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Research Article

Effects of Plant Species on Methane and Nitrous Oxide Emissions from Constructed Wetlands Treating Municipal Wastewater

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Abstract: This study was conducted to quantify emissions of greenhouse gases (GHGs), methane (CH_4) and Nitrous Oxide (N_2O), from free water surface constructed wetlands used for domestic wastewater treatment. All constructed wetlands were monoculture and each plot was planted with *Phragmites* sp., *Cyperus* sp., or *Canna* sp. The average CH_4 and N_2O emissions were in the range of 5.9-11.2 and 0.9-1.8 g/m^2/h, respectively. Seasonal fluctuations of CH_4 and N_2O emissions were observed. The highest fluxes of both GHGs occurred during hot rainy season (July-October) followed by summer and the lowest found in cool season. The mean of CH_4 and N_2O emissions from different plants species were significantly different ($p<0.05$). Average CH_4 emissions from constructed wetlands planted with *Phragmites* sp., *Cyperus* sp. and *Canna* sp. were 11.2, 6.0 and 5.9 mg/m^2/h, respectively, while mean N_2O emissions were 0.9, 1.0 and 1.8 mg/m^2/h, respectively. Calculated of Global Warming Potential (GWP) found that GWP of CH_4 and N_2O flux from constructed wetlands planted with *Cyperus* sp., was the highest (669 mg CO_2 equivalent/m^2/h), followed by *Phragmite* sp., (524 mg CO_2 equivalent/m^2/h) and *Canna* sp., (434 mg CO_2 equivalent/m^2/h), respectively. These results suggested that municipal wastewater treatment by constructed wetlands planted with *Canna* sp. and *Phragmite* sp., had potential of lower GHGs emissions into the atmosphere and *Phragmite* sp., provided the highest removal rate of Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD).

Keywords: Constructed wetlands, methane, nitrous oxide, plant species, wastewater treatment

INTRODUCTION

Constructed Wetlands (CWs) systems are combinations of natural wetlands and conventional wastewater treatment processes and are constructed in order to reduce input of nutrients and organic pollutants to water bodies. Constructed wetland systems, a cost-effective alternative, apply various technological designs, using natural wetland processes, associated with wetland hydrology, soils, microbes and plants (Kadlec and Wallace, 2009; Casselles-Osorio *et al.*, 2011). Tropical countries, like Thailand, could have a great benefit of wastewater treatment by constructed wetlands. When constructed wetlands are used for purification of wastewater, the productivity of the ecosystem could increase as well as the production of greenhouse gases (GHGs), which are by-or end-products of microbial decomposition processes (Inamori *et al.*, 2007; Wang *et al.*, 2008; Liu *et al.*, 2009; Wu *et al.*, 2009). Constructed wetlands, therefore, can be sources of important greenhouse gases.

In constructed wetland microcosm, soil-plant is a highly complex environmental system that acts as a reservoir for microorganisms with their activity varying over space and time. Plants release root exudation, which easily decomposable and preferentially used by microorganisms, increased carbon input into the system (Tanner, 2001). Microbial growth in wetlands soil has been believed to depend upon the plant species and substrate. Furthermore, many species of emergent plants CWs possess a convective flow mechanism when oxygen is transported to the roots and gaseous microbial by-products are emitted from plant roots to the atmosphere (Brix, 1989; Brix *et al.*, 1996). The transport of gases by the convective mechanism is faster than diffusion through water. The presence of plants in constructed wetland system may increase gas emissions from the soil. Therefore, plant species could

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affect microbial ecology and gas emission from treatment processes.

Although constructed wetlands can be beneficial for wastewater treatment they may have an unfavorable environmental impact by increasing the fluxes of greenhouse gases to the atmosphere. Thus, objectives of this study were:

- To quantify CH_4 and N_2O emissions from wastewater treatment constructed wetlands
- To estimate seasonal fluctuations of CH_4 and N_2O emissions from wastewater treatment constructed wetlands
- To investigate the effect of plant species on CH_4 and N_2O emissions

MATERIALS AND METHODS

Study site: The experimental scale of constructed wetlands was built outdoor at Suranaree University of Technology, Nakhon Ratchasima Province in northeastern Thailand (Fig. 1). The geo-location of the study area is 177879N and 1648339E. The region has three-season monsoonal climate, with a relatively cool dry season from November to late February, followed by a hot dry season from March to June and then a hot rainy season from July to October (TMD, 2012).

