wetlands

Treatment wetlands are engineered systems designed to optimize natural processes that can successfully treat raw, primary or secondary treated sewage. They can be subdivided in surface and subsurface flow (SSF) systems. Depending on the direction of water flow, SSF wetlands can be with horizontal flow (HF) or vertical flow (VF). They are commonly used for secondary wastewater treatment. Another type with vertical flow, that provides sludge and wastewater treatment in one construction is the so-called French VF wetland. In Free Water Surface wetlands, the water flows above the media bed. They are generally used for tertiary treatment (Dotro et al. 2017).

In HF wetlands, the wastewater is fed at the inlet, flows through a porous medium under the surface of the substrate in almost horizontal path until it reaches the outlet. In a conventional SSF the conditions are mainly anaerobic and anoxic with aerobic ones only around the roots of the plants (Fig. 3.2). Main removal factor of organic compounds is the microbial degradation. The microbial biomass grows attached to a support medium, forming a biofilm. The support medium can be natural (stones, sand, soil) or artificial (plastic) material. The medium enables a high biomass concentration to be retained in the reactor for long time periods (von Sperling, 2007).

The wetland used in the current experiment is a system with sub-surface water position of the flow, influent flowing beneath the media surface. The system is intensified, with aeration lines on the bottom of the bed to increase oxidation processes and sorptive media, expanded clay, as a substrate to enhance adsorption.

The role of plants in HF wetlands is mainly related to physical processes such as providing increased surface area for attached microbial growth, and for better filtration of TSS. In temperate and cold climates, the litter layer can provide extra thermal insulation during the cold season. For HF wetlands providing secondary treatment of domestic wastewater, the contribution of plant uptake to nutrient removal is minimal. Plant-mediated oxygen transfer occurs, but is minimal in comparison to the oxygen demand exerted by the incoming wastewater (Kadlec and Wallace, 2009; Dotro et al., 2017).

HF wetlands are used for secondary and tertiary treatment of domestic wastewater, as well as for a variety of industrial effluents. For HF wetlands treating domestic wastewater, primary treatment is generally achieved via a septic tank or an Imhoff tank. (Vymazal and Kröpfelová, 2009; Kadlec and Wallace, 2009).

Compared to FWS systems, the contact area of water with bacteria and substrate is much higher, which decreases the area requirement of SSF wetlands per p.e. The lists below gives an overview of the advantage and disadvantages of HF wetlands (Tilley et al. 2014).

Advantages:

- High reduction of BOD_5, suspended solids and pathogens
- Mosquito proliferation is not possible
- No electrical energy is required
- Low operating costs.

Disadvantages:

- Requires a large land area
- Provides little nutrient removal
- Risk of clogging, depending on pre- and primary treatment
- Long start-up time to work at full capacity
- Requires expert design and construction.

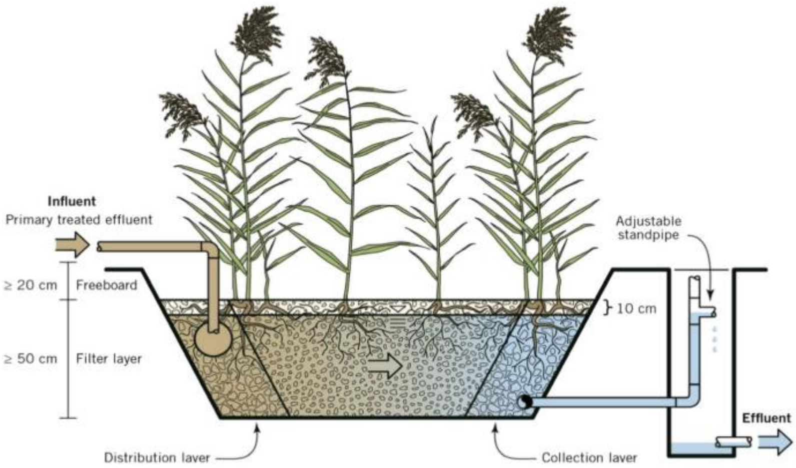


Fig. 3.2: HF wetland (Dotro et al., 2017)

There is one potential problem which may take the HF wetland out of operation quickly. When particles settle in the stagnant zones of the pores or are strained by flow constriction and their decomposition rate is less than the settling rate, the pore volume is reduced. Clogging causes overflow, reduction of the effective volume of the substrate and shortcutting. The rate of clogging initially depends on the solids in the inflow, and in longer term – on solids degradation. To prevent clogging of the filter material, the use of mechanically pre-treated wastewater is essential. Characterization of the wastewater, expert design regarding the water specifics and appropriate hydraulic retention time (HRT) are highly recommended (Knowles et al., 2011; de Matos et al., 2018). Kadlec and Wallace (2009) report frequent flooding of HF wetlands in USA before 1995 the main reasons being clogging and improper hydraulic design.

HF wetlands have high ammonification, very low nitrification, high denitrification, low microbial and plant uptake (Vymazal, 2006). Ammonia removal is limited normally due to constantly waterlogged conditions and therefore lack of oxygen. In improvement designs aeration or alternation of dry and wet periods is introduced. Phosphorus is removed primarily by ligand exchange reactions, where phosphate displaces hydroxyls from the surface of iron and aluminium hydrous oxides (Vymazal, 2010). Typical removal efficiency of HF wetlands as a secondary step treatment is >80% of TSS, 20-30% of ammonium-nitrogen, 30-50% of total N, 10-20% of total P, 2 $\log_{10}$ reduction of Coliforms, and > 80% of organic matter as oxygen demand (Dotro et al., 2017).

HF wetlands are not any more included in the German standards DWA-A 262 for constructed wetlands for secondary treatment of domestic wastewater, unless actively aerated, because VF wetlands provide superior influent quality. The actively aerated HF wetlands can be used for 4-50 p.e. with criteria for specific area of at least 1 m^2/p.e. (Nivala et al., 2018).

3.4 Treatment mechanisms

TWs utilize natural processes involving vegetation, substrate, and microbial assemblages to treat the wastewater. In the TWs the non-water elements are removed by (micro)biological, physical and chemical mechanisms. These include sedimentation, filtration, precipitation, sorption, plant uptake, microbial decomposition (Sundaravadivel and Vigneswaran, 2001). Processes playing role in the organics and N removal are mainly micro(biological), while P and TSS are removed mainly by physical processes (Table 3-2). Changes in temperature, different bioactivity levels, different levels of plant and microbial life and patterns of hydraulic flow can contribute significantly to shifts in removal rates in the same TW.

Table 3-2: Processes playing role in the removal of pollutants (Sundaravadivel and Vigneswaran, 2001)

Pollutant	Process
Organic material	Biological degradation, sedimentation, microbial uptake
Suspended solids	Sedimentation, filtration
Nitrogen	Sedimentation, nitrification, denitrification, microbial and plant uptake, volatilisation
Phosphorus	Sedimentation, filtration, adsorption, plant and microbial uptake
Pathogens	Natural die-off, sedimentation, filtration, predation, adsorption, excretion of antibiotic from plant roots

3.4.1 Organic matter removal

The two main fractions of the organic matter in the wastewater are easily biodegradable fraction and non-biodegradable fraction. Both can be in soluble and particulate form. In a typical domestic sewage, about half of the organic matter is in soluble form and easily degradable (von Sperling, 2007). Organic matter is decomposed in HF wetlands by both aerobic and anaerobic microbial processes as well as by sedimentation and filtration of particulate organic matter. Some of the organic compounds, such as proteins, carbohydrates, and lipids are easily degraded by microorganisms, while other, such as lignin and hemicellulose, are resistant to decomposition (Vymazal and Kröpfelová, 2009).

Biochemical Oxygen Demand in 5 days (BOD_5) is a sum parameter measuring the amount of oxygen which is consumed for oxidation of organic matter present in the water during aerobic decomposition processes carried out by microorganisms. BOD_5 is an indirect measure of the concentration of organic contamination in water. It provides information for the organic materials that are degraded in a period of 5 days at constant temperature of 20°C.

BOD_5 is the most widely used parameter of organic pollution applied to wastewater to:

- determine the approximate quantity of oxygen that will be required to biologically stabilise the organic matter
- determine the size of wastewater treatment facilities
- measure the efficiency of some treatment processes
- determine compliance with wastewater discharge permits.

Chemical oxygen demand is also a bulk parameter and is used to measure the content of all oxidable matter present in a water sample by means of a strong chemical oxidising agent. The COD values include the oxygen demand created by biodegradable as well as non-biodegradable organic substances. Unlike BOD_5, the COD test has the advantage of not being subject to interference from toxic materials.

TOC and DOC are two other means for measuring the organic matter present in water. TOC represent wastewater content of all carbon bound in organic molecules. Most of the carbon in water makes up organic carbon compounds except a few species - carbon dioxide, hydrogen carbonate and bicarbonate, cyanide. DOC together with particulate organic carbon and volatile organic carbon make the TOC.

Carbon is removed from the water mainly by microorganisms that use it as energy source and as cells building material. In HF wetlands light does not play a big role as source of energy because of high TU of the water and its flow below the substrate surface. Therefore, the presence of microorganisms that have light as energy sources (photoautotrophs and photoheterotrophs) is very limited. The organisms of real importance in this case are the chemoautotrophs for whom the energy comes from chemical reaction, carbon comes from CO_2 and the chemoheterotrophs - organic matter is the only carbon source. Two processes, that lead to organic matter decomposition, take place in the microorganism cell – catabolism and anabolism. During catabolism the energy stored in chemical form in the organic compounds is released and during anabolism formation of cellular material happens (von Sperling, 2007). Exoenzymes convert large complex molecules outside the cells in a process hydrolysis. The removal of organic matter can take place through oxidative and fermentative catabolism. The former leads to CO_2 after organic matter was oxidized and the latter produces two final products – CH_4 and CO_2. More energy is released through aerobic than through anaerobic reactions, and the microorganisms use first the one with highest energy. The main reactions for the generation of energy that occur in aerobic (Eq. 3.1) and (Eq. 3.2) anaerobic conditions are:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad \text{Eq. 3.1}$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 3CH_4 + 3CO_2 \qquad \text{Eq. 3.2}$$

The microbial mass involved in the aerobic processes consists mainly of bacteria and protozoa. Other organisms, such as fungi and rotifers, can also be found, but their importance is lower. Bacteria constitute the largest and most important group in biological wastewater treatment systems with heterotrophic bacteria being the most important agents (von Sperling, 2007).

3.4.2 Nutrients removal

Besides the removal of the carbonaceous organic matter, nutrients can also be transformed and removed during the treatment process.

Ammonia, nitrite, nitrate, and Kjeldahl nitrogen are the nitrogen constituents in wastewater usually analysed. A large fraction (up to 100%) of the organic nitrogen is readily converted to ammonia (Vymazal, 2006) in a process that does not consume oxygen. Dissociated ammonium ion is in balance with the NH_3. If the pH of the surface water is in the alkaline range, NH_3 is formed, which is toxic for the fish. Ammonia can be nitrified in the aqueous environment if nitrifying microorganisms and oxygen are present. The nitrifying bacteria consume dissolved oxygen for this process, thus depleting the oxygen content of the water. They are chemoautotrophic bacteria that use CO_2 as building material for their cells. Nitrification happens in two reactions (Eq. 3.3 and Eq. 3.4).

$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$ genus *Nitrosomonas* Eq. 3.3

$2NO_2^- + O_2 \rightarrow 2NO_3^-$ genus *Nitrobacter* Eq. 3.4

The nitrate ion represents a nutrient leading to eutrophication of surface water, and nitrite can react with amines (formed e.g. from amino acids of proteins) to yield N-nitrosamines which represent powerful carcinogens.

Kjeldahl nitrogen is a sum parameter that comprises organic nitrogen compounds and ammonia nitrogen. It is also an important nitrogen parameter because organic nitrogen compounds can be metabolized to ammonia.

The first anoxic oxidation process to occur after oxygen depletion, is the reduction of nitrate to molecular nitrogen. The reduction of nitrate is performed by nitrate-reducing chemoheterotrophic bacteria - the denitrifying bacteria which produce N_2O and N_2 (Eq. 3.5)

$2NO_3^- + 2H^+ \rightarrow N_2 + 2.5O_2 + H_2O$ Eq. 3.5

Another process that leads to the production of nitrogen gas is the anaerobic ammonia oxidation (anammox) in which nitrite and ammonia are directly converted to dinitrogen. It is an autotrophic process (Eq. 3.6).

$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ *Planctomycetes* Eq. 3.6

Phosphorus is another element essential to the growth of biological organisms. The amount of phosphorus compounds present in wastewater discharge must be controlled to avoid noxious algal blooms. The usual forms of phosphorus found in aqueous solutions include the orthophosphate, polyphosphate, and organic phosphate. The sum of all three phosphorus species is designated as total phosphorus. Substrate and plant roots slow down the water movement, thus contributing to sedimentation of P associated with particles. Another physical process for P removal is the sorption. HF systems have higher potential for P removal than VF systems as the substrate is constantly flooded and there is not much fluctuation in redox potential in the bed. However, P sorption depends on the sportive capacity of the substrate and is very low once saturation is reached and plant uptake is low (Vymazal, 2006). In a chemical reaction, P forms salts with metal ions such as calcium, aluminium and iron that can lead to its precipitation (Sundaravadivel and Vigneswaran, 2001). Phosphorus removal can also happen through plants uptake and subsequent harvesting.

Phosphorus and nitrogen from the food products eventually end up in domestic wastewater streams. Globally, nearly 20% of the mineral phosphorous is consumed by humans and therefore ends up in domestic wastewater (Batstone et al. 2015).

By source-separating concentrated blackwater and co-digesting it with wet organic wastes (such as food waste), approximately 90% of the nitrogen, 74% of the phosphorus and 79% of the potassium can be reclaimed and recycled (Jenssen et al., 2003). On-site treatment of this untapped valuable resource using the appropriate level of technology and subsequent resource recovery makes source-separation an attractive domestic wastewater management option and a source of value creation (Moges et al., 2018).

The increased demand for new products and increasing life standards of the population cannot be responded only by reducing the demand for raw materials and energy, or their efficient use. This will eventually lead to depletion of finite resources. About 20 million tons of P are mined every year and P has been included in the Critical Raw Materials list of the EU in 2017 (Robles et al., 2020). Nitrogen, even if not depletable, needs energy intensive process to be fixed from the air. If not treated properly N and P end up in the environment causing pollution problems.

While in the linear economic model, the environmental danger is the main reason for water treatment, CE also considers resources recycling that can greatly reduce the depletion of raw materials.

Potassium is another element that is essential macronutrient for the plants and is part of the compound fertilizers. It is mostly mined from deposits deep underground. Canada, Belarus and Russia are the three biggest producers of mined potash (US Geological Survey, 2021). K soil deficit has been reported for the African continent and significant reduction is observed in many European countries (Ciceri et al., 2015). Recycling of K is not well developed although human excreta are potential source (Öborn et al., 2005). The median load of K in urine is 2.5 $g.p^{-1}.d^{-1}$ and in faeces 0.7 $g.p^{-1}.d^{-1}$ (Meinzinger and Oldenburg, 2009). Theoretical systems to recover nutrients, including potassium, from wastewater have been suggested by (Batstone et al., 2015).

3.4.3 Turbidity and total suspended solids and removal

Solids typically include inorganic matter such as silt, sand, gravel, and clay, and organic matter such as plant fibres, algae, microorganism cells (von Sperling, 2007). TSS is a measurement of all suspended solids - both settleable and non-settleable. TSS are removed because of the low flow velocity and high surface area provided by the substrate. The solids sediment by gravity, get strained or physically captured or adsorbed on the biomass film attached to the substrate and plants roots (US EPA, 2000).

Clarity of water is usually measured by its TU. TU is based on the amount of light that is either absorbed or scattered by suspended material in water – inorganic and organic, dissolved or particulate. Both the size and surface characteristics of the suspended material influence absorption and scattering of light. Low TU is very important if treated wastewater effluent needs to be disinfected with ultraviolet radiation.

3.4.4 Pathogens removal

While microorganisms play an essential role in wastewater treatment, some of them are associated with water-borne diseases. Pathogenic species found in faeces that present a risk for the human health come from the groups of bacteria, viruses, parasitic protozoa and helminths (Table 3-3).

Parameter organisms to describe water quality are so called indicator micro-organisms of faecal pollution such as faecal coliforms*, Escherichia coli, Enterococci* and *Clostridium perfringens*. Coliforms are part of the intestinal flora of mammals and other animals. They can be found in the environment and therefore are not good indicator as faecal pathogens (Sanz and Cawlik, 2014). The great quantity of *E. coli* present in the human digestive tract, together with the fact that it is not usually found in other environments, make this bacterium one of the best faecal contamination indicators (Molleda et al., 2008). The presence of *E. coli* in water proves a recent faecal contamination and the possible existence of pathogens. The use of the indicator bacterial group *Enterococci* is frequently suggested as an alternative to coliforms. Their advantage over *E. coli* lies in their greater resistance and their inability to grow in other environment, such as soil, water, and others (Molleda et al., 2008). Coliforms and *Enterococci* are simple and economical to determine and quantify and they are safer to work with.

Removal of faecal bacteria and pathogens in TWs may occur via physical, chemical, and biological factors, alone or in combination. Physical factors consist of mechanical filtration, sedimentation, and sorption to the TW's matrix. Chemical factors include oxidation and exposure to antimicrobial biocides excreted by plants or UV light. Biological factors comprise, predation by nematodes and protists, activity of lytic bacteria or viruses, retention in biofilms, natural die-off due to starvation or predation, and competition for limiting nutrients (Wu et al., 2016; Alufasi et al., 2017). Stott et al. (2001) found out that predation by free-living ciliated protozoa, which are commonly found in TWs, can be a dominant mechanism for the removal of cryptosporidium oocysts. Protozoa participate in the consumption of organic matter and consumption of free bacteria. They improve the quality of the effluent by consuming the bacteria which did not sedimented as part of the flocs, but are freely suspended (Horan, 1990).

Depending on the system (e.g. surface flow, subsurface horizontal or vertical flow wetlands) and design factors like the used filter media, retention time, plant species, loading rate, water composition etc., bacterial removal efficiency can be quite different. Microbial activity levels will also change with root development and system maturation (Werker et al., 2002)

Table 3-3: Species associated with faeces that can cause diseases in humans (WHO, 2006 adapted)

Type	Microorganism	Disease
Bacteria	*Aeromonas ssp. E. coli (certain strains); Plesiomonas shigelloides*	Enteritis
	Salmonella spp	Salmonellosis
	Shigellia spp.	Shigellosis
	Vibrio cholera	Cholera
Viruses	*Enteric adenovirus 40 and 41* *Astrovirus; Calicivirus; Rotavirus*	Enteritis
	Hepatitis A and E virus	Hepatitis
Parasitic protozoa	*Giardia intestinalis*	Giardiasis
	Entamoeba histolytica	Amoebiasis
Helminths	*Ascaris lumbriocoides*	Ascariasis
	Taenia soliuim/saginata	Taeniasis
	Schistosoma spp.	Bilharzia

In industrialized countries the risks of infection from faecal pathogens are generally small but bacteria like *Salmonella*, *Campylobacter* and enterohaemorrhagic *E. coli* (EHEC), coming with fertilizer products from faeces, sewage sludge and animal manure, can be present in high number. Enteric viruses are considered to be the major cause for gastrointestinal infections in industrialized countries. In healthy individuals the urine is sterile. If pathogens are found in the urine, they come from faecal cross contamination. Therefore, the main risk in the use of excreta is related to faecal and not urinary fraction (WHO, 2006).

When no additional treatment of the faeces is provided, pathogens in excreta and wastewater can survive in the environment long enough to be transmitted to humans through contact or consumption of contaminated products irrigated with wastewater. *Ascaris*, *Giardia* and rotavirus can survive long time in the environment. The most environmentally resistant pathogens are helminth eggs, which can survive for several years in the soil. The risk is especially big when the crops that are more difficult to wash and are eaten raw (WHO, 2006).

3.5 Water reuse and existing regulations

With the rate of water use growing two times faster than the rate of the population in the last century (UN, 2007), more regions in the world suffer from water shortages. The reasons for water scarcity are numerous and not always linked to deficiency of water resources. These include deterioration of water quality, competition between actors, lack of technical and financial means for operation of water supply and treatment infrastructure (Lazarova et al., 2013). Wastewater reuse has gained an importance especially in parts of the world where water is scarce. One of the first civilizations that can be linked to the modern systems of wastewater collection and treatment system were the ancient Greeks. They connected toilets to a closed sewer that conveyed the wastewater to basins outside the city. From those basins the water flew through brick-lines conduits to the agricultural fields and orchards where it was used for irrigation and fertilization. The Romans recycled wastewater from the spas using it to flush latrines before discharging the waste into sewers (Lofrano and Brown, 2010).

Recycled water requires certain quality standards to ensure protection of public health and the environment. When it comes to human health the risk of infection from pathogens and chemicals are of main concern. Heavy metals, emerging pollutants such as pharmaceuticals, personal care products, hormones, household chemicals can have toxic, cancerogenic, mutagenic and various other effects that are still not investigated (Sanz and Gawlik, 2014). In terms of environmental concerns, while nitrogen and phosphorus may have positive effects on crop, excessive amounts can affect plants health, cause eutrophication and contaminate ground water. High salt loads are damaging for the crops causing soil salinization and increased soil water pressure. Suspended solids can lead to sludge deposits and anaerobic conditions as well as clogging of irrigation infrastructure. Excessive organic matter can lead to oxygen depletion providing conditions for microbial growth (Shoushtarian and Negahban-Azar, 2020).

