STUDY OF THE EMISSION OF METHANE FROM THE RICE FIELDS AND WETLANDS OF BANGLADESH AND ITS INHIBITION BY ORGANOMETALLIC COMPLEXES SYNTHESIZED FROM NEEM TREE LEAVES EXTRACTS

BY
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Department of Chemistry
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DECLARATION CERTIFICATE

Certified that the work incoportated in this thesis "STUDY OF THE EMISSION OF METHANE FROM THE RICE FIELDS AND WETLANDS OF BANGLADESH AND ITS INHIBITION BY ORGANOMETALLIC COMPLEXES SYNTHESIZED FROM NEEM TREE LEAVES EXTRACTS" submitted by Md. Sofiqul Islam Mandel has been carried out by the candidate under my supervision. The work was done in the Inorganic Research Laboratory, Department of Chemistry, Dhaka University. Analyses data for this research have been collected from the Chemical Laboratory, Urea Fertilizer Factory Limitted, Ghorasal. The candidate has fulfilled all the terms and conditions for the M. Phil thesis course and has duly passed the Ist year examination.

December, 1995

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ABSTRACTS

The concentration of Methane in Earth's atmosphere is reported to be inoreasing on the basis of the results obtained by means of a variety of independent measurements attributed to the emission from rice growing regions. To understand the global budget of atmospheric methane, intensive research on individual methane sources are required to be investigated and integrated for arriving at a definite conclusion. Attempts have, therefore, been made for measuring methane emission from different rice fields of different places in Bangladesh. Methene emission observed were of highest magnitude in the last 2-3 weeks before harvest. Hourly emissions have been observed to be 27.21 mg. $m^{-2}hr^{-1}$ and the emissions have been found to vary with the paddy varieties, geographical location, soil group, soil texture and structure, soil temperature, soil pH, ambient temperature and various other factors.Effect of different parameters on methane effluxes have been discussed. A correlation of plant growth on methane emissions has been formulated.

In order to study the mitigation measures for the suppression or reduction of methane emission from the rice fields, investigations with the natural pesticides were carried out in the laboratory and then applied in the field experiments. For this purpose the extracts of Neem tree components (Azadirachta Indica)

and some metalcomplexes of the extracted components were used. Petroleum ether, EtOAc, MeOH and also aqueous extracts of AZADIRACHTA INDICA leaf showed insecticidal effect towards house-flies while petroleum ether extract exhibited only repelling effect against mosquitoes and insecticidal effect on lice. Attempts were made to effect complexation of the extracts with metal ions like Ni(II), Cu(II) and Zn(II) and the extracts were subjected to chromatography to yield a number of compounds. The IR spectral data of these compounds have been discussed. In the laboratory experiments the extracts was found to be effective.

CHAPTER-1

INTRODUCTION

The thesis deals with the measurements of methane as a greenhouse gas. In this connection a coordinated research was started for the first time in Bangladesh after receiving training at the National Physical Laboratory (NPL), New Delhi, in the field of methane efflux from the rice fields. The training programme was financed by the Commonwealth Science Council, London which also provided a full set of equipment for use in Bangladesh. The equipment has been developed at the National Physical Laboratory under the guidance of Dr. D.C. Parashar, Director of the Chemistry Division of NPL, New Delhi, India [1]. The data in connection with the methane efflux from rice fields are incorporated in this thesis for the regions of Dhaka and Mymensingh.

The thesis also deals with the extraction of organic components from neem tree leaves, twigs, barks, seeds etc. However, the investigation on the extraction of organic chemicals from the leaves only are included in the thesis.

The purpose of isolation of the organic compound was to investigate the insecticidal and potential toxicity in concentrated form and the possible stabilization of the toxic, species by complex formation with metal ions such as copper, nickel and zinc. In this part the extraction from the leaves with water and with aqueous alcoholic solutions have been investigated.

Methane is an important greenhouse gas which is reported to be continuously increasing in the global atmosphere like CO_2. The potential of methane as a greenhouse gas is 21 times more than CO_2. Each additional molecule of methane added to the atmosphere has been assumed to have higher order of magnitude as greenhouse effect[2]. It has been estimated that the concentration of methane is increasing in the atmosphere at a rate of about 1% per year. It has therefore generated renewed interest in the global cycles of methane particularly the investigations have been directed to unravel the sources of the increased concentration of CH_4 in the atmosphere. The major sources of methane are shown in Table 1 and the magnitude of emissions are also indicated[3].

TABLE 1: Sources of Atmospheric Methane_

Source	CH_4 emissions (Tg $CH_4 Y^{-1}$
Rice Fields	60 - 140
Natural Wetlands	40 - 160
Land fill	30 - 70
Oceans	15 - 35
Ruminants	66 - 90
Natural Gas Exploration	30 - 40
Termites	06 - 42
Coal Mines	35
Biomass Burning	55 - 100

The increase of methane over the past 200 years is probably due to increase of emissions of about 70% where as the depletion may amount to only 30%.

The role of methane in the atmosphere is a complex phenomenon and may be attributed to the following factors :

a. CH_4 has absorption bands in the infrared region of the spectrum.
b. CH_4 is readily oxidized to form OH free radical.
c. CH_4 provides a good source of CO through the oxidation by OH.
d. CH_4 is a source of water vapour in the stratosphere as a result of the reaction.

$$CH_4 + 2O_2 \longrightarrow CO_2 + 2H_2O$$

e. Atmospheric CH_4 may also react with Cl radical forming HCl.

Methane is always produced from the soil by the microbial decomposition of organic materials under an strict anaerobic conditions.

Global methame budgets from 1978 to 1988 was reviewed by Khalil and Rasmussen and noted that rice paddies emission ranged from 18.0 to 280.0 Tg. per year[5]. It has also been observed that during summer in the Northern Hemisphere, CH_4 emission is lower and that a sharp rise occurs in fall indicating seasonal variation of methane efflux.

Thus the main sources of methane include rice fields, wetlands, ruminants, biomass burning, termites, gas drilling, land fills etc. Methane is also produced by the bacterial decomposition of organic matter in flooded rice paddy fields soil and it enters the atmosphere by three routes. As much as 80% passes from roots through plant which act as a chimney. Smaller amount bubble up to the water surface or diffuse slowly from the soil through the water. Most of the diffused methane however, is assimilated in the soil and flood water and never reaches the atmosphere. Several attempts have been made to evaluate the contributions of different sources of methane budget [5-8].

Continuted increases of methane can have global effect because methane interacts strongly with planetary infrared radiation[9,10].

Also its complex roles in atmospheric chemistry is not very clear. Atmospheric methane oxidation produces water vapour, which is most significant[11], in the upper stratosphere. Stratospheric methane also interact with chloride chemistry through its initial reaction with Cl atom to produce HCl.

$$CH_4 + Cl \text{ -----> } HCl + CH_3^{o}$$

Tropospheric reaction destroy perhaps 90% of the methane that enters in the atmosphere; OH radicals initiates this attack. Atmospheric chemical species and processes involved in methane oxidation are also central to the control of the oxidation State of the atmosphere[12,13].

Equations:

$$OH^{\bullet} + CH_4 \text{ ----> } H_2O + CH_3^{o}$$

$$O_3 + hv \text{ ------> } O + O_2$$

$$O + H_2O \text{ ------> } OH^{o} + OH^{o}$$

To understand why atmospheric methane is increasing world-wide, accurate estimates are needed of global input from rice fields and other agricultural sources as well as those of wet-lands. Methane production in water logged soils and other anoxic environments has long been recognized[14,16]. In recent years it has become the subject of intensive studies prompted by the realization of a steady increase in the level of atmospheric methane[17] and concern over the significance of this increase to global heat exchange production[18,19].

Thus it is important to discover the causes of the increasing methane concentrations and to identify the sources and sinks of atmospheric methane and the process that control them. It is in this context that the present work was initiated for the first time in Bangladesh and data have been collected to know the contribution of Bangladesh in the global methane budget.

The experiments in this thesis describe the first quantitative analyses of methane from rice fields in Bangladesh. In general on the basis of several indicator[20] and laboratory studies, it has been postulated[19] that the world's rice paddies would be a major methane sources. Flooded soils are certainly condusive to methane productions. Flooding of flat land results in a rapid lowering of oxidation-reduction potential with the consumption of all available electron acceptors including inorganic ions such as manganese, nitrate, sulphide, in the soil. This sequence of reduction process terminates with the effect on carbondioxide with the formation of methane shown in Fig 1. Methane is also produced by the direct heterotrophic dimethylation of lignin and other organices such as acetate[20].

Schematic diagram of the flow of methane from several representative biological and biogenic sources through various pathways (where oxidation occurs) in sediments, water and the atmosphere is shown in Fig 1. Once in the atmosphere, most methane is destroyed in the troposphere but 10-20% is transported upward to the stratosphere where the remainder is destroyed[21].

Given the growing importance of atmospheric methane and the evidence of methane emissions in the early studies of paddy field experiments, it is important that more observations be made to determine how much variability existed between rice paddy fields and the quality of methane emissions. Factors such as soil organic matter, fertilizers and other environmental factors pertaining to agricultural practice around the world may have possible effect on the methane emissions.

In the present work attempt have been made to measure the emissions of methane from different paddy fields and to correlate the emissions with various factors. The observations during the investigation have been classified into two sectors. The first deals with the measurements in experimental plots. The second consists of collection of data through field experiments in cooperations with a local rice grower. Measurements in the experimental plots were done with respect to the various factors which influence the methane efflux from rice fields mentioned before.

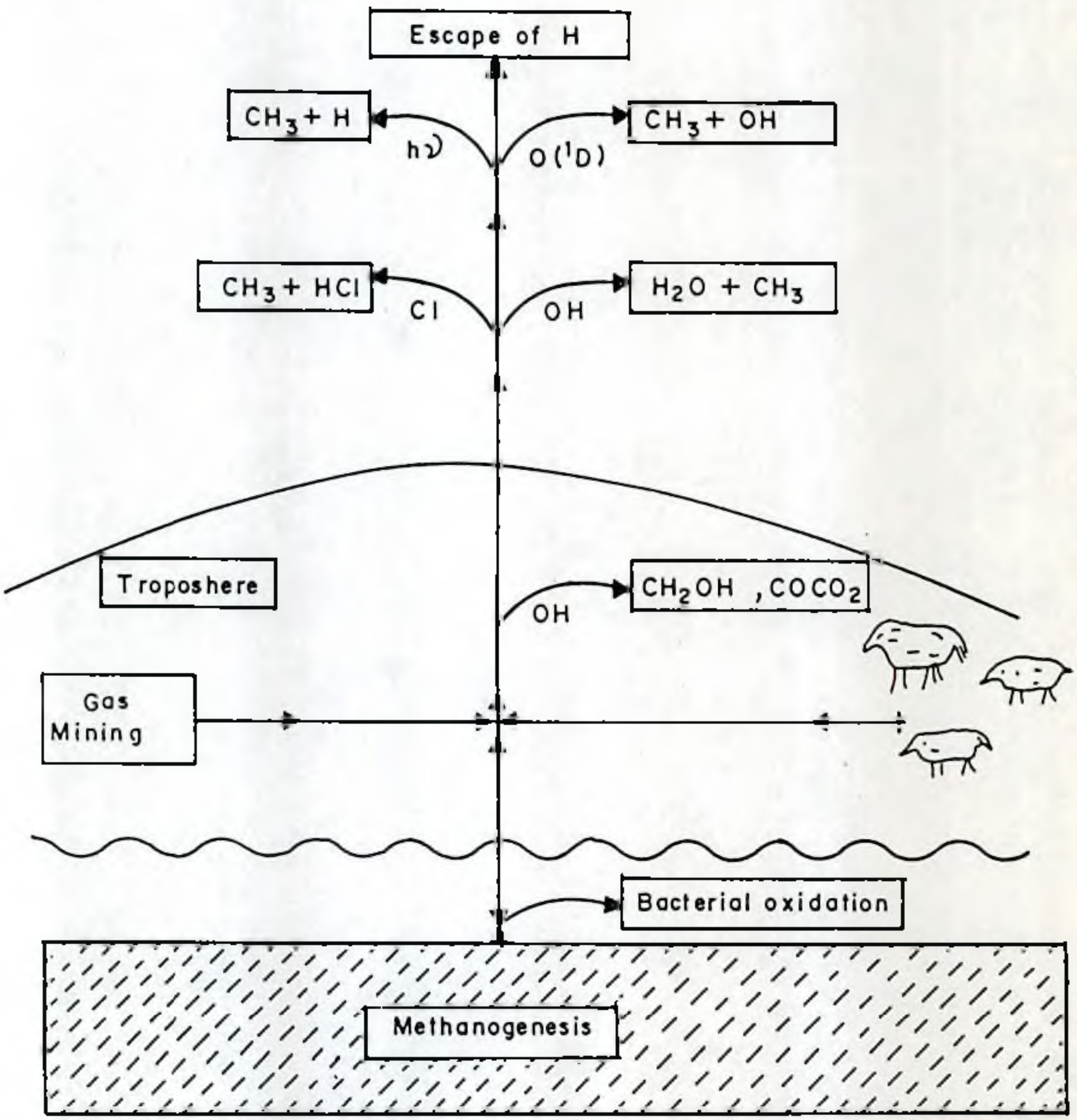


Fig. 1. Flow of methane from biological and biogenic sources

REDUCTION MEASURES FOR METHANE EMISSION

One of the main pathways in CH_4 formation in nature proceeds through CH_3COOH yielding equal amounts of CO_2 and CH_4. In presence of some cations such as Fe^{3+} or anions such as NO_3^- and SO_4^{2-}, methane production is reduced. CH_4 emission can also be reduced by pesticides and insecticides which stop the microbial decomposition of organic matters. Fertilizers and pesticides appear to inhibit methane emission rice field. In view of the fact that long-term use of inorganic fertilizers and pesticide is suspected to have harmful effect on the soil fertility, it was therefore decided to use mild organic pesticides normally obtained from natural sources. For this purpose the most inexpensive material was selected from the Neem Tree leaves.

