3. MATERIALS AND METHODS

3.1 Construction of cement tanks

Brick work cement tanks were constructed under a tiled animal house at Government Arts College (Autonomous), Kumbakonam, for this vermiculture and vermicomposting study.

3.1.1 Large cement tanks

Six rectangular brick work large cement tanks each having the size of 180x75x90cm were constructed free from earthworm invasion and were used as decomposition pits for partial decomposition of organic materials.

3.1.2 Small cement tanks

Eighteen rectangular brick work small cement tanks (size:58x52x36cm) free from other earthworm species or predators invasion were constructed along with the large cement tanks under the same animal house and were used for keeping stock earthworms.

3.2 Procurement of earthworms

The adult specimens of epigeic earthworm, *E.fedida* with the size, 7.8 to 8.7 cm in length and 319 to 389 mg in weight were purchased from the Vermiculture Division of Periyar Maniyammai University, Vallam, Thanjavur District, Tamilnadu. Adult specimens of anecic earthworm, *L.mauritii* with the size, 11.5 to 12.5 cm in length and 847 to 889 mg in weight were collected from the cow dung pits near Vatti Pillaiyar Kovil, Kumbakonam, Thanjavur District, Tamil Nadu.
3.2.1 Maintenance of adult earthworms

Both the species were kept in separate small cement tanks with substrate medium containing 50% cow dung and 50% soil and maintained under the laboratory condition (medium temperature 25 ± 2°C) during the course of this vermiculture study. The culture tanks were covered with cotton clothes to protect the adult earthworms from their predators. Sufficient water was added in these tanks to maintain the optimum moisture condition for better survival and growth of earthworms (Mitchell et al., 1977; Kaplan et al., 1980; Reinecke and Kriel, 1981; Martin, 1982; Loehr et al., 1985; Reinecke and Venter, 1985; 1987; Hallatt et al., 1992; Parthasarathi, 2007).

3.3 Collection of organic materials

3.3.1 Collection of sheep droppings

Fresh sheep droppings of about 300 Kg were collected from sheep herds of Anaikudam Village, Udaiyarpalayam Taluk, Perambalur District, Tamil Nadu.

3.3.2 Collection of press mud

About 500 Kg of fresh press mud was collected from Thiru Aruran Sugar Factory, Thirumandankudi, Thanjavur District, Tamil Nadu.

3.3.3 Collection of Pongamia leaves

Withered dry Pongamia leaves of about 300 Kg were collected from Government Arts College (Autonomous), Kumbakonam.
3.3.4 Collection of cow dung

Fresh cow dung was collected from the dairy farm at Swaminathan Nagar, Palakkarai, Kumbakonam, Thanjavur District, Tamil Nadu, for keeping stock earthworms.

3.4 Collection of soil

Dry alluvium soil collected from the Cauvery river bank near Government Arts College (Autonomous), Kumbakonam, was manually powdered and stored in polyethylene bags.

3.5 Removal of non-degradable matters

The unwanted non-degradable matters such as plastics, glass pieces, polyethylene papers and stones were removed from the organic materials before proceeding to partial decomposition.

3.6 Partial decomposition of sheep droppings

One rectangular brick work cement pit was cleaned with water and filled with sheep droppings. After adding sufficient water, the pit was covered with polyethylene sheets to avoid water evaporation and a possible release of foul smell during decomposition. Once in 3 days the decomposing materials in the pit after adding sufficient water were thoroughly mixed with a spade to ensure uniform decomposition. Ideal semi decomposed sheep droppings were obtained only after 60 days of anaerobic decomposition. About 200 Kg of dry semi decomposed materials were collected after 2 to 3 days of air-drying and stored in polyethylene bags.
3.7 Partial decomposition of press mud

Another cleaned cement pit was used for the partial decomposition of press mud. After adding sufficient water, the press mud was filled in the pit and was covered with polyethylene sheets. The decomposing pit was maintained as per the procedures followed in partial decomposition of sheep droppings. Ideal semi decomposed press mud was obtained only after 45 days of anaerobic decomposition. After air drying for 3 to 4 days, about 300 Kg of dry semi decomposed press mud can be collected and stored in polyethylene bags.

