Passive water purification of pit lakes – a case study from the closed Hammaslahti Cu-Zn-Au mine

Marja Liisa Räisänen[1], Malin Bomberg[2], Jarno Mäkinen[2]

[1]Geological Survey of Finland, P.O. Box 1237 (Neulaniementie 5), 70211 Kuopio, Finland, marja-liisa.raisanen@gtk.fi
[2] VTT Technical Research Center of Finland Ltd., P.O. Box 1000, FI-02044 VTT, Finland

Abstract

The Hammaslahti pit lake study introduces passive water treatment technologies applied in two flooded open pits. Materials consist of physical measurements of the water beds, physical, microbiological and chemical analysis of water samples from selected levels, and microbiological analysis of bottom sediments. Originally the N pit was the pilot site for biological sulphate reduction and it was presumed that sulphate reducing pockets within organic substrate would spread via an adit to the Z pit. Notable differences in geochemistry and the microbial content of waters and bottom sediment of the two pits suggest there is not a hydraulic connection via an adit between the N and Z pit. Water remediation in the N pit is mainly driven by metal sulphide formation, but in the Z pit, the formation of Fe oxyhydroxides and their adsorption capacity facilitate metals retention. The results showed that both oxidizing and reducing bacteria can maintain reactions that retain sulphate and metals via accumulation of stable secondary precipitates at the bottom of the pit. Furthermore, the pit lakes revealed to be challenging sites for biologic sulphate reduction based on-site pit treatment. One reason for inadequate activity of the SRB could be the cold temperature of the pit water, especially at the bottom of the pit. This suggests the need for further study of the behaviour of cold-tolerant microorganisms and their impact on water remediation.

Key words: passive treatment technology, sulphate reducing, pit lake waters, pH, redox

Introduction

The closed Hammaslahti Cu-Zn-Au mine is located in Joensuu, eastern Finland. The open pit mining was started in 1973, followed by underground mining from 1976 to 1986. The Cu-Zn-Au ore is hosted by Proterozoic metaturbidites (arenites, ergillites) subjected to intense silicification and chloritization. In deeper parts of the mine, the black schist is host to remobilized sulphides. The ore body in the N pit mainly consisted of chalcopyrite and the Z ore body in the Z pit of pyrite-pyrrhotite and sphalerite ores with minor chalcopyrite. (Loukola-Ruskeeniemi et al. 1993)

This study presents passive water treatment technologies applied in two flooded open pits (called N and Z pit) of the Hammaslahti mine. The open pits filled with water after mine closure at the beginning of the 1990’s. To promote biologic sulphate reduction, pig manure (sludge) as source of bacteria and nutrients, was added several times in the first (N) pit (in 1998, in 2000 and 2004) and followed by additions of slowly decaying wood bark chips (in 2002) and ethanol (in 2005) as carbon and energy source for the bacteria. Originally, it was presumed that sulphate reducing pockets within the organic substrate would spread via an adit from the first (N) to the second pit (Z).

The aims of this study are to examine the physical, chemical and biological quality of the pit waters, microbiology of the bottom sediments, water remediation mechanisms and the subsequent precipitation of metal sulphides (see also Räisänen et al. 2015). Overall, it is of interest to evaluate the applicability of bacteria addition in form of pig manure to activate sulphate reduction.

Methods

Materials for the N and Z pit study consist of physical measurements of whole pit water columns (measurements taken at depth intervals of every 1 to 2 meters), physical, microbiological and chemical analysis of water samples from selected levels, and microbial analysis of bottom sediments (Räisänen et al. 2015). On-site filtered pit water samples and bottom pit sediment samples from both pits were taken in March and in September in 2014. Sampling methods and the pretreatment of water and sediment samples were described detailed in the web publication by Räisänen et al. (2015).

Alkalinity of the pit water samples was measured on-site using a Hach digital titrator with 0.1600 N or 1.600 N H_2SO_4 to an end point of 4.5. Temperature, pH, redox potential, electrical conductivity (EC), oxygen concentration and oxygen saturation of waters at each sampling site were measured on-site with a multiparameter field meter (Räisänen et al. 2015).

