Materials and Methods
MATERIALS AND METHODS

1. The effluent

The raw and treated effluents (plate I) were collected from a medium sized dyeing and printing factory, Thindal, Erode district, Tamil Nadu and used in the present study. The effluent was brought to the field in 100 litre polypropylene drums whenever required, diluted with well water to obtain the desired concentrations and used as irrigation water. The treated effluent was used separately as irrigation water for comparison.

2. Characterization of the effluent / soil samples

The physico-chemical properties of the effluent was analyzed for three months at monthly interval.

2.1. Colour

The colour of the effluent samples were recorded by visual observation.

2.2. Odour

The odour of the effluent samples were categorized as objectionable or non-objectionable by directly smelling the samples.

2.3. Solids

The solids present in the effluent samples were determined by the filtration method of Therox et al. (1943). An evaporation porcelain disc was cleaned with distilled water, dried for an hour at 104°C, cooled in a desiccator and weighed. The initial weight of the dish was 'c'. A Whatman No.44 filter paper was weighed and its initial weight was referred to as 'a'. A well-mixed 250 ml effluent sample was filtered through the Whatman
Plate I

Different stages of the effluent treatment process

a. Equilizing tank for raw effluent from dyehouse
b. Picture showing
   i. Clarification tank and
   ii. Neutralization / coagulation tank
c. First sedimentation tank. Note the settled sludge
d. Agitation tank
e. Second sedimentation tank
No.44 filter paper into the porcelain dish. The filtrate was evaporated to dryness in a water bath, the dish was dried for 2 h at 104°C and cooled in a desiccator. The filter paper was also dried for 2 h at 104°C and cooled. The final weights of filter paper and porcelain dish were made and marked as 'b' and 'd' respectively. The suspended, dissolved and total solids were calculated using the following formulae:

\[
\text{Suspended solids} = b - a = x
\]
\[
= \frac{X}{250} \times 1000 \text{ mg l}^{-1}
\]

\[
\text{Dissolved solids} = d - c = y
\]
\[
= \frac{Y}{250} \times 1000 \text{ mg l}^{-1}
\]

\[
\text{Total solids} = \frac{X+Y}{250} \times 1000 \text{ mg l}^{-1}
\]

Where,

- \(a\) = Initial weight of filter paper
- \(b\) = Final weight of filter paper
- \(c\) = Initial weight of porcelain disc
- \(d\) = Final weight of porcelain disc

2.4. Determination of soil physical constants

A. Principle

A known quantity of soil is allowed to fully saturate and equilibrate with water and from the water held in the soil the single value constants viz., bulk density, maximum water holding capacity and pore space are determined gravimetrically using Keen Raczkowski Box.
B. Procedure

Keen Raczkowski Box (Circular brass box having an internal diameter of 5-6 cm and a height of 1.6 cm) with perforated bottom having holes of 0.75 mm diameter spaced at 4 mm apart was taken. A thin filter paper (Whatman No.1) was placed on the perforated bottom of the box and fitted in position with the help of the split ring. The box with filter paper was weighed. A small portion of air dried soil to be studied was transferred using a spatula and the soil was packed by tapping the box gently on a hard surface. The addition of soil and tapping the box was continued systematically until it is full. The excess soil was removed by running a sampling knife over the rim of the box. The weight of the box with air dried soil was determined.

The box was placed in a petridish and water was added to the dish until the water level reached about half the height of the box and it was left as such for overnight. The box was removed in the next morning, the water on the outer side of the box was wiped out and the weight was recorded. Then the soil in the box was dried in an oven at 105°C for 10-12 hours or to constant weight, cooled in a desiccator and the weight was recorded. In the case of shrinkage of soil in the box, the total volume of soil was determined by adding sand to fill the box and then subtracting the sand volume from the volume of the box and used in the calculation of pore space. From the weights obtained, the single value constants were calculated.

C. Calculation

\[
\begin{align*}
\text{Weight of box + Filter paper} & = a \quad \text{g} \\
\text{Weight of air dry soil + Box (filter paper)} & = b \quad \text{g} \\
\text{Weight of box + Wet saturated soil} & = c \quad \text{g}
\end{align*}
\]
Weight of box + Wet residue soil = d g
Weight of box + Dry residue soil = e g
The volume of the box = V ml

1. Apparent density (Bulk density) = \( \frac{(b-a)}{V} \) g / cc

2. Maximum water holding capacity (%) = \( \frac{(c-a)-(b-a)}{(b-a)} \) x 100

3. Per cent pore space = \( \frac{(d-a)-(e-a)}{V} \) x 100

2.5. pH

pH of effluent samples was directly determined using a standard Elico pH meter. Soil pH was determined in 1 : 5, soil : water suspension.

2.6. Electrical conductivity

Electrical conductivity was determined in a conductivity bridge and expressed as dSm\(^{-1}\). For soil, it was determined from saturated paste extract.

2.7. Dissolved oxygen

For the determination of BOD, dissolved oxygen (DO) in the samples were estimated following iodometric method described in Standard Methods for the Examination of Water and Waste water (Anonymous, 1981).

A. Principle

When manganous sulphate is added to the sample containing alkaline potassium iodide, manganous hydroxide is formed which is oxidized by the DO of the sample to basic manganic oxide. On addition of
sulphuric acid, the basic manganic oxide liberates iodine equivalent to that of DO originally present in the sample. The liberated iodine is titrated with a standard solution of sodium thiosulphate using starch as the indicator.

B. Reagents

Manganous sulphate solution

Dissolve 91.0 g manganous sulphate monohydrate (MnSO$_4$.H$_2$O) in distilled water. Filter if necessary and dilute to 250 ml. One ml of this solution when treated with 50 ml of acidified potassium iodide solution should not liberate iodine.

Alkali-iodine-azide reagent

i. Dissolve 175 g potassium hydroxide and 37.5 g potassium iodide in distilled water and dilute to 250 ml.

ii. Dissolve 2.5 g sodium-azide in 10 ml of distilled water.

iii. Pour the azide solution to the alkali-iodide solution and mix well.

This solution should not give colour with starch solution when diluted and acidified.

