

**A STUDY ON THE REMOVAL OF SOME DYES USING TERMITE SOIL AS AN ADSORBENT****\*S. Russiarani<sup>1,2</sup> and Dr. K. Balakrishnan<sup>1</sup>**<sup>1,2</sup>Assistant Professor in Chemistry, Arunai Engineering College, Thiruvannamalai.<sup>1</sup>Associate Professor in Chemistry, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur.**\*Corresponding Author: S. Russiarani**

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**ABSTRACT**

The physicochemical analysis revealed that the pH levels of the soil samples are slightly acidic. Hence the soil sample is quite well for plant growth. EC of soil sample found to be normal. The plants that are grown in such type of soil will take water easily from the soil. From the available Nitrogen value of all the soil samples shows that nitrogen deficiency. The available phosphorous and potassium content is high. Proximate analysis showed termite soil content which favors adsorption. Favorable antibacterial studies of after adsorbed termite soil was evident for the removal of methylene blue and malachite green dyes. It is concluded that termite soil can be effectively used for the removal of methylene blue and malachite green.

**KEYWORDS:** Termite Soil, Methylene blue, Malachite Green.**INTRODUCTION**

Soils contain 1500 - 2000 Pg C, representing the third reservoir of C after ocean and fossil fuels. Soil organic carbon (SOC) storage is chiefly controlled by primary production input and organic matter evolution. Changes in land use affect the amount of carbon stored in vegetation and soils and, hence, the flux of carbon between land and the atmosphere.<sup>[1]</sup> Hazardous triphenylmethane dye, Malachite Green from wastewater. The adsorption studies were carried out at 30, 40 and 50 °C and effects of pH, temperature, amount of adsorbent, contact time, concentration of adsorbate, etc. on the adsorption were measured.<sup>[2]</sup> Prepared Arundodonax root carbon (ADRC), a new adsorbent from Arundodonax root by carbonization. The surface area of the adsorbent was determined 158m<sup>2</sup>/g by N<sub>2</sub> adsorption isotherm. Batch adsorption experiments were carried out for the removal of malachite green (MG) from aqueous solution using ADRC as adsorbent.<sup>[3]</sup> The Oil palm trunk fibre (OPTF) were used – an agricultural solid waste as low-cost adsorbent to remove malachite green (MG) from aqueous solutions. The operating variables studied were contact time, initial dye concentration and solution pH. Equilibrium adsorption data were analyzed by three isotherms, namely the Freundlich isotherm, the Langmuir isotherm and the multilayer adsorption isotherm.<sup>[4]</sup> Neem sawdust (*Azadirachta indica*) as an adsorbent for the removal of malachite green dye from an aqueous solution. The studies were carried out under various experimental conditions such as agitation time, dye concentration, adsorption dose, pH and temperature to assess the

potentiality of neem sawdust for the removal of malachite green dye from wastewater.<sup>[5]</sup>

Chloroform is considered as a possible important contributor of reactive chlorine in the troposphere and is therefore one of the subjects in the Reactive Chlorine Emission Inventory.<sup>[6]</sup> Amongst all the treatment processes mentioned, adsorption using sorbents is one of the most popular and effective Processes for the removal of heavy metals from waste water. The adsorption process offers flexibility in design and operation and in many cases produces treated effluent suitable for re-use, free of color and odor. In addition, because adsorption is sometimes reversible, the regeneration of the adsorbent with resultant economy of operation may be possible.<sup>[7]</sup>

**MATERIALS AND METHODS****Analysis of Physicochemical Parameter of Termite Soil****Study site**

The Termite soils were collected from Thennangudi village, Thanjavur district, Tamil Nadu, India. Thennangudi lies on latitude 10.8130°N and longitude 79.0624°E in Thanjavur district and it is located in central Tamil nadu bounded on the northeast by Nagapattinam district, on the east by Tiruvarur district, on the south by the Palk strait, of Bay of Bengal on the west by Pudukkottai district and the north by the river Kollidam, across which lie Tiruchirappalli and Perambalur districts.

### Collection of the soil sample

Before testing the soil, it is essential to collect the soil from the field properly. This is the first phase of soil testing. The soil sample collected should be representative of the area sample. A field can be treated as single sampling unit only if it is appreciable uniform in all respects. Variation in slope, texture, colour, crops grown and management levels followed should be taken into account. Separate sets of composite samples need to be collected from each such areas. Recently fertilized spots, bands, channels, marshy tracts and spots near trees, wells, compost piles (or) other non-representative locations must be avoided during sampling. If fertilizer has been placed in a band, either the field is to be ploughed before sampling (or) sampling done between the crop rows, well away from fertilizer bands. When crops are planted in rows, samples can be drawn in between the lines. The soil samples should be taken in zigzag pattern. A representative composite soil sample can be composed of 8-20 sub-samples from a uniform field. A common error in soil sampling occurs when few cm of soil are dry and are not included in the normal sample.

