

INDUSTRIAL EFFLUENTS CONTAMINATED AND
BIOREMEDIATED SOILS-EFFECT ON HEAVY METAL
CONTENT IN EDIBLE CROPS & HUMAN HEALTH

By

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This is to certify that the thesis entitled “**INDUSTRIAL EFFLUENTS CONTAMINATED AND BIOREMEDIATED SOILS – EFFECT ON HEAVY METAL CONTENT IN EDIBLE CROPS AND HUMAN HEALTH**” submitted in partial fulfillment of the requirement for the degree of **DOCTOR OF PHILOSOPHY IN HOMESCIENCE** of the Acharya N. G. Ranga Agricultural University, Hyderabad is record of the bonafide research work carried out by **Ms. D. SHALINI** under my guidance and supervision. The subject of the thesis has been approved by the student’s Advisory Committee.

No part of the thesis has been submitted for any degree or diploma. The published part has been fully acknowledged by the author of the thesis.


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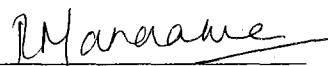
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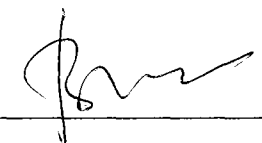
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DECLARATION

I, **Ms. D. SHALINI**, hereby declare that the thesis entitled “**INDUSTRIAL EFFLUENTS CONTAMINATED AND BIOREMEDIATED SOILS – EFFECT ON HEAVY METAL CONTENT IN EDIBLE CROPS AND HUMAN HEALTH**” submitted to Acharya N. G. Ranga Agricultural University for the degree of **DOCTOR OF PHILOSOPHY IN HOME SCIENCE**, is a result of the original research work done by me. I also declare that the thesis or part thereof has not been published earlier elsewhere in any manner.

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LIST OF ABBREVIATIONS

%	Percent
Kg	Kilogram
g	Gram
mg	Milligram
dl	Decilitre
µg	Microgram
cm	Centimeters
Fig.	Figure
Pb	Lead
Cd	Cadmium
Cr	Chromium
Co	Cobalt
Ni	Nickel
Ca	Calcium
P	Phosphorus
Fe	Iron
E.C	Electrical Conductivity
DTPA	Diethylene Triamine Penta Acetic acid
EDTA	Ethylene Diamine Tetra acetic Acid
NCYC	National Collection of Yeast Cultures
AMB	Agricultural Microbiology
BMI	Body Mass Index
FYM	Farm yard manure
°C	Degrees centigrade
dsm ⁻¹	deci siemen per meter
ppm	parts per million
Kg/ha	Kilogram per hectare
GLVs	Green leafy vegetables

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ABSTRACT

Industrialization has provided livelihood and opportunities to millions in urban India. However in recent years, a great deal of concern has been expressed over problems of soil pollution with heavy metals due to rapid urbanization and industrialization. The ecological consequences of heavy metal pollution of soils relate largely to heavy metal mobility and solubility. These chemically related factors determine transmission through the soil to the water table, agricultural crops and animals and ultimately to the human population. Bioremediation is a general term used to describe the destruction of contaminants by biological mechanisms, including micro organisms (eg : Yeast, fungi or bacteria), in contaminated soil and water. Hence the present has been planned to find the effect of industrial effluents contaminated and bioremediated soils on heavy metal content in edible crops and human health

Telangana zone from Andhra Pradesh was selected for the study based on its proximity to metropolitan city, Hyderabad where rapid industrialization had taken place. Two districts from Telangana region namely Ranga Reddy and Medak were selected as they are surrounded by highly polluted industries like synthetic organic chemicals, oil refineries and textiles, tanneries, electroplating units and distilleries etc.

One mandal from each of the districts i.e. Uppal (Ranga reddy district) and Ramchandrapuram (Medak district) were selected for the study as the farmers residing in these mandals use industrial effluent contaminated water for irrigation of crops.

Three villages from polluted areas of mandals were selected i.e. Cherlapalli (Uppal mandal), Patancheru and Ramchandrapuram (Ramchandrapuram mandal). One village i.e. Munagala (Munagala mandal) was selected from Nalgonda district for comparison as it is away from the industrial area.

The farm families from the selected villages were interviewed at random for collection of general information pertaining to agriculture (crops grown, farm practices, irrigation and marketing etc.) and nutritional aspects (consumption pattern, morbidity, cooking practices, food fads and fallacies etc.). BMI was calculated (Weight in kg/height in meters²) and compared with standard classification.

The soil, water and food samples collected from the selected village farms and blood samples collected from the farmer families were analyzed for heavy metals and micronutrient content using standard procedures.

Majority (25%) of the members were suffering from skin rash, which might be due to excessive consumption of nickel through edible crops and is also evident from high concentration of nickel in serum samples of the subjects.

Though soil and water analysis shown to have heavy metal content on upper side of safe limits, the crops grown in the fields found to have significantly higher content of heavy metals.

The poor yield in both polluted and un-polluted land might be due to contamination of land with industrial pollutants and / or scarcity of water for irrigation.

Pot culturing trials were conducted in the laboratory using the soil and irrigated water procured from the land identified to be highly polluted i.e. patancheru from Ramchandrapuram mandal (Medak district) using four yeast strains – *Saccharomyces cerevisiae* NCYC 1190, imported from National Collection of Yeast Cultures, United Kingdom and three locally isolated yeast cultures namely AMB 111, 112 and 113 in liquid form with FYM and varied concentrations of sugar.

A short duration green leafy vegetable crop – fenugreek was grown in the soils and harvested after 10 days of sowing for analysis of heavy metal and micronutrients.

Based on the results, the yeast culture AMB 113 with FYM and 3 percent sugar concentration was found to be effective in remediation of heavy metals and hence used for conducting field trials as is evident from significant reduction in heavy metals and micronutrient concentration of soil and green leafy vegetable and similar findings were obtained in location specific field trials too.

The study thus signifies the usefulness of microbial processes to clean up metallic contaminants, which in turn reduce the entry of toxic heavy metals into human food chain.

INTRODUCTION

CHAPTER I

INTRODUCTION

Industrialization has provided livelihood and opportunities to millions in urban India. However, it was also brought in its wake problem of waste disposal, contamination of environment – air, soil, surface water bodies and ground water aquifer etc., which have resulted in contamination hazard imperiling human beings, livestock and plant life.

Metropolitan region often act as nodes for rapid changes in land use / land cover in their neighborhood causing environmental degradation and pollution of natural resources.

Pollution of ground water and soil is a worldwide problem that can result in uptake and accumulation of toxic chemicals in food chains and contaminate the sites with numerous pollutants, which can constitute a risk to health of humans, plants, animals and or the environment. Many of these sites threaten to become sources of contamination of soil and drinking water supplies and thereby constitute a substantial health hazard for current and future generations (The Hindu, November 6th, 2003).

In recent years, a great deal of concern has been expressed over problems of soil pollution with heavy metals due to rapid urbanization and industrialization (Leeper, 1978).

The ecological consequences of heavy metal pollution of soils relate largely to heavy metal mobility and solubility. These chemically related factors determine transmission through the soil to the water table, agricultural crops and animals and ultimately to the human population (Leeper, 1972 and Emmerich et al., 1982 a&b).

Uptake of metals by plants reflects increasing levels of metals in soils and water used for irrigation (Kabata Pendias and Pendias, 1984). Continuous application of industrial effluents for a number of years results in accumulation of toxicants in topsoil and crop plants being grown thereon (Totawat et al., 1994).

Metal toxicity in plants can cause shortening of roots, leaf scorch, nutrient deficiency, increased vulnerability to insect attack and decrease in yield and or crop quality (Roane et al., 1994).

Contamination of plants by spreading of industrial effluents and by adhering soil splash and soil dust also contributes to the metal uptake by farm animals. Surface contamination of food plants and the direct ingestion of soil together with forage plants can complicate predictions on health hazard to both animals and humans (Healy, 1973).

Human exposure to heavy metals causes a wide range of medical problems such as heart disease, liver damage, cancer, neurological problems and central nervous system damage (Roane et al., 1994).

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Human exposure to heavy metals causes a wide range of medical problems such as heart disease, liver damage, cancer, neurological problems and central nervous system damage (Roane et al., 1994).

The above-mentioned problems pose an urgent need for remediation of toxic compounds. Suggested measures for remediation include amelioration, waste management, recycling, waste minimization, punitive action against defaulters (Biswas, 1997).

Current technologies for the remediation of polluted environments that rely on non-biological, physical-chemical approaches are often cost prohibitive. Microorganisms that can convert toxic compounds to harmless products have been increasingly used as a cheaper alternate for remediation of toxic metals.

Microorganisms said to exist naturally in the environment and have the capability of degrading the contaminants.

Bioremediation is the term that describes the use of microorganisms to remove or reduce human made pollution. It is the process in which organisms metabolize contaminants either through oxidative or reductive processes. Under favorable conditions, microorganisms said to oxidatively degrade contaminants into non-toxic by-products such as water and carbon dioxide (Mac Donald, 1993).

Saccharomyces cerevisiae is said to remove heavy metals such as zinc, cadmium, chromium, nickel and magnesium from industrial waste waters (Dostalek et al., 1996 and Kambe et al., 1998).

Microorganisms need appropriate environmental conditions like appropriate pH, temperature, nutrients and oxygen for their survival and growth.

However, not all sites have competent microbes and typically lack environmental conditions conducive for rapid degradation of the contaminants.

Bioremediation has been used for remediation of polluted water since many years, but literature available on remediation of polluted soils is scanty and hence the present work has been planned to study the effect of selected yeast cultures on heavy metal content in polluted soils used for farming purpose with the following objectives :

General objective :

To find the effect of industrial effluents contaminated and bioremediated soils on heavy metal content in edible crops and human health.

Specific objectives :

- To collect general information on cultivation, consumption practices, health and nutritional status of farm families in the selected areas.
- To analyze the toxic metals (Pb, Cd, Cr, Co & Ni) and micronutrients (Ca, P & Fe) in blood / serum samples collected from the sub-grouped farm families.
- To estimate the heavy metals (Pb, Cd, Cr, Co & Ni) and micronutrients (Ca, P & Fe) in the edible crops grown in the fields.
- To conduct on-farm bioremediation trials using selected microbial cultures.

- To estimate the heavy metals (Pb, Cd, Cr, Co & Ni) and micronutrients (Ca, P & Fe) in soils before and after the bioremediation trials.
- To analyze the heavy metal content in edible crops grown in bioremediated soils.

REVIEW OF
LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Industrial world has contaminated soil, sediment and water sources with hazardous materials. Lead, cadmium, chromium, cobalt and nickel are said to be prevalent at highly contaminated sites.

In recent years, a great deal of concern has been expressed over problems of soil pollution with heavy metals due to rapid urbanization and industrialization (Leeper, 1978).

The ecological consequences of heavy metal pollution of soils relate largely to heavy metal mobility and solubility. These chemically related factors determine transmission through the soil to the water table, agricultural crops and animals and ultimately to the human population (Leeper, 1972 and Emmerich et al., 1982 a&b).

Remediation of contaminated sites is the must to avoid deleterious effects such as heart disease, liver damage, cancer, neurological problems and central nervous system damage. The information reviewed with this regard is presented under following heads:

- 2.1 Pollution Scenario**
- 2.2 Ecological consequences of heavy metal pollution of soils – crop – animal system**
- 2.3 Heavy metals in soils and plants**
- 2.4 Bioremediation**
- 2.5 Heavy metals – effect on human health**

2.1 Pollution Scenario

All things in nature ultimately succumb to decay. Much of this is a natural consequence of the laws of thermodynamics. Many molecules degrade by the action of oxygen, halogens and radicles naturally found in the environment. A large proportion of materials degrade because their components are subject to the action of enzymes. Unfortunately for humans, many of the wastes that we produce do not decay as fast as we would like. They end up polluting the air, land and water. Two major factors prevent our wastes from decaying rapidly. One is that we produce so much at one time that the rate of natural decay is insignificant compared to the amount present. Another factor is that most wastes end up in the areas not conducive to rapid degradation.

Swarup and Patra (2005) reported that environmental pollution is a major global problem posing serious risks to man and animals. It is defined as the human alteration of chemical or physical characteristics of the environment to a degree

that is harmful to living organisms. Rapid pace of urbanization, industrialization and indiscriminate and improper use of chemicals such as pesticides and drugs have resulted in increased contamination and degradation of the environment leading to adverse health effects on living beings and problems associated with residues in food of animal origin. The deleterious health effects may be observed in the form of overt clinical signs and higher morbidity and mortality or as subtle as sub-clinical effects.

Krishna and Govil (2004) reported that rapid industrialization, urbanization and intensive agriculture in India increased contamination of heavy metals in soils. It is also reported that high concentration of heavy metals (Pd, Cd, Cr, Co and Ni) in soil are responsible for the development of toxicity in agriculture products, which in turn affects human life.

The contaminated sites often contain numerous pollutants, which can constitute a risk to health of humans, animals and or the environment. Although substantial progress has been made in reducing industrial releases over recent years, major releases still occur; a considerable number of known polluted sites exist and new ones are continually being discovered. Many of these sites threaten to become sources of contamination of soil and water supplies and thereby constitute a substantial health hazard for current and future generations (The Hindu, November 6th, 2003).

In addition to health hazards, the industries have turned agricultural lands into chemical deserts and turned water bodies into cess-pools of poison. This affected severely not only humans but also animal and agriculture. Long term and indiscriminate application of raw sewage effluent to agricultural field without prior treatment that contains heavy metals in association with suspended solid (sludge) particles may cause accumulation of toxic metals in soils (with subsequent transfer to food chain) – The Hindu, November 6th, 2003.

According to the Union Ministry of Health and Family Welfare, in India, around 5, 63,000 people are affected annually, one fourth of which are children. In the state of Andhra Pradesh around 6000 industries are registered by 2002 – 03, out of which around 2000 are large and medium and around 4000 are small scale industries. Of these registered industries, around 5000 industries are categorized as “Polluting industries” (Jeevananda S, 2006) (Plate 1).

Majority of the polluting industries are located in and around the capital city. Major percentage of industries in these industrial areas consists of synthetic organic chemicals, oil refineries and textiles, tannaries, electroplating units and distilleries (Jeevananda S, 2006).

Hyderabad is one of the fastest growing cities and considered as a cosmopolitan city with its population as varied as Indian population. Hyderabad has a growth rate exceeding 5%. In Andhra Pradesh, other cities like Vishakapatnam, Vijayawada and Tirupathi, do not match this growth. There are

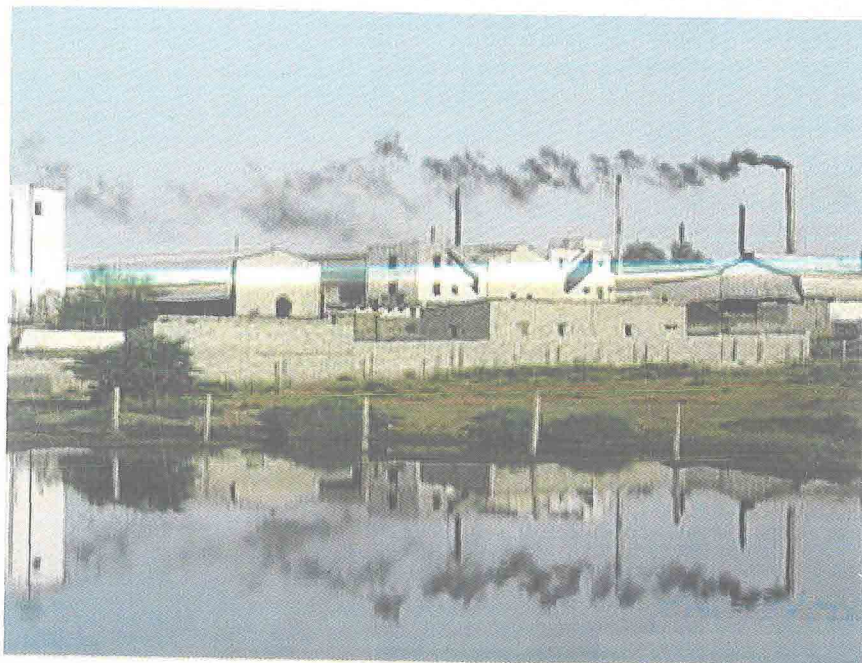


PLATE 1 : VIEW OF HIGHLY POLLUTED INDUSTRIAL AREA

several factors which necessitated such a migration; categorized as 'push' and 'pull' factors. Push factors are those, which induce migration leaving the people no choice, but to migrate to Hyderabad. There are pull factors even. Firstly, it is the seat of power structure. Secondly, it houses several industrial areas like Patancheru, Ramchandrapuram, Cherlapalli, Jeedimetla, Pocharam, Katedan and many more. Thirdly, it has become an important centre for national and international research organizations and institutes like CCMB, IICT, NGRI, NIN, CFTRI etc.

This population shift resulted in enormous pressure for shelter and services fraying the infrastructure. This haphazard growth had its consequential effects on the communities and the environment too (Jeevananda, 2006).

Haphazard growth of Hyderabad has degraded natural resources like land, water and air. Environmental pollution has reached alarming levels in the last 5 to 6 years. This has been chiefly due to industries. Most of the industries are in the midst of residential areas, with no proper drainage system and without any effective monitoring of the industrial discharges. Establishment of IDPL (Indian Drugs and Pharmaceuticals Limited), resulted in the proliferation of chemical and pharmaceutical industries in Jeedimetla and surrounding areas (Jeevananda S, 2006).

There are many highly polluting industries in Patancheru which lack even minimum pollution treatment facilities. Almost 25 villages are affected by the

industrial pollution. Crops are damaged, land is degraded, general health of population has declined sharply and cattle are dying in numbers. Patancheru has been identified by Central Pollution Control Board as one of India's 22nd most critically polluted areas.

Improper and unscientific disposal of urban wastes may pollute air, water and soil in several ways. One of the critical public health hazards from a sanitary landfill is due to the gases emanating from the decomposition of the organic matter under partially anaerobic conditions. Of the gases generated (carbon dioxide, carbon monoxide, methane, hydrogen and hydrogen sulphide), methane and hydrogen can be explosive. Carbon dioxide can cause acidic reactions when dissolved in water and can be the cause for corrosion problems.

Industrial sources of heavy metals include mining, refinement, transport of ores, smelting and metal finishing. High temperature processing of metals such as smelting and castings emit metals in particulate and vapour forms. These may be either dispersed by wind or precipitated in rainfall causing contamination of soil or water bodies. Due to discharge of unregulated industrial effluents, the water quality is degraded and loaded with toxic chemicals, resulting in acidification, increase in total dissolved solids, decrease in dissolved oxygen and decrease in biological diversity. Contamination of soil and water bodies can also occur through runoff from erosion of mine wastes, dusts produced during the transport of crude ores, corrosion of metals and leaching of heavy metals from industries to

soil and ground water. Other industrial sources of pollution include processing of plastics, textiles, micro electronics, wood preservation and paper processing (Kraal and Ernst, 1976).

Soil and water pollution have had a direct impact on crop yield and the food cycle. High total dissolved solids and chemical intermediates have degraded the soil. Yields have suffered due to the increase in salinity, loss of living structure of the soil complex and change in the physical and chemical properties of the soil. Yields have significantly decreased in terms of quality as well as quantity.

Like all organisms, plants are often in a state of dilemma that some heavy metal ions are essential micronutrient, while the same at higher concentrations and even more ion such as cadmium, mercury, arsenic, lead, cobalt, nickel etc. are strongly poisonous to the metabolic activities (Reeves and Baker, 2000).

The dry matter and yield of many higher plants such as pea, wheat, rapeseed, maize and paddy have been reported to decrease under multiple heavy metal stress (Purohit et al., 2003).

It is no surprise that the deadly bio-accumulative chemicals in the environment, are damaging the health of all living things. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, chromium, nickel, mercury, arsenic and many more. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as WHO. Mostly edible parts of plants are the major

source of heavy metal intake for human through consumption, which have long term detrimental effects on human health such as anemia, damage to pancreas, impairment of neurobehavioral and psychological functions, indirect effect on heart, skin damage and many other disorders (Athar and Vohora, 1995).

2.1.1 Water pollution and heavy metals in Drinking water

Earth is a water rich planet. The total available water on the basis of current estimate is $1.4 \times 10^9 \text{ Km}^3$. Ocean water accounts for 97.3% and remaining 2.7% is portable water.

Nanoti and Hasan (1984) reported that surface water in rivers, streams and lakes accounts to less than 0.5% of available fresh water, which constitutes the basic available supply.

Lead content of lake and river waters, world wide has been estimated to range from 1-10 $\mu\text{g/L}$. The levels in drinking can be much higher due to leaching of lead from lead service pipes and household plumbing. In the same water supply area, there may be considerable house-to-house variation in the levels of lead in water because of differences such as, length of pipe; water usage patterns and type of deposit that build-up in pipes.

Contamination of water bodies with the nickel can arise from industrial discharge of effluent containing nickel compounds. Some nickel is removed by

conventional water treatment and thus levels of nickel are generally low in consumer water supplies.

Drinking water normally contains very small amounts of cadmium. There have been no reports of adverse effects from the low levels of cadmium on human and animal health.

Giving due regard to the conditions prevailing in India relating to the provision of safe drinking water supplies, the progress of the schemes under various National and State development programs and the need for prescribing standards of bacteriological and chemical quality which safeguard the health of the consumers and at the same time liberal enough to permit the acceptance of supplies which are satisfactory as regards their sanitary conditions, Bureau of Indian Standards (BIS) have prescribed drinking water standards (Table 1).

Table 1 : Critical permissible limits for Drinking water

Element	Microgram/Liter
Lead	0.05
Cadmium	0.01
Chromium	0.05
Cobalt	0.005
Nickel	0.05
Iron	0.30

Source : BIS, 1991

Levels of chromium in water are usually low due to its poor solubility. It generally exists as trivalent chromium (Cr^{3+}) and hexavalent chromium (Cr^{6+}). Trivalent chromium rarely occurs in drinking water and is considered practically non-toxic. It is assumed that most water borne chromium is in the hexavalent form, which in high doses has been implicated as the cause of cancer of the digestive tract in man.

2.1.2 Ground water pollution

The degree and extent of pollution of ground water depends primarily on the quality and concentration of the pollutants and the porosity, permeability, depth of the water table, storage capacity of the aquifer as well as the time factor.

When applied on the surface, the pollutants travel largely downward till they reach the water table and then move laterally. Shallow water table increases the risk of pollution. The distance to which pollutants travel will vary with each quality component as each constituent of a pollution source may have a different attenuation rate.

An important aspect of ground water pollution is the fact that it may persist under ground for years, decades or even centuries. This is in marked contrast to surface water pollution.

Ayers and Westcot (1976) have prescribed recommended maximum concentrations of trace elements in irrigation waters (Table 2).

Table 2 : Recommended maximum concentrations of trace elements in irrigation water

Element	Parts per million
Cadmium	0.01
Chromium	0.10
Cobalt	0.05
Lead	5.00
Nickel	0.20
Iron	5.00

Source : Ayers and Westcot, 1976

2.1.3 Soil pollution

Soil is a critical component controlling early stages of ecosystem development and is also key element directing ecosystem maturation. Bradshaw, 1997 reported that degraded soils can inhibit natural succession or ecological restoration through physical hostility, such as severely compacted soils, deficiency or occasionally, an excess of nutrients and through toxins, which inhibit plant growth.

Chapman H.D. (1975) have prescribed recommended maximum concentrations of trace elements in soils (Table 3).

Table 3 : Recommended maximum concentrations of trace elements in soils

Element	Part per million
Lead	5.0
Cadmium	0.5
Chromium	2.0
Cobalt	2.0
Nickel	2.0
Iron	10.0
Zinc	2.0
Copper	5.0
Manganese	10.0

Source : Chapman (1975)

Le Riche (1968) first reported metal contamination of soil result in long term use of sewage sludge. Leeper (1978) have stated that in recent years, a great deal of concern has been expressed over problems of soil pollution with heavy metals due to rapid industrialization and urbanization. The heavy metals are part of nutritional chain from soil to plants to animal and human beings.

Lee et al. (2003) observed that larger parts of agricultural soil are contaminated with lead and cadmium. Although most environments are not heavily contaminated, the low levels observed nonetheless pose a high risk of heavy metal accumulation in the food chain.

2.2 Ecological consequences of heavy metal pollution of soils – crop – animal system

The ecological consequences of heavy metal pollution of soils relate largely to heavy metal mobility and solubility. These chemically related factors determine transmission through the soil to the water table, availability to soil microbes, agricultural crops and animals and ultimately to the human population.

Leeper (1972); Emmerich et al. (1982) reported that leeching of heavy metals through soils to ground water, even in soils treated with sewage sludge was not to occur to any appreciable extent.

At moderate levels of heavy metal concentrations, above the deficiency range, most natural soils act as a repository or sink for metals without any obvious effects on soil biological behavior. The important question is the response of plants and animals to increasing concentration of metals that result from natural variability or from pollution. The extent of transfer of metals from soil to plant depends on the kind of plant, the particular metal involved and the soil chemical environment.

Uptake of metals by plants reflects increasing levels of metals in soils, especially the mobile ones. Above certain critical concentrations of heavy metals in plants, yield and or crop quality and animal or human health may be affected. Concentrations of toxic metals require careful monitoring, because potentially dangerous levels may be reached in the plant food diet of animals or humans

without any evidence of deleterious effects on the plants. This is especially so for cadmium and lead for which plant toxicity occurs above the concentrations considered desirable for human intake. Chapman H.D. (1975) prescribed recommended maximum concentrations of trace elements in plants (Table 4).

Table 4 : Recommended maximum concentrations of trace elements in plants

Element	Part per million
Lead	5.0
Cadmium	1.0
Chromium	2.0
Cobalt	2.0
Nickel	2.0
Iron	150.0
Zinc	20.0
Copper	6.0
Manganese	50.0

Source : Chapman (1975)

Page et al. (1981) reported that heavy metal pollutants rarely occur alone. The associated elements, frequently other heavy metals and possibly major nutrients also can affect the biological effectiveness of the metal in question by additive, synergistic, or antagonistic effects.

The impact of heavy metal pollution on health also depends on the nature of the association of heavy metals with plants. The heavy metals associated with

plants are not necessarily absorbed by the root system and trans-located within the plant. In some regions, appreciable amounts of some heavy metals can adhere directly to leaf surfaces after deposition from the atmosphere. Contamination of pasture plants by the spreading of agricultural waste slurries and by adhering soil splash and soil dust also contributes to the metal intake by farm animals.

Healy (1973) reported that surface contamination of food plants and the direct ingestion of soil together with forage plants can complicate predictions on health hazard to both animals and humans.

O'Connor (1998) stated that the DTPA (Diethylene Triamine Penta Acetic acid) soil test generally reflects the labile metal load of a soil. Increases in DTPA extractable metals, however, do not mean increases in plant available metal or increased plant uptake.

Corey et al. (1987) reported that if initial soil nutrient concentrations are already sufficient, further increases in nutrient concentration may increase plant metal concentrations.

Singh and Verloo (1996) observed that prolonged irrigation with metal contaminated sewage effluents from urban sources enhances the accumulation of heavy metals (Pb, Cd, Cr, Co, Ni, Cu, Fe, Mn and Zn) in the top plough layer of soil in fields.

Jeyabaskaran and Ramulu (1996) observed that the DTPA extractable Fe, Cu, Mn, Pb, Cd, Cr, Ni and Co accumulated in the surface of the soil, decreased with both increasing soil depth and distance from the sewage entry point.

The availability of metals and uptake by plants are related both to their total concentrations, to their forms and associations in the soil and to a number of physical and chemical factors operating at the soil-root interface. Different species and indeed different cultivars, regulate metal uptake at both the soil-root and root-shoot interfaces to varying degrees.

Fleming (1965) reported that the trace element content of the whole plant tends to increase with increasing maturity and are also influenced by seasonal changes.

King et al. (1974) observed that levels of cadmium and lead in the rye and corn plants were increased by waste additions but were below levels toxic to the crops or to animals that might consume the crops.

Spittler and Feder (1979) studied growth and lead uptake of plants in garden soils containing a range of lead levels (100 – 2000 ppm). There was a positive correlation between soil lead and plant uptake although uptake varied with the plant species (fruit crops, root crops and leafy vegetables). Plant tops contained a higher lead concentration than roots.

Sit et al. (2002) reported that the uptake of micro nutrients (Fe, Zn, Cu and Mn) and heavy metals (Pb, Cd, Cr and Ni) were greater in rice (*Oryza sativa*) and Lal-saag (*Amaranthus* species) grown under sewage enriched conditions.

Chu and Wong (1987) stated that despite the relatively high heavy metal contents of compost, crops grown on compost treated soils accumulated lower levels of metal than those grown on sludge treated soils.

D'Souza and Ramchandran (1984) studied the uptake of Fe, Mn, Cu, Zn, Co, Pb, Ni, Cd and Cr by maize (*Zea mays* L) grown on a laterite soil (oxisol) and medium black soil (vertisol) treated with different doses (0, 56, 112, 224 and 448 t / ha) of city compost from Mumbai (India). The results revealed that city compost increased the concentration of Co, Pb, Ni and Cd in the plant.

2.3 Heavy metals in soils and plants

2.3.1 Lead (Pb)

The presence of indigenous lead in the earth's crust at concentrations averaging 16 ppm in soil (De Treville, 1964) and the knowledge that lead is highly toxic to various physiological process have brought into focus over the last two decades the question of the effects of lead on higher plants and the consequences of the heavy metal entering the food chain.

Koeppel (1980) reported that lead is available to plants from soil and aerosol sources. The actual uptake and association of lead with plants is affected to varying degrees by almost all environmental factors.

