Removal of Metals from Circum-Neutral Mine Water by Biologically-Induced Diurnal Ph Cycling

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ABSTRACT

In surface freshwater streams the pH is typically controlled by the carbon dioxide, bicarbonate, carbonate buffer system. Photosynthesis during daylight hours leads to removal of carbon dioxide and/or bicarbonate from the water column with an associated increase in pH. Additionally, a locally strongly oxidizing environment can be created owing to production of high dissolved oxygen concentrations. These conditions can lead to enhanced removal of metals from the water column via precipitation of hydroxide and carbonate species, adsorption on sediments, or oxidation to insoluble forms.

This paper describes the findings from field and laboratory investigations of the removal of substantial amounts of manganese (Mn) and zinc (Zn) from a surface stream system, as a final polishing step for partially treated circumneutral metalliferous mine drainage produced from an underground lead/Zn mine. Diurnal pH modulation by the naturally occurring algal assemblage dominated by the filamentous cyanobacterium *Schizothrix calcicola* ,combined with formation of amorphous manganese dioxide coatings on the stream bed, were found to be the drivers of this process. Selective chemical extraction of the filamentous phototrophic biofilm material indicated that Zn was removed by surface adsorption or precipitation of carbonate phases and that Mn was largely removed by oxidation on the surface of the cyanobacterial filaments.

The findings from this work indicate the key role that photosynthetically driven pH cycles can play in metal removal in minewater treatment systems, and highlight the importance of maintaining shallow open water areas. The occurrence of diurnal cycles in metal concentrations downstream of mine water inputs emphasises the need to address this process in the design of water quality sampling programs being used to assess perfomance of these systems.

Keywords: mine drainage, diurnal pH cycles, metal removal, cyanobacteria, passive water treatment

INTRODUCTION

In aquatic systems dissolved metals can be removed from the water column by precipitation, adsorption to suspended sediment (followed by sedimentation), adsorption to the bed sediment, or by uptake by aquatic plants (Chapman, Jones & Jung, 1983). Precipitation, or co-precipitation of another metal present at lower concentration, followed by sedimentation can occur if the hydroxide or carbonate complexes become supersaturated. For trace metals (cations and oxyanions), pH is the key variable that controls the extent of adsorption and/or precipitation in oxic surface waters.

In most aquatic systems the pH is controlled by the carbonate buffer system. When photosynthesis occurs during daylight hours, carbon dioxide and/or bicarbonate is removed from the water column and the pH rises (Lucas & Smith, 1973). Additionally, a strongly oxidizing environment can be created locally as a result of the production of high dissolved oxygen concentrations. Variation in pH and oxygen concentrations due to photosynthetic activity can lead to fluctuations in the concentrations of contaminants in the water column as the solubility and/or state of adsorption-desorption equilibrium changes (Gammons, Nimick & Parker, 2014).

Over the past decade many observations have been made of diurnal cycles in concentrations of zinc (Zn) and manganese (Mn), in particular, in circum-neutral streams impacted by drainage from historic mines (Gammons, Nimick & Parker, 2014). The process is driven by the daily cycling of pH induced by the photosynthesis (light) and respiration (dark) of phototrophs attached to the stream bed. The importance of these processes in controlling metal removal, and their consequent relevance for obtaining meaningful measurements of metal concentrations downstream of mine water sources, has been the subject of a recent review (Gammons, Nimick & Parker, 2014). It is noted by these authors that although observed daily diurnal ranges of pH occasionally exceed one unit, in most locations they vary less than this.

There are few well-documented examples of the importance of diurnal pH cycling in driving metal removal in full scale mine water treatment systems. This study documents the findings from investigations of the passive water treatment system at the underground Hilton lead-zinc-silver mine located 20 km north of Mt Isa in central-northern Australia (Figure 1). Mt Isa Mines Ltd (the owners of the resource in the late 1980s) needed to dewater the underground ore-body in the lead up to developing production from the mine.

