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# AMD-Prevent

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# Abstract

Acid mine drainage (AMD) and acid rock drainage (ARD) are two of the most challenging environmental problems the mining industry faces worldwide. The major concern related to AMD, is its harmful environmental impact due to heavy metal rich, acidic leachates that originate from the mining activities of sulfur rich metal ores reaching ground and surface water.

AMD prevent is a pre-study to take the first step in developing a passive, preventative measure for acid mine drainage. The first step is to map the microbial composition and geochemical conditions of a Swedish mine in a cold climate to determine AMD generating parameters.

A literature study was performed to investigate the current research of AMD generation. Field studies for microbial and waste rock sample collection were carried out on two different occasions, one in autumn and one in spring to capture possible seasonal changes in AMD generation.

It can be concluded that most previous studies investigated tailings and water streams for AMD and few sampled waste rock. It was difficult to sample waste rock, but successful extractions were achieved after some method development. This report contributes to the research gap of microbial composition on waste rock in cold climates for AMD generation.

Key words: Acid mine drainage (AMD), microbial composition, pyrite, prevention

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# Content

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# 1 Introduction

A secure supply of metals is a key factor in advancing sustainable development and moving towards an electrified, net-zero, carbon dioxide emitting society. While the preferential use of recycling and processing secondary metal sources will rise, electrification is expected to increase the global demand for metal resources that will require continued mineral mining. As part of this transition, the Swedish mining industry aims to be at the forefront of sustainable mining practices. However, one of the major environmental and sustainability challenges the global mining industry faces is the generation of waste, with approximately 103 505 000 tonnes generated in 2018 in Sweden, and several hundreds of billion tonnes globally (Bell et al., 1998). To achieve sustainable and responsible mining practices within the industry, the urgent issue of mining waste management must be addressed, and subsequently be improved through improved methods and strategies.

Acid mine drainage (AMD) and acid rock drainage (ARD) are two of the most challenging environmental problems the mining industry faces worldwide[1] and are linked to the waste materials produced in the mining of sulfide ores. The major concern related to AMD, is its harmful environmental impact due to heavy metal rich, acidic leachates that reach ground and surface water, as AMD permeates soil, ore, and rocks leading to a detrimental impact on aquatic ecosystems in adjacent lakes and rivers (Acharya & Kharel, 2020; Bell et al., 1998; Ochieng et al., 2010)

This phenomenon occurs naturally on small scales but is predominantly associated with waste rocks and process tailings of mined sulfide-containing ores from deep geological environments (Park et al., 2019). When these waste products are exposed to oxygen and moisture, a chemical release of sulfuric acid and toxic metals occurs (Vera et al., 2022). This problem is further compounded through the gradual propagation of various iron- and sulfur-oxidizing microorganisms that accelerate these reactions (Vera et al., 2022). The timescales of AMD vary greatly and are dependent on the geology and scale of mining activities, and the various seasonal and geographical weather conditions (Cánovas et al., 2021).

To achieve sustainable and responsible mining practices within the industry, the urgent issue of mining waste management must be addressed, and subsequently be improved through improved methods and strategies. Therefore, a preventative, inexpensive AMD treatment strategy is highly sought after. The aim of this project was to take the first step towards such a treatment, by characterizing the microbial ecology for waste rock deposits in cold climates that generate AMD. This report consists of a literature study, summarizing the current field of AMD microbial consortium characterization in relation to climate and geochemical conditions at mines worldwide. From there, we describe the field studies where microbial and geochemical samples along with weather data were collected at a Boliden mine. The report sums up with suggestion of other preventative measures for AMD and gives recommendations for future work. The project was funded by Åforsk and Boliden and the field studies were taking place at the Kristineberg mining

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[1] From here on, AMD and ARD will be referred to as AMD due to their similar chemical and physical properties. The difference between AMD and ARD is (simplified) in how it is generated i.e., AMD is generally a result of (underground) mining activities whereas ARD is the result of either open pit mining (various types) or naturally occurring oxidation of ore exposed to air while in nature.

site in October 2022 and May 2023. The project consortium was RISE, Boliden, and Linnaeus University.

## 1.1 The origin of AMD

Multiple factors influence AMD that have been categorised into three groups: generation factors (oxygen, water, bacteria), chemical factors (acidity, alkalinity), and physical factors (waste rock/tailing particle size, permeability, weathering, and hydrology) (Acharya & Kharel, 2020). Each of these variables work mutually, but pH is regarded as a leading parameter to AMD and governs metal dissolution. Low pH (<4.5), with respect to oxygen, shows the highest the presence of iron, aluminium, and manganese, whereas increasing alkalinity (pH >6), shows a gradually reduced presence of these metals but higher level of suspended particles, (Acharya & Kharel, 2020). The oxidation of sulfide-containing ores, for example, iron (pyrite), can be considered to be gradually lowering pH levels, and can be summarised through the following equations (Evangelou & Zhang, 1995):

$$2FeS_2 + 2H_2O + 7O_2 \Rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \text{ (eq. 1)}$$

$$4Fe^{2+} + 4H^+ + O_2 \Rightarrow 4Fe^{3+} + 2H_2O \text{ (eq. 2)}$$

$$FeS_2 + 7Fe_2(SO_4)_3 + 8H_2O \Rightarrow 15FeSO_4 + 8H_2SO_4 \text{ (eq. 3)}$$

Equations 1-3 demonstrate the stepwise process of acid formation through pyrite oxidation (Evangelou & Zhang, 1995), that can occur in either biotic or abiotic conditions. Equation 2 is considered a rate limiting step under abiotic conditions, as formation of iron(III) is dependent on $O_2$ oxidation of dissolved iron(II). This however can be significantly accelerated as mentioned previously through acidophilic, iron-oxidising bacteria (e.g., *Acidithiobacillus ferrooxidans*) by a factor $10^6$ (Evangelou & Zhang, 1995). Biotic pyrite oxidation, typically under low pH conditions, can therefore be demonstrated through equations 2 and 3, and forms the principal basis of AMD formation studied globally. These reactions are driven through the oxidising roles of acidophilic bacteria that utilise electrons from oxidation of sulfur, pyrite (e.g.: Fe(II) to Fe(III)) and other sulfide ores, as a source of energy (Dugan, 1972). The biotic, acidophilic oxidation of reduced sulfur can offer up to eight electrons in comparison to a single electron conferred by iron, that when furnished with relevant electron transport machinery, may result in higher ATP production per electron mol than iron(II) (M. Dopson & Johnson, 2012). Despite the relative difficulties of sampling and sequencing these bacteria (Kuang et al., 2013), several full genome sequences are published, see for example the work by Mark Dopson and Okibe (2022) or Teng et al. (2017). This moves towards entire -omics studies to understand the relevant metabolic and survival mechanisms in AMD prevalent species (Mark Dopson & Okibe, 2022). Many reviews have described the communities of sulfur and iron-cycling AMD bacteria and have described an intricate symbiotic relationship which is thought to aid in robust survival and adaptation (L. X. Chen et al., 2016; Kuang et al., 2013).

With this concept in mind, it can be regarded that this subset of bacteria is ubiquitous in nearly all examples of anthropogenic AMD occurrences where sulfidic metals are present. However, a further question arises as to whether these acidophiles are as prevalent in cold climates as they are in temperate ones, with only few published examples showing in-depth study (Auld et al., 2016; Liljeqvist et al., 2015; Zhao et al., 2021). Moreover, typical analysis of microbial diversity in AMD focuses on rock tailings and leachate effluent, instead of waste rock, which is a significant component of mining wastes. Further attention could lead to a clearer understanding of whether symbiotic relationships offer enhanced resilience in cold climates, and whether the nature of waste sources impacts their propagation.

# 2 Current understanding of AMD in cold climates

To determine the current state of knowledge in cold climate AMD, a systematic search of the available literate was performed. From this it was possible to compare literature with the results from the field studies and identify knowledge gaps.

## 2.1 Literature search method

A systematic literature search was conducted to compare the literature on characterization of microbial consortia and abiotic parameters for AMD generation with the field studies performed in this project (Section 3). The search engines used were Google Scholar, Science Direct and Scopus. A two-round search for articles was done, the second round was made with more refined search terms based on the knowledge gained from the first round.

AMD is a very wide research field, with a lot of different topics and published studies. In order to narrow down the search field, a research question was defined. The research question was: “What are the chemical, biological and environmental factors impacting microbial AMD generation in continental and polar climates?”