Concentrations of Br, F, Cl, NO_3 and SO_4 in the water samples were determined with ion chromatography and concentrations of 34 elements were analyzed using ICP-OES or MS-ICP. Concentrations of dissolved organic carbon and total carbon were measured with a C analyzer. The content of solids was determined by gravimetric method (SFS-EN 872:2005). Concentrations of Fe^{2+} were measured with a spectrophotometer. All the laboratory analyses were carried out at the FINAS-accredited testing laboratory of Labtium Ltd.

The microbiology was assessed from the water and sediment samples collected in March 2014. For the characterization of microbial communities, microbial DNA was isolated from the samples using the NucleoSpin Soil DNA extraction kit from two replicates of each sample. First, the microbial biomass was collected on 0.2 µm pore-size cellulose acetate filters (Corning) by vacuum suction, where after the filters were cut out from the funnels using sterile scalpels and forceps, put in to sterile 50-mL plastic screw cap tubes (Corning) and stored at -80°C until DNA extraction. The 1L watery sediment samples were left stationary for several hours until the sediment had set on the bottom of the bottles. The water was carefully decanted and the sediment collected in 50-mL sterile plastic screw cap tubes (Corning). Excess water was separated from the sediment by centrifugation and DNA was extracted from 0.5 g samples. In the DNA extraction, one replicate sample was extracted with buffer SL1 and the other with SL2. Enhancer solution was used in all extractions and the DNA was eluted in 100 µL elution buffer.

The total concentration of bacteria in the pit water was estimated by a DNA based qPCR method, where the number of bacterial taxonomical marker genes for the ribosomal small subunit (ssu) was calculated (as described in Tsitko et al. 2014). The bacterial community composition was determined by characterizing the whole community profile of the bacterial ribosomal ssu genes using high throughput amplicon sequencing on the Iontorrent platform. Bacterial ribosomal ssu genes were amplified with primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Klindworth et. al., 2012), targeting the variable region V3-V4 of the ssu gene. PCR amplification was performed in parallel 25 µl reactions for every sample containing 1x MyTaq™ Red Mix (Bioline, London, UK), 20 pmol of each primer, up to 25 µL molecular-biology-grade water (Sigma) and 2 µL of DNA. The PCR program consisted of an initial denaturation step at 95°C for 3 min, 35 cycles of 15 s at 95°C, 15s at 50°C, and 15 s at 72°C, and a final elongation step of 30 s was performed at 72°C. The PCR products were verified with agarose gel electrophoresis. Amplicons were sent to Ion Torrent sequencing with PGM equipment (Bioser, Oulu, Finland) and amplicons were purified before sequencing at Bioser. The sequence data was analyzed with the QIIME pipeline (Caporaso et al. 2010) using the Greengenes database (DeSantis et al. 2006) for identification of sequence reads.

Results and discussion

Water quality and acid generation in the N and Z pits

The number of bacteria in the N pit water varied between 4.6×10^7 ribosomal ssu gene copies in the oxygenated water and 9.6×10^5 in the anoxic water at 40 m depth in March 2014. In the Z pit water, the number of bacteria varied between 5.1×10^6 - 4.3×10^7, with the lowest number of bacteria at 45 m depth. The bacterial communities in the N and Z pits were very diverse and distinct from each other (figs. 1 and 2). The bacterial community in the N pit was dominated by putative sulphur oxidizing and iron reducing Burkholderiales in the surface water (fig. 1, Farkas et al. 2013). Members of the uncultured and uncharacterized group Sbla14 betaproteobacteria were common at 22 m depth in the N pit water, but their ecological role is not clear. At 40 m depth 8-22% of the bacterial community consisted of epsilonproteobacterial Campylobacterales bacteria, which oxidize sulphides and other oxidized sulphur compounds, and fix carbon from CO_2 (Handley et al. 2014). These bacteria may also be able to oxidize Fe^{2+} (Chan et al. 2013). The water at 40 m depth contained between 6 and 8% SRB (sulphate reducing bacteria) belonging mainly to the Desulfobacterales clade. The bacterial community in the sediment of the N pit belonged almost exclusively (86-93%) to the fermenting Clostridiales bacteria. This group is responsible for the degradation of organic materials, producing acetic and lactic acids, ethanol, and also CO_2 and H_2 gases, which function as carbon and energy sources for the microorganisms.

