# The metal oxides of abandoned tin mining pit waters as an indicator for bacterial diversity

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**Abstract**. This study aimed to provide information about the form of metal oxides of heavy metals identified in abandoned tin mining pit waters with different ages and explain their relationship to the diversity of acidophilic bacteria and the presence of fish in acid mine waters in Bangka Regency, Indonesia. The analysis of metal oxides was carried out using X-Ray Fluorescence (XRF). The 16 oxide forms of heavy metals identified showed that iron (III) oxide ($Fe_2O_3$), tantalum (V) oxide ($Ta_2O_5$), tin (IV) oxide ($SnO_2$), manganese (II) oxide (MnO) were found in high concentrations in all mine waters of different ages. The presence of heavy metals and their oxides affected the water quality, especially the pH value, decreasing it by oxidation processes. This condition contributed to the presence of acidophilic bacterial groups such as phyla Proteobacteria, Bacteriodetes, Planctomycetes, Actinobacteria, Chloroflexi, Firmicutes, Chlorobi, Acidobacteria, Cyanobacteria, etc. They play the important role in biogeochemical processes, changing the environment. The changes of chronosequences in the ecosystem can support the life of organisms such as fish (*Aplocheilus* sp., *Rasbora* sp., *Betta* sp., *Puntius* sp., *Channa* sp., *Oreochromis* sp., *Belontia* sp., *Anabas* sp., and *Trichopodus* sp.). Furthermore, the fish produce organic material. The organic material was decomposed by bacteria in anions and functional groups, which react to protons and cause the neutralization pH.

**Key Words**: acidophilic bacteria, chronosequence, fish, heavy metals, interaction.

**Introduction**. A number of metals have been identified in aquatic ecosystems, especially in artificial lakes (pits) after tin mining activities. These metals become components of ecosystem pollutants (Dinis & Fiuza 2011; Guan et al 2014; Kurniawan 2016), including heavy metals such as Pb, Zn, Mn, Fe, Cr, Cu, Ni, Cd, Sn, and As (Ashraf et al 2011a; Henny 2011; Ashraf et al 2012a; Rosidah & Henny 2012; Daniel et al 2014). The general term "heavy metal" refers to a group of metals and semi-metals (metalloids) associated with contamination, with a density higher than 3.5-5 g $cm^{-3}$, with atomic weights between 63.546 (≈63.6) and 200.59 (≈200.6), and specific gravity higher than 4 (Duffus 2002; Srivastava & Majumder 2008; Aslam et al 2011).

Heavy metals are not always described as dangerous metals (toxic metals), but their chemical structure determines the biological properties and toxicity of these elements (Templeton 2015). Some metals in certain structures are needed by the body of an organism as essential microminerals (trace elements), but can be dangerous in other structures (Kurniawan & Mustikasari 2019). Elements such as chromium in the form of Cr (III) are important trace elements, but Cr (VI) can cause cancer (Govind & Madhuri 2014). Hg (II) is more toxic than Hg (0) (Azimi & Moghaddam 2013), inorganic Mn (III) is more toxic than oxidation forms such as Mn (II) $Cl_2$ and Mn (IV) $O_2$; As (III) is more toxic than As (V); element V (V) is more toxic than V (IV) (Templeton 2015), and Fe (II) is more significantly absorbed by cells than Fe (III) (He et al 2008). The chemical structure is confirmed by ionization. Ionization of heavy metals can potentially be disruptive and dangerous to health and can even damage the vitality of the systems in a body (Abdi & Kazemi 2015).

Microecosystem changes can be indicated by the diversity of microorganisms system because it can be related to variations in water characteristics (Ashraf et al 2011b), which can be determined through weather, geomorphologic, and geochemical conditions (Ashraf et al 2012b). Studies on the relationship between bacterial diversity and its role in the biogeochemical cycle and the interaction of microorganisms with their environment have been widely conveyed (Bhowal & Chakraborty 2015; Fashola et al 2015), including methane-oxidizing bacteria, ammonia-oxidizing bacteria (Sow et al 2014a; Sow et al 2014b), and arsenic-resistant bacteria (Jareonmit et al 2010; Valverde et al 2011). The study of the diversity of microorganisms and their activities as ecological bioindicators is an important step in predicting ecosystem conditions and environmental changes (Niemi & McDonald 2004; Moscatelli et al 2005). This is because microorganisms have the capacity to respond to changes that occur in the environment quickly, including in soil and aquatic ecosystems (Paerl et al 2003; Lau & Lennon 2012).

