APPENDICES

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Appendix 1 Calculation of the amount of nutrients to be added

Data:

Organic Carbon content in the soil = 17.46 gr/100gr

Total kg soil to be adjusted = 125 kg

Total Nitrogen in the soil = 0.22 gr/100gr

Total Phosphorous in the soil = 6.76 mg/kg

Calculation:

Organic C in the 125 kg of experiment = 17.46 gr/100gr x 125 kg x 1000 gr/kg

= 21829.17 gr

Total N in the 125 kg of experiment = 0.22 gr/100gr x 125 kg x 1000 gr/kg

= 275 gr

Total P in the 125 kg of experiment = 6.76 mg/kg x 125 kg

= 845 mg = 0.85 gr

Desired C:N:P = 100:10:1

When organic carbon = 21829.17 gr

So ratio C:N:P should be has in the soil = 21829.17 : 2182.9 : 218.3

P needed = 218.3 gr – 0.85 g = 217.45 gr P

P-source was Diammonium Phosphate (DAP), $(NH_3)_2HPO_4$

gr P/ gr DAP = 0.24

Amount DAP needed = 217.45 gr P : 0.24 gr P/gr DAP

= 906 gr DAP

N needed = 2182.9 gr – 275 gr = 1907.9 gr N

The amount of N supplied by DAP = 906 gr DAP x 0.215 gr N / gr DAP

= 194 gr N

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Primary N-source was Urea, $(CO(NH_2)_2$

gr N / gr Urea = 0.46

Urea needed = (1907.9 gr N – 194 gr N from DAP) : 0.46 gr N / gr Urea

= 3726 gr = 3.7 kg of Urea

Notes:

Percentage of N:P:K in Urea $(CO(NH_2)_2$ = 46:0:0

Percentage of N:P:K in Diammonium-phosphate $(NH_3)_2HPO_4$ = 22:24:0

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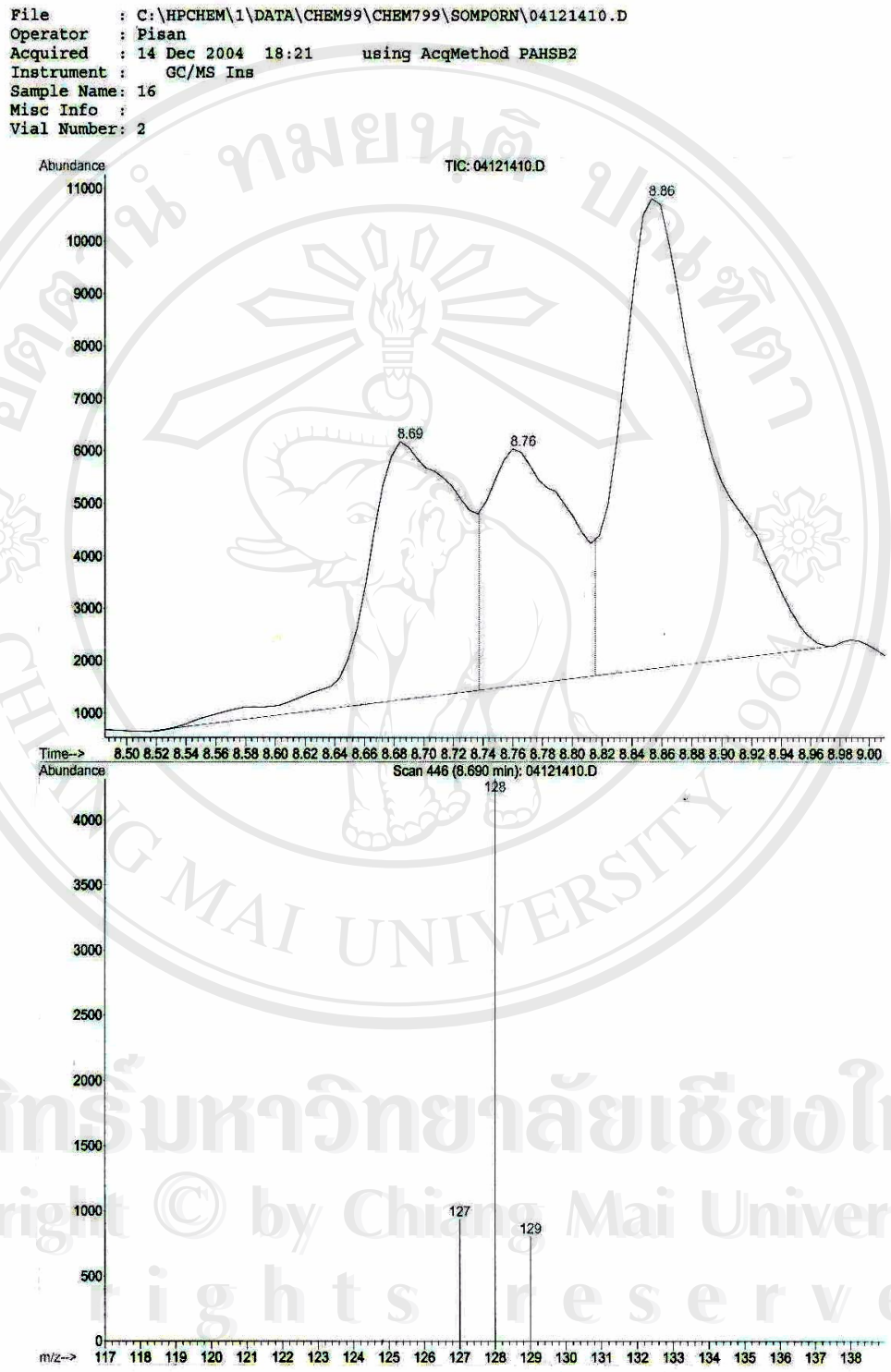
Appendix 2 Gas chromatogram of Naphthalene



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Appendix 3 Calculation of the air flow rate for treated-soil group

Data:

Soil weight = 25 kg

Total hydrocarbon contaminant = 106254 mg/kg

Naphthalene ($C_{10}H_8$) was dominant PAHs contaminant in the soil

The total quantity of reactants can be calculated from a balance reaction:

Half Reaction of electron donor ~ H_D
$1/Z\ (C_aH_bO_cN_d) + (2a-c)/Z\ (H_2O) = a/Z\ (CO_2) + d/Z\ (NH_3) + H^+ + e^-$ Where Z = 4a + b – 2c -3d : a,b,c and d represent the average number of atoms for C, H, O and N, respectively in organic contaminant

Half Reaction of Electron Acceptor ~ H_A
Aerobic : when oxygen is the electron acceptor: $¼\ O_2 + H^+ + e^- = ½\ H_2O$

Cell Synthesis Equation ~ C_S
When ammonia is the nitrogen Equation $¼\ CO_2 + 1/20\ NH_3 + H^+ + e^- = 1/20\ C_5H_7O_2N + 2/5\ H_2O$

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The overall reaction can be given is general term by:

$$H_D + f_eH_A + f_sC_S$$

Where H_D = half reaction for the organic compound oxidation, electron donor

H_A = half reaction for the electron donor

C_S = reaction that provides nutrients requirements for biomass synthesis

f_e = fraction of organic oxidized for energy and,

f_s = fraction associated with conversion to microbial cells

and: $f_e + f_s = 1$

for aerobic system, the f_s factor the energy distribution is found range between 0.12 and 0.6, and f_s was selected 0.12.

