THE UNIVERSITY OF HULL

Trace Metal Dynamics in Mine-Impacted, Circum-neutral Streams

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By

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List of Abbreviations

AMD – Acid Mine Drainage

DTM – Digital Terrain Model

EDAX - Energy Dispersive X-ray analysis

EPS – Extracellular Polymeric Substances

EU – European Union

GPS – Geographical Positioning Satellite

ICP – OES Inductively Coupled Plasma Optical Emission Spectroscopy

ICP – MS Inductively Coupled Plasma Mass Spectrometry

LOD – Limit of Detection

MWD – Mine Waste Discharge

PCA – Principal Component Analysis

SEM – Scanning Electron Microscopy

SI – Saturation Index

UHQ – Ultra High Quality

WFD – Water Framework Directive

XRF – X-ray Fluorescence

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1.0 Introduction

Abandoned non coal (metal) mining sites can represent a major source of metal pollutants to watercourses, which can have negative effects on ecosystems (Lottermoser *et al*, 1998). The extent of the problem in the UK has been recently addressed by Mayes *et al.* (2009), who estimate 6% of surface water bodies appear to be affected nationally. Pollution from such sources has undergone a marked increase in attention by researchers and policy makers over the past decade, with the introduction of the Water Framework Directive (2000/60/EC) (European Commission, 2000), which states as its primary objective that all rivers and streams in the EU should be of “good ecological and chemical status” by 2015. While other sources of aquatic pollution have diminished in recent decades through deindustrialisation and improvements in wastewater treatment (Luoma and Rainbow, 2008), relic sources of pollution from abandoned mines are thus becoming a more prominent constraint on achieving water quality objectives throughout large parts of the UK. As such, they represent a key focus of regulatory authority monitoring and remedial efforts. In recent years national initiatives in the UK to address these legacy sources have made great strides in: (1) collating national datasets and prioritising catchments for possible remediation (e.g. Jarvis and Mayes, 2012), (2) in devising methods for catchment-scale assessment of diffuse and point sources (e.g. Gozzard *et al.,* 2011) and (3) in developing passive mine water treatment systems which are now seeing full scale deployment (e.g. Gandy and Jarvis 2012). However, research efforts are still required to improve the science-base for informing decision making. Specific gaps in both our understanding of fundamental biogeochemical processes and in environmental engineering approaches to address these legacy pollutants concern:

(a) Lack of scale-appropriate monitoring data to understand the relative spatial and temporal importance and impacts of key mine water sources,

(b) Gaps in our understanding of the nature and controls on temporal fluctuations in water quality with diurnal, seasonal or hydrological change, and

(c) a shortage of proven, low cost passive treatment systems that can be deployed for the full geochemical range of mine discharges that exist to effectively remove mobile contaminants (for example zinc and cadmium).

While ongoing data collection efforts will begin to provide a more comprehensive coverage of the extent and impacts of mining-derived contaminants on freshwater systems in time, it is in to the characterisation of the complex transient processes that control solute dynamics in mine-impacted streams where major knowledge gaps remain. These include pronounced cycling of biogeochemical parameters (Spiro and Pentecost, 1991; Bäckström *et al*., 2002) and cycling of trace elements (Brick and Moore, 1996, Nimick, 2005) which has been widely observed in freshwaters in recent decades. While there has been much debate on the controls on such cycling, a theory which is becoming more accepted is that biofilms (laminated microbial mats) are responsible for the uptake and release of trace metals in changing environmental conditions (Nimick *et al*., 2011). However, the exact uptake/release mechanisms are not known for certain, which is an obvious constraint on understanding both catchment-scale processes and the importance of biofilms in natural attenuation of mining-derived contaminants. Such cycling has been shown to lead to baseflow changes in in-stream metal concentrations by up to 500% over daily cycles (Kimball *et al*., 2009), usually in highly acidic mine-impacted streams. Diurnal cycling of trace metals is largely understudied in areas with carbonate-rich bedrock, which are prevalent throughout the UK, in particularly the former mining regions of the Pennines, north Wales, Shropshire and the Mendips. As such, there are large areas of mine-impacted streams where current ambient monitoring fails to effectively characterise the in-stream solute dynamics and key processes governing metal fate. This is a severe impediment to understanding the relative importance of key pollutant sources, downstream impacts of mines and where remedial efforts should be targeted. Furthermore an understanding of the mechanisms crucial in controlling the mobility of key contaminants (e.g. Zn, Cd) in mine-impacted streams is essential for informing both monitoring and potential future remedial technologies.

The overall aim of this thesis is to improve our understanding of the controls on solute dynamics in carbonate-rich mine-impacted streams. This will be achieved by addressing the following objectives;

- *Assess the extent of polluting mine waters across the Yorkshire Dales region:* a reconnaissance survey of mine discharges in the Yorkshire Dales will be undertaken. Geochemical facies will be identified and key sources of trace metal flux to surface waters will be determined. This region is one that is under-represented in recent national surveys (Mayes *et al.*, 2009) and represents a major carbonate-rich region with limited mine solute data. Flux-referenced measurements will be used to put these discharges in national and international context.
- *Catchment –scale surveys: source apportionment and hydro-geochemistry*– The Hebden Beck, a heavily polluted carbonate system in the Pennines will be assessed in detail. The reconnaissance work will give better insight into the geochemical processes present in a carbonate-rich environment. A synoptic sampling approach (e.g. Kimball *et al.* 2009) will be carried out to assess the ambient loadings of metals in the stream, contributions of diffuse and point source pollution, and also to identify the major discharges into Hebden Beck.
- *Identification of cycling trace metals* – Diurnal sampling campaigns will be carried out to identify the metals which cycle, and to what amplitude of change the cycles occur. Attention will also be given to elements which have been hitherto understudied, in this case barium and strontium. This is to establish the

nature of trace metal cycling in carbonate rich systems and to form ideas on what controls on such cycling are in place.

- *Mesocosm testing of Zn uptake rates and geochemical controls* – Laboratory studies will be undertaken to study biogeochemical cycling under carefully controlled conditions and begin to elucidate controls on metal mobility. Mesocosm testing will be focussed on 1) comparing precipitation rates in abiotic and biotic environments and hence uptake of trace metals in these systems 2) identifying mechanisms by which metals are incorporated into calcite 3) identifying ways to manipulate environmental conditions to affect microbiological communities in biofilms to increase trace metal uptake rates.

The aims and objectives are therefore inherently multi-disciplinary and require the careful integration of both laboratory and field experimentation. The flow chart provided (Figure 1.1) aims to provide a brief outline illustrating the key aims for each section, and more importantly, how all the sections link together to achieve the aims of the thesis.

Chapter Two provides the broader context for the study by reviewing key studies on metal mine pollution processes, catchment-scale impacts and remediation while also focussing on our current understanding of transient biogeochemical control on contaminants in mine-affected streams. Chapter Three commences with an overview of generic field and laboratory methods applied in the thesis, before concluding with description and justification of the configuration and operation of the laboratory mesocosms designed to simulate in-stream processes. Chapter Four provides a field sampling campaign observing metal discharges throughout the Yorkshire Pennines, which predominantly discharge into carbonate dominated, circum-neutral pH streams. Also presented in this chapter is a diurnal study observing trace metal cycling in a field setting. Chapter five is the first of two chapters experimenting with biofilm in a Laboratory setting. In chapter five results of batch experimentation with biofilm are presented, where different treatments (light, pH) were applied to biofilm to assess their effects on trace metal removal by biofilms. Novel flume experimentation was also carried out in this chapter, with the focus on the colonisation of biofilm in mesocosms situated in a controlled environment laboratory, and their capability for the long term removal of trace metals from the water column. Chapter 6 continues experimentation in the flume mesocosms, but here focuses on the short term dynamics of trace metals in the presence of biofilm, with a view to establish and understanding of the uptake and release mechanisms controlling the concentrations of trace metals, particularly Zn. Chapter seven concludes the thesis, presenting a summary of the findings, potential directions for future work, and assesses the success of the thesis.

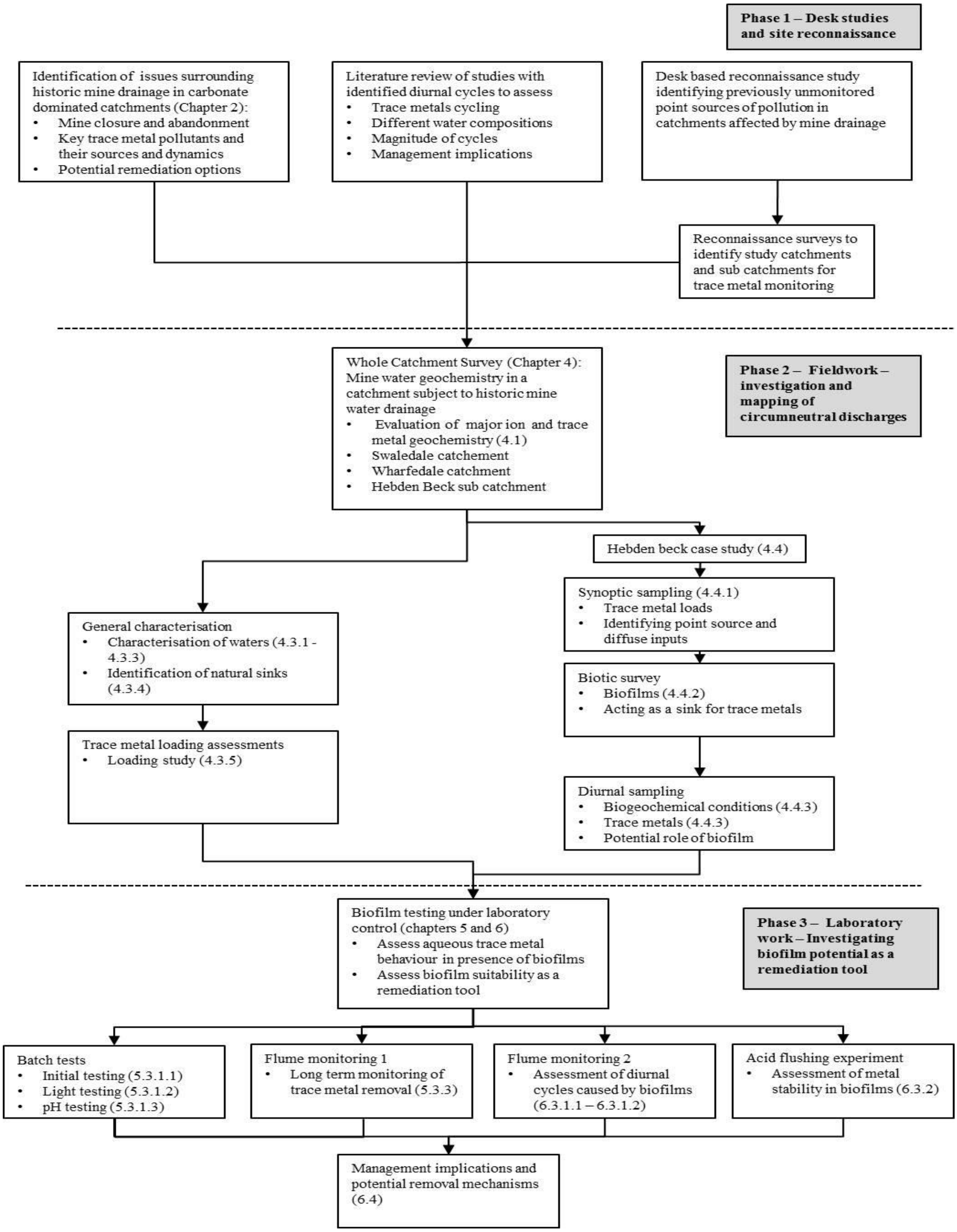

Figure 1.1 Flow chart outlining thesis aims, objectives, and structure. Chapters relevant to section are shown in brackets

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2.0 Circum-neutral pH mine drainage: background, issues and research needs

This chapter provides a review of the available literature, commencing with a general background to the subject of mining pollution. The subsequent focus will then move onto the impacts of metals on the surface water environment and the uptake / release mechanisms that control their mobility with specific reference to orefields with carbonate-rich country rock. While the term Acid Mine Drainage (AMD) or Acid Rock Drainage (ARD) predominate in the international literature, many mine-affected streams are buffered to neutral pH where metal bioavailability and controls on mobility can differ greatly to acidic systems. A critical appraisal of the current understanding of metal attenuation mechanisms in such circum-neutral pH systems therefore concludes the Chapter.

2.1 Mine processes

2.1.1 Mine dewatering, closure and consequent flooding

In order for mines to remain as safe as possible during operation the water in the mine must be excluded through the use of water pumps or under draining. Dry conditions are favoured as safe working conditions can prevail, wear and tear on machinery is reduced, and earth moving costs are reduced; wet working conditions also entail the following problems;

- Decrease in slope stability
- Difficulty in ore handling
- Increased explosives costs
- Floor heave
- Nuisance factor

(Morton and Mekerk, 1993)

A number of techniques exist to exclude water from mines during operation including sump dewatering (groundwater drawn to a low point in the mine and pumped out), and adit dewatering (Younger *et al.*, 2002). In many upland settings in the UK, adit dewatering was the most commonly utilised form of draining productive orefields. Adits are drainage tunnels driven into the area that was being mined, at a slight upwards gradient, to permit water to naturally flow out and drain the area of water, allowing any area above the adit to be dry and minable. The low maintenance costs of adits, and the lack of any mechanical aide required, meant that adits can be used to drain large areas of land. Adits of notable length in the UK are summarised in Table 2.1. Some of these were major civil engineering undertakings during the late 18th and 19th Century which opened up large productive orefields (Dunham, 1985). Such features do lead to largely irreversible changes in local hydrogeology by changing subterranean flow paths and in some cases draining significant portions of large aquifers (e.g. Younger, 2000).

Adit	*Area*	*Length (miles)*	*Water flow (million L/day)*	*Reference(s)*
Dukes Level	Yorkshire Pennines	3.31	0.043	Dunham (1985)
Milwr Tunnel	North Wales (Halkyn Minera)	10	7.730	Ebbs (1993)
Nent Force Level	Cumbria	~5	2.3	Roberts (1981) Nuttall and Younger (2002)
Blackett Level	Northern Pennines	5	4.41	Ward (1962), Gozzard (2008)
Meerbrook Sough	Staffordshire	4+	3.75	BGS (2011)

Table 2.1 Notable UK adits and key features

Modern mines are closed in accordance with a set of procedures that are established by experts, regulatory agencies and governments, which ensures that the mine is shut down in a sustainable manner, leaving the area as environmentally sound as possible (Sheldon *et al.*, 2002). Sheldon *et al.* (2002) outlines the process of mine closure in this manner, discussing economic, environmental and social factors that will affect mines and the community during closure, noting that the mine should not just be shut down and left to its own device. Equipment should be removed alongside any hazardous substances, the used land should be reclaimed and all appropriate measures are taken to ensure there is the lowest amount of possibility that pollution of ground or surface water can occur

(Wolkersdorfer, 2008). Sheldon *et al.* (2002) recommend that an initial mine closure plan is created during the mine licensing phase of development (before mining has begun), noting that this can lead to key economic and remedial decisions being made in such a way that they will lower operational costs of the mine workings overall.

Once the mine has been fully decommissioned, it can become re-flooded as the water table returns to a pre mining condition, or intentionally flooded from a safety view point. This flooding can bring about the beginning of the processes that contribute to the pollution found in mine waters, potentially contravening legislation that prevents degradation of the water environment.

2.1.2 Legislation

Few subject specific restrictions or legal constraints existed to control and regulate the abandonment of mines and future polluted waters emanating from such a mine. The Water Resources Act (HMSO, 1991) gave a broad protection of water bodies in section 85, stating;

“A person contravenes this section if he causes or knowingly permits any poisonous, noxious or polluting matter or any solid waste matter to enter any controlled waters”.

However, section 89(3) goes on to state:

“A person shall not be guilty of an offence under section 85 above by reason only of his permitting water from an abandoned mine or an abandoned part of a mine to enter controlled waters”

This essentially cleared anyone who has allowed mine polluted water to enter a controlled water of any wrong doing, and they cannot be punished under section 85 of the WRA 1991.

From the 31st December 1999 legislation became more stringent in terms of the processes required to shut down or abandon a mine. The Environment Agency requires at least 6 months' notice before the shutting down of a mine under section 58 of the Environment Act 1995 (HMSO, 1998). Failure to give six months' notice can result in prosecution, and if the abandoned mine pollutes any water course the Environment Agency will enforce legal action under section 85 of the WRA 1991 (Environment Agency, 1997).

The overwhelming majority of abandoned mines in the UK were abandoned before 1999. The complex legal situation that has ensued in the area of mining abandonment has meant that there are hundreds of kilometres of water bodies that are affected by mine drainage from abandoned mines, and no person has to accept liability for the pollution, or more importantly, the budget for the remediation of the pollution (Banks, 1997). As such, "the polluter pays" principle that underpins much modern environmental management (Hill, 2010) cannot apply to these long abandoned sites owned by disparate (and now defunct) private interests. The advent of the European Union Water Framework Directive in 2000 has however provided the legislative impetus to develop programmes of measures to improve the chemical and ecological status of water bodies affected by these legacy sources of mine pollution (Mayes *et al*., 2008). This prompted national initiatives to quantify the extent of the pollution problem and prioritise catchments (and mine sites within them) for possible remediation (Mayes *et al*., 2009; Jarvis and Mayes, 2012). Since 2011, legislative changes have seen the

UK Government Coal Authority to expand their remit to incorporate non-ferrous metal mining sites. Also, funding by the Department of Energy and Climate Change (DECC), in which the Coal Authority sit, has also been supplemented by DEFRA, leading to the Coal Authority taking control of efforts to remediate streams and rivers impacted by metal mining. The Coal Authority was originally set-up in 1994 to deal (in part) with the environmental legacy of what was a predominantly nationalised coal mining industry in the UK (Coal Authority, 2014). Since 1994, the Coal Authority have developed a successful environmental remediation programme for a range of polluting coal mine sites across the UK (Coal Authority, 2014).

2.2 The process of trace metal pollution generation from mine drainage

2.2.1 Pollution generation

Disulphide weathering is the first step in the process of producing metal rich leachate, in particular the oxidation of pyrite (and marcasite) by abiotic and bacterial means (Cortecci *et al*, 2008). Equations 1-4 show the process of disulphide weathering using one of the common source minerals, pyrite;

$$FeS_2 + \frac{7}{2}O_2 + H_2O \leftrightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \qquad (2.1)$$

$$Fe^{2+} + \frac{1}{4}O_2 + H^+ \leftrightarrow Fe^{3+} + \frac{1}{2}H_2O \qquad (2.2)$$

$$Fe^{3+} + \ 3H_2O \leftrightarrow Fe(OH) \quad + 3H^+ \qquad (2.3)$$

$$FeS_2 + 14Fe^{3+} + 8H_20 \leftrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad (2.4)$$

The main principle behind the weathering process occurring is the overall net production of H^+ ions, present in the form of sulphuric acidic in the mine water, thus lowering the pH. Generally, natural waters that are in contact with the predominantly pyritic ore or other types of disulphide-rich mine waste, become acidic and become known as acid mine drainage (AMD). Acidity generation enhances weathering rates and crucially increases the solubility of most trace metals (Langmuir, 1997).

Most mines will have in common three factors that are required to keep the process of disulphide weathering; a presence of disulphide ore (pyrite (FeS_2), marcasite (FeS_2), sphalerite (ZnS), galena (PbS), greenockite (CdS)), oxygen, and water. The reaction is exothermic, transferring heat energy to the surrounding water and air, raising the ambient temperature of the air and water. The rise in air temperature causes this to rise, drawing in fresh air which maintains the reaction (Wolkersdorfer, 2008).

Pyrite is stable at the pH and oxygen levels of pre-mining water. However, with the introduced air filling voids in the mine that are not flooded, coupled with the lowered pH from the oxidation of pyrite, the minerals can become unstable, releasing trace metals, as shown in the theoretical formula below, where Me stands for any metal;

$$Me^{n+}S + nO_2 \leftrightarrow Me^{n+} + \frac{2}{n}SO_4^{2-} \qquad (2.5)$$

(Wolkersdorfer, 2008).

The oxidation of sulphide minerals as shown above can also lead to an increase in the dissolved metal available, without the rise in pH. This could be occurring in the case of

ZnS, which would create conditions that constitute high Zn concentrations and high SO_4^{2+} levels, which discharge without being buffered by the bedrock.

Microbial activity is also a key factor in the reaction, as reactions (1) and (2) are catalysed by bacteria. Two species of acidophilic bacteria that a repetitively used in the literature surrounding this topic are *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*, which are regarded as chemolothotrophic species using the inorganic pyrite as an energy source (Bacelar-Nicolau and Johnson, 1999). Both *T. ferrooxidans* and *L. ferrooxidans* are able to convert the pyrite to Fe^{3+}, ensuring a high ratio of Fe^{3+} to Fe^{2+} in order to maintain a high production of H^+ protons (thus more acid) (Luoma and Rainbow, 2008).

Once pH has been lowered due to the weathering of the pyrite, metal dissolution takes place at an accelerated rate. Metalliferous ores often occur in paragenesis with each other, and a selection of commonly mined minerals can be seen in Table 2.2.

Metal	*Mineral Ore Composition*	*Name*	*Total World Production of metal* *(thousand tonnes)*
Barium (Ba)	$BaSO_4$	Barite	n/a
Copper (Cu)	CuS	Covelite	14,676
	$CuFeS_2$	Chalcopyrite	
Lead	PbS	Galena	3,038
	$PbSO_4$	Anglesite	
Zinc	ZnS	Sphalerite	8,922

Table 2.2 Commonly mined metals, their ores, and worldwide production in 2000 (MMSD, 2002)

2.2.2 UK context

Historic mines are prevalent throughout England and Wales (Figure 2.1). Recent reviews have shown areas that have been subject to historic mining are polluting streams and rivers in England and Wales, and have outlined that up to 6% of river catchments are at risk from metalliferous pollution (Mayes *et al.*, 2009). In England and Wales, the most commonly mined metals historically have been lead and iron although other metals have been mined. Table 2.3 shows examples of mined metals, and the areas in which they have been mined.

Mine(s)	*Area*	*Metal(s) mined*	*Ores Present*	*Reference*
Frazer's Grove	North Pennine Orefield	Pb, Zn, Ba, F	PbS, $FeCuS_2$, CaF_2, $FeCO_3$, SiO_2	Younger (2000)
Wheal Jane	Devon/Cornwall	Sn, Cu, Fe, W, As, Ag, Pb	SnO_2, $CuFeS_2$, FeS_2, $(Fe,Mn)WO_4$, FeAsS, PbS	Bowen *et al.*, (1998)
Cwm Rheidol/Cwm Ystwyth	Mid Wales	Pb, Zn, Cu	PbS, ZnS, $CuFeS_2$, FeS, FeS_2	Fuge *et al.*, (1991)
Yarnbury	Pennines	Pb	PbS, $BaSO_4$, $SrCO_3$, CaF_2, ZnS, $Zn_4Si_2O_7(OH)_2 \cdot H_2O$	Dunham, (1985)

Table 2.3 Examples of mines in England and Wales, with mined ores

2.2.3 Mine water: typical constituents and impacts

Both modern and historic metal mining and ore operations are known to yield polluted, metal rich, waters, which are discharged into natural watercourses throughout the UK, and more relevantly to this study, the North Yorkshire Pennine orefield. Waters linked with mine water drainage can usually expect to see increases in the concentrations of Fe, Ca, Mg, Cd, Ba, Al, Mn, Cu, Zn, and Pb and varying amounts of other trace elements (Singh and Rawat, 1985). The various metals present in the water can form different species generally depending on the form the metal takes (e.g. Fe^{2+} or Fe^{3+}), the quantity of available cations present, and the environmental conditions at the time (pH, conductivity, temperature and alkalinity). The various species that the metal takes can have varying effects on the compounds properties, changing its bioavailability, toxicity, solubility, mobility and chemical interaction with other compounds (Dang *et al.*, 2002).

