CHAPTER III

MATERIALS AND METHODS

The heavy metals such as Fe, Al, Mn, Cu, Cd, Ni and Cr in soils of mine areas pose serious problems to the major crops. Their varying proportions interfere in metabolic processes of the plants directly or indirectly. Sometimes the toxicity of these elements does not allow the majority of the field crops to complete their life cycle and farmers have to incur loss. Therefore, the vast mine areas about 7,454 kms in the country is treated as waste lands. Many people are trying to develop these waste lands for agriculture purposes. For economically viable agriculture in these mine soils especially in chromite overburden, the growing of economic plants (aromatic and medicinal) for medicinal uses is given with top priority. With a view to increase the yield and oil content of aromatic grasses with fertilizers, organic manures and lime (CaCO₃), these experiments were planned and the details are presented in foregoing pages.

Seven pot-culture experiments were conducted during 1986 Kharif to 1989 Kharif, in aromatic and medicinal plant garden, Regional Research Laboratory,
Bhubaneswar (Latitude 28°15'N and longitude 85°52'E) in an attempt to evaluate the nutritional studies of economic plants (Cymbopogon species) in chromite overburden (soil) brought from Kaliapani mine area of Cuttack district (Sukinda Block, Orissa). This soil is left over in the adjoining areas after the excavations of chromite ore and is subject to action of rain and sun throughout the year. The high rainfall in Kaliapani area leads to the leaching of essential plant nutrients like N, P, K, Ca, Mg with an increase of toxic elements like Fe, Al, Cr, Mn, etc.. The soil is a silt loam in texture having pH (1.2) 5, organic carbon 0.26%. The details of pot culture experiments and analytical procedures adopted in the present investigation are summarised below.

3.1 POT CULTURE EXPERIMENTS

The pot culture experiments were conducted to evaluate the growth behaviour of Cymbopogon species in chromite overburden (soil) with and without organic soil amendments. The effect of different recommended doses of fertilizers on these species were also studied.

Experiment 1

**Title**: Effect of Nitrogen on Palmarosa (Cymbopogon martinii var. motia) in Chromite Overburden soil.
Season and year: Kharif and Rab 1986
Design: Complete randomised block design
Replication: Three
Crop & Variety: Palmarosa (Cymbopogon martinii)
   Var. Motia. - RRL(B)-49.
Filling of pot with soil: The soil was brought from Kaliapani, and dried under shade. This was
passed through 2 mm sieve and 10 kg soil was filled in each pot. Then 1 litre of tap water
was added and allowed one day before mixing fertilizer.

Treatment: $T_1$: No Nitrogen (control)
   $T_2$: Nitrogen @ 30 kg/ha. as urea
   $T_3$: Nitrogen @ 60 kg/ha as urea
   $T_4$: Nitrogen @ 90 kg/ha as urea
   $T_5$: Nitrogen @ 120 kg/ha as urea.

Fertilizer: 25% N as per the treatments and full
dose of $P_2O_5$ and $K_2O$ @ 50 kg/ha each were added
to and mixed thoroughly with the soil in pot
before planting the slips. The rest 75% N(as
urea) was applied in two splits (50% and 25%)
at 25 and 50 days of the planting respectively.
The addition of N was repeated after each
harvest with $P_2O_5$ and $K_2O$.

Date of Planting: On 24th March, 1986. Two slips
were planted in each pot.
Date of harvesting: September, October, 1986 and
Irrigation: Each pot was added with 500 ml of tap water after 3 days intervals during rainless days.

Plant protection: Appropriate plant protection measures were adopted as and when required.

Biometric observations: Observations in respect of average herb height, number of tillers, herb yield were recorded at the time of harvest.

Experiment 2

Title: Effect of Nitrogen on Palmarosa (*Cymbopogon martinii*) in chromite overburden (soil) amended with farm yard manure (FYM).


Design: Complete randomised block design.

Replication: Five

Crop and Variety: Palmarosa (*Cymbopogon martinii* var Motia.)

Filling of Pots: 10 kg soil (< 2mm) + FYM (2.1) was packed in each pot to which 1 litre of tap water was added and allowed to stand one day before addition of required amendments and fertilizer.