The experiment was employed on a regime of free water surface flow constructed wetland. Eight constructed wetlands with identical dimensions were built based primarily on criteria of Aspect Ratio (AR) or length to width of 4:1 to minimize short circuiting and force the flow to move closely to plug flow hydraulic regime (U.S.EPA, 2000). Each wetland had the dimension of 2.0×0.5×0.8 m (length×width×depth). Brick, cement and mortar were the materials used for the construction of the constructed wetlands. Permanent transparent roof made from clear plastic was also constructed to prevent rain getting into the experiment setup and allow direct sun light exposure. Three emergent plants were used. All constructed wetlands were monoculture with *Phragmites* sp., *Cyperus* sp. and *Canna* sp. Each had two replicate cells.

The compositions of synthetic domestic wastewater consisted of glucose, $FeCl_3$, $NaHCO_3$, KH_2PO_4, $MgSO_4 \bullet 7H_2O$ and urea (Sirianuntapiboon and Tondee, 2000), similar to the domestic wastewater from Thailand's Housing Estates. The concentration of each component is described below:

Glucose	190 mg/L	$FeCl_3$	0.31 mg/L
$NaHCO_3$	6.7 mg/L	KH_2PO_4	6.0 mg/L
$MgSO_4 \bullet 7H_2O$	3.9 mg/L	Urea	9.0 mg/L

Synthetic domestic wastewater was fed daily into the constructed wetlands using gravimetric flow and the flow was controlled by needle valves. The characteristics of the prepared synthetic wastewater are shown in Table 1.

Fig. 1: Map of study area in northeastern Thailand

Table 1: Characteristics of synthetic wastewater

Parameter	Range (mg/L)	Avg. (mg/L)
BOD	115-235	163
COD	17-23	20
TP	0.03-0.33	0.14
TKN	0.28-0.35	0.32

Avg.: Average

Gas flux measurement and analysis: Gases emissions from the constructed wetlands were sampled monthly from June 2010 to May 2011. The measurements of GHGs fluxes were conducted between 09:00 and 15:00 periods. Gas emissions were measured using a static chamber method described by Hutchinson and Mosier (1981). The chambers consist of two parts. The upper part was constructed from 3.0 mm clear acrylic sheet and made gas-tight by heated glue doubling with silicon sealant. The chamber included two gas sampling points, a thermometer and a fan. Size of the acrylic chamber was 0.25×0.25×1.5 m (width×length×height). A small electronic fan was installed to ensure a thorough gas mixing inside the chamber during the measurement. The bottom part was made from aluminum rod. This frame was used as a base for the upper part. Four-side groove was made with 4.0 mm trench to accommodate the 3.0 mm acrylic chamber during the gas sampling. The aluminum frame was firmly inserted into the top soil overnight prior the measurement. During the measurements, the chamber was placed on top of the aluminum frame. In each constructed wetlands, three chambers were installed at the inlet, middle and outlet. Gas flux measurements were performed at these three sampling locations. The acrylic chambers were placed on the aluminum bases and gas measurements began at 0, 15, 30 and 45 min intervals. Soil temperature at the 5-cm depth was also monitored, whereas soil pH, soil ORP (at 5-cm depth) were measures by pH/ORP device.

Methane (CH_4) and nitrous oxide (N_2O) samples were collected from the chambers and were analyzed later in laboratory with Gas Chromatography (GC)

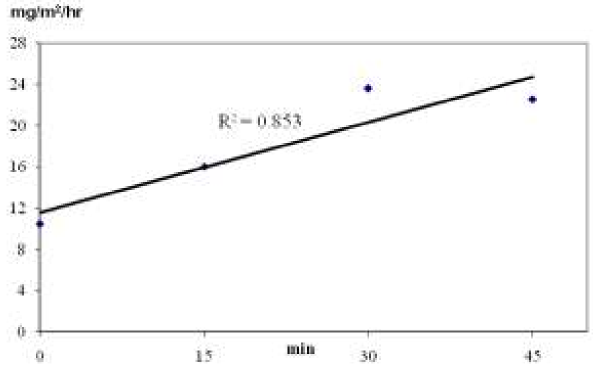

Fig. 2: Linear relationship of greenhouse gas flux response with time

instrument. Gas samples were taken from each chamber with polypropylene syringes and transferred to evacuated glass vial. Air inside the glass vials were evacuated creating negative pressure prior to sampling. Vials were overfilled in order to minimize potential diffusion across the septa. All the samples were labeled and kept cool in ice-packed cooler immediately after the sampling. Then, the samples were transport to a freezer, <4°C, waiting for later GC analysis.