Calculation half reaction

Naphthalene $C_{10}H_8$ so $Z = 4a + b - 2c - 3d = 48$

Since f_s was selected 0.12 so $f_e = 0.88$

for H_D can calculated as following

$0.021C_{10}H_8 + 0.417H_2O = 0.208CO_2 + H^+ + e^-$

for f_eH_A can be calculated as following

$0.22O_2 + 0.88H^+ + 0.88e^- = 0.44\ H_2O$

for f_sC_S can be calculated as following

$0.03CO_2 + 0.006NH_3 + 0.12H^+ + 0.12e^- = 0.006C_5H_7O_2N + 0.05H_2O$

Overall reaction by summation half reaction and cell growth ($H_D + f_eH_A + f_sC_S$).

Left side:

$0.021C_{10}H_8 + 0.417H_2O + 0.22O_2 + 0.88H^+ + 0.88e^- + 0.03CO_2 + 0.006NH_3 + 0.12H^+ + 0.12e^-$

Right side:

$0.208CO_2 + 1H^+ + 1e^- + 0.44H_2O + 0.006C_5H_7O_2N + 0.05H_2O$

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And the stochiometric equation is

$$0.021C_{10}H_8 + 0.22O_2 + 0.006NH_3 = 0.178CO_2 + 0.07H_2O + 0.006C_5H_7O_2N$$

or

$$1C_{10}H_8 + 10.56O_2 + 0.288NH_3 = 8.56CO_2 + 3.42H_2O + 0.288C_5H_7O_2N$$

Molecular weight of Naphthalene : $C_{10}H_8$ = 128; O_2 = 32

Oxygen required for 1 mole Naphthalene is (32/128) x 10.56 = 2.64 kilogram

So: every kilogram of contaminant degraded will be required 2.64 kg of oxygen.

From soil weight = 25 kg and contaminant of hydrocarbon is 106254 mg/kg so the amount of hydrocarbon to be graded is 25 kg x 106254 mg/kg = 2.66 kg.

So amount of O_2 needed is

Oxygen = 2.64 x 2.66 kg = 7.01 kg

Calculation for aeration rate

The rate of degradation using the first-order :

$dL/dt = -kL$

$L_t = Le^{-kt}$

Where L = original concentration of organic compounds

L_t = concentration after degradation for time t in days

k = first-order degradation rate constant, unit per day

The half-lives for PAH degradation is solid-phase systems have been reviewed and the 95% percent confidence intervals establish for specific PAHs. Designed experiment will be conducted as long as 4 month or about 120 days so from that figure it known $L_t/L = 0.10$ and the t = 120 days.

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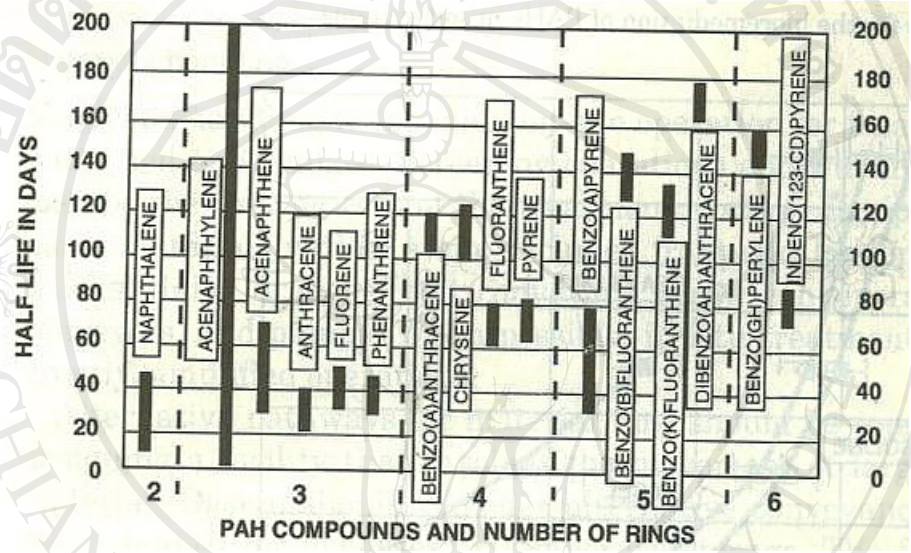
And k = ln(0.1)/-120 = 0.019 days

The rate of oxygen utilization is

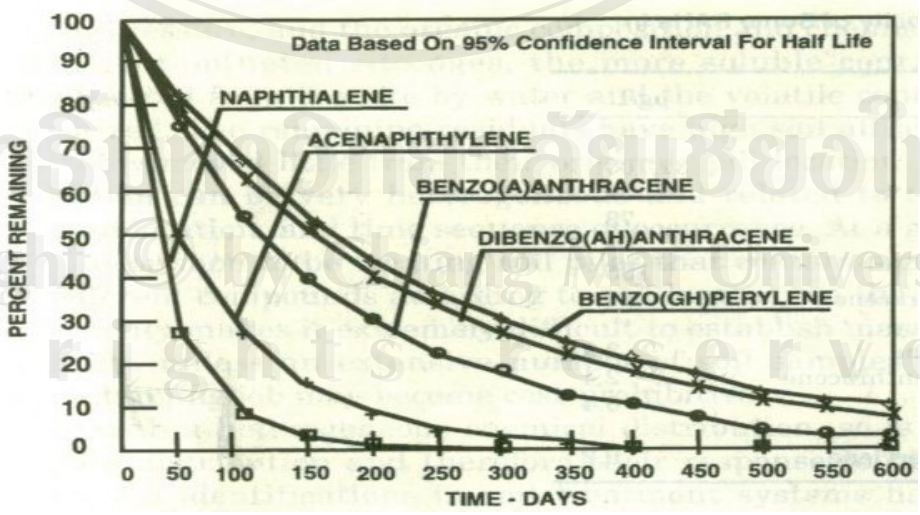
$$dN/dt = -k(R_N)L_t$$

where *dN/dt* = kilogram of oxygen required day

R_N = mass ratio of the oxygen



Picture Half-lives 95 percent confidence interval for polynuclear aromatic hydrocarbon. Source : Cookson, (1995).



Picture: Rates for the bioremediation of PAHs insoil system

Source: Cookson, (1995).

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To calculate the rate of oxygen so the amount of oxygen demand at the midpoint of every day period is established (Cookson 1995).

Table. Estimation of the air will be delivered to the tank

Daily period (1)	t, days (2)	-kt (3)	PAH fraction remaing, Lt/L (4)	Total remaining oxygen requirement (kg) (5)	Maximum rate of oxygen update (kg/day) (6)
0	0			7.01	
1	1	-0.02	0.98	6.88	0.35
2	5	-0.10	0.91	6.37	0.32
3	6	-0.12	0.89	6.25	0.32
4	7	-0.13	0.87	6.13	0.31
5	8	-0.15	0.86	6.01	0.30
6	9	-0.17	0.84	5.90	0.30
7	10	-0.19	0.83	5.79	0.29
8	11	-0.21	0.81	5.68	0.29
9	12	-0.23	0.79	5.57	0.28
10	13	-0.25	0.78	5.46	0.28

Note: The maximum rate of oxygen update shown just for 10 daily period

From the above table can be known the amount of oxygen have to delivered 0.35 kg/day and air volume can be calculated as following:

Average air pressure and temperature yearly in Chiang Mai = 1008.8 mb and 25.7^{0}C

So : Average air density in Chiang Mai = 1.175 kg/m^3 (the calculation may be saw at the below).