Phosphoric acid 85 to 90%

Sodium thiosulphate solution 0.1N

Dissolve 24.82 g sodium thiosulphate (Na$_2$S$_2$O$_3$.5H$_2$O) in boiled and cooled distilled water and make up to 1000 ml in a volumetric flask. Standardize it against standard potassium dichromate solution (0.1N).

Sodium thiosulphate solution 0.025 N

Dilute appropriate volume of 0.1N sodium thiosulphate solution (about 250 ml required) to 1000 ml with distilled water in a volumetric flask.
1.0 ml of exactly 0.025 N thiosulphate = 0.2 mg DO.

Starch solution

Dissolve 1 g starch in a little water and stir with a glass rod to make it as a thin paste. Pour this paste in about 100 ml boiling distilled water and boil for two minutes and cool.

C. Procedure

The sample in a 300 ml BOD bottle was added with 2 ml manganous sulphate solution followed by 2 ml alkali-iodide-azide solution. The tip of the pipett should be below the surface of the liquid. Stopper the bottle without entrainment of air and mix by inverting the bottle at least ten times. Allow the precipitate to settle completely, leaving a clear supernatant liquid. Carefully remove the stopper and add 2 ml of concentrated sulphuric acid by the sides of the bottle. Stopper the bottle and mix thoroughly until the dissolution is complete. Measure 200 ml of the solution from the bottle (which corresponds to 200 ml of the original sample) into a conical flask of 500 ml capacity. Titrate immediately with 0.025 N sodium thiosulphate solution using starch solution as the indicator.

D. Calculation

If the thiosulphate solution is exactly 0.025 N, then,

\[ \text{mg/l dissolved oxygen} = \text{ml sodium thiosulphate} \times 1. \]

2.8. Biochemical oxygen demand (BOD)

Dilution method was employed for BOD determination (Anonymous, 1972).

A. Principle

The dissolved oxygen content of the sample is determined before
and after five days incubation at 20°C. The amount of oxygen depleted is calculated as BOD. Samples devoid of oxygen or containing less amount of oxygen, are diluted several times with special type of dilution water saturated with oxygen in order to provide sufficient amount of oxygen for oxidation.

B. Reagents

**Calcium chloride solution**

Dissolve 27.5 g anhydrous calcium chloride (CaCl₂) in distilled water and dilute to 1000 ml.

**Magnesium sulphate solution**

Dissolve 25 g magnesium sulphate heptahydrate (MgSO₄.7H₂O) in distilled water and dilute to 1000 ml.

**Ferric chloride solution**

Dissolve 0.25 g ferric chloride hexahydrate (FeCl₃.6H₂O) in distilled water and dilute to 1000 ml.

**Phosphate buffer solution**

Dissolve 8.5 g of potassium dihydrogen phosphate (KH₂PO₄), 21.75 g dipotassium hydrogen phosphate (K₂HPO₄), 33.4 g disodium hydrogen phosphate heptahydrate (Na₂HPO₄.7H₂O) and 1.7 g ammonium chloride (NH₄Cl) in 500 ml distilled water and make up to 1000 ml. The pH of this buffer solution should be 7.2. Keep this reagent bottle in refrigerator.

**Standard dilution water**

Aerate 2 l distilled water with a supply of clean compressed air after bringing the temperature to 20°C. Add 1 ml each of calcium chloride solution, magnesium sulphate solution, ferric chloride solution and phosphate buffer solution. Use the dilution water as soon as possible.
C. Procedure

The test was performed as soon as possible after the effluent was collected. The effluent was first brought to 20°C, thoroughly mixed and then diluted in the ratio of 1:99 where, the first numerical indicates the volume of the effluent and the second one denotes the volume of dilution water.

Dilution technique for the effluent

Carefully siphon the standard dilution water into a 1000 ml graduated cylinder and half fill it without entrainment of air. Add 10 ml of well mixed effluent sample into the cylinder by the sides without producing any air bubble. Make up to 1000 ml mark with dilution water. Mixing well with a plunger type mixing rod without entrainment of air, siphon the mixture into two BOD bottles of 300 ml capacity. While siphoning, allow about 50 ml of mixture to waste. Fill the bottles and stopper carefully without allowing any air bubble to remain inside. Water seal both the bottles. Siphon out standard dilution water into two BOD bottles and water seal them after filling completely.

Determination of dissolved oxygen

Keep one set of samples i.e., the effluent mixer and dilution water to find out the initial dissolved oxygen content. Keep the other set of samples into BOD incubator at 20°C (in dark for 5 days). After 5 days, determine the dissolved oxygen content of the samples and dilution water.

D. Calculation

The BOD of the effluent samples were determined using the following formula:

$$\text{BOD (at 20°C for 5 days)} = \frac{(\text{IDoS-FDoS})-(\text{IDoB-FDoB})}{1} \times 100 \text{ mg l}^{-1}$$
Where,

\[
\begin{align*}
IDoS &= \text{Initial dissolved oxygen content of the effluent} \\
FDoS &= \text{Final dissolved oxygen content of the effluent} \\
IDoB &= \text{Initial dissolved oxygen content of the blank} \\
FDoB &= \text{Final dissolved oxygen content of the blank}
\end{align*}
\]

2.9. Chemical Oxygen Demand (COD) (Sundaresan, 1979)

A. Principle

The organic matter of the sample is oxidized to water, carbon-dioxide and ammonia by refluxion with a known excess of potassium dichromate in a 50% sulphuric acid solution. The excess dichromate is titrated with a standard solution of ferrous ammonium sulfate solution.

Usually silver sulfate is added as a catalyst to promote oxidation of certain classes of organic compounds such as straight chain aliphatic compounds like acetic acid, amino acids etc. Mercuric sulphate is added to eliminate the interference due to chlorides.

B. Reagents

**Mercuric sulphate crystals**

**Sulphuric acid-silver sulphate reagent**

Dissolve 10.1 g silver sulphate ($\text{Ag}_2\text{SO}_4$) in 1000 ml conc. $\text{H}_2\text{SO}_4$. Allow 2 days for dissolution.

