CHAPTER-4

MATERIALS AND METHODS

4.1 Selection of study sites and survey on pesticide use pattern

Dimoria region of Kamrup, Assam, which is having a history of repeated pesticide applications, has been selected as the study area. The people of this region practice different land use systems like food agriculture, bamboo plantation, horticulture, tea plantation, agro-forestry, natural forest, shifting cultivation etc. Several agrochemicals (chemical fertilizers, pesticides etc.) of different chemical nature are known to be used in different land-use systems which may be associated with a number of environmental concerns viz. persistence in the environment, accumulation of heavy metals, toxicity in soil, vegetation and water supplies and impact beyond the target organism including bioaccumulation and its implication for human health.

Nowadays, organochlorine (OC) pesticides were not used primarily due to their persistence in the environment. At this time, the use of OPs and CMs glided due to their availability and quick degradation in the environment. Organophosphorus pesticides have been widely manufactured and used in the world. And they replaced the chlorinated pesticides.

A survey of the study area was carried out to assess the extent of widely used pesticides before sample collection and analysis. The survey was a non-experimental, descriptive research method. Each aspect of farming practices was investigated in a variety of ways adopting techniques such as-direct observation, semi-structured interview with the help of questionnaires developed for the purpose.

The survey was undertaken during the period from Dec 2012- Jan 2013. The researcher visited the fields where the farmers were at work, and minutely observed their working environment and the tools they use for various agricultural practices, like application of fertilizers and pesticides. A randomize representative sample size of nearly 20 persons composed mainly of farmers were selected from the study area. They were interviewed as key informants through a well-structured questionnaire.

The farmers' level of awareness on the key issues like proper handling and disposal practices, various environmental and health impacts of pesticide etc. was also ranked accordingly (*Annexure 1 and 2*). To arrive at conclusions regarding impacts of pesticides used on agricultural fields the *Annexure 1 and 2* were designed very carefully with questions relating to various aspects of pesticide use and these were asked to the respondents. The filled up questionnaires were collected and the data were pooled together to represent the population.

Results of the survey showed that many OPs pesticides were used in the area. However, these insecticides (malathion, quinalphos, dimethoate etc.) were widely used in the area on the vegetable crops, paddy fields, tea garden etc. Based on these findings, **malathion** and **quinalphos** were selected for the study.

4.2 Sampling

Soil sampling is perhaps the most vital step for any soil analysis. As a very small fraction of the huge soil mass is used for analysis, it becomes extremely important to get a truly representative soil sample of the field. Soils are naturally variable horizontally as well as vertically, requiring careful consideration in terms of sampling techniques.

4.2.1 Selection of sampling station

For the purpose of this study three different conventionally managed land use systems of the area were selected, along with some organically managed farms. Samples were collected from some selected conventional and organic agro-ecosystems of the area. In all study areas the same method was used to collect soil samples. In total 10 stations were selected. Two stations each from **tea plantation, low land rice field, vegetable farms** which are conventionally managed and one station from **natural forest** representing the (Control sample)were selected and three organically managed systems (viz. **paddy fields, vegetable farms** etc.) were selected. Three soil samples at 0-15 cm depth were collected randomly from each station, comprising 30 soil samples.

4.2.2 Sampling procedure

Proper procedures must be followed to collect representative soil samples. For the present study, Simple Random Sampling Techniques was adopted to collect soil samples. In order to collect soil samples (0-15 cm depth) grasses, mosses, litter and other plant residues were removed from soil surface. Collection of soil samples was done by using an auger. In each case, a triangular block of 0-15 cm depth at random locations of each sampling site was cut with the help of the auger. These samples were mixed well and about 1kg of representative composite samples was prepared. Soils were collected in plastic bags, which were sealed and labelled properly.

Three composite soil samples from a rooting depth of 15 cm were collected randomly from each sampling station. Three samples from each site were also taken from 0 to 15 cm layer for bulk density determination.

