

Zinc Uptake from Circumneutral Mine Drainage in Freshwater Biofilms: New Insights from In Vitro Experiments

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Abstract We present results from in vitro flask and flume experiments using freshwater biofilms sourced and cultivated from a mine-impacted stream in North Yorkshire, UK. Flask experiments showed rapid uptake of Zn from the water column into biofilms. This uptake was not light dependant and suggests that chelation of Zn by negatively charged functional groups in extracellular polymeric substances (EPS) secreted within the biofilm are the key sink for Zn. Solid state analysis of the biofilm from the mesocosm system by scanning electron microscopy highlights the presence of calcite precipitates within the EPS, which may provide another sink for Zn. Long-term monitoring of flume systems showed area-adjusted removal rates of $\approx 0.2 \text{ g Zn m}^{-2} \text{ day}^{-1}$, which is consistent with many other biologically-mediated mine water treatment systems. Diel (24 h) fluctuations in pH and Zn were observed over a 96 h intensive sampling period in the flumes. Practical considerations for establishing and maintaining biofilms under controlled conditions are also highlighted; these include regulation of light intensity and maintenance of flowing, low nutrient status waters.

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Introduction

Although the terms acid mine drainage (AMD) and acid rock drainage (ARD) prevail in the literature, many metal mine discharges are of circumneutral pH due to buffering by carbonate-rich country rock, or weathering of sulphides which do not generate acidic conditions (e.g. sphalerite). Over 6 % of rivers in England and Wales have metal concentrations that exceed aquatic life standards due to metal mine discharges (Mayes et al. 2009a). 85 % of such discharges have a pH between 6 and 8, while about 5 % have a pH <4 (Jones et al. 2013; Mayes et al. 2010). The most common metal pollutants in these circumneutral discharges are $\text{Zn} > \text{Pb} > \text{Cd} > \text{Cu}$ (Jarvis and Mayes 2012). Researchers are currently investigating low-cost technologies to strip such mobile metals from waters; this is especially true of Zn due to its ubiquity and toxicity to aquatic life at modest concentrations (Alabaster and Lloyd 1980). Zinc toxicity is particularly apparent for cyprinid and salmonid taxa, which are considered sentinel indicators of aquatic ecosystem quality (Whitfield and Elliott 2002). Many recent studies highlight the sub-lethal effects of Cu and Zn on salmonid behaviour at concentrations below aquatic life standard limits (McGeer et al. 2000; Taylor et al. 2009). Passive technologies for treating Zn- and Cu-rich waters include bioreactors, sorbent media, and low-cost reactants based on industrial by-products (Gandy and Jarvis 2012; Mayes et al. 2011; Warrender et al. 2011). While all have promise in various settings, treatment technologies that can effectively remove metals from circumneutral, mine water with a low residence time, small land footprint, and for long time periods are still needed.

Biofilms are communities of microbial organisms, which may consist of cyanobacteria, sulphate-reducing bacteria, and photosynthetic diatoms, held together in an extracellular polymeric substance (EPS) matrix (Decho 2010). The biofilm EPS is a product of microbial metabolic reactions, although the EPS components can be highly variable, depending on which microbial species are present (Sutherland 2001). EPS molecules contain ionisable functional groups, which give the EPS a net negative charge (van Hullebusch et al. 2003), which allows the EPS to sequester cationic metals (Me^{2+}) from the water column (Decho 2010). It has been established that biofilms are capable of storing large inventories of trace metals (Rogerson et al. 2008). The EPS effectively protects the ecosystem of microorganisms from external stressors (Flemming and Wingender 2010), and allows the biofilm to be governed by processes that are independent of those occurring in the bulk water column (Bissett et al. 2008).

These micro-environments are also subject to various dynamic processes. During daylight hours, photosynthetic microbes use CO_2 , which increases pH. During night time hours, when respiration predominates, CO_2 is released and thus the pH decreases. Bissett et al. (2008) observed that regardless of changes in ambient water column pH (experiments were run between pH 7.8 and 8.9), the pH at the biofilm surface was 9.4 in the light and 7.8 in the dark. The implications of this internal pH change are that these changes in pH can result in chelated metals being released from the EPS (van Hullebusch et al. 2003); in consort with this, in hard-water systems, increased pH and desorption of Ca^{2+} from EPS can promote carbonate precipitation within the biofilm. This calcite is a potential sink for other divalent metals (Me^{2+}) through sorption or co-precipitation (e.g. Zachara et al. 1991), and this process would not occur under the chemical regime of the ambient water (Rogerson et al. 2008). A recent field assessment of biofilm Zn concentrations in circumneutral streams draining areas of former Pb and Zn mining in North Yorkshire, UK, showed median Zn concentrations of 1,800 mg/kg dry weight (range 800–4,100 mg/kg dry weight), a bioconcentration factor of over 3,500 from the water column under baseflow conditions (Jones et al. 2013). There is, therefore, considerable opportunity to use biofilm as a tool to remediate areas that are affected by mine drainage. However, further research is needed to elucidate the pathways for metal uptake (e.g. biomineralization vs. sorption) and the permanence of the metal removal. This paper aims to assess these processes through in vitro experiments using biofilms cultured from mine-impacted streams. This serves both to improve our understanding of biofilms as a control on contaminant transport in mine-affected streams, and to underpin approaches to potentially harness biofilms for treatment of metal mine discharges.

Methods

Batch Experiments

Static batch experiments are a useful tool for answering specific questions. We used this technique to evaluate the impact that different environmental treatments had on biofilm metal uptake and dissolved metal concentrations. For each experiment, 150 mL of mine water was placed in a clean 250 mL conical flask with 5 g of wet biofilm. Biofilm was collected on the day of the experiment and transported so as to avoid denaturing of the biofilm. Biofilm and water was collected from Dukes Level mine discharge (Lat 54°04'43N, Long 1°57'41W), a major mine drainage level with ambient Zn concentrations of $950 \mu\text{g L}^{-1}$ and major ion chemistry characterised by $\text{Ca}^{2+}-\text{HCO}_3^- - \text{SO}_4^{2-}$ —dominated waters (see Jones et al. 2013). Biofilm (see supplementary Figure 1, which accompanies the on-line version of this paper) had spontaneously colonised on the mine drainage level discharge, which is a concrete structure. The biofilm was sufficiently thick to enable sections of it to be removed without interference from the substrate. Each flask experiment was agitated (to ensure gaseous diffusion) for 21 days, in a laboratory where light, time, and temperature were accurately controlled to simulate summer conditions in the UK (ambient air temperature set at 18 °C (± 1 °C), with 8 h UV light/day). To observe the effects of photosynthesis on metal uptake, each flask was wrapped in a polyamide elastane-based fabric to limit the amount of UV light that passed through to the biofilm, using four treatments that ranged through full light (*FL*: 6.82 kLux), high light (*HL*: 4.83 kLux), low light (*LL*: 2.64 kLux), and no light (*NL*: 0 kLux) exposure, with 6 replicates for each treatment. After the incubation period, the biofilm flasks were sampled for aqueous metal concentrations. A filtered (0.45 μm) 14 mL water sample was taken and acidified immediately with concentrated HNO_3 in preparation for trace metal analysis by a Perkin Elmer Optima 5300DV inductively coupled plasma optical emission spectrometer (ICP-OES). A blank and standard suite were analysed every 15 samples to check instrument calibration, and wavelengths were selected in accordance with standard methods (USEPA Method 200.7).