### Procedure for soil sampling

For surface sample collection, a V-shaped cut was made and scraped 1 (or) 1.5 cm thick slice of soil from the sides of cut. Here the V – shaped cut was made for about 15 cm depth. Since a single sample would never accurately characterize the nutrient status of a field, at random (zigzag) 10 to 16 places in sampling unit were selected and V-shaped cut was made and the sample was pooled in a clean basket. After being quartered and mixed homogenously, the pooled samples were packed for ½ kg in each sample in a clear polythene bags. Sample details slips were inserted in the above bags in which the sample number and the farmer's field from which the sample taken were marked according to the standard methods.<sup>[9]</sup>

### Processing for samples in the laboratory

A sample brought to the laboratory was spread out on an aluminium tray. Coarse concentrations, stones and pieces of roots, leaves and other undecomposed organic residues were removed. Large lumps of moist soils were broken by hand. The soil samples were dried in shadow at 20-25°C and 20-60% HUMIDITY (Jacks on 1958).<sup>[10]</sup> After being dried in air, the soil sample were crushed gently in pestle and mortar and sieved through 2 mm sieve. (If the sample in analyzed for micro nutrients, Iron, Copper, Brass sieves are to avoided). The crushing process was continued till the soil retained on the sieved contains no aggregates. The material larger than 2 mm was discarded. For organic carbon analysis, 25-50 g of 2 mm fraction was further ground with pestle and mortar to pass through 0.5 to 0.2 mm sieve so that 1 g of 0.2 mm could be got. For getting moist samples, after being collected, these were transported immediately to plastic bags. After being removed the stones, these were rubbed through a wire mess sieve with openings about 4-5 mm

across and weighed immediately for analysis and dry weight determination.

These samples were stored in card board, plastic and glass containers. These were mixed before weighed the samples. The soil sample analysis is involved in the following experimental techniques.

1. Estimation of soil texture
2. Estimation of pH
3. Estimation of EC (Electrical Conductivity)
4. Estimation of Nitrogen
5. Estimation of Phosphorous
6. Estimation of Potassium

### Physical Properties

#### Soil Texture

Soil texture of particle size distribution is a stable soil characteristic which influences physical and chemical properties of the soil. The sizes of the soil particles have a direct relationship with the surface area of the particles. Soil particles remain aggregated due to various types of binding forces and factors which included the content of organic matter, other colloidal substances present in the soil, oxides of iron and aluminium and the hydration of clay particles etc. To estimate the content of various sizes of soil particles, the soils sample has to be brought into dispersed state by removing various types of binding forces.

In the dispersed soil samples, the soil particles settle down at a different settling rate according to their size. In the estimation of soil texture, particles below 2 mm diameter are separately determined which constitute sand, silt and clay. Each one of them is characterized as below:

Coarse Sand diameter	:	2.0 – 0.2 mm
Fine Sand diameter	:	0.2 – 0.02 mm
Silt diameter	:	0.02 – 0.002 mm
Clay diameter	:	< 0.002 mm

The soil sample is dispersed by removing the binding force in soil particles. The settling of dispersed particles in water is measured. Large particles are known to settle out of suspension more rapidly than do small particles. This is because larger particles have less specific area and hence have lesser buoyancy than smaller particles. Stoke's law (1851) is used to express the relationship. The law stipulates that the resistance offered by the liquid to the fall of the particle varied with the radius of the sphere and not with the surface. Accordingly, the formula was given as below:

$$V = 2/9 \left[ \frac{dp-d}{\eta} \right] gr^2$$

Where, V is the velocity of the fall in centimetre per second, g is the acceleration due to gravity, dp is the density of the particle, d is the density of the liquid, r is

the radius of the particle in centimetre and  $\eta$  is the absolute viscosity of the liquid. It is obvious that the velocity of fall of the particles with the same density in a given liquid will increase with the square of the radius. With the above principle in view, the particle size distribution is estimated by measuring the amount of different sizes of soil particles present at different calibrated depths in the cylinder containing suspended soil sample.

Generally, two methods are most commonly used for estimation of particles size or soil texture:

1. International Pipette Method
2. Bouyoucos Hydrometer method

Hydrometer method is most commonly used since it is less time consuming and easy to follow in a service laboratory. Dispersion is obtained by using calgon (Sodium hexametaphosphate).

### 1. Bouyoucos Hydrometer method

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### 2. Hydrometer method

#### Reagent

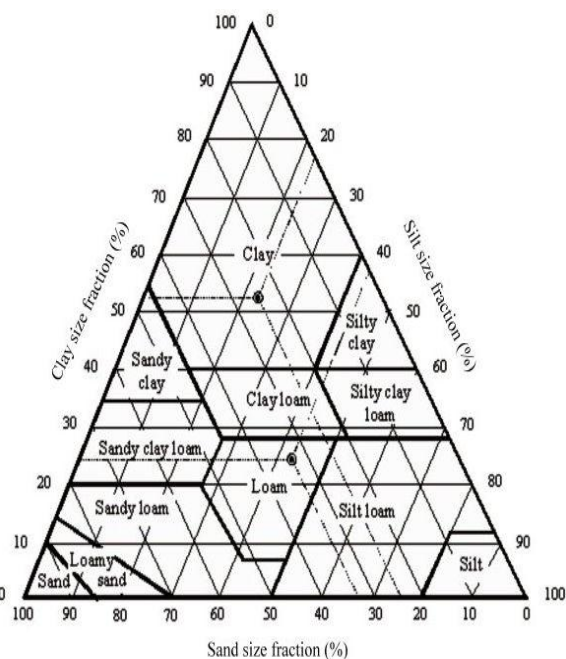
Sodium hexametaphosphate solution containing 50 g salt per litre of water.