Treatments:
- **T<sub>1</sub>**: Control (No Nitrogen)
- **T<sub>2</sub>**: Nitrogen @ 30 kg/ha as urea
- **T<sub>3</sub>**: Nitrogen @ 60 kg/ha as urea
Addition of Fertilizer and FYM:

FYM (Farm Yard Manure) was mixed with the soil in the pot @ 2:1 (soil:FYM) to which 1 litre of tap water was added. This was allowed to stand for 1 day. Then 25% N as per the treatment 1 full dose of $P_2O_5$ and $K_2O$ @ 50 kg/ha each was added and thoroughly mixed with the soil in the pot. The rest 75% N was added in two splits (50% and 25%) at 25 days and 50 days of growth respectively. The same amount of $N$, $P_2O_5$ and $K_2O$ was repeated after each harvest.

Date of planting: Two slips of Palmarosa were planted in each pot on April 12, 1986.

Date of harvesting: Three consecutive harvests were done on early September 1986, late November, 1986 and early January, 1987.

Irrigation: Each pot was added with 500 ml of tap water after three days intervals during rainless days.

Plant protection: Appropriate plant protection measures were adopted as and when required.

Biometric observations: Observations in respect of average herb height, number of tillers and herb yield were recorded at the time of harvest.
Experiment 3

Title: Effect of organic amendments (FYM) on Plamarosa (Cymbopogon martinii) in Chromite overburdens (soil).

Design: Complete Randomised Block Design.
Replication: Five.
Crop and Variety: Palmarosa (Cymbopogon martinii) var. Motia - RRL(B) 49.

Filling of pots: 10 kg (<2 mm) soil + FYM as per the treatment was packed over sieving to which one litre of water (deionised) was added.

Treatment: $T_1$: Local Soil (lateritic surface soil)
$T_2$: Chromite overburden
$T_3$: Chromite overburden + FYM (1:1)
$T_4$: Chromite overburden + FYM (2:1)
$T_5$: Chromite overburden + FYM (3:1)

Addition of fertilizers and FYM: No fertilizer was added. FYM as per the treatment was thoroughly mixed with the soil to which one litre of tap water was added 1 day before planting.

Date of Planting: 2 slips of Palmarosa were planted in each pot on July 10, 1986.

Date of harvesting: Three consecutive harvests were done on 26th September to 21st October, 1986, 22nd January and 10th April, 1987.
Irrigation: Each pot was added with 500 ml of tap water after 3 days intervals during rainless days.

Plant protection: Appropriate plant protection measures were adopted as and when required.

Biometric observations: Observations in respect of average herb height, tiller numbers and herb yield were recorded at the time of harvest.

Experiment 4

Title: Comparative study of aromatic grasses on chromite overburden (soil).


Design: Complete Randomised Block Design.

Replication: Four

Crop and Variety: Aromatic grasses as per the treatments.

Filling of pots: 10 kg soil (chromite overburden) (<2 mm) was packed in each pot.

Treatments:

\[ T_1 \]: Cymbopogon \textit{martini} var. Motia - RRL(B)-49

\[ T_2 \]: Jamrosa (RRL-82) (Hybrid of \textit{C. nardus} var. \textit{confertiflorus} and \textit{C. jawarancusa})

\[ T_3 \]: Cymbopogon \textit{winterianus} (Bangalore local)
\[ T_4 \text{ Cymbopogon pendulus (RRL-16)} \]

\[ T_5 : \text{Cymbopogon flexuosus (SD-68)} \]

Addition of Fertilizers: N, \( \text{P}_2\text{O}_5 \) and \( \text{K}_2\text{O} \) was applied at the rate of 50 kg each per ha. 25\% \text{N} as \( \text{CAN} \) and full dose of \( \text{P}_2\text{O}_5 \) and \( \text{K}_2\text{O} \) was applied at the time of planting. Rest 75\% \text{N} (50\% \text{and} 25\%) as \( \text{CAN} \) was applied at 25 and 50 days of the planting. This dose of fertilizers was repeated after each harvest.

Date of planting: 2 slips as per treatments were planted in each pot on January 6, 1987.

Date of harvesting: Six consecutive harvests were done in April 3rd to 10th, 1987, June 12th to 19th, 1987, August 20th to 27th, 1987, November 11th to 24th, 1987, February 10th to 12th, 1988 and April 8th, 1988.

