CHAPTER 3

RESEARCH METHODS

3.1 Site Selection

Mae Chaem watershed covers a major part of Mae Chaem district, which is a remote district on the western part of Chiang Mai province (Figure 3.1). It is surrounded by mountainous and located about 116 kilometers west of Chiang Mai city. Mae Chaem was established a district in 1904, previously named as Charng Kerng district, and later changed to Mae Chaem in 1956. Boundary of Mae Chaem watershed, the north is adjacent to Pai district, Mae Hong Sorn province and Samerng district, Chiang Mai province. In the south, it connects to Hord district, Chiang Mai province. Samerng, Sanpatong and Jormtong district of Chiang Mai province are adjacent to the east of Mae Chaem. The west of Mae Chaem watershed is adjacent to Khun Yourm, Mae Lar Noi, and Mae Sariang district of Mae Hong Sorn province. The majority of Mae Chaem watershed's 3,344 square kilometers is mountainous and covered by various forest types about 70%, with foothill flat area of 20% and the rest plain area occupies 10%. Elevation ranges from 500 to 2,565 meters above mean sea level. Mae Chaem district consists of 7 sub-districts and 82 villages. The populations are northern Thai and hill tribes including Karen, Hmong, and Lawa. The majority of hill tribe is Karen (Decision Support System in Agricultural Research Unit, unpublished; Intrasuksri, 1983).

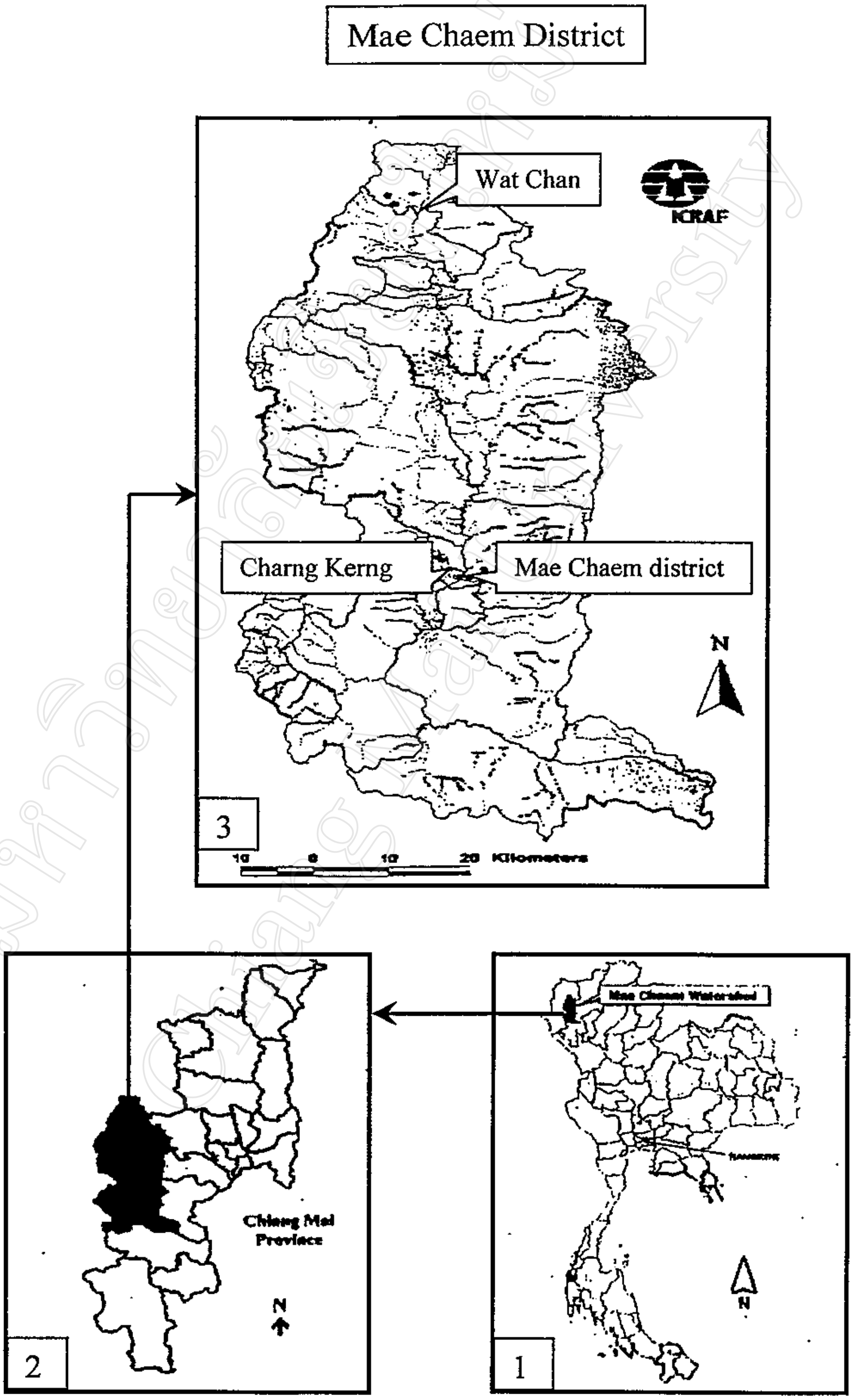

Figure 3.1 Location of Mae Chaem district, (1) map of Thailand, (2) map of Chiang Mai province, and (3) boundary of Mae Chaem district (ICRAF, 2000 unpublished; Salakit, 1997).

3.2 Land Use Types in Mae Chaem Watershed

The selected land uses included two paddy fields, two deciduous forests, one hill evergreen forest, three fallow fields cultivation (one-year, four-year and seven-year fallow fields), one maize field area, one orchard field, and one pine forest (Figure 3.2). Each land use has unique characteristics, with different disturbances and activities.