Liebhardt and Koske (1974) reported that addition of organic matter reduced the uptake of lead into plant root. Miller et al. (1975) found that lead uptake by corn and soy beans were negatively correlated with soil pH.

Miller et al. (1977) reported that the effect of heavy metals together was greater than the sum of the effects of the individual heavy metal on the uptake of plants.

2.3.2 Cadmium (Cd)

Increases in plant cadmium from sewage wastes and refuse applications on crop land are a major issue in the safety of land disposal. Dowdy and Larson (1975a&b) found two to four fold higher levels of cadmium in nearly every crop tissue from large applications of sludge.

John et al. (1972) found that cadmium adsorption capacity, exchangeable cadmium levels, soil pH and soil organic matter content were significantly related to cadmium uptake by oats from cadmium amended soils.

Takijima and Katsumi (1973) found that soil pH had important control on cadmium uptake. Low soil pH favored uptake, while reducing conditions hindered uptake.

Baker and Chesnin (1976) discussed the movement of cadmium into the food chain. It appears that increase in crop cadmium can be kept low by excluding cadmium wastes from sewers.

Chaney (1973) observed that the concentration of cadmium in the edible seeds of fruits generally is lower than in leafy vegetables. The concentration of cadmium in the kernels of corn (*Zea mays* L) has been especially low, even when the corn is grown in soils treated with heavy rates of high cadmium sludge.

2.3.3 Chromium (Cr)

Baetjer (1974) reported that in the earth's crust chromium is fairly abundant, ranking fourth among the 29 elements of biological importance and is more abundant than many essential elements including cobalt, copper, zinc, molybdenum, nickel, lead and cadmium.

Baetjer (1974) also stated that chromium may enter sewage treatment works as chromate from the tannery effluents but is apparently reduced to the chromic form.

Swaine (1955) after a comprehensive review, reported that the chromium in soils is usually in the range of 5 to 1,000 ppm.

De Haan (1978) showed that despite high concentrations of chromium in sludge, plant contents were variable. For example, on a sludge containing 1700 ppm chromium, grass contained 67 ppm. While on another sludge containing 1800 ppm chromium, grass contained only 5.6 ppm chromium.

Lounamaa (1956) reported that lichens and mosses accumulated more chromium than the leaves of higher plants.

Mertz (1969) observed that fruits and seeds are especially low in chromium containing 20 ppb and cereal grains 40 ppb chromium.

2.3.4 Cobalt (Co)

Manson (1966) stated that in the earth's crust, the average concentration of cobalt is 23 ppm. Swaine, 1955 reported that the total cobalt content of soils was with in the range of 1 – 40 ppm.

Mitchell (1945); Hill et al. (1953); Kubota and Allaway (1972) reported that the cobalt content of various plant species, when grown under the same conditions vary markedly. In general, legumes and cereal forages are high in cobalt and underground portions of vegetables are relatively low in cobalt.

Kubota (1965); Handreck and Riceman (1969) reported that a variety of factors effect the cobalt status of plants such as soil composition, soil moisture and pH and plant factors such as stage of growth and plant species.

Vanselow (1966) reported that cobalt produces toxicity to plants when the amounts available exceed certain limit. But that a naturally occurring excess was apparently not toxic. For example *Nyssa sylvatica* can contain thousands of ppm cobalt in their leaves yet show no toxic symptoms, whereas, as low as leaf content

as 11 ppm in citrus plant produced marked growth depression and chlorosis resembling iron deficiency.

2.3.5 Nickel (Ni)

According to Swaine (1955) soils normally contain 5 to 500 ppm nickel with an average of about 100 ppm.

Anderson and Nilsson (1972a&b); Page (1974); Maclean and Dekkar (1978); Soon et al. (1980) have observed that additions of nickel from sewage sludge, even though much of it is organically bound, leads to an increase in both soil and shoot levels of nickel in a wide range of crop plants.

The toxic properties of excessive nickel in soils for plant life was first pointed out by Hoselhoff (1893), who demonstrated its toxicity to corn and bean plants using solution culture techniques. Wolfe (1913) found that 8 ppm nickel in solution rapidly killed barley. Cotton (1930) found that 5 ppm nickel produced chlorosis in wheat. Brenchley (1938) reported 2 ppm nickel to be toxic to bean and barley. Millikan (1949) stated that Flax (*Linum usatissimum*) was found to be sensitive, showing toxicity at 0.5 – 5 ppm.

Vanselow (1966) provided an extensive compilation of data on nickel toxicity from the literature, which showed that for most crop plants, the nickel content is less than 2 ppm of dry weight. Tissue concentrations of greater than 50 ppm could be considered excessive.

Halstead et al. (1969) found a decrease in growth of oats when the nickel content of the grains exceeded 60 ppm.

Mishra and Kar (1974) described the main toxic symptoms of nickel as chlorosis or yellowing of the leaves, followed by necrosis.

Page (1974) reported that nickel concentrations in sludge reach 3000 ppm or more and the plants grown on a sludge treated soils are substantially higher in nickel than control.

2.4 Bioremediation

Metals are naturally found in trace amounts in the soil around the world. Throughout the world, these trace amounts of metals are what help to faster good plant development. Roane et al. (1994) reported that without the trace amounts of metals, organisms would lack optimum development. Thus, plants would be small, have smaller yields, and be more susceptible to diseases. However, metals at high concentrations can also be toxic to micro organisms, impacting microbial growth, morphology and biochemical activities.

Roane et al. (1994) reported that the most toxic metals to the soil are non-essential metals such as cadmium and lead.

Alloway (1990) observed that soil highly contaminated with metals may disrupt the physical, chemical and biological balance of the soil.

Roane et al. (1994) reported that lead, cadmium, chromium, nickel, cobalt, manganese, silver and tin can be found in high concentrations in contaminated soils. He also reported that metal toxicity in plants can cause shortening of roots, leaf scorch, nutrient deficiency and increased vulnerability of insect attack.

There are two main strategies for accomplishing soil decontamination; physical decontamination and bioremediation. Physical decontamination is conducted using techniques such as soil washing and soil vapor extraction. Physical decontamination strategies can be used on a variety of both organic and inorganic toxins, the techniques are readily available commercially, and their efficacy rate is relatively high. However, some drawbacks of physical decontamination techniques are that they are generally conducted ex-situ (outside the site), resulting in greater site disturbance, costs are high and these methods usually require detoxification or immobilization of contaminants that were in the soil and after decontamination are present in solvents or other soil cleaning materials.

Researchers developed bioremediation as a way to accelerate or encourage the degradation of pollutants. Bioremediation is a term that describes the use of organisms to remove or reduce human made pollution. At first bioremediation was a curiosity, today it is seen as a cure-all for all types of pollution, including radioactive isotopes.

Bioremediation is defined by the American Academy of Microbiology (AAM) as “the use of living organisms to reduce or eliminate environmental hazards resulting from accumulation of toxic chemicals and other hazardous wastes” (Gibson and Sayler, 1992).

Jyotsna – Lal (2004) reported that many microbial species exhibit metal binding properties. Both active and passive mechanisms are employed by living cells to accumulate toxic metals.

NABIR (2003) reported that bioremediation was used over 100 years ago with the opening of the first biological sewage treatment plant in Sussex, United Kingdom, in 1891. The study revealed that microbial processes are beginning to be used to clean up radioactive and metallic contaminants – two of the most common and most recalcitrant components of hazardous waste sites.

Microbes can convert contaminants to less harmful products. National Research Council, 1993 reported that in addition to conversion ability, micro organisms can cause contaminants to be demobilized.

Cynthia and David (1997) reported that the mobility of metals is primarily caused by reactions that cause metals to precipitate or chemical reactions that keep metals in a solid phase.

Bioremediation is a general term used to describe the destruction of contaminants by biological mechanisms, including micro organisms (eg : Yeast, fungi or bacteria), in contaminated soil and water.

Bioremediation may rely on either indigenous micro organisms (those that are native to the site) or exogenous micro organisms (those that are imported from other locations). In other case, bioremediation technologies optimize the environmental conditions so the appropriate micro organisms will flourish and destroy the maximum amount of contaminants.

Bacteria, fungi and protista are very good at degrading complex molecules and incorporating the breakdown products into their metabolisms. The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms. Yeasts are good at degrading complex organic compounds that are normally not degraded by other organisms.

Bioremediation can take place under aerobic or anaerobic conditions. Under aerobic conditions, organisms consume atmospheric oxygen in order to function. Under anaerobic conditions, no oxygen is present and organisms breakdown chemical compounds in the soil to release the energy they need.

2.5.1 Technologies of bioremediation :

Technologies using bioremediation treatment include bio-stimulation, bio-augmentation, bio-filters, bio-reactors, bio-venting, composting and land farming.

Bio-stimulation :

Bio-stimulation is the use of nutrients or substrates to stimulate the naturally occurring organisms that can perform bioremediation. Fertilizer and growth supplements are the common stimulant. The presence of small amounts of the pollutant can also act as a stimulant by turning on operons for the bioremediation enzymes.

Bio-augmentation :

This is a general term describing the addition of organisms or enzymes to a material to remove unwanted chemicals. Bio-augmentation is used to remove byproducts from raw materials and potential pollutants from waste.

The targeted organic contaminants either serve as the food source or are co-metabolized. Essential elements are added to the "food source" to provide the required nutrient levels, and water provides the media in which the organism functions.

Bio-filters :

It is the removal of organic gases by passing air through compost or soil containing microorganisms capable of degrading the gases. It has been used to remove volatile organic compounds (VOC's) from air.

Bio-reactors :

It is the treatment of a contaminated substance in a large tank containing organisms or enzymes. Bioreactors are commonly used to remove toxic pollutants from solid waste and soil.

Bio-venting :

This is similar to bio-stimulation. It involves the venting of oxygen through soil to stimulate the growth of natural and introduced bioremediation organisms. This is used predominantly for soils contaminated with petroleum products.

Composting :

This involves mixing contaminated materials with compost containing bioremediation organisms. The mixture incubates under aerobic and warm conditions. The resultant compost can be used as a soil augmentation or be placed in a sanitary landfill.

Land farming :

Land farming is the use of farming tilling and soil amendment techniques to encourage the growth of bioremediation organisms in a contaminated area. It has been used successfully to remove large petroleum spills in soil.

These technologies are classified as either in-situ or ex-situ. In-situ technologies are the ones commonly seen in the media. *In-situ* bioremediation treats the contaminated water or soil where it was found. *Ex-situ* bioremediation processes involve removing the contaminated soil or water to another location before treatment.

Francis (1997) reported that microbial transformation of toxic metals and radio nuclides may affect their solubility, mobility and bioavailability. Several of the key microbial processes may affect mobilization or immobilization of toxic elements by one or more of the following mechanisms :

- Chelating of elements by metabolites,
- Oxidation-reduction of metals which affect the solubility or valence state,
- Changes in pH which affect the ionic state,
- Bio-sorption by functional groups on the cell surface,
- Bioaccumulation by an energy-dependent transport system,
- Immobilization due to formation of stable materials,
- Bio-methylation,
- Bio-degradation of organic complex of metals and radio nuclides.

Poole and Gadd (1989) reported that a wide variety of micro organisms, including bacteria, fungi, yeasts and algae can interact with metals and radio nuclides through several mechanisms to transfer them into non-toxic forms.

Gadd (1988) observed that toxic metals such as lead, cadmium, chromium, cobalt and nickel are carried out by an energy dependent transport system.

The advantages of microbe treatment are : environment friendly, self reproducibility, adaptability, recyclization of bi-products, specificity and good cost / benefit ratio.

2.5.2 Role of yeasts in Bioremediation :

Volesky and Holan (1995) reported that yeast cells are a mediocre metal biosorbent, when compared with algae and fungi.

Yeasts are inexpensive source of biomass, since they are a by-product of large-scale fermentations. Brady and Duncan (1994a); Blackwell et al. (1995); Avery and Tobin (1992) reported that yeasts accumulate a broad range of heavy metals to varying degrees under a wide range of external conditions. They are economically attractive for the treatment of a wide variety of metal bearing industrial effluents.

Metal uptake by yeasts typically occur in two steps : a metabolism – independent and metabolism – dependent step.

Avery and Tobin (1992); Brady and Duncan (1994a) observed that metabolism – dependent step (initial bio-sorption) is rapid and takes place during the first minutes of contact with the metal.

De Rome and Gadd (1987); Blackwell et al. (1995) reported that metabolism – independent step is independent of metabolic energy, temperature and metabolic inhibitors.

Bayhan et al. (2001) reported the removal of heavy metal ions Ni^{2+} , Cu^{2+} and Pb^{2+} by yeast (*Saccharomyces cerevisiae*) as carriers in a cross-flow micro-filtration.

Dahiya (1990) reported that *Saccharomyces cerevisiae* effectively accumulate metals by passive retention at the cell surface or by metabolism dependent intracellular accumulation.

Wilhelmi and Duncan (1995) observed accumulation of metal chlorides (Co, Cu, Zn, Cd, Ni and Cr) to immobilized forms by *Saccharomyces cerevisiae*. Desorption of over 90% was obtained and the biomass was reusable.

Joho et al. (1995) reported that nickel tolerant micro organisms i.e., *Saccharomyces cerevisiae* and *Neurospora crassa* are used for the removal of nickel from polluted environments.

Ashour et al. (1992) studied the tolerance and adsorption of heavy metals by *Saccharomyces cerevisiae*. The organism absorbed Ag^{5+} , Co^{2+} , Cr^{3+} , Ni^{2+} and Pb^{2+} . Nickel binding by *Saccharomyces cerevisiae* has an active transport process with the ratio of 1:10.

Soares et al. (2002) have reported the accumulation of lead and cadmium by *Saccharomyces cerevisiae* NCYC 1190 in metal salt solution. Cadmium

accumulated almost linearly in the first 40 minutes, with an uptake of approximately 214 nmol / mg (dry weight). The uptake of lead was approximately 210 and 344 nmol / mg (dry weight) after 60 and 120 minutes respectively.

2.5 Heavy metals – effect on human health

Mostly edible parts of plants are the major source of heavy metal intake for human through consumption, which have long term detrimental effects on human health. Several heavy metals are considered toxic metals due to adverse human health effects, when taken in excess. Heavy metals pose hazards to human health because these are persistent in nature and have accumulation tendency in biological systems.

2.4.1 Lead

The general population is exposed to Pb from air and food. Children are particularly susceptible to Pb exposure due to high gastrointestinal uptake, and the permeable blood brain barrier leading to neuro-toxic effects at low level of exposure (Jarup, 2003). The toxicity of Pb is caused by its direct interference with activity of different enzymes or displacing essential metal ions from metalloenzymes. The major exposure pathway of inorganic Pb is via ingestion and adsorption through the gastrointestinal tract, respiratory tract and inhalation.

Kidney and liver are considered potential targets of Pb toxicity before storage in bones (Athar and Vohora, 1995). Depending on the level of exposure, Pb has potential to cause a variety of biological effects such as decreased hemoglobin synthesis, impairment of neuro-behavioral and psychological functions, peripheral neuropathy, indirect effect on heart, renal tubular damage and reproductive problems (Brown and Kodama, 1986).

2.4.2 Cadmium

Food and cigarette smoking are the most important sources of Cd apart from water. Cd accumulates within the kidney and liver over long time (McLaughlin et al., 1999). Long term low-level exposure leads to cardiovascular disease and cancer. It is known to primarily affect renal tubular function of reabsorbing protein, sugar and amino acids (McLaughlin et al., 1999).

Cadmium exposure in conjunction with Ca, Fe, Zn, protein, fat and vitamin D deficiencies, led to ostomalacia and bone fractures in postmenopausal women in polluted Jintsu valley, Japan commonly referred to as Itai-Itai disease (Noda and Kitagawa, 1990).

2.4.3 Chromium

The harmful effects of Cr to human are mostly associated with its hexavalent form. Cr toxicity includes liver necrosis, nephritis and gastrointestinal

irritation (Athar and Vohora, 1995). Cr^{+6} can also produce cutaneous, nasal and mucous membrane ulcers and cause dermatitis by skin contact. Cr has potent carcinogenic effects on human beings and other animals (O'Brien et al., 2003).

2.4.4 Cobalt

Cobalt is a natural element found throughout the environment. Acute (short-term) exposure to high levels of cobalt by inhalation in humans and animals results in respiratory effects, such as a significant decrease in ventilatory function, congestion, edema, and hemorrhage of the lung. Respiratory effects are also the major effects noted from chronic (long-term) exposure to cobalt by inhalation, with respiratory irritation, wheezing, asthma, pneumonia, and fibrosis noted. Cardiac effects, congestion of the liver, kidneys, and conjunctiva, and immunological effects have also been noted in chronically-exposed humans (ATSDR, 1992).

2.4.5 Nickel

Nickel induces embryo toxic and nephrotoxic effects, allergic reactions and contact dermatitis (EPA, 2002). Nickel sensitization also occurs in general population from exposure to coins, jewellery, watch cases, clothing and fasteners. It causes conjunctivitis, asthma and local or system reaction to Ni containing

protheses such as joint replacements, cardiac valve replacements, cardiac pacemaker wires and dental inlays (Athar and Vohora, 1995).

Nickel is potential carcinogen for lung and may cause skin allergies, lung fibrosis and cancer of respiratory tract in occupationally exposed populations (Kasprzak et al., 2003)

MATERIALS &
METHODS

CHAPTER III

MATERIALS AND METHODS

The agricultural lands polluted with industrial effluents and bioremediated were studied for heavy metal content in edible crops grown in the soils. The effect of consumption of the crops on human health was studied through blood analysis for heavy metal content and micronutrients. The detailed description of methodology is presented under following heads.

- 3.1 Selection of area under study**
- 3.2 Collection of information from farm families**
- 3.3 Blood analysis for heavy metals and micronutrients of selected farm families**
- 3.4 Testing of soil and water samples from polluted land for pH and Electrical conductivity**
- 3.5 Analysis of heavy metals and micronutrients of polluted soil and water**
- 3.6 Analysis of edible crops for heavy metals and micronutrients from polluted lands**
- 3.7 Pot culturing for bioremediation trials**
- 3.8 Field testing of selected yeast culture for bioremediation trials**
- 3.9 Testing of soil and crop grown from bioremediated land for heavy metals and micronutrients**

3.10 Statistical analysis

3.1 Selection of area under study

Andhra Pradesh constitutes mainly three zones namely Rayalaseema, Telangana and Coastal Andhra. Out of the three, Telangana zone was selected for the study based on its proximity nearer to metropolitan city, Hyderabad where rapid industrialization had taken place. Two districts from Telangana region namely Ranga reddy and Medak were selected as they are surrounded by highly polluted industries like synthetic organic chemicals, oil refineries and textiles, tanneries, electroplating units, distilleries etc.

3.1.1 Selection of mandals

One mandal from each of the selected districts i.e., Uppal (Ranga Reddy district) and Ramchandrapuram (Medak district) was selected for the study as the farmers residing in these mandals use industrial effluent contaminated water for cultivation of crops.

The distance from district Head quarters of the selected three mandals i.e., Uppal, Ramchandrapuram and Munagala are 80, 98 and 120 Kms respectively (Fig 1).

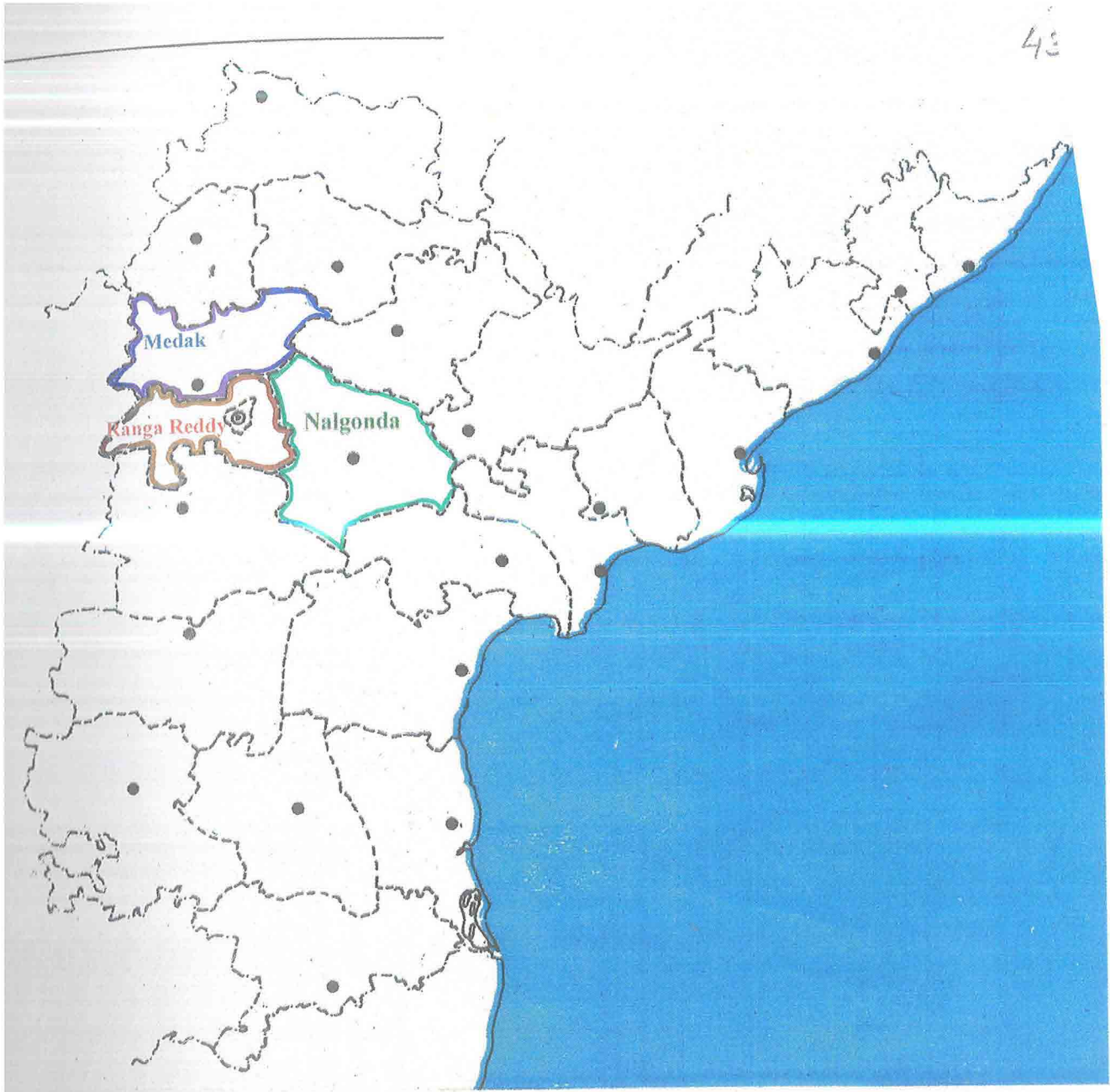


FIG 1: LOCATION PARTICULARS OF THE STUDY AREA

3.1.2 Selection of villages and farm families

From polluted areas, three villages were selected i.e., Cherlapalli (Uppal mandal), Patancheru and Ramchandrapuram (Ramchandrapuram mandal) which are at a distance of 15, 4 and 1.5 Kms from mandal head quarters (Plate 1). Patancheru and Ramchandrapuram were surrounded by industries within the vicinity of 1.5 to 10 kms, whereas in Cherlapalli, the distance was 1 to 7 kms. The industries in the selected polluted villages were established far back in 1980's.

One village i.e. Munagala (Munagala mandal) was selected for comparison from Nalgonda district as it is away from the industrial area. The village is 1 km away from the mandal head quarters.

3.2 Collection of information from farm families

From the available farm households; 156 families (20%) were selected at random for collection of information. Information pertaining to agriculture (crops grown, farm practices, irrigation and marketing etc) and nutritional aspects (consumption pattern, morbidity, cooking practices, food fads and fallacies, etc) was collected using a pre-tested interview schedule from the head of the household and housewife during the period from February to April, 2006. (Appendix I) (Fig 2 and Plate 2).

Height and Weights of all the household members was recorded using the standard equipment i.e., height (cms) by Stature meter 2M and weight (Kgs) by

FIG 2 : SCHEMATIC REPRESENTATION OF SELECTION OF FARM FAMILIES

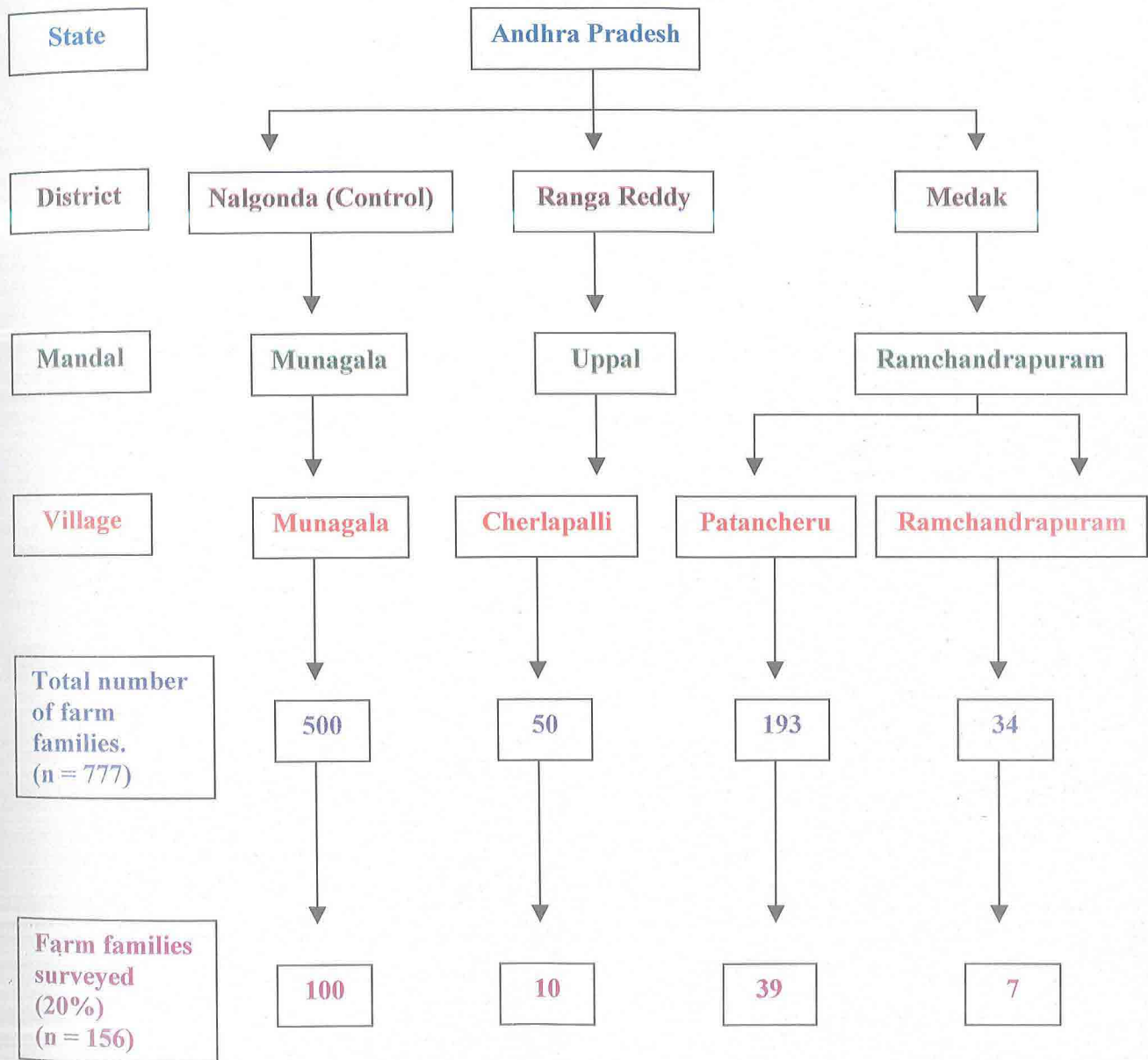




PLATE 2 : COLLECTION OF INFORMATION FROM FARM FAMILIES

standard weighing machine (weigh bird spring balance) with 100 g division. Based on the height and weight measurements, Body Mass Index (BMI) for adults was calculated ($\text{Weight in kg/height in meters}^2$) and compared with standard classification (James et al., 1988). The body weights and heights of preschool children were compared with NCHS standards (Gomez et al., 1956).

3.3 Blood analysis for heavy metals and micronutrients of selected farm families

From the three polluted villages selected, ten farm families from each of Patancheru and Cherlapalli and all the available seven families from Ramchandrapuram village were randomly identified for analysis of blood samples. From the control village i.e., Munagala (Nalgonda district), ten farm families at random were selected for the purpose.

3.3.1 Collection of blood and serum

The farm families were appraised of the purpose of the study and the need for their co-operation was requested. For collection of blood samples the members were requested to assemble at primary health center located in respective villages in the morning hours and the blood was drawn using disposable syringes (Dispovan) with the assistance of medical practitioner (Plate 3).



PLATE 3 : COLLECTION OF BLOOD SAMPLES FROM FARM FAMILIES AT PRIMARY HEALTH CENTERS

Two milliliters of blood was drawn into small plastic vials and were placed in ice box and immediately carried to lab for analysis of heavy metals - lead and cadmium (Kennath and Amy Schmaling, 1999) (Appendix II).

Seventeen milliliters of blood was drawn as explained above and centrifuged in an electric centrifuger for serum separation. Serum was collected in plastic vials and was placed in ice box and carried to lab, and stored at 4°C for further analysis of heavy metals - chromium (Raghuramulu et al., 2003), cobalt (Nadica Todorovska et al., 2003), nickel (Nomoto and Sunderman, 1970) and micro nutrients i.e., calcium (Oser and Summerson, 1957), phosphorus (Oser and Summerson, 1965) and iron (Raghuramulu et al., 2003). The details of the procedures are given in Appendix III to VIII.