3.8 Partial decomposition of *Pongamia* leaves

*Pongamia* leaves soaked with sufficient water were filled in another three cement pits. The pits were covered with polyethylene sheets for proper decomposition. The procedures adopted in partial decomposition of sheep droppings were followed here to get ideal partly decomposed *Pongamia* materials for vermiculture study. About 150 Kg of dry semi decomposed materials can be obtained only after 180 days of anaerobic decomposition. The materials were air dried for 2 to 3 days and stored in polyethylene bags.

3.9 Separation of core particles

The partly decomposed materials stored in polyethylene bags were separately powdered manually using thick wooden rod. The powdered organic materials and the soil were sieved separately through a sieve net (1 mm × 1 mm) to obtain a medium with a particle size less than 1 mm as suggested by Reinecke and Venter (1985) and were stored
in separate polyethylene bags for vermiculture, nutrients analysis and plant cultivation study.

3.10 Preparation of organic mixture

An organic mixture (1:1:1 ratio) was prepared using the above three partly decomposed organic materials for the culture practices of earthworms along with other organic materials.

3.11 Procurement of earthen pots

Two hundred earthen pots of equal size (26 cm diameter and 25 cm height) were purchased from Ammachatram, Kumbakonam, Thanjavur District, Tamil Nadu, for vermiculture and plant cultivation study.

3.12 Culture of earthworms

3.12.1 Preparation and maintenance of vermiculture media

Six sets of eight media, 100, 75, 50, 40, 30, 20, 10 and 0 per cent substrate ratios (PSR) in each organic material were prepared using sieved powders of partly decomposed sheep droppings/ press mud/ Pongamia leaves/ organic mixture and dry soil with volume by volume basis. Four liters of each substrate medium was taken in an earthen pot and sufficient water was added to ensure optimum moisture condition (Mitchell et al., 1977; Kaplan et al., 1980; Reinecke and Kriel, 1981; Martin, 1982; Loehr et al., 1985; Reinecke and Venter, 1985; 1987; Hallatt et al., 1992; Parthasarathi, 2007) and temperature (Edwards and Bater, 1992; Hou et al., 2005). To assess the rate of cocoon production in the said media in each organic material, 12 adult earthworms of *E.fetida* or *L.mauritii* were
introduced into each pot. Regular watering is a must for the growing earthworms. All the pots were covered with cotton clothes to protect the earthworms free from their predators such as frog, ant, rat, squirrel, centipede, millipede and termites.

3.12.2 Collection of cocoons

Cocoons produced by earthworms were collected once in six days and recorded for a period of 30 days. The earthworm body weight was also taken and recorded once in six days at the same time of cocoon collection. Survival of earthworms was also noted during the course of this study.

3.12.3 Maintenance of cocoons

Cocoons collected at 6 days interval for 30 days from the earthworms, *E.fetida* and *L.mauritii* exposed to different PSR media were placed separately in plastic cups containing the same PSR media to assess their hatching ability, incubation time and rate of hatchling production. The cocoons kept in the plastic cups were added sufficient water and observed their hatching time until they were hatched out into hatchlings. In order to maintain the optimum moisture condition and free from predation, if any, the plastic cups were covered with cotton clothes.

3.12.4 Collection of vermicomposts

At the end of 30 days of reproductive study, the PSR media used by earthworms were collected as vermicomposts and stored in separate polythene bags for macro and micronutrients analysis and for raising black gram in pots.
3.13 Culture study of F₁ offsprings

Only twelve F₁ hatchlings were taken from each PSR medium and placed in the earthen pots containing the same PSR medium from where they were collected.

3.13.1 Measurements of length and weight of growing F₁ hatchlings

The length and weight of growing hatchlings were measured at an interval of 10 days until they transformed into mature stage (appearance of clitellum).

