ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Seasonal variation of methane fluxes from rice paddy ecosystems of South India

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ABSTRACT

The constructed wet land paddy ecosystem is a major source of atmospheric methane (CH_4), which is currently increasing at 7ppbv yr^{-1}. Methane emission from rice paddies indicates a global source of 60 Tg yr^{-1}. The CH_4 emission rates from rice paddy ecosystems vary significantly with the soil type, cultivar variety and age, water management. The CH_4 emission from wetlands are influenced by physical processes namely diffusion, ebullition and ventilation and biological processes namely microbial production and consumption. Thus in an effort to reduce uncertainties, diel variation of CH_4 fluxes were measured from control, Pseudomonas and Nemento (biopesticide) amended rice cores for 24 hours at an interval of 30 minutes at tillering, reproductive and harvesting stages of plant growth. Further, to understand the effect of pseudomonas and nemento on CH_4 fluxes the results have been subjected to principal component analysis (PCA). Results are discussed in the paper.

KEY WORDS: Anoxic Soils, Methane Emission, Methane Flux, Methanogenesis, Rice Paddies.

1. INTRODUCTION

Methane came into focus of public and scientific interest because of its contribution to global climatic changes (greenhouse effect). Though it is a relatively minor component of the global carbon cycle, it is of great importance because each molecule of methane stays in the troposphere for 8-11 years and approximately traps 30 times as much heat than CO_2 molecule (Lelieveld, 1993). The CH_4 concentration is growing in the atmosphere due to human activities such as rice paddies, animal husbandry, land use, biomass burning, and fossil fuel production and use. Emissions from paddy ecosystems indicate a global source of 60 Tg yr^{-1} with a range between 20 – 100 Tg yr^{-1}. Methane is produced in wetland ecosystems by anaerobic degradation of complex organic matter by microbial community consisting of different hydrolytic, fermenting, acetogenic, syntrophic and methanogenic bacteria (Stams, 1994; Conrad, 1987; Whitman, 1992). The CH_4 emission from rice paddy ecosystem is the outcome of the balance between production, oxidation, transport and nutrient interaction (Zinder, 1993). The expansion of irrigated cultivation area and new cultivation practices have made rice fields one of the most important anthropogenic sources for atmospheric methane (Pingali, 1997; Wassmann, 1993; Minami and Neue, 1994). Rice plants influence CH_4 emission by 1) by providing substrates in the form of root exudates to the anaerobic food chain; 2) transporting CH_4 from the anoxic soil into the atmosphere via the intercellular space and arenchyma systems (Wang and Shangguan, 1996). Additionally, the agricultural soils also influence methane emissions. The soils with high clay content will have poor structure and affect methane emission as they protect organic matter form mineralization, delayed methanogens (Ghosh, 2003). The methanogens are also sensitive to variation in soil pH and their activity is optimum around neutral or slightly alkaline pH (Wang, 1993). Methanogenesis is initiated after the sequential reduction of oxygen, nitrate, manganese (IV), iron (III) and sulfate. (Ponnamperuma, 1972). The reduction process is paralleled by the decrease of redox potential (Eh). The growth of methanogenic bacteria does not depend on the onset of CH_4 production. High concentrations of soil organic matter and high temperature accelerate CH_4 production (Yagi and Minami, 1990). Methane production also increases exponentially with increase in soil temperature (Sexstone and Mains, 1990). Acetate and hydrogen are the predominant precursors in rice paddy soils for CH_4 production (Conrad, 1989; Krumbock and Conrad, 1991). Methane produced in anoxic rice paddy soil reach the atmosphere by three different pathways namely- (a) diffusion across the soil water interface into the flooding water and from there across the water-air interface into the atmosphere, (b) ebullition into the atmosphere after formation of a gas bubbles with sufficient buoyancy, (c) diffusion into the roots followed by transport through the parenchyma of the rice plants into the atmosphere (Figure 1).