**Potassium dichromate solution 0.125 N**

Dissolve 6.124 g potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) (previously dried at 103°C) for 2 days in distilled water and make up to 1000 ml in a volumetric flask.

\[
1.0 \text{ ml } 0.125 \text{ N potassium dichromate } = 1.0 \text{ mg } \text{O}_2
\]
**Ferroin indicator**

Dissolve 95 mg ferrous sulphate (FeSO$_4$.7H$_2$O) in 100 ml water. Add 1.485 g 1,10-phenaenthroline mono-hydrate and mix thoroughly until dissolved.

**Ferrous ammonium sulphate solution 0.125 N**

Dissolve 49 g of ferrous ammonium sulphate (Fe(NH$_4$)$_2$(SO$_4$)$_2$.6H$_2$O) in distilled water. Add 20 ml concentrated sulphuric acid, cool and make up to 1000 ml in a volumetric flask. Standardize it daily against 0.125 N potassium dichromate solution.

**C. Dilution**

10 ml sample of the effluent was thoroughly mixed with distilled water and made up to 1000 ml. From this, aliquot samples were taken for oxidation.

**D. Procedure**

Refluxing flasks of 250 ml capacity with ground glass 24/40" necks fitted with a 300 mm double surface condensers were used. The condensers were fitted with glass caps. Heating was provided by heating mantles with thermostat control. Place 20 ml sample in the refluxing flask and add 500 mg of mercuric sulphate. Pipette into the flask 10 ml of 0.125 N potassium dichromate solution and mix well. Pour 30 ml concentrated sulphuric acid into the flask by the side carefully while water cooling the flask. Add a few glass bellings into the flask as anti-bumbling granules.

Now fit the flasks in the heating mantle and heat at 70°C while cold water was circulated in the condenser. Switch-off heating mantle after 2 h and allow the condenser to cool down. Add 80 ml of distilled water in the
flask and allow to cool for 8 h. Add 3 drops of ferroin indicator and titrate with 0.125 N ferrous ammonium sulphate solution. The end point was the sharp colour change from blue green to reddish brown. Run blanks with 20 ml distilled water instead of sample and note the volume of ferrous ammonium sulphate solution spent for titration.

\[
\text{COD} = \frac{(\text{Blank titre value} - \text{Sample titre value}) \times N \times 8 \times 1000}{\text{ml of sample taken for determination (20)}} \times \frac{100}{1} \times \text{mg}^{-1}
\]

where,

\(N\) = Normality of ferrous ammonium sulphate

**2.10. Preliminary treatment (dry ash HCl extract) for the estimation of Na, K, Cl and P**

Evaporate 250 ml of the effluent sample in a porcelain dish on a water bath and ignite the content to destroy the organic matter at 500°C in a muffle furnace to get a white ash. Transfer the ash/ignited soil into a 250 ml conical flask using minimum quantity of water. Add 60 ml of 1:1 HCl, cover the mouth of the conical flask with a funnel and digest the content over a sand bath for 8 h. Add 200 ml of water and keep overnight. Filter the digest through Whatman No.3 filter paper and collect the filtrate in a 500 ml volumetric flask after smearing the mouth of the conical flask with a thin layer of vaseline to prevent the solution from being spilt. Transfer all the soil into the filter paper and wash with hot water till the filtrate runs free of chloride. Cool the filtrate and make up the volume to 500 ml with cold distilled water.

**A. Principle**

Making use of EDTA, the concentration of calcium and magnesium is estimated. In the first titration, the amount of calcium and magnesium
is found by elevating the pH with ammonium buffer and restricting the precipitation of magnesium as magnesium hydroxide. For the first titration, erychrome black ‘T’ is used as the indicator. The second titration is carried out in alkaline condition using murexide as indicator. In the second titration magnesium is removed completely as its hydroxide. The first titration answers for both calcium and magnesium and the second titration answers for calcium alone.

**B. Reagents**

**Ethylenediamine tetraacetatedihydrate (disodium salt) (0.1N)**

Dissolve 16.825 g of AR grade EDTA in one litre of distilled water. In order to avoid probable decomposition, it is better to keep the solution in polythene bottle.

- 1 ml of 0.1 N EDTA = 2 mg of calcium
- 1 ml of 0.1 N EDTA = 1.2 mg of magnesium

**Ammonium buffer**

Dissolve 70 g ammonium chloride in 570 ml liquor ammonia.

**Sodium hydroxide**

Dissolve 80 g sodium hydroxide in one litre of distilled water.

**Erychrome black ‘T’**

A fine mixture of sodium chloride and erychrome black ‘T’ is made in proportion of 200:1.

**Murexide indicator**

A solid mixture of murexide and sodium chloride in the ratio of 1:100.

**C. Procedure**

Take 50 ml of the pretreated solution in a conical flask and add a
pinch of eryochrome black 'T'. Warm the solution to 60°C and titrate against EDTA from a burette. The end point of the titration was from salmon red to blue. Take the titre value as 'A'. Take another 5 ml of the sample in a conical flask and add 5 ml of sodium hydroxide followed by a pinch of murexide indicator. Dilute the solution to 100 ml with distilled water and titrate against EDTA until the colour changes from pink to blue. Take this titre value as 'B'.

D. Calculation

Consumption of EDTA due to calcium and magnesium = A
Consumption of EDTA due to calcium alone = B
Consumption of EDTA due to magnesium = A - B
Factor value (F) for calcium (=2 mg) = 2

\[
\text{Amount of calcium present in the sample as CaCO}_3 = \frac{F \times B}{\text{Sample volume}} \times 1000 \text{ mg l}^{-1}
\]

Factor value (F) for magnesium (=1.12 mg) = 1.12

\[
\text{Amount of magnesium present in the sample as CaCO}_3 = \frac{F \times (A-B)}{\text{Sample volume}} \times 1000 \text{ mg l}^{-1}
\]

2.10.1. Sodium and potassium

A. Principle

Certain elements when excited in flame emit radiation. The excitation causes one of the outer electrons of neutral atoms to jump to an outer orbit of higher energy level or the atoms may be excited sufficiently to lose an electron completely. When excited atoms return to lower energy levels, light of characteristic wave length is emitted. The
flame photometer measures this radiation intensity which is proportional to the concentration in a solution.