Flume Experiments

General Features

Continuous flow flume experiments were adapted from previous work by Pedley et al. (2009), with the objective of observing long-term dynamics and diel cycling of biogeochemical parameters, assessing the influences on trace metal mobility in a controlled environment. The novelty of

this system is built around the culture and long-term maintenance of biofilm communities collected from environmentally relevant field sites (in this case, metal mine impacted streams in North Yorkshire, UK). The flume experiments had a recirculating design permitting the experiment to proceed under controlled environmental conditions with no external influence (Fig. 1). The water used in the flumes was first sterilised for 24 h with a UV filter to ensure that naturally occurring microbes would not interfere with the biofilms in the flumes. The flume consisted of a 50 cm long, 7 cm wide polycarbonate gutter, lined with carbonic fabric and small rocks to aid colonisation and, given the low flow rates applied, increase water turbulence throughout the vertical water column. The gutter was housed in a Perspex flume box built for this purpose. Flow was driven by a Waston Marlow 300 series tube pump, with four 313D pump head attachments (Watson Marlow, Falmouth, UK), running at 55 rpm (giving a flow rate of 1 L/min), through peristaltic tubing (1.6 mm wall, 4.8 mm bore). The mesocosm was subjected to a 8:16 day: night light cycle (light: Thorn Lopak 250 W HPS-T Na lamp), in a windowless, air-conditioned laboratory (ambient air temperature 18 ± 1 °C). Two flumes were colonised with biofilm (sourced as per flask experiment), and one was sterilised before the experiment with a 10 % acid rinse (HNO_3), ethanol rinse, and finally ultrapure (18Ω) water rinse, and left uncolonised to act as a control.

Long Term Sampling Campaign

The flume mesocosm was placed in full light conditions (6.82 klux), and allowed to run without samples being taken to allow the system to fully equilibrate. After that, long-term sampling took place over a 66 day period, with 14 mL aliquots of water being taken every 3 days to establish initial trends of metal removal. Water analysis proceeded as per the flask experiments. Non-parametric statistical tests were applied to discontinuous percentage data in Minitab v. 15. Geochemical analysis was undertaken using PHREEQC Interactive 2.18.3 and the WATEQ 4F database. Area-adjusted removal rates were calculated to assess the removal capability of the biofilm over the long-term sampling regime.

$$R_A = \frac{Q_d(Me_i - Me_e)}{A}$$

where A = treatment media area (m^2); Q_d = mean daily flow rate ($\text{m}^3 \text{ day}^{-1}$); Me_i = influent metal concentration (i.e. concentration from previous day; mg L^{-1}); Me_e = effluent metal concentration (i.e. concentration from current day; mg L^{-1}), and; R_A = area adjusted metal removal rate ($\text{g m}^{-2} \text{ day}^{-1}$).

Diel Cycling Experimental Procedure

To characterise diel cycles in bulk physio-chemical parameters and trace metal concentration, a diel sampling

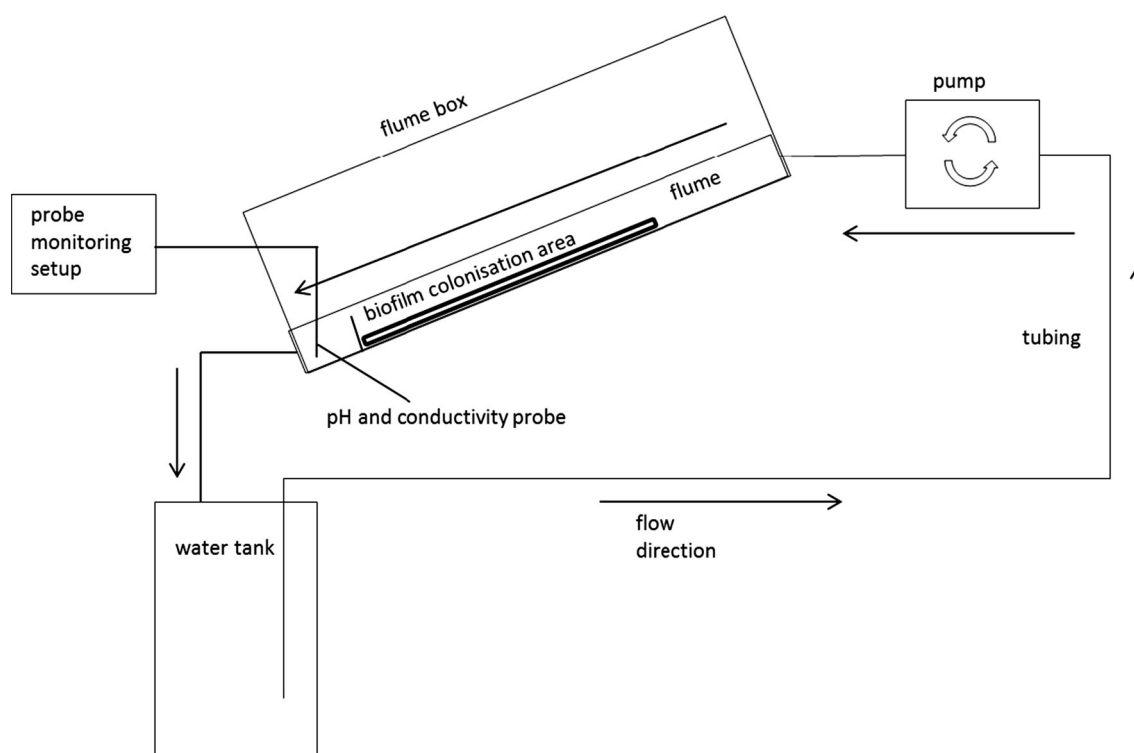


Fig. 1 A schematic diagram of the flume set up in its recirculating format

regime was implemented to assess the control exerted on dissolved trace metal concentrations by biofilms. Electrodes were placed in the flumes to constantly monitor pH and electrical conductivity (EC), using Pinpoint pH and EC—freshwater hardness monitors, American Marine Inc. The pH and EC data was logged every 10 min via a webcam connected to a computer running Yawcam software, and then transferred into written data via Watchman software. Running in conjunction with the pH and EC logging, 14 mL water samples were taken from each of the biofilm flumes and the control flume and filtered with a Millex-HA filter unit, a 0.45 μm MF-Millipore membrane, and immediately acidified with ultrapure concentrated HNO_3 for trace metal analysis via ICP-OES. Samples were taken from an area of the flume where water could be taken without disturbing the biofilm and were filtered to assess the dynamics of dissolved Zn in the system. Samples were then dried for scanning electron microscopy (SEM) analysis. Dried samples of interest were glued to SEM mounts, carbon coated, and observed using the SEM (Zeiss SMG EVO 60).

Results

Batch Experiments

The static batch experiments showed that the biofilm affected Zn removal; high levels of Zn removal were observed in the flasks with the biofilm, with a median removal of 81.68 %, and a maximum removal of 99 %. Some Zn (median of 38.92 %) was also removed in the control flasks (empty flask and water), most likely due to sorption onto glassware or precipitation from the water column (Fig. 2). However, significantly less Zn was removed (Kruskal–Wallis: H : 12.6316, df : 1, p < 0.001) in the control flasks than in those with the biofilm, suggesting that the biofilm was responsible for most of the Zn removal from the water column (Fig. 2). There was a significant difference in Zn removal between the light treatments (Kruskal–Wallis: H : 8.46, df : 3, p = 0.037), but this was due to the no light (NL) treatment, as there was no significant difference in removal between light intensities FL, HL, and LL (Kruskal–Wallis: H : 0.667, df : 2, p = 0.7165).