Azadirachta Indica (Neem tree) is known for the beneficial value since ancient time. It is common knowledge and even laymen are aware of the economic and medicinal benefits of Neem trees. The bark, leaf, flower, seed and even the shade of Neem tree are considered as of medicinal effects by the common people. Neem tree grows well in dry subtropical and hunid-subtropical climatic regions like Bangladesh. It can also be grown on the basis of agroforestry if needed particularly on the pattern of Agrisilviculture [23] which covers all systems in which land is used to produce both forest trees and agricultural crops as in the wheat fields. The insecticidal, pesticidal, fungicidal and germicidal properties of the different part of Neem treel warrants scientific studies for the isolation of the chemical ingredients which are actually responsible for the extraordinary properties. Neem seed oil has been in vogue in the subcontinent since time immemorial and it has been found that neem oil cake, after extraction of oil, contains about 6% nitrogen and about 2% phosphorate. The known beneficial effect of neem constituents may be mentioned as follows:

1. As pest control.
2. In medicine.
3. Protection of plants and agricutural products.
4. Efficient use of nitrogen fertilizers by soil.
5. Inhibits soil bacteria.
6. Neem tree improves the soil fertility.
7. Neem tree hardly needs water for plantation and maintenance.
8. Neem tree is most effective in stopping desertification.
9. Neem leaf extracts have been found to be effective against the mosquito menace and do not produce environmental effects at all.
10. The Neem tree produces hard and durable timber.
11. The fruiting takes place in about 5 years and the tree survives for about 200 years.

In view of the above considerations and on the ground of the fact that the population in the world is increasing enormously. As a result the farmland is diminishing and the maximisation of crop-returns is becoming evermore acute. The crop growers are now using numerous types of synthetic agrochemicals. Some are manures and others are insecticides. All of these are synthetic chemicals and mostly are not biodegradable. The synthetic insecticides[24] are organo-chlorines, organo-phosphates and dinitrophenols etc. These broad spectrum insecticides are toxic towards a wide range of insects. Whilst being effective against the target pest, they often indiscriminantly destroy other beneficial species including the pest's natural enemies. Additionally, as insects gradually in course of time evolve to resist such toxins increasingly, larger amounts of pesticides are required thus causing increased ecological imbalance and pollution of the environment[25]. Furthermore, a large proportion of crops are still lost to predatory insects and other pests. The crop fields also need gradually increasing doses of the fertilizer to keep pace with the increasing demands of crop production. The need for environmentally acceptable methods of protection particularly in respect of the use and pesticides has prompted us to work on insecticidal plants available in Bangladesh. The Neem tree (Azadirachta indica . Juss) is one of them and holds promise as it is popularly used by the rural people to protect grains and cloths from the attack of insects. It has been the source of a large array of biologically active triterpenoids[26,27]. The isolation of these compounds has been reported from its seeds, barks and leaves. Recently it has been also reported that the farmers of Nicaragua(South America) are using the aqueous extract from pest's attack. However, the literature review reveals that of leaves of the Neem tree for the protection of various crops so far little work has been carried out on any part of the Neem tree which abundantly grows in Bangladesh. Therefore, in this search the leaves of the Neem tree have been chosen first to examine its constituents, their efficacy as either inscticidal or insect-repellant and their complexation with Ni(II), Cu(II) and Zn(II) in order to produce compounds of higher potential toxicity. In this paper the preliminary results in this field of research are presented.

CHAPTER-2

2. EXPERIMENTAL :

2.1 Equipments : The equipment used for the collection of methane emission from the rice field consisted of the following parts. The equipment was supplied by the National Physical Laboratory, New Delhi, India.

1. A Perspex chamber (L - 52.0 cms, B- 32.0 cms, H - 70.0 cms) for enclosing a small area of the rice field.
2. A Pulse Pump (Arovironment Inc. Monrovia Ca, USA) for homogenization of the gaseous mixture in the perspex chamber.
3. Aluminium base channel for holding the perspex chamber in the field.
4. Gas sampling bags, Teflon (Arovironment Inc., Monrovia, Ca., USA) 8 bags were used for collecting the gas.
5. Three-way stopcock for use during the collection of gas and circulation of gaseous mixture in the chamber.
6. Thermometer (0-200^0 C) for the measurement of chamber inside temperature and ambient temperature.
7. Soil Thermometer (0-150^0 C) for the measurement of soil temperature 6-10 cms below the soil surface.
8. Gas Chromatograph (Shimadzu, Model 12APT) using Flame Ionization Detector(FID).

2.2 EXPERIMENTAL DETAILS :

A rectangular perspex chamber of length 52.0 cms, breadth 32.0 cms and height 70.0 cms was set on a rectangular aluminium channel which was placed in the paddy field 24 hours before collection of gas samples. This perspex chamber isolates the air inside it from the atmosphere. The perspex chamber has three holes on the top part. Through the two holes rubber tubes are connected to a pulse pump (Arovironment, Inc. Ca., USA) and through the third hole a thermometer (0-200^{o}C) is inserted in a fixed condition. Pulse pump is used to circulate the gases inside the chamber for homogenising the gases mixture inside the chamber. One end of the rubber tube is connected to the inlet of the pulse pump and another rubber tube is connected to the outlet of the pulse pump using a three-way stopcock. A schematic sketch of the apparatus assembly is shown in Fig. 2.

2.3. Sampling:

Initially four gas sampling bags were flushed with nitrogen and then evacuated. First the ambient sample is collected in a sampling bag designated as (Flux-amb.). Just after collection of ambient sample the pulse pump is put on in operation in the collection circuit and immediately another sample is collected (Flux-0, 0.min.). The three-way stopcock is turned to the chamber direction in the circuit and pulse pump is kept on for circulating the gas inside the chamber. Another two gas samples are collected after 15 minutes (Flux -1, 15 min) and 30 minutes (Flux-11, 30 min) by operating the three-way stop-cock toward the sample collection. Simultaneously, the temperature of ambient condition inside the chamber and during the collection of samples are recorded. Sampling from the same location of paddy field have been made twice a day. One set of collection was done at 1000

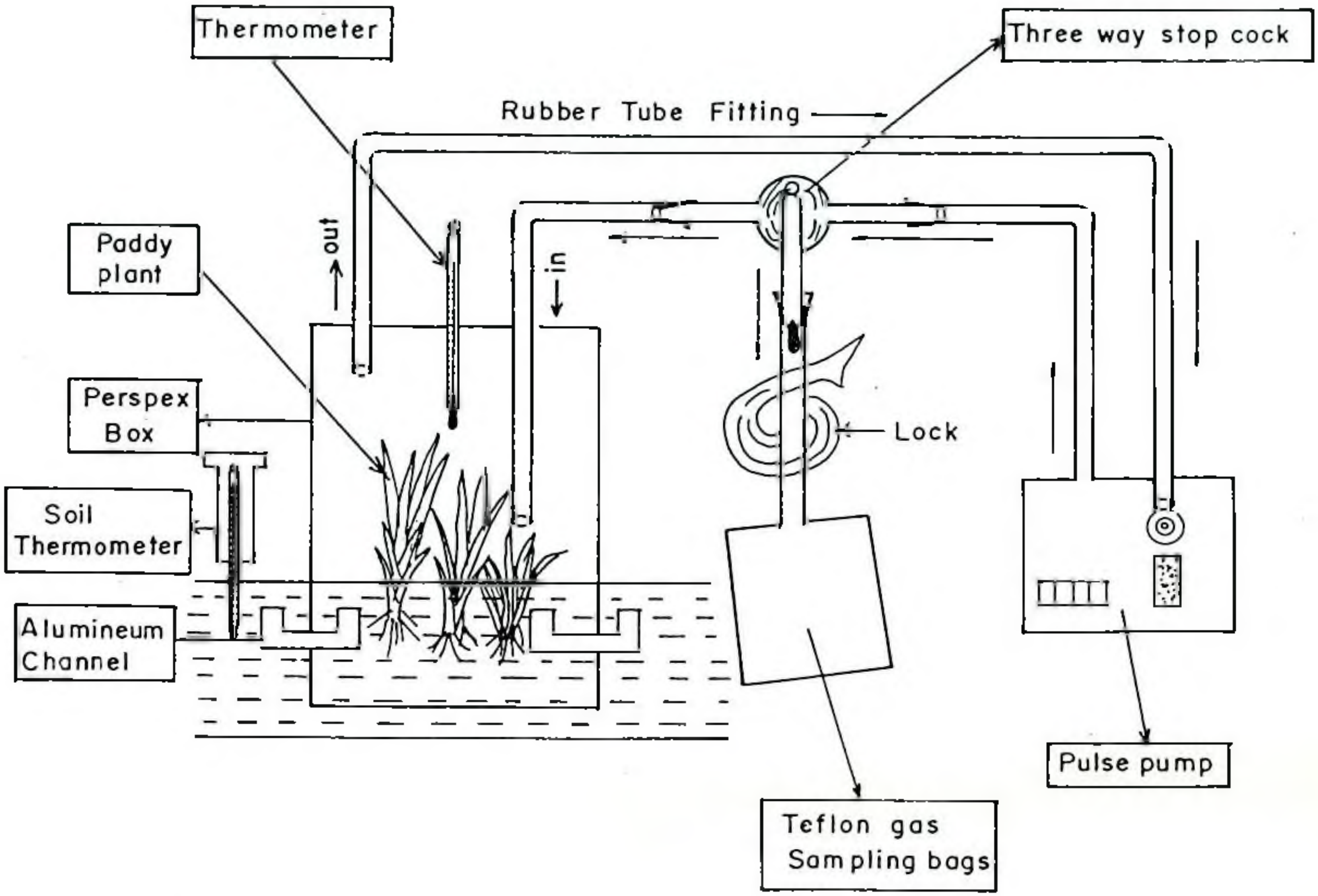


Fig. 2. Experimental arrangement for methane efflux measurement.

hours and the other set at 1600 hours. Gas samples were analysed with the help of Gas chromatograph (Shimadzu model-12 APT) using FID (Flame Ionization Detector) indicator attached with a printed circuit after standardization of the equipments. A stainless steel column 2.9 m long having OD (Outer Diameter) 4.0 m.m fitted with molecular sieve (5A) and the temperature maintained at 120^{o}C with carrier gas hydrogen at a flow rate of 45 ml/min. and FID (Flame Ionization Detector) were used for methane determination. The various parameters of Gas chromatograph are as follows.

Oven temperature	80^{o}C
Column temperature	80^{o}C
Detector temperature	130^{o}C
Injector temperature	100^{o}C
Converter temperature	200^{o}C
Sampling loop volume	1.0 c.c.

Detector FID (Flame Ionization Detector)

Carrier Gas H_2, Flow rate 40 ml/min

Fuel H_2 Gas, flow rate 40 ml/min

Air flow rate 400 ml/min

Different GC condition are set such as Oven temperature, detector temperature etc. used when necessary. Data station receiving the GC singal are also kept ready after installation of CROM software programme. Printer is put on run, switch on GC is pressed and the output of the result are recorded on printer with chromatogram.

The soil pH was measured in the Laboratory taking 30.0 gms. of soil sample well dried in air and roughly powdered and mixed with 75.0 g/wt. distilled water and allowed to stand for an hour with stirring. The temperature of soil was measured in the experimental field 6.0 to 10.0 cms. below the surface of the soil using a soil thermometer. Simultaneously, soil sample was taking to the Laboratory for estimation of organic carbon, NO^{3-}, SO^{-2}, Cl^{-} etc.