2.2.4 Mine water in the Pennines

Base metal mining has been documented as having affected many Pennine catchments primarily through studies on fluvial sediments. Elevated concentrations of many metals, notably Pb, Zn, and Cd have been identified in numerous studies as being exceptionally high (Macklin *et al.*, 2006). The bedrock of the Pennines lends itself to having distinct water chemistry due to its bedrock geology, mainly comprising of Namurian-Dinatanian limestone bedrock overlain with sandstones (Dunham, 1985). This gives rise to the neutral to alkaline pH waters, and also to elevated levels of trace elements that may not be observed in other areas affected by mining. For example, more mineralised barium ($BaSO_4$ and $BaCO_3$) has been observed in carbonate-rich bedrock associated with Pennine strata (e.g. Johnson and Younger, 2002; Nuttall and Younger, 2002). The prevalence of mines in carbonate bedrock catchments is illustrated in Figure 2.1.

2.3 Trace Metals

The composition of mine waters can vary greatly depending on local hydrogeology. This section reviews some of the potential impacts of metals commonly present in mine discharges on both aquatic biota and human health. Also under consideration are some of the other trace elements that can be found in carbonate-rich mining districts that can yield useful information on trace element mobility in the aquatic environment.

2.3.1 Zinc

The transition metal zinc (Zn) is a lustrous, blue-grey metal and is abundant in the environment, occurring at a concentration of around 70mg/kg in the earth's crust (WHO, 2001). Zinc is primarily mined from veins rich in the mineral sphalerite, however, other secondary minerals can also be mined for zinc with variable amounts of zinc present in them as seen in Table 2.4. The most common anthropogenic source of zinc compounds in the environment are from mining and associated processing activities (mining of zinc, smelting, galvanizing works), with zinc commonly found in hydrological systems draining abandoned mining operations (e.g. Grande *et al*, 2010; Casiot *et al*, 2009).

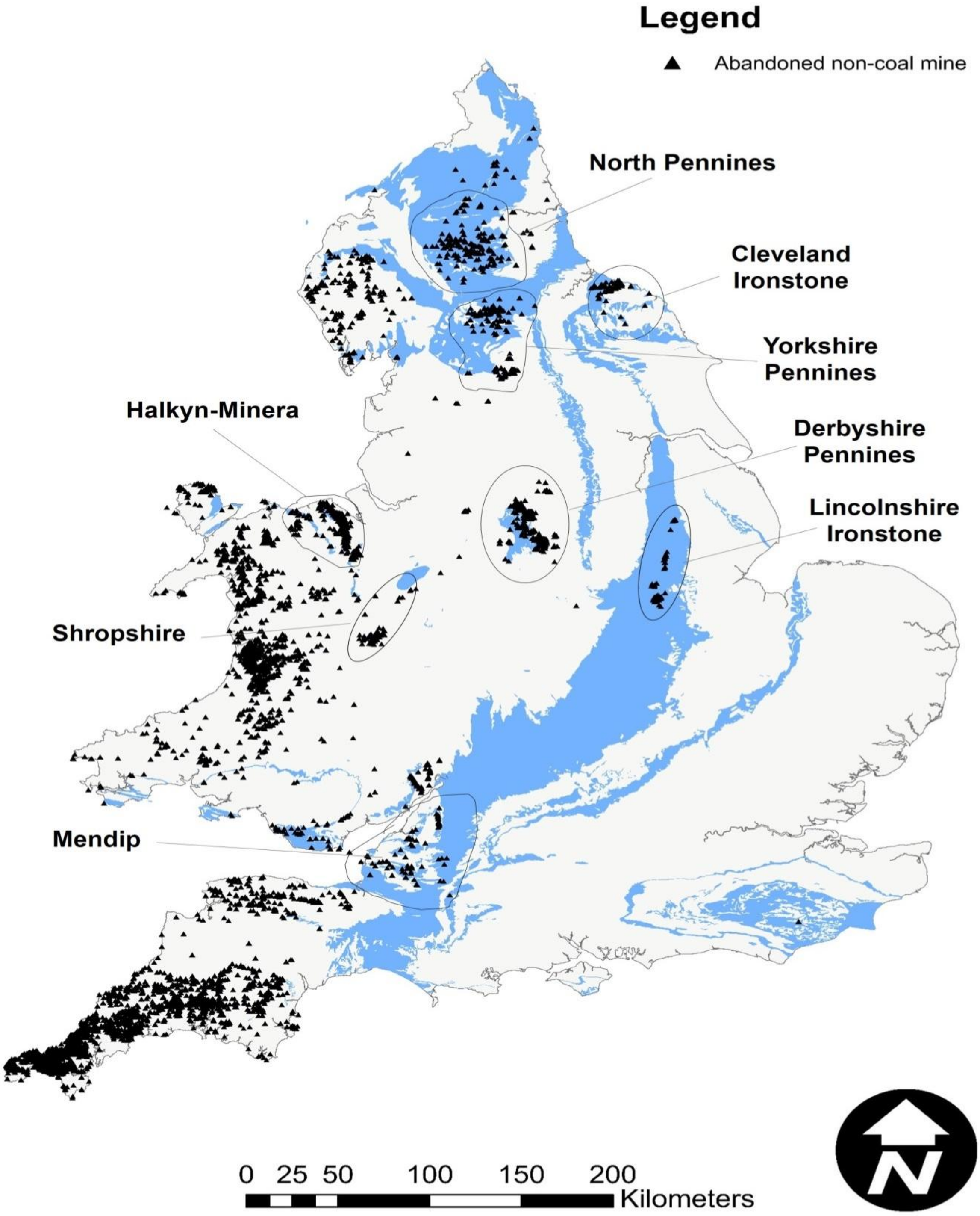

Figure 2.1 The importance of historic mining in carbonate-rich bedrock areas (blue). These are dominated by the Jurassic Ironstones of Cleveland and Lincolnshire, the Carboniferous limestone of Halkyn Mountain, Shropshire, Mendip, the Peak District, the North Pennines and Yorkshire Dales. A total of 4891 non-coal mines are present, with 20% (1014) of these being situated in carbonate rich areas (Mayes *et al.*,2009).

Name	*Composition*	*% Zinc*
Sphalerite	ZnS	67.0
Hemimorphite	$Zn_4Si_2O_7(OH)_2.H_2O$	54.2
Smithsonite	$ZnCO_3$	52.0
Hydrozincite	$Zn_5(OH)_6(CO_8)_2$	56.0
Zincite	ZnO	80.3
Willemite	Zn_2SiO_4	58.5
Franklinite	$(Zn,Fe,Mn)(Fe,Mn)_2O_4$	15-20

Table 2.4 Zinc mineral ores and percentage zinc

2.3.1.1 Impact on key freshwater biota

Bioaccumulation of zinc in species of fish can occur via uptake though the gills, diet, or accidental ingestion of contaminated sediments and various studies have found that increased zinc ingestion can lead to a decrease in the production of eggs by fish species (Calow and Petts, 1994). Acute toxicity, however, affects the gills of fish and their ability to uptake oxygen from the water, and this can lead to death (Todd *et al.*, 2009). Lappivaara *et al.* (1995) explain that the increased levels of zinc create lesions on the surface of the gill, which in turn increases the diffusion distance of oxygen across the secondary lamellae, reducing oxygen transfer across the gill epithelium. Respiratory problems also observed for a fish just prior to death due to long exposure to zinc at 21.5mg/l (96hr LC_{50}) (Taylor *et al*, 1985).

Waters in which salmonid species are, or can be, present are subject to the EC Freshwater Fish Directive. This directive is designed to protect and improve bodies of freshwater for a range of freshwater fish species (European Union Directive, 2006/44/EC) (European Commission 2006), and is embedded within the European

Union Water Framework Directive (2000/60/EC) (European Commission, 2000). Concentrations of zinc should be ≤ 0.3 mg/L in water hardness of 100mg/L $CaCO_3$, although as water hardness changes so will the acceptable level (Table 2.5). It is this sensitivity of sentinel aquatic organisms, via the exposure of sensitive organs (gills), that are therefore the key driver for control of zinc (and copper) pollution from abandoned mines, given the fact that in zinc-rich (>5mg Zn per litre) waters are considered safe to drink by humans (Hellawell, 1988). The difference in safe levels of Zn between humans and invertebrates relies on the sensitivity of the latter to Zn. Indeed, major mine drainage adits have been used for potable water supply in many former orefields with a limestone country rock (Banks *et al.*, 1997), despite Zn concentrations that would be considered an issue for aquatic biota (Mayes *et al.*, 2010).

Fish Type	*Water Hardness (mg $CaCO_3$/L)*		*Level of Zn acceptable*
Salmonid	Soft	0-50	30 µg/L
	Moderate	50-150	300 µg/L
	High	150+	300 µg/L (500 µg/L when hardness exceeds 250 mg CaCO3/L)
Cyprinid	Soft	0-50	300 µg/L
	Moderate	50-150	1000 µg/L
	High	150+	1000 µg/L

Table 2.5 Zn EQS in varying water hardness

2.3.2 Lead

Lead is a metal without odour and with a silver blue – white appearance, and usually exists in nature with a +2 valence. It rarely occurs as a pure element in nature, and galena (PbS), anglesite ($PbSO_4$) or cerrusite ($PbCO_3$) are common minerals in which lead can be found, and subsequently mined. Elevated levels of lead can be found in pollution from both metal (Strosnider *et al*., 2011; Cidu, 2011) and non-metal (Das and Ramanujam, 2011) mines.

2.3.2.1 Impact on key freshwater biota

Studies concerning lead toxicity focus on the role the metal plays in disturbing ion regulatory mechanisms in fish, causing interference with Na^+ and Cl^- regulation and disruption in processes utilising Ca^{2+} (Rogers *et al*., 2003; Patel *et al*., 2006). Patel *et al*., (2006) also identify the gills as the primary site of lead uptake, and that significant lead burden occurs after both acute and prolonged chronic exposure. Both studies note that an acute toxicity level of LC_{50} 1mg dissolved Pb L^{-1} at 96 hours.

The EA provides guidance on lead concentrations, dependent on what is it protecting (e.g. human health, aquatic biota), and environmental conditions. The Drinking Water Directive requires water for human consumption, should contain no more than 25μg Pb/L until 2013, and after 2013 this limit will fall to 10μg Pb/L. The Dangerous Substances Directive (European Commission, 74/464/EEC) provides limits that are intended to safeguard aquatic life. The levels of lead it recommends vary with changes to water hardness, as lead will readily form $PbCO_{3,}$ a less mobile and thus less toxic species of lead (Patel *et al*., 2006). These values are summarised in Table 2.6.

Fish Type	*Water Hardness (mg $CaCO_3$/L)*		*Level of Pb acceptable*
Salmonid	Soft	0-50	4 µg/L
	Moderate	50-150	10 µg/L
	High	150+	20 µg/L
Cyprinid	Soft	0-50	50 µg/L
	Moderate	50-150	125 µg/L
	High	150+	250 µg/L

Table 2.6 Acceptable levels of lead at different water hardness (Environment Agency, 2011)

2.3.3 Cadmium

Cadmium (Cd) is a white-blue group 12 metal, found in the earth's crust in abundances of around 0.3ppm. Greenockite (CdS) is the most common ore, however, it is uneconomical to mine this ore alone, and Cd is usually derived from Zn mining activities given its high concentration, typically in a ratio of 1:100 in Zn based ores (Nordberg *et al*., 2007). Cd has many uses, although low toxicity variants are available for the majority of these uses, and thus, given the high toxicity of Cd, many countries have restricted the use of Cd (Nordberg *et al*., 2007).

2.3.3.1 Impact on key freshwater biota

Cd serves no useful biological purpose and is known to be highly toxic to fish, even at sub-lethal concentrations (as low as 10µg/L) (Sandhu and Vijayan, 2011), and due to low excretion rates can accumulate in the soft tissue of fish (De Conto Cinier *et al*., 1998). Like Pb, Cd interferes with ion regulatory processes within the fish, and although not conclusively proven, Cd is thought to interfere by competing for binding sites with Ca^{2+} at Ca^{2+} channels (Lacrioux and Hontela, 2006).

Cd is a common contaminant in literature surrounding mine water, as many metal mines will have varying levels of cadmium ore in the veins being mined for zinc. An EU directive set up to protect aquatic wildlife has set limits for Cd discharges for varying processes (76/464/EEC) with the acceptable quantity of Cd discharged from “zinc mining, lead and zinc refining, cadmium metal and nonferrous metal industries” prescribed at 0.002mg Cd/L of discharge. Also, on a general note the WHO (2004) state safe limits for human consumption as being 0.003 mg Cd/L. Cd toxicity is dependent on hardness, as illustrated in Table 2.7. Ambient in stream concentrations have been observed (Jones *et al*, 2013). Recent surveys suggest that as much as half of the known Cd emissions to the water environment arise from abandoned non-coal mine sites in England and Wales (Mayes *et al.*, 2010).

Water Hardness (mg $CaCO_3$/L)		*Level of Cd acceptable*
Soft	0-50	0.08µg/L
Moderate	50-150	0.09µg/L
High	150+	0.15 µg/L

Table 2.7 Cd EQS limits in varying hardness of water

2.3.4 Barium

Barium (Ba) is an alkaline earth metal found in group two of the periodic table, it is never found in a free form (Ba^{2+}) naturally, as it reacts with oxygen upon contact. Barium makes up around 0.04% of the earth’s crust, and concentrations found in freshwater vary from 0.07 – 15mg/L (Oskarsson and Reeves, 1996). It is most commonly found as either barite ($BaSO_4$) or, less commonly, as witherite ($BaCO_3$). Barite can be seen to be the most common in the UK with around 6Mt of barite has being mined in the UK compared to 1Mt of witherite (BGS, 2000). BGS (2000) also notes the Pennine orefield as one of the primary producers of these ores. The main commercial use for barite is in the oil and gas industry, where it is used as a weighting agent in drilling muds (this accounts for around 85% of the total barite produced commercially). Witherite as well as barium chloride and nitrate are used as pesticides, witherite is known for being used as a rodenticide (Ghose *et al.*, 2009).

2.3.4.1 Impact on key freshwater biota

Regardless of its toxicity, Ba is not a regularly monitored pollutant in the UK, and this is supported by the apparent lack of literature concerning Ba and its effect on freshwater species. The limited literature produced little on the effects of Ba, barring its interference with the reproductive system of rainbow trout (Tanimoto and Morisawa, 1988). The lack of regular monitoring is reinforced with the lack of EQS for the protection of aquatic species, with only DWS for the protection of human health being put in place (0.7mg Ba/L: WHO, 2004). There are no set guidelines on Ba concentrations in varying water hardness's. Given Ba is not routinely measured in ambient water quality monitoring, there is a lack of published data detailing occurrence and concentrations in mining-affected catchments.

2.4 Transient in-stream controls on metal concentrations

In order to understand the processes affecting the fate of trace metals in streams and rivers that are subject to mine impacted drainage, it is important to gain an understanding in the broader cycling of in-stream biogeochemical parameters.

Diurnal fluctuations of biogeochemical parameters (pH, dissolved oxygen (DO), dissolved carbon dioxide (DCO_2), conductivity, temperature and flow) have been observed in stream waters (Spiro and Pentecost, 1991). Some cycling of these parameters has been observed since the late 70's (Kannan and Job, 1980), although over shorter cycles of 12 hours. More importantly to this study, cycling of trace metals has been observed in streams and rivers receiving waters that are impacted from mining activities (Fuller and Davis, 1989; Brick and Moore, 1996; Bäckström *et al.*, 2002; Kay *et al.*, 2009).

These patterns of cycling are a key issue in the successful management of water bodies exhibiting them, and a poor understanding of such processes can result in an ineffective management strategy (Nimick *et al.*, 2011). At catchment scales, the characterisation of streams can be completely different depending on what time of day samples are taken. For example, Gammons *et al.* (2007) sampled High Ore Creek with two teams simultaneously, each team starting from either end of the creek. One team collected data that suggested Zn concentrations increased with distance downstream, whilst the other collected data that suggested the opposite. The implications of this could lead to errors in the characterisation of the stream, or the behaviour of any in-stream contaminants.

For example, if the loadings of the stream are high at the top of the catchment and lower towards the bottom, it could be assumed that any trace metals are being attenuated, and thus posing a different problem to if the loadings get greater as sampling moves from the top to the bottom of the catchment, which would imply that there are no attenuation processes occurring. Diurnal cycles play there part in this as metal concentrations will change throughout the day, and with sampling campaigns usually taking upwards of 6 hours, even days for larger catchments, the failure to take these changes into account will lead to incorrect load estimates being recorded, and ultimately incorrect conclusions drawn about the catchment. Poor understanding of short term diurnal dynamics can also result in inefficiencies at smaller spatial scales. Littlewood and Marsh (2005) identified issues with sampling carried out by monitoring schemes in the UK. They found that sampling regimes corresponded with a normal working day, where sampling would begin at 0700-0800, have a lull at 1300 and typically cease at 1800 with no sampling undertaken overnight (Figure 2.2).

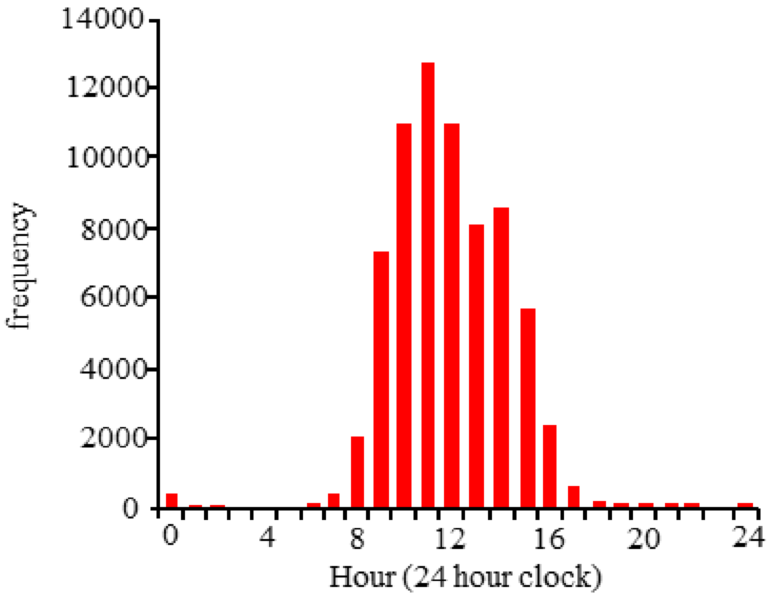

Figure 2.2 Number of samples taken throughout the day in ambient water quality monitoring of rivers by the Environment Agency (after Littlewood and Marsh, 2005)

In streams subject to trace metal pollution, that require a management strategy that incorporates remediation, it is important to take into account the dynamic nature of solutes, particularly cycling of trace pollutants. Failure to take into account the processes and mechanisms of the stream throughout a 24 hour period could lead to the over or under estimation of the level of remediation required. This is particularly relevant in carbonate rich systems, as small changes in environmental parameters lead to trace metals being precipitated out of the water column, and remobilised at different times of the day.

2.4.1 pH

The pH of water is a master variable controlling many biogeochemical processes occurring in-stream. Fluctuations in pH can have an effect on carbonate chemistry, adsorption and desorption kinetics, precipitation and dissolution kinetics, element speciation and microbial growth (Nimick *et al.*, 2011).

Diurnal fluctuation in pH is caused by the daily alterations in microbial and algal consumption of CO_2 for photosynthesis;

$$6CO_2 + 6H_2O \xrightarrow{+hv} C_6H_{12}O_6 + 6O_2 \qquad (2.6)$$

Or releasing CO_2 from aerobic respiration into the stream water;

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad (2.7)$$

During periods of CO_2 release (during dark hours), CO_2 dissolves in the water, forming carbonic acid (Equation 2.8), which lowers the pH of the stream, making it more acidic. When CO_2 is consumed, the pH can return to a lower state due to the buffering effects of calcite, of which carbonic acid is in equilibrium with (Equation 2.9).

Magnitudes of pH cycles are often around 0.5 pH unit (Bäckström *et al.*, 2002; Gammons, 2007), yet the magnitude can be amplified or diminished by changes in the seasons. Nimick *et al*. (2005) found that fluctuations in pH correlated with seasonal temperature increases. Nimick *et al* observed small pH fluctuations of 0.09 – 0.30 units in winter (0.0 - 5.1^{o}C), moderate fluctuations of 0.31 - 0.58 units in snowmelt conditions (March/April) (6.0 – 10.2^{o}C), and larger fluctuations of 0.50 – 0.79 unit during summer conditions (9.2-11.4^{o}C).

Bäckström *et al*. (2002) state temperature dependent factors that potentially affect the pH; the pH of neutrality, solubility of calcite (increases) and solubility of CO_2 (increases) with decreasing water temperature. Bäckström *et al*. (2002) show that pH rises throughout the night as the temperature decreases, and then lowers when the temperature rises throughout the day. Although this can be biologically-mediated, it is important to note that the change in solubility of CO_2 can also contribute to the effect of causing fluctuation in pH.

2.4.2 Dissolved Gasses - Dissolved Oxygen and Dissolved Carbon Dioxide

Concentrations of DO and DCO_2 have been known to diurnally cycle in streams and rivers with biological activity, owing to the photosynthetic reactions at different times of day uptaking CO_2 and releasing O_2 at different rates (Odum, 1956). Decreased levels of DCO_2 have been found during the day, amplified during high productivity seasons and periods of higher temperatures (Guasch *et al.*, 1998), due to temperature dependent gas solubility (Loperfido *et al*., 2009). Gas exchange between the surface of the stream and the atmosphere can also occur, resulting in an elevation in DO concentration.

DO concentrations have a great importance to the wider biota of the stream, and DO concentration data can be used to assess biological health of streams. However, DO concentrations have little effect on the biogeochemistry of a stream, unless the concentrations fluctuate to the extent at which long periods of anoxia can occur (Nimick *et al.*, 2011).

DCO_2, on the other hand, can affect in stream biogeochemistry, potentially controlling the tendency of minerals in the water to precipitate or dissolve. DCO_2 enters the steam during periods of photosynthesis, and upon dissolution in water, forms a weak carbonic acid;

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \qquad (2.8)$$

This is responsible for the pH of water becoming acidic, which is a factor in dictating carbonate chemistry, mineral precipitation and dissolution equilibria and pH dependent sorption reactions (as discussed in 5.2.0). Ground waters from areas with limestone bedrock, such as the study site of this study, will be carbonate-rich and thus will be affected by the above reaction. Calcium and bicarbonate will be present in the groundwater due to the dissolution of limestone, and will be in equilibrium as follows;

$$\mathrm{CaCO_3 + H_2CO_3 \leftrightarrow Ca^{2+} + 2HCO_3^{2-}} \qquad (2.9)$$

The equilibrium will shift towards the right or left, depending on the conditions. For example, a rise in the concentration of carbonic acid (as in reaction 1) will shift the equilibrium to the right, which would result in the dissolution of calcite, yet a rise in the concentration of Ca or HCO_3^- (or a drop in the concentration of H_2CO_3) would result in the equilibrium shifting to the left, promoting the precipitation of calcite (Hiscock, 2005).

Calcite is a mineral in which a host of trace metals can adsorb to, in particularly divalent trace metals, given the negative surface charge of calcite, which allows for adsorption, and there is also scope for trace metals to be incorporated into the crystal lattice of calcite via co-precipitation (Zachara *et al.*, 1998). Hence a change in the carbonate chemistry of the steam water from groundwater inputs and the photosynthetic diurnal changes that can change it could ultimately have an effect on other constituents of the stream water cycling over a 24 hour period.