The first field measurements were done in California (Cicerone and Shetter, 1981), then subsequently in Spain (Seiler, 1984), Italy (Schutz, 1990), Japan (Yagi and Minami, 1990) and Philippines. The findings of these field experiments drastically lowered the global estimate of CH_4 source strength of rice paddies to about 100Tg yr^{-1} and stressed the importance of the rice plant as a conduit for CH_4 transport form soil to atmosphere. However, this estimate is still very tentative. In India, CH_4 campaign was initiated by NPL, New Delhi in 1991 in collaboration with Indian Rice Research Institute (IARI), New Delhi to estimate the contribution of Indian rice paddies to the global methane budget (Mitra, 2002). A more accurate estimate of the global CH_4 source strength of wetland rice fields is needed, not only to evaluate the impact and cost benefit ratio of mitigating technologies on CH_4 from rice fields but also to reduce the uncertainties in the estimates of other CH_4 sources (Purvaja and Ramesh, 2000). Thus in an effort to reduce uncertainties diel variation of methane measurements were performed from rice cores subjected to different amendments for 24 hours at an interval of 30 minutes during different stages of growth of rice plants. The results are analyzed to PCA to understand the effect of amendments on CH_4 mitigation.

1.1. Study area:

1.1.1. Soil and Cultivar Type: Soils contribute to the global budget of many atmospheric trace gases like CH_4, N_2O by acting either as the source or sinks. The soil type Alfisol was used for methane analysis from rice cores to study the effect of various biological and chemical amendments. The agricultural soils for rice cores studies were collected mainly from Medur located on the suburbs of Chennai, Tamilnadu. The physical and chemical characteristics of Alfisol soil types are shown in Table 1. Medur belongs to Ponneri taluk and is geographically located in 80°13’12” E longitude and 13°22’50” N latitude respectively (Figure 2). The agricultural soils were uniformly spread in the shade and dried for about a week after which it was crushed to a size less than 2mm diameter to give a homogenized sample (Bosse and Frenzel, 1997). The soil homogenized soil was then thoroughly mixed with water in the ratio (2:1 w/w). The cylindrical acrylic cores of height 0.2 m and diameter 0.07 m were filled up to the height of 0.14 m. The soil cores prepared with crushed soils were left undisturbed in the shade for about a week and the water level 0.5- 1 cm is maintained above the soil surface. The rice variety IR 50, a hybrid variety of IR 2153-14 x IR 28 x IR 36 and has the cultivation period of 110 days was used to study the methane flux measurement in rice cores.

2. METHODOLOGY

The IR 50 paddy seeds (approx. 100 numbers) were placed in an air tight moistened cloth for about 12 hours. The excess water was then drained and the seeds were spread on the 3-4 layers of moist seedbed made of soft tissue paper. The rice seeds are germinated on the moistened seed bed and grown undisturbed for about three weeks. The seedlings were removed from the seedbed and transplanted in the experimental cylindrical cores (Figure 3). The details of the amendments, its dosage mode of application is given in table 2. The diel variation of CH_4 was measured on 30, 60 and 90 days in rice cores amended with pseudomonas, bio-pesticides (Nemento), and also from control rice cores without any amendments. The acrylic measurement chamber of standard dimensions is placed over the cores with a solitary rice plant and gas samples were taken using nitrogen flushed gas tight syringe for analysis at regular intervals of 30 minutes for 24 hours. The open end of the Perspex chamber and the core containing the plant is connected so that the air inside the chamber was isolated from the outside atmosphere making the system airtight. The samples were analyzed immediately in Gas Chromatograph (HP-5890) fitted with flame ionization detector (FID) and Porapak Q column, detector temperature was maintained at 60, 100 and 250°C. Respectively with high purity N_2 as a carrier gas and the flow rate 30 ml min^{-1} was maintained during analysis for CH_4 emission. The variations in chamber temperature with time were also observed throughout the experiment. The gas chromatograph was calibrated before and after each set of measurements using CH_4 standards (116 ppmv) in N_2 obtained from Bhoruka gases (99.9% pure) and National Physical laboratories (NPL), New Delhi respectively. A regular check for linearity of gas chromatograph was made with the CH_4 standards of concentration 1195 ppmv and with pure CH_4 standards (100% CH_4) at various volumes (0.1- 1 ml) using a gas tight syringe.