3.4 Testing of soil and water samples from polluted land for pH and Electrical conductivity

3.4.1 Collection of soil sample

Five sub samples of soils from the cultivable lands of polluted and control villages were collected from a uniform field to a plough layer i.e., 15cms depth and mixed thoroughly (Plate 4). Quartering was done by dividing thoroughly mixed soil into four equal parts and the two opposite quarters were discarded. Again the soil was mixed and divided into four parts and the procedure was repeated until 500 g of soil sample was left.



PLATE 4: COLLECTION OF SOIL SAMPLE FROM THE FIELDS

The soil thus collected was shade dried at room temperature and packed in polythene bag till further analysis.

3.4.2 Estimation of pH and Electrical Conductivity (E.C.) in soil

Soil samples thus collected from the selected villages were analyzed for pH and E.C. by soil – water suspension method (Jackson, 1973) by weighing 40 gm of soil into a 250 ml Erlenmeyer flask and addition of 80 ml of distilled water in it to prepare 1:2 soil – water suspension. The flask was stoppered and was thoroughly mixed by shaking the mixture on the reciprocating shaker for one hour. Procedural details are furnished in Appendix IX and X.

3.4.3 Collection of water

Samples of water used for drinking purpose were collected from well and municipal tap and that used for irrigation purpose were collected from bore well and canals (Plate 5).

Water samples were collected in 500 ml of plastic bottles after thoroughly rinsing the bottle with same water for three to four times. 2-3 drops of pure toluene was added to avoid bacterial growth and the bottles were refrigerated till further analysis.



PLATE 5 : COLLECTION OF GROUND WATER SAMPLE FROM THE SUMP USED FOR IRRIGATION PURPOSE

3.4.4 Estimation of pH and Electrical Conductivity (E.C.) in water samples:

pH and E.C. of water samples were determined following the standard methods prescribed for testing water (American Public Health Association, 1985). pH was determined by digital pH meter by taking 50 ml water sample in a 100 ml clean beaker and the readings were noted. E.C. was determined by immersing conductivity cell into a clean beaker containing water sample by digital conductivity bridge.

3.5 Analysis of heavy metals and micronutrients of polluted soil and water

Heavy metals in soil samples i.e., Pb, Cd, Cr, Co and Ni were extracted with 0.0005 M DTPA (Diethylene Triamine Penta Acetic acid) in 2:1 ratio i.e., extractant : Soil and were analyzed using Atomic Absorption Spectrophotometer by DTPA extractable method; Lindsay and Norvell, 1978 (Appendix XI).

Iron was extracted with 0.0005 M DTPA (Diethylene Triamine Penta Acetic acid) in 2:1 ratio i.e., extractant : Soil and was analyzed using Atomic Absorption Spectrophotometer by DTPA extractable method; Lindsay and Norvell, 1978 (Appendix XI). Calcium was analyzed by Schollenberger and Simon method, 1945 (shaking and filtration) by Atomic Absorption Spectrophotometer (Appendix XII) and phosphorus (Spectrophotometry) was analyzed by Olsen's method, 1954 (Appendix XIII).

Water samples (drinking and irrigated) were directly analyzed for heavy metals i.e., Pb, Cd, Cr, Co and Ni by Atomic Absorption Spectrophotometer using standard methods (American Public Health Association, 1985).

Iron was directly analyzed using Atomic Absorption Spectrophotometer (American Public Health Association, 1985), Calcium was analyzed by Complexometric titration method using Ethylene Diamine Tetra acetic Acid (EDTA) (Appendix XIV) and phosphorus by Vanadomolybdo – phosphoric acid method (Murphy and Riley, 1962) using Spectrophotometer (Appendix XV).

3.6 Analysis of edible crops for heavy metals and micronutrients from polluted lands

The edible crops that are prominently grown and consumed in the selected areas were collected from farm households at random in amounts of approximately 500g and sub-sample was taken for analysis of heavy metals and micronutrients (Plate 6). The particulars of the food samples that were collected and analyzed are presented in Table 5.

Perishables (fruits and vegetables) and non-perishable food samples were collected and sealed in polythene bags and carried to lab. Perishables were collected direct from the fields and transported on the same day. They were cleaned thoroughly and oven dried at 60°C till constant weight was obtained and pounded to fine powders using motor and pestle.



PLATE 6 : COLLECTION OF FOOD SAMPLES FROM THE
FIELDS

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ACC. No. D-8090
Date 9.1.08

Table 5 : Edible crops prominently grown in selected villages

Name of the village	Crops grown
Munagala (Control)	Paddy
Patancheru	Paddy, wheat, sugar cane, green leafy vegetables, ladies finger, ridge gourd, cluster bean, brinjal, bitter gourd, tomato, mango and guava.
Ramchandrapuram	Paddy
Cherlapalli	Paddy

The fine powders were digested with tri-acid mixture (Appendix XVI) and were stored in plastic bottles and refrigerated for further analysis of heavy metals (Lead, Cadmium, Chromium, Cobalt and Nickel) and micronutrients i.e., calcium and iron (Raghuramulu et al., 2003) by Atomic Absorption Spectrophotometer (Plate 7) and phosphorus by Vanadomolybdo – phosphoric acid method by Spectrophotometer. The details of the procedures are given in Appendix XVI and XVII.

3.7 Pot culturing for bioremediation trials

Pot culturing trials were initiated during the period from July to August, 2006. The details of the experiment are as under i.e. selection of culture, collection of soil and water from the villages (Patancheru and Munagala), preparation of pots and growing of the GLV – fenugreek.

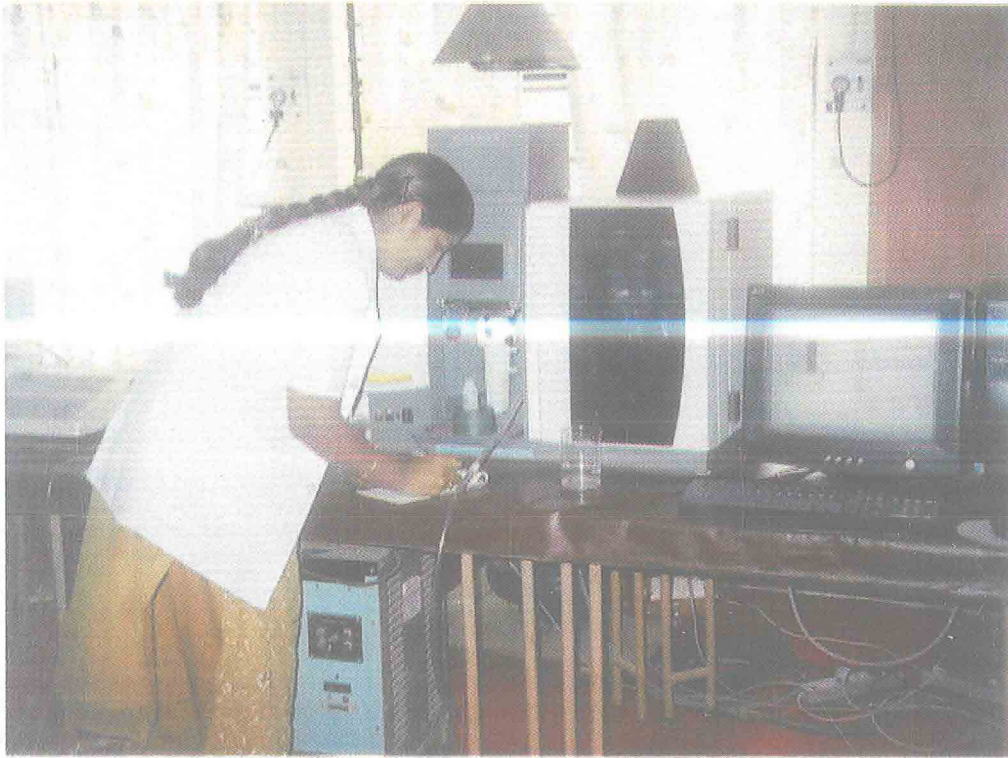


PLATE 7 : ANALYSIS OF HEAVY METALS USING ATOMIC
ABSORPTION SPECTROPHOTOMETER

3.7.1 Selection and preparation of yeast cultures

Four yeast strains - *Saccharomyces cerevisiae* NCYC 1190, imported from National Collection of Yeast Cultures, United Kingdom and three locally isolated yeast cultures namely AMB 111, 112 and 113 were selected for bioremediation trials to be used for pot culturing.

The selected four cultures were tested for its activity by determining the growth curve by Spectrophotometric method (Gopal et al., 2005). Growth curve includes three phases i.e., lag phase, log phase and stationary phase. A fourth phase called death phase follows the stationary phase. This helps in detecting the activity period of the organism. The details are furnished in Appendix XVIII. The number of colonies was enumerated at the highest activity period of organisms (Appendix XIX).

The selected microbial cultures in liquid form were added to soil with the help of farm yard manure which helps in uniform distribution of culture. Farm yard manure was sterilized before use in order to kill the microbes naturally present in it, so as to find the exact effect of selected cultures on remediation of heavy metals.

Four milliliters of liquid culture mixed with sufficient quantity of farm yard manure (6 grams) was added to the top soil of 2.5 cms depth in order to meet the standard biological requirement of 10^6 cells of yeast per gram of soil.

Simple sugar i.e., dextrose (Qualigens) was added at 1, 3 and 6 % concentration of farm yard manure to find the effect of sugars on the activity of micro organisms.

3.7.2 Preparation of pots for crop trials under polluted and control conditions

The soils from polluted land, Patancheru and control village, Munagala were collected from the depth of 8 inch and filled in black colored polythene bags of size 20 cms in height and 8.5 cms in diameter. Each polythene bag was filled up to 17 cms with approximately 1.25 Kgs of soil. Pot culturing was carried out separately by keeping the pots of 20 each for polluted and control soils.

Experiment has been conducted strictly as per the farmer's practice where they are not in the habit of adding chemicals or N P K except FYM at the rate of 300 kgs/acre.

The selected four cultures with different sugar concentrations were mixed in top soil of 2.5 cms depth in each bag (Table 6). From selected four cultures, the most appropriate culture was used for field trials.

Green leafy vegetable i.e., fenugreek was selected for pot culture experiments and field trial as it is short duration crop and also it is commonly cultivated in Patancheru. The fenugreek seeds were collected from the farmers of Patancheru and a table spoonful (approximately 15 grams) of seeds were spread

uniformly in the pot and were covered with a thin layer of soil. The irrigated water collected from designated areas at Patancheru and Munagala was used for maintaining polluted and control trials respectively. Pots were irrigated with sufficient water once in two days, as practiced by the farmers (Plate 8).

Table 6 : Cultures and sugar concentrations used for pot culture experiments

Pot .No.	Name of the culture	Sugar concentration (% of FYM)
1	-	-
2	-	1
3	-	3
4	-	6
5	NCYC 1190	-
6	NCYC 1190	1
7	NCYC 1190	3
8	NCYC 1190	6
9	AMB 111	-
10	AMB 111	1
11	AMB 111	3
12	AMB 111	6
13	AMB 112	-
14	AMB 112	1
15	AMB 112	3
16	AMB 112	6
17	AMB 113	-
18	AMB 113	1
19	AMB 113	3
20	AMB 113	6

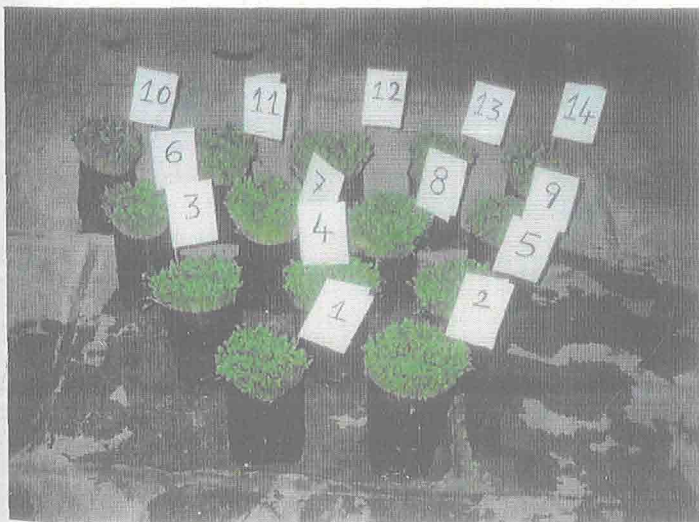
At the time of harvest (after 10 days of sowing as practiced in Patancheru), the plants were carefully uprooted, cleaned and leaves were separated and oven dried at 60°C till constant weight was obtained. The dried leaves were pounded to fine powder and were used for analyzed of heavy metals and micronutrients with the standard methods as mentioned earlier.



**A : PREPARATION
OF CULTURE**



**B : APPLICATION
OF CULTURE IN
THE SOILS**



**C : CROP READY
FOR HARVEST**

PLATE 8 : POT CULTURE TRIALS

A thin slice of soil in cross section was collected with a soil auger to a uniform depth of 10 cm and was analyzed for heavy metals and micronutrients with the standard procedures as mentioned earlier.

3.8 Field testing of selected yeast culture for bioremediation trials

Based on the pot culture trials, AMB 113 with 3 % sugar concentration was found to be ideal for remediation of heavy metals.

Identification trials for genera were carried out in locally isolated yeast culture, AMB 113 (Hupert et al., 1975). Details are furnished in Appendix XX.

Bioremediation trials were initiated during the period from September to October, 2006 in Patancheru village of Ramchandrapuram mandal from Medak district as it was found to be highly polluted among the selected villages based on the tests for soil and water (3.4 & 3.5). Field trials were conducted in 6 x 4 square foot of land. The land was divided into 12 plots each of 1 x 1 square foot for control and experimental trials. Experimental land was treated with microorganism [AMB 113 (4 ml) + Farm yard manure (6 grams) + 3% sugar concentration (0.18 grams)]. The microbe, farm yard manure and sugar mixture were mixed in the top soil of 2.5 cms depth and fenugreek was cultivated (Plate 9).

Trial conditions for control land are same as per experimental land excepting application of microorganism.

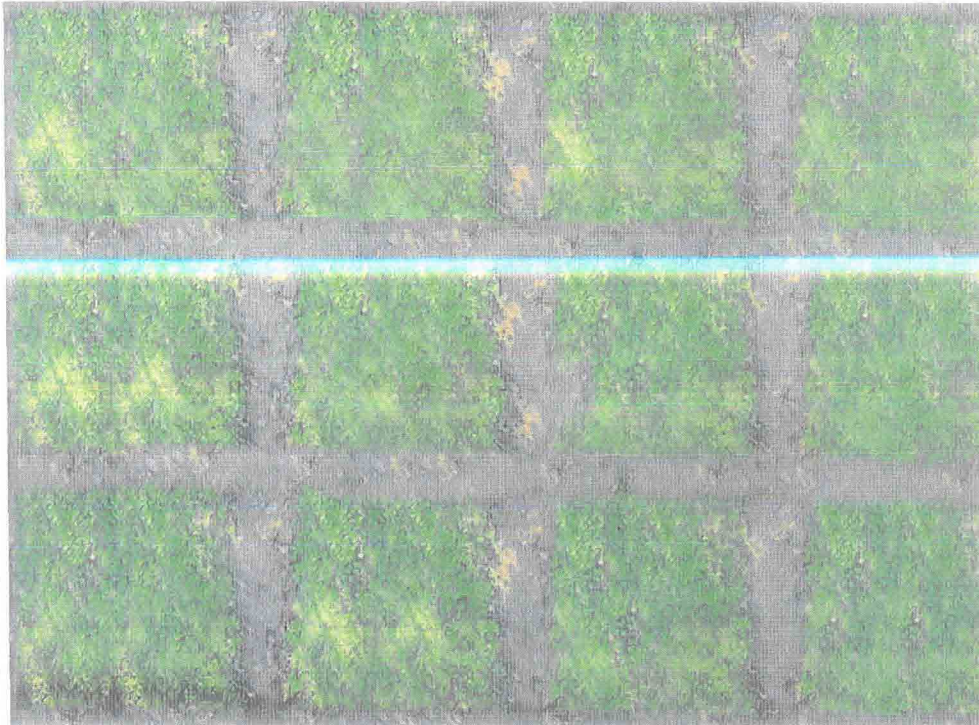


PLATE 9 : EXPERIMENTAL FIELD TRIALS IN POLLUTED FARMS OF PATANCHERU VILLAGE

3.9 Testing of soil and crop grown from bioremediated land for heavy metals and micronutrients

At the time of harvest (after 10 days as practiced in Patancheru), the plants were carefully uprooted, cleaned and leaves were separated and oven dried at 60°C. The dried leaves were pounded to fine powder and were analyzed for heavy metals and micronutrients. A thin slice of soil in cross section was collected with a soil auger to a uniform depth of 10 cm and was analyzed for heavy metals and micro nutrients as per the standard procedures earlier described.

3.10 Statistical analysis

The data was consolidated, tabulated and subjected to statistical analysis i.e., simple and paired t tests (Snedecor and Cochran, 1989) to find the correlation between the selected organisms and remediation of heavy metals in polluted soils and edible crops.

RESULTS

CHAPTER IV

RESULTS

The agricultural lands polluted with industrial effluents and bioremediated were studied for heavy metal content in edible crops grown in the soils. The effect of consumption of the crops on human health was studied through blood analysis for heavy metal content and micronutrients. The results pertaining to the study conducted are presented under following heads.

- 4.1 **Collection of information from farm families**
- 4.2 **Blood analysis for heavy metals and micronutrients of selected farm families**
- 4.3 **Testing of soil and water samples (of land under cultivation) for pH and Electrical conductivity**
- 4.4 **Analysis of heavy metals and micronutrients of polluted soil and water**
- 4.5 **Analysis of edible crops for heavy metals and micronutrients from polluted lands**
- 4.6 **Pot culturing for selection of effective yeast strains**
- 4.7 **Testing of soil and crop grown from bioremediated land for heavy metals and micronutrients**

4.1 Collection of information from farm families

Twenty per cent of the farm families were selected randomly from the selected four villages from 3 mandals for collection of general information. Survey was conducted using pre-tested interview schedule in the farm families regarding family particulars, consumption pattern, health and nutritional status and agricultural practices.

4.1.1 Family particulars

The details pertaining to the family particulars of selected farm families are presented in Table 7. As is seen from Table 7, the maximum percentage (60.3%) of the family members was in the age group of 17-50 years. Majority of the farm families are literates i.e., control (69.3%) and polluted (77.4%) families.

4.1.2 Source of drinking water

The source of drinking water for the farm families is presented in Table 8.

TABLE 8 : SOURCE OF DRINKING WATER IN SELECTED VILLAGES

Village	Source of drinking water	
	Manjeera	Well water
Control (n = 100)	-	100 (100)
Polluted (n = 56)	56 (100.0)	-

(Figures in parenthesis indicate percentages)

TABLE 7 : FAMILY PARTICULARS OF SELECTED FARM FAMILIES

Village	Family size						Educational level			Occupation			
	A	B	C	D	E	F	A	B	C	A	B	C	D
S1 (n=333)	-	6 (1.8)	28 (8.4)	52 (15.6)	201 (60.3)	46 (13.8)	231 (69.3)	40 (12.0)	62 (18.6)	100 (30.0)	113 (33.9)	117 (35.1)	3 (0.9)
S2 (n=222)	1 (0.5)	3 (1.4)	18 (8.1)	28 (12.6)	134 (60.3)	38 (17.1)	172 (77.4)	37 (16.7)	13 (5.9)	60 (27.0)	79 (35.6)	80 (36.0)	3 (1.4)

(Figures in parenthesis indicate percentages)

- | <u>Village</u> | <u>Family size</u> | <u>Educational level</u> | <u>Occupation</u> |
|----------------|--------------------|--------------------------|-------------------|
| S1 = Control | A = 0-12 months | A = Literate | A = Housewife |
| S2 = Polluted | B = 1-3 yrs | B = Semi-literate | B = Student |
| | C = 4-9 yrs | C = Illiterate | C = Labor |
| | D = 10-16 yrs | | D = Others |
| | E = 17-50 yrs | | |
| | F = >50 yrs | | |

Table 8 indicated that cent percent of the farm families residing in control village consume well water, whereas those residing in polluted villages consume manjeera water.

4.1.3 Method of cooking

The cooking practices followed by the farm families are presented in Table 9.

TABLE 9 : METHOD OF COOKING PRACTICED BY SELECTED FARM FAMILIES

Village	Number of Farm families		Method of cooking practiced				
	Non-vegetarians	Vegetarians	Boiling	Pulusu	Frying	Steaming	Fermentation
Control (n = 100)	94 (94.0)	6 (6.0)	100 (100.0)	100 (100.0)	100 (100.0)	100 (100.0)	100 (100.0)
Polluted (n = 56)	53 (94.6)	3 (5.4)	60 (100.0)	60 (100.0)	60 (100.0)	60 (100.0)	60 (100.0)

(Figures in parenthesis indicate percentages)

As is evident from Table 10 that maximum percentage of control (94%) and polluted (95%) farm families were non-vegetarians. The common cooking practices followed by the farm families were found to be boiling, pulusu, frying, steaming and fermentation.

4.1.4 Food fads and fallacies:

The data pertaining to food fads and fallacies are presented in Table 10. As is seen from Table 10 that majority of control (82%) and polluted (60%) farm families follow some food believes during pregnancy i.e., they avoid the consumption of papaya. The control (6%) and polluted (40%) farm families avoid consumption of cucumber and curd during cold and cough. The farm families – control (15%) and polluted (31.6%) avoid cucumber during lactation period.

TABLE 10 : FOOD FADS AND FALLACIES FOLLOWED BY FARM FAMILIES

Village	Life stage / health condition				Foods avoided			
	A	B	C	D	A	B	C	D
Control (n = 100)	5 (5.0)	5 (5.0)	82 (82.0)	15 (15.0)	6 (6.0)	1 (1.0)	6 (6.0)	82 (82.0)
Polluted (n = 56)	19 (31.6)	19 (31.6)	36 (60.0)	19 (31.6)	24 (40.0)	9 (15.0)	24 (40.0)	36 (60.0)
Z-value	1.72 ^{NS}	1.81 ^{NS}	3.82**	0.83 ^{NS}	2.73*	1.69 ^{NS}	2.69*	3.78**

NS = Not significant * = Significant at 5% level ** = Significant at 1% level
(Figures in parenthesis indicate percentages)

Life stage / health condition

A = Cold
B = Cough
C = Pregnant
D = Lactating

Foods avoided

A = Cucumber
B = Guava
C = Curd
D = Papaya

4.1.5 General health profile of the families

The general health profile of the farm families are tabulated in Table 11 and

TABLE 11 : GENERAL HEALTH PROFILE OF ADULTS IN THE SELECTED FAMILIES

Village	Body Mass Index (BMI)						
	A	B	C	D	E	F	G
Control (n = 319)	19 (5.9)	18 (5.6)	51 (15.9)	62 (19.4)	130 (40.8)	32 (10.0)	7 (2.2)
Polluted (n = 219)	9 (4.1)	7 (3.2)	11 (5.0)	17 (7.8)	128 (58.4)	38 (17.4)	9 (4.1)
Z-value	4.2**	4.62**	16.8**	18.63**	0.46 ^{NS}	0.31 ^{NS}	0.52 ^{NS}

NS = Not significant ** = Significant at 1% level

(Figures in parenthesis indicate percentages)

Body Mass Index (BMI)

- A = <16 III degree chronic energy deficiency (CED)
- B = 16-17 II degree chronic energy deficiency (CED)
- C = 17-18.5 I degree chronic energy deficiency (CED)
- D = 18.5-20 Low normal
- E = 20-25 Normal
- F = 25-30 Over weight
- G = >30 Obese

Table 11 revealed that maximum percentage of control (39%) and polluted (57.6%) farm family members were with in the normal range, whereas 5.7% and 4.05% were in the III degree of chronic energy deficiency and 2.1% and 4.05% of control and polluted farm family members were obese.

**TABLE 12 : GENERAL HEALTH PROFILE OF CHILDREN IN THE
SELECTED FAMILIES**

Village	National Centre for Health Statistics (NCHS)			
	A	B	C	D
Control (n = 13)	11 (84.6)	2 (15.4)	-	-
Polluted (n = 2)	2 (100)	-	-	-
Z-value	1.86 ^{NS}	-	-	-

NS = Not significant
(Figures in parenthesis indicate percentages)

National Center for Health Statistics (NCHS)

A = >90 Normal (Normal nutrition)
 B = 75-90 Grade I (Mild malnutrition)
 C = 60-75 Grade II (Moderate malnutrition)
 D = <60 Grade III (Severe malnutrition)

As is evident from Table 12, the preschool children residing in control (84.6%) and cent percent of those residing in polluted villages where in the normal grade and 15.4% of children in control farm families were in the grade of mild malnutrition.

4.1.6 Morbidity status of farm families

The data pertaining to morbidity status are given in Table 13. It is evident that that 9.91 per cent and 18.02 per cent of control and polluted farm family members fell sick. The maximum percentage of subjects who fell sick was in the age group of 17 – 50 years.

TABLE 13 : MORBIDITY STATUS OF FARM FAMILIES (FROM LAST 15 DAYS)

Village	Age group						Type of illness						Duration						Treatment	
	A	B	C	D	E	F	A	B	C	D	E	A	B	C	D	A	B			
Control	-	2 (6.1)	5 (15.2)	11 (33.3)	12 (36.4)	3 (9.1)	14 (42.4)	3 (9.1)	10 (30.3)	6 (18.2)	-	1 (3.0)	22 (66.7)	9 (27.3)	1 (3.0)	16 (48.5)	17 (51.5)			
Polluted	-	1 (2.5)	5 (12.5)	9 (22.5)	21 (52.5)	4 (10.0)	11 (27.5)	6 (15.0)	7 (17.5)	6 (15.0)	10 (25.0)	-	13 (32.5)	17 (42.5)	10 (25.0)	40 (100.0)	-			
Z-value	-	0.20 NS	-	0.27 NS	1.69 NS	0.21 NS	0.29 NS	0.28 NS	0.26 NS	-	-	-	1.70 NS	1.68 NS	1.69 NS	2.76**	-			

NS = Not significant ** = Significant at 1% level
(Figures in parenthesis indicate percentages)

Age group

- A = 0-12 months
- B = 1-3 yrs
- C = 4-9 yrs
- D = 10-16 yrs
- E = 17-50 yrs
- F = >50 yrs

Type of illness

- A = Fever
- B = Eye infection
- C = Cold
- D = Cough
- E = Skin rash

Duration

- A = One day
- B = 2-3 days
- C = 4-7 days
- D = 15 days

Treatment

- A = Taken
- B = Not taken

Majority of the control (42.42%) and polluted (25.5%) farm family members suffered with fever and it was reported that 25 per cent of the polluted farm family members suffered with skin rash which was not found in control farm family members.

4.1.7 Obstetric history

The results related to obstetric history are given in Table 14. As is seen from Table 14, the average number of pregnancies in control and experimental families were 2.08 and 1.27 respectively. It is evident that in control families, 90.87 per cent of pregnancy outcome were alive and 9.13 per cent were dead whereas in polluted farm families, 85.04 per cent were alive and 13.37 per cent were dead.

TABLE 14 : OBSTETRIC HISTORY OF THE FARM FAMILIES

Village	Average number of pregnancies	Pregnancy outcome			Abortions (Average number)
		Alive	Dead	Carrying	
Control (n = 98)	2.08 ± 0.64	189 (90.9)	19 (9.1)	-	0.60 ± 0.53
Polluted (n = 53)	1.27 ± 0.85	108 (85.0)	17 (13.4)	2 (1.58)	0.92 ± 0.49
Z-value	0.20 ^{NS}	5.94**	0.30 ^{NS}	-	1.81 ^{NS}

NS = Not significant ** = Significant at 1% level
(Figures in parenthesis indicate percentages)

4.1.8 Land particulars

The data pertaining to land particulars are tabulated in Table 15.

TABLE 15 : LAND PARTICULARS OF FARM FAMILIES AND SOIL TYPE

Village	Total cultivable area (acres)	Soil type	
		Black soil	Sandy soil
Control (n = 100)	692.0	100 (100.0)	-
Polluted (n = 56)	341.16	-	56 (100.0)

(Figures in parenthesis indicate percentages)

Table 15 indicated that the land area cultivated by the selected farmers in control and polluted villages are 692.0 and 341.16 acres respectively. The soil is of black type in control village and sandy soil in polluted villages.

4.1.9 Crops grown

Crops grown in control and polluted villages are tabulated in Table 16. It is revealed that paddy was the only crop cultivated in Munagala, Cherlapalli and Ramchandrapuram villages whereas farmers residing in patancheru cultivate wheat, sugarcane, fruits and vegetables along with paddy.