Initially the scope focused on cold climate, rocks (specifically sulfidic ore/ waste rock) and biological + chemical interactions driving the system. This focus needed to be broadened along with the search, since very few articles were found in this specific area.

The inclusion criteria for the search were designed to 1) Include both Swedish and English literature. 2) peer reviewed journal articles, 3) non-reviewed articles, 4) book chapters/extracts 5) field studies, lab studies of system view, studies with focus on pH gradients, studies on DNA analysis of ARD. The main idea was to focus the study on cold/temperate climates. However, some exceptions were made regarding the study of seasonal variability in microbial community, long-term monitoring of site and abundant sampling of site(s). This means that some articles were chosen even though being outside of the scope of the inclusion criteria. The following search criteria was excluded in the search: 1) Purely chemical and physical studies of AMD/ARD systems, AMD/ARD streams, treatment/bioremediation, coal/shale related waste rock piles 2) non-online accessible articles, grey papers, conference papers and theses.

Key words used in the first and second search round are found in Table 1.

Table 1: Search terms/keywords used for first and second round

| **Key words 1st round** | **Key words 2nd round** |
|---|---|
| ARD+ "cold climate"+microorganisms | "pyrite oxidation" + "microbiology" |
| "acid rock drainage"+"cold climate"+microorganisms | "microbial ecology model"+"pyrite" |
| "acid rock drainage generation"+bacteria | |
| "acid rock drainage" + biotic + "cold climate" | |
| "acid rock drainage" + "physical properties" + "cold climate" | |
| "acid rock drainage" + biotic | |
| "acid rock drainage" + DNA | |

The articles were selected and evaluated based upon 1) title, 2) abstract and 3) full text.

A couple of drawbacks with the methodology used were identified during the literature study. Many articles found were older, even though some newer ones are also included in the study. It was difficult to get new relevant articles with the search terms used. Six full texts selected were published 2019 or later and 16 full texts were published 2014 or later, meanwhile the remaining 28 full text articles were published before 2014.

## 2.2 Results

Data from the full text articles were collected to analyse trends and the overall results from the topic. Data was collected based on country/site, climate, pH, geochemical data, AMD or ARD, mine related or other AMD generating source, type of study, study period (short, medium or long study), seasonal variation, detected microorganisms and keywords mentioned. The number of articles found for each selection criteria and search round are listed in Table 2.

Table 2: Main results found in articles

| | First round | second round | Total |
|---|---|---|---|
| **Abstract** | 62 | 57 | 119 |
| **Full text read** | 21 | 23 | **44** |
| **Full text uncertain** | 15 | 14 | 29 |
| **Full text rejected** | 26 | 20 | 46 |

Analysing the results from the collected data showed some trends in the literature about AMD. Most articles describing the system, focus on AMD and not ARD – i.e., the processes in a wet environment (e.g., tailings dams, runoff streams, rivers etc.) rather than the processes in waste rock piles with circulation and drainage.

***Acidithiobacillus*** and ***Leptospirillum*** are the most commonly reported genera in AMD-communities. Some report specific species, others report findings as phylum or general metabolic groups (e.g., iron oxidising bacteria, sulfur oxidising bacteria). *Acidithiobacillus* is the most dominating genus found in the reviewed literature, 27 articles identified this strain. The second most common strain is *Leptospirillum*, which is found in 20 articles, often in the same ones as *Acidithiobacillus*. *Acidophilium* was found in seven articles followed by *Ferrovum* found in three articles, finally *Sulfobacillus* was found in three articles. *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* are the most common species found when specifying on strain level. They were found in 13 and 10 articles respectively.

A mix of laboratory/field and review articles were included in the literature study. Laboratory/field studies represented 26 out of the articles and 14 were reviews. There was a large variation of pH and climate in the articles, even though a low temperature and a pH at the tipping point were the focus for the selection criteria of the articles. Twelve articles studied climates below 15 °C. 14 articles were below pH 3 and 19 articles were around pH 3 ±0.5 and ten articles investigated AMD around pH 6 ±0.5. Archaea are found primarily in warmer climate zones and not in colder climates.

Table 3 summarises the most frequent reported microorganisms found for different criteria such as pH intervals, cold climate, seasonal variation and long-term studies. It also shows how many articles are found withing each category of parameter. There are a lot of similarities in all categories and most microorganisms are found in each. The most diverse spectrum of microorganisms is found in the pH range around 3 ± 0.5.

Table 3: Most frequent reported microorganisms found for different pH intervals, cold climate, seasonal variation and long term studies

| Parameters | Number of articles | Most frequent reported microorganisms |
|---|---|---|
| **pH ≤6±0.5** | 10 | *A. ferrooxidans, A. ferrivorans, Acidithiobacillus, A. thiooxidans, Leptospirillum, Leptospirillum ferrooxidans, Thiobacillus, T. thioparus, Acidiphilium, Thiomonas, Sulfobacillus, Iron oxidising (IOB), sulfur oxidising (SOB), SRB, Acidobacteria, Desulfotomaculum spp., Actinobacteria, Proteobacteria, Nitrospira, Firmicutes, Bacteroidetes, Acidiferrobacter, Ferritrophicum* |
| **pH ≤3±0.5** | 19 | *A. thiooxidans, Acidithiobacillus, Acidithiobacillus ferrivorans, Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans, Leptospirillum, T. thioparus, Thiobacillus, Acidiphilium, Thiomonas Sulfobacillus, Sulfobacillus thermosulfidooxidans, Iron oxidising (IOB), sulfur oxidising (SOB), SRB, Acidobacteria, Desulfotomaculum spp., Actinobacteria, Proteobacteria, Candidatus Fodinabacter communificans, Nitrospira, Firmicutes, Ferrimicrobium acidophilum, Bacteroidetes, Alicyclobacillus* |

| Parameters | Number of articles | Most frequent reported microorganisms |
|---|---|---|
| | | *cycloheptanicus, Chloroflexi, Clostridium and Verrumicrobia, , Acidimicrobium* |
| **pH <3** | 14 | *A. thiooxidans, Acidithiobacillus, Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans, Leptospirillum, T. thioparus, Thiobacillus, Acidiphilium, Thiomonas, Sulfobacillus, Iron oxidising (IOB), sulfur oxidising (SOB), SRB, Desulfotomaculum spp., Ferrimicrobium, Acidimicrobium, acidophilic chemolithotrophs* |
| **T ≤ 15 °C** | 12 | *Acidithiobacillus ferrivorans, Acidithiobacillus, A. ferrooxidans, Leptospirillum ferrooxidans, Acidiphilium, Iron oxidising (IOB), sulfur oxidising (SOB), SRB, Acidobacteria, Actinobacteria, Proteobacteria, Candidatus Fodinabacter communificans, Nitrospira, Firmicutes, Ferrimicrobium acidophilum, Bacteroidetes, Alicyclobacillus cycloheptanicus, Chloroflexi, Clostridium, Verrumicrobia* |
| **Seasonal variation** | 11 | *Acidithiobacillus, Acidithiobacillus ferrooxidans, Leptospirillum, Leptospirillum ferrooxidans, Thiobacillus, Acidiphilium, Acidobacteria, Actinobacteria, Proteobacteria, Candidatus Fodinabacter communificans, Nitrospirae, Firmicutes, Bacteroidetes, Acidiferrobacter, Ferritrophicum, thermoplasma, Ferrovum myxofaciens, Ferroplasma acidiphilum, acidophilic chemolithotrophs* |
| **Long term study** | 5 | *Acidithiobacillus, Acidithiobacillus ferrooxidans, Leptospirillum, Thiobacillus, Acidiphilium, IOB, SOB, Acidobacteria, Actinobacteria, Proteobacteria, Candidatus Fodinabacter communificans, Nitrospirae, Firmicutes, Ferrimicrobium, Bacteroidetes, Acidiferrobacter, Ferritrophicum, COT, Acidimicrobium* |

Implications of results found in the literature study are discussed in the context of this reports case study in Section 4.

# 3 Kristineberg case study

Boliden's Kristineberg Mine was selected for the case study since it had the required cold climate conditions, sulfide waste rock piles of different ages, and the area chosen had no active mining activities. The lack of mining activities simplified the field work and gave an opportunity to preserve the site for future sampling. The mine consists of polymetallic Volcanogenic Hosted Massive Sulfides ("VHMS"). Ore from the mine is processed at the Boliden Area Operations plant for production of copper, lead and zinc concentrates.

