Supplementary Material (SM)

SM1 – Greenhouse conditions for fall 2020 and spring, summer and fall 2021

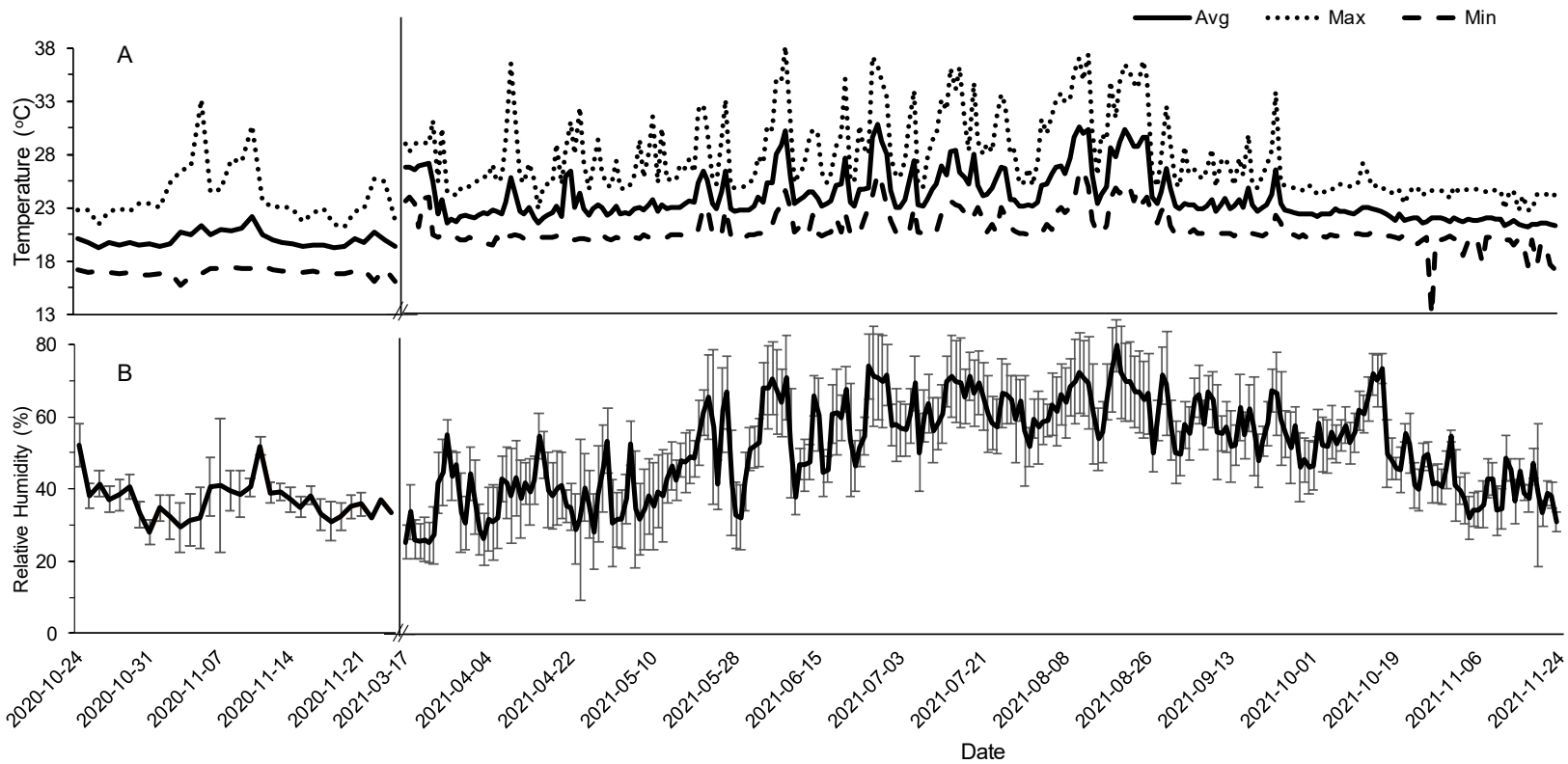

Figure SM1: A) Average, maximum and minimum daily temperature in the greenhouse, B) Average daily relative humidity in the greenhouse, error bars represent standard deviation

SM2 – Feeding regime

The feeding regime is presented in table SM1.

