CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE
FUCLTY OF ENVIRONMENTAL SCIENCES

MASTER THESIS

The effect of harvesting on nutrients accumulation in aboveground biomass of macrophytes

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DIPLOMA THESIS ASSIGNMENT

Koharu Okada, BSc

Nature Conservation

Thesis title

The effect of harvesting on nutrients accumulaion in aboveground biomass of macrophytes

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Objectives of thesis

1. To evaluate available literature on acumulation of nitrogen and phosphorus in wetland macrophytes
2. To setup a field experiment at one constructed wetland planted with Phalaris arundinace and Phragmites australis

Methodology

Literature review on nutrient accumulation in wetland plants

The proposed extent of the thesis

50 pages including attachments

Keywords

macrophytes, nutrients, wetlands, harvesting

Recommended information sources

Vymazal, J. a Kröpfelová, L. 2005. Growth of Phragmites australis and Phalaris arundinacea in constructed wetlands for wastewater treatment in the Czech Republic. Ecological Engineering 25(5): 606-621.

Vymazal, J. a Kröpfelová, L., 2008. Nitrogen and phosphorus standing stocks in Phalaris arundinacea and Phragmites australis in a constructed wetland: 3-year study. Arch. Agron. Soil Sci. 54(3): 297-308.

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Prague on 13. 04. 2018

Declaration:

I hereby declare that I am the sole author of the thesis entitled: "The effect of harvesting on nutrients accumulation in aboveground biomass of macrophytes ". I duly marked out all quotations. The used literature and sources are stated in the attached list of references.

In Prague on 18.04.2018 ______________________

Koharu Okada

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In Prague on 18.04.2018 ________________________

Koharu Okada

Abstract

Considering fresh water supply issue, application of Constructed wetlands (CW) for waster water treatment is one of effective solution. In Czech Republic, there are several active CW and this study picked one in Zbenice applying for domestic water and storm water. One of significant class of contaminant are nutrients (nitrogen and phosphorus) and that causes eutrophication of the aquatic environment.

This study was focused on nutrient uptake into macrophytes aboveground biomass from discharge and measured amount of aboveground biomass and nutrient standing stock. There are *Phalaris arundinacea* and *Phragmites australis* in this CW and harvest of aboveground biomass was conducted on June, July, October and June, September for each macrophytes.

The result showed that biomass for *P. arundinacea* was generally higher in inflow than outflow and biomass, nutrient standing stock of single harvest (1888 gm^{-2}, 3.73 gPm^{-2} and 33.0 gNm^{-2}) in highest growing period was higher than double harvest. For *P. australis* there was no regrowth after first harvest and the difference between inflow and outflow was not significant ($p > 0.05$). Single harvest in September was shown to yield the highest biomass and nutrient accumulation(3943 gm^{-2}, 6.34 gPm^{-2} and 62.3 gNm^{-2}).

Considering on results, aboveground biomass and nutrients *P. arundinacea* in a single harvest was higher than in a double harvest. However, newly grown shoots in October after first harvest was contained high concentration of nutrients. And there were significant differences of biomass and nutrient between inflow and outflow for *P. arundinacea* that were caused by different amount of nutrients load. From these points of view, application of different harvest timing on inflow and outflow can be an effective way to enhance the capability of CW.

Keywords: Macrophytes, nutrients, wetlands, harvesting

Abstract (Czech)

Umělé mokřady jsou vhodnou alternativou pro čištění odpadních vod z malých zdrojů znečištění. V České republice je v provozu několik set umělých mokřadů a jeden z těchto mokřadů ve Zbenicích byl vybrán jako pokusná lokalita pro tuto práci. Živiny (dusík a fosfor) patří mezi hlavní znečištění ve splaškových vodách a tato práce byla zaměřena na posouzení kumulace těchto živin v nadzemní biomase rákosu obecného (*Phragmites australis*) a chrastice rákosovité (*Phalaris arundinacea*). Při experimentech bylo také provedeno porovnání kumulace živin v nadzemní biomase sklízené jednou a dvakrát v roce. Výsledky ukázaly, že biomasa chrastice je výrazně vyšší na přítoku v porovnání s odtokovou zónou. Porovnání jedné a dvou sečí prokázalo vyšší kumulaci biomasy a živin při jedné seči v červenci (1888 gm^{-2}, 3.73 gPm^{-2} and 33.0 gNm^{-2}). Vzhledem k faktu, že u rákosu nebyl zaznamenán opětovný nárůst biomasy po sklizni, nebylo možno porovnat jednu a dvě sklizně. Biomasa a kumulované množství v září byly vyšší než u chrastice (3943 gm-2, 6.34 gPm^{-2} and 62.3 gNm^{-2}). Výsledky prokázaly, kumulace živin v nadzemní biomase u chrastice je výrazně závislé na lokalizaci na čistírně, přičemž na přítoku jsou hodnoty výrazně vyšší především vzhledem k vyšší biomase ve srovnání s odtokem. Z tohoto důvodu by bylo vhodnější umístit chrastici pouze na přítok, protože rákos není tolik závislý na umístění na čistírně.

Klíčová slova: Makrofyta, živiny, mokřad, sklizeň

Table of Contents

1. Introduction

In the world, the arising fresh water issue is expanding day by day because of population growth and intensive agriculture uses of fresh water. Therefore wastewater treatment for conducting sustainable freshwater cycling is increasing in importance.

In Czech Republic, for example, run-off containing high nitrogen and phosphorus drained from fertilizer of agriculture field could cause eutrophic condition into surrounding aquaculture facilities. Especially in regarding to Czech local features, there are many fish ponds that are usually in quite a eutrophic condition and, therefore, it is necessary to find ways to mitigate nutrient inflow into fish pondsConstructed Wetland (CW) is a capable wastewater treatment system that consumes less energy than conventional wastewater treatment. In Czech Republic, this technology has been applied widely and is especially prevalent in rural areas without proper sewage connection.

2. Aim of this study

1) Set up the experiment on Constructed wetland in Zbenice, Czech Republic.
2) Measure aboveground biomass and nutrients (total – nitrogen, phosphate) of *Phalaris arundinacea* and *Phragmites australis.*
3) Consideration the effect of harvesting of harvest frequency and seasonal difference to aboveground biomass and nutrient accumulation.
4) Insight to optimization of efficiency of Constructed wetland.

3. Review of literature

3.1 Concept of Constructed wetland (CW)

3.1.1 General features

As for water resources from the global point of view, maintaining water quality and purification limited water resources is a significant issue. Discharge water from industry, agriculture and residential area are constant concerns, especially in developing countries. Conventional wastewater treatment facilities using, for instance, aeration, filtration, chemical participation for wastewater treatment takes cost and uses energy. Under condition of low infrastructure or area isolated from urban area, even operation is still having many difficulties when it comes to sustainable operation and labor.

Constructed Wetland (CW) is an artificial wetland as its names suggests. And generally it is intended to serve the role of water treatment function to utilize natural process involving wetland vegetation, soils, and associated microbial assemblages (Vymazal, 2005). This technique is mainly used for wastewater treatment, mitigation for run-off from agriculture field, road construction and etc.., ecotone to encourage biodiversity and biotope as well. Furthermore, as for wastewater treatment, this CW technique doesn't require frequent intense maintenance compared with organizing conventional wastewater treatment facility. For example, CW could function at especially small isolated communities in Czech Republic and several are functionally operating.

3.2 Type of Constructed wetland

3.2.1.Free water surface flow wetland (FWS)

Free water surface flow wetland (FWS) is commonly defined as whole water of wetland system exposure to the atmosphere and the state could be said most near to natural wetland such as bog, swamps and marshes (Agency, 2000) and water is passing through over the gravel soil in water from inlet to outlet of the wetland system. In this treatment measure, generally floating aquatic plant like as *Eichhornia crassipes* -water hyacinth, *Pistia stratiotes* - water lettuce (Rezania, 2016) and no root floating plants such as *Lemnaceae* - duckweed, *Azolla pinatta* - water fern, submerged plants like as *Elodea canadensis, Myriophyllium spicatum* (Verhofstad et al., 2017) and emergent plant like as *Typha* spp. – Cattails, *Scirpus* (*Schoenoplectus*) spp – Bulush and *Phragmites australis* – Common reed, are used in this type of CW (Vymazal, 2013). One significant feature to apply it is these floating aquatic plant species contain high dense of root area and it help to enhance diversity of microbial community inhabiting around rhizosphere zone by providing large surface area and provide oxygen to respiration for aerobic communities. On the other hands, compared with other types of wetlands, FWS constructed wetlands occupy larger areas as compared to other types of CWs (Agency, 2000). FWS with the floating plant *Eichhornia crassipes* is common it displays high productivity and and relatively easy to reproduce (Vymazal, 2008). But this plant is among the 100 of the World’s Worst Invasive Alien Species and it is hard to control its explosive growth. Therefore its usage should be under rigid control. When FWS system applied, this method is mainly used for secondly treatment (figure:1) after getting rid of big particle of suspended solid (SS) to function in removal of nutrients and inorganic material like as trace elements, heavy metals and so on. This technique has been used all kinds of climates, but the efficiency of removal for some components will be negativelyaffected by low temperature (Kadlec, 2009).

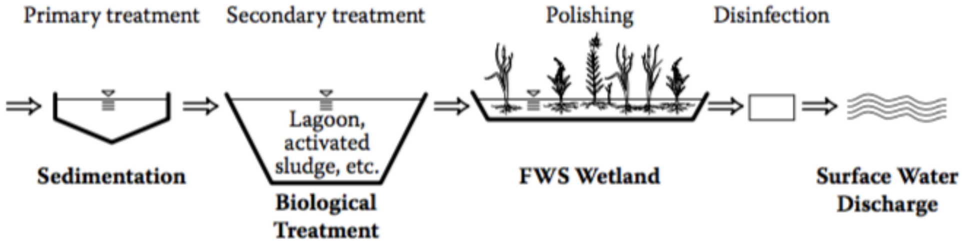
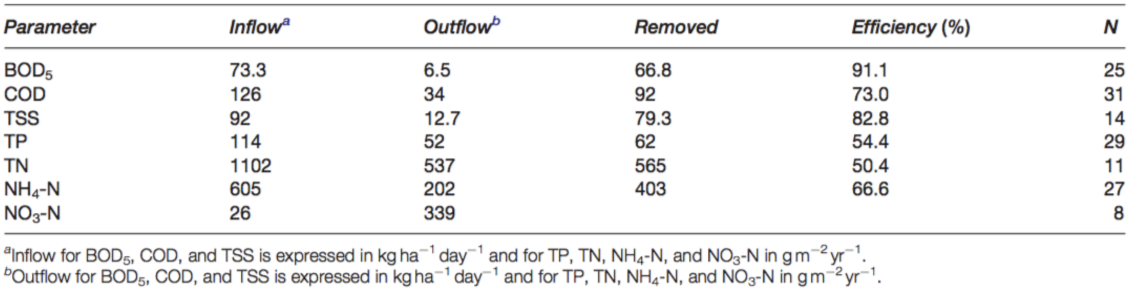


Figure 1: Flow chart of Free water surface wetland for municipal wastewater treatment (Wallace and Knight (2006).)

3.2.2 Vertical flow wetland (VF)

Vertical flow constructed wetland (VF) is defined as artificial wetland designed to pass the water through vertically from top to bottom by filtrating between gravel or the soil. Reed (*Phragmites australis*) beds allow for sludge to dry quickly by providing drainage of water in channels create by reed stems, rhizomes and roots, by creating holes on the sludge surface, and through evapotranspiration and microbiologically forced mineralization. As for specialized removal feature, this system provides more oxygen that help nitrification than other wetlands. Therefore regarding to Total nitrogen removal result generally shows not significant amount of removal (Table 1) (Melidis et al., 2010) In a study conducted by Li and Tao (2017) a VF wetland was introduced for treating water from dewatered active sludge from a wastewater treatment plant. This special type of wetland contains 2 phases, aerobic and anaerobic, inside soil through which the effluent passes. Under this particular condition, combination of nitrification and denitrification occurred can be used for wastewater treatment containing high ammonia (Figure 2). This study indicated availability of removal of nitrogen from the VF wetland by plant uptake and denitrifying bacteria located in soil, and it mentions carbon resource from litter material and root exudates. In general, emergent macrophytes of the genera of *Phragmites*, *Phalaris*, *Iris*, *Schoenoplectus* (*Scirpus*), *Sparganium, Carex, Typha* and *Acorus* are utilized in this system (Vymazal, 2013).

Table 1. Loading of constructed wetlands with horizontal subsurface flow (Vymazal et al., 2008).



Parameter	*Inflow*[a]	*Outflow*[b]	*Removed*	*Efficiency (%)*	*N*
BOD_5	73.3	6.5	66.8	91.1	25
COD	126	34	92	73.0	31
TSS	92	12.7	79.3	82.8	14
TP	114	52	62	54.4	29
TN	1102	537	565	50.4	11
NH_4-N	605	202	403	66.6	27
NO_3-N	26	339			8

[a] Inflow for BOD_5, COD, and TSS is expressed in $kg\,ha^{-1}\,day^{-1}$ and for TP, TN, NH_4-N, and NO_3-N in $g\,m^{-2}\,yr^{-1}$.
[b] Outflow for BOD_5, COD, and TSS is expressed in $kg\,ha^{-1}\,day^{-1}$ and for TP, TN, NH_4-N, and NO_3-N in $g\,m^{-2}\,yr^{-1}$.