3.13.2 Collection of cocoons from F₁ earthworms

Cocoons produced by mature F₁ earthworms were collected and recorded once in six days for a period of 30 days as followed in their parent’s reproductive study.

3.14 Calculation

Data collected during reproductive study with parent earthworms and vermiculture study with F₁ offsprings were used to calculate the following growth and reproductive parameters.

3.14.1 Per cent weight change (PWC)

\[
PWC = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100
\]

3.14.2 Growth rate (GR)

\[
GR \text{ (mg/day)} = \frac{\text{Final body weight (mg)} - \text{Initial body weight (mg)}}{\text{Growth period (days)}}
\]
3.14.3 Cocoon production rate (CPR)

\[ \text{CPR (C/W/D)} = \frac{\text{Total cocoons produced} \div \text{No. of worms used}}{\text{Period of cocoon collection (days)}} \]

3.14.4 Hatchling production rate (HPR)

\[ \text{HPR (H/C)} = \frac{\text{Total hatchlings emerged from the cocoons}}{\text{Total cocoons incubated}} \]

3.14.5 Per cent hatching success (PHS)

\[ \text{PHS} = \frac{\text{No. of hatched cocoons}}{\text{Total cocoons incubated}} \times 100 \quad \text{(or)} \]

\[ \text{PHS} = \frac{\text{Total cocoons incubated} - \text{Unhatched cocoons}}{\text{Total cocoons incubated}} \times 100 \]

3.15 Physico-chemical analysis

The levels of pH, electrical conductivity (EC), macro nutrients (organic carbon, total nitrogen, total phosphorus, total potassium, total sodium and total calcium) and micro nutrients (iron, manganese, zinc and copper) present in the samples taken from soil, partly decomposed and vermicomposted organic materials (sheep droppings, press mud, Pongamia leaves and organic mixture) were measured/estimated at Soil Testing Laboratory, Department of Soil Science and Agricultural Chemistry, Anbil Dharmalingam Agricultural College and Research Institute, Trichirappalli, Tamil Nadu.
3.15.1 Determination of pH

The pH was determined by Potentiometric method of Jackson (1973). The term, “pH” is a notation used to express the acidity or alkalinity of the given sample.

Reagents

Buffer solutions - Buffer tablets of pH 4, 7 and 9.2 are dissolved separately and made upto 100 ml with water. If the buffer tablets are not available, salt may be used. For pH 4.0 - Dissolve 10.210 gm of potassium hydrogen phthalate in water and dilute to one litre. For pH 9.2 - Dissolve 3.810 gm of sodium tetraborate in water and dilute to one litre.

Procedure

Weigh 20 gm of organic sample in a 100 ml beaker and add 50 ml of water to make sample to water ratio as 1:2.5. Stir the suspension with rubber tipped glass rod at regular intervals for 30 minutes. Switch on the pH meter and warm up for about 15-20 minutes. Set the galvanometer reading at zero with the help of zero set knob. Dip the electrode pair into a buffer solution of known pH. Adjust the pH reading with the help of buffer set knob. Dip the electrodes into the sample water suspension and record the pH.

Ratings of pH level in soil

<table>
<thead>
<tr>
<th>Type</th>
<th>pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic</td>
<td>&lt; 6.5</td>
</tr>
<tr>
<td>Normal</td>
<td>6.5 - 7.5</td>
</tr>
<tr>
<td>Saline/calcareous</td>
<td>7.5 - 8.5</td>
</tr>
<tr>
<td>Alkaline</td>
<td>&gt; 8.5</td>
</tr>
</tbody>
</table>
3.15.2 Determination of electrical conductivity (EC)

The EC was determined by Conductometric method of Jackson (1973). It is expressed as reciprocal ohms or mhos per cm. As the values of EC for organic samples and the most irrigation waters are very small, it is convenient to express them as millimhos (m-mhos) per cm or micromhos (µ-mhos) per cm, or deci Siemens per meter (dSm\(^{-1}\)).