Table SM1: Volumes and feeding regimes of UASB reactor, PAFs and VAFs.

Reactor	Influent flowrate avg ± std (n) L/d	Feeding events avg ± std (n) batches/d	Time between batches h:min	Approx. pumping time min	Recirculation rate (Q_R/Q)
UASB	52.7 ± 6.5 (27)	2.62 ± 0.30 (27)	7:45	15	N/A[1]
VAF-C	6.9 ± 1.5 (21)	2.88 ± 0.06 (21)	8:00	0.75	0.91 ± 0.37 (21)
VAF-W	7.9 ± 1.2 (21)	2.73 ± 0.07 (21)	8:00	0.75	0.71 ± 0.19 (21)
PAF-W	7.9 ± 1.4 (23)	2.79 ± 0.09 (23)	7:57	3.5	0.73 ± 0.19 (23)
PAF-VAF-W	7.9 ± 1.4 (23)	2.79 ± 0.09 (23)	8:00	0.75	0.73 ± 0.19 (23)
VAF-S	6.9 ± 1.7 (21)	2.81 ± 0.07 (21)	8:00	0.75	0.88 ± 0.50 (21)
PAF-S	7.6 ± 1.7 (21)	2.73 ± 0.32 (21)	7:57	3.5	0.73 ± 0.22 (21)
PAF-VAF-S	7.6 ± 1.7 (21)	2.73 ± 0.32 (21)	8:00	0.75	0.73 ± 0.22 (21)

[1]The UASB was mixed by a pump that recirculated UASB contents at 0.5 L/min continuously, except for 30 minutes before to 15 minutes after each feeding batch. Abbreviations: UASB: upflow anaerobic sludge blanket reactor; PAFs: pre-anoxic filters; VAFs: vertical flow aerated filters. Note: Letters W and S indicate filter systems testing willows and cordgrass, respectively and n shows the number of samplings taken during the course of the experimentation.

SM3 – Oxygen mass transfer coefficient (k_La) results

During the study, VAFs were aerated continuously by pumps that provided an air flowrate of 2.5 ± 0.2 m^3/h (n = 3) through the VAF. This was measured by installing a large plastic bag of a known volume over the VAF-C and measuring the volume of air captured in the bag during a known period. The volumetric oxygen transfer coefficient (k_La) was measured three times in each VAF (ASCE, 2007) and all VAFs gave similar results each time. Results for all three k_La tests for each VAF, corrected to a temperature of 20 ºC, are presented in Table SM2.

Measurements in clean water ranged from 4.4 to 5.0 h^{-1}, from 20.0 to 31.1 h^{-1} in clean water with gravel and plants before starting the experiment, and from 20.5 to 25.7 h^{-1} in clean water with gravel and plants after the 2020 season. All k_La tests confirmed similar oxygen transfer efficiencies in all VAFs and little decrease in oxygen transfer efficiencies between the 2020 and 2021 operating periods.

Table SM2: k_La test results corrected to a temperature of 20 °C

VAF	$k_La_{1,20}$ (h^{-1})	$k_La_{2,20}$ (h^{-1})	$k_La_{3,20}$ (h^{-1})
VAF-C	4.4	31.1	25.5
VAF-W	4.9	24.1	23.1
PAF-VAF-W	5.0[1]	20.0	27.1
VAF-S	4.5	27.2	20.5
PAF-VAF-S	-	22.0	25.7

$k_La_{1,20}$: k_La measured in clean water only. $k_La_{2,20}$: k_La measured in clean water with gravel and plants before the start of the experiment. $k_La_{3,20}$: k_La measured in clean water with gravel and plants between fall 2020 and spring 2021 operating periods.