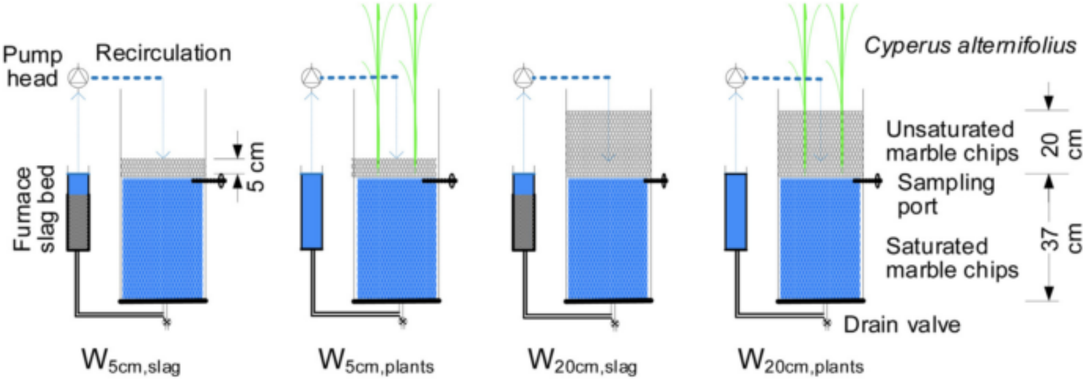


Figure 2: Four configurations of vertical flow wetland that intended for denitrification (Li & Tao, 2017)

3.2.3 Horizontal subsurface flow wetland (HSSF)

A horizontal subsurface flow wetland is consists of gravel and soil and water is passes below the top surface of medium horizontally. The priority of application is to prevent odor and mosquitos by putting the water level below ground (Agency, 2000). The design is typically consists of a rectangular bed planted with a common emergent plant, reed-like as *Phragmites australis* and *Phalaris arundinacea*, Lined with an impermeable membrane. This is most widely used wetland system in Europe is HSSF (Figure 3) (Vymazal, 2005). The shallow soil bed is easily affected by the condition of low tempratures to reduce the removal of nutrients like as phosphoros and nitrogen (Zhai at al., 2016). On the other hands, HSSF have been found to limited in the capacity of removal of nitrogen (TN) removal due to lack of oxygen flux for nitrification(Pan et al., 2012) and in this paper HSSF were introduced for denitrification process. This system requires primary removal of big suspended solids, so that it is usually applied as a secondly treatment system (Vymazal, 2008).Removal of phosphorus occurs in HSSF CW is mainly through ligand exchange reactions in soil media (ex, pea gravel, crushed stones), where phosphate displace water or hydroxyls from the surface of Fe and Al hydrous oxide. However soil in HSSF generally doesn't consist great quantities of these trace elements like Fe and Al, so removal by this reaction is usually low.

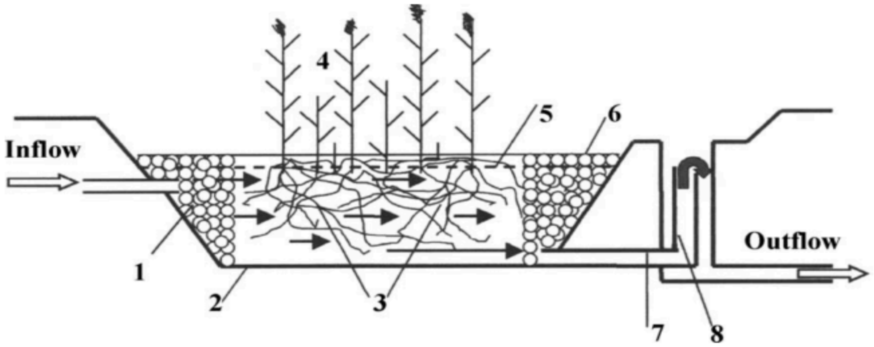


Figure 3: Schematic representation of Horizontal sub-surface flow wetland 1, distribution zone filled with large stones; 2, impermeable liner; 3, filtration medium (gravel, crushed rock); 4, vegetation; 5, water level in the bed; 6, collection zone filled with large stones; 7, collection drainage pipe; 8, outlet structure for maintaining of water level in the bed. The arrows indicate only a general flow pattern.(Vymazal, 2005)

3.2.4 Hybrid constructed wetland

As I mentioned above, one type of constructed wetland (CW) is sometimes not expected to achieve removal of every subjected element when it applied to municipal wastewater treatment. So as to improve the efficiency of CW, combining 2 more types of CW is introduced. Commonly combinations of HSSF-VF or VF-HSSF wetland are shown in the studies as aerobic and anaerobic treatment are conducted at the same time. For example a three-stage wetland applied for wastewater treatment of sewage, food processing and farming showed a high degree of nutrient removal especially total nitrogen and phosphate(Švereš et al., 2017; Serrano et al., 2011; Vymazal, 2008)(Figure 4). There are the wetland integrated with FWS also and if the wastewater site contains pond or reservoir a FWS could be easily converted by these condition (Serrano et al., 2011).

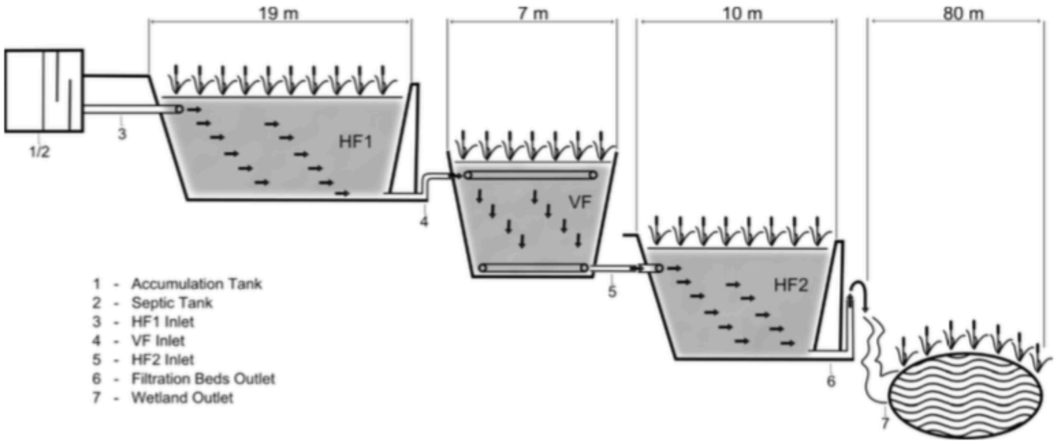


Figure 4: Flow chart of the three stages of a hybrid constructed wetland in Czech Republic. (Seres et al., 2017)

3.3 History of Constructed wetland

The idea of utilizing wetlands for mitigating polluted effluent is derived from the application of natural wetlands, like meadows, swamps and marshes, for such a purpose. An old study was carried out on a great meadows natural wetland near the Concord river in Lexington, Massachusetts, which started to receive wastewater in 1912 (Kadlec and Wallace, 2009). The first experiment intended for investigating possibility of water quality improvement with constructed wetland with aquatic macrophytes was conducted by Käthe Seidel in Germany, in early 1950s at the Max Planck institute in Plön (Seidel, 1955 ;engineering, 2005) Afterward, Reinhold Kickuth from Göttingen University developed the Root Zone Method and investigated the first HF wetland systems put in operation in 1974 in the community of Liebenburg-Othfresen (Kickuth, 1977, 1978, 1981; Brix, 1987a ; engineering, 2005). The Ijssel Lake Polder Authority in Flevoland (The Netherlands) constructed its first FWS CW in 1967, Afterward research about FWS was spread into North America but not significantly into Europe (Vymazal, 2008). Recently HF CW has become used throughout the world, mostly in Germany where the number of these systems may exceed 50,000. Owing to the low cost sand convenience of CW systems, they are starting to be employed in developing country as well. Brix et al. (2011) showed an example of CW application at a site where wastewater treatment hadn't operated well. Remote locations will be ideal site for application of these systems.

3.4 Process of Remediation.

3.4.1 Function of macrophytes

Wetland plants play multiple role in a CW. From the nature conservation view, keeping natural vegetation and visual impact of environment also contribute these concept. However not only conservative point of view, these plants physically affect chemical factors in CW. The root system of macrophytes in CW is always important to stabilize the soil of CW especially HSSF wetlands and preventing erosion by covering surface of wetlands (Brix, 1994). When it comes to purification and wastewater treatment, wetland plants show significant effect on it. Accumulation into plant tissue happens via metabolism. (Haritash et al., 2017). In this paper, *Canna liliy* show significant amount of accumulation of phosphate in each plant tissues especially on flower part that accumulate 4 times higher concentration than initial state. On the other hands, wetland plant plays a role to uptake Nitrogen as well. Hallin et al., (2015) showed unique result that amount of Nitrogen uptake via wetland plant under growth chamber is higher than natural condition. In addition, figure 5 illustrates plant uptake of cation like as calcium, magnesium and potassium from soil then release hydrogen ions than cause acidification (Wortmann, 2015). When it comes to other trace element contained in contaminated soil like post-mining site, in this paper (Puga, et al., 2015) there were results that crop plant

Jack bean and *Mucuna aterrima* could accumulate cadmium, lead and zinc into plant tissue, otherwise (Figure 6) shows *Mucuna aterrima* got interveinal chlorosis and necrotic spots on it.

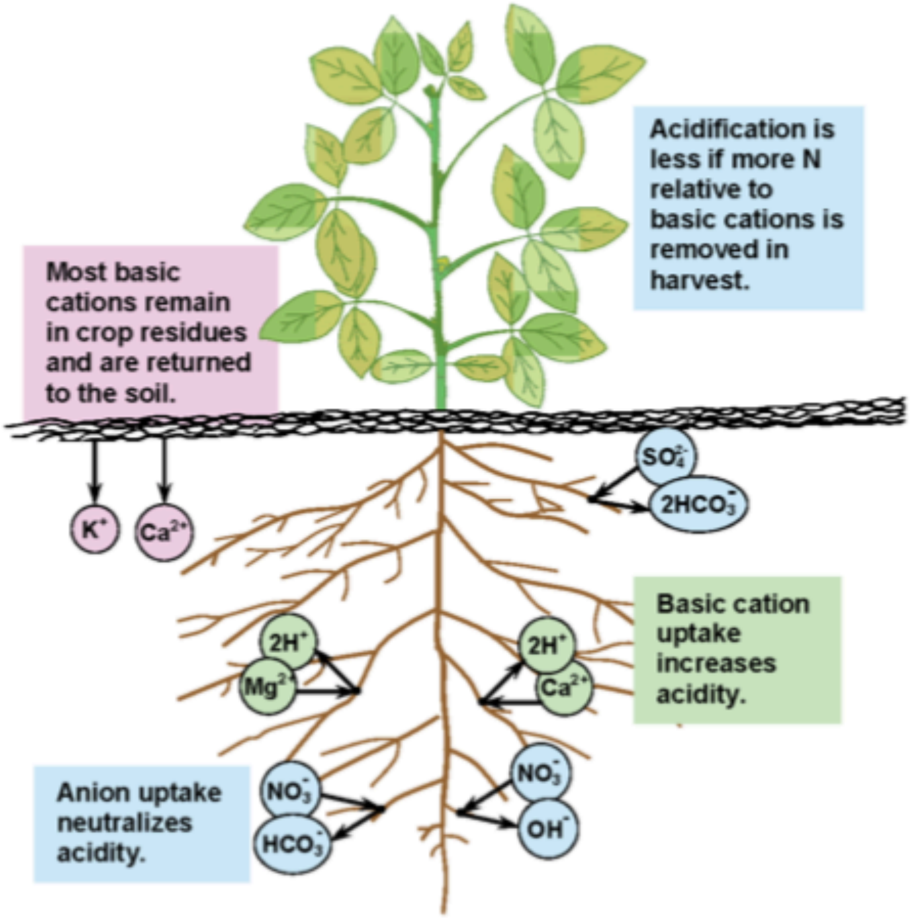


Figure 5: Plant uptake and relation of acidification (Elt et al., 2009)

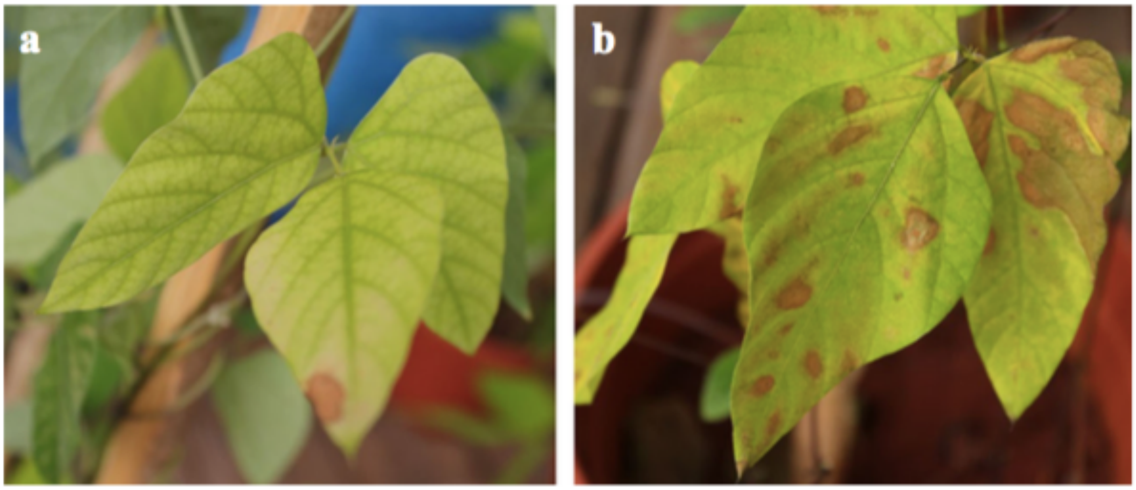
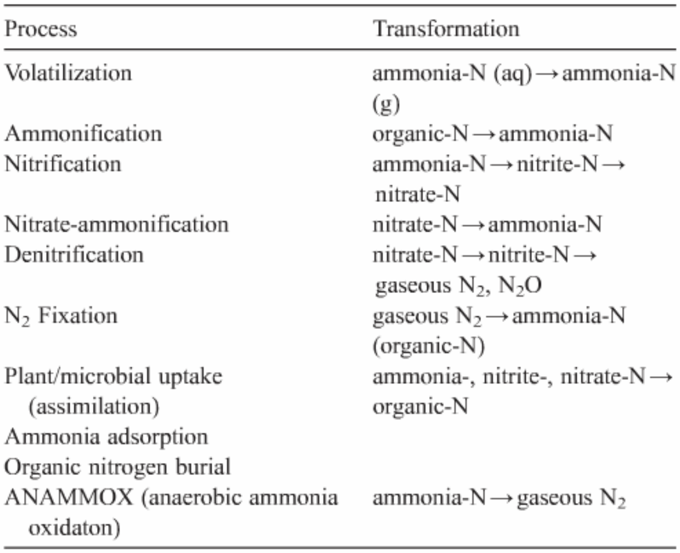


