CHAPTER V
DISCUSSION
Chapter V
DISCUSSION

5.1 Characterization of mine overburden

Coalmine OB or any mine wastelands are basically hostile environment for living organism. Characteristics of coalmine OB varies based on geographical and climatic condition as well as geological features. Therefore understanding the characteristics of a mine OB is of primary importance for understanding eco-restoration process. In general coalmine OB is characterized by sand rock, rock debris, elevated level of trace and heavy metals, lack of moisture holding capacity as well as true soil forming materials and organic matter (Kundu and Ghose, 1994; Cherfas, 1997; Maiti and Saxena, 1998; Maiti, 2007; Gogoi et al., 2007; Juwarkar and Singh, 2007; Deka Boruah et al., 2008a).

The mine OB of Tirap colliery was found to be acidic pH of 2.6 - 3.9 with redox potential Eh of 198 mV at pH 2.6 and 138 mV at pH 3.9. The acidic nature of the mine OB may be due to the maximum leaching of basic cations (Kundu and Ghose, 1994) or due to the high amounts of elemental as well as pyretic sulphur (Boruah et al., 2002). The high concentration of S and the acidic pH in northeastern coalmine OB dumps are unique in character. Both these pH and redox (Eh; oxidation-reduction) potential have long been recognized as critical parameters for assessing the extent of pollutants in environment (Chuan et al., 1995). pH is indeed a very important soil parameter that affects, among other parameters, the diversity and composition of the soil microbial community (Bardgett, 2005). In turn, soil pH has been reported to affect the activity of soil enzymes through different mechanisms. Similarly, soil pH can alter the concentration of inhibitors, activators, and substrates (Wang et al., 2006). The
redox potential of soil is a major indicator of biochemical reactions, such as oxygen demand, decomposition, geochemical equilibrium and plant stress (Moore and Reddy, 1994; Newman et al., 1996; Pezeshki et al., 1996; Newman et al., 1997; Chabbi et al., 2000). It is primarily controlled by microbial activity, which is a function of soil temperature, water levels, and soil carbon and nutrient supply (Fiedler et al., 2007). In the present study, the redox potential of the mine OB was found between the ranges +138 to +198 mV. The moisture content of Tirap mine OB was significantly low compared to un-mined soil. The water holding capacity (10.46%) in mine OB were also significantly less compared to un-mined soil. Moisture content is an important factor influencing soil conditions, thus addition of water is necessary during remediation processes of mine OB degraded wasteland. Therefore, due to the low moisture and water holding capacity the mine OB site suffers drought stress. Similarly, organic carbon (0.005%) was significantly low in mine OB which is of 95% less compared to un-mined soil. The lower level of organic carbon in mine OB might be due to the disruption of ecosystem functioning (Stark, 1977), depletion of soil organic pool (Parkinson, 1979) and also due to the loss of litter layer during mining which is the prime source of organic carbon and exchange site for nutrients. The available phosphorus content was also found significantly low in mine OB (0.009%) compared to un-mined soil. Lack of adequate phosphorus is an obstacle for initial growth of microbes or invasive lower plants since like nitrogen and carbon phosphorus is vitally essential to sustain basic life processes. The main source of available phosphorus in mine spoil is the breakdown of mine OB material and precipitation from atmosphere (Coppin and Bradshaw, 1992). The characteristics feature of Tirap coal is quite similar in physicochemical properties to other coalmine wasteland that have been reported earlier (Machulla et al., 2005; Mandez, 2007; Juwarkar and Jambhulkar, 2008).
Soil microbes are the critical entity of a good quality soil as well as first invader in hostile environment like mine OB. It plays a key role in nutrient cycling in the soil. Besides essential and non-essential elements, a good quality soil always the storehouse of different microbes (Warkentin, 2006). Microbial activities are an integral part of the functions of ecosystems connected with several important reactions necessary for life processes, stabilization of soil structure, decomposition of organic waste, organic matter formation and in nutrient cycling (Dick et al., 1994). Therefore, determinations of microbial population size in any wasteland are considered as good indicators of the nature of soil type (Bezbaruah, et al., 1998; Anderson and Domsch, 1990). In this study, it was found that, microbial population assessed in terms of cfu were significantly low about 100 times less than that of un-mined soil. The assessment of mine OB’s cfu were also done based on visual distinction of mine OB’s. These results indicated that mine spoil was devoid of essential group of microorganisms for productive rhizosphere development, necessary for ecological succession. It is quite obvious that harsh environmental conditions like high acidity, lack of true soil, water, essential nutrients etc. have restricted microbial invasion and growth. Similar results are reported by many workers (Gohain, 2007; Juwarkar and Jambhulakar, 2008).

Soil enzyme activity is also considered good indicators of soil health (Anderson and Domsch 1990; Bezbaruah, et al., 1998). Ghose, (2004) described the effects of opencast mining on the fertility of soils. In the present investigation, very low microbial enzyme activities were found in the mine OB dumping site of the Tirap collieries. No dehydrogenase, urease or phosphatase activities were detected in mine OB, while a ten-fold lower phosphatase activity was seen in the mine OB compared to the un-mined soil. Gohain, (2008) found great co-relation between the number of microbial population and mine OB enzyme activities. As there is a great reduction in microbial
population in mine OB, its enzyme activities are also very low. Thus, this study reveals that number of microbes and their activities are highly affected in mine tailings or overburden dumping areas. Soil enzyme activity is essential in both the mineralization and transformation of organic carbon and plant nutrients. Hence, soil enzyme measurements have been used to determine the effects of unfavourable environment as well as contaminants on soil microorganisms. The measurement of microbial dehydrogenase activity in soils and sediments has been used extensively as dehydrogenases are intracellular to the microbial biomass, common throughout microbial species and are rapidly degraded following cell death (Rossel and Tarradellas, 1991). Furthermore, soil characteristics such as organic carbon and moisture contents can affect the activities of soil enzymes (Atagana et al., 2003; Bogan and Sullivan 2003).

It appears that high acidity, lack of true soil, acute moisture stress, lack of basic nutrients coupled with the presence of pollutants such as toxic heavy metal, sulphur compounds etc are the major hindrances in eco-restoration of coalmine OB dumps of Tirap collieries. Besides these, the ion exchange capacity of the mine OB was very high. Highly acidic cation aluminium (Al), which was recorded maximum in mine OB is highly toxic to plants and a limiting factor for growth of any organism. The other basic exchangeable cations which play a critical role in soil nutrition status was found to be critically low compared to un-mined soil. All these act as deterrent for invasion and growth of microbes necessary for ecological succession.
5.2 Evaluation of the soil formation through microbial biomass and analysis of soil microbial DNA

Soil particularly top soil is the most precious but non-renewable natural resource because it is the top soil which sustain all forms of life directly and indirectly. Typical soil comprises of three major components viz., clay particle (≤ 0.002 mm), silt (0.002 mm to 0.05mm) and sand (0.05 mm to 2.0mm) apart from essential ingredients like soil nitrogen, organic carbon, and essential trace elements etc. In nature and under natural process it takes century to millennium for formation of life sustaining true soil. However due to a number of anthropogenic factors, particularly industrial activities and mining activities waste areas of life sustaining soil turn into life threatening wastelands like coalmine OB. Eco-restoration process can speed up the process of soil formation and convert it habitable. Therefore, in eco-restoration process it is very important to monitor the changes in physico chemical properties of mine wasteland. Being the first invader of such habitat full of abiotic stress, microbial population and microbial activity play an invaluable role in this natural succession process. Microbial population and activity to a great extent play a critical role in restoring and enhancement of soil health and facilitate the growth of higher plants (Petrisor et al., 2004; Addicott, 2006).

