Communication

Effects of solids accumulation on greenhouse gas emissions, substrate, plant growth and performance of a Mediterranean horizontal flow treatment wetland

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Abstract: The aim of this study was to evaluate the effects of solids accumulation on greenhouse gas (GHG) emissions, substrate, plant growth and performance of a horizontal flow (HF) treatment wetland (TW) planted with *Phragmites australis* (Cav.) Trin. ex Steud. subsp. australis. The study was carried out in an eight-year-old full-scale HF-TW located in the Mediterranean region (Sicily, Italy). To collect data inside the HF unit, nine observation points (besides the inlet and the outlet) along three 8.5 m long transects (T1, T2, and T3) were identified. The first transect (close to the inlet zone) showed hydraulic conductivity (*Ks*) reduction of about one order of magnitude higher than the other two. Results highlighted GHG emissions increasing during the summer when temperature and solar radiation were higher than in the rest of the year. Carbon dioxide (CO_2) emissions decreased from T1 to T3, with maximum monthly values in T1 (21.4 g CO_2 m^{-2} d^{-1}) about double with respect to T2 (12.6 g CO_2 m^{-2} d^{-1}) and T3 (10.7 g CO_2 m^{-2} d^{-1}) observed in July. The CO_2 seasonal trend was similar to that of *P. Australis* growth. Theoretical methane (CH_4) emissions followed the trend of volatile solids (VS), which was about 3.5 and 4 times in T1 to T2 and T3. The highest CH_4 emissions in T1 were probably due to anaerobic bacteria (methanogens) that proliferated in the waterlogged, anoxic part of TW. The pore clogging affected the chemical oxygen demand (COD) removal efficiency which decreasing from T1 to T3 for the whole observation period. Notwithstanding this behaviour, the final effluent quality was very satisfactory with the average value of COD removal efficiency above 90%.

Keywords: Treatment wetlands; Greenhouse gas emission; clogging; water quality; *Phragmites australis*

1. Introduction

Treatment wetlands (TWs) are systems increasingly used worldwide to treat different types of wastewaters (WW) [1] by removing mineral and organic pollutants through both physical and biochemical processes [2,3,4]. Besides the reusable effluent, they integrate water service management and reduce the resource demands for freshwater [5]. Anyway, managers often have to face pore clogging, a complex and changeling phenomenon that affects TWs during their operational life [6]. In addition, the new century's challenges as global warming and climate change have pushed several authors to study TWs also in terms of environmental sustainability. It is well recognized that in such nature-based systems, organic matter is removed through carbon dioxide (CO_2) and methane (CH_4) evolution, and they can act as a carbon (C) sink or source. More than 200 papers have been published in international peer-reviewed journals [4,7] considering CO_2 emission and sequestration as well as CH_4 emissions in TW concerning numerous factors: TW types, meteorological [8], hydrological [9], operational and lifespan conditions [10] as well as vegetation [4]. In addition, some authors highlighted that the permeability variation of a TW substrate would affect greenhouse gas (GHG) flows and their interactions with the underlying groundwater [11-12]. Moreover, pore clogging generally causes the rise of the water table. This TW condition creates anaerobic (anoxic) soil, which can store CO_2 and release CH_4 by decreasing the decomposition rate [13]. Also, aerobic degradation is the predominant process responsible for organic matter removal and accumulation of insoluble organic matter in TWs can reduce the organic matter removal rate [14], even if the total treatment capacity of a partially clogged horizontal flow (HF) unit could remain satisfactory [15]. The aim of this study was to evaluate effects of solids accumulation on GHG emissions, substrate, plant growth and performance of a Mediterranean eight-year-old full-scale HF-TW planted with *Phragmites australis.*