#### Procedure

Weigh 50 g oven dried fine textured soil (100 g for coarse textured soil) into a baffled stirring cup. Fill the cup to its half with distilled water and add 10 ml of sodium hexametaphosphate solution. Place the cup on stirrer and stir until soil aggregates are broken down. This usually requires 3-4 minutes for coarse textured soils and 7-8 minutes for fine textured clay. Quantitatively transfer stirred mixture to the settling cylinder by washing the cup with distilled water. Fill the cylinder to the lower mark with distilled water after placing the hydrometer in the liquid. If 100 g of coarse textured sample was used fill to the upper mark on the setting cylinder.

Remove hydrometer and shake the suspension vigorously in a back and forth manner. Avoid creating circular currents in the liquid as they will influence the settling rate. Place the cylinder on a table and record the time. After 20 seconds, carefully insert the hydrometer and read the hydrometer and read the hydrometer at the end of 40 seconds. Repeat step 4 and 5 to obtain hydrometer readings within 0.5 g differences from each other. The hydrometer is calibrated to read grams of soil material in suspension. Record the hydrometer readings on the date sheet. Measure the temperature of the suspension. For each degree below 20°C add 0.36 to the hydrometer reading and for each degree below 20°C, subtract 0.36 from the hydrometer reading. This is the corrected

hydrometer reading. Reshake the suspension and place the cylinder on a table where it will not be disturbed. Take a hydrometer reading exactly 2 hours later. Correct for temperature as described above. From the percentage of sand, silt and clay calculated on the data sheet, use the diagram for textural triangle to determine the textural class of the Soil.



**Figure 1 Soil textural triangle, composed of 12 classes**  
**CHEMICAL PROPERTIES**

### Estimation of pH

pH is an notation used to measure the acidity (or) alkalinity of the soil solution. This term was introduced by Soressen and it is defined as the negative logarithm of hydrogen ion concentration in gram moles per litre.

Mathematically

$$\text{pH} = -\log_{10}[\text{H}^+]$$

### pH meter

#### Principle

A glass rod in contact with  $\text{H}^+$  ions of the solution under test acquired an electrical potential which depends on the concentration of  $\text{H}^+$  ions. This is measured potentiometrically against some reference electrode which is usually a calomel electrode. The potential difference between glass electrode and calomel electrode is expressed in pH units.

#### Procedure

20 g of soil sample was weighed and 40 ml of distilled water was added. Stirred well with glass rod and was kept for 30 minutes. The solution was stirred intermittently and transferred the supernatant liquid to a test tube. Saturated  $\text{CaSO}_4$  was added to remove the turbidity of the solution and the contents were shaken in the test tube and were set aside. The suspended

impurities were settle down by repeating the above process.

### pH meter operation

First of all, the pH range selector switch was kept to zero. The temperature compensation control was set to the solution temperature. The instrument was connected to 220-330 volts. AC supply and tuned the power switch of the instrument in "Start position" and 10-15 minutes were allowed to warm up and turned the switch to operate position as the electrodes were immersed in distilled water.

The zero adjustment knob was adjusted to read the pointer in meter exactly to zero. The electrodes were lifted from the distilled water and wiped dry using filter paper strips and they were dipped in standard buffer solution to know pH.

The function switch was changed to the particulars pH range from 0-7 or 7-14 and the standardisation knob was adjusted till the correct pH value of buffer was read. (The zero adjustment knob should not be distributed during this measurement). The range switch was changed to zero position. The electrodes were removed from buffer solution. They were washed with distilled water and wiped with dry filter paper. They were dipped in a test solution of unknown pH. Now the range switch was changed to the expected pH value to read the pH value exactly on the meter (Range switch should be kept at zero when the electrodes are immersed into (or) removed from the soil suspension to prevent of the glass electrode).

### Reagent required

pH 4.0, pH 7.0, pH 9.0. The buffer tablets each was dissolved in double distilled water and made up to 100 ml. They were stored in neatly labelled polythene bottles. Other standard buffers are,

#### 1. 0.05 M potassium hydrogen phthalate.

Molecular weight of (KH C<sub>8</sub>H<sub>4</sub>O<sub>4</sub> = 204.2) pH was 4.0, 0.3 M stock solution was prepared by dissolving 61.24 g of Analar grade salt in double distilled water and made up to one litre. 3 drops of toluene was added to prevent the growth of mold. 10 ml of solution was diluted with some of water to get 0.05 M solution.

2. A buffer of pH 8.0 was also prepared by mixing 19.45 ml of 0.2 M Na<sub>2</sub>HPO<sub>4</sub> (142.80) with 0.55 ml 0.1 M citric Acid.

### pH by soil-water 1:2 method

20 g of soil was weighed and transferred to 100 ml polythene beaker, 40 ml of distilled water was added, stirred well with glass rod and was allowed for 30 minutes with intermittent stirring. pH meter was adjusted as mentioned above with buffer solution. Then the electrodes were washed with a set of water and wiped carefully with a piece of filter paper. The electrodes were

immersed in the beaker containing soil water suspension (the suspension must be stirred well before the electrodes are immersed). The function switch was changed to particular pH range (0-7 (or) 7-14). The meter reading will directly indicate the pH value of sample.

