RESPONSE OF PLANT SPECIES TO THE MINING REJECTS

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in

BOTANY

By

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To

My Parents

A C K N O W L E D G E M E N T S

I wish to express my sincere gratitude to my guide, Dr.S.G. Torne, Professor and Head, Department of Botany, S.P. Chowgule College, Margao, Goa for suggesting this problem and for his valuable guidance and constant encouragement throughout the period of investigation.

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CERTIFICATE

As required under R. No. 0.19.8 (vi) of the Goa University, I certify that the thesis entitled "Response of Plant Species to the Mining Rejects", submitted by Mr. A.V. Veeresh for the award of degree of Doctor of Philosophy is a record of research work done by the candidate during the period of study under my guidance.

(S. G. Torne)

Signature of the Guide

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S T A T E M E N T

As required under R. No. O.19.8(ii), I state that the research work entitled "Response of Plant Species to the Mining Rejects" is my original contribution and the same has not been submitted for any degree of this or any other University on any previous occasion.

To the best of my knowledge the present studies is the first of this kind.

The research work comprising in this thesis is my original contribution.

1. Iron ore reject was analysed for organic matter and inorganic elements . It contains iron, aluminium and manganese in high concentrations and nitrogen, phosphorus, potassium, calcium and magnesium in low concentration. In addition to this, physical analysis showed that reject contains more amount of clay. These factors do not promote the growth of plants.
2. Application of seaweed and fertilisers to the reject soil helped the growth of the plants on the

mining site. This is confirmed with the present experiment carried out on **Crotalaria juncea**.

3. The uptake of different inorganic metals showed that aluminium and manganese have an antagonistic effect on the uptake of iron, magnesium and calcium. This difficulty can be rectified by the addition of seaweed to the reject soil.

4. Among the tree species (6 in number) studied, **Bombax ceiba** and **Tamarindus indica** were more resistant to the different stress conditions present on the mining dumps.

All these studies will definitely help in restoring the vegetation and environment in the mining area.

The sources on which the present work is based are indicated in "Literature Cited" and also in the Introductory chapter.

The observations and discussions are entirely original and are based on the research carried out by me.

(A.V. Veeresh)

Signature of the Student

(S.G. Torne)

Signature of the Guide

S. P. CHOWGULE COLLEGE,
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CONTENTS

INTRODUCTION

The mining industry has evolved gradually in response to both availability and depletion of resources, technical and technological progress, economics and diseconomics of scale, energy crisis and substitution threat and growing consciousness of workers and public. However, the basic tenets of this industry include maximisation of production and minimisation of wastage. The production, within an economic frame work, is divided into three sectors - primary production of resources, secondary production based on the resources, tertiary services - the intersectoral relations being limited to minor input-output linkage only.

Since most of the mining industry in India is located in the backward areas, the focus is on regional development through employment generation. Contradictory tendencies between the support of production system for a larger economy and the demand for regional development have led to unhealthy relationships between income generation and reinvestment for development. Thus mining has led to the destruction of the resource itself as also of other productive natural resources such as land, water and vegetation. In addition, technological change has often meant both regional unemployment as well as the import of skilled labour from outside the region. Inter-sectoral linkages, contradiction between maximisation of production, minimisation of waste and welfare of the deprived and the long and short-term implications of the industry, both in terms of its impact on nature as well as its effect on society, would provide the background for analysing the question of the environmental impacts of mining. This essentially means that the environment consists not only of natural resources but also includes men and women and the relationship between different strata of society.

With the increasing demands, land has been constantly exploited for raw materials from the natural environment. Land is not a resource which automatically renews itself like rainfall or sunlight. It is a finite resource, being diminished by the spread of industry and urbanisation (Coleman,1979). Mining accounts for a substantial proportion of the total loss of land of primary production due to mining activity. In India, 7,85,000 hectares of land is reported to be under mining operations (Baliga, 1985).

IMPACT OF MINING ON ENVIRONMENT:

Modern industrial, economic and commercial activity depend a lot on the exploitation and consumption of minerals. The process of extraction of mineral resources and its use in various ways generates a wide range of environmental changes sometimes having far reaching consequences.

More than 7,85,000 hectares of land of the country are presently under the stresses of mining activities of various kinds. Much larger area is disturbed by other activities associated with mining. The recovery of minerals and construction material requires removal of vegetal cover with underlying soil mantle and excavating overlying rock masses (overburden) which more commonly exceed the volume of material sought. The result is reshaping of the topography, generation of great volumes of debris (waste) and disruption of surface and ground water circulations.

As demands for minerals grow, the area of mining would expand at a faster rate, threatening increasingly larger areas of landscape with scarification, debris dumps, soil degradation, with widening circle of deforestation and distress to the population affected.

In the strip-mining, the overburden is stripped away to recover the minerals by use of bull dozers, scrapers or by manual operations. The contour-strip mining involves removal of the overburden, starting from the outcrop and proceeding along the hillside so that the cut after another is made and a series of benches formed. The inside walls range in height from a few meters to more than 30m but genrally within 10m. On the outer side, debris is dumped on the slopes. The net result is very ugly disfigurement of the landscape.

In the opencast contour-strip mining in the mountains, rich soil together with overburden is scrapped off and pushed downslope. The sliding of the great volume of debris causes great damage to the vegetation and to the springs and streams. Ugly scarifications and drastic reshaping of the landscape and serious destruction of forests have occurred. Picking only the very high grade materials and discarding the rest, the mine-owners had been casting of more than 30% of the valuable ores to slide down the 30^{o} - 50^{o} slope (Valdiya, 1984). When saturated with heavy rain water, the loose waste material becomes debris flow which descend into the valleys, clog the channels and spread over agriculture fields.

Indiscriminate mining since 1961 has destroyed 50,000 hectares of forests in Goa and against the export of 12 million tonnes/year of high grade iron ores, 30 million tonnes of iron ore rejects are scattered indiscriminately over an area of 10,000 hectares of paddy and coconut groves, thus killing the fertility of the soil. The washing of mineral ores for beneficiation has caused serious water pollution in rivers as well as in wells and springs.

Present Problem:

GOA is situated in the western part of India, lying on 15^{o} 48' 00" N & 14^{o} 53' 54" N Latitude and 74^{o} 20' 13" E & 73^{o} 40' 33" E Longitude. On the Western side is the Arabian Sea, in the North it is bordered by Maharashtra and on the East and South by Karnataka.

Exploitation of mineral deposits of Goa remains the dominant industry, inspite of the various new industrial developments, in diversified fields like small scale cottage industries, electronics, automobiles, tourism, etc., Mining industry still forms the back bone of the state's economy. The iron and manganese ore belt in the western ghats extends from Maharashtra to Karnataka passing through Bicholim, Sattari, Sanguem and Quepem talukas of Goa. Large chunks of land of these taluka were leased out for mining to the private entrepreneurs by the Portuguese ruler in fifties. Subsequently termination of some of the leases by the Government of India reduced the number of leases to 581; which covered 46.12% of Bicholim, 35.83% of Sanguem, 15.8% of Quepem and 14.7% of Sattari talukas. As with any other large scale industry, the mining industry and particularly the iron ore mining activity, has undeservedly earned the stigma of environmental damage and pollution.

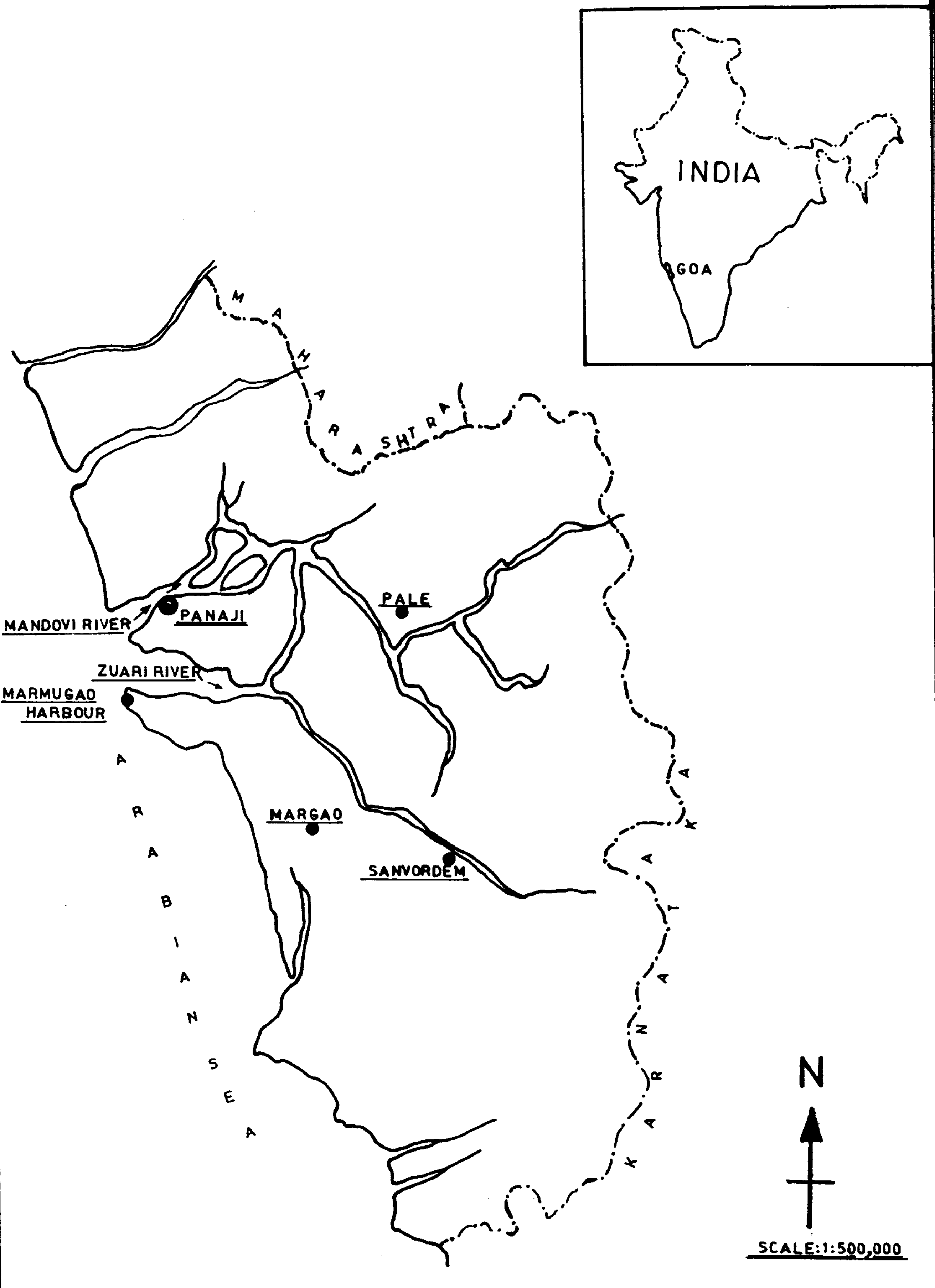
Open cast mining involves deforestation at the mining site, excavation and movement of large volumes of earth's crust. A ton of iron ore mined for instance, produces almost 2/3 tons of rejects. Since 1950, large amount of rejects has accumulated. Dumps of the rejects are spread over hill sides, river sides, and places around other waterbodies, agricultural lands and human habitations. Various mining and processing operations have the potential for pollution and can affect not only the environment but also ecosystem. Mining operations expose relatively large areas of sub-surface rocks to the action of the atmosphere, with the result that abnormal quantities of water soluble compounds of iron, manganese, etc., may contaminate the drainage system. The ore is transported by open trucks to the river sides to load in the barges. During the transport, trucks spill the ore and dust flies. From the reject dumps, dust is blown adding to the dust pollution. During the monsoon, heavy rains wash down the reject from dumps and the ore materials from the mining areas into the agricultural fields and the waterbodies. As such, agricultural yield was affected. Eventhough, the rice plants can withstand high concentrations of iron, soil may become reducing with the addition of organic matter and the plants may develop symptoms of iron toxicity (Ponnamperuma et al. 1955).

Heavy metals, unlike other pollutants are non-biodegradable. Once released into the environment, they pose danger to life for a very long time. Effects of heavy metals on plants has been studied extensively. Heavy metals cause virtual cessation of root growth and formation of short stumpy laterals and ultimately, although not immediately, the death of plants (Bradshaw & Chadwick, 1980).

Ernst (1976) has stated that most significant symptom of heavy metal toxicity is stunted growth. Poor root growth can be the cause of retarded root length (Foy et al. 1978). They also suggested that most characteristic symptom of metal toxicity is stunting and chlorosis. The stunting in plants may be due to (1) specific toxicity of the metals (2) antagonism with other nutrients or (3) inhibition of root penetration in the soil.

Nag P. et. al. (1981), have studied the heavy metals effects on plant tissues involving chlorophylls and proteins. Metals taken up by plants are incorporated into a tissue depending on its mobility within the plant, i.e. translocation (Ernst, 1980). Surplus of heavy metals can severely reduce growth and biomass production of plants. The expression of a metabolic disorder may be the result of interaction of ion uptake and or transport (Ernst, 1972).

The present work deals with a study wherein establishment of vegetation in a stressed environment is being tried. A huge mining complex at Pale in GOA is one



MAP OF GOA SHOWING PALE

of the mining areas where iron ore is extracted. This mining complex is made up of three mines of which one of the mines is operated by M/s.Chowgule & Co. Ltd., Mormugao, Goa. The area occupied by them for the purpose of mining is 159.638 hectares of which 122.00 hectares are under active mining operations and 42 hectares of the area is occupied by the reject dumps. The mine first started functioning from the year 1954 under non-mechanical sector. Later in the years 1960-61 the mine was fully mechanised. The depth of the mine is 160 meters and the height of the dumps is 30 meters. The slope of the dumps is 40°. The age of the inactive dumps range from 5 - 15 years. The annual production of iron ore is approximately 1 million tonnes. Annually approximately 4 million tonnes of ore including the overburden/reject is handled. In the 1960's & 70's the ore exported was lumpy type of the +57 grade. Later from 1980's onwards powdery ore of the grade +60 was exported. Japanese are the main importers of the iron ore from this region. The labour force employed by the company to run the mines is about 390 people.

Annual rainfall at Pale is in the range of 2,800mm to 3,200mm under normal conditions. The monsoons start around the last week of May and end in October. Maximum temperature is observed in the months of April & May is 35^{o}C to 37^{o}C. Minimum temperature is in the month of January ranging from 17^{o}C to 21^{o}C. Relative humidity ranges from 70% to 90%.

From the survey of the areas around the mines showed that it was evergreen type of physiognomy with a more or less similar floral composition (Henry Nyabuto, 1989). Evergreen plant species easily noticed are **Alstonia scholaris**,**Mallotus albus**,**Garcinia indica**,**Tamarindus indica**, **Syzygium cumini** &**Mangifera indica** as trees.

However, some deciduous plant species are frequent like, **Sterculia urens**, **Sapium insigne**, **Strychnos nux-vomica**, **Bombax ceiba**, **Careya arborea** and **Terminalia** species. In some places, **Acacia nilotica** is also found growing. Many different types of herbs and shrubs are found growing around the mining sites. One of the genus found growing around the mining areas is **Crotalaria**, a legume.

During mining activity, it is known to leave huge amounts of wastes in the form of enormous reject dumps.

Information available showed that the vegetation was destroyed and lost for good below the huge dumps and that no new vegetation can survive under the conditions of aridity, instability, nutrient deficiency and heavy metal toxicity which is prevalent on the dumps. It was necessary to study the various aspects of plant reponses to these stresses. Once the nature of responses to these stresses is well understood, the next step is to improve upon the existing conditions.

In the following chapters efforts in this direction are detailed starting with the preparation and analysis of the reject soil used for studying the response of plants. Morphological and biochemical response of **Crotalaria juncea** L. (Sunn hemp), a leguminous plant of economic importance was taken up for study in the nursery with different percentage composition of rejects, and also with the addition of seaweeds and different combinations of fertilizers (NPK) to the reject. Chemical analysis of the different parts of the plant viz. leaf, stem and root was carried out to find the amount of iron, manganese & aluminium taken up by the plant from the reject soil.

Also a study of six tree species used for the purpose of revegetation on the reject dumps were made. The species studied were **Acacia nilotica** **(Linn.) Delile; Azadirachta indica** A.Juss.; **Bombax ceiba** Linn.; **Parkia biglandulosa** Wight & Arn.;**Pithecellobium dulce** Benth. and **Tamarindus indica** **Linn.** Of the above mentioned species taken up for study **Acacia nilotica**, **Bombax ceiba** and **Tamarindus indica** are the native plants and the other three are introduced ones to this region because of their hardy nature and drought resistance.

A survey through the literature showed that no information was available regarding the response of the above mentioned plants to the mining rejects or to the different heavy metals like iron, manganese and aluminium. Hence the present study was taken up to see how they would respond to the mine rejects.

CHAPTER I

SOIL PREPARATION AND ANALYSIS

Physico-chemical factors at the iron ore mining areas undergo change due to the removal of the vegetation cover, top soil and the disturbance of the soil horizons. Leaching and erosion which occur later, contribute to the degradation.

For the reclamation of any degraded area, knowledge of the physico-chemical parameters of the degraded and the undegraded area in the locality is essential. However, the exact assessment of these parameters at the entire area is difficult as the constitution of the soil varies even at the close proximity of the sampling sites due to the interaction of the various factors.

Mining companies estimate some of the chemical constituents of the soil to find the richness of the ore, plan the methods for enrichment of the ore and to assess the concentrations of the impurities which affect adversely sale value of the ore. Studies to find the suitability of the reject for the revegetation were not conducted, as the revegetation was not the responsibility of the mining companies.

During mining operations, different types of clays are obtained viz., Manganiferous, Phyllitic, Limonitic, Intrusive and Lateritic. These clays form the bulk of the overburden/reject during the process of ore extraction from the earth's crust. The characteristics of these clays along with the range of some of the major elements found in them are given in Table.1.1.

Table no. 1.1: Type of Clay and its Characteristc

Sl. No.	Name of Clay	Colour	Composition of elements
1.	Manganiferous	Black,Yellow brown sticky with oily appearance.	Fe=25-40%; Al_2O_3 =05-12% Mn=05-20%;SiO_2=05-15%
2.	Phyllitic	Pink	Fe=15-35%;Al_2O_3=15-25% Mn=0.02-0.5%;SiO_2=20-35%
3.	Limonitic	Yellowish Orange	Fe=40-50%;Al_2O_3=05-10% Mn=0.02-0.5%;SiO_2=10-15%
4.	Intrusive	Pale pink with Yellow spots	Fe=15-20%;Al_2O_3=30-35% Mn=0.02-0.5%;SiO_2=10-15%
5.	Lateritic	Brown pink	Fe=40-45%;Al_2O_3=20-25% Mn=0.02-0.5%;SiO_2=10-20%

The other elements found with the above mentioned ones in lesser quantitites in the clays are

Cu = 4 - 10 ppm

Ni = 10 - 20 ppm

Zn = 10 - 35 ppm

Pb = traces.

The above range of elements were found in the earth's crust ranging from a depth of 10 to 120 meters.

The natural undisturbed/undegraded soil from the surrounding areas of the mine sites showed the following range of elements which were found in the soil ranging from a depth of 15 to 75 cms:

Fe	=	20-30%
Al_2O_3	=	15-30%
Mn	=	0.3-4%
SiO_2	=	25-30%
Cu	=	5 -10 ppm
Ni	=	10 - 20 ppm
Zn	=	5 - 20 ppm
Pb	=	traces

In the present work, different percentage composition of rejects along with good soil and addition of seaweeds were prepared for studying the responses of plants in them. The soil thus prepared was analysed for various chemical constituents and the particle size.

MATERIALS & METHODS:

Garden soil was first prepared by mixing one part farmyard manure with one part of sand for every five parts of soil collected from the nearby hills which was undisturbed by mining activity.

The mining reject was collected from the mines of Chowgule & Co. Ltd., Pale. The type of clay obtained as reject from the mine was of Phyllitic type. Different proportions of mixtures of mine rejects along with garden soil were prepared as given below:

1. 0% reject + 100% garden soil
2. 20% reject + 80% garden soil
3. 40% reject + 60% garden soil
4. 50% reject + 50% garden soil

5. 60% reject + 40% garden soil

6. 80% reject + 20% garden soil

7. 100% reject + 0% garden soil

Also another set of reject containing seaweed was prepared. The seaweed used was Dictyota dichotoma (Huds.) **Lamouroux**, collected from the sea shore of Anjuna, Goa during low tides. This seaweed was thoroughly washed in sea water at the time of collection to remove the sand sticking to it and dried thoroughly in diffused light and then powdered for use. The composition prepared were as follows:

1. Control - 0% reject
2. Reject - 100% reject
3. Reject + Seaweed - 80% reject + 10% sand + 10% seaweed
4. Garden soil + Seaweed - 80% garden soil + 10% sand + 10% seaweed

All the percentage compositions prepared were wt./wt.

The above prepared different percentage composition of reject were analysed for their particle size, pH value, concentrations of iron, alumina, manganese, copper, nickel, zinc, calcium, magnesium, lead, silica, available nitrogen, phosphate and potassium, organic carbon and loss on ignition.

The methods outlined by Allen (1974) and Moore and Chapman (1976) were employed for the estimation of different elements. A known quantity of dried soil sample (100 mg) was taken up in 5 : 1 : 0.5 ml of conc. HNO_3 : perchloric acid : conc. H_2SO_4 in a beaker and digested on a hot plate thoroughly and made to 100 ml by adding distilled water. This solution was taken up directly for the estimation of Fe, Mn, Cu, Zn, Ni, Pb, Ca, Mg & K with the help of Atomic absorption spectra Model No. 2380 - Perkin Elmer make. Alumina was estimated titrimetrically by EDTA Complexometry method (Manual of Procedures for Chemical and Instrumental Analysis of Ores, Minerals and Ore dressing products, 1979) and Silica gravimetrically by fusing with fusion mixture and dissolving the fused sample in dil. HCl and then filtered through Whatman No.1 filter paper. The residue along with filter paper was taken in a silica crucible and ashed it in an oven to get the silica. Nitrogen was estimated following the method of Hawk *et al*.

(1954) and phosphorus was estimated according to the method of Seking *et al.* (1965).

OBSERVATIONS:

Physical analysis of the different percentage composition of the soil is shown in Table 1.2, reveals the particle size of it. From the table it can be observed that the garden soil i.e. 0% reject is mostly made up of gravel (60.33%) and sand (33.84%) with a little of clay and silt (05.83%), with the increasing percentage composition of the reject, it can be seen that there is a decrease in the amount of gravel (7.04%) and a consistant increase in the amount of clay (55.75%) whereas the amount of sand (37.21%) remains almost the same in 100% reject.

The chemical characteristics of the different percentage composition of the soil rejects are given in Table 1.3. In all the cases the soil was acidic in nature having a pH value of 5.5. Concentrations of the major plant nutrients and heavy metals in the soil reflect the low content of organic matter, severe deficiencies of available nitrogen (2.24 Kg/ha.), available phosphorus (2.24 Kg/ha.)

Table No.1.2: Particle size analysis of the different %age rejects.

Sl. No.	% of reject	Textural Class in %		
		Gravel	Sand	Silt + Clay
1.	0%	60.33	33.84	05.83
2.	20%	52.37	34.47	13.16
3.	40%	45.60	35.69	18.71
4.	50%	40.43	35.91	21.66
5.	60%	34.30	36.93	28.77
6.	80%	28.66	36.97	34.37
7.	100%	07.04	37.21	55.75

Textural Class	Particle size diameters (mm)
Gravel	>2
Sand	2 - 0.02
Silt + Clay	<0.02

and available potassium (33.6 Kg/ha.) and high levels of iron (34.18%), alumina (21.34%) and manganese (0.48%) in the 100% rejects than in the normal garden soil (0% reject) where the available nitrogen was 51.52 Kg/ha., available

Table No. 1.3 : Chemical characteristics of the different percentage composition of reject soil.

Sl. No.	Characteristic	Percentage of reject						
		0%	20%	40%	50%	60%	80%	100%
1.	pH value	5.5	5.5	5.5	5.5	5.5	5.5	5.5
2.	Loss on ignition	15.50%	14.5%	13.72%	12.50%	10.45%	08.61%	07.18%
3.	Organic carbon	02.40%	02.12%	01.85%	01.50%	00.82%	00.40%	00.28%
4.	Iron	20.80%	22.80%	24.01%	27.00%	29.54%	32.15%	34.18%
5.	Alumina	15.52%	16.01%	17.07%	17.73%	18.83%	19.54%	21.34%
6.	Manganese	0.20%	0.24%	0.28%	0.31%	0.35%	0.41%	0.48
7.	Copper in ppm	6.0	7.0	7.0	8.0	8.0	9.0	10.0
8.	Zinc in ppm	7.6	8.6	9.0	9.6	13.2	16.4	34.0

Sl. No.	Characteristic	Percentage of reject						
		0%	20%	40%	50%	60%	80%	100%
9.	Nickel in ppm	14.0	14.0	12.0	12.0	14.0	14.0	16.0
10.	Lead	Traces	Traces	Traces	Traces	Traces	Traces	Traces
11.	Calcium	0.15%	0.12%	0.09%	0.08%	0.07%	0.07%	0.05%
12.	Magnesium	0.07%	0.05%	0.04%	0.03%	0.03%	0.02%	0.01%
13.	Available nitrogen in Kg/ha.	51.52	40.32	17.92	17.92	17.92	11.20	2.24
14.	Available phosphorus in Kg/ha.	53.76	26.88	8.96	6.72	6.72	4.48	2.24
15.	Available potassium in Kg/ha.	>448	>448	380.80	369.60	212.80	100.80	33.60
16.	Silica	38.17%	35.03%	34.31%	32.11%	31.45%	30.12%	28.09%

Note: Kg/ha. = kilogram/hectare

phosphorus was 53.76 Kg/ha., available potassium was more than 448 Kg/ha.,iron (20.8%), alumina (15.52%) and manganese (0.2%). Concentrations of the associated metals like zinc increased from 7.6ppm in 0% reject to 34ppm in 100% reject and in the case of nickel ranged from 14ppm (0% reject) to 16ppm (100% reject) with increasing percentage of reject. The amount organic carbon decreased from 2.4% in 0% reject to 0.28% in 100% reject with increasing percentage composition of reject. The values of organic carbon was multiplied by 1.724 to get percent organic matter. The 100% reject had organic matter content of 0.48% which was poor compared to the normal garden soil (0% reject) having 4.15. This low content of organic matter was poor to support vegetation on it. The other nutrients essential for the healthy growth of the plant like calcium and magnesium also decreased with increasing percentage of reject. Calcium decreased from 0.15% in 0% reject to 0.05% in 100% reject and magnesium from 0.07% (0% reject)to 0.01% (100% reject). Lead was found only in traces in all the percentage composition of rejects. Silica decreased from 38.17% (0% reject) to 28.09% (100% reject).