TABLE 2: Gas samples and their Methane Concentration

Gas Sample No.	Sampling time in hour	Temp. (^{o}C)	CH con. (ppmv)	Change in CH_4 con. w.r.t. Flux-0 (in ppmv)	CH_4 efflux mgm-2hr-1
Flux-amb.	0955	38.0	27.45	-	-
Flux-0 (0-min.)	1000	38.0	27.65	-	-
Flux-I (15-min.)	1015	41.0	40.32	12.72	-
Flux-II (30-min.)	10.30	41.0	47.60	20.00	13.75

CH_4 efflux calculated from the above mentioned data using the equation.

2.4. Calculation for Methane Emissions from Paddy Fields:

(1) Perspex chamber volume at STP(BV):STP.
Box dimension are L=52.0 cms. B=32.0 cms. H=70.0 cms.
Water level above channel was 0.0 cms.
Box volume after correction to water
level = (52.0 x 32.0 x 70.0-0.00) = 116480.0 c.c.

Biomass Volume inside the chamber should be deducted from chamber volume 116480.0 c.c.

Biomass Volume - 1320.0 c.c.

Therefore effective Box Volume (116480.0-1320.0) = 115160.0 c.c.

Barometric pressure was 754.0 mm of Hg.

Temperature inside the perspex box were 38.0, 41.0, 41.0 ^{o}C for

samples Flux-o, Flux-I and Flux-II.

Therefore, the $BV_{(STP)}$ would be at 38.0 ^{o}C = 100290.0 c.c.

For 41.0 ^{o}C $BV_{(STP)}$ = 99332.0 c.c.

2.5. Calculation for Methane Efflux:

Methane effluxes is measured by the following formulae;

where,

F = Efflux of CH_4 in mg $m^{-2}hr^{-1}$.

x = Increase in methane concentration in ppmv from o-min. time to t-minutes. (i.e from 15-min and 30 min sampling)

A = Perspex box base area or channel base area

= (52.0 cm x 32.0 cms = 1664.0 cm^2

= 0.1664 m^2

t = Sampling time (15-min, 30-min.)

As the concentration of CH_4 at 0-min. (Flux-0), 15-min. (Flux-I) and 30-min. (Flux-II) are 27.60, 40.32 and 47.60 ppmV;

Therefore, ΔX_1 for 15-min. = 12.72

and

ΔX_2 for 30-min. = 20.00

Therefore, more methane emissions has been observed for 15-min (Flux-I).

Smilarly, F_2 (methane effluxes) for 30-min. has been calculated 17.00 ppmV.

Therefore, methane efflux in morning (1000 hrs) = 19.00 ppmV and in the afternoon (1600 hrs) = 8.52 ppmV.

The average methane efflux per hour has been calculated 13.75 ppmV.

CHAPTER-3

3. MATERIALS AND METHODS FOR THE EXTRACTION OF THE COMPONENTS OF NEEM TREE LEAVES.

3.1 Collection of Neem tree leaves :

The leaves (20.0 Kg) of Azadirachta Indica commonly known as the Neem tree were collected from Mirpur area of Dhaka, dried in the shade for a couple of weeks and powdered in a laboratory crushing mill.

3.2 Extraction

The leaf powder (5.0 Kg) of Azadirachta Indica as extracted in a soxhlet (Fig.3) successively and exhaustively with petroleum ether (60-80^0 C), EtOAc, MeOH and water. Each of the extracts was concentrated *in vacuo*. Thin layer chromatographic studies revealed that the petroleum ether and EtOAc extracts were of similar behavious. These extracts were combined together, concentrated and subjected to Vaccum Liquid Chromatography (VLC) (Fig. 4) over TLC grade silica gel.Elution of the column first with petroleum ether and then with mixtures of petroleum ether and increasing proportion of EtOAc yielded AI-1 (white solid, 32.0 mg, R_f = 0.85 and 0.65; Petrol : EtOAc = 9:1), AI-2 (Yellowish gummy material, 65.0 mg, R_f = 0.62; Petrol : EtOAc = 9:1), AI-3 (amorphous white powder, 95.0 mg, R_f = 0.52, Petrol : EtOAc = 8:1), AI-4 (reddish gummy material, 69.0 mg, R_f = 0.32, Petroleum ether) and AI-5 (white solid, 53.0 mg, R_f = 0.34, Petrol : EtOAc = 8:1).

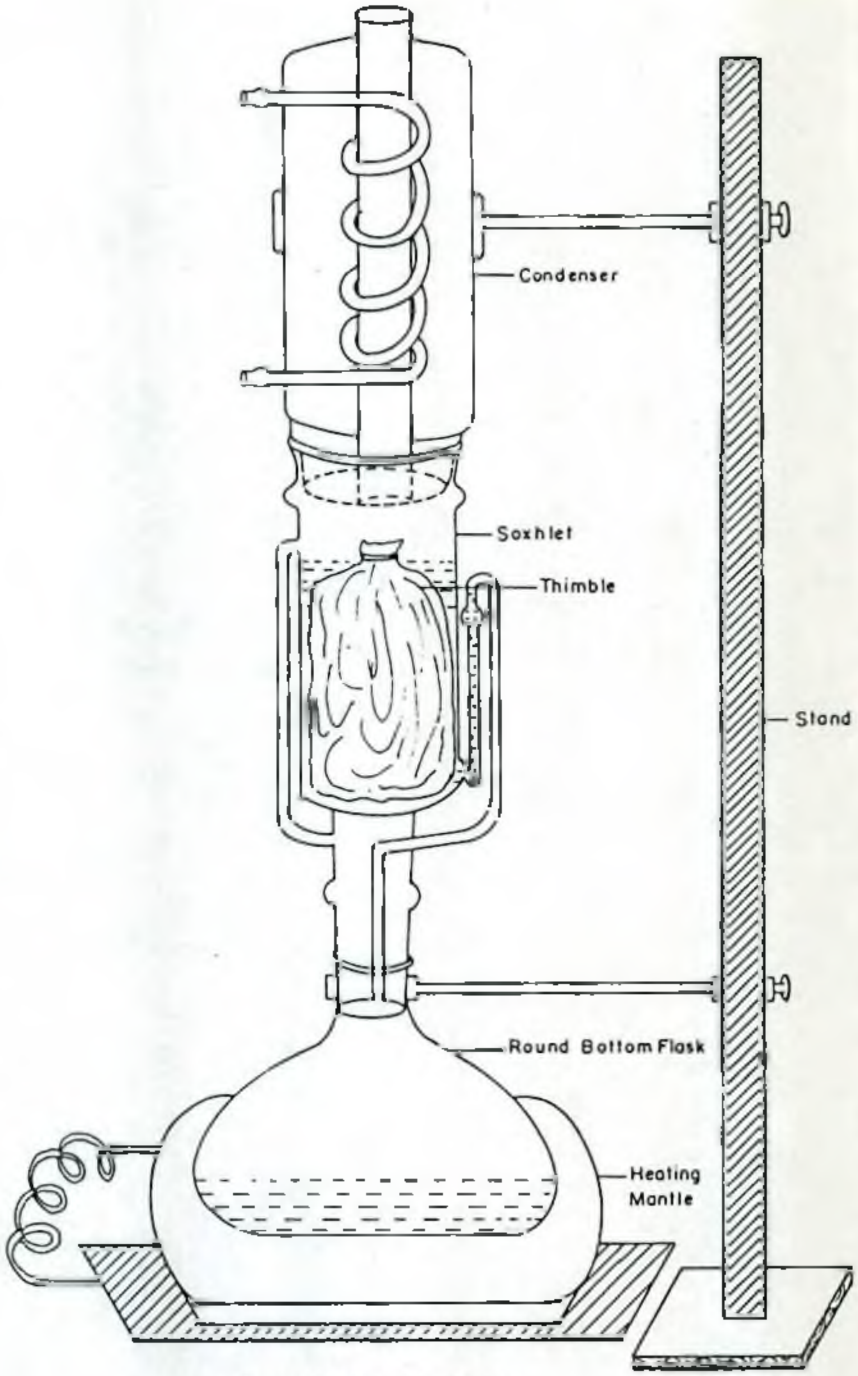


Fig. 3 Soxhlet Apparatus

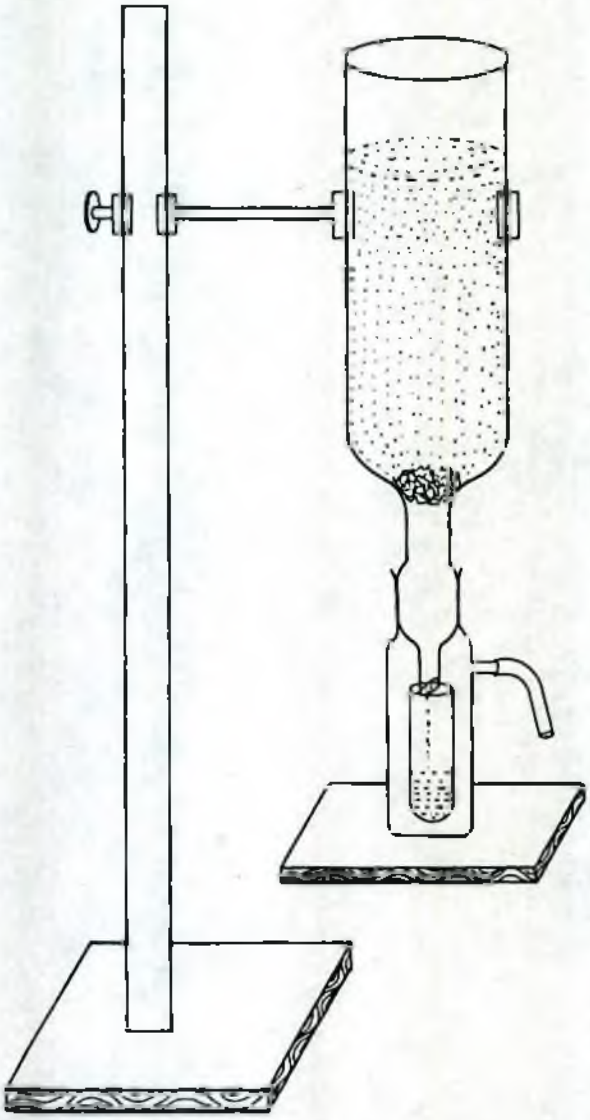


Fig. 4. Vacuum liquid chromatography apparatus.

CHAPTER-4

4. PROPERTIES OF COMPOUNDS:

4.1. Properties of Compound AI-1:

AI-1, white solid, 32.0 mg, m.p.135-139 $^{\circ}$C,

R_f-value 0.85 and 0.65 (petrol: EtOAc= 9:1) (Fig. 5).

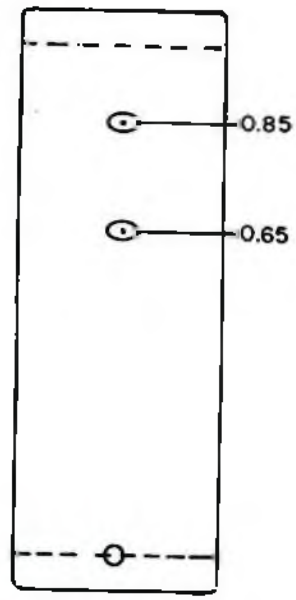


Fig. 5

4.2. IR Spectrum: ν_{max}(KBr):

3650-3200, 2900, 2850, 1630, 1455, 1378, 1100, 725 and 720 cm^{-1} (Fig. 6).