Since percentage O_2 = 21%

So: Volume of air needed = (0.35 kg/day) / (1.175 kg/m^3 x 0.21)

= 0.143 m^3/day = 0.059 m^3/hour

Pump was turn on for 2 hour per day during experiment

so volume air was required : 0.059 m^3/hour x 24 hour/2 hour = 0.713 m^3/hour = 11.8 l/min.

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Air Density Calculations:

From law of the ideal gas:

$$P*V = n*R*T$$

where: P = pressure

V = volume

n = number of moles

R = gas constant

T = temperature

Density is simply the number of molecules of the ideal gas in a certain volume, in this case a molar volume, which may be mathematically expressed as:

$$D = n / V$$

where: D = density

n = number of molecules

V = volume

Then, by combining the previous two equations, the expression for the density becomes:

$$D = \frac{P}{R * T}$$

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where: D = density, kg/m^3

P = pressure, Pascals (multiply mb by 100 to get Pascals)

R = gas constant , J/(kg*degK) = 287.05 for dry air

T = temperature, degK = deg C + 273.15

Based on Chiang Mai City Meteorological the yearly in 2002 air pressure and temperature average is 1008.8 mb and 25.7^{0}C, respectively. So air density average is

$$D = \frac{100880}{287.05 * (25.7 + 273.15)} = 1.175 \text{ kg/ m}^3$$

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Appendix 4 Physical, Chemical and total heterotrophic microbiological determination

Determination of soil porosity

1. Measuring water content by placing the amount of sample to oven.
2. Dried the sample with temperature 105^0C for 24 hour or until the sample shown stable weight.
3. Weighted the sample
4. Calculate the soil porosity by this formula:

$$n = \frac{Vv}{Vt}$$

where n = porosity; may be expressed either as a decimal or as a percentage

Vv = volume of voids in a soil mass = Vw + Va

Vt = total volume of a soil mass = Vs + Vw + Va

Vs = volume of soil solids in a soil mass

Vw = weight of water present in a soil mass

Va = volume of air present in a soil

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Measurement of soil pH

Equipments and Reagents:

1. pH meter equipped with glass (indicating and reference electrode)
2. Automatic pipette
3. Paper cup, 28 g (1 oz)
4. Standard buffers, pH 7 and 4
5. Distilled water

Procedure:

1. weigh or measure 20 g of air-dry soil into a 28-g paper cup.
2. With automatic pipette, add 20 ml of distilled water to each cup
3. Stir thoroughly for 1 min
4. Let stand for 10 min.
5. Insert the electrodes into the container, and stir the soil suspension by swirling the electrodes slightly. Protect the electrodes with a short glass rod attached to the electrode holder and extended just below the tips of the electrodes.
6. Read the pH immediately on the standardized pH meter. Record as soil in water, or pH_w

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Determination of soil texture

Apparatus and Reagents:

1. Standard hydrometer
2. Thermometer
3. Electric stirrer
4. Parafilm
5. Sedimentation cylinder with 1-L mark
6. Metal dispersing cups and 600-ml beakers
7. Sodium-hexametaphosphate (HMP) solution 50g/L
8. Set of sieves wit following openings 1000, 500, 250, 106, 75 and 53 μm
9. electric oven and weighing jars

Procedure:

1. Weighing 40 g of soil into 600 beaker, add 250 ml of distilled water and 100 ml HMP and allow the sample to soak over night.
2. Transfer the HMP-treated sample to dispersing cup and mix for 5 min with the electric mixer.
3. Transfer the suspension to a sedimentation cylinder and add distilled water to bring the volume to 1 L
4. After mixing is completed, lower the hydrometer into the suspension and take readings after 30 s, 1 min, 1.5 min and the end of 2 hrs.
5. Determine soil texture by using USDA classification scheme

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Measurement of soil moisture content

Place sample of 1 to 100 g of soil in metal cans with tight-fitting lids. Weigh (the balancing was AND HR 200 made in Japan series 12304150 max. 210 g, d=0.1 mg) the samples immediately, or store them in such a way that evaporation is negligible. Place the sample in a drying oven (the oven was Melag made in Germany series 401) with the lid off, and dry it to constant weight. Remove the sample from the oven, replace the cover, and place it in a desiccator containing active desiccant e.g. magnesium perchlorate or calcium sulfate) until cool. Weight it again, and also determine the tare weight of the sample container. Compute the water content by one of the following formulas:

$$\theta_{dw} = \frac{(\text{weight of wet soil + tare}) - (\text{weight of dry soil + tare})}{(\text{weight of dry soil + tare}) - (\text{tare})}$$

$$= \frac{(\text{weight of wet soil + tare}) - (\text{tare})}{(\text{weight of dry soil + tare}) - (\text{tare})} - 1$$

$$= \frac{(\text{weight of wet soil })}{(\text{weight of dry soil})} - 1$$

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Measurement of organic carbon

Reagents:

1. Potassium dichromate ($K_2Cr_2O_7$), 1N.

2. Sulfuric acid (H_2SO_4)

3. 4. o-phenanthroline-ferrous complex, 0.025 M

5. Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) solution, 0.2N

Procedure:

Grind the soil to pass through a 100 mesh sieve, avoiding Fe or steel mortars. Transfer a weighed sample, containing 10 to 25 mg of organic C, but not in excess of 10 g of soil, into a digestion tube. Add 5 ml of 1N $K_2Cr_2O_7$, and swirl the flask gently to disperse the soil in the solution. Then rapidly add 7.5 ml of conc. H_2SO_4, warm at 150^0C for about 30 min. then add 200 ml of water to the flask, and filter the suspension if experience shows that the endpoint of the titration cannot otherwise be clearly discerned. Add 3 to 4 drops of o-phenanthroline indicator, and titrate the solution with 0.2N $FeSO_4$. As the endpoint is approached, the solution takes on a greenish cast and then changes to a dark green. At this point, add the ferrous sulfate heptahydrate drop by drop until the color changes sharply from blue to red (maroon color in reflected light against a white background). Make a blank determination in the same manner, but without soil, to standardize the $Cr_2O_7^{2-}$. Repeat the determination with less soil if > 75% of the dichromate is reduced.

Calculate the results according to the following formula:

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$$\%\,organic\,C = \frac{(A)x\left[N\ of\ Fe(NH4)2.6H20\right]x(0.003)\,x(100)}{g\ oven-dry\ soil}$$

$$A = (mlBB - mlsample)\,x\left(\frac{mlUB - mlBB}{mlUB}\right) + (mlBB - mlsample)$$

where: UB = unboiled blank

BB = boiled blank

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Determination of total nitrogen

Apparatus:

1. Micro-Kjedahl flask 100 ml
2. Micro-Kjedahl digestion stand
3. Büchi 322/342 steam distillation apparatus

Reagents:

1. Potassium sulfate-catalyst $CuSo_4.5H_2O$ Se black powder mixture ratio 100:10:1
2. Sulfuric acid (H_2SO_4), concentrated
3. Sodium hydroxide (NaOH) solution , approximately 10N
4. Boric acid-indicator solution
5. Sulfuric acid, 0.02N standard

Procedure

Place sample of soil containing about 0.1-0.5 mg of N in a micro-Kjedahl digestion flask, add 2.2 g of K_2SO_4-catalyst mixture and 6 ml of conc. H_2SO_4 and heat the flask cautiously on the digestion stand. Increased the heat continuously starting from the first temperature 150^0C for 30 min., 256^0C for 30 min., 345^0C for 120 min. and finally 370^0C for 180 min. Regulate the heating during this boiling so that the H_2SO_4 condenses about one third of the way up the neck of the digestion flask.