### Estimation of electrical conductivity

#### Principle

The method is based on the principle that conductivity of a solution of salt is nearly proportional to its concentration of salts. The electrical conductivity is measured in terms of the resistance offered to the flow of current using a conductivity bridge. The conductivity bridge is based on the Wheatstone's bridge principle. When four resistances are offered to the flow of current and three of them are known, then the fourth resistance will be unknown. The resistance at which there is no flow of current through the circuit is a measurement of electrical conductivity ( $C = 1/R$ ).

#### Reagent required

1. Saturated solution of CaSO<sub>4</sub>
2. 0.01 N KCl solution (0.749 g/litre)

#### Procedure

The soil solution prepared for the pH taken for the EC measurement. Now the conductivity bridge is switched on. The saturated CaSO<sub>4</sub> solution (or) 0.01 N KCl solution was fed and checked the conductivity. For saturated CaSO<sub>4</sub>, the conductivity was 2.20 millimhos/cm and for 0.01 N KCl, it was 1.41 millimhos/cm. Hence the cell constant was taken as 1.0

By applying formular  $Q = k/c$

Where Q is cell constant, k is specific conductance of known standard, c is meter reading, K 1.41 millimhos/cm for 0.01 N KCl (standard value)

Hence  $k/c = 1.0$

So in this case, meter reading x cell constant = conductivity i.e., direct reading. (In case where the cell constant differs from the experimental value, the meter reading has to be multiplied by cell constant to give the conductivity).

#### Measurement

The electrode was now washed with water and immersed into clear soil suspension. (If the electrode is one pipette type, get the multiplier switch at an intermediate position and rotate the main dial control unit the magic eye of the null indicator is at its widest (or) if it needle type, it is in centre point). The multiplier was used and knob was rotated to get the correct value. The readings of the scale at this multiplier switch position were taken as the current electrical conductivity. (The conductivity cell has to be periodically cleared by rinsing with 10 % HCl (or) HNO<sub>3</sub> to remove any soil (or) organic matter and having to the cell).



### 3.1.6.2 Estimation of the available Nitrogen

Easily oxidizable organic carbon (OC) and mineralizable N are largely used as measure of variable Nitrogen in Indian soils. Organic carbon is estimated by the modified walkley method.<sup>[11]</sup>

#### Organic carbon by wet digestion method

##### Principle

Organic matter in the soil is oxidized with a mixture of  $K_2Cr_2O_7$  and Conc.  $H_2SO_4$ , utilizing the heat of dilution of Conc.  $H_2SO_4$ . The unused  $K_2Cr_2O_7$  is back – titrated with ferrous sulphate ( $FeSO_4 \cdot NH_4)_2SO_4 \cdot 6H_2O$ ).

##### Reagent required

- Standard potassium dichromate solution (0.1667 M = 1.0 N) 49.04 g Analar grade  $K_2Cr_2O_7$  was dissolved in distilled water (dried at 105°C for w hours) and was diluted to one litre in volumetric flask.
- Ferrous (or) Ferrous ammonium sulphate (0.5 M = 0.5 N) 196.1 g of grade ferrous ammonium sulphate was dissolved in 800 ml water. To this 20 ml of Conc.  $H_2SO_4$  was added and was diluted to 1 litre in volumetric flask.
- Diphenylamine indicator: 0.5 g diphenyl amine was dissolved in a mixture of 20 ml of Conc.  $H_2SO_4$ .
- Sulphuric acid: 96% concentration was prepared (Whose specific gravity is 1.84). (If high amount of  $Cl^-$  is present in the samples,  $Ag_2SO_4$  is added at the rate of 15 g/litre of the Acid).
- 85% of orthophosphoric acid was prepared (Sodium fluoride of pure grade may be used).

##### Procedure

Accurately 1 g 0.2 mm (80 mesh) soil sample was weighed. It was placed in a 500 ml dry Erlenmeyer flask (corning/Pyrex) Two blanks were included to standardize  $FeSO_4$  solution. Exactly 10 ml of dichromate solution was added. The flask was swirled gently and kept on the asbestos sheet. 20 ml of Conc.  $H_2SO_4$  was added rapidly by directing stream into suspension. The flask was swirled again for 2 to 3 times. The flask was allowed to stand on the asbestos sheet for 30 minutes. Then 200 ml of distilled water was added: After the addition of 100 ml phosphoric acid (or) 0.5 g of sodium fluoride) and 1 ml of diphenyl amine indicator, the contents were titrated against Ferrous Ammonium Sulphate solution till the colour hanged from blue violet to green. (If the burette reading is 0-4 ml, repeated this with less soil, if it is 17 ml of higher, repeat with more soil).

##### CALCULATION

$$\text{Organic Carbon (\%)} = \frac{10(B-T)}{B} \times \frac{0.03 \times 100}{B \text{Weight of Soil (g)}}$$

Where, B - volume of ferrous ammonium sulphate solution required for blank titration.

T - volume of ferrous ammonium sulphate solution needed for titration of soil sample.

#### Actual organic carbon

Organic carbon estimated\*1.3 since there is an incomplete oxidation of organic matter and 77 % recovery.