SM4 – Water balance

The first method used to calculate the water balance was using an estimation of evapotranspiration based on plant health factors:

$$\text{Water balance (\%)} = \left(Q_{eff} + ET_c + Q_{aer}\right)/(Q_{leachate})\ x\ 100\% \qquad \text{(SM1)}$$

where Q_{eff} was the effluent flow rate from the reactor (L/d), ET_c was the plant evapotranspiration or evaporation in the VAF-C (L/d), Q_{aer} was the water loss from aeration (L/d) and $Q_{leachate}$ was the influent flow rate to the reactor (L/d). Water loss from aeration was estimated assuming air entered the VAF at greenhouse humidity and left at 100% water saturation. The UASB reactor was not exposed to the atmosphere and had no evaporation nor aeration humidity loss. For VAF-C, evaporation was considered equal to pan evaporation (Allen et al., 1998) measured

using a white bucket filled with approximately 10 L of tap water in the greenhouse. The bucket was emptied and refilled once 3 to 5 L of water had evaporated. For the planted filters, evapotranspiration (ET_c) was estimated from pan evaporation with the addition of a plant health factor, according to Equation SM2,

$$ET_c = ET_0 \ x \ K_c \ x \ plant \ health \ factor \quad \text{(SM2)}$$

where:

$$ET_0 = K_{pan} \ x \ E_{pan} \quad \text{(SM3)}$$

where E_{pan} (mm/d) was the pan evaporation and a K_{pan} value of 0.6 was used (Allen et al., 1998), due to medium humidity and moderate ventilation at the pan location. K_c values of 4.3 for the willows (Frédette et al., 2019b) and 4.5 for the cordgrass (Benli et al., 2006) were used. The plant health factor was determined according to Equation SM4:

$$Plant \ health \ factor = \frac{Number \ of \ living \ plants \ in \ VAF}{Number \ of \ plants \ in \ VAF} \ x \ \frac{Chlorophyll \ fluoresence \ in \ VAF \ plants}{Chlorophyll \ fluorescence \ in \ control \ plants} \quad \text{(SM4)}$$

To confirm this method, water balances were also determined using FS concentrations by Equation SM5, assuming the influent and effluent FS mass rates were equal:

$$Water \ balance \ (\%) = \left(Q_{eff} + Q_{water \ loss}\right)/(Q_{leachate}) \ x \ 100\% \quad \text{(SM5)}$$

where $Q_{water\ loss}$ (L/d) was calculated according to Equation SM6,

$$Q_{water \ loss} = Q_{leachate} - \ (Q_{leachate} \ x \ FS_{leachate})/FS_{eff} \quad \text{(SM6)}$$

where $FS_{leachate}$ and FS_{eff} are the influent and effluent FS concentrations (mg/L), respectively.

During fall 2021, since all the plants in the VAFs were dead and there was no possibility of calculating plant health factor, the water balance of all the 5 systems was calculated by the method used for the VAF-C.