3.2.1 Paddy Fields (RF1 and RF2)

Two rice fields were selected, one in Baan Nong Jed Noui (RF1), Wat Chan sub-district and another in Baan Bon Na, Charng Kerng sub-district (RF2). Rice field in Wat Chan (RF1), gas sampling point was located at 19° 4′ 15.5″ N and 98° 15′ 53.3″ E, with an elevation of 950 meters above mean sea level. This paddy field locates on small foothill plain area, with about 50-100 meters wide between gentle hills. There is a natural canal with running water throughout the year. Farmer grows rice only in the rainy season, they normally transplant in July and harvest in October. The traditional rice varieties are common because of their good environmental adaptation and consumption quality. Pumpkin, which introduced by the Royal Project, is also grown after rice harvesting, therefore cropping system is Rice-Pumpkin-fallow, after they harvest pumpkin they leave the field until the next rice growing season. There is no chemical fertilizer application during rice growing season. The average yield of paddy rice and pumpkin is 2,500 and 6,040 kg ha^{-1}, respectively (Ekasingh *et al.*, 1999).

Paddy field in Baan Bon Na, Charng-Kerng sub-district (RF2), gas sampling point was located at 18° 30′ 20.9″ N and 98° 20′ 45.2″ E, with an elevation of 533 meters above mean sea level. This paddy field is a large flat area compared to the rice field (RF1) in Wat Chan. It is a flat area along the side of Mae Chaem River. Farmers grow only one rice crop per year in the rainy and fallow in the dry season. They used are RD6 and RD10 rice varieties, which are recommended varieties.

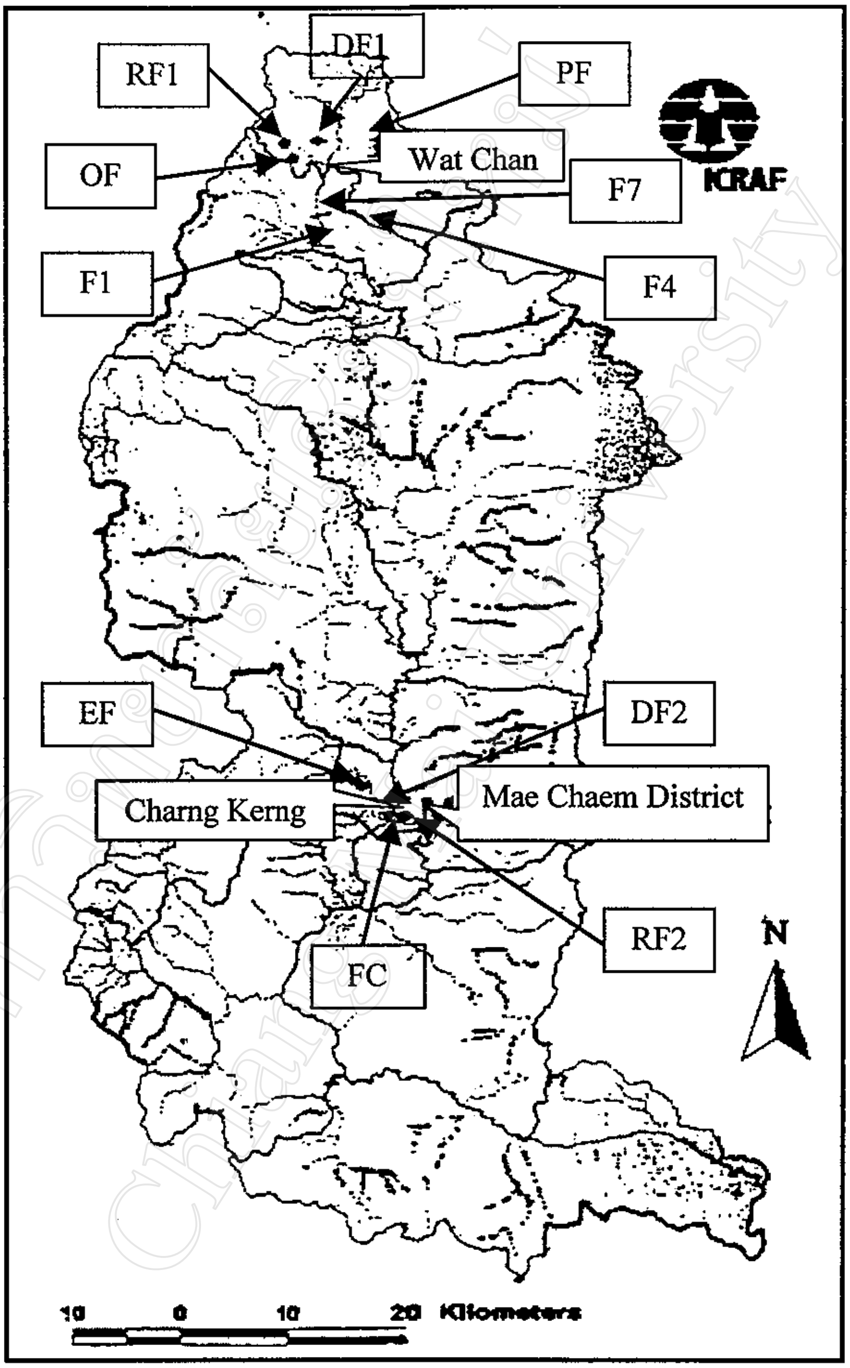

Figure 3.2 Location of eleven selected land uses in Mae Chaem watershed
Note:
RF1= rice field in Wat Chan sub-district, RF2 = rice field in Charng Kerng sub-district, DF1 = deciduous forest in Wat Chan sub-district, DF2 = deciduous forest in Charng Kerng sub-district, EF = hill evergreen forest, F1, F4 and F7 = one-year, four-year and seven-year fallow field cultivation, respectively, FC = field crop area OF = orchard field and PF = pine forest, (ICRAF, 2000 unpublished)

RD6 is a photosensitive variety, harvesting date is around the third week of November, while RD10 is a non-photosensitive variety, and growth duration is about 130 days after planting. Farmers begin to grow rice in late July, transplanting method is still used even labor shortage. Chemical fertilizer is normally applied during rice vegetative phase. Rice is harvested in late November to mid December. If there is enough water supply in dry season, soybean is one additional crop after rice. Fields adjacent to Mae Chaem River, farmer may grow sweet corn, onion or others vegetables for their home consumption and sell extra products to the local market in the dry season.