2.4.3 Temperature

Diurnal temperature fluctuation in stream water can be buffered by a number of internal and external factors. Internal factors include stream morphology (width/depth), where a wider deeper stream would better resist heating and cooling regimes based on solar radiation. Riparian vegetation and alluvial aquifer stratigraphy can also control the resistance of the stream water to heating, ultimately dictating the temperature of the stream in spring fed catchments, whilst external factors are based on weather conditions (controlling solar radiation) (Poole and Berman, 2001).

Temperature is one of the more important parameters to cycle in stream owing to the control it has on many other biogeochemical processes. Solute kinetics (Fe photo reduction in acidic streams (Gammons *et al.*, 2008)), speciation of precipitates (minerals and metals), hydraulic conductivity, and biological quality (fish re-productivity)) are all temperature dependent factors.

Temperature regimes in mine impacted streams are largely consistent over a diurnal time period, exhibiting expected daily fluctuations in tandem with the daily solar cycle and its subsequent increase and decrease in solar radiation. Seasonal changes are evident; however, the cycles in temperature still remain (Nimick *et al.*, 2005).

2.4.4 Stream flow

The main causes in fluctuation of stream flow have been identified as being snowmelt, evapotranspiration, infiltration and diurnal precipitation (Lundquist and Cayan, 2002); although it should be noted that anthropogenic causes such as water abstraction/discharge could also cause temporal fluctuation in flow levels. Gribovszki (2010) describes daily freeze thaw cycles of snow as a cause of diurnal fluctuation of flow in ground waters, which could potentially cause fluctuations in stream flow. Similarly, cycles in precipitation have also been proposed as a cause for diurnal stream flow cycles (Lundquist and Cayan, 2002), and have been observed in tropical climates (Wain, 1994). Diurnal precipitation causing diurnal cycles in stream flow throughout temperate climates have not been observed (Gribovszki, 2010).

2.4.5 Implications on trace metal mobility control

It has been suggested that a combination of the geochemical and/or hydrological controls described are responsible for controlling trace metal mobility. Fluctuations in trace metal concentrations will be dictated by geochemical controls in catchments in the UK, as there are typically no large snowmelt increases in flow, and changes in flow attributed to this are likely to be insignificant. In catchments with large mine drainage levels, patterns of flow derogation in upland areas (due to under-draining) and augmentation below points of discharge may well lead to more consistent flow regimes than without the mine drainage structures (Younger *et al.*, 2002).

The cycling of geochemical conditions would suggest fluctuations in geochemical conditions control the mechanisms responsible for the controlling trace metal mobility, and are described in the next section.

2.5 Mechanisms controlling trace metal mobility

Numerous potential controls on trace metal concentrations have been documented. This section will review these controls, outlining the conditions in which they prevail, and the magnitudes of fluctuation expected in trace metal concentration.

2.5.1 Photo-reduction

Diurnal fluctuations due to redox cycling are well documented, and prevalent in acidic waters, and in particular concerning the fluctuations of iron concentrations (McKnight *et al.*, 1988; McKnight and Durren, 1994; Parker *et al.*, 2007). This in stream reaction reduces Fe^{3+} to Fe^{2+}, maintaining an acidic environment and creating an hydroxide free radical, which in turn reacts with redox sensitive ions, as demonstrated by the equation below;

$$Fe^{3+} + H_2O + hv \rightarrow Fe^{2+} + H^+ + OH \cdot \qquad (2.10)$$

A study into the diurnal fluctuation of Fe^{2+} has ruled out other chemical and biological (i.e. non-physical) reactions exerting a major control on the concentrations of Fe^{2+} and Fe^{3+} (Gammons *et al.*, 2008). However, the role of microbial activity cannot be overlooked, as Fe^{2+} that is a product of photo reduction can be metabolised by bacteria (Johnson and Hallberg, 2003).

2.5.2 Abiotic precipitation

When looking at the subject of carbonate precipitation, a major consideration is the alkalinity of the hydrological system, as carbonate species appear as a major component in the alkalinity. This is of particular importance to this study as it has been outlined that carbonate precipitation in hydrological systems will be able to incorporate metal impurities, effectively removing them from the water column (Forstner and Whittmann, 1981; Al-Degs *et al.*, 2006). Sources of alkalinity in streams can be attributed to the dissolution of carbonate rock, with calcite and dolomite providing the largest sources of freshwater alkalinity. The alkalinity of a stream can be acquired as shown by VanLoon and Duffy (2000):

$$Alkalinity = [OH^-] + [HCO_3^-] + 2[CO_3^{2-}] - [H_3O^+] \qquad (2.11)$$

Water courses that have a higher alkalinity have a greater tendency to precipitate solid carbonate species, and thus, have a greater chance of incorporating trace metal loads as the precipitates can adsorb/co-precipitate trace metals (2.5.3).

In order for the water column to precipitate carbonates, certain environmental parameters have to be met. Primarily, pH dictates the speciation of the inorganic carbons in the system, at the circum-neutral pH that are prevalent in this study, the inorganic carbon will be present as bicarbonate (HCO_3^-) as can be seen in Figure 2.3. Under ambient pH conditions in unpolluted rivers, carbonate and hydroxide are of peripheral importance, only becoming a main focus in highly alkaline streams (Pentecost, 1992).

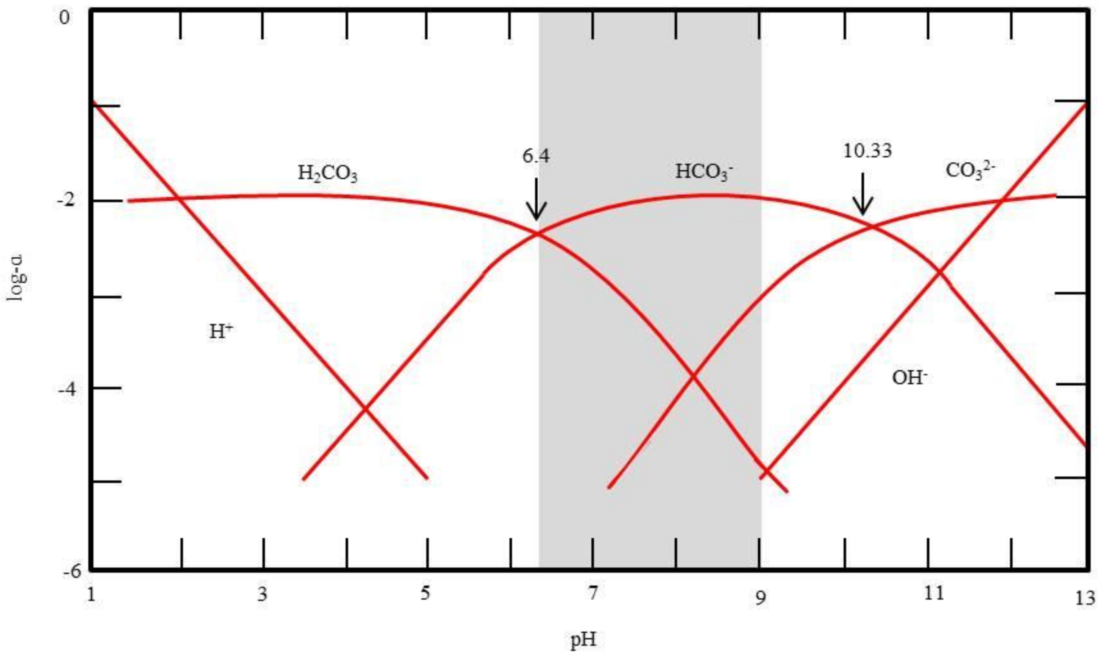

Figure 2.3 Speciation of carbonates through the pH spectrum. pH at which bicarbonate is the dominant species are identified. Shaded area represents common pH range of natural surface waters, and thus this study. (after Kehew, 2001)

Although the pH dictates that bicarbonate is dominant in the waters concerned with this study, other conditions have to be satisfied to enable the precipitation as a solid mineral in the water column. Knowledge of these factors can be used to assess chemical equilibrium in a hydrological system to precipitate a mineral in a solid form; the solubility product constant, ion activity potential and the saturation index.

Ultimately, the saturation index alludes to whether a mineral will be present in solution as a precipitate or dissolved ionic constituents. The saturation index can be used to predict which of these minerals will be under saturated, saturated or super saturated.

2.5.2.1 Ion Activity Product

The ion activity product can be used to determine the direction of the equilibrium and thus whether dissolution or precipitation would be more likely to occur. The process is outlined below in how this is calculated for calcite;

The equilibrium would be displayed as

$$CaCO_3 \leftrightarrow Ca^{2+} + CO_3^{2-} \qquad (2.12)$$

And the IAP;

$$IAP_{Calcite} = [Ca^{2+}][CO_3^{2-}] \qquad (2.13)$$

(Kehew, 2001)

The IAP can be used together with the solubility constant (K_{sp}) in the following equation to calculate the saturation index of the system:

$$SI = \log\left(\frac{IAP}{K_{sp}}\right) \qquad (2.14)$$

(Kehew, 2001)

If the result is negative the water body is under saturated and therefore the mineral calcite will be present in a dissolved form. If the result is positive the water body is oversaturated, thus precipitating calcite. Modelling programmes are able to calculate this value quickly for a whole range of minerals, taking into account other environmental conditions to show which minerals will be present in different forms, an example of this would be the PHREEQC programme (Parkhurst, 2010), used for this study.

2.5.3 Trace metal uptake by carbonate precipitates

Carbonate precipitates can be effective in removing trace metals from solution (see Al-Degs *et al*. 2006). Metals are removed from solution via co-precipitation or adsorption. Co-precipitation is the removal of a trace metal during the formation of the precipitate, essentially, the ion is incorporated into the structure of the precipitate, and co-precipitation of ions can be into both homogenous and heterogeneous precipitates (Lee *et al*., 2002). Trace metals that sorb to a precipitate do so at the precipitate surface/water interface, and thus are not actually incorporated into the structure of the precipitate. Due to the nature of the bonding between the trace metal ion and the precipitate surface, the mechanism that allows for adsorption of ions is controlled by pH (Gozzard, 2008), which itself cycles throughout the day and thus could be an explanation to the cycling of trace metals as ions are preferentially adsorbed or released.

2.5.4 Adsorption/Incorporation into biologically mediated minerals

Abiotic precipitation of minerals out of the water column can occur naturally in highly alkaline freshwater. However, most of the rapid production of these carbonates as a precipitate in circum-neutral freshwater systems is typically attributed to biofilms (Pedley *et al*., 2009). The next section provides insight into both processes.

2.6 Biofilm: Structure, functions and role in metal uptake

The influence of abiotic mineral precipitation, and adsorption processes on trace metal concentrations in the water column have been observed in field and the laboratory conditions (Tesoriero, 1996; Shope *et* al., 2006; Elzinga *et al*., 2006; Rogerson *et al*., 2008). However, the role of biological mediation in these metal uptake processes has only recently become an area of intense research, which has largely focused on quantifying the magnitude of biological trace metal uptake (Kurz et al., 2013; Morris *et al.*,2005). The mechanisms which govern the dynamics of trace metal mobility have by and large been understudied with respect to trace metal pollution in mine impacted streams and thus it is important to gain an understanding of the exact nature and permanence of metal removal mechanisms that are mediatated by indigenous microbial communities.

Biofilms and microbial mats have been credited with being a highly resilient ecosystem, which have been used as geochemical archives to investigate past environmental conditions (Rogerson *et al.*, 2008). Recently, microbial interactions, and more importantly to this study, biogeochemistry (element cycling) and microbe-mineral interaction (precipitation/dissolution of carbonates silicates and oxides) have become areas of intense research. They are found in a wide range of conditions, in soils, marine environments, freshwater environments, hyper-saline environments (Dupraz *et al*., 2009) and even in humans where they can manifest as a microbial infection (Harrison *et al*., 2007). Ubiquitous in the freshwater environment are oxygenic phototrophic biofilms; biofilms with a component that is able to produce energy via photosynthetic reactions, such as benthic diatoms, green algae and cyanobacteria (Roeselers *et al.*, 2008). This section of work will outline the development process, composition and the established research areas of these biofilms, and then more in depth commentary will be given on the biological processes, precipitation and ion uptake mechanisms that of more pertinence to this study.

2.6.1 Development

The initial development of a biofilm requires a lifestyle change for the microbial consortia that will inhabit it. Microorganisms will revert from a planktonic state, which is;

“a suspension of microorganisms that grow primarily as free floating single cells in a liquid medium that is under constant mixing”

To

“a surface adherent assemblage of microorganisms that is encased in an extracellular matrix of self-produced polymers”

(Harrison *et al.*, 2007)

This change is thought to be brought about by a change in gene expressions within active bacteria, where a change would induce a “swimmer” gene to be turned into a “sticker” gene to allow adhesion onto almost any substrate within minutes (Hall-Stoodley *et al.*, 2004), and allow for further growth as a structured community as opposed to a free living mass of individual calls (Lemon *et al*., 2008). The growth of biofilms is a dynamic process and can be reversed, as summarised in Figure 2.4.

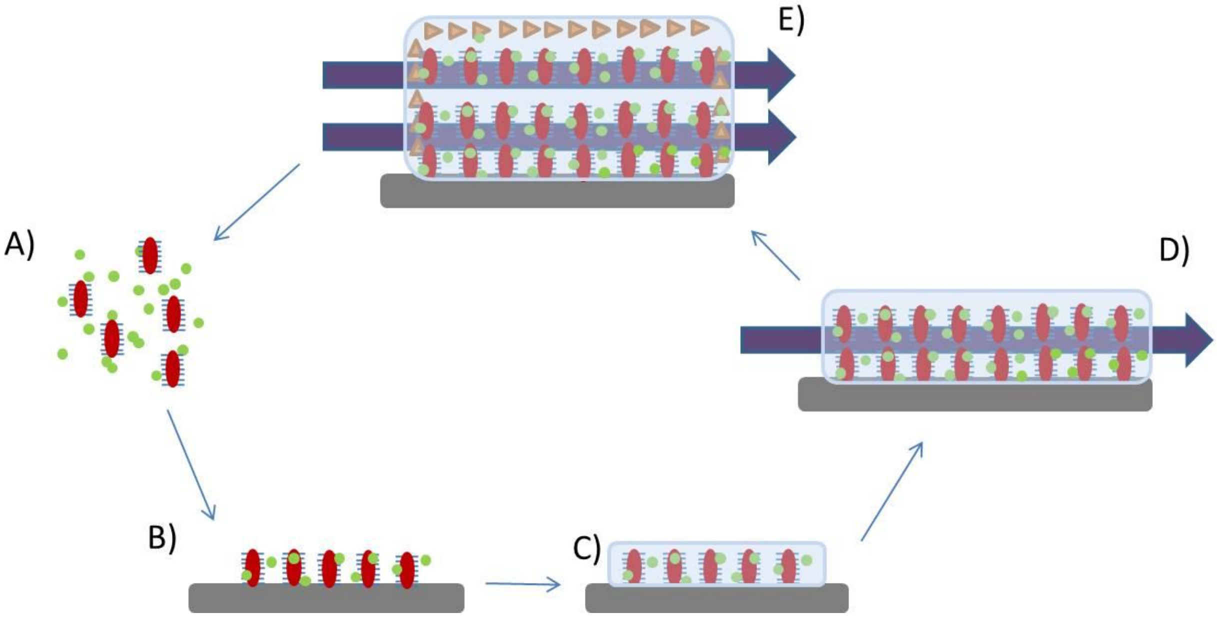

Figure 2.4 Schematic representation of biofilm life cycle A) Free living planktonic microbial consortia approach substrate B) Consortia attaches to substrate via mechanism described by Hall-Stoodley *et al.* (2004). C) Development of attached cells through monolayer growth and consequently development of EPS. D) Further development of biofilm leads to more depth and the ability of EPS to allow influx of water channels, and thus nutrients. E) Biofilm reaches full maturity and chemical gradients created by the influx of nutrients give rise to new microbes differentiated to specific tasks. If environment changes to less favourable conditions, biofilm can disperse and return to A) (although some microbes can remain attached to substrate until ambient conditions return)

A structure-based community has been identifiable since early investigations into biofilms, albeit in a marine settings, where microbes are observed inhabiting defined layers within the biofilm (Stal *et al*., 1985). A laminar structure has been attributed to their freshwater counterparts, given the dependence on light as an energy source some microbes have, which means they will be towards the upper part of the biofilm, where light photons do not have to penetrate as far into the biofilm. Photons of light, however, are not restricted to just the upper most part of the biofilm, the EPS aids in the scattering of photons to deeper parts of the biofilm. Extracellular polysaccharides have a slightly different refractive index than water, which allows for photons of light to be scattered forward (through the biofilm), as opposed to being reflected back, a feature also emphasised by the gelatinous nature of the EPS (Flemming, 2011). This allows photosynthetic components deeper in the biofilm to remain active (Flemming, 2011). The structure must be sufficiently thick to allow for non-photosynthesising layers to form. Typically biofilm thickness ranges from 10 micron to 1 millimetre, although biofilms can reach thicknesses of a few centimetres. It must be noted that due to the number of environments biofilms are able to form in, there is no universal structure to biofilms (Sutherland, 2001b). Thus the EPS matrix over a range of biofilm can be equally diverse.

2.6.2 Composition of Biofilm Matrix

2.6.2.1 Water

Water forms a major part of the biofilm matrix, contributing up to 97% of the biofilm matrix (Zhang *et al*., 1998). Water can be bound to microbial cells, or can exist as a solvent within the biofilm, organised within the fine structure of the biofilm

(Sutherland, 2001a). Water forms channels that penetrate to all areas of the biofilm, allowing nutrient and ion transport throughout the whole biofilm (Costeron, 1995).

2.6.2.2 Bacterial component

Examples of organismsfound in freshwater biofilms are summarised in Melo (2003), but generally consist of diatoms, green algae and cyanobacteria. The bacterial element of the biofilm is responsible for the creation of the EPS (through the creation and excretion of polymeric substances via various metabolic processes), and the creation of microenvironments within the biofilm due to the close proximity of bacterium (10^7 - 10^9 cells/cm^2) and other associated metabolic activity, most importantly respiration and photosynthesis which controls pH and which has a major bearing on geochemical reactions within the biofilm (Bissett *et al*., 2008). Bacterial communities within biofilms vary greatly in terms of diversity, as different environments will create conditions suitable to different strains of bacteria, competition will allow certain bacterial organisms to flourish due to natural selection mechanisms, a point further outlined by the cultivation of heterogeneous biofilms (Jones *et al*., 2013b).

2.6.2.3 Extracellular polymeric substances – EPS

Originally termed as ‘extracellular polysaccharides’, the secretions of microbes within the biofilm were thought to only be polysaccharides, but this was changed to EPS when further investigation into biofilms revealed EPS contained proteins, nucleic acids, lipids and other biopolymers (Flemming and Wingender, 2010). The organic EPS (Extracellular Polymer Substances) matrix forms an environment for microbes to inhabit, and thrive in whilst having protection against the external environment. The

EPS is responsible for up to 90% of the dried mass of a biofilm and can account for up to 90% of the biofilms organic matter (Wingender *et al.*, 1999; Flemming and Wingender, 2010). The composition of natural biofilm EPS is largely determined by its environment, with nutrients, shear forces and temperature dictating the EPS make up (Flemming and Wingender, 2010). The primary constituents are polysaccharides (1-2% of matrix), DNA (<1-2%) and proteins (including enzymes: <1-2%; Hall-Stoodley *et al.*, 2004). The EPS has many functions, mainly in promoting the growth of the biofilm via protection from the bulk water constituents, these functions are summarised in Table 2.8 below.

Function	*Advantage*
Adhesion	The initial step of biofilm formation as planktonic bacteria are immobilised onto the surface of a host strata.
Microbe accumulation	Allows for high microbial densities and thus cell to cell regulation.
Cohesion	Essentially the formation of the biofilm matrix. Maintains the physical structure of the biofilm allowing for internal microenvironments to form.
Water channels	Keeps biofilm hydrated, imperative for maintaining physical structure and thus microbial population. Allows for transport of nutrients and ions though out the biofilm structure.
Refractive properties	Allows light to penetrate through the biofilm, allowing for better development of photosynthetic microbe layer.
Protection	Internal regulation allows an environment independent to that of the bulk water column, so high ion concentrations will not necessarily affect the microbial population within the biofilm. Ions can also be sequestered to EPS molecules, effectively sinking them, and reducing their ecotoxicity.
Nutrient source	Provides carbon nitrogen and phosphorus containing compounds for the microbial community.

Table 2.8 Summary of EPS function (adapted from Flemming and Wingender, 2010)

EPS contain the ionisable function groups, carboxyl, phosphoric, amine and hydroxyl, all of which give the EPS a net negative charge (Hullebusche *et al*. 2003), an important feature that lends itself to ion sequestration. This feature, coupled with the creation of microenvironments and thus geochemical gradients over microscopic distances within the biofilm can allow for the precipitation of minerals in the biofilm structure, which is explained in the following section(s).

2.6.3 General Chelation Theory

Sequestering agents within the biofilm are molecules capable of removing ions through complexation. Chelation is a mode of sequestration, which allows a ligand to remove a metal ion 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). For example, if the metal is bonded in a unidentate manner, the metal can be released if the coordination becomes unstable, allowing the metal to come and go. In bidentate bonding, if one part of the coordination becomes unstable, the other will retain the metal, and allowing the bidentate coordination to be established again. The determining factor in coordination preference appears to be the relationship between hard and soft acids and bases, suggested by Pearson (1963). Pearson’s hard-soft acid-base principle (commonly referred to as HSAB principle) states extra stability is gained in a complex between a hard acid and a hard base (or a soft acid and a soft bases). The concepts of hardness and softness are generally down to the ionic (electrostatic) and covalent (polarising) bonds, and the radius of said ion. Metals or ligands with a high ionic charge density (the relationship between number electrons power) reactions (Chandrakumar *et al*., 2013) and low polarisation are classed as hard, and characterised by large energy gaps between the highest occupied molecular orbit of the base and the lowest unoccupied orbital of the acid, whilst metals or ligands with low ionic charge density and high polarisation power are classed as soft (Saunders, 2012). The distinction is evident in the bonding of the metal ligand complex, with hard metal – hard base complexes bonding in an ionic nature, and soft metal – soft base complexes bonding in a covalent manner, it is also noted that this is not a hard and fast rule of classification, as borderline species also exist (Saunders, 2012). The hard and soft classifications of ions that have relevance to both freshwater chemistry (prevalence throughout discharges in the literature) and biology (ecotoxicity to organisms) in this study are summarised in Table 2.9.

Hard	*Borderline*	*Soft*
H^+, Li^+, Na^+, K^+	Fe^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}	Cu^+, Cd^{2+}, Ag^+, Au^+, Hg^+, Cs^+
Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Sn^{2+}		
Al^{3+}, Cr^{3+}, Fe^{3+}		
Si^{4+}, Ti^{4+}, Zr^{4+}		
Cl^{7+}		

Table 2.9 Classification of hard and soft metals under the HSAB scheme of Pearson (1963)

2.6.4 Chelation via Biofilm EPS

The most important feature of the EPS is that most of the molecules have a negative charge, due to the functional groups present on the EPS molecules. Common functional groups include carboxylic acids (R-COOH), phosphoryl ($R\text{-}PO_4$), hydroxyl groups (R-OH), amino groups ($R\text{-}NH_2$), sulphate ($R\text{-}O\text{-}SO_3H$), sulphonate (R-SOH), and sulfhydryl groups (R-SH) (Hullebusche *et al*. 2003), these negatively charged molecules are capable of sequestering, via chelation, metal ions (e.g. Dupraz *et al*,. 2009). Carboxylic acids and sulphate groups are thought to be the most important functional groups regarding metal uptake, although Zn removal has been attributed to phosphyll groups within the biofilm (Toner *et al*., 2005)

Chelation of metal ions largely benefits the biofilm as a whole. Removal of toxic chemicals protects the biotic component of the biofilm, and chelation also helps maintain the structural integrity of the biofilm through a bidentate chelation mechanism. Ca^{2+} and Mg^{2+} have been identified as forming bidentate coordination between EPS molecules, which is the stabilising factor within the EPS matrix as different EPS molecules can essentially be bridged together by the ions in the case of bidentate chelation (Dupraz *et al*., 2009). Multiple (e.g. bidentate) ligand sites are preferential to form a more stable chelation and thus biofilm, whilst unidentate chelation of metal ions can begin a process of heterogeneous mineral precipitation within the biofilm.