Methane gas samples were analyzed in a laboratory using a gas chromatograph (Agilent GC 6890, USA) equipped with a flame ionization detector, a Poraplot Q capillary column (0.32 mm ID×10 m). Column, injector and detector temperatures were set at 40, 250 and 300°C, respectively, with split ratio 0.7:1 and split flow 15.0 mL/min. Nitrogen was use as carrier and flow rate of 20 mL/min was maintained during analysis.

Nitrous oxide concentrations were determined using a gas chromatograph (Agilent GC 6890, USA) equipped with a 0.53 mm ID×15 m HP-Plot Q column and a Micro Electron Capture Detector (μECD). The temperatures of μECD and the column were set at 300 and 180°C, respectively. Nitrogen was supplied as the carrier gas at a flow rate of 15 mL/min. Data were analyzed by Agilent Chemstation A0803 software (Agilent, USA). The concentrations of methane and nitrous oxide in gas samples were extrapolated by comparing chromatogram (peak area) of gas sample with standard gases (Air Liquid, Ltd. and Scott Specialty Gases, UK).

Gas emission rates were calculated based on the gas and time linear relationship (Fig. 2). Gas concentrations for each sample were plotted against sampling time. Gas emission rate (ppm/h) was converted to flux rates (mg/m^2/h) and corrected for chamber volume and temperature (Healy *et al*., 1996). If the increase/decrease in the gas concentration was non-linear (r^2<0.85), the measurement was rejected (Altor and Mitsch, 2006). Gas flux (mg/m^2/h) was calculated by the following equation:

$$E = \frac{XhM}{RT}$$

where,

E = Emission on the aerial basis (mg/m^2/h)
X = Gas concentration increase in chamber (ppm/h)
h = Height of chamber (m)
M = Molecular weight
R = Gas constant = 0.0821 (atm/K/mol)
T = Absolute temperature (K)
Air pressure = 1 atm

Wastewater analysis: Daily wastewater samples were collected from the influent and effluent points and analyzed for Biochemical Oxygen Demand (BOD) until the steady-state conditions were reached. After the steady-state conditions have achieved, monthly analyses were performed on influent and effluent samples for BOD, Chemical Oxygen Demand (COD), ammonia nitrogen (NH_3-N) and Total Phosphorus (TP). All samples were analyzed according to the standard methods (APHA-AWWA-WEF, 2005).

Data analysis: Statistical analysis was performed with SPSS® and Microsoft Excel® for Windows®. All data entering statistical comparisons were tested for homogeneity of variance and normal distribution using Levene and Kolmogorov-smirnov Test. If assumptions were fulfilled, one-way ANOVA and LSD's post hoc test were carried out. Otherwise, non-parametric Chi-Square (χ^2) Test (Kruskal Wallis) was used instead, followed by Mann-Whitney as Post-hoc test. In all analysis, p<0.05 was considered as a significance level.

RESULTS

Constructed wetlands performance in wastewater treatment: Average removal rates of BOD, COD, NH_3-N and TP were in the ranges of 57-70%, 51-67%, 25-43% and 39-45%, respectively (Table 2). Similar removal rate of BOD was reported by Coleman *et al*. (2001) that used *Typha latifolia* in constructed wetlands. Higher removal rate of COD was found in constructed wetlands planted with *Eriochloa aristata* and *Eleocharis mutate* in Columbia reported by Caselles-Osorio *et al*. (2010) at about 75%. In this present study, the removal efficiencies varied in constructed wetland units with different plants species. High removal rate of BOD occurred in the units planted with *Phragmite* sp., while low removal rate occurred in *Canna* sp. The highest COD removal rate found in the unit planted with *Phragmite* sp., while removal rate was lowest in *Cyperus* sp. NH_3-N was removed in the unit planted with *Canna* sp., more effectively than *Phragmite* sp. Total phosphorus removal rate was high in the unit planted with *Canna* sp., while the rate was lower in the units planted with *Cyperus* sp. Statistical analyses of removal efficiency by one-way ANOVA followed by LSD's post hoc test indicated that average removal rates of BOD, COD and NH_3 were significantly different for all plant species (Table 3).