## 3.1 Method

Samples were taken from the Kristineberg Mine on the 31 of October 2022 and 30 of May 2023. These sampling times allow for assessment of changes in microbiological consortia in the waste rock during winter. Three sampling areas were selected based on the age of the waste rock, where rock surface pH was used as an indicator of age. For the youngest waste rock samples, a pile of fresh rock was created specifically for the purpose of this study. This was done since fresh waste rock typically becomes covered with more fresh rock as mining operations continue. Figure 1 show the three sample locations at Kristineberg.

Figure 1 Sampling areas at Boliden's Kristineberg Mine

### 3.1.1 Weather data

Three Ebro EBI 20-TH1 temperature and humidity loggers were placed at different locations at the Kristineberg Mine. The loggers were set to take a reading every one hour. Humidity sensors are sensitive to water ingress and typically have a low ingress protection (IP) rating for water. To prevent sensor damage from water the sensors were mounted inside a protective PE (Polyethylene) housing designed to shelter the sensors from rain and prevent submersion of the sensor.

The sensor housing was planned to be mounted on a stake above the ground to allow an enough airflow around the bottom of the protective housing. During the October 2022 visit it was found that the ground was frozen, and it was not possible to drive a stake into the ground for mounting the housings. Given this, the housing was mounted in nearby trees (Figure 2). On the May 2023 visit it was found that one of the housings had become dismounted from the tree and water had damaged the sensor in the humidity logger. The two other sensors were in good condition. One was recovered to extract data, while the other has been left at the site to continue collecting data in the event of a follow up study being conducted.

Figure 2 Temperature and humidity loggers in protective housing mounted to nearby trees.

### 3.1.2 Geochemical analysis

In the first sampling campaign, rock samples were taken from different locations in the waste rock dumps near the biological sample locations. After analysis from the first campaign, it was clear that the waste rock dumps were not homogenous in composition with large differences within the same sample age area. These large differences make comparison of the bacterial community to the geochemistry difficult as there is a risk that the rock the bacteria are on is different to the rock analysed to determine geochemical composition. Given this, for the second campaign rock samples were taken in the same place as where biological samples were taken. Results from the first sample campaign are not used in the analysis but are provided in the appendices (Section 7.2).

Rock samples were sieved at 2 mm to separate out coarse material that may not fully represent the environment the bacteria are present in. Both the coarse (>2 mm) and fine (<2 mm) fractions were analysed for metal content. Sample analysis was performed by ALS Scandinavia in Luleå. ICP-SFMS as per SS-EN ISO 17294-2:2016 was used for elemental analysis following sample digestion. Two digestion methods were used depending on the target element. Hot nitric, hydrochloric and hydrofluoric acid digestion (according to SS-EN 13656:2003) was used for As, Be, Cd, Cu, Hg, Mo, Ni, P, Pb, S, Sb, Sn, W, Zn. LiBO2 fusion followed by acid digestion (according to ASTM D3682:2013, ASTM D4503:2008, and An. Chem. 50:679-680) was used for all other elements (Al, Ba, Ca, Co, Cr, Fe, K, Mg, Mn, Na, Nb, Sc, Si, Sr, Ti, V, Y, Zr). Analysis of results was conducted using the Python packages pandas and scipy.

### 3.1.3 Biological analysis

The microbial samples from the Kristineberg mine were taken in the same area as the rock samples shown in Figure 1. The goal of the three sampling areas was to find different progression of AMD with different rock ages. The waste rock was sampled as aseptic as possible to avoid contamination of human DNA. Rocks in the size of 10 cm in diameter were chosen and marked with colouring spray. The rock was flipped, and fine particles were collected in Falcon tubes with sterile spoons and nucleic acid preservation solution was added to the tube. The pH was measured in close proximity of the sampling point, but a couple of centimeters away to avoid contamination. Samples were collected in triplicates and at six different locations of each age of waste rock. The samples were stored at -20°C before processing. The samples were processed at Linnaeus University for DNA extraction and 16S rRNA gene sequence amplification and sent for sequencing at the Science for Life Laboratory, Sweden. Linnaeus University performed bioinformatics on the received sequencing data to compare the results from the two sampling points and the different waste rock piles.

## 3.2 Results

### 3.2.1 Weather data

Daily minimum, average and maximum temperature and relative humidity between November and May are shown in Figure 3 and Figure 4. Temperatures were typically below 0 °C from November 2023 to the end of January 2023. During February and March there was an increase in the number of days with maximum temperatures 0 °C and by April average temperatures were typically above 0 °C. By the end of the measurement period minimum temperatures were still typically near 0 °C, with only one value above 5 °C recorded.

Figure 5 shows intraday temperature and relative humidity difference over the measurement period. A change can be seen around February 2023 where the intraday variation increases for both measurements. The impact of this on microbiology in the waste rock is discussed in Section 4.

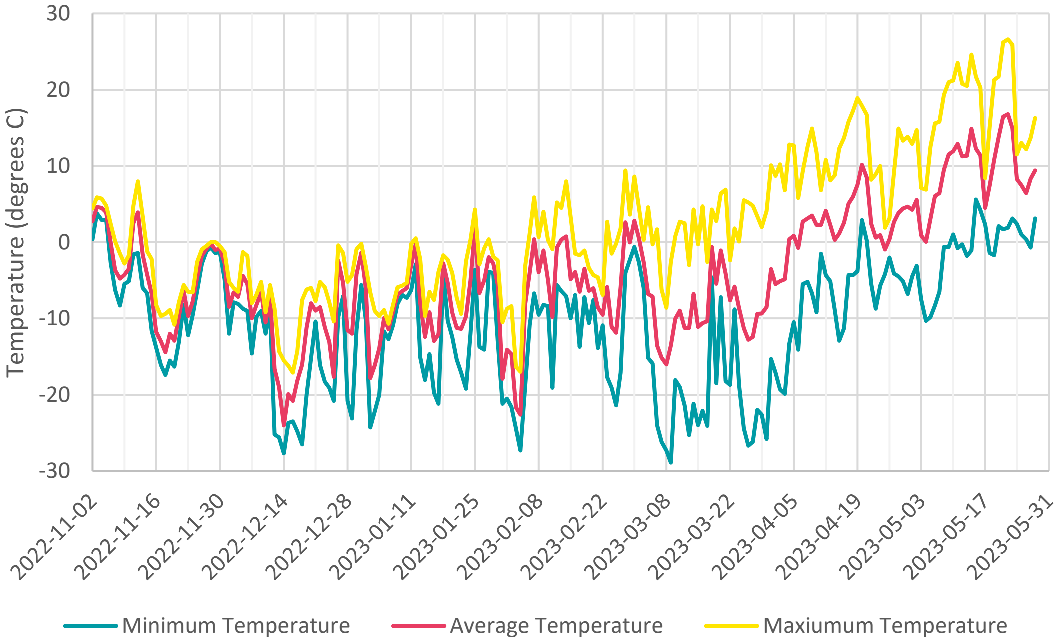

Figure 3 Daily minimum, average and maximum temperatures logged at the Kristineberg mine site from November 2022 to May 2023.

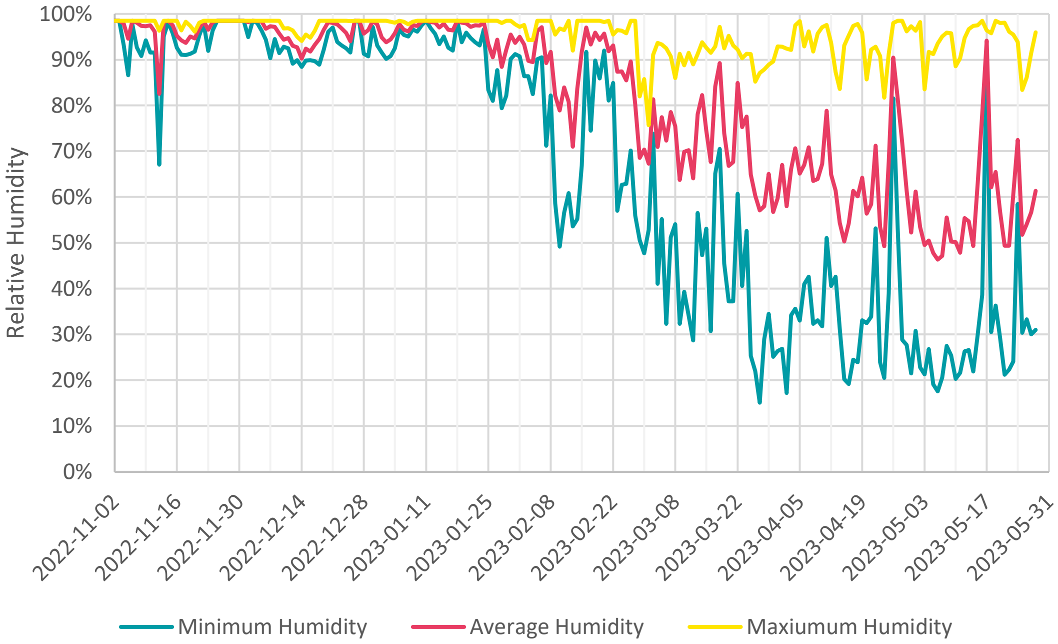

Figure 4 Daily minimum, average and maximum relative humidity logged at the Kristineberg mine site from November 2022 to May 2023.