TABLE 16 : CROP PARTICULARS OF THE FARM FAMILIES

Village	Crops grown	Total area cultivated (acres)	Total yield per acre (Kgs)	Quantity retained (Kgs)		Quantity sold (Kgs)
				Consumption	Seed	
Control (n = 100)	Paddy	692	2500.00	103100.00	34580	1592320
Polluted (n = 56)	Paddy	292	2466.67	62262	14603.13	643402.51
	Wheat	1.563	350.00	35.00	29.50	447.55
	Sugarcane	25	32500.00	7.50	105000.00	707492.50
	Green leafy vegetables	1.16	550.00	2.50	-	635.50
	Ladies finger	1.25	750.00	6.50	-	931.10
	Ridge gourd *	1.5	1200.00	2.00	-	1798.00
	Cluster bean *	1.5	1000.00	1.00	-	1499.00
	Brinjal *	1.0	400.00	10.00	-	390.00
	Cabbage *	1.0	300.00	3.00	-	97.00
	Onion	0.468	10750.00	47.50	-	4983.50
	Tomato	0.719	500.00	15.00	-	344.50
	Mango *	8.0	2500.00	50.00	-	19950.00
	Guava *	6.0	2000.00	20.00	-	11980.00

* Actual values

4.1.10 Usage of manures and fertilizers for crops grown

The data pertaining to usage of manures and fertilizers are tabulated in Table 17. It is evident that cent percent of farmers residing in control and polluted villages use farm yard manure. Majority of the farmers in control (80%) and polluted (82.14%) villages use 500 and 300 Kg of farm yard manure per acre. All farmers residing in control and polluted villages use nitrogen, potassium and DAP as fertilizers. The amount of nitrogen (Urea) used in control and polluted villages

TABLE 17 : USAGE OF MANURES AND FERTILIZERS FOR CROPS GROWN IN SELECTED VILLAGES

Village	Number of families using FYM	Quantity of manure used			Type of fertilizer used				Average amount of fertilizer used / acre (Kgs)		
		A	B	C	Straight		Complex		A	B	Complex
					DAP (N&P)	(100.0)	56	(100.0)			
Control (n = 100)	100 (100.0)	80 (80.0)	1 (1.0)	19 (19.0)	100 (100.0)	100 (100.0)	100 (100.0)	100.0	50.0	100.0	
Polluted (n = 56)	56 (100.0)	10 (17.9)	-	46 (82.1)	56 (100.0)	56 (100.0)	56 (100.0)	174.52	133.21	181.66	
Z-value	-	4.76**	-	2.57**	-	-	-	4.09**	4.87**	4.63**	

** = Significant at 1% level
 (Figures in parenthesis indicate percentages)

Quantity of manure used
 A = 500 Kgs
 B = 400 Kgs
 C = 300 Kgs

Fertilizers used : Straight :
 A = Nitrogen (Urea)
 B = Potassium (MOP)

are 100 and 174.52 Kg per acre respectively; Potassium (MOP) : 50 and 133.21 Kg per acre and the amount of DAP used are 100 and 181.66 Kg per acre respectively.

4.1.11 Plant protection chemicals used for crops

The data pertaining to plant protection chemicals used are tabulated in Table 18. It is revealed that 29 per cent of control and 26.78 per cent of polluted families use monocrotophos and 71 per cent and 73.21 per cent use endosulfon. Cent percent of the farm families use carborylant and phorate granules. The amount of monocrotophos and endosulfon used in control and polluted villages are 150 and 250 ml and carborylant : 50 and 100 ml respectively. In all the villages 4 Kg of phorate granules are used per acre.

It is indicated that cent percent of both control and polluted families use fertilizers namely carbendazim and captan. 12.5 per cent of polluted families use thiram and mancozeb and 30.35 per cent use hexaconazole. Control farm families use 90 ml of carbendazim and captan per acre, whereas polluted farm families use 100 ml per acre.

4.1.12 Marketing of the produce

The data related to the marketing of the produce are given in Table 19.

TABLE 18 : PLANT PROTECTION CHEMICALS USED FOR CROPS IN SELECTED VILLAGES

Village	Pesticides				Fungicides					Amount of pesticides used / acre					Amount of fungicides used / acre (ml)				
	A	B	C	D	A	B	C	D	E	A (ml)	B (ml)	C (ml)	D (Kgs)	A	B	C	D	E	
Control (n = 100)	29 (29.0)	71 (71.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	-	-	-	150	150	50	4	90	90	-	-	-	
Polluted (n = 56)	15 (26.8)	41 (73.2)	56 (100.0)	56 (100.0)	56 (100.0)	56 (100.0)	7 (12.5)	7 (12.5)	17 (30.3)	250	250	100	4	100	100	150	150	200	
Z-value	-	-	-	-	-	-	-	-	-	5.01**	5.03**	3.89**	-	1.87 NS	1.86 NS	-	-	-	

NS = Not significant ** = Significant at 1% level
(Figures in parenthesis indicate percentages)

Pesticides A = Monocrotophos, B = Endosulfon, C = Carborylant, D = Phorate granules
Fungicides A = Carbendazim, B = Captan, C = Thiram, D = Mancozeb, E = Hexaconazole

TABLE 19 : MARKETING OF THE PRODUCE IN THE SELECTED VILLAGES

S.No.	Village	Name of the produce	Marketing of the produce	
			Local	Outside
1	Munagala(control)	Paddy	100 (100.0)	-
2	Patancheru	Paddy, wheat, fruits and vegetables & sugarcane	39 (100.0)	-
3	Ramchandrapuram	Paddy	7 (100.0)	-
4	Cherlapalli	Paddy	10 (100.0)	-

(Figures in parenthesis indicate percentages)

As is evident from Table 19, all the farmers residing in selected four villages (1-control and 3-polluted) market their produce in local rice mill and local fruit and vegetable markets.

4.2 Blood analysis for heavy metals and micronutrients of selected farm families

Heavy metals i.e., lead and cadmium was analyzed in blood samples as they adhere to the RBC – receptors. Whereas the other heavy metals i.e., cobalt, chromium and nickel and micronutrients i.e., calcium, phosphorus and iron were analyzed in serum samples. The subjects selected for the collection of blood samples are tabulated in Table 20.

TABLE 20 : SELECTION OF SUBJECTS FOR BLOOD COLLECTION

S.No.	Name of the village	Number of families	Total subjects	
			Adults & Adolescents (>12 yrs)	Children (\leq 12 yrs)
1	Munagala(control)	10	25	3
2	Patancheru	10	23	2
3	Ramchandrapuram	7	22	2
4	Cherlapalli	10	28	9

Note : Based on the biochemical standards available, age categorization of the selected families was done.

4.2.1 Lead

The blood lead levels analyzed in the selected subjects are tabulated in Table 21.

TABLE 21 : BLOOD LEAD LEVELS OF SELECTED SUBJECTS

S.No	Name of the village (N = 114)	Blood Lead level					
		Adults & Adolescents (> 12yrs)			Children (< 12 yrs)		
		< 20 μ g/dl	> 20 μ g/dl	Z-value	< 10 μ g/dl	> 10 μ g/dl	Z-value
1	Munagala(control)	25 (100.0)	-	-	3 (100.0)	-	-
2	Patancheru	18 (78.26)	5 (21.74)	2.42*	1 (50.0)	1 (50.0)	-
3	Ramchandrapuram	17 (77.27)	5 (22.72)	2.40*	1 (50.0)	1 (50.0)	-
4	Cherlapalli	21 (75.0)	7 (25.0)	2.49*	7 (77.78)	2 (22.22)	2.10*

* = Significant at 5% level
(Figures in parenthesis indicate percentages)

Note : Based on the biochemical standards available, age categorization of the selected families was done.

Table 21 reported that the blood lead levels estimated in both adults and children residing in control village (100%) were within the normal range, whereas those residing in patancheru (21.74%, 50%), ramchandrapuram (22.72%, 50%) and cherlapalli (25%, 22.22%) were above the limit suggested by Department of health and human services, 2006 (Adults = $<20\mu\text{g} / \text{dl}$; Children = $<10 \mu\text{g} / \text{dl}$) (Fig 3 and 4).

4.2.2 Cadmium

The data pertaining to cadmium analysis in blood samples are given in Table 22.

TABLE 22 : BLOOD CADMIUM LEVELS OF SELECTED SUBJECTS

S.No.	Name of the village (N = 114)	Blood Cadmium level		
		$< 1 \mu\text{g}/\text{dl}$	$> 1 \mu\text{g}/\text{dl}$	Z-value
1	Munagala(control)	28 (100.0)	-	-
2	Patancheru	23 (92.0)	3 (8.0)	2.63*
3	Ramchandrapuram	22 (91.67)	1 (4.17)	2.70*
4	Cherlapalli	34 (91.89)	3 (8.11)	2.84*

* = Significant at 5% level

(Figures in parenthesis indicate percentages)

As is evident from Table 22, cent percent of the subjects in munagala village reported normal cadmium levels, whereas those in patancheru (8%), ramchandrapuram (4.17%) and cherlapalli (8.11%) reported higher blood

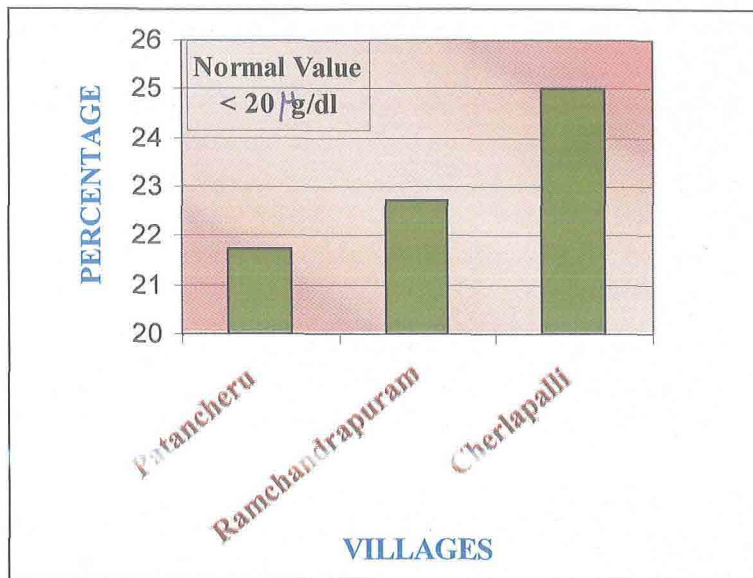


FIG 3 : PERCENTAGE OF SUBJECTS WITH HIGH BLOOD LEAD CONCENTRATIONS IN SELECTED POLLUTED VILLAGES - ADULTS

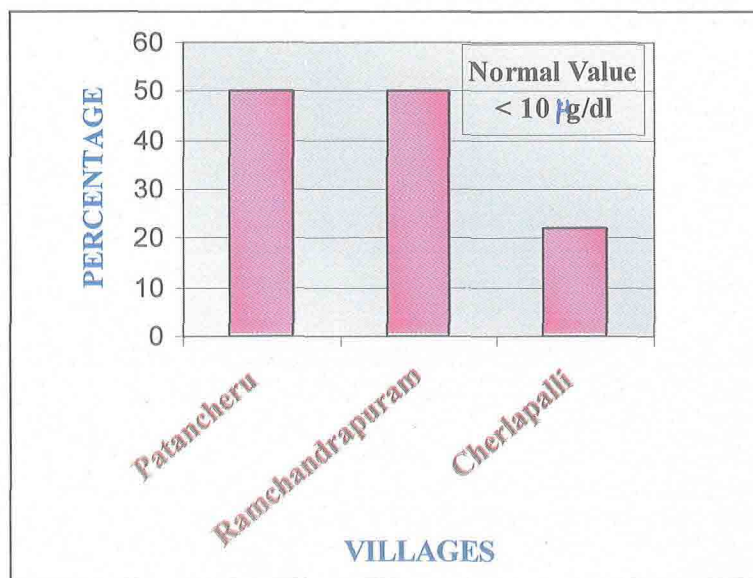


FIG 4 : PERCENTAGE OF SUBJECTS WITH HIGH BLOOD LEAD CONCENTRATIONS IN SELECTED POLLUTED VILLAGES - CHILDREN

cadmium levels than those suggested by Department by health and human services, 2006 ($<1\mu\text{g} / \text{dl}$) (Fig. 5).

4.2.3 Chromium

The data related to chromium levels in serum samples are given in Table 23.

TABLE 23 : SERUM CHROMIUM LEVELS OF SELECTED SUBJECTS

S.No.	Name of the village (N = 114)	Serum Chromium level		
		$< 1 \mu\text{g}/\text{dl}$	$> 1 \mu\text{g}/\text{dl}$	Z-value
1	Munagala(control)	24 (85.71)	4 (14.29)	2.63*
2	Patancheru	16 (64.0)	9 (36.0)	2.97*
3	Ramchandrapuram	18 (75.0)	6 (25.0)	2.41*
4	Cherlapalli	29 (78.38)	8 (21.62)	2.93*

* = Significant at 5% level
(Figures in parenthesis indicate percentages)

Table 23 revealed that 14.29 per cent of subjects residing in munagala village, 36 per cent in patancheru, 25 per cent in ramchandrapuram and 21.62 per cent in cherlapalli reported higher serum chromium levels than those suggested by Department of health and human services, 2006 ($<1\mu\text{g} / \text{dl}$) (Fig. 6).

4.2.4 Cobalt

The data related to serum cobalt analysis in selected subjects are given in Table 24. As is seen from Table 25, cent percent of the subjects residing in munagala village showed normal cobalt levels whereas those residing in

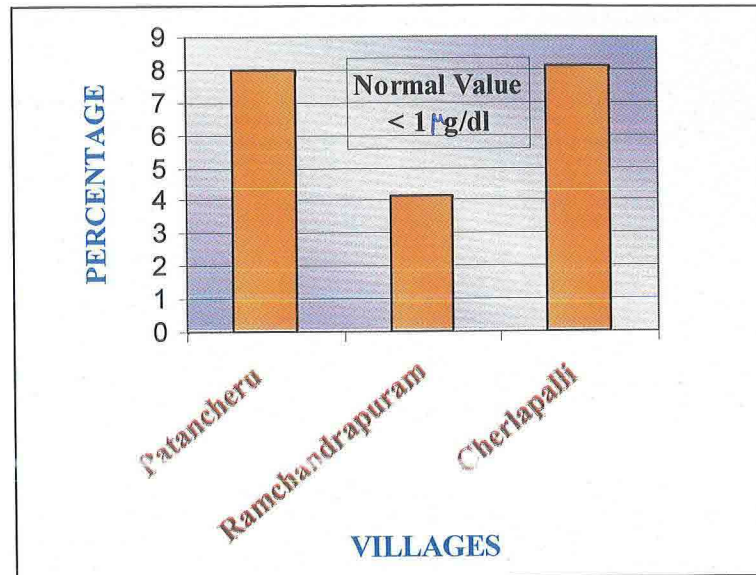


FIG 5 : PERCENTAGE OF SUBJECTS WITH HIGH BLOOD CADMIUM CONCENTRATIONS IN SELECTED POLLUTED VILLAGES

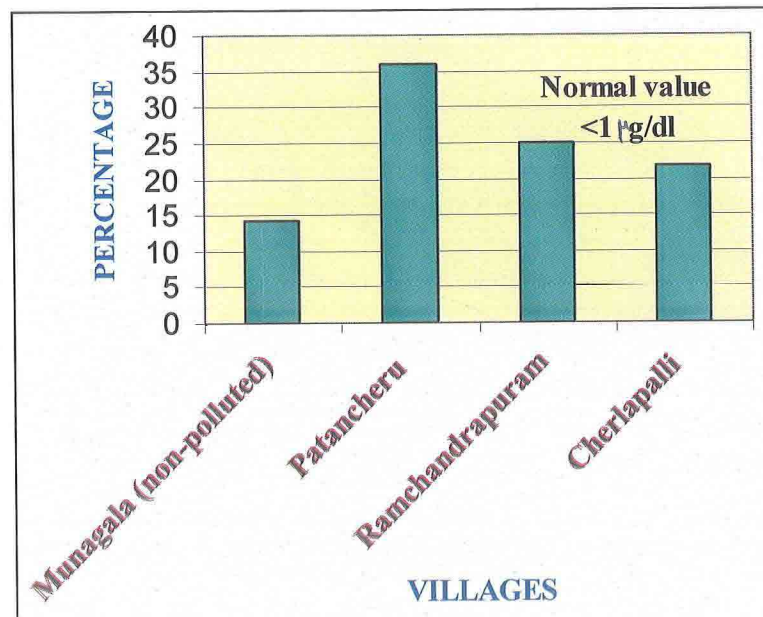


FIG 6 : PERCENTAGE OF SUBJECTS WITH HIGH SERUM CHROMIUM CONCENTRATIONS IN SELECTED VILLAGES

patancheru (16%), ramchandrapuram (8.33%) and cherlapalli (13.51) reported high serum cobalt levels than those suggested by Department of health and human services, 2006 (0.01-0.05 $\mu\text{g}/\text{dl}$) (Fig. 7).

TABLE 24 : SERUM COBALT LEVELS OF SELECTED SUBJECTS

S.No.	Name of the village (N = 114)	Serum cobalt level		
		0.01-0.05 $\mu\text{g}/\text{dl}$	> 0.05 $\mu\text{g}/\text{dl}$	Z-value
1	Munagala(control)	28 (100.0)	-	-
2	Patancheru	21 (84.0)	4 (16.0)	2.61*
3	Ramchandrapuram	22 (91.66)	2 (8.33)	2.73*
4	Cherlapalli	32 (86.49)	5 (13.51)	2.99*

* = Significant at 5% level
(Figures in parenthesis indicate percentages)

4.2.5 Nickel

The data related to serum nickel analysis are tabulated in Table 25.

TABLE 25 : SERUM NICKEL LEVELS OF SELECTED SUBJECTS

S.No.	Name of the village (N = 114)	Serum Nickel level		
		0.11-0.45 $\mu\text{g}/\text{dl}$	> 0.45 $\mu\text{g}/\text{dl}$	Z-value
1	Munagala(control)	28 (100.0)	-	-
2	Patancheru	18 (72.0)	7 (28.0)	2.42*
3	Ramchandrapuram	21 (87.5)	3 (12.5)	2.53*
4	Cherlapalli	33 (89.19)	4 (10.81)	2.98*

* = Significant at 5% level
(Figures in parenthesis indicate percentages)

It is revealed from Table 25 that cent percent of the subjects in munagala village reported normal serum nickel level, whereas those residing in patancheru

(28%), ramchandrapuram (12.5%) and cherlapalli (10.81) reported serum nickel level above the normal range Normoto and Sunderman, 1970 (0.11-0.45 $\mu\text{g/dl}$) (Fig. 8).

4.2.6 Calcium

The data related to serum calcium levels are presented in Table 26.

TABLE 26 : SERUM CALCIUM LEVELS OF SELECTED SUBJECTS

S.No.	Name of the village (N = 114)	Serum Calcium level		
		8.8-10.8 mg/100ml	> 10.8 mg/100ml	Z-value
1	Munagala(control)	26 (92.86)	2 (7.14)	2.63*
2	Patancheru	24 (96.0)	1 (4.0)	2.40*
3	Ramchandrapuram	23 (95.83)	1 (4.17)	2.57*
4	Cherlapalli	35 (94.59)	2 (5.41)	2.77*

* = Significant at 5% level

(Figures in parenthesis indicate percentages)

Table 26 reported that 7.14 percent of subjects residing in munagala and 4 percent in patancheru, 4.17 percent in ramchandrapuram and 5.41 percent in cherlapalli villages reported serum calcium levels above the normal limit suggested by Robert and Denis, 2000 (8.8-10.8 mg/100ml) (Fig. 9).

4.2.7 Phosphorus

The data pertaining to serum phosphorus analysis are given in Table 27. It is evident from Table 27 that 25, 12, 8.33 and 8.11 percent of subjects residing in munagala, patancheru, ramchandrapuram and cherlapalli villages

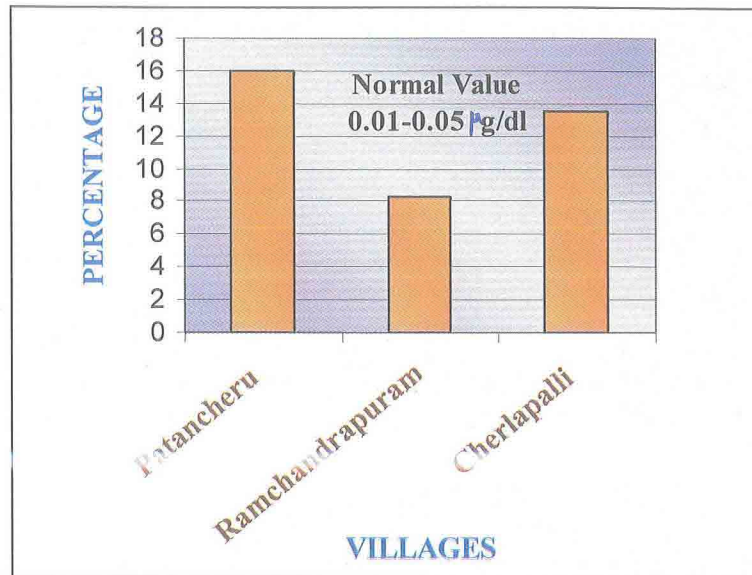


FIG 7 : PERCENTAGE OF SUBJECTS WITH HIGH SERUM COBALT CONCENTRATIONS IN SELECTED POLLUTED VILLAGES

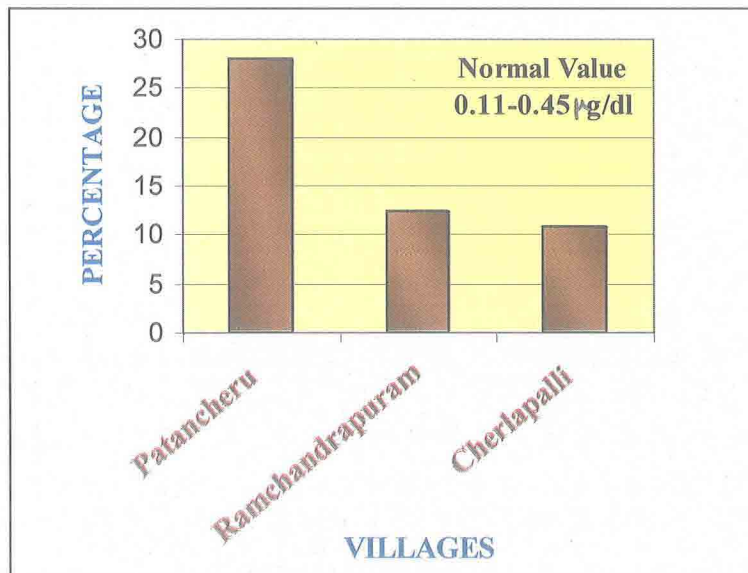


FIG 8 : PERCENTAGE OF SUBJECTS WITH HIGH SERUM NICKEL CONCENTRATIONS IN SELECTED POLLUTED VILLAGES

reported higher serum phosphorus content than those suggested by Robert and Denis, 2000 (2.5-4.5 mg/100ml) (Fig. 10).

TABLE 27 : SERUM PHOSPHORUS LEVELS OF SELECTED SUBJECTS

S.No.	Name of the village (N = 114)	Serum Phosphorus level		
		2.5-4.5 mg/100ml	> 4.5 mg/100ml	Z-value
1	Munagala(control)	21 (75.0)	7 (25.0)	2.53*
2	Patancheru	22 (88.0)	3 (12.0)	2.92*
3	Ramchandrapuram	22 (91.67)	2 (8.3)	2.96*
4	Cherlapalli	34 (91.89)	3 (8.11)	3.01*

* = Significant at 5% level

(Figures in parenthesis indicate percentages)

4.2.8 Iron

The data related to serum iron levels are tabulated in Table 28.

TABLE 28 : SERUM IRON LEVELS OF SELECTED SUBJECTS

S.No.	Name of the village (N = 114)	Serum Iron level	
		50-150 µg/dl	> 150 µg/dl
1	Munagala(control)	28 (100.0)	-
2	Patancheru	25 (100.0)	-
3	Ramchandrapuram	24 (100.0)	-
4	Cherlapalli	37 (100.0)	-

(Figures in parenthesis indicate percentages)

Table 28 indicated that all the subjects residing in selected four villages reported normal serum iron content suggested by Sanberlich, 1999 (50-150 µg/dl).

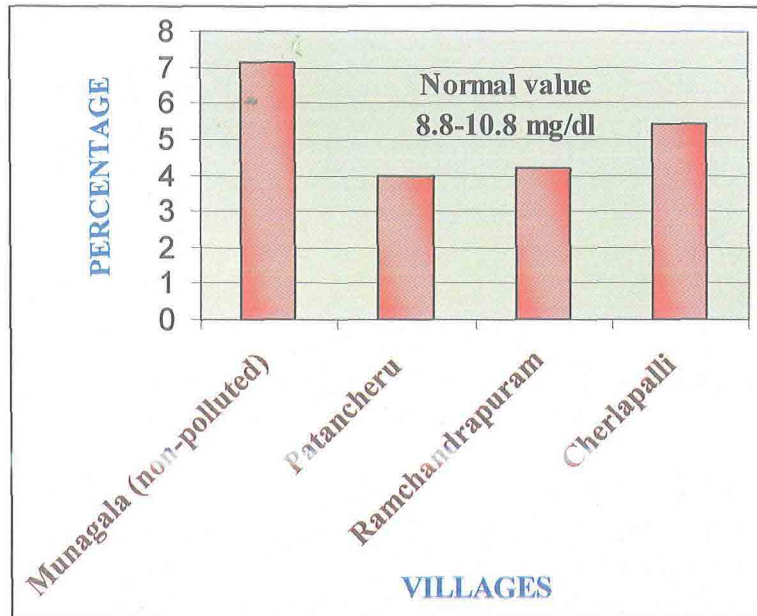


FIG 9 : PERCENTAGE OF SUBJECTS WITH HIGH SERUM CALCIUM CONCENTRATIONS IN SELECTED VILLAGES

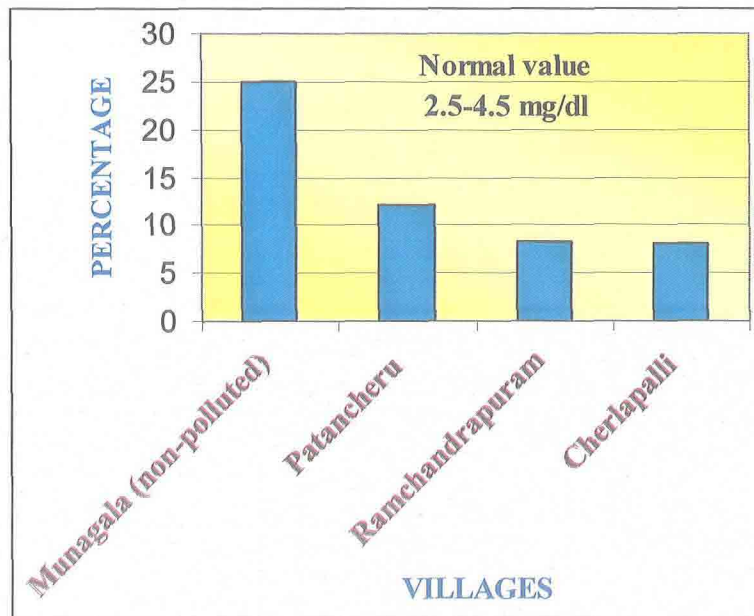


FIG 10 : PERCENTAGE OF SUBJECTS WITH HIGH SERUM PHOSPHORUS CONCENTRATIONS IN SELECTED VILLAGES

4.3 Testing of soil and water samples from polluted land for pH and Electrical conductivity

The data related to the pH and Electrical conductivity of soil and water samples are presented in Table 29, 30 and 31.

TABLE 29 : pH AND ELECTRIC CONDUCTIVITY (E.C.) OF SOIL SAMPLES FROM POLLUTED LAND

S.No.	Name of the village	pH	E.C (ds/m)
1	Munagala(control)	7.36	0.195
2	Patancheru	6.08	0.960
3	Ramchandrapuram	6.50	0.139
4	Cherlapalli	8.19	0.145
F-value	-	521.13**	633.46**

** = Significant at 1% level

As is evident from Table 29 that pH of the soil samples ranged from 6.08 to 8.19 and electrical conductivity ranged from 0.139 to 0.960 ds m^{-1} respectively. It is indicated that patancheru soil has low pH and high electrical conductivity when compared to that of other soil profiles (Fig 11 and 12).