The concentration of Zn at site D3 (Figure 1) needed to meet the water quality discharge limit of 2 mg/L set by the Queensland state regulator. Coincidentally this target for removal of Zn also met the quality requirement for re-use of this water for mineral processing, an important consideration in this semi-arid environment. A 20 km pipeline transported water from the Hilton pump weir at D4 to the mineral processing plant at Mt Isa.

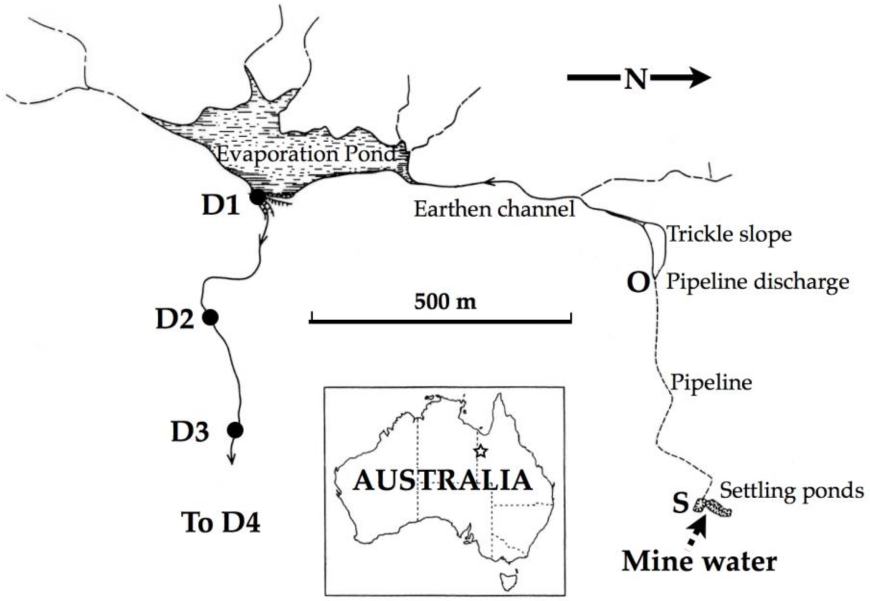

Figure 1 Location (star on map) of the Hilton mine and schematic of the mine water treatment system.

A passive treatment system was constructed to strip the metals from the mine water with the aim of meeting both process water and environmental discharge requirements for Zn. The present study was carried out to identify the specific removal processes involved to:

- facilitate optimization of contaminant removal, and
- to ascertain whether they could continue to operate satisfactorily into the future.

A detailed description of the complete treatment system for the pH circumneutral mine water has been published previously (Jones, Chapman & Jung, 1995). During the 18 month period of this study the total dewatering rate ranged between 2000-6000 m^3/day. There were no diluting inflows from other sources, such as rainfall or stream tributaries, to complicate interpretation of the results.

The focus of this paper is the downstream (from locations D1 to D4, Figure 1) section of the treatment system, wherein the residual dissolved Zn is further removed to acceptable levels. The stream between D1 and D4 was typically shallow (<0.3 m deep at the centre), with a maximum width of 2 m. It comprised sections of interconnected shallow pools punctuated by steeper riffle zones, as the watercourse descended by about 10 m over the reach. The gravel/cobble bed was covered by copious growths of green filaments (Figure 2).

METHODOLOGY

The field work described in this paper was conducted over three campaigns in October 1984, October 1985 and February 1986, with detailed chemical analysis work and laboratory experiments being conducted between the first two field visits and after the last one.

Figure 2 Mats of filamentous biofilm containing *S. calcicola* covering the stream bed

Water chemistry

Measurements of pH were made *in situ*. Water samples for total and dissolved (<0.1 μm) metals were collected in acid leached plastic bottles. All samples were preserved by the addition of nitric acid (to 0.1 %) within 1h of sample collection. The concentrations of major cations were determined by atomic absorption spectroscopy (AAS) or Inductively Coupled Plasma Atomic Emission Spectroscopy (ICPAES), and the concentrations of iron, Mn, and Zn measured by ICPAES. Sulfate was determined by ICPAES or ion chromatography and bicarbonate concentrations were measured in the field by titration with standardised hydrochloric acid.