3.2.2 Deciduous Forest (DF1 and DF2)

Two sites of deciduous forest were selected, the first one is in Wat Chan sub-district (DF1). The sampling point was located at $19^{\circ}\ 4'\ 21.5''$ N and $98^{\circ}\ 18'\ 48.6''$ E, with an elevation of 1,001 meters above mean sea level and the aspect is south facing with a steep slope. The area is covered with deciduous trees, even though it is much lower density of tree species than the hill evergreen forest (EF), but it is very rich in undergrowth species, which become good materials for fire in the dry season. The second one was located in Charng Kerng sub-district (DF2), the sampling point was located at $18^{\circ}\ 31'\ 17.6''$ N and $98^{\circ}\ 18'\ 29.2''$ E, with an altitude of 1,039 meters above mean sea level and the aspect is north facing with a steep slope. This site has a higher upper canopy and lower undergrowth species density than the Wat Chan site (DF1), but fire still occurs in dry season, because of high defoliation biomass.

3.2.3 Hill Evergreen Forest (EF)

The point for taking gas samples in the hill evergreen forest was located in Charng Kerng sub-district at $18^{\circ}\ 31'\ 8.8''$ N and $98^{\circ}\ 17'\ 27.4''$ E, with an elevation of 1,219 meters above mean sea level. The aspect is north facing with a steep slope. It is species-rich plot, crown cover of the upper canopy trees and undergrowth is dense. Soils are deep, with brown to dark color. However soil surface was periodically disturbed by livestock (cattle) and run off after heavy rains.

3.2.4 Fallow Fields Cultivation (F1, F4 and F7)

Three fallow fields were selected, which are one-year, four-year, and seven-year fallow fields in Wat Chan sub-district. The sampling point of one-year fallow (F1) was located at 19° 3′ 29.4″ N and 98° 16′ 25.6″ E. While the four-year fallow (F4) field was located at 19° 3′ 29.2″ N and 98° 16′ 31.4″ E, and the seven-year fallow (F7) was located at 19° 3′ 30.8″ N and 98° 16′ 27.2″ E. All sites are in the same altitude of 984 meters above mean sea level. The original vegetation was pine forest, upland rice was planted after deforestation. The traditional upland rice varieties have been used for many years. The cycle of fallow rotation depends on number of plots each farmer has.

3.2.5 Field Crop Area (FC)

The selected field crop area was located in Charng Kerng sub-district. The gas sampling point was located at 18° 31′ 10.1″ N and 98° 20′ 0.4″ E, with an altitude of 700 meters above mean sea level. The area occupies low to middle terrace adjacent to a rice field. Soil in the area is relatively shallow, with some granular rocks and a steep slope. Its aspect is north facing slope. Seed maize is the major field crop in the area, introduced by a private company. Land preparation is done by household's labors, and hoe is the most common tool for the task since the constraint of high slope. The farmer can grow only one crop per year during June to September, which depending on amount and distribution of rainfall.

3.2.6 Orchard Field (OF)

The selected orchard field was located in Wat Chan sub-district. Gas sampling point was located at 19° 4′ 11.3″ N and 98° 15′ 56.5″ E, with an altitude of 980 meters above mean sea level. The area occupies low terrace to the top of a gentle hill, with a north facing aspect. The vegetation grown in the field was introduced by the Royal Project such as peach, plum, avocado, and Japanese apricot. Temperate vegetables have been grown in between orchard trees, e.g., red cabbage, sweet pepper, pumpkin, parsley, and spinach.

3.2.7 Pine Forest (PF)

Pine forest occupies the top of a gentle hill in Wat Chan sub-district. The coordinate of gas sampling point was located at 19° 4′ 24.9″ N and 98° 17′ 35.9″ E, with an altitude of 1,054 meters above mean sea level and a south facing slope. The majority of vegetation is pine species, e.g., *Pinus merkusii* and *Pinus kesiya*. Sangchyoswat (1998) reported that, in 1996, Wat Chan sub-watershed covered by 4,480 patches of pine forest, with a total of 3,763 ha, equivalent to 30% of the sub-district area. The average patch size is 0.84 ha.

3.3 Research Materials

Materials used in this research consisted of both field and laboratory materials. Field materials were tools for collecting gas samples and measuring auxiliary data. Laboratory materials were components of the Gas Chromatography system and computer software and hardware to develop dynamic models.

3.3.1 Field Materials

- Gas sampling boxes (Chambers)
- Syringes and needles
- Gas sample containers
- Others accessories, i.e., digital thermometer, timing clock, silicone, and plastic bags.

Gas Sampling Boxes (Chambers)

There are two types of gas sampling chambers used in non-flooded and flooded conditions. The first type was used for collecting gas samples in non-flooded soils (including rice field in the dry season). It has been used in the research on

Alternative to Slash and Burn (ASB) sites method. It is made of PVC tubes with 0.2028 m external diameter and a height of 0.10 m. The chambers are not vented and equipped with sampling port fitted with a teflon septum on top of the chambers (Figure 3.3). In order to ensure the minimal disturbance to the soil, with some litter cover, chamber base is needed. During incubation period, the chamber was placed on the base, which is made of metal, insert about 20-30 mm in the soil. It has grooved on the rim, which can be filled with water to ensure a good sealing when the chamber is placed on it. (Palm *et al.*, 1998).

Figure 3.3 The chamber used to collect gas samples in non-flooded soil condition.

Another type of chamber was used in flooded soil condition, designed by IRRI (International Rice Research Institute, Figure 3.4). It was made of Plexiglass, with overall dimension of 0.60 m x 0.60 m x 0.60 m (height x length x width). The top of the chamber is not vented and equipped with a sampling port and a ventilation port. There was a 12 VDC blower accessory (an electric fan) for mixing gas inside the chamber before taking gas sample (Quiamco, 1996).