Burkholderiales bacteria were a major group (14-16%) in the oxygenated water of the Z pit (fig. 2). Gallionellales bacteria were abundant in the water column at the transition zone between oxygenated and oxygen depleted water (fig. 1b). These bacteria are common iron oxidizers that precipitate iron as iron oxyhydroxide (Hallbeck et al. 1993). SRB belonging to deltaproteobacterial clades (Desulfuromonadales and Desulfobacterales) were present only at depths of 35 m and below, and were only below 1% of the total bacterial community. However, their abundance increased slightly in the sediment, where deltaproteobacterial SRB formed 1-2% of the bacterial community. The Bacteroidetes were prominent in the sediment of the Z pit. These bacteria degrade organic matter and thus provide simple organic molecules for the benefit of the whole bacterial community.

In both pits the physical and chemical quality of the surface water above 5 m was better than that of the mid and deep waters (Räisänen et al. 2015). An oxygen deficit occurred below the depth of 20 m in the N pit and below the depth of 30 m in the Z pit (tab. 1). The interface between oxic and anoxic water was about a meter higher in autumn than in spring. The pit water data showed no evidence of spring overturn of pit waters, but partial mixing of surface waters with mid waters occurred in the N pit (Räisänen et al. 2015). The acidification of oxygenated surface waters and especially water at the interface depths of 20 to 22 m, above the anoxic water, indicates the mixing of oxygen rich melt water downwards with relatively reduced waters in spring. Furthermore, the decrease in the pH results in dissolution and hydrolysis of Al at pH<5. The enhanced acidity was followed by oxygen consumption in the water column that can be linked to the oxidation of soluble Fe^{2+} to Fe^{3+} coupled with the release of protons (Nordstrom & Alpers 1999, Bachman et al. 2001).

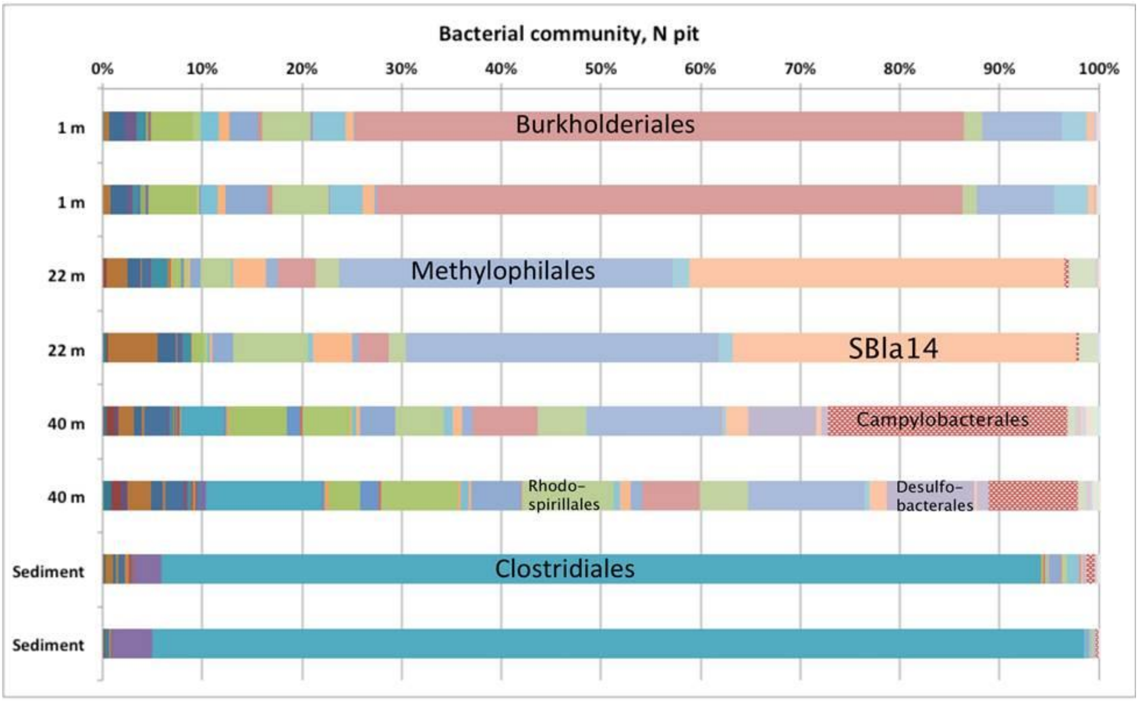

Figure 1 *The bacterial community of the N pit water and sediment resolved by high throughput amplicon sequencing. The bars indicate relative abundance of bacterial clades. Each sampled depth is presented with two replicate samples.*