Collection and analysis of microbial and sediment samples

Samples of the filamentous microbial material were collected from the bed of the stream at D1 and D3, and at several locations between, using plastic scoops. They were stored at 4 ℃ in plastic bottles for two days prior to being processed in the laboratory. Detritus such as leaves and twigs was removed by hand sorting. Filaments were washed by re-suspending them in 0.05 M NaCl solution. The sample was then consolidated by centrifugation. The supernatant was decanted and the washing and centrifugation step repeated.

Subsamples of the wet biofilm were separately extracted at room temperature with 0.1 M pH 4 acetic acid/sodium acetate buffer and 0.1 M hydroxylamine hydrochloride solution in the pH 4 acetate buffer to investigate the modes of binding of Zn and Mn in the algal filaments The extraction methods used were adapted from Tessier, Campbell & Bison, 1979.

Aliquots of supernatant solutions were withdrawn at 10, 20, 40 and 65 min to track the time course of increase in the extracted Zn and Mn concentrations. The concentrations extracted at 65 min were used to estimate the amounts of Zn and Mn associated with the phases targeted by the extract solutions, as the concentrations in solution approached an asymptote value at this time. Total extractable concentrations of Zn and Mn were obtained by digesting finely pulverised freeze-dried samples with a 1:1 mixture of concentrated HNO_3 and HCl acids. A similar digestion was used for the stream sediments. The concentrations of metals in the extract and digest solutions were determined by ICPAES. The oxidation state of Mn in the surface coatings on the stream sediments was determined using the iodometric method of Murray, Balistrieri & Paul, 1984.

Semi-quantitative powder X-ray diffraction (XRD) was used to identify crystalline mineral phases present in finely ground freeze-dried samples of the microbial material and the bed sediments.

Microbial strain identification

Samples of the microbial mat were collected from the stream bed between D1 and D3 were sent to the National Herbarium of New South Wales in Sydney for identification using optical microscopy. The dominant species was found to be a clumped ecoform of the widely occurring cyanobacterium (colloquially known as blue green algae) *Schizothrix calcicola*. It was noted by the phycologist at the Herbarium that this ecoform had a much higher amount of extracellular polymeric substances (EPS) associated with the filaments, than was usual for the species.

RESULTS AND DISCUSSION

Water chemistry

The concentrations of select major ions and of Mn and Zn between sites D1 and D4 in October 1984, are plotted in Figure 3. Only the data for 1984 are shown here as the behaviour of the solutes was found to be the same for the subsequent field campaigns.

