Journal of Applied and Natural Science
11(3): 624- 631 (2019)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Ecobiology of coal mines and spoils

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Article Info
https://doi.org/10.31018/jans.v11i3.2130
Received: June 18, 2019
Revised: July 22, 2019
Accepted: August 21, 2019

How to Cite
Sharma, A. and Sumbali, G. (2019). Ecobiology of coal mines and spoils. *Journal of Applied and Natural Science*, 11(3): 624- 631 https://doi.org/10.31018/jans.v11i3.2130

Abstract
Coal is an important non-renewable source of energy, which is being constantly used by mankind for various purposes. Coal mining activities affect the surrounding ecosystem by contaminating it with traces of toxic metals, which may accumulate and affect the diversity and abundance of biological communities. A number of microorganisms, such as, filamentous fungi, yeasts and bacteria are known to degrade coal by their enzymatic action and use it as the sole source of carbon. In addition, the indoor environments of coal mines possess bioaerosols, which may include living or dead allergens, pathogenic or non-pathogenic bacteria, fungi, viruses, mycotoxins, bacterial endotoxins, peptidoglycans, etc., that may cause skin, respiratory tract and other health problems. This article throws light on the impact of coal mining on the surrounding ecosystem, degradation of coal by the microbial inhabitants and their effects on the health of miners.

Keywords: Coal mines, Coal spoils, Microflora, Ecobiology, Microbial degradation, Miners health

INTRODUCTION

Coal is recognized as an important non-renewable source of energy widely used in metallurgical industries, thermal power plants and other industries (Barooah and Baruah, 1996). The composition of coal is very complex, consisting mainly of carbon (between 60% and 95%) and few other elements like hydrogen, oxygen and sulphur. Its usefulness in different industries is mainly dependent on its volatile matter, combustible and non-combustible constituents. In fact, coal is the most important fossil fuel and a basic source of energy for industrial development (Aseefa *et al*., 2013). Throughout the world, coal deposits are larger than those of the crude oil, which is another source of energy. Therefore, coal acts as a raw material source for future fuel generation.

The formation of coal takes millions of years, which starts with accumulation or rearrangement of organic and inorganic chemical constituents in a low oxygen environment. The organic matter accumulates and forms a bed of peat, which gets buried by other sediments and under heat and pressure begins to transform into low grade coal, known as lignite. Further, more heat and pressure metamorphoses the lignite into bituminous coal and then into anthracite, which is hard shiny, compact and crystalline form of coal (Taylor *et al*., 2009). This whole process of coal formation including metamorphoses of one form of coal into another involves coalification, that is, the degree of change undergone by coal as it matures from peat to anthracite. Coalification has an important bearing on the physical and chemical properties of the coal and is referred to as the 'rank' of the coal. The quality of each coal deposit is determined by the type of vegetation from which the coal has originated, depth of burial, length of time involved in coal formation and few other factors like pressure, humidity, lack of air, microbiological reactions, physical and chemical reactions at those depths (Taylor *et al*., 2009).

According to World Coal Association (WCA), coal reserves are available in almost every country of the world with recoverable reserves in around 70 countries. It has been estimated that there are over 861 billion tonnes of proven coal reserves world-wide and the biggest reserves are in U.S.A, Russia, China and India. In India, there are 44 major coalfields and the total estimated reserves of all types of coal are 315.149 billion tonnes, extending upto depths of 1,200 meters (US Energy Information Administration, 2017). These deposits are mainly distributed in the states of Bihar and Telangana (Chikkatur *et al*., 2009).

Coal mining has multi-dimensional impact on the biological species both directly and indirectly. The present article is an attempt to focus on the ecobiology of coal mines and spoils.

Physico-chemical properties of coal mines and spoils: Coal is basically classified into three major types namely anthracite, bituminous and lignite. Anthracite is considered to be the oldest coal from geological perspective. It is a hard coal composed mainly of carbon with little moisture and volatile content. On the other hand, lignite is considered to be the youngest coal from geological perspective, which is soft coal, composed of volatile matter and moisture content. Much information is available about the physico-chemical properties of coal. For example, physico-chemical properties and microflora of lignite coal from Mirash mine, near Kastriot, Kosovo have been studied by Plakolli *et al* (2010). They analysed these samples for ash, C, O, H, N, S, carbonates and silicates and found that the values of C, H, N and O were approximately similar in the coal and the bacterial biomass. The physico-chemical characterization of Nigerian coal samples have been determined and found to contain major elements like Ca, Fe and S and some minor elements like K, Zn, Ni, Sc, Ti and Zr (Adekola *et al*., 2012). Similarly, Nyakuma (2015) studied physico-chemical properties of three low rank Nigerian coals and determined the elemental composition, proximate analysis, mineral matter and calorific values of the coal. From some Indian coal samples, various physical characteristics like pH and electrical conductivity and some chemical properties like macroelements viz., nitrogen (N), carbon (C), phosphorus (P), potassium (K), sulphur (S) and some microelements viz., zinc (Zn), copper (Cu) manganese (Mn) and iron (Fe) have also been investigated (Sharma, 2016).