TABLE 30 : pH AND ELECTRIC CONDUCTIVITY (E.C.) OF DRINKING WATER SAMPLES FROM POLLUTED LAND

S.No.	Name of the village	PH	E.C (ds/m)
1	Munagala(control)	7.75	0.782
2	Patancheru	7.55	0.238
3	Ramchandrapuram	7.08	0.276
4	Cherlapalli	7.42	0.262
F-value	-	316.01**	414.20**

** = Significant at 1% level

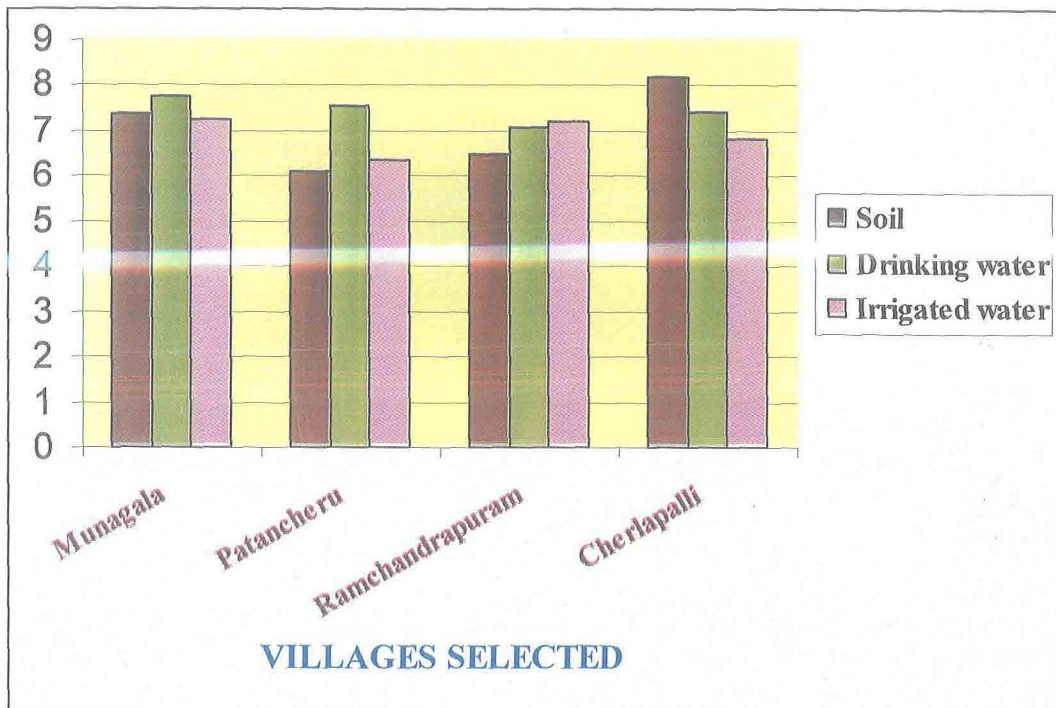


FIG 11 : pH OF SOIL AND WATER SAMPLES COLLECTED FROM SELECTED VILLAGES

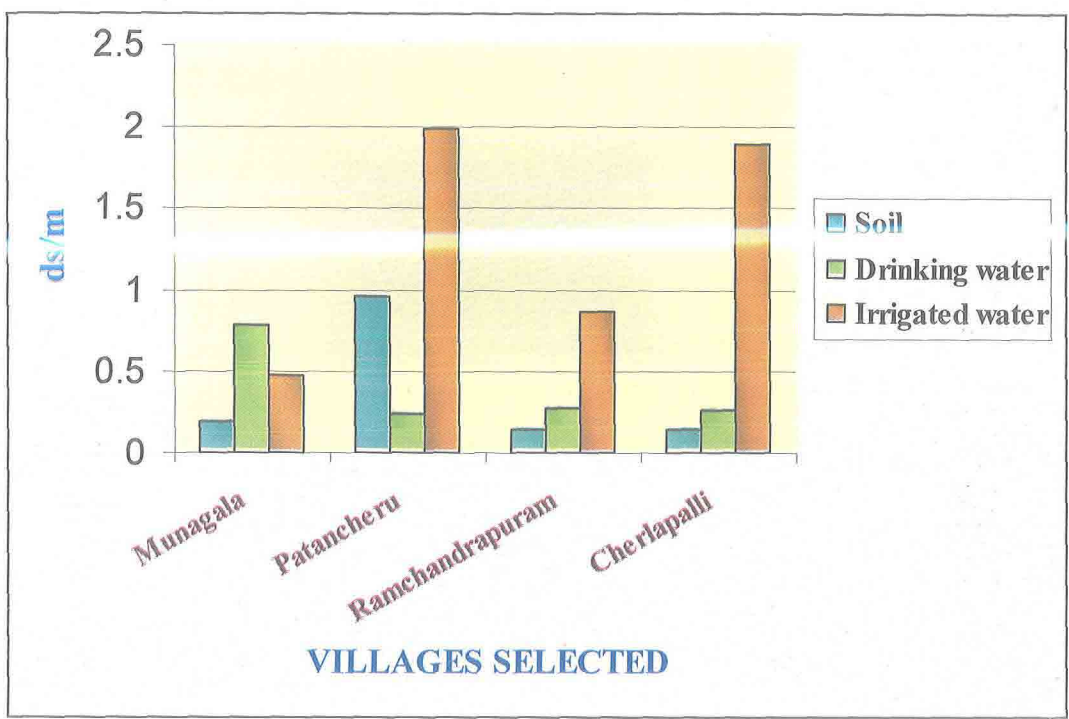


FIG 12 : ELECTRICAL CONDUCTIVITY OF SOIL AND WATER SAMPLES COLLECTED FROM SELECTED VILLAGES

As is seen from Table 30, the pH ranged from 7.08 (Ramchandrapuram drinking water) to 7.75 (Munagala drinking water) and that of electrical conductivity ranged from 0.238 (Patancheru drinking water) to 0.782 (Munagala drinking water) respectively.

TABLE 31 : pH AND ELECTRIC CONDUCTIVITY (E.C.) OF IRRIGATED WATER SAMPLES FROM POLLUTED LAND

S.No.	Name of the village	pH	E.C (ds/m)
1	Munagala(control)	7.25	0.473
2	Patancheru	6.35	1.996
3	Ramchandrapuram	7.21	0.865
4	Cherlapalli	6.83	1.894
F-value	-	324.10**	1014.26**

** = Significant at 1% level

Table 31 revealed that the pH ranged from 6.35 (Patancheru irrigated water) to 7.25 (Munagala irrigated water) and that of electrical conductivity ranged from 0.473 (Munagala irrigated water) to 1.996 (Patancheru irrigated water) respectively.

4.4 Analysis of heavy metals and micronutrients of polluted soil and water

The data pertaining to heavy metal and micronutrient content of soil and water are presented in Table 32, 33 and 34.

As is evident from Table 32, the mean chromium and iron content of control soil was high when compared to that of polluted soils ranging from 0.034

TABLE 32 : HEAVY METAL AND MICRONUTRIENT CONTENT OF SOIL SAMPLES COLLECTED FROM

CONTROL AND POLLUTED FARMS

S.No.	Name of the village	Lead (ppm)	Cadmium (ppm)	Chromium (ppm)	Cobalt (ppm)	Nickel (ppm)	Calcium (ppm)	Phosphorus (Kg/ha)	Iron (ppm)
1	Munagala(control)	0.44	0.021	0.092	0.140	0.226	0.57	15.15	7.82
2	Patancheru	1.24	0.042	0.062	0.182	0.502	2.64	18.96	3.59
3	Ramchandrapuram	0.80	0.040	0.064	0.174	0.444	1.94	15.15	3.46
4	Cherlapalli	0.48	0.022	0.034	0.152	0.240	2.10	12.46	0.87
F-value	-	632.4**	512.43**	496.32**	86.13**	501.2**	1211.13**	9.24**	1816.32**

** = Significant at 1% level

TABLE 33 : HEAVY METAL AND MICRONUTRIENT CONTENT OF DRINKING WATER SAMPLES COLLECTED

FROM CONTROL AND POLLUTED FARMS

S.No.	Name of the village	Lead (ppm)	Cadmium (ppm)	Chromium (ppm)	Cobalt (ppm)	Nickel (ppm)	Calcium (ppm)	Phosphorus (%)	Iron (ppm)
1	Munagala(control)	Trace	Trace	0.010	Trace	Trace	26.22	2.49	Trace
2	Patancheru	Trace	Trace	0.012	Trace	Trace	30.02	1.89	Trace
3	Ramchandrapuram	Trace	Trace	Trace	Trace	Trace	24.23	1.87	Trace
4	Cherlapalli	Trace	Trace	0.000	Trace	Trace	24.66	1.89	0.100
F-value	-	-	-	-	-	-	3012.43**	414.63**	-

** = Significant at 1% level

TABLE 34 : HEAVY METAL AND MICRONUTRIENT CONTENT OF IRRIGATED WATER SAMPLES COLLECTED FROM CONTROL AND POLLUTED FARMS

S.No.	Name of the village	Lead (ppm)	Cadmium (ppm)	Chromium (ppm)	Cobalt (ppm)	Nickel (ppm)	Calcium (ppm)	Phosphorus (%)	Iron (ppm)
1	Munagala(control)	Trace	Trace	0.006	Trace	Trace	29.03	3.14	Trace
2	Patancheru	0.294	0.010	0.024	0.080	0.200	39.86	3.74	Trace
3	Ramchandrapuram	Trace	Trace	Trace	Trace	Trace	35.10	1.81	Trace
4	Cherlapalli	Trace	Trace	0.040	Trace	Trace	31.08	2.12	0.100
F-value	-	-	-	-	-	-	56.21**	563.11**	-

** = Significant at 1% level

to 0.092 ppm and 0.87 to 7.82 ppm respectively. The other elements i.e., Co, Ni, Pb, Cd, Ca and P of patancheru soil were high when compared with other soil profiles. In general, the concentration of heavy metals in soil profiles followed the order of $Pb > Ni > Co > Cr > Cd$.

Table 33 indicated that lead, cadmium, cobalt and nickel were detected to be trace and chromium content of the samples ranged from trace to 0.012 ppm, Calcium : 24.23 to 30.02 ppm, Phosphorus from 1.87 to 2.49 percent and Iron from trace to 0.100 ppm respectively.

It is revealed from Table 34, that lead content ranged from trace to 0.294 ppm, Cd : trace to 0.01 ppm, Cr : trace to 0.024 ppm, Co : trace to 0.08 ppm, Ni : trace to 0.2 ppm, Ca : 29.03 to 39.86 ppm, P : 1.81 to 3.74 percent and Fe : trace to 0.212 ppm respectively. The heavy metal and micronutrient content of patancheru irrigated water was high when compared with that of other water samples.

4.5 Analysis of edible crops for heavy metals and micronutrients from polluted lands

The effect of the use of polluted water on the edible parts is important because this is the first stage of the entry of such constituents which may become toxic in the food chain. The data pertaining to the heavy metal and micronutrient content in the edible plant parts collected from farm families are presented in Table 35 and 36.

TABLE 35 : HEAVY METAL AND MICRONUTRIENT CONTENT OF RICE SAMPLES COLLECTED FROM

CONTROL AND POLLUTED LAND

S.No.	Name of the village	Lead (ppm)	Cadmium (ppm)	Chromium (ppm)	Cobalt (ppm)	Nickel (ppm)	Calcium (ppm)	Phosphorus (%)	Iron (ppm)
1	Munagala(control)	3.17	0.31	3.92	0.12	0.89	1.44	0.57	22.75
2	Patancheru	15.83	1.92	4.03	5.21	9.28	14.39	0.99	26.81
3	Ramchandrapuram	10.17	1.13	3.53	4.93	8.10	6.42	0.69	17.63
4	Cherlapalli	3.83	0.38	1.38	1.97	2.58	5.38	0.71	22.75
F-value	-	2995.08**	957.73**	1096.38**	2375.12**	2876.11**	3216.01**	510.03**	616.34**

** = Significant at 1% level

TABLE 36: HEAVY METAL AND MICRONUTRIENT CONTENT OF OTHER EDIBLE CROPS COLLECTED FROM

POLLUTED PATANCHERU VILLAGE

S.No.	Sample details	Lead (ppm)	Cadmium (ppm)	Chromium (ppm)	Cobalt (ppm)	Nickel (ppm)	Calcium (ppm)	Phosphorus (%)	Iron (ppm)
1	Sugarcane	20.65	1.59	7.71	7.03	11.55	12.91	0.94	32.50
2	Wheat	19.26	1.32	5.13	6.04	9.36	10.57	0.89	28.76
3	Fenugreek leaves	20.99	1.47	6.29	6.45	10.48	9.77	0.96	35.89
4	Tomato	0.83	T	2.19	0.08	1.36	9.81	0.38	9.49
5	Curry leaves	8.16	0.59	3.07	2.38	5.21	4.09	0.66	31.99
6	Brinjal	14.16	0.72	2.13	2.65	4.83	5.08	0.79	19.51
7	Ladies finger	20.48	1.58	5.21	5.91	9.58	1.30	0.44	30.17
8	Gogu	0.01	0.01	1.38	0.25	1.59	0.38	0.32	16.54
9	Ladies finger	11.33	0.23	4.43	3.28	5.01	0.23	0.61	36.42
10	Ridge gourd	7.16	0.42	3.65	2.05	5.08	0.26	0.77	16.96
11	Cluster bean	6.99	0.50	2.33	2.87	5.08	0.55	0.48	31.73
12	Guava	0.33	0.09	0.62	0.68	1.27	0.95	0.20	3.93
13	Mango	0.00	0.06	0.82	0.69	1.05	2.32	0.36	0.43

Table 35 revealed that the lead content of rice samples ranged from 3.17 to 15.83 ppm, Cd : 0.31 to 1.92 ppm, Cr : 1.38 to 4.03 ppm, Co : 0.12 to 5.21 ppm, Ni : 0.89 to 9.28 ppm, Ca : 1.44 to 14.39 ppm, P : 0.57 to 0.99 % and Fe : 17.63 to 26.81 ppm respectively. The heavy metal and micronutrient content of rice sample collected from patancheru were high when compared with other sample profiles.

As is evident from Table 36, the lead concentration of crops cultivated in patancheru village ranged from 0.01 to 20.99 ppm, Cd : 0.01 to 1.59 ppm, Cr : 0.62 to 7.71 ppm, Co : 0.08 to 7.03 ppm, Ni : 1.05 to 11.55 ppm, Ca : 0.23 to 12.91 ppm, P : 0.2 to 0.96 % and Fe : 0.43 to 36.42 ppm respectively.

4.6 Pot culturing for bioremediation trials

4.6.1 Growth curve particulars of the yeast cultures

The data pertaining to growth curve are presented in Table 37. It is revealed that all the selected cultures reached the stationary phase at the time of 24 hours after inoculation into suitable medium which indicates the highest activity period.

4.6.2 Enumeration of the yeast cultures

The data pertaining to the enumeration of the selected microbial yeast cultures at the time of 24 hours after inoculation into suitable medium are tabulated in Table 38.

TABLE 37 : GROWTH CURVE PARTICULARS OF YEAST CULTURES

S.No.	Cultures used	Optical density (O.D.) values									
		2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24 hrs	27 hrs	30 hrs	48 hrs
1	NCYC 1190	0.000	0.011	0.051	0.070	0.184	0.221	0.436	0.429	0.396	0.351
2	AMB 111	0.015	0.025	0.081	0.159	0.236	0.323	0.699	0.677	0.610	0.583
3	AMB 112	0.017	0.043	0.093	0.142	0.202	0.311	0.596	0.582	0.551	0.503
4	AMB 113	0.000	0.020	0.065	0.138	0.294	0.450	1.155	1.120	0.986	0.811

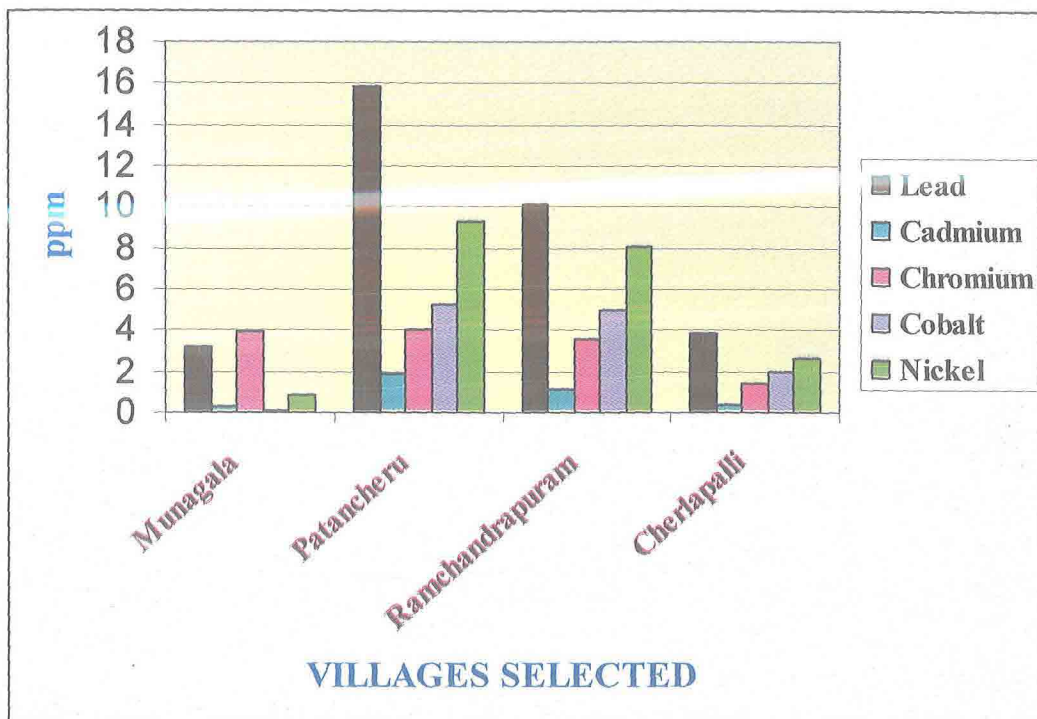


FIG 13 : HEAVY METAL CONTENT IN RICE SAMPLES COLLECTED FROM SELECTED VILLAGES

TABLE 38 : ENUMERATION DETAILS OF THE YEAST CULTURES

S.No.	Cultures used	Colony forming units / ml culture
1	NCYC 1190	50.5 x 10 ⁶
2	AMB 111	40.0 x 10 ⁶
3	AMB 112	31.0 x 10 ⁶
4	AMB 113	48.0 x 10 ⁶

As is evident from Table 38, the number of colonies per milliliter culture ranged from 31x10⁶ (AMB112) to 50.5x10⁶ (NCYC 1190).

4.6.3 Pot culturing trials under polluted and control conditions

The results pertaining to pot culture experiments are presented below.

4.6.3.1 Lead

The lead content of soil and plant samples collected from the pots is tabulated in Table 39. It is evident that the lead content of soils treated with selected four cultures with different sugar concentrations ranged from trace to 0.43 ppm (Control) and 0.444 to 0.844 ppm (polluted) respectively. The lead content of plant samples ranged from trace to 0.4 ppm (control) and 4.78 to 10.3 ppm (polluted) respectively.

4.6.3.2 Cadmium

The cadmium content of soil and plant samples collected from the pots is tabulated in Table 40. It is reported that the cadmium content of control soil and

TABLE 39 : LEAD CONTENT OF SOIL AND PLANT SAMPLES COLLECTED FROM POT CULTURING TRIALS

S.No	Sample details	Lead content (ppm)					
		Soil			Plant		
		C.P.	E.P.	T value	C.P.	E.P.	T value
1	Blank	0.43	0.844	24.21**	0.40	10.30	181.97**
2	Soil + FYM	0.23	0.644	21.03**	0.39	9.70	178.23**
3	Soil + FYM + 1% sugar	0.20	0.466	18.16**	0.30	7.73	162.11**
4	Soil + FYM + 3% sugar	0.19	0.488	22.00**	0.40	7.15	143.92**
5	Soil + FYM + 6% sugar	0.24	0.491	18.24**	0.36	7.96	148.12**
6	Soil + NCYC 1190 + FYM	0.09	0.491	58.12**	0.40	6.01	130.16**
7	Soil + NCYC 1190 + FYM + 1% sugar	T	0.502	-	0.32	5.94	111.02**
8	Soil + NCYC 1190 + FYM + 3% sugar	T	0.478	-	0.00	5.30	-
9	Soil + NCYC 1190 + FYM + 6% sugar	0.08	0.479	56.27**	0.14	5.99	156.12**
10	Soil + AMB 111 + FYM	0.19	0.801	63.24**	0.36	9.26	160.92**
11	Soil + AMB 111 + FYM + 1% sugar	0.22	0.734	43.24**	0.31	8.50	168.14**
12	Soil + AMB 111 + FYM + 3% sugar	0.30	0.796	26.82**	0.29	9.34	169.99**
13	Soil + AMB 111 + FYM + 6% sugar	0.20	0.811	59.24**	0.34	8.62	149.27**
14	Soil + AMB 112 + FYM	0.31	0.723	24.12**	0.31	8.94	170.00**
15	Soil + AMB 112 + FYM + 1% sugar	0.36	0.746	32.08**	0.28	8.88	163.49**
16	Soil + AMB 112 + FYM + 3% sugar	0.29	0.691	26.77**	0.26	8.12	160.27**
17	Soil + AMB 112 + FYM + 6% sugar	0.30	0.682	21.24**	0.30	7.96	164.12**
18	Soil + AMB 113 + FYM	0.003	0.576	166.92**	0.14	4.95	123.67**
19	Soil + AMB 113 + FYM + 1% sugar	T	0.527	-	0.10	5.01	132.63**
20	Soil + AMB 113 + FYM + 3% sugar	T	0.444	-	T	4.78	-
21	Soil + AMB 113 + FYM + 6% sugar	T	0.512	-	0.09	4.94	199.94**

C.P. = Control pot (Munagala soil + Irrigated water)
 E.P. = Experimental pot (Patancheru soil + Irrigated water)
 ** = Significant at 1% level

TABLE 40 : CADMIUM CONTENT OF SOIL AND PLANT SAMPLES COLLECTED FROM POT CULTURING TRIALS

S.No	Sample details	Cadmium content (ppm)					
		Soil			Plant		
		C.P.	E.P.	T value	C.P.	E.P.	T value
1	Blank	0.024	0.410	49.23**	0.08	6.025	96.21**
2	Soil + FYM	T	0.194	-	0.00	4.975	-
3	Soil + FYM + 1% sugar	T	0.202	-	T	4.650	-
4	Soil + FYM + 3% sugar	T	0.204	-	T	4.250	-
5	Soil + FYM + 6% sugar	T	0.211	-	T	4.320	-
6	Soil + NCYC 1190 + FYM	T	0.269	-	T	4.000	-
7	Soil + NCYC 1190 + FYM + 1% sugar	T	0.199	-	T	3.801	-
8	Soil + NCYC 1190 + FYM + 3% sugar	T	0.192	-	T	3.100	-
9	Soil + NCYC 1190 + FYM + 6% sugar	T	0.311	-	T	3.996	-
10	Soil + AMB 111 + FYM	T	0.330	-	T	4.999	-
11	Soil + AMB 111 + FYM + 1% sugar	T	0.240	-	T	4.950	-
12	Soil + AMB 111 + FYM + 3% sugar	T	0.270	-	T	4.313	-
13	Soil + AMB 111 + FYM + 6% sugar	T	0.301	-	T	4.004	-
14	Soil + AMB 112 + FYM	T	0.400	-	T	4.886	-
15	Soil + AMB 112 + FYM + 1% sugar	T	0.393	-	T	4.133	-
16	Soil + AMB 112 + FYM + 3% sugar	T	0.241	-	T	4.082	-
17	Soil + AMB 112 + FYM + 6% sugar	T	0.300	-	T	4.999	-
18	Soil + AMB 113 + FYM	T	0.194	-	T	3.725	-
19	Soil + AMB 113 + FYM + 1% sugar	T	0.201	-	T	3.001	-
20	Soil + AMB 113 + FYM + 3% sugar	T	0.188	-	T	2.350	-
21	Soil + AMB 113 + FYM + 6% sugar	T	0.199	-	T	2.912	-

C.P. = Control pot (Munagala soil + Irrigated water)
 E.P. = Experimental pot (Patancheru soil + Irrigated water)
 ** = Significant at 1% level

plant samples treated with selected four cultures with different sugar concentrations were detected as trace, whereas in polluted soil samples the cadmium content ranged from 0.188 to 0.410 ppm and in plant samples it ranged from 2.35 to 6.025 ppm respectively.

4.6.3.3 Chromium

The chromium content of soil and plant samples collected from the pots are tabulated in Table 41. As is evident from Table 42, the chromium content of control soil samples ranged from trace to 0.094 ppm and those of plant samples ranged from trace to 0.042 ppm. Whereas the chromium content of polluted soil and plant samples were detected as trace amounts.

4.6.3.4 Cobalt

The cobalt content of soil and plant samples is tabulated in Table 42. It is reported that the cobalt content of control soil and plant samples treated with selected four cultures with different sugar concentrations ranged from trace to 0.136 ppm and trace to 0.89 ppm respectively. The cobalt content of polluted soil and plant samples ranged from 0.12 to 0.186 ppm and 2.75 to 4.9 ppm respectively.

TABLE 41 : CHROMIUM CONTENT OF SOIL AND PLANT SAMPLES COLLECTED FROM POT CULTURING TRIALS

S.No	Sample details	Chromium content (ppm)					
		Soil			Plant		
		C.P.	E.P.	T value	C.P.	E.P.	T value
1	Blank	0.094	T	-	0.042	T	-
2	Soil + FYM	0.009	T	-	0.003	T	-
3	Soil + FYM + 1% sugar	0.003	T	-	T	T	-
4	Soil + FYM + 3% sugar	T	T	-	T	T	-
5	Soil + FYM + 6% sugar	T	T	-	T	T	-
6	Soil + NCYC 1190 + FYM	T	T	-	T	T	-
7	Soil + NCYC 1190 + FYM + 1% sugar	T	T	-	T	T	-
8	Soil + NCYC 1190 + FYM + 3% sugar	T	T	-	T	T	-
9	Soil + NCYC 1190 + FYM + 6% sugar	T	T	-	T	T	-
10	Soil + AMB 111 + FYM	T	T	-	T	T	-
11	Soil + AMB 111 + FYM + 1% sugar	T	T	-	T	T	-
12	Soil + AMB 111 + FYM + 3% sugar	T	T	-	T	T	-
13	Soil + AMB 111 + FYM + 6% sugar	T	T	-	T	T	-
14	Soil + AMB 112 + FYM	T	T	-	T	T	-
15	Soil + AMB 112 + FYM + 1% sugar	T	T	-	T	T	-
16	Soil + AMB 112 + FYM + 3% sugar	T	T	-	T	T	-
17	Soil + AMB 112 + FYM + 6% sugar	T	T	-	T	T	-
18	Soil + AMB 113 + FYM	T	T	-	T	T	-
19	Soil + AMB 113 + FYM + 1% sugar	T	T	-	T	T	-
20	Soil + AMB 113 + FYM + 3% sugar	T	T	-	T	T	-
21	Soil + AMB 113 + FYM + 6% sugar	T	T	-	T	T	-

C.P. = Control pot (Munagala soil + Irrigated water)
 E.P. = Experimental pot (Patancheru soil + Irrigated water)
 ** = Significant at 1% level

TABLE 42 : COBALT CONTENT OF SOIL AND PLANT SAMPLES COLLECTED FROM POT CULTURING TRIALS

S.No	Sample details	Cobalt content (ppm)					
		Soil			Plant		
		C.P.	E.P.	T value	C.P.	E.P.	T value
1	Blank	0.136	0.186	35.26**	0.89	4.90	80.06**
2	Soil + FYM	0.112	0.138	31.02**	0.66	4.05	75.57**
3	Soil + FYM + 1% sugar	0.110	0.138	32.00**	0.54	3.50	85.66**
4	Soil + FYM + 3% sugar	0.106	0.148	43.63**	0.61	3.80	83.79**
5	Soil + FYM + 6% sugar	0.113	0.162	34.93**	0.49	3.77	89.38**
6	Soil + NCYC 1190 + FYM	0.100	0.166	51.28**	0.21	4.01	129.79**
7	Soil + NCYC 1190 + FYM + 1% sugar	0.046	0.192	84.93**	0.11	3.92	126.91**
8	Soil + NCYC 1190 + FYM + 3% sugar	0.009	0.132	104.63**	0.03	3.55	161.02**
9	Soil + NCYC 1190 + FYM + 6% sugar	0.013	0.143	106.11**	0.17	3.81	132.97**
10	Soil + AMB 111 + FYM	0.100	0.166	51.32**	0.54	3.99	81.07**
11	Soil + AMB 111 + FYM + 1% sugar	0.090	0.148	81.30**	0.61	3.73	85.63**
12	Soil + AMB 111 + FYM + 3% sugar	0.099	0.155	82.17**	0.59	3.81	82.73**
13	Soil + AMB 111 + FYM + 6% sugar	0.090	0.155	84.16**	0.78	3.66	73.36**
14	Soil + AMB 112 + FYM	0.140	0.196	38.19**	0.81	4.00	83.66**
15	Soil + AMB 112 + FYM + 1% sugar	0.124	0.190	43.66**	0.73	3.99	79.45**
16	Soil + AMB 112 + FYM + 3% sugar	0.123	0.183	47.08**	0.66	3.71	89.41**
17	Soil + AMB 112 + FYM + 6% sugar	0.136	0.177	30.14**	0.49	3.55	84.54**
18	Soil + AMB 113 + FYM	0.049	0.138	70.11**	0.10	3.28	85.46**
19	Soil + AMB 113 + FYM + 1% sugar	0.042	0.147	51.23**	0.04	3.17	157.52**
20	Soil + AMB 113 + FYM + 3% sugar	T	0.120	-	T	2.75	-
21	Soil + AMB 113 + FYM + 6% sugar	0.061	0.139	40.18**	T	3.01	-

C.P. = Control pot (Munagala soil + Irrigated water)

E.P. = Experimental pot (Patancheru soil + Irrigated water)

** = Significant at 1% level

4.6.3.5 Nickel

The nickel content of soil and plant samples is tabulated in Table 43. It is evident that the nickel content of control soil and plant samples treated with selected four cultures with different sugar concentrations ranged from trace to 0.021 ppm and trace to 0.04 ppm respectively. Whereas the nickel content of polluted soil and plant samples ranged from trace to 0.032 ppm and 0.15 to 0.525 ppm respectively.

4.6.3.6 Calcium

The calcium content of soil and plant samples collected from the pots are tabulated in Table 44. It is revealed that the calcium content of control soil and plant samples treated with selected four cultures with different sugar concentrations ranged from 0.29 to 0.51 ppm and 4.0 to 5.43 ppm respectively. The calcium content of polluted soil and plant samples ranged from 1.43 to 2.89 ppm and 8.01 to 9.74 ppm respectively.