2. Materials and methods

2.1 Study site and experimental design

This study was performed in a full-scale HF-TW located in Catania (South Italy, 37° 26′ N; 15° 01′ E) in the Mediterranean basin. The hybrid TW consisting of three in-series connected units, one HF and two vertical flows (VF1 and VF2). The TW has been operating since 2014 as a support for the primary sequence batch reactor (SBR) system. VF1 and VF2 allow for treating WWs and nitrifying ammonia to nitrate. The HF unit, with a surface area of 400 m^2 and a project flow rate of 30 $m^3 \cdot d^{-1}$ (split into two batch phases every day), serves as the secondary treatment step. It has been designed to reduce organic matter and suspended solids (SS) concentrations. The HF filtering unit is 1% slope, 0.6 m deep on average, filled with volcanic gravel (8-10 mm, 0.41 porosity) and planted with *P. australis* at a density of about 4 rhizomes per m^2. During the experiments, the water table was kept constant at 0.30 m from the HF surface to facilitate substrate sampling operations. To collect data, besides the inlet (P0) and the outlet (P10), nine observation points, three 8.5 m long transects (T1 at 8.5 m, T2 at 17 m and T3 at 25.5 m) were considered. Each transect is equipped with three piezometers 0.30 m depth inserted inside the HF unit and placed at a 3 m distance from each other. Observed data were calculated by averaging the three observations points in each transect (from P1 to P9) at the same distance from the inlet, for the reason that no significative difference ($p < 0.05$) has been observed in three sampling points for each distance for studied parameters. The T1 area was afflicted by a severe hydraulic conductivity reduction (Ks = 660 $m \cdot d^{-1}$) in comparison to T2 and T3, which showed a Ks = 6508 $m \cdot d^{-1}$ and Ks = 6104 $m \cdot d^{-1}$, respectively [16]. The HF vegetation has been harvesting every year (at the end of January) when the shoot vegetation is maximum.

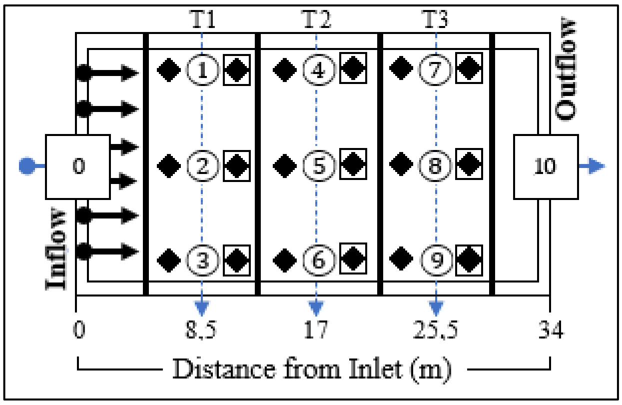

Figure 1. HF experimental setup – black diamonds are bulk substrate sampling points; circles are piezometers, squares are aboveground biomass sampling point and numbers are wastewater sampling point.

2.2 *Weather data*

A weather station (Campbell scientific – General Research-Grade Weather station – GRWS100), able to recorder different climatic variables, was installed close to the TW plant to record the following meteorological data: air temperature, wind speed and direction, rainfall, and relative humidity. The HF influent flow rate and the HF effluent WW discharge volume, combined with precipitation data measured by the meteorological station were used to estimate the evapotranspiration (ET) rates of the *P. australis* during the vegetative period. The ET was calculated using a water balance approach according to the method described in [17].

2.3 Water quality

The water flow rate was daily collected and recorded at inlet and outlet using a flow-measurement device (B-Meters MUT 2200 EL). WW samples were collected at the inflow and outflow wells and at the nine piezometers installed in the HF unit. Biochemical oxygen demand (BOD_5, mg · L^{-1}), chemical oxygen demand (COD, mg L^{-1}), total nitrogen (TN, mg L^{-1}), total phosphorous (P, mg L^{-1}), and total suspended solids (TSS, mg L^{-1}), were estimated according to [18]. The removal efficiency (RE, %) of the system was calculated as follow (1):

$$\boldsymbol{RE}\ (\%) = \left(\mathbf{1} - \frac{C_{out}}{C_{in}}\right) \cdot \mathbf{100} \qquad (1)$$

where C_{out} and C_{in} was the pollutants concentration in the effluent and inflow point, respectively. In particular, the COD, RE was evaluated also for each transect.

