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Application of Microorganisms in Rehabilitation of Degraded Habitats and Mining Industry - Review

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Abstract

Deforestation and habitat loss is not about losing a few plants and animals, but also the survival of man hangs on it (Butler, 2019). Deforestation aids the mechanism of altering the already endangered planet earth; making it dangerous for plants and animals to survive it. The quest for more lands for agricultural activities and human expansions is further reducing the forested areas on the surface on the earth. Environmental education is the key to reversing continuous and deliberate human action through the protection of every natural forest and afforestation where necessary. Soil microbes play an important role in many critical ecosystem processes, but little is known about the effects of land reclamation. A fundamental shift is taking place worldwide in agricultural practices and food production. In the past, the principal driving force was to increase the yield potential of food crops and their productivity. Today, the drive for productivity is increasingly combined with the desire and even the demand for sustainability. Sustainable agriculture involves successful management of agricultural resources to satisfy human needs while maintaining environmental quality and conserving natural resources for future. Improvement in agricultural sustainability requires the optimal use and management of soil fertility and its physico-chemical properties. Both rely on soil biological process and soil biodiversity. This implies management practices that enhance soil biological activity and thereby buildup long term soil productivity and crop health. Such practices are of major concern in marginal lands to avoid degradation and in restoration of degraded lands at regions, where high external input agriculture is not feasible.

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Introduction

Microorganisms are the most ubiquitous living entities on Earth. They are also the most diverse organisms, present in about every nook and corner of the blue planet. The history of mining is as ancient as the history of civilization. Many important cultural eras are identified by specific metals and its derivatives and in other words discovery of metals has shaped the evolution

of civilization (Mudd, 2010). Both agriculture and mining was the basic industry of early human civilization. Mining has a pivotal role in energy/ power industry, for infrastructure development, manufacturing industry and with fertilizer industry (Prior *et al.*, 2012). Besides it benefit mining has been detrimental to environment, and human health. Now a day, the world is plagued with the issue of enormous waste generation from various anthropogenic sources including mining

and industrial activities (Singh *et al.*, 2020). Industrial waste containing diverse heavy metals eventually get accumulated in the soil as they are released out from the dumping sites where these metal containing wastes are indiscriminately dumped (Ghosh *et al.*, 2018; Das and Mishra, 2008;). Metallic pollutants get absorbed by the trees growing in such soils, get biomagnified as a part of the food chain, eventually reaching the human body and causing serious harm.

Since mining and industrial activities have been continuing at a large scale, it has become imperious to manage the generated waste appropriately (Das and Mishra, 2010). Presence of heavy metals in the environment, even at low levels, poses a long-term cumulative health effect leading to major health concerns globally (Biswal *et al.*, 2021).

Causes of habitat destruction

Deforestation leads to habitat loss while preservation and conservation of the natural forest increase biological diversity. The reasons why the issue of deforestation and habitat loss should be seen as global concern and given urgent attention are so many; some of which are the exploding human population, the continuous scientific advancement of new uses for biological diversity and currently the extinction or the gradual disappearance of some rare plants and animals. The poor countries of the world have relatively low rates of deforestation because their available income is limited in carrying out large scale exploitation in their environment; and as the incomes of these countries rises, more lands are being deforested for the purposes of development (Meyer *et al.*, 2003).

Deforestation

Deforestation defined comprehensively to incorporate the change or conversion of a natural forest to a non-forest for purposes of agricultural services and development (Gimah and Bodo, 2019). In a nutshell, we can conclude that deforestation simply means the permanent transition from forest to all other land uses.

Forest Degradation

Forest degradation is a process which negatively affects the structural and functional characteristics of a forest. The degradation of the forest do not happen suddenly (like an earthquake) but a gradual process, that may take a long period before it is visibly ascertained, implying

that the forest degrades over time (Vasquez-Grandon *et al.*, 2018). The gradual degradation of the forest can also occur due to increased disturbance resulting in loss of forest products or the reduction of forest quality; the thickness and structure of the trees, the biological administrations that provided the biomass of plants and animals, the species variety and the hereditary variety.

When the quality of the forest drops, the forest's biotic components affected leading to reduction of the quality of the soil and water, and interactions between the individual components, ultimately affecting forest functioning and diminishing the provision of ecosystem goods and services.

Habitat destruction

Habitat destruction is the cycle in which natural habitat is harmed or wrecked so much that it is no longer capable of supporting the species and biological networks that normally happen there. This scenario brings about the elimination of species and, subsequently, the deficiency of biodiversity (Gimah and Bodo, 2019). Most habitats are greatly destroyed directly by the numerous human activities, where a large portion land is usually cleared for farming, mining, logging, hydroelectric dams, and developmental structures (Bodo and Gimah, 2019b). Very unfortunately, it is reported that humans are currently destroying natural habitats at a rate and spatial extent that exceed the level of at which most species and communities can survive (Gimah and Bodo, 2019). Aside from the human induced factors, habitat destruction can also be caused by natural circumstances like floods, volcanic eruptions, seismic tremors, and atmosphere variances.

Despite the fact that habitat destruction fundamentally causes species eradications, it can likewise open up new living space that may give a climate wherein new species can develop, in this manner exhibiting the versatility of life on Earth (Bodo and Gimah, 2019b). The continual quest of humans to develop the environment has led to habitat degradation. Of the factors that are aiding habitat degradation is urbanisation. On the rise, small villages are the turned into towns and cities; leading to an increase in human population and also the demand for land (Bodo, 2019a). This scenario will lead to a bigger reality where habitat degradation not only affects native species and communities but human populations as well. Human's survival depends on healthy lands, because degraded lands are frequently lost to erosion, desertification, and nutrient depletion.

Causes of Deforestation and Habitat destruction

Multiple factors are responsible for deforestation and habitat loss, which could either be of human or natural origins. Natural causes of deforestation could be as a result of forest fires, droughts, exotic animals, floods, overpopulation of foreign animals and climate change. That notwithstanding, human activities are reported to be among the main causes of global deforestation with agricultural expansion, cattle breeding, timber extraction, mining, oil extraction, dam construction and infrastructure development reported as the primary causes (Bodo, 2019c).

Agricultural activities

According to FAO, around 80% of global deforestation are caused by expansions in agriculture (Gimah and Bodo, 2019). Forested lands of more than 50,000 acres are cleared by farmers and loggers everyday worldwide, and the equivalent of over 10,000 football fields are destroyed daily in the Amazon Basin alone (Meek, T 2019). The constant destruction of our forests threatens biodiversity, decreases carbon absorption, magnifies natural disaster damage, and disrupts water cycles. Agricultural practices the type practiced in the developing countries increases deforestation and habitat loss. Forests are set ablaze to clear space for agriculture, taking vegetation and wildlife with them. This process kills most of the nutrient available in the soil that makes it fertile. The bad agricultural practice makes even the available lands barren, leading to reduction in food production and scarcity. Most of the deforested areas are in the rainforests, which are home to over 50 percent of plants and animals on the earth (Gimah and Bodo, 2019).

Urbanization

Usually in cities, people use more unprocessed or processed materials from the forest especially with the availability of more income for their expenditures (Bodo, 2019c). Firewood, timbers, and herbs are highly needed in bakeries, construction and pharmaceutical industries. There are always greater demands for animal products and processed foods, which in turn drives the quest for more lands for livestock grazing and cultivation of crops.