The chemical analysis of the reject supplemented with seaweed is given in Table 1.4. It was observed that with the addition of seaweed to the reject, amount of available nitrogen increased from 2.24 Kg/ha.(pure reject) to 33.60 Kg/ha. (reject + seaweed), available phosphorus from 2.24 Kg/ha. (pure reject) to 11.20 Kg/ha. (reject + seaweed), available potassium from 33.60 Kg/ha. to more than 448 Kg/ha.,calcium from 0.05% (reject) to 0.15% (reject + seaweed) and magnesium from 0.01% (reject) to 0.07% (reject + seaweed). There was also an increase in the amount of organic matter from 0.48% in the pure reject to 3.26% in reject supplemented with seaweed. The pH remained at 5.5 without any change. There was only a little change in the concentrations of the other elements.

DISCUSSION:

Normal soils consist of an inorganic framework of sand, silt and clay particles intimately mixed with organic material produced by degradation of animal and plant remains. The relative amounts of each, control the physical, chemical and biological properties of the soil. Compared to natural soils, mine wastes 1) are deficient in

Table No. 1.4 : Chemical characteristics of the reject soil along with seaweed.

Sl. No.	Characteristic	Type of reject soil			
		Control	Reject	Reject+Seaweed	Garden soil+seaweed
1.	pH value	5.5	5.5	5.5	5.5
2.	Loss on ignition	15.15%	7.18%	12.08%	15.91%
3.	Organic carbon	2.40%	0.28%	1.9%	2.90%
4.	Iron	20.80%	34.18%	32.05%	20.52%
5.	Alumina	15.52%	21.34%	20.14%	15.39%
6.	Manganese	0.20%	0.48%	0.38%	0.18%
7.	Copper in ppm	6.0	10.0	8.0	5.0
8.	Zinc in ppm	7.6	34.0	18.40	10.6%

Sl. No.	Characteristic	Type of reject soil			
		Control	Reject	Reject+Seaweed	Garden soil+seaweed
9.	Nickel in ppm	14.0	18.0	8.0	6.0
10.	Lead	Traces	Traces	Traces	Traces
11.	Calcium	0.15%	0.05%	0.15%	0.17%
12.	Magnesium	0.07%	0.01%	0.07%	0.09%
13.	Available nitrogen in Kg/ha.	51.52	2.24	23.6	49.28
14.	Available phosphorus in Kg/ha.	53.76	2.24	11.20	61.60
15.	Available potassium in Kg/ha.	>448	33.60	>448	448
16.	Silica	38.17%	28.0%	30.25%	37.83%

most plant nutrients 2) may contain excess salts and heavy metals 3) are composed of unconsolidated sands that will easily erode and 4) have a mineralogy upon weathering will affect levels and availability of plant nutrients and possibly toxic minerals (Shetron, 1983).

Investigations into vegetative stabilisation of the mine wastes should include an examination of the physical and chemical characteristics to assess the suitability of the material for plant growth. The physical nature of wastes can sometimes be assessed by an experienced eye, without recourse to formal tests, but the chemical content of wastes requires analytical investigation. Biological characteristics are rarely investigated but are improved indirectly as the physical and chemical problems are overcome.

Physically, the texture, structure, stability and water availability can present problems; chemically, an extreme pH, lack of nutrients and excess of toxic metals and salts are common, and biologically the materials lack typical soil micro-organisms (the agents of breakdown and decay, and of particle aggregation) and larger organisms such as worms which in a normal soil are responsible for mixing and distribution of decaying plant material (Williamson _et_ _al_., 1982).

The texture of soil influences most of the physical and chemical properties. Sandy material has large pores between the particles which allow good aeration and rapid infiltration, clay in contrast has very small particles with little pore space and poor aeration. It tends to be dense with high proportion of moisture bound to the particles and thus unavailable to the plants. The textural analysis of the different percentage composition of the rejects (Table 1.2) showed that 0% reject is mostly made up of sandy loam type of soil whereas the 100% reject is mostly made up of clay.

Normal soils have 2 - 5% organic matter. Mine materials have none or it may be so low that they cannot sustain vegetation on it. Besides supplying nutrients, organic matter is loose and fibrous, and contributes considerably to soil physical properties. The analysis of the 100% reject showed that it was very poor in organic matter content which has deleterious effects (Williamson _et_ _al_ 1982).

In good soil, particles of various size cluster together as a result of organic matter and the activities of micro-organisms, to form aggregates which confer good porosity, aeration and drainage conditions to the medium. Reject (100%), lacks organic matter and suitable micro-organisms, therefore minimal aggregation of particles occurs and the material lacks structure.

There is a wide range of chemical constraints that can prevent plant growth 1) toxicity 2) extreme acidity or alkalinity 3) low nutrient status and 4) chemical cementation. Extremes of any one of these can prove sufficient to prevent plant growth, but generally several constraints act together to produce a particularly hostile environment (Williamson et al, 1982). The analysis of the soil reveals that there are high amounts of aluminium, iron and manganese with deficiency of nutrients having an acidic pH.

Due to the combination of adverse physical structure and chemical characteristics of the mine reject, an environment hostile to plant growth has resulted. A fertile soil contains major nutrients essential for plants, viz. nitrogen, phosphorus, potassium, calcium, magnesium and small quantities of trace elements. Mine rejects are generally sterile and devoid of vegetation (Bradshaw & Chadwick, 1980) and lack plant nutrients.

By the addition of seaweeds to the reject, to a certain extent some of the adverse effects found in the 100% reject soil can be overcome. The addition of seaweed to the reject enhances the availability of the major nutrients, thus reducing nutrient deficiency in the soil and also increases the organic matter content of the reject soil which leads to the improvement of the soil texture.

CHAPTER II

MORPHOLOGICAL RESPONSES

a) Response of <u>Crotalaria juncea</u> L. grown under different composition of rejects :

<u>INTRODUCTION</u> :

Whenever a plant is affected by diseases or due to adverse environmental conditions, the changes can be first observed in the morphology of the plant. Metal ions are particularly important for healthy plant life. Excesses or deficiencies of metal ions have effects on plant growth and morphology. Excessive concentrations of some metals in soils, producing toxic symptoms, may come about in a variety of ways. They may be the result of natural mineralization caused by the presence of undisturbed ore bodies near the surface, known as geochemical anomalies. High concentrations of metals may be the result of the exploitation of mineral resources, eg. mining activities and ore tailings.

Heavy metals have received considerable attention, partly due to their natural occurrence. High concentrations depress plant growth, although certain metals are required in very small amounts for healthy growth. Metalcontaminated wastes in various parts of the world usually contain more than one metal and these may occur at toxic concentrations, eg.metalliferous mined spoils, smelter wastes, coal spoils, sewage sludge and refuse compost.

Studies on the effect of single toxic metals on plants or comparisons of the toxicity of two metals have been frequently reported by various workers eg. the effect of copper on Agrostis tenuis (Wu and Antonovics, 1975); effect of iron on growth in maize and radish (Agarwala et al., 1965). Investigations comparing the toxicity of a number of metals include: copper, nickel, cobalt, zinc, chromium and manganese in reducing fresh weight in mustard (Sinapis alba) by Dekock (1956); aluminium, cadmium, chromium, copper, iron, mercury, manganese, nickel, lead and zinc on radicle of ryegrass (Wong and Bradshaw, 1982). Metal toxicity studies on plants have been reviewed by Woolhouse (1983), aluminium tolerance of soybean has been studied by Foy et al. (1969). But however, a search through available literature indicates that not enough work has been done to understand the response of plants to iron ore mine rejects which contain iron, manganese and aluminium in large quantities together in the reject soil.

In this chapter, the morphological response of **Crotalaria juncea** L. (Sunn Hemp), a leguminous plant of economic importance was studied. The plant was grown in different percentage composition of reject. Some of the morphological responses studied were height of the plant, thickness of the stem and leaf, leaf size, leaf index (L/B), size of the guard cell and stomatal aperture, stomatal frequency, length and dry weight of the root, time of flowering, duration of flowering,number of flowers per inflorescence, number of pods per inflorescence & weight of 100 seeds.

MATERIALS & METHODS:

The seeds of **Crotalaria juncea** L. were collected from experimental plots. The seeds were soaked overnight and then sown in trays. Five day old seedlings were transplanted into bags containing different percentage composition (0%, 20%, 40%, 50%, 60%, 80% and 100%) of reject.

The plants were monitored for their growth fortnightly after transplantation of the seedlings for a period of 60 days. The final morphological readings were taken after 60 days of growth & pollen grain size and fertility were checked. Ten plants were taken up for study in each case. The experiment was carried out for a duration of five months & ten days (5.7.'87 to 15.12.'87) at the mine site.

OBSERVATIONS :

VEGETATIVE CHARACTERS :

As the percentage of reject increased from 0% to 100% there is a decrease in the height of the plant from 239.00cm to 90.1cm. From Fig. 2.1, it can be seen that at 15 days of growth, the height of all the plants is almost same, but as the plant ages, the height of the plant is retarded with increasing percentage of reject (Plate I).

When other characters like thickness of stem and size of the leaf were observed, it was seen that with increasing percentage of reject there is a decrease in the thickness of the stem and the size of the leaf. The stem thickness decreased from 8.4mm in 0% reject to 2.1mm in 100% reject and the leaf size from 13.0cm x 3.1cm in 0% reject to 6.8cm x 1.00cm in 100% reject. The leaf thickness increased

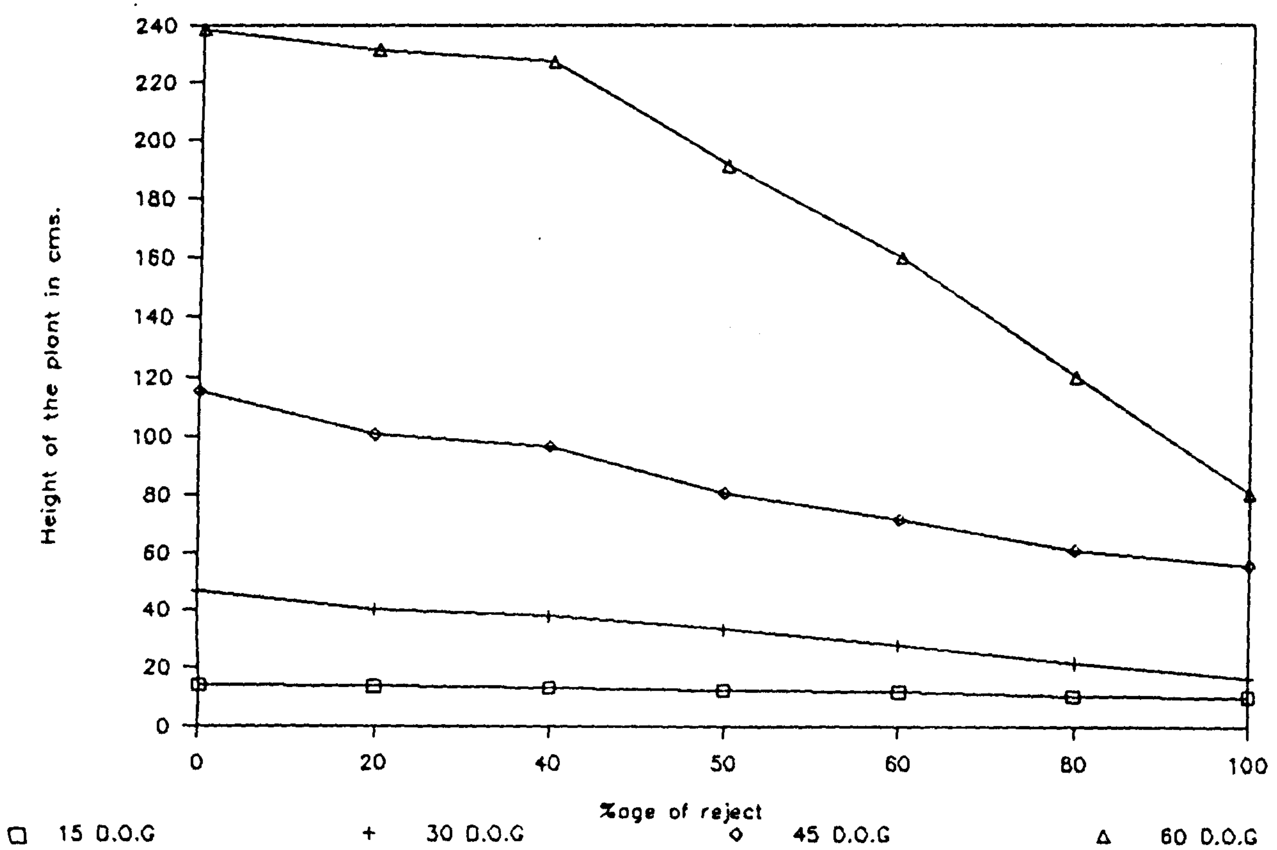


Fig. No.2.1: Growth response of <u>Crotalaria juncea</u> L. to different percentage composition of the mining rejects.

Legend: D.O.G :Days of growth

Table No.2.1 :Morphological responses of ***C. juncea*** to different percentage of mining rejects.

Sl. No.	Response		%age of reject						
			0%	20%	40%	50%	60%	80%	100%
1.	Height of the plant		239.00	231.5**	228.7**	191.4**	159.5**	120.0**	90.1**
	in cm	S.D	2.5	2.48	2.34	2.67	2.63	1.67	1.59
2.	Thickness of stem		8.4	7.4**	5.4**	5.2**	3.4**	3.10**	2.1**
	in mm	S.D	0.3	0.26	0.34	0.41	0.38	0.36	0.23
3.	Size of leaf in cm	L	13.0	12.2**	10.6**	10.2**	9.3**	8.1**	6.8**
		S.D	0.5	0.2	0.3	0.5	0.4	0.3	0.4
		B	3.1	3.0	2.5**	2.1**	1.8**	1.3**	1.0**
		S.D	0.2	0.1	0.2	0.2	0.1	0.1	0.1
4.	Leaf index L/B		4.2	4.07	4.24	4.86	5.17	6.23	6.8
5.	Thickness of leaf in mm		0.17	0.21	0.23	0.25	0.26	0.27	0.28
6.	Stomatal frequency		11.9	11.9	11.8	11.8	11.72	11.2	11.2
	per unit area	S.D	0.83	0.97	1.07	0.94	1.05	0.07	0.87

Sl. No.	Response		%age of reject						
			0%	20%	40%	50%	60%	80%	100%
7.	Size of the guard cell in μm	L	23.76	23.04	21.96	20.88	19.80	18.36	16.92
		B	14.4	14.4	14.4	14.4	14.4	14.4	14.4
8.	Size of the stomatal aperture in μm	L	14.4	14.4	10.8	10.8	10.8	10.8	10.8
		B	3.6	3.6	3.6	3.6	3.6	3.6	3.6
9.	Length of root system in cm		59.0	59.0	53.0	28.0	27.0	19.0	14.0
10.	Dry weight of the root per plant in g		1.21	1.2	1.02	0.95	0.7	0.49	0.3
11.	Age of the plant at the time of flowering		52 days	47 days	52 days	54 days	59 days	59 days	63 days
12.	Period of flowering		75 days	75 days	71 days	65 days	65 days	52 days	50 days

Sl. No.	Response		%age of reject						
			0%	20%	40%	50%	60%	80%	100%
13.	Number of flowers per inflorescence		10+2	8+1	8+2	7+2	6+2	5+2	4+1
14.	Size of std. petal in cm	L	3.5	3.0	2.9	2.5	2.5	2.5	2.2
		B	2.9	2.6	2.5	2.3	2.0	2.0	2.2
15.	Size of the pollen grain (diameter) in µm		7.2	7.2	7.2	3.6	3.6	3.6	3.6
16.	% age of pollen fertility		95%	94%	95%	90%	90%	87%	80%
17.	Number of pods per inflorescence		7+1	5+1	5+2	4+1	4+1	3+1	3+2
18.	Wt. of 100 seeds in g		4.052	3.923	3.856	3.801	3.720	3.515	3.115

Note: Values significant at ** $P < 0.01$ according to student's "t" test.

Explantion for Plate I

Growth of the plants grown in different percentage of reject and addition of seaweed:

1. 0% reject
2. 20% reject
3. 40% reject
4. 50% reject
5. 60% reject
6. 80% reject
7. 100% reject
8. Reject + Seaweed + Sand (80% + 10% + 10%)
9. Garden soil + Seaweed + Sand (80% + 10% + 10%)

Plate I

Explanation for Plate II

Leaves of the plants grown in different percentage of reject and addition of seaweed :

1. 0% reject
2. 20% reject
3. 40% reject
4. 50% reject
5. 60% reject
6. 80% reject
7. 100% reject
8. Reject + Seaweed + Sand (80% + 10% + 10%)
9. Garden soil + Seaweed + Sand (80% + 10% + 10%)

Plate II

from 0.17mm in 0% reject to 0.28mm in 100% reject. The leaves of the plants grown in 100% reject showed crinkling nature with yellowing of the leaves (Plate II). A count of the number of stomata in the leaf epidermis revealed that there was only a slight change in the stomatal frequency of the leaves with increasing percentage of the reject. It decreased from 11.9 in 0% reject to 11.2 in 100% reject. There is also a decrease in the size of the guard cell and the stomatal aperture with increasing percentage of reject. The guard cell was 23.76 μm x 14.4 μm in 0% reject and 16.92 μm x 14.4 μm in 100% reject. The size of the stomatal aperture was smallest in 100% reject (10.8 μm x 3.6 μm) and larger in 0% reject (14.4 μm x 3.6 μm).

It was observed that the root showed a stunted growth in the plants grown in pure reject (Plate III). The length and dry weight of the root decreased with increasing percentage of reject. The length of the root decreased from 59cm in 0% reject to 14cm in 100% reject. The dry weight of the root per plant decreased from 1.21g in 0% reject to 0.3g in 100% reject. Table 2.1 gives comparision of the different characters of plants grown in different percentage composition of reject.

Plate III

REPRODUCTIVE CHARACTERS :

From the Table 2.1, it can be observed that the plants grown in pure reject (100%) matured later and their flowering period was also short compared to the plants grown in 0% reject. At the time of flowering the plants grown in 0% reject were 52 days old with duration of flowering being around 75 days, whereas the plants growing in 100% reject were 63 days old with duration of flowering being around 45 days. The number of flowers reduced from 10 (control) to 4 per inflorescence in the plants growing in 100% reject. The number of pods also decreased from 7 per inflorescence in plants growing in 0% reject to 3 per inflorescence in plants growing in pure reject. The size of standard petal decreased with increasing percentage of reject from 3.5cm x 2.9cm in 0% reject to 2.2cm x 2.2cm in 100% reject. The size of the pollen grain and the pollen fertility decreased with increasing percentage of reject in the soil and also there was a decrease in the dry weight of 100 seeds from 4.052g (0% reject) to 3.115g (100% reject).

DISCUSSION :

VEGETATIVE CHARACTERS :

Results of the soil analysis showed that with increasing percentage of reject there was an increase in the amount of iron, aluminium, manganese and zinc in the soil. It is well known that these elements inhibit the growth of the plants when in excess (Foy *et al.*, 1978).

From the results it can be observed that the growth of *C. juncea* plants is inhibited with increasing percentage of reject. Rees & Sidrak (1961) have shown in their experiment the effects of aluminium on the growth of the spinach plant. With increasing amount of aluminium supplied to the culture media, there is a decrease in the height of the plant. Similar results were also obtained by Clarkson (1966) in *Agrostis*; Lee (1971 a) in potato plants; Mugwira *et al.* (1980) in triticale, wheat and rye; Ahmad & Tan (1986) in soybean plants and Pegtel (1986) in *Succisa pratensis*.

Sideris & Young (1949) showed in pineapple plants that increased amount of manganese in the culture solution depressed the growth of the plant by measuring the plant weight. Also they showed that with high amount of iron and less of manganese in the culture solution lead to the decreased growth of the plants. Lohnis (1951) has shown that excess manganese in the soil depressed the growth of **Vicia sativa, Medicago sativa, Trifolium pratense, Trifolium repens** and Mangold. Also when they were grown in culture solutions, it was observed by Lohnis that the plants grown in highest amount of manganese in the solution were smaller than the control plants. Vlamis & Williams (1964) have shown in rice and barley plants that increasing amount of manganese in the culture solution decreased the growth of the shoot. Bowen (1972) studied the effect of manganese on the growth of sudan grass (**Sorghum sudanese**). He showed that as low concentration as 1 mg/l of manganese in the culture solution affected the growth of the plant. Vlamis & Williams (1973) have shown in lettuce plants that at low concentrations of 0.1 and 0.5 ppm of manganese there is an increase in the growth of the plants, but when further manganese concentrations was increased there was a decrease in the growth of the shoot. Ohki (1976) has shown in soybean plants that the height of the plant decreased in the ones grown in 50,000 μg/l when compared to the plants grown in

1,000 μg/l of Mn. Kuo & Mikkelsen (1981) studied the effect of manganese on Sorghum, it was observed by them that with increasing amount of manganese in the solution decreased the height of the plant. This shows that the concentration of manganese plays an important role in the growth of plants.

Wheeler et al. (1985) showed that excess iron in the soil caused a decreased growth in the shoot of **Epilobium hirsutum**. Pegtel (1986) showed that the growth of **Succisa pratensis** was reduced with increasing amount of iron in the culture solution. The results obtained in the experiments showed that with increasing percentage of reject there is decrease in the growth of the plant. The soil analytical results showed that there is an increase in the amount of aluminium, iron and manganese in the rejects. The observations made here confirms the results obtained by different workers in different plants. The growth of the plants in different percentage composition of rejects showing the reduced growth can be observed in Plate I.

Foy et al. (1978) have also reported that excess of aluminium stunts the growth of the plant thus leading to the thickening of the leaf and decrease in its size. Morris and Pierre (1949) have shown in certain legumes like lespedeza, soyabean, cowpeas and sweet clover that with increasing amount of manganese in the culture solution there was a decrease in the size of leaves with slight chlorotic effect. Lohnis (1951) showed that excess manganese in the culture solution decreased the size of leaf in some vegetable crops. Excess manganese is known to cause chlorosis in the leaves (Foy, 1973 & Foy & Fleming, 1978) and also it causes crinkling of the leaf (Adams & Wear, 1957). Vlamis & Williams (1973) reported that in lettuce plants, excess manganese caused distortion of the leaf shape with marginal chlorosis. The crinkling nature and chlorotic effect is more pronounced in the plants grown in 100% reject which can be observed in the photograph (Plate II). Singh & Sharma (1972) reported that under calcium deficiency conditions there is a decrease in the size of the leaves of potato. The soil analytical results show that there is a decrease in the amount of calcium with increasing percentage of reject. Sharma & Butler (1975), Garg & Varshney (1980) and Mishra (1982) have reported that there is a slight decrease in the stomatal frequency with the reduction in the size of the guard cell and stomatal aperture in many other taxa growing under polluted conditions. Similar type of observations were made in the case of **C. juncea** plants.

There have been frequent reports in the literature which show that aluminium, iron and manganese have inhibitory effects on the plant growth particularly on the growth of the roots. Andrew et al. (1973) have shown in **Glycine wightii** and in **Medicago sativa** the root growth is affected with increasing amount of aluminium in the culture media. Similar results were also obtained by Fageira & Carvalho (1982) in the case of rice plants. Millikan (1949) noted that excess iron, in flax produced a stunted root growth.

Munns (1965) showed that high amount of aluminium in culture solution depressed the root elongation in **Medicago sativa** & **Trifolium subterraneum**. Reid et al. (1971) studied the response of root growth to aluminium treatment in different varieties of barley and found that there was a decrease in the length of the root of plants grown in culture solutions containing 4 ppm of aluminium when compared to control plants. Similar results were also obtained by Mugwira et al. (1978 & 1980) in triticale and wheat; by Wood et al (1984) in the case of cowpeas and by Pegtel (1986) in the case of **Succisa pratensis.**

Wong et al. (1983) studied the root growth of two grass species on iron ore tailings at elevated levels of iron, manganese and copper and found that with increasing amounts of these elements in the soil, there is a decrease in the length of the roots of both the plants.

It was also shown by Munns (1965) in **Medicago sativa** & **Trifolium subterraneum**; by Clarkson (1966) in **Agrostis canina**, **A. stolonifera** & **A. tenuin**; by Lee (1971 a & b, 1972) in potato plants; Mugwira et al. (1978 & 1980) in triticale & wheat and by Pegtel (1986) in the case of **Succisa pratensis** that with increasing amount of aluminium in the growth media there was a decrease in the dry weight of root per plant when compared to control plants.