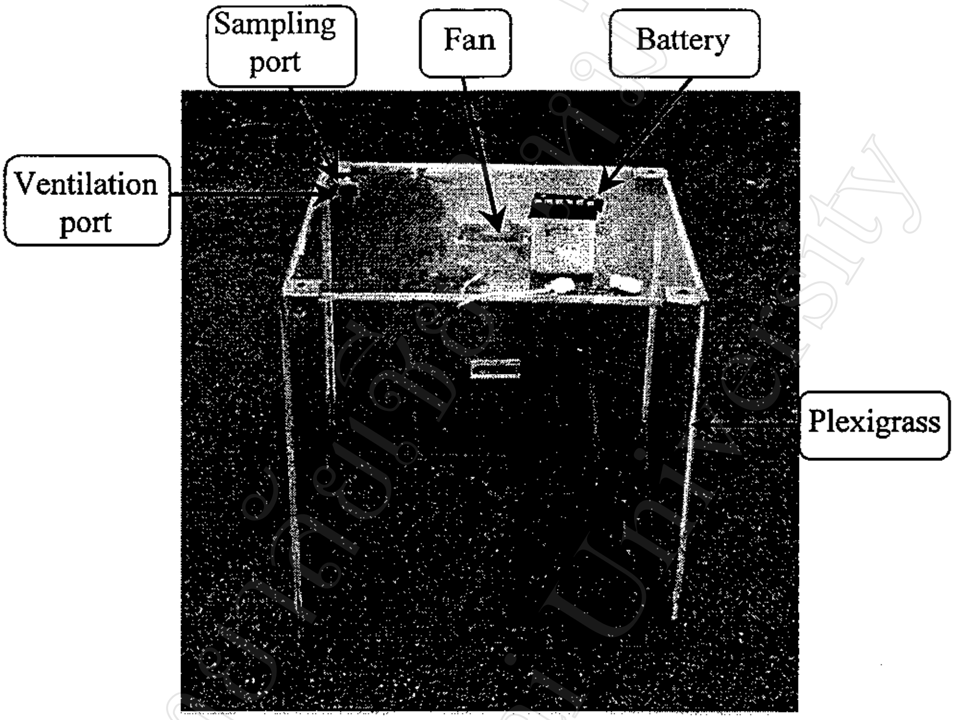

Figure 3.4 The chamber used to collect gas samples in flooded soil condition.

Syringes and Needles

Syringes and needles were used for sucking air to evacuate gas sample containers and for sampling gas samples from gas sampling chambers before injecting into sample containers (Figure 3.5). Syringe volume is 20 ml and needle size is 0.55 x 25 mm. Syringe and needle size is very important. Sampling of gas is more difficult to handle if syringe size is bigger or smaller than this size. Bigger needle may damage the teflon septum of sampling port of the chamber and the gas sample container cap including the septum of the injection port of the Gas Chromatography.

Gas Sample Containers

Screw-cap vials were used to store gas samples. The vials were fabricated from clear Pyrex glass, and featured a combination of open-hole cap and septum to ease preservation and recovery of gas samples (Figure 3.5). Its internal capacity is 16 ml. Evacuation of gas sample containers is necessary before putting gas samples. After vials were closed, inside ambient air was sucked three times using the syringe and then immediately sealed on the septum to avoid leaking.

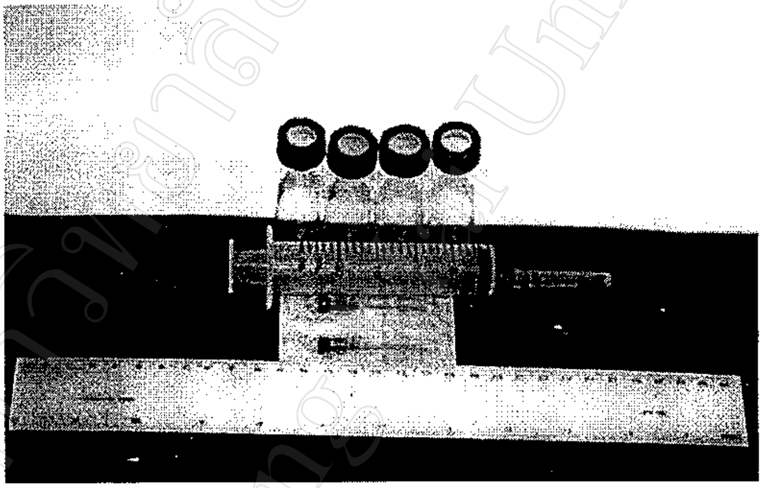

Figure 3.5 Gas sampling materials

Other Accessories

Soil and air temperature were determined by a digital thermometer (Figure 3.6), while time setting for taking gas samples was set by a timing clock. The time was set to be four different taking time steps in each replication (three replications) 0, 10, 20 and 40 minutes after setting chambers. Both chambers and gas sample containers need to be sealed by silicone to prevent leaking. While plastic bags, markers, sticker numbers were necessary for smooth running of research works.

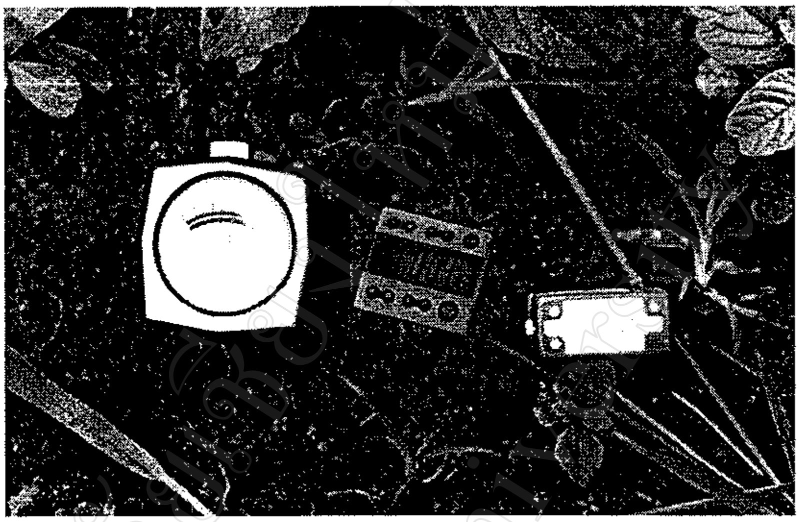

Figure 3.6 Accessories for collecting auxiliary data

Note; (1) tensiometer was used to measure soil moisture content, (2) digital timing clock was set the time for taking gas sample and (3) digital thermometer measured air and soil temperature.