#### Organic matter (%)

Actual C (%) \*1.724 since organic matter contains 58 % of Carbon. (Van Bemmelen Factor)

#### MINERALISABLE NITROGEN

##### Principle

The procedure involves distilling the soil with alkaline potassium permanganate solution and determining the ammonia liberated. This serves as an index of the available (Mineralizable) Nitrogen status of a soil was therefore propose as a soil test for nitrogen (N) by subbaiah and asija (1956).<sup>[12]</sup>

##### Reagent

- 0.32%  $KMnO_4$  solution: 3.2 g reagent grade  $KMnO_4$  was dissolved in distilled water and made up to 1 litre in a volumetric flask and mixed well.
- 2.5 % NaOH solution: 25 g sodium hydroxide was flaked in distilled water and made up to onelitre in volumetric flask and mixed well. I was stored in plastic container.
- Liquid paraffin (Extra pure)
- 0.02 N standard Sulphuric Acid.
- Boric acid- indicator solution: 20 g of pure Boric acid was dissolved in about 700 ml of hot water and then cooled well. The cooled solution was transferred to a 1 litre volumetric flask containing 200 ml of ethanol and 20 ml of mixed indicator solution. After being mixed the contents of flask, 0.05 N NaOH (approximately) was added cautiously till the colour was reddish purple. The solution was diluted to 1 litre volume with water and mixed well.
- Mixed indicator: 0.07 g of methyl red was dissolved with 0.1 g of bromocresol green in 100 ml of 95 % of ethanol.

##### Procedure

20 g of soil sample was placed in a 800 ml Kjeldah 1 flask, to this 20 ml of water was added and swirled. 1 ml of liquid paraffin was added and a few glass beads were also added to prevent frothing and bumping respectively during distillation. Then 100 ml of each 0.32 %  $KMnO_4$  and 2.5 % NaOH solutions were added. The contents in Kjeldah 1 flask were distilled at a steady state and collect the liberate  $NH_3$  gas was collected in Erlenmeyer flask (250 ml) containing 20 ml of boric acid indicator solution. The pink colour of boric (approximately) of distillate was collected in 30 minutes. The contents were titrated with 0.02 N  $H_2SO_4$  to the original shade (pink). Blank correction was made for the final calculations.

##### CALCULATIONS

Mineralizable Nitrogen

$$\text{Kg/ha} = R * 31.36$$

Where R-volume of 0.02  $NH_2SO_4$  required for titration.

**Estimation of available Phosphorous****Olsen's (NaHCO<sub>3</sub>) method**

Sodium bicarbonate solution extracts some exchangeable (or) surface adsorbed Al-p, Fe-p and calcium phosphate and other phosphates.

**Reagents**

- Sodium bicarbonate (Olsen's reagent) (0.5 M NaHCO<sub>3</sub>, pH 8.5). 84 g of NaHCO<sub>3</sub> was dissolved in water and made up to 2 litres and mixed well. The solution was adjusted to pH 8.5 with 1 M NaOH (4g NaOH/100 ml) solution (usually 20-25 ml NaOH solution is required for 2 litres NaHCO<sub>3</sub> solution).
- Reagent A: 12 g of ammonium molybdate (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O) was dissolved in 250 ml of distilled water. 0.2908 g of Antimony potassium tartrate (tartaremetic) (K(Sb) C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>. ½ H<sub>2</sub>O) was dissolved in 100 ml water. These two solutions were added to 1000 ml of 2.5 M H<sub>2</sub>SO<sub>4</sub> and mixed well, then it was made up to 2 Litre. This was then stored in a dark and cool place.
- Reagent B: 1.056 g of ascorbic acid was dissolved in 200 ml reagent A and mixed well (This does not deep more than 24 hrs at room temperature, pre daily as required).
- 2.5 M Sulphuric acid: 140 l of concentrated H<sub>2</sub>SO<sub>4</sub> was made up to 1 litre.
- Standard stock P solution: Exactly 0.439 g of potassium dihydrogen ortho phosphate (KH<sub>2</sub>PO<sub>4</sub>) of a reagent was dissolved in 500 ml of distilled water (the phosphate salt is dried at 60 °C in oven for 1 hour and cooled in desiccator).
- 25 ml of 7 N H<sub>2</sub>SO<sub>4</sub> was added to the above solution and made up to one litre with distilled water. This gave 100 ppm P standard stock solution. Then 2 ppm solution was prepared by diluting the above to 50 times.

**Standard Curve**

1,2,3,4,5 and 10 ml of 2 ppm P solution were in 25 ml volumetric flask to prepare standard curve. To these 5 ml of Olsen's extract was acidified (aliquot) with 2 M H<sub>2</sub>SO<sub>4</sub> to pH 5.0. Distilled water was then added to make the volume of 20 ml and then 41 ml of reagent B was added. Then the distilled water was added to makeup the volume and mixed thoroughly. Then using NaHCO<sub>3</sub> solution, distilled water, and 4 ml of reagent B, a black was prepared. After few minutes, the intensity of blue colour was read in photoelectric colorimeter (Erimameter) using 730-840 nm filter or (on a spectrophotometer at 882 nm, sometimes 730 nm can be preferred as 882 nm is not found sensitive).

**Procedure**

2.5 g of 2 mm air dry soil (0.1 g accuracy) was weighed into a 150 ml Erlenmeyer's flask. A little of Dacron G 60 (or) (p-free activated charcoal) was added to the above material. 50 ml of Olsen's reagent was then added (soil to solution ratio of 1:20) and using the reciprocating shaker, the solution was shake for 30 minutes (180-

Oscillations/min). A blank was made simultaneously without soil. The solution was then filtered through whatmann No.40 (or) 42 filter paper into a clean and dry beaker. The flask was shaken immediately before pouring suspension into funnel. 5 ml of aliquot of the extract was placed in a 25 ml volumetric flask. Then it was acidified with 2.5 M H<sub>2</sub>SO<sub>4</sub> to pH 5.0. Distilled water was added to 20 ml followed by 4 ml of reagent B was added. After 10 minutes, the intensity of blue colour was read on a colorimeter as mentioned above.