In this study, it was found that in the mine OB of the Tirap Colliery is of 0.6% of 0.35 mm grain size particularly constitute only 0.6%, with more than 90% of the grain size being comprised of boulders, pebbles, cobbles etc. The mine OB’s are generally characterized by low organic matter content, low pH (2.5-3.0), high bulk density, and low water holding capacity (Cherfas, 1992). However depending on seasonal changes the changes vary. Maiti, (2007) worked on same mine OB of India and reported similar findings. The mine OB of Tirap collieries are highly acidic and have no true soil characteristics. Therefore, mine OB’s are not a suitable medium for
plant colonization within short span of time. Dobson (1997) postulated that depending on the nature of mine OB's minimum one to five decades is required for establishment of tertiary ecological successions with specific plants in degraded bare land.

The reclamation of mine spoils was attempted by planting different native plant species in a phased manner, and a secondary climax state of succession was achieved (Dowarah et al., 2009). It was found that soil microbial biomass carbon of the reclaimed mine OB significantly enhanced after the third year of reclamation. On the other hand, the bulk density were decreased after three years of reclamation. The decrease in bulk density generally reduces the compaction and facilitate the aeration and better penetration and spreading of roots thereby making the rhizosphere favorable for massive root development. Similar results were observed by Juwarkar and Singh, (2007), on mine OB dump amendment with effluent treatment plant (ETP) sludge and biofertilizer. The organic carbon content reportedly increased during the course of reclamation. The rapid increment of organic carbon level was due to accumulation and decomposition of biomass of the plant material as well as the microbial mass on the surface. As organic matter increases with time, nutrient retention capacity also tends to increase. Similar results were also reported by Singh et al., (2002) and Kumar, (2004). The pH of the reclaimed area reached a moderately acidic level.

The present study clearly indicates that the plantations on mine spoil modify the soil physicochemical characteristics, but the plant species differed in their ability to modify the same. Biomass is considered the main source of soil organic matter and the later is highly correlated with microbial biomass (Schnurer et al., 1985). The plantation of such abiotic stress tolerant plant species maintain the nutrient regeneration due to addition of organic matter and its further decomposition. Increasing availability of organic matter also enhanced N− mineralization, and contributes to essential nutrient
cycling. The plant species found suitable for eco-restoration in the present study viz., *Cassia sp.*, *S. rostrata* and *M. pigra* are leguminous with inherent ability to fix atmospheric nitrogen and this helped to enhance soil nitrogen level and contributed immensely for the success of present study and eco-restoration effort.

The extracted DNA in the OB reclaimed site was appreciably higher and there was concomitant increase in microbial population and diversity. Apparently, higher level of soil DNA is due to increased microbial population. The present findings are in conformity with the earlier work of Petrisor *et al.*, (2004) on co-inoculation efficacy in phytostabilization of phosphogypsum and sulphidic tailings; while Machulla *et al.*, (2005) also reported enhanced microbial growth and activity in mine spoil/material at the initial stage of soil development.

In the reclaimed Tirap colliery, the soil enzyme activities in the mine OB wasteland significantly increased following reclamation activities. Overall, >2 to >24 times more urease, phosphatase and dehydrogenase activities were found for the mine OB reclaimed site towards end of reclamation effort. The improvement of microbial properties in reclaimed mine OB’s were earlier reported in several studies (Petrisor *et al.*, 1999; Stefanescu *et al.*, 2000; Machulla *et al.*, 2005). The enhanced enzyme activities in the high sulphur mine OB reclaimed sites was due to the cumulative effects of microbial population size and plant species grown in the reclaimed mine OB.

5.3 Screening of plant species for phytoremediation

Use of abiotic stress tolerant plant species for environmental cleanup as well as for reclamation of wasteland has been recognized as an efficient technique which is simple requiring unskilled or semi-skilled manpower, very low cost, yet efficient technique and above all it is environmentally self sustaining (Salt *et al.*, 1995; Tordoff *et al.*, 2000). Not all plants can grow in the harsh abiotic stress mine OB. The ability of
certain plants to do so is dependent on their inherent nature and genetic set up. Successful biological reclamation largely depends on the selection of appropriate species for revegetation. Selection of appropriate plant species is very important to ensure a self-sustaining vegetation cover (Wong, 2003). Much progress has been made at the levels of physiology, biochemistry and molecular biology of plant tolerance to heavy metals in past decades (Chaney et al., 1997). In the present study, selected plants were grown under controlled condition (in earthen pots) as well as at site to assess their survival. Selection of plant species for revegetation of OB dumps depends on various parameters such as climate, physical and chemical properties of dump materials, topography, viability and surrounding vegetation (Singh and Jha, 1992). Thus, screening of plants is essential before large scale reclamation. The principle behind the use of native plant species is due to adaptability of such plants to the prevailing climate condition (Cunningham and Ow, 1990). The plant species selection to carry out the phytostabilization of mining wastes must be site specific since besides being tolerant to metal pollution, must be adapted to local climate. Thirty-six different plant species were screened representing twenty different families belonging to the herbaceous, liane, shrub and tree groups. Amongst the herbaceous plant species, Axonopus sp., Saccharum spontaneum, and the economically important essential oil bearing plants Cymbopogon winterianus and C. flexuosus were found to be the most stress-tolerant. These plants were able to resist the stress conditions of mine OB possibly due to the nature of their fibrous root systems and hardy perennial nature. The liane species Mimosa pigra, M. streata, and M. strigillosa were also able to resist OB stress. The present study further revealed that, the shrub species Cassia sp. and S. rostrata and the tree species G. arborea and D. sissoo were resistant to mine OB stress in the Tirap colliery. Similar coalmine OB tolerance of G. arborea and D. sissoo were also reported
by Juwarkar and Singh (2007) and Singh et al (1996). This finding is significant because both the tree species are class one timber plant with good market value. Moreover, they are not food plant and so there is no possibility of any harmful heavy metal or such pollutant entering the food chain. Thus, it is possible to combine eco-restoration with economic benefit.

The goal behind the screening of herbs, lianes, shrubs and tree species was to achieve primary, secondary and tertiary ecological succession within a short period of time in an integrated approach. The herbs support creation of microclimatic conditions in the mine OB environment, and are able to proliferate by generating new tillers after establishment. Thereby, they can cover the exposed area rapidly. On the other hand, lianes (\textit{M. pigra}, \textit{M. streata}, \textit{M. strigillosa}) and shrubs (\textit{S. rostrata} and \textit{Cassia sp.}) are able to produce fruit within a year and thus increase their population size in self-sustaining manner. This allows the plants to maintain their continuity across generations. Selected plant species should have fast growth and spread, and be able to get an effective soil cover (Simon, 2005). In some cases, amendments (e.g., fertilizer, lime) may favour the initial establishment of selected plants (Conesa et al., 2007a; Conesa et al., 2007b). The plant species selection to carry out the phytostabilization of mining wastes must be site specific since besides being tolerant to the abiotic stress must be adapted to local climate.