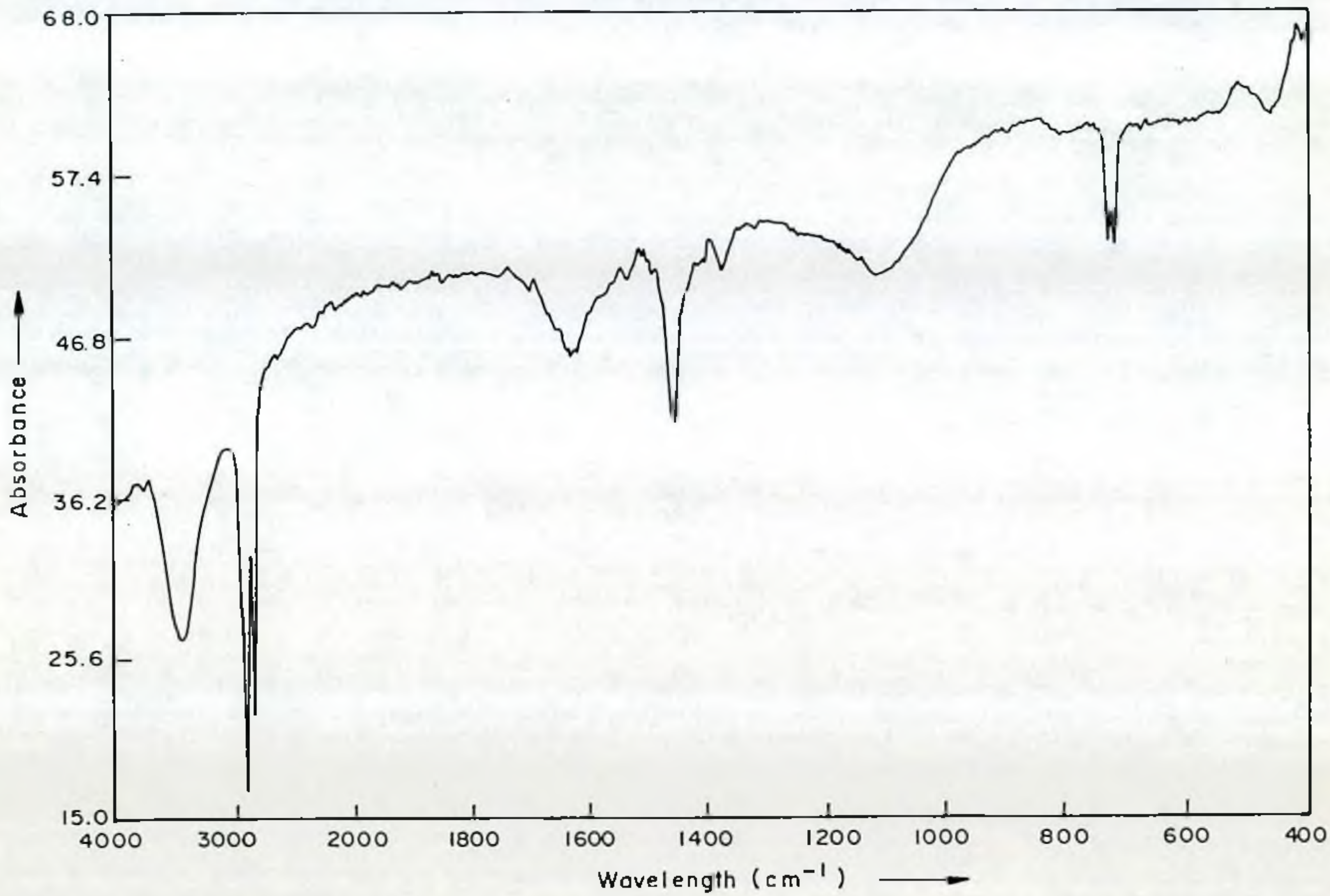


Fig. 6. IR Specirum of compound AI – I

4.3. Properties of Compound AI-2:

AI-2 yellowish gummy material, 65.0 mg,

R_f-value 0.6 (petro): EtoAc=3:1) (Fig.7).

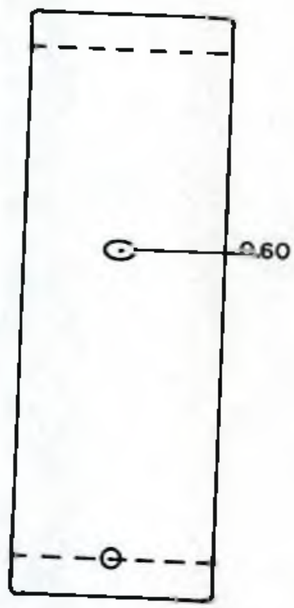


Fig. 7

4.4. IR Spectru: ν_{max}(Nujol):

3445(w), 3178, 2900, 2850, 2710, 1735, 1635, 1458, 1380, 1305, 1240, 1170, 1105, 1032, 1005, 980, 960, 920, 882, 838, 800, 728 and 720 cm^{-1} (Fig. 8).

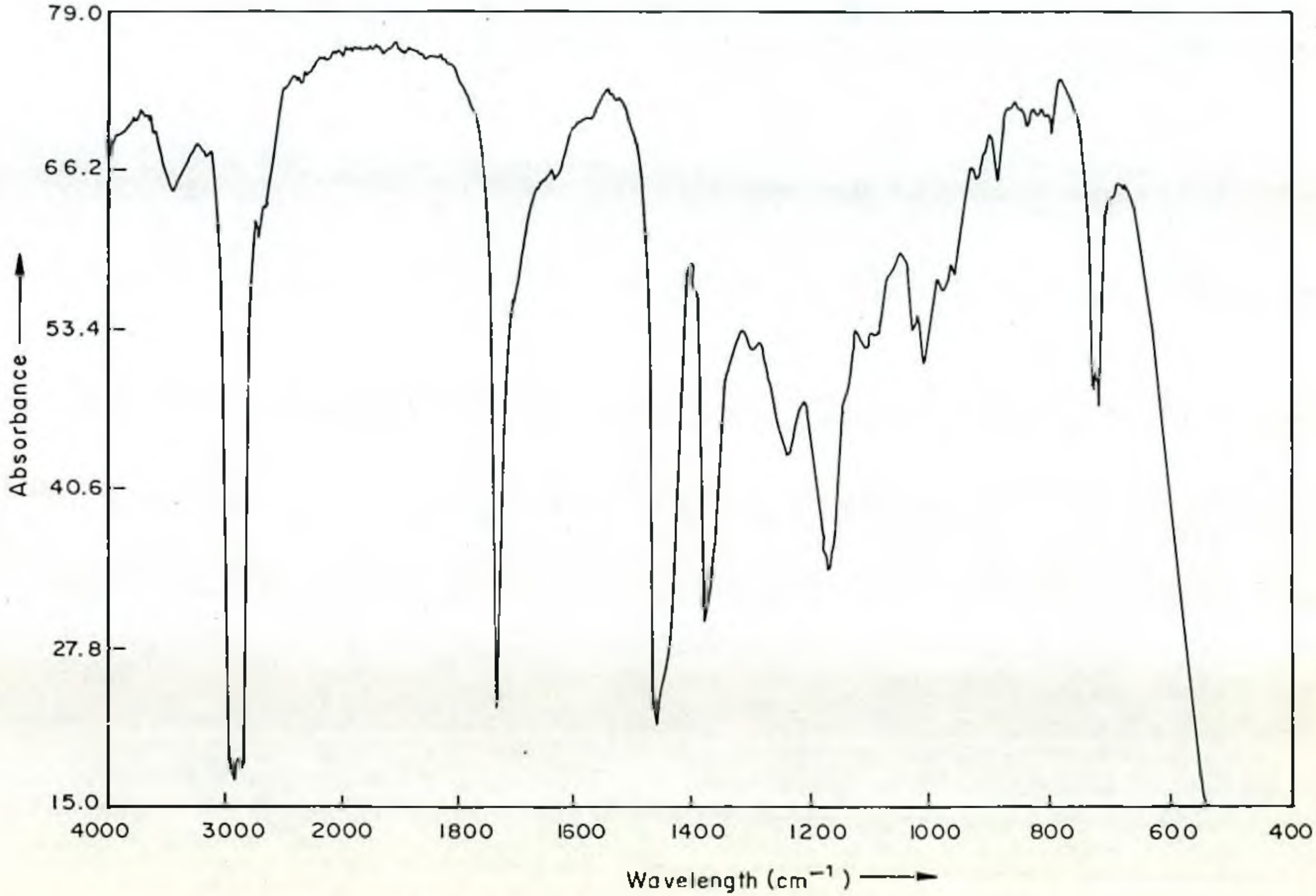


Fig. 8. IR-Spectrum of compound AI -2.

4.5. Propertiesof Compounds AI-3:

AI-3 amorphous white powder, 95.0 mg, m.p. 165-166°C, R_f-value 0.52 (petrol: EtOAc=8:1) (Fig. 9).

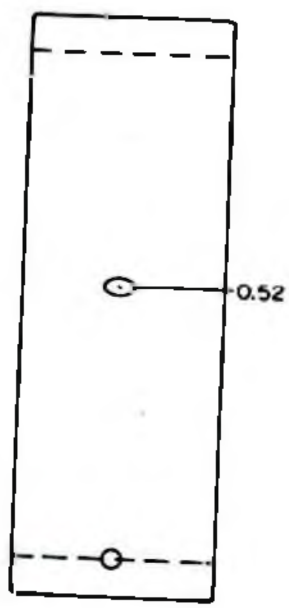


Fig. 9

4.6. IR Spectrum ν_{max} (KBr):

3450, 2900, 2850, 1630, 1455, 1378, 1100, 728 and720 cm^{-1} (Fig. 10).

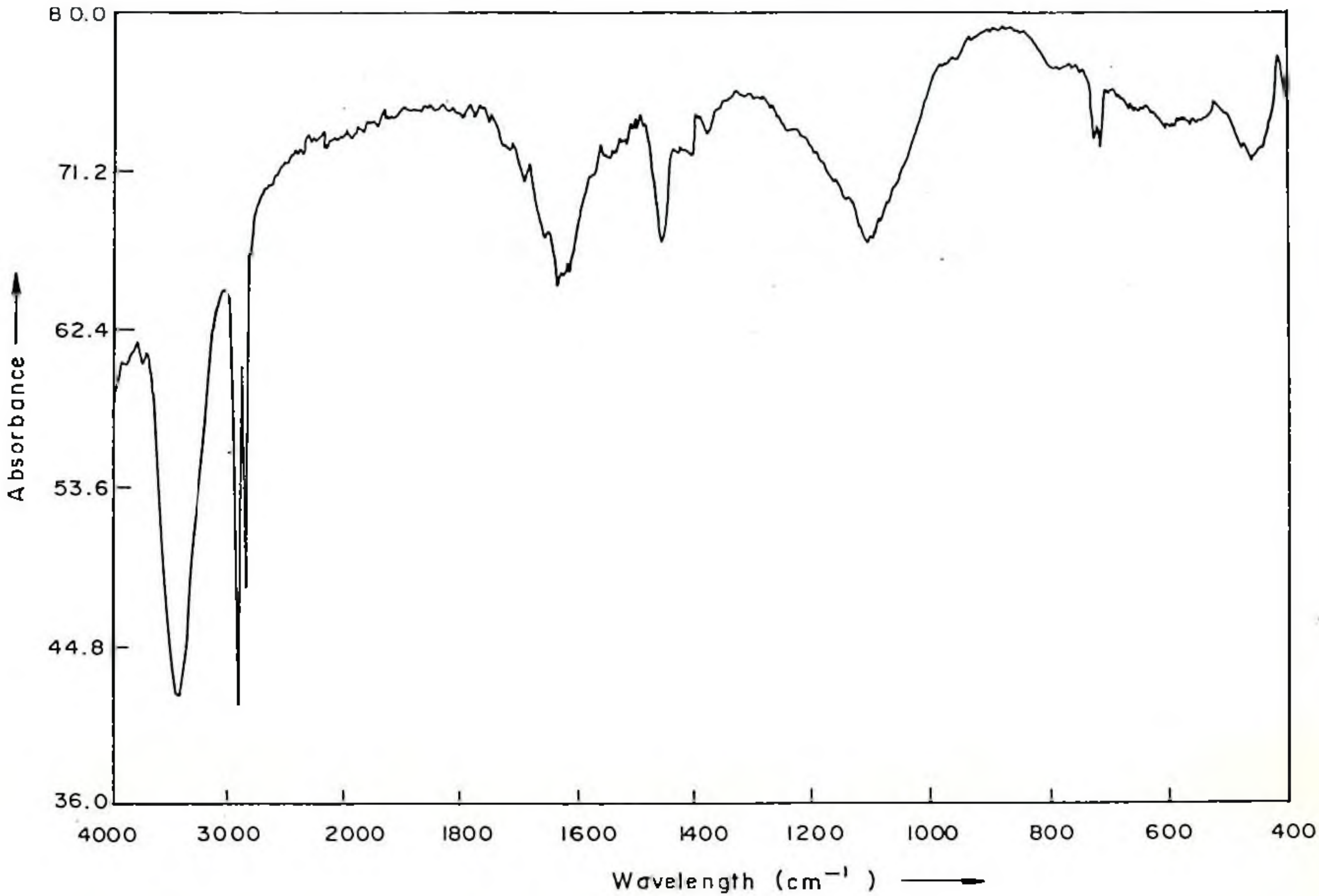


Fig. 10. IR-Spectrum of compound AI-3

4.7. Properties of Compound AI-4:

AI-4 reddish gummy material, 69.0 mg, R_f-value 0.32 (Petroleum-ether) (Fig. 11).

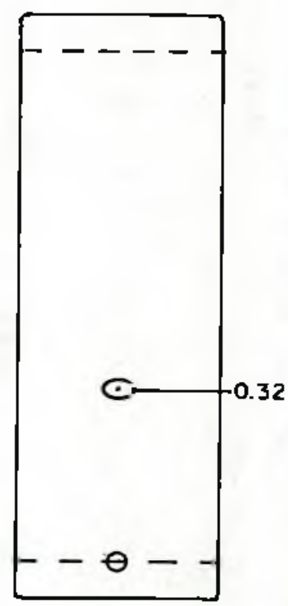


Fig. 11

4.8. IR Spectrum ν_{max} (Nujol):

3400, 2900,2850, 1448, 1378, 1240,1160, 725 and 720 cm^{-1} (Fig. 12).