Vlamis & Williams (1973) showed in lettuce plants that with increasing concentrations of manganese from 0.1 ppm to 10.0 ppm in the culture solution, there was a decrease in the dry weight of the root. Similar observations were also made by Ohki (1976) in the case of soybeans & by Pegtel (1986) in the case of **Succisa pratensis**.

Shetron & Spindler (1983) studied the root pattern of **Medicago sativa** in iron ore tailings and natural soils. They found that the root of the plants growing in iron tailings had a lesser dry weight than the plants grown in natural soils. Similar type of observations were made by Wheeler et al. (1985) in the case of **Epibolium hirsutum** and **Juncus subnodulosus** and by Pegtel (1986) in the case of **Succisa pratensis**.

Emamuelsson (1984) studied the root growth in relation to calcium concentration and found that the length and dry weight of the roots decreased with decreasing calcium concentration to certain extent. The results obtained on the root response of **C. juncea** to the mine rejects where there is a high amount of aluminium, manganese and iron with decreased levels of calcium in the soil confirm to the results obtained by various earlier workers in different taxa.

REPRODUCTIVE CHARACTERS :

Excess aluminium is known to cause delay of flowering in plants (Foy et al.,1978). Halse et al. (1969)

showed in wheat that nitrogen deficiency cause a delay in the flowering of the plants. The soil analytical results show that there is high amount of aluminium and deficiency in nitrogen as the percentage of reject increases. Howard-Williams (1971) has shown that with increased copper levels, there was a reduction in the corolla size and reduction in the seed weight. Mishra (1982) has reported that the size of the petal was smaller in the plants grown under polluted conditions. Gupta et al. (1973) showed that high concentrations of aluminium and manganese affected the yield of kernel in barley plants. The results obtained here show that increased levels of iron, aluminium and manganese and deficiency in the macronutrients affected the reproductive characters also.

II b) Response of plants to seaweed and fertilizer treatments

INTRODUCTION :

Mine wastes are usually deficient in essential plant nutrients. The major nutrients required by plants are nitrogen, phosphorus, potassium, calcium, manganese and sulphur. Minor nutrients required only in trace amounts are iron, manganese, boron, copper, zinc and molybdenum. Nitrogen, phosphorus and sometimes potassium levels are inadequate and deficiencies of calcium and magnesium occasionally arise. Concentrations of the essential minor nutrients are usually satisfactory, but occasional deficiencies occur in some regions.

Sources of plant nutrients are soil minerals, decaying organic matter, and, to a small degree rainfall. Nitrogen, however, is not available from soil minerals and is obtained either from decaying organic matter, or by microbial fixation from the air, mainly by root nodule bacteria of leguminous plants. Both these sources of nitrogen are wholly or almost completely absent in mine wastes. Lack of organic matter, soil micro-organisms and natural plant cover are indications of the extent of nitrogen deficiency in mine rejects.

Conditions of toxicity and paucity of nutrients as well as physical nature of the mine rejects, make normal techniques of vegetation establishment impossible (Hooper and Newton, 1935). The nutrient deficiency in the wastes can be overcome by the use of fertilisers (Bradshaw and Chadwick, 1980), but heavy metal toxicity cannot be so readily overcome. Toxicites could be overcome by the addition of phosphates and organic matter.

Organic matter has the ability to form a stable chelates in which metal ions are held between atoms of complex organic molecule, thereby eliminating toxicity until the organic matter decomposes through natural process. They provide ligands which chelate with toxic metal ions, and these can be important in the movement of toxic metals through soils (Hudgson, 1969).

From the above discussion it is evident that plant's survivability could be studied if the stressful conditions are moderated. By improving the edaphic conditions, severity of stress could be reduced.

In this chapter a study is made to see how the plants would respond to different combinations of fertilizer (NPK) and seaweed treatments to the reject.

Seaweeds have been used as a source of organic matter and manure for paddy and coconut plantations, because they contain many growth promoting hormones, trace elements and micronutrients (Challen & Hemingway, 1966). Beneficial effects from the use of seaweeds have been obtained on plants, their crop yield and their resistance to fungal and insect attack (Brian *et al*., 1973).

MATERIALS & METHODS :

The seeds of ***Crotalaria juncea*** L. were collected from experimental plots. The seeds were soaked in water overnight and then sown in trays. Five day old seedlings were trnasplanted into bags containing garden soil + seaweed and reject + seaweed and also to bags containing reject treated with different combinations of N,P & K. The different combinations of N,P & K treatments given were as follows:

1) N P K - All the three nutrients.
2) N P K_o - Only nitrogen and phosphate.

3) N_o P K - Only phosphate and potassium.

4) N P_o K - Only nitrogen and potassium

5) N P_o K_o - Only nitrogen.

6) N_o P K_o - Only phosphate.

7) N_o P_o K - Only potassium.

The fertilizers were added in the following form : Nitrogen as urea; phosphate as sodium phosphate and potassium as potassium chloride. The plants were treated with 10ppm solutions of the above mentioned fertilizers with the first treatment being given at the time of transplantation and subsequently every 15 days till the end of the experiment.

The plants were monitored for their growth fortnightly after transplantation of the seedlings for a period of 60 days. The final morphological readings were taken after 60 days of growth. Pollen grain size and fertility were checked. The experiment with seaweed was carried out for a duration of five months and ten days (5.7.1987 to 15.12.1987) and with fertilizers for a duration of five months and six days (25.2.1988 to 31.7.1988). Ten plants were taken up for study in each case.

OBSERVATIONS :

SEAWEED TREATMENTS :

The comparitive morphological responses of *C. juncea* to the mine rejects in the presence of seaweeds is given in Table 2.2. It was observed that the height of the plant increased on treatment with seaweed. In the experiment conducted, it was observed that the plants grown only in reject were 90.1cm tall whereas in the presence of seaweed it was 169.8cm. From Fig. 2.2 it can be observed that at initial stages of growth, there is not much difference in the height of the plant in both treated and untreated plants. But as the plant ages it was observed that the height of the plants which were grown in the presence of seaweed were taller than the plants grown in only reject.

Similarly when other vegetative characters like thickness of stem and leaf were observed it was seen that in seaweed treated plants the stem was thicker (5.1mm) and leaf was thinner 0.2mm than the plants grown in only reject where the stem was 2.1mm in thickness with the leaf being thicker 0.28mm. The leaves were larger (10.5cm x 2.3cm) in

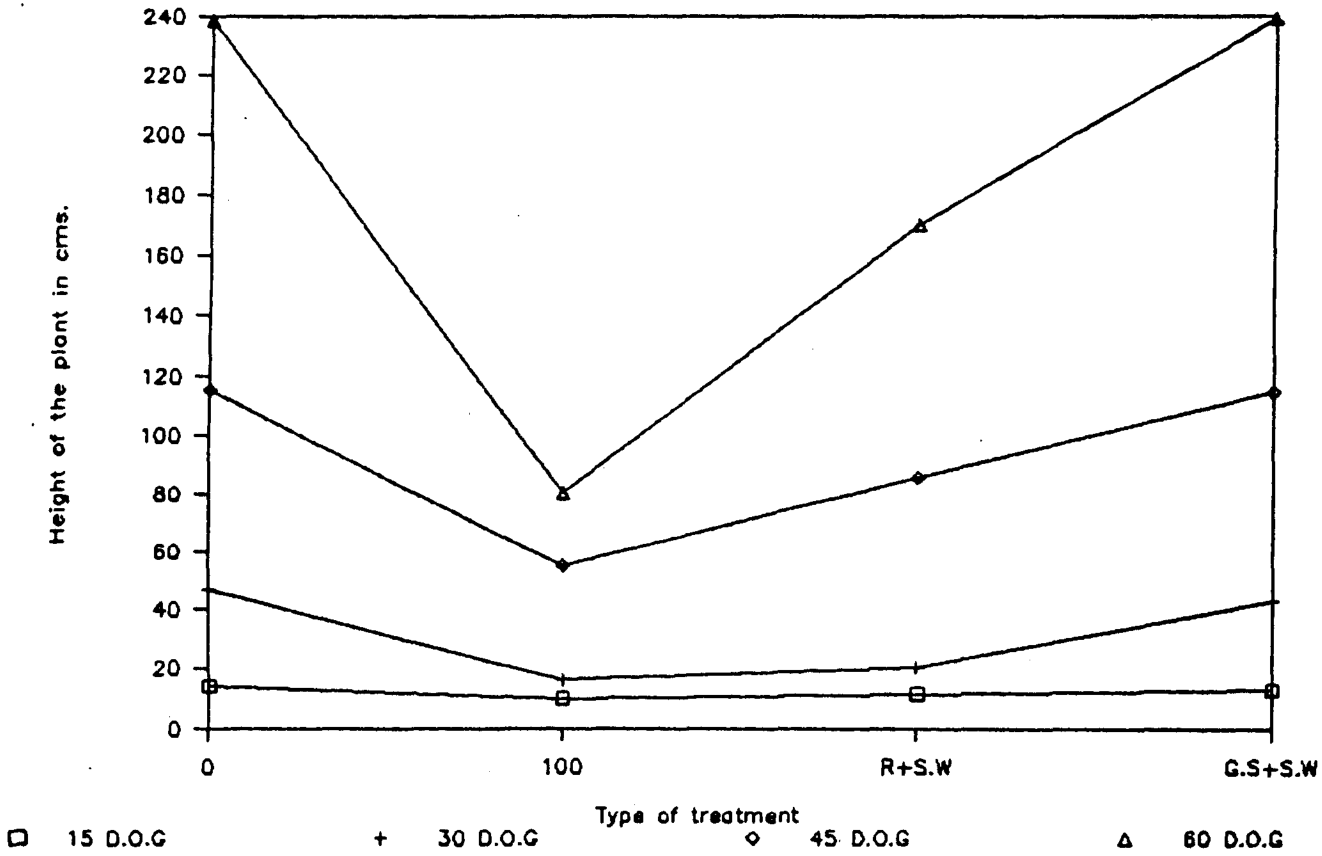


Fig. No. 2.2: Growth response of Crotalaria juncea L. to the mining rejects in the presence of seaweed.

Legend: D.O.G : Days of growth
C - Control
R - Reject
S.W - Seaweed
G.S - Garden soil

Table No. 2.2 : Morphological responses of <u>C. juncea</u> to the mining rejects in the presence of seaweed.

Sl. No.	Response		Control	Reject	Reject + Seaweed	Garden soil + Seaweed
			Type of treatment			
1.	Height of the plant in cm		239.0	90.1**	169.8**	240.5
		S.D	2.5	1.59	2.61	2.29
2.	Thickness of stem in mm		8.4	2.1**	5.1**	8.8**
		S.D	0.3	0.23	0.17	0.29
3.	Size of leaf in cm	L	13.0	6.4	10.5	15.2
		S.D	0.5	0.4	0.4	0.3
		B	3.1	1.0	2.3	3.4
		S.D	0.2	0.2	0.2	0.2
4.	Leaf index L/B		4.2	6.8	4.57	4.47
5.	Thickness of leaf in mm		0.17	0.28	0.20	0.18
6.	Stomatal frequency per unit area		11.9	11.2	11.72	11.8
		S.D	0.83	0.87	1.05	0.87

Sl. No.	Response		Type of treatment			
			Control	Reject	Reject + Seaweed	Garden soil + Seaweed
7.	Size of the gaurd cell in µm	L	23.76	16.92	21.96	23.76
		B	14.4	14.4	14.4	14.4
8.	Size of the stomatal aperture in µm	L	14.4	10.8	12.6	14.4
		B	3.6	3.6	3.6	3.6
9.	Length of root system in cm		59.0	14.0	20.0	57.0
10.	Dry weight of root per plant in g		1.21	0.39	0.5	1.5
11.	Age of the plant at the time of flowering		52 days	63 days	59 days	47 days
12.	Period of flowering		75 days	50 days	70 days	75 days

Sl. No.	Response		Type of treatment			
			Control	Reject	Reject + Seaweed	Garden soil + Seaweed
13.	No. of flowers per inflorescence		10	4	8	11
		S.D	2	1	1	2
14.	Size of std. petal in cm	L	3.5	2.2	3.2	3.5
		B	2.9	2.2	2.8	2.8
15.	Size of the pollen grain (diameter) in μm		7.2	3.6	7.2	7.2
16.	% age of pollen fertility		95%	80%	91%	95%
17.	Number of pods per inflorescence		7	3	5	8
		S.D	1	2	1	1
18.	Wt. of 100 seeds in g		4.052	3.115	3.825	4.102

Note: Values significant at ** $P < 0.01$ according to student's "t" test.

seaweed treated plants than the plants grown in reject (6.8cm x 1.0cm). The stomatal frequency changed very little whereas the guard cell (21.6 μm x 3.6 μm) were larger when compared to the plants grown in only reject. In the case of root, there was only a slight improvement in length (20cm) and dry weight of root per plant was 0.5g when compared to the plants grown in only reject, the length was 14cm and dry weight of root per plant was 0.39g.

When the reproductive characters were observed, it was seen that the plants growing in reject supplemented with seaweed matured earlier (59 days) and their duration of flowering was around 70 days. The number of flowers per inflorescence was 8 in treated plants whereas in untreated plants it was 4. The number of pods per inflorescence was also more in treated plants. The size of the petal increased from 2.2cm x 2.2cm in untreated plants to 3.2cm x 2.8cm in plants treated with seaweed. The pollen grain size and pollen fertility also increased with an increase in the weight of 100 seeds in treated plants.

FERTILIZER TREATMENT :

The comparative morphological response of *C. juncea* to mine rejects in the presence of fertilizers are given in Table 2.3. It was observed that the plants growing in reject without any treatment were shorter (38.0cm). But with the addition of different combinations of fertilizers there was marked change in the growth of the plant. From Fig.2.3, it can be observed that initially the growth is almost the same in all the plants. But with aging of the plant, it was observed that in the presence of NPK (89.1cm), NP (87.3cm) & PK (86.4cm) the height of the plant is almost same and they are taller than the plants grown in NK (77.6cm), N (62.4cm), P (69.8cm) and K (70.8cm).

Similarly when other characters were observed, the stem in fertilizer treated plants was thicker (6.2mm in NPK treatment) than in plants grown in reject without any treatment (2.4mm). The leaves in treated plants were thinner and larger than the plants grown in reject. There was only a slight change in the stomatal frequency with increase in the size of the guard cell and stomatal aperture in plants grown in reject supplemented with fertilizers. The addition of fertilizers showed only a slight improvement

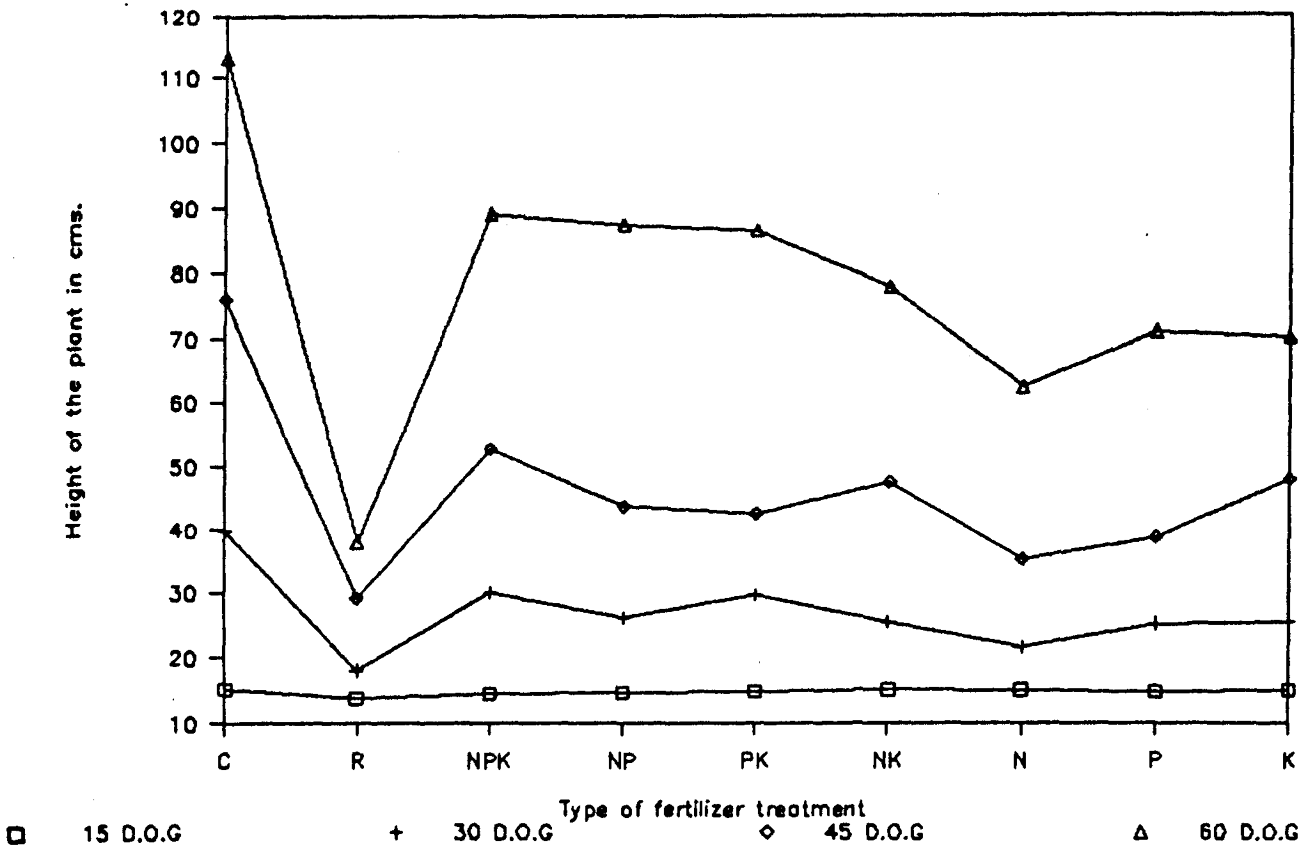


Fig. No. 2.3 : Growth response of <u>Crotalaria juncea</u> L. to the mining rejects with different fertilizer treatments.

Legend: D.O.G.: Days of growth
C - Control R - Reject
NPK - Nitrogen, Phosphorus, Potassium
NP - Nitrogen, Phosphorus
PK - Phosphorus, Potassium
NK - Nitrogen, Potassium
N - Nitrogen
P - Phosphorus
K - Potassium

Table No. 2.3 : Morphological response of C. juncea to the mine rejects in the presence of fertilizers.

Sl. No.	Response		Control	Reject	NPK	NP	PK	NK	N	P	K
			Type of treatment								
1.	Height of the plant in cm		113.2	38.0**	89.1**	87.3**	86.4**	77.6**	62.4**	69.8**	70.8**
		S.D	2.43	1.6	1.75	1.59	1.17	1.05	3.7	2.7	1.5
2.	Thickness of stem in mm		8.5	2.4	6.2	5.8	6.0	6.1	4.8	5.0	4.5
3.	Size of leaf in cm	L	11.6	9.59	12.31	9.78	9.94	9.96	9.36	10.75	9.9
		S.D	0.4	0.4	0.6	0.3	0.5	0.4	0.5	0.4	0.4
		B	2.16	1.66	2.56	1.8	1.98	1.92	1.89	1.86	1.83
		S.D	0.2	0.1	0.3	0.1	0.2	0.3	0.3	0.1	0.1
4.	Leaf index L/B		5.42	5.87	4.91	5.43	5.1	5.47	5.03	5.78	5.41
5.	Thickness of leaf in mm		0.17	0.28	0.20	0.21	0.22	0.21	0.22	0.22	0.23
6.	Stomatal frequency per unit area		11.9	11.2	11.9	11.9	11.8	11.5	11.5	11.5	11.6
		S.D	0.83	0.87	0.83	0.83	1.1	0.80	0.92	1.2	1.1

Sl. No.	Response		Type of treatment								
			Control	Reject	NPK	NP	PK	NK	N	P	K
7.	Size of the gaurd cell in μm	L	25.2	16.9	25.2	21.6	21.96	22.14	21.6	18.0	19.8
		B	14.4	14.4	14.4	14.4	14.4	14.4	14.4	14.4	14.4
8.	Size of the stomatal aperture in μm	L	14.4	10.8	14.4	14.4	14.4	14.4	14.4	14.4	14.4
		B	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
9.	Length of root system in cm		42.0	23.0	30.0	33.0	33.0	29.0	28.0	27.0	26.5
10.	Dry weight of root per plant in g		2.5	0.29	1.8	1.5	1.6	1.6	1.7	1.4	1.3
11.	Age of the plant at the time of flowering		53 days	60 days	55 days	55 days	54 days	56 days	55 days	54 days	57 days
12.	Period of flowering		75 days	50 days	68 days	65 days	66 days	65 days	63 days	64 days	63 days

Sl. No.	Response		Control	Reject	NPK	NP	PK	NK	N	P	K
			Type of treatment								
13.	No. of flowers per inflorescence		10	4	9	8	8	7	7	7	6
		S.D	2	1	1	1	1	1	2	1	1
14.	Size of std. petal in cm	L	3.51	2.66	3.49	3.25	2.89	3.05	2.74	2.76	2.63
		B	2.48	1.97	2.58	2.33	2.18	2.32	2.41	2.36	2.42
15.	Size of the pollen grain (diameter) in µm		7.2	3.6	7.2	5.4	5.4	3.6	3.6	3.6	3.6
16.	% age of pollen fertility		95%	80%	86%	84%	85%	83%	82%	81%	84%
17.	Number of pods per inflorescence		7	3	5	5	5	5	5	5	5
		S.D	1	2	1	1	2	1	1	1	1
18.	Wt. of 100 seeds in g		4.052	3.115	3.750	3.615	3.605	3.685	3.450	3.515	3.485

Note: Values significant at ** $P < 0.01$ according to student's "t" test.

in the length of the root (30cm in NPK treatment and 23cm in untreated plants) but the dry weight of root per plant increased considerably from 0.29g in untreated plants and 1.8g in NPK treated plants.

When the reproductive characters were observed, it was seen that the fertilizer treated plants matured earlier (55 - 57 days) and their flowering duration also increased (64 - 68 days) when compared to the plants grown in reject without any treatment where the plants matured later (63 days) and the duration of flowering also was shorter (50 days). The number of flowers (6-9) and the number of pods per inflorescence (4 - 5) almost doubled in the treated plants whereas in the untreated plants the number of flowers were 4 and the number of pods per inflorescence were 3. There was increase in the size of the standard petal, the pollen grain size and the pollen fertility in treated plants. The weight of 100 seeds also increased marginally with the addition of fertilizers.

DISCUSSION :

SEAWEED TREATMENT :

The observations show that there is an overall beneficial effect seen in the plant on the addition of seaweed to the reject. From the soil analytical results it was observed that the addition of seaweeds has increased the amount of available nutrients viz. N, P & K and also calcium and magnesium in the soil. This additional nutrients now available to the plants and the increase in the organic matter has improved their growth. This improved growth in the plants might be due to the mineral content, trace elements and growth promoting factors present in the seaweed (Boney, 1965). The increase in the leaf size might probably be due to the absorption of micronutrients and cytokinin present in the seaweed, which also enhanced the growth (Blunden *et al*.,1974).

FERTILIZER TREATMENTS :

The results show that in the presence of phosphate in the following combinations - NPK, NP & PK the growth is improved. It is known that the addition of phosphate reduces the toxicity of heavy metal burden on the plants

(Smith & Bradshaw, 1972). The nutrient deficiency is overcome by the addition of fertilizers to the mine wastes (Bradshaw & Chadwick, 1980) which has improved in the overall growth of the plant. The addition of phosphate as a fertilizer might have reduced the toxicity of the metals present in the soil and thus improving the growth of the plants where it was added.

Halse et al. (1969) showed in wheat crop that the addition of nitrogen to the soil improved its growth and the plants matured earlier than the ones grown in soil with nitrogen deficiency. MacLeod et al. (1971) have studied the effect of N, P & K on root yield in Rutbagas. They found that with the addition of nitrogen and phosphorus the dry weight of the root increased. The results obtained here with respect to **C. juncea** showed that with the addition of nitrogen and phosphorus to the reject soil there was an increase in the dry weight of the root per plant. Fleming et al. (1974) showed that with the addition of phosphate to the soil in the presence of aluminium there was increase in the growth of the plant. The phosphate treatment given in combination with different fertilizer like N & K to **C. juncea** plants grown in rejects show that the addition of phosphate has improved the growth of the plants. Mitchell *et al*. (1974) studied the effects of N, P & K on the growth and seed characteristics of sesame. They found that in the presence of added nitrogen and potassium there was an increase in the height of the plant and the dry weight of capsule increased. It was also observed by them that with the addition of phosphorus there was an increase in height of the plant and dry weight of the seeds. Terman *et al*. (1977) showed that the application of N, P & K in varying concentrations enhanced the growth of corn plants. Safaya and Wali (1979) studied the effect of fertilizers on a grass legume mixture grown on Sodic-Coal mine spoil. They found that the addition of NPK to the soil improved the dry matter yield in thickspike wheat grass and yellow sweet clover. Kachi & Hirose (1983) studied the effect of N, P & K in ***Oenothera erythrosepala***. They found that the addition of P in the presence of N, K or NK, there was a considerable improvement in the growth of the plants. Ajakaiye (1986) showed that with the addition of P at high levels of iron there was an increase in the height of cowpea plants with an increase in the leaf area. The observations made on the growth of ***C. juncea*** in the presence of fertilizers showed that responses were similar to the ones obtained by other workers on varied plants.

CHAPTER III

BIOCHEMICAL RESPONSES

INTRODUCTION

Any morphological changes seen in a plant grown under natural or stress conditions are due to the biochemical responses of the plant to the stress. Excesses or deficiencies of metal ions have effects on the metabolic processes of the plant leading to the biochemical changes in the plant system.