2.6.5 Mineral Precipitation by Biofilms

The creation of an environment separate to the geochemical conditions of the bulk water column creates an area in which mineral precipitation can occur; the process of precipitation follows the same format as in the bulk water column, as previously discussed in section 2.5.2.

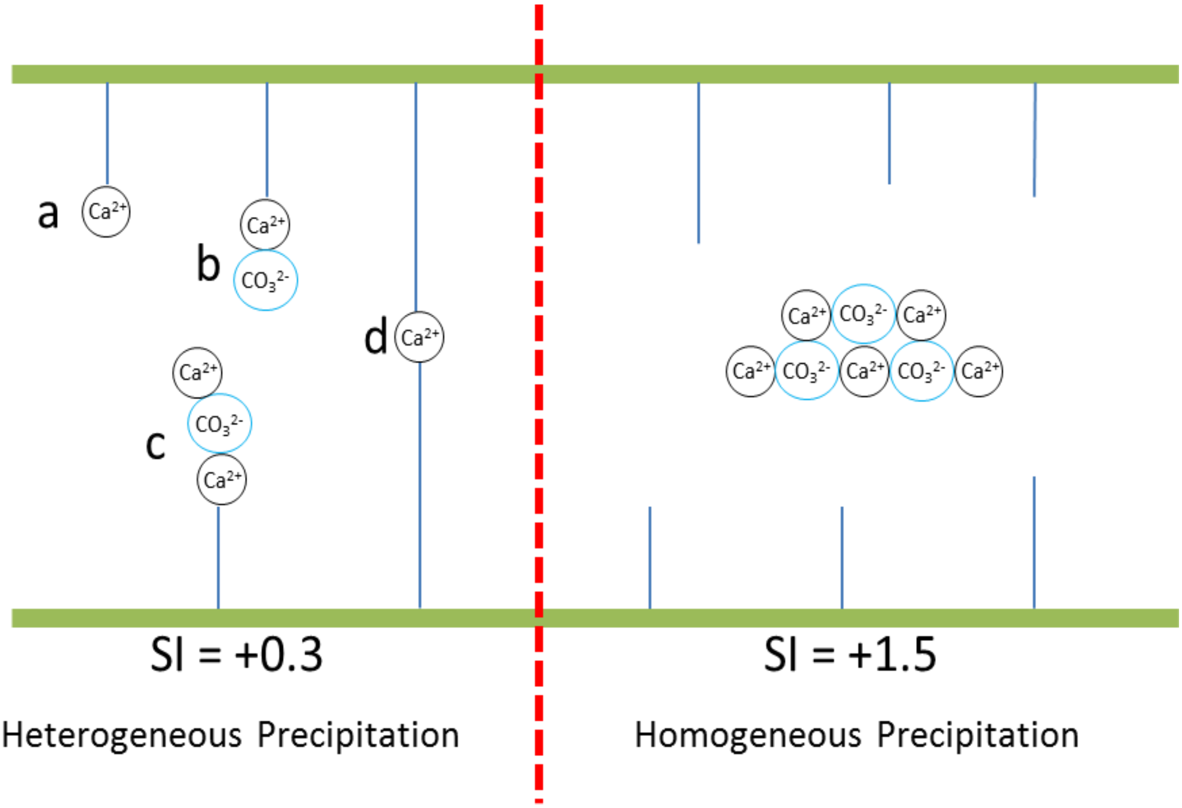

Figure 2.5 Schematic representation of precipitation mechanisms of the example mineral calcite within the biofilm structure. Green lines represent biofilm boundary, Blue lines represent EPS molecules and ions are appropriately labelled Heterogeneous precipitation begins with (a) a Calcium ion nucleating to an EPS molecule. If the process stops at this stage it can be referred to as unidentate chelation, with the ion being free to become labile should conditions change. (b) The nucleation only requires one of the free electrons Calcium has, leaving a carbonate ion to bond with it. (c) the process is continued with the bonding of another Calcium ion. (d) Shows a Calcium ion that has been bonded in a bidentate manner, effectively removing it from solution and any precipitation activity. Homogenous precipitation does not require the nucleation sites and thus precipitation is spontaneous.

Water channels allow for water to propagate through the biofilm structure, These water channels have been observed allowing the passage of 0.3 μm polystyrene beads and remain cell free (Costerton, 1995), meaning ions would be able propagate through to parts of the biofilms other than the biofilm - bulk water column interface. The interaction between EPS and aqueous ions can both inhibit and promote mineral precipitation as outlined below (Figure 2.5), using calcite as an example mineral, as the literature commonly refers to calcite precipitation in biofilms (Rogerson et al., 2008; Rogerson et al., 2013, Gammons et al., 2013), although this theoretically could happen with any mineral, and has been observed with Mn oxides.

2.6.6 Mine drainage and biofilm: scope of this thesis

The uptake of ions into precipitating biofilm has been the focal point of research in many arenas, and there have been studies over the past few decades highlighting the importance of biofilm interactions with trace metals in catchments affected by mine water pollution (Garcia-Mendez *et al*., 2005; Morris *et al.,* 2005; Gammons *et al*., 2013). Biofilm has long been associated with the uptake of trace metals, and the acknowledgement of adsorptive processes removing traces metals in circum-neutral pH streams are evident in earlier work (Ferris *et al*., 1989). More recently, a number of potential uptake mechanisms have been identified, which are outlined throughout the literature review, and summarised in Gadd (2004), and although many studies have focused on the use of biofilm as a potential tool to remove trace metals from the water column, these studies largely focus on the magnitude of removal attributed to the biofilm, without further analysis of the stability of biofilm immobilised metals. The fundamental controls on the trace metal stability have been studied, although to a much lesser extent. Trace metal dynamics in the presence of biofilm has been studied, identifying diurnal cycles of Zn concentrations (Morris *et al.*, 2005) and they have been attributed to the photosynthetic biofilm colonies (Morris *et al*., 2007) in circum-neutral streams affected by mine drainage. However, there has been little focus to understand the underpinning processes responsible for trace metal uptake and dynamics in the presence of biofilm, with a large focus on fieldwork establishing trace metal fluctuations, but little in the way of laboratory work where variable can be controlled to identify the underpinning mechanism responsible for metal cycles. The understanding of these dynamics is something that is of particular importance if biofilm is to be used as a remediation tool to remove trace metals from the water column in mine impacted catchments.

3

3.0 Methodology

3.1 Introduction

Chapter 3 presents the methods employed throughout the study. The Chapter is split into two halves, field work and laboratory methods. The first half will discuss field methods, including physical and geological characteristics of the field study site, providing context for the field sampling campaign, and sampling and analytical procedures for biogeochemical samples. The latter half will focus on laboratory methods, discussing laboratory set up, initial batch experiments with biofilms, flume design, sampling regimes for experiments carried out in batch and flume experiments and laboratory analysis of water and solid samples. Furthermore, data analysis procedures are introduced, which include statistical analysis, geochemical modelling and spatial analysis.

3.2 Field Methods

3.2.1 Introduction

This section of work describes the methodologies of the fieldwork study. Presented is a discussion of methods employed to acquire fieldwork data, consisting of desk studies sample collection, *in situ* biogeochemical analysis, and flow gauging. The methods presented here relate to results presented in Chapters 5 of this thesis.

3.2.2 Study Area Characteristics

The Yorkshire Pennine Orefield covers an upland area of central, northern England. The country rock of the area is of Namurian age (Westphalian to Asbian age Stages of the Carboniferous) and encompasses a Caledonian granite intruded into lower Palaeozoic basement rocks, with a cover of Carboniferous rock around 1.5km deep (Smart and Clayton, 1985). The area exhibits extensive fault controlled mineralisation (Dunham and Wilson, 1985), and mineral transport is aided by the chemical reactivity of carbonate rocks in the presence of mineral bearing solutions (Nuttall and Younger, 2002). The most important economic mineral of the area is galena (PbS), although large quantities of other primary minerals sphalerite (ZnS), fluorspar (CaF_2), barytes ($BaSO_4$) and a relatively large amount of witherite ($BaCO_3$ - quite rare at international scale) are found across the region. A small area of copper ore (chalcopyrite) is apparent in the north east of the region in the vicinity of the town of Richmond (Dunham and Wilson, 1985). The areas of mineralization are found in the upper reaches of four main river systems; the Swale, Ure, Nidd and Wharfe. Outliers of galena and baryte mineralization are apparent to the south west of the region in the upper Aire (Cononley Pb mine and Raygill barytes mine respectively). All the rivers drain into the Humber Estuary in eastern England. Land use in the catchments is dominated by upland grazing, with some areas of afforestation (notably in Wharfedale; Hey and Winterbottom, 2006) and open moorland with extensive peat deposits.

3.2.3 Historic Mining Activity of the Area

The earliest reference to mining activity dates back to the 1st Century, with evidence of lead smelting activities confirmed by geochemical and mineralogical investigations (Macklin *et al*, 1997). Modern mining is traced back to the early 17th Century with accounts dating back to either 1604 (Gill, 1990) or to the discovery of ore deposits at Yarnbury in 1603 (Raistrick, 1973). The opportunity to work the area was seized upon by the Earl of Burlington, who brought miners from Derbyshire and worked the area until 1620, when the area was put out to lease. From 1620 the area was worked by small partnerships exploiting veins and Oreshoots as far as Mossdale, to the point where by 1640 laws and customs similar to the already extensively mined, crown owned, Derbyshire Orefields, were formed (conflicts in land ownership forced a revision of these laws in 1737: Dunham and Wilson, 1985; Gill, 1990), and by the end of the 17th century Dunham suggests that the geological attributes of the area are beginning to become more well understood. This better understanding led to significant investment in the 18th century, with the money contributing horse power to the area and the widening and deepening of workable shafts. However, even with the investment, output of lead in the area was decreasing towards the end of the century (Raistrick, 1973). Perhaps the defining moment of the Orefield came when the Cavendish Estate took control of mineral rights, and put forward the funds for the driving of Dukes Level, a major underdrain to open up the Grassington Moor lead deposits. The project cost £33000 (around £4.2 million today) and the drive began in 1792 (ULSA, 1967). Duke's Level was originally planned to be a horse drawn level, although after the departure of the first mineral agent, Cornelius Flint, in 1818, John Taylor constructed the remainder of the level as a drainage level (Dunham and Wilson, 1985). The project took 28 years to complete, with a length totalling 5.33km, it allowed working down to 130 meters without the need for pumping (ULSA, 1967), across the whole catchment. The driving of Duke's Level stopped the decreasing trend of lead output in the orefield, essentially giving it a new lease of life; just after the completion of the project is described as the most productive period of the orefield, and working of Oreshoots in the area would continue up until 1876.

Year	*Output (Tons Metal)*
1620 – 1734 (estimated)	8 000
1735 – 1790 (1754 – 63 estimated)	23 257
1791 – 1844 (1836 – 44 estimated)	25 424
1845 – 1881	23 258
TOTAL	79 939

Table 3.1 Productivity of the Yorkshire Pennine Orefield (Dunham, 1985)

The main output of the Grassington area was lead, with a breakdown of the total output in Table 3.1, These figures assume a metal recovery rate (from ore) of 60%, so 126, 456 of ore was extracted (Dunham, 1985). Although lead ores (galena) were the primary mined mineral, Dunham notes the abundance of witherite in the area, which is a rare mineral at a global scale, and fluorspar and calcite are also amongst minerals that would have been gained alongside galena. Small amounts of silver were also found, but in such small quantities (~3oz per ton galena), that it was financially unviable to rework the spoil. The area was not worked at all up until the 1970's, when spoil heaps were re-worked for barite and fluorspar (Clark and Clark, 1981); this coincided with the exploitation of North Sea oil reserves as barite is used as a weighting agent in drilling muds. It is worth noting that zinc minerals (notably sphalerite) were not a specific focus of mining activities in the region (with the exception of a calamine mine at Malham, where secondary zinc oxides and carbonates were worked: Dunham 1985). Similar practices were apparent in the adjacent North Pennine Orefield (Dunham and Wilson, 1990), which contributes to high zinc concentrations in many contemporary discharges.

3.2.4 Hebden Beck: sub-catchment study site

Hebden Beck was the sub-catchment chosen for the diurnal study carried out during July 2011. The catchment was chosen after reconnaissance sampling given (a) the abundance of historic and recent mining activity in the catchment, (b) the presence of a permanent flow gauging station at a suitable scale (22km^2), (c) the lack of other major inputs of anthropogenic pollution, (d) the circum-neutral pH typical of the region, and (e) the abundance of microbial biofilms on the streambed in summer months, providing the platform to assess their influence on in-stream solute processes.

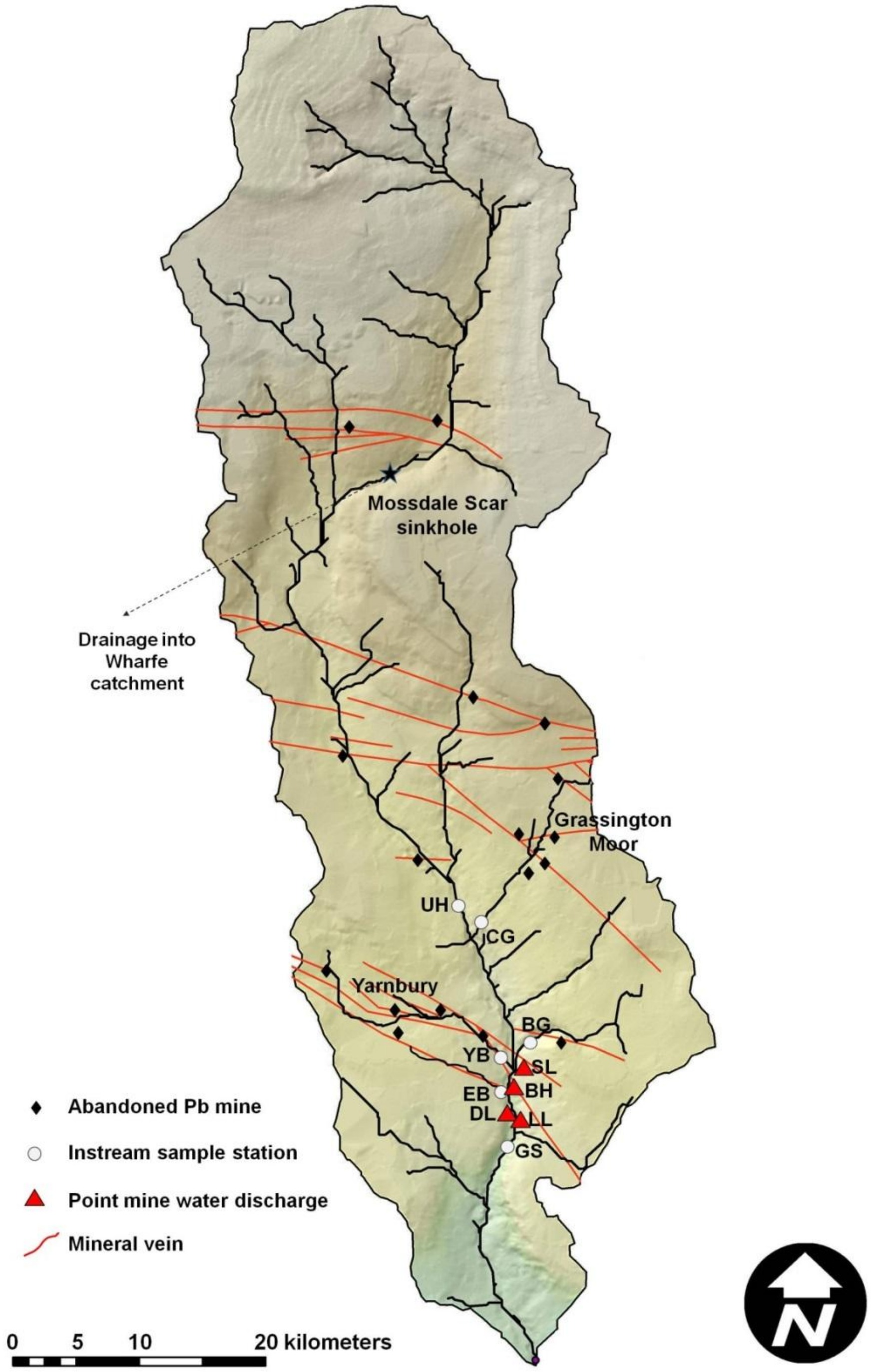

Figure 3.1 Sample stations and physical setting of the Hebden Beck catchment. Colour ramp from green (lowland areas, minimum elevation of 167m AOD at catchment outlet) to brown/white (peak elevation of 696m AOD) (AOD – Above Ordinance Datum).

Historic mining has also impacted the flow dynamics of the catchment, with multiple drainage levels and adits now flowing into the stream (Figure 3.1). Duke's Level is the most notable of the point mine water discharge of the catchment, a gravity driven level stretching 5.33km and draining over 15 million litres of water a year. It is a first order stream (see Strahler, 1957), meaning the effects of the stream diluting the metal concentrations are reduced. To the best of the author's knowledge, no published work exists for similar work at this particular site. The preliminary visit to the catchment revealed a number of point inputs into Hebden Beck, many of which drain abandoned mine workings. Knowledge of all sources, and the constituents of the sources, is necessary in order to carry out sampling campaigns to assess in stream trace metal diurnal cycles (Nimick, 2011). Also, at the bottom of the sub-catchment, is a flow gauging station (Figure 3.2). The Hebden Gauging Station measurements are taken by the Environment Agency (2011b), utilising a V-notch weir thin plate and a Crump Weir system. The V notch weir is highly accurate in low flow events (the local water authority prevented water abstraction when flow fell below 0.1 cumecs). This provided a precise and accurate means to constantly record flow measurements, which was important to facilitate accurate estimates of metal loading.

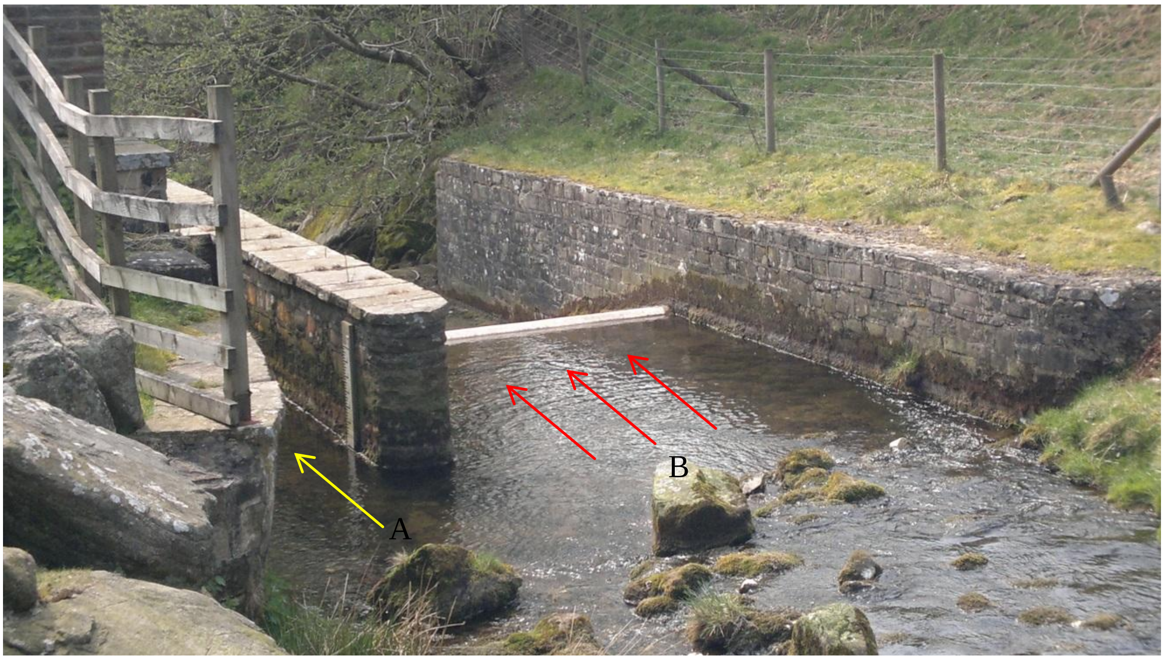

Figure 3.2 Hebden Beck Gauging station – (A) V notch flow gauge installed for low flow conditions (B) Crump weir flow gauge designed as an overflow method of flow gauging.

3.2.5 Field Sampling

Field sampling was carried out between November 2010 and April 2012 to identify the major sources of solutes and trace metals that are entering streams and rivers in a reconnaissance area (Figure 3.3). There is minimal data concerning ambient concentrations of contaminants for the area, a point emphasised by the sampling of over 20 previously un-sampled mine discharges (Figure 3.3), raising the issue of a need for a survey of this kind to be implemented. Mine sites throughout the reconnaissance area were identified through a desk study utilising mineralogical and geological accounts (e.g. Dunham and Wilson, 1985), a range of industrial archaeology resources (e.g. mine plans and online gazetteers: NMRG, 2012) and OS maps.

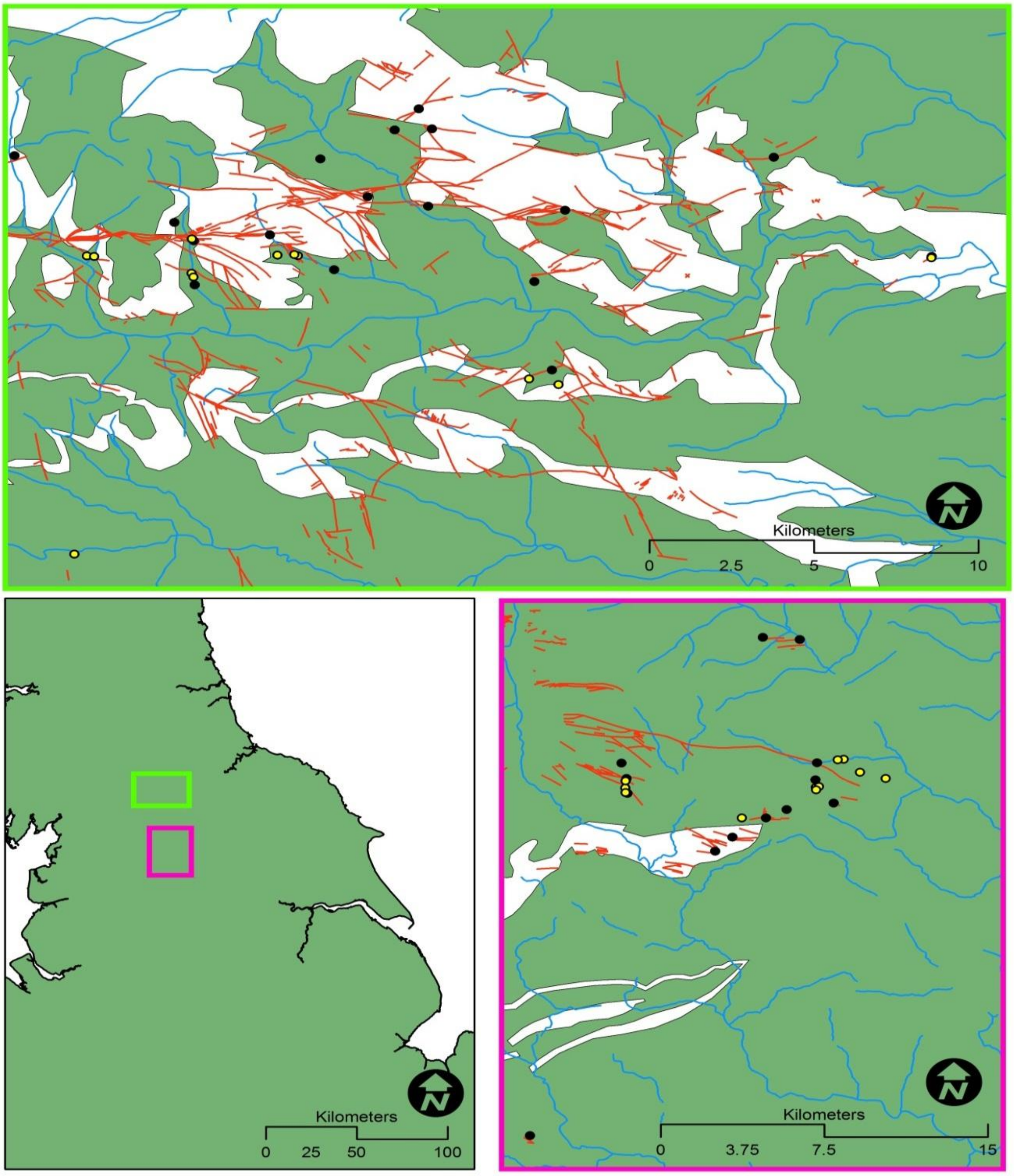

Figure 3.3 GIS plot illustrating sample sites in two catchments. Black points show site previously sampled by DEFRA monitoring programmes. Yellow points represent sites discovered and monitored during this study. Mineral veins and rivers are represented by red and blue lines respectfully. White areas denote carbonate bedrock.