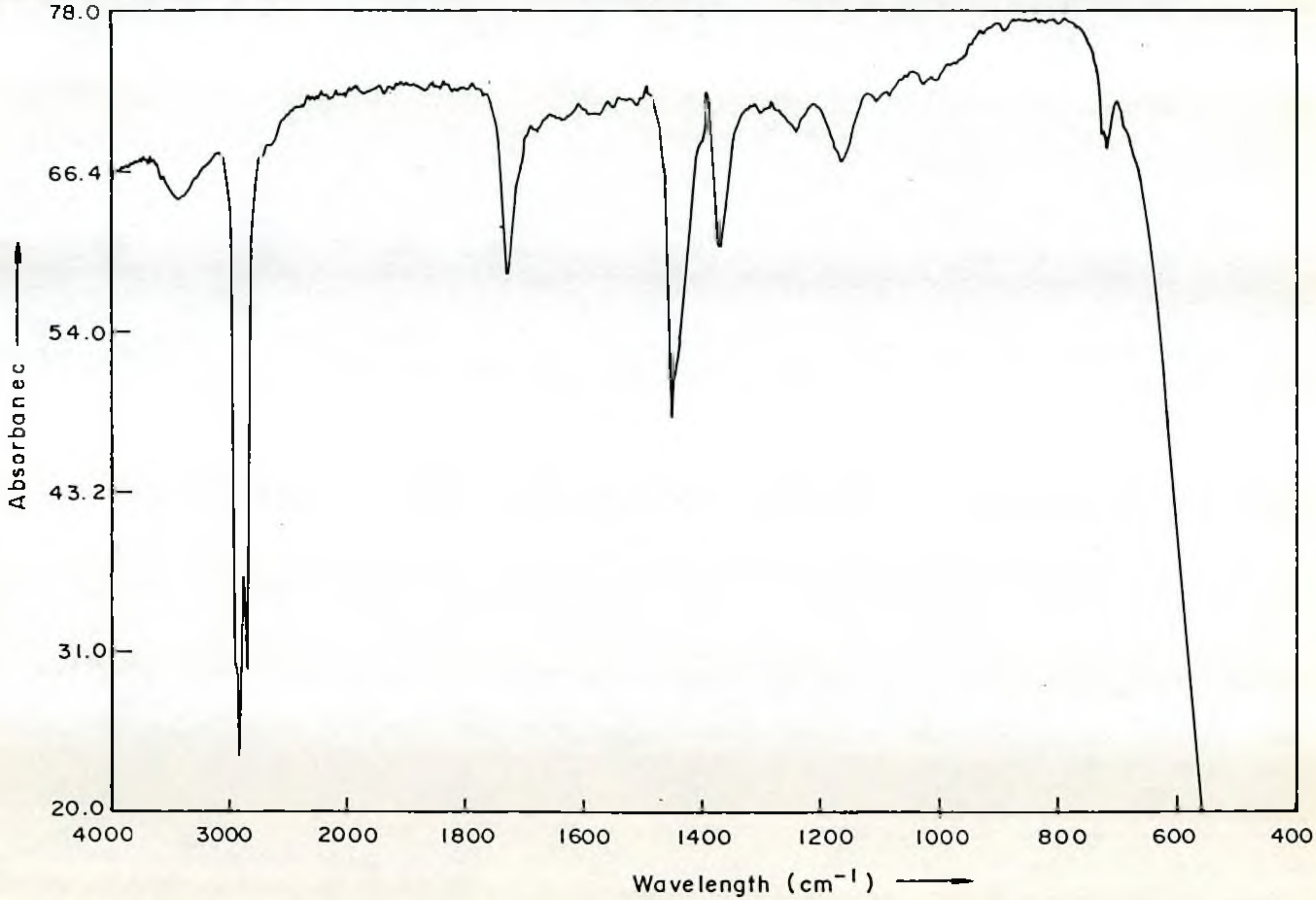


Fig. 12. IR - Spectrum of compound AI -4

4.9. Properties of Compound AI-5:

AI-5 white solid, m.p. 212-214 ^{o}C, 53.0 mg, R_f-value 0.34 (Petrol: EtOAc=8:1) (Fig. 13).

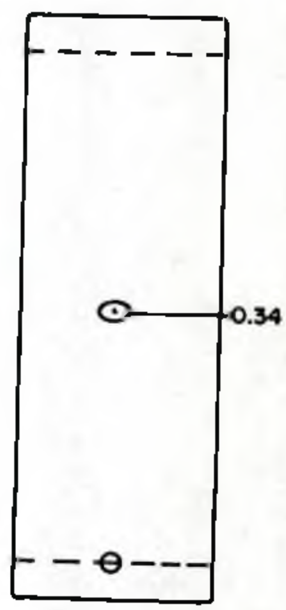


Fig. 13.

4.10. IR Spectrum: ν_{max} (Nujol):

3450, 3008 (sh), 2900, 2850, 1750, 1640, 1460, 1380, 1100(w), 1060, 1020, 970, 960, 800 and 740 cm^{-1} (Fig. 14).

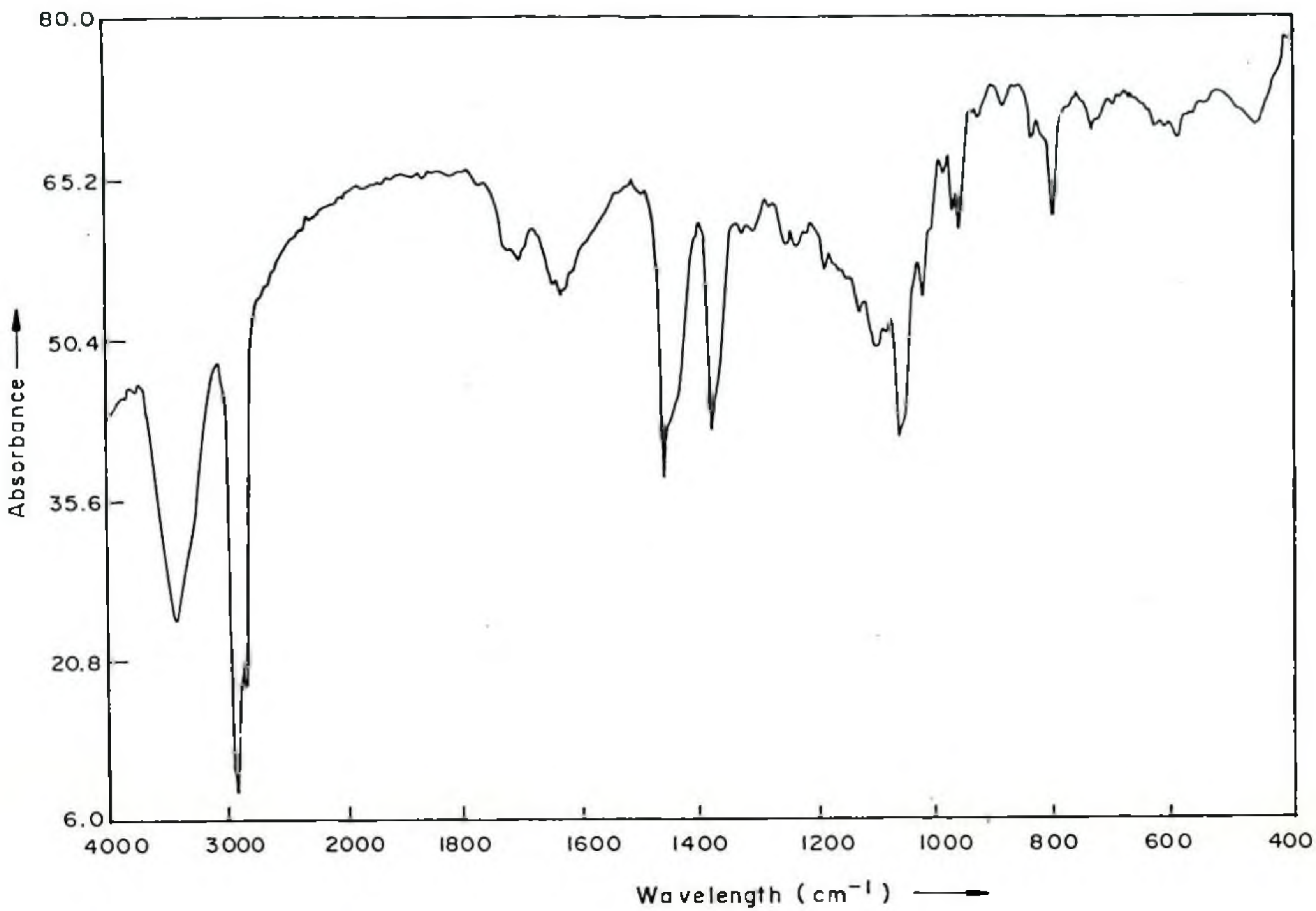


Fig. 14. IR Spectrum of compaund AI – 5

CHAPTER-5

5. ATTEMPTS TO SYNTHESIZE Ni(II), Cu(II) AND Zn(II) COMPLEXES WITH VARIOUS EXTRACTS OF NEEM TREE LEAVES.

Leaf powder (2.0 Kg) of Azadirachta Indica was extracted in a soxhlet successively and exhaustively with petroleum ether, EtOAc and MeOH. Each of the extracts was concentrated in vacuo 50.0 gm of each of the concentrated extract were taken in a beaker. To each beaker 5.0 ml of 5% aqueous Ni(II), Cu(II) and Zn(II) solution was separately added. The contents of each beaker were magnetically stirred for half an hour at the ambient temperature and allowed to stand overnight. No solid materials separated out. However, on keeping the reaction mixtures in a freezer for 48 hours a solid material was formed. The solid material was collected by suction filtration dried in an oven at 95^0 c. Its infrared spectrum was devoid of any absorption bands characteristic for any organic moiety indicating absence of complexation. Further attempts for complexation of some constituents of the various extracts with 10%, 15% and 20% metal ion solutions were carried out following the same procedure.

In the present conditions apparantly the organic extracts do not form complexes with Ni(II), Cu(II) and Zn(II). However, the condition have to be subjected to further investigations for the formation of stable complexes.

5.1 EFFECT OF THE VARIOUS EXTRACTS ON INSECTS :

Two indentical desiccators were cleaned and dried. 2.0 grms of the concentrated petroleum ether extract after complete removal of the solvent were taken on a watch glass. The product was a gummy mass which was placed in the watch glass covered with a piece of mosquito net. The watch glass containing the gummy extract only was then placed inside one of the desiccators. A blank experiment containing the watch glass without the gummy extract was taken in an other desiccator. Six house-flies were kept confined inside each of the desiccators. Within 24 hours all the six files of the desiccator containing the petroleum ether extract were found dead whereas in the other desiccator the flies were found alive. Similar study was carried out with EtOAc and MeOH extracts. In all the cases similar results were observed. All these experiments were conducted thrice and the repeat experiments produced indentical results. A rough measure of the toxicity of the extracts indicate LD_{100} = 24 hours.

5.2 INSECT-REPELLING EFFECT OF THE PETROLEUM ETHER EXTRACT :

About 3.5 gm of the concentrated petroleum ether extract were taken in a small beaker. The beaker was placed underneath the reading table and the reader did not feel any mosquito-bite. Howerver, the same reader has failed to pay proper attention to his study due to servere mosquito-bite when there was no such Azadirachta Indica leaf extract in the vicinity of his study table. This study was repeated during the winter and summer seasons with identical observations indicating strong repellant activity of the Neem extract.

5.3 EFFECT OF THE CONCENTRATED PETROLEUM ETHER EXTRACT ON LICE :

A small quantity (0.5 gm) of the concentrated petroleum ether extract was thoroughly mixed with 50.0 ml of coconut oil. A small portion of this oil was taken on a watch glass. A good number of lice collected from lice affected persons was thrown on to the watch glass containing the oil. It was observed that the mobility of the lice ceased immediately and ultimately all the lice were found dead within a period of about 1(one) hour only.

These observations indicated that the Neem tree leaves extracts can be effectively used to kill the insects found in the rice field and also other environments which are affected with pests and hazardous insects. Therefore, the experiments were conducted to use the Neem tree leaves extracts in the rice fields as a mitigation measures to destroy the insects. It was also assumed that some methane emission is due to the microbiological actions of inset pects in the rice fields, it is surmised that the use of Neem tree extracts may be a useful tool to reduce the emission of methane of it is purely of biological origin and not produced by the rice plants as believed generally. However, there is no concrete proof as yet of the origin of methane emission from the rice field as yet.