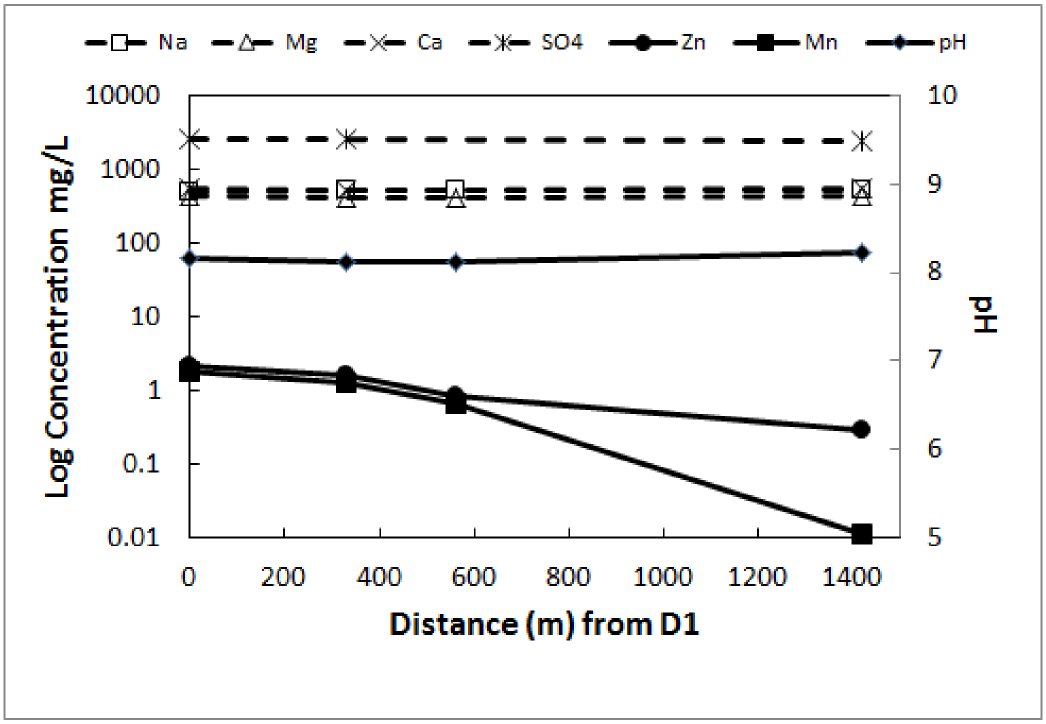

Figure 3 Concentrations of major ions, pH and Zn and Mn downstream of D1 (October 1984)

Only the dissolved concentrations are shown in Figure 3 as there were no substantial differences between the total and dissolved concentrations in this reach. The fact that the concentrations of the major cations did not change significantly between D1 and D4 indicates that there were no significant diluting inflows or losses of water by evaporation. In contrast to the major ions, the concentrations of soluble Zn and Mn decreased substantially (approximately 2.5 fold) between D1

(0 m) and D3 (560 m), with Mn concentrations being below detection limit (0.04 mg/L) at D4 (1420 m).

All of the water samples were collected in daylight hours within 30 min of one another. The possibility of artefacts being introduced due to sampling time, as discussed by Gammons, Nimick & Parker, 2014, was thus minimised.

Mineral Solubility

To determine which solid phases could potentially be removing Mn and Zn from solution, saturation indices (SI) were calculated using the MINEQL chemical equilibrium computer code (Westall, Zachary & Morel, 1976). The saturation index (SI) is defined as the logarithm of the ratio of the activity product of the component ions of the solid in solution to the solubility product for the solid. Values greater than zero indicate super-saturation and values less than zero, under-saturation. The SI calculations showed that calcium (Ca), Zn and Mn carbonates were marginally over saturated (SI values of 0.5, 1 and 1, respectively at D1) in the bulk flowing water downstream of D1, indicating that precipitation of these phases could be controlling the solubility of these metals. The SI for $MnCO_3$ declined below zero downstream of D3.

Investigation of the microbial material and stream sediments by XRD did not show the presence of Zn or Mn carbonate mineral phases, although low concentrations of Ca carbonate were detected. The non-detection of Zn and Mn carbonates does not mean that they were not present, as the limit of detection of XRD is typically around 1-2 wt % in a complex matrix (Chung F & Smith DK, 1999). Reference to Table 1 shows that the total concentrations of Zn and Mn in the microbial material, if all were present as carbonates, would be at or below the detection limit of this technique.

Mechanisms of metal removal– microbial mats

To investigate possible diurnal behaviour (an indicator of photosynthetic activity), the flowing water from mid-stream was sampled over a two-day period in October 1985 at D1 and at D3 (Figure 4). It is noted that the concentration of Zn at D1 at this time was approximately twice that in October 1984, whilst the concentration of Mn was about the same.

The concentrations of both Zn and Mn were considerably reduced (on average by factors of 2 and 4, respectively) along this reach of the stream. Moreover, there was a marked diurnal variation in concentrations which did not originate in the evaporation pond (the overflow of which corresponds to D1), but which occurred during the passage of the water between D1 and D3. Significantly in this reach of stream there was close contact between the water and extensive microbial filaments (primarily the commonly occurring cyanobacterium *Schizothrix calcicola*, hereafter abbreviated to *S. calcicola*) (Figure 2).