Comparatively less Ba was removed in terms of percentage removal of initial concentrations (Fig. 2). Small Ba percentage removal is evident in the control flasks, again probably due to sorption onto glassware or as precipitates in the water column. Ba has a larger ionic radius than Zn, and the literature suggests that ions with larger radii are preferentially taken up by biofilms, whereas our results suggest that Zn was preferentially removed over Ba. While

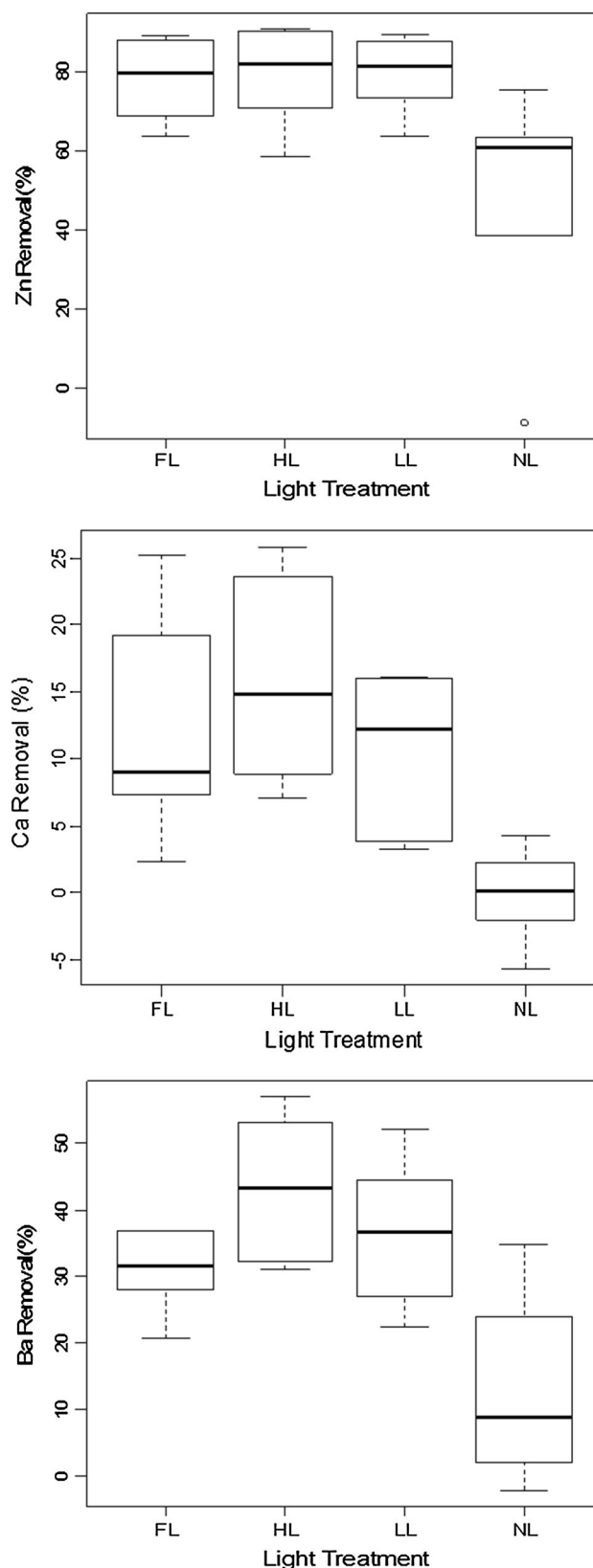


Fig. 2 Metal removal in light experiments; FL full light, HL high light, LL low light, NL no light

Table 1 Summary data for biofilm batch experiment; it can be seen that Zn is preferentially removed in all experimental treatments

Experiment Summary Removal Data (median % removal)		
	Proof of concept batch experiment	Light batch experiment
Zn^{2+}	Active (81.68)	FL (78.05)
	Sterile (38.92)	HL (78.98)
		LL (79.04)
		NL (48.38)
Ba^{2+}	Active (36.40)	FL (30.09)
	Sterile (6.92)	HL (43.29)
		LL (36.57)
		NL (12.67)

the data suggests that metal removal is not affected by changes in light intensity (i.e. metal removal continues with no light present), it is important to note the small volumes of water used and that the amount of Zn and other ions in the sample vessel was finite. The removal of Ca is evident in flasks, although given the comparatively large initial concentrations a percentage removal comparison would not be appropriate. Zn and Ba removal data from the initial batch experiments and are summarised in Table 1.

Long-Term Trends

The flume experiments proceeded for 66 days; Fig. 3 shows the key differences between the control (sterile) flume and colonised flumes for Zn removal. The abrupt spike in flume 2 after day 22 is likely to be due to entrainment of a small amount of organic debris in the sample. As total Zn samples were taken to account for any colloidal Zn exported from the active flumes, Zn concentration can be reasonably variable from one sample day to the next due to entrained particulates. However, consistent patterns can be identified, notably the systematic decline in dissolved Zn in the water column, with 78 % of the initial Zn ultimately being removed by the biofilm treatments. Table 2 summarises the loss of analysed ions in the flume system over the sampling period. The largest loss was Ca ions, with 117 and 126 mg removed in flumes 1 and 2, respectively, which was expected due to the significantly higher amount of Ca ions in the system compared with the other analysed elements. Zn had the second highest loss in terms of mass, which, given that there was more Mg ions available, suggests preferential uptake of Zn regardless of ionic radius. The mass loss of ions from the water column in the flume system are consistent across both of the biofilm colonised flumes, and follows the order $\text{Ca} > \text{Zn} > \text{Ba} > \text{Mg}$.

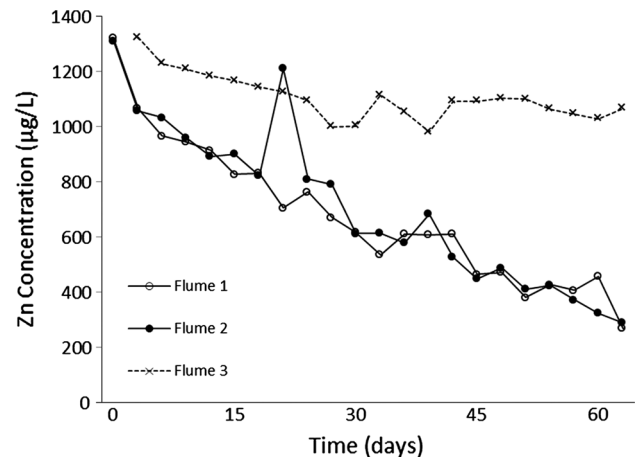


Fig. 3 Long term removal of zinc from the water column in the biofilm mesocosm; flumes 1 and 2 are active, flume 3 is sterile