This short communication aimed to provide information related to the form of metal oxides, especially the heavy metals identified in post mining pit waters with different ages (chronosequences). Furthermore, it shortly reviews some relationships between metals and the diversity of acidophilic bacteria in acid mine waters and the potential of the waters for aquaculture.

**Material and Method**. The study was conducted by testing the metal content in water collected from under the tin mining post with different ages, namely less than 1 year (Station A), 5-10 years (Station B), and more than 15 years (Station C). The coordinates of the research station were: Station A - 01°59'S, 106°06' E; Station B - 01°59'S, 106°06'E; Station C - 01°55'S, 106°06'E (Figure 1) (Kurniawan 2019; Kurniawan et al 2019). Water sampling was carried out in these pits located in Bangka Regency, Bangka Belitung Islands Province, Indonesia, in 2017-2018.

There were 4 water samples, 1.5 L each, collected from depths lower than 4 m and higher than 4 m, with five sampling points for repetitions in each station. The collected water samples were placed into sample containers, transported in a cool box, and analyzed in a laboratory. Analysis of the metal content was carried out using X-Ray Fluorescence (XRF) instruments Rigaku NEX CG (Kodom et al 2012), with cross-section specifications of 3 refracting metals, namely copper (Cu), molybdenum (Mo), and aluminum (Al). The presence of bacteria was identified with Next Generation Sequencing (NGS). Data analysis was performed descriptively with Microsoft Excel 2010 and Origin 8 to explain the concentration of metal oxides identified in the samples and their pattern in the research location.

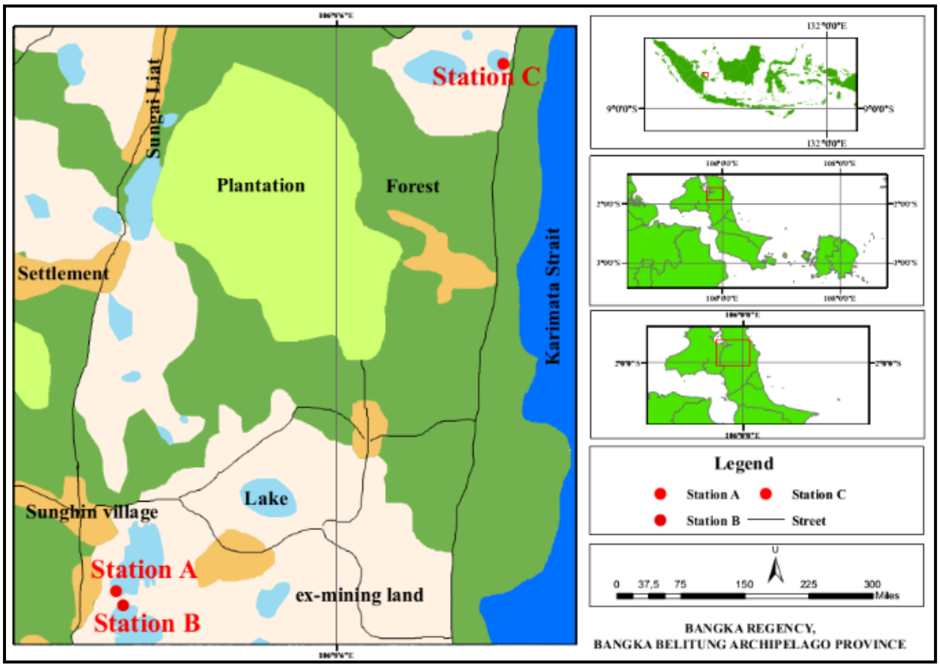

Figure 1. Research stations in ex-tin mining pits in Bangka Regency, Bangka Belitung Archipelago Province, Indonesia.

**Results and Discussion**. This reseach has identified some heavy metals from abandoned tin mining pits. There were 16 heavy metals were identified: As, Co, Cr, Cu, Fe, Ga, Hf, Mn, Ni, Pb, Sn, Ta, Te, Th, V, and Zn. These heavy metals showed oxide forms by XRF analysis (Table 1). The functional properties of metal oxides are strongly dependent on the crystal structure of the oxide, composition, native defects, doping, etc., which govern their optical, electrical, chemical and mechanical characteristics (Grilli 2020).