For over 100 years it has been known that iron is essential for the growth of higher plants and it is involved in some manner with chlorophyll metabolism. Studies on the effect of manganese and zinc in relation to iron on the chlorophyll, total proteins and total sugars have been carried out by Agarwala et al. 1964; Agarwala et al. 1977 and Rosen et al. 1977. Nag et al. 1981 have studied the effect of different heavy metals on chlorophyll and soluble proteins.

Plants have been known to accumulate high levels of proline when subjected to water stress (Aspinall & Paleg, 1981); nutrient deficiencies (Savitakaya, 1976 and Ghildiyal et al. 1986); air pollutants (Godzik & Linslous, 1974 and

Soldatini *et al.*, 1978) and pesticides (Deshpande & Swamy, 1987). The biochemical mechanism leading to the induction of proline accumulation and its physiological significance have not been elucidated although it has been suggested that proline may be involved in osmoregulation (Weimberger *et al.* 1982 and Carcellar & Franschina, 1986).

A survey through the literature showed that very little work has been carried to study how the biochemical processes are affected in the plant. Hence it was of interest to take up the study of chlorophyll, protein, sugars and proline content in the plants grown in mine rejects, which has different heavy metals in high concentrations and also deficient in nutrients. The study was also carried out with and without seaweed and fertilizer treatments.

MATERIALS & METHODS:

Crotalaria juncea plants were grown as mentioned in the previous chapter. Chlorophyll estimations were carried out fortnightly for a period of 60 days. Protein, sugars and proline estimations were carried out after 60 days of growth.

Chlorophyll was extracted and estimated according to the method given by Arnon (1949). Proteins and sugars were extracted by modifying slightly the methods given by Loomis & Shull (1937) and Osborne (1962). Proteins were estimated according to the method outlined by Miller (1959) and sugars by the method given by Dubois et al. (1951). Proline was extracted and estimated according to the method given by Bates et al. (1973).

OBSERVATIONS :

EFFECT OF MINE WASTES ON CHLOROPHYLL CONTENT :

The results of the chlorophyll analysis are given in Figs. 3.1 to 3.3. From the graphs it can be observed that initially there was only a slight difference in the amount of chlorophyll content of the plants grown in different compositions of reject ranging from 0% to 100% and also in plants grown in the presence of seaweed and different fertilizer treatment. But as the plants aged, it was observed that there was significantly less amount of chlorophyll in the plants grown in 100% reject (200 mg/100 g fresh weight of leaves), when compared to the plants growing

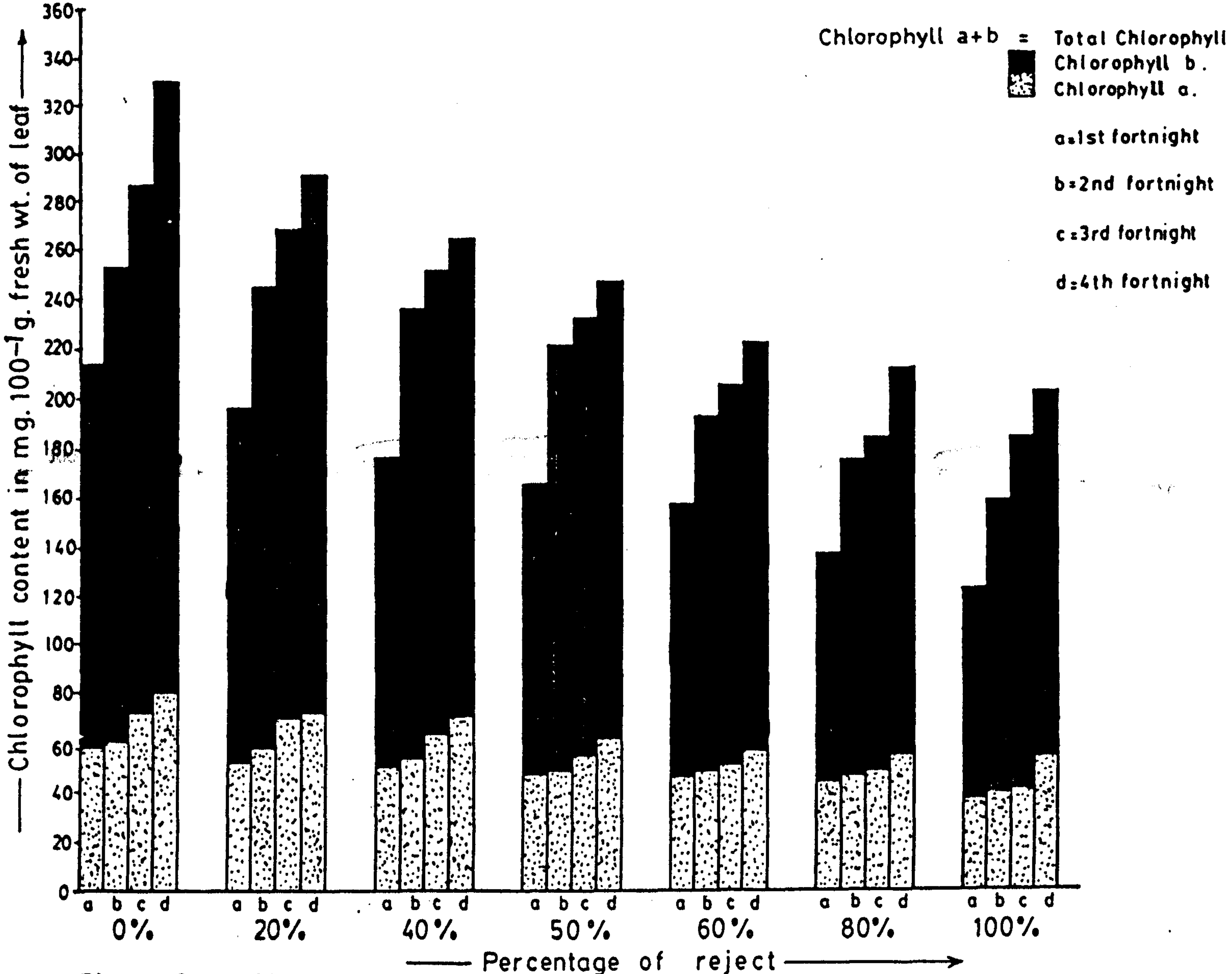


Fig. no. 3.1 Effect of different composition of mining rejects on the Chlorophyll content in *Crotalaria juncea* L.

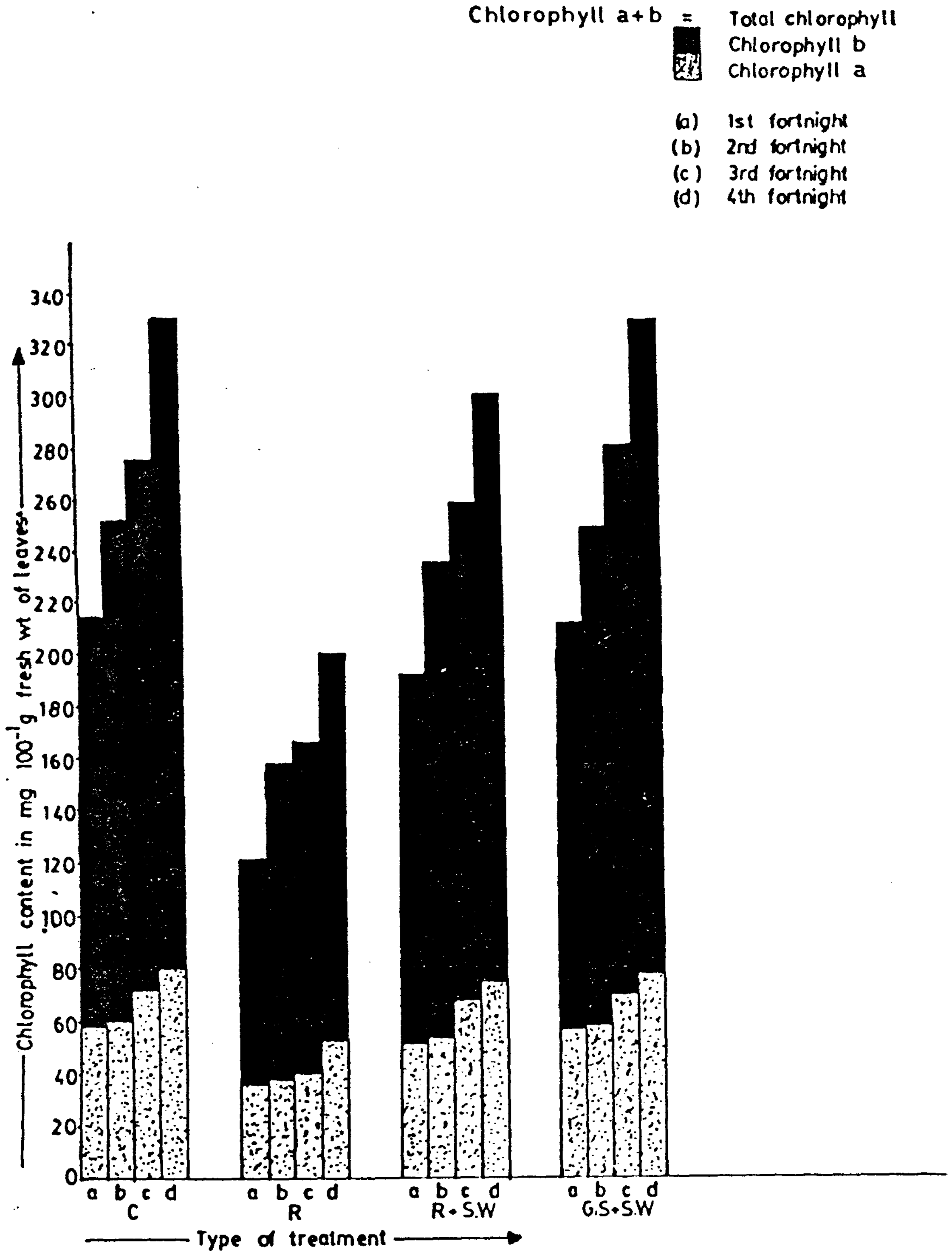


Fig. no. 3.2: Effect of mining rejects in the presence of seaweeds on the chlorophyll content in <u>Crotalaria juncea</u> L.

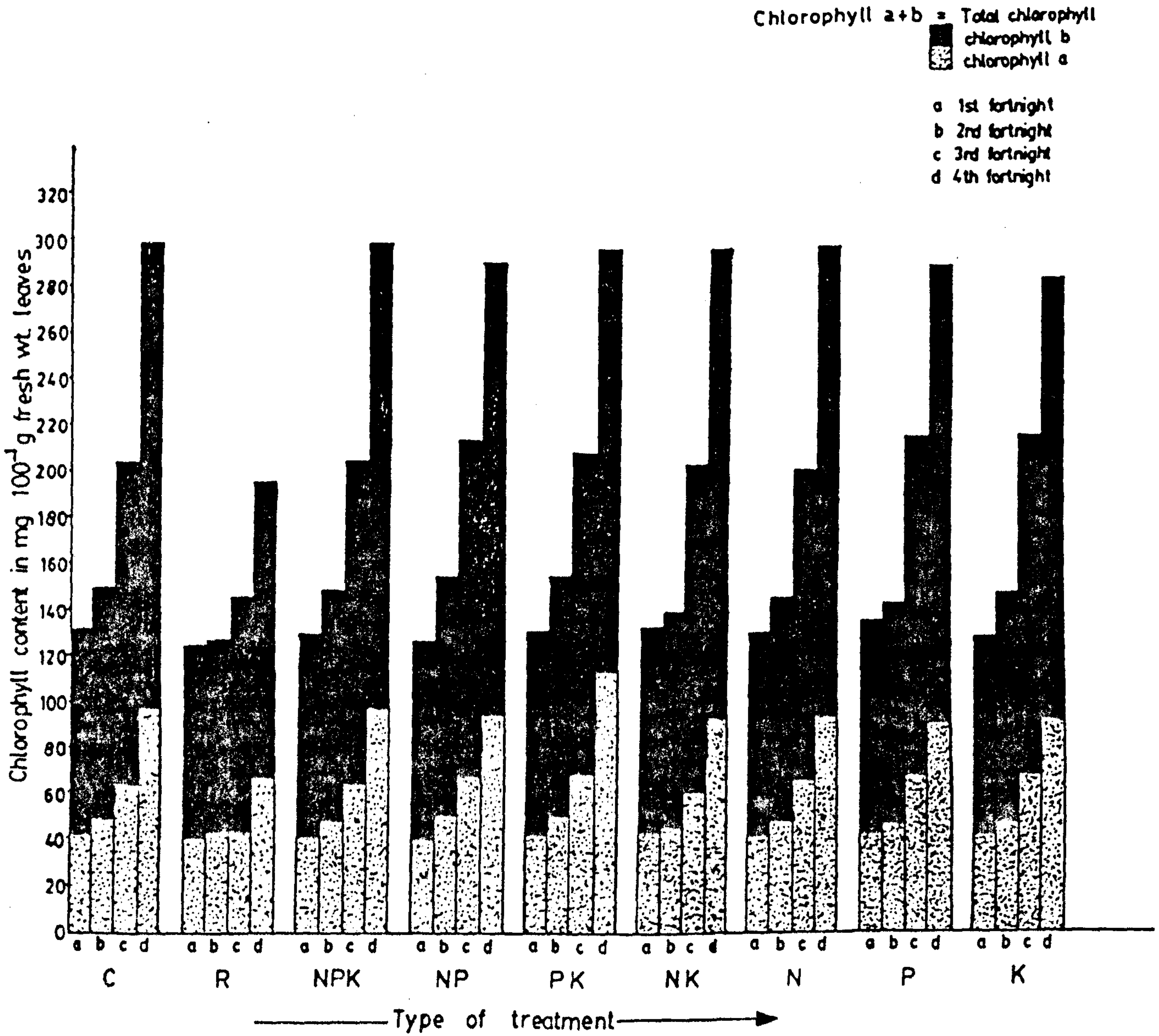


Fig. no. 3.3: Effect of mining rejects in the presence of fertilizers on the Chlorophyll content in *Crotalaria juncea* L.

in 0% reject (330 mg/100 g fresh weight of leaves). There was also change in the chlorophyll a & chlorophyll b contents, they decreased with increasing percentage of reject.

In the presence of seaweed and different fertilizer treatment to the reject, it was observed that the chlorophyll content increased in the treated plants. In seaweed treated plants it was 300 mg/100 g fresh weight of leaves whereas in untreated plants it was 200 mg/100 g fresh weight of leaves. (Fig 3.2). In the case of fertilizer treated plants it was observed that it ranged between 281 - 298 mg/100 g fresh weight of leaves with different treatments and in untreated plants it was 195 mg/100 g fresh weight of leaves (Fig.3.3). In both seaweed and fertilizer treatments, there was increase in the amount of chlorophyll a and chlorophyll b.

EFFECT OF MINE WASTES ON THE TOTAL PROTEIN AND TOTAL SUGAR CONTENT:

The results of the total protein and sugar contents are given in Figs.3.4.1 to 3.4.3. From the graph (Fig.3.4.1) it can be observed that, with increasing

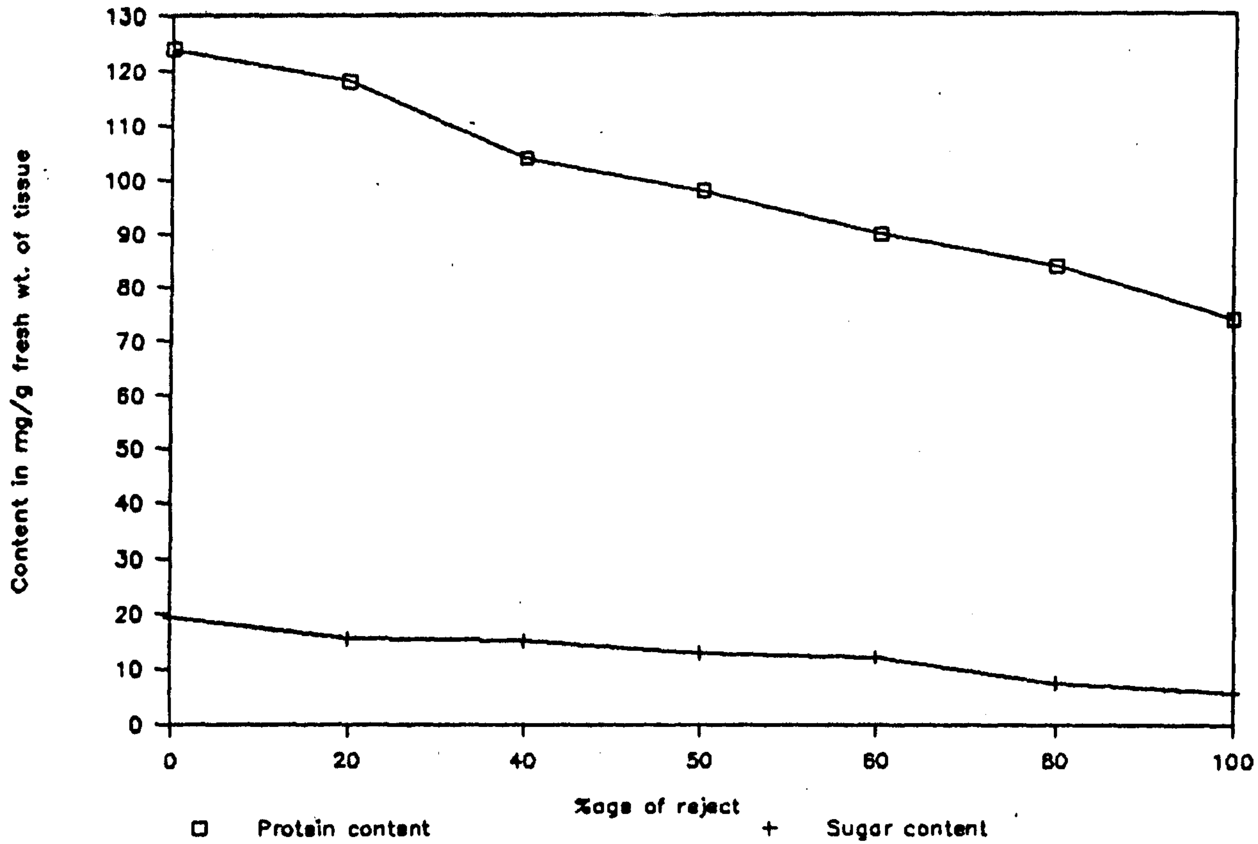


Fig. No. 3.4.1 : Effect of different composition of mining rejects on the Protein and Sugar content in *Crotalaria juncea* L.

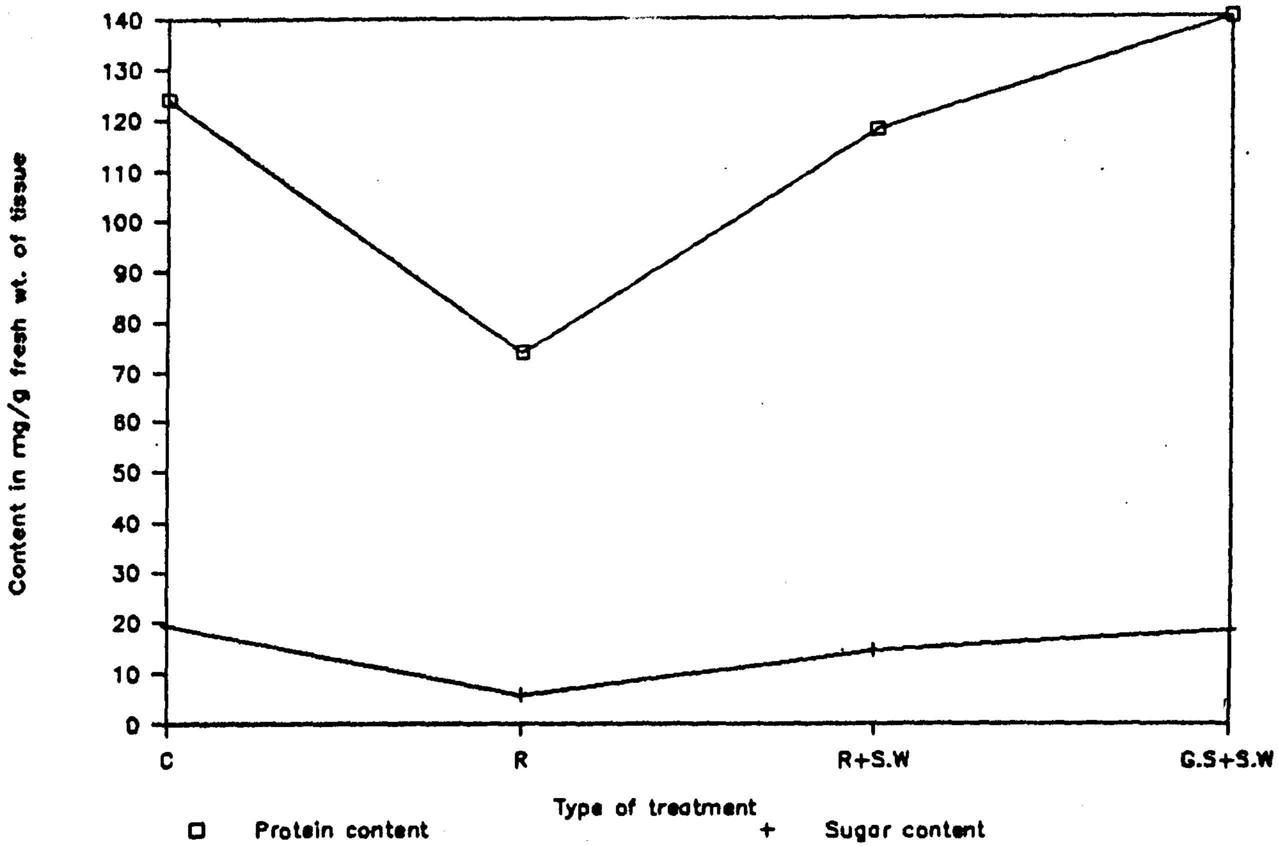


Fig. No. 3.4.2 : Effect of mining rejects in the presence of seaweed on the Protein and Sugar content in <u>*Crotalaria juncea*</u> L.

C - Control
R - Reject
S.W - Seaweed
G.S - Garden soil

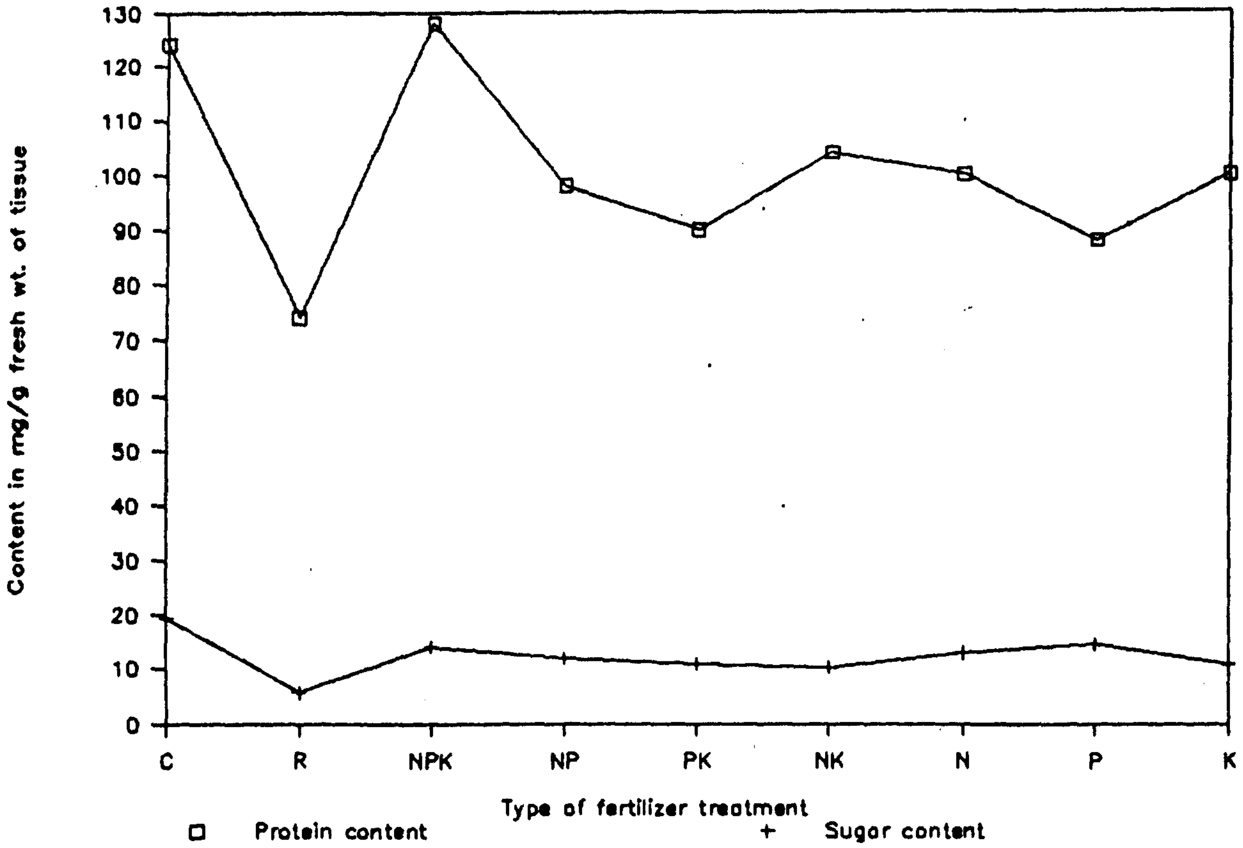


Fig. No. 3.4.3 : Effect of mining rejects with fertilizer treatments on the Protein and Sugar content in <u>Crotalaria juncea</u> L.