Mines with the potential to discharge metal rich discharges into streams and rivers were identified as important sites to visit, as it is important to get all the key inputs into the catchment for detailed flux analysis further in the study.

3.2.6 In- Situ measurements

Environmental parameters; pH, ORP, dissolved oxygen, conductivity, temperature and alkalinity were measured in the field. These are measurements that, to maintain integrity of the data, must be taken in the field, as reactions (e.g. algal, photolytic) can continue to occur in sample bottles, potentially altering the true value of the parameter (APHA, 1998). All the variables excluding alkalinity where recorded on a Hanna HI 9828 multiparameter (Table 3.2). The pH was calibrated before each sampling visit using Fisher Scientific coloured pH buffer 4.0 (phthalate), 7.0 (phosphate) and 10.0 (borate), accurate to ±0.02 pH units and traceable to the National Institute of Standards and Technology (NIST) and BS 1647 standard. Conductivity was calibrated using Fisher Scientific 1413 µS/cm^2 conductivity standard, accurate to ± 1% and traceable to the NIST. Environmental parameters (pH, ORP, conductivity) were also manually recorded with the use of a Myron company Ultrameter as a back-up and cross-check. Generally, the data acquired form the two meters agreed between 5% and 10% and correlated well with each other (Figure 3.4).

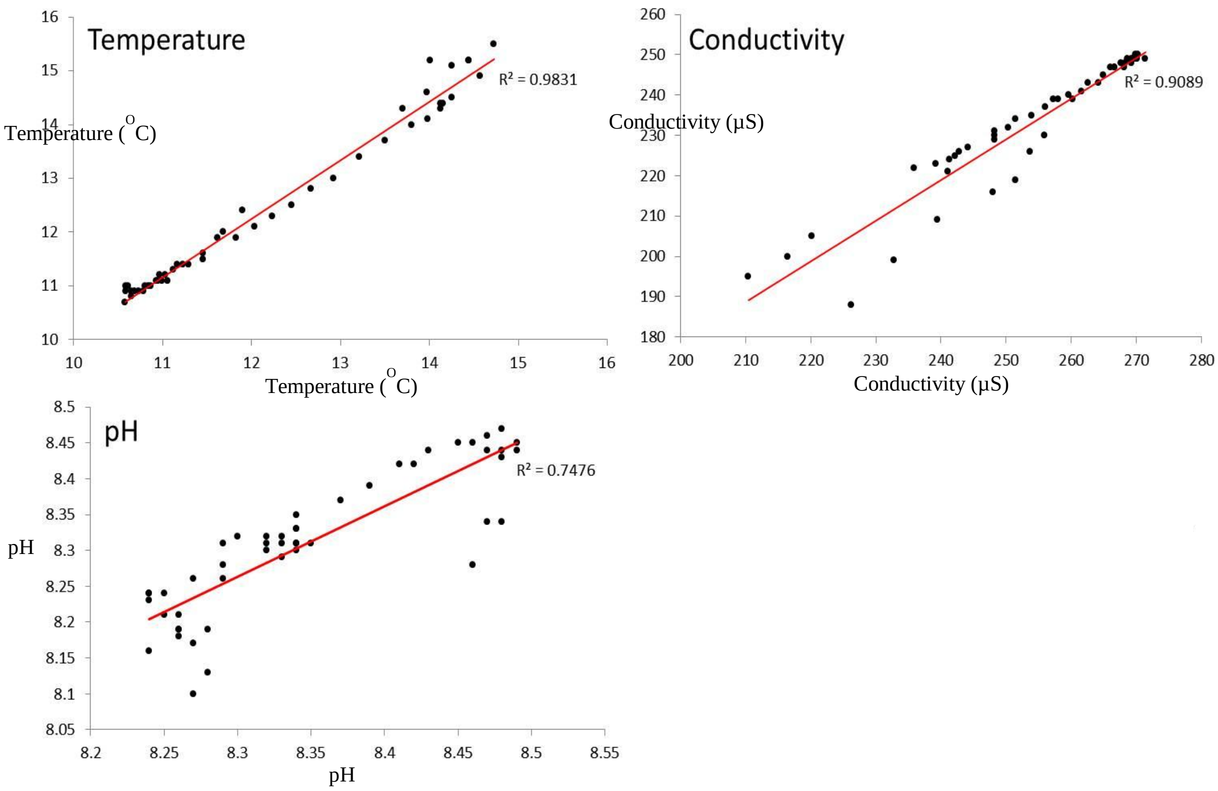

Figure 3.4 Correlations between data acquired via HANNA (y-axis) and ULTRAmeter (x-axis)

Parameter	*Unit*	*Range*	*Accuracy*
pH	arbitrary	0.00 – 14.00 pH	±0.02 pH
ORP	mV	±2000.0 mV	±1.0 mV
Conductivity	µS	0 µS - 200 000.000 µS	±1% / ±0.1 µS (whichever is greater)
Dissolved oxygen	DO%	0.0 – 500.0%	(0.0-300.0%) ± 1.5% (300.0% - 500.0%) ± 3%
Temperature	^{o}C	-5 ^{o}C – 55.0 ^{o}C	±0.15^{o}C

Table 3.2 Specification of the Hanna HI 9828 multiparameter

3.2.7 Sample Collection

3.2.7.1 Water

At each site, three water samples were collected using a Terumo 50ml syringe, and stored in low density polyethylene narrow mouth 30ml leak proof bottles, taken from a point upstream of any *in-situ* measurements or flow measurements to avoid any sediment getting into the sample. The first aliquot was filtered using a Millex-HA filter unit, a 0.45 µm MF-Millipore membrane made of cellulose nitrate and acetate. The second aliquot remained unfiltered. The aliquots where then acidified to less than 1% of the total volume of the sample with concentrated HNO_3 (ROMIL-SpA grade Super purity acid), to a pH of less than 2, so they could be analysed for cation concentrations. A third, un-acidified aliquot was then filtered for anion analysis. Triplicate samples were occasionally and randomly collected to check for and maintain precision in sampling regime (Gozzard, 2008). Samples were stored at 4^{o}C and analysed within 4 days of collection.

3.2.7.2 Biofilm

Biofilm was collected and kept in a container with a small amount of water (taken from where the biofilm had been taken) to prevent it from drying out during transportation. During transportation the biofilm was kept in a cool place to avoid any sudden changes in temperature. Upon arrival to the laboratory biofilm was gently rinsed with distilled water, and used immediately when used for batch experiments and when placed in the flume. For analysis of biofilm via XRF, biofilm was dried in an oven at 105^{o}C, which fully dried the biofilm whilst ensuring it did not carbonise. Biofilm was then grinded up in a pestle and mortar and placed in the sampling units for analysis (see section 3.4.5 for XRF procedure)

3.2.8 Flow Sampling

3.2.8.1 Manual Flow Gauging

The physical nature of the sample sites meant that using the section mean area velocity was a viable and consistently accurate method for gauging flow. The sites at which flow measurements were taken where generally characterised by either an engineered mine discharge (i.e. an adit or a pipe) with smooth flowing, and in some cases (e.g. Duke’s Level) laminar flow, discharge, or streams which at some point had a smooth, bedrock-controlled streambed in which flow was evenly distributed. These characteristics were essential as the method relies on the stream/input having a relatively flat bed, with flow no greater than 5.0m/s. The sampling sites where easily accessible, even in high flow conditions, and the majority of them have a depth of less than 60cm and are less than 2m in width.

Throughout the study a Valeport Model 801 (flat) electromagnetic flow meter (Figure 3.5) was used to gauge flow measurements. The specifications for the impeller can be found in Table 3.3. The process begins with measuring the width of the channel and setting regular intervals at which the meter would be placed in the stream (Figure 3.6), the meter would then take a flow measurements for 30 seconds and provide a value for the mean and standard deviation of the measurement. To ensure the accuracy of the readings, the values where used only when the standard deviation was ±10% of the mean flow value. This was checked in the field and if standard deviation remained

>10%, adjacent reaches with less turbulent flow were sampled. The process is repeated until all the readings are taken over the width of the channel.

Parameter	*Specification*
Range	±5m/s
Accuracy	±0.5% of reading
Sensing Volume	20mm Ø X 10mm
Minimum Depth	5.0cm

Table 3.3 Specifications of the Valeport model 801 (flat) electromagnetic flow meter

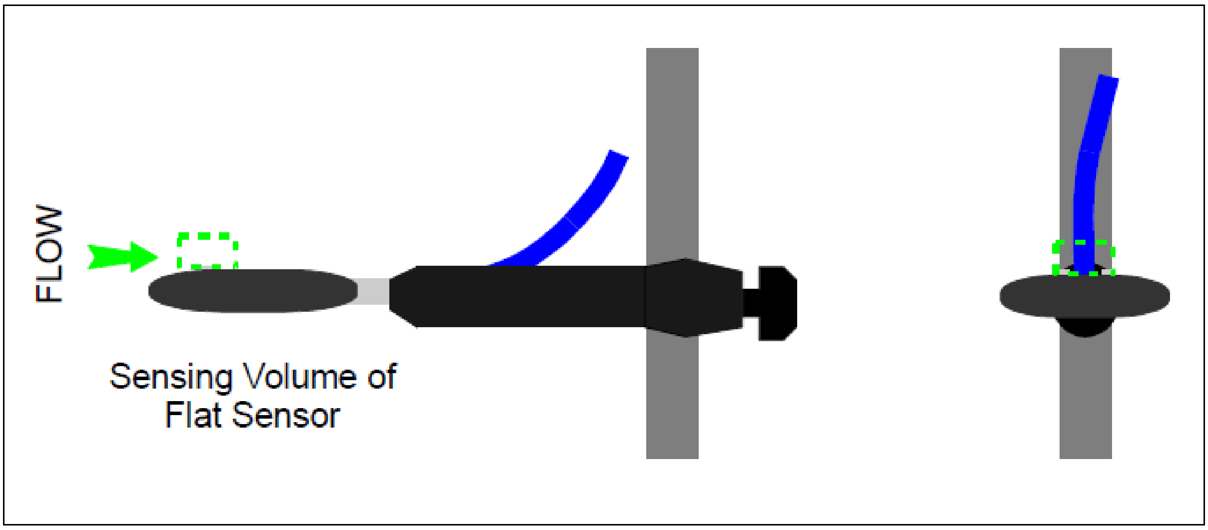

Figure 3.5 Illustration of velocity measurement area (after Gozzard, 2009).

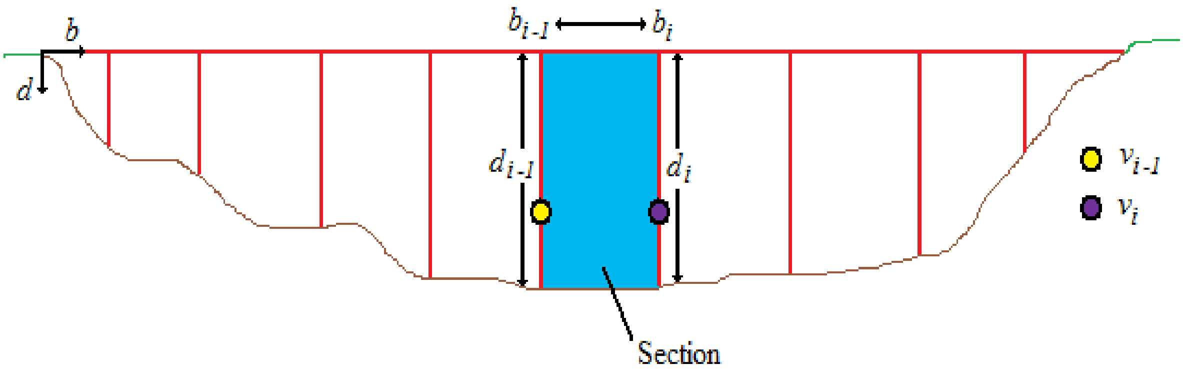

Figure 3.6 Mean section velocity method (Shaw *et al*, 2010)

This method essentially breaks the cross section of the stream into smaller parts, and takes the flow and the area of each of these smaller parts and adds them together. As the stream does not have a rectangular cross section (i.e. the streambed level changes through the cross section), this method provides a more accurate description of the flow. This is illustrated in Figure 3.6. Flow would be gauged at each vertical red line, two thirds of the way down, the depth of the stream and the distance from the stream bank. The derived values are then entered into the equation:

$$Q = \sum_{i=1}^{n} \frac{(\bar{v}_{i-1}+\bar{v}_i)}{2} \frac{(d_{i-1}+d_i)}{2} (b_i - b_{i-1}) \qquad (3.1)$$

Where;

Q = flow

n = total number of sections

v= mean velocity at measuring point

d = depth of water column at measuring point

b = width of section

In practice, minimum intervals in width readings were between 5 – 20 centimetres dependent on the total width of the stream or discharge being measured. Repeat flow measurements at the same site suggest a standard deviation of ±0.09 m/s. Acquiring flow data at numerous streams also facilitates metal loading calculations.

3.2.8.2 Load Estimation

Load estimation of discharges are simply calculated by taking the gauged flow, and multiplying by the concentration in mg/L, which gives a metal load in mg/s, which can then be used to give a figure in terms of kg/day or tonnes/year of metal load in the catchment.

$$\frac{Kg\ Me^{2+}}{day} = Q\ x\ [Me^{2+}] \qquad (3.2)$$

There has been an increasing focus on acquiring load based estimates of nutrients and contaminants in river systems as catchment-scale approaches to management are advocated (e.g. Jarvis and Mayes, 2012). Efficient land management and subsequent remediation needs to be carried out at discharges which are having the most impact on the environment. Concentration data alone does not allude to sources which are having the greatest impact on streams, so total metal load must be calculated to assess the impacts of different contributions to the stream (Kimball and Runkel, 2009). This calculation is particularly pertinent in areas with historic mine drainage, as the vast networks of adits and levels can lead to multiple discharges across a stream's catchment (Kimball, *et al.*, 2010), and also identify the contributions from diffuse sources (Mayes *et al.*, 2008). For example, two mine discharges have a concentration of 0.5mg/L Zn, so concentration data alone would suggest they have the same impact. However, one of the discharges has a substantially higher flow rate, thus giving it an increased metal load of Zn in kg per day, which means the higher flow discharge has more of an impact on in-stream Zn concentrations, thus this would be the discharge that would require remediation.

3.3 Laboratory Methods: Regime, Development and Justification

3.3.1 Introduction

This section of work will discuss laboratory methods. This will include detailed descriptions of initial biofilm batch experimentation, flume set up and subsequent experimentation including long term monitoring for metal removal, long term monitoring for biogeochemical and trace metal cycling and metal release experiments. The methods presented here relate to results presented in Chapters 5 and 6 of this thesis.

3.3.2 Biofilm and Water collection

Originally synthetic water with a similar water composition of major ions and trace metals to a point source mine discharge, such as Duke’s Level, was used in the initial flask experiments. Water was collected from a spring fed from a carbonate bedrock at Welton Beck, East Yorkshire (SE 965 275), in a 5L jerry can that had been acid rinsed and rinsed with the spring water three times before collection. Three 15ml samples were also collected, and immediately acidified with Ultrapure nitric acid for trace element analysis. Individual readings for each experiment are outlined in each experiment specific section below. In general, it was found that the calcium concentrations of the water from Welton Spring were around three times higher than the calcium concentrations at Duke's Level (Table 3.4), so water from the spring was diluted accordingly (1 part Welton Spring water : 2 parts distilled water) and other elements where made up to correct concentrations. Once mixed a sample of the water was immediately acidified and subjected to analysis trace element analysis via ICP-OES.

Ion	*Welton water Concentration (mg/L)*	*Duke's Level Concentration (mg/L)*
Ca	109.5	36.8
Ba	0.044	0.233
Zn	0.012	0.959
Mg	3.077	5.4
SO_4^{2-}	74.456	45.2

Table 3.4 Comparison of water types for experiment, the aim was to synthetically create water with the same concentrations of ions as water from Dukes Level.

3.3.2.1 Alternative water preparation

Due to failure of analytical equipment after the first batch experiments (ICPOES), which prevented instant quality assurance of the Welton Spring water, water was directly taken from Duke's Level (which site monitoring shows to have consistent chemistry under baseflow – see Chapter 4). Water was collected from Duke's Level thereafter for all experiments (Batch experiment 2, 3 and all flume experiments). It was also convenient to harvest biofilm from the Hebden Beck catchment at the same time as bulk sample water was obtained.

3.3.3 Flask experiments

Static flask or batch experiments are a very useful tool for answering a specific question, and were suitable to compare the effect different separate parameters had on metal concentrations in solution. For experiments using an agitated flask method, 150ml of premade solution Duke's Level water was placed into a 250ml conical flask with a set amount of wet biofilm (5g). Care was taken to ensure as much bulk water was removed from the biofilm. Biofilm was collected on the day it would be used to avoid any denaturing of the biofilm quality. Each experiment was left for 21 days in a controlled environment laboratory which was set up to simulate conditions in the UK (ambient air temperature set at 18^{o}C, with 8 hours UV light/day) (Pedley, 2013).

3.3.4 Flask Treatments

Before every experiment glassware was cleaned in a way in which as little contamination as possible occurred. All glassware was left in an oven at 125^{o}C overnight, then when cool enough to handle sterilised with ethanol, rinsed with 10% HNO_3, then washed out with UHQ (Ultra High Quality) water (18MΩ) prior to the experiment. For all flask experiments the shaker was set at 100 oscillations per minute. Flask treatments applied where as follows:

- Abiotic Vs biotic flasks – 4 sterilised flasks and 4 flasks with biofilm
- Light treatment – 4 point light scale ranging from no light to full light
- pH treatment – 3 point pH scale, to reflect pH seen during diurnal study 6.5 – 7.5 – 8.5 (see Chapter 4)
- Preliminary EPS study (not carried out in flasks, see section XXXX)

3.3.5 Flask sampling regime

The water from the flask was carefully decanted in such a way as to not disturb the biofilm. The flask water was sampled at the end of the experiment to determine concentrations of ions in solution. The sample was immediately acidified with concentrated analytical grade HNO_3.

3.3.6 Flume Experiments

It was important to create an environment in which physical conditions could be controlled to maintain biofilm colonies. Studying biofilms in natural settings (e.g. mine-impacted streams) would be ideal, but control of variables (e.g. flow, temperature, light intensity) is sacrificed, rendering potential finding inaccurate as responses of the biofilm to its environment are unpredictable (Pedley, 2013). The controlled environment laboratory suites at the University of Hull provided an area in which systems could be created to support biofilm colonisation, whilst ambient air temperature, hours of light, and flow could be maintained and controlled (i.e. so they can be ruled out as having an effect on precipitation of minerals and potential incorporation of trace metals into both mineral and biological samples).

3.3.7 Flume Design

The experimental set up comprised three flume systems (Figure 3.7), in which separate treatments could be established. The three flumes were identical with the exception of the fact that two of them ran from one large (60L) water butt, and the other one ran from an individual water butt (30L).

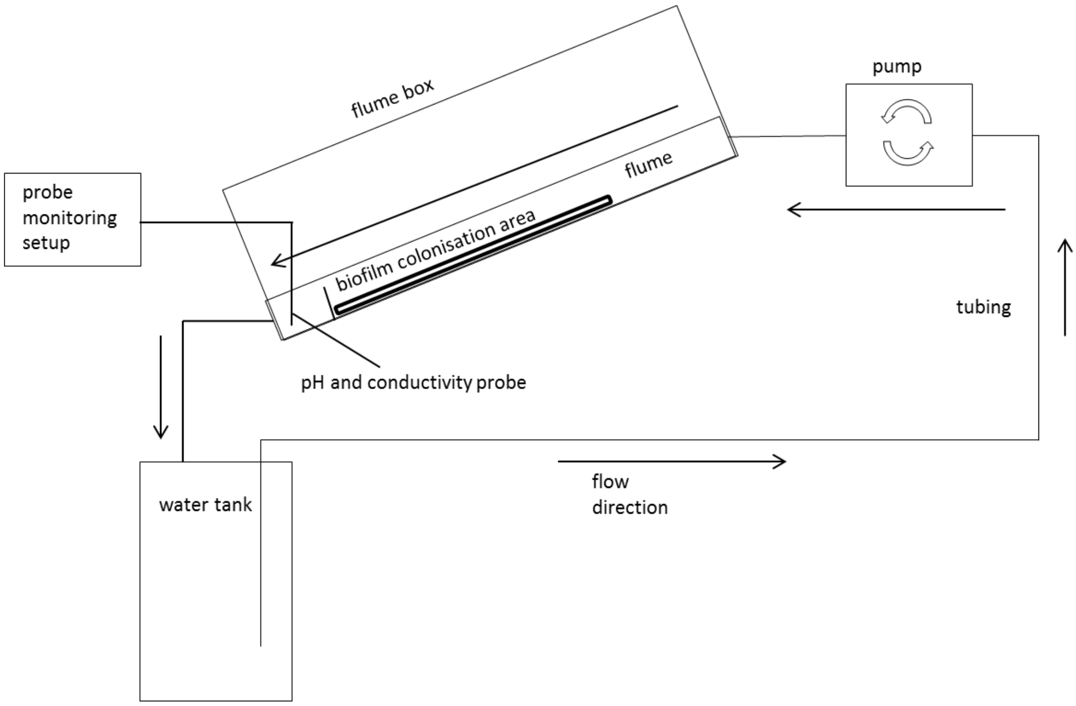

Figure 3.7 A schematic diagram of the flume set up in its recirculating format.