CHAPTER-6

6. RESULTS AND DISCUSSION :

The major goal of this work is to estimate the total annual emissions of methane from paddy fields of Bangladesh that is supposed to contributed to the global warming pool. It has become apparent that it is not an easy task to accurately measure the total amount of methane emitted even by a single field over a full growing season. This is because of the variation with large number of factors such as hour of the day, duration of season, soil temperature, soil pH, soil texture and structure etc. Before estimation of total annual methane emisations can be derive, it is essential to observe the variations of methane emissions occuring at four different locations in Bangladesh.

Factors which determine the control or variations of methane emissions should be elaborately studied. In the present work the patterns of emissions have been observed on the basis of the experimental data. In the light of this assumption, methane efflux occurs in paddy fields at different typical locations have been studied at Bangladesh Agricultural Institute, Dhaka, Housing and Building Research Institute, Dhaka, and at Bangladesh Agricultural University, Mymensingh are shown in Table 3. Soil and ambient temperature of sampling time, paddy plant height morning and afternoon methane emission have been presented in Table 4

It has been found that the methane effluxes found about 0.68 mg $m^{-2}hr^{-1}$ has been occur for a blank field i.e. field before plantation. Therefore it is concluded that methane emissions from paddy field take place due to the rice plant.

Geographical locations, annual rainfall, height from the sea level, are covered Table 5. Different soil parameters including soil pH are presented in Table 6. It has been found that the soil pH varied from 4.40 to 607. Lower pH value 4.40 was observed at BAI, Dhaka paddy field due to the discharge of hospital wastes from the Suhrawardy Hospital nearby situated in a low location.

Figure 15 shows the variation of methane efflux with soil pH. of different paddy fields. It has been observed that maximum

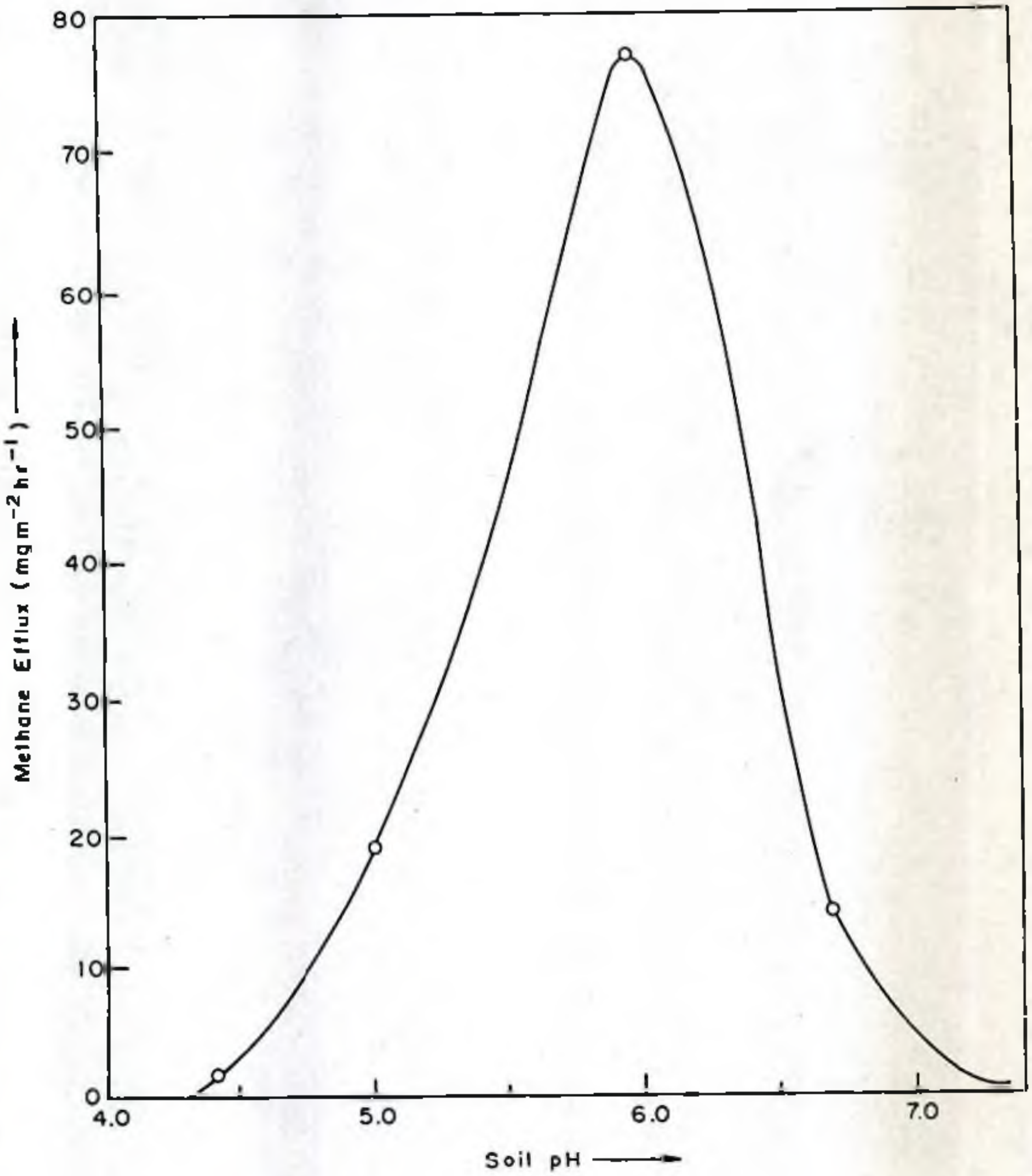


Fig. 15 Variation of methane efflux with Soil pH

TABLE 3: Summary of measurement indicating Dates, locations, Duration Average Values of Soil and Ambient Temperature and methane efflux.

Date	Locations	Time(Hrs)	Average Temp (^{o}C)	
			Soil	Air
09.05.92	Dhaka,BAI	1000-1600	29.0	36.5
21.07.92	HBRI,Dhaka	1000-1600	31.5	32.5
08.09.92	HBRI,Dhaka	1000-1600	32.0	34.0
18.09.92	Mymensingh,BAU	1000-1600	32.5	34.5
07.01.92	HBRI,Dhaka	1000-1600	21.0	23.5
11.01.93	HBRI,Dhaka	1000-1600	21.0	26.5
14.03.93	HBRI,Dhaka	1000-1600	25.0	30.5
25.04.93	HBRI,Dhaka	1000-1600	24.0	32.0
26.05.93	HBRI,Dhaka	1000-1600	28.5	34.0

BAU = Bangladesh Agriculture University
BAI = Bangladesh Agriculture Institute
HBRI = Housing and Building Research Institute

TABLE 4: Summary of measurement indicating Dates, locations, duration, plant height and methane efflux.

Date Time 1992-93	Locations Average	Average (Hrs)	CH_4 efflux height (cm) of plant	Average Morning	Afternoon	CH_4 efflux mg m hr
09.05.92	Dhaka, BAI	1000-1600	70	01.68	01.04	01.36
21.07.92	HBRI, Dhaka	1000-1600	35	16.22	29.28	22.75
08.09.92	HBRI, Dhaka	1000-1600	40	15.55	23.04	19.29
18.09.92	BAU Mymensingh	1000-1600	60	19.00	08.52	19.75
07.01.92	HBRI,Dhaka	1000-1600	00	00.90	00.46	00.68
11.01.93	HBRI, Dhaka	1000-1600	26	42.88	13.50	28.19
14.03.93	HBRI, Dhaka	1000-1600	60	40.50	30.00	35.25
25.04.93	HBRI, Dhaka	1000-1600	70	61.48	31.28	46.38
26.05.93	HBRI, Dhaka	1000-1600	00	113.48	40.77	77.23

BAU = Bangladesh Agriculture University
BAI = Bangladesh Agriculture Institute
HBRI = Housing and Building Research Institute

TABLE 5: Different location with their environmental parameters.

Location	BAI,Dhaka	HBRI,Dhaka	HBRI,Dhaka	BAU, Mymensingh
Dates	May 9,92	July 21,92	Sept 8,92	Sept 18,92
Geographical Location	90.38E 23.77N	90.38E 23.77N	90.37E 23.78N	90.55E 24.91N
Height from Sea level(ft)	250-300	250-300	250-300	300-350
Annual Rain fall(m.m)	1877	1877	1877	2324
Ambient Temp(^{o}C) (Morning & Afternoon	36-37	29-36	31-37	31-38

Location	HBRI,Dhaka	HBRI,Dhaka	HBRI,Dhaka	HBRI,Dhaka	HBRI,Dhaka
Dates -	Jan. 7,92	Jan.11,92	March 4,92	April 25,92	May 26,93
Geographical Location	90.37E 23.78N	90.37E 23.78N	90.37E 23.78N	90.37E 23.78N	90.37E 23.78N
Height from Sea level(ft)	250-300	250-300	250-300	250-300	250-300
Annual Rain fall(m.m)	1877	1877	1877	1877	1877
Ambient Temp(C) (Morning & Afternoon)	23-24	26-27	29-32	27-37	32-37

TABLE 6: Different location with the various soil parameters.

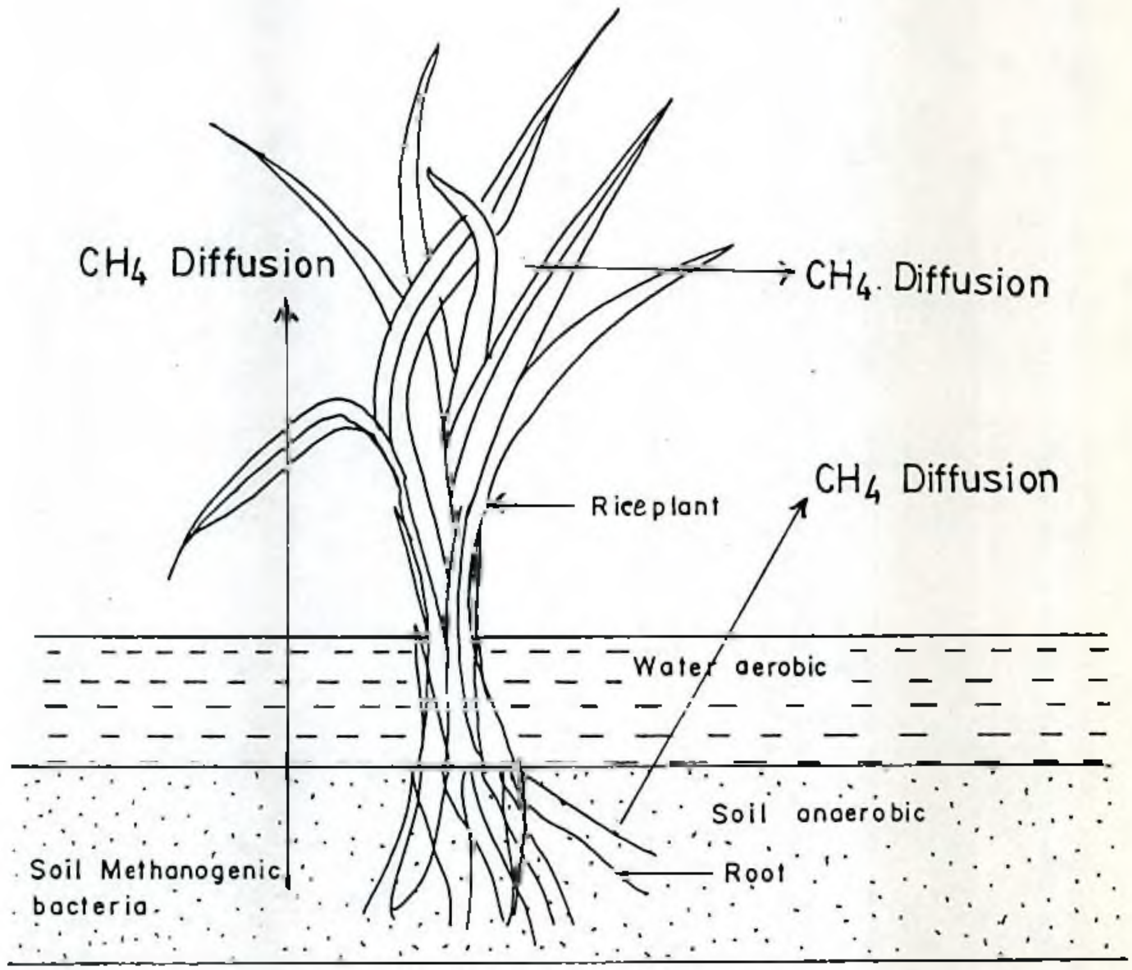
Locations	HBRI, Dhaka	HBRI, Dhaka	HBRI, Dhaka	BAU, Memensing
Dates	May,9,92	July 21,92	Sept.8,92	Sept.18,92
Soil Temp (Morning & Afternoon)	29-29	30-33	29-35	29-36
Soil group	Red loam	Red loam	Red lcam	Alluvial
Soil texture	Silty clay	Silty clay	Silty clay	Sandy laom
Dominant clay mineral	Fe-AL-Silicate	Fe-AL-Silicate	Fe-AL-Silioate	Oa-Al-Silicate
Soil pH	4.40	5.30	5.02	6.70
Sulphate (SO_4^{2-})	-ve	-ve	+ve	-ve
Phosphate (PO_4^{3-})	+ve	+ve	-ve	-ve
Nitrate (NO_3^-)	+ve	-ve	-ve	-ve
Organic oarbon(%)	6.00	7.00	7.00	8.00
Fertilizer applied (Kg/Ha)	NPK(130,90,65)	Farm yard manure	Ncrmal Farm land	Normal Farm land
Methane effluxes (mg $m^{-2}hr^{-1}$)	1.36	22.75	19.29	13.75

Table 6 contd.