Figure 6: *Mucuna aterrima* received the features (a)interveinal chlorosis (b) necrotic spot after cultivation on experimental site (Puga et al., 2015)

3.4.2 Function of rhizospheric microorganism

As for in CW ecosystem microorganism also play significant role for decomposition and remediation via their metabolism. Especially rhizospheric microorganism are interact with host plant by being provided root exudation such as secretion of ions, free oxygen, water, enzymes, mucilage and carbon-containing primary and secondary metabolites (Bais et al.,2006). For instance, nitrification process could cause even under anaerobic condition with oxygen supply to nitrifier microorganism for ammonium degradation. Vymazal, (2007) elucidated nitrogen translocation mechanism under CW soil via microbial activity, but actually the microbial activities involved in nitrogen removal are volatilization, denitrification, plant uptake, microbial uptake, ammonia absorption, ANAMMOX and organic nitrogen burial (Table 2). Integrated transformation is significant to remove actual nitrogen.(Du et al., 2017) mentioned about phosphorus removal by rhizospheric microorganism, in this study wetland with *Canna generalis* showed highest activities of polyphosphate kinase that could digest phosphorus from soil and pseudomonas dominant microbial community got the result. Introducing about assistance of plant growth promoting bacteria in rhizosphere that could bring heavy metals and phosphorous in bioavailable and soluble form, since heavy metals affect nutrient uptake into plant tissue. Furthermore, soil absorption involved by microorganism is also one significant factor of purification, for example, Puga et al., (2015) showed that application of higher ratio of biochar in the soil could immobilize and reduce concentration of heavy metal such as Cd, Pb, and Zn.

Table 2: Nitrogen transformation (Vymazal, 2007)



Process	Transformation
Volatilization	ammonia-N (aq) → ammonia-N (g)
Ammonification	organic-N → ammonia-N
Nitrification	ammonia-N → nitrite-N → nitrate-N
Nitrate-ammonification	nitrate-N → ammonia-N
Denitrification	nitrate-N → nitrite-N → gaseous N_2, N_2O
N_2 Fixation	gaseous N_2 → ammonia-N (organic-N)
Plant/microbial uptake (assimilation)	ammonia-, nitrite-, nitrate-N → organic-N
Ammonia adsorption	
Organic nitrogen burial	
ANAMMOX (anaerobic ammonia oxidaton)	ammonia-N → gaseous N_2

3.5 Improvement of CW

When it comes to improvement on nutrient, organic and trace element removal efficiency from CW, even ongoing studies are struggling to find out best way. CW structure, chemical condition in soil, operation mode, pH, temperature, media (soil) material and other many condition interact efficiency of CW work.

3.5.1 Hydraulic condition

Mode of operation are sorted continuous, batch and intermittent (Meng et al., 2014). Continuous is feeding effluent constantly into CW, batch is sequential feeding during the period of time and intermittent operation is feeding effluent in the manner such that subsurface aeration will occur, which fix aerobic condition and accelerates clogging matter mineralization (Knowles et al., 2011) and it is also called as tidal flow feeding method (Zhao et al., 2004). Wu et al., (2016) introduced aeration system on VF wetland and CW with aeration showed 95.0 - 97.0 % removal of COD and 81.7-85.8 % removal of total nitrogen as well (Figure 7). Clogging in CW is one of major problem occurring during operation. Hydraulic residence time (HRT) is the period how much times media compound is retaining in a storage unit (soil). This paper (Akratos and Tsihrintzis, 2007) is comparing HRT of 6, 8, 14 and 20 days with reed and cattail beds. As a result, CW with 8 days HRT showed most efficient removal rate of organic matter (COD and BOD) than longer HRT ones. Otherwise, regarding to amount of phosphorus and nitrogen removal seem increased according with time lapse.

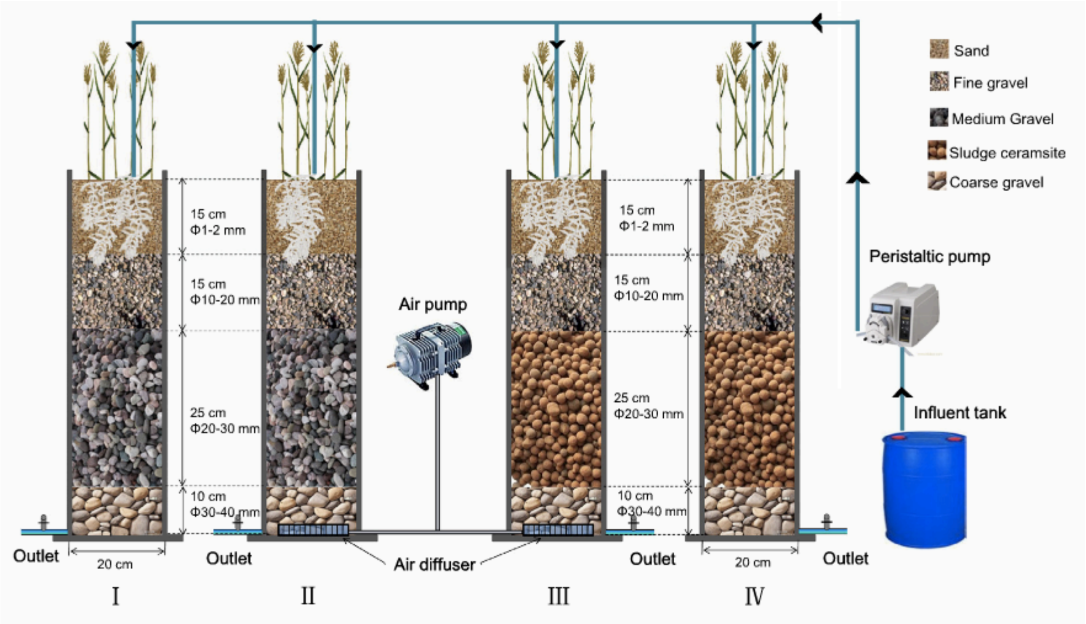


Figure 7: Schematic diagram of experimental laboratory-scale CW (Wu et al., 2016)

3.5.2 Temperature and pH

The climate in CW also affect the efficiency of removal. Even there are many study of CW system in cold region and showing high performance resemble to the one in temperate zone. Wang et al. (2017) indicated that removal of total suspended solid (TSS) and organic contaminant are not affected by low temperature, while regarding to Nitrogen removal optimal temperature of nitrification is range from 25 to 35˚C (engineering, 2005). Moreover it mentioned about regression of oxygen availability which influence redox revel also affect TP removal in cold climate (Table 3). When it comes to pH, microbial process are sensitive to pH in CWs and heterotrophic production rate were higher at around neutral pH values (Meng et al., 2014). In this paper Vymazal,(2007) reported that optimal pH range for denitrification is between pH 6 to 8 and below 4 denitrification will be significantly decreased.

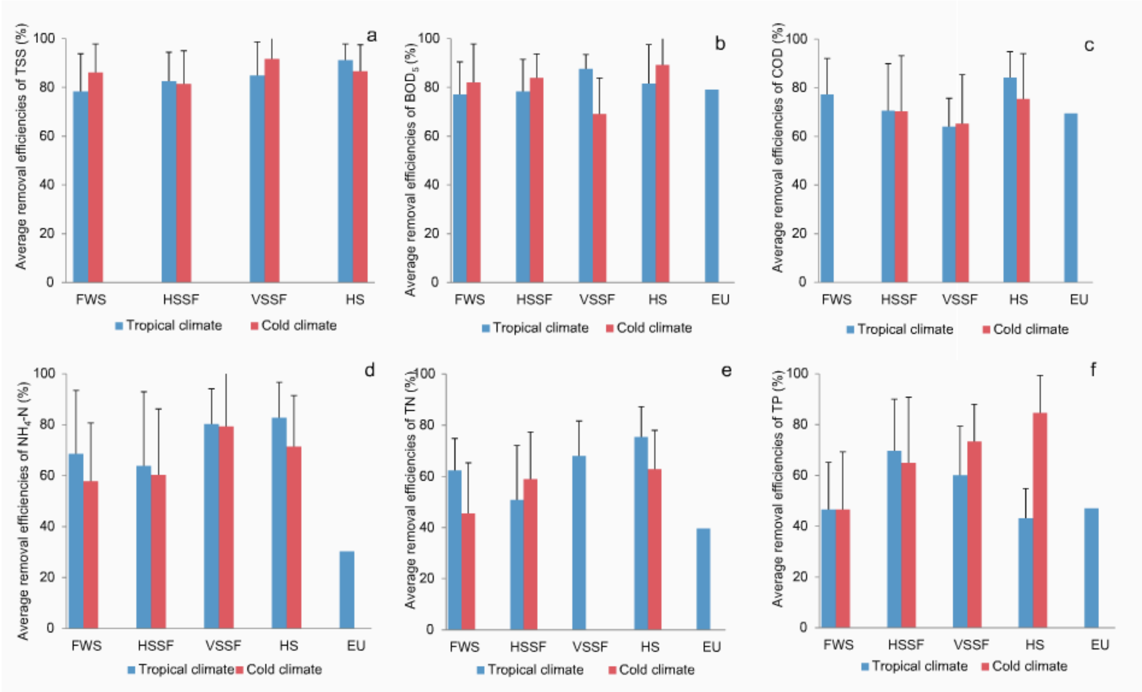


Table 3: Average removal efficiencies of contaminants in various CW systems. (a) TSS removal; (b) BOD5 removal; (c) COD removal; (d) NH4-N removal; (e) TN removal; (f) TP removal. BOD: biological oxygen demand; COD: chemical oxygen demand; HS: hybrid system; EU: European countries; Error bar: standard deviation; TN: total nitrogen; TP: total phosphorous; CW: constructed wetland. (Wang et al., 2017)

3.5.3 Bioaugmentation applied into CW

Bioaugmentation word itself means introduction of microorganism with specific catabolic abilities into contaminated environment to enable or accelerate degradation of targeted contaminants (materials PLOH, 2010; Shao et al., 2014). In this paper (Shao et al., 2014), introduction into CW soil is conducted by adding enrichment medium soil with six bacteria with known denitrifying and phosphorus-removal capabilities isolated from the rhizosphere soil of wetland plants. As a result, reed bed CW with inoculated the bacterial consortium showed high removal ability of COD, TP and TN compared with not inoculated CW. Bioaugmentation is not only introduction of bacteria enriched soil, application specific medium material help bioremediation in CW system. when effluent passing through into porous medium containing large surface area and it provide adhesion for microorganism that could improve CW wastewater treatment function (Lu et al., 2016). This paper conducted experiment of CW with 4 different kinds media and showed different efficiency of removal with ordered as maifanite > steel slag > bamboo charcoal > limestone.

3.5.4 Selection of Wetland macrophytes

Regarding to selecting proper macrophytes for CW, definitely it would be considered suitable condition for them with depending on climate such as temperature mentioned above. J. Vymazal, (2011) mentioned principles for macrophytes in CW should be tolerant of high organic and nutrient loadings, rich rhizospheric structure to fix microbial activity and high above ground biomass for winter insulation. In Czech Republic, mainly *Phragmites australis* and *Phalaris arundinacea* are mainly introduced to CW. In this paper (Vymazal, 2013), *Glyceria maxima*, *Typha latifolia* and *Typha angustifolia* are also used at CW in Czech Republic. Even each wetland plants they showed different ratios of nutrient removal, (Hallin et al., 2015) in this study, for analysis of denitrification ratio that happened via rhizospheric biodegradation, submerged plant *Dichomeris fluitans* was showing significantly higher denitrification ratio. Therefore, application of wetland plant should be selected according to target removal substance. Furthermore, this paper (Toscano et al., 2015) compared efficiency of purification with 4 different macrophytes (*Vetiveria zizanoides*, *Miscanthus* x *giganteus*, *Arundo donax* and *Phragmites australis*.). In the result, *P. astralis* showed significantly highest evapotranspiration ratio and efficiency of removal within four species.

3.5.5 General feature of Macrophytes

In this study site, mainly 2 types of wetland macrophytes *Phalaris arundinacea* (reed canary grass) and *Pharagmites australis* (common reed). They are quite common wetland macrophytes used for CW wastewater treatment. CW system had been used since 1989 in Czech Republic and early system were only planted with *P. australis* (Vymazal, 2013). *P. australis* is perennial and flood tolerant grass with enriched rhizome system reaching to depths of about 0.6 -1.0 m (Vymazal, 2011). This plant tissue contains high ratio of aerenchyma in the stems, helps transferring oxygen into even anoxic root zone and keep buoyancy in saturated soil. Main application of *P. astralis* is Europe, since the use of it in United State is considered as exotic and invasive plant and is limited to introduce (KADELEC and WALLACE, 2009). As Dykyjová (1978); Vymazal et al., (1999) mentioned *P. australis* is growing in nutrient-rich habitats accumulates more mineral nutrients in aboveground shoots than stands growing nutrient-poor habitats, therefore *P. australis* is suitable for exposure against nutrient rich effluent flow.

