2.1 Objectives

Disposal of Guwahati refinery sludge is a perennial problem because of its hazardous nature and space limitation. The volume of sludge goes on increasing day-by-day causing great concern to the authority. So, importance is being laid in this study for management of the generated Sludge in Guwahati refinery. In the light of the above facts the present study was undertaken with the following objectives:

1. Physico-chemical characterization of various types of sludges generated in Guwahati refinery with respect to pH, conductivity (electrical), organic matter, ash content, oil and grease and heavy metals such as Pb, Mn, Ni and Zn.

2. Physico-chemical characterization of Khanapara rice field soil with respect to pH electrical conductivity, organic matter, water holding capacity and heavy metals such as Pb, Mn, Ni and Zn.

3. Natural biodegradation study of the sludge by land farming in a laboratory scale by mixing soil from Khanapara rice field in various proportions with refinery sludges in earthen pots and monitoring oil and grease content periodically.

4. Treatability study of the sludges with respect to biodegradation of hydrocarbons by inoculating native hydrocarbon degrading bacterial strains isolated from petroleum polluted soil of Moran, Upper Assam into different concentrations of sludge mixed soil and monitoring
oil and grease content periodically from the bacterial strains treated plot of sludge mixed soil.

5. Biodegradation study of sludges through plantation of rice crops in different concentrations of sludge mixed soil in earthen pots and monitoring oil and grease content after each harvest.

6. Study on the various sludge mixed soil with respect to pH, conductivity, organic matter and heavy metals (Pb, Mn, Ni and Zn) after each harvest in earthen pots and study on the impact of sludge on rice crops and seeds grown on various sludge-soil mixtures in earthen pots with respect to

i) Survivality of plants

ii) Growth of crops

iii) Yield of grains and

iv) Uptake of heavy metals (Pb, Mn, Ni and Zn) by rice grains and husks.

Standard analytical procedures were used in monitoring all parameters and for this purpose analytical facilities at Institute of Advanced Study in Science and Technology (IASST), Khanapara, Guwahati University, Guwahati, were used. Common statistical tools were used to analyse the data for various correlations.

2.2 Methodology followed

2.2.1 Sludge sampling

Under mentioned sludges were collected in gunny bags from four locations inside the plant area of Guwahati refinery in February 1997, which are shown in (Fig.2.1) and photographs of the sludge pits at the four locations are shown in plates 1 to 4.
Fig. 2.1 BLOCK FLOW DIAGRAM OF GUWAHATI REFINERY WITH SLUDGE AREAS
Plate-1: Old untreated sludge (S1)

Plate-2: Effluent treatment plant (ETP) sludge (S2)
Plate-3: API separator sludge (S3)

Plate-4: Treated tank bottom sludge (S4)
a) Old Untreated Sludge (OUTS): - This is the sludge from the old landfill pits. This is a combined oily sludge of the refinery, which was disposed of several years ago without any treatment, before installation of the melting pits. The disposal site is near the flare area. This sludge is termed as S1.

b) Effluent Treatment Plant Sludge (ETPS): - This is the sludge left in twelve drying beds after the treatment of the refinery effluent treatment plant. This sludge is termed as S2.

c) API Separator Sludge (ASS): - This is the sludge left after the sludges collected from API separators is treated in a separate set of melting pit. The API separators receive all the junks from the refinery including washing, run off, spills accidental and otherwise etc. This sludge is termed as S3.

d) Treated Tank Bottom Sludge (TTBS): - This is the sludge remaining after the tank bottom sludges from crude oil tanks and other product storage tanks is treated at melting pit for recovery of oil by steam circulation. This sludge from melting pit is termed as S4.

e) Mixed sludge (MS): - In addition to the above sludges a mixture of the three generated sludges [b), c) and d)] were prepared for this study. The preparation was made in the ratio 60:3:1, which is the generation ratio of sludges (S2, S3 and S4). This sludge is termed as S5.

2.2.2 Soil Sampling

For lab scale biodegradation and cultivation study, soil was collected from a rice field at Khanapara,
Guwahati in gunny bags. The collected soil was air dried, broken and sieved with a 2mm sieve. The soil sample was collected in February 1997.

2.2.3 Sample preparation for natural biodegradation (Landfarming) study

Sludges of all the categories (S1, S2, S3, S4 and S5) were mixed with Khanapara soil in the following proportion in earthen pots (2.0 Kg in each pot) with three replications. The samples were prepared in the month of February 1997.

<table>
<thead>
<tr>
<th>Sludge (%wt.)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil (%wt.)</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

2.2.4 Sample preparation for inoculated bacterial degradation study

The sludge-soil mixtures were prepared as above (2.2.3) for biodegradation study by inoculating three types of bacterial strains named *Pseudomonas aerogenosa* (Pa), *Pseudomonas stutzeri* (Ps) and *Serratia marcescens* (Sm) isolated from Upper Assam oil fields. This experiment was initiated in earthen pots in the month of February 1997.