**Procedure**

Weigh 20 gm of organic sample and transfer to a 100 ml beaker. Add 50 ml of water stir it well and allow to stand for half an hour. Switch on the conductivity bridge. Check the instrument with saturated CaSO\(_4\) solution (EC 2.2 dSm\(^{-1}\)) or 0.01 N KCl solution (EC 1.41 dSm\(^{-1}\)) before proceeding with the sample. Wash the electrodes with distilled water. Immerse them into the sample suspension or suck the clear supernatant solution into the electrode bulb if the electrode is of pipette type. Balance the galvanometer of the magic eye of the conductivity meter and read directly the specific conductance of the sample solution.

**Ratings of EC level in soil**

- Harmless : 0.0-1.0 dSm\(^{-1}\)
- Injurious : 1.0-3.0 dSm\(^{-1}\)
- Critical : >3 dSm\(^{-1}\)
3.15.3 Macronutrients analysis

3.15.3.1 Estimation of organic carbon (OC)

OC was estimated by chromic acid wet digestion method of Walkley and Black (1934).

Principle

OC in the sample is oxidized by chromic acid (formed during the reaction of K$_2$Cr$_2$O$_7$ with H$_2$SO$_4$) which releases CO$_2$. The un reduced chromic acid in this reaction is estimated by back titration with ferrous ammonium sulphate or ferrous sulphate solution.

$$4\text{Cr}^{6+} + 3\text{C} \rightarrow 4\text{Cr}^{3+} + 3\text{C}^{4+}$$

$$2\text{H}_2\text{Cr}_2\text{O}_7 + 3\text{C} + 6\text{H}_2\text{SO}_4 \rightarrow 2\text{Cr}_2(\text{SO}_4)_3 + 3\text{CO}_2 + 8\text{H}_2\text{O}$$

$$2\text{H}_2\text{Cr}_2\text{O}_7 + 6\text{H}_2 + 6\text{H}_2\text{SO}_4 \rightarrow 2\text{Cr}_2(\text{SO}_4)_3 + 14\text{H}_2\text{O}$$

Reagents

1. 85% phosphoric acid
2. Conc. H$_2$SO$_4$
3. 1 N potassium dichromate solution (49 gm K$_2$Cr$_2$O$_7$/litre)
4. 0.5 N ferrous ammonium sulphate solution (196 gm/litre)
5. Diphenylamine indicator (90.5 gm in 100 ml conc. H$_2$SO$_4$)
Procedure

Transfer (without loss) 0.5 gm of finely powdered sample (to pass through 0.5 mm × 0.5 mm sieve) into a 500 ml conical flask. Add 10 ml of 1 N potassium dichromate solution followed by 20 ml of conc. H₂SO₄. Shake the contents for two minutes and set aside on an asbestos pad for half an hour. At the end of 30 minutes, add 200 ml of distilled water, 10 ml of phosphoric acid and 1 ml of diphenylamine indicator. The contents of the flask attain a blue colour. Titrate the contents against 0.5 N ferrous ammonium sulphate solution until the contents turn to green colour. The change of blue colour to green is the end point. Run a reagent blank (without any sample) simultaneously.

Calculation

Volume of 1 N K₂Cr₂O₇ taken = 10 ml

Volume of 0.5 N ferrous ammonium sulphate used in the blank titration = X ml

Volume of 0.5 N ferrous ammonium sulphate used in the sample titration = Y ml

Volume of 1 N K₂Cr₂O₇ required for oxidizing the organic carbon = (X-Y)/X × 10

1 ml of 1 N K₂Cr₂O₇ = (0.003 gm of C)

Organic carbon in the sample (%) = (X-Y)/X ×10×0.003×100/0.5

Percentage of organic matter = % of organic carbon × 1.724
Ratings of OC level in soil

Low : < 0.5 %

Medium : 0.5 - 0.75 %

High : > 0.75 %

3.15.3.2 Estimation of total nitrogen (TN)

The levels of TN present in the samples were estimated using Kjeldahl method of Jackson (1973). This method was carried out by two steps (1) digestion of the given sample to convert N compound to NH$_4^+$ form and (2) distillation and determination of NH$_4^+$ in the digest.