All those concerns have been addressed by adopting standards and guidelines, that regulate wastewater reuse. Shoushtarian and Negahban-Azar (2020) analysed 70 regulations, guidelines, standards, and criteria for water reuse in agriculture from all over the world. Agriculture water reuse is by far the most practiced use of reclaimed wastewater. The first water reuse standards were issued in the State of California in 1918. WHO is the first international organization that issued standard in 1973, last updated in 2006. Other international organizations that have put efforts in developing standards are the Food and Agriculture Organizaton and the World Bank. In 2010 “Guidelines for treated wastewater use for irrigation projects” were issued by the International Organization for Standardization. On a global basis majority of the documents were issued after 1998 and most come from the US, Australia, and countries from the Middle East. WHO guidelines and Australian Guideline for water recycling make also reference to greywater recycling.

Some guidelines have established a multiple barrier approach for risk reduction linked e.g. to type of irrigation technique and the post irrigation practices. Drip irrigation is less risky than spraying and sprinkling. A period without irrigation before harvest can allow die-off of bacteria and viruses or reclaimed water can be replaced with fresh water for a period before harvest (Sanz and Gawlik, 2014).

In Europe, Italy was the first country to issue a regulation in 1977, followed by France, Greece, Cyprus, Spain, and Portugal. Sanz and Gawlik (2014) make detailed analysis of all European standards. In all European countries except Portugal, the regulations are legally binding. Most of the documents intend to use the reclaimed wastewater for agricultural, urban, and industrial uses. The Spanish regulation contains the highest number of intended use categories and is the only one that also covers irrigation of private gardens, aquaculture, silviculture and uses such as wetland maintenance, minimum stream flow etc. All regulations include at least two microbiological and at least two physico-chemical parameters but their limits usually differ among countries and depend on the intended use or on population equivalent, when included in the discharge limits. All standards meet the EU legislative requirements for physico-chemical parameters, but most have included additional parameters or put lower maximum limits, compared to the EU rules. The strictest limits for *E. coli* and *Salmonella* belong to Italy with less than 10 CFU/100 ml and absence, respectively. Greece has the only standard that has limit for total coliforms to 2 CFU/100 ml and France sets the requirement for 2-4 log reduction for faecal *Enterococci*. TSS limits vary from 2 to 35 mg/l, BOD_5 between 10 and 70 mg/l and COD between 60 and 100 mg/l.

European Directive 91/271/EEC states in Article 12 that “treated waste-water shall be reused whenever appropriate”. Since its publication a number of reports, assessments, analysis were conducted at EU level before in 2020 the European Commission came out with the EU Regulation

2020/741(EU, 2020) on the minimum quality requirements for water reuse. The main purpose of the regulation is to secure safe water is used for agricultural irrigation but also to encourage the application of recycled water and contribute to the WFD for good quantitative and qualitative status for surface and ground water bodies. This regulation is in effect the only legally binding document in most EU member states and should be applied from June 2023. The regulation separates crops in different categories depending on whether they are meant for human consumption or not. Human consumption crops are divided to raw and processed consumption. Non-food crops are industrial, energy and seeded crops. Based on those categories, different quality requirements are set ranging from ≤ 10 to ≤ 10 000 for *E. coli*. The strictest category A has specific requirements for BOD_5 ≤ 10 mg/l, TSS ≤ 10 mg/l and TU ≤ 5 NTU. The other categories refer to the requirements of Directive 91/271/EEC. For all categories there are limits for *Legionella* spp. and helminth eggs. The regulation requires routine monitoring with minimum frequency depending on the parameter. Additional validation monitoring is also needed before new facility is put in operation and includes additional microbiological criteria for coliphages, and *Clostridium perfringens*.

A number of EU legislative pieces, when complied with, will reduce the risk of reclaimed water negative effects on humans and environment. Among them, in relevance to nitrogen pollution is the nitrate directive, which sets concentration limits for ground water 50 mg nitrates per l in Nitrate Vulnerable Zones.

US EPA (2012) provids a guidance on the degree of reclaimed water treatment when human exposure is likely (Table 3-4). Water reuse after only primary treatment should not be practiced at all and the closer it comes to contact with human use, the more treatment steps it should undergo. Direct potable consumption is not foreseen in any case.

Table 3-4: Type of reuse appropriate for increasing level of treatment (US EPA, 2012, adapted)

Treatment level	**Increasing level of treatment** →			
	Primary	Secondary	Filtration and disinfection	Advanced
Process	Sedimentation	Biological oxidation and disinfection	Chemical coagulation, biological or chemical nutrient removal, filtration, and disinfection	Activated carbon, RO, advanced oxidation processes, soil aquifer treatment, etc.
End use	No uses recommended	Surface irrigation of orchards and vineyards	Landscape and golf course irrigation	Indirect potable use including ground water recharge of potable aquifer and surface water reservoir augmentation and potable reuse
		Non-food crop irrigation	Toilet flushing	
		Groundwater recharge of non-potable aquifer	Vehicle washing	
		Wetlands, wildlife habitat, stream augmentation	Food crop irrigation	
		Industrial cooling process	Industrial systems	
Human exposure	Increasing acceptable level of human exposure →			
Costs	Increasing costs →			

Lazarova et al. (2013) give examples of urban water reuse from different parts of the world from decentralized and semi-centralised systems. All secondary treated effluents were additionally treated via micro- (0.2 μm), ultrafiltration (0.035 μm) or RO and then disinfected with UV radiation, ozone, and often chlorinated before the distribution. In Honolulu treated effluent from the WWTP was treated again chemically with sand filtration and UV disinfection, or for higher standards, with microfiltration and RO. The water could then be used for watering golf courses and landscape irrigation. In Australia dual reticulation water system is often used, as the water is ultrafiltered and then UV disinfected and chlorinated before it is used for toilet flushing, garden watering and car washing. For urban uses in USA the treated water receives high-level disinfection and special attention was paid to the nutrients and salts with potential risk for nitrates percolation (US EPA, 2012).

In general, the water requirements for reuse are higher than the requirements for discharge in surface water bodies, i.e. if the wastewater effluent taken from WWTP is to be reused, it needs to go through additional treatment steps in order to reduce possible risks. TWs are one of the possible technologies for water reclamation, but the most adequate technology will depend on the aim and the specific circumstances (Sanz and Gawlik, 2014). Despite the availability of various technologies, and the acknowledged benefits that water reclamation offers, there are still social, economic, and regulatory challenges. Additional treatment levels and regular monitoring increase the price and energy consumption significantly and the social acceptance is often a reason for failure of water reuse projects. Water reuse should be part of integrated water planning and management considering the local situation.

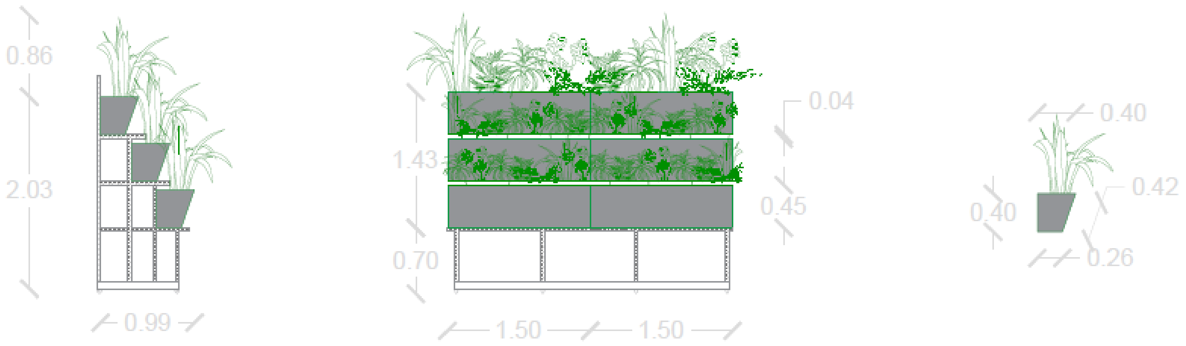
4. Material and methods

4.1 Experimental design

4.1.1 Wastewater treatment system

The current research investigates the performance of an indoor domestic blackwater treatment system. The treatment system was developed by alchemia-nova GmbH and the company is the only holder of the system design intellectual property rights. The model of the system used in current experiment is located in company's laboratory in Vienna. It consists of three horizontal subsurface flow beds connected in series, each bed with two stainless steel containers linked through a short cylindrical connection (Fig. 4.1). Each container has three intermediate semi-walls, perpendicular to the flow direction that are designed to direct the vertical way of the flow. All three beds are identical. One cubic meter substrate is equally split between the three beds. Further in this study the treatment beds are called "upper", "middle" and "effluent" referring to the outflow after the first, second and third bed respectively. The upper and the middle treatment beds are constantly aerated, while the effluent bed lacks aeration, with the intention to create anoxic conditions for nitrogen removal. Due to the vertical position of the three beds, the system is named VertEco. The water level is maintained at height of approximately 25 cm via in- and outflow positioning of every bed. Inlet structure is designed in a way to allow flow distribution across the entire cross-section via pored wall.

a)



b)

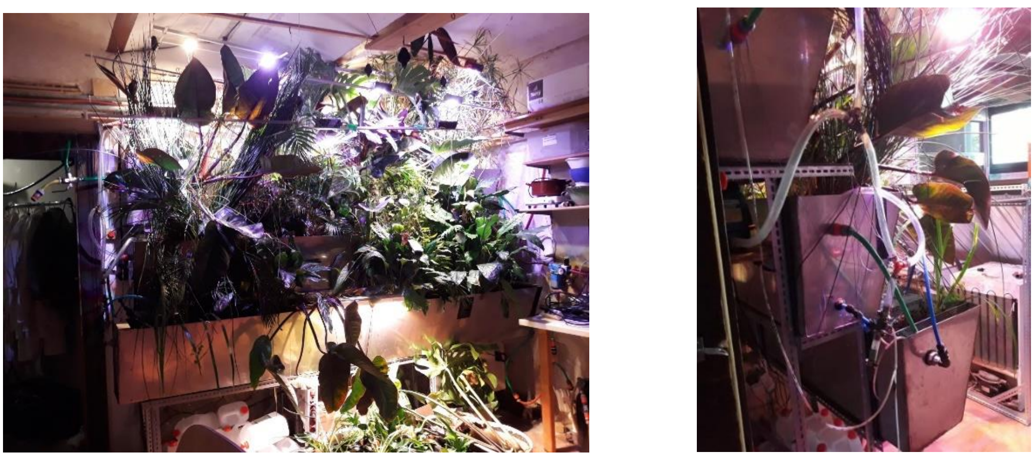


Fig. 4.1: Front and side view of the system a) source: alchemia-nova b) photo: V. Ferdinandova

The air temperature in the laboratory is around 21˚C and relatively constant throughout the year. Due to the lack of natural sunlight at the location, artificial light is used to ensure the viability of the plants. Light emitting diodes lights in the wavelength range of 400-730 nm irradiate for a period of 14 hours per day from 6.00 to 20.00 h.

4.1.2 Wastewater collection and storage tank

The inflow is collected in a 1000 l tank that is fed by two toilets. One of the toilets is used by the alchemia-nova GmbH office and the other one by a family of 4. The preliminary treatment is done by centrifugal and gravity separator “Aquatron” that separates the solids before the water enters the collection tank. After the separation, 98% of the water together with the suspended solids enters the tank. The tank is constantly aerated with aeration pump. Primary sedimentation takes place in the tank - the grit settles while lighter weight material stays suspended. The settling reduces the concentration of suspended solids that move further into the system, thus, reducing the organic load. The mixing effect of aeration maintains small amount of the heavier solids in suspension. From the tank the water is fed to the treatment system via submersible pump, connected to a timer, so that the water is supplied intermittently (Fig. 4.2).

Since the tank is out of the house, the water temperature follows the temperature of the environment. Therefore, the water entering the treatment system bears different temperature depending on the season. The temperature of the effluent changes in much narrower range of about 16 - 20°C since it warms up during the passage through the treatment beds.

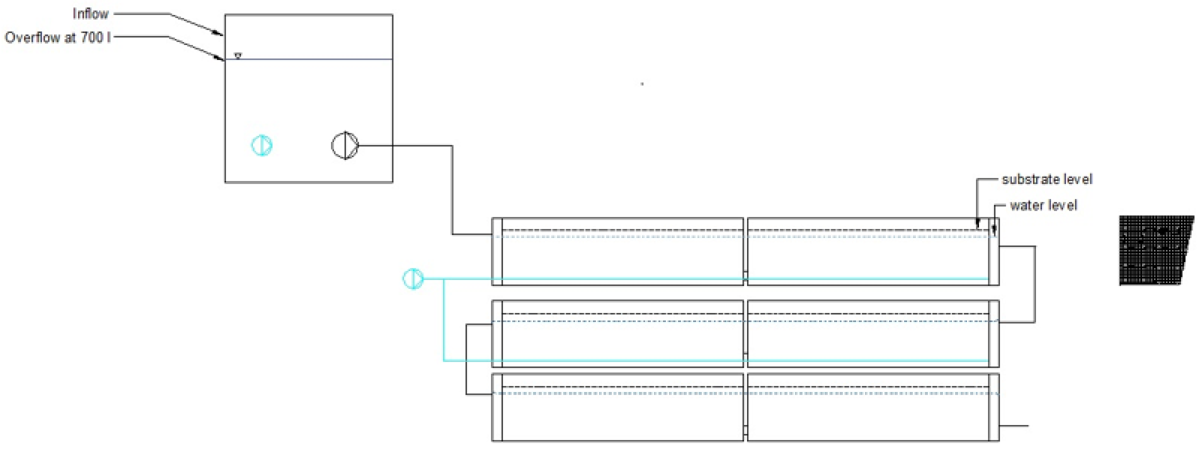


Fig. 4.2: Schematic view of the whole installation

4.1.3 Plants and substrate

The treatment substrate is expanded clay pellets, sized 8 - 16 mm. It has theoretical density of 500 kg.m^{-3} with 55% intragranular pores. The intergranular porosity was experimentally measured to be 46%. Thus, the active volume was calculated to 0.3 m^3.

All three beds are planted with angiosperms. The plants' role is to facilitate the cleaning performance. Since many of them are also ornamental they create the decorative effect of VertEco, which is often intended to be used as a green wall. *Monstera deliciosa, Philodendron erubescens, Chamaedorea elegans, Spathiphyllum sp, Cyperus alternifolius*. *Nephrolepis exaltata and Heliconia sp.* are plants from the tropical regions widely cultivated as house plants in Europe. They require minimum temperature of 12 - 14°C. As epiphytes and lianas most of them are shade tolerant with medium level of water requirements. Only a few prefer high nutrients concentrations. *Ficus pumila* is an evergreen liana that was used to cover the surface of the substrate as an aesthetic element rather than as water purification plant.

Eleocharis palustris, Carex acutiformis, Schoenoplectus lacustris are hydrophytes swamp plants widely distributed in wetlands in Europe with capacity to filter the water.

4.1.4 Pumping regimes

The treatment system was installed in 2015 and since then it was operated mostly with tap water. Before the current experiment it was tested for a period of 6 months with synthetic urine. Additional tests were run with greywater. There was no previous information on how this treatment design will perform with blackwater, except for the expectation of high conductivity of the influent due to high proportion of urine in the blackwater.

Two phases were run in the course of the study. Phase I was a test phase lasting from the end of May to beginning of August. During this time the calibration of the instruments was done, the measurements were initiated, and different influent feeding regimes were tested. A pumping regime of 2 min every 15 min was selected which makes 85 pumping events per 24 hrs or 323 l of wastewater per 24 hrs on average.

At the end of the first phase results from the toilet use frequency showed that the blackwater produced was 152.6 l/2 hrs on an average day. Thus, it was found out that not the entire pumped water volume of 323 l/24 hrs was conveyed to system. Moreover, the amount in the collection tank did not drop, which suggested that the system had clogged. The second phase was carried out with pumping regime of 1 min every 1.5 hrs. This comes to 15.8 pumping events per 24 hrs with 9 l per event, or 142.2 l/24 hrs. This phase lasted one month.

With 323 l/24 hrs HLR was estimated at 5.3 $cm.d^{-1}$ during phase I, and at 2.3 $cm.d^{-1}$ during phase II, using equation Eq. 4.1 (Kadlec and Wallace, 2009):

$$q = Q/A \qquad \text{Eq. 4.1}$$

where

q = hydraulic loading rate ($m.d^{-1}$)

A = 6,1 m^2 wetland area (wetted land area), m^2

Q = water flow rate, $m^3.d^{-1}$

The information collected from the toilet users revealed that the average toilet use per day was 10.9 times in the first and 10.1 times in the second phase. The old toilet water tank assumes 14 l water per flush, which makes total of 152.6 l/24 hrs and 141.4 l/24 hrs fed to the tank daily respective to the phase. Twenty-six and twenty-four percent of the times, respective to the phase, the toilet was used also for faecal excretion.

Based on the daily amount of 152.6 l, it could be estimated that the maximum HLR during the first phase was 2.5 $cm.d^{-1}$ and it was gradually decreasing, reaching at the end of the first phase ca. 0.8 $cm.d^{-1}$. This decrease was due to progressive clogging in the front part of the system that would not allow the water conveyance.

Nominal detention time in saturated media can be calculated using influent flow Q and the water volume of the wetland, taking porosity into account (Eq. 4.2) (Dotro et al., 2017). It should be noted that this is only theoretical value based on clean substrate. Should roots and organic and mineral matter be considered, the detention times would be different.

$$\tau_n = \varepsilon V/Q_i, \text{ days} \qquad \text{(Eq. 4.2)}$$

where

ε – porosity of wetland bed media

V – estimated water volume of the wetland, m^3

Thus, in the first phase the nominal detention time was estimated to a max τ_n = 2.2 days and τ_n in the second phase τ_n = 2.3 days.

The cross-sectional organic load is the mass loading divided by the cross-sectional area of the wetland (Dotro et al., 2017). For phase 1 the maximum OLR would be 37.5 g BOD_5 $m^{-2}.d^{-1}$ and during phase 2 - 67.5 g BOD_5 $m^{-2}.d^{-1}$. Both are well below the recommended maximum for HF of 250 g BOD_5 $m^{-2}.d^{-1}$ (Dotro et al., 2017).

In order to conduct a tracer experiment, the wastewater had to be exchanged with tap water. This reduced significantly the background conductivity and allowed lower amount of the tracer to be used. Otherwise the risk for damaging the plants would have been too high. That is why the end of the phase was chosen to conduct the experiment. Three tracer experiments were run but peak was not detected presumably because of the unstable hydraulics.

4.2 Measurement instruments

4.2.1 UV/VIS spectrometer

A submersible UV/VIS spectrometer called *spectro::lyser* produced by the scan Messtechnik GmbH, Vienna, Austria was used. It records the absorbance spectrum between 190 and 750 nm. The absorbance is resolved to 256 wavelengths into so-called “Fingerprint”, which is then analysed for several parameters (Fig. 4.3). A light beam is emitted by a xenon lamp and a second beam is guided across a comparison pathway thus compensating for the instrumental effects that could influence the measurement. The available instrument had an optical path length of 5 mm, which is recommended for use of surface water measurements, while the wastewater would be measured better with 0.1 mm path length.

The spectrometer is in the shape of a probe made of 0.6 m long heavy-duty stainless-steel housing, with a diameter of 44 mm (Fig. 4.4). On-board electronics controlled the measurement procedure.

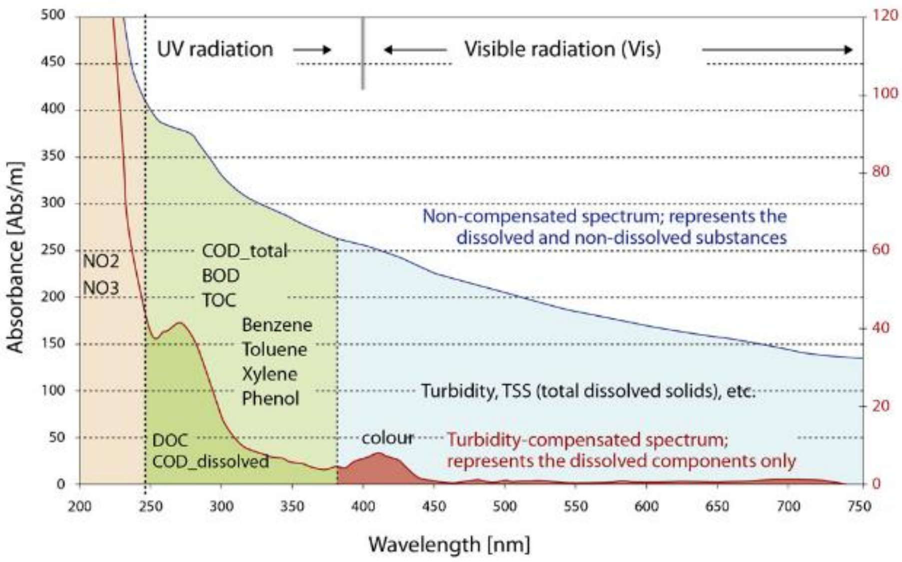


Fig. 4.3: Absorbance of light with different wavelength (www.s-can.at)

The instrument is meant to take measurements directly without sampling or samples treatment, which is a big advantage in real-time monitoring. Also, measurement errors due to sampling, transport, storage, dilution etc. do not pertain (Langergraber et al., 2003). In the current study the samples were taken manually and measured individually but most of the mentioned advantages still applied.