4.2.3 Soil sample preparation

Preparation of soil samples is based on the **ISO 11464 methods** (Soil quality- pre-treatment of samples for physico- chemical analysis).

Drying: Collected samples were brought to the laboratory for analysis. Before analysis, the samples were spread out thinly on a piece of hard paper for drying in air in a shade.

Crushing/Grinding: Following drying, the big lumps were broken down by hand using a wooden pestle, and visible plant roots, pebbles and other undesirable matters were removed.

Sieving: After the soil become completely dry, and after homogenization, a portion of each sample was passed through a 2-mm mesh screen and preserved in clean sealed polythene bags for various physico-chemical analyses. Some samples were preserved in clean sealed polythene bags and stored in sealed polythene boxes to avoid air contamination at 4°C before microbial and biochemical analysis.

The rest was sieved through a 500 μm mesh (Fritsch laboratory sieving set used) and used for determining soil "total" Cd, Cr, Cu, Ni, Pb, and Zn.

4.2.4 Soil sample labeling

In total 30 composite soil samples were collected comprising 10 sampling sites. The collected and prepared samples for various analyses were labelled as follows:

S1 (S1A, S1B, S1C) - Tea Garden 1

S2 (S2A, S2B, S2C) - Tea Garden 2

S3 (S3A, S3B, S3C) - Vegetable farm 1

S4 (S4A, S4B, S4C) - Vegetable farm 2

S5 (S5A, S5B, S5C) - Rice field 1

S6 (S6A, S6B, S6C) - Rice field 2

S7 (S7A, S7B, S7C) - Natural forest (Control sample)

OF-1 (OF1A, OF1B, OF1C) -Vegetable farm (Organic)

OF-2 (OF2A, OF2B, OF2C) - Paddy field (Organic)

OF-3 (OF3A, OF3B, OF3C) - Sugarcane farm (Organic)

The soil samples were collected for two consecutive years **(2013, 2014)** at the same time period of the year except in the case of organically managed farms where sampling was done only once.

4.3 Analytical procedures

4.3.1 Soil quality parameters and methodology for their study

A large number of parameters are generally used to characterize the soil quality criteria. The most important consideration should be those properties of soil, which influence the movement and retention of water that contribute to store and supply of nutrients. In this study some selected physical and chemical parameters were selected. The soil samples were analyzed for those parameters that influence the quality of soil, in context to soil productivity. The available experimental facilities were also taken into account in selecting the parameters.

4.3.1.1 Physical parameters

- Temperature
- Texture
- Bulk density
- Moisture content
- Water holding capacity

4.3.1.2. Chemical parameters

- pH
- Electrical Conductivity
- Organic matter
- Nitrate nitrogen
- Phosphorus
- Potassium

The methodology adopted for analysis of different parameters that were used to assess soil quality of different land uses are shown in Table 4.1.

Table 4.1 Soil properties under study with their methods of measurement

Soil properties	**Methods**
Bulk density	Core sampling method (Blake and Hartge, 1986)
Soil Texture	Feel method
Soil Temperature	Soil thermometer
Moisture content	Gravimetric method.
Water-holding capacity	Gravimetric method.
pH	Potentiometrically in 1:2.5 (v/v) soil suspension in water. Digital pH meter (Systronics μP^H System 361)
Electrical Conductivity	Conductivity meter (Systronics Conductivity meter, 306)
Organic matter	Titrimetric method (Walkley and Black, 1934).
Nitrate nitrogen	Spectrophotometric method (ELICO, SL-159)
Available phosphorus	Spectrophotometric method (ELICO, SL-159)
Available potassium	Flame photometer method (ELICO, CL 22 D)

Temperature:

Soil temperature plays an important role in many processes, which take place in the soil such as chemical reactions and biological interactions. Soil temperature is of greater ecological significance to plant life than air temperature. Soil temperature influence germination of seed, the functional activity of roots, the rate and duration of plant growth, and occurrence and severity of plant disease. Extremely high soil temperature also has harmful effects on roots and may cause destructive lesion on the stems of plant. Low soil temperature impedes the plant minerals intake. Persistently cold soil results in dwarfed growth. Soil temperature also influences on other soil properties such as organic matter accumulation, weathering of parent materials and nutrient availability.