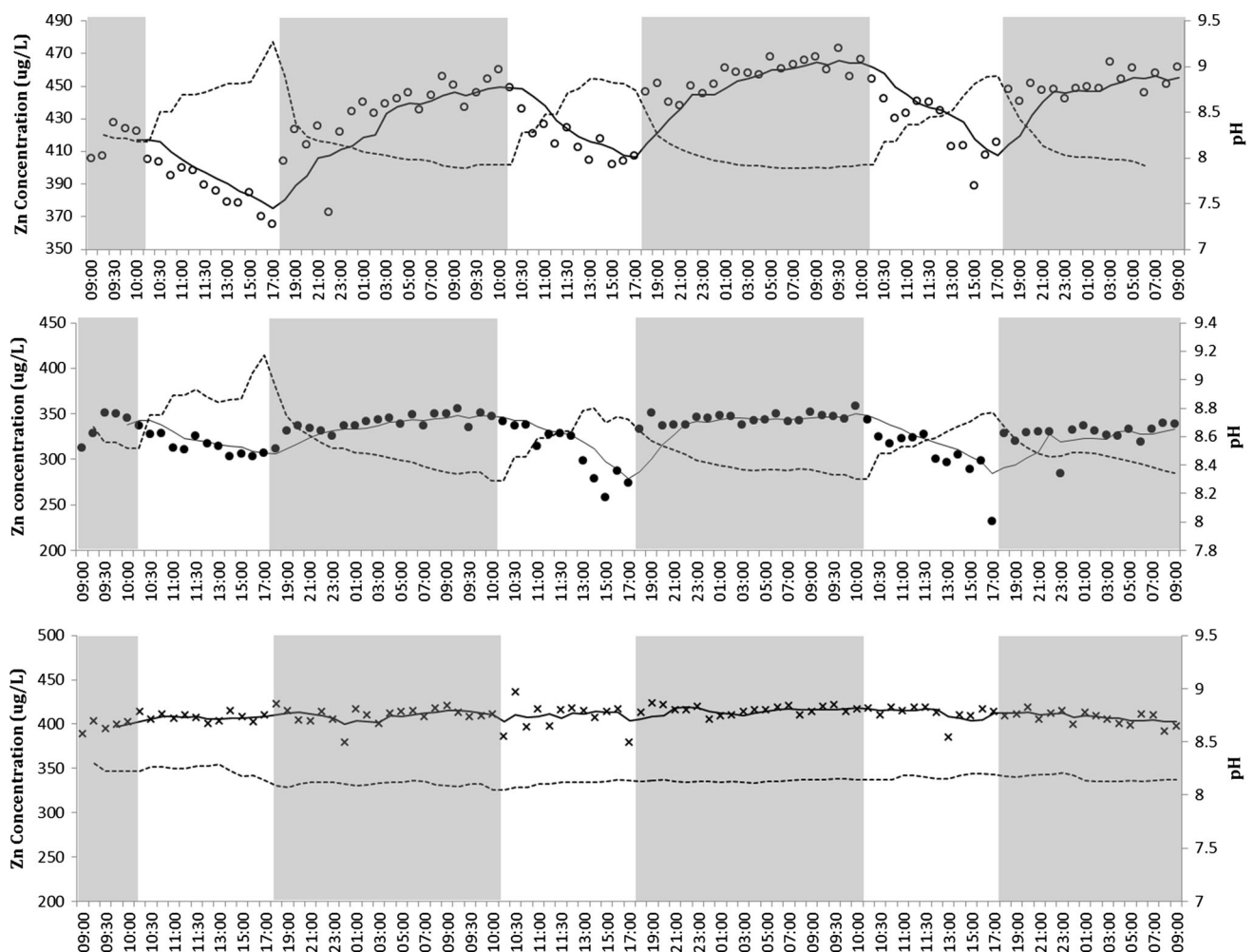
The systematic decline can best be illustrated by calculation of area-adjusted removal rates. The area-adjusted removal rates rely on a calculation between residence time (the time taken for a volume of water to flow through the flume system) and flow (L/s), which accounts for the metal load removed per unit surface area of biofilm per day ($\text{g m}^{-2} \text{day}^{-1}$). The removal rates quoted were achieved with a 9.6 min mean residence time. The initial high removal rates in the active flumes (which equate to 1.02 and $0.67 \text{ g m}^{-2} \text{day}^{-1}$ of Zn) were likely supplemented by sorption to the surfaces of the flume and the carbonic pads used to allow the biofilm community to colonise, as evidenced by the pattern in the control flume (sterilised of biofilm; Fig. 3), which also shows modest initial Zn removal (maximum of $0.25 \text{ g m}^{-2} \text{day}^{-1}$ of Zn). Removal rates stabilise after about 3 weeks. Once the control flume reached a steady state, both biofilm colonised flumes (numbers 1 and 2) showed consistent Zn removal ($0.1\text{--}0.2 \text{ g m}^{-2} \text{day}^{-1}$), which continued until the end of the trial.

Diel Cycling

The flumes with biofilm colonisation had daily fluctuations in both pH and Zn concentrations, as opposed to the sterilised flume where no discernible cycle was established (Fig. 4). The median daytime pH obtained from sensors within the flume was 8.75 in flume A (range 7.93–9.31, $n = 43$); the median night time pH was 7.99 (range 7.88–8.48, $n = 101$). Flume B showed a similar trend, yet had less pronounced changes in pH from day to night [daytime: median = 8.71 (range 8.29–9.23, $n = 43$); night time: median = 8.45 (range 8.33–8.78, $n = 101$)]. Flume C (control flume) exhibited no diel cycle in pH (daytime: median = 8.16 (range 8.05–8.29, $n = 43$); night time: median = 8.13 (range 8.07–8.30, $n = 101$). It took very

Table 2 Mass loss of ions in the system (mg) across the biofilm colonised flumes (1 and 2) and control flume (3)

	Ca			Mg			Ba			Zn		
	1	2	3	1	2	3	1	2	3	1	2	3
Starting mass	1,179	1,173	1,173	75.6	74.1	75.6	10.5	10.5	10.2	39.6	39.3	39.6
End mass	1,062	1,047	1,170	74.4	72.3	78.3	8.1	7.8	8.7	8.1	8.4	31.8
Mass removed	117	126	3	1.2	1.8	−2.7	2.4	2.1	1.5	31.5	30.6	7.5

**Fig. 4** Graphs showing diurnal fluctuations of pH (dashed) and zinc concentrations in biofilm colonised mesocosms (1-open circle, 2-filled circle) and sterilised (3-cross) flumes; shaded areas denote times of darkness

little time after the lights came on for the pH to change, so that there appears to be no lag time in Fig. 4, although the true lag time was around 5 min. This indicates a rapid transition in the photosynthetic activity in the biofilm that is likely far more abrupt in the laboratory than in natural systems, given the gradual transition of the photocycle in natural settings, which was not replicated in the laboratory.

Both biofilm-colonised flumes also exhibited diel cycles in Zn that are comparable to other studies, with Zn

concentrations in flumes A and B fluctuating by up to 77 and 64 %, respectively. The control flume exhibited no diel Zn cycles, confirming that it was the biofilm that was affecting aqueous Zn concentrations.

To assess how the diel cycle affected Zn concentrations, the potential precipitation of key mineral phases were modelled for the flume environments over the same time-scales. Initial modelling suggested that no Zn minerals were close to saturation in the flume environment, with