C - Control R - Reject
NPK - Nitrogen, Phosphorus, Potassium
NP - Nitrogen, Phosphorus
PK - Phosphorus, Potassium
NK - Nitrogen, Potassium
N - Nitrogen
P - Phosphorus
K - Potassium

percentage composition of reject there is a decrease in the total content of both proteins and sugars. Protein decreased from 124 mg/g fresh weight of tissue in 0% reject to 74mg/g fresh weight of tissue in 100% reject and the sugar content from 19.36 mg/g fresh weight of tissue to 5.6 mg/g fresh weight of tissue. But with the addition of seaweed and fertilizers to the reject there was an increase in their content. In the case of total protein it increased from 74 mg/g fresh weight of tissue in reject to 118 mg/g fresh weight of tissue in the presence of seaweed (Fig 3.4.2) and ranged between 88 - 128 mg/g fresh weight of tissue with fettilizer treatment (Fig.3.4.3). The total sugar content increased from 5.6 mg/g fresh weight of tissue in reject to 14.47 mg/g fresh weight of tissue in the seaweed treated plants and ranged from 10.27 - 14.7 mg/g fresh weight of tissue in fertilizer treated plants.

EFFECT OF MINE WASTES ON THE PROLINE CONTENT :

The results of the proline analysis are shown in Figs. 3.5.1 - 3.5.3. From Fig. 3.5.1 it can be observed that with increasing percentage of reject there was an increase in the proline content from 30 µg/g fresh weight of

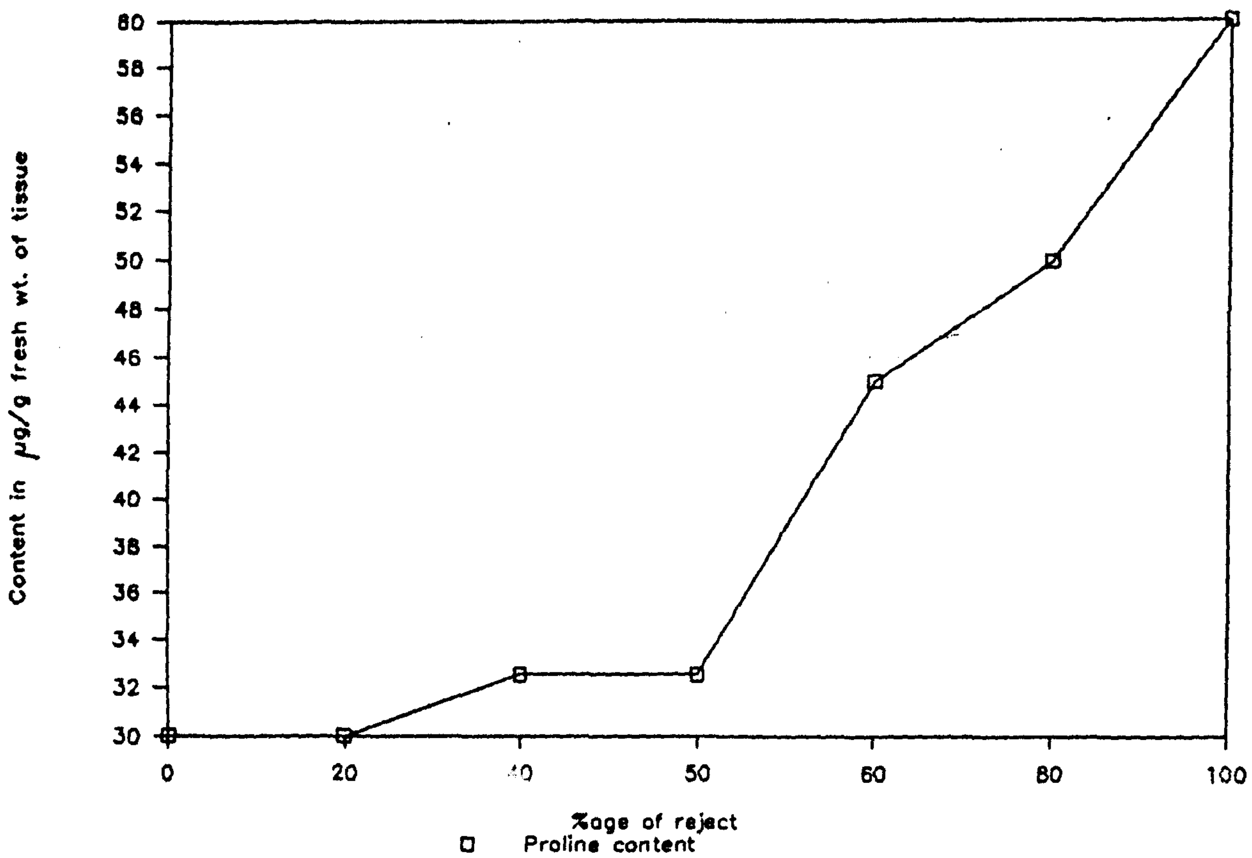


Fig. No. 3.5.1 : Effect of different percentage composition of mining rejects on the Proline content in ***Crotalaria juncea*** L.

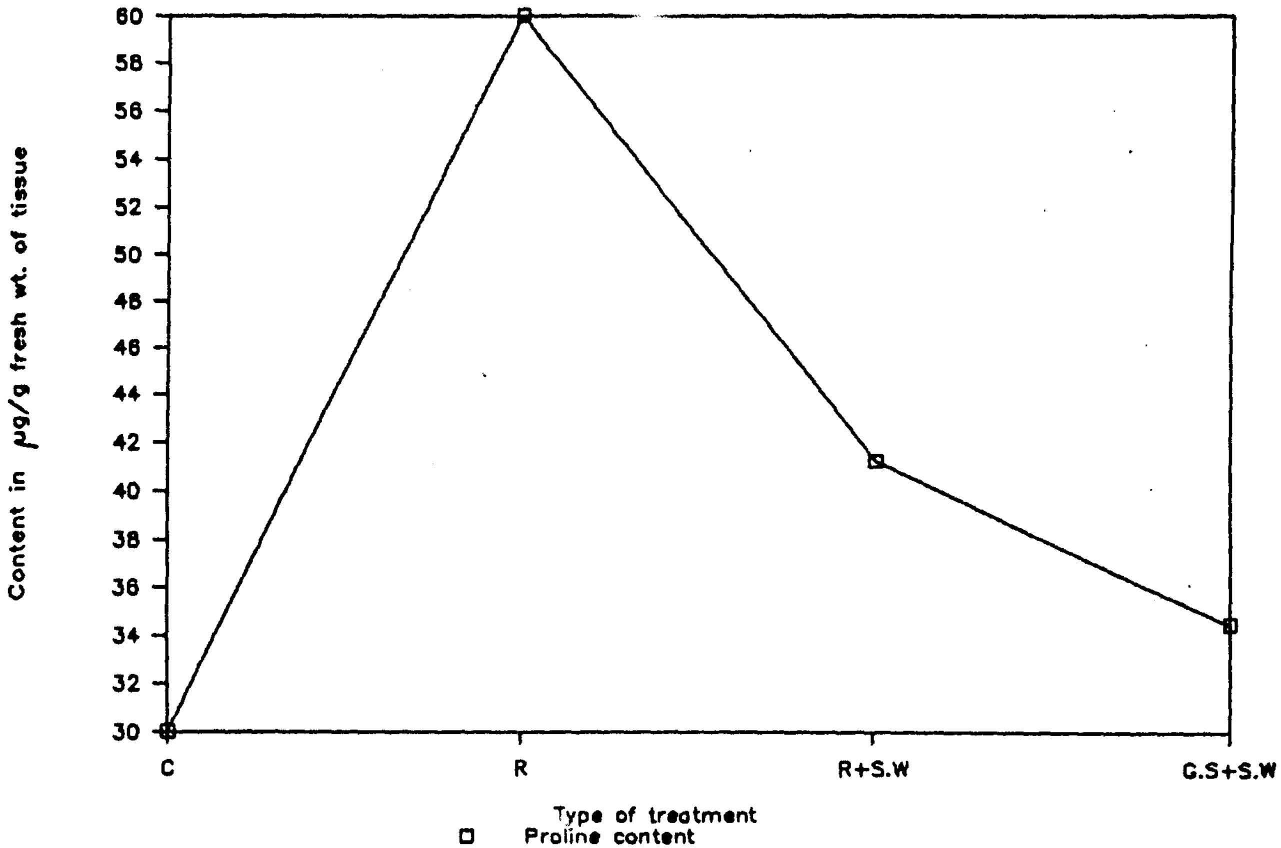


Fig. No. 3.5.2 : Effect of mining reject in the presence of seaweed on the Proline content in *Crotalaria juncea* L.

C - Control
R - Reject
S.W - Seaweed
G.S - Garden soil

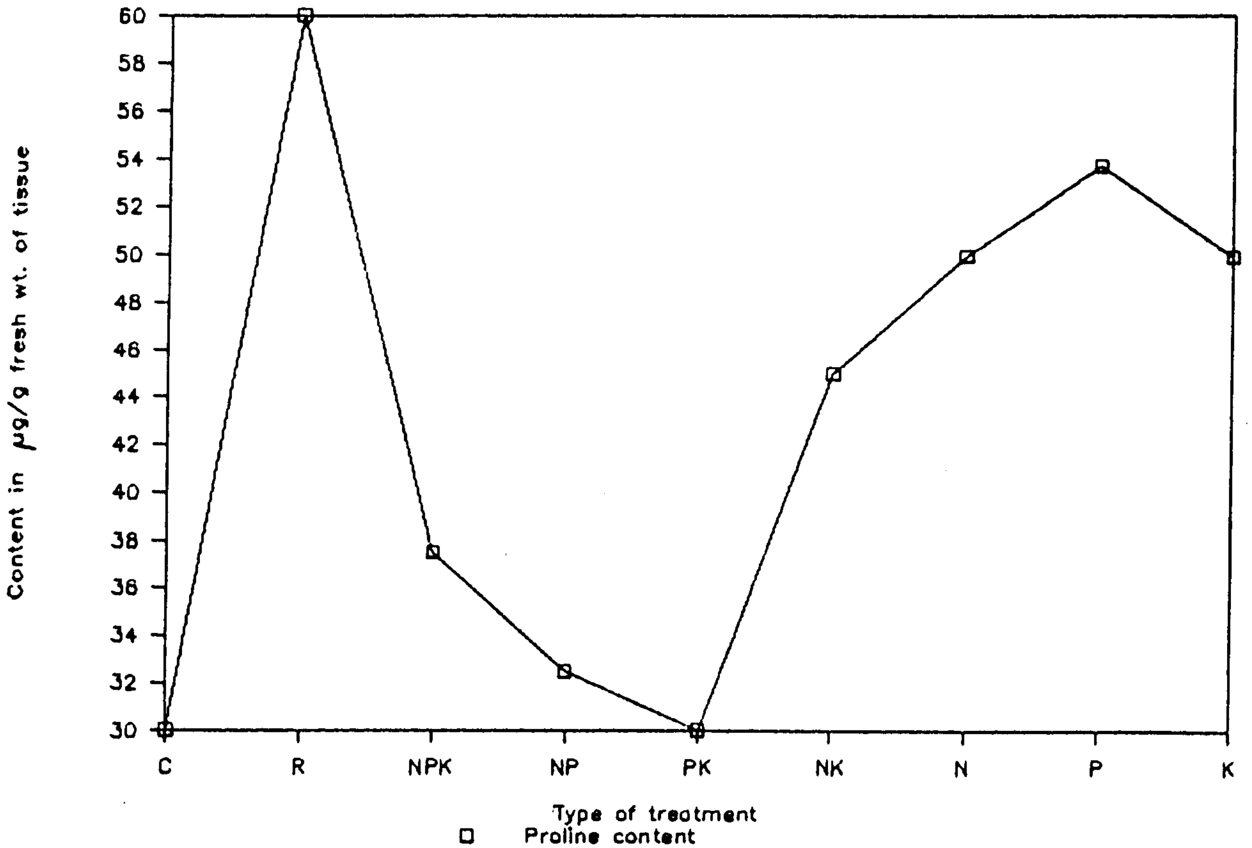


Fig. No. 3.5.3 : Effect of mining rejects with fertilizer treatments on the Proline content in <u>*Crotalaria juncea*</u> L.

C - Control R - Reject
NPK - Nitrogen, Phosphorus, Potassium
NP - Nitrogen, Phosphorus
PK - Phosphorus, Potassium
NK - Nitrogen, Potassium
N - Nitrogen
P - Phosphorus
K - Potassium

tissue (0% reject) to 60 µg/g fresh weight of tissue (100% reject). But with the addition of seaweed to the reject it decreased from 60 µg/g fresh weight of tissue (in reject) to 41.25 µg/g fresh weight of tissue (reject + seaweed) (Fig.3.5.2) and it ranged between 30 - 53.75 µg/g fresh weight of tissue in fertilizer treated plants (Fig 3.5.3).

DISCUSSION :

EFFECT OF MINE WASTES ON CHLOROPHYLL CONTENT :

From the soil analytical results, it was seen that the amount of aluminium, manganese and zinc increased with increasing amount of reject. Sideris and Young (1949) showed that in pineapple plants that excess manganese in the culture solution led to the decrease in chlorophyll content of the plants. Kelley as early as 1912 and Johnson in 1924 have shown that excess manganese at pH values above 5.5 deprived the availability of iron and thus failed to produce chlorophyll. Agarwala *et al*.(1964) have shown in their studies that excess manganese led to decrease in the amount of chlorophyll in barley leaves also. The results obtained here also confirm to the observations made by the different workers. White *et al*. (1974) hypothesized that manganese interfered with iron utilization in leaves for chlorophyll synthesis. Rosen *et al*. (1979) have shown in corn that increasing amount of zinc affected the chlorophyll content of the corn plants also. Sarkunan *et al*. (1984) have shown in rice plants that with increasing amount of aluminium level in the culture solution maintained at an acidic pH led to the decrease of chlorophyll content. The decrease in the chlorophyll development by heavy metals may be due to the interference with the synthesis of proteins, the structural component of chloroplasts (Nag *et al*. 1981). Treatment with heavy metals presumably block either the synthesis or the activity of the enzyme proteins reponsible for chlorophyll biogenesis. Experimental data on the reduction in the chlorophyll content with concurrent inhibition of photosynthesis by treatment with mercury, copper and zinc are available with unicellular alga chlorella (Braden and Winget, 1974).

The addition of seaweed and fertilizers to the reject might in some way be promoting the synthesis of chlorophyll by making available iron for the metabolic processes. It is known that by the addition of phosphate in the presence of excess aluminium, to a certain extent the toxicity of aluminium, is overcome (Fleming et al. 1974). Due to the reduction of toxicity, there is an increase in the chlorophyll content of the plants.

EFFECT OF MINE WASTES ON THE TOTAL PROTEIN AND TOTAL SUGAR CONTENT:

The soil analytical results showed that the amount of heavy metals like manganese and aluminium increased in the soil with increasing amount of reject. Sideris and Young (1949) have reported that excess manganese affected the protein content of the pineapple plants. Anderson and Worthington (1971) showed that by the application of manganese to the soil, there was tendency to decrease the seed protein content. Sarkunan et al. (1984) have shown in rice plants that with increasing concentration of aluminium, there was a decrease in the protein content of the plant. Hojjati and Maleki (1972) showed in wheat plants that with decreasing amount of nitrogen in the soil, there was a decrease in the protein content of the grain. Jellum et al. (1973) have shown in corn grain also that by decreasing content of the nitrogen, there is decrease in the protein content of grains. Novoa & Loomis (1981) in their review article on "Nitrogen and Plant Production" reported that with increasing amount of nitrogen availabiltiy, there is an increase in the protein content of the grains. Similar type of studies have been carried out to study the relationship between the nitrogen supply and protein content of the plants. The analysis of the soil showed that with increasing percentage of reject there is a decrease in the nitrogen content and other major nutrients. The addition of seaweed and fertilizers to the reject showed that the increased amount of available nutrients in the soil increased the amount of the total protein content. The results obtained here with reference to C. juncea revealed that the observations made here were similar to the results obtained by several earlier workers in different plants.

Sideris and Young as early as 1949 have shown that the manganese content influenced the sugar content of pineapple plants. They found that with high manganese content there is a decrease in the total sugar content in the leaves of the plant. Foy (1974) showed that aluminium decreased the sugar content of the leaves from several plants grown in acidic soils. Also Sarkunan et al. (1984) have reported in rice plants that one of the effects of aluminium toxicity was the reduction in the total sugar content in the leaves of the plants. The results showed that by the addition of seaweed and fertilizers to the reject, to a certain extent there is an increase in the sugar content of the plants, showing that to some extent the toxicity of manganese and aluminium are overcome.

EFFECT OF MINE WASTES ON THE PROLINE CONTENT :

Substantial quantities of the amino acid proline are known to accumulate under stress conditions. It is known that proline accumulates in plants grown in nuitrient deficient soils (Savitskaya, 1976). Ghildiyal *et al*. (1986) showed that under zinc deficiency, one of the essential micronutrients for the growth of plants, there is an accumulation of proline in the plants. The soil analysis showed that with icreasing percentage of reject, there is a decrease in the macronutrient content of the soil. Also proline is known to accumulate under water deficit conditions (Aspinall and Paleg, 1981). The physical analysis of the soil showed that with increasing percentage of reject, there is an increase in the clay content of the soil. It is well known that in the presence of high clay content, the availability of water to the plants decreases as the clay particles adhere to the water particles making it unavailable to the plants. The observations made here showed that with increasing percentage of reject, there is an increase in the proline content of the plants due to nutrient deficiency and the water stress. The addition of seaweed and fertilizers to the reject, there is decrease in the amount of proline content in the plants, thus showing that the availability of nutrients has reduced the stress on the plants.

CHAPTER IV

CHEMICAL ANALYSIS FOR INORGANIC METALS:

INTRODUCTION :

In the previous chapters it was seen how the mining rejects, having different heavy metals affected the growth and the biochemical composition of the plant. After having known how the plants respond to the rejects, a study was made to know the uptake of the different elements from the reject soil. Various authors have studied the translocation of different elements in the presence of various concentrations of aluminium (Andrew et al. 1973, Lee & Pritchard, 1984); translocation of manganese, iron, cobalt and zinc (Tiffin, 1967); the effect of zinc on iron uptake (Ambler et al. 1970) and the inter-relationship of aluminium and manganese on the uptake of different minerals (Rees & Sidrak, 1961).

In this chapter a study of the uptake of aluminium, iron, manganese, calcium, potassium and zinc were made on plants grown in different percentage composition of reject and with seaweed treatment.

MATERIALS & METHODS :

C. juncea plants grown in different percentage composition of reject and with seaweed treatment were taken up for chemical analysis after 60 days of growth. The plants were thoroughly washed in detergent to remove the dust sticking to it and then washed in distilled water. Leaf, stem and root were separated and dried in oven at 100°C constantly till there was no change in the dry weight. The dry material was powdered in a pulveriser.

The powdered material was taken up for the analysis of the different minerals. 100 mg of the dry powdered material was digested in a mixture of nitric acid : perchloric acid : sulphuric acid in the ratio of 5 : 1 : 0.5 ml according to the method given by Allen (1974). Thus obtained solution was analysed for iron, manganese, aluminium, magnesium, calcium, potassium and zinc by Perkin Elmer Atomic absorption spectra.

OBSERVATIONS :

The analysis of the plant material for inorganic metals are given in Tables 4.1 to 4.3. From soil analytical results, it was seen that with increasing percentage composition of reject there was an increase in the amount of iron, manganese, alumina, zinc and decrease in the amount of magnesium, calcium, and potassium in the soil. The analysis of leaf showed that with increasing percentage of reject there is gradual decrease in the uptake of iron from 6ppm/g dry weight in 0% reject to 2.5 ppm/g dry weight in 100% reject, magnesium from 36 ppm/g dry weight in 0% reject to 12 ppm/g dry weight in 100% reject and calcium from 510 ppm/g dry weight in 0% reject to 375 ppm/g dry weight in 100% reject. There was a gradual increase in the amount of manganese from 0.1 ppm/g dry weight in 0% reject to 5 ppm/g dry weight in 100% reject, aluminium from 6.5 ppm/g dry weight in 0% reject to 14.7 ppm/g dry weight in 100% reject, potassium from 83 ppm/g dry weight in 0% reject to 116 ppm/g dry weight in 100% reject and in zinc from 0.5 ppm/g dry weight in 0% reject to 1 ppm/g dry weight in 100% reject.

The detailed results of the analysis in different percentage composition of reject are given in Table 4.1.

Table No. 4.1 : Different elements in the leaf of the plants grown in different percentage of reject and addition of seaweed (in ppm/g dry wt. of tissue).

Sl. NO.	Percentage of reject	Fe	Mn	Al	Mg	Ca	K	Zn
1.	0%	6.0	0.1	6.5	36.0	501.0	83.0	0.5
2.	20%	5.3	0.1	7.2	35.0	479.0	88.0	0.5
3.	40%	4.8	1.0	8.1	29.0	470.0	90.0	0.6
4.	50%	4.1	1.0	9.5	24.0	430.0	97.0	0.8
5.	60%	3.6	1.0	11.6	21.0	418.0	105.0	0.8
6.	80%	3.0	1.0	13.5	17.0	402.0	110.0	0.9
7.	100%	2.5	5.0	14.7	12.0	375.0	116.0	1.0
8.	Reject + Seaweed	4.6	0.5	11.5	25.0	409.0	90.0	0.6
9.	Garden soil + Seaweed	5.8	0.1	6.7	28.0	495.0	85.0	0.6

Table 4.2 gives the elemental content in the stem. From the analysis it was observed that the trend was same as in the leaf. Here also there is a decrease in the magnesium from 23 ppm/g dry weight in 0% reject to 9 ppm/g dry weight in 100% reject and in the calcium content from 130 ppm/g dry weight in 0% reject to 92 ppm/g dry weight in 100% reject. There was an increase in the manganese (traces), aluminium (3.7 ppm/g dry weight), potassium (66 ppm/g dry weight) and zinc (0.4 ppm/g dry weight) in 0% reject plants to manganese (0.9 ppm/g dry weight), aluminium (8.7 ppm/g dry weight), potassium (109 ppm/g dry weight)and zinc (0.7 ppm/g dry weight) in plants growing in 100% reject. Iron was found in traces in all the plants.

With the addition of seaweed to the reject, it was seen that there was slightly more amount of iron, magnesium and calcium and less of aluminium, manganese, potassium and zinc in both leaf and stem (Tables 4.1 & 4.2) when compared to the plants grown in reject.

In the root it was seen that (Table 4.3) with increasing percentage of reject, there was an increase in the amount of iron, manganese, aluminium content and decrease in the amount of potassium, zinc, magnesium and

Table No. 4.2 : Different elements in the stem of the plants grown in different percentage of reject and addition of seaweed (in ppm/g dry wt. of tissue).

Sl. No.	Percentage of reject	Fe	Mn	Al	Mg	Ca	K	Zn
1.	0%	Traces	Traces	3.7	23.0	130.0	66.0	0.4
2.	20%	Traces	Traces	4.1	19.0	127.0	70.0	0.4
3.	40%	Traces	0.3	4.9	19.0	120.0	80.0	0.5
4.	50%	Traces	0.3	5.8	19.0	113.0	85.0	0.5
5.	60%	Traces	0.5	6.3	14.0	97.0	92.0	0.6
6.	80%	Traces	0.6	7.5	13.0	96.0	96.0	0.6
7.	100%	Traces	0.9	8.7	9.0	92.0	109.0	0.7
8.	Reject + Seaweed	Traces	0.3	5.7	16.0	96.0	95.0	0.5
9.	Garden soil + Seaweed	Traces	Traces	3.9	20.0	128.0	65.0	0.6

Table No. 4.3 : Different elements in the root of the plant grown in different percentage of reject and addition of seaweed (in ppm/g dry wt. of tissue).

Sl. No.	Percentage of reject	Fe	Mn	Al	Mg	Ca	K	Zn
1.	0%	39.0	0.6	35.4	15.0	56.0	86.0	0.21
2.	20%	40.0	0.6	41.4	13.0	53.0	76.0	0.15
3.	40%	40.0	0.7	43.5	13.0	50.0	70.0	0.14
4.	50%	43.0	0.8	48.1	12.0	49.0	66.0	0.13
5.	60%	53.0	0.8	51.3	10.0	47.0	61.0	0.13
6.	80%	55.0	0.9	58.7	9.0	43.0	59.0	0.10
7.	100%	69.0	2.0	65.9	7.0	40.0	53.0	0.09
8.	Reject + Seaweed	46.0	0.7	51.6	9.0	49.0	81.0	0.15
9.	Garden soil + Seaweed	35.0	0.5	33.7	14.0	56.0	87.0	0.19

calcium. Iron increased from 39 ppm/g dry weight in 0% reject to 69 ppm/g dry weight in 100% reject; manganese from 0.6 ppm/g dry weight in 0% reject to 2 ppm/g dry weight in 100% reject and alumnium from 35.4 ppm/g dry weight in 0% reject to 65.9 ppm/g dry weight in 100% reject. Potassium decreased from 86 ppm/g dry weight in 0% reject to 53 ppm/g dry weight in 100% reject, zinc from 2.1 ppm/g dry weight in 0% reject to 0.09 ppm/g dry weight in 100% reject, magnesium from 15 ppm/g dry weight in 0% reject to 7 ppm/g dry weight in 100% reject and calcium from 56 ppm/g dry weight in 0% reject to 40 ppm/g dry weight in 100% reject. With the addition of seaweed it was found that there was less of iron, manganese and aluminium with more of magnesium, calcium, potassium, and zinc than the plants grown in only reject.