During the process of coal mining, the overlying soil and the fragmented rocks that are removed are usually heaped in the form of overburden dumps. These dumps are usually nutrient poor, loosely adhered particles, which contain high concentration of heavy metals. These overburden dumps create mine spoils when deposited in an un-mined area, affecting landscape and cause environmental pollution (Makdoh and Kayang, 2015). The physico chemical properties of overburden dump materials are site specific and differ from one dump to another dump due to different geological deposit of rocks (Lovesan *et al*., 1998). Ghose (2001) analysed soil dumps around Raj Mahal coalfield areas of Eastern Coalfield Ltd. and found them to change greatly with age, which ultimately became biologically sterile.

In India, studies have been conducted on the physico-chemical characteristics of overburden dump soil in selected coal mining areas of Jharia, Jharkhand (Rai *et al*., 2011), Sundargarh, Odisha (Maharana and Patel, 2013), Chirimiri, Chattisgarh (Nigam *et al*., 2015), Khliehriat, East Jantia Hills district, Megahalaya (Makdoh and Kayang, 2015) and Raniganj, West Bengal (Kar and Palit, 2017). These investigations revealed that the overburden spoils were poor in nutrient content but rich in heavy metals. Ralte (2017) studied the impact of coal mining on soil physico-chemical properties of Nokrek Biosphere Reserve, Meghalaya and found that physico-chemical properties of mine spoils were different from those of the core zone of the biosphere reserve.

Microbial inhabitants of coal mines: The first pure culture of a microbe was that of bacteria, which was found to be growing on brown coal samples (Galle, 1910). Later, more bacteria were shown to be associated with coal by Schroeder (1914) and Lipman (1937). Some microbiologists, geochemists and fuel scientists consider that micro-organisms might be able to metabolize the physico-chemical structure of coal (Hofrichter *et al*., 1997).

Fungal species inhabiting various types of coal was first given by Fischer and Fuchs (1927). They surprisingly observed white and green mycelial growth on untreated moist coal, which led to the discovery of various filamentous fungi. Later, Lieske and Hofmann (1928) carried out detailed investigations on the micro-flora of coal deposits and a large number of microorganisms were detected from the mining areas. From India, some mycologists have surveyed fungal flora of coal mines. Prasanth *et al*. (2011) investigated airborne mycoflora near Neyveli lignite mine in Tamil Nadu and recovered predominantly the mitosporic fungi. Tulsiyan *et al.* (2017) studied fungal diversity of coal mines near Hazaribagh, Jharkhand and recovered 11 fungal species from Class Ascomycetes and Zygomycetes whose optimal growth temperature was 45º C or above. Similarly, from J&K State, Sharma and Sumbali (2017) recovered 33 fungal species belonging to 19 genera from the lignite and anthracite coal mines of Jammu province. They observed lignite type of coal to favour the growth of 30 fungal species, whereas 28 fungal species were recovered from anthracite coal. Of these, *Aspergillus* accounted for 27% of the total mycodiversity in both types of coal, followed in decreasing order by *Curvularia* and *Penicillium* species (Sharma, 2016).

Microbial degradation of coal: The idea that microorganisms might be able to degrade coal is not new. In the early 20th century, bacteria were reported to act as biocatalysts in the oxidation of coal (Potter, 1908). It has been observed that there is a close connection between microbial activity and coalification as in this process, a number of bacteria and fungi are known to degrade the organic matter and cellulose content by their enzymatic action (Laborda *et al*., 1997). However, some microbiologists, geochemists and scientists are of the opinion that most of the microorganisms may not be able to modify the chemical structure of coal because they usually prefer simple sugars and organic acids for their activity and avoid the use of complex materials, such as coal (Fakoussa and Hofrichter, 1999). Secondly, less is known to the geochemists about the physico-chemical processes and extreme conditions involved in the genesis of coal, that is, coalification. By studying this process, an idea has emerged that coal might be acted upon by microbes or utilized as growth substrate as it contains 60-95% of carbon. In fact, coal and different types of micro-organisms have co-existed since the deposition of coal millions of years ago and they have survived in a dormant state, which slowed down their metabolism in order to survive in the unfavourable conditions (Fakoussa and Frost, 1999).