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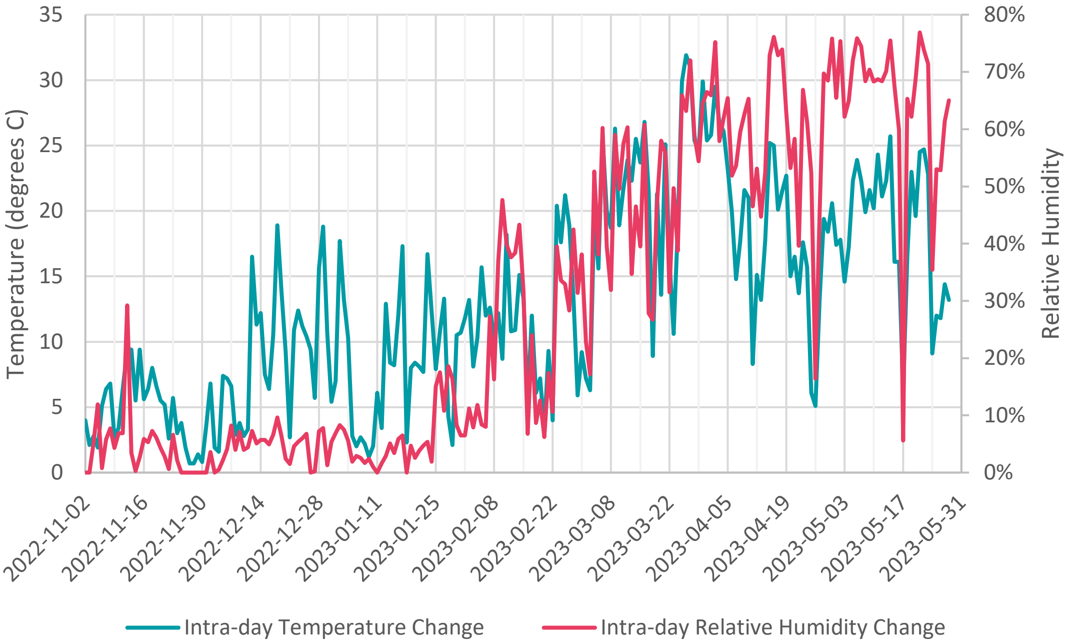

Figure 5 Intra-day difference between minimum and maximum temperature and humidity logged at the Kristineberg mine site from November 2022 to May 2023.

## 3.2.2 Geochemical analysis

Two geochemical samples have been identified as potential outliers compared to samples taken in the same area. In the old rock the rejected sample had notably higher iron content of 35.6% compared to an average of 5.3% ± 0.6% once the sample is removed. This iron value suggests this material is predominantly iron oxide. In the mid aged rock, the rejected sample had notably higher calcium content of 17.0% compared to an average of 0.6% ± 0.3% once the sample is removed. Elemental composition of the waste rocks for key elements are shown in Table 4.

Table 4 Key results of geochemical analysis from the May 2023 sampling campaign.

| Sample area | Old | Mid | Young |
|---|---|---|---|
| pH | 4.0 ± 0.3 | 7.6 ± 2.2 | 9.8 ± 1.1 |
| Fe coarse fraction | 5.3% ± 0.6% | 6.3% ± 3.7% | 4.0% ± 0.3% |
| S coarse fraction | 0.5% ± 0.1% | 4.4% ± 5.3% | 1.9% ± 0.4% |
| Fe:S molar ratio coarse fraction | 6.0 ± 1.3 | 1.7 ± 1.3 | 1.2 ± 0.2 |
| Al coarse fraction | 6.8% ± 0.4% | 6.6% ± 0.4% | 6.6% ± 0.1% |
| Ca coarse fraction | 0.6% ± 0.1% | 0.6% ± 0.3% | 0.9% ± 0.1% |
| K coarse fraction | 1.6% ± 0.1% | 1.8% ± 0.4% | 2.3% ± 0.2% |
| Mg coarse fraction | 3.3% ± 0.3% | 2.6% ± 1.2% | 1.9% ± 0.3% |

| Sample area | Old | Mid | Young |
|---|---|---|---|
| Na coarse fraction | 1.2% ± 0.3% | 0.8% ± 0.5% | 0.2% ± 0.0% |
| Si coarse fraction | 31.5% ± 0.5% | 30.2% ± 5.9% | 32.3% ± 0.4% |
| Other coarse fraction | 48.8% ± 1.2% | 46.0% ± 4.3% | 49.2% ± 0.6% |
| LOI coarse fraction | 4.9% ± 0.3% | 7.1% ± 4.7% | 4.1% ± 0.3% |

Iron and sulfide content was not significantly different between the three age groups (Fe p=0.204, S p=0.140). Iron to sulfide molar ratio (Figure 6), which can be an indicator of oxidation through AMD mechanisms, was significantly different between the age groups (p<0.001). The old material had a considerably higher iron to sulfide ratio (6.0±1.3) compared to the mid aged (1.7±1.3, Tukey HSD p<0.001) and young rocks (1.2±0.2, Tukey HSD p<0.001). This suggests a higher degree of oxidation in the old rocks. No significant difference was seen between min and young rock (Tukey HSD p=0.804).

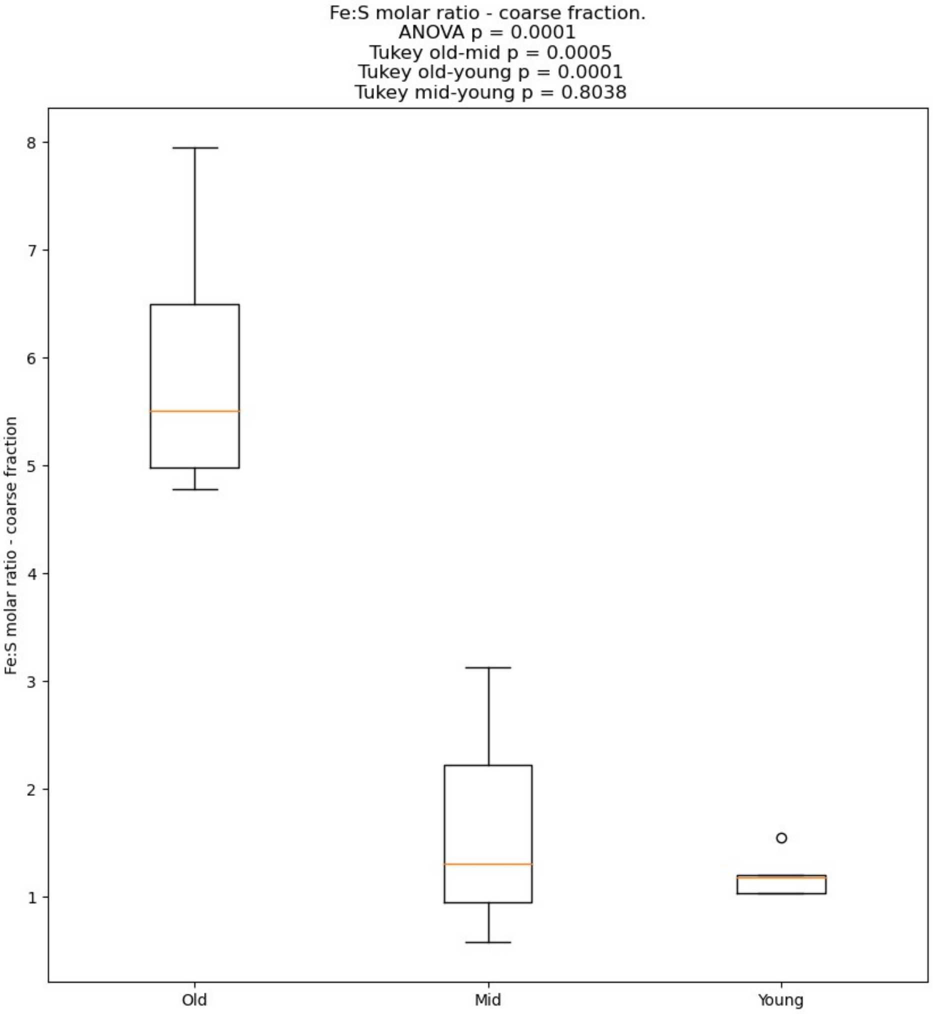

Figure 6 Box plot of iron to sulfide molar ratio in the waste rock grouped by age.