**B. Estimation**

Fifty ml of the pretreated sample was used for estimation of the Na and K using flame photometer referring to the respective standard solution.

**2.10.2. Chloride**

Chloride in the samples was estimated following the titrimetric method described by Sundaresan (1979).

**A. Principle**

Chloride ion is determined by titration with standard AgNO$_3$ in which AgCl$_2$ precipitates out. The end of titration is indicated by formation of red silver, silver chromate from excess silver nitrate. Potassium chromate is used as an indicator in neutral to slightly alkaline solution.

**B. Reagents**

**Potassium chromate indicator**

Dissolve 50 g K$_2$CrO$_4$ in distilled water. Add AgNO$_3$ till definite red precipitate is formed. Allow to stand for 12 h, filter and dilute to 1000 ml.

**Silver nitrate (0.0141 N)**

Dissolve 2.395 g of AgNO$_3$ in distilled water and dilute to 1000 ml. Standardize against NaCl, 0.0141 N.

\[ 1 \text{ ml of 0.0141 N AgNO}_3 = 0.5 \text{ mg Cl.} \]

**Sodium chloride (0.0141 N)**

Dissolve 824.1 mg of NaCl (previously dried at 140°C) in distilled water and dilute to 1000 ml in a volumetric flask.
Special reagent to remove colour and turbidity

Dissolve 125 g AlK(SO₄)₂.12H₂O or AlNH₄(SO₄)₂.12H₂O and dilute to 100 ml. Warm to 60°C and add 55 ml concentrated ammonium hydroxide slowly. Allow to stand for 1 h. This solution should be free from Cl.

C. Procedure

a. Pre-treatment

i. Take 100 ml pretreated sample in a conical flask and add 3 ml special reagent. Mix well and allow to settle. Filter the supernatant for titration purpose.

ii. To the sample having sulfite, neutralize the sample and add about 1 ml H₂O₂.

iii. In case, if sulfide or thiosulfate is present, raise the pH of the sample to 8.3 or more, add 1 ml H₂O₂ and then again neutralise the sample.

b. Titration

i. Adjust the pH of the sample between 7.0 and 8.0.

ii. Take 50 ml of well mixed sample in a conical flask, adjusted to pH 7.0-8.0 and add 1 ml K₂Cr₂O₇.

iii. Titrate with standard AgNO₃ solution till AgCrO₄ starts precipitating (A).

iv. Standardize AgNO₃ against standard sodium chloride.

v. For better accuracy, titrate distilled water (50 ml) in the same way to establish reagent blank (B).
D. Calculation

\[
Cl = \frac{(A-B) \times N \times 35.45 \times 1000}{\text{ml of sample}} \text{ mg l}^{-1}
\]

where,

A = ml of AgNO\text{3} for sample
B = ml of AgNO\text{3} for blank
N = Normality of AgNO\text{3} used

2.10.3. Estimation of total phosphorus (Pemberton, 1945)

A. Principle

Phosphorus is precipitated as ammonium phosphomolybdate in nitric acid medium. The precipitate is filtered, washed free of acid, dissolved in a known excess of standard alkali and the excess alkali is determined by back titration with a standard acid using phenolphthalein indicator.

B. Procedure

Pipette out 200 ml of HCl extract into a 400 ml beaker and evaporate to a small bulk. Transfer it to a silica basin using hot water and evaporate to dryness over a water bath. Keep the silica basin in an air oven at 105 to 110°C for 3 h to dehydrate the silica and rendering it insoluble (or roast over a wire gauze till the residue becomes brown using a rose head flame, taking care to see that there is no loss of residue by spurting). Dissolve this residue again by adding a small quantity of 1:1 hydrochloric acid and evaporate to dryness over a water bath. Dissolve the residue again in nitric acid, adding just sufficient amount of nitric acid, to dissolve the same. Allow the insoluble silica to settle overnight.
Filter through Whatman No.42 filter paper and wash the residue in the silica basin and on the filter paper with small quantities of 1:4 nitric acid till no yellow colour is left either in the basin or in the filter paper. Collect the filtrate and washings in a 250 ml beaker (collect preferably not more than 100 ml).

Make the extract alkaline with concentrated ammonium hydroxide (to suppress all other acids, if any, present) and then distinctly acidify with concentrated nitric acid (test-with red litmus paper). Add 5 g of solid ammonium nitrate and keep it on a thermostat at 65°C for 15 min. In the meantime prepare the precipitant mixture by taking 7 ml of concentrated nitric acid and 3 ml of distilled water in a 100 ml beaker and adding 10 ml of 20% ammonium molybdate to this solution drop by drop with constant stirring. The precipitant mixture should be clear without any turbidity. Add 10 ml of this precipitant mixture to the beaker in the thermostat drop by drop with constant stirring, taking care not to touch the sides of the beaker. Keep the beaker in the thermostat for another half an hour at 65°C and allow the precipitate to settle well, leaving a clear supernatant liquid.

Filter through Whatman No.40 filter paper by decantation, pouring only the supernatant liquid to the filter paper and retaining as much of the precipitate as possible in the beaker itself. Wash the precipitate with cold distilled water till the filtrate runs free of acid (1 drop of 0.1619 N KOH + 1 drop of phenolphthalein indicator + 2/3 filtrate in test tube - appearance of faint but permanent pink colour shows free of acid). Transfer the filter paper with the precipitate to the same beaker in which precipitation was done and add enough water to make the filter paper into a pulp. Now add from the burette, 0.1619 N KOH till the yellow precipitate
is completely dissolved leaving a colourless solution. Add another 5 ml of 0.1619 N KOH to keep the alkali in fair excess quantity. Note down the volume of alkali added. Add a drop of phenolphthalein indicator and titrate the excess alkali against 0.1619 N nitric acid. Disappearance of pink colour indicates the end point.