Calculations

$$CH_4\ fluxes\ from\ rice\ cores\ (mg\ \ m^{-2})\ =\ \frac{16*CH_4\ (\mu\ mol)*100}{1000*chamber\ volume}$$

$$CH_4\ (\mu\ mol) = \frac{Chamber\ volume}{Molar\ volume*CH_4\ concentration\ in\ sample\ (ppmv)}$$

$$CH_4(ppmv) = \frac{Standard\ CH_4\ concentration\ of\ sample}{Area\ of\ standard}$$

$$Molar\ Volume = Gas\ Constant*Chamber\ temperature\ (K)$$

$$Chamber\ volume = \pi r^2 h(m^3)$$

Where, $16 = Atomic\ mass\ of\ CH_4$

DISCUSSION

Rice paddies are the important source of the greenhouse gas, namely CH_4. The magnitude of CH_4 emission from rice paddies reflects the balance between methanogens and methanotrophy. The use of bacterial inhibitors on mitigating methane emission is still to be understood and its common practice is by farmers is subjected to technical and economical constraints (Houghton, 1997). In our present study the diel methane emission rates over a period of 24 hours from control, pseudomonas and nemento amended rice cores showed similar patterns at different growing stages and the emission characteristics varied significantly. (Table 3). In general, the CH_4 emission rates increased at accelerated rates and were maximum during the early afternoon (2.59 – 9.35 mg m^{-2}) and decreased rapidly and remained constant during night (8.19 -7.71 mg m^{-2}).

Methane flux at different plant growth stages: At the tillering stage the CH_4 emissions in all the rice cores were lower (2.18 – 7.17 mg m^{-2}) probably due to higher soil Eh during the tillering stage of the plant and also the younger tillers contribute less to methane emission (Table 3). The comparatively low CH_4 flux may be attributed to the under developed microbial population during the initial stages of flooding (Debnath, 1996). At this stage the organic matter breaks down into simple substrates which will be utilized by the methanogens. During the reproductive stage the CH_4 emission is higher (2.59 – 9.50 mg m^{-2}) because of the result of release of root exudates, root lysates and root litter from rice plants (Schutz, 1989; Adhya, 1995; Ghosh, 2003). The root exudates are correlated with the extension of the root mat and might show seasonal variation with a maximum value occurring at the end of the heading and flowering stage. The gradual decrease in methane flux was observed soon after the maturity of the crop (0.98 – 5.04 mg m^{-2}) because the rice plants and root mats start to decay during the ripening stage of the plants. This causes a reduction in the release of root exudates and consequently substrates for anaerobic mineralization and methanogenesis are reduced.

Methane flux during the 24 hour period: In the forenoon the methane emission rates increased at accelerating rates (Table 3) and were maximum during the early afternoon as the light intensity may enhance the emission rates by accomplishing a shift from diffusion driven emission to pressure driven transport by increasing stomatal conductance and increasing photosynthesis coupled methane production rates (Brynes, 1995). The CH_4 emission was lower in night due to low soil temperature and higher ambient CO_2 concentration in the canopy which reduces the CH_4 transport through plants (Pathak, 2003). In the morning up to early afternoon the soil temperature was a major factor driving the CH_4 emission rate, while in the afternoon the CH_4 concentration in soil was the major factor limiting CH_4 emission although soil temperature decreased slowly.

Methane flux from Nemento (Biopesticide) amendment: The CH_4 emission rates decreases in Nemento amended cores than Control cores at all the three growth stages of rice plant (Table 3). The nemento (neem extract-biopesticide) reduces CH_4 production in soil as it can be an effective soil nitrification inhibitor. The use of nitrification inhibitors is being increasingly recommended for rice agriculture to minimize fertilizer N losses (Pathak, 2003). Nitrification inhibitors such as calcium carbide and nitrapyrin have been shown to inhibit CH_4 emission from flooded rice soil (Keerthisinghe, 1993). The use of nitrification inhibitors minimize fertilizer N losses and by limiting the formation of NO_3^- from NH_4^+ (Bharati, 2000). The methane production is linked to a decrease in redox potential and an increase in pH of inundated soils. The application of Nemento probably would have caused high soil redox potential and low soil pH status thereby inhibiting methane production. Secondly the application of nitrifying inhibitor may check the growth of methanogenic population by inhibiting the enzymes like mono oxygenase and cytochrome oxidase involved in oxidation or by binding a metal like copper, which is essential for the activity of monooxygenase.