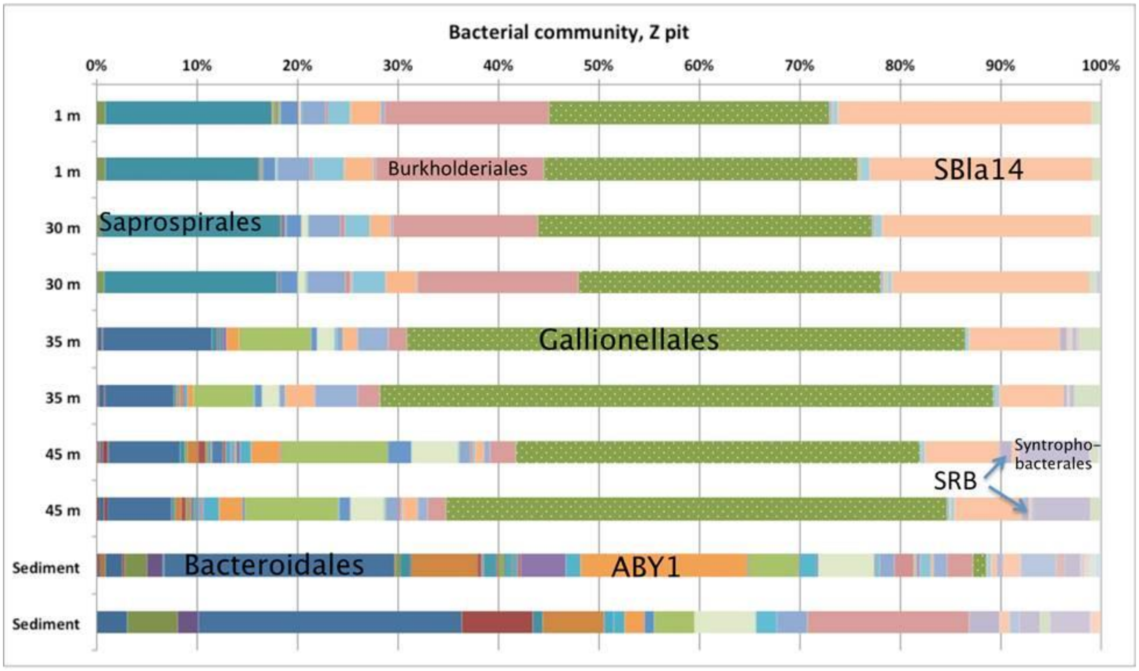

Figure 2 *The bacterial community of the Z pit water and sediment resolved by high throughput amplicon sequencing. The bars indicate relative abundance of bacterial clades. Each sampled depth is presented with two replicate samples.*

The relatively high abundance of methylotrophic bacteria in the water column of the N pit, especially at 22 m indicates high organic matter degradation with the production of methylated compounds and methanol. Methanol is produced by the degradation of, for example pectin, which is abundant in plant cell tissue, such as coniferous tree bark, added to the N pit in 2002. Other sources of methanol and methylated compounds may include the pig manure additions (1998, 2000, 2004), hydrolysis of added absolute ethanol (2005) (Vestola & Mroueh, 2008), or recalcitrant carbon compounds trapped in the black shale of the rock. The high abundance of Clostridiales bacteria fermenting organic matter in the bottom sediments probably reflects the addition of high amounts of organic matter in the N pit. The fermentation process supports putative methanogenesis in the anoxic parts of the N pit.

In the Z pit, the pH of surface and mid waters stayed pretty constant, near 6, and the pH of deep waters varied a little from spring to autumn (Räisänen et al. 2015). On the basis of alkalinity, waters throughout the Z pit had excellent buffering capacity (tab. 1). Presumably, pit waters have been acidified, or are acidifying actively by Fe sulphide oxidation on pit walls. However, potentially because there was only a single sampling site in each pit, the results did not reveal active sulphide oxidation. Additionally, results do not show that acidic water from the N pit is flowing into the Z pit. The interpretation of active acid generation is based on the marked increase in alkalinity, especially in the deep waters of the Z pit.