Water was stored in the water butt under the flume tanks, and water was drawn up to the flume itself through peristaltic tubing (1.6mm wall, 4.8mm bore) (Fischer Scientific) via a Waston Marlow 300 series tube pump, with four 313D pump head attachments (Watson Marlow, Falmouth, UK). The pump was set to 55rpm (0.1L/m), which allowed a thin coverage of biofilm and a smooth flow of water, ideal for flow estimations throughout the system and thus more accurate calculation of any metal removal rates. This speed also reduced the likelihood of the tubing ripping, and thus reduced the risk of potentially contaminating the experiments. 22mm pipe fittings where used to drain the flume box under gravity, and the water was returned to the water butt. The flume was housed in a Perspex flume box. The flume itself was uPVC guttering (Width: 14 cm, Length: 50cm), lined with scourer pads and rocks to allow an environment in which the biofilm could root and inhabit. Rocks placed in the flume were tested with acid to ensure they were not carbonate based (to prevent them dissolving in the flume), then cleaned in a 10% HNO_3 acid rinse, kept at 250^oC for twelve hours and finally rinsed thoroughly in UHQ water. The flume systems where subjected to a 8:16 day: night light cycle (light: Thorn Lopak 250 W HPS-T sodium lamp), in a windowless, air conditioned laboratory (ambient air temperature $18^oC \pm 1^oC$), which gave the best assurance that external environmental conditions would not interfere with the experiments. Before the flumes were put into use, everything was thoroughly cleaned to remove any impurities and sterilise the flumes of any bacteria that where present from the construction of the flumes. The water butts were scrubbed out with tap water, acid rinsed with 10% HNO_3, rinsed with ethanol, the swilled out with UHQ (18MΩ) water. Immediately the experimental water was put in it and the butt was sealed until it was ready to use. All tubing and connectors were also rinsed in ethanol and then immediately after UHQ (18MΩ) water was passed through it to remove any particles that could have settled in the tube. The flume boxes and flumes were completely cleaned with ethanol wipes and sealed before use. Once the whole cleaning procedure was complete the systems where connected, the biofilm placed in the flumes and the pump switched on.

3.3.7.2 Water Preparation and renewal strategy

Water for the flumes was directly taken from the mining discharge Duke's Level, as this would give the best representation of natural conditions, in 30L containers that had been acid rinsed and rinsed with the sample water three times before collection. Three 15ml samples were also collected, and immediately acidified with Ultrapure nitric acid for trace element analysis. The water was passed through a recirculating UV filter for 24 hours prior to introduction to the flume to remove any microbes that would compromise the integrity of the study. The flumes were all set-up with a recirculating configuration (i.e. the effluent would be recycled to become the influent) for both logistical reasons (i.e. the volumes of mine water required would be unmanageable if it was not recirculating) and scientific reasons. The latter include the value of testing metal removal rates over a range of influent concentrations, which with any attenuation within the system would obviously be apparent. However, it was necessary to renew the water periodically to maintain ion concentrations in the water typical of mine discharge sites. The water was renewed when new experiments were due to take place, to ensure the concentrations of ions were as close to actual discharge concentrations (at Duke's Level) as possible, most importantly that Zn concentrations reached between 1-1.5mg/L. Water would be collected and introduced to the flume with the same method as above before each experiment.

3.3.8 Justification: Zn and Ba as elements of interest

Waters used throughout all experiments had elevated levels of Zn and Ba, given the area from which waters where sourced having high concentrations of the elements. Initially Zn was the solitary choice as an element of interest, as the ion has already been shown to be highly toxic to aquatic life in mobile forms, also at these concentrations the stream can expect to fail WFD quality guidelines (see Chapter 2). Elevated Zn concentrations are ubiquitous throughout the wider study area, and discharges in the Hebden Beck catchment account for the majority of all Zn pollution in the Wharfdale catchment (Chapter 4).

It was also decided that Ba should be an element of specific focus, particularly for the fieldwork. Ba is also highlighted as being potentially toxic to aquatic life, although the lack of monitoring data and EQS concentrations has resulted in a relevant lack of literature surrounding this topic. Ba is ubiquitous throughout the fieldwork area, owing to barite (Ba_2SO_4) and a relatively rare (on the international scale) witherite ($BaCO_3$) mineralisation throughout the ore field.

The idea of both of these elements being of interest arises from their chemical variations, more specifically in terms of their ionic radii (or charge density). This leads to the two elements having distinctly different geochemical behaviour. Ba is also one of the more important ions, in terms of concentration (after Ca, Mg, and Na). The sulphate concentrations in Hebden Beck readily allow for barite precipitation out of the water column (PHREEQC (Parkhurst and Appelo 1999)), whereas there are no minerals Zn will readily form precipitates. Rogerson (2008) suggest chemoselective uptake based on ionic charge density, showing ions with lower charge densities are preferentially accumulated into the EPS matrix. Elzinga and Reeder (2002) further elaborate on uptake mechanisms into precipitates, and although on a laboratory-produced artificial calcite, they showed ions with a smaller radius than Ca will be incorporated into different areas of the calcite to ions with a larger radius.

Thus, choosing two ions that have different chemical traits will help to differentiate between the pathways for accumulation within the biofilm environment. Zn has a higher charge density (Charge/Radius (pm)) than barium, and their respective radii represent both smaller and larger radii than that of the Ca^{2+} (Table 3.5), thus making them ideal ions to analyse throughout the study.

Ion	*Charge*	*Radius (pm)*	*Charge Density*
Ba	2+	135	0.015
Zn	2+	74	0.027
Ca	2+	114	0.018
Mg	2+	86	0.023

Table 3.5 Descriptive data for ions of interest in experiments in descending order based on charge density.

3.3.9 Flume Sampling Strategy

3.3.9.1 Long term flume monitoring

The initial strategy for sampling the flume set up was to identify long term trends in metal removal from the water. An unfiltered 14 mL water sample was taken every three days to establish initial trends of total metal removal in the system. The sample was immediately acidified with a few drops of concentrated HNO_3 in preparation for trace metal analysis via ICP-OES. The control flume was also sampled in the same manner for comparison.

3.3.9.2 Diurnal Sampling Experiment

Diurnal cycles are prevalent in nature, as discussed in Chapter 2. The aim for this part of the experiment was to observe the same effects on trace metal concentrations in the flume water, as well as biogeochemical cycling of pH and conductivity. Conductivity and pH were monitored with electrodes being placed in the flumes to constantly monitor pH (Pinpoint pH monitor, American Marine Inc.) and conductivity (Pinpoint Conductivity Monitor – Freshwater Hardness, American Marine Inc.). pH and conductivity data was logged every 10 minutes via a webcam connected to a computer running Yawcam software, and then transferred into written data (into a .txt file) via Watchman software (Middleton, 2007). Running in conjunction with the pH and conductivity logging, 14ml water samples were taken from each of the biofilm flumes and a 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 ready for trace metal analysis via ICPOES.

3.3.9.3 Metal Release experiments

After the flumes had been operated to a point where biofilm had matured, experiments to test the permanence of metal removal were undertaken. Metal release and reversibility experiments relied on the mixing of “surface runoff” water with mine water discharge water to simulate a rainfall event. Surface runoff events in the Hebden catchment are acidic due to the peat land of the catchment surrounding the beck. A rainfall event was simulated by running the acidic “rain water” through the flume. This was achieved by the addition of a longer pipe running into the flume, allowing for two capillary tubes to enter it, and thus allowing for the control of water into the flume. The water was sourced from a surface stream (SE02677 64606). This simulation was carried out for 5 hours and then the acidic water was stopped from entering the flume and conditions were allowed to return to normal. The sampling regime was carried out as per the diurnal cycling sampling strategy.

3.3.10 EPS extraction and metal uptake study

The purpose of the EPS extraction was to examine at a molecular level how trace metals interact with the matrix. The introduction of chemicals to the extraction process increased the risk of contamination, which could have potentially hindered the investigation given the scale at which we investigated the EPS uptake properties with regard to trace metals.

Extraction techniques can be basic, with immersion and agitation in ultrapure water being considered as the most basic technique (Nielsen and Jahn, 1999), with some more complicated extraction techniques requiring days to perform (e.g. EDTA method: Comte *et al.,* 2006)). Issues begin to arise when the complex nature of biofilms are considered. EPS composition can vary greatly depending on microbial consortia, temperature and available nutrients (Flemming and Wingender, 2010), and biofilm type can also vary in different environmental settings, examples being sediment bound biofilms (Takahashi *et* al., 2009), sludge’s (Frølund *et al*., 1996; Park and Novac, 2007) and freshwater biofilms (Rogerson, 2009; Pedley, 2013). Given this large range of variability, which is reflected in Table 3.6, there is no universal method for EPS extraction (Nielsen and Jahn, 1999).Early studies identified differences in physical and chemical methods of EPS extraction on activated sludge, synthetic sludge and a *Klebsiella aerogenes* culture (Brown and Lester, 1980), it was noted that physical methods where capable of producing EPS yields whilst maintaining molecular integrity, whilst chemical methods tended to interfere with the EPS at such scales. Physical isolation of EPS from biofilm separates the two factions with minimal interference at the molecular level, interference which becomes an issue with chemical methods of extraction. Chemical methods of extraction rely on chemicals breaking down chemical bonds in the EPS matrix resulting in the separation of the EPS and microbial factions of the biofilm. A common problem with chemical methods is the contamination EPS samples, which could hinder further investigations with them. Flemming and Wingender (2010) note that extraction techniques utilising NaOH are “almost certain” to lead to contamination of EPS components, whilst Saunders (2012) noted that extraction using H_2SO_4 would lead to removal of $Ca^{2+}_{(aq)}$ through precipitation of $CaSO_4$, which would impact on further experiments which would use EPS. It was also noted that ultrasonication, whilst not releasing enough EPS to be an efficient EPS extraction tool on its own, did aid in releasing EPS so maybe be used as a preliminary treatment in conjunction with another method (Brown and Lester, 1980).

Extraction technique		*Method*	*Yield*
Physical	Ultrapure water	• mix 12ml ultrapure water and 5g biofilm • leave for 24 hours • filter through 11um and 0.2um filter papers	(1.5)[3]
	Centrifugation	• centrifuge cell suspension at 20000rpm at 4^{o}C for 20 minutes • filter supernatant through 0.22 um membrane	(7.20)[1]
		• mix 12g of ultrapure water and 5g biofilm in a centrifuge tube • agitate every 15minutes for 2 hours • centrifuge at 3300rpm for 15 minutes •	(4.1)[3]
	Heat treatment	• Heat biofilm at 80^{o}C for 1 hour • Centrifuge at 20000G for 20 minutes • Centrifuge at 10000G for 15 minutes • Purification by dialysis machine at 4^{o}C for 24 hours	(62-64)[2]
		• mix 24ml of ultrapure water with 10g biofilm and shake to mix • leave for 2 hours • place in oven (130^{o}C) for 2 hours (agitate every 20 minutes) • allow to cool and centrifuge at 3300rpm for 15 minutes • filter through 11um and 0.2um filter papers	(11.1)[3*]
	Ultrasonication	• Cell suspension sonicated at 40W in ice bath for 2 minutes • Centrifuge suspension at 20000rpm for 20 minutes at 4^{o}C • Filter supernatant through 0.22um filter membrane	(12.70)[1]
		• Sonicate at 40W for 2 minutes • Add to cation exchange resin and centrifuge at 600rpm for 1 hour • Centrifuge at 20000G for 20 minutes • Centrifuge at 10000G for 15 minutes • Purification by dialysis machine at 4^{o}C for 24 hours	(27-33)[2]
Chemical	Centrifugation plus H$_2$SO$_4$	• Complete centrifuge method [3] as above, set aside EPS • Remove centrifuged biofilm and add to 10ml flask • Add 8ml ultrapure 0.2M H$_2$SO$_4$ • Heat at 95^{o}C for 30 minutes and then allow to cool • Centrifuge at 3300rpm for 15 minutes • filter through 11um and 0.2um filter papers • add filtrate to previously acquired EPS and shake to homogenise	(11.3)[3]
	EDTA	• Subject cell suspension to 2% EDTA for 3 hours at 4^{o}C • Centrifuge suspension at 20000rpm for 20 minutes at 4^{o}C • Filter supernatant through 0.22um filter membrane	(164.50)[1]
		• Subject cell suspension to 2% EDTA for 3 hours at 4^{o}C • Centrifuge at 20000G for 20 minutes • Centrifuge at 10000G for 15 minutes • Purification by dialysis machine at 4^{o}C for 24 hours	(96-100)[2]
	Formaldehyde	• Subject Cell suspension to 36.5 formaldehyde at 4^{o}C for 1 hour • Centrifuge suspension at 20000rpm for 20 minutes at 4^{o}C • Filter supernatant through 0.22um filter membrane	(14.20)[1]
	Formaldehyde-NaOH	• Subject Cell suspension to 36.5 formaldehyde at 4^{o}C for 1 hour • Subject mixture to NaOH at 4^{o}C for 3 hours • Centrifuge suspension at 20000rpm for 20 minutes at 4^{o}C • Filter supernatant through 0.22um filter membrane	(114.70)[1**]
		• Subject Cell suspension to 36.5 formaldehyde at 4^{o}C for 1 hour • Subject mixture to NaOH at 4^{o}C for 3 hours • Centrifuge at 20000G for 20 minutes • Centrifuge at 10000G for 15 minutes • Purification by dialysis machine at 4^{o}C for 24 hours	(284-318)[2]

Table 3.6 Common EPS extraction methods and EPS yields. [1](Pan *et al*., 2010) (mg/g EPS/biofilm) [2](Comte *et al*., 2006) (mg/g dry weight EPS/suspended sludge solid) [3](Saunders, 2012) (mg of EPS extracted from 5g freshwater biofilm) [*]method taken from Barranguet *et al*. (2006)method taken from Liu and Fang (2002)**

Given the wide range of biofilms that the extraction techniques where used for, it was decided it would be beneficial to opt for the heat technique used in Saunders (2012) with the addition of sonication prior to the centrifugation step of the extraction technique. The biofilm used in that study are freshwater biofilm and inhabit natural freshwaters in a karst environment, which are of similar water composition to the biofilms used for this study. In order to assess the EPS uptake mechanisms is to assess the chemical interactions between the solution and the EPS molecules independent of any effects from the biotic part of the biofilm.

3.3.11 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 for effective metal removal. During the development of the laboratory flume system there were a number of practical obstacles that had to be overcome which offer useful insight to future field testing of biofilm systems. For the biofilm to colonise, a suitable substrate should be made available. The first issue encountered with the mesocosm set up was biofilm colonisation. Initially when developing this system, carbonic pads where 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 three 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 flooding events during summer 2012 led to the scouring (and thus removal) of biofilm communities from the roof slates left to colonise. Biofilm communities did colonise the stream within a month of the high flooding events ending, 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 used in the flume, as the porous nature of the pads allowed biofilm to attach quickly due to the high surface area. This stable substrate permitted rapid colonisation of biofilm within a few weeks. Biofilm colonisation of the flume mesocosm environment throughout the study period is shown (Figure 3.8).


A
B
C
D

Figure 3.8 (Page 96) Colonisation of biofilm over time (around 3 months). (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), testament to the ability of biofilm to attach and develop onto numerous strata. Note that this photo was taken from another angle hence the different flow direction. (C) Further biofilm development sees the biofilm "thicken" and grow into the bulk water column. (D) Biofilm at full maturity in this system. Not clear from the photo is the fact the biofilm has now developed as a thick layer in the bulk water, with no areas of bulk water remaining that are not subjected to biofilm colonisation. Also, the addition of the pH probe (blue) and conductivity probe (black) are shown.

The flow of water should also be controlled with high accuracy, as any changes in water flow rate can change functioning of the biofilm (Pedley and Rogerson, 2010). When low flow rates are applied to the flumes, care was taken not to allow water to "pool", as biofilm tends to ecologically and physically degenerate in stagnant water.

As previously outlined in the methodology, the laboratory was equipped with a UV lamp to simulate a day/night cycle. Both the length of cycle and light intensity had to be factored into the mesocosm set up, as the biofilm integrity could be affected if the light intensity was too strong for long periods of time, potentially killing the biofilm completely. Initial trial periods allowed for altering the UV light's position above the mesocosms and the length of the "day" period of the simulation. The light was fixed in position allowing for a maximum light intensity of 6.82 kLux, and was set for a period of 8 hours daylight, around that of a UK summer day. Although mid-latitude summer days are considerably longer than this, it is important to note there was no control on light intensity over time, meaning the light was either on or off, with no way of simulating light intensity changes over a natural photo-cycle. An important consideration was the effect of the light on the temperature of the flume set up, as to tease out the key, underpinning processes of the biofilm it was important that diurnal temperature fluctuations in the flume occurred. This could interfere with a range of factors in the flume tank, including viscosity of the water, changes in evaporation rates throughout a 24hour period and, probably the most important factor to this study, the precipitation kinetics of the water. The flume water maintained a temperature of 17^{o}C (±1 ^{o}C). The large water butts that stored the water would play a role in buffering the temperature of the water, as they placed out of the light and where in the laboratory for a long time prior to the experiment, to allow them to equilibrate with the ambient air temperature. Foil wrapping was added to the capillary tubes which were situated in the path of the light to stop them heating up in the UV light. Also, care was taken to place the light as far away from the flume as possible over the top of the flumes, in the beginning with a view of preventing denaturing the biofilm, but this would have also helped maintain the temperature of the flume. The nutrient requirements of biofilm are also an important factor in their use as a passive remediation option, as high nutrient requirements could require additional maintenance given the remote, upland location of many historic orefields in areas of low agricultural intensity with generally low nutrient status. At the Hebden Beck site where biofilms were collected ambient nutrient concentrations were low in both the stream and mine waters (NO3- 3.2mg/L; soluble reactive P: <0.01mg/L; K: 3.5mg/L: Environment Agency, 2013), yet the stream would be dominated by biofilm in summer months (see Supporting Information), a characteristic feature 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 utilising 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. The EPS itself is a complex element of the biofilm, meaning it is slow to naturally biodegrade (Decho, 2010), and certain factions of bacteria have the ability to depolymerise their own EPS and reutilise it as a food source (Saunders, 2012).

3.4 Laboratory methods

3.4.1 ICP-OES Reconnaissance samples

Analysis for concentrations of all major and mine related cations in water samples taken through all studies were carried out using a Perkin Elmer OES optima 5300DV Inductively Coupled Plasma Optical Emissions Spectrometer (ICP-OES). All samples were analysed undiluted, the ICP OES can select different wavelengths so concentrations of individual isotopes will not go above detection limits. The ICP OES is capable of either radial or axial viewing, which, given the wide range on concentrations available from the samples was necessary to prevent saturation of the detector (particularly a problem with Ca wavelength 393.33)

Before every analysis, a 2 point calibration (0 – 10ppm) of 69 elements was undertaken. Standards were made using ROMIL Primag®-xtra element reference solutions (1000ppm ±3ppm) which are traceable to the United Kingdom Accreditation Service approved ROMIL laboratories (subject to ISO17025). All element reference material contained in a 0.5M HNO_3 matrix. There could not be only one standard made up from all 69 elements as there would be a risk of elements precipitating out of solution. To avoid this 5 standards were made (Table 3.7); all of the elements used in each standard were used in 10ppm aliquots, except in standard 2 where Cs was used as a 100ppm aliquot due to the ICP machine having a low sensitivity. Detection limits for each wavelength of every individual element where calculated from the blanks using the Relative Standard Deviation (RSD). The RSD was calculated by taking the average of the three blank calibration sample means, and multiplying it by three to acquire a Limit Of Detection (LOD) (measured in ppm).

3.4.2 Laboratory samples

The experimental samples were analysed using the Perkin Elmer OES optima 5300DV Inductively Coupled Plasma Optical Emissions Spectrometer (ICP-OES) as well. However, a new method was employed that would focus the attention of the machine onto the cations of interest.

A 4 point calibration was employed with standards containing all the elements that were of interest to the flask and flume experiments, this would help to improve the accuracy of the machine. A concentrated sample was created which was then diluted in a 1:10 ratio to create the “upper” standard, 0.5:10 ratio to create the “middle” standard, and 0.1:10 ratio to create the “lower” standard (Table 3.7). All standards were contained in a 2% ultrapure HNO_3 matrix. The final point on the calibration was 2% ultrapure nitric acid (point zero).

Cation	*Standard concentrations (mg/L)* *Concentrate*	*Upper*	*Middle*	*Lower*
Ca	500	50	25	5
Mg / S	100	10	5	1
K / Na/ Zn	50	5	2.5	0.5
Ba / Sr / Fe / Pb	10	1	0.5	0.1
Mn / Al / Cu / Cd	1	0.1	0.05	0.01

Table 3.7 Standards and subsequent concentrations used for calibration

3.4.3 Diurnal Cycling samples

The diurnal cycling experiment samples where then subjected to Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) analysis via an ICP-MS ELAN DRC II for Zn concentrations. The ICP-MS is capable of lower detection limits (0.1 - 1ppb) than the ICPOES, thus giving a more precise result after data analysis (in terms of the graph). Another advantage of the ICP – MS is the internal standard (10ppb Ga, In, Bi in a 2% HNO_3 matrix), which can account and rectify instrument drift throughout the analysis, increasing instrument precision. It accounts for changes in viscosity of water samples. A sample with a higher conductivity is more viscous, which creates bigger water droplets in the aerosol delivery of the machine. With a constant internal standard being introduced to the sample the ICPMS can compensate for this discrepancy. A 5 point calibration was employed with standards containing Zn, in order to achieve the highest accuracy possible, only Zn concentrations where analysed for the samples. A concentrated standard of 10ppm Zn was created which was then diluted accordingly to create the standards listed in Table 3.8. All standards where contained in a 2% ultrapure HNO_3 matrix. The final point on the calibration was 2% ultrapure nitric acid (point zero).

Cation	*Standard concentration*				
	Concentrate (ppm)	*Upper (ppb)*	*Upper Middle (ppb)*	*Lower Middle (ppb)*	*Lower (ppb)*
Zn	10	1000	750	500	250

Table 3.8 Standards and subsequent concentrations used for ICP – MS calibration

3.4.4 SEM (Scanning Electron Microscopy)

Samples were taken at different points of analysis to be dried for visual analysis under a Zeiss SMG EVO 60 microscope (Carl Zeiss SMT Ltd., Cambridge, UK) with an Oxford Instruments INCA energy 350 energy dispersive X-Ray spectrometer probe (Oxford Instruments, High Wycombe, UK). Dried samples of interest were then glued to SEM mounts, carbon coated (around 10nm thick) to remove charging effects to the sample and then observed under an SEM.

3.4.5 XRF (X-ray fluoroscopy)

XRF was performed on various dried biofilm samples for identification of element concentrations in a solid form. Samples where over dried at 50^{o}C for 24 hours then ground to a fine power using a pestle and mortar, the powder was then transferred to a sample vessel. The sample vessel consists of a plastic casing with an area in which the sample powder can be placed. The powder is contained in the vessel with a packing material (cotton wool), and a Mylar TF-160-255 6µ gauge X-Ray film. This film is designed to produce as little interference as possible when the sample is submitted to X-rays. Prior to the analysis the pestle and mortar is given a 10% nitric acid rinse, a rinse with deionised water and finally a rinse with ethanol. In between each sample the pestle and mortar are rinsed with ethanol to avoid any cross contamination between samples.

3.4.6 Charge Balance

In order to maintain the accuracy of analysis of the concentrations of anions and cations, a charge balance formula can be employed. The charge balance formula is;

$$\text{charge balance}(\%) = \left(\frac{\sum \text{cation} - \sum \text{anion}}{\sum \text{cation} + \sum \text{anion}}\right) \text{x} 100 \qquad (3.3)$$

The percentage balance should equal zero, which would indicate that there are equal amounts of cations and anions in the water sample. However, a charge balance of 0% can be unobtainable, so a charge balance of ±5% is considered to be an ideal outcome for this quality control test, with a charge balance of ±10% considered to be acceptable (Appelo and Postma, 2005). A selection of the SI data acquired via PHREEQC is available (Appendix 1).

3.5 Data Analysis

3.5.1 Correlations

Correlations were performed on data using Minitab v15. All data subjected to correlations where first tested for normality using the Kolmogorov- Smirnov test for normality. Data conforming to normality ($p>0.05$) were correlated using Person's correlation and data not conforming to normality ($p<0.05$) using Spearman's rank correlation. For assessing differences in metal removal in flask experiments, average removal rates between different treatments were tested using the Kruskal Wallis test, given removal rate data did not conform to a normal distribution, even after log transformation (Kolmogorov-Smirnov $p<0.05$).