Principle

Organic form of nitrogen is converted into ammonium sulphate by digestion with conc. H$_2$SO$_4$. When this is distilled with excess alkali, ammonia is liberated which is absorbed in known excess of standard acid. The unused acid is found out by back titration with standard alkali.

Reagents

1. Conc. H$_2$SO$_4$        2. Salicylic acid
3. Sodium thio sulphate   4. Potassium sulphate
5. Copper sulphate        6. 40% NaOH
7. 10% Na$_2$S            8. Methyl red
9. 0.1 N H$_2$SO$_4$      10. 0.1 N KOH
Procedure

Digestion of the sample

Transfer 0.5 to 1.0 gm of organic material depending upon N content into Kjeldahl flask, add 30 ml of conc. H$_2$SO$_4$ containing 1.0 gm of salicylic acid and mix well and leave it for 30 minutes. Add 5.0 gm of sodium thio sulphate and allow 5 minutes for reaction under cold condition. Heat it gently and add the digestion mixture containing 10 gm of potassium sulphate and 1.0 gm of copper sulphate crystals and digest the contents till the contents turn into apple green colour.

Distillation

Dilute the contents in the digestion flask without any loss of solution. Rinse the Kjeldahl flask three or four times with water and transfer the washing also into the distillation flask. The volume of the contents in the distillation flask should be about 300 ml. Add few porcelain and zinc bits. Add 120 to 150 ml of 40% NaOH. Then add about 10 ml of 10% sodium sulphide and start the distillation after placing a beaker containing a known excess of 0.1 N H$_2$SO$_4$ with 2 to 3 drops of methyl red. After the distillation is over, remove the beaker and back titrate the excess acid against 0.1 N KOH till colour changes from pink to straw yellow. Calculate the percentage of N from the volume of acid consumed.

Calculation

\[
\text{Weight of organic matter taken} = 1.0 \text{ gm}
\]
Volume of 0.1 N H₂SO₄ taken in
the beaker to absorb ammonia = A ml

Volume of 0.1 N KOH consumed
in the back titration = B ml

Actual volume of 0.1 N H₂SO₄ consumed = (A - B) ml

1 ml of 0.1 N H₂SO₄ = 0.0014 gm of N

(A-B) ml 0.1 N H₂SO₄ = 0.0014 × (A-B) gm of N

1.0 gm of organic matter contains = 0.0014 × (A-B) gm of N

3.15.3.3 Estimation of total phosphorus (TP)

The levels of TP present in the organic samples were estimated using ammonium phospho-molybdate method of Pemberton (1945).

Principle

TP is extracted by digestion with conc. HCl and HNO₃. The phosphorus is then precipitated as ammonium phospho-molybdate in nitric acid medium, filtered, washed free of acid and dissolved in a known excess of alkali and the excess alkali is back titrated against nitric acid using phenolphthalein as indicator. From the volume of alkali consumed, TP content of the sample is arrived at.
Reactions

The reaction between ammonium phospho-molybdate and alkali takes place according to the equations given below.

\[
\text{Na}_2\text{HPO}_4 + 12 \text{NH}_4\text{MoO}_3 + 23 \text{HNO}_3 \rightarrow (\text{NH}_4)_3\text{PO}_4.12 \text{MoO}_3 + 12 \text{H}_2\text{O} + \\
23 \text{NH}_4\text{NO}_3 + 2\text{NaNO}_3 \\
2 (\text{NH}_4)_3\text{PO}_4. 12 \text{MoO}_3 + 46 \text{KOH} \rightarrow 23 \text{K}_2\text{MoO}_3 + (\text{NH}_4)_2\text{MoO}_4 + \\
2 (\text{NH}_4)_2\text{HPO}_4 + 22 \text{H}_2\text{O} \\
2 (\text{NH}_4)_2\text{HPO}_4 \rightarrow \text{P}_2\text{O}_5 + 4\text{NH}_3 + 3 \text{H}_2\text{O}
\]

From the equation it is seen that two molecules of \((\text{NH}_4)_3\text{PO}_4. 12 \text{MoO}_3\) will be equivalent to one molecule of \(\text{P}_2\text{O}_5\). Further 46 gm molecules of KOH are required to completely dissolve 2 gm molecules of ammonium phospho-molybdate corresponding to a gm molecule of \(\text{P}_2\text{O}_5\).

