



# Characterization of iron and manganese minerals and their associated microbiota in different mine sites to reveal the potential interactions of microbiota with mineral formation



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## HIGHLIGHTS

- Microbiota in mine water was associated with the formation of iron and manganese minerals.
- Iron oxidizing bacteria of *Gallionella* was predominant in iron rich environments.
- Manganese oxidizing bacteria (*Hyphomicrobium*) was found in birnessite forming environment.
- Iron and manganese minerals can be used to remediate metal(loid)s in mine water.

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## ABSTRACT

Different environmental conditions such as pH and dissolved elements of mine stream induce precipitation of different minerals and their associated microbial community may vary. Therefore, mine precipitates from various environmental conditions were collected and their associated microbiota were analyzed through metagenomic DNA sequencing. Various Fe and Mn minerals including ferrihydrite, schwertmannite, goethite, birnessite, and Mn-substituted  $\delta$ -FeOOH ( $\delta$ -(Fe<sub>1-x</sub>, Mn<sub>x</sub>)OOH) were found in the different environmental conditions. The Fe and Mn minerals were enriched with toxic metal(loid)s including As, Cd, Ni and Zn, indicating they can act as scavengers of toxic metal(loid)s in mine streams. Under acidic conditions, Acidobacteria was dominant phylum and *Gallionella* (Fe oxidizing bacteria) was the predominant genus in these Fe rich environments. Manganese oxidizing bacteria, *Hyphomicrobium*, was found in birnessite forming environments. *Leptolyngbya* within Cyanobacteria was found in Fe and Mn oxidizing environments, and might contribute to Fe and Mn oxidation through the production of molecular oxygen. The potential interaction of microbial community with minerals in mine sites can be traced by analysis of microbial community in different Fe and Mn mineral forming environments. Iron and Mn minerals contribute to the removal of toxic metal(loid)s from mine water. Therefore, the understanding characteristics of mine precipitates and their associated microbes helps to develop strategies for the management of contaminated mine water.

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## 1. Introduction

Mining activity exposes pyritic minerals to atmosphere, increasing surface contact with oxygen and water, resulting in oxidation of the mineral and production of acid mine drainage (AMD). Acid mine drainage generally refers to discharged mine

waters with acidic pH, but the pH of the water could be above 6 at the point of discharge (Johnson and Hallberg, 2005). Acid mine drainage contains elevated levels of sulfate (SO<sub>4</sub><sup>2-</sup>) and metals such as iron (Fe), manganese (Mn), aluminum (Al) and other heavy metals such as cadmium (Cd), lead (Pb), and zinc (Zn) (Sheoran and Sheoran, 2006). Iron and Mn are present in reduced forms (Fe<sup>2+</sup> and Mn<sup>2+</sup>) which are more stable than the fully oxidized forms (Fe<sup>3+</sup> and Mn<sup>4+</sup>) at higher pH in anoxic AMD (Johnson and Hallberg, 2005).

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When the environmental conditions change, dissolved metals such as Fe, Mn and Al precipitate. Rapid oxidation of  $\text{Fe}^{2+}$  results in the formation of ferrihydrite which converts over time to crystalline forms such as goethite or hematite (Cudennec and Lecerf, 2006). Goethite and ferrihydrite have been documented in acid mine drainage precipitates (Cull et al., 2014). Poorly crystalline Fe oxyhydroxysulfates such as schwertmannite were the main constituents of ochreous precipitates in a mine stream with a pH range of 2.5–4.0 (Bligham et al., 1990). The schwertmannite can transform to goethite mediated by Fe(II) pathway in anoxic aquatic environments (Burton et al., 2008). However, naturally occurring Fe oxides vary in morphology and crystallinity and are strongly influenced by the physical and chemical nature of the depositional environment (Ferris et al., 1989). Bigham et al. (1996) found particular mineral precipitates formed under different solution pH. Manganese is more soluble than other transition metals in slightly acidic to neutral waters, and is more difficult to be precipitate under natural conditions. Manganese oxides and hydroxides precipitate from mine waters at slightly alkaline pH (Larsen and Mann, 2005). Oxidation of  $\text{Mn}^{2+}$  precipitated poorly crystalline hexagonal birnessite, triclinic birnessite and todorokite in coal mine drainage treatment systems (Tan et al., 2010).

Iron and Mn minerals are effective in the removal of toxic metal(loid)s from mine water. Especially, arsenic (As) have high affinity for iron minerals. When schwertmannite was formed arsenic co-precipitated with schwertmannite and adsorbed onto goethite transformed from schwertmannite and ferrihydrite (Park et al., 2016). Arsenic(V) sorption to schwertmannite was high at low pH, while As(III) sorption was favored at high pH. Release of sulfate from schwertmannite with both As(III) and As(V) sorption indicated incorporation of As in schwertmannite structure (Burton et al., 2009). In anoxic condition, iron sulfide minerals such as mackinawite play an important role in the removal of arsenic. The removal of As by mackinawite was not affected by the presence of phosphate (Niazi and Burton, 2016). Therefore, characterization of minerals formed under different environmental conditions helps to understand metal(loid) attenuation in contaminated water.