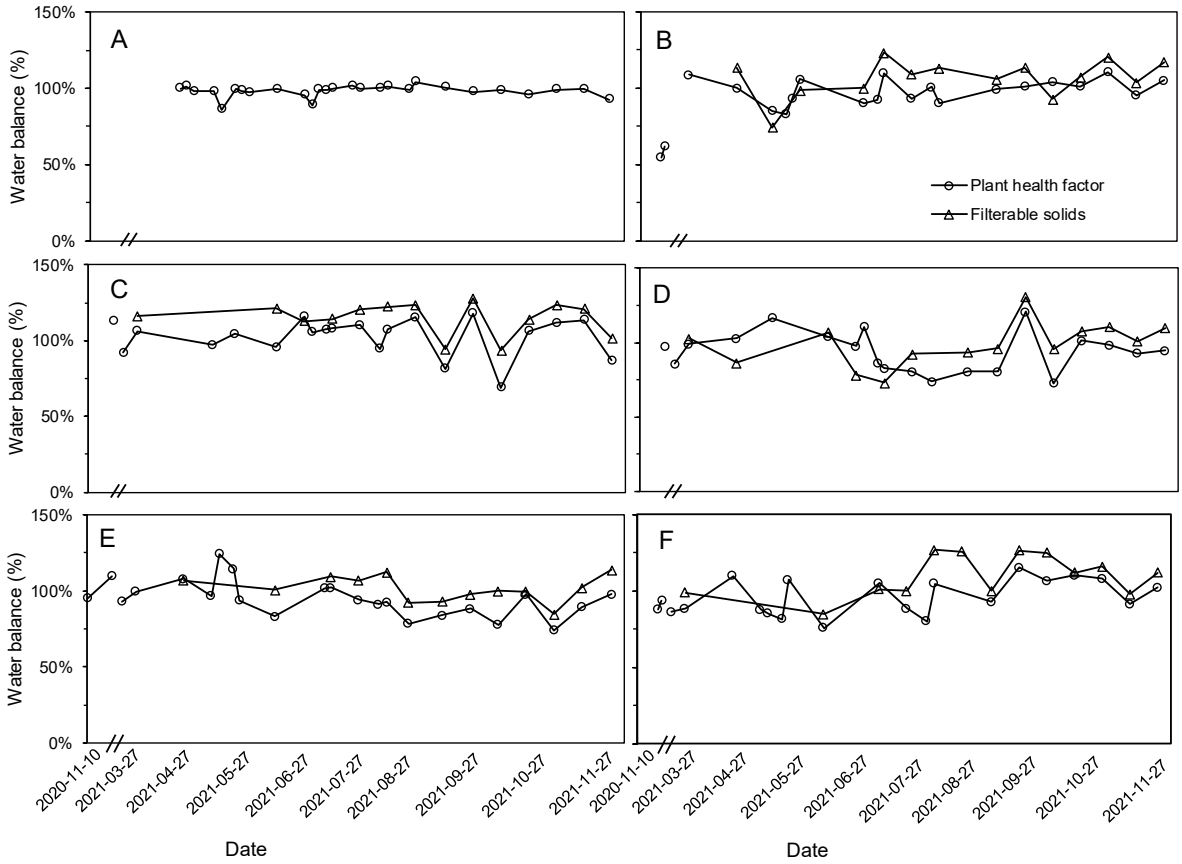

Figure SM2: Water balances (left to right, top to bottom: A) UASB, B) VAF-C, C) VAF-W, D) VAF-S, E) PAF-VAF-W, F) PAF-VAF-S. Circle markers represent water balances measured by evapotranspiration corrected with plant health factors. Triangle markers represent those measured by filterable solids (FS) mass balance determination of water loss.

SM5 – Gravel Characteristics

Table SM3: Gravel characteristics

Parameter	Units	Small Gravel	Large Gravel
Approximate diameter range	mm	10 - 20	20 - 40
SiO_2 composition	%	67	63
Al_2O_3 composition	%	15	16
FeO composition	%	5	5
CaO composition	%	4	4
K_2O composition	%	4	4
Na_2O composition	%	3	3
Dry, packed density	kg/m^3	1580	1530
Dry, packed void space	%	42	44
Effective diameter	mm	9.4	21.8
Coefficient of uniformity	mm/mm	1.9	1.8

SM6 – Plant nutritional content

Plant nutritional content was measured in VAF and control cordgrass leaves by inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis following acid digestion of dry plant biomass once in August 2021. This test was not performed for willows due to a lack of leaves. Cordgrass leaves were cut from the base of the VAF and control plants and wiped clean using a wet cloth before being packaged in a plastic bag and shipped on ice to Agro Enviro Lab (Sainte-Anne-De-La-Pocatiere, Quebec) for analysis. There, samples were rinsed, dried at 75 ^{o}C for 18 hours, crushed and sifted (2 mm). 250 mg were taken of each sample and digested with 6 mL of HNO_3 for 10 minutes at 80 ^{o}C, then 2 mL of H_2O_2 were added after samples had returned to room temperature. Samples were then further digested for 1 hour at 130 ^{o}C. Digested samples were then filtered on a 2 µm pore size filter and analyzed by ICP-AES for sodium, iron, zinc and boron. These measurements allowed a comparison of plant uptake of different elements in VAF versus control cordgrass.