The temperature of the soil samples were measured at the time of collection using mercury thermometer graduated between 0° and 100°C.

Texture:

Soil texture is a soil property used to describe the relative proportion of different grain sizes of mineral particles in a soil. Particles are normally grouped into three main classes: sand, silt and clay. Texture of soil was qualitatively determined by rapid method which involves scrubbing the moistened soil between thumb and forefingers. In this procedure proficiency is gained through practice, and making comparison with samples of known textural class determined by some quantitative methods. Each soil separate has a distinct texture that can be distinguished by touch.

Table 4.2 Classification of soil texture by feel method

Experience	Type	Textural class
1. Very gritty, does not form ball, does not stain finger.	Very light	Sand
2. Very gritty, form ball but very easily broken, stains finger slightly.	Light	Loamy sand
3. Moderately gritty, forms fairly firm ball which is easily broken, definitely stains finger.		Sandy loam
4. Neither very gritty nor very smooth, forms firm balls but does not ribbon, strains finger appreciably.	Medium	Loam
5. Smooth or sticky, buttery feel, forms firm ball, strains and has a slight tendency to form ribbon with flaky surface		Silt loam
6. Moderately sticky, slightly gritty feel, forms moderately hard ball when dry, stains, ribbons out on squeezing but the ribbon breaks easily.	Heavy	Clay loam
7. Same as above but very smooth, shows flaking on ribbon surface, very sticky feel, forms ball which when dry cannot be crushed by fingers, stains heavily, squeezes out at right moisture into long (2-5 cm) ribbon.		Sandy clay loam

(Source: Ghosh, A.B., Bajaj, J.C., R and Shing, D. (1983), Soil and Water Testing Methods: A Laboratory Manual, IARL, New Delhi)

Moisture Content:

Calculation and reporting of the results of soil analysis is done on basis of "oven-dry" soil. The moisture content of air-dry soil is determined prior to soil analysis. To recalculate the analysis results on dry weight basis, the moisture content of the sample has to be determined by oven-drying a sample to constant mass. The difference in mass is used to calculate water content on a mass basis. Moisture regulates the physical, chemical and biological properties of soil. The moisture content of soil was determined in the laboratory from the difference in weights of the wet soil, and the dry soil and the result were calculated on the basis of dry weight.

Bulk Density:

Bulk density is defined as the mass (weight) of a unit volume of oven dry soil. The volume includes both solids and pores. In mineral soils with no coarse fragment content the bulk density of the total mineral soil is equal to the bulk density of the fine earth. The dry bulk density (BD) is the ratio between the mass of oven dry soil material and the volume of the undisturbed fresh sample. The ISO defines dry bulk density as the ratio of the oven-dry mass of the solids to the volume (the bulk volume includes the volume of the solids and of the pore space) of the soil. The bulk density of the soil is the dry weight of a unit volume of it and it is expressed as gm/cm^3.

Bulk density of soil is determined on core samples, which are taken by driving a bamboo corer into the soil at the desired depth. The samples are then oven dried and weighed.

Results are computed by using the formula:

$$Bulkdensity(gm/cm^3) = \frac{Weightofsoil\ (gm)}{Volumeofsoil\ (cm^3)}$$

Water holding capacity:

Water holding capacity of soils is controlled primarily by:

1) The number of pores and pore-size distribution of soils; and
2) The specific surface area soils.

Water holding capacity increased with addition of organic fertilizers, but increases vary with soil texture.

Water holding capacity of the soil usually refers to amount of maximum water, which can be held in the saturated soils. It is generally measured as the amount of water taken up by unit weight of dry soil when immersed in water under standardized conditions.