C. Calculation

Weight of soil taken = W g
Volume of HCl extract prepared = 500 ml
Volume of HCl extract pipetted out for analysis = 200 ml
Volume of 0.1619 N KOH added in excess = a ml
Volume of 0.1619 N HNO₃ used for back titration = b ml

Therefore, actual volume of 0.1619 N KOH used to dissolve the precipitate = (a-b)

1 ml of 0.1619 N KOH = 0.0005 g P₂O₅

(a-b) ml of 0.1619 N KOH = 0.0005 x (a-b) g P₂O₅

This is present in 200 ml of HCl extract

Therefore, in 500 ml = 0.0005 x (a-b) x \( \frac{500}{200} \) g

This is present in W g of soil

Therefore, in 100 g = 0.0005 x (a-b) x \( \frac{500}{200} \times \frac{100}{W} \) g

Percentage of P₂O₅ on moisture free basis

\[ = 0.0005 \times (a-b) \times \frac{500}{200} \times \frac{100}{W} \times \frac{100}{(100-M)} \]

(M = Moisture content of the soil)
2.11. Estimation of total nitrogen (Jackson, 1973)

A. Principle

The nitrogen in the soil sample is converted into ammonium sulphate by digestion with sulphuric acid-salicylic acid mixture in the presence of sodium thiosulphate, potassium sulphate and copper sulphate. The ammonium sulphate is distilled with excess alkali and the ammonia liberated is collected in a known excess quantity of standard acid. The excess acid is determined by back titration with standard alkali.

B. Procedure

a. Digestion

Weigh accurately 10 g of soil (10 ml in the case of effluent) and transfer into a clean dry kjeldahl flask and add 30 ml of concentrated sulphuric acid containing 1 g salicylic acid. Shake the contents well and allow to stand for atleast 15 minutes. Add 5 g of sodium thiosulphate, shake well and allow to stand for half an hour. Then add 10 g of potassium sulphate – copper sulphate mixture and digest the contents over a bunsen burner till the contents of the flask become apple green or colourless (copper sulphate acts as a catalyst and also serves to indicate the completion of digestion. Potassium sulphate raises the boiling point).

b. Distillation

Cool the Kjeldahl flask and add about 50 ml of distilled water. Shake the contents well and transfer the supernatant liquid alone to the distillation flask. Take sufficient care not to transfer the soil to the distillation flask. Add another 50 ml of water. Shake the contents well and transfer the liquid to the distillation flask. Repeat this process for atleast 6 times to ensure complete transfer of the digested material to the distillation flask. Pipette out 25 ml of 0.1 N sulphuric acid into an ice
tumbler, add 2-3 drops of methyl red indicator and keep it at the delivery end. The delivery end should be completely immersed inside the acid, otherwise a part of gaseous ammonia will escape. Now add few zinc and porcelain pieces to the distillation flask followed by 120 ml of 40% sodium hydroxide till the contents become distinctly alkaline as tested with litmus paper and 10 ml of 10% sodium sulphide. Close the mouth of the flask immediately and start the distillation. Collect the ammonia evolved in 0.1 N sulphuric acid, kept in the ice tumbler. (If the content turns yellowish during the course of distillation, it indicates that the standard acid taken for absorbing ammonia is insufficient in which case pipette out another 25 ml of 0.1 N sulphuric acid into the ice tumbler). Test for completion of distillation by collecting a drop of distillate on a moist red litmus (the red litmus must retain the red colour). When the distillate runs free of ammonia, remove the ice tumbler and titrate the excess acid with 0.1 N potassium hydroxide. The end point is the change of colour from pinkish red to straw yellow. From the volume of 0.1 N sulphuric acid actually consumed for absorbing the ammonia, calculate the percentage of nitrogen in the soil.

**C. Calculation**

\[
\begin{align*}
\text{Weight of soil taken} & \quad = \quad 10 \text{ g} \\
\text{Volume of 0.1N sulphuric acid taken} & \quad = \quad a \text{ ml} \\
\text{Volume of 0.1 N potassium hydroxide consumed for back titration} & \quad = \quad b \text{ ml} \\
\text{Actual volume of 0.1 N sulphuric acid consumed for absorbing the ammonia} & \quad = \quad (a-b) \text{ ml} \\
1 \text{ ml of 0.1 N sulphuric acid} & \quad = \quad 0.0014 \text{ g of N}
\end{align*}
\]
Therefore, (a-b) ml of 0.1 N sulphuric acid = 0.0014 x (a-b) g N

This is present in 10 g of soil

Therefore, percentage of N on moisture free basis

\[
\frac{0.0014 \times (a-b)}{10} \times \frac{100}{100-M}
\]

(M = Moisture percentage)

2.12. Nitrate nitrogen

Nitrate nitrogen was estimated following phenol disulphonic acid method described in Standard Methods for the Examination of Water and Wastewater (Anonymous, 1981).

A. Reagents

Phenol disulphonic acid

Dissolve 25 g white pure phenol in 150 ml of conc. H\(_2\)SO\(_4\) and 75 ml of fuming sulphuric acid. Heat for about 2 h on a steam bath and keep in a dark bottle. In place of 75 ml of fuming sulphuric acid, 85 ml of concentrated sulphuric acid can also be added.

Silver sulphate solution

Dissolve 4.4 g silver sulphate in distilled water and make up the volume to one litre.

Liquid ammonia 30%

Standard nitrate solution (1 mg N/l)

Dissolve 0.7222 g potassium nitrate in distilled water to prepare one litre of solution. This solution contains 100 mg N/l. Dilute to 100 times to prepare a solution having 1 mg N/l.

B. Procedure

Extract 10 g of dry soil in distilled water, filter and make up to 100 ml. Take 50 ml of the extract (or an aliquot containing not more than
1 mg/l of NO₃N) in a conical flask. To remove the interference of chloride, add an equivalent amount of silver sulphate solution (1 mg/l of Cl⁻ = 1 ml of 0.0141 N Ag SO₄ solution). Heat slightly and filter the precipitate of AgCl. Evaporate the filtrate in a porcelain basin to dryness, cool and dissolve the residue in 2 ml phenol disulphonic acid. Dilute the contents to 50 ml and add 6 ml of liquid ammonia to develop a yellow colour. Take the absorbance of this colour at 410 nm and calculate the NO₃⁻ N from the standard curve.