Table 2: Pollutants removal rates in constructed wetlands with different plant species

		Pollutants removal rates (%)				
Pollutant	Plant species	N	Mean	S.D.	Min.	Max.
BOD	*Phragmite* sp.	12	69.97	9.26	54.8	81.9
	Canna sp.	12	56.85	8.45	43.4	68.7
	Cyperus sp.	12	59.53	7.83	46.8	68.4
	Total	36	62.11	10.08	43.4	81.9
COD	*Phragmite* sp.	12	67.17	5.54	58.2	75.0
	Canna sp.	12	58.83	7.91	45.8	70.8
	Cyperus sp.	12	50.71	8.41	37.4	64.6
	Total	36	58.90	9.90	37.4	75.0
NH_3-N	*Phragmite* sp.	12	25.24	7.44	9.6	36.3
	Canna sp.	12	42.52	8.08	29.3	59.8
	Cyperus sp.	12	41.13	9.94	28.6	59.9
	Total	36	36.30	11.50	9.6	59.9
TP	*Phragmite* sp.	12	40.16	13.09	22.2	57.4
	Canna sp.	12	45.23	20.09	19.8	78.6
	Cyperus sp.	12	39.05	14.97	19.8	67.9
	Total	36	41.48	16.08	19.8	78.6

S.D.: Standard deviation; Min.: Minimum; Max.: Maximum

Table 3: Comparison of pollutants removal rates among constructed wetlands with different plant species by one-way ANOVA

Pollutant CW		S.S.	df	M.S.	F	Sig.
BOD	Between groups	1152.92	2	576.46	7.914	0.002**
	Within groups	2403.82	33	72.84		
	Total	3556.74	35			
COD	Between groups	1626.85	2	813.42	14.875	0.000**
	Within groups	1804.62	33	54.69		
	Total	3431.46	35			
NH_3-N	Between groups	2212.44	2	1106.22	15.111	0.000**
	Within groups	2415.75	33	73.20		
	Total	4628.19	35			
TP	Between groups	260.97	2	130.48	0.490	0.617
	Within groups	8788.59	33	266.32		
	Total	9049.56	35			

**: Significant at the 0.01 level; S.S.: Sum of square; M.S.: Mean square

Table 4: Seasonal variation of CH_4 and N_2O emission from constructed wetland with different plant species

	CH_4 emission (mg/m^2/h)		N_2O emission (mg/m^2/h)	
Plant-season	Mean	S.D.	Mean	S.D.
Phragmite sp.				
Hot rainy	19.88	16.23	1.32	1.15
Cool	3.92	3.32	0.32	0.12
Summer	6.29	4.42	0.90	0.89
Canna sp.				
Hot rainy	10.29	7.67	1.41	1.19
Cool	2.40	2.48	0.31	0.14
Summer	3.17	2.72	1.42	1.26
Cyperus sp.				
Hot rainy	10.83	10.58	2.60	2.44
Cool	2.65	2.07	0.32	0.12
Summer	2.12	1.47	2.46	1.71

S.D.: Standard deviation

Seasonal variation of environmental factors in constructed wetlands: Monthly average of air temperature reached maximum in September (36.7°C) while the lowest mean was found in March (24.7°C). It should be noted that lower air temperature in March was unusual in the area of northeastern Thailand but it occurred in March 2011 during the experiment. Soil temperatures at all constructed wetlands were measured at 5 cm depth. The graphic shown in Fig. 3 illustrates that soil temperatures were in the range of 20-29°C, which was in optimum range for plants and microorganisms to grow (Iasur-Kruh *et al*., 2010) and narrowly changed during three seasons. Soil temperature peaked in December with the average of 29°C and reached its minimum value in January (19.9°C) before it rise again in summer season (March-June).

Average soil pH during the experiments ranged between 6.7 and 8.0, in different constructed wetlands. However, soil pH remained in the optimum range for methanogenic bacteria (Iasur-Kruh *et al*., 2010).