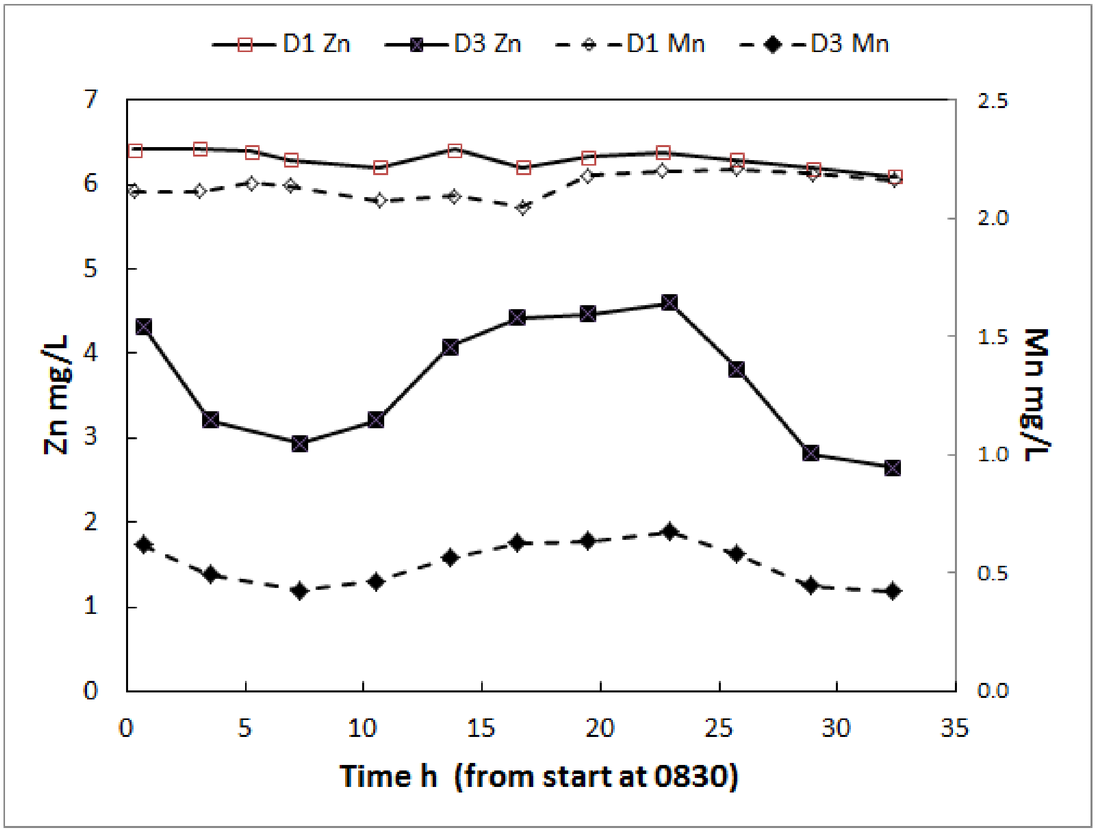

Figure 4 Concentrations of Zn and Mn at D1 and D3 over a two day period (October 1985)

A subsequent study was undertaken at D1 in February 1986 to test the hypothesis that the increase in pH associated with photosynthesis was a driver for metal removal. At this time the Zn concentrations were much lower than in October 1984 and 1985 because there was a lower proportion of the Zn-rich water derived from the orebody in the total volume of water being discharged.

The pH and concentrations of Zn close to a microbial mat were compared with that in mid-stream between sunrise and sunset (Figures 5 and 6). At sunrise, the pH values of both the stream water and the water close to the microbial mat were close to pH 7.9, with similar Zn concentrations (0.88 mg/L) at both locations. As the sun rose, the pH in the water close to the filaments rose steadily to a maximum value of 8.6-8.8, whereas the water in mid-stream remained close to 7.9. Coincidentally with the rise in pH near the *S. calcicola* filaments, the concentration of Zn in solution fell to 0.6 mg/L, whilst that in the bulk flowing water remained at its initial concentration. As the sun was setting, the pH near the *S. calcicola* filaments began to decline rapidly until, by nightfall, it had returned to its original early morning value. The pH increase measured in the field was consistent with *in vitro* laboratory measurements in a closed system that showed that the pH in vessels containing the *S. calcicola* varied by as much as 1.5 pH units over a simulated day/night cycle.