While *P. arundinacea* is also common application, Vymazal, (2013) mentioned benefits well germination ability from the seeds, easy planting, fast growth and provision of good insulation during the winter. This plants is also invasive species in certain region such as UK or US, but in Czech republic both of wetland macrophytes is native (Vymazal et al., 1998; KADELEC and WALLACE, 2009). The peaks of biomass is in July for *P.arundinacea* and in the period of August or beginning of September for *P. australis* (Vymazal and Krőpfelová, 2005). And *P. arundinacea* start to sprout in May and *P. arundinacea* start to sprout in the period end of March or beginning of April under the climatic condition of the Czech Republic. Before sampling on 23th May, there were both of macrophytes had been sprouted on the ground (photo). However quantity of sprouting of *P. astralis* is still not enough for harvesting.

Figure 8: Early sprouting of *P. arundinacea* (left, well-grown strip) and *P. australis* (right, spontaneous grown strip) on 23th May 2017 at Zebenice.

3.5.6 Biomass harvesting

Plant growth itself is limited by the actual CW area, therefore macrophytes harvesting is considered as a way to manage this situation or also available to use as biomass resource. Some research showed significant correlation between nutrient removal and amount of plant biomass in CW as they require nutrient for growth. The potential rate of nutrient uptake by the wetland plant is limited by its net productivity and nutrient concentration in plant tissue (Vymazal and Kröpfelová, 2008). Verhofstad et al. (2017) applied harvest on submerged plant, *Myriophyllum spicatum* and 2 times harvesting on beginning and end of growing season achieved highest nutrient standing stock in total biomass. While emergent plants *Scirpus grossus* and *Typha angustifolia* are used in this study (Lu et al., 2016). Maximum biomass is depending on wetland plant species that have proper frequency of harvesting and location from inlet zone is also related to amount of biomass. And regarding to nutrient uptake, repeated harvesting CW show relatively high standing stock. Uptake of trace element like as Al, Fe, Mn and so on showed higher result in double harvest than single one. However some trace element like as Ni and Pb are less in double (Vymazal et al., 2010). Cropped biomass is expected to utilize as green manure, feed supplement for animals, raw material of paper industry or especially fuel production. Ciria et al., (2005) indicated capability of macrophytes (*Typha latifolia*) biomass use as a fuel in thermo-chemical processes. Regarding to standing stock of biomass, biomass of *P. astralis* is shown relatively high range from 788 gm^{-2} to 5000 gm^{-2} (Vymazal snd Krőpfelová, 2005; Vymazal, 2011) and expected to be nice resource for biomass yielding.

4. Material and Methods

4.1. Description of the site

As In this study, sampling of plant biomass was conducted Sub-surface horizontal flow constructed wetland in Zbenice village and this location is isolated 70km south-west from the city of Prague (Figure 9). There are not there are well-organized sewer connection with big wastewater treatment system. This CW was built in 1996 and started its operation since 1. 1. 1997 by local authorization for treatment of domestic wastewater and storm water (Table 4). The outflow is directly connected to fishpond next to CW (figure 10). There are 2 parallel beds 21.2 m long and 20 m width with a total are 444 m^2. *Phalaris arundinacea* are planted in both adjacent to inflow and outflow zone (3.6 m and 5.4 m long respectively). *Pharagmites astralis* are planted in central part of CW (13.2 m) (Figure 10). As a pretreatment, horizontal grit chamber with screening system and Imhoff tank is used. Horizontal grit chamber is intended for removal of solid inorganic particles. Pretreatment units are needed to remove solid particles which may clog the filtration beds. In this system, heavier solid material in effluent are sunk into the diches on bottom by gravity (Environmental Protection Agency, 2003). In the Imhoff tank, the separation of liquid and solid phase of wastewater with V-shaped settling compartment above a tapering sludge digestion chamber occurs (Tilley et al., 2014). Basic information of effluent from the community (1997 - 2016) is shown on Table 5. The inflow and outflow concentrations of TN, TC, TOC and TP during 2017 are presented in Table X and the inflow and outflow concentrations of BOD_5, COD, TSS, NH_4-N and TP during the period 1997 - 2016 are presented in Table 6.

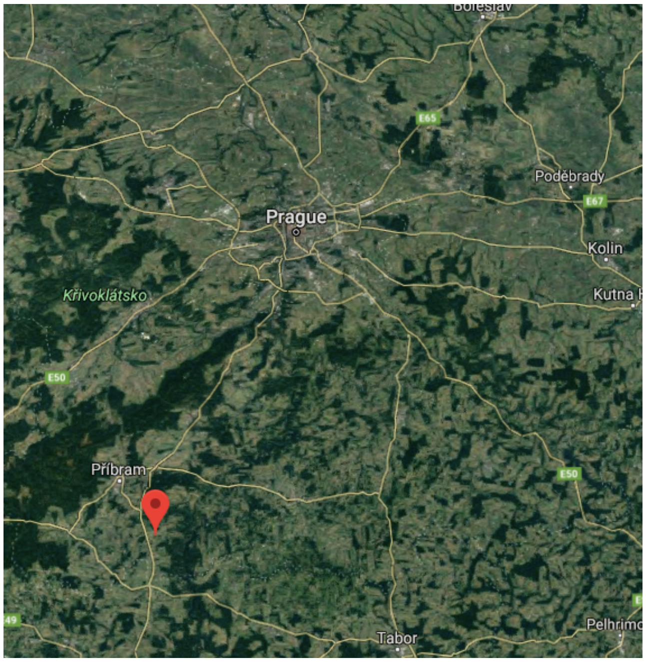
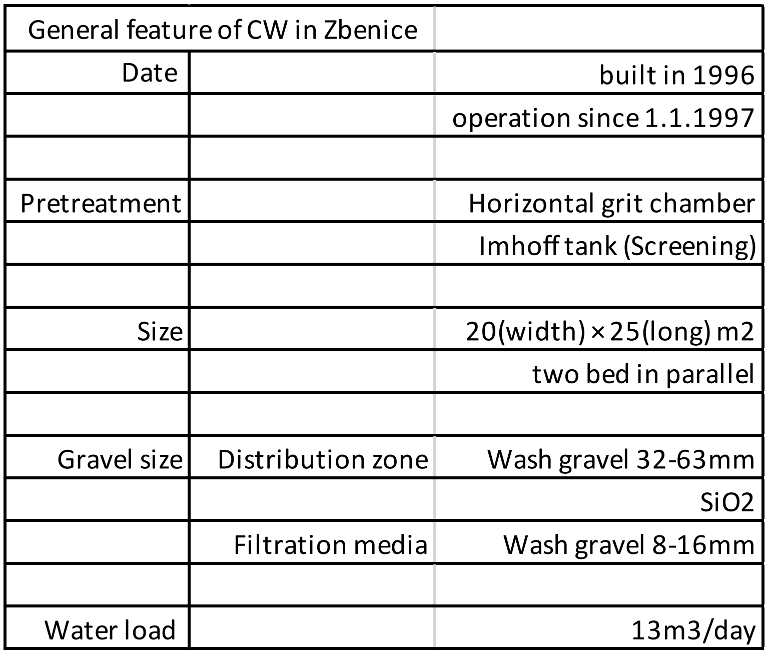


Figure 9 : Location of Zbenice (Red pin showing)

Figure 10 : Outflow of CW adjacent to local fish pond at Zbenice on 23th May 2017.

Figure 11: Overview of CW in Zbenice taken from inflow side on 4th July 2017.

Table 4. General information of CW in Zbenice



General feature of CW in Zbenice		
Date		built in 1996
		operation since 1.1.1997
Pretreatment		Horizontal grit chamber
		Imhoff tank (Screening)
Size		20(width) × 25(long) m2
		two bed in parallel
Gravel size	Distribution zone	Wash gravel 32-63mm
		SiO2
	Filtration media	Wash gravel 8-16mm
Water load		13m3/day

Zbenice	BOD (mg/l)		COD (mg/l)		TSS (mg/l)		N-NH$_4$ (mg/l)		P celk. (mg/l)	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT
1997	101.4	6.9	214.5	19.8	134.0	8.7				
1998	145.8	20.0	345.5	76.3	153.5	23.5	12.84	20.35	2.64	3.13
1999	114.8	24.5	245.5	74.5	115.0	22.5				
2000	136.6	14.6	278.0	32.3	142.5	10.7	15.95	11.96	20.44	3.42
2001	93.3	13.0	197.7	53.0	113.6	12.4	16.01	11.49	6.35	4.53
2002	114.7	7.3	217.7	28.3	241.7	3.0	8.99	5.87	3.04	1.56
2003	132.0	12.0	212.7	38.0	96.7	7.6	38.14	24.36	7.66	7.66
2004	73.2	5.4	145.3	24.0	35.0	6.3	13.81	12.17	4.87	2.66
2005	96.3	9.0	194.3	24.7	85.0	6.0	28.71	15.44	5.39	3.46
2006	269.0	12.7	483.3	41.3	235.3	8.8	31.87	10.93	7.14	5.55
2007	503.3	10.3	978.0	39.0	853.0	5.3	53.01	25.37	11.39	4.25
2008	175.8	16.0	330.0	60.3	221.0	7.3	22.13	18.28	6.13	4.30
2009	164.8	11.5	297.5	38.0	215.0	3.9	5.03	3.12	29.50	20.53
2010	100.8	6.8	235.0	28.8	159.5	5.5	22.90	14.85	4.18	2.56
2011	75.0	4.1	172.5	32.5	111.0	5.1	15.65	10.05	5.45	1.43
2012	122.8	8.6	320.0	38.3	168.5	8.8	33.10	12.43	6.25	3.57
2013	77.5	6.6	214.3	38.5	102.5	7.8	12.78	7.53	7.25	1.13
2014	67.0	5.7	157.0	29.3	35.0	4.5	15.10	13.50	4.20	2.30
2015	83.0	5.0	205.0	26.0	76.0	3.8	28.60	11.00	6.40	3.80
2016	109.0	5.8	255.0	35.0	72.0	2.5	24.30	18.00	6.00	2.50
Total average	137.8	10.3	284.9	38.9	168.3	8.2	22.16	13.71	8.02	4.35
Removal rate		93%		86%		95%		38%		46%

Table 5 : Inflow and outflow concentrations of BOD$_5$, COD, TSS, NH$_4$-N and TP at Zbenice CW during the period 1997-2016.

Table 6: Concentrations of TN, TC, TOC and TP in inlflow and outflow at Zbenice during 2017.

Zbenice	TN (mg/l)		TC (mg/l)		TOC (mg/l)		TP (mg/l)	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT
2017/06/20	35.6	16.1	147.0	96.5	83.8	20.9	7.30	7.30
2017/07/04	26.4	16.2	138.0	72.4	85.4	19.1	6.41	6.35
2017/07/24	48.3	17.2	258.0	100.0	196.0	21.6	7.14	7.35
2017/08/07	52.6	21.9	266.0	107.0	51.4	22.1	6.77	4.27
2017/09/01	16.7	35.1	91.8	81.2	61.5	37.0	8.01	5.61
2017/09/04	52.8	20.3	228.0	62.2	147.0	18.7	8.60	6.28
2017/10/04	21.4	28.9	71.6	108.9	20.6	21.8	1.85	2.00
2017/10/17	46.9	25.8	116.0	88.4	30.1	18.9	3.00	3.10
2017/11/03	30.3	21.6	97.0	59.0	34.0	11.0	1.84	1.78
2017/11/21	34.6	18.7	111.4	63.5	45.6	20.4	11.65	7.75
2017/12/06	20.3	22.0	68.1	73.4	21.3	23.7	5.80	4.80
2017/12/15	24.8	20.2	79.7	72.3	25.1	10.6	6.20	7.90
2017/12/28	20.7	21.7	72.1	72.4	24.3	11.4	7.20	6.20
Total average	33.2	22.0	134.2	81.3	63.5	19.8	6.29	5.44
Removal rate		34%		39%		69%		14%

4.2. Biomass harvesting and analysis

Harvesting of aboveground macrophytes biomass was conducted 4 times during the year of 2017. The time schedule of harvesting is shown in Table 7 . For *P. arundinacea*, four quadrants (0.5 × 0.5 m^2) were samples in four replicates at both inflow and outflow bands (Figure 13). For *P. australis*, four quadrants of the same size were randomly selected at the begging at the end of 13m long band (Figure 12). Aboveground biomass was clipped at the ground level and transported immediately to the laboratory. In the laboratory, 10 shoots of both macrophytes were separated (Figure 13) into stems, leaves including leaf sheaths and inflorescence and the samples were dried in the oven at 60°C until constant weight (Vymazal and Kröpfelová, 2005). All other plant material was cut and dried under the same conditions.

After drying the samples were weighed and recalculated to square meter. The results were expressed in g dry mass m^{-2} (Vymazal and Krőpfelová, 2005). These separated samples were ground through 1 mm mesh using a Fritsch Pulveristte 15 mill (Idar-Oberstein, Germany). Each sample was used for three subsamples which were then analyzed separately (total of 96 samples). Total nitrogen (TN) and total carbon (TC) were analyzed directly by using The Skalar Primacs SNC Analyzer (Breda, the Netherlands). Afterward result (%) were acquired via software. Total Phosphorus was determined by a colorimetric analysis after digestion in nitric-perchloric acids (Sommers and Nelson, 1972; Březinová and Vymazal, 2015). For acid-digestion Digi PREP HT was used. To colorize phosphorus, molybdate-blue method (Vymazal and Kröpfelová, 2008) was used and spectrometer Agilent Technologies Cary60 UV-Vis was used. For nitrogen and phosphorus, NIST 1547 Peach Leaves was use as the standard (National Institute of Standards and Technology, Gaithersburg, MD, USA) (Vymazal, 2016). The statistical analysis was performed by using Turkey HSD test was used (Vymazal and Kröpfelová, 2008).

Table 7. Time schedule of macrophytes harvesting. For *P. arundinacea*, the squares harvested in June were re-harvested in October. On July, the squares were harvested only once during the peak biomass next to the quadrants designed for re-growth harvesting..