While iron to sulfide ratio is potentially an indicator of the degree of iron sulfide oxidation in the rocks, care must be taken with this interpretation since sulfur may be present in any non sulfide minerals as sulfates. Nonetheless, the pH data (Figure 7) from the different sample sites aligns with the interpretation of the old rock being more oxidised since it has a lower pH (4.0±0.3) compared to the mid aged (7.6±2.2, Tukey HSD $p=0.007$) and young (9.8±1.1, Tukey HSD $p<0.001$) rocks. The pH difference between the mid and young age rock was marginally significant (Tukey HSD $p=0.084$). Visual inspection of the area also aligns with these conclusions.

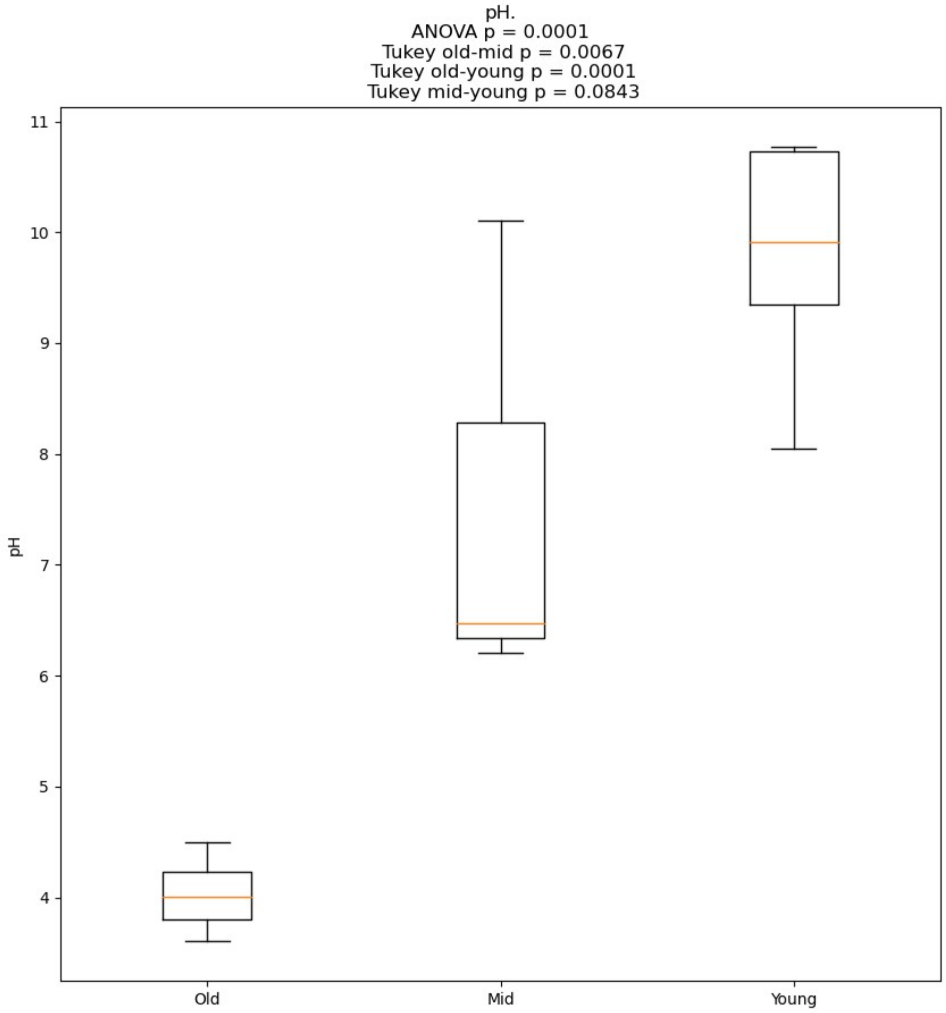

Figure 7 Box plot of pH on the waste rock grouped by age.

Finally, if an iron sulfide such as pyrite ($FeS_2$) is converted to haematite ($Fe_2O_3$) there will be a reduction in mass of the mineral due to the higher iron content in pyrite (47%) versus haematite (70%). Given this, any trace metals with low solubility will become concentrated. One such metal is arsenic which requires low pH values to be soluble and is not mobile during the AMD process (Tabelin et al., 2020). Figure 8 shows that arsenic follows the same trends as iron. The increasing iron concentration, decreasing pH values, and increasing arsenic concentrations all point towards different degrees of oxidation of the waste rock assuming there initial compositions were similar.

Cobalt, which can be an indicator of AMD occurring showed significant results with one way ANOVA (p=0.0465). Nonetheless, Tukey HSD did not show significant differences between the groups. Given this, cobalt is not a good indicator of differences in the mineralogy with respect to age.

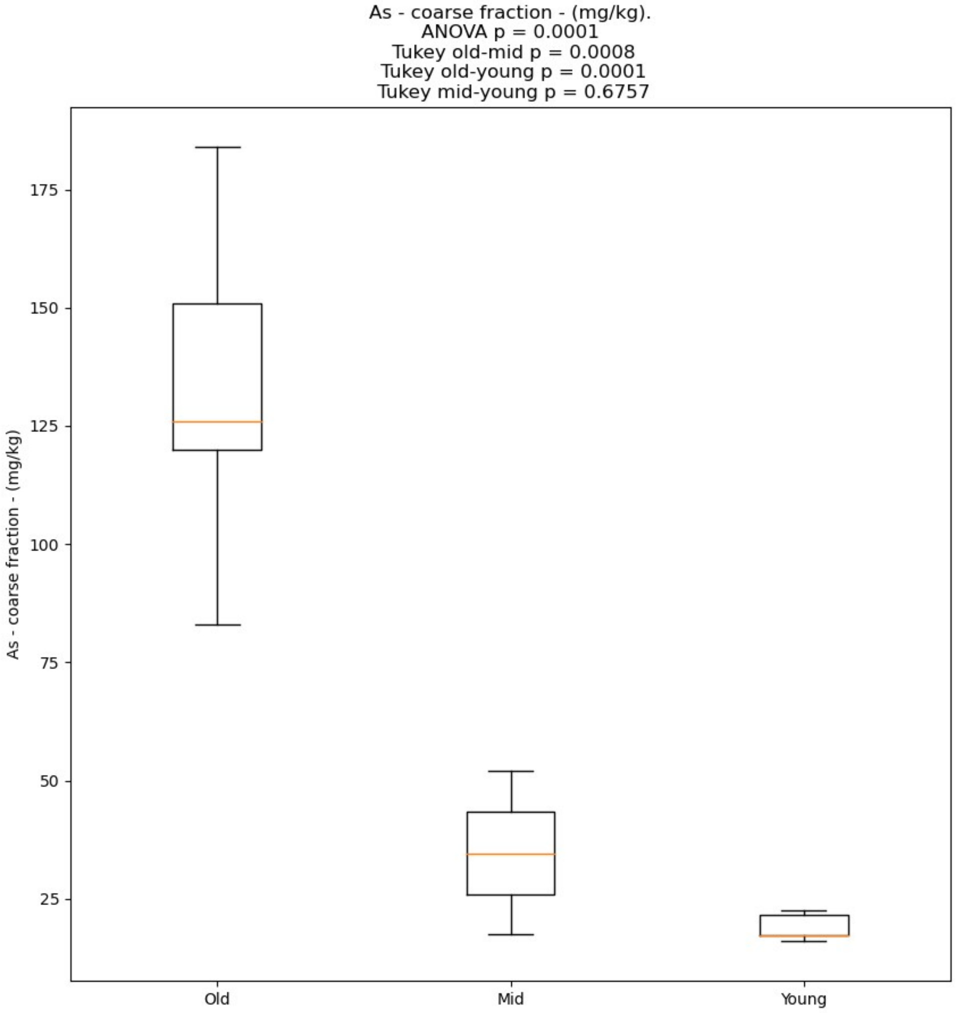

Figure 8 Box plot of arsenic ratio in the waste rock grouped by age.