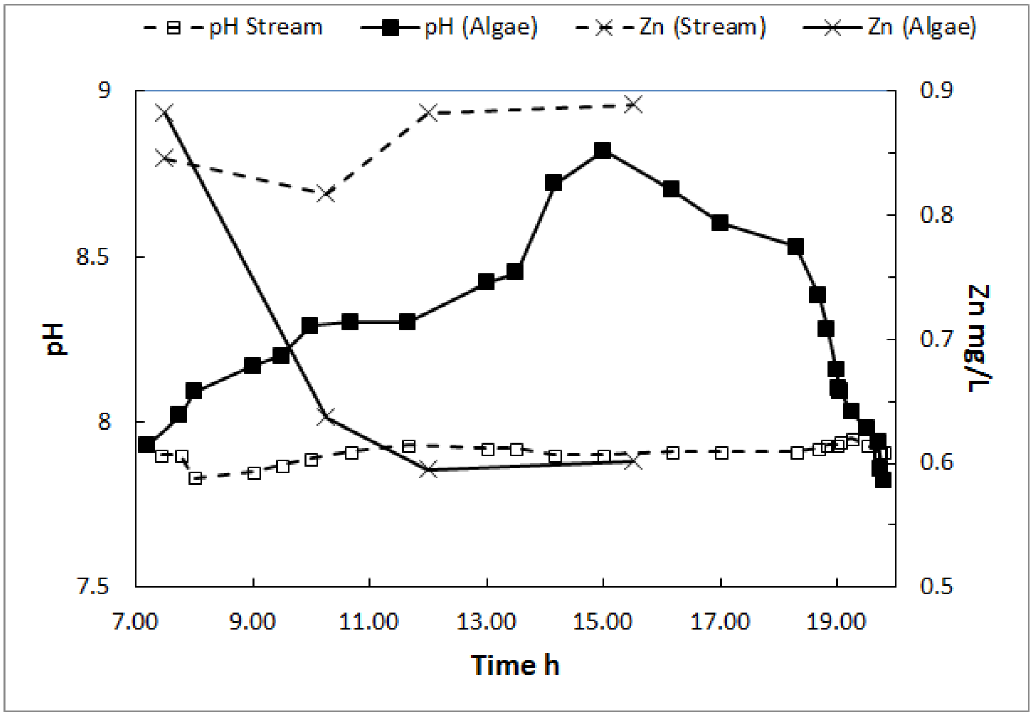

Figure 5 Comparison of pH and Zn concentrations in the middle of the stream and in a microbial mat at D1

Some properties of the microbial material collected at D1 are summarised in Table 1. The rapid rates of dissolution of the metals from intact *S. calcicola* filaments material by extraction reagents (pH 4 acetate buffer and 0.1 M hydroxylamine hydrochloride at pH 4) indicates that a high percentage of the total amounts of these metals are located on the surface of the *S. calcicola* filaments. In contrast to Zn, only 30 % of the Mn was extracted with the pH 4 buffer. However, the majority of the Mn was rapidly dissolved at pH 4 in the presence of a reducing agent (hydroxylamine) that is selective for Mn dioxide and Mn oxyhydroxides (Tessier, Campbell & Bison, 1979).

The extraction results are consistent with) metal removal by surface adsorption/complexation on and/or by enhancement of pH-dependent (precipitation, oxidation) removal processes on the surface of the cyanobacteria filaments. The EPS produced by the cyanobacteria may provide increased surface area for metal binding. The potential importance of EPS in binding and concentrating Zn on the surface of algal cells has been noted by others (Podda et al, 2000).

The increases in pH and oxygen concentrations produced as a result of photosynthesis may favour the precipitation of Zn and Mn carbonates and the oxidation of Mn(II) to produce insoluble Mn(III)/Mn(IV) oxides on the microbial filaments. Whilst concentrations of dissolved oxygen were not measured in the field, the occurrence of copious small bubbles on the surfaces of the microbial mats during daylight hours suggested the presence of elevated concentrations of oxygen in the vicinity of the filaments.