The predominance of ferrous Fe in the total soluble Fe content of the surface and acidified waters somewhat contradicted the above interpretation of the water acidification mechanism at the N pit (tab. 2). Furthermore, the predominance of bacterial groups that degrade organic matter and potentially reduce Fe oxyhydroxides (consuming, not producing acid) suggest an alternate acidity source, such as the desulphurization of decaying organic material (e.g. chips) via photosynthetic oxidation in oxygenated waters (Sánches-España et al. 2008, NCSU 2015).This mechanism is supported by the discrepancy in total soluble sulphur and sulphate-sulphur results (tab.2), which indicate the occurrence of reduced soluble sulphides potentially in both oxygenated and reduced water layers.

***Table 1** Physical property of the waters at the depths sampled in the N and Z pits, the closed Hammaslahti mine area, eastern Finland. Keys: < below the lowest detection limit, n.d. = no data.*

	Depth m	Temp °C	pH	Redox mV	Oxygen mg/l	EC mS/m	Alkalinity1) mmol/l	Solids mg/l
N pit								
March 24th	1	1.6	4.9	276	12.4	10.4	n.d.	<10
Sept 8th	1	15.2	5.6	216	8.86	10.5	0.04	< 2
March 24th	22	4.5	3.9	463	1.29	32.5	n.d.	<10
Sept 8th	20	4.6	4.0	516	3.33	22.3	n.d.	< 2
March 24th	40	5.5	5.6	-58	-0.05	49.9	0.21	128
Sept 8th	37	5.5	5.7	-3.8	0.12	34.6	0.28	4.7
Z pit								
March 25th	2	3.5	6.3	143	5.29	85.9	0.50	<10
Sept 9th	2	15.2	7.2	147	9.05	81.2	1.00	< 2
March 25th	30	3.4	6.3	135	4.99	85.8	0.50	<10
Sept 9th	30	5.0	6.4	120	1.30	61.6	1.04	< 2
March 25th	35	4.9	6.2	-4.6	-0.01	188	2.36	28
Sept 9th	35	4.7	6.4	-58	0.12	137	3.66	68
March 25th	45	5.2	6.2	-31	-0.06	219	0.53	<102)
Sept 9th	45	5.1	6.5	-82	0.10	155	5.43	342)

1)no = non measurable alkalinity (pH ≤ 4.5)

2)The duplicate sample from the depth of 45 m had solids 110 mg/l in March and 120 mg/l in September.

***Table 2** Concentrations of organic carbon, and soluble main and trace elements in water samples taken from the N and Z pit, the closed Hammaslahti mine area, eastern Finland. Keys: < below the lowest detection limit, n.d. = no data, Duplic. =duplicate sample.*

N pit	Depth m	TOC mg/l	DOC mg/l	$S\text{-}SO_4$1) mg/l	S mg/l	Ca mg/l	Al mg/l	Fe mg/l	Fe^{2+} mg/l	Mn mg/l	Zn µg/l	Cu µg/l
March 24th	1	12	11	21.4	26.5	18.8	0.97	0.45	0.51	0.24	327	41.9
Sept 8th	1	8.6	7.9	23.7	24.1	19.2	0.32	0.16	0.16	0.19	226	41.5
March 24th	22	1.4	0.93	76.8	87.1	49.5	4.28	0.75	0.66	1.32	1810	42.8
Sept 8th	20	1.7	1.3	76.8	75.9	47.7	3.77	0.46	0.07	1.29	1750	60.3
March 24th	40	3.7	1.4	130	152	79.4	1.25	38.2	41.2	1.83	20.4	<0.1
Sept 8th	37	2.0	1.3	129	132	77.3	1.02	36.6	37.4	1.81	21.9	0.28
Z pit												
March 25th	2	1.7	0.81	267	322	287	0.003	1.11	1.25	2.83	91.7	0.15
Sept 9th	2	1.9	1.5	264	268	278	0.17	0.05	<0.02	1.10	46.4	<0.1
March 25th	30	1.6	1.3	270	321	291	0.005	1.15	1.26	2.84	89.8	0.23
Sept 9th	30	1.8	1.5	260	265	276	0.17	0.05	<0.02	2.76	84.0	<0.1
March 25th	35	6.9	4.0	634	786	547	0.004	259	n.d.	12.4	44.8	<0.1
Sept 9th	35	5.3	3.9	624	620	503	0.17	179	181	10.1	44.3	<0.1
	45	2.1	1.2	267	334	298	0.003	5.12	6.00	3.01	88.0	<0.1
March 25th	Duplic.	6.2	5.1	701	927	549	0.003	411	n.d.	15.5	18.3	<0.1
	45	7.5	5.6	731	724	508	0.17	302	324	13.7	22.4	<0.1
Sept 9th	Duplic.	6.4	5.4	721	708	494	0.17	294	n.d.	13.6	24.2	<0.1