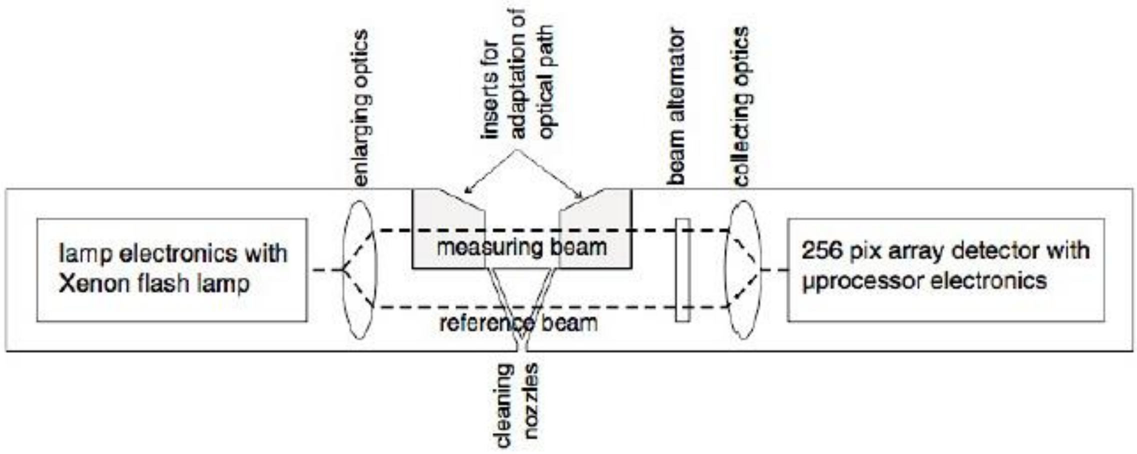


Fig. 4.4: UV/VIS spectrometer probe (Langergraber et al., 2003)

The spectrometer was used to measure six surrogate parameters, namely TSS_{eq}, COD_{eq}, BOD_{5eq}, $TUeq_{eq}$, TOC_{eq} and DOC_{eq} and nitrate as a single substance. The measurements are equivalent values because the light absorption is a proxy of the real value that is measured with direct methods.

Global calibrations were used to measure the composition of the wastewater. Those calibration calculate the concentrations from the Fingerprint based on specific factory settings. The global calibration is provided by the manufacturer as a default configuration of the spectrometer. It is based on wastewater samples from different WWTPs and has been continuously improved by increasing the number of samples (Rieger et al., 2006).

The relationship between absorbance and concentration is given by the Beer-Lambert Law. It is a linear relation of a single determinant, i.e. the concentration of a substance in solution is directly proportional to the absorbance of the solution. However, the law applies only if all spectra constituents are known. The wastewater has a matrix of numerous dissolved and suspended compounds, where not all constituents are known, there is strong correlation between various parameters and the reference measurements are not error-free. Global calibrations are therefore done with Partial-Least-Square regression. It accounts for correlations between the variables and leads to robust results for concentration-spectra relationships (Rieger et al., 2006).

In this study, three different global calibrations were used to measure the wastewater composition:

- INF300N0V20T intended for the treatment plant influent monitoring
- EFFFLUBODV150 intended for the treatment plant effluent monitoring
- GROH2SNTV160 monitors TU, TOC and DOC and was created for the use in groundwater monitoring.

Due to chemical and physical differences between the studied blackwater and the wastewater typical for WWTPs, local calibrations were performed to fine-tune the accuracy and precision of the specific water matrix based on a linear regression.

4.2.2 Hach-Lange DR-1900

DR-1900 is a portable spectrophotometer that was used for measuring parameters that were currently not possible to measure with the UV/VIS spectrometer (Table 4-1). These were ammonium nitrogen, orthophosphate and potassium. Each parameter was measured 7 times during each phase.

Table 4-1: Measurement methods used with Hach-Lange DR-1900 spectrophotometer

Parameter	Name	Number	Measurement range
Nitrogen Ammonia	Salycilate Method	Method 8155	0.01 to 0.50 mg/l NH_3–N
Orthophosphate	Ascorbic Acid Method	Method 8048	0.02 to 2.50 mg/l PO_4^{3-}
Potassium	Tetraphenylborate Method	Method 8049	0.1 to 7.0 mg/l K

4.3 Calibration process

The wastewater composition required a local calibration in order to take account of the specific water matrix and bring the sensor values closer to true values. It was done based on linear regression models between the spectrometer and laboratory values. As prescribed by Rieger et al. (2006) for successful local calibration the following conditions should be observed:

1) The grab samples measured in-situ and in the laboratory should be identical. This was ensured by mixing the samples (inflow and its dilutions) in 5-liter jerry cans and preparing samples for the laboratory analysis and in situ measurements from the same mixture.
2) The errors induced by sampling and samples storage should be minimized. To ensure this, the samples were brought to the lab within 3 hours in a cooling box.
3) The entire concentration range should be covered by reference samples. This condition was not fully ensured - the reference values were not equally distributed. Most of them were confined in the lower half of the concentration range and a few values in the upper end of the concentration range (not diluted influent), creating a small gap in between. The reason for this was that during the period the reference samples were taken, the influent concentrations were gradually decreasing.
4) The samples were taken throughout the span of the measurement – May to August and October, i.e. they include the temporal variability.

4.3.1 Sampling

For the UV/VIS spectrometer calibration 6 measurement events took place. TSS, COD, BOD_5 and NO_3-N have two different dataset corresponding to the two different global calibration methods used to measure them, namely effluent and influent global calibrations (EGC and IGC). TU, TOC and DOC equivalent values are measured with the groundwater global calibration (GWGC).

The following sample types were taken for the calibration:

- Influent - undiluted influent was taken immediate next to inflow to the treatment system
- Influent diluted with distilled water
  - 1:1 = 1 part influent + 1 part distilled water
  - 1:3 = 1 part influent + 3 parts distilled water
  - 1:7 = 1 part influent + 7 parts distilled water
- Effluent – taken from the outlet of the treatment system

Immediately after preparing the samples, the bottles were stored in a refrigerator. After the in-situ measurement was finished, the sample bottles were put in a cooling box with cooling elements and brought to the laboratory. For each sampling point, two samples for the laboratory were prepared, of which one was acidified to pH < 2 for preservation purposes with 1 mL concentrated sulphuric acid (H_2SO_4).

In total, 18 samples were taken and analysed for each parameter. Not all these samples could be used for the calibration since some parameters showed values below the quantification limit.

4.3.2 On-site measurements

A cleaning routine was followed before every on-site measurement with the spectrometer probe to ensure internal consistency of the measurements by removing organic and inorganic accretion from the lens, including:

- Removal of “multifunctional slide”
- Brushing the measuring path and rinsing with tap water
- Rinsing and wiping with ethanol
- Thoroughly rinsing with distilled water
- Brushing and rinsing of “multifunctional slide” with distilled water

From the prepared samples, approximately 100 ml were poured into a beaker for the spectrometer measurement. The measurement chamber of the probe was rinsed two times with the sample, and then filled up. The “multifunctional slide” was turned 90 degrees with respect to the SL cabinet. Between each sample measurement, the probe was rinsed with distilled water.

4.3.3 Laboratory analysis

The samples prepared for the SIG laboratory were analysed according to standardised methods listed in Table 4-2. The results of these measurements are presented in Table 9-1.

Table 4-2: Analysis methods of SIG laboratory with LOQ (limit of quantification)

Parameter	norm	LOQ
TSS	DIN 38409 (H2) : 1987	1 mg/l
NH_4-N	DIN 38406 (E5) – 1 : 1983	0.03 mg/l
BOD_5	DIN EN ISO 5815-1:2018-01 (H 50)	3 mg/l O2
COD	DIN 38409 (H41)-1:1980	10 mg/l O2
DOC	DIN EN 1484 (H3) : 2019-04	1.0 mg/l
NO_2-N	EN 26777 (D10) : 1993	0.003 mg/l
PO_4-P	DIN EN ISO 6878 (D11): 2004	0.02 mg/l
TNb	DIN EN 12260 (H34): 2003	0.1 mg/l
TOC	DIN EN 1484 (H3) : 2019-04	1.0 mg/l
TU	DIN EN ISO 7027-1:2016-11 (C21)	0.07 NTU
NO_3-N	EN ISO 10304-1 (D19) : 1995	0.1 mg/l
	EN ISO 10304-1 (D20) : 2009-07	
	EN ISO 10304-3 (D22) : 1997	
K	EN ISO 14911 (E34) : 1999	0.2 mg/l

4.3.4 Local calibration models

The calibration measurements were carried with the UV/VIS spectrometer user interface of the monitoring terminal (*con::cube, s::can*) and the manufacturer-specific software (*moni::tool,* *s::can*).This procedure is described in the moni::tool manual V3.1 (s::can Messtechnik, s-can.at, 2018). The Sample & Calibration button of the Service main window provides access to taking and managing sample measurements. After a name is given to the sample “Take sample” is pressed and the value is stored allowing the user to enter reference values later. The samples are given a number and a time stamp in the database, to be able to find the corresponding data pairs later an accurate measuring protocol is necessary.

The option “Multi calibration” could be used since we had more than two reference samples per parameter, but the software integrated local calibration was not used in the current study. The regular measurements were done only with global calibrations and different methods for local calibration were tested using MS Excel and R software. The following linear regression models were create in order to find the relationship between the lab values (true/reference values) and the s::can measurements, namely:

1) Lab – UV/VIS spectrometer entire EGC dataset for TSS, COD, BOD_5 and NO_3-N
2) Lab – UV/VIS spectrometer entire IGC dataset for TSS, COD, BOD_5 and NO_3-N
3) Lab – UV/VIS spectrometer entire GGWC dataset for TOC, DOC, TU
4) Lab – UV/VIS spectrometer effluent values range for all parameters
5) Lab – UV/VIS spectrometer influent values range for all parameters

In the last two, the influent and effluent values were split in two different models. The division however is not clear-cut. E.g. the BOD_5 lab values for the influent in the experimental phase are between 10 and 39 mg/l, therefore it makes little sense to allocate all of them to typical wastewater influent values. As orientation for the division line, the legal concentration limits for wastewater effluent were used, where such exist, as everything above the limit was allocated to the influent values.

All calibration datasets had lognormal distribution. For the statistical analysis they were transformed to normally distributed with natural logarithm function. Normality was necessary in order to calculate reliable residual standard errors (RSE), prediction intervals and R-squared. For building a linear regression model, normal distribution and constant variance of the residuals is assumed. All residuals were checked and showed homoscedasticity and normal distribution with two exceptions. TSS_EGC and TSS_IGC showed slight deviations from those requirements (Fig. 4.5). This is a sign that the model predictions cannot be fully trusted and there is a bias in predicted values. RSE is absolute measure of the lack of fit of linear regression model. It is the average amount that the predicted values deviate from the regression line (Eq. 4.3) (James et al., 2013). In other words, RSE is an estimate of the standard deviation of the Residual Sum of Squares (RSS), or the average amount, that the response (prediction) will deviate from the true regression line.

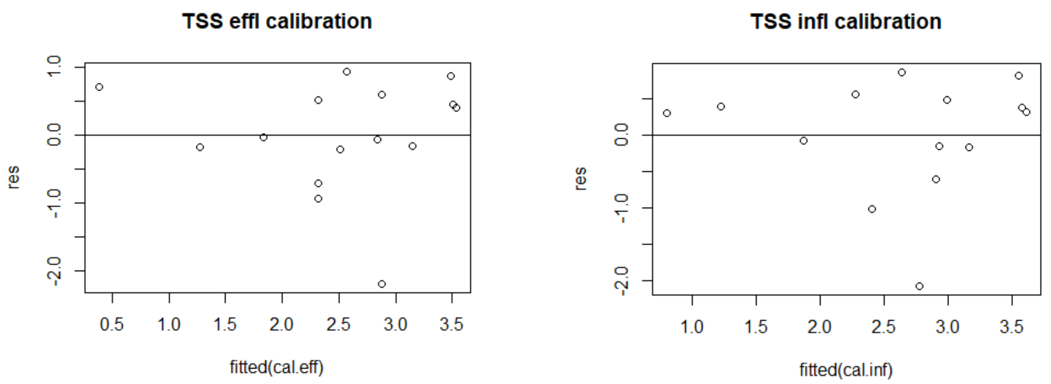


Fig. 4.5: Residuals vs fitted residuals in the TSS models

RSE was calculated from the transformed data and it is used to compare the models.

$$\text{RSE} = \sqrt{\frac{1}{n-2} RSS} = \sqrt{\frac{1}{n-2} \sum_{i=1}^{n} (Yi - \hat{Y}i)^2} \qquad \text{Eq. 4.3}$$

where

Y_i is the actual value, $\hat{Y}_i$ – the predicted value, i.e. the standard deviation of the errors of prediction.

For the goodness of fit the R-squared was considered. It is the proportion of variability in the predicted lab data that can be explained using sensor data. Additionally, p-values for the slope are presented allowing us to decide if we can assume that with certain probability there is a relationship between the predictor and predicted variables. The 5% probability that we can be wrong rejecting the Null hypothesis is used as a criterion for deciding on whether to use the regression model or not.

The percent error was calculated with the original data. The latter gives the accuracy of the measurement by difference between the measured (experimental) value E and the accepted true value A. It is very informative when one wants to know how far the predicted from the true values are.

$$\% \text{ Error} = \frac{|E-A|}{A} * 100 \qquad \text{Eq. 4.4}$$

4.4 Measurements

4.4.1 Spectrometer measurements

Samples were taken at four sampling points – influent – in the beginning of the treatment system and after each treatment bed - upper, middle and effluent. In the first phase a total of 26 measurement events took place. In the second phase 20 measurements were performed.

Various combinations of the models described in Table 4-3 were used to predict laboratory data from the spectrometer values.

Table 4-3: Approaches for prediction of laboratory from spectrometer values, EGC = Effluent Global Calibration, IGC = Influent Global Calibration, GWGC = Groundwater Global Calibration

Approach	Influent	Upper	Middle	Effluent
I	IGC/ GWGC	IGC / GWGC	EGC / GWGC	EGC / GWGC
II	IGC	2/3 IGC + 1/3 EGC	1/3 IGC + 2/3 EGC	EGC
III	2/3 IGC + 1/3 EGC	1/3 IGC + 2/3 EGC	EGC	EGC
IV	EGC	EGC	EGC	EGC
V	Influent values range	Influent values range	Effluent values range	Effluent values range

The IGC and EGC signifies that the entire dataset models were used in the respective approach. In the second and third approaches a proportion of the influent model predicted values were combined with a proportion of the effluent model predicted values. The assumption is that the two intermediate beds do not clearly belong either to the influent or the effluent of the system. The fifth approach uses the datasets split in influent and effluent values that come from regression models 4 and 5 (section 4.3.4).

For the prediction of the data the models were created with natural logarithm transformed values. The back transformation was calculated with equation Eq. 4.5:

$$C_{lab} = e^{(\beta o + \beta 1 \ln(Cs))} \qquad \text{Eq. 4.5}$$

where

C_{lab} are the predicted values, C_s sensor values

4.4.2 Analysis of the predicted data

Tests for statistically significant differences between the inflow and the outflow of a constructed wetland are normally not performed due to the assumption that they are different. In the current study we measured three treatment beds and one of the goals was to analyse the treatment process along the system. The fact that the system was divided in three beds was an opportunity to gain a better understanding of how treatment process works. For this reason, tests for significant differences among the three beds and the between the latter and influent were performed.

The predicted datasets showed non-normal distribution. For testing the differences between the spectrometer predicted values first Kruskal–Wallis one-way analysis of variance by ranks test was used. In order to find out between which groups the difference are, the Conover-Iman Test of Multiple Comparisons Using Rank Sums was used. The Conover-Iman test is strictly valid if and only if the corresponding Kruskal-Wallis null hypothesis is rejected (Dinno, 2017). Both tests are non-parametric, i.e. there is no assumption that the data come from a particular distribution. The null hypothesis is that there are no differences between the groups. The tests were run for all five approaches.

The bootstrap method was used to calculate the confidence interval of the data. The practical meaning of 95% confidence interval is that it shows with 95% probability the range of the mean value. This is important because of legal restrictions for maximum concentration that can be discharged in the environment. Due to the unknown distribution of the datasets, they could not be used for calculating a mean and CI. Bootstrapping is a process that is used when the sample size is small, and the distribution is non-normal. It is a method that creates multiple random samples with replacement from a single sample. These repeated samples are called resamples. Each resample is the same size as the original sample. The final sample is made up of the means of each resample and tends towards normal distribution. The influent and effluent datasets were bootstrapped and mean values and 95% confidence intervals calculated and presented graphically (Orloff, 2014).

Analysis where conducted with the statistical softwar R (R Core Team, 2013). All graphs were prepared with MS Excel® and R package "ggplot2" (Wickham, 2016) and colour pallet package (Neuwirth, 2014).

4.4.3 Photometer measurements and data analysis

As described before on-site measurements were performed for ammonium nitrogen, orthophosphate and potassium with a Hach-Lange field photometer. Each parameter was measured seven times during each phase. The method requires necessary dilution to be applied, so that the results fall within the respective measurement range of the device. The respective reagents are added to the sample resulting in a specific sample colour. Colour intensity is proportional to the concentration in question. The accuracy of photometer was checked with the Hach-Lange standard solutions.

For the statistical analysis, the values for NH_4-N below the quantification limit are accepted as the lower detection threshold of 0.01 mg/l. Conover-Iman Test of Multiple Comparisons as described in 4.4.2 was used for comparison of group differences.

The interquartile range (IQR) was used to express statistical dispersion around the median. It is less precise than standard deviation and tells only where the middle 50% of the data is located but in case of lack of normal distribution and small samples it is the preferred measure.

4.4.4 Treatment performance of the wetland system

The removal efficiency of the wetland system was calculated subtracting the outflow concentration of the parameter from its inflow concentration and dividing the result to the inflow concentration. To express it as a percentage it is multiplied by 100 (Eq. 4.6).

$$R\ (\%) = ((C_{in} - C_{out})/C_{in})*100,\ \% \qquad \text{Eq. 4.6}$$

C_{effl} - median of the effluent concentration, C_{infl} - median of the influent concentration.

In the current study the efficiency is calculated for each treatment bed in the wetland system, i.e. the removal between the influent after the pre-treatment step and the effluent after each treatment bed, as the last treatment bed represents the total of the whole HF wetland system.

The removal of the entire system including both the pre-treatment step and the secondary treatment step is calculated with the theoretical loads for raw blackwater (DWA, 2014) in g per person per day. The concentrations in the raw black water are calculated based on the average toilets uses frequency, and the daily generated amount of raw black water. It is assumed that one person visits a toilet 6 times per day. Eq. 4.6 is then used with inflow concentration of the raw blackwater and outflow concentrations of the HF last bed effluent.

4.5 Microbiological parameters

4.5.1 General procedure

Culturable microorganisms, *Escherichia coli* and total coliforms as well as *Enterococci* were tested four times during each phase. Two of the tests were conducted in the SIG laboratory and the 6 in the laboratory of alchemia-nova, using the same methods. Two sampling points were tested – the influent and effluent of the wetland system. Samples were taken in sterile bottles and stored in a fridge at 4°C. Conventional serial dilution method up to 10^{-7} was used for CFU/mL of colonies in the water samples before and after the treatment. Each dilution test was duplicated. After incubation, colonies that appeared were enumerated and the CFU of each colony was then calculated using Eq. 4.7:

$$\text{CFU/ mL} = \text{number of colonies x dilution factor/ volume of sample} \qquad \text{Eq. 4.7}$$

The inoculation procedure was performed in a laminar flow for sterile working environment treated beforehand with UV radiation and alcohol. The following methods were used for the analysis:

4.5.2 Enumeration of culturable micro-organisms

Method (EN ISO 6222:1999). The method includes aerobic bacteria, moulds, yeasts, which are capable under specific conditions to form colonies.

Yeast extract was prepared by suspending 24 g of the medium in 1 l distilled water, heated until complete dilution and sterilization at 121ºC in an autoclave for 15 minutes. Of each dilution 1 ml is pipetted into separate sterile 100 mm petri dishes. Subsequently 15 – 20 ml of liquified culture medium is poured into each petri dish. After mixing medium and sample the mixture is allowed to solidify and subsequently the petri dishes are inverted and incubated at two temperatures at 37º C for 44 hours and at 22º C for 68 hours. The number of colonies per plate was counted and CFU calculated per 1 ml sample.

4.5.3 Enumeration of *Escherichia coli* and coliform bacteria

Method (ISO 9308-1:2014 + Amd. 1:2016) - Part 1: Membrane filtration method for waters with low bacterial background flora.

Chromogenic coliform agar 26.45 g was suspended in 1 l distilled water. The solution was heated until fully solved. Petri plates 60 mm diameter were poured with the medium and stored at 4º C. 100 ml of the sample was filtered through 0.45 μm pore size, 0.47 mm diameter filter, and then incubated at 37º C for 21-24 hours in the prepared petri plates. Dark-blue to violet colonies are counted as *E. coli*. The total coliforms were counted as all red colonies (presumed to be coliforms) + dark-blue colonies.

4.5.4 Detection and enumeration of intestinal *Enterococci*

Method (ISO 7899-2:2000) – Part 2: Membrane filtration method.

Slanetz-Bartley agar was prepared from 41.5 g medium and 1 l distilled water. Bile Esculin Azide agar was prepared from 56.6 gram of medium and 1 l distilled water. Heated until fully dissolved then sterilized in autoclaved 121 C for 15 min.

Petri plates 60 mm in diameter were poured from both agars, solidified and then stored at 4º C. 100 ml sample was filtered and the filter was placed on the solidified medium in the petri plate. The plates were incubated at 37º C for 44 hours. After the incubation, filters with colonies that turned red, brown or rose are transferred to Bile Esculin Azide agar and incubated for 2 hours at 44º C. All colonies that turn yellow-brown to black are reported as intestinal *Enterococci*.