The role of photosynthesizing cyanobacterial or other types of phototrophic filaments in facilitating the precipitation of hydrozincite (a zinc hydroxy-carbonate phase) on their surfaces has been documented by Kwong et. al., 1994, and Podda et al, 2000. Support for these types of processes occurring downstream of D1 is provided by the findings from the selective extractions described above.

Table 1 Chemical extraction of Zn and Mn from D1 *S. calcicola* samples

Extract medium	Likely Phases	Zn	Mn
pH 4 acetate, 10 min	Adsorbed Zn and Mn, labile Zn and Mn carbonates (I)	62 %	23 %
pH 4 acetate, after 65 min	Adsorbed Zn and Mn, labile Zn and Mn carbonates(I)	77 %	30 %
pH 4 hydroxylamine, 10 min	(I) plus Mn oxyhydroxides and Mn oxides	65 %	84 %
pH 4 hydroxylamine, 65 min	(I) plus Mn oxyhydroxides and Mn oxides	90 %	98 %
Aqua regia digest (total)		17200 mg/kg dry weight	8330 mg/kg dry weight

The effectiveness of photosynthetically driven processes for metal removal will be a function of maximum day time temperature, day length and solar radiation intensity. The climate data (available from the Australian Bureau of Meteorology: www.bom.gov.au) for the Mt Isa region show that these parameters do not change substantially over the year at the Hilton mine. Consequently, it is not expected that there would be substantial variation in the effectiveness of biological removal processes through the year. This situation contrasts strongly with that existing in the temperate latitudes from which much of the published literature on diurnal cycling of metal removal has been produced.

Mechanisms of metal removal – inorganic sediments

Samples of the gravelly streambed sediment were collected 60 m downstream of D1. Gravelly soil was also collected from the banks of the stream at this location to provide a control for experiments to investigate the role that the bed material could play in removing dissolved Zn and Mn from the water. The only obvious physical difference between the two types of solids was that the gravel substrate from the channel bed was covered with a soft black coating. Samples of each (on-bank and in-stream) of these solids were agitated with aliquots of water from D1, and the decrease in metal concentrations monitored. Both sediment types removed similar amounts of Zn from solution (50 % after 20 h), but only the material collected from the stream bed removed Mn (97 % after 10 h for stream sediment, 0 % after 72 h for bank material).

Bacteria are known to catalyse the oxidation of soluble Mn(II) to insoluble Mn(IV) (Diem & Stumm, 1984). However, in this case the addition of the biocide sodium azide did not inhibit the removal of Mn by the stream sediment, indicating that the reaction must have been an abiotic process. X-ray diffraction, and chemical and redox speciation (Murray, Balistrieri & Paul, 1984) analysis of the black material coating the stream sediment particles showed it to be primarily amorphous manganese dioxide with considerable associated Zn (Mn/Zn=4). Pre-formed Mn(IV) oxides are known to catalyse the oxidation of initially adsorbed Mn(II) (Hem, 1978). The ability of Mn oxide coatings on stream beds to remove Mn(II) from solution at pH values for which the abiotic rate of oxidation in solution would otherwise be very slow has been noted by others (Gordon & Burr, 1988) as has the ability of these types of coatings to absorb substantial amounts of Zn (Shope, Xie & Gammons, 2006).

Conclusions and significance of findings

Both abiotic (formation of Zn-rich manganese oxides on bed substrate) and biologically-mediated (adsorption and/or precipitation on *S. calcicola* filaments) processes have been shown to contribute to the reduction in Zn and Mn concentrations downstream of site D1 at the Hilton mine. The diurnal variations observed in the downstream concentrations of these metals occurring in concert with the daily cycling in pH adjacent to the *S. calcicola* filaments, imply that a significant proportion of the metals are removed as a result of the photosynthetically-induced pH oxygen concentration increases in the water close to the filamentous biomass in the stream channel.