Table 1

Concentrations of metal oxides in abandoned tin mining pit waters

| *Metal form* | *Metal oxide form* | *Name of oxide form* | *Average metal oxide concentration in the each station (ppm)* | | |
|---|---|---|---|---|---|
| | | | *A* | *B* | *C* |
| As | $As_2O_3$ | Arsenic (III) oxide | 5.7 | 8.49 | 3.04 |
| Co | $Co_2O_3$ | Cobalt (III)) oxide | 14.2 | ND | 9.34 |
| Cr | $Cr_2O_3$ | Chromium (III) oxide | 13.8 | 14.5 | 1.96 |
| Cu | CuO | Cupric (II) oxide | 7.03 | 7.83 | 6.91 |
| Fe | $Fe_2O_3$ | Iron (III) oxide | 1424.3 | 2307.67 | 1571.93 |
| Ga | $Ga_2O_3$ | Gallium (III) oxide | 11.5 | 12 | 12.65 |
| Hf | $HfO_2$ | Hafnium (IV) oxide | 7.74 | 8.76 | 11.07 |
| Mn | MnO | Manganese (II) oxide | 33.7 | 35.1 | 39.05 |
| Ni | NiO | Nickel (II) oxide | 10.4 | 7.32 | 4.64 |
| Pb | PbO | Lead (II) oxide | 14.5 | 13 | 9.4 |
| Sn | $SnO_2$ | Tin (IV) oxide | 89.73 | 64.77 | 74.53 |
| Ta | $Ta_2O_5$ | Tantalum (V) oxide | 987.33 | 1373.33 | 888.73 |
| Te | $TeO_2$ | Tellurium (II) oxide | 13 | 9.48 | 14.5 |
| Th | $ThO_2$ | Thorium (II) oxide | 10.5 | 9.97 | 15.75 |
| V | $V_2O_5$ | Vanadium (V) oxide | 3 | ND | 2.38 |
| Zn | ZnO | Zinc oxide | 3 | ND | ND |

Note: ND - not detected; Station A (age of pit < 1 year), Station B (age of pit between 5-10 years), and Station C (age of pit > 15 years).

The form of oxides and their concentrations (Table 1) indicated that there was potential of contamination with heavy metals in abandoned tin mining pit waters. The highest concentration found was $Fe_2O_3$, which was identified at Station B. This oxide form was also found with the highest concentration in all abandoned tin mining pits. Other oxide forms such as $Ta_2O_5$, $SnO_2$, and MnO also presented high concentrations in all stations, while others had low values.

$Fe_2O_3$, $Ta_2O_5$, $SnO_2$, MnO also showed their distribution patterns in the waters. $Fe_2O_3$ and $Ta_2O_5$ had increasing patterns of concentration in abandoned tin mining pit waters with age between 5-10 years compared those with age less than 1 year. The values decreased in pits with ages above 15 years. $SnO_2$ had a decreasing pattern of concentration in abandoned tin mining pit waters with age between 5-10 years compared to those with ages less than 1 year, and the values increased in pits with ages above 15 years. MnO showed an increasing pattern of concentration among the chronosequence of abandoned tin mining pit waters. The presence of these oxides indicates a high potential for heavy metal contamination, although the chronosequences of abandoned tin mining pits were more than 15 years. In addition, other heavy metals also had high values in pits with ages above 15 years, namely $Ga_2O_3$, $HfO_2$, $TeO_2$, and $ThO_2$. $As_2O_3$, $Cr_2O_3$, CuO, NiO, PbO, and ZnO had decreasing concentration patterns. The values indicate that the concentrations decrease during chronosequences above 15 years.

The presence of heavy metals is correlated with pH conditions. According to Kurniawan et al (2019) in a previous study in this research location, a number of metals and their oxides contribute to water quality parameters such as pH. In water with an age below 15 years, a low pH value (3) occurred, while water with an age above 15 years had a neutral pH value (7). A number of minerals undergoing chemical reactions that form acidic pH are categorized as potentially acid forming: Cu, Fe, Pb, and Zn (Celebi & Oncel 2016). The acidic condition also can be formed by associations of Al, As, Cd, Co, Cr, and Mn with environment material (Campaner et al 2014). Oxidation and hydrolysis reactions of elements such as S, Fe, Cu, Zn, Ni, Cr, and Pb cause cation formations of $Cu^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Cr^{3+}$, and $Pb^{2+}$. The increase in proton $H^+$ contributes to the increase in acidity. An increasing number of $H^+$ ions can cause more acidic pH conditions in these environments (Gaikwad & Gupta 2008; Hatar et al 2013). The existence of these metals can directly or indirectly affect the pH value (De Saedeleer et al 2010; Zhao et al 2010; Fernandes et al 2011; Strom et al 2011; Huang et al 2012; Sadeghi et al 2012; Zhang et al 2014) and form acidity in mining waters known as acid mine drainage (Bigham & Nordstrom 2000; Kolmert & Johnson 2001; Tan et al 2007; Ashraf et al 2011b; Kurniawan 2019).