### 3.2.3 Microbiological analysis

The DNA extraction followed by the sequencing of the microbial communities for the two sampling points (October 2022 and May 2023) showed no significant difference in the composition of the microbial communities for the mid and old waste rock pile. There was a difference from the sampling points in the new rock pile. This result suggested that the difference depended on a change of environment for the new rock pile, it was freshly sampled from the mine in the first sampling. For the second sampling the microbial community had been allowed to adapt to the new environment with a surface exposed to air and water, something that is depleted in the lower parts of a mine. In one of the samples from the mid aged waste rock pile contained significant amounts of the order *Acidithiobacillales*, something not found in the other samples or waste rock of other ages. This suggests a tipping point for AMD generation with a microbial community promoting AMD formation. Other AMD generating microbial communities were found in the old waste rock pile, corresponding to the visual identification that the areas are generating AMD. The different microbial communities at order level for the young, mid and old waste rock pile for the first and second sampling point can be found in Figure 9 and Figure 10.

Figure 9: Microbial communities at order level in young, mid and old waste rock pile, first sampling October 2022

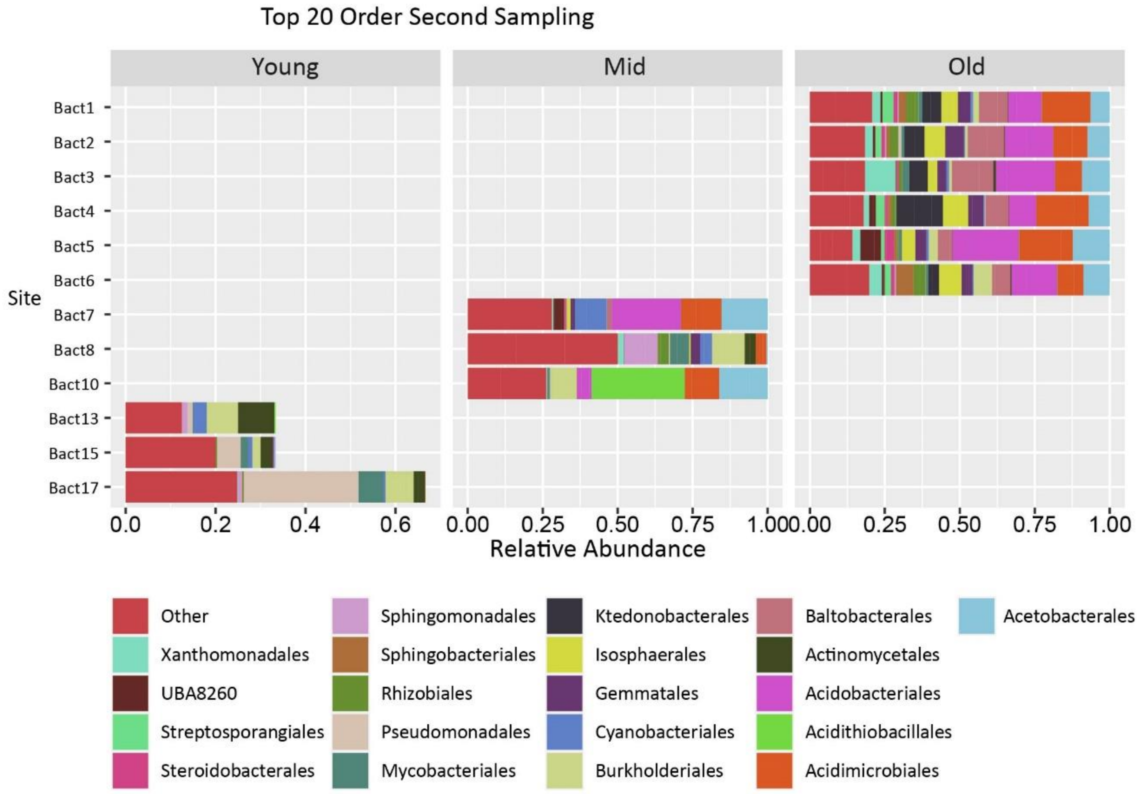

Figure 10: Microbial communities at order level in young, mid and old waste rock pile, second sampling May 2023

Figure 11 shows the Beta distribution of the microbial community of the two sampling points for all waste rock ages. It is a clear significance in each distribution depending on rock age and it can be seen that the young waste rock is getting more similar to the mid aged waste rock by the second sampling point.

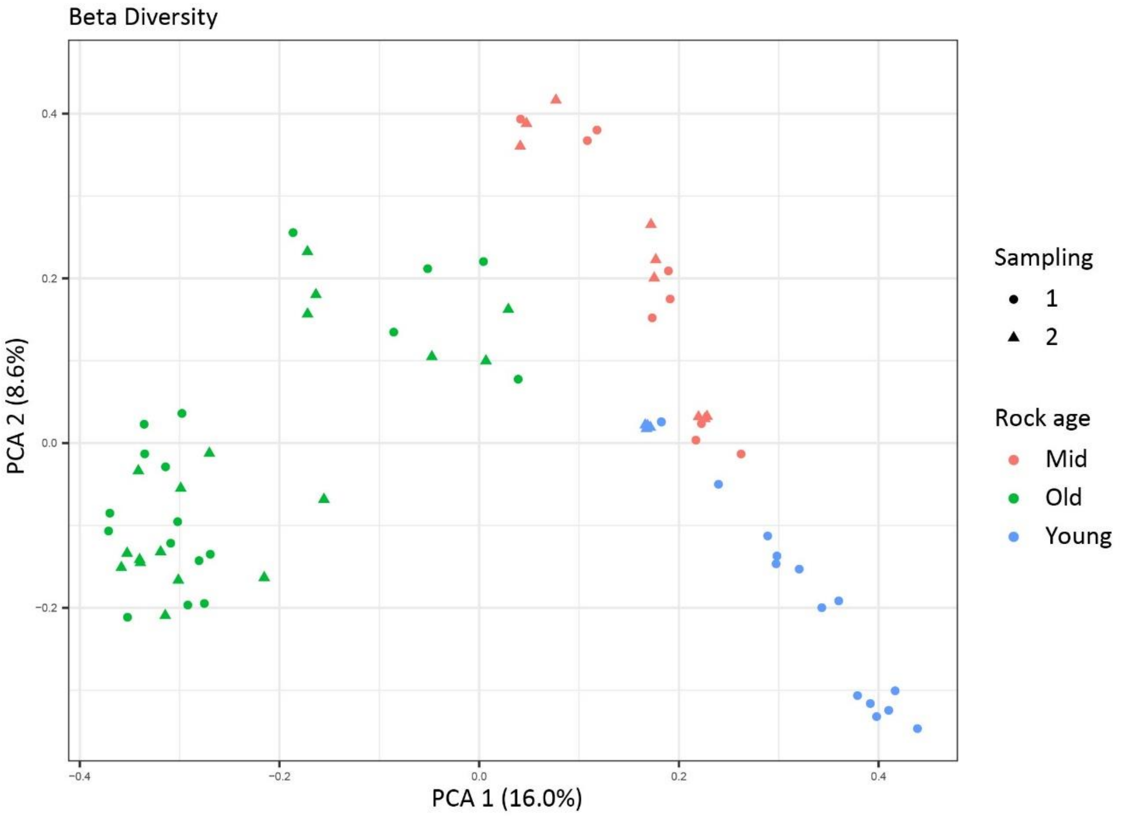

Figure 11: The Beta Diversity of the microbial community for the two sampling points for all waste rock ages.

# 4 Discussion

A key result from the work is that there is a limited number of studies specifically on AMD from waste rock piles. Most studies discuss tailings and water streams rather than waste rock. Typically, tailings are made up of fine particles as a result of crushing and grinding in mineral processing operations. These fine particles are typically less than 1 mm and are often in ranges of 50 to 500 µm. The fine nature of tailings offers significantly more surface area for microbial attack of the minerals. Further, hydrological aspects are potentially different for tailings and waste rock dumps given the difference in particle size distribution and use. For example, water flow and ground freeze will be different in a waste rock dump versus an active tailings storage facility.