Results are computed by using the formula:

$$WHC(\%) = \frac{(W_2 - W_1) - (W_1 - W_0)}{(W_1 - W_0)} \text{ x } 100$$

Where, WHC = water holding capacity.

W_0 = weight of empty box (gm).

W_1 = weight of box with dried soil (gm)

W_2 = weight of box with water saturated soil (gm)

For this purpose, approximately 10 g of oven-dried soil (100-105°C) was taken in a Whatman No 1 filter paper, placed on the bottom of a perforated brass box and weighed. The mass of the circular box along with the dry filter paper and the mass of the same with wet filter paper were determined separately. During the drying period the temperature was maintained between 100-105° C, and the sample was dried for a uniform period of at least 10 hours. The soil samples were preserved overnight in the box in contact with water up to one fourth. Next day, the sample was taken out and allowed to stand to drain off the excess water before mass was determined (Nath, T.N., 2010).

pH:

The pH is a measure of the hydrogen ion activity of the soil water system and expresses the acidity and alkalinity of soil. The pH is a very important property of soil as it determines the availability of nutrients, microbial activity and physical condition of the soil.The pH range normally found in soils varies from 3 to 9.

The pH of the soil is potentiometrically measured in the supernatant suspension of a 1:5 soil : liquid (v/v) mixture. This liquid is made up of a 0.01 mol/l solution of calcium chloride in water pH ($CaCl_2$) or water pH (H_2O).

pH of the soil suspension was determined with the help of a digital pH meter.

The United States Department of Agriculture Natural Resources Conservation Service, formerly Soil Conservation Service classifies soil pH ranges as in table 4.3

Table 4.3 Soil pH range classification by The United States Department of Agriculture Natural Resources Conservation Service

Denomination	**pH range**
Ultra acid	< 3.5
Extreme acid	3.5–4.4
Very strong acid	4.5–5.0
Strong acid	5.1–5.5
Moderate acid	5.6–6.0
Slight acid	6.1–6.5
Neutral	6.6–7.3
Slightly alkaline	7.4–7.8
Moderately alkaline	7.9–8.4
Strongly alkaline	8.5–9.0
Very strongly alkaline	> 9.0

ICAR-Indian Institute of soil science in 2015 presented the rating chart for soil pH mentioned in the table 4.4

Table: 4.4 Rating chart for soil pH (ICAR, 2015)

Soil pH value	**Soil reaction**	**Rating**
>8.5	Strongly alkaline	Poor
7.1-8.5	Moderately alkaline	Fair

7.0	Neutral	Good
6.6-6.9	Slightly acid, maximum availability of all the essential plant nutrients.	Good
5.6-6.5	Moderately acid	Fair
4.6-5.5	Strongly acid	Poor
3.5-4.6	Extremely acid soils of warm to humid and high rainfall areas. (Laterite soils)	Poor
Less than 3.5	Acid sulphate soils (Kerala coastal belt)	Poor

Soil Electrical Conductivity (E.C.)

The electrical conductivity of a solution is the reciprocal value of its electrical resistivity. Soils possess at least small amounts of various soluble salts. These salts may be acidic, neutral or basic. Soluble salts present in soil dissociate into their respective cations and anions in soil solution. These cations and anions carry current and impart conductivity. Higher the concentration of ions in solution more is its electrical conductance.

For the measurement of E.C., the dried soil sample was grounded finely using mortar and pestle and the coarse fraction was separated out using 2 mm sieve. The soil sample (10g) was mixed with boiling distilled water (40 mL) in the ratio of 1**:**5 (The International standard NF ISO 10390, 1994). Then the entire mixture was kept on an oscillating table for 5 minutes to secure proper agitation. The mixture was then allowed to settle and decanted to obtain the clear layer of water. By using a standard

HACH sensor (conductivity meter), the E.C. the clear layer was measured three times and an average of these three readings was taken to minimize the error. The electrical conductivity of soil sample was recorded in (µS/cm).