There were differences in soil Oxidation-Reduction Potential (ORP) across the seasons. The lowest soil ORP occurred in January with the average of -258±5 mV whereas the highest soil ORP occurred in May with the average of -127±6 mV.

Seasonal variation of CH_4 gases emissions from constructed wetlands: Methane emissions from all constructed wetlands observed during August and September were noticeable higher than those in other months (Fig. 3). The highest CH_4 emissions from all constructed wetlands with different plants occurred in September with the average of 58.3, 44.9 and 21.6 mg/m^2/h for *Phragmite* sp., *Canna* sp. and *Cyperus* sp., respectively. The lowest CH_4 emissions occurred during December and March. CH_4 emissions from constructed wetlands planted with *Phragmite* sp. and *Canna* sp., were lowest in March with the average of 2.4 and 1.1 mg/m^2/h, respectively, whereas low CH_4 emissions from *Cyperus* sp., occurred in December with the average of 0.9 mg/m^2/h. When seasonal variation was taken into account, CH_4 emissions from all constructed wetlands were highest in hot rainy

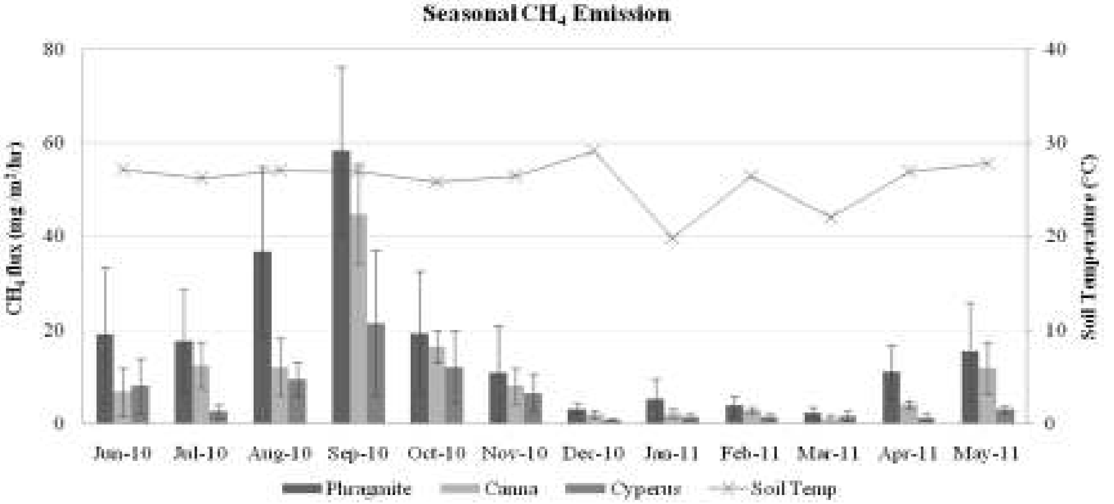

Fig. 3: Seasonal variation of CH_4 emission from constructed wetland planted with *Phragmite* sp., *Canna* sp. and *Cyperus* sp.,