**CALCULATION**

$$\begin{aligned} \text{Available phosphorous} &= \frac{R \times \text{Volume of extract}}{\text{Volume of aliquot}} \frac{2.24 \times 10^6}{\text{Weight of soil} \times 10^6} \\ &= \frac{R \times 50 \times 2.24}{5 \times 2.5} \\ &= R \times 8.96 \end{aligned}$$

R = ug P in aliquot obtained from standard curve

**Bray and Kurtz P<sub>1</sub> Method****Principle**

The dilute acid fluoride extract removes easily acid solute 'P' from phosphate bout to Al, Fe, Ca. Phosphate in the extracts is determined calorimetrically a phospho molybdenum blue with citric acid as a reducing agent and Antimony (Sb) (From tartar emetic) added to give a stable Mo-P-Sb compound (complex).

**Reagents**

- Bray and Kurtz No. 1 extracting solution: 22.2 g of NH<sub>4</sub>F was dissolved in 41.6 ml of Con. HCl and made up to 500 ml. (this makes the solution of 0.03 M NH<sub>4</sub>F in 0.02 M of HCl). It can be stored in a glass bottle for more than a year without appreciable deteriorations but should in polythene bottle.
- Reagent A (as Olesen's methods)
- Reagent b (Olsen's methods)
- Standard P solution ( as described in Olsen's method)
- Standard curve: (as Olsen's process)

**Procedure**

2.5 g of 2 mm air dry soil (0.1 g accuracy) was weighed into a 150 ml Erlenmeyer flask 25 ml of extracting solution (soil to solution 1:10) was added. The suspension was shaken for exactly 5 minutes using reciprocating shaker (180 Oscillations/min). The solution was filtered immediately through whatmann 42 filter paper. (If the filterate is turbid, quickly pour the filterate back through the same filter). To avoid interference of F, 7.5 ml of 0.8 M boric acid (50 g H<sub>3</sub>BO<sub>3</sub> per litre) was added to 5 ml of extract. 5 ml of aliquot of the extract was placed in a 25 ml volumetric flask. Distilled water was added to 20 ml and then 4 ml of reagent B was added. After 10 minutes, the intensity of blue colour was read in colorimeter.

**Calculation**

$$\begin{aligned} \text{Bray's P (Kg/ha)} &= \frac{R \times 25 \times 2.24}{5 \times 2.5} \\ &= \text{ug P} \times 4.48 \text{ (from standard curve)} \end{aligned}$$

Where R = ug P in the aliquot

### Estimation of Potassium

#### Principle

The term available potassium (K) conventionally refers to exchangeable K. The exchangeable K constitutes the major portion of available K except in saline (or) saline-sodic soils. Available K (or) exchangeable K, along with Ca and Mg are usually determined in neutral 1 N (NH<sub>4</sub>OAc), ammonium acetate extract of the soil. The extraction is carried out by shaking followed by filtration (or) centrifugation. The 'K' is estimated by using a flame photometer. In soils with appreciable amount of K, Ca, Mg, these cations are estimated in a saturation extract (Jackson 1958)<sup>[110]</sup> and deducted from 1N NH<sub>4</sub>OAc extractable K, Ca and Mg to obtain respective exchangeable cations.

#### Reagent

- Ammonium acetate: 1 N, pH 7.0. To 100 ml of distilled water, 57 ml of 9.5 % glacial acetic acid (CH<sub>3</sub>COOH) and 69 ml of Conc. NH<sub>4</sub>OAc were added. The solution was diluted to 900 ml and was adjusted pH to 7.0 by the addition of 3 N NH<sub>4</sub>OAc (or) 3 N CH<sub>3</sub>COOH and made up to one litre. It was stored in a Pyrex (or) poly propylene bottle. Alternatively 154 g of NH<sub>4</sub>OAc was dissolved in water and diluted to 1.8 litre and mixed very well. It was then adjusted pH 7.0 with dilute NH<sub>4</sub>OH or HOAc as required and made up 2 litre.
- Potassium was made by dissolving 1.908 g of AR grade KCl (direct at 60 °C for an hour) in distilled water and made up to one litre. 100 ppm standard was prepared by distilled water and made up to one litre with the extracting solution.
- Standard Curve: 0.5,10,15 and 20 ml of 100 ppm solution was pipetted out into 100 ml volumetric flasks and made up to the mark with extracting solution. The solutions now contained 0.5,10,15,20 ppm K respectively.