Certain bacterial strains are known to grow on coal by utilizing hard coal as the sole source of carbon. For example, *Pseudomonas fluorescens* has been used for bioconversion of coal, which could alter several coal characteristics like colour, wettability and extractability (Fakoussa, 1981). Assumptions have been made that secreted enzymes convert the coal substance to more hydrophilic status, that is, it becomes more water soluble and is then taken up by the bacterium (Fakoussa, 1981). Few investigations have also been carried to find out whether yeasts and filamentous fungi could utilize the coal as a sole source of carbon and energy (Fakoussa and Hofrichter, 1999). Interestingly, they found that when the fungal spores germinated, there was no affinity to the coal, but in mature stage many coal particles adhered to the fungal cell wall and the older hyphae became completely coated with the coal layer. Cohen and Gabriele (1982) found that the two white rot fungi, *Trametes versicolor* and *Poria monticola* were able to convert leonardite, a low rank coal deposit in North Dakota. Few more researchers have also shown that the fungi and actinomycetes are able to solubilise small amount of 3.2 N nitric acid oxidized coals in liquid culture (Quigley *et al*., 1988). Later, Stewart *et al.* (1990) also investigated colonization of pretreated bituminous coal by a number of filamentous fungi and found *Penicillium* and *Cunninghamella* species to be the most active. By using SEM, they observed extensive surface colonization of coal, which included conidia formation and a tight attachment of fungal hyphae to the coal particles. Further, they found that only the pretreated coal samples, which had previously been exposed to 150º C for 7 days, showed luxuriant growth of molds, whereas the untreated particles showed little colonization. According to Machnikowska *et al.* (2002), microbial degradation of coal has been considered as an economic and effective way of transforming macromolecules into simpler, low molecular weight products. Rajak *et al.* (2014) carried an investigation on the bio-desulphurization of high sulphur coal by using the bacterium, *Thiobacillus ferrooxidans* and studied different parameters such as variation of pH, reduction of sulphur, ash and nitrogen.

Most of the earlier studies have demonstrated that bacteria and fungi are able to metabolize the low rank coal but less is known about the transformation of hard coal by microorganisms. Osipowicz *et al.* (1994) found that the white-rot fungus, *Piptoporus betulinus* and two bacterial strains showed bio-transformation of Polnish hard coal. Monistrol and Laborda (1994) demonstrated liquefaction of Spanish hard coal by forming a tar-like substance using a newly isolated mold. Later on, Hofrichter *et al.* (1997) screened different fungal strains for their ability to metabolize German hard coal. It was realised that both non-enzymatic and enzymatic mechanisms may be involved in coal degradation/ solubilisation by the same microorganism (Holker *et al*., 2002; Yuan *et al*., 2006). Biological desulphurisation of coal was carried out from North Eastern Coalfields of Assam, India by using *Aspergillus* species isolated from coal itself and as high as 70-80% of total sulphur removal was achieved (Acharya *et al*., 2005). Later, Silva-Stenico *et al.* (2007) reported increase in coal solubilisation efficiency of *Trichoderma atroviride* ES11 by the addition of nitrogen source such as ammonium sulphate to the medium. Biodepolymerization of low rank Indian coals by using *Pleurotus djamor, P. citrinopileatus* and *Aspergillus* species were studied and the findings showed that as compared to sub-bituminous and bituminous coal, lignite coal has higher rate of solubilization (Selvi *et al*., 2009). Similarily, bio-solubilisation of lignite (a low-rank coal) into humic acid was investigated by using fungal organisms and it was found that the microbial dead cells and their metabolites present on the surface of lignite particles reduce the rate of lignite solubilisation (Tripathi *et al*., 2009). In North China, a fungus, *Hypocrea lixii* strain TZ1 isolated from coal mine soil at the Fushunxi Colliery, Liaoning Province, has been used for the bioconversion of Chinese lignite (Tao *et al*., 2010). It is speculated that strain TZ1 produces extracellular enzymes such as polyphenol oxidase, which can utilize amide (–$CONH_2$), carboxyl (-COOH) and carboxylate (-COO^-) group (Holker *et al*., 2002).

Investigations have also been carried out to study the effect of some filamentous fungi on bio-liquefaction of low rank Indian coals, its chemical composition, surface characteristics of the products and the microbial mechanism of coal conversion. Among the fungal strains used, mixed cultures of *Aspergillus niger* and some *Penicillium* species caused significant removal of ash and minerals (Balachandran and Elcey, 2010). It has been reported that extracellular enzymes produced by *Aspergillus niger*, such as laccase, lignin peroxidase and manganese peroxidase play a role in the solubilisation of bituminous coal (Balachandran and Elcey, 2010). Studies have also been done on the bio-demineralization of Indian bituminous coal by *Aspergillus niger* and characterization of its products. In this process, bio-demineralization significantly reduced the ash content in the coal and the leaching process removed minerals like silicates and pyrites, whereas aluminates were decreased considerably (Balachandran, 2013).