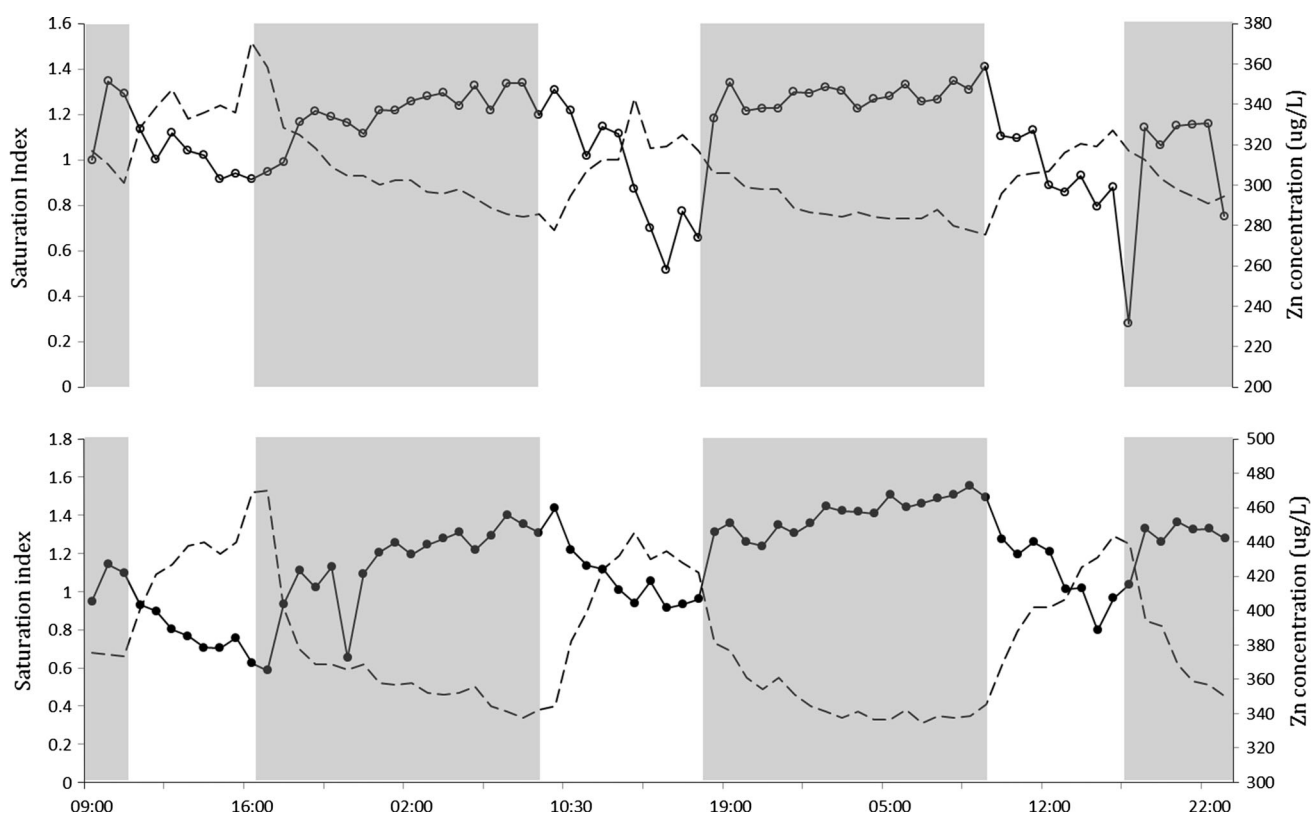


Fig. 5 Graphs illustrating the fluctuation of saturation state (calcite) (*dashed*) and Zn concentrations across the two biofilm colonised flumes (1-*open circle*, 2-*filled circle*); shaded areas denote dark hours

$SI_{ZnO} = -0.18$ at its peak, and $SI_{ZnCO_3} = -0.71$. SI_{CaCO_3} however did exhibit periods of higher (max = +1.52, min = +0.67) and lower saturation (Fig. 5).

Scanning Electron Microscopy

SEM analysis performed on the flume biofilm confirmed the presence of precipitated calcite (Fig. 6). The calcite particles were generally small and were sparsely distributed throughout the biofilm. The calcite is an additional potential sink for divalent metals within the biofilm, as it provides additional surface area that metal ions could sorb on to, although the small timescales over which environmental factors (e.g. pH) vary might mean that the ions could also quickly be released. Longer, more stable removal of the metal ions would occur if it precipitated as its own mineral, or co-precipitated as an impurity within the calcite.

Discussion

Flume Development—Experience for Cultivating and Sustaining Biofilm Communities

The potential application of engineered biofilm treatment systems will ultimately demand a systematic approach to

cultivating and maintaining biofilms. During the development of the laboratory flume system, there were a number of practical obstacles that had to be overcome. These offer useful insight for future field testing of biofilm systems. For the biofilm to colonise, a suitable substrate should be made available. When initially developing this system, carbonic pads were attached to roof slates and deployed at the mine-affected Hebden Beck stream in North Yorkshire (Jones et al. 2013) and left in-stream to allow the colonisation biofilm over a 3 month period, which is the optimal length of time to allow development of a biofilm to full maturity (Rogerson et al. 2010). However, high flow events during summer 2012 led to the scouring (and thus removal) of biofilm communities from the colonized roof slates. Biofilm communities did colonise the stream within a month of the high flow events, leading to biofilm simply being collected and transported back to the laboratory to try and manually colonise the film. Carbonic fibre pads were therefore attached to the gutter in the flume, as the high surface area of the porous pads allows biofilm to attach quickly. This permitted rapid colonisation of biofilm within a few weeks. The biofilm colonisation and subsequent increase in terms of mass within the flume mesocosm environment throughout the study period is

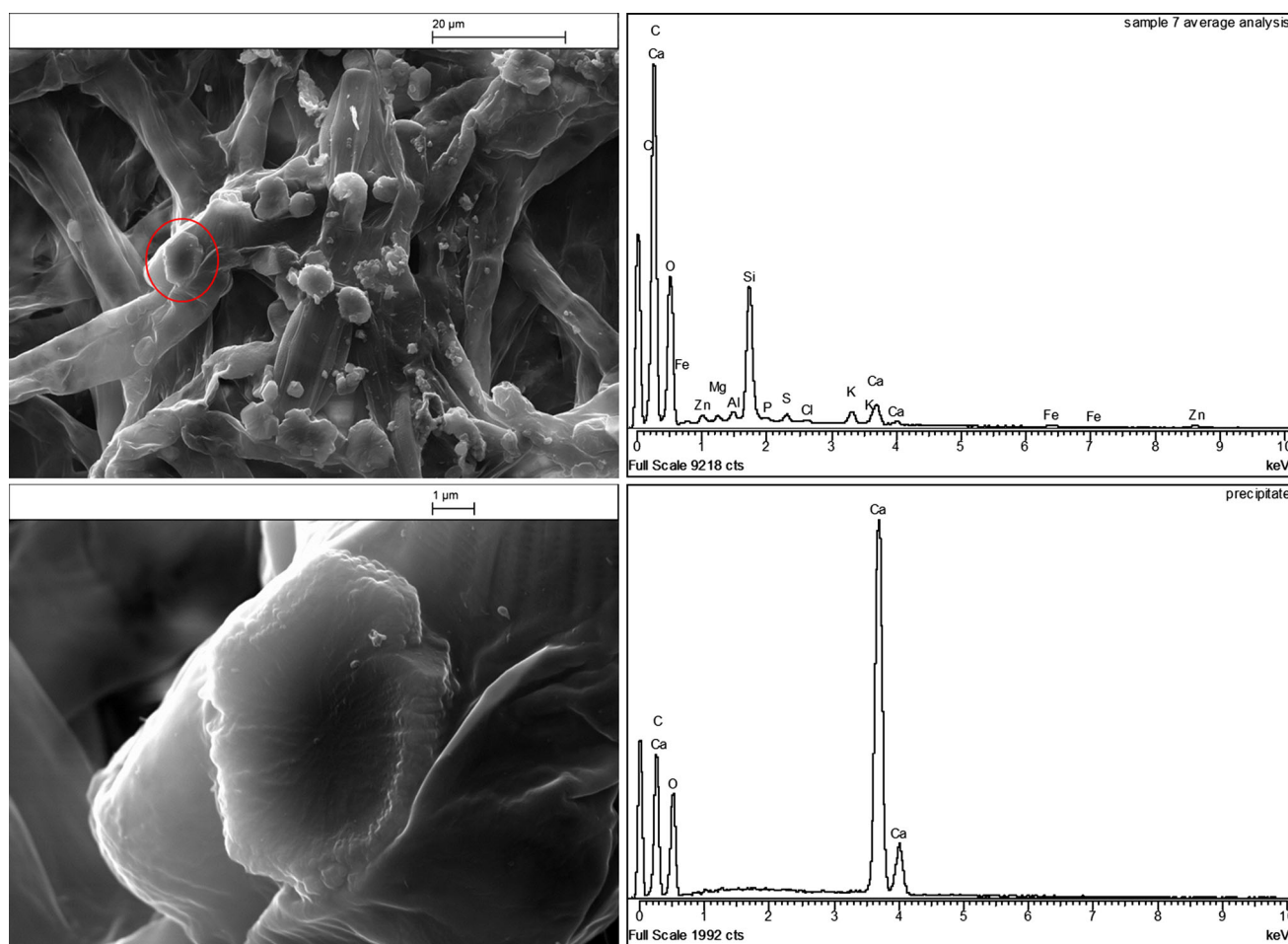


Fig. 6 SEM of calcite precipitates within the biofilm and corresponding EDAX spectra. Biofilm taken from the flume shows the small precipitates observed throughout the biofilm (*top*). Magnification and EDAX confirmation of calcite crystal (*bottom*)

described in Fig. 7. Water flow should also be controlled, as any changes in water flow rate can affect the functional activity of the biofilm (Pedley and Rogerson 2010).