2.4 *Accumulated material characterization and vegetation study*

Substrate samples mixed with belowground biomass and organic matter was collected at 2 points around each piezometer (*n=18* samples). A depth of 0.30 m was explored as most of the plant root apparatus concentrated in the system's upper layer [19]. At each sampling point, a 0.20 m in diameter by 0.30 m long sharp-end steel tube was inserted in the unit substrate to avoid a collapse of the lateral wall inside the hole and to collect the material samples. The steel tube was inserted in the unsaturated zone of the HF system surface. Then, the bulk sample inside the tube was extracted by a soil scoop (0.005 m3). Laboratory analysis was performed according to the conventional methods described in [20] to characterize the sampled material in terms of concentrations of accumulated total solids (TS, g · m^{-3}), volatile solids (VS, g · m^{-3}), and plant root biomass (PRB, g · m^{-3}). Moreover, in each transect, three 1 m^2 – parcels were outlined to study *P. australis* aboveground volume in terms of number, height and circumference of culms from January to December 2021.

2.5 Greenhouse gases emissions

The monitoring activities were performed from January to December 2021. Daily CO_2 emissions were measured after plant cutting when shoot vegetation coverage was = 0% up to the end of the year. The *static stationary chamber technique* [3,21] was used to estimate in situ CO_2 emissions in T1, T2, and T3 of the HF unit. Further details about constructive and operational features, apparatus setting and calibration, are described in [3,22]. The chamber was positioned with its bottom part (0.2 m) permanently inserted in each fixed HF sampling point to calculate cumulative CO_2 daily emission. For each transect, two replicated measures around each piezometer were acquired. Theoretical CH_4 emissions were calculated as a function of the BOD_5 loaded into HF unit and its related emission (EF) as suggested by [23]. This method, as defined good practice approach for countries with limited data [24]. The EF was obtained using the following equation (2).

$$\boldsymbol{EF} = \boldsymbol{B_0} \cdot MCF \tag{2}$$

Where B_o indicates the maximum CH_4 generation capacity. The [25] suggests a default value of 0.6 (kg · CH_4) · (kg · BOD_5)$^{-1}$. *M*CF indicates the CH_4 correction factor for TW type (*MCF* = 0.1 for HF-TW as shown in the [25]).

2.6 Data analysis

Statistical analysis made in this study was performed using *Minitab* software. CO_2 emissions and organic biomass fraction among T1, T2 and T3 were evaluated by analysis of variance (ANOVA). The non-parametric Kruskal-Wallis's test (R Core Team 2014) with $p < 0.05$ was performed to check the CO_2 emissions differences and the aboveground biomass growing in the three transects. Statistical significance between two average values of TS, VS, PRB was tested by a two-tailed t test ($p = 0.05$) assuming a normal distribution for these variables. The multiple linear regression model was applied to check the relationship between observed CO_2 emissions and the weather variables. According to the influent and effluent concentration of BOD_5, COD, NH_4^+-N, TN, TP and TSS the statistical difference of the average RE values was figured out using ANOVA.

3. Results and Discussion

3.1 Weather data

The meteorological data recorded during the experimental period showed typical characteristics of Mediterranean environments with an average annual rainfall of about 626 mm and an average annual air temperature of 18.3 °C, ranging from a minimum of 9.8 °C up to a maximum of 31.6 °C, with an average relative humidity of 39.8%. The discussed time span was characterized by a cumulative solar radiation of 214.34 MJ · m^{-2} · d^{-1} and an average wind speed of about 1.72 m · s^{-1}, with a prevailing wind direction of 247.50° north. The average daily ET was 6,80 mm · d^{-1} and showed the highest value (14.61 mm · d^{-1}) at the end of July. The lowest value was recorded at the end of January (0.96 mm · d^{-1}). As highlighted in several studies [4,8,26,27], the environmental conditions since are able to influence directly and indirectly the vegetation development, the microbial communities and their level of activity. The linear regression analysis performed in this study suggests a linear association of observed CO_2 emissions with both the average air temperature ($R^2 = 0.75$) and the average solar radiation ($R^2 = 0.63$), recorded during the observation period (Figure 2). This result is in agreement with the positive correlation highlighted in more than 200 paper reviewed by [4].