Although some fungi can specifically grow on untreated coals, several research studies have shown that the brown rot fungi in comparison to white rot fungi are weakly active or inactive to solubilise lignite under the same test conditions (Catcheside and Mallett,1991). It has been demonstrated that some filamentous fungi and streptomycetes have the ability to attack low rank coal (leonardite) and modify the physico-chemical structure of coal (Faison, 1991; Fakoussa, 1992; Crawford, 1993). Hofrichter *et al.* (1997) also demonstrated modification of hard coal by fungi. They screened more than 750 fungal strains for their ability to attack German hard coal and of these; selected six strains could modify the physico-chemical properties of hard coal pieces placed on the overgrown surface of petri-dishes. The fungal strains used for this screening programme were isolated from soil, coal and also included some wood and litter decaying Basidiomycetes. Following this, intensive research was conducted by various other research groups also with an aim of establishing better understanding of coal bioconversion and the production of value added products (Polman *et al*., 1994; Fakoussa and Frost, 1999; Fakoussa and Hofrichter, 1999; Gotz and Fakoussa, 1999; Machnikowska *et al*., 2002; Igbinigie *et al*., 2008; Jiang *et al*., 2013). Recently, Sharma (2016) observed that the fungal isolates recovered from some Indian coal mines possessed the ability to utilize coal as the sole source of carbon.

Effect of coal microflora on the health of miners: The health of coal miners is always at risk as bioaerosols are ubiquitously present in the coal mines. Bioaerosols may include allergens, pathogenic or non pathogenic bacteria, fungi, viruses and their toxins. Transmission of bioaerosols can occur by the airborne droplets or dust through the skin, respiratory tract and mucous membrane (Rdzanek *et al*., 2015). Working conditions inside the coal mines are hard due to the depths of the mines, which can be upto thousands of metres, high temperature reaching over 30ºC and relative humidity ranging between 70-100% (Drenda, 2012). These prevailing conditions and additionally the presence of organic substances create a congenial environment for microfungal organisms, which can be found on any organic material used in the mining activities especially on timber, organic waste, insulators, machinery tyres or other rubber material surfaces (Piontek and Bednar, 2010). The coal miners while working in the mines, go down and take spores of various microbes into the depths of mine. Some of the airborne microfungi like *Aspergillus*, *Penicillium, Cladosporium* and *Stachybotrys* species, which usually occur in the mine excavations and galleries can cause allergies and fungal infections in some of the exposed workers (Gamboa *et al*., 1996; Obtulowikz, 2006; Cabral, 2010). Spores of *Aspergillus fumigatus* are known agents of cystic fibrosis and allergic bronchopulmonary aspergillosis (Frisvad *et al*., 2004). In addition, these spores also pose a serious risk for miners as they are inhaled along with the coal dust and cause occupational diseases, such as pneumoconiosis, silicosis and emphysema (Yoltas *et al*., 2015). Another species, *A. flavus* is also known to be allergenic and causal agent of aspergillosis. Both these fungal species are even known producers of some mycotoxins and other secondary metabolites, which may cause disorders like irritation of the mucous membrane, nausea, immune system deficiency, cancer and acute or chronic damage to the liver and central nervous system (Dutkiewicz, 1997).

Since coal is being used in some of the electric plants even today, coal miners continue to suffer from lung diseases due to coal mine dust. After the passage of Federal Coal Mine Health and Safety Act in 1969 (FCMHSA, 1969), the exposure to coal dust was limited and a decline in the prevalence of pneumoconiosis among coal workers of USA was observed between 1970 and 2000 (Cohen *et al*., 2016). However, since then, pneumoconiosis among miners has increased significantly and incidence of several forms, such as, massive fibrosis, silicosis and rapidly progressive pneumoconiosis have increased due to modernization of the mining technology, which generates clouds of respirable dust particles containing toxic silica (quartz), silicate particles and the less toxic coal (carbon) particles (Pollock *et al*., 2010; Perret *et al*., 2017). Recently, a study demonstrated that the silica/ silicate particles from coal mine dust get accumulated inside the alveolar macrophages and cause a disease called as coal mine dust desqamative chronic interstitial pneumonia, which is a precursor of both coal dust related diffuse fibrosis and emphysema (Jelic *et al*., 2017).