Reagents

1:1 HCl, conc. HNO₃, 0.1619 N HNO₃, 0.1619 N KOH, 20% ammonium molybdate, solid \(\text{NH}_4\text{NO}_3\), dil. \(\text{NH}_4\text{OH}\) and phenolphthalein indicator.

Procedure

a. Digestion and extraction of the sample

Weigh 2 gm of the sample into a 250 ml conical flask and add 30 ml of 1:1 HCl and 3 ml of conc. HNO₃ and digest the contents on a sand bath for about an hour. Dilute the contents with 100 ml of distilled water and filter through Whatman No.40 filter paper and collecting the filtrate in a 250 ml volumetric flask. Wash the residue with hot water till
about 240 ml of the filtrate is collected. Cool the filtrate and make up the volume to the mark.

b. Precipitation

Pipette out 10 ml of the above extract into a 250 ml beaker and add about 20 ml of distilled water. Add NH₄OH until the solution is alkaline to litmus, followed by dilute nitric acid until it is distinctly acidic. Add about 2 gm of solid NH₄NO₃ and warm the contents to about 70°C on a water bath. Prepare the precipitant mixture by adding 10 ml of 20% ammonium molybdate to 10 ml of 7:3 HNO₃ and water in a 100 ml beaker. Add 20 ml of the precipitant mixture slowly to the aliquot of phosphate solution. Gently stir the contents without touching the sides of the beaker. Keep the beaker on a thermostat maintained at 65°C for half an hour and allow the canary yellow precipitate to settle, leaving a clear supernatant liquid.

c. Filtration and titration

Filter through Whatman No. 40 filter paper, each time transferring only the supernatant liquid and retaining as much of the precipitate as possible in the beaker itself. Give washings with cold water alternatively to the filter paper and to the precipitate in the beaker, using only about 10 ml of distilled water for each washing. Add the next installment water to the filter paper after the previously added water drained off completely. Repeat this process until the filtrate runs free of acid (test by collecting half test tube fall of the filtrate and adding a drop of 0.1619 N KOH and a drop of phenolphthalein: pink colour shows free of acid). Precipitation was done and dissolves the yellow precipitate by adding known excess of 0.1619 N KOH. Add 2 or 3 drops of
phenolphthalein and titrate against 0.1619 N HNO₃. From the actual amount of KOH required to react with the precipitate calculate the percentage of P₂O₅.

**Calculation**

Weight of sample taken = 2 gm

Volume of the extract made up after digestion = 250 ml

Aliquot pipetted out = 10 ml

Known excess of 0.1619 N KOH added to dissolve the precipitate = X ml

Volume of 0.1619 N HNO₃ used for back titration = Y ml

Actual volume of 0.1619 N KOH utilized to dissolve the precipitate = (X-Y) ml

1 ml of 0.1619 N KOH = 0.0005 gm P₂O₅

Percentage of P₂O₅ in the sample = (X-Y) (0.0005) (250/10) (100/2)

**3.15.3.4 Estimation of total potassium (TK) and total sodium (TNa)**

The levels of TK and TNa present in the organic samples were estimated by using Flame photometry method of Stanford and English (1949).
Principle

Certain elements when excited in flame emit radiations. The excitation causes the electrode of natural atom to jump to an outer orbit of higher energy level and the atom return to lower energy, light of characteristic wave length is emitted. The flame photometer measures this emission intensity which is proportional to the concentration of the element in solution.