Prepare standard curve between concentration 0.0 to 1.0 mg/l of NO₃⁻ N at interval of 0.1. Find out the absorbance of standards using the same procedure described for the sample above except the removal of chlorides by addition of silver sulphate. Plot a curve between concentration and absorbance.


A. Principle

The sulphate in sample is precipitated as barium sulphate by the addition of barium chloride in hydrochloric acid medium. The precipitate is filtered, washed free of chloride, ignited and weighed as barium sulphate.

B. Procedure

Filter the effluent in Whatman No.42 filter paper. Pipette out 50 ml of it into a clean 250 ml beaker. Add 10 ml of dilute hydrochloric acid and 1 g of solid ammonium chloride. Heat to boiling and add 10 ml of 10% barium chloride solution drop by drop with constant stirring. Continue boiling for another 2 to 3 minutes. Allow the precipitate to settle and test for completion of precipitation by adding a small amount of barium chloride solution through the sides of the beaker. If any turbidity is
noticed, add sufficient quantity of barium chloride to precipitate all the sulphate. Transfer the contents to a sand bath and digest for half an hour to promote granulation of the precipitate. Filter through Whatman No.42 filter paper and wash with boiling water till the filtrate runs free of chloride (test with silver nitrate solution). Transfer the filter paper along with the precipitate to a weighed silica crucible and dry it in an air oven. Ignite over a low flame initially, taking care to ash the filter paper completely, then ignite strongly over a rose head flame to constant weight. From the weight of barium sulphate obtained, calculate sulphate content.


A. Principle

Organic carbon present in organic matter is oxidized by chromic acid in the presence of conc. H₂SO₄. Potassium dichromate on reaction with H₂SO₄ provides nascent oxygen, which combines with carbon and forms CO₂. The H₂SO₄ enables easy digestion of organic matter by rendering heat of dilution. Only a certain quantity of chromic acid is used for oxidation. The excess chromic acid left unused by the organic matter is determined by back titration with 0.5 N ferrous sulphate or ferrous ammonium sulphate using diphenylamine indicator.

B. Reagents

N-Potassium dichromate solution

Dissolve 49.03 g potassium dichromate in water and make up to 1 litre.

Sulphuric acid (atleast 96%)

Add 1.25 g silver sulphate to each 100 ml of acid.

Phosphoric acid 85%

Diphenylamine indicator
Dissolve 0.5 g diphenylamine in a mixture of 100 ml of conc. H₂SO₄ and 20 ml of distilled water.

N-Ferrous ammonium sulphate solution

Dissolve 393.13 g ferrous ammonium sulphate in water and add 15 ml conc. H₂SO₄ to prevent hydrolysis of the ferrous salt. Dilute to 1 litre and standardize against 10.5 ml of N potassium dichromate.

C. Procedure

Take a weighed amount of oven dried soil (2 g) in a 500 ml Erlenmeyer flask and add with exactly 10 ml of normal potassium dichromate solution and 20 ml of conc. H₂SO₄ successively. Shake the flask for 2-3 min and allow to stand on a sheet of asbestos for 30 min. After 30 minutes, dilute the contents of the flask with 200 ml distilled water and add 10 ml phosphoric acid and 1 ml diphenylamine indicator. Titrate the mixture against normal ferrous ammonium sulphate until the colour of the mixture flashes to green. Add an excess of 0.5 ml of dichromate and complete the titration by adding sulphate solution drop by drop until the last traces of blue colour disappear.

D. Calculation

The following formula was used to calculate the percentage of carbon:

\[ \frac{V_1 - V_2}{W} \times 0.003 \times 100 \]

Where,

\[ V_1 = \] Volume of N potassium dichromate in ml
\[ V_2 = \] Volume of N ferrous ammonium sulphate in ml
\[ W = \] Weight of soil taken
3. **Experiments to study the impact of short-term effluent irrigation**

3.1. **Percentage germination of seeds**

Sand culture method was followed to find out the influence of the effluent on germination under in vitro conditions. Different concentrations of the effluent (i.e., 25, 50, 75 and 100%) were prepared. One litre from each concentration was added to enamel trays of 30 cm x 40 cm x 6 cm size containing 10 kg sand and used for the experiment. Control was maintained using tap water treated effluent was used for comparison.

Uniform seeds of *Vigna radiata* (L.) R. Wilczek obtained from Tamil Nadu Agricultural University, Coimbatore were surface sterilized with 0.1% HgCl₂ and washed thoroughly. Hundred seeds were sown in each tray containing the effluent treated sand. The moisture content was maintained by spraying tap water. Observations were made on the seventh day and for each treatment the percentage inhibition of germination was calculated.

3.2. **Field experiment**

The field experiment designed to study the impact of dyehouse effluent irrigation on crop plants was performed in an agricultural field in Thindal, Erode.

3.2.1. **Preparation of experimental plots**

The experimental field was ploughed twice and partitioned into 25 plots of 1 x 1 m size with a gap of 50 cm between them so as to avoid leaching between adjacent plots. The effluent collected from a medium sized dyehouse in Erode was brought to the experimental field. Dilutions of the effluent with well water in the ratio of 1:3, 1:1, 3:1 were prepared to get 25, 50, 75 per cent effluent concentrations respectively while the raw
effluent was considered as 100% concentration. A randomized complete block design was followed for the effluent drench of different concentrations. Each plot was drenched with 30 litres of effluent and for each concentration five replicates were maintained. Five plots drenched with the well water served as control. The soil was thoroughly mixed up to a depth of ca.20 cm with the help of a spade and left as such for a week.

3.2.2. Raising of the test crop

Green gram (*Vigna radiata* (L.) R. Wilczek, seeds procured from Tamil Nadu Agricultural University, Coimbatore, were surface sterilized with 0.1% HgCl₂ solution and sown in rows at an equidistance of 25 cm from each other, a week after the effluent drenching. Originally 2 seeds were sown in each hole and subsequently thinned to one after germination. The plots were irrigated with the respective concentrations of the effluent at the rate of 30 litres per plot at fortnightly intervals, until harvest with intermittent irrigation with well water whenever needed. Weeding and tilling of the plots were done regularly at 20-day interval and no manures or insecticides were applied during the experimental period.

