



Microbes from mined sites: Harnessing their potential for reclamation of derelict mine sites[☆]



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ABSTRACT

Derelict mines pose potential risks to environmental health. Several factors such as soil structure, organic matter, and nutrient content are the greatly affected qualities in mined soils. Soil microbial communities are an important element for successful reclamation because of their major role in nutrient cycling, plant establishment, geochemical transformations, and soil formation. Yet, microorganisms generally remain an undervalued asset in mined sites. The microbial diversity in derelict mine sites consists of diverse species belonging to four key phyla: Proteobacteria, Acidobacteria, Firmicutes, and Bacteroidetes. The activity of plant symbiotic microorganisms including root-colonizing rhizobacteria and ectomycorrhizal fungi of existing vegetation in the mined sites is very high since most of these microbes are extremophiles. This review outlines the importance of microorganisms to soil health and the rehabilitation of derelict mines and how microbial activity and diversity can be exploited to better plan the soil rehabilitation. Besides highlighting the major breakthroughs in the application of microorganisms for mined site reclamation, we provide a critical view on plant–microbiome interactions to improve revegetation at the mined sites. Also, the need has been emphasized for deciphering the molecular mechanisms of adaptation and resistance of rhizosphere and non-rhizosphere microbes in abandoned mine sites, understanding their role in remediation, and subsequent harnessing of their potential to pave the way in future rehabilitation strategies for mined sites.

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1. Introduction

A mine site is considered abandoned when metal mining and other processing activities have completely ceased, and the site is exposed to toxic wastes as a result of dumping in pit cavities. It includes the entire soil, water, and surrounding watersheds contaminated by mineral extraction process (www.abandonedmines.gov). Based on their applications, minerals associated with mining are of six types: energy minerals, precious metals, ferrous metals, non-ferrous metals, specialty metals and

industrial metals (Venkateswarlu et al., 2016). According to UNEP estimates in 2001, there are around one million derelict mines around the world of which >50,000 are in Australia alone and this alarming number of abandoned mine sites is a major environmental concern (Venkateswarlu et al., 2016). A major challenge in rehabilitation of mined land is the re-establishment of a self-sustaining vegetative cover. The relation between mining and microbiology dates back to thousands of years ago when ancient Romans found microbial activity in mine tailing where the fluids appeared to be blue because of the bacterium, *Thiobacillus ferrooxidans*. This bacterium is known for its ability to convert low quality ore to valuable metal, which accounts for 25% of copper (Cu) produced worldwide with more than one billion US\$ in revenue (Devasia and Natarajan, 2004). In the case of gold mining, the microbes increased the rate of metal recovery up to 70–95%, whereas some bacterial species like *Pseudomonas cepacia* and *Erwinia*

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herbicola were able to remove phosphate from its ore even at room temperatures without using sulphuric acid (Liu et al., 1992; Goldstein et al., 2003).

Some of the recent reports show the nature of microbes with unusual activities in mines. Over the past couple of decades, scientists have been working towards engineering microbial species such as *Thiobacillus ferrooxidans* that can remediate soil contaminated with toxic metals like mercury and cadmium. Unfortunately, there is very little information available on the complex survival mechanisms of the indigenous microbiota in mined soil and rhizosphere. Microbial community profiling of mined sites is currently possible after the advent of high-throughput techniques such as 16S rRNA gene amplicon sequencing where the microbial communities or microbiomes of complex environments have been simplified. The genes involved in resistance to various toxic elements, heavy metals and plant growth promotion can be elucidated using comparative genomic analysis (Xie et al., 2015). Hence, the ecological function of a specific microbial community can be studied in relation to phylogeny despite its environmental constraints (Fierer et al., 2012).

Microbial activity in abandoned mine sites can be found in (i) top soil environment (20–30 cm), (ii) waters and drainage, (iii) tailings and sediments, (iv) rhizosphere environment associated with the roots of plants, and (v) phyllosphere environment of plants where the microbes mostly reside as endophytes or epiphytes. Microbes are ubiquitous that have evolved novel mechanisms to resist toxic pollutant stress and thereby thrive on noxious environments which are not hospitable to higher organisms. The currently used reclamation strategies at most of the abandoned mine sites are based on chemical amendments to neutralise the acidity, and these are un- or less sustainable. Although considerable research has been conducted on the bioleaching of metals such as copper, silver, gold, uranium, etc. from mineral ores, very little work has been done on the potential application of microbes for mine site rehabilitation. Recent advances in omics have helped to discover novel microorganisms and their genes involved in various biogeochemical cycling of elements with great potential for their application in mine site rehabilitation. Certainly, the application of microbes in mine site rehabilitation is a cost effective, green and sustainable approach. In spite of these advances in microbial physiology and genetics on the potential of these tiny organisms, very little has been realized in terms of their application in mined site rehabilitation. Currently, remediation strategies such as revegetation and phytoremediation have been employed predominantly in heavy metal-contaminated sites (Sheoran et al., 2010; Nirola et al., 2016; Venkateswarlu et al., 2016); however, more emphasis must be given in understanding the role of plant–microbiome interactions in mined sites for developing sustainable remediation methods. Therefore, in this review, we present the recent knowledge and advances in (i) microbial profiling of diverse communities at mined sites and acid mine drainage, (ii) plant–microbiome of mined sites, (iii) signalling plant–microbiome interactions in contaminated mine sites, (iv) the role of rhizosphere microbes and plant–microbiome interactions, (v) strategies for microbial reclamation of mined sites, (vi) microbe-assisted revegetation in mine site remediation, (vii) role of genetic transfer in remediation of mined sites, and (viii) the technological gaps and future perspectives for application of microbes in mine site rehabilitation. We hope the present review will serve as a valuable resource for researchers/practitioners involved in mined site rehabilitation.