In the present investigation, it was found that the seedling growth rate was low, but sufficient to maintain the subsequent plant generations. The major goals of the revegetation are to reduce erosion, stabilize mine spoils and develop processes for suitable post mining landuse (Roberts et al., 1988; Jones et al., 1975).
5.4 Top layer refilling with un-mined soil: Amendment as first step of eco-restoration

A number of earlier works corroborate the findings of the present study that coalmine OB’s represent very high abiotic stress, which normally cannot support average living organism (Deka Boruah et al., 2004; Deka Boruah, 2006a and Gogoi et al., 2007). Even the exceptional ones, which can tolerate such harsh environment, face problem with initial establishment and anchorage. A possible solution to this problem is amendment. Basically, it is digging small pits in rows at regular intervals and filling the same with normal soil with or without inorganic, organic fertilizers, biofertilizers etc. All these create microenvironments for initial establishment of selected plants. Cow dung, poultry litter and pig manure are commonly used as tailings amendments because the addition of organic matter can significantly improve the physical characteristics and the nutrient status of mine soil (Ye et al., 1999). In addition, fertilizers are an essential ingredient for successful restoration of mine wastes (Bradshaw and Chadwick, 1980). Top soil is an essential component for land reclamation in coal mining areas (Ghose, 2004; Ghose and Singh, 2005; Ghose, 2005). In this study an attempts has been made to assess the bare minimum requirement of top soil refilling with un-mined soil (soil from farm land) instead of other chemical or organic fertilizer for quick establishment of vegetation.

In this investigation against no germination under mine OB, a significantly higher rate of germination was found for top layer refilling with un-mined soil. The germination percentage was higher when the seeds were shown in top in 25 mm and 30 mm depth. This indicates that amendment with un-mined soil can provide necessary microenvironment for initial establishment of selected crop.
Seedling growth, shoot length, root length, nature of root growth, synthesis of chlorophyll pigment and biomass in one week old seedlings (seeds sowed in at the top and middle layer of un-mined soil coverage) are described in Table 4.13 and 4.14; Figure 4.4, 4.5 and 4.6. Mine OB amended with un-mined soil did not significantly enhance the shoot height and root length of *S. rostrata* compared to no germination under mine OB alone. Considered as control overall, seeds sowed in mine OB after top soil refilling with un-mined soil improve the germination and growth of *S. rostrata* seedlings compared to no germination under mine OB alone. Among the different depth of un-mined soil amendments, overall higher growth (higher shoot length, higher secondary root, higher SRL and biomass) was observed in the 30 mm depth of top layer refilling.

Compared to control (plant grown in normal soil) overall growth of plants grown in amended mine OB was lesser. However, it is still significant, because it demonstrate that amendment is effective way for initial establishment of vegetation. Similar to seeds sowed in the top, while seeds sowed in the middle of the un-mined soil refilling, significantly higher growth was observed in 25 and 30 mm depth of top layer refilling.

Among the various amendments shoot length in 25 mm depth category was significantly higher. Significantly, increased girth of main root, root surface area, and SRL and total biomass was found in 30 mm depth category of amendment. Higher chlorophyll content, better root growth etc were observed in both 25 mm and 30 mm depth categories of amendments. On the other hand, highest accumulation of total biomass, total number of secondary root, root length and total chlorophyll contents were found in 30.0 mm depth category of amendments.
Therefore, the low rate of germination, lower root biomass and other growth parameters in amended mine OB compared to control clearly correlates the limitations of the mine OB physical and chemical characteristics. The findings of the present study indicate that, the nutrient enriched un-mined soil acts as a source and sink of plant available nutrients. It also provides a reservoir of essential macronutrients and provided a habitat for microorganisms in energy and nutrient cycling. All these contribute to eco-restoration process.

5.5 Amelioration effect on AMD using beneficial rhizobacteria

Coalmining not only generate huge wasteland in its vicinity it also generate liquid waste related to acid mine drainage (AMD). Since AMD is liquid, it flows down in all directions far and wide according to geological gradient and creates additional wasteland. Thus, AMD is more damaging than coalmine OB. It is one of the most common problem associated with mining of coal which is a global problem affects the soil and water in the neighborhood of the mining site (Charles, 1998). AMD generated from opencast and underground high sulphur containing coalmine causes environmental damage in and around colliery of Northeastern coalfields (Deka Boruah, 2006; Boruah et al., 2006). In the present study the damage due to the AMD in neighbouring areas of the colliery of NE region were also found to be similar. Amount of sulphur (2-12%) found in coal and mine OB accelerates the formation of AMD. Generally, the coals contain three forms of sulfur, mainly sulfate, pyretic, and organic. However, another two forms of sulfur, elemental and secondary sulfur, has also been reported for the high sulfur coals of Northeast India (Boruah et al., 2002). The problems of AMD in the coalmine areas of NE India have been highlighted by several workers (Boruah et al., 2005; 2006; Bhattacharjee et al., 2007; Khare and Boruah, 2008). These AMD is highly acidic in nature contain many toxic compound and hence
it is highly damaging to living organisms. However, in this study, the effect of AMD on plant was tried to be mitigated by co-inoculation of rhizobacteria *Pseudomonas* and *Rhizobium* that can withstand abiotic stress of AMD. Recent advances in understanding the role and application of bacteria for the remediation of toxic contaminated terrestrial environment have come from several avenues. Several workers reported about the usefulness of microbes for bioremediation (Kloepper, *et al.*, 1989; Kumar, *et al.*, 1995; Glick, 1995; Glick, *et al.*, 1999; Sayler and Ripp, 2000). For the present study, involving the deleterious effect of AMD and the amelioration effect on AMD through supplementation with plant nutrient solution (PNS) and rhizobacteria. *S. rostrata* was taken as the test plant, because among the selected plant species its performances were better than the rest.