In most developing countries, the forested areas in the cities are being cleared to give way to developmental projects like the construction of markets, schools, parks, bridges, roads and industries. The governments of the developing countries focus more on developing and

modernizing their environment than the preservation of the forest. It is against this backdrop that some researchers have observed that urbanisation do not always lead to positive changes in the environment (Bodo, 2019c).

Consequences of Deforestation and Habitat Loss

Loss of Biodiversity

The most pronounced consequence of deforestation is the destruction of biodiversity. The forests house some of the most veritable hubs of biodiversity, covering mammals, birds, insects, amphibians or plants, the forest shelters many rare and fragile species. When humans engage in deforestation, they put the entire ecosystems in danger, creating natural imbalances, and putting their own lives at risk. The forest is a huge support system or a web of connectivity. For instance, the trees provide shade and colder temperatures for animals and smaller trees or vegetation which may not survive with the heat of direct sunlight. Besides, trees also feeding animals with their fruits while providing them with food and shelter they need to survive. Deforestation is very disastrous for plants and animals, as many may not survive the effect. Deforestation that is accompanied by bush burning, can wipe out an entire species.

Soil erosion

Deforestation usually reduces the quality of the soil. The soil in the forest is very rich in organic matter and also very resistant to erosion, bad weather, and extreme weather events. On the other hand, deforestation simply exposes the soil making it increasingly fragile, leaving it more vulnerable to natural disasters such as landslides and floods.

Deforestation will also pose a serious erosion problem to the environment. These eroded soils can lead to disastrous mudslides. Large amounts of soil can wash into local streams and rivers, clogging waterways and causing damage to hydroelectric structures and irrigation infrastructure. In certain areas, soil erosion issues caused by deforestation lead to farming problems and loss of reliable electric power (Cook, 2018). The truth is that, deforestation causes the soil erodes and washes away, causing farmers to keep moving to another land by deforesting more areas in quest of searching for a fertile land for agriculture. When a soil is eroded, it leaves the land barren and more susceptible to flooding, specifically in coastal regions.

Climate change

Trees, on a daily basis specialize in trapping and absorbing excess carbon dioxide (CO₂) from the atmosphere which could have been harmful to man. The fact remains that when we cut down trees, we are releasing the already trapped CO₂ back into the atmosphere. Very unfortunately, these trees are cut down for the purposes of agriculture; as records show that food and agriculture account for 24% of greenhouse gas emissions, while deforestation is estimated to be responsible for 10-15% of all anthropogenic CO₂ emissions (Cook, 2018). Global climate changes are result of greenhouse gases such as methane and carbon dioxide are gases that trap heat in Earth's atmosphere. Trees provide human with what they need most to survive which through the release of oxygen and water into the atmosphere, aside from eliminating the excess carbon dioxide through absorption. Global climate change can change survival of wild animals, plants and humans through drastic weather changes and increased possibilities of natural destructions. Every year forest clearing is contributing to the excess greenhouse gases in the atmosphere, leading to several habitat loss on earth because of the continuous cutting down of trees that acted as valuable carbon sinks (Gimah and Bodo, 2019).

Water cycle disruption

Water from Earth's oceans as well as from the surface of trees evaporates and condenses into clouds. Trees extract water from under the ground and release the same water into the atmosphere through a process called photosynthesis. Subsequently, this water in the atmosphere forms clouds that produce rain, which falls back on the earth forming run-offs on the surface, with some percolating downwards to produce groundwater and eventually ocean water again. Deforestation simply implies that there will be no tree to extract, store and release into the atmosphere. This means that cleared forests, which once had moist, fertile soil and plenty of rain become barren and dry (Cookey *et al.*, 2019). When the natural forest is cleared, there is usually less water in the air to be returned to the soil. This causes the soil to become dryer, reducing the ability of the crops to grow.

Outbreak of new disease

The invasion of the forest by human for food or for games has led to the emergence of tropical diseases and outbreaks of new diseases, including deadly hemorrhagic

fevers like ebola and lassa fever, which are consequences of deforestation (Butler, 2019). These exploiters, who are pushing deeper into the thick forest, usually encounter dangerous microorganisms that they can transmit to those outside the forest on their return. Unfortunately, this bad practice of forest invasion and destruction could lead to a massive epidemic that could kill many innocents on our planet (Butler, 2019). A scientific report reveals that land alterations especially the invasion of thick forests, brings humans in contact with pathogens (such as malaria and snailborne schistosomiasis). Most of these invasions are mainly for the purposes of development, leaving behind breeding grounds for mosquitoes because of the proliferation of artificial pools of water like dams, rice paddies, drainage ditches, irrigation canals, and puddles created by tractor treads (David and Bodo, 2019). Malaria has become a common problem in deforested and degraded areas, and far less is experienced in the forested zones (Butler, 2019).

Destruction of renewable resources

Valuable renewable resources are destroyed yearly through deforestation leaving behind barren lands. The forests are the source of renewable resources that can significantly contribute to the economic growth of a country on a continuing basis. When practiced properly, logging can be a sustainable activity, generating huge source of revenue without diminishing the resource base. According to World Bank, an estimate of about US\$5 billion in revenues is being lost annually as a result of illegal logging. Ecotourism of a nation also suffers from deforestation as no tourists, will want to travel in order to see polluted rivers, stumps of former forests, barren wasteland, animal carcasses, and abandoned settlement of former inhabitants (Gimah and Bodo, 2019).

Over grazing

Overgrazing is regarded as a serious pressure on the natural environment and a well-known desertification driver in areas where morphology, climate, vegetation cover, and soil are unsuitable for intensive agricultural use (Brunner *et al.*, 2008). Overgrazing can lead to many negative impacts, including decline in vegetation cover, biomass, species diversity and increase in undesirable vegetation. Unsustainable grazing is one of the most diffused land management problems at the global scale and associated with marginal, depopulated, and disadvantaged rural areas whose landscape is dominated by pastures, low value-added cropland, and fallow land (Papanastasis, 2004). Unsustainable grazing is a factor in

the degradation of agro-forest. Mediterranean landscapes (Simpson *et al.*, 2001). A sustainable management of pastures impacts positively on soil erosion and mitigates land degradation (Papanastasis, 2004). Moderate grazing contributes to soil preservation from water and wind erosion, creating, at the same time, favorable conditions for vegetation and thus improving soil fertility (Peco *et al.*, 2006). Poor vegetation cover reduces the water infiltration capacity of the soil and indirectly triggers land degradation (Carmona *et al.*, 2013). It was indicated that the rate of soil erosion decreases with vegetation cover (Elwell and Stocking, 1986) indicate 40% plant cover as the critical threshold under which accelerated soil erosion occurs.

Land degradation

Inadequate grazing management can lead to extensive land degradation. Land resource degradation in the Himalayan region occurs mainly due to landslide, mudslide, man-made terraces, and soil loss from steep slope, and decline of forest/pasture areas (Shrestha *et al.*, 2004). Degradation is broadly natural and anthropogenic. In general, soil erosion, flood, and landslides caused by torrential rain, forest fire, slash and burn cultivation, inappropriate agriculture practices, uncontrolled grazing and overgrazing, encroachment and unplanned settlements, and so forth are the common problems of the Churia hills (Paudel and Kafle, 2012). Land degradation by salinization and sodication is one of the most important environmental problems under unprecedented regional and global climate (Wong *et al.*, 2010). Accelerated soil degradation and soil loss resulting from agricultural activities have been observed globally and are increasingly recognized as a threat to food production systems landscape stability water quality and ecosystem functioning (Kroon *et al.*, 2016).