Irrigation: Each pot was added with 500 ml of tap water after three days intervals during rainless days.

Plant Protection: Appropriate plant protection measures were adopted as and when required.

Biometric observations: Observations in respect of average herb height, tiller numbers and herb yield were recorded at the time of harvest.
Experiments 5 and 6

Title: Effect of lime (CaCO₃) and fertilizers on yield and nutrient uptake of aromatic species in chromite overburden (soil).


Design: Factorial Complete Randomised Block Design.

Replication: Three

Crop and Variety: As per treatments.

Filling of pots: 10 kg soil (< 2mm) was packed in each pot to which 2 lits. of tap water was added.

Treatments: Main & subplot treatments:

(a) Main plot treatments (Aromatic species)
   \( V_1 \) - Cymbopogon martini Var. Motka.
   \( V_2 \) - Jamrosa (Hybrid).
   \( V_3 \) - Cymbopogon pendulus (RRL-16).
   \( V_4 \) - Cymbopogon flexuosus (SD-68).

The species \( V_4 \) (Cymbopogon flexuosus) was not taken up in Experiment No.6.

(b) Sub-plot treatments (Lime and fertilizers)
   \( T_1 \) - \( L_0 F_0 \) - No lime, No fertilizer
   \( T_2 \) - \( L_0 F_0.5 \) - No lime+Fertilizer @ 100kg N, 50 kg \( P_2O_5 \) and 50 kg \( K_2O \) per ha
   \( T_3 \) - \( L_0 F_1.0 \) - No lime+Fertilizer @ 200kg N, 100kg \( P_2O_5 \) and 100 kg \( K_2O \) per ha.
 Addition of Lime • Required amount of lime (CaCO₃) as per treatments was accurately weighed and mixed thoroughly with the presoaked soil in the pot seven days before planting.

Addition of fertilizers: Out of required fertilizer dose as per treatment 25% N, full dose of P₂O₅ and K₂O was added to the pots and thoroughly mixed with the soil before planting. Rest 75% N (50% + 25%) was applied in two splits at 25 and 50 days of the planting. After each harvest only N was applied as per the treatment without P₂O₅ and K₂O.
Date of planting: Two slips from each species were planted in each pot on 14th December, 1987 and the same on 5th December, 1988.

Date of harvesting: Three consecutive harvests were made on April 14th, July 21st and October 26th, 1988 and the same on 30th May, 13th July and 15th September, 1989.

Irrigation: About 500 ml of tap water was added to each pot at three days intervals during rainless days.

Plant protection measures: Appropriate plant protection measures were adopted as and when required.

Biometric observations: Observations in respect of herb height, tiller numbers and herb yield were recorded at the time of harvest.

**Experiment 7**

**Title**: Effect of lime and organic amendments on yield and nutrient uptake of aromatic plant, Jamrosa in chromite overburden (soil).


Design: Complete Randomised Block design.

Replication: Three

Crop and variety: Jamrosa (RRL 82)

Filling of pots: 10 kg soil (< 2mm) was packed in each pot to which two litres of tap water was added.
Treatments:

- $T_1$ - No lime
- $T_2$ - Lime @ 1 LR (6t CaCO$_3$/ha)
- $T_3$ - Lime @ 1.5 LR (9t CaCO$_3$/ha)
- $T_4$ - Lime @ 2 LR (12t CaCO$_3$/ha)
- $T_5$ - FYM @ 10 t/ha + No lime
- $T_6$ - FYM @ 10 t/ha + Lime @ 1LR (6t CaCO$_3$/ha)
- $T_7$ - FYM @ 10 t/ha + Lime @ 1.5LR (9t CaCO$_3$/ha)
- $T_8$ - FYM @ 10 t/ha + Lime @ 2LR (12t CaCO$_3$/ha)
- $T_9$ - Leucinia leaves @ 1 t/ha + No lime
- $T_{10}$ - Leucinia leaves @ 1 t/ha + Lime @ 1LR (6t CaCO$_3$/ha)
- $T_{11}$ - Leucinia leaves @ 1 t/ha + Lime @ 1.5LR (9t CaCO$_3$/ha)
- $T_{12}$ - Leucinia leaves @ 1 t/ha + Lime @ 2LR (12t CaCO$_3$/ha)

Addition of Lime and Organic amendments: Required amount of lime (CaCO$_3$), FYM, and Leucinia leaves as per treatments were mixed thoroughly with the soil in pots seven days before planting.