Mineral precipitation can be mediated by abiotic and biotic processes. Abiotic pathways include oxidation by reactive oxygen species, manganese, and chemodenitrification. Biotic oxidation involves Fe oxidizing bacteria and phototrophic microorganisms (Melton et al., 2014). Iron and sulfate oxidizing bacteria contribute to the oxidation of sulfide minerals such as pyrite, arsenopyrite, chalcopyrite, sphalerite and marcasite, thereby releasing sulfur and metals to the surrounding environment (Baker and Banfield, 2003; Nordstrom et al., 2015). The activity of these bacteria and passive sorption and nucleation reactions precipitate biogenic minerals (Fortin and Langley, 2005). Mine streams can be characterized by low pH and high metal concentrations. Distinct species of microbes, such as acidophilic and neutrophilic bacteria, populate acid mine drainage due to the extreme conditions of acid mine waters (Baker and Banfield, 2003; Fortin and Langley, 2005). Although microbial community of acid mine sediments in a specific mine drainage was studied, the comparison of microbial community in mine precipitates in relation to different mineral forming environments was not conducted.

This study hypothesizes that mine precipitates possess different characteristics depending on the environmental conditions of the mine water, and as a result, different microorganisms interacts with the mine precipitates. Therefore, the objectives of this study were to compare acid mine drainage precipitates collected from various mines and their associated microorganisms among samples. Since Fe and Mn minerals have high affinity for metal(loid)s such as arsenic, understanding the characteristics of minerals formed and microbial community can lead to develop strategies for mitigation

of metal mining influenced water.

## 2. Methods

### 2.1. Sampling of mine precipitates

Mine water and precipitate samples were collected from six different abandoned mine sites (5 coal mines and 1 metal mine) in Korea. The five coal mines included Buguk, Gapjeong, Chungsan, Danbong and Samcheok; Ilkwang was the metal mine. Sampling locations are marked in Fig. 1. Yellowish red or brown black colored precipitates were collected from mine stream sediments. Three to four composite samples (40 g each) from each site were collected in sterile conical tubes and kept in a  $-80\text{ }^{\circ}\text{C}$  freezer before microbial community analysis. Samples for chemical analysis were freeze-dried. Water samples were collected in 1 L sterile water sample bottles.

### 2.2. Mine water analysis

Water samples were analyzed for pH and electrical conductivity (EC) using a calibrated pH and EC meter (Orion Star A211, Thermo Scientific). Anion ( $\text{F}^{-}$ ,  $\text{Cl}^{-}$ ,  $\text{Br}^{-}$ ,  $\text{NO}_3^{-}$ ,  $\text{PO}_4^{3-}$ , and  $\text{SO}_4^{2-}$ ) concentrations in water samples were measured by ion chromatography (IC, Metrohm) and other elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Si, and Zn) were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer), after passing through a  $0.45\text{ }\mu\text{m}$  syringe filter. The analytical data were used to calculate saturation indices (SI) using PhreeQC version

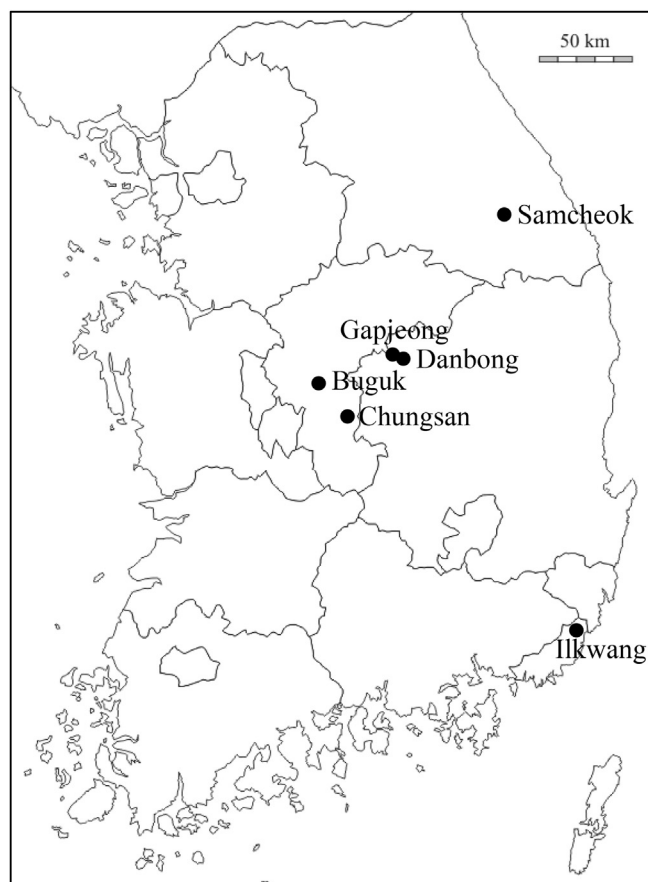


Fig. 1. Sampling locations (Buguk, Gapjeong, Samcheok, Chungsan, Danbong and Ilkwang mines).

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