Locations	HBRI,Dhaka	HBRI,Dhaka	HBRI,Dhaka	HBRI,Dahak	HBRI,Dhaka
Dates	Jan.7,92	Jan.11,92	Mar.4,93	Apr.25,93	May 26,93
Soil Temp($^{\circ}$C) (Morning & afternoon)	19-23	19-23	25-25	25-25	28-29
Soil group	Red lcam	Red loam	Red loam	Red loam	Red loam
Soil texture	Silty clay	Silty clay	Silty clay	Silty clay	Silty clay
Dominant clay mineral	Fe-Al-Silicate	Fe-Al-Silicate	Fe-Al-Silicate	Fe-Al-Silicate	Fe-Al-Silicate
Soil pH	5.80	5.90	5.90	6.03	6.07
Sulphate (SO_4^{2-})	+ ve	- ve	- ve	- ve	- ve
Phosphate (PO_4^{3-})	- ve	- ve	- ve	- ve	- ve
Nitrate (NO_3^-)	-ve	-ve	-ve	-ve	-ve
Organic carbon(%)	6.62	8.10	9.40	8.80	8.00
Fertilizer applied (Kg/Ha)	Farm yard manure	Farm yard manure	Farm yard manure	Farm yard manure	Farm yard manure
Methane effluxes (mg $m^{-2}hr^{-1}$)	0.68	28.19	35.25	46.38	77.23

effluxes were observed at pH 6.0-7.0 therefore this pH values may be the most favourable condition for methanogenesis process in the soil. It is also observed that methane emissions has been found to have low value below pH 6.0 and greater emissions occur above pH 7.0. But the question still remains unsolved whether the methane emission is due to microbiological action at the roots of the rice plants in the soil or the rice plant evolve methane gas through the plant stem and leaves. The soil arameters of Bangladesh varies from region to region. The dominant clay minerals and texture at Dhaka and Mymensingh are different. The soil groups and soil texture at Mymensingh zone are alluvial and sandy loam but in Dhaka, HBRI soil group and exture are red loam and silty clay possibly of lateritic type. Although Mymensingh zone is alluvial but higher methane emissions has been found at HBRI, Dhaka 77.23 mg $m^{-2}hr^{-1}$. Soil texture plays an important significant role in methane effluxes.

A possible mechanism of methane emission rice fields may be postulated according to the scheme in Fig. 16. Organic matter in the soil at the roots of the rice plant is decomposed by microorganisms in consecutive steps. The hydrogen acceptors are used up in a sequence of reaction involving aerobic respiration, general fermentation, oxidizing agents, reduction and microbial oxidation-reduction processes leading to the formation of a mixture of



$$CH_3COOH \xrightarrow{\text{root}} CH_4 + CO_2$$

$$CO_2 + H_2 \xrightarrow{\text{anaerobic}} CH_4$$

Fig. 16 Schematic production of methanc from the root system of rice plant.

CO_2 and CH_4. One of the main pathways in the formation of CH_4 in nature proceeds via acetic acid which yields equal amounts of CO_2 and CH_4.

$$\begin{tikzcd}
\text{Rice plants roots} \arrow[r, dashed] & \text{Organic carbon} \arrow[r, dashed] \arrow[d, dashed] & \text{Acetate} \arrow[d, dashed] \\
& CO_2+H_2 \arrow[r, dashed] & CH_4
\end{tikzcd}$$

It has been mentioned that the methane effluxes has been collected in the day time but there is need to measure the emissions at night also to observe the variation due to sunlight and darkness. Normally it is observed that at morning time methane effluxes are higher than that of afternoon time.

6.1. Effect of Fertilizers:

The average value of methane effluxes for different paddy varieties grown under identical environmental and soil parameters conditions are shown in Fig. 17. It is observed that the rate of methane emissions is greatly influenced by paddy variety. It has also been found that IRRI paddy variety effluxes are comparatively higher than any other variety. The presence of SO_4^{-2} bearing fertilizers reduce the methane effluxes and application of green leaves manure enhance the methane emission by some orders of magnitude[28]. The effect of different nitrogen fertilizers on methane emission has also been studied . It has been observed that low methane effluxes with ammonium sulphate as ferti

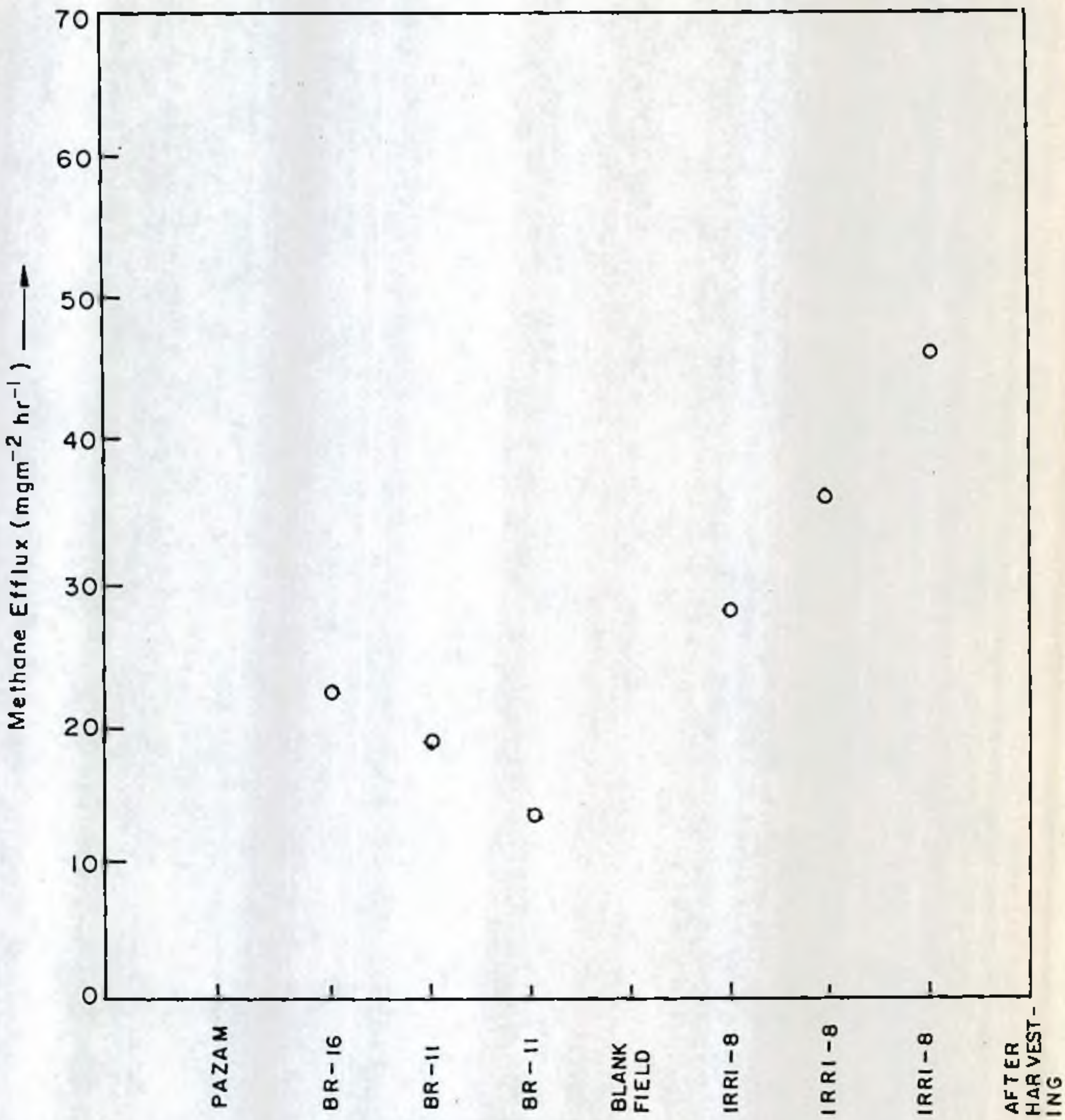


Fig. 17. Variation of methane efflux with paddy variety.

lizer is due to the presence of methane. The presence of SO_4^{-2} appear to enhance the methanogenesis(28) process. Fig. 18 shows the variation of methane effluxes with soil temperature in different paddy fields. It has been observed that the percentage increase in methane effluxes occur due to soil temperature with the range from 21.0°C to 32.5°C. Obviously as expected the 15.0 minutes methane effluxes is higher than 30 minutes effluxes.

6.2. Plant Growth Rate and Methane Emissions:

Attempt has been made to observe the variation of methane emission right from the sowing upto harvesting. It has been found that during the flowering period just before harvesting period. The emission in fields wherethe chmical fertilizers NPK in the ratio of (130, 90, 65) as fixed to be minimum i.e. 0.68 mg m^{-2} hr^{-1}. This aspect needs furhter study. It may be generally concluded that the rice paddy field have not been producing alarming emissions of methane for the whole duration of growth but it is only for a particular period just before harvesting. This point needs elaborate study in different locations and the various parameters. Fig. 19 show the variation of methane emission with plant growth.

The other new aspects of methane emission from rice field is to study the efflux in rice field where chemical insecticides have been used. It is quite obvious that insecticides and pesti-

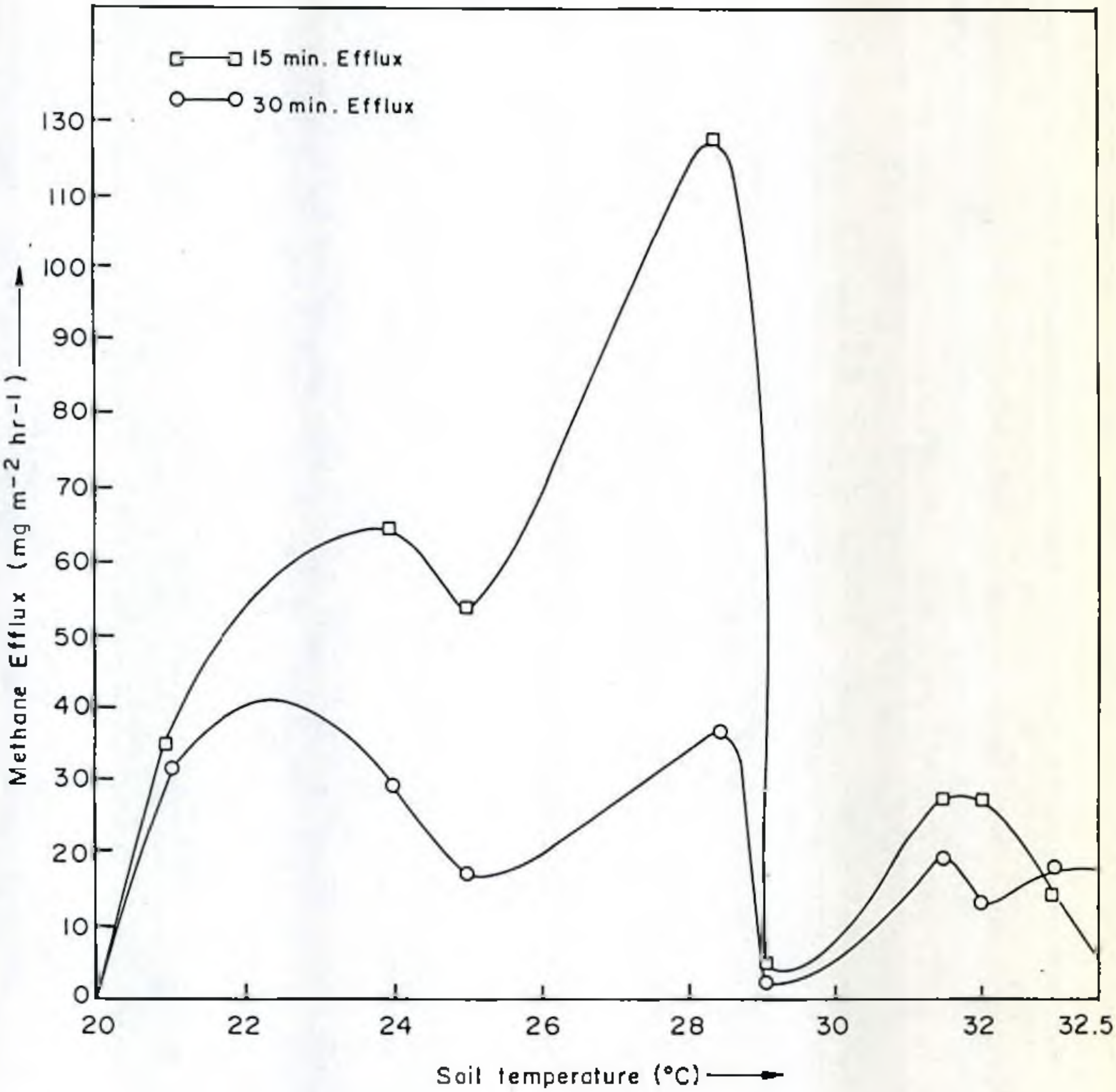


Fig. 18 Variation of methane efflux with Soil temperature (°C).