A significantly retarded seed germination of *S. rostrata* was observed when treated with AMD and simulated AMD compared to plant nutrient solution and beneficial rhizobacteria *Pseudomonas* 373 and *Rhizobium* treatment. Seedling grown in AMD amended with PNS and with co-inoculation of beneficial *Rhizobium* and *Pseudomonas* showed enhance seedling survival and seed germination of *S. rostrata*. These may be due to amelioration of deleterious effect of AMD by the beneficial *Rhizobium* and *Pseudomonas* sp. 373. Higher seed germination following PGPR treatment was reported for India gooseberry by Aseri and Rao, (2004). A number of studies have shown the improvement in plant growth and development in response to inoculation with various microbial inoculants capable of producing plant growth regulators (Kloepper, 1992; de Silva *et al.*, 2000; Sudhakar *et al.*, 2000; Esitken *et al.*, 2002; 2003; Kloepper, 2004a; Zahir *et al.*, 2004; 2006; Orhan *et al.*, 2006; Aslantas *et al.*, 2007).
5.6 Biochemical basis of AMD amelioration by PNS and rhizobacteria

The role of phytohormones in plant growth and development is well documented. Plant growth promoting rhizobacteria produce plant hormones, such as auxins (Jeon et al., 2003; Egamberdiyeva, 2005; Aslantas et al., 2007), cytokinins (Garcia de Salamone et al., 2001; Aslantas et al., 2007), gibberellins (Gutierrez-Manero et al., 2001), and ethylene (Glick et al., 1995). The increase in plant growth might be associated with secretion of auxins, gibberellins and cytokinins (Ramamoorthy and Samiyappan, 2001). In the present investigation, a significantly higher amount of GA accumulation in *S. rostrata* was found for the seedlings in root (21.7 ng g⁻¹) and stem (20.3 ng g⁻¹) of PNS-Rhizobium-Pseudomonas treatment. In leaf, the higher GA synthesis was observed in PNS-Pseudomonas treatment (24.4 ng g⁻¹). The synthesis of ABA in all cases (root, stem and leaf) of *S. rostrata* were found to be higher in simulated AMD-Rhizobium treatment (root, 9.39 ng g⁻¹ stem, 3.38 ng g⁻¹ and leaf 9.46 ng g⁻¹). These enhanced hormone synthesis in PGPR and AMD treatment may be due to the effect of PGPR on hormone synthesis. Abscisic acid (ABA), well known as a plant stress hormones, plays an important role in the improvement of plant tolerance to adverse environmental conditions.

A significant difference in enzyme activity nitrate reductase (NR) and catalase were found for the seedlings grown in AMD, *Rhizobium* and *Pseudomonas* treatment. Higher amount of NR activity in leaf (170 mM mg⁻¹ protein) and root (679.0 mM mg⁻¹ protein⁻¹ was found in PNS- *Rhizobium-Pseudomonas* and PNS-Pseudomonas.

However little is known about the ability of these PGPR strains to stimulate higher levels of defence enzymes. An appreciably lower amount of catalase activities were found in simulated AMD-*Pseudomonas* (root, 16.1 mM g⁻¹ h⁻¹ and leaf, 9.3 mM mg⁻¹ protein). Highest catalase activity in root (149 mM mg⁻¹ protein) and leaf (133
mM mg\(^{-1}\)protein) were recorded for simulated AMD-Rhizobium grown seedlings. It appears that induction of suppression of catalase activity is determined to a great extent by the rhizobacteria used for amelioration.

The plant pigment chlorophyll is the most essential component of the photosynthesis. Photosynthetic characteristics are very much correlated to the nutrient status of leaves (Sobhana et al., 1996). Highest chlorophyll content of *S. rostrata* was found in PNS treated seedlings and comparatively lowest was found in *rhizobium* treated AMD sample. The growth characteristic of *S. rostrata* was determined for the seedling grown in gnotobiotic condition and observations were recorded after 15 days of growth in a plant growth chamber. A significantly reduced leaf size (0.198 cm\(^2\)), root length (6.1 cm) and shoot height (4.0 cm) were found for the seedlings of *S. rostrata* grown in AMD and simulated AMD. On the other hand seedling grown in AMD and simulated AMD with the co-inoculation of beneficial rhizobacteria showed the enhanced growth. Hence, these strains are collectively called PGPR (Kloepper et al., 1980). Plant Biomass accumulations for the plant seedling of *S. rostrata* were also found to be significantly higher upon PGPR application. Similar findings of enhanced biomass accumulation due to PGPR application was reported by Bevivino et al., (2000). Inoculations with PGPR increased shoot and root growth of canola and sugar beet (Bertrand et al., 2001), enhanced germination and dry weight of rape seed seedlings (Kloepper et al., 1991), and increased the mass of root-adhering soil on wheat (Bezzate et al., 2000).

5.7 Amendment of mine OB with various fertilizers and impact on plant growth parameters

Mine OB is basically a solid waste covering huge area and in huge amount thereby creating an uninhabitable wasteland. The present study as well as several
earlier works by many other researchers in various parts of the world confirm that physically it is lacking in true soil characteristics; chemically it is virtually devoid of essential nutrients like organic carbon soil nitrogen phosphorus etc. These apart there is acute deficiency of moisture and there is presence of toxic heavy metals and inorganic pollutants. All these constitute severe abiotic stress and a limiting factor for plant growth giving it a barren look. Even except some exceptional bacteria, most microbes can not survive there.

Remediation of such a hostile wasteland is extremely difficult. A part of the present study described earlier showed that amendment of mine OB with normal fertile soil by digging small pits and filling the same with normal soil to various depths is quite helpful in establishment of vegetation with carefully selected plant. However, it appears possible that by supplementing it with organic and inorganic fertilizers together with biofertilizer in different combination will make the technique more effective. A number of workers tried this approach with organic and inorganic fertilizer supplements with good degree of success (Corriea et al., 1995; Bellamy et al., 1995; Guerrero et al., 2001). Others also recorded good degree of success with biofertilizers, particularly PGPR (Kumar et al., 2007).

5.7.1 Improvement in plant growth due to amendment with various fertilizers

Like the experiment involving AMD affect, for mine OB amendment study, also S. rostrata was taken as the test plant since among the selected plants its performances were most satisfactory.

After one week of growth highest root biomass was 97 mg in FYM-U-MOP-Pseudomonas-Rhizobium, shoot biomass 101 mg and leaf biomass 224mg in FYM-U-MOP-Pseudomonas. Likewise, higher root length 4cm was observed in FYM-U-MOP Pseudomonas compared to 3.5 cm in un-mined soil. FYM-U-MOP combination
resulted in highest secondary root (19 nos) was observed. As against this in control condition 2.8 cm root length, 2.6 cm shoot length and 12 numbers secondary root were observed. After seventh week of growth highest root biomass (740 mg), shoot biomass (1170 mg) and leaf, biomass (2030 mg) was observed in FYM-U-MOP-Pseudomonas-Rhizobium combination. Highest root length (7.6 cm) was found in FYM-U-MOP-Pseudomonas and FYM-U-M-Pseudomonas-Rhizobium. Shoot length (18.8 cm) and total number of secondary root (24 nos) were found to be higher in FYM-U-MOP-Pseudomonas-Rhizobium. So from this study, it was found that overall growth of *S. rostrata* was significantly enhanced by the amendment with farmyard manure, urea MOP and plant growth promoting rhizobacteria *Rhizobium* and *Pseudomonas*. Several studies have sown that treatment with PGPR enhances root growth, leading to a root system with large surface area and increased number of root hairs (Mahaffee and Kloeper 1994; Mantelin and Touraine, 2004). The positive effects of PGPR on plant growth are always correlated with remarkable changes in root morphology, namely increased lateral root length and root hair number and length and can have influence on nutrient uptake potentials (Okon and Vanderleyden, 1997; Bertrand *et al.*, 2000).