	June 20th	July 24th	September 4th	October 4th
Phalaris		xxxx		
Phalaris-regrowth	xxxx			xxxx
Phragmites		xxxx	xxxx	

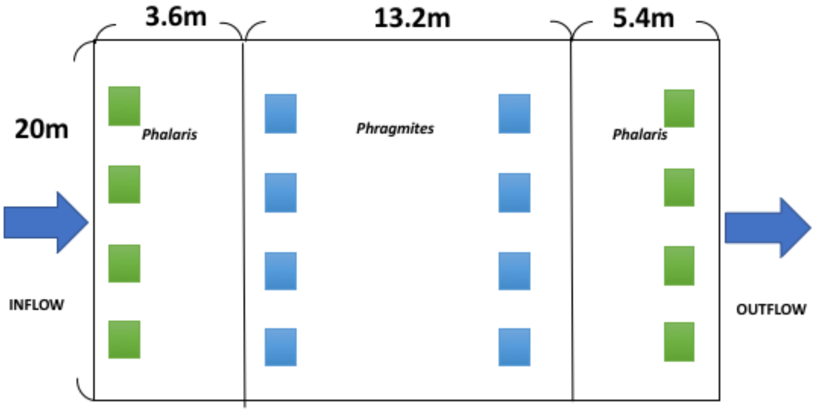


Figure 12 : Schematic layout of macrophytes harvesting during study in 2017

Figure 13: Regrowth of *P. arundinacea* inside the quadrant in the outflow zone at Zbeniceon on October 4th, 2017

Figure 14 : Separation procedure of *P. arundinacea* into leaf, stem and flower.

5.Results

5.1.Aboveground biomass

5.1.1. *Phalaris arundinacea*

Aboveground biomass of *P. arundinacea* was highly variable from minimum of 519 gm^{-2} at outflow plot in June to 1883 gm^{-2} at inflow plot in July (Figure 15). According to t-test ($p < 0.05$), biomass at inflow plot were significantly higher than the outflow plot in each harvest timing except for second harvest in October (Figure 16, 17). Otherwise second harvest conducted in October exhibited relatively small amount of biomass during the study like as 245 gm^{-2} at inflow and 116 gm^{-2} at outflow. The maximum difference of 1248 gm^{-2} between inflow and outflow biomass was measured in July. The amounts of biomass in July and sum in June + October were not significant different. With the exception of inflow plot in June, amount of biomass was in this order like as leaf > stem > flower. When it comes to inflorescence it was not recorded at the outflow plot throughout the season. In July, the aboveground biomass in the inflow was three times higher than in the outflow. Leaf to stem ratio was quite similar in both inflow and outflow in June and July (Table 8) and varied between 1.05 (June. Inflow) and 1,45 (June, outflow). However, the value calculated in October for the *P. arundinacea* regrowth were much higher and varied between 3.04 (inflow) and 3.99 (outflow).

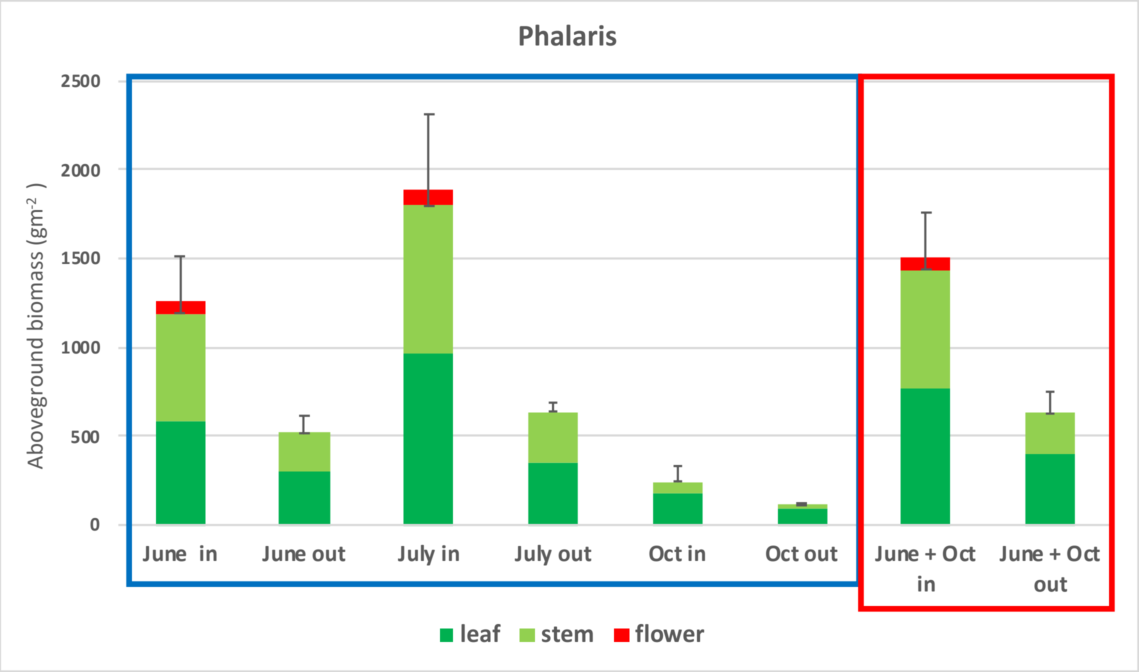
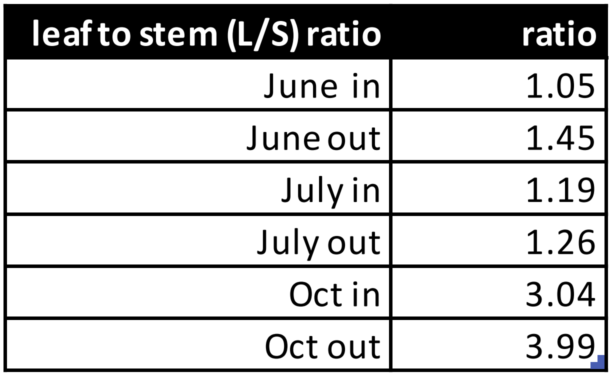


Figure 15 : Aboveground biomass of *Phalaris arundinacea* (gm^{-2}) at constructed wetland Zbenice. In= inflow zone ,out = outflow zone. June, July, Oct = single harvest (blue). June + Oct = double harvest (red).

Figure 16: Outflow (left) and inflow (right) aboveground biomass of *Phalaris arundinacea* at the constructed wetland Zbenice in June 2017. taken by J,Vymazal

Figure 17: Outflow (left) and inflow (right) aboveground biomass of *Phalaris arundinacea* second growth at constructed wetland Zbenice in Ocotober 2017.

Table 8: Leaf to stem ratio for *P. arundinacea* harvested in June, July and October 2017 at inflow (in) and outflow (out) zones of a constructed wetlands at Zbenice.



leaf to stem (L/S) ratio	ratio
June in	1.05
June out	1.45
July in	1.19
July out	1.26
Oct in	3.04
Oct out	3.99

5.1.2. *Phragmites australis*

The amount of *P. australis* biomass was minimum at outflow in June (1290 gm^{-2}) and maximum at outflow plot in September (3943 gm^{-2}). According to t-test ($p > 0.05$), there was no significant difference between biomass at inflow and outflow plots in both harvesting periods in June and September (Figure 18, 19). On the other hand, there was a significant difference between inflow and outflow biomass in June and September. In general, the amount of biomass at inflow plot was higher than that at the outflow, otherwise difference of biomass between inflow and outflow were less than the difference found for *P. arundinacea.* With the exception of the outflow plot in September, amount of biomass was found in the order of leaf > stem > flower. The leaf to stem ratio (Table 9) was ranged from 0.90 to 1.48 with the lower values found in September.

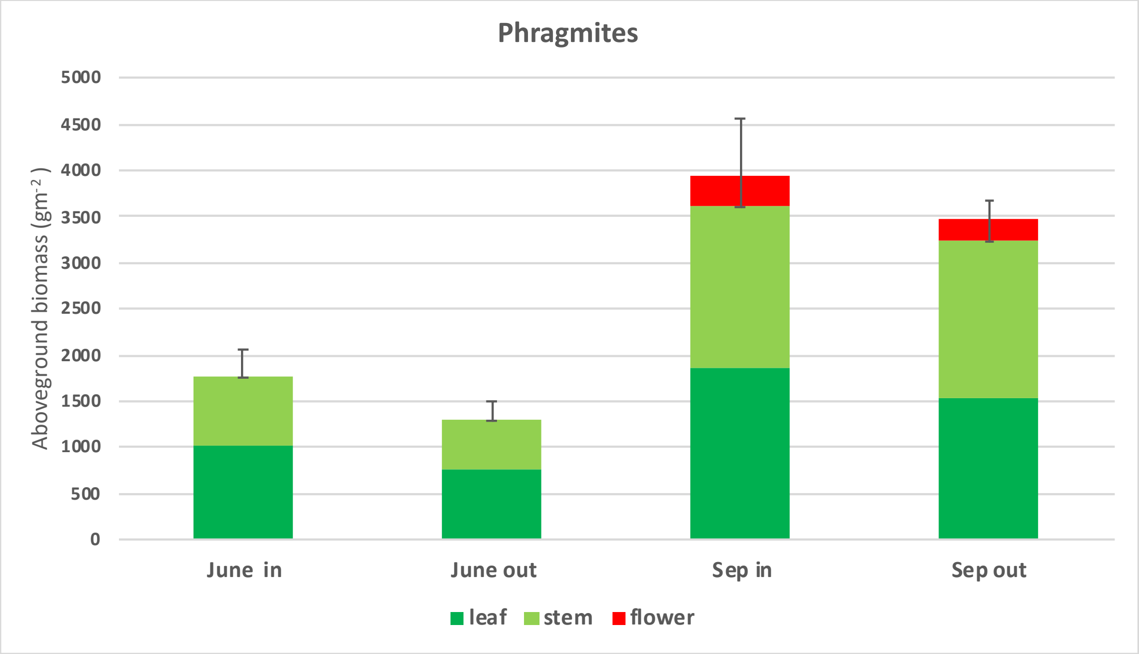


Figure 18: Aboveground biomass of *Phragmites australis* (gm^{-2}) at constructed wetland in Zbenice. In= inflow, out= outflow.

Fig. 19. Inflow (left) and outflow (right) aboveground biomass of *Phragmites australis* harvested in June 2017 at constructed wetland Zbenice. Taken by J.Vymazal.

Table 9: Leaf to stem ratio for *P. arundinacea* harvested in June, July and October 2017 at inflow (in) and outflow (out) zones of a constructed wetlands at Zbenice.

leaf to stem (L/S) ratio	ratio
June in	1.39
June out	1.48
Sep in	1.08
Sep out	0.90

5.2 Concentration

5.2.1. *Phalaris arundinacea*

The concentration of phosphorus in various parts of P. *arundinacea* is presented in Table 10. The concentration decreased in the order of leaf ≥ flower > stem. The highest phosphorus concentrations were found in leaves in October in re-growing plants (3106 mg kg^{-1} at the inflow and 3123 mg kg^{-1} at the outflow). However, this is not surprising as the highest concentration of nutrients are usually found in the newly growing shoots. The Difference in phosphorus concentration between inflow and outflow was not significant but stem parts of the outflow showed higher concentration than the one at the inflow.

Table 10: Phosphorus concentration in various parts of *Phalaris arundinacea* (mg/kg ; Standard deviation) at constructed wetland Zbenice.

Phosphorus concentration mg/kg	leaves	stem	flower
June in	2680(±221)	1695(±166)	2417(±155)
June out	2682(±264)	1952(±157)	NA
July in	2657(±134)	1333(±192)	1425(±220)
July out	2673(±216)	1888(±142)	NA
Oct in	3106(±249)	1659(±294)	NA
Oct out	3123(±216)	2206(±135)	NA

The result presented in Table 11 indicate much higher concentration of TN in the leaves as compared to stem and flowers. Similarly to phosphorus, the highest concentration of nitrogen were found in October in leaves for newly growing shoots in the plots harvested in June.

Table 11: Nitrogen concentration in various parts of *Phalaris arundinacea* (% ; standard deviation) at constructed wetland Zbenice.

TN concentration (%)	leaf	stem	flower
June in	2.96(±0.33)	1.14(±0.09)	1.88(±0.24)
June out	2.1(±0.15)	0.71(±0.05)	NA
July in	2.75(±0.27)	0.74(±0.15)	0.89(±0.11)
July out	2.28(±0.28)	0.38(±0.08)	NA
Oct in	3.37(±0.26)	0.86(±0.17)	NA
Oct out	2.61(±0.26)	0.59(±0.22)	NA

5.2.2. *Phragmites australis*

The phosphorus concentration in various parts of *P. australis* are shown in Table 12. The phosphorus concentration decreased in the order of flower ≥ leaf > stem. Furthermore, highest concentration was found in flower part in September with the concentration of 2405 mg kg^{-1} and 2569 mg kg^{-1} at the inflow and outflow, respectively. In June there was no significant difference between the phosphorus concentration in leaves and stem, otherwise in September this difference was significant and concentration of leaves was much higher than stem.

Table 12: Phosphorus concentration in various parts of Phragmites *australis* (mg/kg; Standard deviation) at constructed wetland Zbenice.

Phosphorus concentration	leaves	stem	flower
June in	2067(±150)	1855(±140)	NA
June out	2120(±122)	2176(±220)	NA
Sep in	1913(±226)	1152(±126)	2408(±284)
Sep out	1659(±164)	1070(±143)	2569(±203)

The TN concentration in *P. australis* is shown in Table 13. The concentration were substantially higher in leaves than in stems in June as well as in September. The highest concentration was found in June in the leaves (3.3 % at inflow and 3.23 % at outflow). Difference between concentration at inflow and outflow was not significant.