3.3. Experiment under potted condition

Earthen pots (30 cm x 20 cm) filled with field soil (ca. 6 kg) were drenched with different concentrations of the effluent and left as such for 1 week. Ten replicates were maintained for each treatment. The pots were sown with surface sterilized, healthy seeds of *V. radiata* at the rate of 5 seeds per pot and watered at fortnightly interval with the respective effluent concentration. Well water was used for intermittent watering whenever necessary. No fertilizer / pesticide was applied to the plants during the course of the study.
3.4. Sampling

From the periphery of each plot, one plant was uprooted each time (at the rate of 5 plants per treatment) along with their entire root system on 20, 40, 60 and 80th days after sowing. In the case of potted plants, 5 plants at the rate of one per replicate pot was uprooted each time. Their root systems were washed free of soil particles and the plants were used for the observations of growth parameters. Few, randomly collected, fine feeder root samples were preserved in FAA for assessing the arbuscular mycorrhizal colonization. When the plants came to yield, the mature pods were separated and used for studying yield parameters.

Whenever plant samples were made, soil samples were also collected from A1 layer (0-10 cm depth) from the replicate plots/pots, composited and used for the bacterial, fungal and arbuscular mycorrhizal spore populations.

For chemical analysis, soil samples were made on the day 0 (just before sowing) and on 80th day after harvest.

3.5. Growth parameter
3.5.1. Shoot and root lengths

The shoot and root lengths of five plants collected from the replicate plots were measured and the mean values were expressed in cm.

3.5.2. Root nodulation

The number of root nodules in the root system was counted and expressed as individuals plant\(^{-1}\).

3.5.3. Plant biomass

The sampled plants were dried for 48 h in an incubator at 80°C, weighed and the biomass was expressed in g plant\(^{-1}\).
3.6. Yield

Plant yield was expressed as the mean pod length, number of grains per pod, weight of 1000 grains, number of pods per plant, grain yield per plant and contents of seed proteins, soluble carbohydrates and reducing sugars.

3.7. Analysis of biochemical constituents of leaves

The fourth unfolded leaf from the apex of each branch from all the five replicate plants were removed, washed in running tap, cut into small pieces, mixed well and used for biochemical analysis. Each time a known quantity of the sampled fresh leaves was dried for 48 h in an incubator to determine the dry weight. All the biochemical values except for chlorophylls were expressed on leaf dry weight basis. The values obtained were analyzed statistically by ANOVA and the means separated by Duncan’s multiple range test.

3.7.1. Chlorophylls (Arnon, 1949; Yoshida et al., 1976)

Five hundred mg of leaf bits were washed in water and homogenized in a mortar with excess of acetone and a pinch of calcium carbonate was added to prevent pheophytin formation. The homogenate was filtered through Whatman No.1 filter paper and the filtrate was made up to 25 ml with 80% acetone and read at 645 and 663 nm in a spectrophotometer.

Total chlorophyll =

\[
\left( (20.2 \times A_{645}) + (8.02 \times A_{663}) \right) \times \frac{50}{1000} \times \frac{100}{5} \times \frac{1}{2} \text{ mg g}^{-1} \text{ fresh weight}
\]

where, \( A \) = optical density
3.7.2. Proteins (Lowry et al., 1951)

500 mg washed fresh leaf bits / powdered seeds were homogenized in 10% trichloro acetic acid (TCA), squeezed through 4 layers of muslin cloth and centrifuged at 2000 x g for 20 min. After decanting the supernatant the residue was added with 10 ml of 0.1 N NaOH and the extract used for protein estimation.

0.5 ml of protein extract was added to 5 ml of alkaline copper solution and allowed to stand for 10 min at room temperature. To this 0.5 ml of diluted Folin phenol reagent was added, mixed thoroughly and allowed to stand for 30 min and read at 500 nm in a spectrophotometer. Alkaline copper solution was made fresh daily by mixing 0.5% CuSO₄·5H₂O in 1% potassium sodium tartrate with 50 ml of 2% Na₂CO₃ in 0.1 N NaOH. Bovine serum albumin was used as standard protein.

3.7.3. Estimation of soluble carbohydrates

One gram of leaf bits/powdered seeds were homogenized in a mortar and pestle with 5 ml of 80% ethanol and centrifuged at 5000 x g for 10 min. The supernatant was combined and reduced to 1-2 ml by evaporation.

3.7.3.1. Total soluble carbohydrates (Clegg, 1956)

Anthrone reagent was prepared by dissolving 1 g of anthrone in 500 ml of conc. H₂SO₄ and stored at 4°C. Fresh solution was prepared for every 2 days.

To a test tube kept in an ice bath, 1 ml of alcohol extract was pipetted out. Five ml of anthrone reagent was added to the tube allowing the reagent to run down to the side of the test tube and stirred slowly with a glass rod. Then, the tube was kept in a boiling water bath for exactly 7.5 min, cooled immediately in an ice bath and the blue colour
developed was read at 620 nm. The total soluble carbohydrate was calculated from a glucose standard.

3.7.3.2. Reducing sugars (Nelson, 1944)

A. Reagents

Copper reagent ‘A’

To 800 ml of distilled water, 25 g of anhydrous Na$_2$CO$_3$, 25 g of sodium potassium tartrate (Rochelle salt), 20 g of NaHCO$_3$ and 20 g of anhydrous Na$_2$SO$_4$ were dissolved and diluted to 1,000 ml with distilled water.

Copper reagent ‘B’

To 100 ml of distilled water, 15 g of CuSO$_4$ and 1 or 2 drops of conc. H$_2$SO$_4$ were added.

Arsenomolybdate colour reagent

To 450 ml of distilled water, 25 g of ammonium molybdate, 21 ml of conc. H$_2$SO$_4$ and 3 g of Na$_2$HAsO$_4$.7H$_2$O dissolved in 25 ml of distilled water were added and the mixture was kept in an incubator at 37°C for 48 h. The reagent was stored in a glass stoppered brown bottle.