3.3.2 Laboratory Materials

Materials used in the laboratory were tools for analysis concentration of methane gas in gas samples. The materials included Gas Chromatography (Injection port, Column, and Flame Ionize Detector), Recorder or Integrator, carrier gas (nitrogen, N_2), hydrogen (H_2), compress air (pump) and standard methane (known methane concentration).

Gas Chromatography and Accessories

Gas Chromatography (GC, model GC-8A, Shimadzu CO., Inc. Japan) was the machine used to separate the components of gas sample (Figure 3.7). The components of GC are injection port, column, Flame Ionize Detector (FID) and electrometer (Figure 3.8). Injection port was the point where the samples were

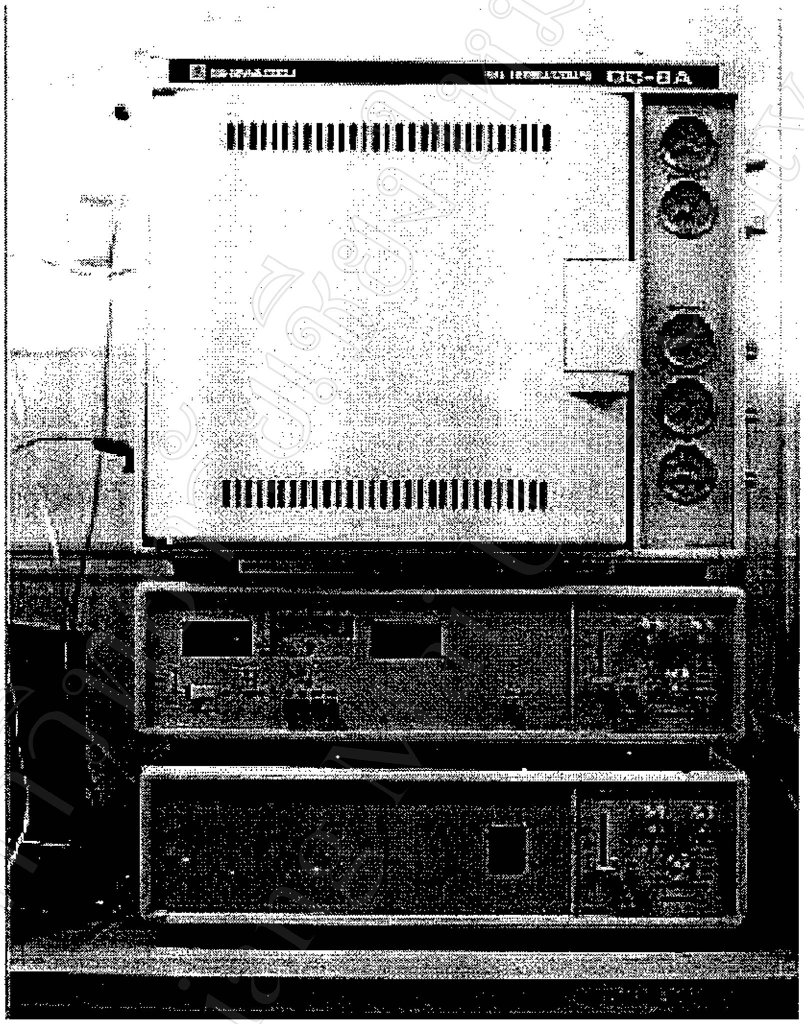

Figure 3.7 Gas Chromatography (model GC-8A, Shimadzu CO., Inc. Japan)

Note: The GC was provided to analyze gas samples by the collaboration of Prachin Buri Rice Research Center, Rice Research Institute.

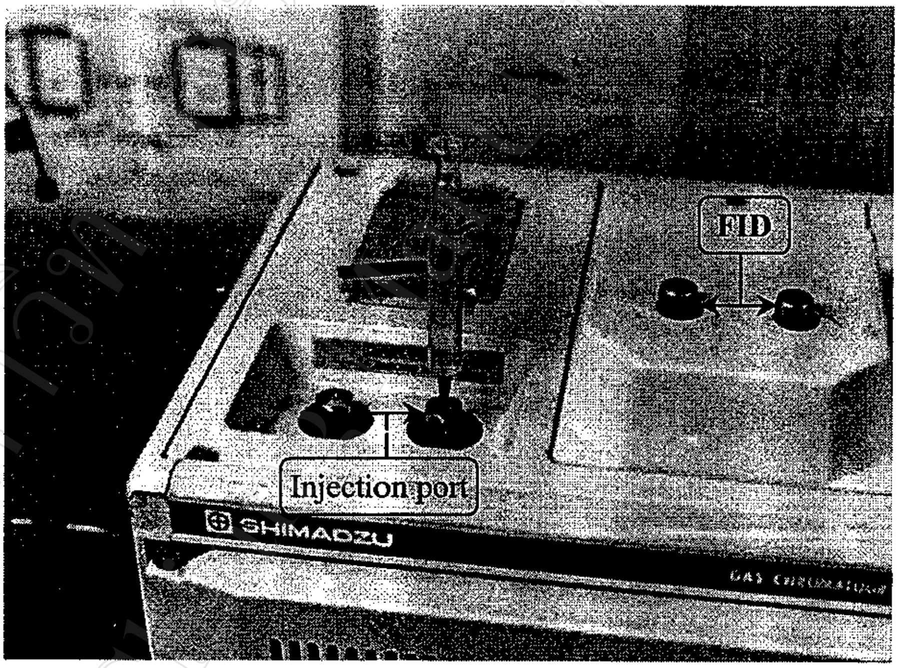

Figure 3.8 Injection port and FID of GC-8A

introduced into the GC system. The temperature of the injection zone must be slightly higher than the oven temperature so those samples will be instantaneously vaporized upon injection. But temperature should not be so high as to cause gas decomposition. Two common methods for injecting the gas samples into GC system by hand and automatic injection, hand injection is handle in this research.