Organic Matter:

Soil organic matter (SOM) (or soil organic carbon (SOC)) is commonly recognized as one of the key chemical parameters of soil quality.

Organic carbon or soil organic carbon (SOC) is the carbon associated with soil organic matter. Soil organic matter is the organic fraction of the soil that is made up of decomposed plant and animal materials as well as microbial organisms, but does not include fresh and un-decomposed plant materials, such as straw and litter, lying on the soil surface. Soil carbon can also be present in inorganic forms, e.g. lime or carbonates in some soils in the drier areas. Sedimentary organic matter includes the carbonaceous component of sediments. A major portion of nitrogen (95-99 % of the total), phosphorus (33-67 % of the total) and sulphur (75% of the total) in soil occur in organic combination, which mineralize to release the nutrients in inorganic form to be used by plants. The level of soil organic matter is largely determined by the addition of surface litter (fallen leaves, manure and dead organisms) and root material and the rate at which microbes break down organic compounds (NRMMC, 2005).

The organic carbon (%) of the soil samples was determined by titrimetric method, Walkley and Black, 1934. Thereafter, the soil organic matter is measured.

% Soil organic matter =% organic carbon x 1.724 (Allison, L.E., 1965)

Nitrate nitrogen (mg/kg):

Soil nitrate (NO_3^-) is a form of inorganic nitrogen that is available for use by plants. It forms from the mineralization (by microorganisms) of organic forms of N (i.e, soil organic matter, crop residue, and manure) in the soil. The rate of N mineralization is dependent on the amount of soil organic N, water content, temperature, p^H and aeration.

The determination of this ion was done with the help of spectrophotometer (ELICO, SL-159). The NO_3-N in soil is expressed in mg/kg of soil.

Phosphorus (kg/ha):

Phosphorus is second key nutrient found in soil. Phosphorus occurs in soil in both mineral and organic forms. Phosphorus is essential to plant growth as it promotes plant metabolism. Phosphorus occurs in soil in both mineral and organic forms. The phosphorus in organic matter is tied in compounds and is unavailable to plants until the organic material decomposes. Inorganic phosphorus comes from the mineral apatite, $Ca_3 (PO_4)_3F$.

Phosphorus in soil is generally determined as available phosphorus, which can be extracted from soil with 0.002 N H_2SO_4. After extraction, the phosphorus was estimated spectrophotometrically. The available phosphorus in soil is expressed in Kg/ha of soil.

Potassium (kg/ha):

Potassium in soil is known to be required by plant in large quantities and it contributes largely in the productivity of crops. The higher level of potassium nutrition enhances amino acid translocation from the vegetative plant parts to grains, thereby promotes protein synthesis.

The K^+ in the filtrate (NH_4- acetate extract) was measured by the Flame photometric method. The available potassium in soil is expressed in Kg/ha of soil.

4.3.2 Heavy metal analysis of soil samples

For determining metal concentration in soil, at first the soil samples were prepared for digestion. For digestion, 1gm of soil from each sample was taken and mixed with 1ml sulphuric acid, 10ml nitric acid and 4ml perchloric acid and heated in a hot plate in digestion chamber at temperature 100°C. When a white fume appears it indicates the completion of digestion process. Then the samples were filtered by Whatman filter paper and kept in a narrow mouth plastic bottle. The total volume of prepared samples was made upto10ml and analyzed by Atomic Absorption Spectroscopy (AAS) (VARIAN, Model: AA240). During digestion process all the apparatus was cleaned properly and glass wares was washed with tap water and then distilled water to prevent cross contamination.

In AAS, for the determination of heavy metals Cd, Cr, Cu, Ni, Pb, and Zn the calibration is done by standard solution of each metal.