Table 13: Nitrogen concentration in various parts of *Phragmites australis* (% ; standard deviation) at constructed wetland Zbenice

TN concentration (%)	leaf	stem	flower
June in	3.3(±0.21)	1.76(±0.14)	NA
June out	3.23(±0.18)	1.86(±0.11)	NA
Sep in	2.4(±0.07)	0.54(±0.11)	2.4(±0.30)
Sep out	2.53(±0.19)	0.57(±0.13)	2.45(±0.12)

5.3. Standing stock

5.3.1. *Phalaris arundinacea*

The total standing stock of *P. arundinacea* was variable from minimum of 0.10 gm^{-2} at the outflow plot in October to maximum of 3.73 gm^{-2} at inflow plot in July (Figure 20). Second harvest in October were shown relatively low standing stock that were 0.65 m^{-2} at the inflow and 0.34 gm^{-2} at the outflow, that were mainly consisted by leaf parts. According to t-test ($p < 0.05$), there was significant difference between standing stock of inflow and outflow. But between sum of June, October and single harvest in July didn't have any significant difference. Generally standing stock in inflow plot was higher than outflow plot. Standing stock decreased in the order of leaf > stem > flower. For the amount of phosphorus in July and sum in June, October there was no significant difference.

The range of total TN standing stock of *P. australis* was between 2.5 gm^{-2} at outflow plot in October to maximum of 33.0 gm^{-2} at inflow plot in July closely 32.1 gm^{-2} that is sum of inflow in June and October as well (Figure 21). The smallest standing stock at the outflow in October was almost consisted by leaf part 2.4 gm^{-2} against 0.1 gm^{-2} of stem. According to t-test ($p < 0.05$), there were significant difference between standing stock of inflow and outflow. Highest difference was 26.3 gm^{-2} and standing stock of leaf is nearly 4 times higher than stem in the maximum growing period of July. Standing stock decreased in the order of leaf > stem > flower. The standing stock of TN at inflow in July and sum in June, October were no significant by t-test, however regarding on outflow sum was slightly higher than the standing stock at outflow in July.

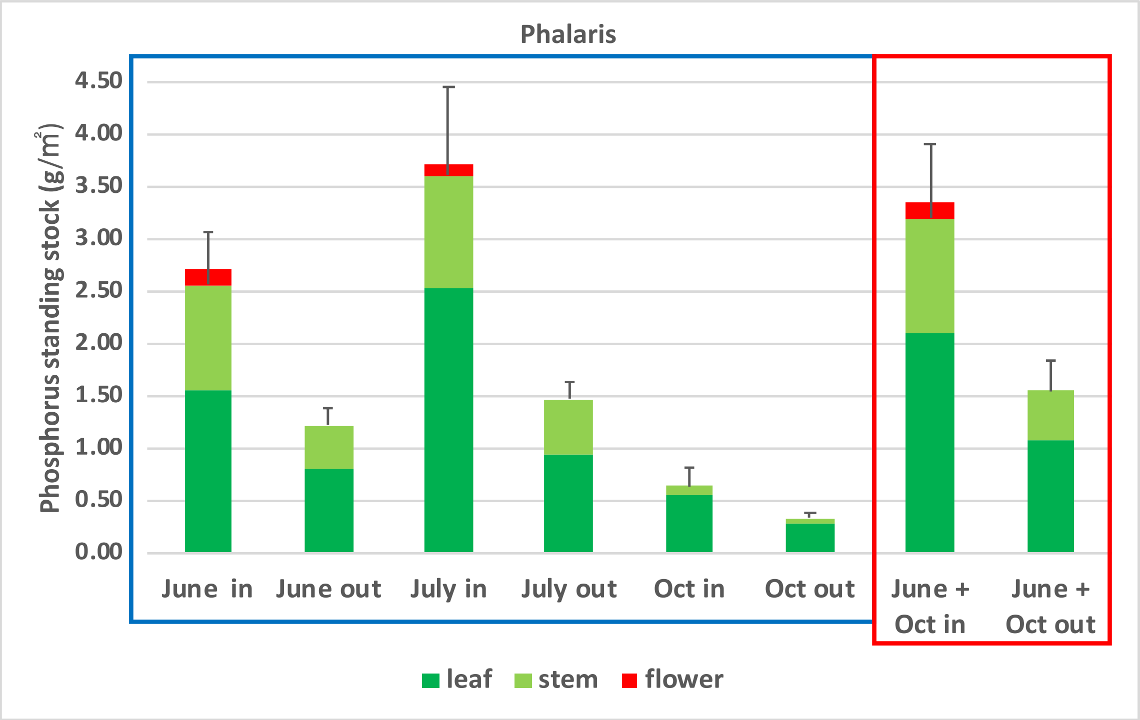


Figure 20: Phosphorus standing stock (gm^{-2}) in aboveground biomass of *Phalaris arundinacea* at constructed wetland Zbenice. In = inflow zone, out = outflow zone, June, July, Oct = single harvest (blue). June + Oct = double harvest (red).

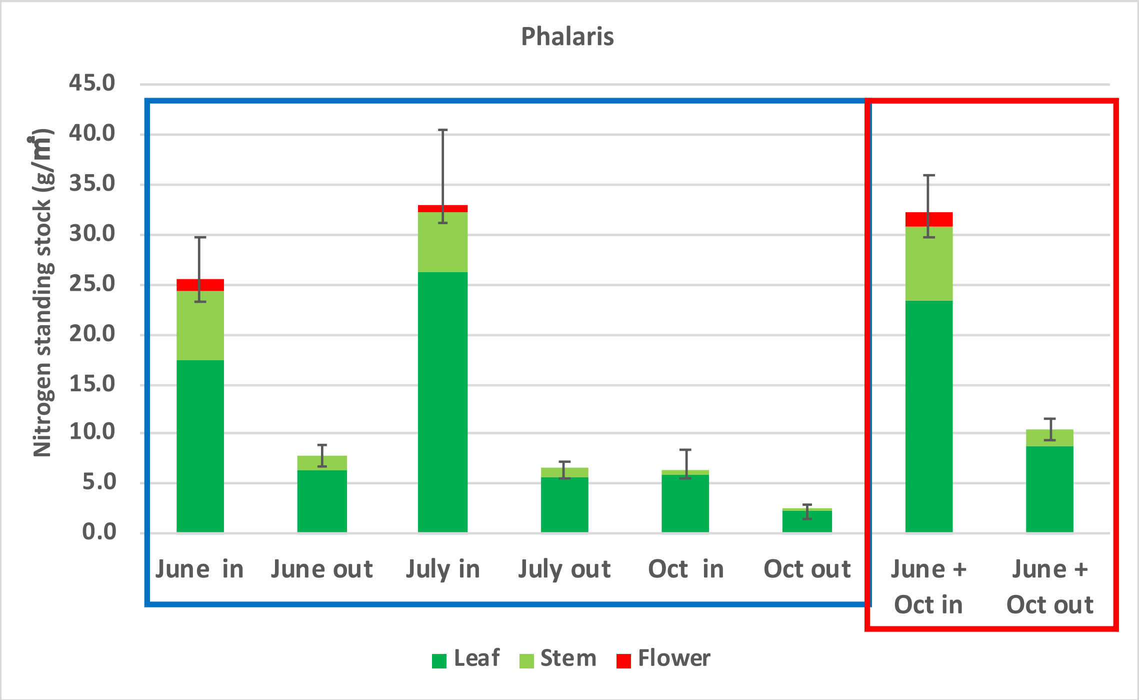


Figure 21: Total nitrogen (TN) standing stock (gm^{-2}) in aboveground biomass of *Phalaris arundinacea* at constructed wetland Zbenice. In = inflow zone, out = outflow zone, June, July, Oct = single harvest (blue). June + Oct = double harvest (red).

5.2.2. *Phragmites australis*

For *P. australis* (Figure 22), the range of total standing stock was between 2.78 gm^{-2} at the outflow plot in June and 6.34 gm^{-2} at inflow plot in September. There were no significant difference between phosphorus standing stock of inflow and outflow at each harvest periods with the result of t-test ($p > 0.05$). However Standing stock at inflow plot for both harvest were higher than outflow in both periods and difference was relatively smaller than the one of *Phalaris.* As same as *P, arundinacea*, standing stock decreased in the order of leaf > stem > flower.

The nitrogen standing stock was minimum 34.4 gm^{-2} at the outflow plot in June to maximum 62.3 gm^{-2} at inflow plot in September (Figure 23). There was no significant difference between standing stock of inflow and outflow in June and September according to the result of t-test ($p > 0.05$). However standing stock at inflow plot for both harvest were higher than outflow ones. As same as *P. arundinacea*, Amount of standing stock is such order like as leaf > stem > flower. The highest part of nitrogen standing stock was found in leaves.

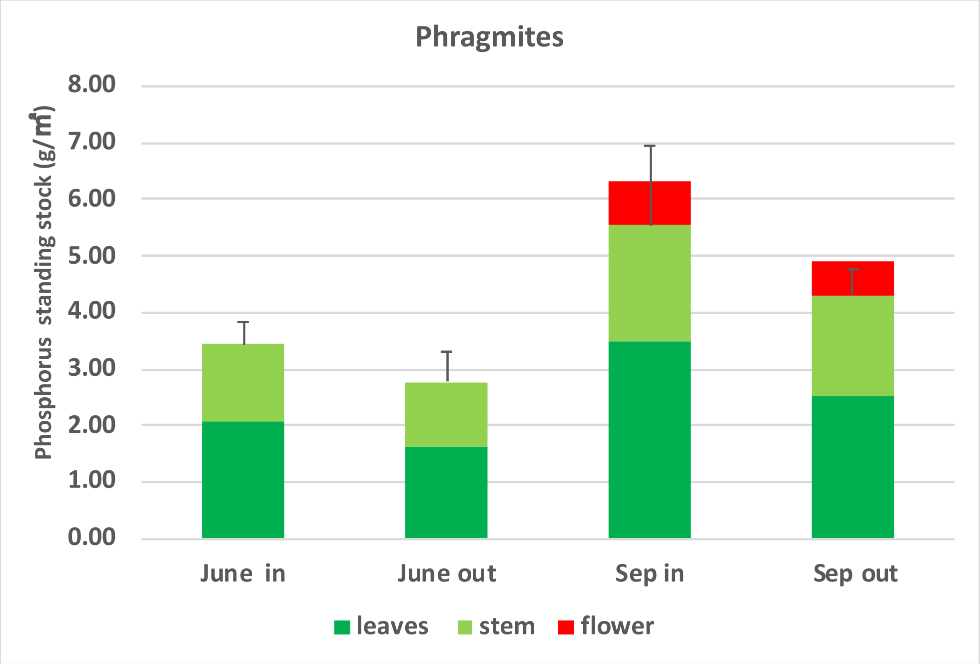


Figure 22 : Phosphorus standing stock (gm^{-2}) in aboveground biomass of *Phragmites australis* at constructed wetland Zbenice,

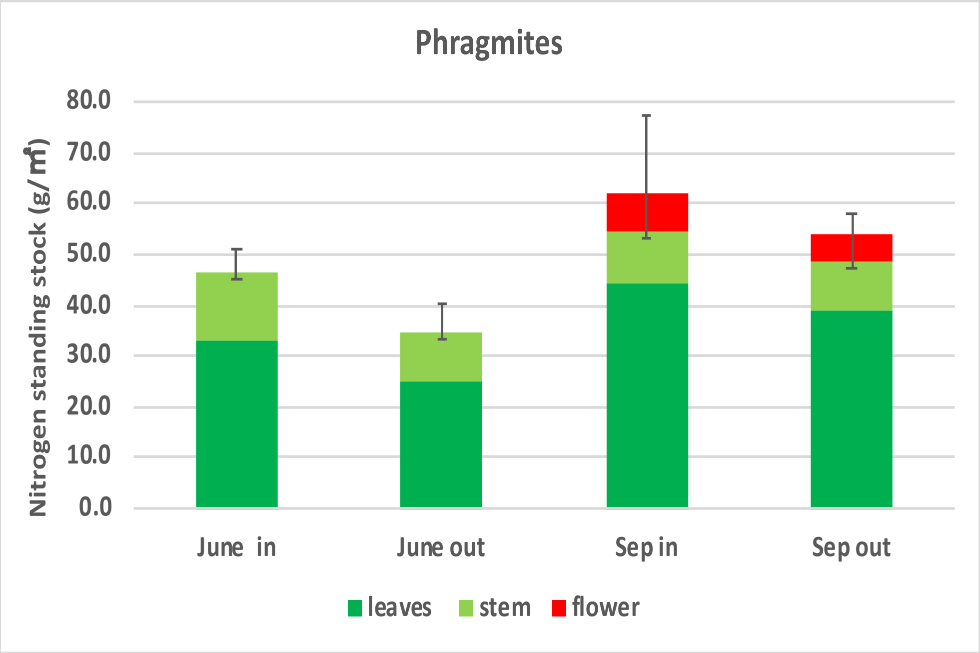


Figure 23: Total nitrogen (TN) standing stock (gm^{-2}) in aboveground biomass of *Phragmites australis* at constructed wetland Zbenice,

6.Discussion

6.1. Aboveground biomass

6.1.1. Case of *Phalaris arundinacea*

The aboveground biomass varied from 519 gm^{-2} to 1883 gm^{-2} and average amount was 777 gm^{-2} (± 588 gm^{-2}) in 2017. Vymazal and Krőpfelová, (2005) reported that aboveground biomass of *P. arundinacea* varied between 345 gm^{-2} at Cista (other CW in Czech Republic) and 1902 gm^{-2} at Zbenice with an average value of 1286 gm^{-2} (± 477 gm^{-2}). Therefore the figure on this study in Zbenice wasn't outside the range of aboveground biomass of *P. arundinacea* since table is showing figure in 2017 was middle with comparison of other case studies (Table 13). Regarding on factors of difference between inflow and outflow, Green and Galatowitsch, (2001); Maurer and Zedler, (2002); Březinová and Vymazal, (2015) mentioned that high nutrient concentration increased biomass of *P. arundinacea* and increased allocation to aboveground growth. In the results, biomass of leaf was higher than stem part, however in 2 papers (Vymazal and Kröpfelová, 2008) and (Vymazal and Krőpfelová, 2005), the stem biomass of *P. arundinacea* was generally higher than that of leaves.

Table 13: Maximum aboveground biomass of *P. arundinacea*, comparison with previous studies in the Czech Republic and abroad.