Methane flux from Pseudomonas amendment: The Pseudomonas amended cores resulted in less CH_4 emission than Control cores (Table 3). In general the bacterial enzyme monooxygenase oxidize the CH_4 formed in the anoxic soil to methanol (Higgins, and Quayle, 1970). In paddy soils, the oxygen consumption by microbial community is dominated by heterotrophic and methanotrophic respiration. The heterotrophs can make use of diverse carbon sources, mainly acetate which are rich in agricultural soils. During the growth of the rice plant the CH_4 availability increases with distance from the rice root while oxygen availability increases with distance from the rice roots (Gilbert and Frenzel, 1998). The heterotrophs (Pseudomonas) present around the rhizosphere consume more oxygen close to the root surface. The pseudomonas being denitrifying bacteria helps reducing CH_4 emission because the denitrification of NO_3^- leads to accumulation of intermediates (nitrite, NO, N_2O) to different extents that are toxic to methanogenic Archaea (Kluber and Conrad, 1998). In anoxic paddy soil, the addition of NO_3^-, Fe^{3+}, and SO_4^{2-} lead to the suppression of methane production. The case of suppression by nitrate has not been as thoroughly studied as that by sulfate.

Principal component (PC) analysis: This is essentially a statistical method that is used to determine components that are linear combinations of the original variables. In this method a set of p correlated variables is transformed to a smaller set of uncorrelated hypothetical constructs called principal components. For this a correlation matrix is used. The first PC is the linear combination of the variables with maximal variance. This represents the largest variability of the original data set. The second component is the linear combination with the next largest variability that is orthogonal to the first component. The correlation matrix is obtained from the above data and it is provided in the Table 4.

The Eigen values can be obtained from characteristic equation which is provided in the Table 4. Thus Nemento amendment contributes 97 % in the tillering stage, 99.7% in the flowering stage and 93 % in the Harvesting stage (Table 4).

Table.1. Physical and chemical characteristics of Alfisol soil

Soil Type	Colour	pH	Sand (%)	Silt (%)	Clay (%)	Organic carbon (%)	Water holding capacity (%)	Moisture (%)
Order: Alfisol Family: Fine loamy Udic Haplustalfs	Dark Brown	6-6.7	50-60	-	30-40	0.08-0.4	-	1.8-2.8

Table.2. Dosage levels of different organic and inorganic amendment

Treatments	Concentration	Possible effects
Pseudomonas	2 ml of culture was applied to rice cores after transplantation	It forms chelated compounds with Fe, which is one of the principal electron acceptors in CH_4 production
Nemento	2 ml of culture was applied to the rice cores after transplantation	Due to the presence of azadiractin and other natural products it may have inhibitory effects on CH_4 emission