DISCUSSION :

Lee & Pritchard (1984) have shown in their experiment on Trifolium repens that with increasing amount of aluminium in the culture media, there is a decrease in the amount of iron, magnesium and calcium in the tops of the plant and an increase in the potassium content. Rees & Sidrak (1961) have shown in spinach and barley that with the addition of both aluminium and manganese in the form of sulphates, there is a decrease in the amount of iron, calcium and magnesium with an increase in the potassium content. Also the amount of aluminium and manganese increased with increasing levels of them in the culture solution. Lingle et al. (1963) have shown that manganese became effective as an interfering ion when the concentration of this element exceeded 2 x 10^{-6} M, for the uptake of iron in soybean plants. Foy & Brown (1963) have shown in cotton plants that aluminium at pH 5.0 affected the uptake of calcium in the tops and roots, with increasing aluminium there was a decrease in calcium content. Johnson & Jackson (1964) have shown in wheat seedlings that the uptake and absorption of calcium was inhibited by high aluminium content in the culture solution. Vlamis & Williams (1964) have shown in rice and barley plants that with increasing concentration of manganese in the culture solution, there was a decrease in the iron levels in the leaves of the plants. Lee (1971 b) has shown in potato plants that with increasing amount of aluminium in the culture solution, there was an increase in the aluminium content in both tops and roots, iron content decreased with increasing concentration of aluminium in the tops and increased in the roots. Also it was observed by him that the calcium content decreased in both tops and roots with increased concentration of aluminium. Singh & Sharma (1972) have shown in potato plants that iron content is low in the leaves and more in the roots of calcium deficient plants when compared to control plants. Bowen (1972) has shown in sudan grass that with increasing concentration of manganese in the culture solution, there is an increase in the manganese content in the tops of the plant. Andrew et al. (1973) have also shown in six tropical legumes that with increasing amount of aluminium there is a decrease in the amount of magnesium and calcium and an increase in the potassium content in the tops of the plants except in the case of **Medicago sativa** where there was a decrease in the potassium content. The results obtained here were similar to the observations made by other workers in different plants. Cumbus et al. (1977) have shown in watercress, that increasing levels of manganese in the culture medium reduced the total translocation of iron. They showed that with increasing amount of manganese in the culture solution, there was an increase in the amount of manganese in the leaves. Tiffin (1967) has shown in tomato that with increasing amount of manganese in the nutrient solution, the uptake of iron was reduced in the exudate of the plant.

Andrew *et al.* (1973) have shown that in ***Medicago sativa*** there was a decrease in the potassium content of the root at a higher level of aluminium. Lee (1972) has shown in his experiments that increasing amount of aluminium in culture solution, there was an increase in the amount of iron in the roots. Also it was shown in the experiment that there was a decrease in the amount of calcium and magnesium in the roots of plants with increasing levels of aluminium and manganese in the culture solution.

The relatively low concentrations of aluminium in the plant tops compared with those in the roots is well documented (Clarkson, 1967; MacLeod & Jackson, 1967). The dominant effects of aluminium on the uptake of cations was to decrease the uptake of calcium. Many investigators have reported similar results (Munns, 1965 ; MacLeod & Jackson, 1965 & 1967).

The data on the major elements presented in Tables 4.1 & 4.2 indicate that the reduction in calcium and magnesium concentrations in the plant leaf and stem was balanced approximately by reciprocal increase in potassium with increasing percentage of reject. MacLeod & Jackson

(1965) and Andrew *et al.* (1973) suggested that the increased uptake of potassium might be attributable to high levels of aluminium, which may not be direct effect but brought about by the capacity of the plant to preserve cationic balance (van Itallie, 1938 & 1948). The effect of aluminium was mainly on cation absorption, rather than trasnlocation. Whereas Tiffin (1967) showed that manganese affected the uptake of iron in soybean plants. The results obtained with respect to *C. juncea* confirm to the observations made by other workers on the uptake of elements in different taxa.

CHAPTER V

RESPONSE OF SIX TREE SPECIES PLANTED ON THE MINE WASTE DUMP :

INTRODUCTION

In the previous chapters the different responses of Crotalaria juncea L. was studied in nursery with different percentage composition of reject, with seaweed and fertilizer treatements. In this chapter studies on the reponse of six tree species used for the purpose of revegetation on the dumps was carried out. The plant species studied were Acacia nilotica (Linn.) Delile; Azadirachta indica A.Juss;Bombax ceiba Linn.; Pithecellobium dulce Benth; Parkia biglandulosa Wight & Arn. and Tamarindus indica Linn.

For successfully revegetating the dump it is of utmost importance to know the response of plants planted to the conditions, on the site and which plants are suitable for revegetation. The above mentioned plants were chosen for study because of their sturdy and drought resistant nature. Of the above plant species selected, Acacia nilotica, Bombax ceiba & Tamarindus indica are the local tree species and the remaining three are introduced to this region.

Before carrying out the studies on the plants, the analysis of dump soil was carried out for different characteristics and also of garden soil in which plants as control were grown. The plants were monitored regularly for their growth after plantation for a period of one year. The different morphological characters studied were height of the plant, thickness of stem and leaf, size of leaf and leaf index and size of the guard cell and stomatal pore. Chlorophyll and proline analysis of the plant material was carried out. Also chemical analysis for iron, manganese, aluminium, magnesium, calcium and potassium was carried out from dry leaf and root to know their uptake by the plant.

MATERIALS AND METHOD :

Garden soil was prepared as mentioned in the first chapter. Reject soil sample was collected from various points on the dumps, bulked together and then final sample prepared for analysis. Analysis of the soil was carried out according to standard methods as mentioned in the earlier chapter.

Seeds of *Acacia nilotica*, *Bombax ceiba* and *Tamrindus indica* were collected from the trees during their flowering and fruiting period, brought to the nursery and dried thoroughly. *Parkia biglandulosa* seeds were collected from the campus of Shivaji University, Kolhapur and Karnatak University, Dharwad. <u>Azadirachta indica</u> seeds were also collected from the campus of Karnatak University, Dharwad. Seeds of <u>Pithecellobium dulce</u> were collected from a tree growing in a park in Panjim.

The seeds were presoaked overnight before sowing them in beds. After germination they were transplanted into small bags before being planted on the dumps. At the onset of monsoons, the saplings were transplanted on the dumps and in the nursery as control plants in the big bags of the size 24" x 12" filled with garden soil. At the time of plantation the age of the different saplings varied from 15 days to 2 months. The different morphological parameters were monitored regularly. Analysis of chlorophyll and proline was carried out after a period of one year growth. Chlorophyll was analysed according to the method of Arnon (1949) and proline according to the method of Bates <u>et al</u>. (1973).

The plants were studied for a period of one year from 25.6.1987 to 24.6.1988. Chemical analysis for iron, manganese, aluminium, calcium, magnesium and potassium from the leaf and root were carried out after a period of one year growth of the plants. The elements were analysed by Atomic Absorption Spectra and the method followed for extraction was accroding to Allen (1973).

OBSERVATIONS :

SOIL ANALYSIS :

Physical analysis of the garden and dump soil shown in Table 5.1 reveal the particle size of them. From the table it can be observed that the garden soil is mostly made up of gravel and sand with little of clay and silt, whereas in the case of the dump soil, it can be seen that there is less amount of gravel with more of sand, along with silt and clay.

The angle of the slope was found to be between 30^{o}-40^{o}. Chemical analysis of the soil revealed that both the soils were acidic in nature having a pH value of 5.5 (Table 5.2). Concentrations of the major plant nutrients and heavy metals in the soil reflect the absence of organic matter with severe deficiencies in nitrogen, phosphorus and

Table No.5.1: Particle size analysis of the garden soil and dump soil.

Textural Class	% amount present	
	Garden soil	Dump reject
Gravel (<2 mm)	60.33	35.30
Sand (2 - 0.02 mm)	33.84	36.93
Silt + Clay (>0.02 mm)	05.83	27.77

potassium with high levels of iron (46%) and manganese in the dump reject than in the garden soil. The amount of organic carbon was less in the dump reject, than in garden soil. The dump reject had organic matter content of 1.38% which was poor compared to the garden soil having 4.15%. This low content of organic matter was poor to support vegetation on the dump reject. There was deficiency of other plant nutrients like calcium, zinc and magnesium in the dump reject.

Table No. 5.2 : Chemical characteristics of the garden soil and mine waste dump.

Sl. No.	Characteristic	Garden soil	Dump reject
1.	pH value	5.5	5.5
2.	Loss on ignition	15.5%	6.23%
3.	Organic carbon	2.40%	0.80%
4.	Iron	20.80%	46.00%
5.	Alumina	15.52%	7.13%
6.	Manganese	0.20%	0.78%
7.	Copper in ppm	6.0	4.0
8.	Zinc in ppm	7.6	18.6
9.	Nickel in ppm	14.0	10.0
10.	Lead	Traces	Traces
11.	Calcium	0.15%	0.08%
12.	Magnesium	0.07%	0.01%
13.	Available nitrogen in Kg/ha.	51.52	15.68
14.	Available phosphorus in Kg/ha.	>448	33.60
15.	Available potassium in Kg/ha.	53.76	2.24
16.	Silica	38.17%	34.17%

PLANT RESPONSES :

1) *Acacia nilotica* (Linn.) Delile :

The different comparative morphological and chemical responses of the plant are given in Table 5.3. From Fig. 6.1 it can be observed that the plants growing in garden soil have a better growth rate than the ones growing on dumps during one year period they were studied from the time of transplantation. The plants growing in garden soil were 146.5 cm in height when compared to the plants growing on dump were 140.1 cm in height. The stem (1.01cm) and leaf (0.16mm) were thicker in the plants growing on dumps when compared to the plants growing in garden soil where the stem was 0.88 cm in thick and the leaf was 0.14 mm thick. The leaf size showed that the plants growing on dumps were smaller (1.03cm x 0.48cm) when compared to garden plants (2.0cm x 0.95cm). The length of the root in the plants grown in garden soil was 25.6 cm whereas in the plants grown on dumps it was 19.5 cm. The dry weight of the root per plant was also less in dump plants (2.3 g) when compared to control plants (3.5 g).

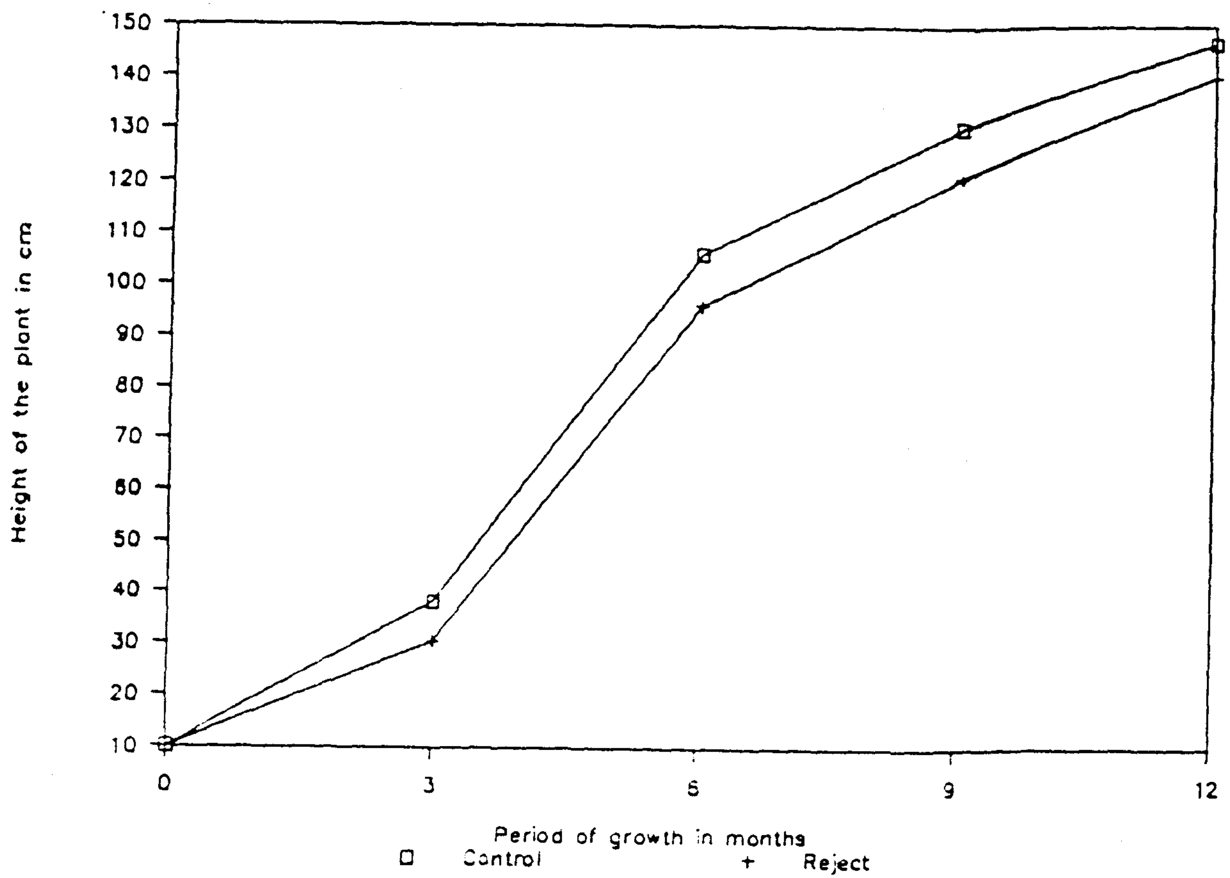


Fig. No. 5.1.1: Growth response of <u>Acacia nilotica</u> (Linn.) Delile to the mine waste.

Table No. 5.3 : Response of <u>Acacia nilotica</u> to garden soil and dump reject.

Sl. No.	Response		Garden soil	Dump reject
1.	Height of the plant in cm		146.5	140.1**
		S.D	4.1	2.5
2.	Thickness of stem in cm		0.88	1.01**
		S.D	0.03	0.03
3.	Size of leaf in cm	L	2.00	1.03**
		S.D	0.04	0.03
		B	0.95	0.48**
		S.D	0.04	0.02
4.	Leaf index L/B		2.5	2.18
5.	Thickness of leaf in mm		0.14	0.16**
		S.D	0.003	0.003
6.	Length of root system in cm		25.6	19.5
7.	Dry weight of root per plant in g		3.5	2.3
8.	Total chlorophyll in mg per 100g fresh weight of leaves		196.08	166.9
9.	Chlorophyll a in mg per 100g fresh weight of leaves		60.64	56.28
10.	Chlorophyll b in mg per 100g fresh weight of leaves		135.42	110.70
11.	Proline content in μg per g fresh weight of tissue		52.5	75.0

Note: values significant at ** $P < 0.01$
according to student's "t" test.

Analysis of chlorophyll showed that the amount decreased in plants grown on reject dumps (166.9 mg/100g fresh weight of leaf) whereas in the case of control plants it was 196.08 mg/100 g fresh weight of leaf. There was a decrease in both chlorophyll a & b content also. It was observed that the proline content increased in the plants growing on dumps (75 µg/g fresh weight of tissue) whereas in control plants it was 52.5 µg/g fresh weight of tissue.

The chemical analysis of leaf and root for inorganic metals are given in Table 5.4. It was observed that there is accumulation of all the three major elements i.e. iron, manganese and aluminium and also potassium in the leaves of the plants growing on the dumps whereas there was a decrease in the amount of calcium and magnesium when compared to control plants. In the case of root, iron and manganese content was more or less same both in control plants and plants growing on dumps whereas aluminium was more in the plants growing on dumps. Calcium, magnesium and potassium content was more in the roots of control plants when compared to the plants growing on dumps.

Table No. 5.4 : Different elements in the leaf and root of <u>Acacia nilotica</u> grown in garden soil and on dump (in ppm/g dry wt. of tissue).

Sl. No.	Type of plant material		Fe	Mn	Al	Ca	Mg	K
1.	Leaf	Garden soil	10.0	05.0	42.0	199.0	13.0	119.0
		Dump reject	13.0	08.0	59.3	69.0	06.0	135.0
2.	Root	Garden soil	18.0	01.0	18.2	158.0	09.0	138.0
		Dump reject	17.0	00.8	31.1	145.0	05.0	64.0

2) <u>Azadirachta</u> <u>indica</u> A. Juss. :

It was observed that the plants growing in garden soil had a better growth rate when compared to the plants growing on dumps (Fig.6.2). The control plants were taller (98.2cm) than the plants growing on dumps (85.5cm) after a period of one year growth. Stem and leaf were both thicker in the plants growing on dumps than the control plants (Table 5.5). The leaf size was almost same in both the control plants as well as plants growing on dumps. Epidermal peeling of the leaf showed that there was a decrease in the size of the guard cell and the stomatal aperture in the plants grown on dumps when compared to the control plants. The root showed a stunted growth with shorter length (20.6 cm) and decrease in dry weight of root per plant (2.8 g) in the plants growing on dumps, whereas the root length in control plants was 26.3 cm and the dry weight of root per plant was 3.5 g.

The chlorophyll analysis showed that the control plants had more chlorophyll (170.64 mg/100 g fresh weight of leaves) than the plants grown on dumps (82.26 mg/100 g fresh weight of leaves). There was a decrease in both chlorophyll

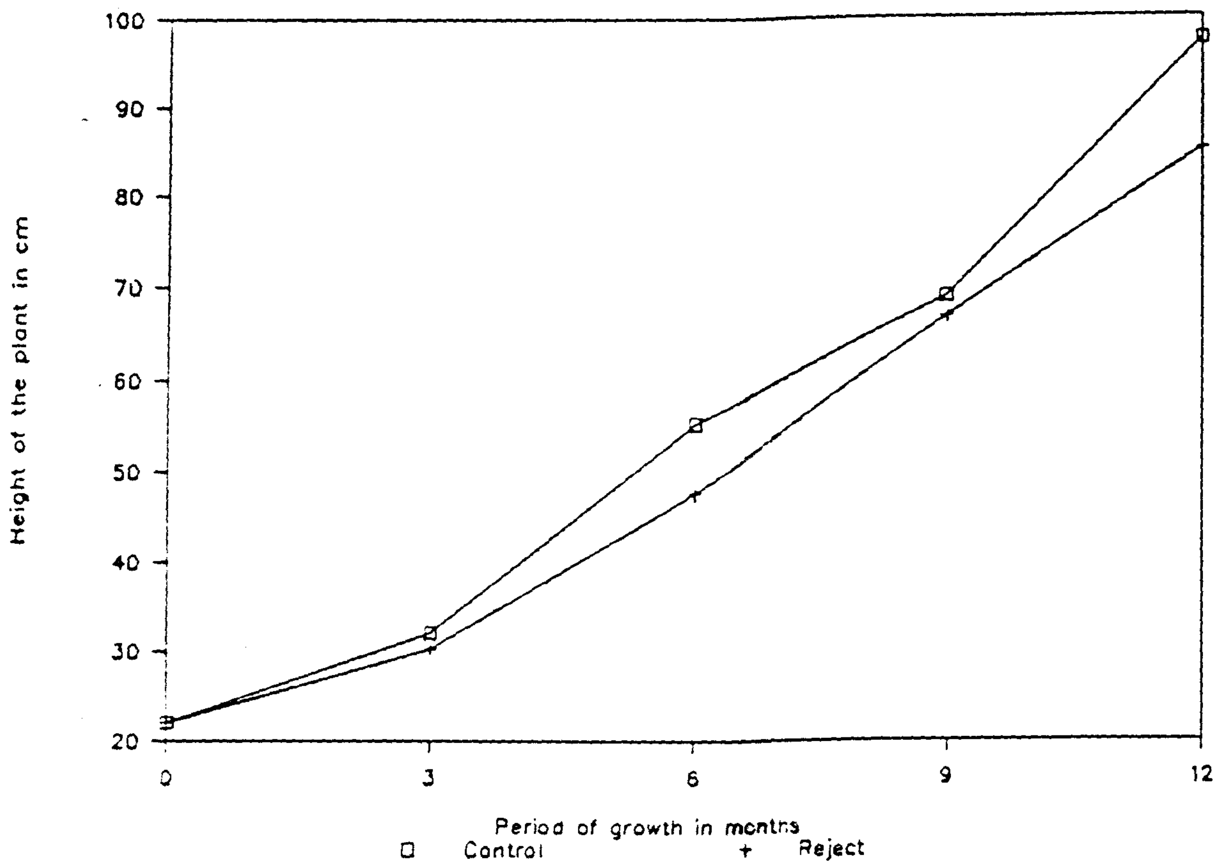


Fig. No. 5.1.2: Growth response of *Azadirachta indica* A.Juss. to the mine waste.

Table No. 5.5 : Response of **Azadirachta indica** to garden soil & dump reject.

Sl. No.	Response		Garden soil	Dump reject
1.	Height of the plant in cm		98.2	85.5**
		S.D	2.8	3.6
2.	Thickness of stem in cm		0.48	0.57**
		S.D	0.03	0.02
3.	Size of the leaf in cm	L	4.77	4.74
		S.D	0.05	0.14
		B	1.80	1.81
		S.D	0.07	0.06
4.	Leaf index L/B		2.68	2.65
5.	Thickness of leaf in mm		0.16	0.19**
		S.D	0.003	0.003
6.	Size of the guard cell in µm	L	25.2	18.72
		B	16.56	16.20
7.	Size of the stomatal aperture in µm	L	12.60	8.64
		B	5.76	5.76
8.	Length of the root system in cm		26.3	20.6
9.	Dry weight of the root per plant in g		3.5	2.8
10.	Total chlorophyll in mg/100g fresh weight of leaf		170.64	82.26
11.	Chlorophyll a in mg/100g fresh weight of leaf		51.63	26.22
12.	Chlorophyll b in mg/100g fresh weight of leaf		118.98	56.04
13.	Proline content in µg/g fresh weight of tissue		167.5	168.5

Note: values significant at ** $P < 0.01$ according to student's "t" test.

Table No. 5.6 : Different elements in the leaf and root of **Azadirachta indica** grown in garden soil and on dumps (in ppm/g dry wt. of tissue)

Sl. No.	Type of plant material		Fe	Mn	Al	Ca	Mg	K
1.	Leaf	Garden soil	09.0	01.0	44.9	548.0	21.0	68.0
		Dump reject	14.0	02.0	67.3	484.0	07.0	90.0
2.	Root	Garden soil	29.0	02.0	21.7	168.0	09.0	71.0
		Dump reject	09.0	01.8	28.0	124.0	07.0	22.0

a & b content in the plants grown on dumps when compared to control plants. Analysis of proline content showed that it was almost same in both the plants.

Chemical analysis of the leaf and root for inorganic metals are given in Table 5.6. It was observed that iron, manganese, aluminium and potassium was more in the leaves of the plants grown on dumps and less of calcium and magnesium when compared to the control plants. In the case of roots, iron was more and aluminium less in the control plants than the plants growing on dumps whereas manganese content was more or less same. Calcium, magnesium and potassium content were more in the roots of the control plants than the plants growing on dumps.

3) *Bombax ceiba* Linn. :

It was observed that the plants growing on dumps were shorter (59.1 cm) than the control plants (68.8 cm) which had a better growth rate (Fig.6.3) afer a period of one year growth. The stem was thicker in the plants growing on dumps (1.8 cm) compared to the control plants (1.16 cm). The leaf was larger in the plants growing on the dumps

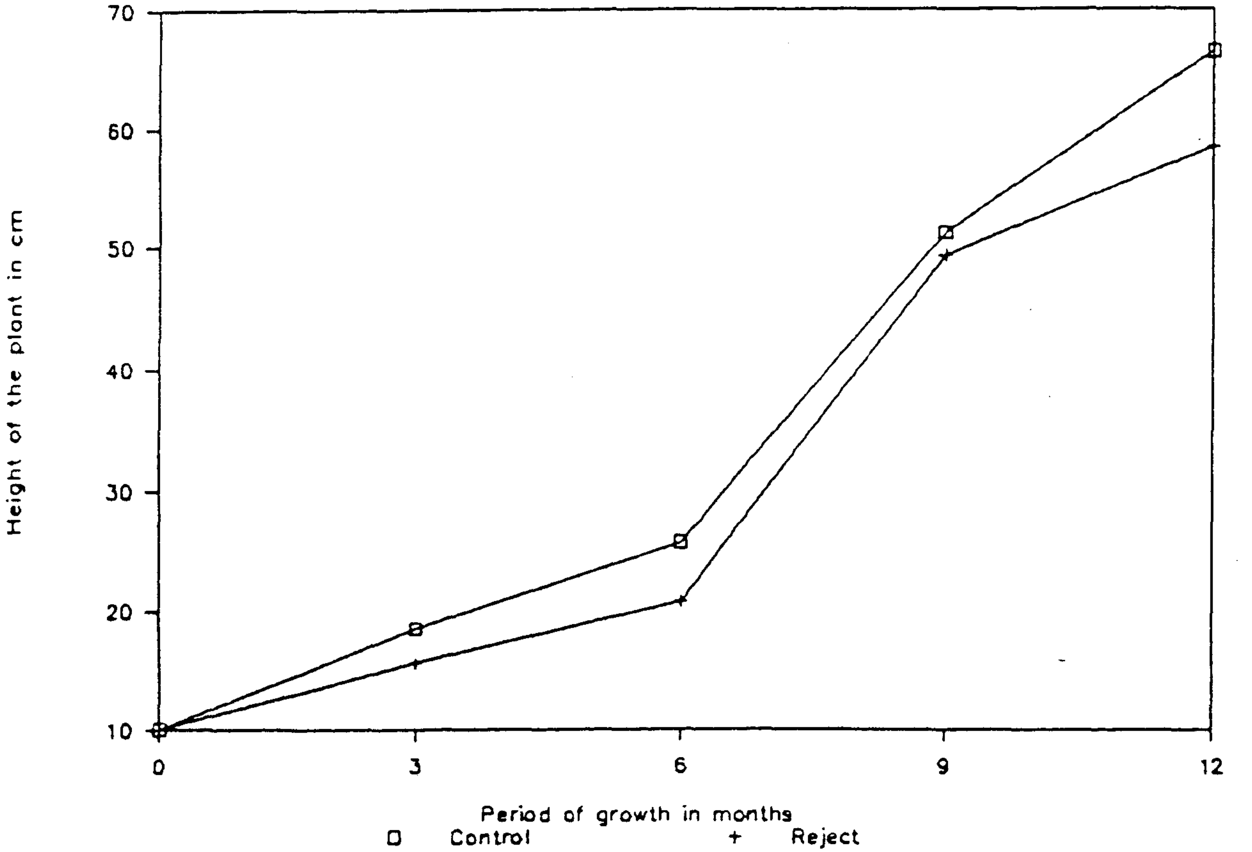


Fig. No. 5.1.3: Growth response of *Bombax ceiba* Linn. to the mine waste.