5. Results and discussion

5.1 Calibration

The linear regression models with equation $Y = \beta_o + \beta_1 X + \varepsilon$ applied for the local calibration, as described in section 4.3.4, are presented in this section. The basis for the modelling were the data from laboratory measurements and their corresponding spectrometer values (Table 9-1). The slope (β_1) and the intercept (β_o) of each model are given in Table 5-1. For all water quality parameters, the modelling with the entire dataset EGC, IGC and GWGC, shows that the slopes are statistically different from 0, i.e. there is a strong proof for a relationship between the predictor and predicted variables. When it comes to the models constructed from the effluent and influent values range, there is not sufficient statistical evidence that relationship exists between the predicted and predictor variables for BOD_5 and TU influent values range and TSS, TOC and TU effluent values range. Therefore, those regression models were not be used for data prediction.

The number of reference points per parameter are different because some values were below the laboratory quantification limit techniques and could not be used for the calibration.

Table 5-1: Regression models slope, intercept and coefficient of determination (based on ln values), # - number of points, EGC = Effluent Global Calibration, GWGC = Groundwater Global Calibration, IGC = Influent Global Calibration

	#	EGC / GWGC (TU, TOC, DOC)			IGC			Effluent values range			Influent values range		
		R2	**slope**	**offset**	**R2**	**slope**	**offset**	**R2**	**slope**	**offset**	**R2**	**slope**	**offset**
COD	16	0.93	0.92^{***}	0.61	0.95	0.96^{***}	-0.50	0.69	0.73^{**}	1.01	0.86	0.85^{**}	0.09
BOD_5	14	0.80	0.95^{***}	-0.02	0.75	1.16^{***}	-3.55	0.70	0.84^{**}	0.10	0.91	$0.87^{\dagger}$	-1.62
TSS	15	0.53	1.04^{**}	-0.33	0.53	0.83^{**}	-0.10	0.26	$0.41^{\dagger}$	0.94	0.72	0.53^{*}	1.68
NO_3-N	18	0.73	1.44^{***}	-2.31	0.80	1.74^{***}	-2.28	0.80	1.33^{*}	-1.19	0.66	1.23^{***}	-1.57
TOC	18	0.80	0.99^{***}	0.06		na		0.36	$0.45^{\dagger}$	0.92	0.77	1.21^{**}	-0.45
DOC	18	0.93	0.8^{***}	0.78		na		0.72	0.65^{***}	0.87	0.96	0.87^{***}	0.67
TU	17	0.56	0.82^{***}	-0.49		na		0.13	$0.32^{\dagger}$	0.21	0.08	$0.22^{\dagger}$	2.25

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, † $p>0.05$

For comparison of the goodness-of-fit of the regression models for each parameter, the adjusted coefficient of determination and the residual standard error (RSE) were used (Table 5-2). Adjusted R-squared reduces the value of R-squared until it becomes an unbiased estimate of the population value. While R-squared provides the relative measure of the percentage of the dependent variable variance that the model explains, RSE provides the absolute measure of the distance that the data points fall from the regression line. That is why when comparing two model we should consider both parameters. The precision of the models was estimated with the 95% PI. 95% PI is the range where, with a probability of 95%, a single new observation will fall given values of the independent variable. Therefore, narrower prediction intervals indicate more precise prediction. Fig. 5.1 shows graphical examples for the parameters with narrow and large prediction intervals. The width of the prediction interval is given for the mean sensor value. It should be noted that PI, RSE and R-squared adjusted are calculated from the natural logarithm transformed data, therefore they can only be used for comparing the calibration models.

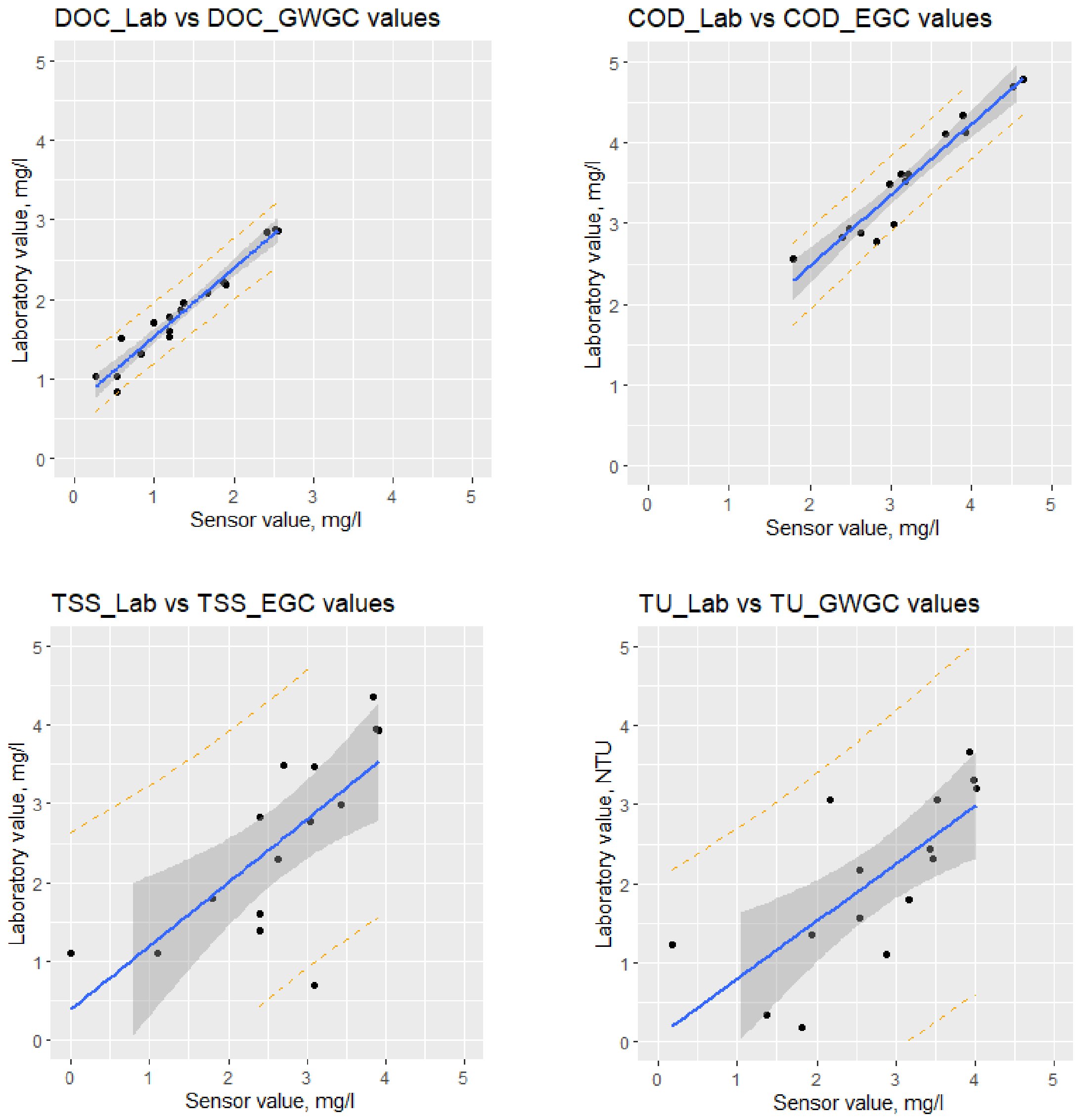


Fig. 5.1: 95% Prediction intervals for selected parameters

The goodness-of-fit is lowest for the TU and TSS models. The laboratory methods for TSS measurements are based on the solids weight, which can be measured very accurately. The spectrometer values coming from light absorption will depend on how well the sample was mixed. A spectrometer measurement takes around 15-20 seconds during which time some of the solids can start sedimenting and thus they will be “missed”. That explains the higher discrepancy between the sensor values and the fitted values. If we classify the goodness-of fit in very good, good and fair, we can put the COD and DOC in the first category, BOD_5, NO_3-N and TOC in the second and TSS and TU in the third one (Table 5-2). COD and DOC models based on EGC and IGC values have higher goodness-of-fit, compared to the other parameters. COD models are given as an example in Fig. 5.2.

EGC and IGC models for the TSS, COD and BOD_5 have very similar PI, R-squared adj and RSE. Based on these small differences it is not possible to say which calibrations are better. Generally, when the modelling is based on effluent and influent ranges, the coefficients of determination drop, the PI becomes larger and the residuals become higher, compared to the EGC and IGC that are based on entire dataset. This is not the case with NO_3-N. Here the effluent values model is more precise and accurate, and the influent values model is more precise but with slightly lower accuracy. TSS influent values range model also performs better than the models based on entire dataset, but it cannot be used for the prediction since its effluent counterpart model is not reliable.

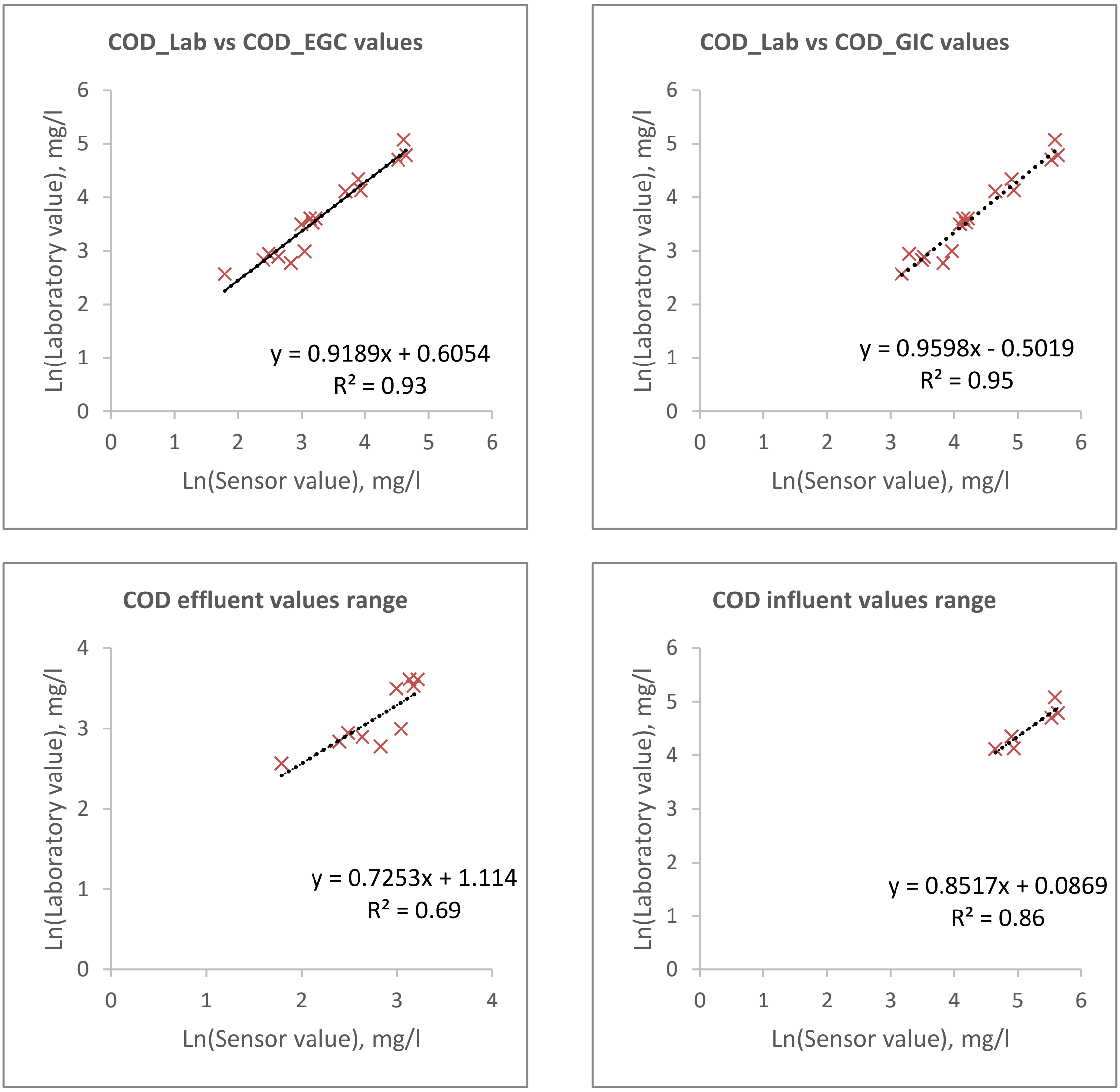


Fig. 5.2: COD linear regression models based on the natural logarithm transformed data

To calculate how far away the equivalent from true values were, the percent error was calculated. It shows the accuracy of the global calibrations for this specific water matrix in case local calibrations are not available. There was no general pattern showing if equivalent values were higher or lower than true values. On average TSS_{eq}, BOD_{5eq} and NO_3-N_{eq} overestimated the true values in both global calibrations. All DOC true values were underestimated, and all TU true values were overestimated. COD_{eq} EGC underestimated the true values and COD_{eq} IGC overestimated them.

Table 5-3 shows that for the IGC equivalent values the percent error was higher for all parameters than the error for the EGC equivalent values, with the exception of NO_3-N. In other words, EGC equivalent values were much closer to the true values.

We can assume that the spectrometer factory IGC is based on samples with higher concentrations in the influent than the ones in the current experiment. Therefore, for the current water matrix, in case a local calibration is not available, COD_{eq}, BOD_{eq} and TSS_{eq} will give results closer to the true values if measured with EGC. For TSS_{eq}, TU_{eq} and NO_3-N_{eq} the error is too big to allow using the spectrometer values without local calibration.

Table 5-2 Goodness-of-fit and precision of the models, based on ln values. # - number of points, EGC = Effluent Global Calibration, GWGC = Groundwater Global Calibration, IGC = Influent Global Calibration

	#	EGC / GWGC			IGC			Effluent values range			Influent values range		
		95% PI	R2 adj	RSE	95% PI	R2 adj	RSE	95% PI	R2 adj	RSE	95% PI	R2 adj	RSE
COD	16	0.93	0.93	0.212	0.82	0.94*	0.187	1.14	0.65	0.234	0.99	0.82	0.165
BOD_5	14	1.7	0.79*	0.374	1.91	0.73	0.422	1.93	0.66	0.408	3.52	0.83†	0.120
TSS	15	3.78	0.50	0.847	3.72	0.50	0.826	3.64	0.17†	0.748	1.3	0.64*	0.214
NO_3-N	18	3.73	0.72	0.857	3.25	0.79	0.75	2.67	0.74*	0.397	3.01	0.63*	0.656
TOC	18	1.59	0.79*	0.365		na		1.41	0.28†	0.299	1.67	0.72	0.302
DOC	18	0.75	0.93*	0.173		na		0.96	0.69	0.205	1.27	0.95	0.088
TU	17	4.28	0.53*	0.912		na		4.27	0.03†	0.898	2.77	-0.1†	0.501

† - models without significant relationship; * - models that perform better for specific parameter

Table 5-3: Percent error based on the original values. EGC = Effluent Global Calibration, GWGC = Groundwater Global Calibration, IGC = Influent Global Calibration

GC	COD	BOD_5	TSS	NO_3-N	TU	TOC	DOC
IGC	100	1550	198	209			
EGC	28	37	111	361			
GWGC					338	28	39

An increased number of calibration points will add more robustness to the models and will make them more reliable. Adding validation data would fine-tune our confidence in the models. For the current work the multiple points local calibration gave more accurate results than the global calibrations would but validation data were not collected.

5.2 Prediction of laboratory values based on s::can spectrometer equivalent values and analysis

Twenty-six measurements were conducted during the test phase and 20 measurements during the experimental phase at 4 points of the system. Five different approaches were used to predict laboratory values from the models and the spectrometer measurements. The prediction approaches (Table 4-3) are based on the selected models from the previous section. The results for the two phases are presented below.

5.2.1 Phase I

Approaches 1, 2, 3 and 4 (Table 4-3) use EGC and IGC and their combinations for predicting the laboratory values. All four approaches give very similar median values (Table 9-2). Approach 5 showed differences in the median values, notably for NO_3-N_{eq}. The lack of significant difference among the treatment beds and the influent are not the same for every approach (Table 5-4) due to differences in dataset structures in terms of data dispersion and number of outliers. Most of the parameters showed no difference between the effluent and the middle treatment bed, i.e. the treatment at the last bed did not lead to a significant change of the concentrations. The graphical representations and further analysis consider only the results from one approach. The choice of approach is justified as follows: for TSS_{eq} and DOC_{eq} the results do not differ among approaches, so any approach can be taken; for COD_{eq} the prevailing result was selected; the result from approach 4 was selected for BOD_{5eq} due to the low values of both influent and effluent concentrations and better performance of EGC model; for $NO_3\text{-}N_{eq}$ the models used in approach 5 performed best; TU_{eq} and TOC_{eq} could only be analysed for one approach.

Table 5-4: Concentration and NTU lacking statistically significant differences among influent and treatment beds; "-" signifies that there were differences among all treatments beds and the influent

	Approach 1	**Approach 2**	**Approach 3**	**Approach 4**	**Approach 5**
TSS_{eq}	Effl-mid*	Effl-mid	Effl-mid	Effl-mid	na
COD_{eq}	-*	Effl-mid	-	-	-
BOD_{5eq}	Effl-mid Mid-up	Mid-up	Mid-up	Effl-mid* Mid-up	na
$NO_3\text{-}N_{eq}$	Effl-mid Up-infl	Effl-mid Up-infl	Effl-mid	Effl-mid	Effl-mid* Up-infl
TOC_{eq}	Effl-mid* Mid-up Effl-up	na	na	na	na
DOC_{eq}	-	na	na	na	-
TU_{eq}	Effl-mid* Mid-up Effl-up	na	na	na	na

* results to be considered in further analysis

The boxplot graphs visualize the median concentrations and the differences among treatment beds and influent concentrations (Fig. 5.3). For organic matter, TSS and TU, the highest concentration reduction takes place in the first treatment bed and even if there are significant differences among other beds, they are relatively smaller. In case of $NO_3\text{-}N_{eq}$, the concentration increases sharply in the middle bed. While the nitrate concentration did not change in the upper bed, $NH_4\text{-}N$ has been reduced significantly (Fig. 5.11). The question is what happened to $NH_4\text{-}N$ if it was not converted to nitrates? Loss of ammonia through volatilization is considered negligible. Ammonia nitrogen is more reduced energetically than nitrate, therefore it is readily incorporated into amino acids by many autotrophs and microbial heterotrophs being a preferable source of nitrogen for assimilation (Vymazal, 2006). This might have contributed to the decrease of ammonia but still not in substantial amount. The first bed lacked good oxygen supply due to replacement of the voids by solids, so there was not enough oxygen for the nitrifying bacteria, which are also outcompeted by carbon decomposing bacteria. Studies have shown that anaerobic ammonia oxidation (anammox) could also be the removal route for ammonia in HF TWs. In environment with nitrite and ammonia present a reaction to dinitrogen occurs (Dong and Sun, 2007; Tao and Wang, 2009; Van de Graaf *et al.*, 1995) (Eq. 3.6). This process requires only 1.94 g O_2 per gram $NH_4\text{-}N$ (less than half that in the conventional denitrification process) and is autotrophic, i.e. there is no organic carbon requirements (Kadlec and Wallace, 2009). The $NH_4\text{-}N$ in the upper bed might have been lost through anammox, due to good conditions for it.

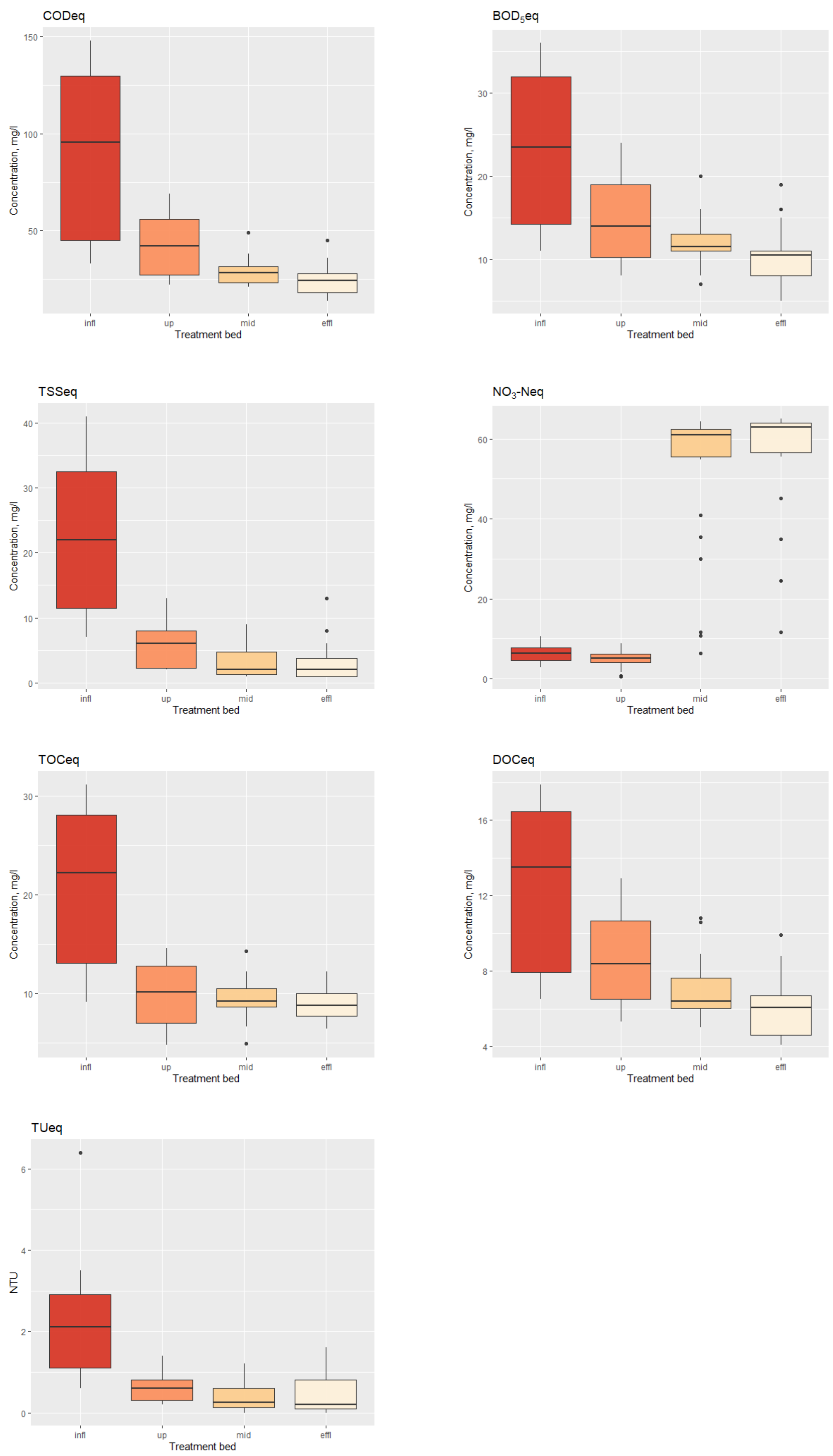


Fig. 5.3: Parameters concentrations by treatment bed

The lack of difference between the middle and effluent bed in nitrates concentration can be explained by the lack of aeration in the last treatment bed, hence low or no conversion of NH_4-N. Moreover, the NH_4-N levels were already very low before the last bed – 0.01 mg/l and therefore there was no NH_4-N available for conversion (Fig. 5.11). The lack of change of nitrates at the third bed shows on one hand that no ammonium was converted but on the other, that no denitrification took place despite the lack of oxygen supply. Denitrification process is done by heterotrophic bacteria and despite the environment rich in nitrate it was poor in carbon and this might have prevented the denitrification process. For the heterotrophic denitrification to be possible a ratio COD/N should be more than 7.6 (Todt et al., 2015). In the current study the ratio COD:NH_4-N was 4.8.