4.6.3.7 Phosphorus

The phosphorus content of soil and plant samples is given in Table 45. As is evident from Table 45, the phosphorus content of control soil and plant samples treated with selected four cultures with different sugar concentrations ranged from 13.964 to 15.001 Kg/ha and 0.39 to 0.63 percent respectively. Whereas in polluted

TABLE 43 : NICKEL CONTENT OF SOIL AND PLANT SAMPLES COLLECTED FROM POT CULTURING TRIALS

S.No	Sample details	Nickel content (ppm)					
		Soil			Plant		
		C.P.	E.P.	T value	C.P.	E.P.	T value
1	Blank	0.021	0.032	17.92**	0.04	0.525	56.48**
2	Soil + FYM	T	0.016	-	0.000	0.575	-
3	Soil + FYM + 1% sugar	T	0.010	-	T	0.475	-
4	Soil + FYM + 3% sugar	T	0.006	-	T	0.450	-
5	Soil + FYM + 6% sugar	T	0.008	-	T	0.421	-
6	Soil + NCYC 1190 + FYM	T	0.003	-	T	0.390	-
7	Soil + NCYC 1190 + FYM + 1% sugar	T	0.001	-	T	0.342	-
8	Soil + NCYC 1190 + FYM + 3% sugar	T	T	-	T	0.275	-
9	Soil + NCYC 1190 + FYM + 6% sugar	T	0.009	-	T	0.299	-
10	Soil + AMB 111 + FYM	T	0.026	-	T	0.512	-
11	Soil + AMB 111 + FYM + 1% sugar	T	0.012	-	T	0.500	-
12	Soil + AMB 111 + FYM + 3% sugar	T	0.008	-	T	0.500	-
13	Soil + AMB 111 + FYM + 6% sugar	T	0.019	-	T	0.506	-
14	Soil + AMB 112 + FYM	T	0.024	-	T	0.499	-
15	Soil + AMB 112 + FYM + 1% sugar	T	0.020	-	T	0.500	-
16	Soil + AMB 112 + FYM + 3% sugar	T	0.031	-	T	0.482	-
17	Soil + AMB 112 + FYM + 6% sugar	T	0.026	-	T	0.489	-
18	Soil + AMB 113 + FYM	T	0.003	-	T	0.450	-
19	Soil + AMB 113 + FYM + 1% sugar	T	0.001	-	T	0.399	-
20	Soil + AMB 113 + FYM + 3% sugar	T	T	-	T	0.150	-
21	Soil + AMB 113 + FYM + 6% sugar	T	0.001	-	T	0.281	-

C.P. = Control pot (Munagala soil + Irrigated water)
 E.P. = Experimental pot (Patancheru soil + Irrigated water)
 ** = Significant at 1% level

TABLE 44 : CALCIUM CONTENT OF SOIL AND PLANT SAMPLES COLLECTED FROM POT CULTURING TRIALS

S.No	Sample details	Calcium content (ppm)							
		Soil				Plant			
		C.P.	E.P.	T value	C.P.	E.P.	T value	C.P.	T value
1	Blank	0.51	2.89	82.10**	5.43	9.74	35.91**		
2	Soil + FYM	0.52	2.56	63.85**	5.40	9.53	31.23**		
3	Soil + FYM + 1% sugar	0.50	2.59	58.10**	5.36	9.56	32.14**		
4	Soil + FYM + 3% sugar	0.46	2.43	54.45**	5.32	9.48	31.97**		
5	Soil + FYM + 6% sugar	0.49	2.66	73.73**	5.40	9.40	34.13**		
6	Soil + NCYC 1190 + FYM	0.52	2.03	93.51**	5.31	9.12	29.13**		
7	Soil + NCYC 1190 + FYM + 1% sugar	0.50	1.98	98.41**	4.37	9.00	29.76**		
8	Soil + NCYC 1190 + FYM + 3% sugar	0.32	1.62	96.08**	4.12	8.39	24.66**		
9	Soil + NCYC 1190 + FYM + 6% sugar	0.36	1.69	99.70**	4.18	8.56	26.21**		
10	Soil + AMB 111 + FYM	0.43	2.00	93.77**	4.96	9.32	50.66**		
11	Soil + AMB 111 + FYM + 1% sugar	0.53	2.43	79.05**	4.82	9.21	48.12**		
12	Soil + AMB 111 + FYM + 3% sugar	0.51	2.59	59.21**	4.56	9.04	51.24**		
13	Soil + AMB 111 + FYM + 6% sugar	0.49	2.66	70.87**	4.77	9.38	49.98**		
14	Soil + AMB 112 + FYM	0.55	2.83	79.74**	4.83	9.11	48.73**		
15	Soil + AMB 112 + FYM + 1% sugar	0.50	2.50	80.05**	5.00	8.92	22.13**		
16	Soil + AMB 112 + FYM + 3% sugar	0.51	2.03	65.32**	4.90	8.90	52.01**		
17	Soil + AMB 112 + FYM + 6% sugar	0.48	1.94	75.92**	4.83	9.00	53.21**		
18	Soil + AMB 113 + FYM	0.36	1.63	98.96**	4.24	8.63	41.17**		
19	Soil + AMB 113 + FYM + 1% sugar	0.34	1.53	91.50**	4.16	8.42	43.12**		
20	Soil + AMB 113 + FYM + 3% sugar	0.29	1.43	90.86**	4.00	8.01	40.93**		
21	Soil + AMB 113 + FYM + 6% sugar	0.34	1.50	90.29**	4.03	8.19	40.81**		

C.P. = Control pot (Munagala soil + Irrigated water)
 E.P. = Experimental pot (Patancheru soil + Irrigated water)
 ** = Significant at 1% level

TABLE 45 : PHOSPHORUS CONTENT OF SOIL AND PLANT SAMPLES COLLECTED FROM POT CULTURING TRIALS

S.No	Sample details	Phosphorus content					
		Soil (Kg/ha)			Plant (%)		
		C.P.	E.P.	T value	C.P.	E.P.	T value
1	Blank	15.074	18.980	49.33**	0.63	0.99	5.79**
2	Soil + FYM	15.001	18.342	48.37**	0.60	0.93	5.01**
3	Soil + FYM + 1% sugar	15.012	18.414	47.24**	0.59	0.90	5.00**
4	Soil + FYM + 3% sugar	15.067	18.303	48.78**	0.61	0.89	2.89**
5	Soil + FYM + 6% sugar	15.061	18.096	48.34**	0.52	0.88	2.70*
6	Soil + NCYC 1190 + FYM	14.983	17.000	25.66**	0.50	0.83	2.71*
7	Soil + NCYC 1190 + FYM + 1% sugar	14.677	16.031	15.53**	0.43	0.74	5.00**
8	Soil + NCYC 1190 + FYM + 3% sugar	14.010	15.110	9.63**	0.32	0.52	2.20*
9	Soil + NCYC 1190 + FYM + 6% sugar	14.999	15.964	8.12**	0.40	0.68	2.26*
10	Soil + AMB 111 + FYM	15.001	18.016	46.38**	0.53	0.77	2.54*
11	Soil + AMB 111 + FYM + 1% sugar	15.014	18.000	28.93**	0.50	0.83	2.71*
12	Soil + AMB 111 + FYM + 3% sugar	15.048	17.948	21.90**	0.61	0.69	1.84*
13	Soil + AMB 111 + FYM + 6% sugar	15.007	18.122	26.51**	0.49	0.80	5.01**
14	Soil + AMB 112 + FYM	15.066	18.246	34.30**	0.60	0.72	1.99*
15	Soil + AMB 112 + FYM + 1% sugar	15.042	18.333	46.24**	0.55	0.79	2.56*
16	Soil + AMB 112 + FYM + 3% sugar	15.001	18.148	51.06**	0.58	0.80	4.63**
17	Soil + AMB 112 + FYM + 6% sugar	14.968	18.093	37.78**	0.60	0.83	2.69*
18	Soil + AMB 113 + FYM	14.029	16.031	39.44**	0.42	0.79	4.92**
19	Soil + AMB 113 + FYM + 1% sugar	14.011	15.003	8.00**	0.40	0.62	2.41*
20	Soil + AMB 113 + FYM + 3% sugar	13.964	14.900	9.59**	0.33	0.48	1.97*
21	Soil + AMB 113 + FYM + 6% sugar	14.001	14.963	6.89**	0.39	0.58	2.18*

C.P. = Control pot (Munagala soil + Irrigated water)

E.P. = Experimental pot (Patancheru soil + Irrigated water)

* = Significant at 5% Level ** = Significant at 1% level

soil and plant samples, the phosphorus content ranged from 14.9 to 18.98 Kg/ha and 0.48 to 0.99 percent respectively.

4.6.3.8 Iron

The iron content of soil and plant samples collected from the pots is tabulated in Table 46. It is revealed that the iron content of control soil and plant samples treated with selected four cultures with different sugar concentrations ranged from 1.942 to 7.619 ppm and 14.010 to 20.104 ppm respectively. The iron content of polluted soil and plant samples ranged from 1.114 to 3.586 ppm and 17.6 to 26.025 ppm respectively.

4.6.3.9 Enumeration of yeasts in soils collected from pot culturing trials

The data pertaining to enumeration of yeasts in soils collected from pots are tabulated in Table 47 and 48. Table 47 indicated that the colony forming units per gram of control soil on zero day ranged from 1×10^3 to 4×10^3 ; on 2nd day – 2×10^3 to 151×10^3 ; 4th day – 1×10^3 to 121×10^3 ; 6th day – 2×10^2 to 79×10^3 ; 10th day – 1×10^2 to 40×10^2 and on 15th day – 1×10^2 to 9×10^2 respectively.

It is evident from Table 48 that the colony forming units per gram of polluted soil on zero day ranged from 1×10^3 to 4×10^3 ; on 2nd day – 2×10^3 to 158×10^3 ; 4th day – 1×10^3 to 122×10^3 ; 6th day – 1×10^2 to 78×10^3 ; 10th day – zero to 38×10^2 and on 15th day – zero to 5×10^2 respectively.

TABLE 46: IRON CONTENT OF SOIL AND PLANT SAMPLES COLLECTED FROM POT CULTURING TRIALS

S.No	Sample details	Iron content (ppm)					
		Soil			Plant		
		C.P.	E.P.	T value	C.P.	E.P.	T value
1	Blank	7.619	3.586	99.97**	20.104	26.025	65.23**
2	Soil + FYM	4.142	1.984	83.58**	20.000	25.200	69.28**
3	Soil + FYM + 1% sugar	4.008	1.682	84.54**	18.146	25.275	87.05**
4	Soil + FYM + 3% sugar	4.362	1.716	86.32**	15.142	24.600	88.32**
5	Soil + FYM + 6% sugar	4.198	1.999	92.01**	16.199	24.112	81.42**
6	Soil + NCYC 1190 + FYM	3.266	2.001	71.53**	15.190	20.121	69.32**
7	Soil + NCYC 1190 + FYM + 1% sugar	1.992	1.946	0.94	15.003	20.100	69.99**
8	Soil + NCYC 1190 + FYM + 3% sugar	1.990	1.248	19.09**	14.242	19.175	70.67**
9	Soil + NCYC 1190 + FYM + 6% sugar	2.246	1.613	28.04**	15.146	21.124	68.89**
10	Soil + AMB 111 + FYM	5.142	4.011	22.38**	18.124	24.001	83.75**
11	Soil + AMB 111 + FYM + 1% sugar	4.117	3.442	64.92**	18.946	23.225	80.15**
12	Soil + AMB 111 + FYM + 3% sugar	4.942	3.998	70.35**	18.078	22.012	74.43**
13	Soil + AMB 111 + FYM + 6% sugar	4.006	4.000	0.15	19.111	23.119	72.38**
14	Soil + AMB 112 + FYM	3.983	2.781	66.10**	19.782	24.099	80.17**
15	Soil + AMB 112 + FYM + 1% sugar	3.946	2.040	80.89**	18.990	23.177	81.11**
16	Soil + AMB 112 + FYM + 3% sugar	4.000	2.005	76.63**	19.246	23.009	80.66**
17	Soil + AMB 112 + FYM + 6% sugar	3.879	2.611	69.56**	19.517	22.163	76.97**
18	Soil + AMB 113 + FYM	3.014	1.972	71.30**	15.482	20.300	66.13**
19	Soil + AMB 113 + FYM + 1% sugar	2.568	1.840	54.84**	15.001	18.449	68.58**
20	Soil + AMB 113 + FYM + 3% sugar	1.942	1.114	27.13**	14.010	17.600	60.87**
21	Soil + AMB 113 + FYM + 6% sugar	2.412	1.339	70.82**	15.672	19.621	66.01**

C.P. = Control pot (Munagala soil + Irrigated water)

E.P. = Experimental pot (Patancheru soil + Irrigated water)

** = Significant at 1% level

**TABLE 47 : ENUMERATION OF MICROBES IN THE SOILS COLLECTED FROM POT CULTURING TRIALS
(CONTROL POTS)**

S.No.	Sample details	Colony forming units / gm soil								
		0 day	2 nd day	4 th day	6 th day	10 th day	15 th day			
1	Blank	2 x 10 ³	3 x 10 ³	2 x 10 ³	2 x 10 ²	-	-	-	-	-
2	Soil + FYM	1 x 10 ³	2 x 10 ³	1 x 10 ³	2 x 10 ²	-	-	-	-	-
3	Soil + FYM + 1% sugar	1 x 10 ³	6 x 10 ³	1 x 10 ³	3 x 10 ²	1 x 10 ²	-	-	-	-
4	Soil + FYM + 3% sugar	2 x 10 ³	10 x 10 ³	3 x 10 ³	4 x 10 ²	1 x 10 ²	-	-	-	-
5	Soil + FYM + 6% sugar	3 x 10 ³	11 x 10 ³	5 x 10 ³	5 x 10 ²	3 x 10 ²	-	-	-	-
6	Soil + NCYC 1190 + FYM	2 x 10 ³	128 x 10 ³	105 x 10 ³	60 x 10 ³	25 x 10 ²	1 x 10 ²	-	-	-
7	Soil + NCYC 1190 + FYM + 1% sugar	3 x 10 ³	131 x 10 ³	114 x 10 ³	61 x 10 ³	24 x 10 ²	1 x 10 ²	-	-	-
8	Soil + NCYC 1190 + FYM + 3% sugar	1 x 10 ³	134 x 10 ³	121 x 10 ³	75 x 10 ³	33 x 10 ²	4 x 10 ²	-	-	-
9	Soil + NCYC 1190 + FYM + 6% sugar	1 x 10 ³	136 x 10 ³	120 x 10 ³	73 x 10 ³	28 x 10 ²	3 x 10 ²	-	-	-
10	Soil + AMB 111 + FYM	2 x 10 ³	117 x 10 ³	100 x 10 ³	67 x 10 ³	15 x 10 ²	1 x 10 ¹	-	-	-
11	Soil + AMB 111 + FYM + 1% sugar	1 x 10 ³	119 x 10 ³	98 x 10 ³	47 x 10 ³	5 x 10 ²	1 x 10 ¹	-	-	-
12	Soil + AMB 111 + FYM + 3% sugar	2 x 10 ³	120 x 10 ³	112 x 10 ³	58 x 10 ³	7 x 10 ²	3 x 10 ¹	-	-	-
13	Soil + AMB 111 + FYM + 6% sugar	1 x 10 ³	122 x 10 ³	110 x 10 ³	55 x 10 ³	8 x 10 ²	4 x 10 ¹	-	-	-
14	Soil + AMB 112 + FYM	3 x 10 ³	110 x 10 ³	92 x 10 ³	47 x 10 ³	6 x 10 ²	3 x 10 ¹	-	-	-
15	Soil + AMB 112 + FYM + 1% sugar	3 x 10 ³	111 x 10 ³	95 x 10 ³	46 x 10 ³	9 x 10 ²	5 x 10 ¹	-	-	-
16	Soil + AMB 112 + FYM + 3% sugar	1 x 10 ³	113 x 10 ³	100 x 10 ³	44 x 10 ³	7 x 10 ²	1 x 10 ¹	-	-	-
17	Soil + AMB 112 + FYM + 6% sugar	1 x 10 ³	115 x 10 ³	97 x 10 ³	45 x 10 ³	6 x 10 ²	1 x 10 ¹	-	-	-
18	Soil + AMB 113 + FYM	3 x 10 ³	140 x 10 ³	99 x 10 ³	71 x 10 ³	29 x 10 ²	9 x 10 ²	-	-	-
19	Soil + AMB 113 + FYM + 1% sugar	4 x 10 ³	146 x 10 ³	110 x 10 ³	74 x 10 ³	34 x 10 ²	5 x 10 ²	-	-	-
20	Soil + AMB 113 + FYM + 3% sugar	2 x 10 ³	149 x 10 ³	114 x 10 ³	77 x 10 ³	36 x 10 ²	6 x 10 ²	-	-	-
21	Soil + AMB 113 + FYM + 6% sugar	2 x 10 ³	151 x 10 ³	120 x 10 ³	79 x 10 ³	40 x 10 ²	8 x 10 ²	-	-	-

**TABLE 48 : ENUMERATION OF MICROBES IN THE SOILS COLLECTED FROM POT CULTURING TRIALS
(EXPERIMENTAL POTS)**

S.No.	Sample details	Colony forming units / gm soil								
		0 day	2 nd day	4 th day	6 th day	10 th day	15 th day			
1	Blank	1 x10 ³	2 x10 ³	2 x10 ³	1 x10 ²	-	-	-	-	-
2	Soil + FYM	2 x 10 ³	3 x10 ³	2 x10 ³	2 x10 ²	-	-	-	-	-
3	Soil + FYM + 1% sugar	1 x10 ³	7 x10 ³	1 x10 ³	3 x10 ²	1 x10 ²	-	-	-	-
4	Soil + FYM + 3% sugar	2 x10 ³	9 x10 ³	2 x10 ³	4 x10 ²	1 x10 ²	-	-	-	-
5	Soil + FYM + 6% sugar	4 x10 ³	10 x10 ³	3 x10 ³	4 x10 ²	2 x10 ²	-	-	-	-
6	Soil + NCYC 1190 + FYM	2 x10 ³	130 x10 ³	102 x10 ³	63 x10 ³	22 x10 ²	1 x10 ²	-	-	-
7	Soil + NCYC 1190 + FYM + 1% sugar	3 x10 ³	136 x10 ³	110 x10 ³	65 x10 ³	29 x10 ²	2 x10 ²	-	-	-
8	Soil + NCYC 1190 + FYM + 3% sugar	1 x10 ³	138 x10 ³	122 x10 ³	70 x10 ³	31 x10 ²	3 x10 ²	-	-	-
9	Soil + NCYC 1190 + FYM + 6% sugar	2 x10 ³	137 x10 ³	122 x10 ³	71 x10 ³	30 x10 ²	3 x10 ²	-	-	-
10	Soil + AMB 111 + FYM	3 x10 ³	120 x10 ³	101 x10 ³	54 x10 ³	12 x10 ²	2 x10 ¹	-	-	-
11	Soil + AMB 111 + FYM + 1% sugar	1 x10 ³	124 x10 ³	100 x10 ³	49 x10 ³	4 x10 ²	1 x10 ¹	-	-	-
12	Soil + AMB 111 + FYM + 3% sugar	2 x10 ³	126 x10 ³	116 x10 ³	56 x10 ³	8 x10 ²	2 x10 ¹	-	-	-
13	Soil + AMB 111 + FYM + 6% sugar	1 x10 ³	126 x10 ³	114 x10 ³	54 x10 ³	9 x10 ²	2 x10 ¹	-	-	-
14	Soil + AMB 112 + FYM	2 x10 ³	111 x10 ³	98 x10 ³	46 x10 ³	8 x10 ²	2 x10 ¹	-	-	-
15	Soil + AMB 112 + FYM + 1% sugar	3 x10 ³	114 x10 ³	99 x10 ³	45 x10 ³	8 x10 ²	1 x10 ¹	-	-	-
16	Soil + AMB 112 + FYM + 3% sugar	1 x10 ³	116 x10 ³	102 x10 ³	47 x10 ³	9 x10 ²	2 x10 ¹	-	-	-
17	Soil + AMB 112 + FYM + 6% sugar	2 x10 ³	117 x10 ³	100 x10 ³	46 x10 ³	8 x10 ²	2 x10 ¹	-	-	-
18	Soil + AMB 113 + FYM	3 x10 ³	141 x10 ³	110 x10 ³	69 x10 ³	29 x10 ²	3 x10 ²	-	-	-
19	Soil + AMB 113 + FYM + 1% sugar	4 x10 ³	150 x10 ³	114 x10 ³	72 x10 ³	32 x10 ²	4 x10 ²	-	-	-
20	Soil + AMB 113 + FYM + 3% sugar	2 x10 ³	156 x10 ³	119 x10 ³	75 x10 ³	34 x10 ²	4 x10 ²	-	-	-
21	Soil + AMB 113 + FYM + 6% sugar	1 x10 ³	158 x10 ³	122 x10 ³	78 x10 ³	38 x10 ²	5 x10 ²	-	-	-

4.6.3.10 Identification of yeast culture, AMB 113

The results are schematically represented in Plate 10. The results revealed that AMB 113 culture isolated from molasses showed ascospore formation similar to that of NCYC 1190 (*Saccharomyces cerevisiae*).

4.7 Testing of soil and crop grown from bioremediated land for heavy metals and micronutrients

The results pertaining to the bioremediated farm trials practiced in Patancheru with selected yeast strain are presented below :

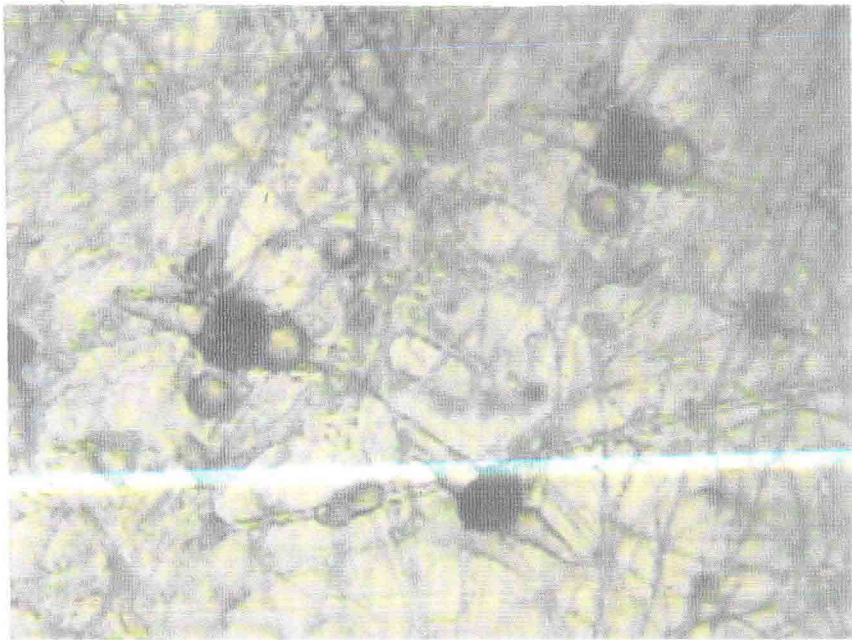
4.7.1 Lead

The lead content of soil and plant sample collected from the control and bioremediated soils of patancheru are tabulated in Table 49.

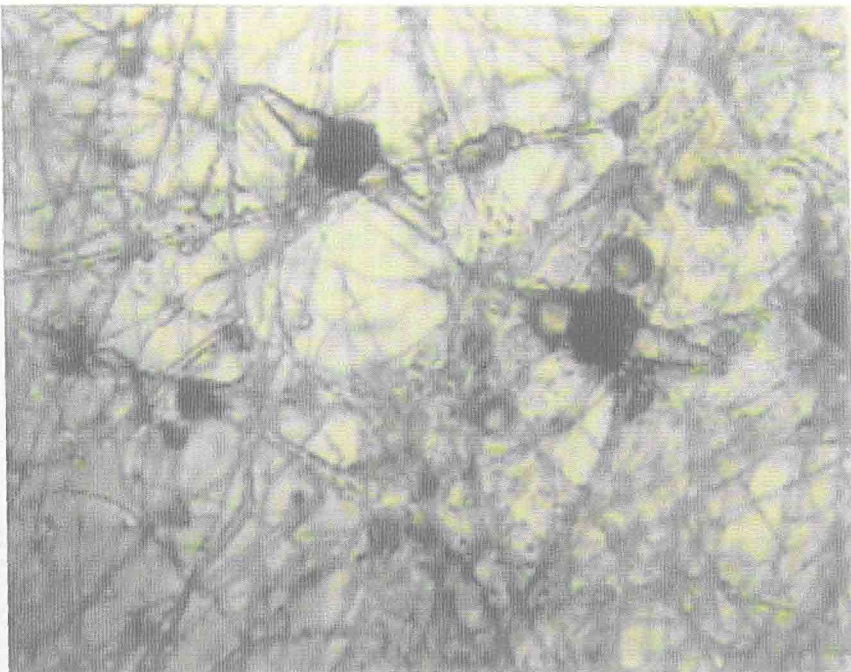
**TABLE 49 : LEAD CONTENT IN SOIL AND FENUGREEK LEAVES
COLLECTED FROM BIOREMEDIATED FARM**

Sample details	Lead content (ppm)	
	Soil	Edible leaves
Control	0.856	10.41
Bioremediated soil	0.438	5.48
T-value	7.24**	5.012**

** = Significant at 1% level



A: Saccharomyces cerevisiae NCYC 1190



B : Saccharomyces species AMB 113

PLATE 10 : ASCOSPORE FORMATION IN YEAST CULTURES

Table 49 revealed that the lead content of soil and plant samples collected from bioremediated field (patancheru) ranged from 0.856 to 0.438 ppm and 10.41 to 5.48 ppm respectively.

4.7.2 Cadmium

The cadmium content of soil and plant samples collected from the bioremediated field are tabulated in Table 50.

**TABLE 50 : CADMIUM CONTENT IN SOIL AND FENUGREEK LEAVES
COLLECTED FROM BIOREMEDIATED FARM**

Sample details	Cadmium content (ppm)	
	Soil	Edible leaves
Control	0.590	6.16
Bioremediated soil	0.187	3.11
T-value	7.82**	5.92**

** = Significant at 1% level

As is evident from Table 50, the cadmium content of soil and plant sample ranged from 0.590 to 0.187 ppm and 6.16 to 3.11 ppm respectively.

4.7.3 Chromium

Trace amount of chromium was detected in soil and plant samples collected from the bioremediated field (patancheru).

4.7.4 Cobalt

The cobalt content of soil and plant samples collected from the field are tabulated in Table 51. It is evident that the cobalt content of soil sample ranged from 0.221 to 0.138 ppm and that of plant sample ranged from 5.01 to 3.64 ppm respectively.

**TABLE 51 : COBALT CONTENT IN SOIL AND FENUGREEK LEAVES
COLLECTED FROM BIOREMEDIATED FARM**

Sample details	Cobalt content (ppm)	
	Soil	Edible leaves
Control	0.221	5.01
Bioremediated soil	0.138	3.64
T-value	2.781*	2.982*

* = Significant at 5% level

4.7.5 Nickel

The data pertaining to nickel content of soil and plant samples collected from bioremediated field are presented in Table 52. As is seen from Table 52, the nickel content of soil sample collected from bioremediated (patancheru) field was detected as trace and that of plant sample ranged from 0.625 to 0.310 ppm respectively (Fig 14 and 15).

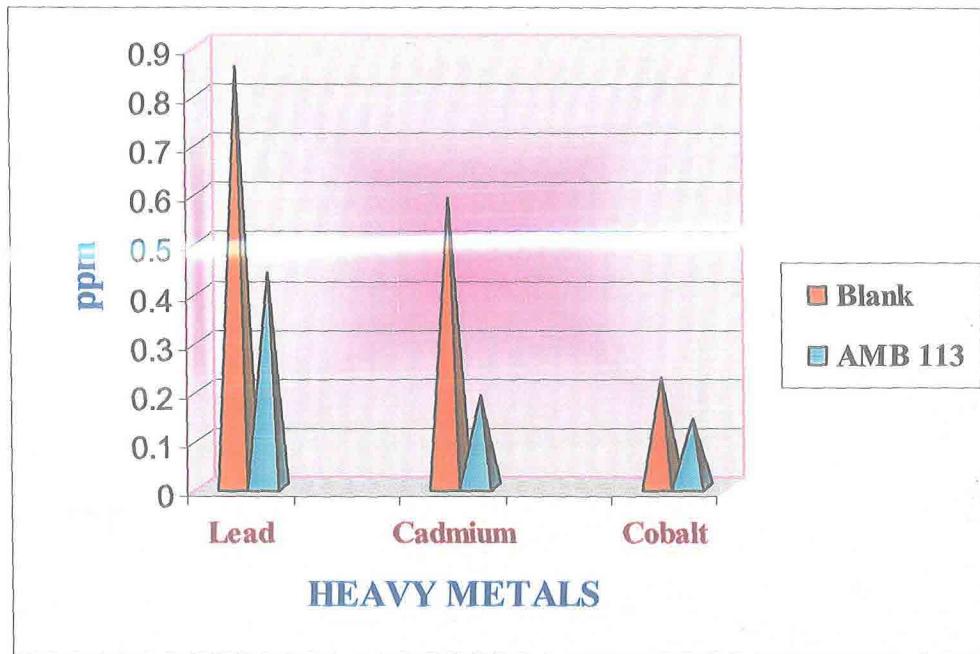


FIG 14 : HEAVY METAL CONTENT OF SOIL SAMPLES COLLECTED FROM BIOREMEDIATED SOILS USING YEAST CULTURE (AMB113)

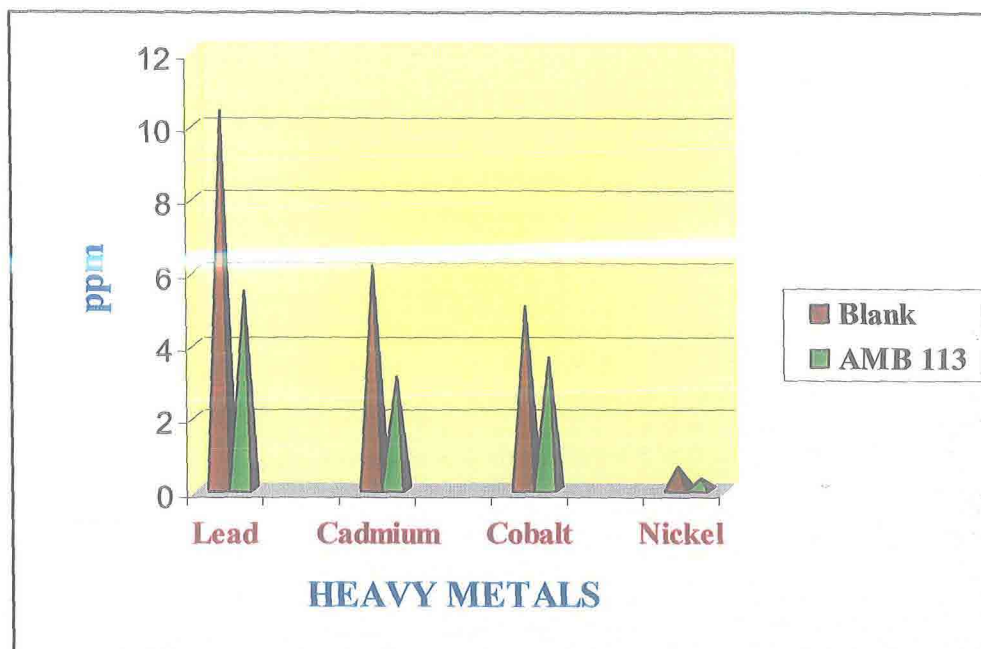


FIG 15 : HEAVY METAL CONTENT OF FENUGREEK LEAVES COLLECTED FROM BIOREMEDIATED SOILS USING YEAST CULTURE (AMB113)

**TABLE 52 : NICKEL CONTENT IN SOIL AND FENUGREEK LEAVES
COLLECTED FROM BIOREMEDIATED FARM**

Sample details	Nickel content (ppm)	
	Soil	Edible leaves
Control	Trace	0.625
Bioremediated soil	Trace	0.310
T-value	-	5.12**

** = Significant at 1% level

4.7.6 Calcium

The calcium content of soil and plant samples collected from the fields were presented in Table 53.