#### Procedure

Shaking and filtration (Schoolenberger and simon 1945) 5g of soil was placed in a 150 ml Erlenmeyer flask and was poured in 25 ml of neutral 1 N NH<sub>4</sub>OAc (pH 7.0). Using a reciprocating shaker (180 + oscillations/min) the soil sample was shaken for 5 minutes and immediately, it was filtered through whatmann No.1 filter paper. First few ml of the filtrate was rejected. Shaking and centrifugation: (Knudsen et al., 1982) 10 g of less than 2 mm air dried soil (or use 5 g of soil contains 500 ppm K) was placed in a 50 ml centrifuge tube. 25 ml of NH<sub>4</sub>OAc was added and the tube was shaken for 10 minutes. The tube was centrifuged at 2000 rpm for 10 minutes until the supernatant liquid clear. The supernatant liquid was decanted into 100 ml volumetric flask. Three additional extractions were made in the same way. These three combined extracts was diluted to 100 ml with NH<sub>4</sub>OAc. The solution was mixed and K in the extract was determined in the flame photometer using potassium

filter being set and calibrated the instrument (use 0 and 20 ppm working K concentrations). Similarly the concentrations was read for K in the flame photometer and a standard curve was obtained by plotting reading against different concentrations of K.

#### Calculations

$$\text{K content (Kg/ha)} = \frac{\text{R x volume of soil x } 2.24 \times 10^6}{\text{Weight of soil taken x } 10^6}$$

Where R-ppm of K in the extract (obtained from standard curve) - ppm of K x 11.2

#### Antibacterial study

##### Test microorganisms

The test organisms used were clinical isolates viz., gram positive bacteria *Streptococcus pyogenes*, *Staphylococcus aureus* and gram negative bacteria *E. Coli*, *Klebsiella pneumoniae*. which were obtained from Department of Microbiology, Raja Muthaiyah medical college, Annamalai University. The bacterial and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively.

#### Growth and Maintenance of Test Microorganism for Antimicrobial Studies

The bacterial cultures were maintained on nutrient broth (NB) at 37°C and fungus were maintained on Potato dextrose agar (PDA) at 28°C.

**Table: 1. Composition of Nutrient agar medium**

Peptone	5.0 g
Beef extract	3.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	7.0

**Table 2. Composition of PDA medium**

Potato	200.0 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1000 ml
Ph	6.2

#### Preparation of Inoculum

The gram positive bacteria *Streptococcus pyogenes*, *Staphylococcus aureus* and gram negative bacteria *E. Coli*, *Klebsiella pneumoniae* were pre-cultured in nutrient broth over night in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A<sub>610</sub> nm).

#### Anti-bacterial Activity (Anonymous, 1996).<sup>[13]</sup>

The samples were tested by the well diffusion method. Different concentration of the extracts (100 µg/ml) was prepared by reconstituting with methanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After



solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Chloramphenicol (10 µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24hrs. The diameters of the inhibition zones were measured in mm.

## RESULTS AND DISCUSSION

### Physical and chemical properties of Termite soil

The soil sample collected from Thenangudi village, Texture is sandy clay loam. The pH value measures the ratio of H<sup>+</sup> ions to OH<sup>-</sup> base ions in the soil. If the soil solution has more H<sup>+</sup>, the soil is acidic. If the OH<sup>-</sup> dominates, the soil is alkaline. The equal balance between them is neutral and its value 7.0. Brady found that a pH range of 6.5 to 7.5 is optimal for plant nutrient availability.<sup>[14]</sup> In the present study, the pH level of the soil samples is found to be slightly acidic (pH 6.50) in nature. The EC value of the soil sample results are good condition (ranges from 0.0 to 1.0 dsm<sup>-1</sup>) and

within the normal range (0.225). Therefore electrical conductivity does not produce any harmful effect to soil samples.

The results shows that available Nitrogen (Organic carbon) in the soil sample collected from Thenangudi village were found to be low (56.4), (<113-low, 113-180 Medium, >180-High) shows that Nitrogen deficiency. The result shows that available Phosphorous content (25) are generally high level in Thenangudi village (Low 0-9.9, Medium 10-20, > 20 High). It is inclining. Phosphorus is often recommended as a row-applied starter fertilizer increases growth even if P does not increase grain yield.<sup>[13]</sup> The results show that there is high amount of potassium content (1350) in Thenangudi village. Potassium is essential element. The main role of K is to provide the ionic environment for metabolic processes which regulates various processes including growth regulation.<sup>[14]</sup>