Table.3. Diel methane flux in 30, 60 and 90 day rice cores

S.No	Time	Hours	Tillering stage (mg m^{-2})			Flowering stage (mg m^{-2})			Harvesting stage (mg m^{-2})		
			Control cores	Pseudomonas amendment	Nemento amendment	Control cores	Pseudomonas amendment	Nemento amendment	Control cores	Pseudomonas amendment	Nemento amendment
1	8:30	0	2.18	2.18	1.69	2.59	2.42	2.58	0.98	0.82	0.9
2	9:00	0.5	2.4	2.47	1.87	2.59	2.62	2.77	1.43	1.05	0.92
3	9:30	1	2.4	2.63	2.41	2.81	2.62	2.79	1.94	1.36	0.98
4	10:00	1.5	2.52	2.65	2.64	3.53	2.86	3.07	2.48	1.48	1.13
5	10:30	2.0	2.52	2.67	2.82	4.08	3.09	3.11	2.9	1.58	1.3
6	11:00	2.5	2.56	2.76	2.82	5.06	3.65	3.43	3.36	1.67	1.48
7	11:30	3.0	3	2.78	2.95	5.67	4.1	3.98	3.68	1.78	1.51
8	12:00	3.5	3.85	3.22	2.98	5.86	4.56	4.49	4.19	1.88	1.52
9	12:30	4.0	4.55	3.24	2.98	6.56	4.83	4.77	4.61	1.93	1.82
10	13:00	4.5	5.57	3.54	3.01	6.96	5.35	5.18	4.87	2.1	1.85
11	13:30	5.0	6.06	3.92	3.29	7.35	5.69	5.61	4.96	2.42	2.16
12	14:00	5.5	6.39	4.28	3.35	8.2	6.09	5.83	5.21	2.77	2.19
13	14:30	6.0	5.98	4.58	3.79	8.47	6.9	6.31	5.43	3.32	1.91
14	15:00	6.5	6.27	4.61	3.93	8.91	7.24	6.52	5.35	3.55	2.25
15	15:30	7.0	7.17	4.71	4.27	9.35	7.58	6.92	5.2	3.82	2.5
16	16:00	7.5	6.99	5.07	4.39	9.41	7.98	7.3	5.2	4.15	2.72
17	16:30	8.0	6.75	5.47	4.61	9.27	8.12	7.63	5.2	4.19	2.57
18	17:00	8.5	6.47	5.44	4.89	9.21	8.11	7.66	5.71	4.05	2.43
19	17:30	9.0	6.63	5.58	4.85	9.5	8.05	7.74	5.73	4.14	2.82
20	18:00	9.5	7.05	5.42	5.27	9.45	8.12	7.74	5.82	3.99	2.99
21	18:30	10.0	6.45	5.41	5.18	9.26	8.11	7.74	6.58	4.06	3.00
22	19:00	10.5	6.39	5.24	5.14	8.98	8.08	7.7	6.88	4.00	3.07
23	19:30	11.0	6.39	5.55	5.16	8.7	8.09	7.71	6.57	4.04	3.1
24	20:00	11.5	6.7	5.12	5.07	8.61	8.10	7.67	6.64	4.05	3.1
25	20:30	12.0	6.54	4.84	5.31	8.62	7.69	7.6	6.62	4.03	3.06
26	21:00	12.5	6.82	4.84	5.02	8.47	7.67	7.57	6.6	4.01	3.2
27	21:30	13.0	6.32	4.98	4.96	8.36	7.74	7.45	6.62	3.98	3.19
28	22:00	13.5	6.32	4.81	4.69	8.37	7.7	7.29	6.67	3.97	3.22
29	22:30	14.0	6.10	4.64	4.62	8.2	7.45	6.84	6.43	3.83	3.3
30	23:00	14.5	6.47	4.61	4.37	8.2	7.31	6.83	6.44	3.92	3.39
31	23:30	15.0	6.23	4.62	4.10	8.19	6.97	6.79	6.37	3.91	3.45
32	24:00	15.5	6.26	4.48	4.03	8.18	7.09	6.62	6.40	3.87	2.96
33	00:30	16.0	6.21	4.67	4.03	7.95	7.09	6.46	6.28	3.83	3.55
34	1:00	16.5	5.98	4.29	4.03	7.96	7.08	6.49	6.32	3.77	3.34
35	1:30	17.0	5.98	4.35	4.15	7.9	7.06	6.48	6.41	3.86	3.43
36	2:00	17.5	6.17	4.11	3.98	7.71	6.75	6.2	6.33	3.88	3.73
37	2:30	18.0	6.01	4.14	3.65	7.6	6.6	6.19	6.39	3.83	3.71
38	3:00	18.5	6	4.33	3.73	7.61	6..64	6.15	6.14	3.75	3.64
39	3:30	19.0	5.87	3.92	3.71	7.73	6.57	6.15	6.09	3.77	3.5
40	4:00	19.5	5.53	3.91	3.44	7.52	6.56	6.22	5.98	3.73	3.69
41	4:30	20.0	5.46	4.03	3.63	7.29	6.42	5.93	5..90	3.72	3.69
42	5:00	20.5	5.53	3.87	3.57	7.33	6.34	5.92	6.09	3.7	3.63
43	5:30	21.0	5.22	3.72	3.48	7.28	6.35	5.93	6.00	3.67	3.54
44	6:00	21.5	5.39	3.8	3.39	7.04	6.35	5.9	5.91	3.52	3.54
45	6:30	22.0	5.41	3.68	3.4	7.07	6.27	5.74	5.13	3.52	3.22
46	7:00	22.5	5.28	3.6	3.39	6.99	6.1	5.74	5.36	3..57	3.09
47	7:30	23.0	5.18	3.63	3.37	6.86	6.08	5.68	5.11	3.48	3.38
48	8:00	23.5	5.12	3.56	3.32	6.61	5.93	5.58	5.04	3.46	3.14
Minimum			7.17	5.58	5.31	9.5	8.12	7.74	6.88	4.19	3.73
Maximum			2.18	2.18	1.69	2.59	2.42	2.58	0.98	0.82	0.9
Mean			6.005	4.28	3.79	7.815	6.75	6.22	5.82	3.77	3.08
Median			6.39	3.92	4.03	8.2	2.62	7.74	5.2	3.83	3.1
Average			11.02	4.16	3.85	7.40	6.37	6.05	5.35	3.30	5.94
Standard deviation			1.40	0.90	0.90	1.79	1.66	1.49	1.44	0.98	0.85
Standard error			1.26	0.37	0.29	0.87	0.39	0.16	0.64	0.39	0.42
KURT			0.72	-0.62	-0.40	1.57	0.34	0.17	1.93	0.15	-0.50