Table No. 5.7 : Respnse of Bombax ceiba to garden soil & dump reject.

Sl. No.	Response		Garden soil	Dump reject
1.	Height of the plant in cm		68.80	59.10**
		S.D	3.70	3.50
2.	Thickness of stem in cm		1.16	1.80**
		S.D	0.06	0.05
3.	Size of the leaf in cm	L	9.81	10.54**
		S.D	0.12	0.08
		B	1.78	3.28**
		S.D	0.08	0.04
4.	Leaf index L/B		5.65	3.22
5.	Thickness of leaf in mm		0.20	0.20
		S.D	0.004	0.004
6.	Size of the guard cell in µm	L	27.72	23.04
		B	19.08	12.96
7.	Size of the stomatal aperture in µm	L	15.84	13.32
		B	4.68	4.68
8.	Length of the root system in cm		18.6	18.3
9.	Dry weight of root per plant in g		2.1	2.2
10.	Total chlorophyll in mg/100g fresh weight of leaf		147.66	99.24
11.	Chlorophyll a in mg/100g fresh weight of leaf		46.43	32.22
12.	Chlorophyll b in mg/100g fresh weight of leaf		101.22	67.02
13.	Proline content in µg/g fresh weight of tissue		41.25	45.00

Note: values significant at ** $P < 0.01$ according to student's "t" test.

(10.54 cm x 3.28 cm) than the control plants (9.81 cm x 1.78 cm), whereas the thickness of leaf was almost same in both. There was not much of a change in the length and dry weight of root per plant.

The chlorophyll analysis showed that the total chlorophyll content was less in the dump plants (99.24 mg/100 g fresh weight of leaf) when compared to the control plants (147.66 mg/100 g fresh weight of leaf). There was a decrease in both chlorophyll a and chlorophyll b content also. There was very little change in the proline content of the plants grown both on the dumps and in the garden soil. The comparative morphological and chemical responses are given in Table 5.7.

The chemical analysis of leaf and root for inorganic metals are given in Table 5.8. It was observed that iron, manganese, aluminium and potassium content was more in the leaves of plants growing on dumps and less of calcium and magnesium when compared to the control plants. In the case of roots, it was observed that iron, potassium, calcium and magnesium content was more & aluminium less in the control plants when compared to the plants growing on

Table No. 5.8 : Different elements in the leaf and root of <u>Bombax</u> <u>ceiba</u> grown in garden soil and on dumps (in ppm/g dry wt. of tissue)

Sl. No.	Type of plant material		Fe	Mn	Al	Ca	Mg	K
1.	Leaf	Garden soil	06.0	02.0	51.4	348.0	29.0	88.0
		Dump reject	09.0	04.0	57.3	266.0	23.0	132.0
2.	Root	Garden soil	21.0	02.0	23.2	186.0	15.0	126.0
		Dump reject	06.0	01.8	35.8	179.0	07.0	83.0

dumps. Whereas manganese content was almost same in both the plants.

4) *Parkia biglandulosa* Wight & Arn. :

The comparative morphological and chemical responses of the plants grown on dumps and in garden soil are given in Table 5.9. The plants growing in garden soil had a better growth and were taller (136.5 cm) than the plants growing on the dumps (110.1 cm) (Fig. 6.4). The stem (1.57cm) and leaf (0.17 mm) were thicker in the plants growing on dumps when compared to the control plants where stem was 1.07cm thick and leaf was 0.11 mm thick. The leaves of the plants growing on dumps were larger (13.6 cm x 1.7 cm) than the control plants (9.84 cm x 1.3 cm). The root was shorter (18.6 cm) and dry weight of root per plant (2.6 g) was less when compared to the control plants where the length was 26.4 cm and the dry weight of root per plant was 3.1 g.

The chlorophyll content decreased in the plants growing on dumps (189.96 mg/100 g fresh weight of leaf) when compared to the control plants (221.46 mg/100 g fresh weight

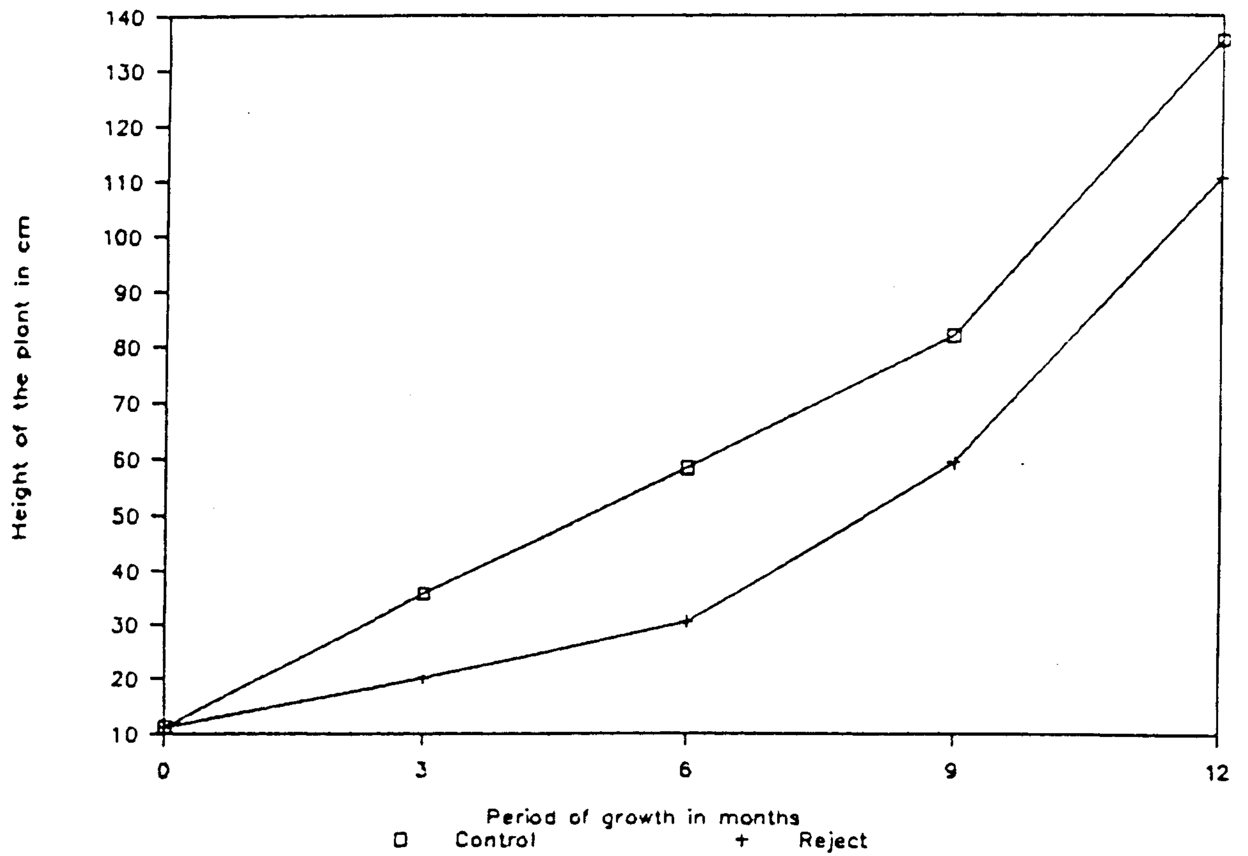


Fig. No.5.1.4: Growth response of Parkia biqladulosa Wight & Arn. to the mine waste.

Table No. 5.9 : Response of *Parkia biglandulosa* to garden soil & dump reject.

Sl. No.	Response		Garden soil	Dump reject
1.	Height of the plant in cm		136.5	110.1**
		S.D	4.8	5.6
2.	Thickness of stem in cm		1.07	1.57**
		S.D	0.04	0.04
3.	Size of the leaf in cm	L	9.84	13.6**
		S.D	0.18	0.43
		B	1.3	1.7**
		S.D	0.03	0.07
4.	Leaf index L/B		7.76	8.15
5.	Thickness of leaf in cm		0.11	0.17**
		S.D	0.002	0.003
6.	Length of root system in cm		26.4	18.6
7.	Dry weight of root per plant in g		3.1	2.6
8.	Total chlorophyll in mg/100g fresh weight of leaf		221.46	189.96
9.	Chlorophyll a in mg/100g fresh weight of leaf		69.66	61.44
10.	Chlorophyll b in mg/100g fresh weight of leaf		151.80	128.52
11.	Proline content in $\mu g/g$ fresh weight of tissue		41.25	41.25

Note: values significant at ** $P < 0.01$ according to student's "t" test.

Table No. 5.10 : Different elements in the leaf and root of <u>Parkia biglandulosa</u> grown in garden soil and on dumps (in ppm/g dry wt. of tissue)

Sl. NO.	Type of plant material		Fe	Mn	Al	Ca	Mg	K
1.	Leaf	Garden soil	15.0	01.0	49.5	164.0	22.0	46.0
		Dump reject	17.0	02.0	51.7	108.0	12.0	65.0
2.	Root	Garden soil	19.0	00.8	25.3	142.0	11.0	95.0
		Dump reject	20.0	01.0	33.9	108.0	09.0	35.0

of leaf). Proline content in both the control and as well as plants growing on reject was 41.25 µg/g fresh weight of tissue.

The chemical analysis of leaf and root for inorganic metals are given in Table 5.10. In leaf, the amount of iron, manganese, aluminium and potassium content was more with less of calcium and magnesium in the plants growing on dumps when compared to the control plants. In the case of roots, iron and manganese content was more or less same in both control as well as plants growing on dumps with less of aluminium and calcium, magnesium and potassium being more in the control plants.

5) <u>Pithecellobium</u> <u>dulce</u> Benth.:

The comparitive morphological and chemical responses are given in Table 5.11. The plants growing in garden soil had better growth and were taller (136.5 cm) when compared to the plants growing on dumps (125.6 cm) after a period of one year (Fig 6.5). The thickness of the stem was almost same in both the cases, whereas leaf (0.2mm) was thicker in dump plants than the control plants (0.15mm).

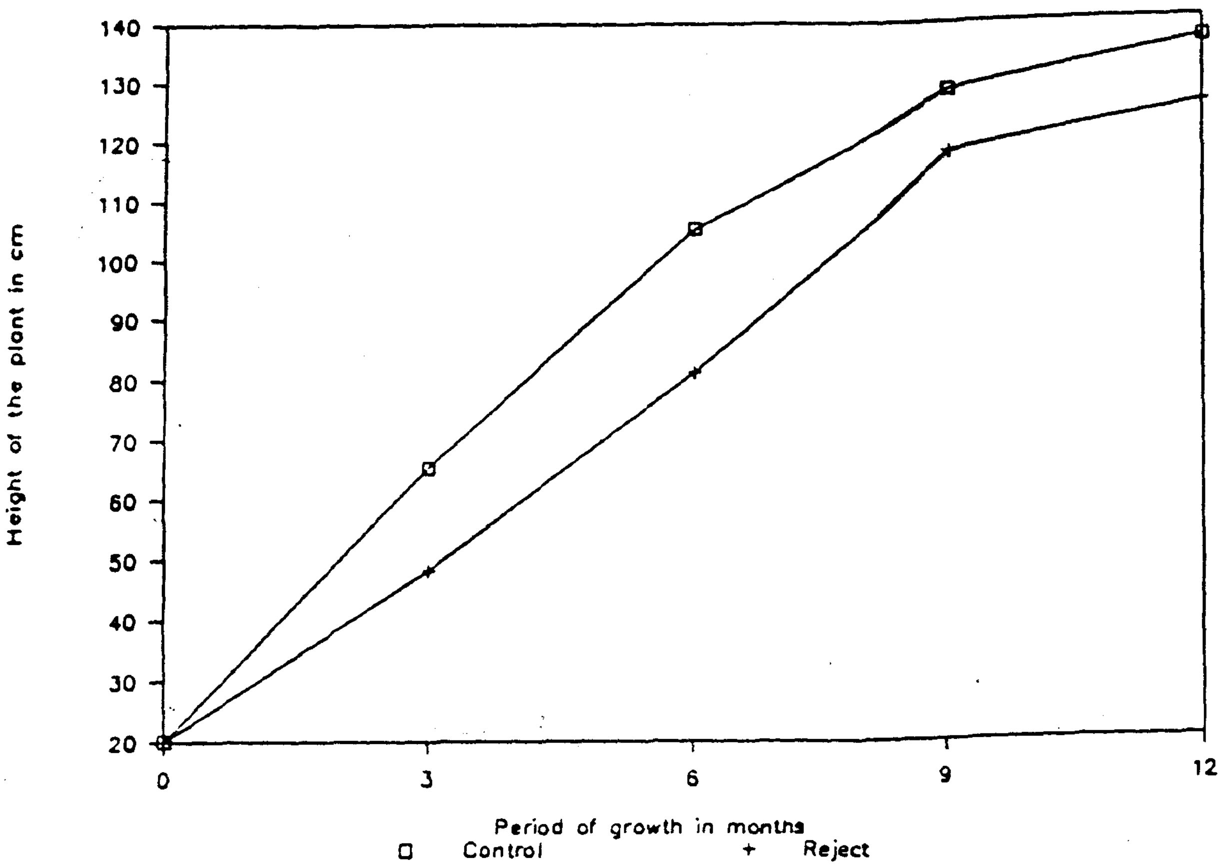


Fig. No. 5.1.5: Growth response of *Pithecellobium dulce* Benth. to the mine waste.

Table No. 5.11 : Response of <u>Pithecellobium</u> <u>dulce</u> to garden soil & dump reject.

Sl. No.	Response		Garden soil	Dump reject
1.	Height of the plant in cm		136.5	125.6**
		S.D	4.6	4.2
2.	Thickness of stem in cm		1.02	1.03*
		S.D	0.03	0.04
3.	Size of leaf in cm	L	2.77	2.06**
		S.D	0.07	0.05
		B	1.06	0.71**
		S.D	0.03	0.05
4.	Leaf index L/B		2.64	3.09
5.	Thickness of leaf in mm		0.15	0.20**
		S.D	0.003	0.002
6.	Size of guard cell in μm	L	23.04	23.4
		B	13.32	14.4
7.	Size of the stomatal aperture in μm	L	15.84	16.2
		B	3.6	3.6
8.	Length of the root system in cm		27.4	21.5
9.	Dry weight of root per plant in g		3.2	2.4
10.	Total chlorophyll in mg/100g fresh weight of leaf		158.46	166.98
11.	Chlorophyll a in mg/100g fresh weight of leaf		53.22	56.28
12.	Chlorophyll b in mg/100g fresh weight of leaf		105.24	110.70
13.	Proline content in μg/g fresh weight of tissue		120.00	167.50

Note: values significant at ** $P < 0.01$, * $P < 0.05$ according to student's "t" test.

The leaf was larger in the control plants (2.77 cm x 1.06 cm) when compared to the plants growing on dumps (2.06 cm x 0.71 cm). The size of the guard cell and stomatal aperture was almost same in both the plants. The root was longer (27.4 cm) and dry weight per root was more (3.2 g) in control plants when compared to the plants growing on dumps where the length was 21.5 cm and dry weight was 2.4 g.

Chlorophyll analysis of the leaf showed that chlorophyll content was slightly more in dump plants (166.98 mg/100 g fresh weight of leaf) when compared to control plants (158.46 mg/100 g fresh weight of leaf). Also proline was more in the dump plants (167.5 μg/g fresh weight of tissue) when compared to control plants (120 μg/g fresh weight of tissue).

Chemical analysis for inorganic metals in leaf and root are given in Table 5.12. It was observed that there is accumulation of iron, manganese, aluminium and potassium in the leaves of plants growing on the dumps when compared to the control plants. Whereas there was a decrease in the amount of calcium and magnesium in the plants grown on dumps. In the case of root there was less of aluminium &

Table No. 5.12 : Different elements in the leaf and root of **Pithecellobium dulce** grown in garden soil and on dump (in ppm/g dry wt. of tissue).

Sl. No.	Type of plant material		Fe	Mn	Al	Ca	Mg	K
1.	Leaf	Garden soil	05.0	03.0	56.2	255.0	25.0	54.0
		Dump reject	13.0	17.0	61.5	162.0	16.0	68.0
2.	Root	Garden soil	10.0	02.9	19.8	119.0	08.0	73.0
		Dump reject	09.8	03.0	34.8	91.0	06.5	34.0

more of calcium, magnesium and potassium in the control plants whereas iron and manganese content was more or less same in both control plants as well as in plants grown on dumps.

6) **Tamarindus indica Linn.** :

The comparative results of the morphological and chemical responses are given in Table 5.13. It was observed that the plants growing in garden soil had a better growth and were taller (121.5 cm) than the plants grown on dump (109.5 cm) (Fig.6.6) at the end of one year growth. The stem (1.75 cm) and leaf (0.2mm) were thicker in the plants grown on dumps when compared to the control plants where the stem was 1.07 cm thick and leaf was 0.18mm thick. The leaf was also larger (6.57 cm x 2.34 cm) in the dump plants when compared to control plants (5.94 cm x 1.95 cm). Epidermal peeling of the leaf showed that the guard cell (21.6 μm x 18.72 μm) and the stomatal aperture (14.4 μm x 3.6 μm) were larger in the plants grown on dumps when compared to the control plants where both were smaller-guard cell (18.0 μm x 15.84 μm) and stomatal aperture (10.8 μm x 3.6 μm). There was not much of a change in the length and dry weight of root per plant.

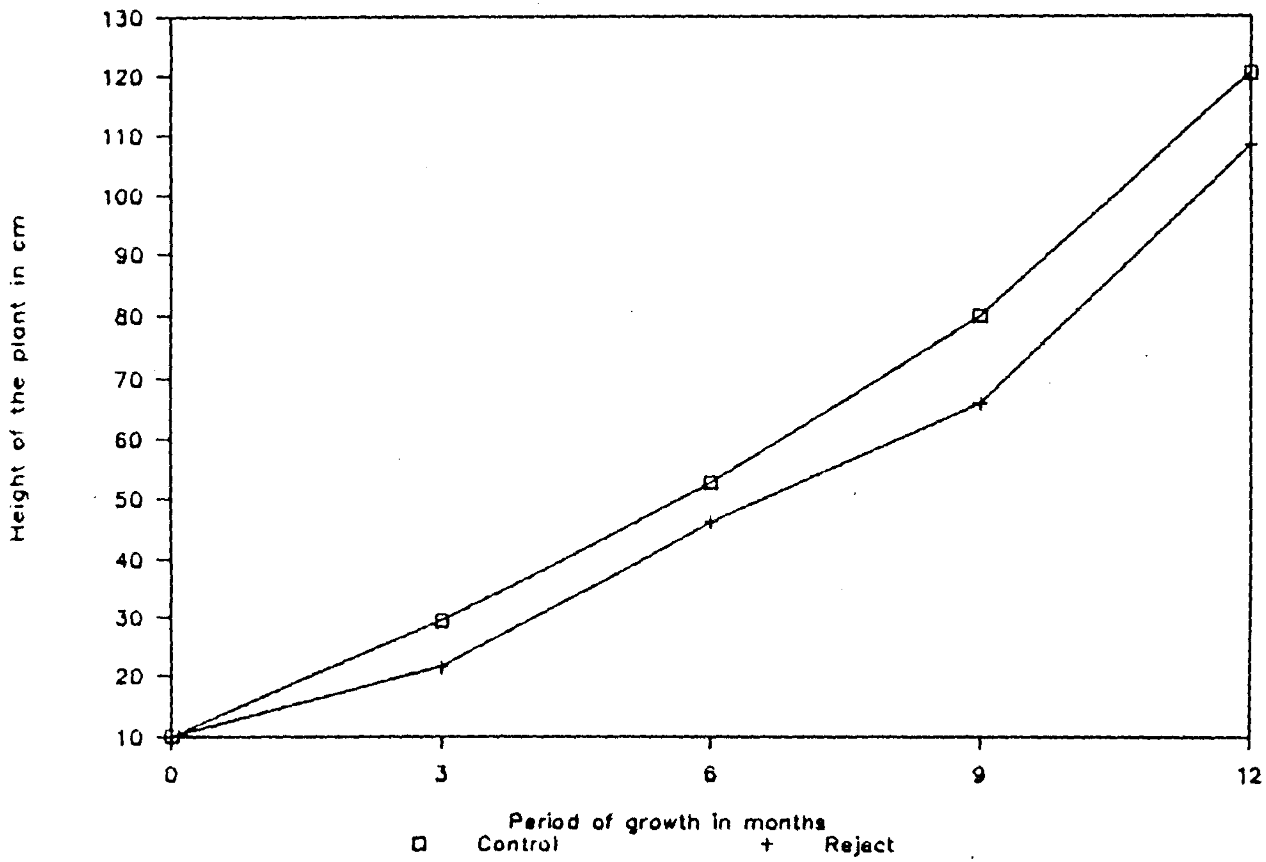


Fig. No.5.1.6: Growth response of <u>Tamarindus indica</u> Linn. to the mine waste.

Table No. 5.13 : Response of **Tamarindus indica** to garden soil & dump reject.

Sl. No.	Response		Garden soil	Dump reject
1.	Height of the plant in cm		121.5	109.1**
		S.D	4.5	3.4
2.	Thickness of stem in cm		1.07	1.75**
		S.D	0.03	0.04
3.	Size of leaf in cm	L	5.94	6.57**
		S.D	0.05	0.08
		B	1.95	2.34**
		S.D	0.04	0.05
4.	Leaf index L/B		3.06	2.82
5.	Thickness of leaf in mm		0.18	0.20**
		S.D	0.004	0.003
6.	Size of the guard cell in um	L	18.0	21.6
		B	15.84	18.72
7.	Size of stomatal aperture in μm	L	10.8	14.4
		B	3.6	3.6
8.	Length of the root system in cm		28.5	27.8
9.	Dry weight of root per plant in g		2.9	2.87
10.	Total chlorophyll in mg/100g fresh weight of leaf		212.94	124.62
11.	Chlorophyll a in mg/100g fresh weight of leaf		66.60	41.22
12.	Chlorophyll b in mg/100 fresh weight of leaf		146.34	83.4
13.	Proline content in μg/g fresh weight of tissue		41.25	45.00

Note: values significant at ** $P < 0.01$ according to student's "t" test.

There was a decrease in the chlorophyll content of the plants grown on dumps (124.62 mg/100 g fresh weight of leaf) when compared to the control plants (212.94 mg/100 g fresh weight of leaf). Proline analysis showed that there was only a slight difference between the two. In the plants growing on dumps it was 45 μg/g fresh weight of tissue whereas in the case of control plants it was 41.25 μg/g fresh weight of tissue.

The chemical analysis of leaf and root for inorganic metals are given in Table 5.14. In leaf it was observed that there was more of iron, manganese, aluminium and potassium with less of calcium and magnesium in the plants grown on dumps when compared to control plants. In the root of the plants grown on dumps the amount of calcium, magnesium and potassium was less than in the control plants, whereas iron and manganese was almost same and aluminium was more in the plants grown on dumps.

Table No. 5.14 : Different elements in the leaf and root of **Tamarindus indica** grown in garden soil and on dump (in ppm/g dry wt. of tissue).

Sl. No.	Type of plant material		Fe	Mn	Al	Ca	Mg	K
1.	Leaf	Garden soil	10.0	01.0	39.5	84.0	31.0	53.0
		Dump reject	12.0	06.0	50.9	162.0	21.0	68.0
2.	Root	Garden soil	09.8	01.0	22.7	260.0	06.0	67.0
		Dump reject	10.0	00.95	31.2	158.0	04.5	32.0

DISCUSSION :

SOIL ANALYSIS :

Normal soils consists of an inorganic frame work of sand, silt and clay particles intimately mixed with organic material produced by degradation of animals and plant remains. The relative amounts of each control the physical, chemical & biological properties of the soil. Compared to natural soils, mine wastes 1) are deficient in most plant nutrients 2) may contain excess salts and heavy metals 3) are composed of unconsolidated sands that will easily erode and 4) have a mineralogy upon weathering which will affect levels and availability of plant nutrients and possibly toxic minerals (Shetron, 1983).

Investigations into vegetative stabilisation of mine wastes should include an examination of the physical and chemical characteristics to assess the suitability of the material for plant growth. The physical nature of wastes can sometimes be assessed by an experienced eye, without recourse to formal tests, but the chemical content of wastes requires analytical investigation. Biological characteristics are rarely investigated but are improved indirectly as the physical and chemical problems are overcome.

Physically, the texture, structure, stability and water ability can present problems ; chemically, an extreme pH, lack of nutrients and excess of toxic metals and salts are common, and biologically the materials lack typical micro-organisms and larger organisms such as worms which in a normal soil are responsible for mixing and distribution of decaying plant material (Williamson et al., 1982).