Land degradation has a wider scope definition than both soil erosion and soil degradation in that it covers all negative changes in the capacity of the ecosystem to provide goods and services. Soil degradation caused by human activity involves: physical, chemical and biological mechanisms (Lal, 2001).

Soil erosion

Soil erosion is the removal topsoil due to natural, animal, and human activity. Natural activity includes the removal of soil by the action of water and wind. Soil erosion is the natural phenomenon in which removal and transportation of soil material occur through the action of

erosive agents such as water, wind, gravity, and human disturbances (Aksoy and Dirim, 2009). It is the biggest threat to soil fertility and productivity, mostly due to human disturbances (Kefi and Yoshino, 2010). Soil erosion causes negative impacts on the environment and the economy, removes organic matter and important nutrients, and prevents vegetation growth, which negatively affects overall biodiversity.

Loss of soil fertility in arable lands and decline in the soil quality resources are the consequences of soil erosion (Kagabo *et al.*, 2013). Soil erosion process alters the physical, chemical, and biological characteristics of soil, which creates a reduction in agricultural productivity and arable land size and causes facing the problem of food scarcity in the context of growing world population. It created severe problems for human sustainability on agricultural productivity, water quality, hydrological system, soil fertility, and environments (Lal, 2015). Soil erosion has impacts both at the point where soil is detached (on-site) and at the point where the soil is deposited (off-site). On-site impacts of soil erosion include the reduction in soil quality resulting in the loss of the nutrient-rich upper layers of the soil, and reduced water-holding capacity of many eroded soils.

Climate change/global warming

The overcutting of trees can change the global and local climate, not only through the micrometeorological process but also by increasing the concentration of carbon dioxide in the atmosphere (Pinker, 1980). Consequence of felling trees will lead to climatic variation and unsustainable land use. It causes global warming which includes; desertification, temperature shift, precipitation, ozone depletion and atmospheric pollution. Climate change poses a direct as well as indirect impact on soil processes, where soil moisture portrays an illustrious part. It regulates the accessibility of water and nutrients to plants, govern soil thermal regimes, and drive the biological activity of soil (Medhi *et al.*, 2021).

The climate change tempted consequences to lead to rapid mineralogical as well as chemical changes in soil comprising steady dehydration of goethite to haematite in response to elevated temperatures or severe drying, loss of nutrient cations in regions where leaching increases, and salinization where net uphill water drive follows, owing to it to amplified evapotranspiration rate or decline in rainfall or water supplication through irrigation (He *et al.*, 2018).

Fire

The impacts of fire on ecosystems depend on a set of interactions at spatial and temporal scales. The natural fire regimes are characterized by fire intensity, duration, and extent, as well as the time of the year and frequency at which the disturbance occurs (Cochrane, 2009). Fire frequency and severity affect the distribution and abundance of species (Chia *et al.*, 2016), population sizes, and the availability of food and refuge resources as well as several ecological interactions, such as competition and predation (Letnic *et al.*, 2013).

Effects Fire on Wildlife

Fire has influenced composition, structure and landscape patterns of animal habitat. Wildlife may be affected by fire both through direct mortality or habitat alteration (Lyon *et al.*, 2000b). Some fires alter the vegetation structure of forest, which is work as shelter and hiding cover for wild-animals and vegetation structure spatially arranged all the resource needed to live and reproduce. Dead wood on the ground is an essential habitat component for many birds, small mammals and even large mammals, including bears (Bull and Blumton, 1999). Fire cause large dead logs on the ground, harbor many invertebrates and are ants; they also provide shelter and cover for small mammals, amphibians and reptiles. Ground-nesting birds could be killed prior to fledging (Reinking, 2005) and forest floor arthropods in the egg or larval stages may be more vulnerable to loss (Niwa and Peck, 2002). Dark-eyed juncos (*Junco hyemalis*) often choose nest sites in unburned patches within prescribed fire. Amphibians are also likely to be more active with the moister conditions under which prescribed fires are typically conducted. The longer term responses of many bird species are thought to be due primarily to structural changes of vegetation or changes to food resources, as affected by fire severity (Kirkpatrick *et al.*, 2006).

Fire Effects on Animal Populations

Bird populations respond to changes in food, cover, and nesting habitat caused by fire. The season of burning is important to birds in two ways: Fires during the nesting season may reduce populations more than fires in other seasons; and migratory populations may be affected only indirectly, or not at all, by burns that occur before their arrival in spring or after their departure in fall. Fire effects on insect- and plant-eating bird populations depend on alterations in food and cover. The canyon

towhee, which eats insects and seeds, increased after stand-replacing fire in chaparral, foraging for food in the recent burn (McClure, 1981). Large mammals may move into burned habitat simply because of familiarity with the area before fire.

Fire Effects on Animal Communities

Many animal-fire studies depict a “reorganization” of animal communities resulting from fire, with increases in some species accompanied by decreases in others. Descriptions of faunal communities after fire, however, are much less prevalent than descriptions of population changes. The literature about fire and bird communities is more complete than the literature about other kinds of animals. In this chapter, we use the literature about fire and birds to search for response patterns in the relationship between fire regime and changes in bird community composition. The literature does not at this time provide enough studies of mammal communities to complete a similar analysis. Each animal species in a community is likely to respond differently to fire and subsequent habitat changes. To synthesize information about these responses, we modified Rowe’s (1983) classification of plant responses to fit animal responses to fire. Rowe’s approach was to assign to each plant species an adaptation category based on reproduction and regeneration attributes in the context of fire. Using similar categories in our evaluation of the animal-fire literature, we classified species’ responses (not species themselves) for a given study location using observed changes in animal abundance. Mean changes in species abundance before and the first few years after fire, or in burned versus unburned areas, can be classified into one of six categories.

Fire Effects on Fauna at Landscape Scales

Landscapes are spatially heterogeneous, characterized by structure, function, and temporal variation (Forman and Godron, 1986). Landscape structure encompasses the spatial characteristics of biotic and abiotic components in an area and is described by the arrangement, size, shape, number, and kind of patches (homogeneous units). Landscape function is defined by interactions among biotic and abiotic components. Temporal variation of a landscape is expressed by changes in structure and function over time. Configuration of patches affects the occurrence and spread of subsequent fires, so landscape-level feedback is an important part of fire effects at landscape scales (Agee, 1998). Fire’s most obvious function in landscapes is to create and maintain a mosaic

of different kinds of vegetation (Mushinsky and Gibson, 1991). This includes size, composition, and structure of patches, as well as connectivity (linkages and flows) among patches.

When fire increases heterogeneity on the landscape, animal species have increased opportunities to select from a variety of habitat conditions and successional stages. Fires often burn with varying severity, increasing heterogeneity. Adjacent unburned areas (which may surround or be embedded in the burn) serve as both sinks and sources for animal populations, and also influence animal emigration and immigration patterns (Pulliam, 1988). Bird diversity after stand-replacing fire may be higher on patchy or small burns than on large, uniform burns because the small areas are accessible to canopy and edge species as well as species that use open areas. Some animals require habitat that contains different features at different scales.