The nutrient requirements of the biofilm are also an important factor in their potential use as a passive remediation option, as high nutrient requirements could require additional maintenance. At the Hebden Beck site where the biofilms were collected, ambient nutrient concentrations were low in both the stream and mine water [NO_3^- 3.2 mg/L; soluble reactive $p < 0.01$ mg/L; K: 3.5 mg/L (Environment Agency 2013)], yet the stream was dominated by biofilm in summer months (see supplemental Fig. 1), which is characteristic of many of these upland streams (Armitage et al. 2007; Gozzard et al. 2011). Biofilms are able to concentrate nutrients from the bulk water column (Jones et al. 2013), so the biofilm could have been using previously sequestered minerals from the freshwater environment. In conjunction with this, certain biotic elements of the biofilm are able to recycle nutrients from the EPS.

Batch Experiments

Our results suggest that Zn was preferentially removed by the biofilm over Ba, a conclusion that contradicts current literature. Other studies have shown how divalent metal uptake into biofilms is chemoselective via chelation of divalent metals by extracellular polymeric substances (EPS); a process that usually favours ions with a low charge density (z_i^2/r_i ratios; Rogerson et al. 2008). Ions with a low charge density, such as Ba, have been identified as being readily chelated (Rogerson et al. 2008), which would suggest ions with a high charge density, such as Zn, would not be preferentially chelated over Ba.

The replenishment of EPS requires a light source in order to keep the phototrophic component of the biofilm alive and maintain its structural integrity over long periods of time. By the end of the experiment, the NL biofilm was completely degraded and dispersed, with no structure, and completely mixed with the water. However, the FL, HL, and LL biofilms were still functioning, with normal

Fig. 7 Colonisation of biofilm over time: **a** biofilm in the initial stages of colonisation, biofilm coats rocks and carbonic fibre (*centre*) and signs of new growth can be seen with small amounts of biofilm (*top*). **b** Due to the biofilm colonisation on the carbonic fibre (*centre*), the biofilm now begins to attach to the polycarbonate guttering (*top*), illustrating the ability of biofilm to attach and develop on numerous strata. Note that this photo was taken from another angle, hence the different flow direction (*blue arrow*). **c** Further biofilm development sees the biofilm “thicken” and grow into the bulk water column. **d** Biofilm at full maturity in this system. Also, the addition of the pH probe (*blue*) and conductivity probe (*black*) are shown



structural integrity. The removal of Zn from the NL flask suggests it is possible that metal removal may have occurred before biofilm degradation in the NL flask. This removal of Zn in the absence of growth is consistent with ion chelation within the EPS matrix, as opposed to direct uptake within or as a consequence of photosynthesising organisms (e.g. Rogerson et al. 2008).

Flume Experiments

Long-Term Trends

These patterns reveal that a mechanism in addition to sorption on the surfaces within the flume (i.e. rock surface,

gutter, carbonic fibre) is continuing to remove Zn from the water column. This is most likely ascribable to extra sorption sites made available by the presence of biofilm in the active flumes. Production of new EPS (and thus new sorption sites) in active flumes may be responsible for maintaining removal over the longer term. Encouragingly, there is no significant correlation between volumetric removal rate and influent concentration across the two active flumes ($r = 0.16$; $p = 0.32$; d.f. = 40). This consistent performance over a range of influent concentrations typical of circumneutral metal mine discharges (Mayes et al. 2010) is promising for potential deployment in polishing operations at a range of sites. Comparisons of our preliminary data with other mine water treatment schemes reveal

similar metal removal rates in terms of area-adjusted removal rates. Pilot and field studies using algal mats achieved a similar order of magnitude of Zn removal, with removal rates of 0.47 and 0.25 g m⁻² day⁻¹, respectively (Kalin 1998). The removal rates documented here are also well above the range of those quoted for wetland systems (e.g. Gillespie et al. 1999; Song et al. 2001).

Diel Trends

Superimposed onto the longer term trend of Zn uptake are transient fluctuations associated with the simulated daily photocycle. The pH fluctuations are a key indicator of biological productivity in the flume. Photosynthesis during light hours produces O₂ and removes CO₂ (increasing pH); the opposite occurs in the dark when respiration occurs (decreasing pH). The fluctuations in biofilm mesocosm water column pH are consistent with in situ studies (Backstrom et al. 2002; Morris et al. 2005; Nimick et al. 2005) and with the observed geochemical micro-environment in other calcifying biofilm mesocosms (e.g. Bissett et al. 2008), where daytime pH was far higher than in the bulk water column, which is a feature of photosynthetic carbon utilisation. However, it must be stressed that these previous studies observed whole systems under ambient conditions, as opposed to the controlled conditions of our experiments. In this study, the fluctuation can be assumed to be solely due to the biofilm, given the utilisation of a control flume, which exhibited no pH cycle over the diel timescale, ruling out any other potentially contributing factors. The flume study pH data clearly illustrates the extent to which biofilms influence the aquatic environment they inhabit. This influence is not limited to biogeochemical factors such as pH. Note that the pH cycle and Zn cycle are mirrored in Fig. 4; when pH reaches its highest value, Zn reaches its lowest, which suggests that the pH is exerting some form of control over Zn concentrations. To better understand the cycling of Zn in the system, the processes of chelation/sorption and precipitation (both precipitation of Zn minerals and co-precipitation with calcite), both of which are underpinned by pH, have to be considered.

Potential Removal Mechanisms

Mineral Precipitation

Observations of changes in water column pH highlight the influence that biofilms have on the surrounding water column, which can also affect the microenvironment of the biofilm, potentially shifting saturation of key mineral phases towards solid phase precipitation, given that the right conditions are met in terms of the saturation index

(SI). Given the high bicarbonate alkalinity of the flumes and the availability of Ca²⁺ in the flume systems, calcite was likely to be a key mineral phase, as well as potentially ZnO and ZnCO₃. Precipitation and dissolution of solid phase minerals have been identified in numerous studies as a contributor to trace metal cycles over a diel timescale (Brick and Moore 1996; Nimick et al. 2003; Scott et al. 2002). It should be noted that different minerals have different thresholds at which precipitation occurs. Calcite, for example, typically begins to precipitate in a heterogeneous manner at an SI of +0.3, and in a homogeneous manner at an SI of +1.5 (Ford and Williams 2007). Heterogeneous precipitation was observed in the active flumes, with modelled precipitation of calcite in the water column consistently in the region of +0.3 to +0.4 in its lowest range (Fig. 5). Conditions for widespread, homogeneous precipitation of calcite were not routinely met in the flumes (Fig. 5). Precipitation of minerals in the water column is low in most in-stream locations with modest concentrations of ions and at circumneutral pH (Jones et al. 2013), but the ability of biofilms to concentrate ions within the biofilm matrix can allow the saturation of specific ion minerals (e.g. calcite and the aforementioned Zn minerals) within a biofilm. The combined effect of ion bioaccumulation and the biofilm's internal pH, independent from water column influences, leads to regions of the biofilm becoming supersaturated in calcite, which could play a key role in direct Zn uptake, through co-precipitation onto calcite (e.g. Zachara et al. 1991). The SEM evidence of mineral precipitates within the biofilm also supports the role of biofilm in the precipitation of mineral phases.