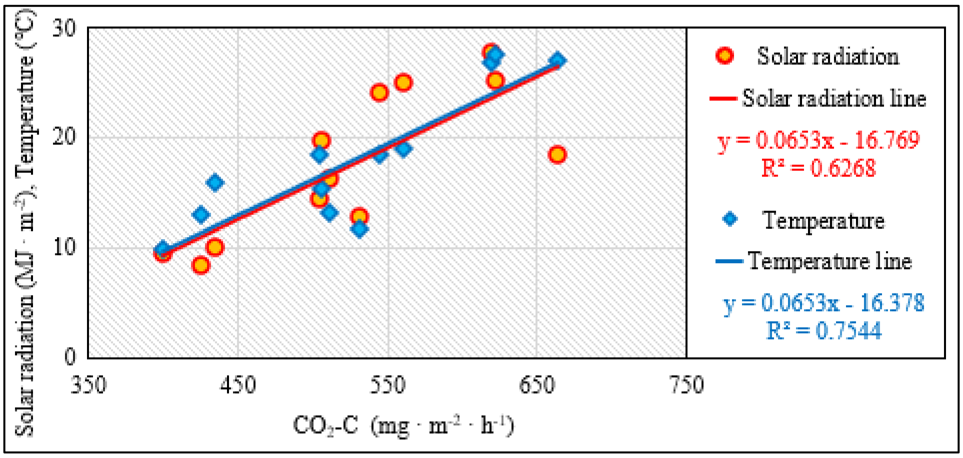

Figure 2. Linear regressions between the average monthly CO_2 emission from the HF unit with the average air temperature values (blue line) and the average solar radiation values (red line) documented during the observation period (January - December 2021). Yellow circles and blue diamonds are solar radiation and temperature data, respectively.

Moreover, [3-4] found out a significant correlation between average air temperature and CO_2 emission in a pilot-plant scale HF-TW vegetated with *Chrisopogon zizanioides* and *P. australis.* Also [28] reported a positive correlation of CO_2 flow rates through the culms with solar radiation in a HF-TW unit vegetated with *P. australis*. Regarding CO_2, similarly [3-4] highlighted a positive correlation with solar radiation but only for *Cyperus papyrus*, supporting [29-30] which suggest that not only the vegetation presence has a significant impact on the GHG emissions from TW, but also the plants genotype.

3.2 Water quality

COD removal increased significantly from T1 to T2 to T3 for the whole observation period. In T1, the range of COD removal monthly variability was 6-14% with a mean value of 10%; in T2, it was 14-40% with a mean value of 24%; in T3, it was 17-58% with a mean value of 33% (Figure 3). The lowest COD-RE observed in the T1 transect could be due to the pore clogging phenomenon, which causes a unit useful volume loss and a rising of the water table, generating anaerobic (anoxic) zones. As well-known aerobic degradation is the predominant process responsible for COD removal, and the accumulation of insoluble organic matter in the HF unit may reduce the COD removal rate [14]. This behaviour is in agreement with [32] which found out that the amount of COD degradation is related to the effective porous volume of the filler. Notwithstanding the lower COD-RE in the first part of the HF unit, the effluent quality was good during the whole observation period, with an average value of COD-RE above 90% [33]. In fact, in agreement with [15], the treatment performance of a partially clogged HF unit can remain satisfactory. The Table 1 shows the average concentrations and the RE of the main pollutants obtained from the water samples analysis collected at the inflow and outflow of the HF unit during the monitoring campaign (2021).