Microbiology of Biomining

By their mode of nutrition, chemolithoautotrophic bacteria and archaea, heterotrophic bacteria and heterotrophic fungi are used for biomining process. These microorganisms are referred as mesophilic because they carry out their activity at a temperature in the order of 40 °C or less. Chemolithoautotrophs used in biomining are acidophilic microorganisms, which are having the ability to fix CO₂ by oxidizing the ferrous iron or reduced sulfur and produces ferric iron or H₂SO₄. Solubilization of metal sulphides by ferric iron or H₂SO₄ decreases the pH of surrounding and as a result further enhance the solubilization of metals, because low pH (1.5-3.0) is best for microbial leaching due to the fact that at low pH most of the metals stay in solution (Bosecker, 1997). Acidithiobacillus thiooxidans being a facultative aerobe also thrives in anaerobic conditions by using reduced compounds of sulphur and ferric iron as an alternate acceptor of electron (Sugio *et al.*, 1985).

Mechanism and Techniques of Biomining

The microbial (bacteria and fungi) ability of metal leaching and mobilizing from solid substances has three principles. They are (i) redox (oxidation and reduction) reactions, (ii) acid formation (organic or inorganic acids), and (iii) the secretion of complexing agents (Brandl, 2001).

In terms of the contact between microbes and the minerals to be leached, the facilitation by redox reaction

is either on direct or indirect bioleaching. In direct bioleaching electrons are transferred directly from the reduced minerals (Metal sulfide) to bacterial cells. In this case close contact between bacterial cell and reduced mineral is needed. The adherence of cells to metal sulfide takes few minutes to hours with the help of electrochemical process (Sugio *et al.*, 1985; Mishra *et al.*, 2005). Microbial growth and metabolism can be negatively affected by dissolved metals, which in turns limit productivity. Ferric sulfate is produced by oxidation of pyrite in this mechanism. Indirect bioleaching method is most appropriate for lower sulfur and sulfide minerals as sources for H₂SO₄ production by autotrophs (Anjum *et al.*, 2012).

Bioremediation in mineral beneficiation

Microorganisms play a significant role in management of these toxic wastes without damaging the environment through biotransformation of these pollutants from their toxic to nontoxic form. Bacterial cells are capable of catalyzing metal dissolution from minerals and bacteria mediated leaching processes are faster in comparison to the chemical processes at ambient temperature and atmospheric condition (Sanket *et al.*, 2017; Mohanty *et al.*, 2018; Prabhakar *et al.*, 2019).

Through biomining process microorganisms are applied to recover precious minerals and metals of commercial significance from mining and industrial waste residues. This technology is applied for environmental clean-up sites contaminated with heavy metals and other pollutants. At present biomining technology is used to extract minerals valuable from metals like manganese, lithium, copper, lead and gold from mining and industrial wastes (Mohanty *et al.*, 2016). Diverse industrial scale bioleaching operations are currently adopted for the extraction metals from waste mining residues and in-situ bioleaching. Microbiological contribution in metal solubilization process explored in the last few decades and their utility has been very well recognized. bacterial strains have the capability of using the oxidation of inorganic compounds to generate energy, which is essential for their growth and viability making them a chemolithotroph (Pal *et al.*, 2010).

Bioremediation and its application

The utility of microbes in mineral beneficiation, recent biotechnological developments have hinted at the possibility of coming up with absolute solution for the bioremediation of environmental problems. One such

study reports the remediation of mining wastes in which bioleaching for recovery of manganese was carried out by bacterium *Lysinibacillus* (Ghosh and Das, 2020). Bioremediation of crude oil and heavy metal contaminated waters was also achieved by microbial intervention. Other processes of remediation include Bio-stimulation and Bioaugmentation. Bio-stimulation involves managing naturally occurring microbial population for providing an environment for optimization of growth, viability and activity of microorganisms.

Bio-stimulation methods include bio-venting, air sparging, nutrient addition and oxygenation. Microbial biomining process mostly utilizes the native microbial communities which are common in the environment by guaranteeing that the operation is conducted under controlled conditions. Microorganisms therefore provide a means for recovering and recycling precious mineral from low grade ores, mining scraps and Industrial solid waste residues and effluents (Seifelnassr and Abouzeid, 2013). The applicability of microbes in the process of remediation of industrial wastes gets hampered due to the unavailability of sufficient research data correlating to industrial applications. The metal load ends up being too high for the microbes to sustain naturally and extensive research for optimization of the process for optimum output is the need of the hour. Investigation of microbial bioleaching process and the use of microbial metabolites could help in elevated mineral recovery from mining, industrial and natural resources.

Advances in bioleaching technology

The advancement of bioleaching technology has been investigated for different applications including polluted wastewater, industrial effluents, end of life batteries, and many more through biological approaches and received significant consideration (Mishra and Das, 2021).

Omics technologies is the most powerful upcoming tool that can extend a great aid in understanding the genomic and proteomic complexities of bioleaching microorganisms leading to the discovery of novel genes, enzymes, metabolic pathways, and also novel species. multi-omics analysis consisting of metagenomics, meta proteomics and meta transcriptomics can aid in linking data at different biomolecular levels, leading to complete understanding of bio-mining mechanisms (Ghosh and Das, 2018; Martinez *et al.*, 2015). Bioleaching using nanoparticles and biosensor-mediated bioleaching is also expected to give this technology a major boost in the

future and the ongoing research on commercial scale applications of this process have paved way for this technology to pick up high momentum in the near future.

Paper contribution to special issue

Bioleaching technology is mostly used for cleaning environmental pollutants and recovery of minerals from different wastes. Extractions of valuable minerals from mining residues, low grade ores, and metal deposits have drawn the attention of scientific investigators.

Microbial Activity and Soil Degradation

Soil organisms, especially, the microbiota, play a huge role in the cycling of elements and stabilisation of soil structure. The mineralization of organic matter is carried out by a large community of microorganisms and involves a wide range of metabolic processes. Biological processes are intimately linked with the maintenance of soil structure and fertility and are potentially more sensitive to changes in the soil than indicators based on physical and chemical properties (Nannipieri *et al.*, 1990; Brookes, 1995). Biological indicators can provide early warning of system collapse and allow us react before irreversible damage occurs.

Microbial Biomass and Their Habitat

Microbial status in ecosystem can be assessed through the genetic characteristics of the soil microbial community and gives the idea about quality of the soil and the progress of restoration after degradation (Harris, 2003; Singh, 2015). To control the microbial community, soil organic carbon and pH were the most important factors and soil total nitrogen was a potentially important factor for soil microbial composition and function. In addition, soil moisture, CEC and physical structure to a lesser extent. The changes in vegetation, management practices and other anthropogenic activities in the process of reclamation impose different impacts on the soil micro-environments in which microbes exist and that the variation in edaphic environmental conditions would be the most crucial factor affecting the soil microbial community.

Soil microbes

Microbial biomass is both the agent of biochemical changes in soil and a repository of plant nutrients that are more labile than the bulk of soil organic matter (Patra, 1994). The level of soil microbial biomass and the

activity of top soil organisms are important factor in determining the soil health. Microorganisms play all important role in regulating ecosystem processes ranging from nutrient mineralization and cycling, to soil carbon storage, trace gas fluxes, transformation of aqueous solutes and processing of water pollutants and for driving above ground ecosystem (Ovreas, 2000).

Micro-fauna recycle OM that is trapped in bacteria, fungi, protozoa and they create more surface area for fungi and bacteria to act upon by breaking down organic matter. Soil microbe populations are one of the important soil components. Soil microbes play a great role in aggregate stabilization which consequently maintains suitable structural conditions for cultivation and porosity for crop growth (Ghose, 2005). Their activity declines when soil layers are disrupted and is slow to resume independently. Soil microbes produce polysaccharides that improve soil aggregation and positively affect plant growth (Williamson and Johnson, 1991). Sites with an active soil microbe community exhibit stable soil aggregation, whereas sites with decreased microbial activity have compacted soil and poor aggregation (Edgerton, 1995).