Subsequently, this creates two differences between studying tailings and waste rocks:

- A difference in the microbial consortia on the rocks/minerals
- More difficulty in sampling of waste rocks due to lower bacterial count and possibly a difference in the attachment of bacteria to the surface of the harsh waste rock than in cavities of tailings.

For point 1, more studies of waste rock dumps are required to determine if there are differences in the microbial consortia of waste rock dumps in cold climates versus tailings storage facilities. For point 2, the difficulty of sampling rock arose in this study where the waste rock could not only be swabbed on the surface, but the rock samples needed to be washed to allow DNA extraction in the relative low biomass to waste rock ratio.

During the literature search articles relating directly to the Kristineberg mine site were found (Kock & Schippers, 2008). The field studies from these articles were performed in 2003. These articles were investigating tailings and not waste rock. It found genera such as *Acidithiobacillus*, *Leptospirillum* and *Sulfobacillus*, which are common genera of microorganisms found in the literature. The sampling of tailings rather that waste rock suggests a difference in microbial sampling techniques and findings compared to our field study, corresponding to the discussion above related to the differing physiochemical conditions in the two sample types.

In general, *Acidithiobacillus* is reported in many AMD microbial analysis studies in the scientific literature. At Kristineberg, *Acidithiobacillus* was only found in one of the samples in the mid-aged rock pile. In the oldest rock pile, a lot of the other microbial communities were found corresponding to literature findings about AMD/ARD, such as Acidobacteria and Acetobacteria. It is suggested that the mid-age pile might be reaching the tipping point and are still oxidizing whereas the old pile have other driving forces for the AMD generation with a different microbial community. Not many AMD generating microorganisms were found in the fresh waste rock pile, which corresponds to the literature of AMD being a slow progression over time and environmental factors has a large impact on the system. It can be seen in the study that the new pile moves towards the mid-age pile when comparing the two seasonal sampling points. The microbial community gets more similar to the mid-age pile with time.

During the study large intraday temperature variations were observed from February 2023. It is expected that such temperature variation can have an impact on microbiology in the waste rock. Nonetheless, temperatures in the waste rock pile are expected to stabilise with depth and with temperature variations occurring over longer time periods. The British Geological Survey state that once a depth of 10 to 15 meters is reached temperatures can be expected to be the same as the yearly average (for example see British Geological Survey (2011)). It should be noted this may be different in Nordic climates were there may be deeper frost depth. Temperatures also impact the movement of water through the waste rock pile due to freezing and thawing cycles (for example see Y. Chen et al. (2022)). Finally, sulfide rocks undergoing AMD process can generate heat that will may change temperature profiles compared to other rocks or soils. Given this, modelling of AMD in cold climates should consider how temperature and water mobility is impacted at different depths. This is unique to cold climate AMD generation. Any modelling of AMD generation in cold climates needs to capture these aspects.

## 4.1 Old and new mitigation strategies

It can also be concluded that not many passive, preventative methods were found for waste rocks, most focus on treatment of AMD with neutralization. A summary of other mitigation strategies found in the literature is presented below.

AMD begins as a slow abiotic process which is initiated with exposure of sulfide-rich minerals to air and water, resulting in sulfuric acid and metal leaching, both of which are discharged into nearby water sources. The process is accelerated biotically through the propagation of acidophilic, iron-oxidising bacteria that thrive in extreme environments. As AMD is primarily governed by oxygen, water and microbial activity, excluding these variables could offer long-term solutions. Creating passive oxygen and moisture barriers, as well as pH neutralisation are well-established strategies of AMD mitigation in mining operations which effectively suppress pyrite-oxidation. Addressing AMD before significant sulfuric acid production takes place would reduce suitable conditions for acidophilic bacteria that further accelerate AMD. Usage of tailing dams, or clay impoundments, allows for the effective removal of both oxygen, or both (Park 2019). Moreover, increasing pH is another strategy that mixes and aerates lime or other alkaline material to neutralise mining wastes, thereby mitigating AMD. This process can involve co-blending wastes from pulp or steel mills, concrete waste residue, biochar and even shellfish residues which elevate pH and immobilise metals, providing an effective amendment strategy to already AMD-affected areas (Anawar et al., 2015; Jia et al., 2023; Park et al., 2019). These methods are widely adopted and remain a relatively cost-effective means of preventing and mitigating significant onset of AMD. However, there are still inherent risks to these solutions with respect to the duration of their efficacy, as unmanaged/abandoned operations without containment plans raise questions of problem ownership, which can remain long after operations have ceased and may instead be the burden of local governments.

To fully address initial and advanced stages of AMD, creative strategies should be considered. One concept could directly target AMD-accelerating microbial communities, through the use of bactericides, or disrupting essential biochemical systems. Bactericides come in many different forms and are not a new means of curbing the problem of AMD and can include the usage of anionic surfactants (e.g.: sodium lauryl sulfate, sodium dodecyl sulfate, sodium dodecylbenzene sulfonate), acids (e.g.: acetic, lactic, pyruvic), which disrupt the bacterial cell membrane, have been reported in reducing acid production and metal leaching up to 95% (Park et al., 2019; M. Zhang & Wang, 2016; S. Zhang et al., 2018). However, this approach cannot be considered a standalone solution, whereby an application regimen is required to maintain the desired effects.

In recent years, several AMD-accelerating bacteria have been characterised allowing for an in-depth study of their metabolism. Genera that include *Acidithiobacillus*, *Leptospirillum, Sulfobacillus* and *Acidiphilium*, all contain species that possess signalling systems which grant spatial and environmental awareness with respect to local cell concentrations (Huang et al., 2022). This phenomenon, known as quorum sensing, is a chemical language ubiquitous in bacteria and controls many important cell duties in which species can gain advantages in competition with each other. Regarding AMD, it can be reasoned that disrupting these systems (via quorum quenching) could offer solutions in reducing or eliminating AMD, example outcomes of quorum quenching could lead to removing resistance to high metal concentrations, disabling ability to chemically sense metals (chemotaxis) or being able to form biofilm adhesion to rock ores (Brune & Bayer, 2012; Ruiz et al., 2008; G. Zhang et al., 2012). Much is left to explore in manipulating such QS systems, but examples exist where quorum quenching has shown promise such as aquaculture and membrane anti-biofouling (Grandclement et al., 2016).

Usage of biotechnological methods have also been applied through bioreactors that contain sulfate-reducing bacteria that precipitate sulfide, as well as remove of toxic metals (Alexandre et al., 2022; M. Zhang & Wang, 2016), which has been demonstrated in RISE-led projects such as “SO4-Biored” (Vinnova project no.: 201804610), which aimed to develop a low temperature, hydrogen sulfide removal process, that would dramatically reduce process water from Boliden's operations. Building upon this concept, bioelectrochemical technology adopted in waste-water treatment, can be applied in sulfate-rich AMD effluent treatment which can be used as a negative-value feedstock that could provide a source of electricity and metal recovery (Alexandre et al., 2022; Mark Dopson et al., 2016). Using this effluent as a substrate in microbial fuel cell (MFC), with the addition of sulfate-reducing and oxidising bacteria, that convert sulfide to sulfur for complete precipitation and removal, with an additional production of electricity (Lee 2012). However, such a system may require additional carbon and electron sources to reach its full potential, and studies have suggested using organic waste effluents from other sources (industrial, agricultural, domestic). Alternatively, MFCs using Fe(II) oxidation can utilise AMD-causing bacteria directly, and by increasing pH, allow for iron precipitation (Cheng et al., 2007). Performed under laboratory conditions, a maximum power density of 290 $mW/m^2$ was achieved, which is considered a respectable result in comparison to other laboratory-scale MFC reactors. Despite this, techno-economic analysis is required to show that AMD remediation potential and electricity production are viable at scale, whilst remaining a relatively cost-effective.

In summary, future AMD prevention/mitigation strategies can performed through combined established and novel methods. By adopting a multi-step approach in tackling on-going and established AMD a step-by-step guide can be outlined:

- Firstly, analysis should take place on the extent of AMD damage, location, seasonal variation, and nature of the waste dumps (e.g.: tailing dams vs waste rock piles), it also would be advised to have prior knowledge of the microbial communities persistent in established AMD zones.
- Secondly, newly established mine dumps could be pretreated with specific cost-appropriate surfactants to delay onset of bacterial proliferation. Then wastes could be co-blended with other negative-value waste substrates (e.g.: biochar, cement waste, food residues), which maintain a high pH and sequester metals.
- Finally, with additional dilution of co-blended wastes, soil amendments can take place that would be able to sustain plant life, and further reduce water run-off. Ore waste with high pyrite content could be segregated and placed into lined processing pits to encourage sulfur and metal precipitation, and potential electrical production through electrochemical method.