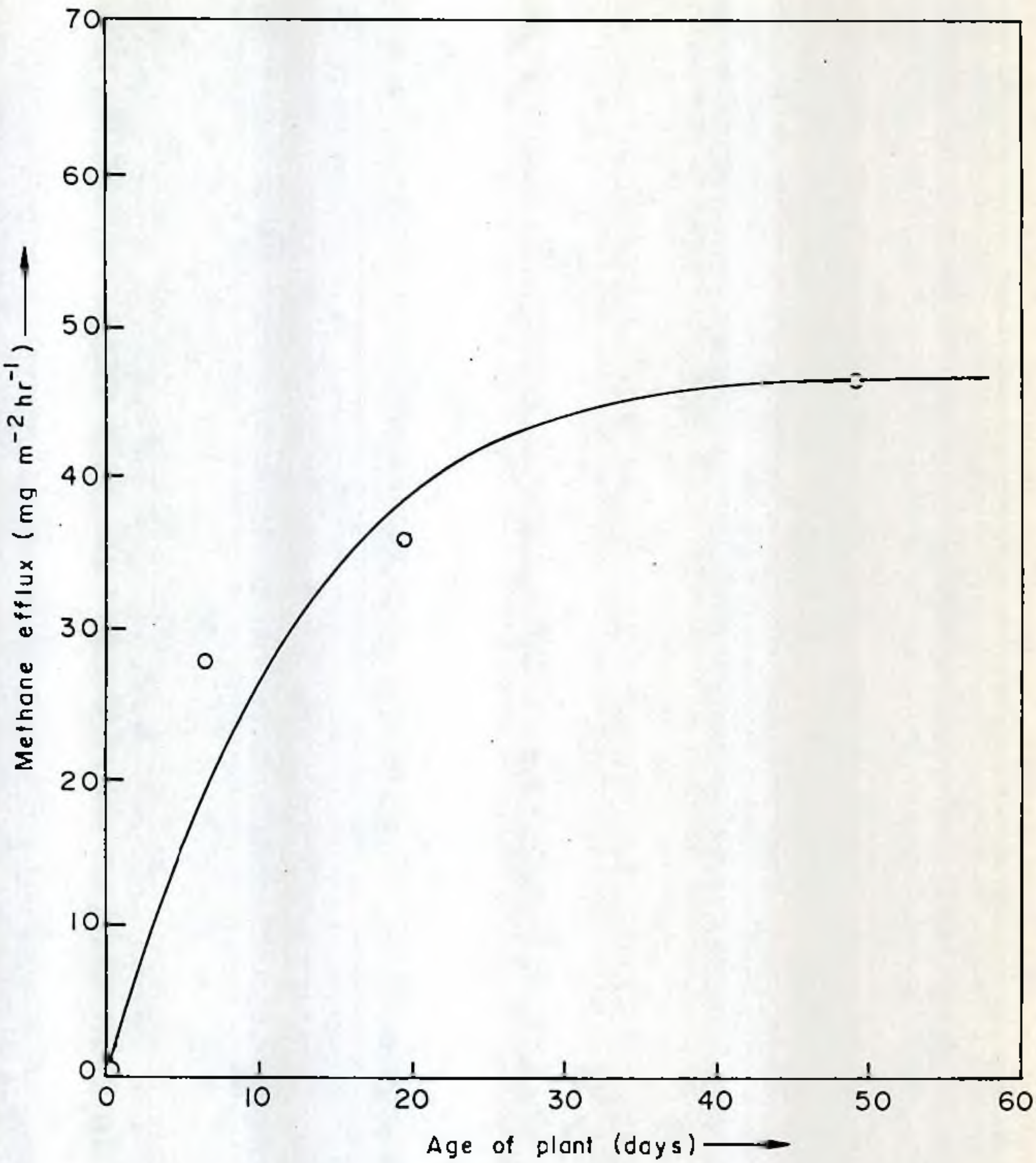


Fig. 19 Variation of methane efflux with plant Growth(days).

cides may act on methanogenic bacteria in the soil and the rice plant thereby decreasing the emission of methane. The mild insectes isloated from plant sources such as neem tree may possibly behave in a different manner. These aspects of the influences of various types of fertilizers and insecticides are subject matter of further investigations. For this purpose, extraction of insecticides from neem tree leaves has been carried out and is ready for subjecting the same for utilization in rice fields and to investigate the comparative characteristics in methane emission from rice fields.

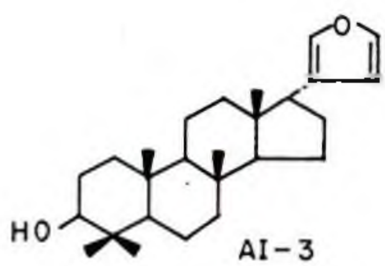
Since the methane emission from rice fields takes place through the microbiological activities in the soil, attempt has been made to reduce the emission of methane by using natural pesticides extracted from Neem tree leaves. It is already found through experimental results that the use of fertilizers such as Urea, Phosphate and Potash in the rice field reduces the emission of mathane. It is inferred that the chamical action of fertilizers as well as the chmical pesticides have the quality of reducing methane emission from rice fields. But such fertilizers and fertilizers also are harmful to environmentally friendly microorganisms. The Neem leave extracts may be fruitfully used to suppress the methane emisstion but are not harmful to the environment. Accordingly, a plan has been drawn up to study the effect of the natural extracts in the suppression of methane emission. However, the detailed experiment could not be carried out within the time frame allowed for the research during the M.Phil degree programme. The research is therefore, to be continued in the future. At present attempts have been made to identify the active functional groups in the extracts and the metal-complexes with the infrared spectra. Several compounds designed as AI-1, AI-2 etc. have been isolated from the Neem tree leaves extracts for the complexation with Ni(II), Cu(II) and Zn(II) as considered to be effective as pescides to be used for suppression of methane emission from rice fields.

6.3 Identification of Compounds in the Neem Tree Leaves Extracts:

The compound AI-1 showed in its IR spectrum (Fig. 6) at 3650-3200 cm^{-1} with its peak centred at 3450 cm^{-1}. This peak together with its another IR absorption band at 1100 cm^{-1} is indicative of a secondary hydroxyl group. Its IR spectrum also showed a band of strong intensity at 1455 cm^{-1} demonstrative of a good number of methylene groups. The presence of these methylene groups also receives support from the doublet-like bands at 725 and 720 cm^{-1}. The higher intensity of the band at 1455 cm^{-1} compared to the band at 1378 cm^{-1} indicated the presence of more methylenes than methyl groups within the molecule [29]. The presence of methylene and methyl groups is also supported by the bands at 2900 and 2850 cm^{-1}. The information obtained from its IR spectrum suggests that the compound AI-1 may be triterpenoid. This compound is considered to be a different triterpenoid as its IR spectral data are not in conformity with those of the reported one from A. Indica.

The compound AI-2 was isolated as a yellowish gummy material. Its IR spectrum (Fig. 8) showed absorption bands at 3178 and 1635 cm^{-1} suggestive of a hydrogen attached to a carbon-carbon double bond. The band at 1735 cm^{-1} together with a tiny peak at 2710 cm^{-1} is indicative of an aldehydic group[30]. The higher intensity band at 1380 cm^{-1} are typical for more methylenes and less methyls in the molecule. The bands at 1240, 1170, 1105, 1032 and 1005 cm^{-1} are demonstrative of an ether linkage. All these information suggest that the compounds is an aldehydic compound ornamented with an aromatic moiety. But such an aldehydic compound has not been previously encountered in this species.

The compound AI-3 was isolated as amorphous white solid. It melted at 165-66 $^{\circ}C$. Its IR spectrum (Fig. 10) had a sharp band at 3450 cm^{-1} indicative of a hydroxyl group and a broad band at 1630 cm^{-1} demonstrative of a carbon -carbon double bond enriched moiety. The wide band at 1100 cm^{-1} was suggestive of an ethereal functionality. The higher intensity band at 1455 cm^{-1} and the lower intensity band at 1378 cm^{-1} are typical for more methylenes and less methyl groups respectively. These spectral data suggest that the compound AI-3 may have the following structure:



AI-3

The isolation of this compound has not been reported from this species prior to this investigation.

The compound AI-4 was isolated as reddish material from the combined petroleum ether and ethyl acetate extracts. Its IR spectrum (Fig. 12) showed a band at 3400 cm^{-1} indicative of a hydroxyl group and a band at 1730 cm^{-1} suggestive of a carbonyl group. The other bands at 2900, 2850, 1448, 1378 and 1240 cm^{-1} are typical for methyl and methylene groups. All of these IR spectral data are in good agreement with those published for an acetoxy triterpenoid[30] previously encountered in this specis.

The compound AI-5 was white solid in appearance and melted at 212-14 ^{0}C. Its IR spectrum (Fig. 14) showed a band at 3450 cm^{-1} typical for a hydroxyl group and another band at 1705 cm^{-1} indicaative of a carbonyl group. The bands at 1640 cm^{-1} together with a band at 800 cm^{-1} suggests the presence of a vinylidene group[29]. The other IR absorption bands at 2900, 2850, 1460 and 1380 cm^{-1} were assignable for methyl and methylene groups. The spectral data were in close agreement with those published data for tetranortriterpenoid lactum[30] isolated from this species. The compounds presently encountered in this species are found to contain hydroxyl and carbonyl groups. These functionalities are believed to have propensity for effecting complexation with metal ions like Cu(II), Ni(II) and Zn(II). But under the condition the reaching do not appear to fake place. The inability of the constituents of the leaf extract for undergoing complexation with metal ions may be due to either of the following reasons or both :

(i) The oxygenated functionalities of the compounds exist either in different planes or in positions far away from each other to span a 5-membered or 6-membered ring structures and also no replacable hydrogen atom occur in the structure.

(ii) The compounds extracted may remain encapsulated with chlorophylls, organic pigments, oil, fatty acids and phenolic materials.

(iii) The complexes are formed but appear to be polymerised. However, the insecticidal and insect repelling effects of the extracts have been found to be quite promising. Further work is in progress to establish the following facts :

(a) the identification of the isolated compounds by the use of modern spectroscopic methods such as ^{1}H NMR, 2D-NMR, ^{13}C NMR, EIMS etc.

(b) the insecticidal and insect-repelling effect of the pure compounds as compared with those with the metal incorporated compounds particularly those of zinc.

(c) Complexation of the purified compounds with other metal ions having different oxidation states

Comparision of the estimated emission of CH_4 of Bangladesh, China and India are given in Table - 7. No other experimental data is available for Bangladesh except the present research. Other authors have made an estimate without any field work in Bangladesh.

Bachelet and Neue[4] using FAO technique of country statistics and crop calenders determined and land area under rice cultivation each month and assuming a constant emission rate, the Asian rice fields alone emit about 82.0 Tg methane per year. However, the Asian rice fields are reported to produce an estimated emission of methane from 57.0 - 82.0 Tg per year. The comparative values of methane emission from the rice fields and wetlands of various countries have been estimated by several authors.

Table - 7 : Comparatives areas of rice cultivation and methane emission Tg. Yr^{-1}

	Countries		
	Bangladesh	China	India
Mitra (1991)	-	5.00	3.50
Mathews et. al (1991)	6.70	21.62	27.64
Mathews et. al (1991)	5.81	13.46	18.35
Neue et. al (1990)	4.13	21.32	18.54
Taylor et.al (1991)	5.81	21.62	18.35
This work (Bangladesh)	1. Full cycle measurements from sowing to harvesting period 0.03 Tg. Yr^{-1} 2. Sporadic measurements at different sites 0.11 Tg. Yr^{-1} (maximum efflux)		

There are considerable deviation in the estimation of methane. The annual emission values of Bangladesh, India and China are give in Table-7. The present research indicate that estimated value of CH_4 from rice fields of Bangladesh based on the experimental results is much less than those reported on the basis of theoretical estimation possibly using data of other countries and applying the same for the soil of Bangladesh.

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