SM7 – UASB function and contaminants removal

The UASB was designed to remove influent dissolved ferrous iron as FeS after its reaction with H_2S produced by sulfate-reducing bacteria (Török, 2021). Influent dissolved iron concentrations were expected to be around 16 mg/L but were only 2.2 ± 1.2 mg/L (n = 21). Based on leachate pH and ORP values, influent iron was present as aqueous ferrous iron, or as solid Fe_2O_3 or $Fe(OH)_3$ (Fermoso et al., 2019), which may explain why influent dissolved iron concentrations were lower than expected. UASB REs for multiple contaminants are presented in Figure SM3, including varying irons REs, presented in Figure SM3D. The trends for total and dissolved iron REs seem to be inversely related, with an increase in dissolved iron when total iron REs are high and a decrease in dissolved iron when total iron REs are low. The pH decreased by 0.13 on average in the UASB, which may have changed a certain amount of solid Fe_2O_3 or $Fe(OH)_3$ to aqueous ferrous iron and resulted in negative dissolved iron REs.

While limited research has been conducted on the use of UASB reactors to remove iron, one study achieved 50 to 80% total iron removal with influent concentrations of 15 to 30 mg/L (Kettunen & Rintala, 1998). The COD loading rate applied to the pilot-scale UASB in this study was lower than typically applied for full-scale UASB reactors, but similar to that used in the Kettunen & Rintala (1998), as presented in Table SM4. The sludge in this study was never wasted and led to a very long sludge retention time of 196 days, which may have impacted the reactor's ability to effectively remove organic matter and iron.