After completion of digestion, allow the flask to cool, and add about 150 ml of water (slowly, and with shaking). Then swirling the flask to bring any insoluble material into suspension, and transfer the contents to the distillation chamber of the

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Büchi apparatus via the funnel of the apparatus. Rinse the Kjedahl flask four times with a total of about 15 ml of water to complete the transfer. Add enough water to the distillation chamber to bring the level of the solution to a mark made previously to indicate a volume of 250 ml, and close the stopcock connecting the funnel and distillation chamber. Add 15 ml of H_3BO_3-indicator solution to a 250 ml beaker, and place the flask under the condenser of the distillation apparatus so that the end of the condenser is deeply in H_3BO_3 solution. Then add 20 ml of 10N NaOH to the funnel of the apparatus, and run the alkali slowly into the distillation chamber by opening the funnel stopcock. When about 1 ml of alkali remains in the funnel, rinse the funnel rapidly with about 15 ml of water. Allow enough of this water to run into the distillation chamber to bring the level of the solution to a mark made previously to indicate a volume of 80 ml. Then close the funnel stopcock, and immediately commence distillation by closing the steam by pass tube at the base of the steam jacket of the distillation chamber. (It is not necessary to interrupt the flow of steam to the steam jacket of the distillation chamber before addition of the digest and alkali, but the bypass tube of the steam jacket must be kept open during these addition). When the distillate reaches the 150-200-ml mark on the receiver beaker, stop the distillation by opening the steam bypass tube, rinse the end of the condenser, and determine NH_4^+-N in the distillate by titration with 0.02 M H_2SO_4 (1 ml of 0.01N H_2SO_4 = 0.14 mg of NH_4^+--N). The color change at the endpoint is from green to pink.

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Determination of phosphorous-availability Indices

Reagents:

1. Ammonium fluoride (NH_4F), 1N
2. Hydrochloric acid (HCL), 0.5N
3. Extracting solution: add 15 ml of 1.0N Ammonium fluoride (NH_4F) and 25 ml of 0.5N Hydrochloric acid (HCL) to 460 ml of distilled water. This gives a solution 0.03N in NH_4F and 0.025N in HCl.
4. Stannous chloride dyhydrate ($SnCl_2.2H_2O$) stock solution
5. Ammonium paramolybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$]
6. Stannous chloride ($SnCl_2$), dilute solution
7. Standard phosphate solution

Procedure:

Weigh 1 g soil into an extraction bottle or tube, and add 7 ml of the extracting solution. Shake the container 1 min, and filter the contents through Whatman no. 42 paper. If the filtrate is not clear, pour the solution back through the filter. To 2 ml of the filtrate, add 5 ml of distillate water. Add 2 ml of the ammonium paramolybdate solution, and 1 ml Stannous chloride and mix the contents well. After 5 or 6 min and before 20 min, measure the color photometrically using 660-nm incident light.

Prepare a standard curve including the 2 ml of extracting solution in the range of 0.1 to 1 μg of P/ml. Plot the transmittances of the standards against the micrograms of P per milliliter on semilogarithmic graph paper.

Calculate the concentration of extratable P as follows:

ppm of P in soil = ppm of P in solution x 35

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Media and enumeration of total heterotrophic bacterial

Material and Equipment

1. Autoclave and oven
2. Balance
3. Medical bottle
4. Flask glass
5. Dilution bottles
6. Mechanical shaker
7. Vortex mixer
8. Pipettes
9. Water bath
10. Glass spreaders (“hockey sticks”):
11. Petri plates
12. Media (see sec. media.)
13. Diluent (see sec. diluent)

Sterilize all equipment in sterilizer

Media

Media was PTYG (peptone tryptone yeast extract glucose agar) medium

PTYG Medium

peptone	: 5.0 g
tryptone	: 5.0 g
yeast extract	: 10.0 g

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glucose : 10.0 g

$MgSO_4.7H_2O$: 0.6 g

$CaCl_2.2H_2O$: 0.07 g

agar : 15.0 g

distilled water : 1000 ml

Diluent

Diluents used is peptone-water

Preparing Peptone-water:

Dissolve 1.0 g of peptone in 1000 ml of distilled water in a large flask, mix and autoclave the suspension for 30 min to 1 hr.

Procedure of Preparing of Sample Solution

1. Prepare solution of 90 ml of peptone water for sample solution in flask glass. The number of sample solution is 5 (4 is for treatment and 1 is preservation).
2. Weigh out a 10 gr soil sample and put to 90 ml peptone water in flask glass containing 20 to 40 3-mm glass beads.
3. Shake the bottle vigorously by hand for 30 to 60 sand then palce it on a shaker for 20 min. at 150 to 200rpm. This solution is 10^{-1} dilution.

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Procedure of Media Preparation

1. Weighing each ingredient of media and mix well then put in the flask glass.
2. Autoclave media for 15 minute at 121^0C and then cooled at 45^0C.

Procedure of Dilution Solution

1. Prepare 80 ml of peptone water in medical bottle for ten fold dilution. The number of bottle of the solution is 5 (4 is for treatment and 1 is preservation).
2. Prepare 6 of dilution bottle then Pipette 9 ml of peptone water from medical bottle to dilution bottle.

Procedure Ten-fold Dilution

1. Prepare 4 number of plates and pour the PTYG agar to it. Allow them to “dry” prior to using them in the enumeration procedure.
2. Label the plates for each sample. Label the bottom half of the dish and include sample ID, dilution, date and medium.
3. Preparing 10^{-2} dilution

   Using the sterile pipette 9 ml of peptone water and 1 ml of sample solution, resulting 10^{-2} dilution of sample.
4. Preparing 10^{-3} dilution

   Using a sterile 1-ml pipette, remove 1 ml from the middle region of the suspension (10^{-2}) and transfer it to a 9-ml blank to achieve the 10^{-3} dilution of sample.
5. Preparing 10^{-4} dilution

   Using a sterile 1-ml pipette, remove 1 ml from the middle region of the suspension (10^{-3}) and transfer it to a 9-ml blank to achieve the 10^{-4} dilution of sample.
6. For preparing 10^{-5} dilution, 10^{-6} dilution, 10^{-7} dilution repeat these steps as procedure above to prepare ten fold dilution.

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7. When the dilution series is completed, pipette 0.1 ml of 10^{-4} dilution to petri plate
8. Repeat again the procedure for preparing 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.
9. Once the inocula have been delivered to the plate, use a sterile or freshly flamed glass spreader and spread the inoculum over the agar surface by moving the spreader back and forth while rotating the plate with a manual for approximately 15 second.

   Flame the spreader between successive dilutions. If after spreading the plates, some liquid remains visible on the agar surface, allow the plates stand right side up until the moisture is absorbed.
10. Afterward, invert the plates for incubation at 25 to 28^0C.
11. Count the colonies on each plate containing between 30 and 300 colonies. Count the colonies (use the a colony counter, hand lens, or dissection microscope if necessary) and calculate the number of bacteria present. Average the numbers of colonies appearing on the plates and multiply the mean colony number by the reciprocal of the dilution. Examples, if an average of 150 colonies was observed on plates from the 10-6 dilution, the count would be expressed as 1.5 x 108 colony forming units (CFU). This figure must be adjusted to a soil dry weight basis.