Studies by various workers shows that PGPR works in more than one way simultaneously which ultimately culminate in improvement of plant growth. These bacteria promote plant growth and health by various means such as mineralization of nutritional elements, nodulation and nitrogen fixation (Bashan *et al.*, 2004), synthesizing phytohormones etc (Khalid *et al.*, 2004; Lucy *et al.*, 2004; Shaharooma, 2008). Benefits to plants from plant-PGPR interactions have been shown to include increases in seed germination rate, root growth, yield, leaf area, chlorophyll content, nutrient uptake, protein content, tolerance to abiotic stress, shoot and root weights, biocontrol, and delayed senescence (Mahaffee and Kloeper, 1994; Raaijmakers *et al.*, 2000).
Application of sludge, compost, soil like material results in remarkable increase in microbial population and activity and typically increases the level of activity of soil enzymes in OB, which in turn enhance the plant growth and development (Beyer, et al., 1993). The findings of the present study corroborate with the observations and views of those workers. Similar findings involving enhanced root biomass, more secondary root etc were also reported by Vivas et al., 2003; Han and Lee, 2005 etc.

5.7.2 Impact of mine OB amendments with fertilizer on basic physiological parameters of test plant

Along with growth parameters, it is equally important to observe the impact of mine OB amendments on basic physiological parameters. Because increase or decrease in physiological activity is directly related to growth and development.

In this study, observations were recorded with respect to SLW, NAR, RGR and LA following different amendment combinations. An incremental increase of leaf area over the time of observation was found for all the treatments. Compared to mine OB alone the FYM amended mine OB promote more than two times higher leaf growth except first and second week for FYM-U-MOP. The cumulative increase of leaf area in treated plant to that of mine OB grown plants are 52% to 81%. Among all the treatments, the FYM-U-MOP-Pseudomonas-Rhizobium treated plant showed highest leaf area.

The SLW for the plant species grown in mine OB conditions showed a significant increase up to the of 6th week. There was an increase in leaf thickness and leaf tissue biomass per unit area. Higher SLW is generally associated with greater photosynthetic potentiality of leaves. Thus, these characters are associated with the adaptation mechanism of plants to mine OB conditions. Contrary to enhanced SLW,
the NAR values were significantly low under OB stress. Plant species in unfavorable environments often shows inherently low RGRs compared to species from in favourable environments (Chapin 1980; Lambers and Poorter 1992; Lambers et al. 1998). Here in this investigation the cumulative relative growth rate was found to be highest in FYM-U-MOP-Rhizobium-Pseudomonas. The trend of affect of amendment on RGR of S. rostrata were control ≥ FYM-U-MOP < FYM < FYM-U-M-Rhizobium < FYM-U-MOP < FYM-U-MOP-Pseudomonas-Rhizobium. In woody and non-woody species, an inherently low RGR is often strongly associated with a lower foliage area per unit foliage dry mass (Poorter and Remkes 1990; Garnier 1992; Atkin and Lambers, 1998; Atkin et al., 1998). For the present study, the lower RGR may be due to the lower leaf area in mine OB stress. Likewise, NAR, the dry matter increment per unit foliage area per day, is a function of the daily integral of photosynthesis i.e., the fraction that is utilized in respiration by the plant for growth, ion uptake and maintenance. Net assimilation rate (NAR) recorded over the different period is described in Figure 13. A variation in net assimilation rate was observed for the treated mine OB grown plants. A cumulative enhancement of NAR of 10%, 5.8% and 67% were found for the treated plants treated with FYM, FYM-U-MOP-Pseudomonas, FYM-U-MOP- Pseudomonas- Rhizobium and un-mined soil respectively. Here also the lower NAR in mine OB stress may also be related with the lower leaf area. The present findings are in corroboration with similar reports involving stress induced decline in physiological parameters and their augmentation following amendment treatments (Anjum et al., 2003a; Bhatt and Srinivasa Rao, 2005; Kusaka et al., 2005; Shao et al., 2008; Specht et al., 2001; Wu et al., 2008).
5.7.3 Impact of mine OB and mine OB amendments on biochemical parameters in test plant

Understanding biochemical basis of tolerance or susceptibility to mine OB stress is of paramount importance. Because tolerance or susceptibility is an inherent nature of a plant species and its understanding can lead to development of tolerant plants. In the present study effort was made to see how mine OB stress affect certain biochemical parameters and how they respond to various amendment.

In the present study, antioxidant enzyme peroxidase activity was found significantly higher in case of mine OB plant saplings compared to un-mined soil while lowest was found in FYM-U-MOP-Pseudomonas-Rhizobium. The peroxidase activity is considered as stress indicating enzyme. On the other hand, compared to un-mined soil, nitrate reductase activity was found to be lowest in the leaf and root samples of S. rostrata grown in mine OB and increased activity was observed in PGPR treated sample.

The total essential element viz., carbon, hydrogen and nitrogen accumulation were also enhanced by the amendment of organic matter and beneficial rhizobacteria. Higher level of carbon, hydrogen and nitrogen imply better nutrient accumulation. Therefore, it appears that amendment with organic matter and *Rhizobium* and beneficial *Pseudomonas* *sp* may enhance the accumulation or absorption of nutrients from amended mine OB. The beneficial affect of rhizobacteria on nutrient accumulation were reported by many workers (Kloepper *et al.*, 1991; Kloepper, 1994; Kloepper *et al.*, 1998; Glick and Pasternak, 2003; Siddiqui, 2004; Khalid *et al.*, 2004).

In the present study endogenous hormone levels were also estimated and higher gibberellic acid (GA) accumulation was found in FYM-U-MOP-Pseu.-Rhiz combination (11.86 ngg⁻¹) compared to only 4.69 ngg⁻¹ for mine OB control. In case of root highest GA accumulation was found in FYM-U-MOP-Rhiz-Pseu combination.
(23.29 ng g\(^{-1}\)) while lowest level of (5.09 ng g\(^{-1}\)) was recorded for FYM-U-MOP. For the other treatment combinations an enhancement was found which were highly significant statistically.

It appears that organic fertilizers and PGPR works separately with own mechanism. There are reports that PGPR can act by enhancing the synthesis of the enzyme 1-amino cyclopropane-1-carboxylate (ACC) deaminase, which lowers plant levels of ethylene, thereby reducing environmental stress on plants (Uchiumi et al., 2004; Belimov et al., 2005; Gutierrez et al., 2001; Glick et al., 2007).