Principal Component Analysis (PCA) is a technique that can be employed to identify important factors and sources contributing to differences in water quality, and demonstrating the geochemical processes responsible for water composition (Parizi and Samni, 2013). Specifically for this study, PCA analysis was useful in grouping water types over large areas, as has been done in the reconnaissance part of this study, where different geological conditions can bring about differences in water composition. Data were log-transformed prior to PCA as is standard for the procedure (e.g. Mayes *et al*., 2011).

3.5.2 Spatial analysis

ArcGIS v9.1 and 9.3 was used for all spatial analysis and mapping throughout the study. Catchment areas where acquired using multiple Digital Terrain Models (DTMs) which were subsequently merged to cover the whole area. Catchment extractions were carried out to identify all possible sources it contained, and was acquired using the procedures;

- Fill Sinks – Tells the model that rivers will not flow up hill, and that "sinks" will occur in the DTM
- Flow direction – Identification of flow paths in the area throughout the DTM
- Flow accumulation – further characterisation of flow. Identifies areas of preferential flow across the DTM (channels).
- Stream network definition – Defines streams with relevant flows, which can then be given a stream order (Strahler, 1957).
- Catchment extraction – creation of a "pour point" (the bottom of the catchment/ catchment outlet). All streams and rivers contributing flow to the pour point are identified as the catchment, which can be extracted as a shapefile for mapping purposes and spatial analysis.

GPS co-ordinates were acquired for discharges, and subsequently mapped, alongside shape files for watercourses, bedrock and superficial geology and mineral veins. Calculations of Oreshoot density were also performed in catchments (Chapter 4).

3.5.3 Geochemical modelling

Solute data were analysed using the geochemical code PHREEQC v.1.5.10 (Parkhurst and Appelo, 1999) with the WATEQ4F database (Ball and Nordstrom, 1991) to calculate saturation indices of relevant mineral phases. The WATEQ4F database is particularly suitable for this study given it was derived largely from mine-impacted systems and, crucially, contains thermodynamic data for an extensive range of relevant Zn mineral phases.

4

4.0 Mine water geochemistry and metal flux in a major historic Pb-Zn-F orefield, the Yorkshire Pennines, UK

4.1 Introduction

As Chapter 2 highlighted, mining processes expose sulphide minerals (e.g. pyrite (FeS_2), galena (PbS), sphalerite (ZnS)), which are weathered in the presence of oxygen and water, to release sulphate and the associated metal ion in solution. The oxidation of pyrite (Eq. 1) creates an acidic environment in the mine (Wolkersdorfer, 2008), which can both increase further dissolution of metal-bearing ores and the mobility of trace metals in the water column (Younger *et al*., 2002). While the terms *acid rock drainage* and *acid mine drainage* prevail in the literature (e.g. Younger *et al*., 2002; Sarmiento *et al*., 2012) this is not a true reflection of drainage chemistry in areas with carbonate-rich bedrock. Here, the acidic metal rich water can be buffered during transit to the surface from dissolution of carbonates (notably limestone and dolomite) (Eq. 2), resulting in metal rich, circum-neutral pH discharge (Nuttall and Younger, 2002; Rudall and Jarvis, 2012).

$$FeS_{2(s)} + \frac{7}{2}O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4{}^{2-} + 2H^+ \qquad [4.1]$$

$$CaCO_{3\,(s)} + H^+ \rightarrow Ca^{2+} + HCO_3^- \qquad [4.2]$$

Recent reviews have shown that up to 6% of river catchments in England and Wales are impacted by abandoned mine discharges (Mayes *et al.*, 2009), with many catchments in Cornwall and Devon (Bowen *et al*., 1998), Wales (Fuge *et al*., 1991) and the North Pennine Orefield (Younger, 2000) shown to have elevated in-stream metal concentrations (notably Zn and Cd). While such national or regional exercises have provided useful data for identifying the most polluted catchments, there are numerous research challenges that remain to ensure that limited public funds can be effectively directed to maximise the length of streams remediated and deliver a healthier water environment. These include overcoming data disparities between regions, which currently limit the effectiveness of prioritisation methods. Some UK regions have far more detailed site inventories and data availability (in terms of contaminant loadings) than others, reflecting efforts of local agencies or research institutions (e.g. Environment Agency, 2002). Prioritisation tools typically rank catchments based on known mine sites and chemical and ecological impacts (e.g. Jarvis and Mayes, 2012). Where scale-appropriate data are not available from ambient monitoring records, catchments that are in fact heavily impacted such impact assessment methods will fail to register as high priorities. Similarly, lack of information about the character of pollution sources in terms of geochemical composition, contaminant loading can disguise the real state of impact of specific sites on in-stream contaminant fluxes. It is clear that accurate characterisation of sources is essential to underpin design of effective remedial tools (e.g. Jarvis and Mayes, 2012) and to discern the relative significance of point discharges to diffuse sources (e.g. Mighanetara *et al*., 2009; Banks and Palumbo-Roe, 2010; Gozzard *et al*., 2011).

This chapter provides a multi-scale assessment of mine water and in-stream geochemistry across a globally important, yet understudied lead-zinc-fluorite orefield: the Yorkshire Pennines, UK. A range of approaches from regional mine water quality surveys through to catchment-scale mass balance estimates and analysis of mine waters at source are used to: (1) assess the geochemical nature and extent of pollution from metal mines across the Yorkshire Pennine orefield, (2) highlight key sources of metal flux arising from mine sites, and (3) describe key catchment-scale solute processes in a circum-neutral pH mine-impacted catchment.

4.2 Methods

The methods presented here are chapter specific, and a full range and in depth explanation of all methods used can be found in the methodology chapter (Section 2.0).

4.2.1 Study site

The Yorkshire Pennine Orefield covers an upland area of central, northern England (Figure 4.1). The country rock of the area is of Namurian age (Westphalian to Asbian Stages of the

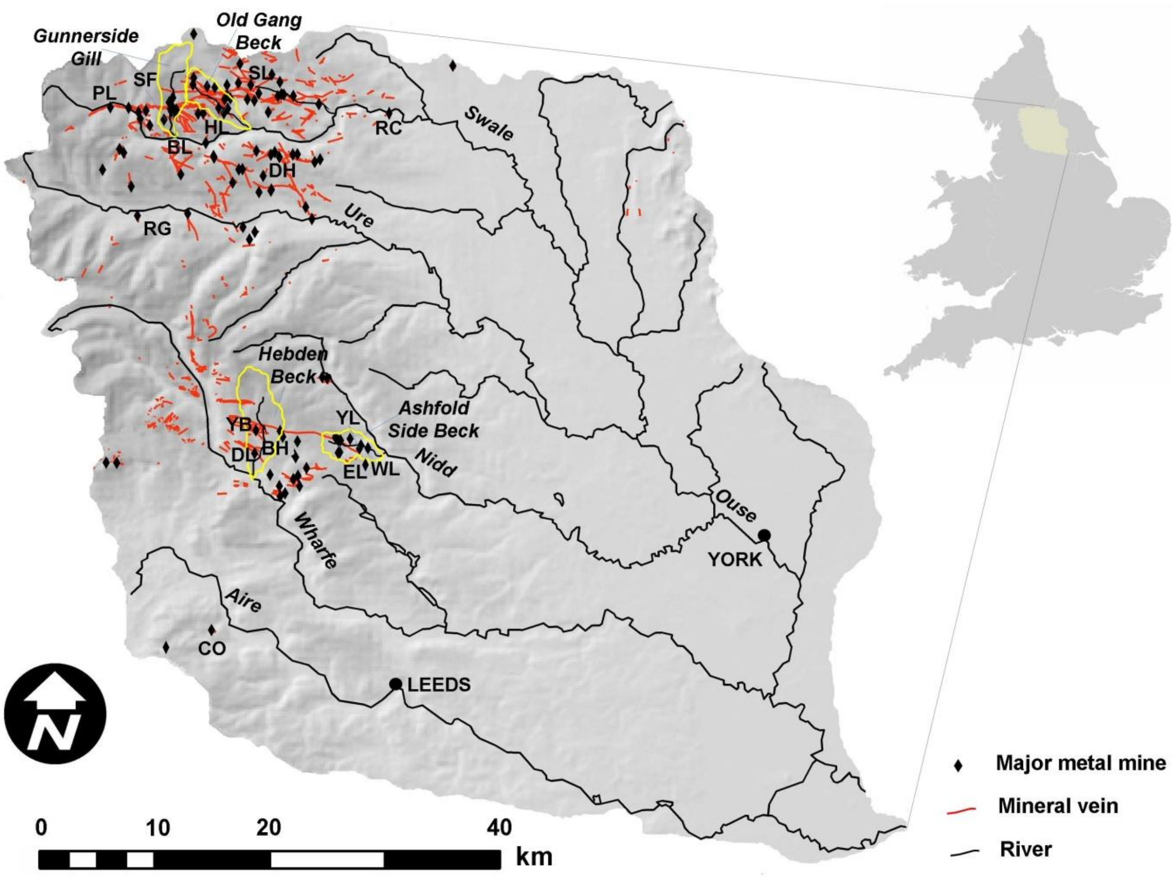

Figure 4.1 Physical setting of the Yorkshire Pennine Orefield. Abbreviations for selected annotated mines referred to in text or figures: BH: Bolton Haw Level; BL: Bunton Level; CO: Cononley Pb Mine; DH: Devis Hole Level; DL: Duke's Level; EL: Eagle Level; HL: Hard Level; PL: Parkes Level; RC: Richmond Copper Mines (Billybank Level); RG: Raygill Level; SF: Sir Francis Level; SH: Shaw Level; WL: Wonderful Level; YB: Yarnbury surface runoff; YP: Yarnbury Pond; YL: Yorke Level.

Carboniferous) and encompasses a Caledonian granite intruded into lower Palaeozoic basement rocks, with a cover of Carboniferous rock around 1.5km deep (Smart and Clayton, 1985), dominated by limestone with subordinate sandstone and argillaceous rock, the former of which is heavily karstified in places. The area exhibits extensive fault controlled mineralisation (Dunham and Wilson, 1985), and mineral transport is aided by the chemical reactivity of carbonate rocks in the presence of mineral bearing solutions (Nuttall and Younger, 2002). The most important economic mineral of the area is galena (PbS), although large quantities of other primary minerals sphalerite (ZnS), fluorite (CaF_2), barite ($BaSO_4$) and a relatively large amount of witherite ($BaCO_3$ - quite rare at international scale) are found across the region. A small area of copper ore (chalcopyrite) is apparent in the north east of the region (Dunham and Wilson, 1985). Over a million tonnes of metal ores, predominantly lead with smaller amounts of zinc and copper, have been mined in the area since Roman times (Dunham and Wilson, 1985). The majority (~1 million tonnes) of this was lead concentrates, with 140,000 tonnes of fluorite as well as smaller tonnages of sphalerite, barite and witherite being worked (Palumbo-Roe and Colman, 2010). The areas of mineralization are found in the upper reaches of four main river systems, the Swale, Ure, Nidd and Wharfe. Outliers of galena and barite mineralization are apparent to the south west of the region in the upper Aire (Cononley Pb mine and Raygill barite mine respectively). All the rivers drain into the Humber Estuary in eastern England (Figure 4.1). Land use in the catchments is dominated by upland grazing, with some areas of afforestation (notably in Wharfedale; Hey and Winterbottom, 2006) and open moorland with extensive peat deposits.

4.2.2 Sampling Regime

Mine site identification was undertaken through a desk study utilising mineralogical and geological accounts (e.g. Dunham and Wilson, 1985), a range of industrial archaeology resources (mine plans and online gazetteers: e.g. Northern Mines Research Group, 2012) and Ordnance Survey topographic and British Geological Survey geological maps to identify mines which were potentially discharging metal rich water into streams in the area.

Synoptic sampling (as per Mayes *et al*., 2008) was undertaken through synchronous flow and water quality measurements in the Hebden Beck sub-catchment of the Wharfe to identify the contribution of point discharges to in-stream contaminant flux. Flow was also recorded at each site so flux of trace metals (i.e. the product of concentration and flow) entering the stream could be calculated, for this a mean section velocity-area method was employed (Shaw *et al*., 2010). A Valeport 801 electromagnetic flow meter with flat sensor was used and is particularly suited to the nature of the discharges which predominantly arose from engineered mine drainage structures with smooth, shallow flow (see Chapter 3).

At each sample station, environmental parameters; pH, oxygen reduction potential, dissolved oxygen, conductivity, temperature and alkalinity were measured *in situ*. All the variables excluding alkalinity where recorded on a Hanna HI 9828 multiparameter meter, calibrated before each sampling visit. The in-field total alkalinity measurement is by titration following the fixed endpoint method (Hach reference 8203) using 1.6N H_2SO_4 with bromocresol green-methyl red indicators (to pH 4.6). Three water samples were collected in low density polyethylene leak proof bottles; one unfiltered sample for total cations and two filtered (0.45µm) samples for dissolved cation and anions. The two cation samples where immediately acidified to preserve metal concentrations. Cation samples were analysed using a Perkin Elmer OES optima 5300DV Inductively Coupled Plasma Optical Emissions Spectrometer (ICP-OES). The ICP-OES was calibrated using standards made on the same day, with concentrations ranging from 0 – 10ppm. A blank (2% UHQ HNO_3 (Romil Spa)) and standard suite were analysed every 10 samples, to check the calibration was maintained throughout, while Certified Reference Material was used to check instrument accuracy. Detection limits for each wavelength (selected according to USEPA Method 200.7) of every individual element where calculated from the blanks using the Relative Standard Deviation (RSD). Anion (F^- and Cl^-) concentrations were determined using a Palintest 8000 photometer. In order to maintain the accuracy of analysis of the concentrations of anions and cations, charge balance testing was employed (as in section 3.4.6)

4.2.3 Data analysis

Solute data were analysed using the geochemical code PHREEQC v.1.5.10 (Parkhurst and Appelo, 1999) with the WATEQ4F database (Ball and Nordstrom, 1991) to calculate saturation indices (SI) of relevant mineral phases. All statistical analyses were undertaken in Minitab v15. Data were not normally distributed even after log-transformation (Kolmogorov-Smirnov: $p < 0.05$) so non-parametric tests were used. Arc GIS® v9.3 was used for spatial analyses which included terrain analysis (as per Mayes *et al.*, 2008) for calculations of drainage area, Oreshoot density (total number of mineral veins / catchment area) and stream order (Strahler, 1957).

4.3 Results and Discussion

4.3.1 Major ion and physical chemistry

Discharge waters throughout the area are fairly consistent in terms of major ion geochemistry, and reflect conditions that are indicative of groundwater from a carbonate host rock (Figure 4.3). Temperatures recorded ranged from 6-12^{o}C, and all had circum-neutral pH (6.18 – 7.96). The pH distribution is typical of many metal mining regions in the UK and particularly those in carbonate-rich country rock (Figure 4.2), which include all the Pennine orefields, Halkyn-Minera in north Wales, West Shropshire and Mendip (Mayes *et al*., 2009a).

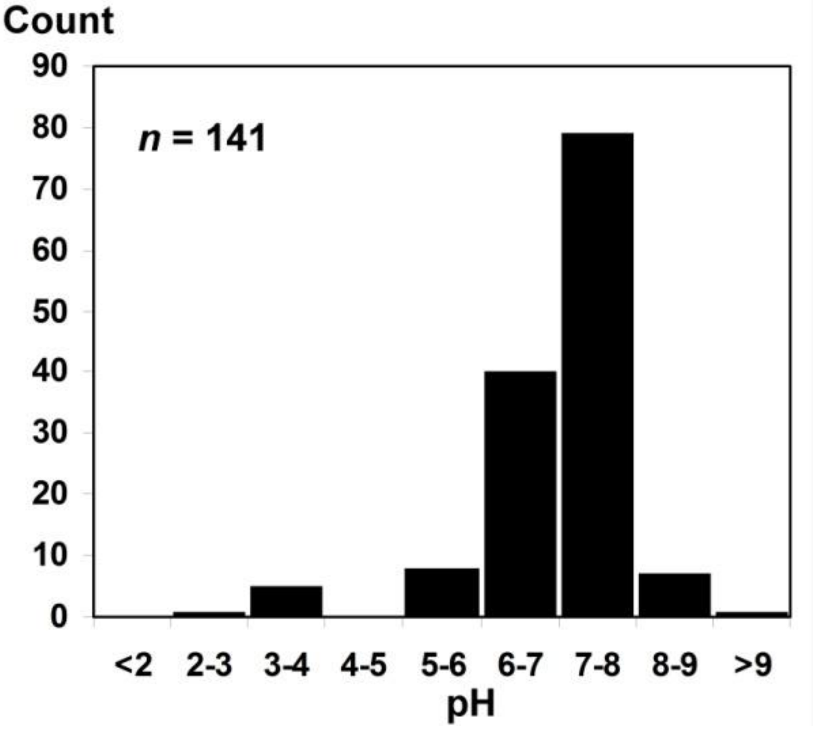

Figure 4.2 Frequency distribution of metal mine water pH for discharges across England and Wales (data adapted from Mayes *et al*., 2010, incorporating data from this study)

Throughout the Orefield there is a predominance of Ca–HCO_3–SO_4 type waters (Figure 4.3), although slight differences are apparent between the two main areas of mineralization, which occur over two separate catchments; Swaledale and Wharfedale. Swaledale discharges typically have a lower total alkalinity and Ca^{2+}, with higher sulphate concentrations than Wharfedale discharges (Figure 4.3; Table 4.2).

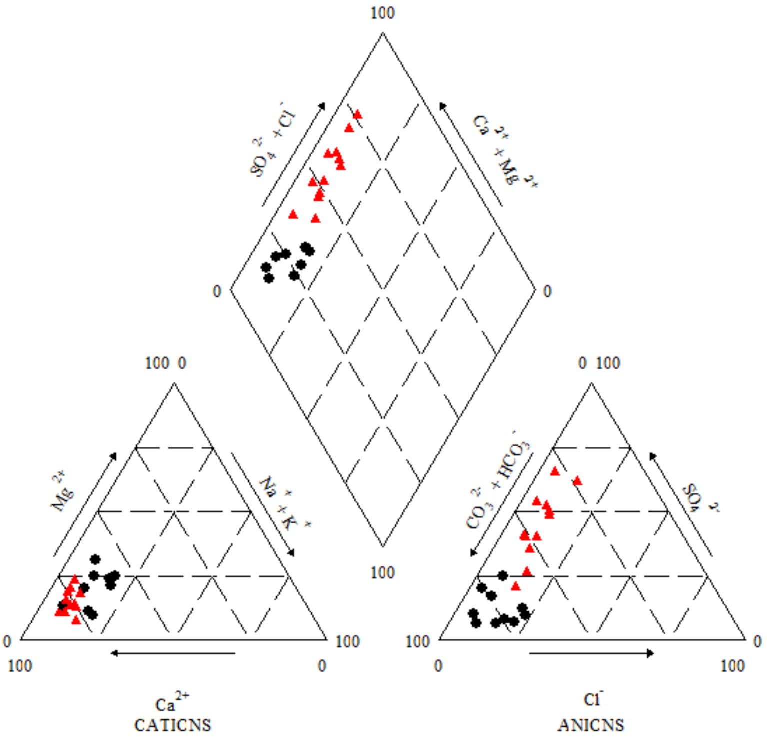

Figure 4.3 Piper Diagram showing difference in major ion water composition between two catchments: Swaledale (Red) and Wharfedale (Black).

Ore-bearing strata differ between these areas, with mineral veins penetrating more recent strata (Grassington Grit of Late Pendleian (Silesian) Age) in the Ashfold Side / Hebden area. This may be due to the absence of a thick shale band at the intra-Pendleian unconformity that prevails across the northern part of the region and is thought to have prevented rising mineral fluids penetrating into younger strata in the Swale and Ure catchments (Dunham and Wilson, 1985). However, the Namurian country strata do not differ significantly in mineral facies and the differences are likely to be controlled by the greater Oreshoot density in the Swale (mineral veins/km^2) and Ure compared to the Wharfe (as seen in Table 4.1) and the subsequent products of sulphide mineral weathering. This is also alluded to in the conductivity data, which shows higher conductivities in the Swaledale catchment, which would be expected given the presence of a higher Oreshoot density. Other major ions are present in consistently low concentrations (Na^+: 5-25mg/L; K^+ 0.5-5mg/L; Cl^-: 7-40mg/L) with peak concentrations occurring at the Raygill Barytes mine and Cononley Pb mine, where urban sources (e.g. highways runoff) are likely to be of greater prominence.

4.3.2 Minor elemental composition

The key metal contaminants consistently present in the mine water discharges by dissolved concentration are Zn > Pb > Cd and again, distinctions can be made based on mine location over the two catchments. Piper plots are a useful tool for characterising waters in terms of major ion geochemistry, however, on their own they do not identify key differences in mine water composition due to the lack of minor ion analysis. PCA analysis can further help in differentiating water types between catchments as seen in Figure 4.4. Generally, mine water discharges in the Swaledale catchment tend to be characterised by higher concentrations of trace metals (Cd, Zn, Pb) and sulphate, whilst Wharfedale waters are typically lower in Cd, Zn and Pb but characterised by higher Fe and Mn concentrations.

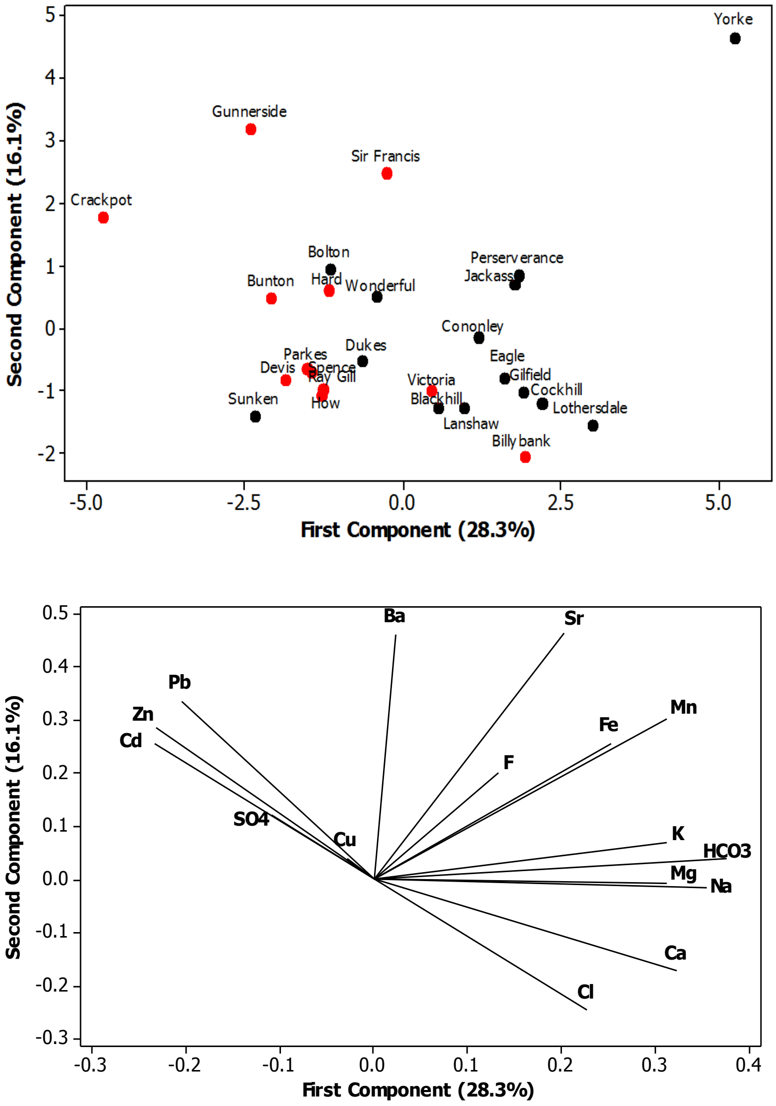

Figure 4.4 Principal component analysis of trace metal concentrations in catchment waters. Figures show PCA by site (top image), also distinguishing between two catchments (Red – Swaledale, Black – Wharfdale) and eignenvectors for analysed elements (bottom image).