    Soil water content is calculated as follows:

$$\%H_2O = \frac{\text{wet wt.} - \text{dry wt.}}{\text{oven - dry wt. of soil}} x100$$

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Measurement of total petroleum hydrocarbon (TPH)

Sample extraction:

1. Thoroughly homogenize the sample and if necessary, reduce the particle size of the sample, to less than 1mm.
2. Weigh 10 g of sample in a tared beaker and record the weight to $\pm$ 0.1 g. Add 10 g of anhydrous sodium sulfate to form a free-flowing mixture. Smaller sample aliquots may be used for samples with high concentrations of hydrocarbons.
3. Place the mixture in an extraction thimble. An acceptable alternative to the thimble is a pair of glass wool plugs which are placed above and below the sample in the soxhlet extractor.
4. Transfer 300 ml of fluorocarbon-113 to a 500 ml round bottom flask. Place one or two clean boiling chips in the flask. Assemble the extraction apparatus, place it on a water bath at approximately 600C, and adjust the temperature so that the solvent boils vigorously. Continue the extraction for 16 to 24 hours.
5. Cool the extract to ambient temperature.
6. Dry the extract by passing it through a 10 cm column of sodium sulfate. Collect the dried extract in a distillation flask. Wash the extractor flask and drier column with 100 to 125 ml of pure fluorocarbon-113 and combine the wash solution with the dried extract.

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Sample concentration:

1. Assemble the flask which contains the dried extract, two clean boiling chips, and a three ball macro Snyder column. Prewet the Snyder column with approximately

80 ml is completed in 10 to 20 minutes.

2. Remove the Snyder column and rinse its inside surface with a few ml of fluorocarbon-113. Combine the wash with the concentrated extract. Transfer the extract to a 100 ml volumetric flask. Wash the inside surface of the boiling flask with small quantities of fluorocarbon-113 and combine the wash solution with the concentrate. Adjust the volume of the concentrate to 100 ml.

Sample analysis :

The sample is ready for analyses by EPA Method 418.1 (TPH by IR) or EPA method 413.2 (oil and grease by IR)

Calculation :

1. Record the initial weight of the sample and the final extract volume for calculating the TPH or oil and grease concentration.

2. $$\%moisture = \frac{(\text{wt. of sample}) - (\text{wt. of dry sample}) \times 100}{(\text{wt. of sample})}$$

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Analyses of Total Petroleum Hydrocarbon (TPH)

Reagents

1. TCTFE (1,1,2-trichloro-1,2,2-trifluoroethane), reagent grade, boiling point 48^0C.
2. Sodium sulfate, Na_2SO_4, anhydrous crystal.
3. Wesson or other pure cooking oil for preparation of spikes

4. Hydrochloric acid 1:1 HCl; Mix equal volumes of concentrated HCl and ASTM Type II water.

Sample preparation:

Mark the meniscus on the sample bottle for later determination of sample volume. If the sample was nor previously acidified, add 5 ml of 1:1 HCl to the sample and mix. Check the pH with pH paper to insure that the pH is less than 2. If necessary, add more 1:1 HCl and note on the oil and grease log that the sample pH was adjusted.

Sample analyses

1. Set the hot plate temperature or water bath temperature at 70^0C.
2. Pour the entire acidified sample into a 2 L separatory funnel. Since oil and grease sometimes separate from the water, an accurate result depends on an analytical transfer of the entire sample. The method recommends that the entire contents of the sampling container be analyzed (i.e. 1 L of sample). If the sample extremely oily, then it is not possible to analyze the entire sample. Therefore, the sample should be shaken vigorously and an aliquot taken immediately in order to prevent separation of oil and grease from the water.
3. Weigh the dried, cooled flask to the nearest mg and record the empty weight and ID for each flask.
4. Rinse the internal walls of the sample bottle with 30 ml of TCTFE. Transfer the wash to the separatory funnel. Shake it vigoursly for 2 minutes while intermittently venting the funnel. This procedure is more safely accomplished in a ventilated hood. Eye protection should be worn throughout the procedure. Allow

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the separatory funnel to stand until the nonmiscible layers separate. Filter the solvent layer into the weighed flask through a funnel fitted with filter paper pre-wetted with solvent containing 1 g Na2SO4.

5. Repeat the extraction procedure twice more with fresh TCTFE solvent. Combine the solvent washes for each sample into the one labeled flask dedicated to that specific sample. Rinse the filter paper with 10-20 ml of TCTFE solvent and add the wash to the dedicated flask.
6. Evaporate all of the solvent by heating the flask in a water bath or on a hot plate at 70^0C. Cool the flasks in a desiccator. Weigh them to the nearest mg and record the combined weight of each flask and its residue. Substract the weight of each empty flask (initial weight) from the weight of the combined flask and residue (final weight). Record the difference in weight as the “residue weight”.
7. For each sample, refill the sample bottle to the marked lline with water, then the water to a graduated cylinder in order to measure the sample volume.

Calculation

$$Oil\ and\ grease\,(mg/L\ or\ mg/kg) = \frac{(A-B)-C}{D}\ x1{,}000{,}000$$

where:

A = final weight of the extraction flask and residue in grams

B = initial weight of the empty extraction flask in grams

C = weight of solvent balnk in grams

D = volume of sample extracted in mL (liquids) or g (solids).

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Appendix 5 Temporal variation of soil temperature during biopile experiment (^{0}C)

	1/17	1/20	2/1	2/3	2/4	2/5	2/6	2/7	2/8	2/9	2/10	2/12	2/14	2/16	2/17	2/18	2/19	2/19	2/20	2/24	2/25	2/26
Tank 1	29.5	28	29	30	30	29.5	28	26	27	29	30	26	27.5	29	27	28	27	28	27	33	33	29
Tank 2	27	25	26	27	28	27	25	24	25	26.5	28	25	27	27	25	27	26	26	25	29.5	29	25
Tank 3	27	26	26	27	27.5	27	26	24	25	26.5	28	24	26.5	28.5	26	26.5	25	26	25	29	29	25
Tank 4	28.5	26	28	28	28.5	28.5	26	24.5	26	27	29	25	27	28	26	27	26	25	26	30	30	26
Tank 5	27	26	26	26.5	27	27	25.5	23	24.5	26	27	24	26	27.5	24.5	25	24.5	26	25	29	28	24.5
Tank 6	27	26	26.5	25	28	27	26	24	25	26.5	29	24.5	26.5	28	26.5	27	25	24	25.5	30.5	29	24

	2/27	3/10	3/12	3/14	3/15	3/17	3/19	3/20	3/22	3/23	3/25	3/28	3/30	4/1	4/6	4/8	4/9	4/14	4/19	4/22	4/23	4/25
Tank 1	30	29	29	31.5	30	32	32	31	32.5	32	32	31.5	27.5	34	31.5	34	35	31.5	29	29	34	33
Tank 2	26	27	27.5	27	26	27	28.5	28	28	28	28	28	25.5	30	28	29	30	29	27	27	30	30
Tank 3	26	27	27	26.5	26	27.5	28.5	28	28	27.5	28	28	25	29	28	29	30	29	27	27	30	30
Tank 4	27	29	28	27.5	27	28.5	29.5	29	29	29	29.5	29	27	30	29	30.5	31	30	28	28	31	31
Tank 5	25.5	28	27	26	25	27	28	27.5	27.5	27	28	27	26	29	27	29	30	28	26	25.5	29	29
Tank 6	26	28	28	27	26.5	28	29	29	29	27.5	29	28	26	30	28.5	30	31	29.5	27	26.5	30	30