Role of microbes in the soil

Microbes contribute to soil formation through nutrient cycling and organic matter production. Microbial products are critical to soil aggregation, improved soil structure making soil more habitable for plants. Soil microbial communities constitute a diverse group of microorganisms whose activities can positively or negatively impact the growth and productivity of plants.

Soil biological activities are mainly performed in the top portion of the soil, up to a depth of 25–30 cm. In this top portion, biological fraction comprises a small part (<1%) of the total volume of soil, which is lesser than 1/tenth of the total soil organic matter (Kumar *et al.*, 2017).

Soil microorganisms play an indispensable role in ecosystem services, such as C sequestration and nutrient cycling, by degrading and transforming plant debris and organic compounds (Martin *et al.*, 2013). The growth, activity, size, and composition of soil microorganisms are affected by abiotic and biotic factors, including tree species, the quality and quantity of organic matter input, soil properties (such as soil pH, moisture, temperature, and nutrient), and physical disturbance (Smith *et al.*, 2015). Various soil-dwelling arthropods alter soil microbial communities and associated soil functions.

Microbes living in the soil develop active, diverse, and often underappreciated ecosystem. Soil microorganisms can be classified nutritionally based on the nature of the energy source for generating adenosine triphosphate (ATP) and the nature of the principal carbon source used for cell growth. Microbial communities in soil Microorganisms affect the structure and fertility of different soils and contribute to nutrient availability in soil (OM decomposition, humus formation, N-fixation, seed germination) manage soil stability by different biochemical processes Degrade pesticides and chemicals in soil Contribute the growth and success of the plants and overall ecosystem of a soil environment.

Soil Microbial Role in Restoration of Degraded Lands

The mineral content and its physical structure are important for balanced condition of soil. In native soil the soil biota includes vast numbers of microorganisms that naturally reside in soil and perform a wide range of functions which are essential for a normal and healthy soil, but in a disturbed soil the micro-organism decreases. Soil microbe regulates the production and destruction of pollutant like nitrous oxides, methane, nitrates and other biologically toxic compounds (Doran and Linn, 1993). They also form the symbiotic associations with roots. Many human activities such as urban development, agriculture, mining, use of pesticides and pollution can affect soil microbial diversity.

Microbial biomass is one of the components to measure the restoration progress of the degraded areas. To assess soil development, the microbial properties such as the amount of soil microbial biomass, soil respiration rate and metabolic quotient have been used (Grahm and Haynes, 2004; Frouz and Novakova, 2005). With increase in soil organic carbon and microbial biomass the functional diversity of soil microbial communities may increase that consequently increases the functionality and stability of soil ecosystems (Yan *et al.*, 2000; Lynch *et al.*, 2004).

The soil microbial population consisting of bacteria, fungi and microfauna (Micro means microscopic that one can't see with naked eyes and fauna means animals) are termed as soil microbial biomass (SMB) and it is closely related to the soil organic matter (SOM) (Dwivedi and Soni, 2011). During decomposition, the SMB assimilates complex organic substrates for energy and biomass carbon with excess inorganic nutrients being released to the soil. During restoration of degraded lands, it is important to establish and maintain a vegetation cover

without the use of top soils or other bulky amendments (Rimmer, 1982). To recover the fundamental functionality of the soil ecosystem, it is requisite to make a strategy to catalyze the natural return of some of the basis for more restoration processes. Authors suggested, in the initial stage of the soil restoration, mineralization rate of soil OM is dependent of substrate supply and the size of microbial population (Ross *et al.*, 1990).

The ratios of microbial biomass C: total C and respiration: microbial C has been taken as measures of the success of reclamation efforts (Insam and Domsch, 1988). The soil microbial biomass and its activities are dependent on the quality, quantity and turnover of detrital organic matter in the forest floor.

Bacteria

Bacteria play an important role in decomposition of organic materials, in the early stages of decomposition when moisture levels are high. In the later stages of decomposition fungi tend to dominate. Rhizobia are single celled bacteria, belongs to family of bacteria Rhizobiaceae, form a mutually beneficial association, or symbiosis with legume plants.

These bacteria take nitrogen from air and convert it into a form of nitrogen called ammonia (NH_4^+) used by plants (Gil-Sotres *et al.*, 2005). Free living as well as symbiotic plant growth promoting rhizo-bacteria can enhance plant growth directly by providing bioavailable P for plant uptake, fixing N for plant use, sequestering trace elements like Fe for plants by siderophores, producing plant hormone like auxins, cytokinins and gibberlins, and lowering of plant ethylene levels (Khan, 2004). When soil layers are removed and stockpiled, the bacteria inhabiting the original upper layers end up on the bottom of the pile under compacted soil. A flush of activity occurs in the new upper layer during the first year as bacteria are exposed to atmospheric oxygen. After two years of storage there is little change in the bacterial numbers at the surface, but less than one half the initial populations persist at depths below 50 cm (Williamson and Johnson, 1991).

Fungi

These organisms are responsible for the important process of decomposition in terrestrial ecosystems as they degrade and assimilate cellulose, the component of plant cell walls. Fungi are constituted by microscopic cells that usually grow as long threads or strands called

hyphae of only a few micrometres in diameter but with the ability to span a length from a few cells to many metres.

Algae

Algae are present in most of the soils where moisture and sunlight are available. Their number in soil usually ranges from 100 to 10,000 per gram of soil. They are photoautotrophic, aerobic organisms and obtain CO_2 from atmosphere and energy from sunlight and synthesize their own food.

Protozoa

Soil protozoa belonging to the class ciliate / ciliophora are characterized by the presence of cilia (short hair-like appendages) around their body, which helps in locomotion. They are abundant in the upper layer (15 cm) of soil. Protozoa can be split up into three categories: flagellates, amoebae, and ciliates. Soil moisture, aeration, temperature and pH are the important factors affecting soil protozoa. Most of protozoans derive their nutrition by feeding or ingesting soil bacteria belonging to the genera *Enterobacter*, *Agrobacterium*, *Bacillus*, *Escherichia*, *Micrococcus*, and *Pseudomonas* and thus, they play important role in maintaining microbial or bacterial equilibrium in the soil and some of them are recently used as biological control agents against phytopathogens. Several soil protozoa cause diseases in human beings which are carried through water and other vectors like Amoebic dysentery caused by *Entamoebahistolytica*.

Actinomycetes

Actinomycetes are clubbed with bacteria, the class of Schizomycetes and confined to the order Actinomycetales. They are unicellular like bacteria, but produce a mycelium which is nonseptate (coenocytic) and more slender, like true bacteria they do not have distinct cell wall and their cell wall is without chitin and cellulose (commonly found in the cell wall of fungi).

Actinomycetes are numerous and widely distributed in soil and are next to bacteria in abundance. They are heterotrophic, aerobic and mesophilic (25-30 °c) organisms and some species are commonly present in compost and manures are thermophilic growing at 55-65° c temperature. They egrade/decompose all sorts of organic substances like cellulose, polysaccharides, protein fats, organic- acids and organic residues /

substances added to soil are first attacked by bacteria and fungi and later by actinomycetes, because they are slow in activity and growth than bacteria and fungi.