Table 1. Average concentrations and removal efficiencies with standard deviation (±SD) of the physicochemical parameters detected at the inflow (in) and outflow (out) of the HF- TW during the experimental period (January - December 2021**)**

Water quality parameters	HF in (mg· L^{-1}) (±SD, *n=12*)	HF out (mg· L^{-1}) (±SD, *n=12*)	Removal efficiency (%) (±SD, *n=12*)
COD	164.4 (±172.1)	38.4 (±13.1)	76.6 (±7.3)
BOD_5	129.11 (±28.5)	8.2 (±4.3)	93.6 (±1.8)
TSS	62.2 (±39.4)	4 (±5.8)	99 (± 0.8)
$N\text{-}NH_4$	12.8(±10.6)	0.1 (±0.1)	99 (± 0.4)
Total N	76 (±28.5)	26.9 (25.8)	74.3 (±30)
Total P	16.6 (±9.1)	10.2 (±11.1)	54 (±15)

Pollutant concentrations of the final effluent were low (4 ± 5,8 mg · L^{-1} of TSS, 0,1 ± 0,1 mg · L^{-1} of $N\text{-}NH_4^+$, 26,9 ± 25,8 mg · L^{-1} of N_{tot} and 10,2 ± 11,1 mg · L^{-1} of P_{tot}), notwithstanding the high initial concentrations at the inlet stage (62.2 ± 39,4 mg · L^{-1} of TSS; 12.8 ± 10,6 mg · L^{-1} of $N\text{-}NH_4^+$, 76 ± 28,5 mg · L^{-1} of N_{tot} and 16,6 ± 9,1 mg · L^{-1} of P_{tot}). Therefore, results evidenced the key role of the HF unit which provided an efficient reduction of TSS (up to 99 ± 0,8%), $N\text{-}NH_4^+$ (up to 99 ± 0,4%), N_{tot} (up to 74,3 ± 30%) and P_{tot} (up to 54 ± 15%). The effluent quality was outstanding, and the BOD_5, COD and TSS values, in particular, were below the Italian law discharge limits (35 and 125 mg · L^{-1}, respectively). The HF unit provided a very high average reduction of TSS, BOD_5, allowing for the limits fixed by the Italian law to be respected. The high TN reduction confirming that both processes (nitrification and denitrification) were efficient.

3.3 Accumulated material characterization

In the T1, TS concentrations varied between 3088.61 and 5646.41 g · m^{-3} with an average value of 4320.48 ± 471.45 g · m^{-3} (CV = 0.10). The VS concentration varied from 1550 to 2157 g · m^{-3} with an average value of 1355 ± 115.15 g · m^{-3} (CV = 0.08), the volatile fraction accounted for 51% of the total TS concentration. The T2 showed a TS concentration ranging between 656.01 and 1152.43 g · m^{-3} with an average value of 920.48 ± 77.28 g · m^{-3} (CV = 0.08), meanwhile T3 is characterized by a TS concentration that ranging between 467.89 and 1055.08 g · m^{-3} with an average value of 846.48 ± 61.4 g · m^{-3} 107.18 (CV = 0.12). The volatile fraction in T2 and T3 accounted 27,7% and 25% of total TS concentration, respectively. The higher VS average concentration value in T1 with respect to the rest of the HF unit may explained as an effect of the organic matter accumulation close to the inlet area. Moreover, the VS average values trend with respect to distance from inlet has a strong negatively correlation (R^2 = - 0.98) with the K_s one (Figure 4). Similarly, [20,34] observed a significative increasing in VS close that zone. This result is in line with the *Ks* reduction observed by [16] close the inlet zone (T1) which has been highlighted as an expected consequence of organic matter accumulation due to wastewater type and supply also in others studies [33-34]

3.4 Phragmites Australis growth

Monthly aboveground vegetation volume (calculated from the number of culms, height and circumference) showed an expected increasing trend from February to July in the HF unit. This trend was almost similar in the three transects from February to May (Figure 3). However, a higher growth rate was observed in T1 starting from June and it raised up until August (Figure 3). Lowest values of the monthly above vegetation volume were observed in T1 area which was affected by pore clogging. The PRB observed at 0.3 m belowground depth followed the same trend with values decreasing from T1 (5646.8 g m^{-3}) to T2 (1650.2 g m^{-3}) and finally to T3 (656.0 g m^{-3}).