TSS and partly TU depend on the solids quantity in the water which is mostly reduced in the beginning of the first treatment bed. TU did not show significant difference among the upper middle and effluent beds and for TSS the difference between upper and middle bed was very small. The solids movement in the substrate is prevented as soon as the influent enters the system, because the velocity of the flow sharply falls, and they tend to accumulate in the front part. That also often becomes the reason for clogging of the system.

COD:BOD ratio of the influent was 3.4 which means the prevailing amount of organic matter was slowly biodegradable. Almost half of the BOD_5 reduction happens in the upper treatment bed, where the microbial processes are most intensive. The differences in BOD_5 concentrations between in- and outflow of the next two beds are not significantly different.

DOC has been reduced gradually along the system, but most of its reduction still happens in the first bed. At the effluent bed, where oxygenation is not applied, DOC concentrations hardly changed. A reason for the small change is that DOC may have also non-biodegradable fraction that leaves the system without being altered. TOC is reduced mostly in the first bed, where also most of the solids (particulate matter) is being trapped and most of the readily biodegradable carbon is being consumed.

So far, we looked at the median values since they are more representative for samples with unknown distribution than mean values and are not affected by outliers. In the approach below we have calculated the mean concentrations and 95% confidence intervals around the means

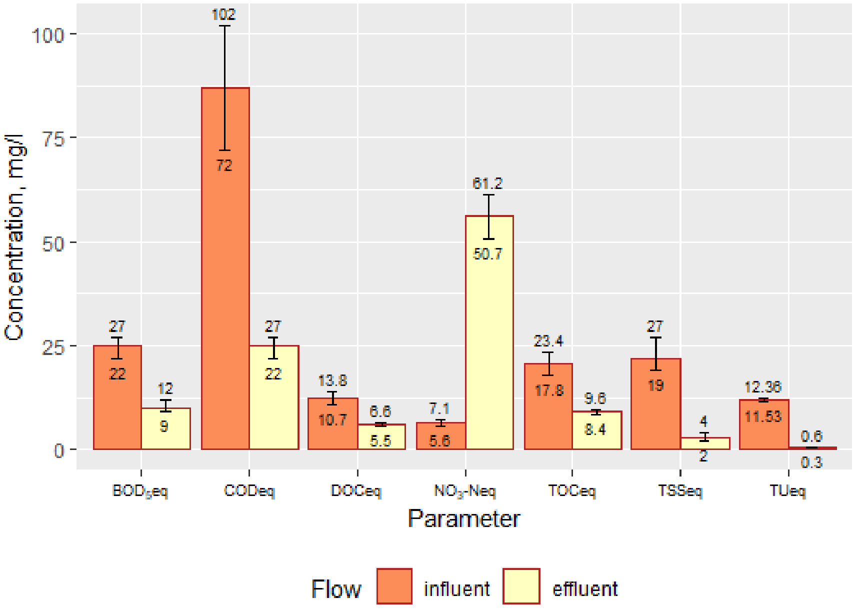


Fig. 5.4: Mean concentrations of in- and outflow and the respective 95% confidence intervals (error bars), based on bootstrapped data

derived from bootstrapping of the influent and effluent datasets of the parameters. Bootstrapping allows normal distributions to be derived and CI calculated. The CI tells us with 95% probability that the mean value will fall within the interval. This information is necessary when considering legal concentration limitations. E.g. our results show that the effluent concentration of BOD_5 will be in the interval between 9 and 12 mg/l (Fig. 5.4). If the upper CI is below the strictest requirements in effluent discharge, we can make sure that the probability of exceeding the limit concentration is lower than 2.5%. Further compliance with legal requirements will be discussed in phase 2, which represents better the cleaning performance of the system.

Fig. 5.5 shows the single concentration measurements during the test period. The parameters have decreasing trend except for the effluent turbidity and solids concentrations. The reasons for the decreasing trend in the influent can be explained with the increased quantity of solids in the beginning of the treatment system that clogged the pores and prevented the water from freely moving through. This process was happening progressively during the measurement period leading to less and less water being conveyed to the system. Even though the pumping regime stayed the same, less water was taken up for treatment. Thus, the water stayed longer in the tank, where it was aerated, and was becoming cleaner. Since increasing amount of water was blocked, the collection tank stayed full to the outflow level all the time. The fresh wastewater with higher concentration entered the tank 10 cm above the overflow and was flowing out without reaching the area around the pump.

For a wetland with horizontal subsurface flow most critical operation problem is the clogging. One of the maintenance requirements is regular emptying of solids from the settling tank upstream. There were several reasons for the clogging in the current case (Fig. 5.6). Even though big amount of the solids settled in the collection tank the wastewater did not pass a stage of zero velocity and still a lot of solids went further in the cleaning system. Even in systems with primary sedimentation, around 1/3 of the suspended solids are not removed in this stage and enter the biological reactor (von Sperling, 2007). Secondly, in order to reduce the clogging potential, the geometrical shape of the HF wetland should have an aspect ratio length to width between 2:1 and 4:1 (Dotro et al., 2017). In the current system this ratio was more than 8:1 if we consider the length of the upper bed.

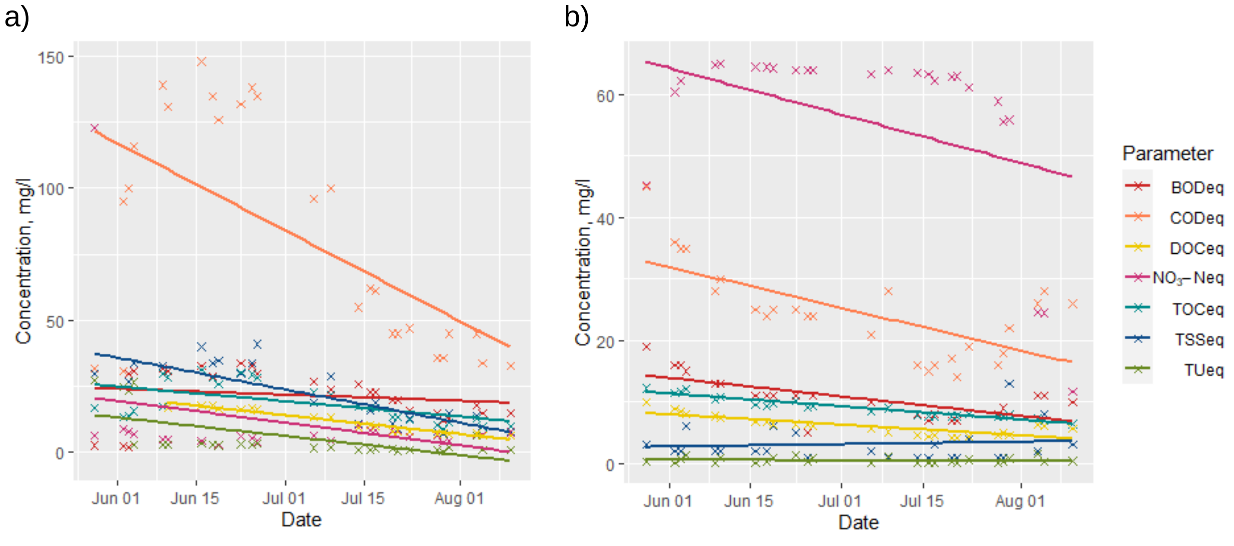


Fig. 5.5: Individual concentration values a) influent; b) effluent

Additional obstacle for the flow movement was the separation of each bed in two containers linked only with the cylindrical connection about 10 cm in diameter, which was easily blocked by the growing root system. The walls perpendicular to the flow movement were meant to direct and slow down the flow. However, they contributed further to the reduced movement of solids, which eventually blocked the front part of the wetland.

In a simulation of 4 wetlands with different depths and aspect ratios Sanchez-Ramos et al. (2017) found that the clogging progressed faster in shallow HF wetland (0.27 m) because of lower pore volume available for solids accumulation. This depth is similar to the water depth of the current experiment and shows that together with other factors the clogging is inevitable.

Fig. 5.6: Removal of the clogged substrate at the end of phase I

5.2.2 Phase II

The laboratory values prediction with the first four approaches gave very similar medians for all parameters (Table 9-2). Approach 5 is the only one that shows differences, most pronounced in NO_3-N_{eq} concentrations (Table 5-5). Here, significant difference between the effluent and middle bed were not detected for TSS_{eq}, TU_{eq} and NO_3-N_{eq} (the same approaches as in the previous phase are considered). All the other parameters showed significant difference among treatment beds and the influent. Here, as in the first phase, the biggest differences between concentrations were observed after the first treatment bed and less concentration reduction took place at second and third beds (Fig. 5.7).

Table 5-5: Concentration and NTU lacking statistical difference among influent and treatment beds

	Approach 1	**Approach 2**	**Approach 3**	**Approach 4**	**Approach 5**
TSS	Effl-mid	Effl -mid	Effl -mid	Effl -mid	na
COD	-	Effl -mid	-	-	-
BOD_5	Effl -up Mid-up	Mid-up	-	-	na
NO_3-N	Effl -mid	Effl -mid	Effl -mid	Effl -mid	Effl -mid
TOC	-	na	na	na	na
DOC	-	na	na	na	-
TU	Effl -mid	na	na	na	na

The organic materials, the food for microorganisms, come with the high influent concentrations. The closer to the inlet the higher the organics concentrations. Therefore, unless pushed further down the wetland by solids, the maximum amount of microorganisms will be also found in the front part of the wetland (Sanchez-Ramos et al., 2017). Logically the highest consumption of oxygen and carbon will take place in the first part of the system.

The ratio COD:BOD_5 in the influent was 4. In a blackwater influent in dormitory in Norway the ratio ranged from 2.7 to 3.4, an indication that the blackwater contains higher share of inert organic materials (Todt et al., 2015). Typical values for the ratio COD:BOD_5 for untreated blackwater are in the range from 2.0 to 3.6 (Table 5-6). If the COD:BOD_5 ratio for untreated wastewater is 2 or lower, the waste is considered to be easily treatable by biological means. The ratio changes significantly with the degree of treatment the waste has undergone. Normally the ratio increases in the effluent because the biodegradable organic material is consumed in the treatment process. In the current case, the effluent ratio drops to 3.1. The COD concentration reduction in the system is much higher than that of BOD_5 (Fig. 5.8; Table 5-10). There is a probability that a big amount of particulate non-biodegradable organic matter was trapped in the system and that reduced a lot the COD concentration but did not affect BOD_5. (Todt et al. 2015) reported about 70% of the COD bound to particulate matter in blackwater. Knerr et al. (2011) found a high fraction of COD in blackwater being inert. However, Hocaoglu et al. (2010) studied biodegradation characteristics of blackwater and found out that the inert soluble and particulate COD was only 4.8% of the total COD. The nature of the solids and the reason for their low bio-degrability in the studied system needs to be investigated further. It should be kept in mind that if the black water is going to be reused in a closed cycle, e.g. for toilet flushing, this inert fraction would accumulate in the system and will impede the biological treatment further.

The blackwater in other studies was much more concentrated (Table 5-6). To make the data comparable the concentrations were recalculated to 14 l per toilet flush. The organic matter in the blackwater in the literature is much higher than in the current experiment, while the nutrients are lower. The differences could be ascribed to the solid fraction separation in the current study.

Table 5-6: Mean concentrations in blackwater for residential households (* calculated concentrations for 14 l toilet flush).

	Current study	**Todt et al. (2015)**	**Knerr et al. (2011)**	**Graaff (2010)**	**Hocaoglu et al. (2010)**	**Sakurai et al. (2021)**
L per flush	14	1.2	9	1	na	na
EC, $mS.cm^{-1}$	1.9		2.6			
BOD_5, mg O_2 L^{-1}	38	3100-3600 265.7-308.5*	323 207.6*		338	455.8
COD, mg O_2 L^{-1}	152	8900-11400 763-814*	720 463*	9800 700*	1225	1215.7
NH_4-N $mg.L^{-1}$	200		202 129.5*	1400 100*	147	174.6
NO_3-N $mg.L^{-1}$	0.3		1.8 1.2*			
PO_4-P $mg.L^{-1}$	35	150-200 (total P)	23.3 14.9*	79 5.6*	25 (total P)	24.7
TOC $mg.L^{-1}$	30.9		306 196.7*			
TSS $mg.L^{-1}$	52		67 43*		625	
E. coli, CFU/100 ml	$2.6x10^5$		$1.7x10^6$			

High conductivity in the blackwater, which comes from nutrient salts in the urine - mostly sodium compounds such as sodium chloride and sodium nitrate, was reported by Knerr *et al.* (2011). It is slightly higher than the one measured in the current experiment but falls in the same range.

The reason for the low nitrification in the first bed could be that bacteria involved in the nitrification process require more time to reproduce and are more sensitive to environmental conditions than the heterotrophic bacteria involved in the stabilisation of the carbonaceous organic matter, so the

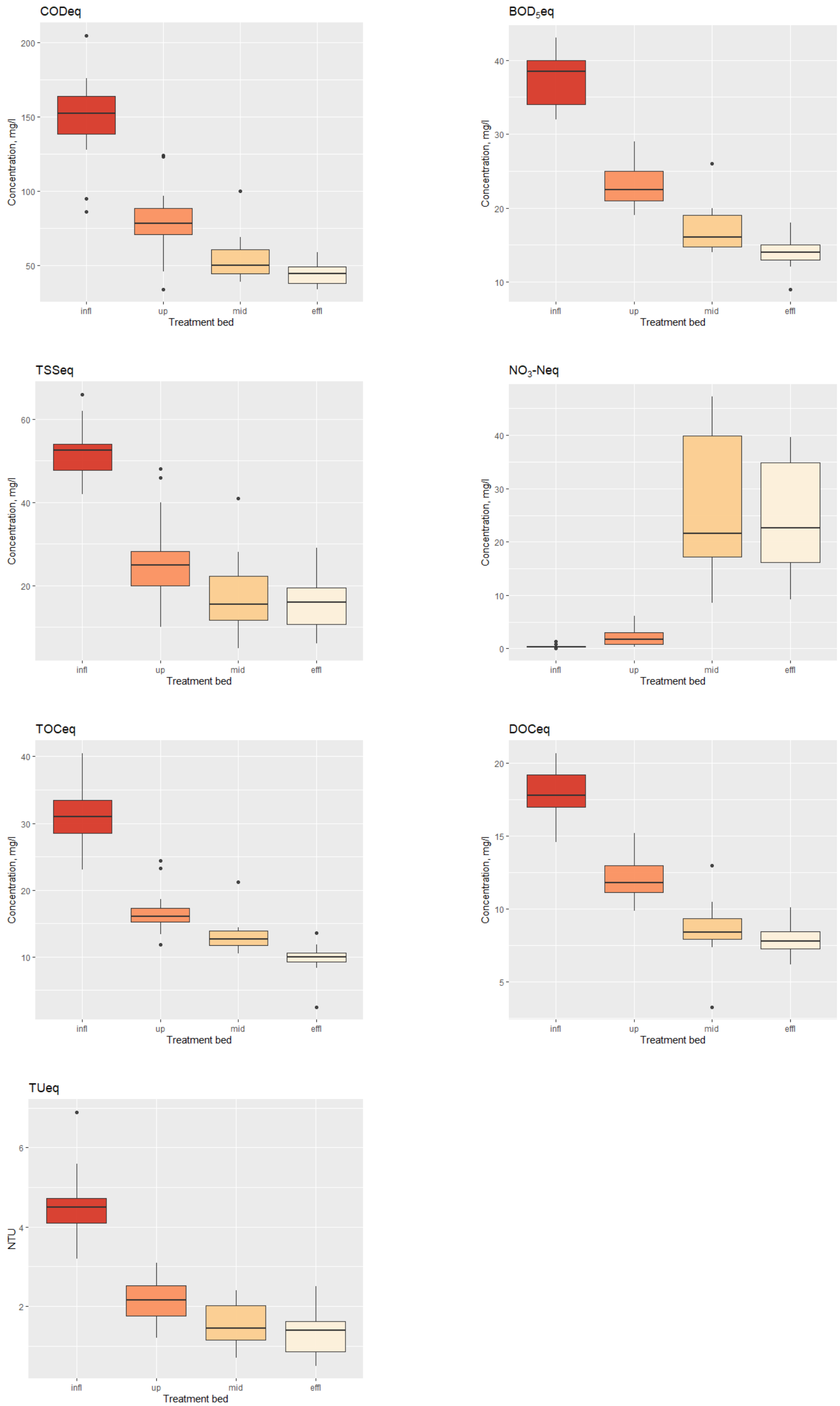


Fig. 5.7: Parameters concentrations by treatment bed

latter outcompete the former (von Sperling, 2007). In the first treatment bed, high reduction rate of the organic substances can be observed as a result of high activity of chemoheterotrophic bacteria. Nitrates concentrations do not show significant difference between the outflows of the middle and effluent beds, either i.e. there is no conversion between the nitrogen constituents in the last bed. The likely reason for the absence of nitrification is the lack of oxygen. The lack of significant difference of nitrate concentration in the third level means also that no denitrification took place, although the opposite was expected. This is most probably due to very low COD:N ratio and lack of food for the heterotrophic denitrifying microorganisms. Before entering the last bed BOD_5 was as low as 16 mg/l.

The mean concentrations received from data bootstrapping showed higher values than in the first phase (Fig. 5.8). This can be explained with the much shorter HRT of the wastewater in the collection tank due to higher conductivity of the treatment beds substrate.

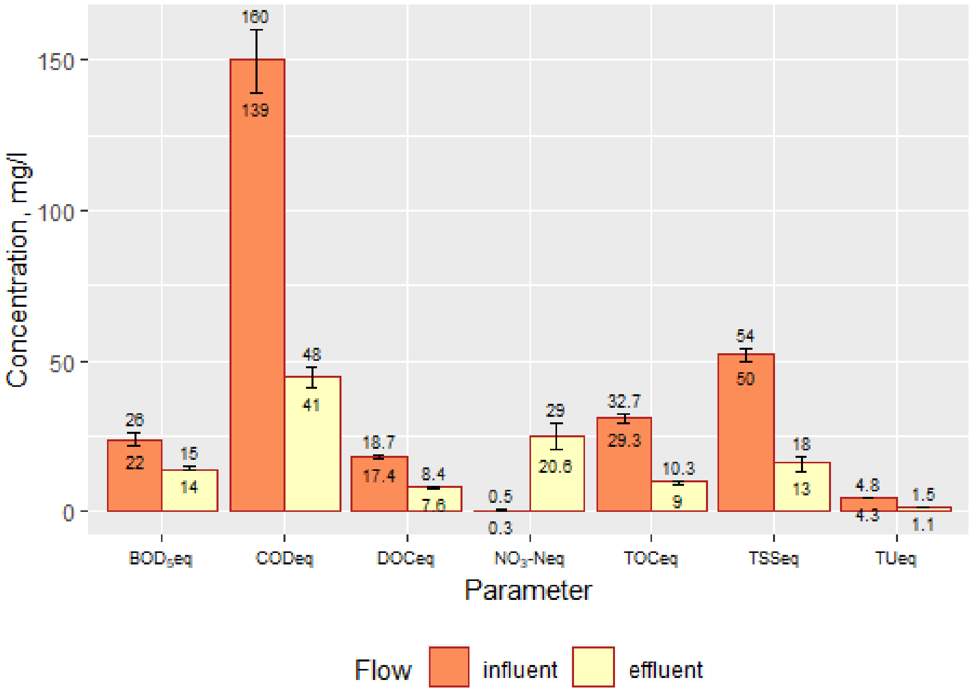


Fig. 5.8: Mean concentrations of in- and outflow and the respective 95% confidence intervals (error bars), based on bootstrapped data

COD_{eq}, BOD_{5eq}, TSS_{eq} and TOC_{eq} effluent values are below the legislation limits of the EU Directive 91/271/EEC and of the Austrian wastewater treatment act (AEVkA (1996). Total N limits in the EU Directive 91/271/EEC for discharge in sensitive areas is 10 mg/l. Total N = Total organic N + Total ammonia N + NO_x. In the current study only the effluent nitrate value was higher than the limit for total N. This makes the treated water not suitable for discharge in sensitive areas. Only conversion of nitrogen took place in the treatment process but little N reduction, if any.