Procedure

a. Preparation of sample solution

Weigh 1 gm of organic sample accurately and transfer it to a 1000 ml volumetric flask. Dissolve it in water and make up the volume to the mark. Pipette out 10 ml of this solution into a 250 ml volumetric flask and make up the volume to the mark with water. Shake it well to get homogeneous solution and measure the concentration of K and Na in the solution using flame photometer.

b. Preparation of standard solution for K and Na

Accurately weigh 1.907 gm of KCl and 2.338 gm of NaCl separately and transfer them into separate 1000 ml volumetric flasks and dissolve them in distilled water and make up to 1000 ml. These solutions give 1000 ppm of K or Na. From these 1000 ppm stock solutions, various standard solutions ranging from 10 to 100 ppm K or Na are prepared.
c. Recording the readings

Adjust the galvanometer to read 0 for blank and 100 for 100 ppm K or Na solution. Then introduce the standards and record the readings. Construct the standard graph for both K and Na with the readings recorded for various standard solutions of K and Na.

Now transfer about 5 to 10 ml of the organic sample solution to a vial. Insert it into the aspirator and note down the galvanometer reading. Deduce the concentration of potassium and sodium present in the sample solution from the standard graph and calculate the percentage of K and Na in the given sample.

Calculation

\[
\begin{align*}
\text{Weight of sample taken} & = 1 \text{ gm} \\
\text{Volume make up in first dilution} & = 1000 \text{ ml} \\
\text{Volume of aliquot taken for second dilution} & = 10 \text{ ml} \\
\text{Volume made up in second dilution} & = 250 \text{ ml} \\
\text{Concentration of K or Na in the solution as deduced from the standard curve} & = X \text{ ppm} \\
\text{Therefore percentage of K or Na in the given sample of KCl/K}_2\text{SO}_4 \text{ or NaCl} & = \left(\frac{X}{1000000}\right) \left(\frac{250}{10}\right) \left(100\right) \left(100\right)
\end{align*}
\]
3.15.3.5 Estimation of total calcium (TCa)

The levels of TCa present in the organic samples were estimated by adopting the EDTA or Versenate method of Jackson (1973).

Principle

The most widely used salt for EDTA is the disodium salt with the formula Na$_2$H$_2$Y. 2H$_2$O where Y is the tetravalent anion of EDTA. When Ca$^{2+}$ is treated with H$_2$Y$^{-2}$ a very stable complex is formed. The generalized reaction of EDTA with Ca$^{2+}$ ion is shown as

\[ \text{Ca}^{2+} + \text{H}_2\text{Y}^{-2} \rightarrow \text{CaY}^{-2} + 2\text{H}^+ \]

Reagents

(1) 10% NaOH

(2) 0.02 N EDTA

(3) Murexide indicator

Procedure

Weigh 10 gm of organic sample in a 500 ml beaker. Saturate the sample with neutral normal ammonium acetate. Filter the sample solution in stages in a buchner funnel under suction. Wash the sample continuously with ammonium acetate solution. Make up the volume to 500 ml with distilled water and use it for calcium estimation.

Pipette out 10 ml of aliquot into a dry clean 100 ml conical flask. Add 5 ml of 10% NaOH solution. Add a pinch of murexide powder. Titrate against 0.02 N EDTA till colour
changes from red to violet. A blank is run by pipetting 10 ml of water instead of calcium solution. Deduct the blank reading from sample reading.

Calculation

Volume of 0.02 N EDTA used for

sample titration = A ml

Volume of 0.02 N EDTA used for

blank titration = B ml

Corrected titre value = (A-B) ml

Meq. of Ca in V₁ ml of aliquot = (A-B) × 0.02

Meq. of Ca in V ml of the extract = (A-B) × 0.02 × V/V₁

10 gm of sample contains = (A-B) × 0.02 × V/V₁ × 100/10 Meq. Ca

100 gm of sample contains = (A-B) × 0.02 × V/V₁ × 100/10 Meq. Ca

3.15.3.6 C:N ratio

The ratio of the percentage of carbon to that of nitrogen (C/N ratio) was arrived at by dividing the percentage of carbon with the percentage of nitrogen estimated in the given organic sample.
3.15.4 Micronutrients analysis

3.15.4.1 Estimation of micronutrients

The levels of micronutrients such as iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) present in the organic samples were estimated by Atomic Absorption Spectrophotometry method of Lindsay and Norwell (1978).