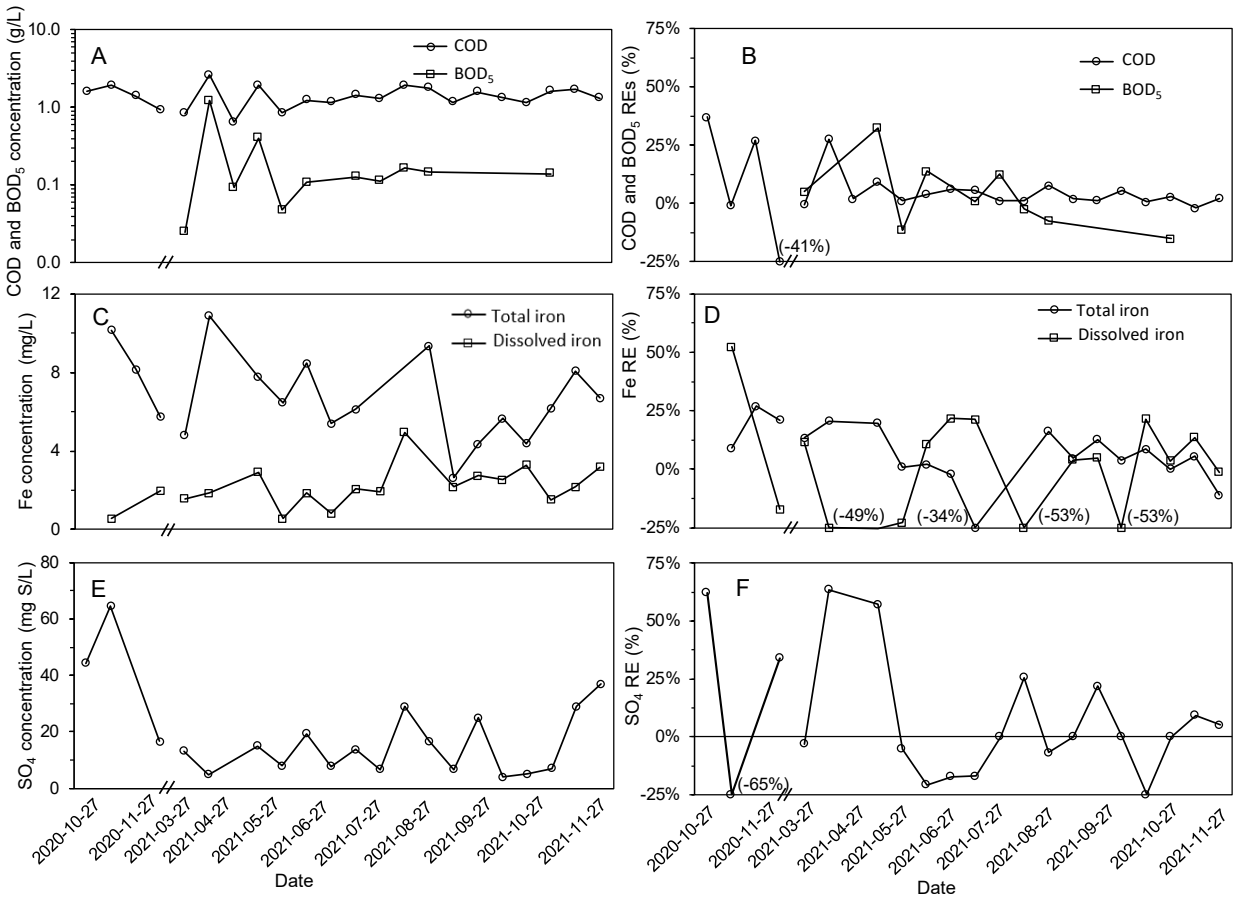

Figure SM3: UASB effluent quality and REs for COD and BOD_5 (A: effluent quality with y-axis on logarithmic scale, B: REs), for iron (C: effluent quality, D: REs) and for SO_4 (E: effluent quality, F: REs)

To remove ferrous iron with H_2S produced by sulfate-reducing bacteria, 0.58 g of SO_4-S is required per g of Fe^{2+}, thus the influent sulfate concentration of 27 mg S/L on average should have been sufficient for dissolved iron removal. BOD_5 removal efficiencies varied (Figure SM3B) but were typically between 0 and 25% suggesting some bacterial activity. While NOx concentrations in UASB influent were about 40 mg N/L, NOx removal efficiency was relatively low, varying between -14 and 20% with an average removal of 0%, suggesting little denitrifying activity overall. Some bacterial activity occurred in the UASB, suggesting a possible

competition between anaerobic and denitrifying bacteria, however it is thought that short-circuiting may have occurred in this reactor and led to low and varied iron and organic matter REs.

Table SM4: UASB characteristics compared to other studies and typical values

Parameter	Units	This study	Kettunen & Rintala (1998)	M&EA (2014)
Influent iron concentration	mg/L	7	15 to 30	
Total iron REs	%	-35 to 30	50 to 80	
Sludge retention time	d	196		> 30
HRT	H	28	13 to 35	4 to 8
COD loading	kg COD m^{-3} d^{-1}	1.5	2 to 4	5 to 20[1]

[1]: *In high strength industrial wastewater applications (food and beverage, pulp and paper, etc.) and municipal wastewater applications.*

SM8 – VAF effluent quality and Quebec discharge standards

Table SM5: Average effluent concentrations of parameters monitored by Quebec provincial government, with their subsequent average monthly discharge standard values.[1]

Parameter	BOD_5	TSS	NH_4	Phenolic compounds	Zn	Fecal coliforms[2]	pH
Units	mg/L	mg/L	mg N/L	mg/L	mg/L	UFC/100 mL	-
VAF-C	3.4 ± 2.3 (13)	5.0 ± 5.0 (18)	< 2.6 ± 3.4 (21)	0.0046 ± 0.0036 (17)	0.34 ± 0.19 (20)	approx. 50	7.8 ± 0.9 (21)
VAF-W	3.2 ± 3.4 (12)	3.3 ± 1.7 (18)	< 4.3 ± 5.3 (22)	0.0046 ± 0.0042 (18)	0.32 ± 0.13 (19)	< 250	7.5 ± 1.1 (22)
PAF-VAF-W	2.0 ± 0.5 (13)	3.7 ± 2.7 (18)	< 3.0 ± 3.9 (22)	0.0037 ± 0.0033 (18)	0.27 ± 0.11 (19)	< 10	8.1 ± 0.8 (21)
VAF-S	2.9 ± 1.8 (13)	5.0 ± 3.5 (16)	< 1.6 ± 0.6 (22)	0.0043 ± 0.0035 (19)	0.41 ± 0.33 (20)	< 10	8.0 ± 0.8 (22)
PAF-VAF-S	3.6 ± 3.1 (11)	5.2 ± 4.3 (17)	< 3.0 ± 4.1 (20)	0.0052 ± 0.0044 (16)	0.29 ± 0.13 (17)	< 10	8.2 ± 0.8 (20)
Maximum average monthly value (MELCC)	65	35	10	0.030	0.07	1000	6.0 – 9.5

Average, standard deviation and number of data points are presented

VAF effluent concentrations presented are the average of concentrations measured over the course of the whole study.