However, the lack of a cycle in Ca concentrations in the water column suggests that the biofilm precipitates are not the product of a transient sink, as also evidenced by the large amount of Ca removed from the biofilm systems as a whole (Table 2). This long-term removal mechanism could also be removing Zn, through co-precipitation, as demonstrated in laboratory settings (Elzinga and Reeder 2002), although the small particle size of the calcite precipitates, and thus the small amount of Zn removed by them would need more sophisticated techniques to confirm this mechanism. This leaves open the possibility that co-precipitation is acting as a long term sink, and suggests other controls (e.g. pH dependent sorption and chelation mechanisms) affecting Zn concentrations within the biofilm on the shorter, diel cycles. Given that no Zn minerals appear to be precipitating in the water column, or within the biofilm film matrix (from SEM evidence), it appears likely that the precipitation and dissolution kinetics of solid phase minerals are not exerting a major transient control on the mobility and thus bioavailability of Zn, other than in the sorption/release to the mineral phase surface.

Chelation/Sorption

The Zn concentrations appear to be fluctuating in diel cycles that mirror the pH cycles (Fig. 4), which suggests that a pH dependent transient mechanism is responsible for the diel dynamics of Zn. Chelation is a mode of sequestration, which allows a ligand to remove a metal ion from solution through coordination with “ligation sites”. Strength of bonding largely depends on the affinity of the metal to the ligand, and the number of ligation sites (Moore et al. 2013). The biofilm EPS contains carboxylic, phosphoryl, hydroxyl, sulphate, sulphonate, and sulphhydryl functional groups (van Hullebusch et al. 2003), and it is these negatively charged groups that are capable of chelating ions, often as bidentate complexes with Ca^{2+} and Mg^{2+} (Saunders 2012). Sorption to surfaces within the biofilm also contributes to metal sequestration, which includes sorption to cell walls and minerals within the biofilm (Tercier-Waeber et al. 2009). Fluctuations of pH have been shown to allow the release of trace metals from chelation in biofilms in freshwater settings (Warren and Haack 2001), and sorption to surfaces is well known to be pH-dependent. A pH-dependent chelation or adsorption mechanism would help explain the transient uptake and release of Zn, and the fact that the diel fluctuations in Zn concentrations mirror the fluctuations in pH (Fig. 4).

Management Implications

Previous attempts to remove Zn from mine discharges have had mixed results. Adaptation of anoxic limestone drains to encourage smithsonite (ZnCO_3) precipitation was investigated at field scale and although it showed initial promise, the removal rates were low (10–30 %) due to the difficulty of maintaining narrow geochemical conditions in the field (Nuttall and Younger 2000). Sulphate-reducing bioreactors have typically been focussed on highly acidic mine waters (where alkaline media and action of sulphate reducing bacteria help buffer the pH), but there have been successful applications of bioreactors for circumneutral waters. For example, Gandy and Jarvis (2012) report average area-adjusted Zn removal rates of $0.4 \text{ g m}^{-2} \text{ day}^{-1}$ of Zn in pilot-scale field systems in northern England. However, maintaining efficient flow distribution through dense reactors and concerns over the waste status of spent organic and metal-rich substrate remain issues to be overcome. Indeed, the most successful bioreactor applications for metals removal typically demand dosing with a refined carbon source to maintain suitable geochemical conditions for sulphate reduction (e.g. Tsukamoto and Miller 1999), which means a truly passive approach is sacrificed. Sorbents (e.g. zeolites: Oren and Kaya 2006) or reactive media are another option. Generally sorbents are capable of high

metal removal rates (up to 99 % Zn removal), which initially sounds promising. However, this level of uptake can only be maintained for small volumes of water, as the functional sites for metals to adsorb to become saturated, and then removal efficiency drops (Warrender et al. 2011). Furthermore, while modest volumetric removal rates have been maintained for reasonable timescales in field sorbent-based systems, these effects have been ascribed to the colonisation of biofilm communities on the surface of the sorbent media (e.g. Mayes et al. 2009b). The advantage of a biofilm-based approach is that it is a community of living organisms, so the EPS that they create is constantly being replenished. This effectively means that sites for metal uptake are constantly being produced, resulting in higher efficiencies for longer time periods.

Conclusions and Further Work

Biofilms are ubiquitous in circumneutral pH, metal-rich waters and play a crucial role in the diel and seasonal cycling of trace elements (e.g. Nimick et al. 2011). Our laboratory studies highlight how natural biofilm communities can be readily cultured and maintained under controlled conditions. Under these conditions, Zn removal was consistent and long-lived; the production of EPS appears to replenish sites for metal uptake within the biofilm. The Zn removal rates are consistent with many other systems, but it is the low residence time and longevity of the treatment without maintenance that are most promising for developing biofilms into a treatment option. Future work is concentrating on elucidating these uptake mechanisms in greater detail through a range of solid phase assessments, assessing the permanence of Zn removal, and evaluating the scope for engineering systems at full scale. The latter will also include laboratory evaluation of synthetic EPS for Zn removal. As such, there are significant engineering barriers to overcome to demonstrate the technology at full scale. These centre on: (1) biofilm response to seasonal changes in temperature, (2) determining maintenance frequencies for field systems (e.g. clearance to maintain hydraulic efficiency), and (3) assessing the resilience of biofilm to scouring under high flow events. Some of these uncertainties could be minimised via strategic implementation of units at point sources where thermal regime of mine waters are generally consistent and flow rates are unlikely to change as dramatically as within main river channels.

The results obtained in this study suggest that the Zn uptake is only indirectly controlled by photosynthetic processes and it is the EPS production that maintains effective removal through chelation of Me^{2+} . Thus, there may be considerable scope for enhancing metal removal

using synthetic EPS. Determining specific metal uptake pathways in EPS, the structure of the biofilm communities, and optimal operating conditions in field settings will be crucial to underpin field development of effective biofilm-based treatment systems in the future. Harnessing these low-energy biological processes in remote locations, where modest Zn concentrations adversely impact headwater streams of otherwise high amenity value, could provide a new approach in mine water treatment.