2.2.5 Preparation of inoculum

Conical flasks of 250ml containing 100ml of nutrient broth media (peptone 5.0g, beef extract 3.0g, NaCl 5.0g and distilled water 1 liter) were plugged with non-absorbent cotton and sterilized in autoclave at 15lb pressure for half an hour. After sterilization, the media were kept for cooling and then inoculated the respective bacterial strains by an inoculating needle in to the above cold and sterilized media separately. Inoculations were made aseptically in laminar flow. After inoculation the conical flasks were incubated at 30 ± 2° C and shaked at 150 RPM for two hours/day in shaking incubator for three days. This bacterial broth was used for mixing in the experiments.
2.2.6 Inoculum mixing procedure

2.0ml inoculum of each strain such as bacteria (Pa), bacteria (Ps) and bacteria (Sm) was mixed separately with 248.0ml of dug well water and then entire amount of 250.0ml water solution of each strain was mixed thoroughly with each pot sample of 2.0 Kg. with three replications.

In case of natural degradation study the prepared samples were aerated by tilling and moisturized by spraying water at regular interval and degradation study was conducted by monitoring oil and grease content at an interval of 12 months for three consecutive periods, first in the month of February, 1998 then in February 1999 and finally in February 2000.

Similarly in biodegradation study bacterial strains were inoculated and tilling and spraying of sludge and water were done at regular interval of time as above and samples were collected at an interval of 12 months for three consecutive periods starting from 1997. Each sample of the natural degradation study has been taken as to be the 'Control' sample for respective biodegradation study.

2.2.7 Mixing procedure for plantation study

Soil sample from Khanapara rice field was mixed with each category of sludge sample in the following proportion for rice cultivation in earthen pots (4.0 Kg mixture in each pot) with three replications.

<table>
<thead>
<tr>
<th>Sludge (%wt.)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil (%wt.)</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Khanapara soil was treated as 'Control' sample (sludge 0% and soil 100%) for rice cultivations. Four numbers of rice seedling of Masuri (Aljong) variety were planted in each pot and water level in the pots were continuously kept 5-10 cm above the soil surface as observed in rice fields. The
The experiment was conducted for three consecutive years starting from 1997. The growth of seedlings was constantly monitored and heights were recorded periodically at an interval of 30 days.

The plantation and harvest of rice crops were done for the first season during July-October in 1997, for the second season during July-October in 1998 and for the third season during July-October in 1999. The matured seeds were collected and dried at oven at 45°C. Dry weight of the seeds was recorded. Rice grain and husks were separated from the seeds. Rice grain and husks were analyzed with respect to heavy metals (Pb, Mn, Ni and Zn).

2.3 Physico-chemical parameters and their analysis

The methods of estimation of physico-chemical parameters of all the categories of collected sludges, soil and soil mixed sludge prepared for both biodegradation as well as cultivation studies are discussed below.

2.3.1 Soil

pH

Soil pH is an indicator of the acidity or basicity of the soil. The soil pH greatly affects the solubility of minerals. The soil pH can influence plant growth by stimulating the activity of microorganisms. Most agricultural crops do best in slightly acidic mineral soil (pH 6.5) or acidic organic soil (pH ~ 5.5). Plants on soil of pH > 9.0 usually have reduced growth or even die. Some plants, of course, are tolerant to high salt or pH. Soil becomes acidic by the leaching effects of rain water which replaces basic cations (Ca²⁺, Mg²⁺, Na⁺, K⁺) with H⁺ from the carbonic acid formed from water and dissolved CO₂. The exchange mechanisms may be as follows (Townsend, 1973):

\[
\begin{align*}
\text{Clay Na}^+ + \text{H}^+ + \text{OH}^- & \rightarrow \text{Clay Na}^+ + \text{H}^+ + \text{OH}^- \\
\text{Na}^+ + \text{H}^+ + \text{OH}^- & \rightarrow \text{Na}^+ + \text{H}^+ + \text{OH}^- \\
\end{align*}
\]
Soil pH was determined by using the model L1-120 Elico Digital pH meter in 1:5 soil water proportion.

**Conductivity (Electrical)**

The electrical conductivity value is depended upon the concentration of various cations (Na⁺, Ca⁺⁺, Mg⁺⁺, K⁺) and anions (HCO₃⁻, NO₃⁻, CO₃²⁻). If the concentration of these ions is more electrical conductivity will be increased. Soil conductivity was determined by using conductivity bridge Cm-82t (Elico Pvt. Ltd.) by dipping the conductivity cell (cell constant 1.0) in 1:5 soil water mixtures.