[1]*According to the 2020 Environment Quality Act, (EQA, 2020)*

[2]*Fecal coliforms were only measured once during the experiment. The MELCC only regulates a monthly average value discharge standard, and the fecal coliform concentration measured in leachate was well below this standard.*

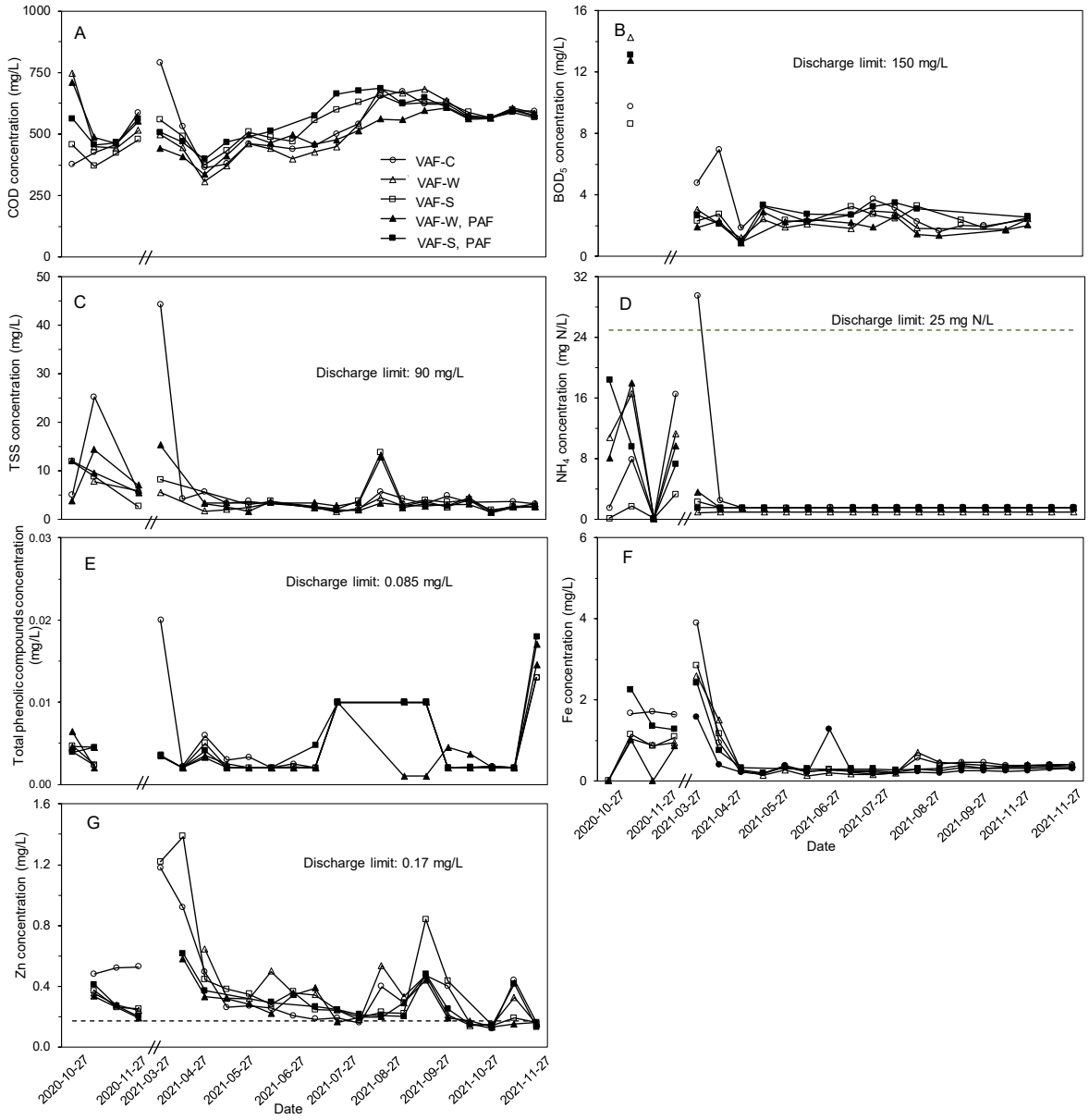

Figure SM4 : VAF effluent qualities: A) COD, B) BOD_5, C) TSS, D) NH_4, E) total phenolic compounds, F) iron, G) zinc. Horizontal dotted lines represent discharge limits per Quebec government.