**TABLE 53 : CALCIUM CONTENT IN SOIL AND FENUGREEK LEAVES
COLLECTED FROM BIOREMEDIATED FARM**

Sample details	Calcium content (ppm)	
	Soil	Edible leaves
Control	2.98	9.82
Bioremediated soil	1.48	8.15
T-value	2.514 ^{NS}	2.712 ^{NS}

NS = Not significant

Table 53 reported that the calcium content of soil and plant samples collected from bioremediated field ranged from 2.98 to 1.48 ppm and 9.82 to 8.15 ppm respectively.

4.7.7 Phosphorus

The data pertaining to phosphorus content of soil and plant samples collected from field are given in Table 54. It is evident that the phosphorus content of soil and plant samples collected from bioremediated field (patancheru) ranged from 18.701 to 14.925 Kg/ha and 0.98 to 0.46 percent respectively.

**TABLE 54 : PHOSPHORUS CONTENT IN SOIL AND FENUGREEK LEAVES
COLLECTED FROM BIOREMEDIATED FARM**

Sample details	Phosphorus content	
	Soil (Kg/ha)	Edible leaves (%)
Control	18.701	0.98
Bioremediated soil	14.925	0.46
T-value	7.482**	9.268**

** = Significant at 1% level

4.7.8 Iron

The data related to iron content of soil and plant samples collected from field are presented in Table 55. It is revealed from Table 55 that the iron content of soil and plant samples collected from bioremediated field (patancheru) ranged from 4.66 to 1.987 ppm and 27.108 to 17.948 ppm respectively.

TABLE 55 : IRON CONTENT IN SOIL AND FENUGREEK LEAVES
COLLECTED FROM BIOREMEDIATED FARM

Sample details	Iron content (ppm)	
	Soil	Edible leaves
Control	4.66	27.108
Bioremediated soil	1.987	17.948
T-value	3.863**	163.41**

** = Significant at 1% level

DISCUSSION

CHAPTER V

DISCUSSION

Soil, water and food samples collected from agricultural lands highly polluted with industrial effluents were analyzed for heavy metals and micronutrients. Consumption of crops grown in the polluted soils on human health was also studied through blood and serum analysis. The effect of bioremediation of the polluted lands on heavy metal and micronutrients was investigated.

General survey of the farm families

The selected farm families have been residing in the polluted villages for the past 25 years and are exposed to industrial effluents through soil, water and air pollution. The major industries within the vicinity of the selected villages include oil refineries, textile industries, tanneries and distillery industries and synthetic organic chemical and electroplating manufacturing industries and the soils and water of the selected villages are found to have heavy metals in high concentration.

The farm families are well aware that nearby industries are affecting the soil, water, environment and also their health. They are also under the misconception that use of higher doses of pesticides and fungicides increase the crop yield.

The major source of drinking in the selected polluted villages of Medak and Ranga Reddy districts is Manjeera water and for the selected control village in Nalgonda district (which is far away from the industrial area) in addition to municipal water, well water also forms the major drinking source (Table 8).

Nutritional status of the farm family members (adults and children) in the selected villages as assessed by BMI found to be normal as compared with the standards (Table 11 and 12).

It is observed that families from the polluted villages were affected with skin rash, which was not found in families residing in non-industrial area (Table 13). The causative factors for skin allergies may be due to excessive consumption of nickel through edible crops (Table 35 and 36).

It was reported that plants grown in the industrial areas uptake the heavy metal nickel and the edible portions also said to accumulate the heavy metal (Jeevan Rao, 1992). Excessive consumption of the heavy metal nickel might have resulted in skin allergies (Kasprazak et al., 2003). It is also evident from the data (Table 14) that number of farm women undergoing abortions was high in polluted villages as compared to the village far away from the industrial area. Accumulation of heavy metal lead was said to cause abortions in occupationally exposed population (Kasprazak et al., 2003). This could be attributed due to high concentrations of lead found in soil (Table 32), edible crops (Table 35 and 36) and blood (Table 21) in the affected villages.

Variety of crops such as paddy, wheat, sugarcane, fruits and vegetables are cultivated in the selected villages. The total yield was less in polluted farms, which was attributed to the use of industrial effluent contaminated water for irrigation purpose (Table 16). Similar findings were reported with vegetables grown in nutrient solution with different levels of heavy metals (Du Ying Qiong et al., 2003). The crop yield in control village was also said to be lower (Table 16) mainly due to scarcity of water for irrigation.

Agricultural produce grown on industrial contaminated soil and water is mainly marketed in village local markets and also raithu bazaars in wide distribution.

Blood analysis for heavy metals and micronutrients

Blood analysis for heavy metal lead in both adults and children of farm families residing in industrial areas shown to have significant high blood lead levels than those suggested by ATSDR, 2006 (Adults = $<20\mu\text{g}/\text{dl}$; Children = $10\mu\text{g}/\text{dl}$) (Table 21). Similar findings were observed in children residing in industrial areas of Kanpur (Mukesh et al., 2005), Lucknow (Ahamed et al., 2005), Brazil (Santos et al., 1993), Sweden (Hallen et al., 1995), Mexico (Carrizales et al., 2006) and Turkey (Sanl et al., 2006).

Chronic mild blood lead elevation may impair psychological, behavioral and intellectual function of children (Mahaffey, 1981). Lead absorption is said to

be decreased by regular consumption of a well balanced diet and hence measures may be taken by the government for supply of balanced meal through Supplementary Nutrition Program or Mid Day Meal to the children in the polluted areas.

Similar results with regard to blood cadmium and serum chromium levels, higher than those suggested by ATSDR, 2006 ($< 1 \mu\text{g/dl}$) (Table 22 and 23) was found in farm families residing in industrial polluted areas. Similar findings were reported in Singapore (Xu et al., 1994), Sweden (Hallen et al., 1995) and Korea (Choi Mikyeong, 2000).

With regard to heavy metals cobalt and nickel, serum concentrations of farm families in industrial areas found to be significantly higher as compared to ATSDR (2006) for cobalt - 0.01 to $0.05 \mu\text{g/dl}$ (Table 24) and Normoto and Sunderman (1970) - 0.11 to $0.45 \mu\text{g/dl}$ (Table 25). Similar observations were reported in students of Korea (Choi Mikyeong, 2000).

However the farm families residing in village far away from industrial area found to contain normal levels of Pb, Cd, Co and Ni except Cr, where serum chromium levels were found to be significantly higher than those suggested by ATSDR (2006). The causative factor for high chromium might be the increased concentration of Cr in soil, water and edible crops grown (Table 32 to 36).

Serum analysis for micronutrients calcium and phosphorus of farm families residing in the selected villages shown to have significantly high levels than those

suggested by Robert and Denis (2000) for calcium - 8.8 to 10.8 mg/dl (Table 26) and for phosphorus - 2.5 to 4.5 mg/dl (Table 27). However the chronic ill effects of the high concentration of calcium and phosphorus in blood need to be further studied. With regard to micronutrient iron, serum concentrations of farm families in the selected villages found to be within the normal range (Table 28) suggested by Sauberlich (1999) (50 – 150 µg/dl).

The high concentrations of heavy metals in serum and blood samples of subjects residing in polluted areas reflects rapid industrialization and the effect of these toxic metals can range from subtle symptoms to serious diseases. The worst part about heavy metals is that once they build up in the body, they can cause irreversible damage. Further damage can be prevented by removing the metals, but this can be a slow and difficult process, hence prevention is the best defense with regard to metal poisoning.

Dietary intake of many heavy metals through consumption of plants reported to have long term detrimental effects on human health (Rajesh and Madhoolika, 2005).

It is reported that human exposure to a variety of metals causes a wide range of medical problems such as heart diseases, liver damage, cancer, neurological problems and central nervous system damage (Roane et al., 1994).

The chemicals accumulate in the body with time are said to pass on to the next generation often in high concentrations posing health problems (Jeevananda, 2006)

Soil analysis

Surface soil samples (0-15 cm) collected from different industrial areas clearly indicated that the soils located near to effluent stream (Patancheru and Ramchandrapuram) were acidic and became alkaline (Cherlapalli) in pH as the distance from the effluent stream increased. High E.C. was found in soils located nearer to the effluent streams (Table 29). Similar findings were reported by Seeda et al. (1990); Singh et al. (1991); Basnal et al. (1992); Jeevan Rao (1992) and Palaniswami and Sreeramulu (1994).

The DTPA-extractable heavy metals (Pb, Cd, Cr, Co and Ni) and micronutrients of soils collected from polluted areas were within the safe limits as per the standards of Chapman (1975) and Gupta (2004) (Table 32). Similar observations were reported by Madhavi (1996) and Deshmukh et al. (2006).

Even though the heavy metal content in the soil samples were found within the safe limits, they pose to be toxic to life even at very low concentrations. Heavy metals said to bind certain proteins and essential elements thereby rendering them in exhibiting normal functions in metabolism.

Water analysis

The polluting effluent streams changed the ground water used for irrigation towards the acidic range, increased the E.C. and heavy metal concentrations, which have adverse effects on the agricultural crops grown and human health. Patancheru irrigated water reported low pH and high E.C. than other sample profiles (Table 34). Deterioration of ground water quality, due to industrial effluents has been reported by Muller (1985); Govardhan (1989) and Golimowski et al. (1985).

The heavy metal and micronutrient content of both drinking and irrigated water samples collected from the villages were within the safe limits as per the standards of BIS (1991) and Ayers and West Cot (1976) (Table 33 and 34) except cobalt content of Patancheru irrigated water.

The heavy metals namely Pb, Cd, Cr, Co, Ni, As, Mo, Mn were identified in water bodies polluted with industrial wastes. Many of these are non-essential and are toxic to plants, animals and human beings (Kanwar and Sandha, 2000).

Edible crops collected from fields

The heavy metal content i.e. Pb, Cd, Cr, Co and Ni of rice samples collected from Patancheru and Ramchandrapuram villages were above the limits suggested by Chapman H.D. (1975) (Table 35). This indicates the effect of industrial effluent contaminated water used for irrigation. Similar findings were

observed in vegetable crops grown in Punjab (Kanwar and Sandha, 2000), Kanpur and Varanasi (Singh et al., 2004). Similar results with lead and cadmium in paddy samples grown on toxic heavy metal stress was reported (Sharma et al., 2006).

The acidic pH of soils also contributed to the heavy metal accumulation in agricultural crops. Similar findings were reported in Hungary (Kovacs et al., 1998).

The heavy metal content of fruits and vegetable crops collected from Patancheru were above the limit suggested by Chapman (1975) (Table 36). High concentrations of heavy metals in edible crops were also reported by Jeevan Rao (1992).

The uptake of heavy metals in plant tissues increased as soil heavy metals increased although uptake varied with the plant species. The heavy metal content in fruits was found to be lower than that of the vegetable crops (Table 36). Similar findings were observed by Jeevan Rao (1992) and Chaney (1973).

The micronutrient content of fruit and vegetable crops collected from Patancheru was within the limits suggested by Chapman (1975) (Table 36).

Though soil and water analysis shown to have heavy metal on upper side of safe limits, the crops grown in the fields found to have significantly higher levels of heavy metals (Table 35 and 36).

It is also reported that heavy metals are also absorbed directly to the leaves due to particles deposited on the foliar surfaces. Heavy metals enter the human

body either through inhalation or ingestion, which is the main route of entry for general population. However, urbanization and traffic, industrial and agricultural activities, waste incineration and mining have significantly contributed to the entry of heavy metal through inhalation in human body (Rajesh and Madhoolika, 2005).

Heavy metal toxicity to plants said to vary with plant species, specific metal, concentration, chemical form and soil composition and pH as many heavy metals are considered to be essential for plant growth (Rajesh and Madhoolika, 2005).

Heavy metals constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions. Heavy metals are kept under environmental pollutant category due to their toxic effects on plants, animals and human being. Heavy metal contamination of soil results from anthropogenic as well as natural activities. Anthropogenic activities such as mining, smelting operation and agriculture have locally increased the levels of heavy metals in soils up to dangerous levels. Heavy metals are persistent in nature, therefore get accumulated in soils and plants. Heavy metals interfere with physiological activities of plants such as photosynthesis, gaseous exchange and nutrient absorption, and cause reductions in plant growth, dry matter accumulation and yield.

Pot culture experiments

Pot culture trials were done using one of the highly polluted soils from the village Patancheru of Medak district and by the application of four selected *Saccharomyces* cultures (NCYC 1190, AMB 111, AMB 112 and AMB 113) with FYM and different sugar concentrations. Green leafy vegetable, fenugreek was selected for pot culturing trials as it is a short duration crop.

Significant difference (at 1 % level) was observed between control and polluted soil and plant samples with regard to the heavy metals – Pb, Co and micronutrients i.e. Ca, P and Fe (Table 39, 42, 44, 45 and 46). The other heavy metals were found to be trace in control soil and plant samples.

The application of *Saccharomyces* cultures have markedly reduced the heavy metal and micronutrient content in polluted soil and plant samples (Table 39 to 46). The bioaccumulation of metal chlorides (Cu, Zn, Co, Ni and Cr) to immobilized *Saccharomyces cerevisiae* was studied in packed bed continuous flow columns. Desorption of over 90% was obtained and the biomass was reusable (Wilhelmi and Duncan, 1995). Removal of heavy metal ions i.e. Ni²⁺, Cu²⁺ and Pb²⁺ using yeast (*Saccharomyces cerevisiae*) as carriers in a cross flow microfiltration were observed by Bayhan et al. (2001).

Dahiya (1990) reported that *Saccharomyces cerevisiae* effectively accumulate metals such as Pb, Cd, Cr, Ni by passive retention at the cell surface or by metabolism dependent intracellular accumulation.

Ashour et al. (1992) studied the tolerance and adsorption of heavy metals by *Saccharomyces cerevisiae*. The organism absorbed Ag^{5+} , Co^{2+} , Cr^{3+} , Ni^{2+} and Pb^{2+} . Nickel binding by *Saccharomyces cerevisiae* has an active transport process with the ratio of 1:10.

Joho et al. (1995) reported that nickel tolerant microorganisms i.e., *Saccharomyces cerevisiae* and *Neurospora crassa* are used for the removal of nickel from polluted environments.

Saccharomyces cerevisiae NCYC 1190 absorbed 66 and 46 percent of cadmium and lead from metal salt solution. Cadmium accumulated almost linearly in the first 40 minutes, with an uptake of approximately 214 nmol / mg (dry weight). The uptake of lead was approximately 210 and 344 nmol / mg (dry weight) after 60 and 120 minutes respectively (Soares et al., 2002).

Saccharomyces cerevisiae absorbed heavy metals viz. cobalt, chromium, nickel and lead by the addition of 0.2 – 2.0 percent glucose from sabouraud medium containing metal ions (Ashour et al., 1992).

Enumeration of yeasts in soils collected from pots indicated that the microbes were detected till the harvest period, indicating their efficiency to bind heavy metals and make them unavailable for soil and plant samples (Table 47 and 48).

Pot culturing trials revealed that AMB 113 with FYM and 3 percent sugar concentration were found to be more efficient in remediation of heavy metals

when compared with other microbial cultures used. Hence the same was used for bioremediation trials on polluted agricultural land in Patancheru village.

The destruction of manmade contaminants by microorganisms said to depend on three factors: the type of organism, the type of contaminated and the geological and chemical conditions at the contaminated site (Kerr and Robert, 1994).

The interest of commercial business in utilizing microorganisms to detoxify effluents, soils etc. is reflected in "bioremediation" having become a common buzzword in waste management. Companies specializing in bioremediation will need to develop a viable integration of microbiology and systems engineering.

Bioremediation trials on polluted patancheru land

Bioremediation trials on polluted Patancheru land reported significant difference (at 1 & 5% level) for heavy metals i.e. lead, cadmium, cobalt, nickel and for micronutrients i.e. phosphorus and iron. Trace amounts of chromium was found in control and bioremediated soil and plant samples, whereas no significant difference was observed with regard to calcium content (Table 49 to 55).

Remediation of heavy metals and micronutrients in bioremediated land were not as efficient as those reported in pot culture experiments. This might be due to environmental conditions, presence of field in Patancheru i.e. under the

vicinity of polluted area and due to diffusion of heavy metals into soil from adjacent untreated areas.

The study thus signifies the usefulness of microbial processes to clean up metallic contaminants, which in turn reduce the entry of toxic heavy metals into human food chain.

Microorganism said to be ideally suited to the task of contaminant destruction because they possess enzymes that allow them to use environmental contaminants as food and because they are so small that they are able to contact contaminants easily (Karr and Robert, 1994).

Thus microbial bioremediation can be used as a cost-effective secondary treatment scheme to decrease the concentration of contaminants to acceptable levels. At first bioremediation was a curiosity, today it is seen as a cure all for all types of pollution, including radioactive isotopes.

SUMMARY &

CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

Soil, water and food samples collected from agricultural lands highly polluted with industrial effluents were analyzed for heavy metals and micronutrients. Consumption of crops grown in the polluted soils on human health was also studied through blood and serum analysis. The effect of bioremediation of the polluted lands on heavy metal and micronutrients was investigated.

Industrialization has provided livelihood and opportunities to millions in urban India. However in recent years, a great deal of concern has been expressed over problems of soil pollution with heavy metals due to rapid urbanization and industrialization. The ecological consequences of heavy metal pollution of soils relate largely to heavy metal mobility and solubility. These chemically related factors determine transmission through the soil to the water table, agricultural crops and animals and ultimately to the human population. Bioremediation is described as the use of living organisms to reduce or eliminate environmental hazards resulting from accumulation of toxic chemicals and other hazardous wastes.

Andhra Pradesh constitutes mainly three zones namely Rayalaseema, Telangana and Coastal Andhra. Out of the three, Telangana zone was selected for the study based on its proximity nearer to metropolitan city, Hyderabad where rapid industrialization had taken place. Two districts from Telangana region

namely Ranga reddy and Medak were selected as they are surrounded by highly polluted industries like synthetic organic chemicals, oil refineries and textiles, tanneries, electroplating units, distilleries etc.

One mandal from each of the selected districts i.e., Uppal (Ranga Reddy district) and Ramchandrapuram (Medak district) was selected for the study as the farmers residing in these mandals use industrial effluent contaminated water for cultivation of crops. From polluted areas, three villages were selected i.e., Cherlapalli (Uppal mandal), Patancheru and Ramchandrapuram (Ramchandrapuram mandal). One village i.e. Munagala from Nalgonda district was selected for comparison as it is away from the industrial area.

From the available farm households; 156 families (20%) were selected at random for collection of information pertaining to agriculture (crops grown, farm practices, irrigation and marketing etc) and nutritional aspects (consumption pattern, morbidity, cooking practices, food fads and fallacies, etc) was collected using a pre-tested interview schedule from the head of the household and housewife.

The farm families are well aware that nearby industries are affecting the soil, water, environment and also health. They are also under the misconception that use of higher doses of pesticides and fungicides increase the crop yield.

The plants grown in industrial areas shown to accumulate heavy metals as evinced by significant high concentration of the heavy metals in the edible parts. Excessive consumption of the heavy metal nickel might have resulted in skin

allergies through food, which can be attributed to high concentrations of nickel in soils of polluted villages. Repeated high incidence of abortions by the farm women could be attributed to high concentration of lead in soils of polluted villages.

Variety of crops such as paddy, wheat, sugarcane, fruits and vegetables are cultivated in the selected villages. The total yield was said to be less in polluted farms, which was attributed to the use of industrial effluent contaminated water for irrigation purpose. Agricultural produce grown on industrial contaminated soil and water is mainly marketed in village local markets and also nearby raithu bazaars (market yards), which aid in wider availability of produce with high heavy metals for consumption.

The blood was drawn with the assistance of medical practitioner and analyzed for heavy metals (pb, Cd, Cr, Co & Ni) and micronutrients (Ca, P & Fe). The high concentrations of heavy metals in blood and serum samples of subjects residing in polluted areas reflects rapid industrialization and the effect of toxic metals can range from subtle symptoms to serious diseases and may even lead to death. The worst part about heavy metals is that once they build up in the body, they can cause irreversible damage to vital organs.

Soil and water samples were collected from the selected villages and analyzed for heavy metals and micronutrients using standard procedures. The heavy metal content of soil and water were within the limits but pose to be toxic to life even at very low concentrations as the heavy metals bind certain proteins and

essential elements thereby rendering them in exhibiting normal functions in metabolism. Low pH and high E.C. in soil and water samples of patancheru indicates the high degree of pollution among the polluted villages.

The heavy metal content of edible crop samples grown in polluted areas were above the upper safe limits. The heavy metal content in fruits were lower than that of vegetable crops. The uptake of heavy metals in plant tissues increased as soil heavy metals increased although uptake varied with the plant species.

Four yeast strains – *Saccharomyces cerevisiae* NCYC 1190, imported from National Collection of Yeast Cultures, United Kingdom and three locally isolated yeast cultures namely AMB 111, 112 and 113 were selected for bioremediation trials. Pot culturing experiments were conducted in the laboratory using the microbial cultures in liquid form. The cultures were added to soil with the help of farm yard manure which helps in uniform distribution. The selected four cultures with different sugar concentrations were mixed in top soil of 2.5 cm depth in each pot.

Green leafy vegetable i.e. fenugreek, a short duration crop was selected for pot culture experiments. Soil and edible leaves of fenugreek harvested after 10 days of sowing were analyzed for heavy metals and micronutrients.

Out of the four strains used, AMB 113 was found to be more efficient in remediation of heavy metals and micronutrients through pot culturing trials.

Bioremediation trials with AMB 113, location specific field trials on polluted Patancheru land reported significant reduction in the heavy metal - Pb

(48.8 %), Cd (68.3%) and Co (37.6%) content in agricultural soils and also with regard to edible leaves, the reduction was found to be 47.4, 49.5, 27.3 and 50.4 percent respectively for Pb, Cd, Co & Ni.

The interest of commercial business in utilizing microorganisms to detoxify effluents, soils etc. is reflected in “bioremediation” having become a common buzzword in waste management. Companies specializing in bioremediation will need to develop a viable integration of microbiology and systems engineering.

Bioremediation shown to have significant positive effect in remediation of soil polluted with industrial effluents which in turn reduce the entry of toxic heavy metals into human food chain.

Heavy metals pose a number of hazards to human health. Therefore their concentration in the environment and their effects on human health must be regularly monitored. More researches are required to understand the mechanisms involved in heavy metal tolerance in plants. Metal induced defense response at molecular level need to be worked out for understanding the cascade of chemical mechanisms of heavy metal tolerance.

Thus microbial bioremediation can be used as a cost-effective secondary treatment scheme to decrease the concentration of contaminants to acceptable levels.

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*** Originals not seen**

APPENDIX

Appendix I**SCHEDULE FOR COLLECTING INFORMATION OF FARM FAMILIES**

District : _____ Date : _____
 Mandal : _____
 Village : _____

I. FAMILY PARTICULARS :-

Name of the head of the family : _____

Name of the house wife : _____

Family composition :

Name of the family member	Age (yrs)	Sex	Relation to the head	Educational level	Occupation

II. What is the source of drinking water _____

III. COMMON METHOD OF COOKING PRACTICED :-

Name of the food group	Common preparation	Cooking method adopted

IV. FOOD FADS AND FALLACIES :-

Life stage / health condition	Foods given	Foods avoided	Reason

V. GENERAL HEALTH PROFILE OF THE FAMILY (> 1year – Adults) :-

Name	Age (yrs)	Height (cms)	Weight (Kgs)	Abnormalities if any

VI. MORBIDITY STATUS (last 15 days):-

Name of the family member	Age (yrs)	Type of illness	Duration	Treatment taken / not taken

Duration : 1. One day 2. 2-3 days 3. One week
 4. 15 days 5. One month 6. Any other

VII. OBSTETRIC HISTORY :-

Number of pregnancies	Complications if any	Pregnancy outcome – Alive / Dead

VIII. HEALTH PROFILE OF INFANTS :-

Name	Age (yrs)	Premature / Full term	Birth weight (Kgs)	Abnormalities if any

III. USAGE OF MANURES AND FERTILIZERS FOR CROPS GROWN :-

Crop	Type of manure used	Quantity of manure used per acre	Type of fertilizer		Amount of fertilizer used / acre	
			Straight	Complex	Straight	Complex

Manures :

1. FYM
2. Poultry manure
3. Sheep manure
4. Any other

Fertilizers :**Straight**

1. Nitrogen (Urea)
2. Phosphorus (SSP)
3. Potassium (MOP)
4. Any other

Complex

1. DAP (N&P)
2. 15 :15 :15 (N&P&K)
3. 20 : 20 (N&P)
4. Any other

IV. PLANT PROTECTION CHEMICALS USED FOR CROPS :-

Crop	Pesticides			Fungicides		
	Name	Dosage	Stage*	Name	Dosage	Stage*

* Stage : Crop stage at which pesticides or fungicides used

V. MARKETING OF THE PRODUCE :-

Name of the produce	Quantity marketed (Kgs)		
	Locally	Outside markets	Any other

Appendix II

Estimation of Lead and Cadmium in serum

Reagents :

- **Stock solutions, 1 mg/ml :** Dissolve 1 gm of lead and cadmium mineral equivalent of mineral salt in a minimum amount of hydrochloric acid (about 10 ml). Dilute to 1 liter with deionized water.
- **Working standard solutions :** Dilute aliquots of the stock solutions with deionized water to make at least 4 standard solutions of each mineral within the range of determinations (Pb – 217.0 and Cd – 228.8 nm).

Procedure :

- Take 1 ml of whole blood into a small graduated test tube.
- Add equal volume of supra pure nitric acid.
- Digest the mixture in a micro oven at 150°C for 1 hour and make up the mixture to a known volume (4 ml) with deionized water and filtered to get clean sample solution.
- The digested samples are directly introduced into the graphite furnace.
- Prepare calibration curve from the readings of standards.
- Determine the concentration of samples from the standard graph.

Appendix III

Estimation of Chromium in serum

Reagents :

- **Stock solutions, 1 mg/ml :** Dissolve 1 gm of chromium mineral equivalent of mineral salt in a minimum amount of hydrochloric acid (about 10 ml). Dilute to 1 liter with deionized water.
- **Working standard solutions :** Dilute aliquots of the stock solutions with deionized water to make at least 4 standard solutions of each mineral within the range of determinations (Cr – 357.9 nm).

Procedure :

- Serum is diluted 5 times with 8% butanol in deionized water for chromium determination. The butanol is checked for its mineral contamination which serves as a blank.
- The solution is aspirated into the flame directly.
- The optimum operating conditions recommended by the instrument manual should be used.
- Read at least 3-4 ranges of standard solutions before and after sample readings.
- Flush burner with deionized water between samples and check for zero setting.
- Prepare calibration curve from the readings of standards.
- Determine the concentration of samples from the standard graph.

Calculation :

$$\mu\text{g (mineral) / ml} = \text{ppm mineral} \times \frac{\text{ml of aliquots} \times \text{ml sample}}{\text{dilution factor}}$$

Appendix IV

Estimation of Cobalt in serum

Reagents :

- **Stock solutions, 1 mg/ml :** Dissolve 1 gm of cobalt mineral equivalent of mineral salt in a minimum amount of hydrochloric acid (about 10 ml). Dilute to 1 liter with deionized water.
- **Working standard solutions :** Dilute aliquots of the stock solutions with deionized water to make at least 4 standard solutions of each mineral within the range of determinations (Co – 242.5 nm).

Procedure :

- The collected serum samples were acidified up to pH 2.0.
- 10 μ l of serum were directly introduced into the graphite furnace.
- Prepare calibration curve from the readings of standards.
- Determine the concentration of samples from the standard graph.

Appendix V

Estimation of Nickel in serum

Principle : Addition of trichloroacetic acid to serum disassociates nickel from serum proteins and simultaneously precipitates the proteins. Nickel in the protein-free supernatant fraction is converted to a pyrrolidine dithiocarbamate complex and is extracted into methylisobutylketone. The concentration of nickel in the methylisobutylketone extract is measured by atomic absorption spectrophotometer.