Addition of fertilizers: The recommended doses of fertilizer were 100 kg N, 50 kg P$_2$O$_5$ and 50 kg K$_2$O/ha. Out of this 25% N, full dose of P$_2$O$_5$ and K$_2$O was added as basal at the time of planting. Rest 75% N (50%
+ 25%) was applied in two splits at 25 and 50 days of planting. The same recommended fertilizers were applied after each harvest.

Date of Planting • December 22, 1988.

Date of harvesting • Three consecutive harvests were made in 6th April, 7th July and 28th September, 1989.

Irrigation • About 500 ml of tap water was added to each pot at three days intervals during rainless days.

Plant protection • Appropriate plant protection measures were adopted as and when required.

Biometric observations • Observations in respect of herb height, tiller numbers and herb yield were recorded at the time of harvest.

3.2 ANALYTICAL METHODS

The methods followed for the analysis of soil, plant and oil are described below. In general, methods outlined in the text, "Soil Chemical Analysis" by Jackson (1962) and "Soil and Plant Analysis" by Piper (1949) were adopted.

3.2.1 Analysis of soil

Collection and preparation of soil samples:
Loose soil samples were collected before start of the
experiments from the surface layers. Also soil samples were collected from each treatment (pot) replication-wise. These samples were dried under shade and passed through a 2 mm sieve. The processed soil samples were preserved for analysis.

**Collection and preparation of plant samples**

After taking required biometric observations (height, tiller number, etc.), the plant samples were harvested, treatment-wise, washed with deionised water and dried with blotting paper. The fresh weight was taken. Two to three tillers out of the entire harvest were put to hot air oven after taking fresh weight for 48 hours at 70±5°C. 2-3 leaves were cut (third leaf from top) and made into small pieces by scissors. 0.2 g of (cut) leaf was put to 10 ml (80%) acetone for chlorophyll analysis. The rest plant samples were dried under shade and preserved for oil analysis.

**\( \text{pH}_w \) Values**

The \( \text{pH}_w \) of the homogenized soil samples (< 2 mm sieve) collected before and after the experiment was measured in 1:2 soil–water suspension. 10 g soil samples were taken in a polythene beaker (50 ml capacity) containing 20 ml of distilled water. The contents were stirred for 10 minutes and equilibrated for 30 minutes. The \( \text{pH}_w \) reading was taken with the help of Elico glass electrode pH meter (model LI-10).

**Lime requirement value of the soil**

The lime requirement value of the soil was determined by means
of the Woodruff's buffer method (1948). This buffer was prepared by mixing 10.0g calcium acetate, 12.0g p-nitro-phenol, 10.0g salicylic acid, and 4.0g calcium hydroxide in 900 ml of distilled water and the final volume was made up to 1000 ml after the pH of the solution was adjusted to 7.0.

To 10g sample of soil taken in a 50 ml plastic beaker, 10 ml of distilled water and 10 ml of Woodruff's buffer solution were added. The contents were thoroughly stirred with help of a glass rod at 10 minutes interval until 30 minutes elapsed. The pH of the suspension was then determined with a properly calibrated glass electrode pH meter. Every 0.1 pH unit less than pH 7.0 is equivalent to 1.0 me of neutralizable acidity per 100 g of soil. The lime requirement value is calculated as CaCO$_3$ equivalent to neutralizable acidity and expressed in kilograms of pure, finely ground CaCO$_3$ per hectare.

Cation Exchange Capacity: The cation exchange capacity of the soil was determined by 1N NH$_4$OAc methods.

Organic Carbon: Organic carbon of the soil was determined by Walkley and Black's rapid titration method.

Available Nitrogen: The available nitrogen of the soil was determined by alkaline permanganate method described by Subbiah and Asija (1956).
Available Phosphorus: The available phosphorus content of the soil was analysed by NaHCO$_3$ (pH 8.5) extraction (Olsen's method) followed by the chloro-stannous acid reduced molybdo-phosphoric acid method.

Available Potassium: Available potassium was determined from the ammonium acetate leachate by means of a systronics flame photometer.