**Water holding capacity**

Water is held in soil because of its polar character with the two hydrogens forming the positive end of the dipole and oxygen making the negative end. When fatty or oily substances, which are low in oxygen, coat the soil particles water is not attracted to and held to the coated surface. These soils are called water repellent soil (Donahue et al. 1990).

Clay soil holds more water than sandy soil (White, 1987). Water holding capacity (WHC) of the collected soil sample was determined by using circular brass box of known weight (a). The perforated bottom plate of the box was supported on a Whatman No. 1 filter paper. 10.0g of soil was added to the brass box and weighed (b). The box with the soil was kept overnight. Next day the box was removed and the excess water was allowed to drain off. Then the box was weighed (c). The weight (m) of the moist Whatman No. 1 filter paper was also measured. The value of

\[
\text{Soil} + \text{Ca(HCO}_3\text{)}_2 \rightarrow \text{SoilCa}^{++} + 2\text{H}_2\text{O} + 2\text{CO}_2
\]
WHC is calculated as follows—

\[
\text{WHC} \% = \frac{c - (b + m)}{(b - a)}
\]

**Organic matter**

The presence of organic matter is of great importance in the formation and stabilization of soil structure. Organic compounds with a suitable spatial arrangement of reactive groups (-OH, \(-\text{C}_6\text{H}_5\text{OH}\) and \(-\text{COOH}\)) can form co-ordination complexes with metallic cations by displacement of some of the water molecules from the cation's hydrations shell. Chelates formed with certain di- and polyvalent cations are the most stable, the stability constant falling in the order Cu > Fe

\(~\text{Al} > \text{Mn} > \text{Co} > \text{Zn}\). For example

![Diagram](image)

Polymers of Fulvic acid and Humic acid fractions are adsorbed onto mineral surfaces by a variety of mechanisms. Primarily involving the functional groups - carboxyls, carbonyls (\(-\text{C} = \text{O}\)), alcoholic -OH, phenolic -OII, secondary amines (\(-\text{NH}\)) and primary amines\((-\text{NH}_2\)) Polysaccharide and polyurinide gums also function as bonding agents between mineral particles. The total organic carbon in the soil was determined first by Walkley and Black method.

50.0g of air-dried soil were mixed with 10.0ml of 1N \(\text{K}_2\text{Cr}_2\text{O}_7\) solution and 20.0ml of concentrated \(\text{H}_2\text{SO}_4\) in a 500ml conical flask. Solid AgSO\(_4\) was added to it by gentle stirring so that the AgSO\(_4\) goes into the solution completely. The contents were diluted to 200ml. The colour of the solution turned bluish purple on addition of 1.0ml of phosphoric acid and 1.0ml of diphenylamine indicator.
The solution was titrated with ferrous ammonium sulphate till colour changes to brilliant green.

Calculation -

Organic carbon % \(= (x-y) \times (0.003) \times \frac{100}{w}\)

Where,

\(x = \) Volume of \(\text{K}_2\text{Cr}_2\text{O}_7\) solution

\(y = \) Volume of ferrous ammonium sulphate required for titration

\(w = \) Weight of soil sample

Organic matter % \(= \) Organic carbon \(\times 1.724\)

Na and K

Na and K in soil were determined by extraction with 1N ammonium acetate as per established procedure (Jackson, 1967). The Na and K in the filtrate was determined using flame photometer.

Pb, Mn, Ni and Zn (Extraction)

Air-dried soil sample was grounded and screened through 80-mesh sieve. 1.0g of sieved sample were digested with 25.0ml of 2N HNO\(_3\) for about 4 hours. The acid extraction was evaporated to near dryness, then 1.0ml of 6N HNO\(_3\) and 10.0ml of deionised distilled water were added. The mixture was warmed, allowed to cool and filtered in a 50ml volumetric flask. Volume of the flask was made up to the mark by deionised distilled water. The solution was then analysed for heavy metals (Pb, Mn, Ni and Zn) by Atomic Absorption Spectrophotometer (SHIMADZU) by using the following calculation

Metal content, mg/kg \(= \) \(p \times q \times r \div w\)

Where,

\(p = \) Concentration of metal in digested solution

\(q = \) Final volume of digested solution, ml.

\(r = \) Further dilution ratio

\(w = \) Sample weight, g

57
2.3.2 Refinery sludge

Parameters like pH, conductivity and organic matter were determined as were done in case of Khanapara soil (2.3.1), in all the five categories of sludges.

Ash content

Ash content was determined by taking 1.0g of each sludge sample in silica crucible. The crucible was first oven dried at 45°C for evaporation of lighter products and moisture for one hour. The oven-dried samples were then heated in muffle furnace at 450-500°C for eight hours. White ashes were observed. After cooling the ashes were weighed.