There are two types of column, packed and capillary columns. Metal packed column is used in this research. Column acts as sample's components separator. The components of sample are separated by the principle of selective adsorption and stationary phase.

Flame Ionized Detector (FID) is a type of detector, which sensitive to types of carrier gas used and to carrier flow rate. FID is one of the most popular detectors for routine of organic and flammable compounds. The sensitive of FID to organic compounds is roughly proportional to the number of carbon atom. The FID signal depends on the efficiency of the flame (flame temperature), which determined by the ratio of hydrogen and air mixture. FID has a good sensitivity, wide range of application, and it is very reliable. The signal from FID is collected by electrometer and sent to recorder to be further converted to methane peak. Recorder reports the results as peak areas of difference gases.

Gases Used in the GC System

Three gases were used to run the GC system, consisting of carrier gas (nitrogen, N_2), hydrogen (H_2) and ambient air (from pumping). Carrier gas carries gas sample from injection port via column before flamed in FID with flow pressure 0.118 MPa. Hydrogen and air mixture was used for flame in FID to burn gas samples. Flow pressures of hydrogen and air were 0.049 and 0.039 MPa, respectively.

3.4 Methods

3.4.1 Methane Emission Measurement

Gas Sampling

Gas samples were monthly undertaken from eleven land uses in Mae Chaem watershed throughout the year of 1999. Three replications with four time steps of gas samples were collected from each land use type by using gas-sampling chambers. Four gas samples were taken from each replication at time 0, 10, 20 and 40 minutes after setting the chamber. Eighteen milliliters of gas samples were withdrawn from the chamber and injected into pre-evacuated gas sample containers. Taking into account of the variability within each land use type, each chamber (or each replication) was positioned about 20 – 30 m apart from each other to obtain the best representative samples. Gas samples were transported and analyzed by using Gas Chromatography in Prachin Buri Rice Research Center (PRRC), Ban Sang Prachin Buri province.

Methane Gas Concentration Analysis (at PRRC)

Injection Techniques

Gas samples were analyzed by the GC system (Shimadzu CO., Inc. Japan). For the efficient analysis, appropriate temperature of column and detector was set to 50 and 100 °C, respectively. Carrier gas pressure influences on injection, high pressure may cause leaking of septum of the injection port and difficult to inject. After setting GC system, standard gases were injected, follow by the samples with site by site. Three millimeters of gas sample was withdrawn from gas sample container and immediately injected into the GC system via injection port through column and burned in FID. The priority of gas samples injection in each site was ambient air, and then the samples at time 0, 10, 20 and 40 of replication one fallow by replication two and three, respectively.

Parameters Setting

Actually, there was not only methane peak that comes out from the GC system, but also ghost peaks (non-methane peaks). Analysis parameters file in the recorder must be defined to eliminate those ghost peaks. Parameters to be set were Atten, Width, Minimum area, Slope, and Speed. Atten parameter was used to controls peak size. The Atten adjusting scale ranged from 1 to 10. Low Atten scale gives long peak, and the appropriate Atten used was 6. Width is the Width of the peak at half-height. The scale was also ranged from 1 to 10, and the optimum was 5. Minimum area eliminates peak areas, which were smaller than methane peak. For example, if the area of methane peak was greater than 2,500, so that the minimum area was set at 2,500 to discard unneeded peaks. Slope was set to separate peaks and Speed was the movement of recording paper.

After selecting gas-sampling sites, taking gas samples, and setting of GC system, then three milliliters of a gas samples were systematically injected into the GC system. Gas sample was carried by the carrier gas (Nitrogen) via injection port, column and burnt at Flame Ionization Detector (FID). Electrons from the burning sample was collected by the electrometer and transformed into gas signal. The signal was converted into the specific peak and reported as peak areas by the recorder. Methane peak areas were further calculated into methane emission rates.

3.4.2 Methane Emission Calculation

Results from the recorder reported as peak areas (Table 3.1), these areas must be calculated into methane concentration (Table 3.2) base on standard (known concentration methane peak) and obtained methane concentration of each time step of gas samples were further converted into slope. The slope of each sampling site in each month was calculated to be methane emission rate with unite of milligrams methane per meter square per hour (mg CH_4 m^{-2} h^{-1}).

Table 3.1 Identification of a report from recorder

Rep	TIME	AREA	CONC
1	0	12939	1.7253
1	10	10846	1.4462
1	20	10234	1.3646
1	40	8781	1.1708
2	0	13536	1.8049
2	10	11525	1.5367
2	20	11572	1.5430
2	40	10693	1.4258
3	0	12016	1.6022
3	10	12196	1.6262
3	20	11945	1.5927
3	40	10349	1.3799

Source: Data from seven-year fallow sub-system in Wat Chan sub-district, July 1999

Table 3.2 Average and Standard Deviation (SD) of methane concentration from three replications

Time	Average	SD
0	1.7108	0.1021
10	1.5364	0.0900
20	1.5001	0.1199
40	1.3255	0.1359

Source: Data from seven-year fallow sub-system in Wat Chan sub-district, July 1999.