DTPA extractable Cr and Fe from Soil: The DTPA (0.005 M) extractable Cr and Fe was estimated by the methods of Lindsay and Norvell (1969). The DTPA (0.005M) extracting solution was prepared by mixing 1.965g of DTPA (Diethelene triamine pentacetic acid) with 13.3 ml of TEA (0.1M) (triethanol amine) and 1.118g of CaCl$_2$.2H$_2$O (0.01M) in 900 ml of double distilled water adjusted to pH 7.3 with 0.01 NH$_4$Cl. The volume was made upto 1000 ml with double distilled water.

To 10g soil twenty ml of DTPA solution was added in a 100 ml conical flask and was shaken for 2 hours with mechanical shaker. The contents were filtered through No.1 Whatman filter paper to 50 ml sample bottle and stored for analysis of Cr and Fe.

The concentration of Cr and Fe in the original extract was estimated by a Varian Techtron Atomic Absorption Spectrophotometer, Model AA/100.
Soil Texture: The relative percentage of sand, silt, and clay was determined from loose soil (< 2 mm) by the process of mechanical analysis using Bouyoucos hydrometer.

3.2.2 Chemical Analysis of the herb samples:

The oven dried herb sample (70±5°C) was ground in a plant sample grinder and was stored in desiccator for further analysis.

Analysis of total Nitrogen: A 0.2g plant sample was accurately weighed and transferred into 100ml digestion flask. To it 5ml of Conc. H₂SO₄+2% salicylic acid mixture was added. After lapse of a time of 5 minutes one globule of sodium-thio-sulfate was added and allowed to react for a period of 30 minutes. Then the contents were digested at a temperature of 200±10°C till a white fume is evolved. One g digestion mixture (1g CuSO₄ + 10g K₂SO₄) was added to it and digestion was continued till a clear bluish green colour obtained.

The contents were transferred to a distillation flask for distillation of NH₃ into 2% boric acid medium as described by Piper (1949).

Analysis of P, K, Ca, Mg, Fe, Cr: For determination of mineral constituents in dry matter (leaves, shoot, top) 1g sub-sample of the ground material was digested in conc. HNO₃ + HClO₄ mixture (5:2) at
200±20°C following overnight predigestion in 15 ml Concentrated HNO₃ (Johnson and Ulrich, 1959).

The digestate was made to 100 ml after filtration. Taking suitable aliquots, various mineral constituents such as P, K, Ca, Mg, Fe and Cr were determined as follows:

**Phosphorus:**
Vanado-molybdo phosphoric yellow colour method in HClO₄ system outlined by Jackson (1962) for the determination of plant phosphorus was adopted.

**Potassium:**
A suitable aliquot was diluted and the concentration of potassium was read in Systronics make flame-photometer.

**Calcium and Magnesium:**
The concentration of calcium and magnesium in the original aliquot was read in a Varian Techtron Atomic Absorption Spectrophotometer, Model AA/100.

**Fe and Cr:**
The concentration of Iron (Fe) and Chromium (Cr) in the original aliquot was estimated by a Varian Techtron Atomic Absorption Spectro-photometer model AA/100.
3.2.3 Analysis of Chlorophyll:

2-3 leaves were removed from each pot and made small pieces with the help of a scissor. 0.2g of this was accurately weighed and transferred to a 50 ml sample bottle to which 10 ml of 80% acetone was added and allowed to react for 24 hours. The contents were decanted to a measuring cylinder and the volume was made to 20 ml in 80% acetone. The concentration of chlorophyll was estimated in Spectronic 20 as per the methods outlined by McKinney (1941).

3.2.4 Analysis of Oil:

The oil present in the plant material was estimated through the process of hydrodistillation. To isolate essential oil from aromatic plants, these plant materials were packed in a still to which sufficient quantity of water was added for complete immersion. The water was boiled by the help of heating elements. Under the influence of hot water and steam the essential oil was vapourised from the oil glands of the plant tissue. The vapour mixture of liquid water and oil was passed through a connecting tube into a condenser where it was condensed by external cooling system (cold water). The condensate from the condenser was flown into a receiver where the oil was separated automatically from distillation.
water. The distillation was considered to be complete when no oil was seen in the condensate.

The concentration of oil was calculated on moisture free basis.