### Antibacterial activity of dye adsorbed Termite soil

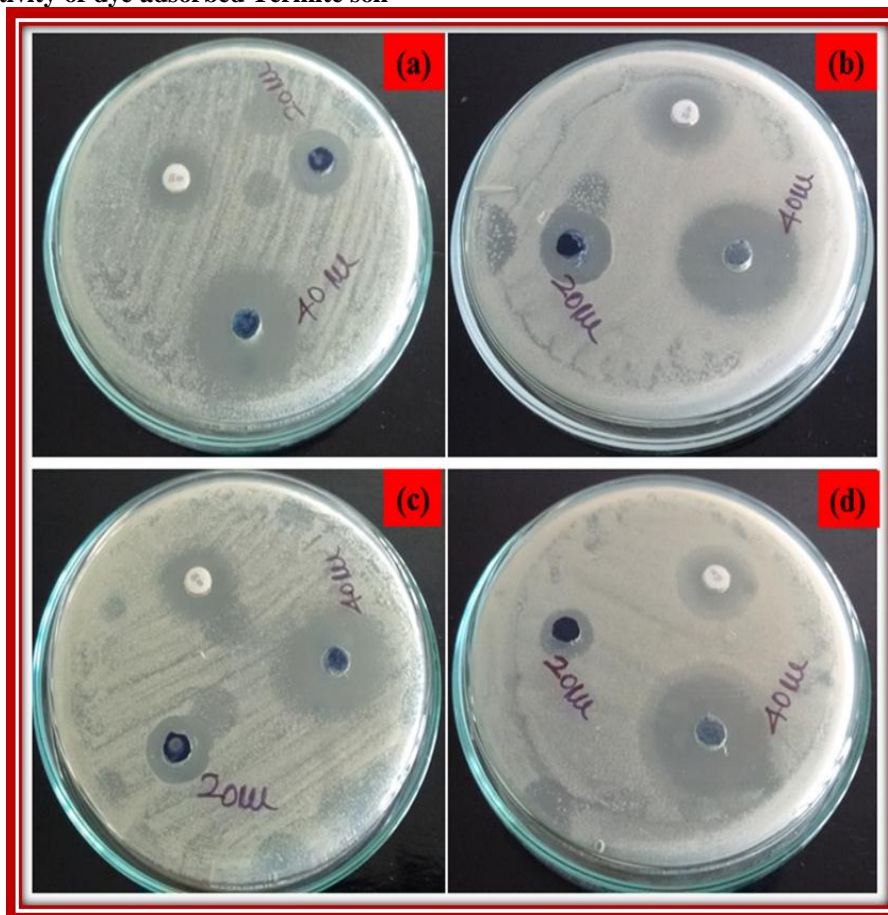
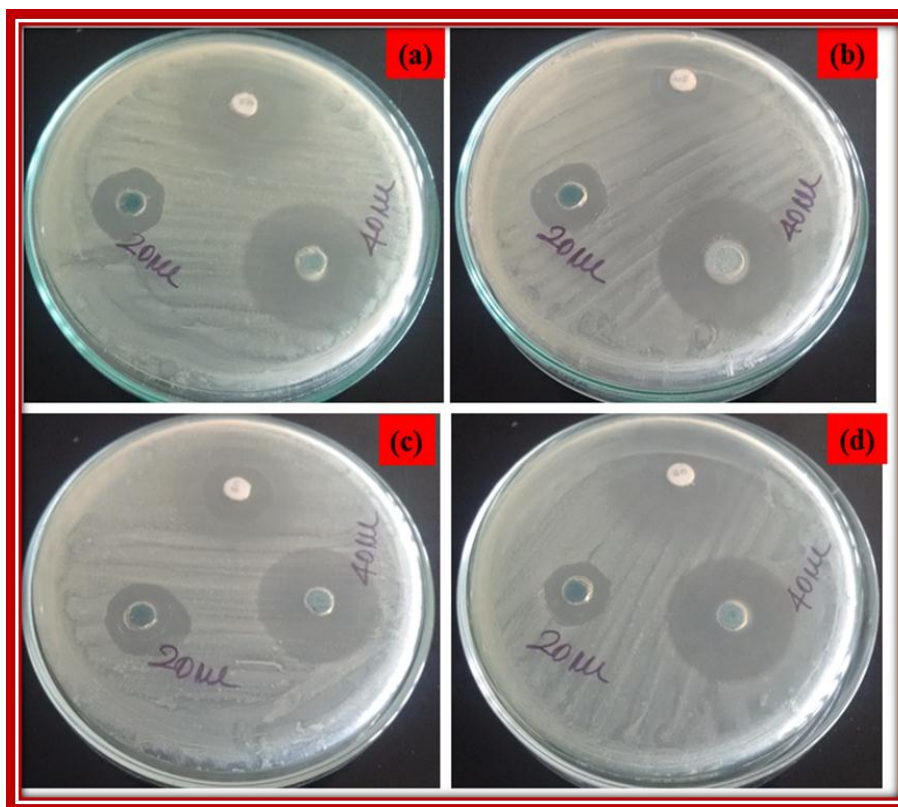


Figure 2 Antibacterial activity of methylene blue adsorbed termite soil against (a) *Streptococcus pyogenes*, (b) *Staphylococcus aureus*, (c) *Escherichia coli*, (d) *Klebsiella pneumoniae*.





**Figure 3** Antibacterial activity of malachite green adsorbed termite soil against (a) *Streptococcus pyogenes*, (b) *Staphylococcus aureus*, (c) *Escherichia coli*, (d) *Klebsiella nemoniae*

Antibacterial activity is another evidence for adsorption studies of termite soil. Methylene Blue and malachite green were shown to have antimalarial activity in the last decade of the 19<sup>th</sup> century.<sup>[15]</sup> From the figure 2 and 3 shows that the antibacterial activity of methylene blue and malachite green adsorbed termite soil. The figure shows that the moderate antibacterial activity against the bacterial strains *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella nemoniae*.

### CONCLUSION

The physicochemical analysis revealed that the pH levels of the soil samples are slightly acidic. Hence the soil sample is quite well for plant growth. EC of soil sample found to be normal. The plants that are grown in such type of soil will take water easily from the soil. From the available Nitrogen value of all the soil samples shows that nitrogen deficiency. The available phosphorous and potassium content is high.

- ❖ Proximate analysis showed termite soil content which favors adsorption.
- ❖ Favorable antibacterial studies of after adsorbed termite soil was evident for the removal of methylene blue and malachite green dyes.
- ❖ The finally came to conclusion that termite soil can be effectively used for the removal of methylene blue and malachite green.

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