The consequences of the acidity of waters include the disruption of the life of some organisms. Organisms at a microscopic level such as bacteria and archaea that are acidophilic have the ability to survive and live optimally in extreme acidic conditions, including in acid mine waters (Navarro et al 2013). Acidophilic groups have the capacity to modify the physical and chemical conditions of waters by detoxifying or exploiting their metabolism and play an important role in the biogeochemical cycles of iron and sulfur (Fashola et al 2015). Acidophilic bacteria can be true acidophil (extreme acidophiles), which live at pH lower than 2.7, even lower than 1, with optimum growth under a pH of 3, and moderate acidophil that live in a pH range of 3-7.2, with optimum growth in pH of 3-5 (Johnson & Hallberg 2008; Mendez et al 2008; Oren 2010). Various studies explain that some bacteria found in mining areas are derived from the following phyla: Proteobacteria, Acidobacteria, Chloroflexi, Cyanobacteria, Actinobacteria, Nitrospirae, Firmicutes, Planctomycetes, Bacteroidetes, and Chlorobi (Gupta 2000; Lefebvre et al 2010; Hua et al 2015; Mesa et al 2017; Teng et al 2017; Cesario Fernandes et al 2018). In these research locations, the presence of bacteria was identified and presented in Table 2.

Some species of these phylum can be grouped as acidophilic bacteria and they have the ability to reduce and oxidize metals, sulfur and other minerals (Islam et al 2004; Hallberg 2010; Harahuc et al 2000; Yli-Hemminki et al 2014). They also help the carbon cycle flow (Wegner & Liesack 2017; Hausmann et al 2018; Sun et al 2018), are nitrogen retarders (Gargaud et al 2011; Sun et al 2015), and play a role in decomposing organic matter (Khare & Arora 2015). This capability is generally used in the process of detoxification of contaminated waters (Davis-Belmar & Norris 2009; Johnson et al 2009; Murali et al 2014; Shivlata & Satyanarayana 2015; Hu et al 2018).

The presence and biological activity of microbial groups in environments containing sulfide minerals can accelerate the formation of acidic conditions in the environment (Rawlings 2005). Acidic pH conditions involve oxidation processes and complex chemical reactions to produce $H^+$ ions, sulfates ($SO_4^{2-}$), $Mn^{3+}$, and other ions. More and more of these ions that form in an environment cause an increase in acidity (Gaikwad & Gupta 2008; Hatar et al 2013; Nurofiq et al 2016). The acceleration of the formation of acidic conditions can involve biological interactions such as microbial metabolic activity (Violante et al 2010).

The oxidation processes of iron and sulfur in acidic waters produce an energy reaction used by acidophilic microbes for growth and metabolic functions. The acidophilic bacteria use sulfur metabolic enzymes to oxidize sulfur (sulfur dioxygenase, sulfur oxygenase reductase, and Hdr-like complex). They also use thiosulfate oxidizing enzymes such as sulfuroxidizing enzyme and thiosulfate dehydrogenase, sulfide oxidizing enzymes such as sulfide quinone oxidoreductase (Wang et al 2019). They present iron oxidizing enzymes (Li et al 2017) to utilize the iron cycle under acidic pH conditions for ferrous iron ions ($Fe^{2+}$) as electron donors and ferric iron ions ($Fe^{3+}$) as electron acceptors (Lei et al 2016).

The presence of acidophilic bacteria in low pH values occurs due to its capability to survive and use sulfide minerals such as sulfur and iron as their energy source for growth by oxidation processes (Korehi et al 2013). Some examples are $S^0 + O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$ or $Fe^{2+} + O_2 + 2H^+ \rightarrow Fe^{3+} + H_2O$ (Rawlings 2005). The relative abundance of acidophilic bacteria in long chronosequences can contribute to the decomposition of organic material such as water plants, metabolism products of water organisms, and dead organisms. The decomposing processes can increase $CO_2$ levels, which can interact with $H_2O$ to form carbonic acid ($H_2CO_3$) by the following reaction $CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$ (Loerting & Bernard 2010; Ghoshal & Hazra 2015). The dissociation of $H_2CO_3$ to ion carbonate ($HCO_3^-$) can neutralize ion hydrogens ($H^+$) so it can increase the pH value to neutral (Andersen 2002). The decomposition product of organic acid from organic materials has the functional group R-COOH as dissociated organic anion and it can use $H^+$ cations to make the concentration of $H^+$ ions decrease in environment, and the pH to reach neutral condition (Rukshana et al 2010).