Reagents :

- **Nickel stock standard, 50 μg of Ni per ml :** Powdered nickel, 50 mg, is placed in a 50 ml beaker and dissolved with heating in a mixture of 5 ml of concentrated nitric acid and 5 ml of water. The solution is quantitatively transferred to a 1 liter volumetric flask and diluted to volume with water.
- **Nickel working standard, 0.05 and 0.10 μg of Ni per ml :** One ml portions of the nickel stock standard solution are transferred to 1 liter and 500 ml volumetric flasks, and diluted to volume with water.
- **TCA, 15 gm/100 ml :** 150 gm of metal free TCA is dissolved in 1 liter of water. This solution is stored, refrigerated and is prepared every two weeks.
- **MIBK certified grade solvent.**
- **MIBK saturated with TCA :** This is used in establishing the spectrometer baseline. Into a 1 liter separating funnel are placed 400 ml of MIBK and 100 ml of TCA solution. The mixture is shaken and allowed to stand in a refrigerator for 1 hour. The MIBK phase is removed and centrifuged to eliminate traces of aqueous phase. This solution is prepared each week.

- **APDC, 2 gm/100 ml** : One gram of ammonium pyrrolidine dithiocarbamate is dissolved in 50 ml of water, and the solution is extracted twice with 5 ml of MIBK. The solution is prepared each day.
- **Phthalate buffer, pH 2.5** : To approximately 400 ml of water in a 1 liter volumetric flask are added 10.2 gm of potassium hydrogen phthalate and 39 ml of 1 N hydrochloric acid. The contents of the flask are dissolved, diluted to volume with water and transferred to a 2 liter separating funnel. The pH of the solution is tested with a pH meter, and adjusted, if necessary, to pH 2.5 by adding potassium phthalate or hydrochloric acid. 10 ml of the APDC solution is added, and the mixture is shaken vigorously. The buffer is extracted at least 5 times with 25 ml of MIBK, the nickel content of the final MIBK extract, as measured by atomic absorption, should not differ from that of pure MIBK.
- **Concentrated ammonium hydroxide, 25 gm/100 ml.**
- **Ammonium hydroxide, 2.5 gm/100 ml** : 25 ml of the concentrated reagent is diluted to 250 ml with water.
- **Sulfuric-nitric acid** : One part of concentrated sulfuric acid is mixed with 5 parts of concentrated nitric acid.
- **Perchloric acid, 70 gm/100 ml.**
- **Hydrochloric acid** : 300 ml of concentrated hydrochloric acid is diluted to 1 liter with water.

Procedure :

- 2 ml of serum are transferred in duplicate to 50 ml centrifuge tubes. 1 ml of each of the two nickel working standard solutions and 9 ml of water are transferred in duplicate to additional 50 ml tubes. These standard samples are equivalent to 0.5 and 1.0 μg of Ni /100 ml of serum. 2 ml of water are placed in duplicate 50 ml tubes to serve as the reagent blanks.
- 20 ml of TCA solution is added slowly to all of the tubes, with constant mixing. The glass stoppered tubes are agitated for 30 minutes by a mechanical apparatus and then centrifuged for 15 minutes.
- 20 ml aliquots of the protein free supernatant phases are transferred into clean 50 ml centrifuge tubes, and 2 ml of phthalate buffer is added.
- Concentrated ammonium hydroxide solution is added drop-wise with constant mixing until the pH reaches approximately 2.0, as monitored with a pH meter. Then the pH is adjusted to 2.5 by drop-wise addition of dilute ammonium hydroxide solution.
- 2 ml of APDC solution is added and the contents are mixed. 3 ml of MIBK is added and each tube is mixed with a vortex mixer for 20 seconds, and placed in an ice-water bath for 10 min. the tubes are then centrifuged for 5 minutes.
- MIBK extracts are aspirated into the burner, and the absorbances of the samples are recorded at 232 nm. Heights of the absorbance peaks are measured, and the concentrations of nickel in the serums are calculated by proportionality with the standard samples.

Appendix VI

Estimation of Calcium in serum

Principle : Calcium is precipitated as oxalate and is titrated with standard potassium permanganate.

Reagents :

- 4 % ammonium oxalate solution
- Dilute ammonia solution : 2 ml of liquor ammonia with 98 ml water.
- 1 N Sulphuric acid
- 0.01 N Potassium permanganate solution
- 0.01 N Oxalic acid : Sodium oxalate is dried in an oven at 100-105°C for 12 hours. Exactly 0.67 gms is dissolved in redistilled water. 5 ml concentrated sulphuric acid is added and solution made up to 1 liter after it is cooled down.

Standardization of potassium permanganate solution : 25 ml of 0.01 N oxalic acid is transferred to an Erlenmeyer flask. 1 ml of concentrated sulphuric acid is added, warmed to about 70°C and titrated against potassium permanganate solution, till the faint pink color remains.

The normality of potassium permanganate solution = $\frac{25 \times 0.01}{\text{Titre value (ml)}}$

Procedure :

- 2 ml of sample is taken into a 15 ml centrifuge tube.
- Add 2 ml of distilled water and 1 ml of ammonium oxalate solution and mix thoroughly and leave overnight. Again the contents are mixed and centrifuged for 5 min at 1500 rpm.

- The supernatant liquid is poured off and the centrifuge tube drained by inverting the tube for 5 min on a rack.
- The mouth of the centrifuge tube is wiped with a piece of filter paper.
- The precipitate is stirred and the sides of the tubes are washed with 3 ml of dilute ammonia. It is centrifuged again and drained as before.
- The precipitate is washed once more with dilute ammonia to ensure the complete removal of ammonium oxalate.
- The precipitate is dissolved in 2 ml of 1 N sulphuric acid.
- The tube is heated by placing it in a boiling water bath for 1 min and titrated against 0.01 N potassium permanganate solution to a definite pink color persisting for at least 1 min.

Calculation :

1 ml of 0.01 N potassium permanganate solution is equivalent to 0.2004 mg of calcium

$$\text{mg of calcium} / 100 \text{ ml serum} = (x-b) \times 0.2004 \times \frac{100}{2}$$

Where, x = volume in ml of 0.01 N potassium permanganate solution required to titrate the sample.

 b = volume in ml of 0.01 N potassium permanganate solution required to titrate 2 ml of sulphuric acid (blank).

Appendix VII

Estimation of Phosphorus in serum

Principle: Phosphate reacts with ammonium molybdate to give rise to phosphomolybdate complex which is reduced to a more stable color complex with ascorbic acid.

Reagents :

- a) 10 % trichloro acetic acid
- b) 10 % Ascorbic acid : 10 gm of ascorbic acid is dissolved in distilled water and dilute to 100 ml. store under refrigeration at 2-4°C. the solution is stable for about 7 weeks.
- c) 2-5 % Ammoniummolybdate : Dissolve 2.5 gm of ammonium molybdate in distilled water and dilute to 100 ml.
- d) 6 N sulphuric acid : Dilute 18 ml of concentrated sulphuric acid to 108 ml with distilled water.
- e) Reagent A : Mix 1 volume of reagent d with 2 volumes of distilled water and 1 volume of reagent c, then add 1 volume of reagent b and mix well (prepare fresh each day).

Standard phosphate : Exactly 351 mg of anhydrous mono potassium phosphate is dissolved in water. 10 ml of 10 N sulphuric acid is added and the solution diluted to 1 liter water and mixed. This solution contains 0.4 mg of phosphorus in 5 ml. it is stable indefinitely.

Procedure :

- Add 0.5 ml of whole blood, plasma or serum to 2 ml of 10 % trichloro acetic acid. Mix well, centrifuge and pipette off the filtrate.
- For routine analysis use 0.5 ml of filtrate.
- Pipette the phosphorus standards up to 8 μg of phosphorus, and trichloro acetic acid filtrate (blood, plasma or serum) and adjust the volume to 4 ml with distilled water.
- The reagent blank consists of 4 ml distilled water.
- Pipette 4 ml of reagent A into each tube, cap with parafilm, mix and place the rack with all tubes at 37°C for 1.5 – 2 hours. Remove, allow a few minutes to cool to room temperature and read the absorbance at 820 nm against a blank in a spectrophotometer.

Appendix VIII

Estimation of Iron in serum

Principle : Iron in serum is present as Fe³⁺—bound to tranferin. In estimations of serum iron, the iron is released by mild acid treatment, the proteins are precipitated and the released iron is reduced to Fe²⁺—by reducing agents and then this Fe²⁺—is reacted with bathophenanthroline to form a pink colored complex, whose intensity can be measured at 540 nm.

Reagents :

- **Protein precipitant :** Aqueous solution made to contain 100 gm Trichloro acetic acid, 30 ml of thioglycolic acid and 2 ml Hydrochloric acid per liter. This should be stored in a dark brown bottle and is stable for at least 2 months.
- **Chromogen solution :** Sodium acetate (2M) containing 250 mg bathophenanthroline sulfonate per liter.
- **Iron standard solution, Stock solution :** 70.2 mg of ferrous ammonium sulphate, dissolved in water containing 0.2 ml of 2 N sulphuric acid, and made up to 1 liter. The iron concentration of the solution is 1 mg per 100 ml.

Working standard : 40 ml of stock solution is diluted to 100 ml with glass distilled water. This working standard contains 400 µg per 100 ml.

Procedure :

- To 1 ml of plasma / serum are added 1 ml of water and 2 ml of the protein precipitant solution, and mixed thoroughly and allowed to stand for 5 minutes and centrifuged at 2000 rpm for 15 minutes to get an optically clear supernatant solution.

- 2 ml of the clear supernatant is transferred to a clean tube and 2 ml of the chromogen solution is added, mixed and allowed to stand for at least 5 minutes. The optical density of the color developed is measured in a photoelectric colorimeter using 54 filter or in a spectrophotometer at 535 nm.
- A reagent blank is prepared by substituting water for serum. The optical density of the blank should not exceed 0.015 against distilled water.
- A standard curve is constructed using 0.1 – 2.0 ml of working standard and treating them similar to the test sample.

Calculation : Read the unknown concentration from the standard graph. Plasma / serum iron concentration is expressed as μg per 100 ml.

Appendix IX

pH in 1:2 soil – water suspension

Weigh 40 gm of soil into a 250 ml Erlenmeyer flask and add 80 ml of distilled water in it to prepare 1:2 soil – water suspension. Stopper the flask and shake the mixture on the reciprocating shaker for one hour.

Procedure :

- Take 1:2 soil – water suspension in which pH is to be determined.
- On the pH meter, set the temperature compensating knob and confirm that the electrode is completely filled with the standard potassium chloride (KCl) solution. Allow the pH meter to warm up for 15 minutes to eliminate asymmetric potential of the instrument.
- Place known standard buffer solution in a beaker, say pH 7 and immerse both the electrodes or one electrode (in case combined electrode is provided) into the buffer solution. With the help of knob, adjust the instrument reading at the known pH of buffer i.e., 7. The buffer is then removed and the electrodes are carefully flushed with distilled water.
- Now take another buffer solution of known pH i.e., 9.2. The pH meter must read 9.2. The second buffer is then removed and electrodes are again flushed with distilled water.
- The electrodes are then immersed in the beaker containing soil – water suspension and read pH on the dial and record it.

- Remove the electrodes from the soil – water suspension, clean them with distilled water and then dip into a beaker of distilled water. The electrodes are maintained in working condition by keeping them immersed in distilled water.

Appendix X

Electrical conductivity in 1:2 soil – water suspension

Weigh 40 gm of soil into a 250 ml Erlenmeyer flask and add 80 ml of distilled water in it to prepare 1:2 soil – water suspension. The filtrate must be cleared, if not, it may again be filtered to obtain a clear filtrate.

Procedure :

- Take 1:2 soil – water suspension in a 25 ml beaker.
- Warm up the electrical conductivity bridge for 20 minutes.
- Use 0.01 M KCl solution to calibrate the meter.
- Rinse the conductivity cell with distilled water and then with the sample.
- Connect conductivity cell to meter and dip in the sample. Pass the current and adjust the current by rotating the dial in such a way that maximum sensitivity is obtained.
- Read the conductivity value in d s/m.

Appendix XI

DTPA extractable method

Heavy metals i.e., lead, cadmium, chromium, cobalt, nickel and micro nutrient i.e., iron can be extracted by DTPA and can be analyzed using Atomic Absorption Spectrophotometer.

Extracting solution : Dissolve 13.1 ml reagent grade Triethanolamine (TEA), 1.967 gm Diethylene Triamine Penta Acetic acid (DTPA) and 1.47 gm of calcium chloride in 100 ml of glass distilled water. Allow sometime for the DTPA to dissolve and dilute to approximately 900 ml. Adjust the pH to 7.3 ± 0.05 with 1:1 hydrochloric acid while stirring and dilute to 1 liter. Addition of approximately 4 ml of 1 N hydrochloric acid will bring the pH of the solution to 7.3. This solution is stable for several months.

Procedure :

- Place 10 gm of air dried soil in a 125 ml Erlenmeyer flask.
- Add 20 ml of the DTPA extracting solution and shake for 2 hours.
- After shaking, filter through a Whatman No. 42 filter paper. Re-filter if filtrate is turbid.
- Analyze heavy metals i.e., lead, cadmium, chromium, cobalt, nickel and micro nutrient i.e., iron in filtrate by atomic absorption spectrophotometer and note the readings as parts per million (ppm).

Appendix XII

Estimation of Calcium in soil

Reagent :

Ammonium acetate : 1.0 N, pH 7.0 : To 700 ml of distilled water, add 57 ml of 99% glacial acetic acid and then 69 ml of concentrated ammonium hydroxide. Dilute to a volume of 900 ml and adjust pH to 7.0 by the addition of 3 N ammonium hydroxide and 3 N glacial acetic acid and make up to 1 liter. Store in pyrex or polypropylene bottle.

Procedure :

- Place 5 gm soil in a 150 ml Erlenmeyer flask and pour in 25 ml of neutral N ammonium acetate.
- Shake on a reciprocating shaker for 5 minutes and immediately filter through Whatman No. 1 filter paper. First few ml of the filtrate may be rejected.
- Analyze calcium in filtrate as ppm by Atomic Absorption Spectrophotometer.

Appendix XIII

Estimation of available Phosphorus in soil

Principle : Sodium bicarbonate solution extracts some exchangeable or surface absorbed Aluminium-phosphate, Calcium-phosphate and other phosphates.

Reagents :

- 1) **Sodium bicarbonate (Olsen's reagent) 0.5 M, pH 8.5** : Dissolve 84 gm sodium bicarbonate in water and make up to 2 liters. Mix thoroughly. Adjust to pH 8.5 with 1 M sodium hydroxide (4 gm NaOH / 100 ml) solution. Usually 20 – 25 ml NaOH solution is required for 2 liter sodium bicarbonate solution. Store in glass or polyethylene bottle.
- 2) **Reagent A** : Dissolve 12 gm ammonium molybdate in 250 ml of distilled water. Dissolve 0.2908 gm antimony potassium tartrate in 100 ml water. Add these two solutions to 100 ml of 2.5 M sulphuric acid, mix thoroughly and make to 2000 ml. Store in pyrex bottle in a dark and cool place.
- 3) **Reagent B** : Dissolve 1.056 gm ascorbic acid in 200 ml reagent A and mix. This does not keep for more than 24 hours at room temperature. Prepare daily as required.
- 4) **Sulphuric acid, 2.5 M** : Dilute 140 ml concentrated sulphuric acid to 1 liter with distilled water.
- 5) **Standard stock phosphorus solution** : Dissolve exactly 0.439 gm potassium dihydrogen orthophosphate in half a liter of distilled water. Add 25 ml of 7 N sulphuric acid and make to 1 liter with distilled water. This gives 100 ppm

phosphorus standard stock solution. From this, a 2 ppm solution is made by diluting it 50 times.

Standard curve :

To prepare the standard curve, 1, 2, 3, 4, 5 and 10 ml of 2 ppm Phosphorus solution are taken in 25 ml volumetric flasks. To these 5 ml of the extracting solution is added. Acidify 5 ml aliquot with 2.5 M sulphuric acid to pH 5.0. Add distilled water to make the volume to 20 ml and then add 4 ml of reagent B. Make the volume with distilled water and mix. Prepare a blank with sodium bicarbonate solution, distilled water and 4 ml of reagent B. After waiting for 10 minutes, read the intensity of the blue color in a photoelectric colorimeter using 730 – 840 nm filter or on a spectrophotometer at 882 nm.

Procedure :

- Weigh 2.5 gm air dry soil into a 150 ml Erlenmeyer flask.
- Add a little of Darco G 60 or equivalent grade P-free activated charcoal.
- Then add 50 ml of Olsen's reagent and shake on the reciprocating shaker for 30 minutes.
- Similarly run a blank without soil.
- Filter through Whatman No. 40 or 42 filter paper into a clean and dry beaker. Shake the flask immediately before pouring suspension into funnel.
- Place a 5 ml aliquot of the extract in a 25 ml volumetric flask and acidify with 2.5 M sulphuric acid to pH 5.0. Add distilled water to 20 ml and then add 4 ml

of reagent B. After awaiting for 10 minutes, read the intensity of blue color on a spectrophotometer or colorimeter as described for standard curve above.

Calculation :

$$\text{Available Phosphorus (Kg/ha)} = \frac{\text{R x Volume of extract}}{\text{Volume of aliquot}} \times \frac{2.24 \times 10^6}{\text{Wt (g) of soil} \times 10^6}$$

Where R = μg phosphorus in the aliquot (obtained from standard curve)

$$= \frac{\text{R} \times 50 \times 2.24}{5 \times 2.5}$$

$$= \text{R} \times 8.96$$

Appendix XIV

Estimation of Calcium in water

Principle : EDTA is one of the most versatile complexone which strongly complexes and forms chelate with a number of polyvalent cations such as those of Ca, Fe, Mg, Cu, Mn, Ni, Cd and Zn.

Reagents :

- 1) **Sodium Hydroxide (4 N) :** Weigh 160 gm of pure sodium hydroxide in water and make volume to 1 liter.
- 2) **Ethylene Diamine Tetra acetic Acid (EDTA) solution, 0.01 N :** Weigh 2 gm of versenate (disodium dihydrogen ethylene diamine tetra acetic acid). Dissolve it in distilled water and make the volume to 1 liter. Standardize it against 0.01 N calcium solution by using separately both ammonium perpurate and Erichrome Black T (EBT) as indicators. Calculated normality of EDTA with ammonium perpurate may be 3 – 5 % higher than that of EBT.
- 3) **Standard calcium solution (0.01 N) :** Weigh 0.5 gm of pure, dried calcium carbonate and dissolve in 10 ml of 0.2 N hydrochloric acid. Heat till the solution boils and carbon dioxide is completely driven off. Cool and make the volume accurately to 1 liter. This solution is for standardizing EDTA.
- 4) **Ammonium perpurate powder :** Weigh 0.2 gm of ammonium perpurate and 40 gm of potassium sulphate. Mix both the reagents thoroughly in a pestle motor and keep it as powder in a clean dark colored bottle.

5) **Erichrome Black T (EBT)** : Weigh 0.5 gm of EBT dye and 4.5 gm of hydroxylamine hydrochloride and dissolve both in 100 ml of ethyl alcohol. Add 5 ml of 2 % sodium cyanide solution.

Procedure :

- Pipette 5 ml of aliquot (water sample) in 100 ml conical flask and dilute it by adding 25 ml of distilled water.
- Add approximately 5 ml of NaOH and 25 mg of ammonium perchlorate powder. Shake the contents well. Here the original color is orange red.
- Start titrating it against EDTA till the color changes to purple. Since the color change is not spontaneous, keep blank or standard sample after adding indicator for noting the end point more accurately. Note the volume (ml) of EDTA used.

Calculation :

$$\text{Concentration of calcium, ppm} = \frac{\text{ml EDTA used} \times 0.01 \times 1000}{\text{ml of aliquot}}$$

Appendix XV

Estimation of Phosphorus in water

Principle : Vanadate, molybdate and orthophosphates react together to give a yellow color complex in nitric acid medium.

Reagents :

- 1) **Ammonium molybdate – ammonium vanadate in nitric acid :** Dissolve 22.5 gm ammonium molybdate in 400 ml distilled water. Dissolve 1.25 gm ammonium vandate in 300 ml boiling distilled water. Add the vandate solution to the molybdate solution and cool to room temperature. Add 250 ml of concentrated nitric acid and dilute to 1 liter.
- 2) **Phosphate standard solution :** Dissolve 0.2195 gm of analytical grade phosphate and dilute to 1 liter. This solution contains 50 µg of phosphorus per ml (50 ppm).

Preparation of standard curve :

Transfer 0, 1, 2, 3, 4 and 5 ml of standard solution to 50 ml volumetric flask to get 0, 1, 2, 3, 4 and 5 ppm of phosphorus respectively. Add 10 ml of vanadomolybdate reagent to each flask. Make up the volume with deionized water and shake thoroughly. Read the transmittance or absorbance of solution after 30 minutes at 420 nm with spectrophotometer or colorimeter using blue filter. Plot the absorbance against concentration.

Once the linear calibration curve is established, the slope of the curve is determined and then the concentration of the unknown solution can be calculated by using the equation $A = mc$, where

A = Absorbance

M = Slope

C = Concentration

Appendix XVI

Processing of the food sample for Atomic absorption spectrophotometric estimation

Wet digestion :

Organic matter in food materials or solids can be oxidized by boiling with sulphuric acid, perchloric acid and nitric acids. The method is as follows :

- Weigh about 3 gm of ground food sample in a 100 ml kjeldahl flask.
- Add 25 ml of 3:2:1 nitric acid, perchloric acid, sulphuric acid mixture and mix well ensuring that no dry food lumps remain. Add a clean glass bead (acid washed).
- Leave aside for 3-4 hours or preferably overnight in fume cup board.
- Heat for about 30 minutes cautiously until the initial vigorous reaction has subsided (dense yellow fumes will evolve).
- Heat more strongly for 4 hours until most of the nitrous fumes are removed.
- Continue heating until white fumes of perchloric acid evolve. If charring occurs or flask contents tend to become dry, remove from heat, cool and add 5 ml of nitric acid and continue heating.
- Carry a blank with each set of samples.
- When cool, transfer quantitatively with 3-4 washings of deionized water the contents of digestion flask to 15 ml graduated test tube. Make up to the 10 ml mark with deionized water and mix thoroughly. Allow the heated solution to cool and make up to the 10 ml mark again, if necessary.
- The tubes are then centrifuged for 30 minutes.

- Transfer the ash solution to acid washed polythene bottles and store in a cool place prior to mineral analysis by atomic absorption spectrophotometer.

Atomic Absorption Spectrophotometer determination :

Representative sample in a suitable liquid form is sprayed into the flame of an atomic absorption spectrophotometer and the absorption or emission of the mineral to be analyzed is measured as ppm at a specific wavelength.

Reagents :

- Stock standard, 1 mg / ml :** Dissolve 1 gm mineral equivalent of mineral salt in a minimum amount of redistilled hydrochloric acid (about 10 ml). dilute to 1 liter with deionized water.
- Working standard solution :** Dilute aliquots of the stock solutions with deionized water to make at least 4 standard solutions of each element within the range of determination.

Operation parameters

Minerals	Wavelength (nm)
Cr	357.9
Co	240.7
Pb	217.0
Ni	232.0
Cd	228.8
Ca*	422.7
Fe	248.3

* Dilute with 0.1 % Lanthanum chloride solution

Procedure :

- Depending upon the mineral to be determined, standards and sample solutions may be aspirated into flame directly or after suitable dilutions to attain working range of the instrument.
- The optimum operating conditions recommended by the instrument manual should be used.
- Read at least 3-4 ranges of standard solutions before and after the sample readings. Flush burner with deionized water between samples.
- Note the readings in ppm.

Appendix XVII

Estimation of Phosphorus in food samples

Digest the sample with tri-acid mixture as given in Appendix---

Principle : Vandate, molybdate and orthophosphate react together to give a yellow color complex in nitric acid medium.

Reagents :

- a) **Ammonium molybdate – ammonium vandate in nitric acid :** Dissolve 22.5 gm ammonium molybdate in 400 ml distilled water. Dissolve 1.25 gm ammonium vandate in 300 ml boiling distilled water. Add the vandate solution to the molybdate solution and cool to room temperature. Add 250 ml of concentrated nitric acid and dilute to 1 liter.
- b) **Phosphate standard solution :** Dissolve 0.2195 gm of analytical grade phosphate and dilute to 1 liter. This solution contains 50 μg of phosphorus per ml (50 ppm).

Preparation of standard curve :

Transfer 0, 1, 2, 3, 4 and 5 ml of standard solution to 50 ml volumetric flask to get 0, 1, 2, 3, 4 and 5 ppm of phosphorus respectively. Add 10 ml of vanadomolybdate reagent to each flask. Make up the volume with deionized water and shake thoroughly. Read the transmittance or absorbance of solution after 30 minutes at 420 nm with spectrophotometer or colorimeter using blue filter. Plot the absorbance against concentration.

Estimation of phosphorus in plant extract :

The plant digest containing phosphorus is transferred to volumetric flask and the procedure described for standard curve is followed. The phosphorus concentration is calculated using standard curve.

Calculation :

$$\text{P in \%} = \frac{\text{Sample concentration (ppm)}}{\text{Wt. of sample (gm)}} \times \frac{1}{\text{aliquot (ml)}} \times \frac{100}{10000} \times \frac{\text{Final volume (ml)}}{10000}$$

Appendix XVIII

Determination of Yeast Growth Curve

Principle : Growth curve includes three phases i.e., lag phase, log phase and stationary phase. A fourth phase called death phase follows the stationary phase. This helps in detecting the activity period of the organism.

Requirements : Test tubes, conical flasks, measuring cylinder, autoclave, spectrophotometer, incubator.

Procedure :

- Prepare 150 ml of Sabouraud's glucose broth medium by using the following composition :- Peptone : 1% and Glucose : 4%.
- Disperse about 10 ml of the medium into each of the 12 test tubes.
- Carry a blank with each set.
- Sterilize the tubes by autoclaving at 121°C temperature and 12 lbs pressure for 15 minutes.
- After sterilization, cool the test tubes under running tap water.
- Incubate the tubes at 37 °C for 24 hours in an incubator to check the purity.
- Inoculate the tubes with loopful of yeast suspension in front of a spirit lamp, in an inoculation chamber. Then incubate them at 28 °C in an incubator.
- At bihourly intervals take one test tube and observe the growth of yeast by measuring the optical density of the culture at 600 nm using a spectrophotometer.
- Tabulate the optical density values at bihourly intervals, up to 48 hours.
- Plot a graph, by taking the time in hours on X-axis and optical density values on Y-axis.

Results :

S.No.	Hours after inoculation	Optical density
1	2	
2	4	
3	6	
4	8	
5	10	
6	12	
7	24	
8	27	
9	30	
10	48	

Appendix XIX

Enumeration of colonies by pour plate method

Principle : Each living cell multiplies and grows into a colony on a suitable medium under optimal conditions.

Materials required : 6-10 sterile dilution blanks, 7-10 sterile 2.2 ml pipettes, 250 ml molten sabouraud agar medium and 6 sterile petriplates.

Procedure :

- Arrange for required number of dilution blanks containing 9 ml of sterile normal saline. Label them as 10^0 , 10^{-1} , so on as required for the sample / culture.
- Transfer 1 ml or 1 gm of sample / culture into first dilution blank using a sterile pipette or a sterile spatula respectively, under aseptic conditions. Mix the contents thoroughly.
- Transfer 1 ml from the first dilution blank into the second dilution blank using a fresh sterile 2.2 ml pipette.
- Mix the contents thoroughly.
- Continue serial dilution up to the last dilution blank using a fresh pipette at each transfer.
- Mix the contents thoroughly before pipetting out and after adding into the next dilution blank.
- Label duplicate sterile plates with dilution factor for last three dilutions.
- Mix the contents, and transfer 1 ml of diluted samples from the last three dilution blanks into the pre-sterilized petriplates using fresh sterile pipette at each transfer.

- Pour sabouraud agar medium in molten condition (around 45°C) into the plates, to cover nearly 3/4th of the bottom plate.
- Mix the contents gently by rotating the plates (with medium and sample) clockwise and anti-clockwise for 3 or 4 times.
- Incubate the plates at 37°C for 2-3 days.
- The colonies are readily visible to the naked eye.
- Total number of colony forming units, CFU / ml or gm can be calculated by the equation : $n = y / dv$.

Where, n = number of colony forming units, CFU per ml or gm.

y = number of colonies on the plate

d = dilution used for plating

v = volume used for plating

Results :

Dilution factor	Number of colonies		Average number
	Plate I	Plate II	

Appendix XX

Identification of yeast culture – Ascospore induction and detection

Principle : Ascospore media contain small amounts of carbohydrates, this restricts vegetative growth while enhancing ascospore formation.

Reagents : Yeast malt agar, V8 juice agar, acid fast stain, glass slide, microscope.

Procedure :

- Inoculate yeast to yeast malt agar for enrichment. Incubate for 2-3 days.
- Inoculate the yeast from yeast malt agar to a V8 juice agar slant. Incubate aerobically at 20-25°C.
- Most freshly isolated strains begin forming ascospores in 1-2 days. Older stock cultures usually require a longer period of time.
- Examine the culture in 3-5 days and weekly thereafter for 3 weeks. Prepare wet mounts of the yeast in distilled water.
- Examine the wet mounts using the oil immersion lense. Ascospore form, surface topography, size, color, brims, number of ascospores for ascus, and the presence or absence of inclusion bodies are characteristics used in part to identify the various species.
- If ascospores cannot be readily seen in a wet mount, perform an acid fast stain. Ascospores are acid fast.
- Each time yeast is inoculated to a V8 juice agar slant, *Saccharomyces cerevisiae* will be inoculated concurrently as a control.

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