In terms of water reuse, the only existing legal requirement in Austria currently is the EU Regulation 2020/741 on minimum requirement for agricultural irrigation. According to it, only TU_{eq} falls into class A for all food crops consumed raw. BOD_{5eq} and TSS_{eq} requirements of 10 mg/l for class A are not met by the effluent concentrations of our study but the latter are lower than the requirements for classes B, C and D, referring to the limits of Directive 91/271/EEC. The classes are categorized by crops consumed raw, crops with or without contact with the irrigation water, processed crops, crops for feeding animals and industrial and energy crops. The irrigation method is also important for the categorization.

The individually measured concentrations are presented in Fig. 5.9. Slight decrease of COD concentrations can be noticed while the rest of the parameters stay mostly constant. The effluent shows nitrates increase over the one-month measurement period. Since the system was mature and supposed to work at steady-state, these trends were not expected. Further investigation is needed to find out if the trends are related to a clogging process and decreasing loading rates.

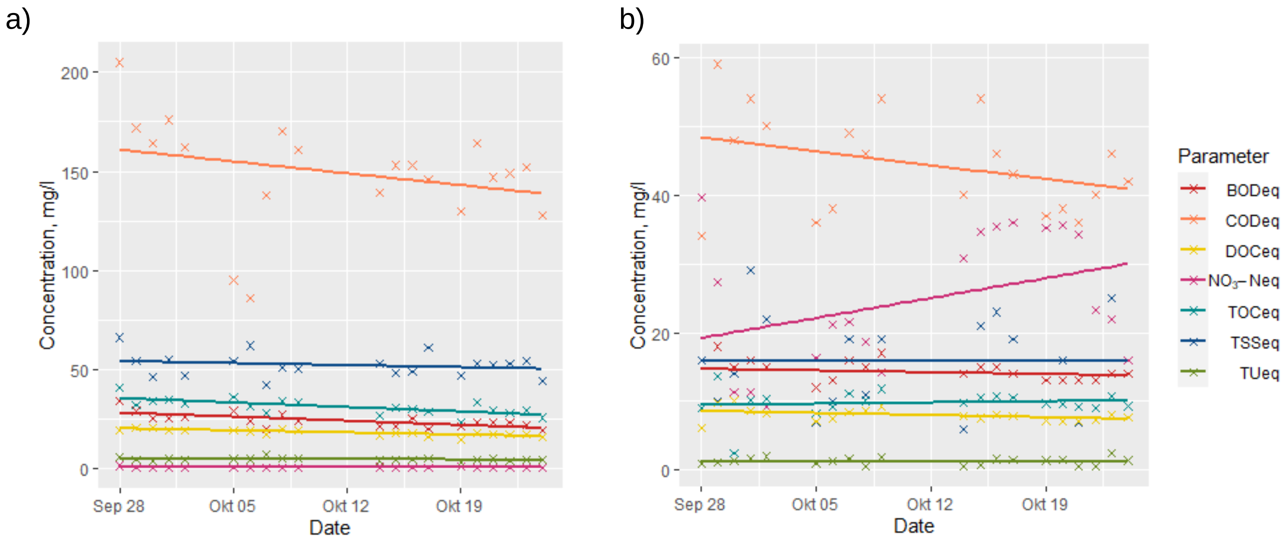


Fig. 5.9: Individual concentration values a) influent; b) effluent

5.3 Ammonium nitrogen, orthophosphate and potassium

Ammonium nitrogen, orthophosphate and potassium were sampled and measured at the same points where the spectrometer measurements took place. Because of the low number of measurements and the lack of normal distribution, non-parametric tests were used for their comparison. For the same reasons we used the median value and IQR to visualize the dispersion (Fig. 5.10). The concentrations for all three parameters are higher in the second than in the first phase. This is due to constant fresh wastewater inflow in the treatment system and shorter HDT in the tank. Additionally, the outside temperature during the second phase was half that of the first phase. In this case microbial processes or any volatilization in the outside tank were lower. (Kadlec and Wallace, 2009) reported that high removal of ammonia occurs at temperatures of 25 °C.

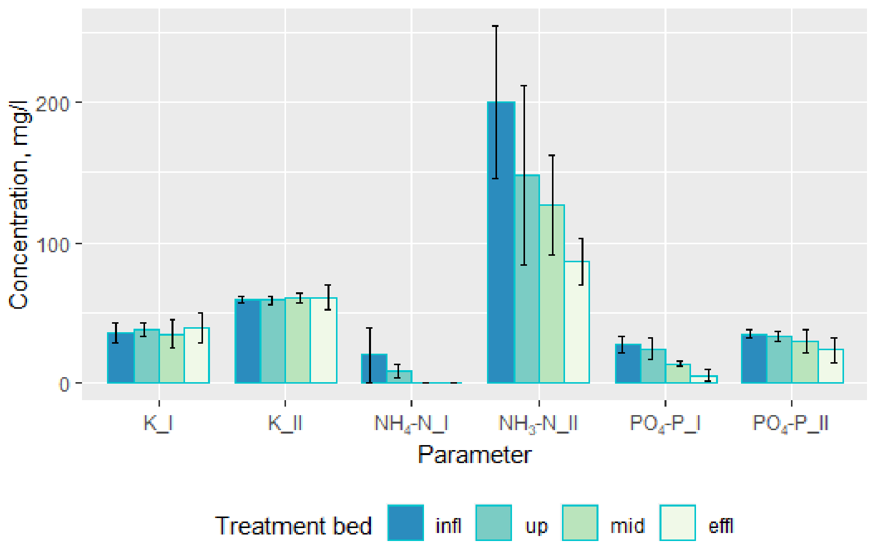


Fig. 5.10: Median concentrations and interquartile range (error bars) for potassium, ammonium nitrogen and orthophosphate in the first (I) and second (II) phase

During the second phase, there was no significant transformation of ammonium nitrogen in the first bed perhaps because nitrifying bacteria were outcompeted by the heterotrophic bacteria, as discussed earlier. The only significant decrease of ammonium concentrations happened in the second bed (Fig. 5.11). Nitrification has been typically associated with the chemoautotrophic bacteria, i.e. carbon dioxide is used as a carbon source for synthesis of new cells. Therefore, the lower amount of carbon substances at the second bed would not impede the metabolism of those bacteria. Oxygen was also supplied to the second bed, so this would not be a limiting condition either. The limiting factor for nitrification process in the third bed was the oxygen supply.

Austrian regulation (AEVkA, 1996) requires a maximum ammonium nitrogen effluent concentration of 10 mg/l (if effluent water temperatures are higher than 12 °C). The effluent level of 86.7 mg/l is well above this restriction.

In the current study P was measured in the form of orthophosphates. Bigger amount of P was removed in the first phase than in the second one and the removal was more equally distributed along the system. In the second phase significant concentration difference was observed only between upper and middle bed (Table 5-7). Orthophosphates, or dissolved inorganic phosphate ions, are the most reactive form of P. They are the only form of phosphorus to be utilized directly by macrophytes (Vymazal, 2006). Plants uptake of phosphorus is very low compared to the phosphorus loading in the wastewater. Even with plants harvesting, the amount of phosphorus removed will still be very low. In modelling the plant nutrients uptake in HF wetland Langergraber (2005) found that phosphorus uptake, when phosphorus content is 0.2% of dry weight of the plant, was 3.0 g $P.m^{-2}.yr^{-1}$. In the current experiment, especially because of the short time for plants growth, we cannot ascribe any role to the plants in phosphorus removal.

Table 5-7: Concentration and NTU lacking statistical difference among influent and treatment beds

	NH_4-N	**PO_4-P**	**K**
Phase I	Effl-mid	-	No difference between any of the beds
Phase II	Effl- Mid, Up-infl	Effl- Mid, Up-infl	No difference between any of the beds

Microbial communities play an important role in remobilization and cycling of nutrients but their life cycle is very short. Hence, the phosphorus taken up for microbial mass building is quickly released in the form of dissolved organic phosphorus and particulate P (Kadlec and Wallace, 2009). Therefore, microbial communities cannot store P for a long time.

The main mechanism in HF TW for phosphorus removal is sorption. Orthophosphates are dissolved in water and they are not removed through settling and filtration mechanisms as the P associated with particulate matter, except when precipitated in chemical reactions (Kadlec and Wallace, 2009). Soluble phosphorus will move with the water flow and will be adsorbed by the bed aggregates. Many factors influence the sorption capacity of a TW. Different phosphorus forms will affect the rate and degree of sorption. Different materials will have different potential adsorption sites for removal of phosphorus. The amount of phosphorus that can be sorbed is also function of the inlet phosphorus concentration: when the equilibrium concentration and the influent concentration are the same the driving force for sorption ceases and no more phosphorus will be sorbed unless there is an increase in influent concentration (Kadlec and Wallace, 2009).

Finer materials have more surface area per unit volume and more surface area results in higher phosphorus sorption. Apart from the grain size, the type and age of the material are also important. (Zhu et al., 2003) studied the sorption of phosphorus by light weight aggregate made of expanded clay. In a solution of 320 mg P L^{-1} and 2–4 mm granule size, sorption was 0.42 g P kg^{-1}. This was low compared to smaller grain sizes but when substrate is chosen the advantages of bigger grains also should be considered. Moreover, P desorption did not occur when the P content in the loading solution decreased, which makes leigh weight aggregate stable and promising medium for horizontal subsurface flow filters. In a review of 30 main categories of P sorption materials expanded clay removed 89% of total P (Vohla et al., 2011).

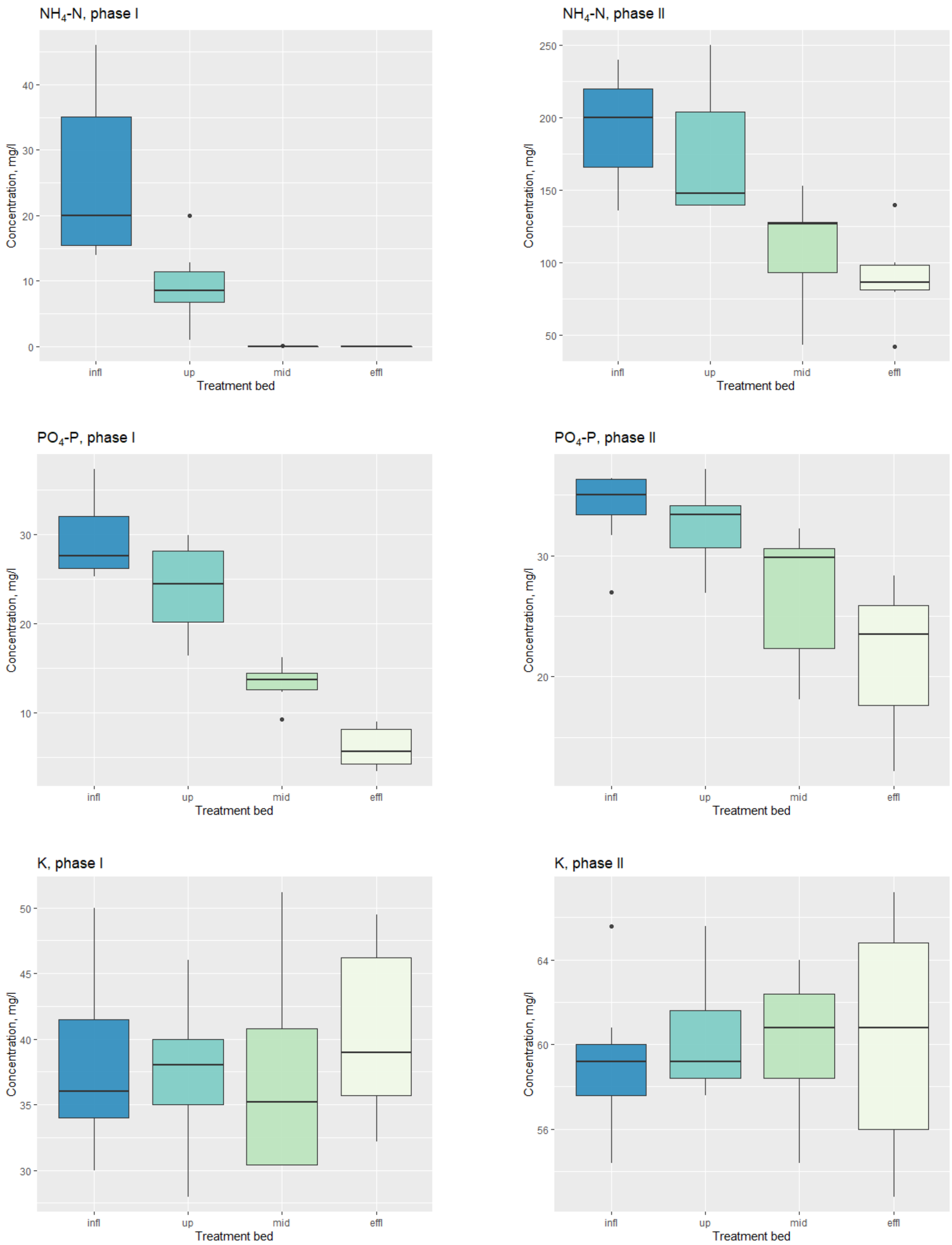


Fig. 5.11: Parameters concentrations by treatment bed

The expanded clay used in the current experiment had bigger granular size – 8-16 mm. Based on the studies above, it can be considered good material for P sorption, still it should be taken into account that it is meant for industrial application and not for wastewater treatment. Only a few companies produce special expanded clay material for TWs. It usually has more calcium additions meant to improve the P sorption. It also should be considered that the sorption storages have finite capacities, which place an upper limit on the amount of P sorbed, i.e. the substrate has limited lifetime for P storage.

There are no statistically significant differences in potassium concentrations reduction at any level.

5.4 Microbiological analysis

Five microorganism groups were tested 4 times during each phase. The *Enterococci* were not found in the influent tests conducted in alchemia-nova laboratory during the first phase but were detected in SIG laboratory. Assumingly this was due to error in the inoculation or the media. Therefore, only 2 counts are available for this phase. In one test during the second phase all *Enterococci* influent dishes were overgrown and could not be counted, that is why there are only three counts from that phase.

In the first phase, similar to the results from the chemical analysis, due to the differences in the influent characteristics, a smaller number of microorganisms was counted in the influent than in the second phase (Fig. 5.12). The log reduction rate for *E. coli* and *Enterococci* was similar in the two phases but since the initial amount was higher in the second phase, they were not entirely eliminated in the effluent (Table 5-8). Compared to other microorganisms, the reduction rate of these two was higher.

Table 5-8: Individual colony counts ($\log_{10}$) of the colony forming units (CFU) in 4 tests, 2 phases

		Phase I				**Phase II**			
Count		1	2	3	4	1	2	3	4
CFU 22	influent	5.4	4.7	4.6	4	6	7.7	6.5	6.2
	effluent	4.2	3.6	3	3.5	3.9	4.4	5	4.1
CFU 37	influent	5.9	4.1	4.6	3.5	6.3	5.1	5.8	5.8
	effluent	3.4	3.4	3.1	3	5.6	3.9	4.6	4
Total coliforms	influent	3	3.4	5.7	4	6.7	6.4	5.8	5.2
	effluent	0	2.1	2.7	2.9	4.2	4.9	5	4.7
E. coli	influent	2.7	3.1	5.6	3.9	5.5	5.7	4.7	5
	effluent	<1	<1	<1	<1	2.4	2	2.7	<1
Enterococci	influent	1.7	3.4			5	4.5	5.4	
	effluent	<1	<1			2.1	2.8	1.5	

Reinoso et al. (2008) used several treatment stages system of facultative pond, free surface and subsurface flow for treating domestic raw wastewater. Only the cumulative treatment of the three parts reached removal of 97.1% for total coliforms and 99.3% for *E. coli*. The facultative pond was more efficient than surface and subsurface flow in bacterial removal, while the SSF wetland was significantly more efficient in the removal of coliphages and protozoan cysts. In a review on the removal of human pathogens and faecal indicators Wu *et al.* (2016) found out that generally, HF wetlands have better reduction capacity than FSW, but hybrid wetland systems were the most efficient. The concentration of the main faecal indicator bacteria in the effluent was found to be exponentially related to the loading rate (Williams, 1995). Additional step in the treatment process needs to be added or the HLR reduced for a better performance in the studied system. Examples of pathogens removal efficiency from domestic wastewater by HF wetlands are shown in Table 5-9.

Table 5-9 Pathogen indicators microorganisms removal rates, examples from the literature

Study	***E. coli***	**Total coliforms**	***Enterococci***
Molleda et al. (2008)[1]	96.7%	91.6%	
Reinoso et al. (2008)[2]	72.02% (99.3)	69.27% (97.12)	
Wu et al. (2016)[3]	2.2-2.5 log	0.5-2.0 log	
Hench et al. (2003)[4]			2.8 log
Thurston et at. (2001)[5]		98.8%	
Giácoman-Vallejos, et al. (2015)[6]		80-82%	85%

[1] – Pre-treated water flows through two free surface basins and a combination of surface and subsurface flow stages; [2] - Without brackets – only HF wetland, in brackets – the whole system of facultative pond, surface and subsurface flow; [3] -HF wetlands performance review; [4] – HF wetland, primary treated domestic water; [5] - HF wetland, secondary treated domestic water; [6] - pre-treated domestic wastewater with a low OLR

Higher number of culturable microorganisms developed in incubation at 22°C than at 37°C, i.e. slow growing microorganisms are prevailing.

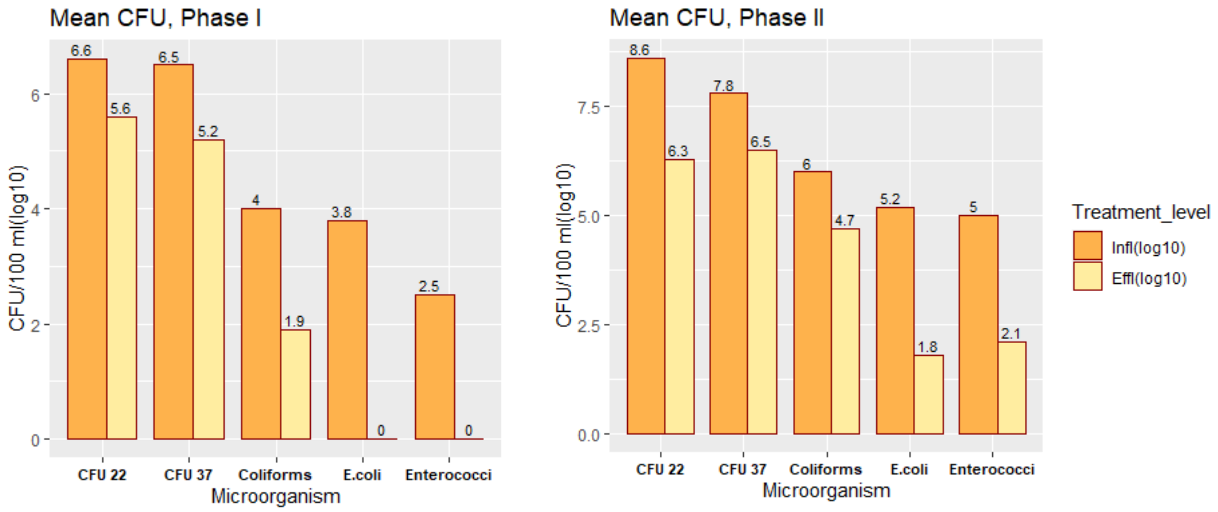


Fig. 5.12: Mean colony forming units in 100 ml of sample

Microorganisms are not included in the legal surface water discharge limits. One directive that stipulates the max count of *E. coli* and *Enterococci* is the Directive 2006/7/EC on bathing water quality. It regards water of excellent quality of inland waters when E. coli < 500 and Enterococci < 200 CFU/100 ml. The Enterococci in the effluent of the current study exceeds this value with 50 but *E. coli* is less than half the limit (Fig. 5.12). Both parameters satisfy the requirements for good quality bathing water of the Directive.

The quality class A of the EU Regulation 020/741 allows less than 10 *E. coli* CFU/100 ml. The average number of *E. coli* in the current effluent is 210 CFU/100 ml. The effluent can only be used with classes C – raw food crops not in direct contact with reclaimed water and fodder and D – industrial, energy and seed crops. The same Regulation requires a minimum *E. coli* removal rate for validation of newly established facilities > 5 log_{10}. This is not achieved by the system that has average removal of 3.5 log_{10}.

Table 5-9: Mean reduction rate (log_{10}) of culturable and faecal indicator microorganisms

	Phase I					Phase II				
	CFU 22°C	CFU 37°C	Total coliforms	*E. coli*	*Enterococci*	CFU 22°C	CFU 37°C	Total coliforms	*E. coli*	*Enterococci*
Mean	1.1	1.3	2.1	3.8	2.6	2.3	1.2	1.3	3.5	2.8
Min	0.5	0.5	1.1	2.7	1.7	1.5	0.7	0.5	2	1.7
Max	1.6	2.5	3	5.6	3.4	3.3	1.8	2.5	5	3.9

5.5 Treatment performance of the wetland system

General assumption is that the treatment performance obtained in TWs with SSF is good in removing TSS. BOD_5 removal and nitrification depend on microbial activities and growth, which on their own depend on the carbon matter quality, C:N ratios and oxygen availability. Oxygen availability is usually low due the constant saturation of the substrate and oxygen deficiency (IWA Specialist Group, 2000; Vymazal, 2009). Without additional oxygenation, HF TWs tend to have good nitrate removal, as they provide good conditions for denitrification, but cannot remove ammonia due to limited ability to nitrify it (Todt et al., 2015).