1)calculated by dividing SO_4 concentration with 2.996

The profile of the bacterial community in the N pit water supports the reduction of Fe^{3+} in the water at least in the uppermost, oxygenated water layer where the Burkholderiales iron-reducing and sulphur-oxidizing bacteria dominated. This feature may also be present at 22 m depth, but the role of the betaproteobacterial SBla14 cluster is not yet clear. At greater depths Fe^{2+} is probably oxidized by epsilonproteobacteria. In addition, these epsilonproteobacteria detected in the N pit at 40 m depth are sulphur oxidizers, which could affect the acidification of water as well as prohibit the formation of Fe sulphides. SRB clades were generally detected only at relatively low abundances in the most diverse bacterial population at 40 m depth, and these bacteria coincided with the sulphur oxidizing epsilonproteobacteria. Thus, the results indicate that an active sulphur cycle is present at this depth. Chemical investigations indicated that sulphate reduction occurs in the anoxic parts of the pit. While the bacterial community reveals only low abundances of SRB in these parts of the N pit, it is, however, possible that a small but efficient population of SRB is responsible for the formation of Fe sulphides (Räisänen et al. 2015).

The high abundance of the iron oxidizing Gallionellales bacteria in the Z pit water strongly supports a biological iron oxidation process where iron is precipitated as Fe oxyhydroxides. In the oxygenated water from 1 to 30 m depth resultant Fe^{3+} may be reduced to Fe^{2+} by Burkholderiales bacteria, thus maintaining an iron reduction/oxidation cycle. The high diversity of bacterial groups present in the Z pit sediment indicates that the pit has not been influenced by anthropogenic organic carbon additions, as there is not a predominance of fermenting bacteria.

Water remediation mechanisms in the N and Z pits

Boundary conditions for biological sulphate reduction in the bottom water layer of the mine pit are characterized by anoxic water conditions, organic substrate, and SO_4^{2-} (Lu 2004, Vestola & Mroueh 2008). Other recommended parameters are pH >5.5, Eh (redox) potential <-100 mV (García et al. 2001), and temperature >6 °C (Vestola and Mroueh 2008). When observing the Hammaslahti N pit (which has been the project site for biologic sulphate reduction) the bottom water was clearly anoxic in March, and almost completely anoxic in September, indicating suitable conditions for SRB. However, the observed redox-potentials were rather high for biologic sulphate reduction. SRB have the ability to generate negative potentials (Sheoran et al. 2010), but this has not happened during the 15 years of treatment in the N pit. However, SRB can also perform successfully under positive Eh conditions when anoxic, reducing "pockets" in organic substrate exist (Sheoran et al. 2010). It is very possible that these pockets are found in the deepest water, just above the bottom sediments, although microbiological sampling found only minimal SRB in the sediments (Räisänen et al. 2015).