5.7.4 Impact on mine OB characteristics following amendments

Apart from its positive impact on plant growth parameters that can facilitate eco-restoration, amendment with various organic, inorganic and biofertilizers had positive impact on mine OB characteristics. This was assessed in terms of enzyme activities and the levels of organic carbon and phosphorus. The soil enzyme phosphatase activity was found to be significantly higher (1.65 mg g\(^{-1}\) h\(^{-1}\)) in FYM-U-MOP - *Pseudomonas-Rhizobium* amended mine OB. Similarly the urease (84.5 mg g\(^{-1}\) h\(^{-1}\)) and dehydrogenase (1.74 mg g\(^{-1}\) h\(^{-1}\)) activity was found to be higher in FYM-U-MOP and FYM-U-MOP- *Pseudomonas-Rhizobium* respectively. Organic carbon content was also increased (1.28 %) in FYM-U-MOP-*Pseudomonas-Rhizobium* amended mine OB. Amendment of FYM enhanced the level of available phosphorus content (0.051 %) in mine OB substrate. Moisture content was found higher (19.88 %) in FYM-U-MOP-*Pseudomonas-Rhizobium* amended mine OB. All these are possible because amendments as well as plant growth which enhance microbial population and activity. It is clear that amendments improved the quality of mine OB.
5.8 Biochemical indicators and biochemical changes following OB stress

Abiotic stress like coalmine OB severely retard plant growth and development and in many cases, it may be fatal. Studies by various workers has established that abiotic stress induces a number of deleterious effect at cellular, biochemical and molecular level and survival of plants depend upon its biochemical defence mechanism to cope up with the cellular and biochemical damages. Notable among them are membrane damage due to lipid peroxidation and generation of free radicals; activities of antioxidative enzymes; biochemical indicators like accumulation of proline, soluble sugar etc. For this study, seven test plants viz., Cassia sp., S. rostrata, M. pigra, G. arborea, D. sissoo, C. winterianus and C. flexosus were taken and the plants were grown under mine OB stress and in un-mined soil (stress free condition).

5.8.1 Biochemical indicator

5.8.1.1 Impact of mine OB on lipid peroxidation and oxidative damage

Lipid peroxidation is estimated in terms of malondialdehyde (MDA) generated and electrolyte leakage was considered as indicative of oxidative damage. It is well documented that MDA is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Ohkawa et al., 1979). Thus, cell membrane stability has widely been utilized to study effects of stress on plants. The present study showed that mine OB stress causes a significant increase in MDA production in root and old leaf. In young leaf, there was considerable amount of MDA but compared to young leaves, in old leaves MDA generation was much more. Therefore, the increased production of MDA in leaf and root of all tested plant in mine OB stress indicated the oxidative damage of membrane. Membrane lipids and proteins are especially prone to attack by free radicals and are considered reliable indicators of oxidative stress in plants (Halliwell and Gutteridge, 2007). This high lipid peroxidation
correlated with the increased rate of electrolyte leakage from the cell and the low dry matter accumulation by the plants. The present finding shows that electrolyte leakage significantly increased in leaf and root due to mine OB stress. Lipid peroxidation of biological membrane might lead to structural alterations in drought stress plants. The increased electrolyte leakage and the increased formation of MDA in mine OB stress condition indicated that the mine OB stress induces oxidative damage in the tolerant plant species. The findings of the present study are in agreement with similar findings by several other workers (Mano, 2000; Reddy et al., 2004; Shankar et al., 2004; Kang et al., 2007). Cell membranes are the first targets of many plant stresses and maintenance of their integrity and stability under water stressed condition is a major component of drought tolerance in plants (Bajji et al., 2002).

5.8.1.2 Impact of mine OB stress on antioxidative and other enzymes

Lipid peroxidation and electrolyte leakage inevitably generate higher level of free radicals particularly reactive oxygen species (ROS). Under normal metabolism, also ROS are generated but these are neutralized by plants in-built defence mechanism which comprise of both enzymes as well as non-enzymatic compounds which are collectively called antioxidants. However among them antioxidative enzymes play a dominant role. Glutathione reductase (GR) is one such enzyme which can effectively scavenge free radicals through conversion of GSSG to GSH (Smith et al., 1989; Feng et al., 2009). Foyer et al. (1994) have shown that GR is one of the key enzymes that helps in reduction of GSSG to GSH by oxidizing NAD(P)H to NAD(P)⁺ and suggested its crucial role in combating oxidative stress in leaves. In the present study, the GR activity was appreciably higher in both young leaf, old leaf and root in mine OB stressed condition compared to un-mined soil condition. In the test plant *G. arborea* in both leaves (53.33 μM mg⁻¹ protein in young leaf and 102 μM mg⁻¹ protein in old leaf)
and root (5.5 μM mg⁻¹ protein) the GR activity was found to be higher than control (un-mined soil). The overall increase of GR activity of young leaf in mine OB grown plants were 20.21% to 43.55% compared to that of un-mined soil. Compared to the leaves almost six times higher GR activity was observed in root. Higher GR activity in root compared to leaf was reported by Satyakala and Jamil (1992). The authors found decreased GR activity in leaves and increased activity in roots of two Cr⁶⁺ treated aquatic weeds. However, some workers reported decreased GR activity in root compared to leaf in different stress conditions (Prasad et al., 1999; Pandey et al., 2005).

Peroxidase (POD) plays an essential role in hydrolyzing H₂O₂ to water and molecular oxygen (O₂) thus averting the cellular damage under un-favorable condition like water stress (Hernandez et al., 2000; Noctor et al., 2000; Sairam and Saxena, 2000; Hamilton and Heckathorn, 2001; Lin and Kao, 2001; Ushimaru et al., 2001; Chaitainya et al., 2002). POD activity significantly increased in young leaf and root of mine OB stressed plant species. Although there were species to species variation among the test plant the overall trend was that compared to old leaves in young leaves POD activities were higher due to mine OB stress. In roots also POD activities increased to different extent among the test plants due to mine OB stress, however compared to young leaf it was lesser. A young leaf is metabolically highly active where the likelihood of generation of more free radicals is more. The increased activity in all the organs particularly young leaf indicate that the plants try to overcome oxidative stress by raising the activity of POD to a higher level.

Ascorbate peroxidase (AXP) is chloroplastic or cytosolic enzyme that act in tandem with SOD to hydrolyse H₂O₂. Ascorbate peroxidase utilizes ascorbic acid as an electron donor in neutralization of H₂O₂ both in the cytosol and molecular components (Noctor and Foyer, 1998; Shigeoka et al., 2002). Ascorbate peroxidase is most efficient
in destroying the reactive oxygen species, which is present throughout the cell and it has higher substrate affinity in presence of ascorbic acid as a reductant (Shankar et al., 2004). The findings of the present study reveal that following mine OB stress AXP activity increases considerably in young and old leaves as well as root of all the test plants. However on a comparative basis AXP activity were significantly higher in root; particularly in some species there were two fold increase AXP activities. From the increased AXP activities, it can be inferred that following mine OB stress the plants experience elevated oxidative damage and like POD the plants try to overcome the same through increased activities of AXP.

Two other antioxidative enzymes viz., superoxide dismutase (SOD) and catalase (CAT) also exhibited enhanced activities due to mine OB stress in all the test plants. In case of SOD, compared to young and old leaves root exhibited much higher level of enzyme activity. SOD is considered as body's first line defence against free radical and is particularly efficient in scavenging ROS type free radicals. However, main function of catalase is hydrolysis of the reactive H$_2$O$_2$. The findings of the present work is in agreement with several earlier works which reported elevated level SOD activity under stress condition (Shanker et al., 2004; Feng et al., 2009; Bai et al., 2010).

Unlike the other enzymes nitrate reductase (NR) activities were found to be lower in all the test plants following mine OB stress. However, unlike the antioxidative enzymes NR is a growth related enzyme not associated with bodys biochemical damage. There are reports that following toxic heavy metals stress NR activity significantly goes down in root and leaves in lemongrass (Handique, 2000), paddy (Sharma, 2002) etc. Moreover, with reduction in NR activity there were concomitant reduction in plant growth. In the present study all, the test plants exhibited reduce
growth under mine OB stress condition, which appear to be due to reduction nitrate reductase activity.