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References

- Alabaster JS, Lloyd R (1980) Water quality criteria for freshwater fish. Butterworths, London
- Armitage PD, Bowes MJ, Vincent HM (2007) Long-term changes in macroinvertebrate communities of a heavy metal polluted stream: the river Nent (Cumbria, UK) after 28 years. *River Res Appl* 23:997–1015
- Backstrom M, Borjesson E, Karlsson S (2002) Diurnal variations of abiotic parameters in a stream, recipient for drainage water in Ranstad, southwest Sweden. *J Environ Monit* 4:772–777
- Bissett A, Reimer A, De Beer D, Shirasishi F, Arp G (2008) Metabolic microenvironmental control by photosynthetic biofilms under changing macroenvironmental temperature and pH conditions. *Appl Environ Microbiol* 74:6306–6312
- Brick CM, Moore JN (1996) Diel Variation of Trace Metals in the Upper Clark Fork River, Montana. *Environ Sci Technol* 30:1953–1960
- Decho AW (2010) Overview of biopolymer-induced mineralization: What goes on in biofilms? *Ecol Eng* 36(2):137–144
- Elzinga EJ, Reeder RJ (2002) X-ray absorption spectroscopy study of Cu^{2+} and Zn^{2+} adsorption complexes at the calcite surface: Implications for site-specific metal incorporation preferences during calcite crystal growth. *Geochim Cosmochim Acta* 66:3943–3954
- Environment Agency (2013) Interactive Maps. Accessed 15 Oct 2013 http://maps.environment-agency.gov.uk/wiyby/wiybyController?ep=maptopics&lang=_e
- Flemming HC, Wingender J (2010) The biofilm matrix. *Nat Rev Microbiol* 8:623–633
- Ford DC, Williams PW (2007) Karst geomorphology and hydrology. Unwin Hyman, London
- Gandy CJ, Jarvis AP (2012) The influence of engineering scale and environmental conditions on the performance of compost bioreactors for the remediation of zinc in mine water discharges. *Mine Water Environ* 31:82–91
- Gillespie WB, Hawkins WB, Rodgers JH, Cano ML, Dorn PB (1999) Transfers and transformations of zinc in flow-through wetland microcosms. *Ecotox Environ Safe* 43:126–132
- Gozzard E, Mayes WM, Potter HAB, Jarvis AP (2011) Seasonal and spatial variation of diffuse (non point) source zinc pollution in a historically metal mined river catchment, UK. *Environ Pollut* 159:3113–3122
- Jarvis AP, Mayes WM (2012) Prioritisation of abandoned non coal mine impacts on the environment. Environment Agency Report SC030136/R14, Bristol, UK
- Jones A, Rogerson M, Greenway G, Mayes WM (2013) Mine water geochemistry and metal flux in a major historic Pb-Zn-F orefield, the Yorkshire Pennines, UK. *Environ Sci Pollut Res* 20(11):7570–7581
- Kalin M (1998) Biological polishing of zinc in a mine waste management area. In: Geller W, Klepper H, Salomons W (eds) *Acidic mining lakes: acid mine drainage. Limnology and Reclamation*, Springer, pp 321–334
- Mayes WM, Johnston D, Potter HAB, Jarvis AP (2009a) A national strategy for identification, prioritisation and management of pollution from abandoned non-coal mine sites in England and Wales. I. Methodology development and initial results. *Sci Total Environ* 407:5435–5447
- Mayes WM, Potter HAB, Jarvis AP (2009b) Novel approach to zinc removal from circumneutral mine waters using pelletised recovered hydrous ferric oxide. *J Hazard Mater* 161:512–520
- Mayes WM, Potter HAB, Jarvis AP (2010) Inventory of aquatic contaminant flux arising from historic metal mining in England and Wales. *Sci Total Environ* 408:3576–3583
- Mayes WM, Davis J, Silva V, Jarvis AP (2011) Treatment of zinc-rich acid mine water in low residence time bioreactors incorporating waste shells and methanol dosing. *J Hazard Mater* 193:279–287
- McGeer JC, Szebedinszky C, McDonald DG, Wood CM (2000) Effects of chronic sublethal exposure to waterbourne Cu, Cd or Zn in rainbow trout. 1: iono-regulatory disturbance and metabolic costs. *Aquat Toxicol* 50:231–243
- Moore LR, Durand JR, Strickland F (2013) Copper removal from mine effluents: from lab to field evaluations. *Mine Water Environ* 32:239–246
- Morris JM, Nimick DA, Farag AM, Meyer JS (2005) Does biofilm contribute to diel cycling of Zn in high ore creek, Montana? *Biogeochemistry* 76:233–259
- Nimick DA, Cleasby TE, McCleskey RB (2005) Seasonality of diel cycles of dissolved trace-metal concentrations in a Rocky Mountain stream. *Environ Geol* 47:603–614
- Nimick DA, Gammons CH, Cleasby TE, Madison JP, Skaar D, Brick CM (2003) Diel cycles in dissolved metal concentrations in streams: Occurrence and possible causes. *Water Resour Res* 39:1247
- Nimick D, Gammons CH, Parker SR (2011) Diel biogeochemical processes and their effect on the aqueous chemistry of streams: a review. *Chem Geol* 283:3–17
- Nuttall CA, Younger PL (2000) Zinc removal from hard, circum-neutral mine waters using a novel closed bed limestone reactor. *Water Res* 34:1262–1268
- Oren AH, Kaya A (2006) Factors affecting adsorption characteristics of Zn^{2+} on two natural zeolites. *J Hazard Mater B* 131:59–65
- Pedley HM, Rogerson M (2010) In vitro investigations of the impact of different temperature and flow velocity conditions on tufa microfabric. *Geol Soc Spec Publ* 336:193–210
- Pedley M, Rogerson M, Middleton R (2009) Freshwater calcite precipitates from in vitro mesocosm flume experiments: a case for biomediation of tufas. *Sedimentology* 56:511–527
- Rogerson M, Pedley M, Wadhawan JD, Middleton R (2008) New insights into biological influence on the geochemistry of freshwater carbonate deposits. *Geochim Cosmochim Acta* 72:4976–4987
- Rogerson M, Pedley HM, Middleton R (2010) Microbial influence on macroenvironment chemical conditions in alkaline (tufa) streams: perspectives from in vitro experiments. *Geol Soc Spec Publ* 336:65–81
- Saunders P (2012) Can Tufa Mg/Ca Ratios be Used as a Palaeoclimate Proxy? PhD Thesis, Univ of Hull, UK
- Scott DT, McKnight DM, Voekler BM, Hrcir DC (2002) Redox processes controlling manganese fate and transport in a mountain stream. *Environ Sci Technol* 36:453–459

- Song Y, Fitch M, Burken J, Nass L, Chilukiri S, Gale N, Ross C (2001) Lead and zinc removal by laboratory-scale constructed wetlands. *Water Environ Res* 73:37–44
- Sutherland I (2001) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* 147:3–9
- Taylor LN, McGeer JC, Wood CM, McDonald DG (2009) Physiological effects of chronic copper exposure to rainbow trout (*Oncorhynchus mykiss*) in hard and soft water: evaluation of chronic indicators. *Environ Toxicol* 19:2298–2308
- Tercier-Waeber M-L, Hezard T, Masson M, Schäfer J (2009) In situ monitoring of the diurnal cycling of dynamic metal species in a stream under contrasting photobenthic biofilm activity and hydrological conditions. *Environ Sci Technol* 43:7237–7244
- Tsukamoto TK, Miller GC (1999) Methanol as a carbon source for microbiological treatment of acid mine drainage. *Water Res* 33:1365–1370
- van Hullebusch ED, Zandvoort MH, Lens PNL (2003) Metal immobilisation by biofilms: mechanisms and analytical tools. *Rev Environ Sci Biotechnol* 2:9–33
- Warren LA, Haack EA (2001) Biogeochemical controls on metal behaviour in freshwater environments. *Earth Sci Rev* 54:261–320
- Warrender R, Pearce NJG, Perkins WT, Florence KM, Brown AR, Sapsford DJ, Bowell RJ, Dey M (2011) Field trials of low-cost reactive media for the passive treatment of circumneutral metal mine drainage in Mid-Wales, UK. *Mine Water Environ* 30:82–89
- Whitfield AK, Elliott M (2002) Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. *J Fish Biol* 61:229–250
- Zachara JM, Cowan CE, Resch CT (1991) Sorption of divalent metals on calcite. *Geochim Cosmochim Acta* 55:1549–1562