	4/26	4/27	4/28	4/29	4/30	5/1	5/2	5/4	5/5	5/7	5/9	5/10	5/11	5/13	5/15	5/17	5/18	5/19	5/20	5/22	5/24	5/26
Tank 1	33.5	35	32.5	34	34	32.5	30	34	33	29.5	30	31	31	30	32	33	33	33	32	31	32.5	31
Tank 2	30	32	30	31.5	30	30	26	30.5	30	28.5	29	28.5	29	28	30	31	31	31	30	30	31	30
Tank 3	30	31	30	31	30	30	26	30.5	30	28.5	29	28	29	28	30	31	31	31	30	29.5	31	30
Tank 4	30.5	32	30.5	32	30	31	27	31	31	29	30	29.5	30	29	31	32	32	32	31	30.5	32	30
Tank 5	29	31	29.5	31	29.5	29	25	30	29	27	28	28	29	27	29	30	30	30.5	29.5	29	30	29
Tank 6	30	31.5	30	32	30	30	26	31	30	28	29	29	30	28.5	30	31	31	32	30.5	30	31	28.5

Note : The experiment was done in 2005 years and for 17 weeks

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Appendix 6 Temporal variation of moisture content (%) during biopile experiment

	17-Jan-05	10-Feb-05	12-Feb-05	21-Feb-05	12-Mar-05	18-Mar-05	25-Mar-05	31-Mar-05
Control / Tank 1	19.9	14.7	14.9	14.6	6.6	6.8	6.0	10.1
Tank 2	21.1	14.5	17.1	17.1	11.1	9.3	8.9	9.7
Tank 3	21.5	17.7	17.0	16.3	10.9	9.3	8.2	9.1
Tank 4	21.6	16.4	17.4	15.8	9.2	8.0	9.1	7.7
Tank 5	20.6	20.3	15.5	15.6	11.7	9.2	8.5	9.1
Tank 6	20.0	23.7	19.3	16.2	10.1	8.8	9.7	8.1

	4-Apr-05	7-Apr-05	19-Apr-05	22-Apr-05	28-Apr-05	30-Apr-05	3-May-05	5-May-05
Control / Tank 1	5.6	5.21	2.16	4.06	3.16	2.75	1.94	2.12
Tank 2	7.3	6.51	4.15	5.82	2.74	2.68	3.32	3.05
Tank 3	6.7	5.62	4.81	3.77	2.37	2.09	3.03	2.6
Tank 4	6.7	5.95	3.19	3.66	2.53	2.38	3.26	2.83
Tank 5	7.0	6.24	4.94	5.38	3.49	3.61	4	4.16
Tank 6	6.1	6.16	5.08	6.15	3.81	3.19	4.42	4.11

	7-May-05	9-May-05	12-May-05	14-May-05	16-May-05	19-May-05	25-May-05
Control / Tank 1	2.49	5.6	3.6	3.1	3.7	2.7	2.4
Tank 2	2.91	3.2	3.0	2.6	2.4	2.5	3.0
Tank 3	2.8	3.2	2.8	2.4	2.5	2.2	2.5
Tank 4	2.83	3.2	4.0	2.4	3.6	2.2	2.7
Tank 5	3.14	5.0	4.1	3.5	2.6	3.4	3.7
Tank 6	3.94	4.9	3.1	3.9	3.6	3.2	3.8

Note: The experiment was done for 17 weeks

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Appendix 7 Temporal variation of heterotrophic microbial number during biopile experiment (CFU/g of dry soil)

	17-Jan-05	10-Feb-05	12-Feb-05	15-Feb-05	18-Feb-05	21-Feb-05	4-Mar-05	8-Mar-05	13-Mar-05	19-Mar-05
Tank 1	5.6E+06	4.9E+07	1.8E+07	2.7E+08	5.5E+08	4.4E+08	6.6E+08	1.7E+08	1.7E+08	1.3E+08
Tank 2	5.5E+06	6.1E+06	5.5E+06	4.3E+06	4.9E+06	4.0E+06	4.9E+06	2.3E+06	2.1E+06	2.1E+06
Tank 3	6.2E+06	5.0E+06	4.1E+06	4.4E+06	4.3E+06	3.8E+06	4.7E+06	3.2E+06	2.6E+06	1.8E+06
Tank 4	6.0E+06	6.4E+06	4.8E+06	4.8E+06	4.7E+06	1.4E+07	4.5E+06	2.6E+06	2.1E+06	1.9E+06
Tank 5	8.0E+06	6.4E+06	7.4E+06	4.5E+06	4.1E+06	5.1E+06	4.9E+06	2.7E+06	2.5E+06	1.6E+06
Tank 6	9.0E+06	5.4E+06	5.5E+06	4.7E+06	4.8E+06	2.4E+06	4.9E+06	1.8E+06	2.3E+06	2.0E+06

	23-Mar-05	1-Apr-05	5-Apr-05	8-Apr-05	19-Apr-05	22-Apr-05	29-Apr-05	1-May-05	4-May-05	6-May-05
Tank 1	2.0E+08	1.8E+08	1.6E+08	1.0E+08	1.6E+07	1.6E+07	3.6E+07	1.9E+07	1.5E+07	1.8E+07
Tank 2	2.7E+06	2.5E+06	2.1E+06	2.5E+06	2.3E+06	2.4E+06	1.9E+06	1.8E+06	1.5E+06	1.3E+06
Tank 3	2.1E+06	2.0E+06	2.0E+06	2.5E+06	2.0E+06	2.0E+06	1.9E+06	1.2E+06	1.9E+06	6.3E+05
Tank 4	1.8E+07	2.1E+07	1.6E+06	7.2E+06	1.5E+06	1.5E+06	1.5E+06	8.4E+05	1.4E+06	1.2E+06
Tank 5	1.7E+06	8.0E+06	2.1E+06	1.2E+07	1.8E+06	1.8E+06	1.5E+06	1.3E+06	1.9E+06	1.4E+06
Tank 6	2.8E+06	2.8E+06	1.3E+06	6.4E+06	2.1E+06	2.1E+06	1.3E+06	1.4E+06	1.5E+06	1.3E+06

	8-May-05	10-May-05	13-May-05	15-May-05	17-May-05	20-May-05	22-May-05	25-May-05
Tank 1	1.2E+07	3.1E+07	4.4E+07	6.2E+07	2.5E+07	1.2E+07	1.7E+07	1.3E+07
Tank 2	1.3E+06	1.1E+06	1.3E+06	1.3E+06	1.2E+06	7.4E+05	4.9E+05	1.0E+06
Tank 3	1.1E+06	8.2E+05	9.4E+05	5.3E+05	1.0E+06	5.0E+05	4.7E+05	7.1E+05
Tank 4	1.0E+06	9.7E+05	7.5E+05	5.7E+05	8.8E+05	4.0E+05	4.7E+05	9.7E+05
Tank 5	1.2E+06	1.0E+06	1.5E+06	1.2E+06	1.4E+06	5.6E+05	5.3E+05	1.8E+06
Tank 6	1.3E+06	1.2E+06	8.9E+05	9.7E+05	9.2E+05	5.5E+05	7.7E+05	1.3E+06

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Appendix 8 Analyses of variances (ANOVA) for soil pH, soil temperature, soil nutrients, soil moisture, total heterotrophic bacterial and TPH

ANOVA of soil pH

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Variable PH
By Variable TREATMEN

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	5	1.5294	.3059	705.8769	.0000
Within Groups	6	.0026	.0004		
Total	11	1.5320			

Multiple Range Tests: LSD test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle

Mean	TREATMEN	G G G G G G r r r r r r p p p p p p 1 2 3 4 5 6
6.0150	Grp 1	
6.8550	Grp 2	*
6.9200	Grp 3	* *
6.9350	Grp 4	* *
7.0350	Grp 5	* * * *
7.0400	Grp 6	* * * *