5.8.1.3 Response of biochemical indicators following mine OB stress

The role of proline as osmoprotectant and as biochemical indicator of stress condition, particularly drought stress is well documented (Hagemann and Main, 1999; Xiang and Rajashekar, 2001). In the present study, following mine OB stress proline accumulation significantly increased in young and old leaf and not all the test plant species. Particularly in young and old leaves of some test plant species there were three to four fold increase in proline accumulation. Although, species to species variation occurred as a matter of general trend mine OB stress invariably induced proline accumulation in all the organs of all the test plant species. Studies of various worker revealed several function attributed to proline accumulation. Handique and Handique, 2009 reported proline accumulation in C flexosus following toxic heavy metal stress where possibly it acts as scavenger of free radicals. There are evidence that accumulated proline function by protecting vital enzymes and through osmoregulation (Sharma and Dietz, 2006). Whatever may be the mechanism in the present study proline accumulation appear to be a protective device for abiotic stress and hence can be considered as a biochemical marker for stress response. Similar findings of proline accumulation adaptive response. Similar findings of proline accumulation adaptive response to stress have been reported by other workers (Hanson et al., 1979; Marin, 1999; Hagemann and Yang et al., 2000; Xing and Rajashekar, 2001; Girija et al., 2002; Gill et al., 2003).

Soluble sugars, which are commonly defined as mono-and disaccharides, are osmotically active compound and respiratory substrate. Therefore, they play a critical role in maintaining osmotic balance apart from being cellular energy source. Hence,
their accumulation or non-accumulation is generally considered as indicative of metabolic status of plant. In the present study following mine OB stress soluble sugar content increased significantly in both the young and old leaf. Although species to species variation occurred the general trend was that mine OB stress enhance sugar accumulation in leaves. There are reports that soluble sugar plays a critical role in balancing the oxidative stress in plants (Coue et al., 2006) by scavenging ROS. For this, many workers opine that soluble sugar accumulation act biochemical indicator for stress response (Roitsch, 1999). Several workers reported enhanced sugar accumulation under abiotic stress condition (Leborgne et al., 1995; Tumanov and Trunova, 1957; Tumanov et al., 1968) and the findings of the present study appear to be in conformity with them.

Chlorophylls are the most important component of photosynthetic machinery and any increase or decrease in chlorophyll content is bound to increase or decrease the growth of plant. In the present study as a consequence of mine OB stress chlorophyll a contents were significantly reduced in young leaves of all the test species with one or two exception. Chlorophyll b which constitute a smaller fraction also exhibited similar trend. In case of old leaves, also chlorophyll contents were reduced. The main difference between young and old leaves was that in old leaves reduction in chlorophyll content was lesser compared to young leaves. There are reports that different stress induces changes of chlorophyll content of plant. Drought stress produce changes in the ratio of chlorophyll ‘a’ and ‘b’ and carotenoids (Anjum et al., 2003a). Several studies reported the reduction of chlorophyll content in drought stress in different plant species (Tahkokorpi et al., 2007; Massacci et al., 2008; Jaleel et al., 2008a; Jaleel et al., 2008b; Jaleel et al., 2008c; Jaleel et al., 2008d; Kiani et al., 2008). Significant reduction of chl
a: b ratio under water limiting regimes was reported by Estill et al., 1991 and Ashraf et al., 1994.

5.9 Accumulation of major elements, essential and trace metals and toxic heavy metals

Study on accumulation of vitally important elements like carbon, nitrogen, hydrogen, alkali metals like sodium, potassium, magnesium etc are of paramount important for stress biology and phytoremediation effort. The basic elements are the constituents of all important biomolecules that in turn constitute the plant body. Alkali metals and trace metals have a regulatory role in all metabolic activities. Therefore accumulation of such elements, metals etc in terms of increase or decrease reflect the overall nutrient status as well as status of metabolism etc. Mine OB or such hostile habitat represent acute abiotic stress where growth of even tolerant plant species are retarded. However, with progress of time and phytoremediation process growth and development shows improvement to varied extent. Against this background monitoring the accumulation of these elements, metals etc generate valuable information.

5.9.1 Accumulation of major elements and essential alkali metals

In the present study elemental analysis reveal that for any of the test plant species and for any organ out of carbon, hydrogen and nitrogen, carbon alone constitute 75% to 85% on an average. Hydrogen and nitrogen accounts for the rest. It has been observed that mine OB stress resulted in slight to significant reduction in carbon content for all the species with exception in *D. sissoo* and *Cassia sp*. Like carbon, accumulation of nitrogen and hydrogen varied from species to species but the general trend was that following mine OB stress nitrogen and hydrogen varied from content were reduced to different extent for most cases except *D. sissoo* and *Cassia sp*. A general reduction of carbon, nitrogen and hydrogen imply reduction in nutrient level
within plant body. This correlate well with overall reduction of plant growth for most species under mine OB stress.

Unlike major elements, alkali metal sodium exhibited a different trend. In case of stem and root for most species mine OB induced slight to significant increase in sodium accumulation. For leaf, however there was increase for three species while the rest exhibited reduction. The average trend is therefore towards increase following mine OB stress. Among the alkali metals in the present study potassium occurred in relatively much higher proportion. The response of potassium to mine OB stress was similar to that of sodium. The general trend observed was that mine OB stress induced increased accumulation of potassium in most cases.

Unlike sodium and potassium, however magnesium did not exhibit any well defined trend while for same species there were increase following mine OB stress for others there were decrease. The only exception was *M. pigra*, which consistently exhibited increased accumulation in all the organs.

5.9.2 Accumulation of essential trace metals

Trace metals like zinc, iron, manganese, copper etc are needed in trace amount but they play a major role in cellular metabolism since they constitute active constituent of many enzymes as activator. In the present study zinc accumulation did not show any general trend and rather species specific pattern was evident. While three species viz., *M. pigra*, *D. sissoo* and *C. winterianus* exhibited, increased accumulation of zinc following mine OB stress to other exhibited the opposite trend. The mine OB of Tirap colliery had zinc level nearly twice that of normal un-mined soil which cannot be considered as highly elevated level. It appears that zinc uptake mechanism and ability is different from species to species and zinc content in the soil doesn’t influence the same. Like zinc, iron accumulation did not exhibit general trend following exposure to mine
OB stress. Iron content in mine OB of Tirap colliery was three times more than normal
un-mined soil. Despite this species like *S. rostrata* and *C. flexosus* exhibited increased
level of iron following mine OB stress while the others were characterized by
decreased level of iron due to mine OB stress. Therefore, iron uptake and accumulation
was species specific. Unlike other metals, manganese accumulation exhibited a definite
trend. Following mine OB stress manganese accumulation increased in all the test
plants and in all the organs. However, on a comparative basis accumulation in leaf was
more than that of root and stem.