They decompose the more resistant and indecomposable organic substance/matter and produce a number of dark black to brown pigments which contribute to the dark color of soil humus. They are also responsible for subsequent further decomposition of humus (resistant material) in soil as well as responsible for earthy /musty odor / smell of freshly ploughed soils.

Viruses

Viruses are the smallest known organisms in the soil. Very little is known about them as compared with other soil organisms. We do know that all viruses are parasitic, feeding off other flora and fauna.

Plant, insect and human viruses can be found in most soils, and are influenced mostly by soil moisture, along with soil structure and plant roots. Soil viruses are thought to greatly influence soil microbes via an ability to transfer genes from host to host, and as a potential cause of microbial mortality.

Plant Growth Promoting microbes

Rhizobacteria and endophytes

In the land rehabilitation process, plant growth promoting bacteria deserve special attention as they are actively involved in plant and soil interactions. Generally, the bacteria that are plant associated migrate from the bulk soil to the rhizosphere of living plant and aggressively colonize the rhizosphere and roots of plants.

Rhizobacteria; *Achromobacter*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas* and *Serratia* (Gray and Smith, 2005), and *Streptomyces* sp. have been found to have beneficial effects on various soils (Dimkpa *et al.*, 2009).

Other compounds produced by rhizobacteria that are beneficial include enzymes, osmolytes, biosurfactants, siderophores, nitric oxide, organic acids and antibiotics. These may be responsible for suppression of pathogenic and deleterious organisms, improved mineral uptake, associative nitrogen fixation (Dobbelaere *et al.*, 2003) tolerance to abiotic stresses or production of phytohormones (Vessey, 2003). Therefore, for knowing

the status of rehabilitation of degraded lands, for promoting plant growth and health, extensive research efforts are to be made to explore microbial diversity, their distribution, as well as function in soils of degraded lands.

Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular Mycorrhizal fungi stabilize the soil and enhance plant growth by alleviating nutrient and drought stress. Evaluation of the mycorrhizal status of degraded land is recommended as a first step in rehabilitation and restoration. It contributed the restoration process by stabilizing windborne soil that settles under dense plant canopies and enhancing establishment of colonizer plants in bare soils of disturbed areas (Bashan and Bashan, 2010). Mycorrhizal fungi strengthen soil structure in physical and chemical manner.

Physically, the hyphal network of these fungi link soil particles to each other and to plant roots. Chemically, AM fungi produce glomalin, a sticky substance that is important in soil aggregation (Wright and Upadhyaya, 1998). Glomalin binds soil aggregates together while still allowing water, nutrients, roots and soil fauna to move within the soil (St John, 1998). The results suggested that AM fungal inoculum potential in hot desert soils and concluded that AM fungal inoculum density is not the primary factor for the establishment of cactus seedlings and that favorable edaphic factors probably play a more important role (Bashan *et al.*, 2000). AM fungi are common in harsh and limiting environments because they mitigate plant stress. Their hyphae permeate large volumes of soil, interconnect the root systems of adjacent plants to facilitate exchange of nutrients between them, and contribute to soil structure. AM fungi are an essential component of plant–soil systems of deserts and have been detected worldwide. Mycorrhizal colonization enhances water and nutrient uptake in dry environments for the succulent *Agave deserti* and the cacti *Ferocactus acanthodes* and *Opuntia ficus-indica*.

Artificial inoculation of these plants with field-collected AM fungi increased the phosphorus content of roots and shoots compared with uninoculated plants. The destruction of mycorrhizal fungal network in soil system is the vital event of soil disturbance, and its reinstallation is an essential approach of habitat restoration. Successful revegetation of severely disturbed mine lands can be achieved by using “biological tools” mycorrhizal fungi inoculated tree seedlings, shrubs, and grasses.

Fig.1 Effect of different degree of disturbance on soil ecosystem

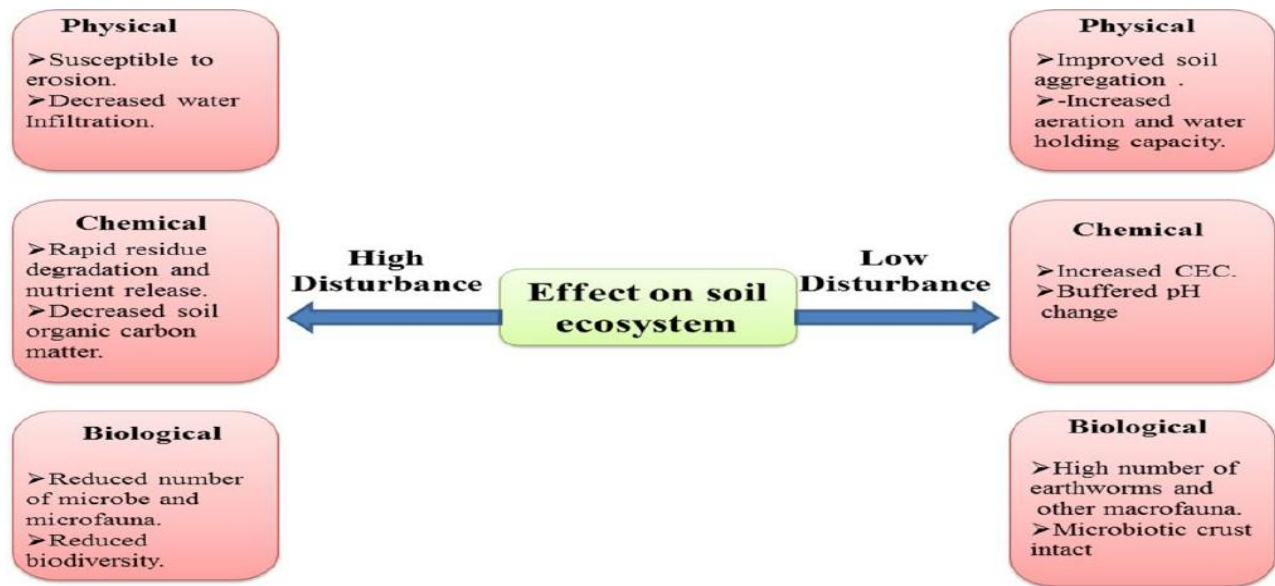


Fig.2 Some examples of application of bio-mining/ bio-leaching technology & its advantage over conventional pyro-metallurgical and hydro-metallurgical technology