In the N pit, the redox potential ranged from -58 mV to 0 mV when measured with Ag/AgCl electrode, equivalent to values between +140 mV and 200 mV (SHE). According to Glasby & Schultz (1999) FeS_2 tends to dissociate to Fe^{2+} and SO_4 under these conditions. Furthermore, SRB groups coincided with sulphur oxidizing epsilonproteobacteria that resulted in an active sulphur cycle and moderately high solubility of sulphate in deep waters. These findings suggest that FeS_2 may be unstable, especially during the summer when overturn can increase redox potential. On the other hand, the low water temperature (<+6 °C) and slightly unfavourable redox potential (>-100 mV) are obvious reasons for a great decrease in SRB activity. Nevertheless, biologic sulphate reduction seems to be more active than the dissolution of FeS_2, as concentrations of SO_4 and Fe have clearly decreased during the treatment (Räisänen et al. 2015). Improvements to the N pit biological treatment can most likely be achieved by the addition of organic substrate in the future to promote the development of the sulphate reducing bacterial community. One possibility would also be to introduce more cold-tolerant bacteria to the pit.

In the Z pit, the source for excellent buffering capacity (i.e. alkalinity) is obviously carbonate weathering followed by sulphide oxidation of pit walls (Räisänen et al. 2015). That explains additional release of soluble Ca in deep waters (See tab. 2). However, crystalline gypsum ($CaSO_4 \times 2H_2O$) was identified from the bottom sediments (Räisänen et al. 2015). The precipitation of gypsum is one of the main neutralizers of acidity. According to Räisänen et al. (2015), the bottom sediments were rich in Fe oxyhydroxides but no Fe sulphides was identified. This suggests that sulphate is first fixed with Fe oxyhydroxides, forming schwertmannite and/or ferrihydrite, which then transforms into crystalline goethite in deep waters and/or bottom sediments (Bigham et al. 1992, Kumpulainen et al. 2007, Sánchez-España et al. 2011). The transformation includes dissolution of sulphate and it can contribute to pH-buffering (Nordstrom & Alpers 1999). According to Bigham et al. (1992), the precipitation of Fe oxyhydroxides controls the formation of Fe sulphides. The above findings can explain the excess sulphur in relation to Ca and Fe in deeper waters. Furthermore, the abundance of soluble Mn may have restricted Fe sulphide formation in slightly acidic and reducing waters (Bigham et al. 1992, Kerrick & Horner 1998).

Conclusions

This study revealed marked differences in physical, chemical and biological contents of the waters in two open pits (N, Z) in the closed Hammaslahti Cu-Zn-Au mine. Bacterial communities in the N and Z pits were very diverse and distinct from each other. These findings indicate that there is no adit connection between the N and Z pits.

The physical and chemical quality of the surface water above 5 m in both pits was better than that of the mid and deep waters. An oxygen deficit occurred below 20 m in the N pit and below 30 m in the Z pit in spring as well as in autumn. Deep waters and bottom sediments of both pits were characterized by year-round slightly reducing and $pH \geq 5.5$ conditions.

In the oxygenated waters of both pits, the sulphur reducing and iron oxidizing Burkholderiales bacteria were common, whereas the deep water and bottom sediments contain a small but efficient population of sulphate reducing bacteria (SRB). Overall, it can be concluded that the sulphate reducing bacteria addition together with organic carbon feed (pig mature, wood chips) has promoted Fe sulphide formation in the Hammaslahti mine N pit. In contrast to waters of the Z pit, the oxygenated water of the N pit showed acidification, most perceptibly, as a result of spring overturns. Unfortunately, the pH of the system has not increased to neutral, possibly due to insufficient activity or abundance of SRB. Indeed, very low amounts of SRB were detected from the sediments and waters during sampling. This is most likely due to the low temperature of the N pit waters. Furthermore, the rocks in the N pit, in contrast to the Z pit, do not bear carbonate minerals and such silicates that can increase buffering capacity via weathering. One possibility to improve the N pit biological treatment would be to introduce more cold-tolerant sulphate reducing bacteria to the pit.

In the Z pit, Fe and SO_4 were mostly precipitated as Fe oxyhydroxides, and the mineralogy of the bottom sediments did not reveal the occurrence of Fe sulphides, despite the fact that the S results revealed some soluble sulphide in waters. However, it is expected that crystalline goethite and other ferrihydroxides adsorbed trace metals, and therefore the retention rate for Zn and other metals was quite good. However, the formation of Fe oxyhydroxides, as well as the buffering reactions resulting from carbonate weathering, increases the solubility of base cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) and sulphate. Therefore the discharge from the Z pit is characterized by elements that increase the water salinity but not the content of trace metals downstream.

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