Table 2

The presence of bacteria in abandoned tin mining pits

| *No* | *Phylum* | *The presence of phylum* | | |
|---|---|---|---|---|
| | | *Station A* | *Station B* | *Station C* |
| 1 | Proteobacteria | + | + | + |
| 2 | Actinobacteria | + | + | + |
| 3 | Chloroflexi | + | + | + |
| 4 | Firmicutes | + | + | + |
| 5 | Acidobacteria | + | + | + |
| 6 | Planctomycetes | + | + | + |
| 7 | Bacteroidetes | + | + | + |
| 8 | Chlorobi | + | + | + |
| 9 | Cyanobacteria | + | + | + |
| 10 | Gemmatimonadetes | + | + | + |
| 11 | OD1 (candidate of Phylum Parcubacteria) | + | + | + |
| 12 | Spirochaetes | + | + | + |
| 13 | Thermi | - | + | + |
| 14 | Nitrospirae | + | + | + |
| 15 | Verrucomicrobia | + | + | + |
| 16 | Armatimonadetes | + | - | + |
| 17 | Chlamydiae | + | + | + |
| 18 | Elusimicrobia | + | - | + |
| 19 | Caldiserica | - | - | + |
| 20 | Chaldithrix | - | - | + |
| 21 | Lentisphaerae | - | - | + |
| 22 | Fibrobacteres | - | - | + |

The presence of heavy metals and their oxide, and the pH change in environment can impact to life of macroorganisms such as fish. There were some fish found in the abandoned tin mining pits (Table 3). The presence of fish in abandoned tin mining pits indicated that some species of fish can survive in the environment. Their metabolism products are decomposed by Bacteriodetes to produce $CO_2$ and carboxylate functional groups ($COOH^-$), bringing the pH values to neutral (Andersen 2002; Loerting & Bernard 2010; Rukshana et al 2010; Ghoshal & Hazra 2015).

In addition, the presence of $CO_2$ in the water can be changed into complex organic molecules and oxygen ($O_2$) in aerobic photosynthesis by the following reaction $H_2O + CO_2 \rightarrow CH_2O + O_2$ (Johnson 2016). The organic molecules can be feed for organisms and the optimum $O_2$ also can supported their life. The $CO_2$ can also be used as a carbon source for some bacteria in anaerobic photosynthesis, such as Green Sulfur Bacteria like *Chlorobium*, Green Non-Sulfur Bacteria like *Chloroflexus*, Purple Sulfur Bacteria like *Thiospirillum*, and Purple Non-Sulfur Bacteria like *Rhodobacter*. The anorganic photosynthesis uses $H_2S$, $H_2$, and S as electron donors with the following reaction: $6CO_2 + 12H_2S \rightarrow C_6H_{12}O_6 + 6H_2O + 12S$ (Nisbet & Fowler 2003). The organic materials resulting from this reaction are used as nutrients in an environment and can improve water quality.

Table 3

The presence of fish in abandoned tin mining pits

| *No* | *Genus* | *The presence of fish* | | |
|---|---|---|---|---|
| | | *Station A* | *Station B* | *Station C* |
| 1 | *Aplocheilus* sp. | - | - | + |
| 2 | *Rasbora* sp. | + | + | + |
| 3 | *Betta* sp. | + | + | + |
| 4 | *Puntius* sp. | + | + | - |
| 5 | *Channa* sp. | + | + | + |
| 6 | *Oreochromis* sp. | - | + | - |
| 7 | *Belontia* sp. | + | + | + |
| 8 | *Anabas* sp. | + | + | - |
| 9 | *Trichopodus* sp. | + | + | + |

The information about the environmental conditions from abandoned tin mining pits showed there was an interaction between the presence heavy metals and their oxides, pH values, acidophilic bacteria and fish life. The capability of acidophilic bacteria and some fish in acid mine waters can be optimized by studying their biochemical characteristics as an effort to improve the condition of the aquatic environment.

**Conclusions**. The presence of a number of metals and their oxides in ex-tin mining or abandoned tin mining pit waters showed an association with the pH in the environment. The change in pH, especially acidic pH causes the presence of a number of acidophilic groups. Acidophilic groups have the ability to live in this extreme environment and then move biogeochemically. The potential ability of these acidophilic bacteria can be utilized to carry out detoxification processes for inorganic and organic contamination found in some waters. The changes of environment quality can impact organism life such as fish. Furthermore, the presence of fish can produce organic materials as a product of their metabolism and it can support biogeochemical processes for chronosequences in this environment.

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