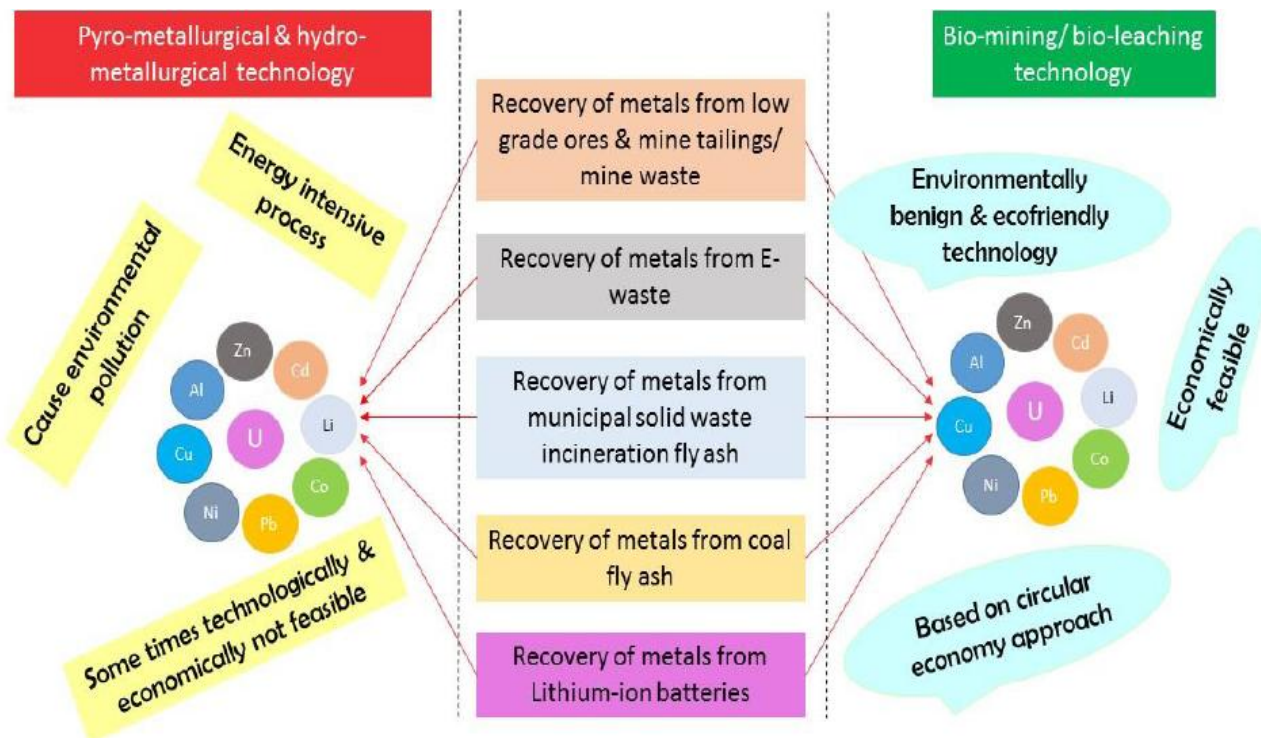
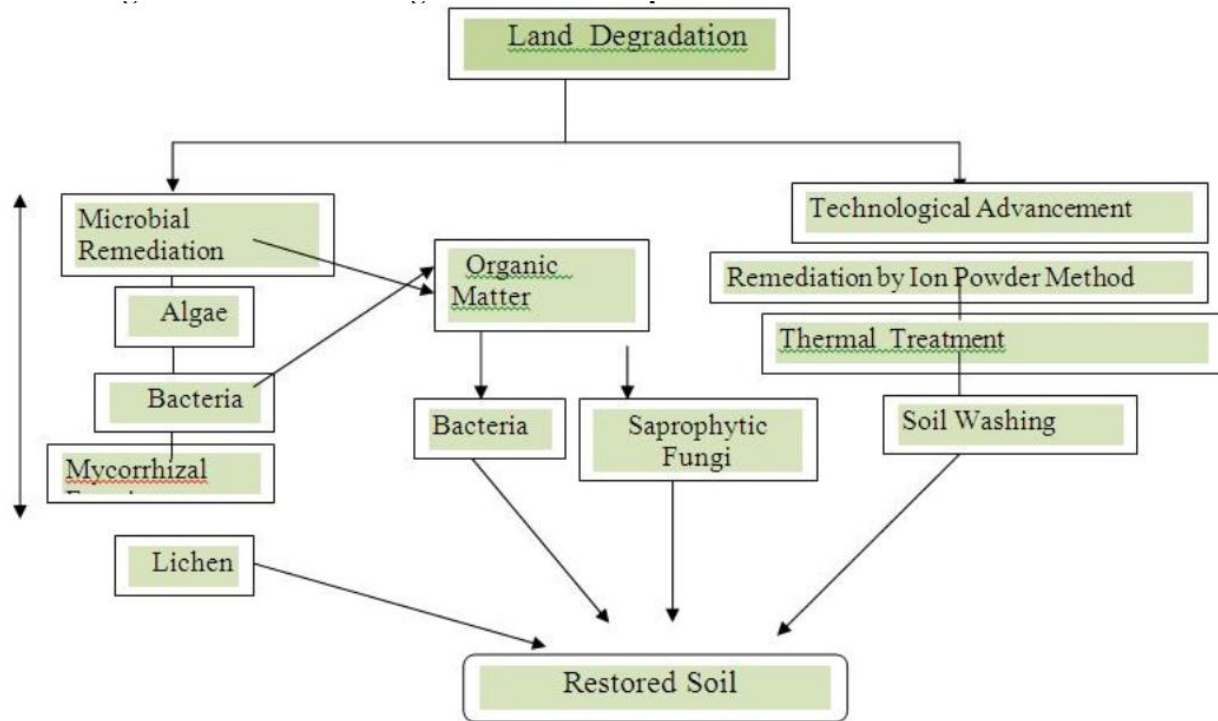


Fig.3 Schematic diagram to show the processes involved in soil restoration



Microbial Biomass

The soil microbial biomass is the eye of the needle through which all natural organic materials that enter the soil must pass, more than once as they degraded to the organic compounds from which they came (Patra, 1994).

The soil microbial population consisting of bacteria, fungi and microfauna (Micro means microscopic that one can't see with naked eyes and fauna means animals) are termed as soil microbial biomass (SMB). Soil microbial biomass is a vital soil component, acting as a source and a sink for plant available nutrients, as well as catalyzing the transformations of these nutrients in soil.

On the basis of detailed analysis, it is reasonable that future research would be focused on the impact of land use change on soil microbial biomass in tropical and subtropical ecosystems (Zhang Jiang-shan *et al.*, 2005).

The status of microbial ecosystem can be assessed through the genetic characteristics of the soil microbial community and it also gives the idea about quality of the soil and the progress of restoration after degradation (Singh, 2015). There are spatial and temporal variation in type of microbes and its community involved in land restoration.

Microbial Biomass and Soil Organic Carbon in Different Land Uses Across the Globe

The players in the decomposition process are the microbial population; the bacteria, fungi and viruses. Although the quantification of soil microbes is difficult because of different of reasons yet various scientists quantify the microbes in a cumulative form that is microbial biomass in different land uses and plantation. As a very broad generalization, the amount of microbial biomass in a soil reflects the total organic matter content, with the living component forming a low proportion of total (Sparling, 1997).

Role of Soil Microbial Biomass in Ecological Restoration of Degraded Lands

All organisms in the biosphere depend on microbial activity because it leads to the degradation of organic materials and provide food (Pace, 1997). The mineral content and its physical structure are important for balanced condition of soil. In native soil the soil biota includes vast numbers of microorganisms that naturally reside in soil and perform a wide range of functions which are essential for a normal and healthy soil, whereas in a disturbed soil the microorganism decreases. At the early stages of ecosystem development soil act as

a critical controlling component, without the natural processes of soil development ecosystem would remain in degraded condition. Soil is a natural medium in which microbes live, multiply and die. Organic matter, mineral nutrients and microbial nutrients decrease and drastically disturbed due to surface mining (Soni and Rawat, 2005). Mine spoil heaps are composed of coarse rocks due to the deep mining operation and mineral processing. These spoils are not suitable for plant and microbial growth because of low organic matter content, unfavorable pH, and drought arising from coarse texture or oxygen deficiency owing to compaction. The other limiting factor for revegetation of mine spoil may be salinity, acidity, poor water holding capacity, inadequate supply of plant nutrients and accelerated rate of erosion (Jha and Singh, 1991).