Table.4. Correlation matrix for Pseudomonas and Nemento amended rice cores

Correlation	Tillering Stage		Flowering stage		Harvesting stage	
	Pseudomonas amendment	**Nemento amendment**	**Pseudomonas amendment**	**Nemento amendment**	**Pseudomonas amendment**	**Nemento amendment**
	1	**0.947143**	**1**	**0.994493**	**1**	**0.872798**
	0.947143	**1**	**0.994493**	**1**	**0.872798**	**1**
	1-2λ+λ^2-(0.947143)^2=0		1-2λ+λ^2-(0.994493)^2=0		1-2λ+λ^2-(0.872798)^2=0	
	1-2λ+λ^2-0.89707=0		1-2λ+λ^2-0.98901=0		1-2λ+λ^2-0.76177=0	
Characteristic equation	λ^2-2λ+0.1029=0		λ^2-2λ+0.0109=0		λ^2-2λ+0.2382=0	
Root of the equation **λ1**	1.9471		1.9945		1,8727	
λ2	0.0524		0.0055		0.1271	
$\lambda_1 + \lambda_2$	1.9995		2.0000		1.9970	
Percentage	97	3	99.70	0.30	93	7

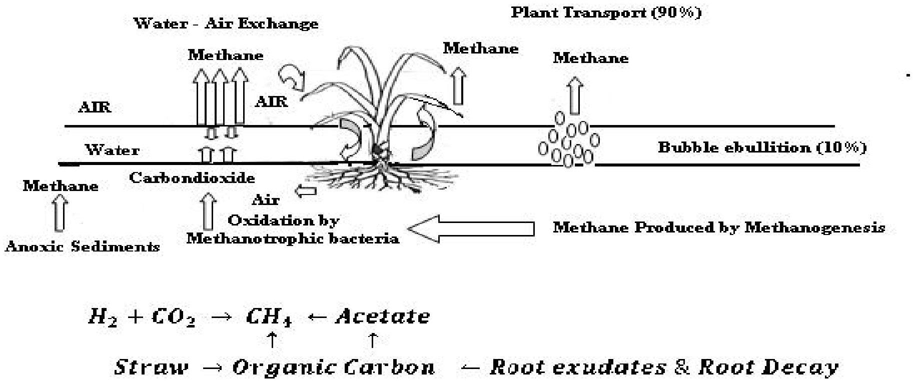

Figure.1. Transport pathway of CH_4 from the anoxic rice paddy soil to the atmosphere