B. Estimation

To 1 ml of alcohol extract in a test tube, 1 ml of the mixture of reagent ‘A’ and ‘B’ (prepared by mixing 25 parts of reagent ‘A’ with 1 part of reagent ‘B’) was added. The mixture was heated for 20 min on a boiling water bath, cooled in tap water and 1 ml of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25 ml with distilled water. Reagent blank contained 80% ethanol. The resulting blue colour was read at 720 nm. Reducing sugars of unknown were calculated from glucose standard.
3.8. Plant tissue nutrients (N, P and K)

Five gram dried and powered plant tissue was digested with nitric, perchloric and sulphuric acid mixture (10:4:1) till it became colourless. The digest was made up to 1 litre and used for the assay of P and K contents as described earlier for soil and effluent samples. For the assay of N content, the plant tissue was digested with perchloric and sulphuric acid mixture (4:1).

3.9. Estimation of soil microbial activity

3.9.1. Soil respiration (Isermeyer, 1952; modified by Jaggi, 1976)

A. Principle

Soil samples are incubated in a closed vessel at 25°C. The CO$_2$ produced is absorbed in sodium hydroxide and quantified by titration.

B. Reagents

Sodium hydroxide solution (0.05M)
Diluted hydrochloric acid (0.1M)
Barium chloride solution (0.5M)

Dissolve 10.4 g of BaCl$_2$ in distilled water and adjust the volume to 100 ml with distilled water in a volumetric flask.

Indicator solution

Dissolve 0.1 g of phenolphthalein in ethanol (60% v/v) and make up the volume to 100 ml with ethanol in a volumetric flask.

C. Procedure

Weigh 20-25 g of field-moist soil into three centrifuge tubes or test tubes (samples). Pipette 20 ml of sodium hydroxide solution into the laboratory bottles, and insert the tubes into the bottles. Close the bottles and incubate for 24 h at 25°C.
Remove the tubes, and add 2 ml of barium chloride solution to precipitate the absorbed CO$_2$ as barium carbonate. Add 3-4 drops of indicator solution, and titrate the remaining sodium hydroxide with diluted HCl.

To prepare the controls, perform the procedure without soil. Use at least three sample and control replicates.

**D. Calculation**

\[
\frac{(C-S) \times 2.2 \times 100}{SW \% \, dm} = mg \, CO_2 \, g^{-1} \, dm \, 24 \, h^{-1}
\]

- $C$ = mean volume of HCl consumed by controls (ml)
- $S$ = mean volume of HCl consumed by samples (ml)
- 2.2 = conversion factor (1 ml of 0.1 M HCl corresponds to 2.2 mg CO$_2$)
- SW = initial soil weight (g)
- 100.$\%$-$^1dm$ = factor for soil dry matter

**3.9.2. Enumeration of microbial population**

Dilution plate method was employed for the enumeration of microbial population in the soil samples. The soil samples were serially diluted in sterile water and 0.5 ml aliquots were dispensed aseptically into pre-sterilized petridishes. About 20 ml of molten, warm (ca. 45°C) medium was poured into each of these petridishes, the petridishes rotated gently so that the diluted sample is uniformly dispersed in the agar medium and incubated at room temperature in inverted position. A dilution of $10^{-3}$ was adapted for the enumeration of fungi where as $10^{-6}$ dilution was used for bacteria. The bacterial colonies appeared on the Nutrient Agar Medium
after 2 days incubation while the fungal colonies were counted on Potato Dextrose Agar Rose Bengal medium after 5 days incubation. The microbial populations were expressed as propagules per gram dry soil for fungi and colony forming units per gram dry soil for bacteria.

\[
\text{Microbial (fungal/bacterial) population} = \frac{\text{Mean number of colonies per plate}}{\text{Volume of diluted sample plated}} \times \text{Dilution factor}
\]

**Media composition**

**a. Potato Dextrose Agar – Rose Bengal Medium for fungi**
- Potato: 200 g
- Dextrose: 20 g
- Agar agar: 20 g
- Distilled water: 1000 ml
- 1% Rose bengal: 3 ml

**b. Nutrient Agar Medium for bacteria**
- Beef extract: 10 g
- Peptone: 10 g
- Sodium chloride: 5 g
- Agar agar: 20 g
- Distilled water: 1000 ml

The media were sterilized in an autoclave at 1.5 kg square cm⁻¹ pressure for 20 minutes.

**3.9.3. Assessment of arbuscular mycorrhizal (AM) status**

**3.9.3.1. Spore density**

Rhizosphere soil samples collected from pots as well as from A₁
layer of the study plots were used to determine AM spore density. The samples were stored at 4°C until subjected to wet sieving and decanting technique prescribed by Gerdemann and Nicholson (1963). One hundred gram soil sample was suspended in 500 ml distilled water and the soil particles were allowed to settle down for 5 min. The suspension was passed through 780 μm and μm sieves. The residues of 38 μm sieve were resuspended in water, transferred to a burette and left undisturbed for 3-5 min. The soil particles settled at the bottom were removed by opening the stopper. The spores in soil suspension were collected over gridded filter paper by passing the suspension over it. The inner surface of the burette was washed two to three times to collect the adhering spores. The filter papers were spread over glass plates and the spores were counted under the magnification of 100X. The population of spores was expressed as number of individuals 100 g⁻¹ dry soil.

3.9.3.2. Root colonization

The procedure described by Phillips and Hayman (1970) was used for the rapid assay of mycorrhizal colonization in plant roots. The feeder root segments were cut into 1 cm pieces and washed thoroughly in distilled water. The tissues were softened by boiling in 5% KOH at 90°C for 15 min to an hour depending upon the hardness of the material, then washed three to four times in distilled water, acidified with 5N HCl for 10 min and stained with 0.05% trypan blue (in lactophenol) for 15 to 30 min. The excess stain, if any, was removed by washing in lactophenol. Stained root bits were mounted on glass slides in lactophenol and examined under a low power compound microscope for the presence of AM infection structures such as arbuscules and vesicles along with hyphae. For each
plant species, at least one hundred root bits were examined and the extent of AM fungal colonization in roots was expressed as per cent root colonization.

Percentage root colonization =

\[
\frac{\text{Number of root bits showing structures of AM}}{\text{Total number of root bits examined}} \times 100
\]