Frankia

Frankia is a gram-positive nitrogen-fixing actinobacterium that forms a symbiotic association with actinorhizal plants. It is a filamentous free-living bacterium (Normand *et al.*, 1996) found in root nodules or in soil. Frankia are filamentous nitrogen-fixing Gram-positive actinobacteria that are found as free-living microbes in the soil and in symbiotic associations with actinorhizal plants. These bacteria fix nitrogen by converting atmospheric N₂ into biologically useful ammonia and supply the host plants with a source of reduced nitrogen. Genomic studies have been conducted to characterize Frankia (Persson *et al.*, 2011) and to better understand the functioning of actinorhizal symbiosis. Frankia are developmentally complex and form three cell types: vegetative hyphae, spores located in sporangia, and vesicles. In liquid culture and depending on the condition of culture, Frankia forms hyphae and multilocular sporangia which are located on hyphae either terminal or intercalary (Obertello *et al.*, 2003). Ultrastructure showed that the hyphae, free living structures are septate, and sporangia are multilocular and contain the spores, the effective propagules of the bacteria. Vesicles are the site of nitrogen activity and are formed when nitrogen is very limited in the medium (Zhang and Benson, 1992). Due to the presence of resistant structure in culture, Frankia inoculum is simpler to conserve than Rhizobium inoculum (Brunck *et al.*, 1990). Frankia morphology in the nodule varies according to host plant. The symbiosis between actinorhizal plants and Frankia induces the formation of a perennial root organ called nodule, wherein bacteria is hosted and nitrogen is fixed (Perrine- *et al.*, 2011). In the field, actinorhizal nodule can have variable forms and

colors. Comparison of actinorhizal and leguminous nodules shows that morphology, anatomy, origin, and functioning of nodules are different for these two nitrogen-fixing plants (Hocher, *et al.*, 2009). Two types of nodule formation occur in actinorhizal symbiosis: the intercellular and the extracellular infection.

Actinorhizal symbiosis and Plants growth

Actinorhizal plants are grouped in the clade of Rosid I (Soltis and Soltis, 2000). With the exception of two species belonging to Datisceae family, actinorhizal plants are mostly trees or woody shrubs. Casuarina and Alnus are the most important and widely spread actinorhizal plants due to their uses in soil reclamation, agroforestry systems, dune stabilization, and windbreaks (Dommergues, 1997). They are pioneer species that colonize disturbed environments with low soil fertility and facilitate the establishment and development of subsequent plant communities (Gtari and Dawson, 2011). Casuarina species are able to grow well under a range of stresses such as drought, flooding, salinity, and sites polluted by heavy metal (Tani and Sasakawa, 2003). Casuarina plantations improve physical and microbiological quality of degraded soils and these plants have an important ecological and economical role in tropical countries. They contribute to the improvement of soil fertility by fixing nitrogen and producing thick leaf litter from needles that can be used as compost by farmers (Sayed, 2011). Association with Frankia increased Casuarina growth and biomass (Sayed, 2011). Furthermore, in this symbiotic relationship, bacteria confer to plants a high resistance to abiotic and biotic stresses. Like Casuarina, Betulaceae contains members that play an important role in improving soil fertility (Kohls *et al.*, 1999). They are used in the production of firewood, pulp, and timber. These species are also used in land reclamation, agroforestry, and as windbreak to avoid erosion and promote the establishment of the more nutrient demanding plants (Roy *et al.*, 2007).

Inoculation with the nitrogen-fixing bacteria Frankia improves the nutrient status and enhances actinorhizal plant development (Sayed, 2011) and to optimize association between the actinorhizal/Frankia, an efficient combination in nitrogen fixation well adapted to environmental conditions is recommended. However, the successful establishment and development of actinorhizal plants in nutrient stressed and/or marginal soils depends on the formation of the symbiotic relationship between plant and the nitrogen-fixing bacteria. In fields, inoculation with Frankia is commonly carried out with

crushed nodule, Frankia suspension, Frankianrobed in alginate bead, soil containing Frankia, or leaf litter from around nodulated plants (Mansour, 1990). The response to Frankia inoculation is strongly linked to factors such as provenance source, Frankia strain, and nutrients status of the site such as nitrogen (Landis and Dumroese, 2006).

Actinorhizal symbiosis and environmental stress

Actinorhizal plants are generally tolerant of abiotic stresses. This tolerance can be improved when plants are associated with Frankia (Bélanger *et al.*, 2011). The actinorhizal plant-Frankia system is widely used for reclaiming lands affected by abiotic stresses (Salt *et al.*, 1995). Most of the Frankia strains were resistant to an elevated level of several heavy metals and also to salinity. Some Frankia strains are very tolerant to salinity and can be used as biofertilizers in land affected by salt (Srivastava *et al.*, 2012). Actinorhizal symbioses are a biological tool used for the remediation and revegetation of soils affected by salt, heavy metal, oil, and so forth (Mehda, 2006). It has been demonstrated that alder-Frankia symbiosis improves remediation capabilities and enhances soil quality by improving soil nutrients, pH, and cation exchange capacity and enhancing plant performance in these harsh conditions (Baumer *et al.*, 1990). Inoculation with Frankia has a beneficial effect on restoration and reforestation of bauxite mine spoils and some studies showed that in bauxite mine spoils the growth and nutrients uptake (N, P, K) of plants inoculated with Frankia was higher than those of non-inoculated plants.

Approaches to rehabilitation

Rehabilitation comprises the design and construction of landforms as well as the establishment of sustainable ecosystems or alternative vegetation, depending upon desired post-operational land use. Restoration ecology has been dogged by semantic issues with myriad detailed definitions and much pedantic discussion – a trap into which we may be about to fall (Elliott *et al.*, 2007). In contrast to the term ‘conservation’ which typically relates to maintaining a favourable state, ‘restoration’ relates to the active re-creation of such conditions, and can interchangeably be used with ‘rehabilitation’ which are sometimes used to indicate less comprehensive or complete restoration actions or those that create novel habitats without natural analogues. Deforestation, extensive irrigation and drainage have been around since agriculture commenced. Usually it is dictated by

pragmatic rather than idealistic considerations, and target definitions should involve a variety of stakeholders and end-users and be made explicit at the onset of any proposed restoration scheme (Geist, 2015). Some ecosystem services and reverses biodiversity loss, then such remediation or rehabilitation can be viewed as a positive intervention. Microorganisms are increasingly recognized as being important for successful reclamation.

Measurement of Habitat Restoration

The measurement of ecological processes is not so easy it is evaluated by the presence of mycorrhizae or nutrient pools. Mycorrhizae colonization can significantly affect plant growth and patterns of succession after a disturbance (Ruiz-Jaen and Aide, 2005). By measuring the soil microbial community, the degradation process and restoration success can be assessed. For measuring restoration success, the Society of Ecological Restoration International produced a primer that provides a list of nine ecosystem attributes as a guideline (Society for Ecological Restoration International Science (SER) & Policy Working Group. 2004).

1. Similar diversity and community structure in comparison with reference sites
2. presence of indigenous species
3. presence of functional groups necessary for long-term stability
4. capacity of the physical environment to sustain reproducing populations
5. normal functioning
6. integration with the landscape
7. elimination of potential threats
8. resilience to natural disturbances and
9. Self-sustainability

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