Selective chemical extractions of the filamentous material indicated that Zn was removed by surface adsorption or precipitation of carbonate phases and that Mn was largely removed by oxidation. Uptake of Mn and Zn by manganese oxide coatings on the stream bed also contributed to the attenuation of these metals. Whilst it was not possible to definitively apportion the extent of Mn and Zn removal between the biotic and abiotic pathways, consideration of the physical context suggests that the phototrophic pathway may be the dominant one, as the *S. calcicola* filaments are in continuous intimate contact with the water column and the microbial mats provide a diffusion barrier to the Mn oxide coating on the underlying stream bed.

One of the primary objectives of water quality monitoring programs in surface water, whether it be a pristine or an impacted water body, is to obtain an accurate representation of the chemical status of the system. In the case of a mine-water treatment system, both regulatory compliance and metal removal efficiency typically need to be assessed. However, if diurnal variations in water quality are not adequately accounted for in the design of the monitoring program, then potentially flawed conclusions may be drawn. Indeed very different conclusions about system performance can be drawn depending on when in the day a system is sampled. This aspect has been well illustrated by a recent review (Gammons, Nimick & Parker, 2014). In the case of the Hilton Mine passive treatment system, substantive net removal of both Mn and Zn occurs throughout the diurnal cycle.

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REFERENCES

Chapman, B, Jones, D & Jung, R (1983) Processes controlling metal ion attenuation in acid mine drainage streams. Geochimica Cosmochimica Acta, 47, 1957-1973.

Chung F & Smith DK (1999) Industrial Applications of X-Ray Diffraction, CRC Press, 1024p.Diem, D & Stumm, W (1984) Is dissolved Mn^{2+} being oxidised by O_2 in absence of Mn-bacteria or surface catalysts? Geochimica Cosmochimica Acta, 48, 1571-1573.

Gammons, C, Nimick, D & Parker S (2014) Diel cycling of trace elements in streams draining mineralize areas-a review, Applied Geochemistry, in press, http://dx.doi.org/10.1016/j.apgeochem.2014.05.008.

Gordon, J & Burr J (1988) Manganese treatment by two novel methods at abandoned coal strip mines in North Alabama. Proceedings of the 42nd Industrial Waste Conference, Purdue University, pp. 907-918.

Hem, J (1978) Redox processes at surfaces of manganese oxide and their effects on aqueous metal ions. Chemical Geology, 21, 199-218.

Jones, D, Chapman, B & Jung, R (1995) Passive treatment of mine water, in Proceedings of Sudbury '95 - Mining and the Environment, Hynes, T.P. and Blanchette, M.C. (eds), May 28 - June 1, 1995, Sudbury, Ontario, Canada, Vol. 2, pp. 755-763.

Kwong, Y.T.J, C. Roots, and W. Kettley(1994) Lithochemistry and aqueous metal transport in the Keno Hill mining district, central Yukon territory. In: Current Research 1994-E; Geological Survey of Canada, pp.7-15.

Lucas, WJ & Smith, FA (1973) The formation of alkaline and acid regions at the surface of *Chara corallina* cells, Journal of Experimental Botany, 24, 1-14.

Murray, J, Balistrieri, L & Paul, B (1984) The oxidation state of manganese in marine sediments and ferromanganese nodules. Geochimica Cosmochimica Acta, 48, 1237-1247.

Podda, F, Zuddas, P, Minacci, A, Pepi M & Baldi, F (2000) Heavy metal coprecipitation with hydrozincite [$Zn_5(CO_3)_2(OH)_6$] from mine waters caused by photosynthetic microorganisms, Applied and Environmental Microbiology, 66, 5092-5098.

Shope, C, Xie, Y & Gammons C (2006) The influence of hydrous Mn-Zn oxides on diel cycling of Zn in an alkakine stream draining abandoned mine lands, Applied Geochemistry, 21, 476-491.

Tessier, A, Campbell, P & Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals, Analytical Chemistry, 51, 844-851.

Westall, J, Zachary, J & Morel, F (1976) Technical Note 18. Water Quality Laboratory, Dept. of Civil Engineering, MIT, Cambridge, Massachussets.