Reference	Biomass (g/m^2)	Locality
Vymazal and Krőpfelová, (2005)	345	HSSF CW Čistá, Czech Republic
Vymazal et al., (1999)	507	HSSF CW, inflow Chmelná, Czech Republic
Vymazal et al., (1999)	731	HSSF CWs (mean) Czech Republic, 2
Edwards et al., (2006) September, 2002	897	HSSF CW, outflow Ostrolovský Újezd, Czech Republic
Březinová and Vymazal, (2015)	1727	HSSF CW Čičenice, Czech Republic
Study in 2017, inflow in July	1883	HSSF CW Zbenice, Czech Republic
Vymazal and Krőpfelová, (2005) 2003	1902	HSSF CW Zbenice, Czech Republic,
Vymazal and Kröpfelová, (2008) 2005	2265	HSSF CW Břehov,Czech Republic
Behrends et al., (1994)	831	HSSF CW Alabama, USA
Březinová and Vymazal, (2015)	1226	Minnesota, USA, stands treated with wastewater
Bernard and Lauve, (1995)	1713	New York, USA, HSF CW, landfill leachate
Hurry and Bellinger, (1990)	2458	England, overland flow wetland
Rodriguez and Brisson (2016),	2707	Quebec, Canada

2012 fall		HSSF, fish warm sludge

6.1.2. Harvesting effect

There was an expectation that the biomass gained by double harvesting should overwhelm the single harvest in July in this study. However, according to result there were no significant difference between single and repeated harvests, and even single harvest in July was slightly higher than double harvest of June and October. Vymazal et al., (2010) also observed that single harvest in growing period showed slightly higher biomass than the sum of first and second harvest. Otherwise Hurry and Bellinger, (1990) mentioned about CW in England acquired 2458 gm^{-2} by multiple harvest. Březinová & Vymazal, 2015) got the conclusion that harvest at inflow and outflow should be conducted on different periods, since in CW in Mořina, Czech Republic the peak above ground biomass in the inflow occurred in June while in outflow occurred in August. This different timing of harvest between inflow and outflow could be utilized for optimizing harvesting aboveground biomass (Figure 24). From the point of study in 2017, according to the other CW data of aboveground biomass from Czech Republic (Březinová and Vymazal, 2015), for optimizing amount of biomass multi harvest should initiate on around July at inflow and on after September at outflow. The study by Kolodziej et al., (2016) conducted in Poland introduced different amount of swage sludge into *P. arundinacea* field. However in this paper has been shown the highest aboveground biomass occurred during the with second harvests (late May and October). Therefore early harvest in spring and late harvest in autumn may optimize efficiency of CW.

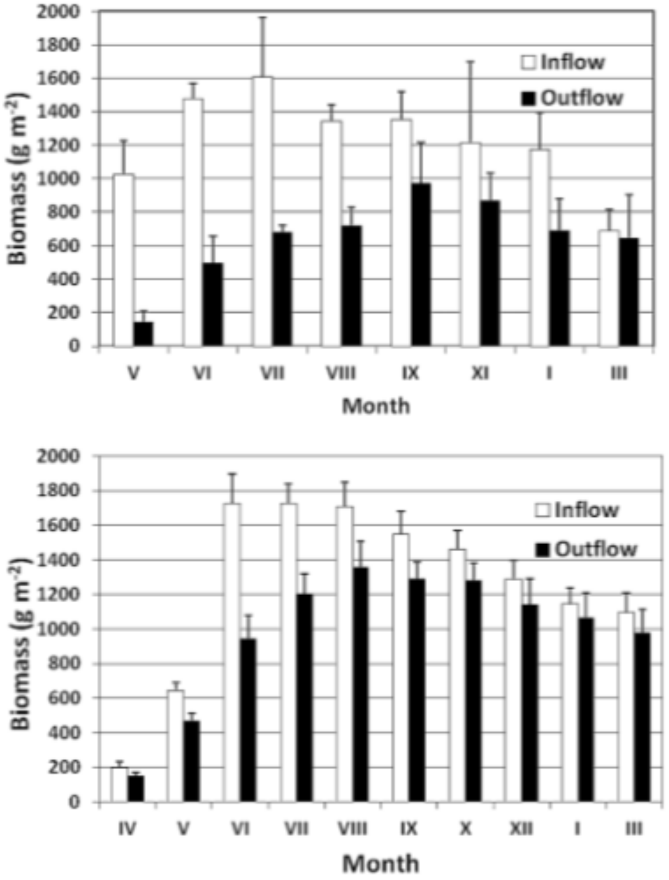


Figure 24: Aboveground biomass of *P. arundinacea* development at CW Cicenice (top) and CW Morina (bottom). Bars represent standard deviations, n = 3. (Březinová and Vymazal, 2015)

6.1.3 Case of *Phragmites australis*

The aboveground biomass was variable from 1290 gm^{-2} to 3943 gm^{-2} and average amount was 2615 gm^{-2} (± 1114 gm^{-2}) in 2017. Vymazal and Krőpfelová (2005) reported that aboveground biomass of *P. australis* varied between 1652 gm^{-2} at Spálené Poříčí (other CW in Czech Republic) and 5070gm^{-2} at Nezdice with an average value of 3266 gm^{-2} (± 1050 gm^{-2}). There are comparison aboveground biomass between other CW and Zbenice in 2017 (Table 14) and study in 2017 was in middle of the range. Compared with difference between inflow and outflow of *P. arundinacea*, that of *P. australis* is not significant. Toet et al., (2005) mentioned that aboveground biomass of *P. australis* was not affected by nutrients supply rate from discharge water flowing CW and growth will not be limited by nutrients load even at low loading rate. So in this study in 2017 also, *P. australis* should not have been significantly affected by the position inside the constructed wetland.

Table 14: Aboveground biomass of *P. australis*, comparison with previous studies in the Czech Republic and abroad.

	Biomass (g/m^2)	Locality
Vymazal and Krőpfelová, (2005) 2003	1652	HSSF CW Spálené Poříčí Czech Republic
Vymazal and Krőpfelová, (2005)	1286	Average aboveground biomass in 13 HSSF CW in Czech Republic.
Vymazal et al., (1999)	2088	HSSF CW (mean) Czech Republic, 5
Vymazal and Kröpfelová, (2008)	2532	HSSF CW, 2005 Břehov,Czech Republic,
Study in 2017, inflow in September	3943	HSSF CW Zbenice, Czech Republic
Vymazal and Krőpfelová, (2005)	5266	HSSF CW, 2003 Nezdice, Czech Republic
Adcock and Ganf, (1994)	788	HSSF CW, Austria
Gries and Garbe, (1989)	1360	HSSF CW, Germany
Obarska-Pempkowiak and Ozimek, (2003)	2353	FWS CW, Storm water runoff, Poland
Toet et al, (2005)	2850	FWS CW, Netherlands
Rodriguez and Brisson (2016), 2012 fall	2942	HSSF, fish Farm sludge Quebec, Canada
Haberl and Perfler, (1990)	3100	HSSF CW, Austria
Behrends et al., (1994)	4046	Mesocosm HSSF CW Alabama, USA

Barbera et al., (2009)	4701	HSSF CW, Sisily, San Michele di Ganzaria
Wrigley and Toeriwn, (1988)	6334	Small-scale model, HSSF CW South Africa,

6.1.4. Harvesting effect

In this study, second harvest of *P. australis* was not possible (figure 25). These squares were surrounded by well grown other *P. australis* and the condition for sprouting was not suitable to regrow after first harvest because of lack of light and spatial capability. Suggestion for success of regrowth, it should be made wider square (2 x 2 m^2) and conducted harvest inside the square at the same time for preventing of surrounded effects.

Figure 25: The square without any sprouting of *P. australis* on 4th October 2017 in Zbenice.

6.2. standing stock

1.1. *Phalaris arundinacea*

1.1.1 Phosphorus

Phosphorus standing stock varied from 0.34 gm^{-2} to 3.73 gm^{-2} and average amount was 1.88 gm^{-2} (± 1.17 gm^{-2}) in 2017. Vymazal and Kröpfelová, (2008) got standing stock from 1.93 gm^{-2} to 4.05 gm^{-2} in HSSF CW Břehov in Czech Republic during two years experiments. In the literature, phosphorus standing stock in *P. arundinacea* growing in treatment wetlands has been reported between 0.4 and 10.5 gm^{-2} (Braxton 1981; Hurry and Bellinger, 1990; Behrend et al., 1994; Vymazal and Kröpfelová, 2008). Compared with other studies, the phosphorus standing stock is in the middle of range (Table 15) Kröpfelová and Vymazal, (2008) have shown that the double harvest (15th June + 12th August) exhibited standing stock of 5.44 g P m^2 and single harvest (27th July) was 3.51 g P m^2, this result exhibited the possibility of optimizing phosphorus standing stock by multiple harvesting. However, from the result of higher phosphorus concentration in newly growing shoots and first harvest sound effective to conduct on early spring that is sprouting season.

Table 15: Aboveground phosphorus standing stock (gm^{-2}) of *P. arundinacea* in constructed wetland, comparison with previous studies in the Czech Republic and abroad.

Reference	Phosphorus standing stock (gm^{-2})	Locality
Vymazal et al., (1999)	1.8	Maximum from HSSF CWs in Kolodeje, Ondrejov and Čičenice.
Vymazal and Kröpfelová, (2008) July, 2004	1.93	HSSF CW Břehov, Czech Republic,

Vymazal and Krőpfelová, (2005) 27th July,2003	3.51	HSSF CW Spálené Poříčí Czech Republic, Single harvest
Březinová and Vymazal, (2015), June, 2011	3.7	HSSF CW, inflow, Čičenice, Czech Repulic
Study in 2017, inflow in July	3.73	HSSF CW Zbenice, Czech Republic
(Vymazal & Kröpfelová, 2008) July, 2006	4.05	HSSF CWs Břehov, Czech Republic,
Vymazal, (1995)	4.8	HSSF CW Spálené Poříčí Czech Republic,
Březinová and Vymazal, (2015), June, 2011	5.13	HSSF CW inflow, Mořina, Czech Republic
Vymazal and Krőpfelová, (2005) Double harvest, 15th June + 12th August	5.44	HSSF CW Zbenice, Czech Republic
Behrends et al., (1994)	1.66	HSSF CW, USA
Hurry and Belinger, (1990)	10.5	CW, North Yorkshire
Hurry & Bellinger, (1990) Multiple harvest	10.9	FWS CW, UK
Rodriguez and Brisson, (2016), 2012 fall	13.5	HSSF CW, fish farm sludge Quebec, Canada
Bernard and Laube, (1995)	32.5	HSSF CW, landfill leachate New York, USA

Total removal of phosphorus inflow load through aboveground biomass aboveground biomass standing stock was calculated as the amount of phosphorus sequestered in the aboveground biomass using the area vegetated by particular plant species. In the case of *P. arundinacea*, the surface area overgrown with this plant was 180 m^2. The total phosphorus amount in the aboveground biomass amounted to was 4044 g yr^{-1} by the difference of total phosphorus between the discharge of inflow (29846 g yr^{-1}) and outflow (25801 g yr^{-1}). Removal standing stock was variable between 83.4 g P in October and 428.7 g P in July (Table 16). The amount of phosphorus standing stock as percentage of annual inflow load varied between 0.28 % for re-harvested biomass in October and 1.44 % in July. If removed amount of phosphorus is taken into consideration, the values vary between 2.1 % and 10.6 % in October and July, respectively. The study conducted by Březinová and Vymazal, (2015) amount of phosphorus standing stock taken into aboveground biomass was ranged between 379 and 540 g P (for comparison converting into 180 m^2 that is same scale as Zbenice). To achieve the maximum phosphorus removal via harvesting, the flow should be harvested in July while outflow in September.

Table 16: *P. arundinacea* Phosphorus standing stock of annual removal (g P yr^{-1}) and removal rate (%) from inflow load and removal amount (inflow load – outflow load).

Phosphorus annual removal (g P)	Inflow	outflow	Total	from inflow	from removal amount
June	196.1	131.6	327.7	1.10%	8.1%
July	269	159.7	428.7	1.44%	10.6%
Oct	46.6	36.8	83.4	0.28%	2.1%
June + Oct	242.7	168.4	411.1	1.38%	10.2%

6.2.3. Nitrogen

Nitrogen standing stock was varied from 2,5 gm^{-2} to 33.0 gm^{-2} and average amount was 15.6 gm^{-2} (± 11.7 gm^{-2}) in 2017. Vymazal and Kröpfelová, (2008) observed nitrogen standing stock from 18.9 gm^{-2} to 32.3 gm^{-2} in Břehov CW in Czech Republic during 2 years, also defined standing stock in *P. arundinacea* growing in treatment wetlands varies between 3.7 and 47.7 gm^{-2} (Vymazal et al., 1999; Kröpfelová et al., 2008). This study in 2017 could not exhibit higher standing stock in double harvest than single and maximum acquired standing stock showed in middle range compared with other studies (Table 17). Kröpfelová and Vymazal, (2008) have shown that double harvest (15th June + 12th August) exhibited 44.2 g N m^2 and single (27th July) was 31.5 g N m^2. This result suggested the possibility for optimizing nitrogen standing stock by multiple harvesting of *P. arundinacea*.

Table 17: Aboveground Nitrogen standing stock of *P. arundinacea* in constructed wetland, comparison with previous studies in the Czech Republic and abroad.