SM9 – Plant health development - photos

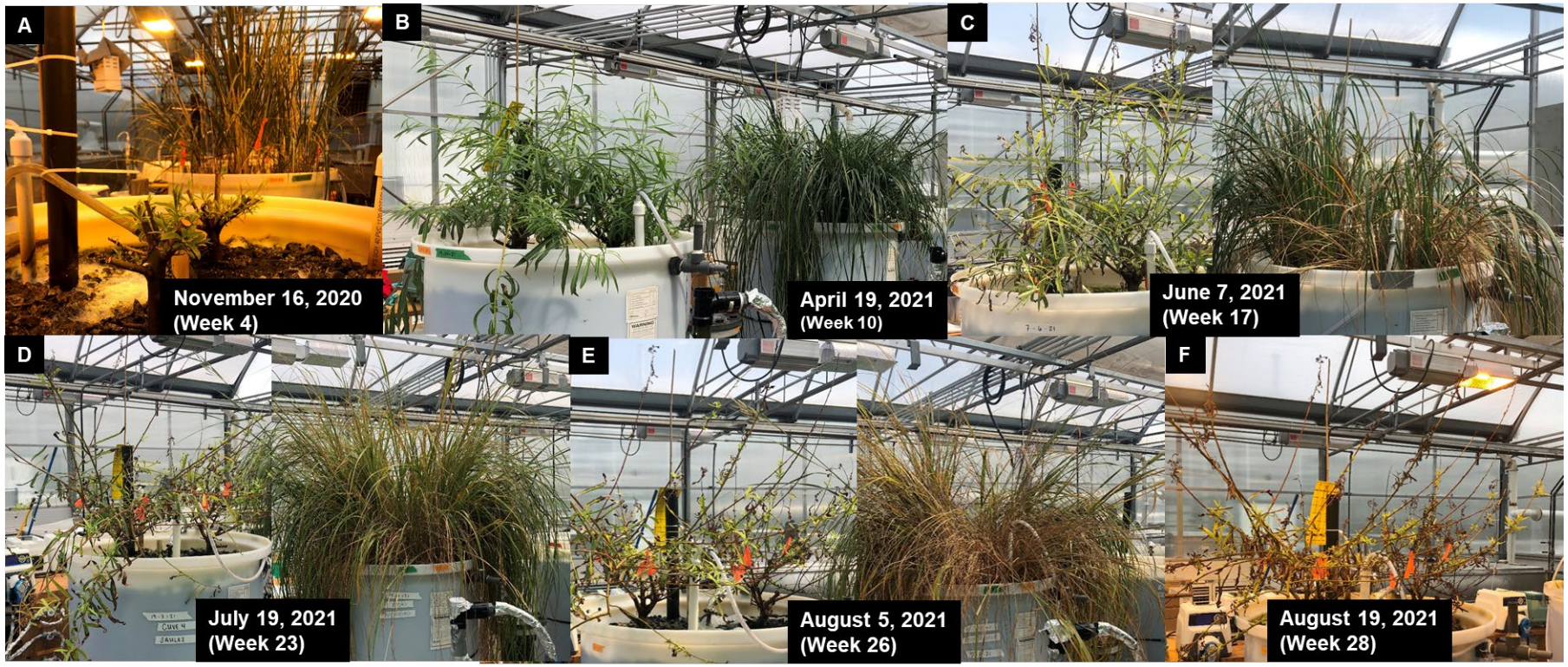

Figure SM5: Change in plant health determined visually throughout the experiment. A) willows are closer and cordgrass can be seen in the background, B through E) willows are on the left and cordgrass on the right, and F) only willows shown because the cordgrass were cut back on that day to perform nutrient content analyses.

Supplementary Material References

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