Homogeneous Subsets (highest and lowest means are not significantly different)

Subset 1
Group Grp 1
Mean 6.0150

Subset 2
Group Grp 2
Mean 6.8550

Subset 3
Group Grp 3 Grp 4
Mean 6.9200 6.9350

Subset 4
Group Grp 5 Grp 6
Mean 7.0350 7.0400

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ANOVA of soil temperature

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- - - - - O N E W A Y - - - - -

Variable TEMP
By Variable TREATMEN

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	5	7.7500	1.5500	12.4000	.0041
Within Groups	6	.7500	.1250		
Total	11	8.5000			

Multiple Range Tests: LSD test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle

Mean	TREATMEN	G G G G G G r r r r r r p p p p p p	
		6 5 2 3 4 1	
28.2500	Grp 6		
28.7500	Grp 5		
29.7500	Grp 2	* *	
29.7500	Grp 3	* *	
29.7500	Grp 4	* *	
30.7500	Grp 1	* * * * *	

Homogeneous Subsets (highest and lowest means are not significantly different)

Subset 1

Group	Grp 6	Grp 5
Mean	28.2500	28.7500

Subset 2

Group	Grp 2	Grp 3	Grp 4
Mean	29.7500	29.7500	29.7500

Subset 3

Group	Grp 1
Mean	30.7500

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ANOVA of soil nutrient

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Variable PHOSP
By Variable TREATMEN

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	5	126295.0468	25259.0094	20.4196	.0011
Within Groups	6	7421.9845	1236.9974		
Total	11	133717.0313			

Multiple Range Tests: LSD test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle

Mean	TREATMEN	G G G G G G r r r r r r p p p p p p 1 6 3 5 4 2
31.7150	Grp 1	
192.9500	Grp 6	*
204.9750	Grp 3	*
241.5850	Grp 5	*
269.2650	Grp 4	*
374.4350	Grp 2	* * * * *

Homogeneous Subsets (highest and lowest means are not significantly different)

Subset 1
Group Grp 1
Mean 31.7150

Subset 2
Group Grp 6 Grp 3 Grp 5 Grp 4
Mean 192.9500 204.9750 241.5850 269.2650

Subset 3
Group Grp 2
Mean 374.4350

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Variable NITROGEN
By Variable TREATMEN

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	5	1.7073	.3415	45.7835	.0001
Within Groups	6	.0447	.0075		
Total	11	1.7521			

Multiple Range Tests: LSD test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle

Mean	TREATMEN	G G G G G G r r r r r p p p p p 1 4 3 5 6 2
.2950	Grp 1	
1.1850	Grp 4	*
1.1900	Grp 3	*
1.2600	Grp 5	*
1.3250	Grp 6	*
1.4400	Grp 2	* * *

Homogeneous Subsets (highest and lowest means are not significantly different)

Subset 1

Group	Grp 1
Mean	.2950

Subset 2

Group	Grp 4	Grp 3	Grp 5	Grp 6
Mean	1.1850	1.1900	1.2600	1.3250

Subset 3

Group	Grp 5	Grp 6	Grp 2
Mean	1.2600	1.3250	1.4400

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ANOVA of soil moisture

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Variable MOISTURE
By Variable TREATMEN

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	5	3.6027	.7205	34.4756	.0002
Within Groups	6	.1254	.0209		
Total	11	3.7281			

Multiple Range Tests: LSD test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle

Mean	TREATMEN	G G G G G G r r r r r r p p p p p p 1 3 4 2 5 6
2.3800	Grp 1	
2.5400	Grp 3	
2.6900	Grp 4	
2.9900	Grp 2	* *
3.7300	Grp 5	* * * *
3.7600	Grp 6	* * * *

Homogeneous Subsets (highest and lowest means are not significantly different)

Subset 1

Group	Grp 1	Grp 3	Grp 4
Mean	2.3800	2.5400	2.6900

Subset 2

Group	Grp 4	Grp 2
Mean	2.6900	2.9900

Subset 3

Group	Grp 5	Grp 6
Mean	3.7300	3.7600

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ANOVA of total heterotrophic bacterial

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Variable BACTERIA
By Variable TREATME

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	5	3.4207E+14	6.8413E+13	460.2618	.0000
Within Groups	12	1.7837E+12	1.4864E+11		
Total	17	3.4385E+14			

Multiple Range Tests: LSD test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle

Mean	TREATME	G G G G G G r r r r r r p p p p p p 3 4 2 6 5 1
714456.6369	Grp 3	
973135.9649	Grp 4	
1018413.743	Grp 2	
1338212.232	Grp 6	
1805280.184	Grp 5	* * *
12831154.46	Grp 1	* * * * *

Homogeneous Subsets (highest and lowest means are not significantly different)

Subset 1

Group	Grp 3	Grp 4	Grp 2	Grp 6
Mean	714456.6369	973135.9649	1018413.7434	1338212.2317

Subset 2

Group	Grp 6	Grp 5
Mean	1338212.2317	1805280.1837

Subset 3

Group	Grp 1
Mean	12831154.457

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ANOVA of total petroleum hydrocarbon(TPH)

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Variable TPH
By Variable TREAT

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	5	135258805.7	27051761.13	14.0897	.0029
Within Groups	6	11519778.00	1919963.000		
Total	11	146778583.7			

Multiple Range Tests: LSD test with significance level .05
*) Indicates significant differences which are shown in the lower triangle

Mean	TREAT	G G G G G G r r r r r r p p p p p p	
		4 5 6 3 2 1	
33858.0000	Grp 4		
34723.0000	Grp 5		
35605.0000	Grp 6		
36590.0000	Grp 3		
37078.0000	Grp 2		
44103.0000	Grp 1	* * * * *	

Homogeneous Subsets (highest and lowest means are not significantly different)

Subset 1

Group	Grp 4	Grp 5	Grp 6	Grp 3	Grp 2
Mean	33858.0000	34723.0000	35605.0000	36590.0000	37078.0000

Subset 2

Group	Grp 1
Mean	44103.0000

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Appendix 9 The goodness of fit (R^2) of linear line regression for tank 1 until tank 6

The goodness of fit (R^2) of linear line regression of tank 1

22 Aug 05 SPSS for MS WINDOWS Release 6.0

* * * * M U L T I P L E R E G R E S S I O N * * * *

Equation Number 1 Dependent Variable.. TANK1

Block Number 1. Method: Enter DAYS

Variable(s) Entered on Step Number
1.. DAYS

Multiple R	.92568
R Square	.85688
Adjusted R Square	.80917
Standard Error	.16321

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	.47841	.47841
Residual	3	.07991	.02664

F = 17.96107 Signif F = .0241

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
DAYS	-.006986	.001648	-.012232	-.001740	-.925677
(Constant)	11.713735	.137934	11.274774	12.152695	

End Block Number 1 All requested variables entered.

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The goodness fit (R^2) of linear line regression of tank 2

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* * * * M U L T I P L E R E G R E S S I O N * * * *

Listwise Deletion of Missing Data

Equation Number 1 Dependent Variable.. TANK2

Block Number 1. Method: Enter DAYS

Variable(s) Entered on Step Number
1.. DAYS

Multiple R	.94549
R Square	.89394
Adjusted R Square	.85859
Standard Error	.15261

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	.58891	.58891
Residual	3	.06987	.02329

F = 25.28690 Signif F = .0152

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
DAYS	-.007751	.001541	-.012657	-.002846	-.945486
(Constant)	11.671179	.128977	11.260723	12.081635	

End Block Number 1 All requested variables entered.