Slope Calculation

Slope of methane production or consumption curve was calculated by using the change of concentration and the sampling time steps using the following equation:

$$Y = a + bx \tag{3.1}$$

Where; ***Y*** is the gas concentration, and the independent variable ***X*** is the time of measurement. Methane concentrations are regressed with times and the slope of the equation also known as regression coefficient (***b***), correspond to the change of gas concentration with time (*dc/dt*) and ***a*** is *Y* axis intercept which is the methane concentration of ambient air (1.72 ppm). The equation 3.2 shows slope calculation from *X* and *Y* variables via several steps (Table 3.3).

$$b = \frac{\sum x_i y_i}{\sum x_i^2} = \frac{\sum x_i y_i - (\sum x_i)(\sum y_i)/n}{\sum x_i^2 - (\sum x_i^2)/n} \tag{3.2}$$

Table 3.3 Slope calculation of methane concentration and sampling time steps

	Time	*Conc.*	*Deviation from means*		*Square*		*Product*
	X_i	Y_i	x_i	y_i	x_i^2	y_i^2	$x_i y_i$
	0.0000	1.7108	-17.5000	0.1926	306.2500	0.0371	-3.3703
	10.0000	1.5364	-7.5000	0.0182	56.2500	0.0003	-0.1363
	20.0000	1.5001	2.5000	-0.0181	6.2500	0.0003	-0.0452
	40.0000	1.3255	22.5000	-0.1927	506.2500	0.0371	-4.3353
Total	70.0000	6.0729	0.0000	0.0000	875.0000	0.0749	-7.8871
Mean	17.5000	1.5182					

Note; 1. The table was developed from Snedecor and Cochran (1987).

2. Data from seven-year fallow system, Wat Chan, July 1999.

3. X is the time after setting the chamber, and Y is the methane concentration.

$$b = \frac{-7.8871}{875} = -0.009$$

$$a = (\bar{Y} - b\bar{X}) = 1.5182 - (-0.009 * 17.500) = 1.676 \qquad (3.4)$$

Calculation of r

$$r = \sum_{i=1}^{n} x_i y_i \Big/ \sqrt{(\sum_{i=1}^{n} x_i^2)(\sum_{i=1}^{n} y_i^2)} \qquad (3.5)$$

$$r = -7.8871 / \sqrt{875 * 0.0749} \qquad (3.6)$$

$$r = -0.9744 \qquad (3.7)$$

The result of calculation is that;

$$Y = -0.009X + 1.676 \quad (3.8)$$

The seven-year fallow site in July 1999, the negative slope (*b* or *dc/dt*) and *r*-value indicate that a tendency of methane concentration and the time of measurement is negative relationship. It means that methanogenic bacteria are using limited methane in the chamber for their activity causing reduction of methane concentration in the box. The rate of decrease is 0.009 ppm per minute.

Slope (dc/dt) of methane emission rate can be both positive and negative. For instant, if methane concentration at time 0 is lower than the concentration at 40 minutes after setting gas-sampling box, the slope will be positive, because the concentration increases overtime. Vise versa, if methane concentration at time 0 is higher than the concentration at 40 minutes after setting gas-sampling box, the slope will be negative, because the concentration was decreased (Figure3.9).

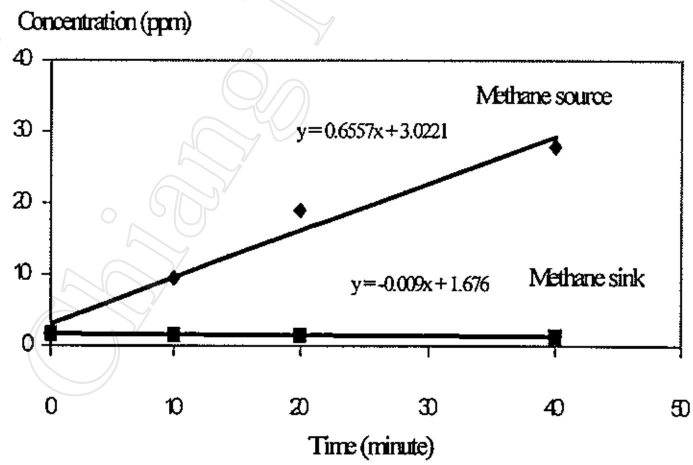

Figure 3.9 Classification of methane production (source) and consumption (sink)
Source: Methane source was the methane production in Wat Chan rice field, while the methane sink was the methane consumption in seven-year fallow field cultivation. The samples from both sites were collected in July, 1999.

Methane Emission Rate Calculation

Obtained slopes from the calculation are further calculated into the rate of methane emission. Variation of methane emissions are quantified by using flux rate formula as follows (Quiamco, 1996);

$$E = \frac{dc}{dt} x \frac{(h)(M_w)(T_{st})}{(W_v)(T_{st} + T)} \tag{3.9}$$

Where:

E	= methane emission/uptake; mg CH_4 m^{-2} h^{-1}
dc/dt	= change of gas concentration; ppm h^{-1}
h	= effective height of chamber; m
Mw	= molecular weight of methane gas = 16.123 x 10^3 mg
Mv	= molecular volume of methane gas = 22.41 x 10^{-3} m^3
T	= temperature; °C
Tst	= standard temperature = 273.2 °K

Methane emission from the seven-year fallow sub-system in July 1999 is calculated as follows;

$$E = (-0.009 x 10^{-6} x 60) x \frac{(0.1)(16.123 x 10^3)(273.2)}{(22.41 x 10^{-3})(273.2 + 22)} \tag{3.10}$$

E = -0.0358 mg CH_4 m^{-2} h^{-1}

Minus sign in front of methane emission rate means that there was methane consumption in the area at the rate of 0.0358 mg CH_4 m^{-2} h^{-1}. After setting the chamber, there was no aeration between inside and outside chamber, therefore, methane concentration in the box depended on activities of methanotrop in the soil that was covered.

3.4.3 Modeling of Methane Production and Consumption

Stella program (High Performance Sytems, Inc., 1994) was employed to construct simple methane production and consumption models (Figure 3.10; Figure 3.11). The methane production model was constructed base on the function of the rice leaf area index (LAI), amount of absorbed Photosynthesis Active Radiation (PAR), quantity of rice root exudate, soil organic matter and decomposable fraction. The rice root exudate and soil organic matter were used to estimate methane produced by methanogenic bacteria under a given environment, e.g., soil pH, Eh, temperature, and water depth. A part of methane was oxidized at rice rhizosphere before emitting into the atmosphere.