Although trace metals have well defined biological role and plays important
role in metabolic activities yet beyond a threshold limit they can be toxic. For
manganese normal range is 20-50 mg kg\(^{-1}\) dry weight and excess of this amount is
toxic (Reeves and Baker, 2000; Lie *et al.*, 2007). In the present study, manganese
contents were found to be higher than normal range and this may be one of the reasons
for oxidative stress observed. Similar adverse effect of manganese, due to excess
accumulation was observed by several workers (Chaney, 1973; Davis, *et al.*, 1978;
Sikora *et al.*, 1980; Mathews and Thornton, 1980; Faber and Niezgoda 1982; Andrea,
1984; Davis, 1997).

**5.9.3 Accumulation of toxic heavy metals**

While certain heavy metals are required by plants in trace amount for their
normal metabolism, some others like nickel, lead, cadmium, mercury, chromium etc
have no biological function; rather even at small concentration they adversely affect
metabolism and hence toxic even at very low concentration (Salt *et al.*, 1995). Coalmine OB often contain such toxic heavy metal at various concentration and hence
it is possible that the mine OB tolerant plant absorbs and accumulate such metals.
In the present study, Ni accumulation was found higher in leaf of *Cassia sp.*, *M. pigra* and *G. arborea* grown in mine OB compared to that of un-mined soil grown plant. In stem, significantly higher accumulation was found in all the plants except *S. rostrata* and *C. winterianus* compared to plant grown in un-mined soil. In root, significantly increased Ni accumulation was observed for mine OB grown plants were found in *M. pigra* for mine OB grown plants to that of un-mined soil grown plants. Compared to the root and shoot (leaf+stem) part higher accumulation was found in shoot part. Ni accumulation was found to be species specific and a general trend was tacking in some species particularly for root there were both significant increase as well as decrease. At high concentrations of the available form in soils (e.g., 10 μM in solution for winter wheat), in some plants it induces toxic effects such as growth and photosynthetic activity reduction, chlorosis, and necrosis (Foy et al., 1978). There are conflicting reports about the status of nickel for plant nutrition. The general opinion is that it is toxic (Salt et al., 1995) while other opine that it is needed as micronutrient for some plant species like legumes (Eskew et al., 1984; Brown et al., 1987).

Unlike nickel, lead accumulation exhibited a definite trend- there were increased accumulation in all the organs in all the test plants. However compared to leaf and stem root accumulation were comparatively lower. This indicate that although root is the absorbing organ it does retain in it, rather it is translocated to aerial part where it is accumulated. The present study revealed pronounced species specific variation in lead accumulation. While species like *S. rostrata* and *C. flexosus* characterized by much lower accumulation in all organs. *M. pigra* and *G. arborea* were characterized by very high level of accumulation in all the organs. This signify that tolerance mechanism vary from species to species. While *S. rostrata* and *C. flexosus* tolerate the lead toxicity by avoiding or minimizing its absorbance. *M. pigra* and *G.*
arborea tolerate by accumulating them in different body parts. However all the test plants in the present study exhibited accumulation above phytotoxic level indicating that all of them are tolerant to varied extent. Lead toxicity is known to be associated with retarded growth and disturbance in normal metabolism in C. winterianus (Deka Boruah, 1999); C flexosus (Handique, 2000) and paddy (Sharma 2002). The retarded growth for the test plants under mine OB stress appear to be in conformity with these earlier works.

Among the heavy metals with no known biological function and rather detrimental to living organism; Cd is known to be among the most toxic (Majare and Bulow, 2001). Like lead, it is also a soil and water pollutant emanating from industrial activities and mining operations and a matter of public concern. In the present study mine OB of Tirap colliery was found to contain 4.76 μgg⁻¹ cadmium and it is high enough to have toxic effect on plants. In the present study, there were pronounced species to species variation in cadmium accumulation but the general trend was that there were increased accumulation in all the parts in all the test plant species. The fact that plants response to cadmium is highly variable is evident from the fact that while in some cases there was no induction at all (eg. Root of D. sissoo) in other cases there were nearly ten fold increase (stem of D. sissoo) eight fold increase (stem of Cassia sp). Cadmium toxicity in plant has attracted the attention of many researcher and lot of information are available about its mechanism of function. Basically, it severely affects the function of various vital enzymes and depending upon concentration may indicate it (Sharma and Dietz, 2006). This finally manifest in retardation in growth and development and even fatality (Deka Boruah, 1999).
The use of trees as a vegetation cover for eco-restoration and phytoremediation of land contaminated by mine OB does seem to have considerable potential. There is plenty of evidence from the natural establishment of trees on contaminated sites that some types of trees can survive under such adverse conditions. This implies that plants with natural ability to survive and grow under harsh abiotic stress exist in nature. Natural revegetation and restoration takes several decades to more than a century depending upon the degree of abiotic stress, local climatic condition and availability of local tolerant plant species. The purpose of eco-restoration is to spend up the process and cut down the time period to less than a decade for productive use of land. The outcome of eco-restoration depends on the nature of the plant distribution after restoration of the sites. Therefore, the record of ecological succession and processes is important to prove the success of eco-restoration (Eamus et al., 2005). In addition, the general challenge of eco-restoration is in developing a site-specific strategy, as all sites around the world are not alike in terms of local climatic and geographical condition and richness of local biodiversity (Hariis et al., 2006). The ecological succession measures studied were plant diversity, vegetation structure and ecological processes.

The most challenging task in eco-restoration is initial establishment of selected plant species. Once initial establishment of selected plants is successful, secondary and tertiary succession can occur naturally and in a speeded up manner. In the present study, a number of technologies were devised and applied with remarkable success. The most important and effective was local amendments. This involves digging rows of small pits in selected pots filling the same with normal un-mined soil collected from farmland and supplements the same with FYM. Second important consideration was use of several plant species at the same time. In the present study apart from the
selected seven plant species taken for control experimentation, a number of other plants which showed good degree of tolerance were used for initial plantation. The idea was that if one or few species died others will survive and the continuity of the process will be maintained.

Additionally the dead plants were replaced with fresh planting. Third important consideration was timing of initial plantation. Under NE climate condition pre-monsoon period which correspond to April-May is most ideal since after plantation rain water help in establishment of vegetation. Conversely, during dry water period there is no chance of success.

The present study and eco-restoration effort yielded remarkable success. After three years of observation, period 80% to 100% vegetation cover was observed. The frequency of species density increased from 8% to 50%. This apart biomass production was found to be 700 cm².

In the present study as secondary succession a number cryptograms viz., bryophytes (*Riccia sp.*, *Marchantia sp.*, *Anthoceros sp.*, and moss species), pteridophytes (*Lycopodium sp.*, *Selaginella sp.*, *Pteris sp.*), herbs, shrubs and trees appeared in the restored mine OB. Overall, a secondary sere ecological succession was observed in the restored mine OB site.

The vegetation height in the restored site was determined by both understory and tree species. Soil organic matter is considered to be an indicator of total biomass production in restored sites; in the present investigation, following eco-restoration effort there were remarkable increase in soil organic matter from 0.005% to 1.5% after the observation period. Dobson *et al.*, (1997) reported a similar strategic approach to achieve a climax state of succession within a short span of time. In the present
investigation, a 100% succession of primary and secondary ecological states was achieved by cultivating various plant species.