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3.5 Greenhouse gases emission

CO_2 emissions increased during the summer when temperature and solar radiation were higher than in the rest of the year (Figure 2). CO_2 emissions were significantly different among T1, T2 and T3, with maximum monthly values in T1 (21.4 g · CO_2 · m^{-2} · d^{-1}) about double with respect to T2 (11.3 g · CO_2 · m^{-2} · d^{-1}) and T3 (10.7 g · CO_2 · m^{-2} · d^{-1}) observed in July (Figure 3). Minimum monthly values (10.8 g · CO_2 · m^{-2} · d-1) in T1, 7.4 g · CO_2 · m^{-2} · d^{-1} in T2 and 4.8 g · CO_2 · m^{-2} · d^{-1} in T3) were observed mainly in November. T2 and T3 hand back a similar trend, with lower differences observed between summer and winter months compared to those observed for T1 (Figure 3). The seasonal trend observed for CO_2 in T1 agrees with that reported by several authors [7,35,36,37,38]. In semi-arid Mediterranean conditions [3] observed an average CO_2 daily emission varying between 0.8 ± 0.1 g · CO_2 · m^{-2} · d^{-1} (winter season) and 24.9 ± 0.6 g · CO_2 · m^{-2} · d^{-1} (summer season). Also, [39] found similar CO_2 emissions (varying from 11.1 to 49.0 g · CO_2 · m^{-2} · d^{-1}) in a Mediterranean HF-TW vegetated with *P. australis* under anaerobic conditions. Moreover, [29] reported CO_2 emissions varying between 0.4 and 27.2 g · CO_2 · m^{-2} · d^{-1} during summer and fall in an HF-TW with *P. australis* that treated combined sewage and stormwater runoff, but no significant differences were highlighted by this author comparing the inlet and the outlet zones. In this study, the seasonal trend observed for CO_2, and *P. Australis* volume was similar, with an R^2 equal to 0.74 for T1, 0.65 for T2 and 0.74 for T3. This could indicate that vegetation growth is responsible for the CO_2 emissions increasing recorded during the summer season.

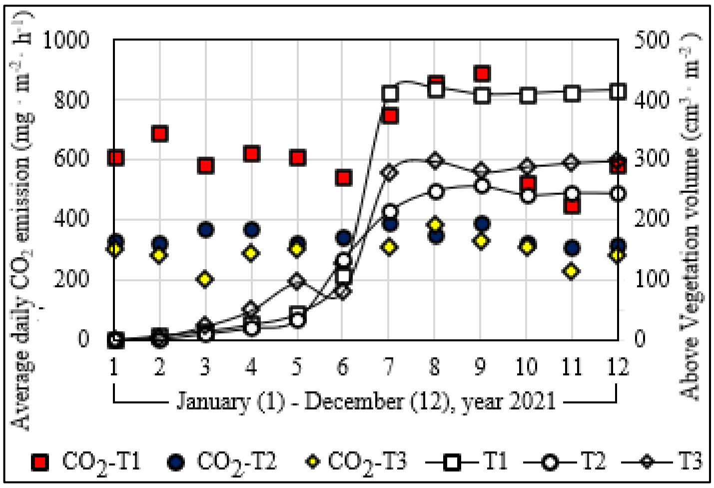

Figure 3 - Temporal trend and comparison between the aboveground vegetation volume (cm^3 per m^2) and CO_2 emissions (mg · m^{-2} · h^{-1}) documented in the three transects during the experimental campaign. Red squares, blue circles and yellow diamonds are the average values of CO^2 emissions observed in T1, T2, and T3 respectively during the 2021. White squares, circles and diamonds are average values of the aboveground vegetation volume of *Phragmites australis* documented in the 2021.