Since 1990's, some of the developed countries have even implemented the occupational safety and health management system (Tian, 2012; Ma and Dai, 2017; Nie *et al*., 2018; Zhou *et al*., 2018). Recently, software system has also been developed for coal mine occupational safety and health management in order to enhance the occupational safety, health management and risk control standards (Cao *et al*., 2013; Zhou *et al*., 2018).

Coal mining and its impact on ecosystem: Coal mining activities cause changes in the landscape and degradation of stable ecosystems. Increasing demand for coal is known to destabilize the ecosystem around the mining area, which is contrary to the objectives of sustainable development (Kar and Palit, 2017). During the process of mining, variety of materials are washed away as discharge into the waterways, which flows downstream and can contaminate water bodies with various metals (Ali *et al*., 2017, 2018). These heavy metals discharged from coal mines may be consumed by some aquatic organisms, whereas the low soluble trace metals easily get adsorbed in sediments (Mishra and Shukla, 2016). This continuous process leads to high accumulation of contaminants in the body of living organisms (Alvarez *et al*., 2011). However, those trace metals, which are not permanently bonded to the sediment can be easily released in the water under different conditions (Wang *et al*., 2016). Some of the trace metals that are not essential for the biological functions can be toxic at very low concentrations (Pagenkopf, 1983). Such trace metals may accumulate in the body of various organisms including humans (Allinson *et al*., 2015).

Certain plant and animal species have their own system to maintain the intake of contaminants and capability to accumulate them, irrespective of the available trace metals in the aquatic system (Johnstone *et al*., 2016). The inhabitants of the coal mining surrounding areas have their characteristic affinity towards the available trace metals. In the presence of certain trace metals, the uptake of other trace metals by inhabitants get reduced and photosynthesis in plants also get affected (Volland *et al*., 2014). Coal mining also has an impact on the miners and other people residing in and around the mining area.Therefore, in view of the mining activities, concern regarding the local habitats and ecosystem is very important. In this direction, angle of slope of overburden dumps, safe disposal drains and other safe techniques are necessary to reduce the environmental impact (Goswami, 2015).

In addition, burning of coal releases harmful gases, such as, carbon dioxide, nitrogen oxide and sulphur dioxide whose emission has been correlated with many health problems and different cancers of skin, brain, heart, lungs and blood (Badman and Jaffe, 1996, Cornell, 2016). High exposure to sulphur dioxide also leads to suffocation, coughing, wheezing and reduction in lung functioning by affecting mucous and cellular mucins (Kelsall *et al*., 1997). Sulphur dioxide is also known to damage plants by causing leaf injury and affecting plant growth, thus reducing the diversity of plant species (Barretti and Benedict,1970; Winner *et al*., 1985). It has been reported that there is a substantial increase in eye and throat irritation, respiratory problems, anxiety related to the smoke and ash and other problems like headaches, nausea, and blurred vision during the periods of fire (Brook, 2014). Coal mine fires also release partially oxidised by-products such as benzene, toulene and xylene (Engle *et al*., 2013). Mining activities may even result in soil erosion, destruction of vegetation and thus alteration of associated microbial communities (Goswami, 2015).

Further, the soot and sublimates associated with coal mine fires show the presence of some harmful trace elements such as mercury, arsenic and selenium (Silva *et al*., 2012; Tulsiyan *et al*., 2017). Recently, a study has been conducted to assess the impact of coal mining on the aquatic environment using macroinvertebrates and chlorophyll as indicators of industrial pollution. This comparative study showed that in the upstream and downstream locations of two coal mines, invertebrates and chlorophyll do not behave identically to the contaminants in the environment for their survival (Ali *et al*., 2018). Researchers have examined that, on an average, streams affected by coal mining were 32% lower in taxonomic richness and 53% lower in total abundance than unmined streams (Giam *et al*., 2018).

Conclusion

The microbial inhabitants of coal mines and their ability to attack the physico-chemical properties of coal is an interesting area of research, which is worth pursuing. Bioconversion of coal that is, biodemineralization, bio-depolymerization, desulphurization, etc., by fungi, actinomycetes and bacteria is of great industrial application. Infact, mining is expected to affect the environment and ecology of a region and the greatest impact is the deterioration of soil quality. The process of bioconversion is also applicable for the management and reclamation of topsoil of coal mining areas. Additionally, microbial flora of coal mines have a major impact on the health of miners. Therefore, in-depth investigations are needed in this particular field so that preventive measures can be taken to control the health related problems of the miners.

ACKNOWLEDGEMENTS

The authors are highly thankful to the DST PURSE SAP-II, New Delhi for providing financial assistance.

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