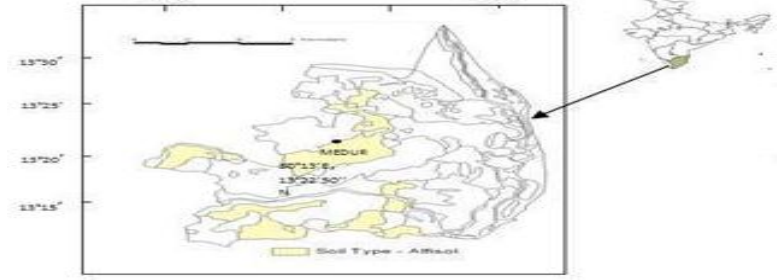

Figure.2.Schematic representation of agricultural soil selected for rice microcosm studies from Medur

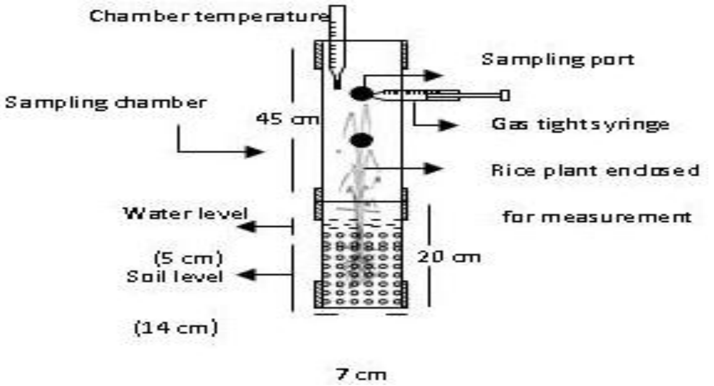

Figure.3. Schematic representation of CH_4 flux measurements in rice cores

4. CONCLUSION

The methane fluxes from rice paddy ecosystem are reduced when it is subjected to nemento amendment. Nemento is a biopesticide obtained from neem tree. Further to understand the effect of this biopesticide the results are subjected to statistical analysis.

5. ACKNOWLEDGEMENT

The author expresses his sincere gratitude to Jawaharlal Nehru Memorial Fund (JNMF) New Delhi for their sponsorship to carry out our research programme. The author expresses his sincere gratitude to Dr R. Rangarajan, Founder & Chairman, Vel Tech group of Institutions for his immeasurable support and encouragement.

REFERENCES

Adhya TK, Rath AK, Gupta PK, Rao VR, Das SN, Parida KM, Prashar DC, Methane emission from flooded rice fields under irrigated conditions, Biol. Fertil. Soils, 18, 1994, 245.

Bharati K, Mohanty SR, Padmavathi PVL, Rao VR, Adhya TK, Influence of six nitrification inhibitors on methane production in a flooded alluvial soil, Nutr. Cycl. Agroecosys., 58, 2000, 389.

Bosse U.P, Frenzel P, Activity and distribution of methane oxidizing bacteria in flooded rice microcosms and in rice plants (*Oryza sativa*), Appl. Environ. Microbiol., 63, 1997, 1199.

Brynes BH, Austin ER, Tays BK, Methane emission from flooded rice soil and plants under controlled conditions, Soil Biol. Biochem., 27, 1995, 331.

Cicerone RJ, Shetter JD, Sources of atmospheric methane measurements in rice paddies and a discussion, J Geophys. Res., 86, 1981, 7203.

Conrad R, Bak F, Seitz F, Thebrath B, Mayer HP, Schutz H, Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment, FEMS Microbiol. Ecol., 62, 1989, 285.

Conrad R, Schutz H, Babbel M, Temperature limitations of hydrogen turnover and methanogenesis in anoxic paddy soils, FEMS Microbiol. Ecol., 45, 1987, 281.

Debnath G, Jund MF, Kumar S, Sarkar K, Sinha SK, Methane emissions from rice fields amended with biogas slurry and farm yard manure, Climate Change, 6, 1996, 97.