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The goodness fit (R^2) of linear line regression of tank 3

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* * * * M U L T I P L E R E G R E S S I O N * * * *

Listwise Deletion of Missing Data

Equation Number 1 Dependent Variable.. TANK3

Block Number 1. Method: Enter DAYS

Variable(s) Entered on Step Number
1.. DAYS

Multiple R	.81041
R Square	.65676
Adjusted R Square	.54235
Standard Error	.30695

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	.54084	.54084
Residual	3	.28265	.09422

F = 5.74034 Signif F = .0962

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
DAYS	-.007428	.003100	-.017295	.002438	-.810410
(Constant)	11.781605	.259420	10.956028	12.607182	

End Block Number 1 All requested variables entered.

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The goodness fit (R^2) of linear line regression of tank 4

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* * * * M U L T I P L E R E G R E S S I O N * * * *

Listwise Deletion of Missing Data

Equation Number 1 Dependent Variable.. TANK4

Block Number 1. Method: Enter DAYS

Variable(s) Entered on Step Number
1.. DAYS

Multiple R	.77491
R Square	.60048
Adjusted R Square	.46731
Standard Error	.35463

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	.56708	.56708
Residual	3	.37729	.12576

F = 4.50910 Signif F = .1238

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
DAYS	-.007606	.003582	-.019005	.003793	-.774909
(Constant)	11.796170	.299718	10.842348	12.749992	

End Block Number 1 All requested variables entered.

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The goodness fit (R^2) of linear line regression of tank 5

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Listwise Deletion of Missing Data

Equation Number 1 Dependent Variable.. TANK5

Block Number 1. Method: Enter DAYS

Variable(s) Entered on Step Number
1.. DAYS

Multiple R	.97593
R Square	.95244
Adjusted R Square	.93658
Standard Error	.10641

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	.68021	.68021
Residual	3	.03397	.01132

F = 60.07298 Signif F = .0045

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
DAYS	-.008330	.001075	-.011751	-.004910	-.975928
(Constant)	11.625255	.089932	11.339055	11.911456	

End Block Number 1 All requested variables entered.

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The goodness fit (R^2) of linear line regression of tank 6

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* * * * M U L T I P L E R E G R E S S I O N * * * *

Listwise Deletion of Missing Data

Equation Number 1 Dependent Variable.. TANK6

Block Number 1. Method: Enter DAYS

Variable(s) Entered on Step Number
1.. DAYS

Multiple R	.96259
R Square	.92658
Adjusted R Square	.90211
Standard Error	.15135

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	.86727	.86727
Residual	3	.06872	.02291

F = 37.86205 Signif F = .0086

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
DAYS	-.009406	.001529	-.014271	-.004541	-.962591
(Constant)	11.638554	.127911	11.231488	12.045619	

End Block Number 1 All requested variables entered.

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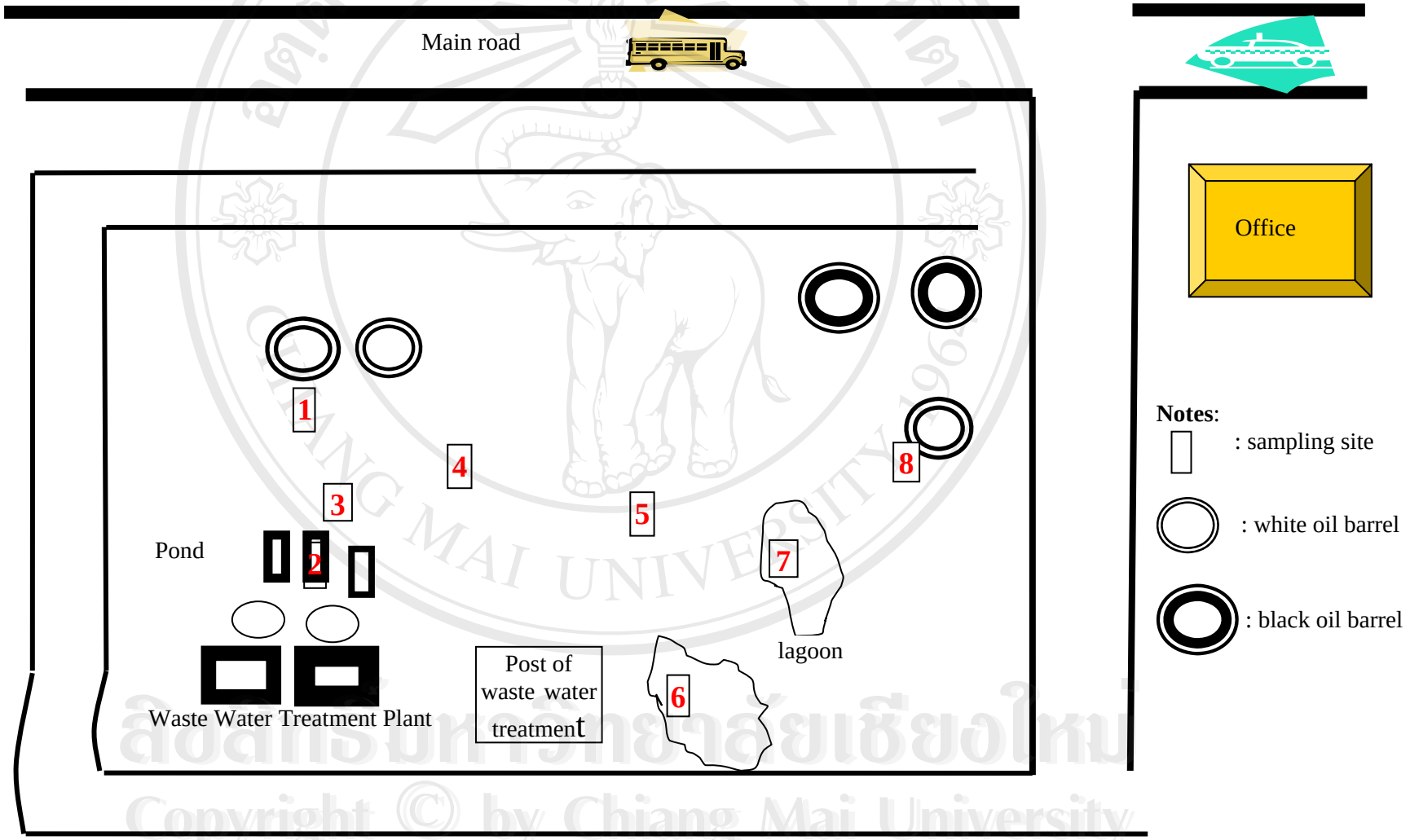
Appendix 10 Figure of the map sampling sites in Fang Petroleum Production



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Appendix 11 Figure of sampling sites

Picture of the sampling site 1

Picture of the sampling site 2

Picture of the sampling site 3

Picture of the sampling site 4

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Picture of the sampling site 5

Picture of the Sampling Site 6

Picture of the sampling site 7

Picture of the sampling site 8

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Appendix 12 Picture of the site 7, site 3, removing large roots, stones, macrofaunas plastics from sample and weighing sample 3 and 7 to get ratio 40:60

Picture of the location of the site 7

Picture of taking sample from site 7

Picture of the location of the site 3

Picture of taking sample from site 3

Picture of stones, plastics, large roots, macrofaunas was removed from the sample

Picture of weighing soil to get ratio site 3 and site 7 was 40:60

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