Reference	Nitrogen standing stock (gm^{-2})	Locality
Vymazal et al., (1999)	12.9	Maximum from HSSF CWs Kolodeje, Ondrejov and Čičenice.
Vymazal and Kröpfelová, (2008) July, 2004	18.9	HSSF CW Břehov, Czech Republic,
Vymazal and Krőpfelová, (2005) Single harvest 27th July, 2005	31.5	HSSF CW Břehov, Czech Republic,
Study in 2017, inflow in July	33.0	HSSF CW Zbenice, Czech Republic
Vymazal and Kröpfelová, (2008) July, 2005	32.3	HSSF CW Břehov, Czech Republic,
Vymazal, (1995)	32.7	HSSF CW Spálené Poříčí Czech Republic,
Březinová and Vymazal, 2(015) June, 2011	41.3	HSSF CW inflow Čičenice, Czech Republic
Vymazal and Krőpfelová,(2005) 15th June + 12th August,2005	44.2	HSSF CW Double harvest, Břehov, Czech Republic
Behrends et al., (1994)	13.3	HSSF CW, Alabama USA
Bernard and Laube, (1995)	16.2	HSSF CW, landfill leachate New York, USA
Hurry and Belinger, (1990)	46.7	CW North Yorkshire
Rodriguez and Brisson, (2016) 2012 fall	98.0	HSSF, fish farm sludge Quebec, Canada

Total removal nitrogen standing stock CW from effluent was 53108 g yr^{-1} by the difference of total phosphorus between the discharge of inflow (157406 g yr^{-1}) and outflow (104299 g yr^{-1}). Total standing stock from whole above ground biomass was calculated with actual surface area (180 m^2) in Table. Removal of standing stock was variable between 740.7 g P in October and 3430 g P of sum between June and October. The amount of nitrogen standing stock as percentage of annual inflow load varied between 0.47% for re-harvested biomass in October and 2.18 % for sum harvest of June and October. If removed amount of nitrogen is taken into consideration, these value vary between 1.39 % for in re-harvested biomass in October and 6.46 % for sum harvest of June and October (Table 18). In this case, nitrogen standing stock removal ratio by double harvest was higher than single harvest. Suzuki et al., (1989); Vymazal and Krőpfelová, (2005) mentioned double harvest enhance the N removal by 22% to 34 %.

Table 18: Nitrogen standing stock of annual removal (g Nyr^{-1}) and removal rate (%) from inflow load and removal amount (inflow load – outflow load).

Nitrogen annual removal (g N)	Inflow	outflow	Total	from inflow	from removal
June	1842.5	847.6	2690.1	1.71%	5.07%
July	2372.7	721.8	3094.4	1.97%	5.83%
Oct	467.7	273.0	740.7	0.47%	1.39%
June + Oct	2310.2	1120.6	3430.8	2.18%	6.46%

6. 3. *Phragmites australis*

6.3.1.6 Phosphorus

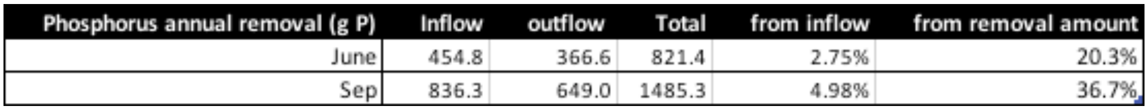
As a results, phosphorus standing stock of *P. australis* varied from 3.45 gm^{-2} to 6.34 gm^{-2} and average amount was 4.37 gm^{-2} (± 1.37 gm^{-2}) in 2017. Vymazal and Kröpfelová, (2008) got standing stock from 1.9 gm^{-2} to 5.16 gm^{-2} in Břehov CW in Czech Republic during 2 years, also general phosphorus standing stock in *P. australis* growing in treatment wetlands varies between 0.56 gm^{-2} and 5.0 gm^{-2} (Kröpfelová and Vymazal, 2008).Table 19 is shown the comparison between the result in 2017 and other studies. Concentration of phosphorus in the biomass in June was higher than that in September and concentration in the biomass growing closer to the outflow in June was higher than that of inflow. It should have caused because of different peak of growing season and shoots in outflow was sprouted later than inflow shoots sprouting. Because newly grown shoots are containing relatively high concentration in biomass. Dykyjová and Kvet (1978); Vymazal et al., (1999) mentioned that maximum biomass and maximum nutrient concentration in the biomass do not occur at the same time of growing season. In contrast of biomass, the lower nutrient availability to CW will reduce nutrient uptake by plants resulting in lower concentration in shoots (Toet et al., 2005)

Table 19: Aboveground phosphorus standing stock (gm^{-2}) of *P. australis* in constructed wetland, comparison with previous studies in the Czech Republic and abroad.

Reference	Phosphorus standing stock (gm^{-2})	Locality
Vymazal et al., (1999)	4.9	Maximum from HSSF CW in Koloděje, Ondřejov and Čičenice.
Vymazal and Kröpfelová, (2008), July, 2006	5.16	HSSF CW Břehov, Czech Republic,
Study in 2017, inflow in July	6.34	HSSF CW Zbenice, Czech Republic
Vymazal, (1995)	9.8	HSSF CW Spálené Poříčí Czech Republic,
Peverly et al.,(1993)	0.56	HSSF CW,USA,
Adcock and Ganf, (1994)	1.42	HSSF CW, Australia,
Gries and Garbe, (1989)	1.63	HSSF CW, Germany
de jong, (1976)	3.5	FWS CW, Netherlands
Behrends et al., (1994)	4.05	Mesocosm HSSF CW Alabama, US
Wrigley and Toerien, (1988)	7.6	HSSF CW, Small-scale model, South Africa
Rodriguez and Brisson, (2016) 2012 fall	7.8	HSSF CW, fish farm sludge Quebec, Canada
Haberl and Pefler, (1990)	13.0	HSSF CW, Mannersdorf Austria

Table 20 is shown that total phosphorus standing stock from all *P. australis* in CW was variable between 821.4 g P yr^{-1} in June and 1485.3 g P yr^{-1} in September that was calculated with 264 m^2 of *P .australis* vegetation bed (inflow and outflow are adjacent each other). Removal ratio form phosphorus in inflow ranged from 2.75 in June to 4.98 % in September and removal ratio from removed load ranged between 20.3 % in June and 36.7 % in September. As compared the phosphorus removal percentage from the inflow and from the removed load, *P. australis* exhibited much higher values than *P. arundinacea.*

Table 20: Phosphorus standing stock of annual removal (g P yr^{-1}) and removal rate (%) from inflow load and removal amount (inflow load – outflow load).



Phosphorus annual removal (g P)	Inflow	outflow	Total	from inflow	from removal amount
June	454.8	366.6	821.4	2.75%	20.3%
Sep	836.3	649.0	1485.3	4.98%	36.7%

6.3.2 Nitrogen

As a results, nitrogen standing stock of *P. australis* was varied from 34.4 gm^{-2} to 62.3 gm^{-2} and average amount was 49.3gm^{-2} (± 10.3 gm^{-2}) in 2017. Vymazal and Kröpfelová, (2008) got standing stock from 18.4 gm^{-2} to 48.2 gm^{-2} in Břehov CW in Czech Republic during 2 years, also general phosphorus standing stock in *P. australis* growing in treatment wetlands varies between 8.5 gm^{-2} and 84 gm^{-2} (Kröpfelová and Vymazal, 2008). Similar to phosphorus concentration in June was higher than that in September and concentration of outflow in June was higher than that of inflow. The table 21 is shown that comparison between nutrients from the study in 2017 and previous studies.

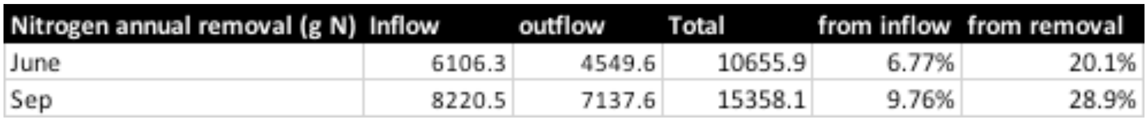
Table 21: Aboveground nitrogen standing stock (gm^{-2}) of *P. australis* in constructed wetland, comparison with previous studies in Czech Republic and abroad.

Reference	Nitrogen standing stock (gm^{-2})	Locality

Jan Vymazal et al., (1999)	42.7	Maximum from HSSF CWs in Koloděje, Ondřejov and Čičenice.
Study in 2017, inflow in July	62.3	HSSF CW Zbenice, Czech Republic
Vymazal and Kröpfelová, (2008), July, 2006	65	Břehov, Czech Republic, HSSF CWs
Vymazal, (1995b)	84.0	HSSF CW, Spálené Poříčí Czech Republic,
Adcock and Ganf, (1994)	18.1	HSSF CW, Australia
Gries and Garbe, (1989)	23.1	HSSF CW, Germany
Peverly et al.,(1993)	26.9	HSSF CW, US
de jong, (1976)	27	HSSF CW, Netherland
Obarska-Pempkowiak and Gajewska, (2003)	29.2	HSSF CW, Poland
Haberl and Pefler, (1990)	35.0	HSSF CW Mannerdorf Austria
Behrends et al., (1994)	51.2	HSSF CW, Alabama, US
Wrigley and Toerien, (1988)	75	HSSF CW, South Africa
Rodriguez and Brisson, (2016), 2012 fall	98	HSSF, fish farm sludge Quebec, Canada

Total nitrogen standing stock from all *P .australis* in CW was variable between 10655.9 g P yr^{-1} in June and 15358.1g P yr^{-1} in September. Removal ratio form nitrogen in inflow ranged between 6.77 % in June to 9.76 % in September and removal ratio from removed load ranged from 20.1 % in June to 28.9 % in September (Table 22).

Table 22: Nitrogen standing stock of annual removal (g N) and removal rate (%) from inflow load and removal amount (inflow load – outflow load).



Nitrogen annual removal (g N)	Inflow	outflow	Total	from inflow	from removal
June	6106.3	4549.6	10655.9	6.77%	20.1%
Sep	8220.5	7137.6	15358.1	9.76%	28.9%

6.4. Suggestion

Considering the possibilities for optimization of efficiency of CW in Zbenice, there were some suggestions. First of all, relocation of macrophytes would be suggested and relocate *P .arundinacea* into front line and *P. australis* should be located in latter parts of CW. Because of result, on aboveground biomass and nutrient standing stock of *P. arundinacea* was affected by nutrient load from discharge, while *P. australis* was not affected much by nutrient load from discharge. If *P. arundinacea* got less nutrient load situation, rhizome structure got much dense and it could clog discharge water(Rodriguez & Brisson, 2016). During study in 2017, there was weedy species *Urtica dioica* found especially at outflow zone and it was causing unpleasant to operate the maintenance of CW. Vymazal, (2013) explained similar situation that *Urtica dioica* showed denser growth in CW with *P. arundinacea*, but if there was long-term flooding location like as inflow zone, *Urtica dioica* could not survive.

About harvesting periods, for *P. arundinacea* located in inflow seemed enough shoots biomass to crop in late on May (Figure 26), but outflow should be conducted at same period in the end of June as this study. Second harvest at inflow should be conducted in July and outflow should be October or later. For *P. australis*, first harvest should be conducted later than June, because peak of *P. australis*, growth is also happened later (Vymazal and Krőpfelová, 2005) (Figure 27).

Figure 26: Dense growth of *P. arundinacea* on 23th May 2017 in Zbenice.

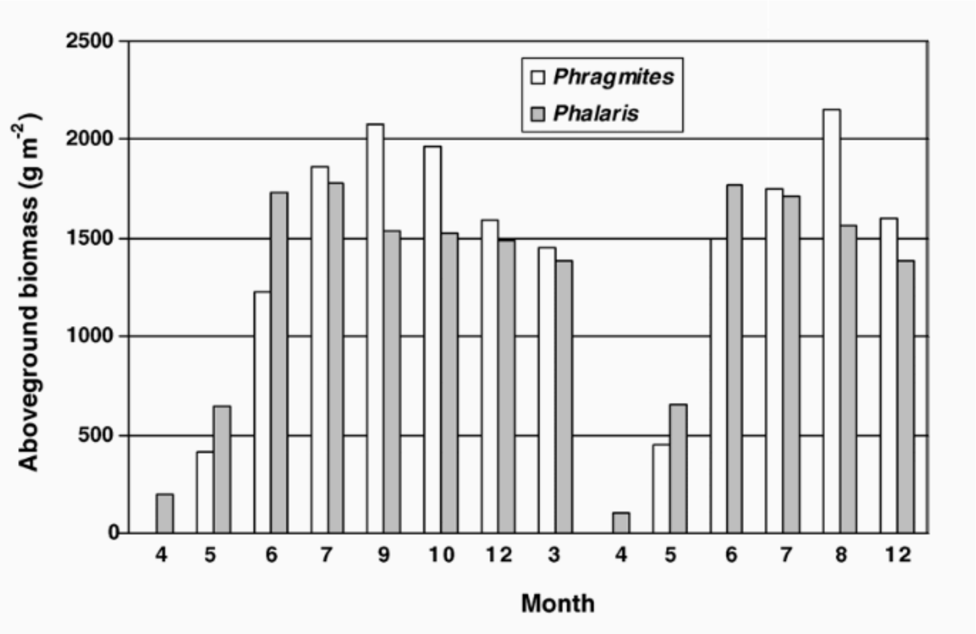


Figure 27: Aboveground biomass of *P. arundinacea* and *P. australis* during the period April 2002- December 2003 in the CW Morina, Czech Republic. (Vymazal and Krőpfelová, 2005)

7. Conclusion

- Aboveground biomass of *Phalaris arundinacea* by single harvest in July (1883 gm^{-2}) is higher than double harvest in June and October (1506 gm^{-2}).
- Nutrients concentration of newly grown plants on early spring or regrowth shoots of *Phalaris arundinacea* and *Phragmites australis* was relatively high (ex: regrowth of *P, arundinacea* was shown 3106, 3323 P mg/kg in leaf part but other month showing around 2600 P mg/kg)
- Nutrient Removal via *Phragmites australis* (maximum removal of phosphorus standing stock 33.9% and nitrogen was 26.7 %) australis was relatively high.
- Position (nutrients load) affected aboveground biomass of *Phalaris arundinacea*, in contrast *Phragmites australis* is less affected against nutrients load.
- Different timing of harvest at inflow and outflow may optimize amount of aboveground biomass and removed nutrient load of *Phalaris arundinacea*.

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