2. Microbial diversity at mined sites

The microbial diversity of mined sites differs across regions

because of the nature of metal extraction process. It varies from Eubacteria, Archaea to the dominating phylum, Proteobacteria (Table 1). Most of the species possess heavy metal resistance, and are extremophiles tolerating such unusual pH of 2–4 as in Rio Tinto, Spain or 0.5–2 in Iron Mountain, California (Edwards, 1999; García-Moyano and Gonzalez-Toril, 2012) and extreme temperatures. In metalliferous mine waters of Rio Tinto, Spain, the bacterial species diversity, classified using 16S rRNA amplicon sequencing, belong to iron-oxidizing *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and a new *Ferrovum*-like bacterium, '*Ferrovum myxofaciens*' (García-Moyano and Gonzalez-Toril, 2012). Consequently, the water flowing across Rio Tinto also appears reddish (ferruginous sedimentation). Brown et al. (2012) used fluorescence *in situ* hybridization (FISH) technique for understanding the bacterial diversity in an acid mine drainage at Pennsylvania in US, and reported that β - and γ -Proteobacteria are the dominant in microbial communities. The bacterial diversity in a mine water treatment plant in Germany was highly dominated by a single class of β -Proteobacteria affiliated to '*Ferribacter polymyxa*' (Heinzel et al., 2009).

Some bacteria have the ability to produce acid streamers (biofilms) in mine waters. Similar microbes were found in acidic and metal-rich waters of abandoned Cu mines in North of Wales and China, and most of them belong to the class β -Proteobacteria (Kay et al., 2013). The bacterial diversity in La-Zarza Perrunal mine in Spain was found to be mostly of sulphate-reducing bacteria such as *Desulfosporosinus* spp. and iron-reducing bacterium, *Acidobacterium* sp. (Gonzalez-Toril et al., 2011). Some bacterium with interesting metabolic activity of iron oxidation and reduction was found at a lignite mine in Germany which is likely to be involved in the formation macroscopic iron-rich aggregates called 'Iron snow' (Reiche et al., 2011; Lu et al., 2013). The geochemical properties such as pH, temperature, and elements in soil have direct effects on microbial communities and their diversity in soil as revealed from analysis performed in a Cu mine at the China, Iberian Pyrite Belt, and Spain (He et al., 2007; Kuang et al., 2013; Santofimia et al., 2013). In an extreme environment of pH 1.0 and temperature above 35 °C of a pyrite mine at Iron Mountain, California, the dominant species is a chemoautotroph, *Thiobacillus ferrooxidans* (Edwards, 1999). Thus, the occurrence of diverse microbes reported from all the mine sites suggests that these communities can either thrive or adapt in extreme conditions.

The microbial diversity in these sites is abundant with complex life cycles, nutrient uptake, and metabolism. Recently, a β -Proteobacterium, *Ferrovum myxofaciens* strain P3G, was isolated from an extremely acidic (pH 2–4) ferruginous mine waters from an abandoned Cu mine in Wales (Johnson et al., 2014). It is extremely acidophilic, psychrotolerant, and an obligate autotroph, which uses ferrous ion as an electron donor and oxygen as an electron acceptor, and also has the ability to catalyze oxidative dissolution of pyrites and many cationic transition metals. Similarly, 13 Cu-resistant bacteria isolated from rhizosphere of a tolerant plant species from a Cu mine in China, exhibited tolerance to heavy metals as well as plant growth-promoting activity (He et al., 2010). Navarro-Noya et al. (2010) performed a comparative analysis using denaturing gradient gel electrophoresis (DGGE) patterns of metagenomic DNA from the rhizosphere of *Bahia xylopoda* and *Viguiera linearis* in a silver mine located in Mexico, and reported a diverse set of 12 distinct bacterial phyla, all having complex modes of nutrition and metabolism in the mine environment.

Soil rhizobacteria, in particular, are crucial in accumulating heavy metals (Cervantes and Campos-García, 2001). The processes of heavy metal accumulation by fungi also must be identified in order to use defined strains with known properties in remediation approaches. Microbiome analysis in soil can be performed using metagenomics and metatranscriptomics approaches by comparing

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