In the current study two thirds of the wetland system was artificially aerated and this provided better condition for the aerobic treatment. The BOD_5 removal was not high - 39%, owing to the already low BOD_5 concentration in the influent of 38 mg/l. An advantage of the treatment systems with attached biofilm is that they can successfully treat wastewaters with very low concentrations of organics, less than 50 mg/l BOD_5, which is not the case with conventional treatment systems (Vymazal, 2009). COD is removed at a rate of 70%, i.e. higher than BOD_5 rate. As mentioned earlier, the assumption is that there was a lot of inert organic matter that was associated with particles, that could be filtered and sedimented in the system but could not be biologically degraded (Table 5-10).

All carbon parameters, TSS and TU have the highest removal rates the first treatment bed. There are small removal rates for these parameters in the last treatment bed, on one hand because the concentrations were already low and on the other because of the lack of oxygenation.

In the first phase NH_4-N was almost entirely converted in the upper and middle treatment beds. In the second phase there was conversion of NH_4-N all along the system. The conversion in the last bed, where no significant increase of nitrates took place, can be explained with conditions created for the annamox process.

Orthophosphates removal is relatively equally distributed along the system. In the first phase removal is much higher than in the second phase – 83 vs 39 %. The accumulation of solids in the first phase might have provided additional sorption places for P (Table 5-10). Generally, removal of phosphorus in all types of constructed wetlands is low unless special substrates with high sorption capacity are used. Removal of total phosphorus varied between 40 and 60% in all types of constructed wetlands (Vymazal, 2006). Due to limited sorption sites P removal is time dependent – it reduces with time, which could be also a reason for lower removal rate during the second phase, when many sorption sites have already been taken. Potassium is not removed from the system - its removal rates sway from slightly positive to slightly negative without a trend.

Sakurai et al. (2021) tested anaerobically digested blackwater. They studied a hybrid system of horizontal and vertical flow wetlands. The HF wetland had similar size, water temperature and EC to the ones in the current study. At HRT 2 days the HF wetland alone achieved removal efficiencies of COD 44,8%, BOD5 47,4 %, and orthophosphate 16%. The average removal of *E. coli* and total coliforms in the HF was low - 1.1 log and 0.9 log respectively. Increased HRT with 24 hrs significantly increased the removal of total coliforms. Ammoniacal N removal was only possible with the VF wetland.

Table 5-10: Median treatment performance at each treatment bed in %, # - number of samples

Parameter	TSS	COD	BOD_5	TOC	DOC	TU	NH_4-N	PO_4-P	K
#	26	26	26	26	26	26	7	7	7
PHASE I					Influent - upper bed				
Median	**77**	**47**	**47**	**53**	**27**	**73**	**59**	**20**	**11**
Min	-50	24	37	41	14	-56	92.9	37	26
Max	88	64	78	62	43	96.9	24	15	-27
					Influent - middle bed				
Median	**84**	**63**	**51**	**51**	**40**	**79**	**99.9**	**53**	**4**
Min	47	18	0	12	6	35	100	67	20
Max	97.5	81	79	69	57	100	99.6	41	-17
					Influent – effluent bed				
Median	**89**	**70**	**59**	**55**	**50**	**82**	**99.9**	**83**	**-7**
Min	13	83	25	17	6	40	100	90.7	11
Max	97.1	18	85	70	63	100	99.9	64	-28
PHASE II					*Influent - upper bed*				
#	**TSS**	**COD**	**BOD_5**	**TOC**	**DOC**	**TU**	**NH_4-N**	**PO_4-P**	**K**
Nr of samples	20	20	20	20	20	20	7	7	7
Median	**51**	**48**	**38**	**45**	**33**	**57**	**27**	**5**	**5**
Max	15	29	14	27	22	28	42	15	5
Min	82	64	50	65	43	74	-44	-4	-9
					Influent - middle bed				
Median	**69**	**66**	**31**	**57**	**52**	**65**	**35**	**17**	**-3**
Min	38	47	5	47	33	44	79	40	6
Max	90.7	76	52	71	84	85	7	11	-8
					Influent – effluent bed				
Median	**70**	**70**	**39**	**67**	**55**	**71**	**47**	**39**	**0**
Min	47	56	20	58	51	39	80	55	8
Max	89	83	74	93	68	89	33	22	-17

Blackwater treatment is usually based on a combined treatment systems, with a goal to recover nutrients and energy. Oarga-Mulec et al. (2017) separated the liquid part of blackwater through peat filter, as the non-liquid part (particle bigger than 1mm) were composted. The liquid part was treated in biofilter followed by sanitation and evaporation. Sahondo et al. (2020) treated the liquid part of blackwater through four granular activated carbon filters and disinfected it by electrochemical oxidation. Jin et al. (2020) measured removal efficiency of COD 81.6%, ammonium nitrogen 42.2% and total phosphorus 73.7% of pre-treated in a septic tank blackwater followed by bio-contact oxidation tank and TWs with filter material activated carbon treatment. The above studies show that the blackwater requires several treatment steps to give the necessary results.

When only HF wetland is considered, BOD_5, COD and TSS removal efficiency is slightly below the legal requirements of the EU Directive 91/271/EEC and well beyond the Austrian wastewater treatment act (AEVkA, 1996). AEVkA limits refer to p.e. between 50 and 500 but the same are also applied for < 50 p.e. (Langergraber et al. 2018).

EU Directive 91/271/EEC put requirements for effluent concentrations of total N and total P only for sensitive areas and with settlements of more than 10 000 p.e. with minimum percent of reduction 70-80% for N and 80% for P. These requirements are not met for N and met only by the P reduction rate in the first phase in our treatment system.

5.6 Treatment performance of the overall blackwater treatment system

The pre-treatment step was not experimentally examined in the current study. The reduction rate in the pre-treatment step was calculated based on loads from DWA, 2014, the frequency of toilets use and the toilet flush volume. The average toilet use that supply the raw blackwater to the treatment system per day is 10.1 times. With an average toilet use by person of 6 times per day, on average the toilets were used by 1.7 persons. This value was lower than it would be in a regular office attendance, but due to the pandemic in 2020 the office was much less attended than normally.

When the pre-treatment step is added to the HF removal rate, the total removal rates comply with the discharge limits for both EU and Austrian legislation for COD, BOD_5 and TSS. Nitrogen and phosphorus concentrations in raw blackwater in the literature are lower than the ones measure after the pre-treatment step in the current system. Therefore, for these two parameters, the HF wetland treatment was considered only (Table 5-11).

Table 5-11: Loads and concentrations in the current study raw blackwater based on literature values (DWA, 2014) and HF and overall removal rates

	g/p.d DWA, (2014)	g/p.d (current study)	mg/l (current study)	HF wetland removal, %	Overall removal, %
BOD_5	37	62.9	444.8	39	97
COD	50	85.0	601.1	70	93
TSS	61	103.7	733.4	70	98
NH_4-N	12	20.4	144.3	47	40
PO_4-P	2	3.4	24.0	39	2.3

6. Conclusion and outlook

The calibration models based on the entire reference dataset generally perform better than the ones based on division between effluent and influent values. Different models combinations to predict true values show small differences in the predicted values. For the particular blackwater matrix used in the current experiment, when local calibration is not available, COD, BOD_5 and TSS values are much closer to the true values if measured with EGC. TSS, TU and NO_3-N measured without local calibration show more than 100% errors. Measurements without local calibration are not recommended for any of the parameters.

The typical blackwater concentrations given in the literature are higher than the current experiment influent concentrations in the pre-treated blackwater with values COD 152 mg/l, BOD_5 38 mg/l, TSS 52 mg/l, TOC 30.9 mg/l, DOC 17.8 mg/l, TU 4.5 NTU, due to the high dilution effect of the flush water and solids separation. The removal efficiency of the entire system including pre-treatment step meets the EU and Austrian legislative requirements for COD, BOD_5 and TSS removal efficiency for discharge in surface waters. HF system alone falls below these removal rates. The effluent concentrations for these parameters satisfy the legally binding limits of the two regulations. The nitrification of the HF system is limited, the ammonium nitrogen in the effluent is 86.7 mg/l, which makes it non-compliable with the limits of the Austrian legislation of 10 mg/l. The maximum allowed concentrations of total N and total P in the effluent to be discharged in sensitive areas according to EU Directive 91/271/EEC are 10 and 1 mg/l respectively. This is much below the concentrations of N and P constituents measured in the current study – 22.7 mg NO_3-N/l and 86.7 mg NH_4-N/l and 23.5 mg PO_4-P/l. For P the removal rate is also below the legal requirements Nitrogen removal in the current system is very low, if any. One reason for the low denitrification is the low COD:N ratio.

The results showed high COD:BOD_5 ratio of 4, which is higher than the typical values for blackwater of up to 3.6. This assumes a lot of non-biodegradable organic matter. The reason for this high ratio should be further investigated.

In terms of the reclaimed water reuse only TU is suitable for class A of the EU Regulation 020/741, BOD5 and TSS limits are met for crop classes B, C and D and. E. coli concentration is only suitable for crop classes C and D.

The treatment could be improved if the HRT is increased and any possible shortcuts prevented. In this case, however, the problem arises that not the whole amount of wastewater produced can be treated. Aerating the lowest bed would help transform the NH_4-N to NO_3 -N but will not contribute to the removal of nitrogen from the water. The high N and P concentrations offer an opportunity for water reuse as fertilizer, but care should be taken that it does not damage the irrigated plants and it does not reach groundwater or surface water streams.

The system is easily prone to clogging, partly due to design, and partly high solids load and high COD:BOD ratio. The design prevents the solids movement and they easy accumulate in the front part of the upper bed. Clogging is a common problem with HF wetlands and more attention should be paid to the pre-treatment level, especially in the presence of low-biodegradable organic materials.

The VertEco design overcomes some of the disadvantages of the HF wetlands – it requires little space since it can be installed along a building wall at the same time improving the microclimate and aesthetics. With the additional aeration the energy need increases but it provides efficient carbon removal and better ammonium transformation than without oxygen supply. Depending on the destination of the effluent, with the current treatment it can be either discharged or additionally treated for specific reuses.

7. Summary

Europe is not an arid continent, but its freshwater resources are non-uniformly distributed in space and time. Mediterranean and densely populated countries have the lowest water availability per capita in Europe. With the objectives of minimizing the waste as well as resources and energy use, the EU adopted the circular economy concept. Recognizing the opportunities for reclaimed water reuse, the EU issued a regulation on minimum requirement for reclaimed water for agricultural irrigation in 2020.

Cities are the biggest consumers of resources, producing the corresponding amount of waste. Especially with the tendency for increasing urbanization, more attention is directed to the opportunity for water, energy, and nutrients recovery in the cities.

The performance of an indoor horizontal flow wetland system was tested for its capacity to treat the liquid part of source separated blackwater. The system is installed in a single-family house in the city of Vienna and treats the blackwater of two toilets with daily generation of 141.2 l. The system consists of 3 beds connected in a series with total expanded clay substrate volume of 1 m^3, HLR of 2.3 $cm.d^{-1}$ and OLR 67.5 g BOD_5 $m^{-2}.d^{-1}$. Before entering the treatment system, the water is separated from the solids with centrifugal-gravity separator and collected in 1 m^3 tank outside the house. Most of the plants planted in the substrate are ornamental tropical plants.

An UV/VIS spectrometer was used for measuring COD, BOD_5, TSS, NO_3-N, TOC, DOC and TU. Multiple points linear calibration models were developed for the specific water matrix with parameters reference values measured in SIG laboratory in BOKU. Twenty-six measurements spectrometer measurements were done in the tests phase when also the calibration was done and 20 measurements in an experimental phase. The measurements were taken at four sampling points – influent – right before it entered the treatment system, the effluent point of each treatment bed. Because of the small sample sizes and non-normal distribution, the median values and non-parametric tests were used in the analysis. The influent and effluent data were bootstrapped to calculate the mean values and the 95% confidence interval of the mean.

Ammonium nitrogen, orthophosphates and potassium were also measured at the same sampling points like for spectrometer measurements. The median values were used in the analysis and non-parametric tests were run to detect statistical differences between the influent and the three effluents. These values were used to compare the effluent concentrations with different legal requirements.

Almost half of the concentration reductions of the organic matter parameters, TSS and TU took place in the upper treatment bed, followed by middle bed. Very little, reduction took place in the effluent bed. The most intensive nitrification happened in the middle bed, while in the upper and effluent beds it was insignificant. Phosphorus was removed more efficiently in the middle and effluent beds. Potassium concentration did not change significantly throughout the system.

Culturable microorganisms at 22°C and 37°C along with three pathogen indicators – *Total coliforms*, *E. coli* and *Enterococci* of the influent and effluent were tested according to ISO standards. The log removal for pathogen indicators are highest for *E. coli* followed by *Enterococci* and total coliforms, respectively 3.5, 2.8 and 1.3.

In Table 7-1 the EU and Austrian legal requirements for wastewater effluent discharge and EU limits for agricultural water reuse are compared with the results from the current study. The overall organic matter and solids removal rates meets the legislative limits for surface water discharge. Nutrients, N and P, have not been efficiently removed from the system and their high concentrations in the effluent might cause environmental problems. Considering the studied parameters, the effluent water can be used for classes with lower risk for human health, C and D, of the EU Regulation 020/741.

Table 7-1: Effluent concentrations and removal rates of the current HF system and legal requirements

	Current study			Directive 91/271/EC		EU Regulation 020/741		AEVkA, 1996	
	Effluent concentration (confidence interval)	Removal rate of HF %	Overall removal rate %	Effluent concentration mg/l	Removal rate %	Effluent concentration mg/l	Removal rate $\log_{10}$	Effluent concentration[8] mg/l	Removal rate %
COD, mg/l	45 (41-48)	70	93	125	75			90	85
BOD_5, mg/l	14 (14-15)	39	97	25	70-90	10[3] 91/271/EC[7]		25	95
TOC, mg/l	9.9 (9.0-10.3)	67						30	80
DOC, mg/l	8.0 (7.6-8.4)	55							
TU, NTU	1.3 (1.1-1.5)	71				5[3]			
TSS, mg/l	16 (13-18)	70	98	60[1]	70[1]	10[3] 91/271/EC[7]			
NO_3-N, mg/l	24.3 (20.6-29.0)								
PO_4-P, mg/l	23.5	39		2 (total P)[2]	80				
NH_4-N, mg/l	86.7	47		15 (total N)[2]	70-80			10	
E. coli, nr/100 ml	210	3.5				10[3] 100[4] 1000[5] 10 000[6]	5 .0		

[1] – between 2000 and 10 000 p.e.; [2] between 10 000 – 100 000 p.e.; [3] – class A [4] – Class B; [5] – class C; [6] – class D,
[7] acc. to Directive 91/271/EC; [8] – used also for < 50 p.e.

8. References

AEVkA (1996): 1. Abwasseremissionsverordnung für kommunales Abwasser (Austrian regulation for emissions from municipal wastewater). BGBl.210/1996, Vienna, Austria [*in German*].

Almuktar, S. A.A.A.N., Abed, S. N., Scholz, M. (2018). Wetlands for Wastewater Treatment and Subsequent Recycling of Treated Effluent: A Review. *Environmental Science and Pollution Research* 2 25:23595–23623.

Alufasi, R., Gere, J., Chakauya, E., Lebea, P., Parawira, W., Chingwaru, W. (2017). Mechanisms of Pathogen Removal by Macrophytes in Constructed Wetlands. *Environmental Technology Reviews* 6 (1): 135–144.

Batstone, D. J., Hülsen, T., Mehta, C. M., Keller, J. (2015). Platforms for Energy and Nutrient Recovery from Domestic Wastewater: A Review. *Chemosphere* 140:2–11.

Brix H. (1993) Wastewater treatment in constructed wetlands: system design, removal processes, and treatment performance. Constructed Wetlands for Water Quality Improvement, A.G. Moshiri, ed., CRC Press, Boca Raton, Florida, pp. 9-22.

Ciceri, D., Manning, D. A. C. Allanore, A. (2015). Historical and Technical Developments of Potassium Resources. *Science of the Total Environment* 502: 590–601.

Daigger, G. T. (2009). Evolving Urban Water and Residuals Management Paradigms: Water Reclamation and Reuse, Decentralization, and Resource Recovery. *Water Environment Research* 81 (8): 809–823.

de Matos, M. P., von Sperling, M., de Matos, A. T. (2018). Clogging in Horizontal Subsurface Flow Constructed Wetlands: Influencing Factors, Research Methods and Remediation Techniques. *Reviews in Environmental Science and Biotechnology*. 17:87–107

Dinno A. (2017). Conover.test: Conover-Iman Test of Multiple Comparisons Using Rank Sums. R package version 1.1.5. https://CRAN.R-project.org/package=conover.test

Dong, Z., and Sun, T. (2007). A Potential New Process for Improving Nitrogen Removal in Constructed Wetlands-Promoting Coexistence of Partial-Nitrification and ANAMMOX. *Ecological Engineering* 31 (2): 69–78.

Dotro, G., Langergraber, G., Molle, P., Nivala, J., Puigagut, J., Stein, O., Von Sperling, M. (2017). *Treatment Wetlands, Volume 7*. Biological Wastewater Treatment Series. IWA Publishing.

DWA (2014). Arbeitsblatt DWA-A 272: Grundsätze für die Planung und Implementierung Neuartiger Sanitärsysteme (NASS) (Translation: Standard DWA-A 272, Principles of Planning and Implementation of Innovative Sanitation Systems (NASS). Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e.V. (DWA), Hennef, Germany. [*In German*]

EC (2007). Water scarcity and Droughts. In-depth assessment. Second Interim Report.

EC (2012a). Report on the Review of the European Water Scarcity and Droughts Policy. *Communication From the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions*. COM(2012) 672 final. Brussels.

EC (2012b). A Blueprint to Safeguard Europe’s Water Resources. *Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of Regions*. COM(2012) 673 final. Brussels.

EC (2015). Closing the Loop - An EU Action Plan for the Circular Economy. *Communication From the Commission To the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions.* COM(2015) 614 final. Brussels.

References

EC (2016): Policy Topics: Nature-Based Solutions, https://ec.europa.eu/info/research-and-innovation/research-area/environment/nature-based-solutions_en (date of visit: 21 February 2021)

EC (2020). Regulation (EU) 2020/741 of the European Parlament and of the Council on Minimum Requirements for Water Reuse. *Official Journal of the European Union* 2019 (May): L 177/32-L 177/55.

EEA, 2009. Water resources across Europe confronting water scarcity and drought. EEA Report No 2/2009. European Environment Agency, Copenhagen, Denmark.

EEA (2012). European Waters — Current Status and Future Challenges. Synthesis report No 9/2012. European Environmental Agency, Copenhagen, Denmark.

Masi, F., Langergraber, G., Santoni, M., Istenic, D., Atanasova, N., Buttiglieri, G. (2020). Possibilities of Nature-Based and Hybrid Decentralized Solutions for Reclaimed Water Reuse. In *Advances in Chemical Pollution, Environmental Management and Protection, Verlicci, P., Barcelo, D. (editors)* Vol 5.

Faivre, N., Fritz, M., Freitas, T., de Boissezon, B., Vandewoestijne, S. (2017). Nature-Based Solutions in the EU: Innovating with Nature to Address Social, Economic and Environmental Challenges. *Environmental Research* 159: 509–518.

Geissdoerfer, M., Savaget, P., Bocken, N. M. P., Hultink, E. J. (2017). The Circular Economy – A New Sustainability Paradigm? *Journal of Cleaner Production* 143: 757–768.

Graaff, M. S. (2010). *Resource Recovery from Black Water*. Doctoral Thesis. Wageningen University, Wageningen, NL.

IWA Specialist Group on Use of Macrophytes in Water Pollution Control. (2000). Constructed Wetlands for Pollution Conctrol. Processes, Performance, Design and Operation. *IWA Publishing*.

Hench, K. R., Bissonnette, G. K., Sexstone, A. J., Coleman, J. G., Garbutt, K., Skousen, J. G. (2003). *Fate of Physical, Chemical, and Microbial Contaminants in Domestic Wastewater Following Treatment by Small Constructed Wetlands*. *Water Research*. Vol. 37: 921-927.

Hocaoglu, S. M., Insel, G., Cokgor, E. U., Baban, A., Orhon, D. (2010). COD Fractionation and Biodegradation Kinetics of Segregated Domestic Wastewater: Black and Grey Water Fractions. *Journal of Chemical Technology and Biotechnology* 85 (9): 1241–1249.

Horan, N. J. (1990). Biological Wastewater Treatment Systems : Theory and Operation. 1990. Print. John Wiley & Sons, Chichester. 310 p.

IPCC (2012). Summary for Policymakers. In: Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation [Field, C.B., Barros, V., Stocker, T.F., Qin, D., Dokken, D.J., Ebi, K.L., Mastrandrea, M.D., Mach, K.J., Plattner, G.-K., Allen, S.K., Tignor, M., Midgley, P.M. (eds.)]. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, and New York, NY, USA, pp. 1-19.

Jahangir, M. M.R., Richards, K. G., Healy, M. G., Gill, L., Müller, C., Johnston, P., Fenton, O. (2016). Carbon and Nitrogen Dynamics and Greenhouse Gas Emissions in Constructed Wetlands Treating Wastewater: A Review. *Hydrology and Earth System Sciences* 20 (1): 109–123.