Ghosh S, Majumdar D, Jain MD, Methane and nitrous oxide emissions from irrigated rice of North India, Chemosphere, 51, 2003, 181.

Gilbert B, Frenzel P, Rice roots and methane oxidation: The activity of bacteria, their distribution and the microenvironment, Soil Biol Biochem., 30, 1998, 1903.

Higgins IJ, Quayle JR, Oxygenation of methane by methane grown *Pseudomonas methanica* and *Methanomonas methanooxidans,* Biochem. J., 118, 1970, 201.

International Rice Research institute, IRRI, Rice Almanac. 2nd ed., Los Banos, Philippines, 1997, 181.

Keerthisinghe DG, Freeney JR, Mosier AR, Effect of wax coated calcium carbide and nitrapyrin on nitrogen loss and methane emission from dry-seeded flooded rice, Biol. Fertl. Soils, 16, 1993, 71.

Kluber HD, Conrad R, Effects of nitrate, nitrite, NO and N_2O on methanogenesis and other redox processes in anoxic rice field soil, FEMS Microbial Ecol., 22, 1998, 301.

Krumbock M, Conrad R, Metabolism of position labeled glucose in anoxic methanogenic paddy soil and lake sediments, FEMS Microbiol. Ecol., 85, 1991, 247.

Lelieveld J, Curtzen PJ, Bruhl C, Climatic effects of atmospheric methane, Chemosphere, 26, 1993, 739.

Minami K, Neue HU, Rice paddies as the methane source, Climate change, 27, 1994, 13.

Mitra AP, Prabhat Gupta K, Sharma C, Refinement in methodologies for methane budget estimation from rice paddies, Nutr. Cycl. Agroecosys., 64, 2002, 147.

Pathak H, Prasad S, Bhatia A, Shalini-Singh, Kumar S, Singh J, Jain MC, Methane emission from rice wheat cropping system in the indo- Gangetic plain in relation to irrigation, farmyard manure and dicyandiamide application Agriculture, Ecosystems and Environment, 97, 2003, 309.

Pingali PL, Hossain M, Gerpacio RV, Asian rice bowls- the returning crisis, Wallingford, UK, 24, 1997, 29.

Ponnamperuma FN, The chemistry of submerged soils, Adv. Agr., 24, 1972, 29.

Purvaja R, Ramesh R, Natural and anthropogenic methane emission from coastal wetland of South India, Environ. Manage, 27, 2000, 547.

Schutz H, Seiler W, and Conrad R, Influence of soil temperature on methane emission from rice paddy fields, Biogeochem., 11, 1990, 77.

Schutz H, Seiler W, Conrad R, Process involved in the formation and emission of methane in rice paddies, Biogeochem., 7, 1989, 33.

Seiler W, Holzapfel-Pschorn A, Conrad R, Scharfee D, Methane emission from rice paddies, J.Atmos. Chem., 1, 1984, 241.

Sexstone AJ, Mains CN, Production of methane and ethylene in organic horizons of spruce forest soils, Soil Biol. Biochem., 22, 1990, 135.

Stams AJM, Metabolic interactions between anaerobic bacteria in methanogenic environments, Ant. Leeuwenhoek, 66, 1994, 271.

Wang M, Shangguan XJ, CH_4 emission from various rice fields in P.R. China, Theor. Appl. Climatol, 55, 1996, 129.

Wang Z, De Laaune RD, Masscheleyn PH, Patrick WH Jr, Soil Redox and pH effects on methane production in flooded rice spoil, Soil Sci. Soc. Am. J., 57, 1993, 382.

Wassmann R, Papen H, Rennenberg H, Methane emission form rice paddies and possible mitigation strategies, Chemosphere, 26, 1993, 201.

Whitman WB, Bowen TC, Boone DR, In: The Prokaryotes. Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH (eds), Springer Verlag, Berlin, 1992, 716.

Yagi K, Minami K, In: Soil and the Greenhouse Effects Bouwman AF, John Wiley and Sons, New York, 1990, 467.

Zinder SH, In: Physiological ecology of methanogen. J.D. Ferry JD, Chapman & Hall, New York, 1993, 128.