Oil and grease

1.0g of sludge sample were taken for oil and grease extraction by using petroleum spirit (40-60°C). The extraction was carried out in Soxhlet apparatus for a period of eight hour for one sample. After extraction, the oil and grease coming out along with petroleum spirit was taken in a pre weighed beaker. The solvent was evaporated in room temperature and final weight was taken. The difference of weight was the concentration of oil and grease.

Calculation

\[
\text{Oil and grease, ppm} = \frac{(x-y) \times 10000}{w}
\]

Where

- \( x \) = Final weight of beaker + oil, g
- \( y \) = Initial weight of beaker, g
- \( w \) = Sample weight, g
Table 2.1  Analytical conditions for Atomic Absorption Analysis

<table>
<thead>
<tr>
<th>Element</th>
<th>Wave Length (nm)</th>
<th>Mode</th>
<th>Operating Working range (ppm)</th>
<th>HC Lamp Current (mA)</th>
<th>Slit Width (nm)</th>
<th>Type of Flame</th>
<th>Fuel Gas Flow rate (l/min.)</th>
<th>Air Flow rate (l/min.)</th>
<th>Burner Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>BGC</td>
<td>(0.5 - 8)</td>
<td>5</td>
<td>0.4</td>
<td>Air-C$_2$H$_2$</td>
<td>1.9</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Ni</td>
<td>352.4</td>
<td>BGC</td>
<td>(0.1 - 5)</td>
<td>4</td>
<td>0.15</td>
<td>-do-</td>
<td>1.7</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Pb</td>
<td>283.3</td>
<td>BGC</td>
<td>(0.2 - 2)</td>
<td>5</td>
<td>1.0</td>
<td>-do-</td>
<td>2.0</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>BGC</td>
<td>(0.1 - 4)</td>
<td>4</td>
<td>0.5</td>
<td>-do-</td>
<td>2.0</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>
Na, K and heavy metals (Pb, Mn, Ni and Zn)

1.0g of sludge was taken in a silica crucible and placed in a muffle furnace at 450-500°C for eight hours. The crucible was then cooled and ash was dissolved in 10.0ml of 0.1M HCl and swirled to dissolve the residue. 5.0ml of deionised water was added and the solution was filtered a number of times by washing with small amount of deionised water. Filtrate was collected in a 50ml volumetric flask and the volume was made up to the mark by deionised water. Metals were estimated by Atomic Absorption Spectrophotometer (AAS).

Calculation

Metal, ppm = \frac{x.y}{w}

Where,

x = Concentration of metal in digested solution

y = Final volume of digested solution, ml

w = Sample weight, g

2.3.3 Rice grains and husks

Metal extraction [Pb, Mn, Ni and Zn]

The collected grains were separated from husks manually. The husk and seeds were dried separately in an oven at 45°C and grounded finely by a grinding machine. Metals like Pb, Mn, Ni and Zn were analysed from the aliquot of ashed sample, which were done according to Jackson [1967].

1.0g of finely ground rice seed and 1.0g of husk were kept separately in crucible and then placed in to the muffle furnace at 450-500°C for about four hours. Crucibles were then cooled and residues were treated with excess of 1N HNO₃ evaporate to near dryness. Evaporation to dryness was repeated thrice. The samples were then treated with 5.0ml of 1N HNO₃ again swirled to dissolve the residue with addition of 5.0ml deionised water.

The mixtures were filtered number of times by washing the residue with small amount of deionised
water. Collected filtrate in 50ml volumetric flask was made up to the mark by adding deionised water.

Calculation

Metal, ppm = AAS reading. L. M/W

Where,  

L = Fill up volume in sample dissolution, ml
M = Further dissolution ratio
W = Weight of sample, g

2.3.4 Sludge mixed soil used for cultivation

Extraction of Pb, Mn, Ni and Zn

Extraction of heavy metals such as Pb, Mn, Ni and Zn were carried out in the following set of samples

a) After first harvest
b) After second harvest and
c) After third harvest

Procedure of sample preparation and determination of metals by AAS method was same as in the case of Khanapara soil sample (2.3.1).

Oil and grease content

Estimation of oil and grease of the above samples [a), b) and c)] was done as it was done in refinery sludge samples (2.3.2).

pH, conductivity and organic matter of the above samples [a), b) and c)] were also estimated in a similar procedure as was done in case of refinery sludges (2.3.2).
The data collected in the field were subjected to statistical analysis to find out the variations and interrelationship of various parameters. Observations were done in replicates and Mean were calculated and presented in tabular form as well as graphs.

Regression analysis was performed to find the relationship of degradation over time by the regression line, \( y = mx + C \), where "m" is the slope of the line. Slope (m) was calculated as:

\[
m = \frac{\sum xy - (\sum x)(\sum y)}{\sum x^2 - (\sum x)^2}
\]

Statistical analysis was performed using SPSS 6.0 and graphs were generated using Microsoft Excel.