Principle

The method consists of shaking few grams of sample with a buffered solution containing diethylene triamine penta acetic acid (DTPA). This chemical acts a mild chelating agent which extracts the easily soluble iron, manganese, zinc and copper. The extracting solution is buffered at pH 7.3 by triethanolamine (TEA) and calcium chloride is added to prevent the dissolution of calcium carbonate. These conditions permit the right amount of Fe, Mn, Zn and Cu to be dissolved and CaCl$_2$ is to stabilize the pH of the extractant. The dissolved elements in the extract are then measured by the Atomic Absorption Spectrophotometer (AAS) wherein the extracted sample is converted first into an atomic vapour, usually by a flame and irradiated by the metal being sought. The absorption of the light by the vaporization samples is related to the concentration of the desired metal in it.

Reagents

1) DTPA extracting solution - It contains 0.005 M DTPA, 0.001 M CaCl$_2$, 0.1 M TEA and was adjusted to pH 7.3. It is prepared by dissolving 13.1 ml of TEA, 1.47 gm of CaCl$_2$. 2H$_2$O, 1.967 gm of DTPA in 100 ml distilled water. Dilute to 900 ml with water. Adjust the pH to 7.3 with 1:1 HCl and dilute to one litre.
2) Standard solution for Fe, Mn, Zn and Cu - Prepare one litre of 100 ppm stock solution in each element respectively with - Fe- 0.4977 gm, Mn- 0.3602 gm, Zn- 0.4398 gm and Cu- 0.3929 gm. From the stock solution, prepare at least 10 working standards in each element with range from 0 to 30 ppm for Zn and Cu and from 0 to 20 ppm for Fe and Mn.

**Procedure**

Weigh 10 gm of organic sample and transfer it to 100 ml polythene shaking bottle. Add 20 ml of extracting solution and shake vigorously for 2 hours. Filter the solution through Whatman No.42 filter paper. The filtrate is used for the estimation of Fe, Mn, Zn and Cu using appropriate hallow cathode lamp in AAS. Construct a separate calibration curve for each element by preparing standard solutions of varying concentrations after setting the AAS with suitable hallow cathode lamp. Measure the concentration of particular element present in the sample using the above curve.

3.16 Cultivation of black gram

3.16.1 Procurement of black gram seeds

The certified ADT-3 black gram (*Vigna mungo*) seeds were purchased from the Mercury Agencies, Kumbakonam, Thanjavur District.

3.16.2 Preparation and maintenance of different PSR media

For each organic manure, 24 earthen pots were taken and to each 5 litres of 75, 50, 40, 30, 25, 20, 15, 10, 7.5, 5.0, 2.5 and 0 PSR of partly decomposed and vermicompost of sheep droppings/ pressmud/*Pongamia* leaves/ organic mixture was transferred separately.
Duplicate the above experiment with another 24 earthen pots in each organic matter for comparison.

Six seeds were placed in each pot at 2.5 cm deep and sufficient water was poured in all the pots for proper germination of seeds. After seven days of seed sowing, only three plants were allowed to grow in each pot. These pots were kept in the terrace roof of Zoology Department for direct sunlight. The seedlings in the pots were regularly poured with sufficient water to ensure proper growth until all of them get harvested (60 days). Care was taken to see that the plants growing in the pots must be protected from predation, if any.

3.16.3 Collection of growth data

Twenty days after seed sowing, the plants in the pots were measured their shoot height and petiole length, and counted their leaves once in 10 days. Parameters such as total flowers, total pods, pod length, total seeds and total seed weight per plant were also noted in all the plants during the course of this study. Although, measurements were made at 10 days interval, only the measurements taken at 60th day (harvest day) were given in result section.

3.17 Statistical analysis

Basic statistics such as mean and standard deviation for various parameters were calculated. Student’s test was applied wherever necessary to test the statistical significance through window based statistical package (SPSS) at P<0.01 and P<0.05 levels.