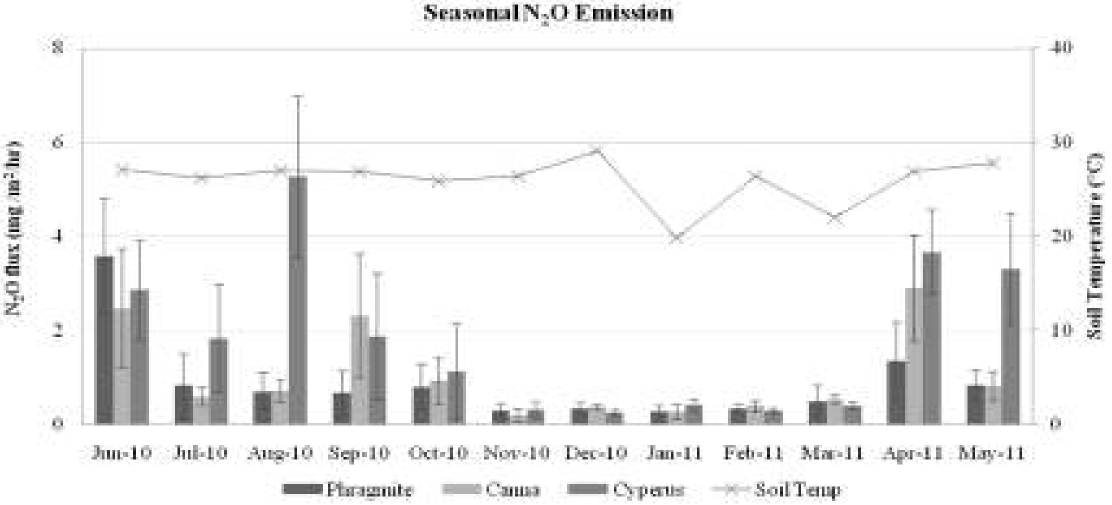

Fig. 4: Seasonal variation of N_2O emission from constructed wetland planted with *Phragmite* sp., *Canna* sp. and *Cyperus* sp.,

season (July-October) with the average of 10.3-19.9 mg/m^2/h and lower in summer season (March-June) with the average of 2.1-6.3 mg/m^2/h and cool season (November-February) with the average of 2.4-3.9 mg/m^2/h. The CH_4 emission was lower in summer season that may because the lower air temperature in March was unusually low indicating that local climate fluctuation was influence CH_4 emissions from constructed wetlands in the area. Seasonal variation of CH_4 emissions is summarized in Table 4.

Seasonal variations of N_2O gases emissions from constructed wetlands: High Nitrous oxide emissions from all constructed wetlands were found during April and October (Fig. 4). The highest N_2O emissions from constructed wetlands planted with *Phragmite* sp., were highest in June with the average of 3.6 mg/m^2/h while high emissions from *Canna* sp. and *Cyperus* sp., occurred in April with the average of 2.9 and 3.7 mg/m^2/h, respectively. The lowest N_2O emission from constructed wetlands planted with *Phragmite* sp. and *Canna* sp., occurred in November with the average of 0.3 and 0.2 mg/m^2/h, respectively. Low N_2O emissions from *Cyperus* sp., occurred in February with the average of 0.3 mg/m^2/h. When seasonal variation was taken into account, N_2O emission from all constructed wetlands were highest in hot rainy season (July-October) with the average of 1.3-2.6 mg/m^2/h, followed by summer season (March-June) with the average of 0.9-2.5 mg/m^2/h and lowest in cool season (November-February) with the average of 0.3 mg/m^2/h.

Influence of plant species on CH_4 and N_2O emissions: Data on CH_4 and N_2O emissions from seasonal study were used to evaluate the influence of plant species within constructed wetlands on greenhouse gas

Table 5: Comparison of CH_4 and N_2O emission from constructed wetland with different plant species using chi-square (χ^2) test (Kruskal Wallis)

		Emission rate (mg/m^2/h)						
GHG/CW		N	Mean	S.D.	Min.	Max.	χ^2	Asymp. Sig. (2-tailed)
CH_4	*Phragmite* sp.	72	11.16	16.10	0.00	113.87	9.88	0.007**
	Canna sp.	72	6.01	6.70	0.00	39.96		
	Cyperus sp.	72	5.92	9.82	0.00	71.56		
N_2O	*Phragmite* sp.	72	0.88	1.17	0.08	6.06	7.06	0.029*
	Canna sp.	72	1.04	1.20	0.02	6.67		
	Cyperus sp.	72	1.80	2.06	0.03	7.32		

*: Significant at the 0.05 level; **: Significant at the 0.01 level; S.D.: Standard deviation; Min.: Minimum; Max.: Maximum

emissions. Descriptive statistics for CH_4 and N_2O emissions are shown in Table 5. Data screening showed that gas emissions were neither a normal distribution nor homogeneity of variance. Thus, chi-square was suitable for differentiating greenhouse gas fluxes among different plant species within constructed wetlands. The means of CH_4 and N_2O emissions from different plants species were compared by chi-square (χ^2) test (Kruskal Wallis). The results showed that the mean of CH_4 and N_2O emissions from different plants species were significantly different ($p<0.05$). Constructed wetlands planted with *Phragmite* sp., emitted the highest CH_4 with the average of 11.2 mg/m^2/h but emitted the lowest N_2O with the average of 0.9 mg/m^2/h. Constructed wetlands planted with *Cannas* sp., emitted CH_4 and N_2O with the average of 6.0 and 1.0 mg/m^2/h. Constructed wetlands planted with *Cyperus* sp., emitted CH_4 with the average of 5.9 and highest N_2O with the average of 1.8 mg/m^2/h.