4.3.3. Determination of pesticide residues in soil

4.3.3.1 Soil sample preparation

Collected samples were brought to the laboratory for analysis. Before analysis, the samples were spread out thinly on a piece of hard paper for drying in air in a shade. The big lumps were broken down, and visible plant roots, pebbles and other undesirable matters were removed. After the soil become completely dry, and after homogenization, a portion of each sample was passed through a 2-mm mesh screen and preserved in clean sealed polythene bags for pesticide residue analysis.

4.3.3.2 Analysis of presence of pesticide residues

Extraction procedure:

About ½ kg of soil sample was mixed with 500 ml HPLC grade hexane: acetone solution in the ratio of 80:20 and left overnight to decant and then filtered. The residue thus obtained was further mixed with 250 ml of the same solution i.e hexane: acetone and filtered. The process of filtration is repeated at least for 3 times and the filtrates are mixed together and air-dried. 1.5 ml of the hexane: acetone solution is again added to air dried filtrate and then transferred into a 2ml capacity glass vial for GC analysis.

GC analysis:

The above prepared soil extract is injected in gas chromatograph (DANI Master G.C). Organophosphorus pesticides were detected with NPD (Nitrogen Phosphorus Detector) fitted with cross linked Methyl Silicon gum capillary (HP-5) column (30 m x 0.32 mm x 0.25 μm). The operating conditions were: injector port temperature 290°C, detector

temperature 350°C, initial oven temperature 160°C for 1 min, raised to 250°C at 10°C/min and then held at 250°C for 4 min, again raised to 270°C at 10°C/ min and finally held at 270°C for 4 min. The carrier gas was nitrogen at 10 ml/min and the injection volume was lml.

4.4. Microbiological procedures

4.4.1 Pesticides used:

Pesticides considered in this study are:

- Malathion
- Quinalphos

4.4.2 Isolation of malathion degrading bacteria

For isolation and selection of malathion degrading bacteria, microbial colonies were isolated from collected soil samples. Pour plate technique was used for the isolation of pesticide degrading bacteria in Nutrient agar (Singh et al., 2009).

Five gms of each soil sample except control sample was mixed in 100 ml autoclaved water in 6 different conical flasks and kept at 100rpm for 24hrs at 37◦C.

A selective medium (M1) was prepared containing the following composition:

M1 Media composition	Quantity (g/l)
Malathion (commercial grade 50%)	0.5
KH_2PO_4	0.1
$MgSO_4$	0.02
NH_4NO_3	0.5
Agar	1.5

To this solution 15µl of a mineral solution (MS) containing the following composition was added.

Mineral Solution composition	Quantity(g/l)
$FeSO_4$	10
$CaCl_2$	10
$CuNO_3$	0.5
$MnCl_2$	0.4

From each processed samples plating was done using **M1 media** and the inoculated plates were subsequently incubated at 37°C for 48 hrs.

Colonies obtained were further cultured in nutrient medium (NM) containing the following composition.

Nutrient medium (NM) composition	Quantity(g/l)
Yeast extract	0.07
Peptone	0.05
Glucose	0.05
K_2HPO_4	0.03
$MgSO_4.7H_2O$	0.007
Malathion (commercial grade 50%)	0.5

Serial transfer of bacterial isolates was made by streaking and inoculating to nutrient medium containing malathion. Selection of pure culture is done by repeating sub culturing for 4-6 times. The isolated strains were maintained on Nutrient agar and King's B agar slants and stored at 4°C.

4.4.3. Isolation of quinalphos degrading bacteria

The bacterial cultures capable of degrading quinalphos were isolated from soil samples collected from the study area using mineral salt medium (MSM), with some concentration of quinalphos. Standard analytical grade solution of quinalphos (25% E.C.) was purchased from the local market. 1gm of each soil sample was inoculated into 6 different 500ml Erlenmeyer flask containing 100ml of mineral salt medium(MSM) supplemented with 5 mg/l concentration of quinalphos.