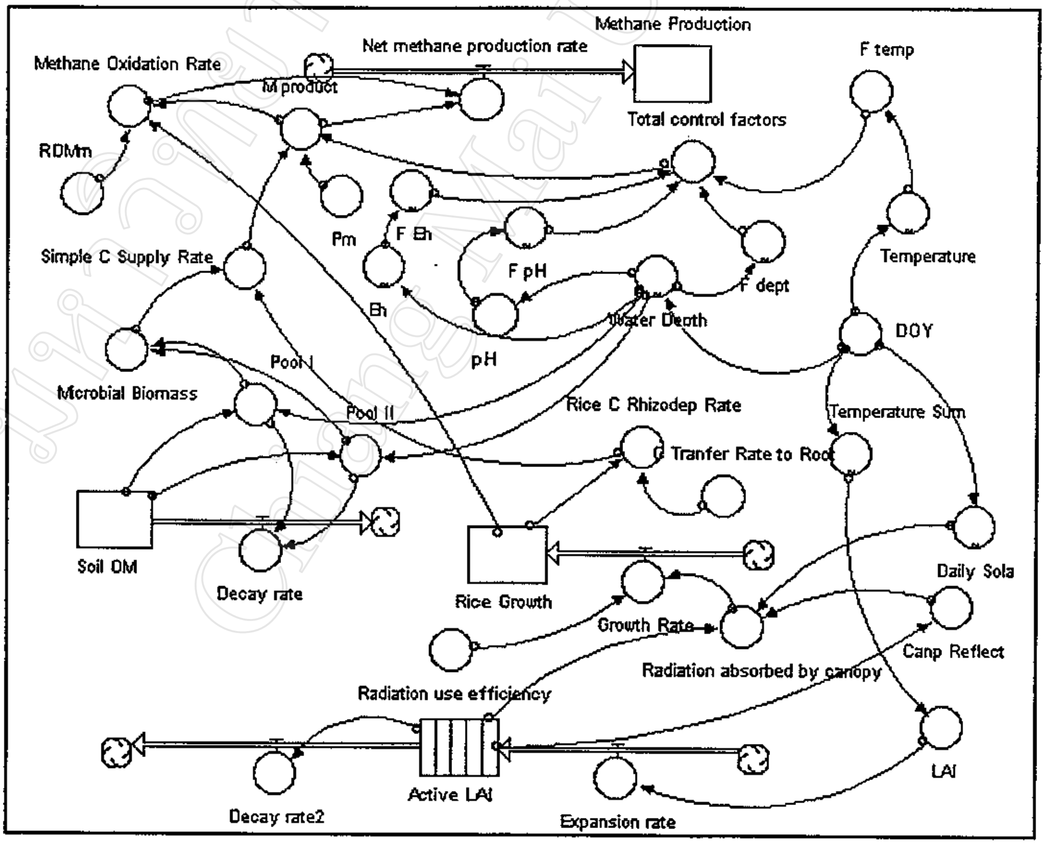

Figure 3.10 Model of methane production in paddy soils.

From the Figure 3.10, methane production is controlled by the function of rice leaf area, which absorb PAR for generating growth rate and root exudate, which was carbon source for methane producing. Soil organic matter was another carbon source for methane producing via non-methane producer biomass, which firstly used the organic material. Efficiency of methane production depends on a given physical environment, soil pH, Eh, water depth in rice field, and temperature. There was a sub-model to simulate methane oxidation at rice rhizosphere to reduce primary methane production before emitting.

The methane consumption model was constructed from functions of soil environment and soil organic matter under atmospheric O_2 and CH_4. The environment factors comprise of soil pH, temperature, and soil moisture (Figure 3.11).

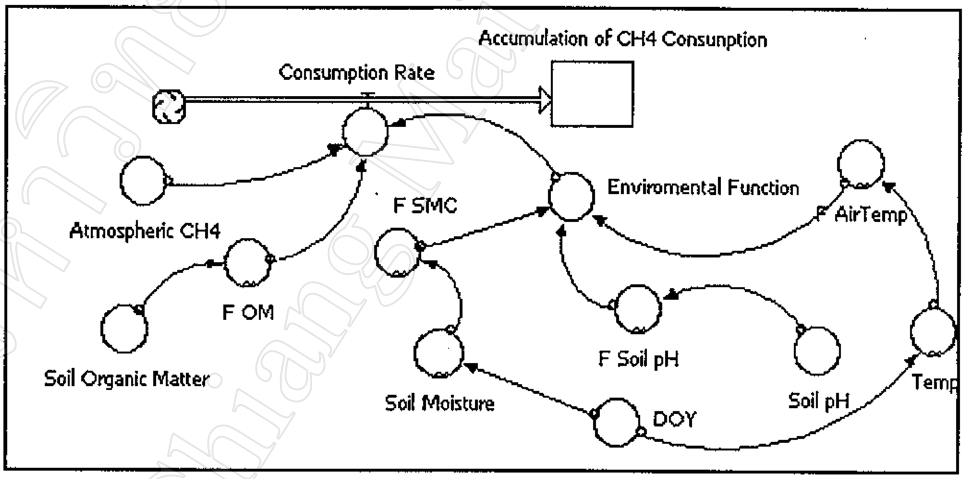

Figure 3.11 Model of methane consumption in non-flooded soils.

Stella program was used to simulate methane consumption in non-flooded soil. Atmospheric concentration of O_2 and CH_4, and soil organic matter were input of the model. Methane consumption controlling factors included, soil moisture content, soil pH, and air temperature.

3.4.4 Auxiliary Data Collection

Data of factors affecting on methane emission were collected. Related factors were weather and soil data. Weather data was collected by manual measurement, when taking gas samples and by secondary weather data collection from the Royal Project and Mae Chaem Watershed Research Station. The data comprised of minimum/maximum air temperature, rainfall. Soil profile descriptions were made couple with taking soil samples for soil physical and soil chemical characteristics analysis by collaboration of Department of Soil Science and Conservation, Faculty of Agriculture, Chiang Mai University.