Estimated Global Warming Potential (GWP) of CH_4 and N_2O emissions from constructed wetland planted with different plants: Comparison of CH_4 and N_2O characteristics in terms of Global Warming Potential (GWP), were corresponded to the average GWP of CH_4 and N_2O at 23 and 296 times of CO_2, respectively (IPCC, 2001). Our estimate showed that the GWP of constructed wetlands planted with *Phragmite* sp., had the average CH_4 and N_2O emissions about 11.2 and 0.9 mg/m^2/h, respectively. These numbers were about 258 and 266 mg CO_2 equivalent/m^2/h (or 524 mg CO_2 equivalent/m^2/h in total). Constructed wetland planted with *Canna* sp., had the average CH_4 and N_2O emissions of 6.0 and 1.0 mg/m^2/h, respectively, corresponded to the average GWP of 138 and 296 mg CO_2 equivalent/m^2/h, for CH_4 and N_2O emissions, respectively (or 434 mg CO_2 equivalent/m^2/h in total).

In the case of constructed wetland planted with *Cyperus* sp., the average CH_4 and N_2O emission were 5.9 and 1.8 mg/m^2/h, respectively. Estimated mean GWP of CH_4 and N_2O emissions were 61 and 325 mg CO_2 equivalent/m/h, respectively, or approximately 669 mg CO_2 equivalent/m^2/h. Therefore, the results indicated that estimated GWP of CH_4 and N_2O emissions from constructed wetland planted with *Cyperus* sp., was the highest, followed by *Phragmite* sp. and *Canna* sp., constructed wetlands, respectively.

DISCUSSION

Average CH_4 and N_2O fluxes from wastewater treatment constructed wetlands, planted with three emergent plants, ranged between 5.9-11.2 and 0.9-1.8 mg/m^2/h, respectively. The average fluxes were comparable to those reported by Inamori *et al.* (2007) which used experimental scale free water surface flow constructed wetland treating non-point sewage at Tsukuba, Japan. However, average N_2O emission was lower than the studies of 2.39 and 3.4 mg/m^2/h reported by Wu *et al.* (2009) and Inamori *et al.* (2007). Seasonal fluctuations of CH_4 and N_2O emissions were observed in this present study. The average CH_4 and N_2O emissions were highest in hot rainy season, followed by summer season and lowest in cool season. However, the pattern of seasonal CH_4 and N_2O emission variations and their relations to environmental factors were not obvious. These may because important environmental factors such as soil temperature, soil pH and soil ORP were in the optimum range for gas production and changed in a narrow range during the experiment. Additionally, CH_4 and N_2O emissions were not the result of one-factor action but of the interaction of more biotic and abiotic factors.

Means of CH_4 and N_2O emissions from different plants species were significantly different ($p<0.05$). Average CH_4 and N_2O emission from constructed wetlands planted with *Phragmite* sp., *Cyperus* sp. and *Canna* sp., were 11.2, 6.0 and 5.9 mg/m^2/h and 0.9, 1.0 and 1.8 mg/m^2/h, respectively. Estimated GWP showed that CH_4 and N_2O emissions from constructed wetlands planted with *Cyperus* sp., was highest (669 mg CO_2 equivalent/m^2/h), followed by *Phragmite* sp., (524 mg CO_2 equivalent/m^2/h) and *Canna* sp., (434 mg CO_2 equivalent/m^2/h), respectively.

For pollutant removal, it was found that constructed wetland planted with different plants had significantly different in efficiency of BOD, COD and NH_3-N removal although TP removal rates indicated no significantly different. The constructed wetland planted with *Phragmite* sp., had the highest removal rate of BOD and COD but had lowest efficiency on NH_3-N removal. *Cyperus* sp., or *Canna* sp., had the higher removal efficiency on NH_3-N. Despite the benefit of constructed wetlands on wastewater treatment, it is important to considered by-product of constructed wetlands in low carbon era. The use of *Phragmite* sp., should be of benefit in reducing GWP from these greenhouse gases when it is used in constructed wetlands for wastewater treatment.

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