James, G., Witten, D., Hastie, T., Tibshirani, R. (2013). *An Introduction to Statistical Learning - with Applications in R*. Springer.

Jenssen, P. D., Heyerdahl, P. H., Warner, W. S., Greatorex, J. M. (2003). Local Recycling of Wastewater and Wet Organic Waste – a Step Towards the Zero Emission Community. Proceedings of the 8th International Conference on Environmental Science and Technology Lemnos Island, Greece, 8 – 10 September 2003, Vol. A, pp. 357 - 364

Jin, Z., Zheng, Y., Li, X., Dai, C., Xu, K., Bei, K., Zheng, X., Zhao. M. (2020). Combined Process of Bio-Contact Oxidation-Constructed Wetland for Blackwater Treatment. *Bioresource Technology* 316, 123891.

Kadlec, R. H. and Wallace, S. D. (2009). Treatment Wetlands. 2nd edition, CRC press, Boca Raton, Florida, USA

Knerr, H., Rechenburg, A., Kistemann, T., Schmitt, T. G. (2011). Performance of a MBR for the Treatment of Blackwater. *Water Science and Technology* 63 (6): 1247–1254.

Knowles, P., Dotro, G., Nivala, J., García, J. (2011). Clogging in Subsurface-Flow Treatment Wetlands: Occurrence and Contributing Factors. *Ecological Engineering* 37: 99-112.

Kujawa-Roeleveld, K., and Zeeman. G. (2006). Anaerobic Treatment in Decentralised and Source-Separation-Based Sanitation Concepts. *Reviews in Environmental Science and Biotechnology* 5 (1): 115–139.

Langergraber, G., Haberl, R. (2001). Constructed Wetlands for Water Treatment. *Minerva Biotecnologica* 13: 123–134.

Langergraber, G., Fleischmann, N., Hofstädter, F. (2003). A Multivariate Calibration Procedure for UV/VIS Spectrometric Quantification of Organic Matter and Nitrate in Wastewater. *Water Science and Technology*, 47:63–71.

Langergraber, G. (2005). The Role of Plant Uptake on the Removal of Organic Matter and Nutrients in Subsurface Flow Constructed Wetlands: A Simulation Study. *Water Science and Technology* 51 (9): 213–223.

Langergraber, G., Pressl, A., Kretschmer, F., Weissenbacher, N. (2018). Small Wastewater treatmetn Plants in Austria – Technologies, Management and Training of Operators. *Ecological Engineering* 120: 164-169.

Lazarova, V., Asano, T., Bahri A., Anderson, J. (2013). *Milestones in Water Reuse: The Best Success Stories*. IWA Publishing.

Lofrano, G., and Brown, J. (2010). Wastewater Management through the Ages: A History of Mankind. *Science of the Total Environment*, 408: 5254-5264.

MED-EUWI (2007). Mediterranean Wastewater Reuse Report. Mediterranean Wastewater Reuse Working Group. November, 2007.

Meinzinger, F., and Oldenburg, M. (2009). Characteristics of Source-Separated Household Wastewater Flows: A Statistical Assessment. *Water Science and Technology* 59 (9): 1785–1791.

Moges, M. E., Todt, D., Heistad, A. (2018). Treatment of Source-Separated Blackwater: A Decentralized Strategy for Nutrient Recovery towards a Circular Economy. *Water (Switzerland)* 10 , 463.

Molleda, P., Blanco, I., Ansola, G., de Luis, E. (2008). Removal of Wastewater Pathogen Indicators in a Constructed Wetland in Leon, Spain. *Ecological Engineering* 33 (3–4): 252–257.

Kabisch, N., Korn, H., Stadler, J. Bonn, A. (2017). *Nature-Based Solutions to Climate Change Adaptation in Urban Areas*. Linkages between Science, Policy and Practice. Springer International Publishing AG, Switzerland.

Neuwirth, E. (2014): RColorBrewer: ColorBrewer Palettes. R package version 1.1-2. https://CRAN.R-project.org/package=RColorBrewer.

Nivala, J., Van Afferden, M., Hasselbach, R., Langergraber, G., Molle, P., Rustige, H., Nowak, J. (2018). The New German Standard on Constructed Wetland Systems for Treatment of Domestic and Municipal Waste Water. *Water Science and Technology* 78 (11): 2414–2426. IWA Publishing.

Nordin, A., Göttert, D., Vinnerås, B. (2018). Decentralised Black Water Treatment by Combined Auto-Thermal Aerobic Digestion and Ammonia – A Pilot Study Optimising Treatment Capacity. *Journal of Environmental Management* 207: 313–318.

Oarga-Mulec, A., Jenssen, P. D., Klemenčič, A. K., Uršič, M., Bulc, T. G. (2017). Zero-Discharge Solution for Blackwater Treatment at Remote Tourist Facilities. *Journal of Cleaner Production* 166: 798–805.

Öborn, I., Andrist-Rangel, Y., Askegaard, M., Grant, C.A., Watson, C.A. Edwards, A.C. (2005). Critical Aspects of Potassium Management in Agricultural Systems. *Soil Use and Management* 21 (1): 102–112.

Orloff, J. and Bloom, J. (2014). Bootstrap Confidence Intervals. *MIT OpenCourseWare*.

Otterpohl, R., Albold, A., Oldenburg, M. (1999). Source Control in Urban Sanitation and Waste Management: Ten Systems with Resource Separation. Water Science and Technology, 39 (5): 153-160.

Otterpohl, R., Grottker, M., Lange, J. (1997). Sustainable_water_and_waste Management in Urban Areas. Water Science and Technology, 35 (9): 121-133.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reinoso, R., Torres, L. A., Bécares, E. (2008). Efficiency of Natural Systems for Removal of Bacteria and Pathogenic Parasites from Wastewater. *Science of the Total Environment* 395 (2–3): 80–86.

Rieger, L., Langergraber, G., Siegrist. H. (2006). Uncertainties of Spectral in Situ Measurements in Wastewater Using Different Calibration Approaches. *Water Science and Technology* 53 (12): 187–197.

Robles, Á., Aguado, D., Barat, R., Borrás,L., Bouzas, A., Giménez, J. B., Martí, N., Ribes, J., Ruano, M. V., Serralta, J., Ferrer, J., Seco, A. (2020). New Frontiers from Removal to Recycling of Nitrogen and Phosphorus from Wastewater in the Circular Economy. *Bioresource Technology*, 300, 122673.

Sahondo, T., Hennessy, S., Sindall, R. C., Chaudhari, H., Teleski, S., Lynch, B. J., Sellgren, K. L., Stoner, B. R., Grego, S., Hawkins, B. T. (2020). Field Testing of a Household-Scale Onsite Blackwater Treatment System in South Africa. *Science of the Total Environment* 703, 135469.

Sakurai, K. S. I., Pompei, C. M. E., Tomita, I. N., Santos-Neto, Á. J., Silva, G. H. R. (2021). Hybrid Constructed Wetlands as Post-Treatment of Blackwater: An Assessment of the Removal of Antibiotics. *Journal of Environmental Management* 278 (2), 111552.

Sanz, L. A. and Gawlik, B. M. (2014). *Water Reuse in Europe: Relevant Guidelines, Needs for and Barriers to Innovation*. *Science and Policy Report by the Joint Research Centre*. Luxembourg: Publications Office of the European Union.

Sanchez-Ramos, D., Agulló, N. Samsó, R., García, J. (2017). Effect of Key Design Parameters on Bacteria Community and Effluent Pollutant Concentrations in Constructed Wetlands Using Mathematical Models. *Science of the Total Environment* 584–585: 374–380.

Shoushtarian, F., and Negahban-Azar, M. (2020). World Wide Regulations and Guidelines for Agriculturalwater Reuse: A Critical Review. *Water* 12, 971.

Stott, R., E. May, E. Matsushita, and A. Warren. (2001). Protozoan Predation as a Mechanism for the Removal of Cryptosporidium Oocysts from Wastewaters in Constructed Wetlands. *Water Science and Technology* 44 (11–12): 191–198.

Sundaravadivel, M. and Vigneswaran, S. (2001). Constructed Wetlands for Wastewater Treatment. Critical Reviews in Environmental Science and Technology. In *Natural Science*

Collection, 31(4): 351-409.

Tao, W. and Wang, J. (2009). Effects of Vegetation, Limestone and Aeration on Nitritation, Anammox and Denitrification in Wetland Treatment Systems." *Ecological Engineering* 35 (5): 836–842.

Thurston, J. A., Gerba, C. P., Foster, K. E., Karpiscak, M. M. (2001). Fate of Indicator Microorganisms, Giardia and Cryptosporidium in Subsurface Flow Constructed Wetlands. *Water Research* 35 (6): 1547–1551.

Tilley, E., Ulrich, L., Lüthi, C., Reymond, Ph., Schertenleib, R. and Zurbrügg, C., 2014. Compendium of Sanitation Systems and Technologies. 2nd Revised Edition. Swiss Federal Institute of Aquatic Science and Technology (Eawag). Duebendorf, Switzerland.

Todt, D., Heistad, A., Jenssen, P. D. 920150. Load and Distribution of Organic Matter and Nutrients in a Separated Household Wastewater Stream. *Environmental Technology* 36 (9-12): 1584–1593.

UN (2018). UN-Habitat Strategic Plan 2020-2023.

UN Water Thematic Initiative (2007). Coping with Water Scarcity. A Strategic issue and priority for sytem-wide action. https://www.un.org/waterforlifedecade/pdf/2006_unwater_coping_with_water_scarcity_eng.pdf (date of visit: 28 Februray, 2021)

US EPA (2000). *Manual Constructed Wetlands Treatment of Municipal Wastewaters*. Office of Research and Development Cincinnati, Ohio.

US EPA (2012). Guidelines for Water Reuse. *Office of Research and Development, National Risk Management Research Laboratory. Cincinnati, Ohio.*

US Geological Survey (2021). Mineral commodity summaries 2021: US Geological Survey, 200 p.

Valipour, A., and Ahn, Y-H. (2016). Constructed Wetlands as Sustainable Ecotechnologies in Decentralization Practices: A Review. *Environmental Science and Pollution Research* 23 (1): 180–197.

Van de Graaf, A. A., Mulder, A. , De Bruijn, P., Jetten, M.S.M., Robertson, L. A., Kuenen, J. G. (1995). Anaerobic Oxidation of Ammonium Is a Biologically Mediated Process. *Applied and Environmental Microbiology* 61 (4): 1246–1251.

Vohla, C., Kõiv, M., Bavor, H. J., Chazarenc, F., Mander, Ü. (2011). Filter Materials for Phosphorus Removal from Wastewater in Treatment Wetlands-A Review. *Ecological Engineering* 37 (1): 70–89.

Von Sperling, M. (2007). *Basic Principles of Wastewater Treatment*. Biological Wastewater Treatment Series, Vol. 2. IWA Publishing.

Voulvoulis, N. (2018). Water Reuse from a Circular Economy Perspective and Potential Risks from an Unregulated Approach. *Current Opinion in Environmental Science and Health*, 2:32-45

Vymazal, J. (2006). Removal of Nutrients in Various Types of Constructed Wetlands. *Science of the Total Environment* 380 (1–3): 48–65.

Vymazal, J. (2009). The Use Constructed Wetlands with Horizontal Sub-Surface Flow for Various Types of Wastewater. *Ecological Engineering* 35 (1): 1–17.

Vymazal, J., and Kröpfelová, L. (2009). Removal of Organics in Constructed Wetlands with Horizontal Sub-Surface Flow: A Review of the Field Experience."*Science of the Total*

Vymazal, J. (2010). *Constructed Wetlands for Wastewater Treatment*. *Water, 2: 530-549.*

Environment 407 (13): 3911–3922.

Werker, A. G., Dougherty, J. M., McHenry, J. L., Van Loon, W. A. (2002). Treatment Variability for Wetland Wastewater Treatment Design in Cold Climates. *Ecological Engineering* 19 (1): 1–11.

West, C., Kenway, S., Hassall, M., Yuan, Z. (2016). Why Do Residential Recycled Water Schemes Fail? A Comprehensive Review of Risk Factors and Impact on Objectives. *Water Research* 102 (October): 271–281.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Williams, .J., Bahgat, M., May, E., Ford, M., Butler, J. (1995). Mineralisation and pathogen removal in Gravel Bed Hydroponic Constructed Wetland for Wastewater Treatment. Water Science and Technology 32(3): 49-58.

WHO (2006): Guidelines for a safe use of wastewater and excreta in agriculture and aquaculture. 2nd edition, World Health Organisation, Geneva, Switzerland

Wu, S., Carvalho, P. N., Müller, J. A., Manoj, V. R., Dong, R. (2016). Sanitation in Constructed Wetlands: A Review on the Removal of Human Pathogens and Fecal Indicators. *Science of the Total Environment* 541: 8-22.

Zeeman, G. and Kujawa-Roeleveld, K. (2011). Resource Recovery from Source Separated Domestic Waste(Water) Streams; Full Scale Results. *Water Science and Technology* 64 (10): 1987–1992.

Zhu, T., Mæhlum, T., Jenssen, P. D., Krogstad, T. (2003). Phosphorus Sorption Characteristics of a Light-Weight Aggregate. *Water Science and Technology* 48 (5): 93–100.

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9. Appendix

Table 9-1: Calibration data – laboratory and their corresponding on-site measurements, IGC – Influent Global Calibration, EGC – Effluent Global Calibration, GWGC – Groundwater Global Calibration

Sampling date	Sample	TSS_Lab	TSS_IGC	TSS_EGC	COD_Lab	COD_IGC	COD_EGC	BOD_5_Lab	BOD_5_IGC	BOD_5_EGC	NO3-N_Lab	NO3-N_IGC	NO3-N_EGC	TU_Lab	TU_GWGC	TOC_Lab	TOC_GWGC	DOC_Lab	DOC_GWGC
19.05.2020	100% influent	78	85	47	160	268	100	21	372	38	1.5	10.0	19.5	39.2	50.6	37.1	26.2	17.9	12.5
	50% influent	32	43	22	77	135	49	4	192	21	0.96	5.3	9.2	21.4	34.1	22.3	14.4	9.3	6.5
	25% influent	17	18	11	37	63	23	5	94	11	0.50	2.8	3.7	8.7	12.8	9.5	6.8	4.6	3.3
	12,5% influent	6	11	6	17	33	11	< 3	48	5	0.29	1.6	1.3	3.9	6.9	4.7	3.6	2.3	1.7
	100% effluent	3	0	1	13	24	6	< 3	363	7	88	30.7	55.8	3.4	1.2	3.8	8.4	2.8	1.7
22.06.2020	100% influent	52	88	48	120	280	104	39	455	43				27.5	53.6	28.5	30.1	17.7	12.9
	50% influent	16	40	21	62	140	51	20	240	22				10.2	32	13.5	15.7	9.0	6.7
	25% influent	4	21	11	37	68	25	12	121	11				4.8	12.6	8.9	7.7	5.0	3.3
	100% effluent	< 1	6	7	16	46	17	< 3	324	12				0.91	4.3	6.8	8.7	6.5	3.8
22.07.2020	100% influent	20	53	31	61	105	40	22	250	18	7.7	16.7	29.1	11.4	30.7	11.8	13.1	8.0	5.3
	50% influent	10	39	14	33	60	20	12	139	9	2.1	8.7	14.7	6.0	23.8	7.1	7.7	5.5	2.7
	25% influent	2	33	22	18	34	14	7	70	5	1.0	4.5	6.7	3.0	17.8	3.9	4.1	2.8	1.3
	100% effluent	< 1	36	2	< 10	41	18	< 3	294	8	62	32.4	54.7	0.56	16.8	4.6	8	3.7	2.3

Sampling date	Sample	TSS_Lab	TSS_IGC	TSS_EGC	COD_Lab	COD_IGC	COD_EGC	BOD_5_Lab	BOD_5_IGC	BOD_5_EGC	NO3-N_Lab	NO3-N_IGC	NO3-N_EGC	TU_Lab	TU_GWGC	TOC_Lab	TOC_GWGC	DOC_Lab	DOC_GWGC
04.08.2020	100% influent							19	259	16	4.3	17.7	31.2						
	50% influent							7	116	7	5.9	8.4	13.6						
	25% influent							3	70	4	1.4	5.0	7.5						
	100% effluent							< 3	172	13	26	16.6	27.1						
11.08.2020	100% influent	33	28	15	34	66	24	10	230	14	18	19.3	33	21.2	8.7	9.9	9.7	7.1	3.9
	50% influent	5	5	11	19	27	12	3	124	6	8.2	10.7	17.4	1.4	3.9	5.4	4.9	4.5	1.8
	25% influent	3	3	3	< 10	11	4	< 3	50	2	5.8	4.8	6.6	0.82	1.2	3.2	1.9	2.5	0.8
	100% effluent	< 1	22	18	20	53	21	< 3	122	12	15	10.2	16	1.2	6.1	5.9	6.1	5.9	3.3
19.10.2020	100% influent	51	92	50	110	253	92	29	284	37	1.0	3.1	6.5	24.6	55.4	40.9	23.4	17.1	11.2

Table 9-2: Influent treatment level predicted equivalent median values

Phase I	TSS_effl	NO_3_N_effl	COD_effl	BOD_5_effl	TU_effl	TOC_effl	DOC_effl	TSS_mid	NO_3_N_mid	COD_mid	BOD-5_mid	TU_mid	TOC_mid	DOC_mid	TSS_up	NO_3_N_up	COD_up	BOD_5_up	TU_up	TOC_up	DOC_up	TSS_infl	NO_3_N_infl	COD_infl	BOD_5_infl	TU_infl	TOC_infl	DOC_infl
Approach1	2	32.2	25	11	0.2	8.8	6.1	2	31.1	29	12	0.25	9.2	6.4	6	9.3	42	14	0.6	10.1	8.4	22	12.7	96	26	2.1	22.2	13.5
Approach2	2	32.2	25	11				3	34.7	26	15				6	9.1	43	15				22	12.7	96	26			
Approach3	2	32.2	25	11				2	31.1	29	15				6	8.8	43	14				23	12.6	95	26			
Approach4	2	32.2	25	11				2	31.1	29	12				4.5	8.9	44	14				25	12.9	93	24			
Approach5		63.0	24				5.5		61.1	27				5.8		5.1	43				8.5		6.35	90				
Phase II																												
Approach1	16	10.6	44	14	1.4	9.9	7.8	16	10.1	50	16	1.5	12.6	8.4	25	2.0	79	15	2.2	16.0	11.8	52	0.2	152	23	4.5	30.9	17.8
Approach2	16	10.6	44	14				17	10.3	47	17				25	2.2	79	18				52	0.2	152	23			
Approach3	16	10.6	44	14				16	10.1	50	17				25	2.5	79	20				55	0.4	152	28			
Approach4	16	10.6	44	14				16	10.1	50	16				26	2.7	79	22				62	0.8	153	38			
Approach5		22.7	38				6.8		21.6	42				7.2		1.7	76				12.2		0.3	136				19.1

10. Curriculum Vitae

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Consultancies 2016-2020

- Evaluation of ecosystem services of two modified lagunes at the Black Sea
- Urban forest management guidelines development
- Evaluation study of the forestry measures under Rural Development in Bulgaria
- Volunteering for Rwanda Wildlife Conservation Association on project proposals development, Grey Crowned Crane Conservation Strategy development, field activities.

Forest Conservation Officer, 2013-2016

Bulgarian Society for the Protection of Birds, Birdlife Bulgaria, bspb.org

Implementation of forest conservation activities within a EU LIFE+ funded project:

- National and Eu policy and advocacy work
- Communications and outreach
- Day-to-day work with state forest departments
- Research and field activities

Biodiversity Project Officer, 2010-2013

International Union for Conservation of Nature Programme Office for South-Eastern Europe, iucn.org

- Project overall management and coordination
- Project proposals development
- Building capacity on environmental topics
- Transboundary cooperation development in Western Balkans
- Input to the preparation and implementation of IUCN programmes, strategies and positions

BirdLife European Forest Task Force coordinator, 2005-2009

Bulgarian Society for the Protection of Birds, Birdlife Bulgaria, bspb.org

- Administration and facilitation of forest conservation work of BirdLife International Partners in Europe
- EU and national level advocacy and lobbying
- Representation of BirdLife at forest-related fora: EC expert meetings, EU level conferences, European NGOs forest conservation events
- Project coordination and steering, staff recruitment

Research associate, 2003-2004

Forest Research Institute – Bulgarian Academy of Sciences, fri.bas.bg

- Maintenance and renovation of forest hydrological stations
- Collection and processing of meteorological and hydrological data
- Inventory of old-growth forests

Additional experience:

- Development of the FSC national forest certification standard
- Development of a Strategic Plan for management of mountain pastures on the territory of Central Balkan National Park
- Small-scale projects evaluation
- EU NGO bio-energy working group expert meetings
- Voluntary nature conservation work in Macedonia, Poland, Mongolia, Malta, Rwanda and Bulgaria.

Master Programme Natural Resource management and Ecological Engineering

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Master thesis "Restoration of open cast mine substrate with mycorrhizal seedlings of Scots Pine", prepared at the Brandenburg Technical University, Germany

Additional trainings:

- Permaculture Design, Uganda, 2017.
- Ecological Monitoring, online course by IUCN and École Polytechnique Fédérale de Lausanne, 2017
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12. Affirmation

I certify, that the master thesis was written by me, not using sources and tools other than quoted and without use of any other illegitimate support.

Furthermore, I confirm that I have not submitted this master thesis either nationally or internationally in any form.

Vienna, April, 1st, Veronika Ferdinandova