The crucial role of vegetation growth in CO_2 emissions has been reported by numerous authors [4,40]. In fact, [29] observed that CO_2 emissions gradually declined toward the end of the growing season. Additionally, he demonstrated that plants are an essential source of available carbon for the microorganism in TWs. This carbon is further transformed into gaseous forms and increases carbon emissions from TWs. In this study has been highlighted that, CH_4 emissions followed the trend of VS (Figure 4), with values decreasing from T1 (equal to 19.8 kg · CH_4 · $year^{-1}$) to T2 (3.3 kg · CH_4 · $year^{-1}$) and T3 (6.5 kg · CH_4 · $year^{-1}$).

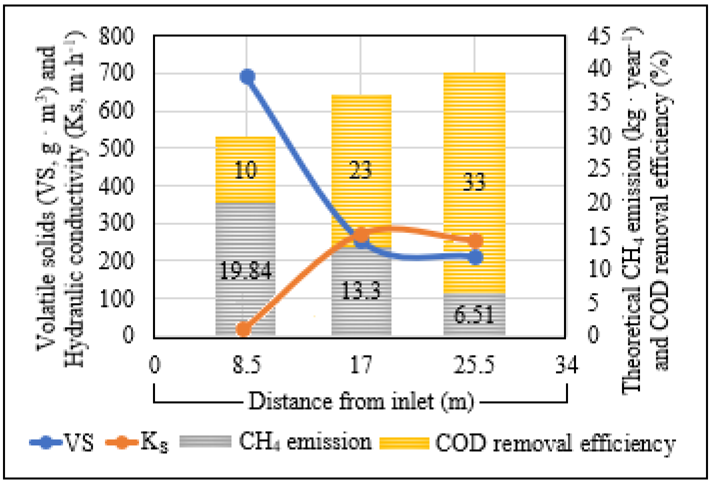

Figure 4 – Data comparison with the distance from the inlet of volatile solids average values (VS, g · m^{-3}); *Ks* average values (m· h^{-1}) reported [16] (m · h^{-1}), COD removal efficiency (%) and theoretical CH_4 emission (kg · year^{-1}).

The highest theoretical CH_4 emissions in T1 are probably due to anaerobic bacteria (methanogens) that increase in HF unit waterlogged anoxic part. Similarly, [8] found higher methane emissions in the HF inlet zone (10 mg · CH_4 · m^{-2} · d^{-1}) than in the HF outlet zone (4.4 mg · CH_4 · m^{-2} d^{-1}). This result may be explained by the HF influent loading [41] also resulting in greater availability of organic substrates for the bacterial biomass growth associated with the inlet zone (140–195 g · VS).

4. Conclusion

Both contributes to C emissions (CO_2-C and CH_4-C) were the highest in the inlet zone (T1). This behaviour may be explained by different processes acting contemporarily in the TW. In particular, CO_2 emissions were significantly different among T1, T2 and T3, with maximum monthly values in T1 about double with respect to T2 and T3 observed in July, but the seasonal trend was similar. CO_2 emissions increased during the summer when temperature and solar radiation were higher than in the rest of the year. The CO_2 seasonal trend was also similar to that of *P. Australis,* indicating that vegetation growth is responsible for the CO_2 emissions that increased during the summer season. The increasing monthly aboveground vegetation volume trend was almost similar in the three transects from February to May; an increasing rate higher in T1 was instead observed starting from June, and it raised up in July. Likewise, to monthly aboveground vegetation volume, PRB observed at 0.3 m belowground depth also decreased from T1 to T3 and T2. CH_4 emissions followed the trend of VS, which was about 3.5 and 4 times in T1 to T2 and T3. The highest CH_4 emissions in T1 were probably due to anaerobic bacteria (methanogens) that proliferated in this waterlogged, anoxic part of TW. The T1 zone was afflicted by pore clogging, which caused a *Ks* reduction, about one order of magnitude, compared to T2 and T3. Also, pore clogging caused the observed COD removal increasing from T1 to T2 to T3 for the whole observation period. Notwithstanding the lower COD removal efficiency observed in the first part of the HF unit, the effluent quality was very satisfactory over the entire observation period, with the average value of COD removal efficiency above 90%. Further investigations will be carried out with the aim to assessing the potential effects of pore clogging on TW carbon balance.

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