AMD is a case specific problem that largely depends on operational, geographic, and seasonal conditions. It can be asserted that with proper management, AMD can be significantly reduced, with mining wastes being blended with pH stabilising substrates and reintroduced back into the environment.

# 5 Conclusions

- Sampling of waste rock for biological analysis can be difficult due to the relatively low cell count. This is not discussed in the literature, with many studies focusing on tailings rather than waste rock.
- Archaea were not present in low temperature environment at Kristineberg. This result is consistent with the literature study. Archaea are more abundant in higher temperature climates.

- When waste rock is brought to the surface there is a rapid change in the microbial consortia tied to the change in environment or the rock from underground to the suface. Less bacteria was also observed since the old consortia died off.
- For the mid and old rock samples there was no large change in microbial consortia during the winter.
- Geochemical analysis of the waste rock in the study is consistent with AMD processes occurring at the site.
- The microorganisms identified on older waste rock included moderately acidophilic and extremely acidophilic populations.
- Daily temperature variations at the Kristineberg site can be significant. An understanding of temperature profile in the waste rock pile, and the impact of water freezing a thawing should be developed for cold climates.
- Mitigation strategies are diverse and case dependent. Many solutions have shown promising results in a controlled laboratory environment but have yet to be demonstrated on a large-scale. With further research in real world conditions, novel methods could hold the key to stepwise AMD management, mitigation, and elimination.

# 6 Next steps

Development of a passive, inexpensive preventative measure against AMD generation should have the following long-term goals:

1. Zero environmental impact from AMD caused by mining activities.
2. Reduced AMD treatment costs for mining companies.
3. Reduced chemical use for AMD treatment and associated CO2-footprint.
4. Improved environmental status for the mining industry.
5. Reduced long term cost to society for remediation of legacy mine sites.

To achieve these goals there is a need to have a deeper and longer term understanding of the factors that impact the tipping point from abiotic to biotic AMD generation. A long-term study with regular sampling of the same waste rock would offer more detailed insight into the development of microbiological consortia involved in AMD generation in cold climate. This will also allow studying of the tipping point for AMD generation and the microbial composition at the tipping point. Further, if different waste rocks are used an understanding of complexities around mineralogy impacts can also be developed.

A better understanding of temperature fluctuation in waste rock piles and its subsequent impact on microbiological consortia at different depths is required. A study of temperature at different depths could be established using different temperature models available commercially and in academic literature. Once temperature profiles are established the impacts of temperature and temperature fluctuation could be explored in a laboratory environment.

By establishing the long-term development of microbial consortia in waste rock piles, coupled with an understanding of temperature effects and freeze thaw cycles, more complex models can be developed around AMD generation. These models could extend on existing AMD models (for example see Muniruzzaman et al. (2020)) by including microbial aspects or water freezing at low temperatures. By including microbial aspects in models, more novel AMD prevention techniques can be explored in a more cost-effective manner.

In terms of AMD prevention techniques, most existing techniques focus on blending of neutralising materials with the water rock or limitation or prevention of either oxygen or water diffusion into the waste rock pile. Interestingly, most techniques for AMD prevention do not focus on microbiological aspects, instead staying with traditional approaches. There is, however, potential for more novel prevention techniques. Of note, quorum sensing/quenching techniques may offer a preventative measure. It should be noted that such techniques are low TRL, and while they offer a potential solution, more development work is required to move to a level where these approaches can be implemented.

# 7 Appendicies

## 7.1 References

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## 7.2 Rock analysis data - October 2022

| Sample | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| Area | Old | Old | Old | Mid | Mid |
| Fe fine fraction | 6.0% | 43.9% | 5.0% | 3.2% | 4.7% |
| S fine fraction | 0.7% | 3.5% | 0.5% | 0.6% | 1.0% |
| Fe:S molar ratio fine fraction | 5.12 | 7.13 | 5.23 | 3.04 | 2.75 |
| Al fine fraction | 7.0% | 1.4% | 6.3% | 4.6% | 6.7% |
| Ca fine fraction | 0.5% | 0.1% | 0.3% | 12.1% | 0.5% |
| K fine fraction | 1.7% | 0.2% | 1.6% | 1.4% | 1.5% |
| Mg fine fraction | 3.2% | 0.4% | 3.2% | 1.6% | 2.6% |
| Na fine fraction | 0.9% | 0.1% | 0.7% | 0.7% | 0.9% |
| Si fine fraction | 26.7% | 3.5% | 28.8% | 17.7% | 29.6% |
| Other fine fraction | 47.3% | 53.4% | 47.0% | 42.6% | 48.0% |
| LOI fine fraction | 6.3% | 26.0% | 5.9% | 15.1% | 6.2% |

## 7.3 Rock analysis data - May 2023

Rock chemical analysis with outliers (sample 3 and 9) removed.

| Sample area | Old | Mid | Young |
|---|---|---|---|
| pH | 4.0 ± 0.3 | 7.6 ± 2.2 | 9.8 ± 1.1 |
| Fe coarse fraction | 5.3% ± 0.6% | 6.3% ± 3.7% | 4.0% ± 0.3% |
| S coarse fraction | 0.5% ± 0.1% | 4.4% ± 5.3% | 1.9% ± 0.4% |
| Fe:S molar ratio coarse fraction | 6.0 ± 1.3 | 1.7 ± 1.3 | 1.2 ± 0.2 |
| Al coarse fraction | 6.8% ± 0.4% | 6.6% ± 0.4% | 6.6% ± 0.1% |
| Ca coarse fraction | 0.6% ± 0.1% | 0.6% ± 0.3% | 0.9% ± 0.1% |
| K coarse fraction | 1.6% ± 0.1% | 1.8% ± 0.4% | 2.3% ± 0.2% |
| Mg coarse fraction | 3.3% ± 0.3% | 2.6% ± 1.2% | 1.9% ± 0.3% |
| Na coarse fraction | 1.2% ± 0.3% | 0.8% ± 0.5% | 0.2% ± 0.0% |
| Si coarse fraction | 31.5% ± 0.5% | 30.2% ± 5.9% | 32.3% ± 0.4% |
| Other coarse fraction | 48.8% ± 1.2% | 46.0% ± 4.3% | 49.2% ± 0.6% |
| LOI coarse fraction | 4.9% ± 0.3% | 7.1% ± 4.7% | 4.1% ± 0.3% |

Rock chemical analysis with outliers (sample 3 and 9) removed.

| Sample area | Area 1 | Area 2 | Area 3 |
|---|---|---|---|
| pH | 4.0 ± 0.3 | 7.6 ± 2.2 | 9.8 ± 1.1 |
| Fe fine fraction | 3.6% ± 0.6% | 3.8% ± 0.9% | 3.2% ± 0.3% |
| S fine fraction | 1.3% ± 0.8% | 2.2% ± 1.6% | 1.6% ± 0.4% |
| Fe:S molar ratio fine fraction | 3.5 ± 4.6 | 1.4 ± 0.8 | 1.2 ± 0.2 |
| Al fine fraction | 5.8% ± 0.7% | 6.1% ± 0.4% | 5.5% ± 0.5% |
| Ca fine fraction | 0.7% ± 0.5% | 0.4% ± 0.1% | 0.3% ± 0.2% |
| K fine fraction | 1.1% ± 0.4% | 1.5% ± 0.5% | 1.9% ± 0.3% |
| Mg fine fraction | 2.4% ± 0.8% | 2.5% ± 0.9% | 1.3% ± 0.1% |
| Na fine fraction | 1.4% ± 0.9% | 0.8% ± 0.4% | 0.2% ± 0.2% |

| Sample area | Area 1 | Area 2 | Area 3 |
|---|---|---|---|
| Si fine fraction | 35.6% ± 1.6% | 35.6% ± 1.8% | 35.4% ± 1.3% |
| Other fine fraction | 47.7% ± 1.3% | 46.8% ± 0.8% | 50.2% ± 1.0% |
| LOI fine fraction | 3.0% ± 0.9% | 3.8% ± 2.0% | 2.9% ± 0.4% |

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