The composition of Mineral Salts Medium (MSM) is given below:

MS Medium composition	**Quantity (g/l)**
$NaNO_3$	0.3
$MgSO_4$	0.05
KCl	0.05
K_2HPO_4	0.1
KH_2PO_4	0.05
$FeSO_4$	0.001
Yeast extract	0.05
Glucose	1.0

The flasks were incubated on a rotary shaker at 150 cycles per rpm for 7 days at room temperature (25-30˚C). At daily intervals, one loop full of enrichment culture from the flask was streaked on nutrient agar plates supplemented with quinalphos (5mg/l) and incubated at room temperature for 24-48hrs. (Pawar and Mali, 2014).

Nutrient agar media was prepared by adding 7g of agar and 5g quinalphos in 250ml water. Individual colonies of bacteria that varied in shape and color were picked up and were sub cultured onto nutrient agar plates containing same concentration of quinalphos until pure culture1 was obtained. The isolated strain was maintained at 4◦C.

4.4.4. Microscopic study of bacterial cultures

The bacterial isolates were studied for their various microscopic characters such as

4.4.4.1. Colony morphology: Study of colony morphology includes colour, size, margin, elevation etc.

4.4.4.2 Gram's staining: Gram's staining of the cultures was performed by the method as described by Cappuccino et al. (1999).

***4.4.4.3. Motility test*:** Motility test was performed by hanging drop method as described by Cappuccino et al. (1999).

4.4.5 Effect of Temperature, pH, carbon sources and nitrogen sources on the growth of pesticide degrading bacterial isolates:

4.4.5.1. Effect of temperature on growth of pesticide degrading bacterial isolates

To study the efficacy of the bacterial isolates for the biodegradation of pesticides (malathion and quinalphos), each isolates were inoculated in an Erlenmeyer flask containing 100ml of sterilized minimal salt broth supplemented with 50ppm of pesticides. The flasks were then incubated at different temperatures (**25°C, 35°C, 45°C** and **55°C**). After 24 hours, 5ml of culture, from each culture was drawn and centrifuged at 5000rpm for 10 minutes and the pellets were discarded and the supernatants were collected to evaluate the growth of pesticide degrading bacteria. The optical density was measured by a UV-Vis spectrophotometer (ELICO, SL-159) at 560nm (Karunya and Reetha, 2012).

4.4.5.2. Effect of pH on growth of pesticide degrading bacterial isolates

In order to analyze the effect of pH variations on the growth of the organism, an experiment was conducted in Erlenmeyer flasks containing 100 ml MS Broth, supplemented with 50ppm of pesticides (malathion and quinalphos) and adjusted the pH of the media to **pH 4, pH 5, pH 6, pH 7** and **pH 8**. After sterilization by autoclaving the flasks were allowed to cool down and inoculated with the bacterial cultures after inoculation. The flasks were then incubated at 37°C separately for24 hrs. To analyze the effect of pH the growth of pesticides degrading bacterial isolates were assessed by using UV Spectrophotometer(ELICO, SL-159) at 560nm (Karunya and Reetha, 2012).

4.4.5.3 Effect of carbon sources on growth of pesticide degrading bacterial isolates

To evaluate the growth of pesticides degrading bacteria, the bacterial isolates were cultured in 50ml of MS Broth with 50ppm of pesticides and 0.5gmof various carbon sources such as **lactose, dextrose, and fructose** and incubated at 37°C for 24 hrs. The growth of pesticides degrading Bacterial isolates was assessed by using UV spectrophotometer (ELICO, SL-159) at 560nm (Karunya and Reetha, 2012).

4.4.5.4 Effect of nitrogen sources on growth of pesticide degrading bacterial isolates

The effect of various nitrogen sources for the maximization of pesticide biodegradation was tested. The bacterial isolates were cultivated in 50 ml Minimal salt broth with 50ppm of pesticides and 0.5g of various nitrogen sources **(peptone, beef extract and yeast extract)** and incubated at 37°C. The growth of pesticides degrading Bacterial isolates were assessed by using UV spectrophotometer (ELICO, SL-159) at 560nm (Karunya and Reetha, 2012).