Normal soils have 2 - 5% organic matter. Mine materials have none or it may be so low that they cannot sustain vegetation on it. Besides supplying nutrients, organic matter is loose and fibrous, and contributes considerably to soil physical properties, the analysis of the reject showed that it was very poor in organic matter content which has deleterious effects (Williamson et al., 1982.)

The soil analysis reveals that there are high amount of iron & manganese with deficiency of nutrients having an acidic pH.

Due to the combination of adverse physical structure and chemical characteristics of the mine reject, an environment hostile to plant growth has resulted. A fertile soil contains major nutrients essential for plants, viz., nitrogen, phosphorus, potassium, calcium, magnesium and small quantities of trace elements. Mine reject are generally sterile and devoid of vegetation (Bradshaw & Chadwick, 1980) and lack plant nutrients.

PLANT RESPONSES :

From the soil analytical results it can be seen that there is a high amount of iron and manganese in the dump soil which are known to inhibit the growth of the plant when present in excess (Foy et al., 1978). In the observations made here it was seen that the plants growing on dumps were shorter than the control plants which showed that high amount of iron & manganese in the reject affected their growth (Tables - 5.3 ; 5.5 ; 5.7 ; 5.9 ; 5.11 ; 5.13).

Sideris & Young (1949) showed that increased amount of manganese in the culture solution depressed the growth of the plant. Also they showed that with high amount of iron in the culture solution lead to the decreased growth of the plants. Vlamis & Williams (1964) have shown in rice and barley plants that at higher concentrations of manganese the height of the plant decreased when compared to control plants. Kuo & Mikkelsen (1981) studied the effect of manganese on sorghum, it was observed by them that with increasing amount of manganese in the solution decreased the height of the plant. Wheeler et al., (1985) showed that excess iron in the soil caused a reduced growth in the shoot of **Epibolium hirsutum**. Pegtel (1986) showed that high amount of iron reduced the growth of **Succisa pratensis**. The results obtained in the experiments carried out here show that there is a decrease in the growth of the plants in the presence of high percentage of iron in the reject. The observations made here confirmed to the results obtained by different workers in different plants.

High aluminium content is known to cause stunting of the plants leading to thicker stem and leaf, with smaller leaves (Foy et al., 1978). Also in the absence or low content of phosphorus, aluminium affects the plant (Fleming et al., 1974). Morris & Pierre (1949) have shown in certain legumes, that with more of manganese in the culture solution, there was a decrease in the size of the leaves, Lohnis (1951) showed that excess manganese in the culture solution decreased the size of leaf in some vegetable crops. Singh & Sharma (1972) reported that under calcium deficiency conditions there is a decrease in the size of the leaves of potato.

The observations made here showed that the stem of all plants growing on dumps was thicker than the control plants, whereas the leaf was thick in all plants except Bombax ceiba where it was almost same. The leaves of Acacia nilotica and Pithecellobium dulce were smaller in the plants grown on dumps. Whereas in the case of Bombax ceiba, Parkia bigladulosa and Tamarindus indica the leaves of plants growing on the dumps were larger than the control plants, and in the case of Azadirachta indica it was almost same in both. This showed that the above plants were not affected by the heavy metals present in the reject soil.

There have been frequent reports in the literature which show that aluminium, iron and manganese have inhibitory effects on the plant growth, particularly on the growth of roots. Millikan (1949) noted that excess iron, in flax produced a stunted root growth. Lohnis (1951) showed in garden crops that manganese affected the growth of the roots. Mugwira _et al_. (1978 & 1980) reported the aluminium injury to roots of triticale & wheat was characterised by decrease in root length and root dry weight per plant. Ohki (1976) studied the effect of manganese toxicity on soybeans and found that at high levels of manganese there is a decrease in the dry weight of the roots. Shetron & Spindler (1983) studied the root pattern of **_Medicago sativa_** in iron tailing & natural soil and found that the dry weight of the roots grown in tailings was less than the plants grown in natural soil. Wong _et al_., (1983) studied the root growth of two grass species on iron ore tailings at elevated levels of iron, manganese and copper and found that with increasing amounts of these elements in the soil, there is a decrease in the length of the roots. Wheeler _et al_.,(1985) reported that with excess of iron in the soils there was a decrease in dry weight of the root in **_Epibolium hirsutum_** & **_Juncus subnodulosus_**. The studies of Pegtel (1986) on the response of **_Succisa pratensis_** to aluminium, manganese & iron showed that there was a reduction in the length and dry weight of root per plant with increased level of these elements. Emanuelsson (1984) studied the root growth in relation to calcium concentration and found that the length & dry weight of roots decreased at low levels of calcium concentrations.

The soil analytical results showed that there is a higher percentage of iron & manganese and less of aluminium and the nutrients in the reject soil than the garden soil. The affect of aluminium was mainly due to the low content of phosphorus in the reject which helps in reducing toxicity of aluminium.

The observations made here show that not all plants were affected by the high amount of iron, manganese, and aluminium in the soil, only **Acacia nilotica**, **Azadirachta indica**, **Parkia biglandulosa** and **Pithecellobium dulce** were affected. There was no effect on **Bombax ceiba** and **Tamarindus indica**.

Presence of high amount of manganese and aluminium in the absence or deficiency of nutrients are known to affect the chlorophyll content in the plants. Kelley (1912) and Johnson (1924) in the first quarter of the present century itself have shown that excess manganese at pH values above 5.5 deprived the availability of iron & thus failed to produce chlorophyll in the plants. Sideris & Young (1949) reported in pineapple plants that excess manganese in the culture solution led to the decrease in the chlorophyll content of the plants. Agarwala et al. (1964) have shown in their studies that excess manganese led to decrease in the chlorophyll content of barley leaves. Sarkunan et al., (1984) have shown in rice plants that aluminium affected the chlorophyll content of the plants. White et al., (1974) hypothesized that manganese interefered with iron utilization in leaves for chlorophyll synthesis. Fleming et al., (1974) have shown that, in the absence or deficiency of phosophorous in the presence of aluminium led to a decrease in the chlorophyll content, but by the addition of phosphorus the effect of aluminium on the chlorophyll content was overcome. The observations made here showed that there was a decrease in the amount of chlorophyll of all the plants except in **Pithecellobium dulce** grown on dumps, where there was high amount of manganese, and also deficient in nutrient with the presence of aluminium in the dump soil.

Proline is known to accumulate in plants under stress conditions or due to deficiency in nutrients

(Savitskaya, 1976 & Ghildiyal *et al.*, 1986). In the analysis of the plants growing on dumps for proline, it was seen that only two plants were under stress - **Acacia nilotica** and **Pithecellobium dulce**. The remaining plants had adapted to the adverse conditions and growing well without being under stress.

It is known that high amount of aluminium available in soluble form affects the uptake of calcium and magnesium and promotes the uptake of potassium by plants to the tops (Andrew *et al.*, 1973 and Lee & Pritchard, 1984). The analysis showed that in the leaves of plants grown on dumps there is accumulation of iron, manganese, aluminium and potassium whereas in the roots it was found that aluminium, magnesium, calcium and potassium were more in the control plants. This showed that even though there was high amount of alumina in the garden soil than the dump reject it was not readily available to the plants, whereas from the reject it was in a soluble form which was readily taken up by plants.

SUMMARY :

The thesis begins with a general introduction to the problem followed by the soil preparation and analysis of it, for growing the plants. Next chapter deals with the morphological response of **Crotalaria juncea** to the different percentage composition of rejects and the treatments with seaweed and fertilizer followed by chapters on the biochemical responses and chemical analysis for inorganic metals. The last chapter deals with the reponses of some tree species to the mining rejects.

The chemical analysis of the different percentage composition of reject soil show that with increasing percentage of reject from 0% to 100% there is an increase in the iron, alumina and manganese content, whereas there is a decrease in the macronutrients. The reject soil is poor in organic matter also. The pH of the soil in all cases is 5.5. The particle size analysis of the different percentage composition of reject shows that with increasing percentage of reject there is an increase in the amount of clay. The chemical analysis of the reject supplemented with seaweed showed that the amount of available nutrients increased considerably in the reject soil with also an increase in the organic matter content. There was only a slight change in the concentration of iron, alumina and manganese.

The analysis of the soil revealed that due to the combination of adverse physical structure and chemical characteristics of the mine reject, an environment hostile to plant growth has resulted. Usually a fertile soil contains major nutrients essential for plant growth and small quantities of trace elements. By the addition of seaweeds to the reject, to a certain extent, some of the adverse effects found in the 100% reject soil are overcome, the availability of the major nutrients is also enhanced.

The morphological response of <u>C. juncea</u> grown under different composition of rejects show that increasing percentage of reject, the height of the plant decreases. Initially at 15 days of growth this effect is not seen, but as the plant ages, the effect of the mine wastes on the plant is more pronounced. Similarly when other characters were observed at the end of 60 days of growth, it was observed that the thickenss of the stem decreased, size of the leaf decreased and leaf thickness increased with increasing percentage of reject. There was a decrease in the size of the guard cell and the stomatal aperture with a slight decrease in the stomatal frequency with icreasing percentage of reject. The root length and root dry weight per plant also decreased with increasing percentage of reject.

The response of the reproductive characters showed that with increasing percentage of reject, there was delay in the flowering of the plant with the flowering period being short compared to plants grown in 0% reject. The number of flowers and pods per inflorescence also decreased with increasing percentage of reject. There was a decrease in the size of the standard petal also with the reduction in the size of the pollen grains. The pollen fertility and weight of 100 seeds also decreased with increasing percentage of reject.

With the addition of seaweed and fertilizers to the reject, it was observed that there was an overall improvement in the growth of the plant. The height of the plant and size of the leaf increased with a decrease in the thickness of the leaf of the plants grown in reject supplemented with seaweed and fertilizers. The size of the guard cell and stomatal aperture also increased. The root growth showed that there was only a marginal improvement in their length but the root dry weight per plant increased considerably in fertilizer treated plants. The treated plants matured earlier and their duration of flowering was also more compared to plants grown without treatment. The number of flowers and pods per inflorescance also increased in treated plants. The size of the standard petal and the pollen grain size was larger with increase in the pollen fertility of the treated plants. Similiarly the dry weight of 100 seeds was more in the case of treated plants.

The biochemical analysis of the plants for chlorophyll, total protein and sugars and proline was carried out. The results showed that with increasing percentage of reject, there was a decrease in the amount of total chlorophyll and also in the chlorophyll a and chlorophyll b contents. Similarly there was a decrease in the total protein and sugar content of the plants with increasing percentage of reject. But with the addition of seaweed and fertilizers to the reject, the plants grown in them showed that there was an increase in the content of all the three viz.chlorophyll, protein and sugar. The analysis for proline showed that with increasing percentage of reject there was an increase in its content, but with the addition of seaweed and fertilizer, it was found to decrease slightly.

The chemical analysis for inroganic metals showed that with increasing percentage of reject there was a decrease in the amount of iron, calcium and magnesium with an increase in the amount of manganese, aluminium, potassium and zinc in the leaves and stem of the plants with iron being found only in traces in the stem region. Whereas in the case of root it was observed that with increasing percentage of reject, iron, aluminium and manganese accumulated and there was decrease in the amount of calcium, magnesium, potassium and zinc. But with the addition of seaweed to the reject, it was observed that there was a slight increase in the amount of iron, calcium and magnesium with decrease in the manganese, aluminium, potassium and zinc content of the leaf, the trend was similar in the stem also. Whereas in the case of root it was observed that the amount of iron, aluminium and manganese was less compared to untreated plants and slightly more of calcium, magnesium, potassium and zinc.

The response studies of the six tree species viz. **Acacia nilotica**, **Azadirachta indica**, **Bombax ceiba**, **Parkia**

biglandulosa, **Pithecellobium dulce** and **Tamrindus indica** planted on the dumps were shorter than the plants grown in garden soil. The stem of all the plants growing on dumps were thicker than the control plants whereas the leaf of all plants growing on dumps were thicker than control plants except in the case of **Bombax ceiba**. The size of the leaf was small only in **Acacia nilotica** and **Pithecellobium dulce** which were grown on dumps when compared to control plants whereas in others the leaf was not much affected. It was observed that roots of only **Acacia nilotica**, **Azadirachta indica**,**Parkia bigladulosa** and **Pithecellobium dulce** growing on the dumps were affected. Their length and dry weight per plant was less than the plants grown in garden soil. Whereas in the case of **Bombax ceiba** and **Tamarindus indica**, the length of the root and dry weight of root per plant was almost same showing that there was no effect of the mine reject. Chlorophyll analysis showed that there was a decrease in the chlorophyll content of all the plants grown on reject dump except in **Pithecellobium dulce**. The analysis for proline content showed that, it accumulated only in **Acacia nilotica** and **Pithecellobium dulce** grown on dumps showing that only these two were under stress and the remaining plants were not much affected by the adverse conditions found on the dumps.

The chemical analysis for inroganic metals showed that, in the leaves of plants grown on dumps there is accumulation of iron, manganese, aluminium and potassium when compared to control plants. The uptake of the elements by the plants showed that, even though the amount of alumina is more in the garden soil than the dump reject, it was not readily available to the plants, whereas in the reject it was in a soluble form which was readily taken up by the plants.

CONCLUSIONS:

The physical analysis of the mine reject reveals that it is mostly made up of clay which hinders the growth of the plants especially the penetration of the roots into the soil because of its compact nature (Williamson _et al_., 1982). The chemical analysis shows that the reject has high concentration of iron, alumina, & manganese which have deleterious effects on the growth of the plants (Foy _et. al_. 1978). It is deficient in the three macro-nutrients viz., nitrogen, phosphorus and potassium and also deficient in the micro-nutrients like, calcium, magnesium which are very much essential for the proper growth of the plants. The reject is also very poor in organic matter content which has deleterious effects (Williamson _et al_., 1982).

The response of **_Crotalaria juncea_** to the reject shows that it is not the effect of a single metal ion, but the effect of all the elements on its growth. The presence of the excess metal ions like Fe, Mn & Al together affect the growth of the plant as revealed by the results. Also by the addition of seaweed and fertilizers it was observed that the growth of the plants improved. This showed that the effect on the plant was not just by the high concentrations of the heavy metals present in the reject, but because of nutrient deficiency also. Safaya & Wali (1979) have shown, how by the addition of fertilizers to a coal mine waste there was an improvement in the growth of the plants. Also, Ajakaiye (1986) has shown that by the addition of phosphorus at high levels of iron, there was an increase in the growth of cowpea plants. Similarly Fleming et al., (1974) showed that with the addition of phosphate to the soil in the presence of aluminium, improved the growth of the plant.

The mine wastes also affected the chlorophyll, total protein and sugar and proline content of the plants. The presence of high concentration of aluminium and manganese affected the chlorophyll and protein content of the plants, whereas only aluminium affected the sugar content. The addition of seaweed & fertilizers overcome the toxic effects of the heavy metals to a certain extent and increased their contents. Whereas in the case of proline, it accumulated due to the deficiency of nutrients and also due to the compact nature of clay. The addition of seaweed and fertilizers helps in overcoming the stress conditions to a certain extent.

The uptake of different inorganic metals showed that aluminium & manganese affected the uptake of iron, magnesium & calcium. They have an antagonistic effect on the uptake of iron and other micronutrients. Even though zinc was present in low concentrations it was found to accumulate in the leaves & stem, thus showing that it was more readily absorbed by the plants in the absence of the nutrients, iron, manganese and aluminium accumulated in the roots of plants grown in reject when compared to the controls, whereas manganese and aluminium was found to accumulate in the leaves & stem also. Calcium also affected the uptake of iron by plants, which were grown in calcium deficient soils. The addition of seaweed to the reject showed that due to the availability of nutrients, the effect of aluminium and manganese on the uptake of other elements was overcome to a certain extent.

The response studies of the tree species showed that, Bombax ceiba and Tamarindus indica were more resistant to the different stress conditions present on the dump rejects compared to the other plants studied. These two being the local plant species were better adapated for the conditions found on the mine waste dumps. This shows that these two should be used in large numbers & popularised to revegetate the mine waste dumps.

The addition of seaweed to the rejects would help for the better growth of the plants and also in improving the conditions of the reject soil by providing organic matter and nutrients which are found in deficiency in the reject soil.

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SYNOPSIS OF THE THESIS :

Response of plant species to the mining rejects

Exploitation of mineral deposits of Goa remains the dominant industry, inspite of the various new industrial developments in diversified fields like small scale cottage industries, electronics, automobiles, tourism, etc. Mining industry still forms the back bone of the state's economy. The iron and manganese ore belt in the Western Ghats extends from Maharashtra to Karnataka passing through Bicholim, Sattari, Sanguem and Quepem talukas of Goa. As with any other large scale industry, the mining industry and particularly the iron ore mining activity, has undeservedly earned the stigma of environmental damage and pollution.

Open cast mining involves deforestation at the mining site, excavation and movement of large volumes of earth's crust. A ton of iron ore mined for instance, produces almost 2/3 tons of rejects. Since 1950, large dumps of rejects have accumulated. Various mining and processing operations have the potential for pollution and can affect not only the environment but also ecosystem. Mining operations expose relatively large areas of sub-surface rocks to the action of the atmosphere, with the results that abnormal quantities of water soluble compounds of iron, manganese, etc., may contaminate the drainage from the mines, and in turn the local surface drainage system.

Extensive work has been done to study the effect and tolerance of heavy metals in plants by many workers for eg. iron toxicity by Ponnamperuma et al. (1955), manganese toxicity by Robinson and Hodgson (1961). But, however, a search through available literature indicates that not enough work has been done to understand the responses of plants to the mine wastes which contain different heavy metals in combined form. Hence an attempt has been made to study the responses of plant species to the mine wastes. In this context **Crotalaria juncea L.** (Sunn Hemp), a leguminous plant of economic importance has been taken up for study.

I. Soil Preparation and Analysis:

During mining operations different types of clays are obtained viz. Manganiferous, Phyllitic, Limonitic, Intrusive and Lateritic. The range of some of major elements found in these clays are as follows:

Fe = 18 - 40%

Al = 15 - 30%

Mn	=	0.02 - 20%
Si	=	25 - 40%
Cu	=	4 - 10 ppm
Ni	=	10 - 20 ppm
Zn	=	10 - 35 ppm
Pb	=	Traces

The above range of elements were found in the earth's crust ranging from a depth of 10 to 60 meters.

The natural undisturbed soil from the surrounding areas of the mine sites showed the following range of the elements in the soil.

Fe	=	20 - 30%
Al	=	15 - 20%
Mn	=	0.3 - 4%
Si	=	25 - 30%
Cu	=	5 - 10ppm
Ni	=	10 - 20ppm
Zn	=	5 - 20ppm
Pb	=	Traces

The above range of elements were found in the soil ranging from a depth of 30 to 75 cms.

Different soil compositions of mine wastes ranging from 0% to 100% rejects were prepared along with garden soil to study the response of **C. juncea**. Garden soil was prepared by mixing 1 part of farm yard manure + 1 part of sand for every 5 parts of soil collected from nearby undisturbed areas. Analysis of the different soil composition was carried out by Atomic absorption spectrophotometer to know the quantity of different metals present. The results of 0% to 100% rejects were as follows:

	Fe	Al	Mn	Si	Cu	Ni	Zn	Pb
0%	20.8%	15.52%	0.2%	38.17%	6ppm	14ppm	7.6ppm	Traces
100%	34.18%	21.34%	0.48%	28.09%	10ppm	16ppm	34.0ppm	Traces

Also a soil composition containing 80% reject + 10% sand + 10% seaweed and 80% garden soil + 10% sand + 10% seaweed were prepared to study the effect of seaweed on the growth of C. juncea plants. The seaweed used was **Dictyota dichotoma (Huds.) Lamouroux.**, collected from the sea shore of Anjuna, Goa.

Fertilizer treatments of NPK combinations were given to the plants growing in pure rejects (100%) at the rate of 10ppm every fortnight for a period of 60 days.

II. Morphological responses:

(a) Plants of *C. juncea* grown under different composition of rejects.

Several morphological parameters like growth rate height of the plant, thickness of stem and leaf, leaf size, leaf index (L/B), length and dry weight of the root, stomatal size and stomatal frequency, time of flowering, number of flowers per inflorescence, duration of flowering, size of standard petal, palynological studies, number of pods per inflorescence and weight of 100 seeds were studied.

The results showed that the plants of *C. juncea* grown in 100% reject had a ht. of 100.1 cm whereas the plants grown in 0% reject were of 249 cm. The root of the plant grown in 100% reject was stunted showing the length of 14 cm with a thinner stem whereas the 0% reject sample showed the root length of 59 cm with a stem of 8.4mm thick. The leaves in the plants of 100% reject were thicker with smaller sized guard cells. The number of flowers per inflorescence was 3-4 in the case of plants grown in 100% reject and 8 - 12 in the case of plants grown in 0% reject.

The standard petal was comparatively larger (3.5 cm L x 2.9 cm B) in plants grown in 0 % reject while the size of the same was 2.2 cm L x 2.2 cm B in those plants grown in 100 % reject.

II. (b) Response of plants to seaweed and fertlizer treatments:

The plants (**C. juncea**) responses were studied in the presence of seaweed and with different fertilizer treatments of NPK. In the presence of 10% seaweed preparation the height of the plants was 179.8cms while the ht. of the plants grown in 100% reject was 100.1cms. Stem also was thicker having a diameter of 5.1cm with the root length being 20cms in those plants after the application of seaweed treatments as compared to the plants grown in pure reject. The leaves were slightly thinner (200 μm) with guard cells being larger (25 μm) than those plants grown in pure reject. The number of flowers per inflorescence was ranging from 7 - 10 in those plants grown in the presence of seaweed treatment.

With the fertilizer treatments of NPK to plants grown in reject, it was observed that the height of the plant was 86.4 cm in NPK treated plant whereas in untreated plant it was 38 cm, i.e. almost double the height. The leaves were larger with the size being 12.31 cm x 2.56 cm in treated plants whereas in rejects it was 9.59 cm x 1.66 cm. The root length in NPK treated plants was 30 cm which is larger than the root of plants grown in reject which was 23 cm. The number of flowers per inflorescence in NPK treated plants was 8 - 12 whereas they were 3 - 4 in untreated plants.

III. Biochemical Responses:

(a) Effect of mine wastes on chlorophyll content:

The plants grown in different composition of rejects were analysed for their chlorophyll content. The analysis was carried out every fortnight for a period of 60 days after planting. It was observed that initially there was no significant difference in the chlorophyll content of the plants grown in different compositions of reject ranging from 0% to 100% and also in plants grown with the addition of seaweed (10% wt/wt) and NPK treatments (10ppm). But as the plants grew it was observed that there was significantly less amount of chlorophyll (200mg/100g fresh wt.of leaves) in the plants grown in pure rejects whereas in plants grown in 0% reject the chlorophyll content was 330mg/100g fresh wt.of leaves. But with the addition of seaweed preparation the chlorophyll content was 300mg/100g fresh wt. of leaves and with the NPK treatment the chlorophyll content was 298mg/100g of fresh wt. of leaves. This shows that with the addition of seaweed and fertilizer there was an increase in the amount of chlorophyll.

(b) **Effect of mine wastes on the total protein and total sugar content:**

The plants were analysed for their protein and sugar content after a period of 60 days of growth. It was seen that the protein and sugar content decreases with the increasing percentage composition of the reject. With the addition of seaweed and fertilizer treatments it was seen to have improved their content only to a marginal extent.

(c) **Effect of mine wastes on the proline content:**

Proline is an amino acid which accumulates in the plants under stress conditions. This proline content was analysed after a period of 60 days of growth after planting.

The plants were grown in different composition of rejects and in the presence of seaweed and fertilizers. With the increasing percentage composition (0% - 100%) of the reject, there was an increase in the amount of proline accumulated by the plants. In the presence of seaweed and fertilizer treatments the proline content was less than in the plants grown in pure reject without any treatment. This shows that the addition of seaweed and fertilzer overcomes the stress on plants to a certain extent.

IV. <u>Chemical analysis for inorganic metals</u>:

Analysis of plant materials for iron and manganese showed that the iron content was more in the leaves of the control plants than the plants grown in rejects. But the roots of plants grown in reject had more iron than the control plants. Whereas in the stem region only traces of iron was seen, while manganese was found to be more in all the three parts of the plant, viz. leaf, stem and root of those plants grown in rejects.

V. Response of 6 tree species planted on the mine waste dumps.

Response of 6 tree species used for the purpose of revegetation on the dumps was studied. The plants were monitored every fortnight for a period of one year from 25.6.'87 to 24.6.'88 for their growth after plantation. Some morphological studies like height of plant, size of leaf, leaf index (L/B), thickness of leaf and stem and stomatal size were calculated.

The age of the plants at the time of transplanting varied from 15 days old saplings to 2 month old saplings. The species studied were Acacia nilotica (Linn.) Delile; Azadirachta indica A.Juss;Bombax ceiba Linn.;Pithecellobium dulce Benth.; Parkia bigloandulosa Wight and Arn. and Tamarindus indica Linn.

It was observed that the plants growing on the dumps were slightly shorter than the plants grown in nursery with garden soil composition.

Plants of B. ceiba, P. biglandulosa, and T.indica grown on dumps have bigger leaves than the ones grown in garden soil but **A. nilotica** and **P. dulce** plants have smaller leaves and **A. indica** had almost the same size of leaf. Leaves of all the plants except **B. ceiba** were thicker in the plants grown on dumps than the ones grown in garden soil. The guard cells were smaller in size in all the plants grown on dumps than the plants grown in nursery.

Chlorophyll analysis of the plants was also carried out. It was observed that except for **P. dulce** plants all the other plants grown on dumps had lesser amount of chlorophyll content than the plants grown in garden soil.

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