To explain this difference in water composition, knowledge of Oreshoot density and mineralogy in the catchment should be considered. Oreshoots are numerous throughout the catchments, owing to the karstic nature of the bedrock which allows for transportation of mineral rich solutions, primarily due to the reactive nature of carbonate rocks associated with karst geology. Spatial analysis of the two catchments revealed although similar number of worked veins exists, the oreshoots in Swaledale are considerably longer in their totality (1793.5 km) than in the Wharfedale catchment (884.0 km), and in a smaller area, leading to a higher concentration of oreshoots in Swaledale (3.6 km/km^2) as opposed to Wharfedale (1.1 km/km^2).

Catchment	*Catchment area (km^2)*	*Oreshoots (count)*	*Mineral Veins (total length km)*	*Mineral Vein density (km/km^2)*
Swaledale	499.4	236	844.0	3.6
Wharfedale	758.9	246	1793.5	1.1

Table 4.1 Oreshoot densities across two catchments.

PCA analysis did reveal some exceptions to the previous statement. For example, discharges in the Hebden catchment (Duke's Level, Bolton Haw, Sunken level) have recorded high concentrations of trace metals; Duke's Level in fact records up to 87 – 98% of the point source contribution of metal loadings for the Wharfedale catchment, primarily due to the productivity of this section of the orefield (Jones *et al*. 2013; Dunham, 1985). It is interesting to note the eigenvector analysis for trace metals over the two catchments being generally dictated by oreshoot density. Trace metals associated with oreshoots of Pb and Zn sulphides can be seen to be on the left of the plot along, whilst trace metals associated with incorporation into calcite as impurities are on the right of the plot with Ca and HCO_3^-. The first four components are significant (based on scree analysis and a 10% individual threshold: Atanackovic *et al*., 2013) account for 67.9% of the variance on the data, which is typically of geochemical datasets (e.g. Mayes et al., 2011). On component one (which plots on the x axis), major ions Ca, Mg, Na, K, and HCO_3^- account for most the variability (based on eigenscores

>0.3). Much of the variation in sites on component 2 (y axis) relates to differences in Sr and Ba concentrations (eigenscores >0.4).

Only occasionally (Yorke Level, Gillfield and Jackass Level are notable exceptions) is Fe present in elevated concentrations above 500μg/L at values at which benthic smothering by

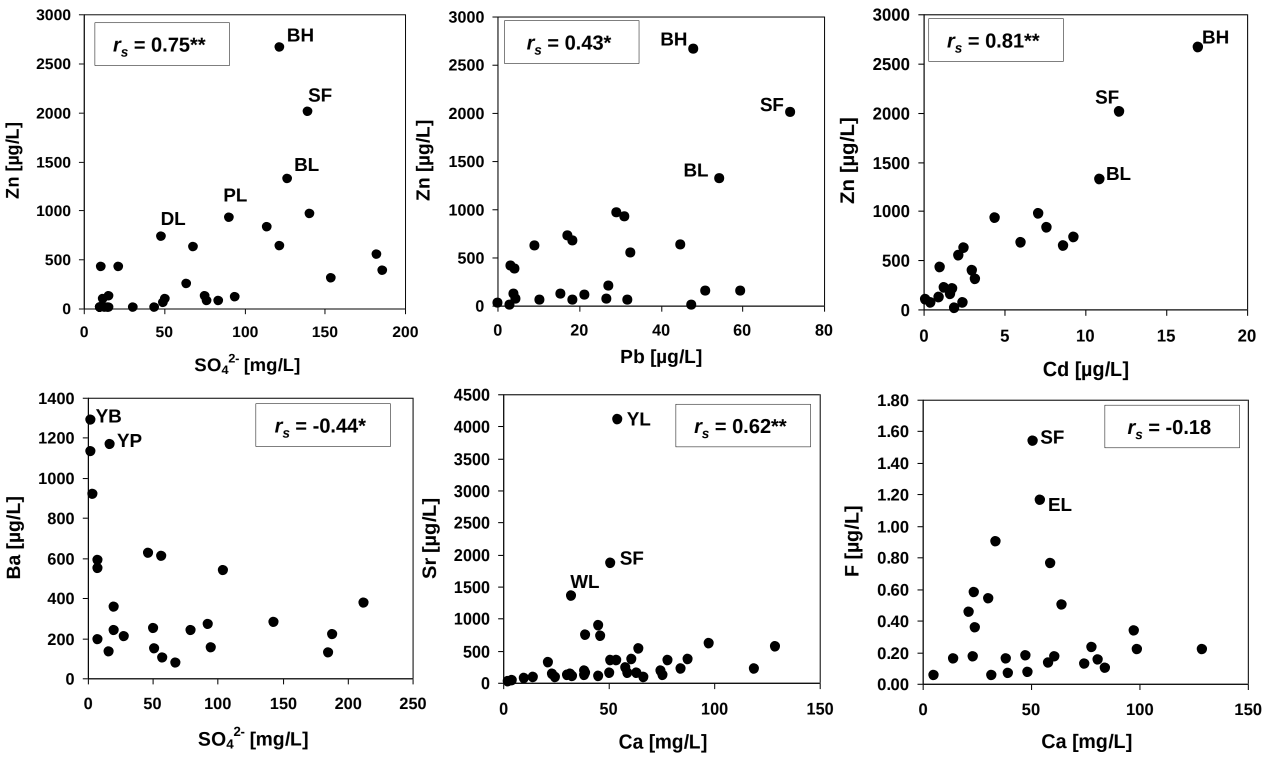

Figure 4.5 Relationships between selected common contaminants and major ions in monitored mine water discharges. * denotes correlation significant at p <0.05; ** denotes correlation significant at p <0.001; n = 28 for all. (values reported below LOD are included at detection limits in correlations).

ferric oxyhydroxides may be anticipated (Younger *et al*, 2002). While pyrite, marcasite (FeS_2) and to a lesser extent bravoite ($(Fe, Ni, Co)S_2$) are abundantly distributed through the orefield, they are usually in minor amounts in veins and flots (Dunham and Wilson, 1985) and in far lesser quantities than adjacent orefields (Dunham, 1990) where Fe-rich mine discharges are more common (e.g. Nuttall and Younger 1999). Mn and Fe correlate well (r_s 0.85; p<0.001) (Figure 4.6) with peak concentrations occurring in the discharges of Ashfold

Site	*Flow*	*pH*	*E.C.*	*Ca^{2+}*	*Mg^{2+}*	*SO_4^{2-}*	*HCO_3^-*	*F^-*	*Fe*	*Mn*	*Zn*	*Cd*	*Pb*	*Sr*	*Ba*	*Cu*	*Calcite*	*Barite*	*Hematite*	*$Fe(OH)_{3(a)}$*	*$Fe_3(OH)_8$*
Billybank	0.16	7.9	564	85.8	16.9	55.1	218	0.1	<1	<1	74	3	<1	381	70	25	0.5	0.2	n/a	n/a	n/a
Blackhill Level	5.2	6.18	337	51.7	9.1	12.2	149	0.8	216	18	102	2	<1	84	78	3	0.2	-0.2	18.8	3.1	1.2
Bolton Haw	0.18	8.48	210	51.0	6.4	15.4	161	-	22	53	2900	16	30	832	203	3	0.76	0.55	16.7	2.1	3.3
Bunton Level	2.0	7.22	243	39.0	3.1	95.1	109	0.6	<1	1	1285	10	54	718	145	<1	-0.8	0.9	n/a	n/a	n/a
Cock Hill Level	5.2	7.3	514	75.2	6.2	18.4	175	0.9	37	55	135	<1	26	107	350	<1	-0.1	0.5	16.9	2.2	0.0
Cononley Pb mine	4.8	8.02	447	55.8	6.2	35.2	145	0.1	64	35	686	10	18	273	239	3	0.32	0.62	15.8	1.51	2.27
Crackpot	0.01	6.83	200	27.6	2.2	56.8	28	0.1	<1	2	2914	32	91	242	213	3	-1.9	1.	n/a	n/a	n/a
Devis Hole Level	31.2	7.1	159	24.5	2.5	70.8	90	0.2	240	2	112	<1	28	82	115	1	-1.2	0.7	18.4	3.0	2.9
Duke's Level	14	6.38	324	53.8	5.4	45.2	200	0.2	3	4	959	6	15	352	220	2	-1.4	0.3	10.4	-1.1	-7.8
Eagle Level	54	7.61	406	51.7	16.4	12.7	191	1.2	89	9	94	2	<1	253	212	<1	0.0	0.1	17.9	2.6	0.5
Gillfield Level	3.4	7.82	439	63.2	5.9	21.0	153	0.5	227	147	63	<1	17	152	239	<1	0.3	0.4	18.8	3.1	1.5
Gunnerside Gill discharge	0.1	6.99	228	35.1	3.6	79.3	95	0.9	18	2	679	6	183	446	971	21	-1.1	1.5	15.9	1.5	-1.6
Hard Level	12.2	7.76	280	43.5	5.2	212.2	147	0.6	12	6	541	3	33	720	372	2	-0.2	1.5	16.4	2.0	-1.6
How Lead Level	2.0	7.3	216	33.0	3.6	51.8	121	0.5	20	3	246	<1	1	105	145	<1	-0.8	0.7	16.5	2.1	-0.4
Jackass Level	0.8	7.2	195	35.5	9.2	20.3	184	0.2	900	175	50	5	73	465	92	2	0.0	0.0	19.4	3.0	2.4
Lanshaw Level	1.5	6.2	358	53.0	9.3	12.2	264	0.1	<1	1	59	2	<1	388	194	<1	-0.5	0.1	13.4	n/a	n/a
Lothersdale	12.2	8.18	525	75.2	5.0	36.1	198	0.4	54	50	75	2	20	445	129	3	0.74	0.27	16.8	1.87	3.14
Parkes Level	5.7	6.5	312	47.4	7.0	65.2	106	0.2	30	19	951	4	29	282	70	3	-1.2	0.4	15.5	1.6	-0.59
Perseverance Level	1.2	7.3	325	48.2	11.5	26.7	147	0.3	470	224	78	3	<1	425	428	<1	-0.4	0.8	19.1	3.2	2.9
Ray Gill Level	37.9	7.8	201	31.8	1.8	31.5	109	0.1	262	7	7	<1	47	148	44	3	-0.2	0.0	18.9	3.0	1.2
Sir Francis Level	12.4	6.75	362	52.4	8.1	104.7	211	0.8	14	3	2003	12	72	1807	544	<1	-0.9	1.4	15.1	1.3	-1.6
Spence Level	1.09	7.8	207	33.5	2.7	56.8	106	0.2	28	8	74	<1	4	172	395	2	-0.3	1.1	17.1	2.3	-0.9
Sunken Level	0.08	7.51	147	28.4	1.2	6.9	43	-	15	1	26	<1	<1	89	181	1	-0.87	-0.03	16.3	1.79	-1.86
Victoria Level	0.1	7.96	403	62.2	10.3	185.6	221	0.3	5	4	9	<1	<1	520	125	1	0.3	1.00	15.7	1.6	-3.2
Wonderful Level	0.9	6.64	273	32.0	7.8	28.3	118	0.6	7	56	811	7	<1	1274	205	<1	-1.5	0.6	1.6	1.3	0.1
Yorke Level	3.2	6.78	439	53.2	11.5	46.5	277	0.8	903	334	281	<1	<1	4033	619	<1	-0.7	1.1	18.9	3.1	3.7

Table 4.2 Major physico-chemical characteristics, major and minor elemental composition and selected saturation indices for spot samples of mine discharges under baseflow condition. Flow in L/s; E.C: electrical conductivity (µS/cm^2); Major ions (Ca, Mg, SO^4, HCO^3, F) in mg/L; Minor ions (Fe, Mn, Zn, Cd, Pb, Sr, Ba, Cu) in µg/L.

Side Beck (up to 224 µg/l Mn at Yorke Level).

4.3.2.1 Zinc

Zn is ubiquitous at modest to high concentrations (up to 2,900µg/L) at all mine discharges (Table 4.2). Zn exceeds current Environmental Quality Standards (EQS: 50-75µg/L) at the hardness encountered in many of the receiving streams (Unpublished Environment Agency data, 2012). Significant positive correlations are apparent between Zn and Cd, Pb and SO_4 (Figure 4.6), which is consistent with the weathering products of galena and sphalerite, the latter of which is a common uneconomic mineral in galena deposits. Cd is consistently found as an impurity in sphalerite (up to 1.4% Cd: Dunham and Wilson, 1985), while the very strong correlation between Zn and Cd is also indicative of their similar geochemical mobility in oxidised waters (Langmuir, 1997). Cd levels exceed 10µg/L at discharges in Gunnerside Gill (Sir Francis Level and Bunton Level) and Hebden Beck (Bolton Haw Level; Table 4.2), and is an issue of particular regulatory focus given its status in Europe as a Priority Hazardous Substance (European Commission, 2008) which must therefore be phased out to background levels. The annual average EQS for Cd in these rivers is 0.08-0.09mg/L (hardness-related).

4.3.2.2 Lead

Pb is found in dissolved concentrations up to 70µg/L, with peak concentrations again occurring at Sir Francis Level in the upper Swale catchment. This is evident in the PCA analysis (Figure 4.4) with the eigenvectors for Pb being largely associated with Swaledale discharges, e.g. Crackpot and Gunnerside. While galena is the most widely abundant mineral in the orefield, Pb mobility is limited in oxidised mine workings by the formation of surficial secondary minerals such as cerussite ($PbCO_3$) and anglesite ($PbSO_4$) which limits further weathering (Dunham and Wilson, 1990). Furthermore, the low solubility of Pb minerals (Langmuir, 1997) means that even when it is released in modest to high concentrations at point of discharge, these do not necessarily translate to high in-stream concentrations. Environment Agency data show that Pb exceeds the EQS (7.2μg/L) in several local mining-impacted rivers (unpublished Environment Agency data). Furthermore, concerns have been raised about the extensive legacy of Pb-contaminated floodplain sediments across the study region (e.g. Dennis *et al.*, 2009, Coulthard and Macklin, 2003; Hudson-Edwards *et al.*, 2000, Macklin *et al.*, 1997). Recent estimates suggest that 155,000 tonnes of Pb are stored in alluvial sediments in the Swale catchment (Dennis *et al.*, 2009).

4.3.2.3 Copper

Cu is present either in modest or below detectable concentrations (10 μg/L) at the majority of sites (Table 4.2). While chalcopyrite ($CuFeS_2$) is widely distributed within other sulphides, it is usually present in volumes less than 0.1% (Dunham and Wilson, 1985). At the Billy Bank (near Richmond) discharge which drains the most notable Cu deposit in the region, Cu concentrations are 25 μg/L. Cu is a regulatory concern primarily due to the sensitivity of salmonids to Cu, which can affect olfactory cues at concentrations close to or below Environmental Quality Standards (e.g. Sandahl *et al*. 2007). However, water quality issues in this area as a result of Cu mine discharges are likely to be minimal given the low point source flux (6.5g Cu/day), the hardness of the water (which limits bioavailability) and the high dilution capacity of the Swale at the

Billy Bank discharge (mean flow of 10.4 m^3/s: CEH, 2012), giving in-stream concentrations of Cu that are well below EQS and detection limits (<1 µg/L).

4.3.2.4 Barium

The concentrations of Ba in mine discharges are in the range of 140 to 1290 µg/L (Table 4.2). Although direct comparisons are limited by data availability (Ba is not ordinarily measured during ambient monitoring by many environmental regulators) the concentrations of Ba are raised relative to other sites in the UK and further afield (Table 4.3). $BaSO_4$ appears to control Ba solubility, as evidenced by the super-saturation with respect to $BaSO_4$ at most mine

Ba (mg/L)	*Discharge*	*Reference*
0.04-0.970	Range from Pb-Zn-Ba mines in Yorkshire Dales, UK	This study
0.002-0.039	Bituminous coal mine discharges, Pennsylvania, USA	Cravotta (2008)
0.002-0.035	Zn-Pb mine, Santa Lucia, Cuba	Romero *et al*. (2010)
0.083-0.316	Pb-Zn-Ba mine discharges in Sunart, Scotland, UK.	Davidson *et al*. (2005)
0.279 (range: <0.01-23.0)	Close House $BaSO_4$ mine, Upper Teesdale, UK	Environment Agency ambient monitoring data (median of 143 samples 1982-2000)
<200	Coal mine discharges, Silesia, Poland	Pluta (2001)
0.001-0.1	Various coal and Pb mine discharges, Derbyshire, UK	Banks *et al.* (1997)
0.1-3.5	Natural mineral waters, Central Romania	Tudorache *et al*. (2010)
0.4-7.8	$BaSO_4$ precipitating spring, Stinking Springs, Utah, USA	Bonny and Jones (2007)

Table 4.3 Barium concentrations in various mining (and related) discharges.

discharges (Table 4.2) and the significant, albeit rather noisy negative correlation between Ba and SO_4^{2-} (Banks e*t al.*¸1997). As such there are unlikely to be any immediate quality issues associated with elevated Ba at the ambient pH and redox conditions of the streams. Similar to Pb, there may be potential for downstream issues of remobilisation from sediments under suitable redox conditions (e.g. Merefield, 1976). However, such conditions are likely to be limited to estuarine sediments where the highly reducing conditions required for elevated barite solubility are likely to be encountered, as has been seen in areas contaminated by $BaSO_4$ when deployed for fluid density control in drilling operations (e.g. Carbonell *et al.*, 1999).

4.3.2.5 Strontium

Figure 4.6 shows a strong and significant positive correlation between Sr and Ca which reflects a consistent ratio between the elements in the Carboniferous limestone and Millstone Grit country rock and is likely to be an indicator of the groundwater residence time for the discharges (i.e. subterranean "prior precipitation" of calcite and aragonite; Fairchild *et al.*, 2000). At a small number of sites, particularly in the Hebden Beck and Ashfold Side Beck sub-catchments of the upper Nidd, outliers associated with exceptionally high Sr concentrations above 4000 μg/L are apparent (Table 4.2). This probably reflects areas of barite mineralization, into which Sr is found to be substituted into, across the region (Dunham and Wilson, 1985) and to a lesser extent localised strontianite mineralization in Wharfedale (Dunham and Wilson, 1985). This is illustrated in the PCA analysis, which shows the eignenvectors for Sr being associated with the Wonderful, Yorke and Sir Francis levels, all of which had high concentrations of Sr. Such high strontium contamination are not typically encountered in metal mine discharges (e.g. Davidson *et al.*, 2005), and reflects the unusual geochemical characteristic of the mine waters in the region. There is no correlation between Ca and F although there are notable outliers of F enrichment occurring in the sub-catchments of Hebden Beck, Ashfold Side Beck and Old Gang Beck. These are areas of notable fluorite deposits and locations where some re-working of legacy lead mining spoil for CaF_2 and $BaSO_4$ took place during the mid to late 20th Century (Dunham and Wilson, 1985).

4.3.3 Mine workings: Secondary minerals as internal sinks?

Mine	*Moles of SO_4^{2-}/ day*	*Moles of Zn /day*	*Molar ratio SO_4^{2-}: Me^{2+}*	*Kg Zn leaving mine per day*	*% Zn lost from mine per day*
Wonderful Level	22.6	1.0	20.8	0.06	4.8
Yorke Level	131.9	1.2	11.8	0.08	8.5
Duke's Level	346.1	30.4	10.1	2.0	9.9
Cockhill Level	85.4	0.9	31.6	0.06	3.2
Eagle Level	616.8	6.7	21.3	0.44	4.7
Devis Hole	663.2	4.6	21.7	0.30	4.6
Sir Francis	389.4	32.8	10.2	2.15	9.8
Bunton Level	57.1	3.4	15.7	0.22	6.4
Hard Level	773.2	8.7	63.5	0.57	1.6
Parkes Level	264.0	5.7	41.6	0.37	2.4

Table 4.4 Metal deficits in selected metal mine water discharges in the Yorkshire Pennines

A useful tool for assessing the nature of the weathering products of metal-rich minerals is the molar deficit approach proposed by Younger (1999) and Nuttall and Younger (2002). Dissolution of sulphide minerals should yield molar concentrations of sulphate and associated metals stoichiometrically consistent with weathered minerals (e.g. pyrite, as a bisulphide mineral, would yield two moles of sulphate for one mole of iron). As such, assessment of molar ratios in discharged mine waters can shed light on mobility of key contaminants within the oxidation zone. By aggregating the molar quantities for all metals predominantly occurring from sulphide ores (in this case Fe, Zn, Pb and Ba: Dunham and Wilson, 1990) and comparing those with the sulphate released (SO_4: Me_{total}) it is apparent that not one of the primary discharges in the region have ratios close to unity (Table 4.4). Although there are discharges with reasonably low ratios (SO_4: $Me_{total} < 15$ was apparent at Duke's Level, Yorke Level, and Sir Francis Level), they still suggest large excess sulphate fluxes leaving the mines. These observations are consistent with those of other workers (Younger, 1999; Nuttall and Younger, 2002) and are usually ascribed to attenuation of metals on/within secondary minerals (Zn-bearing phases in particular) within mine sites, given the more conservative geochemical behaviour of sulphate compared to most divalent metals at ambient pH (Younger, 1999). Barite would represent the major feasible sink for sulphate within the mines (Table 4.2: gypsum and goslarite (not tabulated) were well below saturation in all samples with highest values of -1.4 and -6.1 respectively) but this would have a corresponding effect on the SO_4: Me_{total} ratio. The sinks for metals are likely to be more widespread. While geochemical modelling suggests mine discharges are saturated on occasion with only calcite, barite, and iron oxide minerals (Table 4.2) this does not preclude saturation of phases such as hydrozincite and smithsonite (which are mildly under-saturated upon emergence: values -4 to -1.5) within the mine workings. This is consistent with mineralogical accounts of the area which document extensive secondary Zn mineral deposits (notably smithsonite), which were themselves worked in areas to the west of the study region (Dunham and Wilson, 1985). Furthermore, secondary Fe minerals (such as hydrous ferric oxides and goethite), present in some of the discharges, are noted in mineralogical surveys of the mines (Dunham and Wilson, 1985) and are also known to be an efficacious sink for divalent metals (Dzombak and Morel, 1990). Such secondary minerals are clearly important at limiting concentrations at point of discharge with the deficit data suggesting a median of 95.3% (range = 90.1% - 98.4%) of metals being attenuated within the mine workings.

4.3.4 Metal flux

In many mine pollution surveys, the principal focus has traditionally been solely on water quality, and rarely are synchronous flow measurements obtained (e.g. Piramid Consortium, 2003, Younger *et al*., 2002). This limits our ability to compare the significance of mine pollution sources on in-stream metal fluxes. Consequently, many workers have recommended approaches to mine risk assessment and remediation planning that include routine flux measurements (e.g. Kimball *et al*., 1999) particularly as part of catchment scale studies. Flow rates at the discharges had a median of 2.7 L/s (range 0.1-22 L/s) under baseflow conditions which is within the typical range for many upland metal mine discharges (Mayes *et al*, 2010). To highlight the significance of individual sources, the cumulative frequency of Zn flux (given the ubiquity of Zn in the discharges) is shown in Figure 4.7. The frequency distribution is heavily skewed by a small number of sites that discharge the bulk of the metals to these headwater rivers.

Such patterns are typical of other orefields, where large gravity-driven drainage levels are used to dewater overlying mine sites (e.g. Younger *et al*, 2004; and Fanfani, 2002; Shepley, 2007; Gozzard *et al*., 2011) and are preferential contributors to in-stream metal flux. In this case, the Sir Francis Level and Duke's Level in particular are prominent. The latter is a major drainage system about 5 km in length that was driven in the 18th Century to underdrain numerous lead mines in Hebden Beck and on Grassington Moor (Dunham and Wilson, 1985).

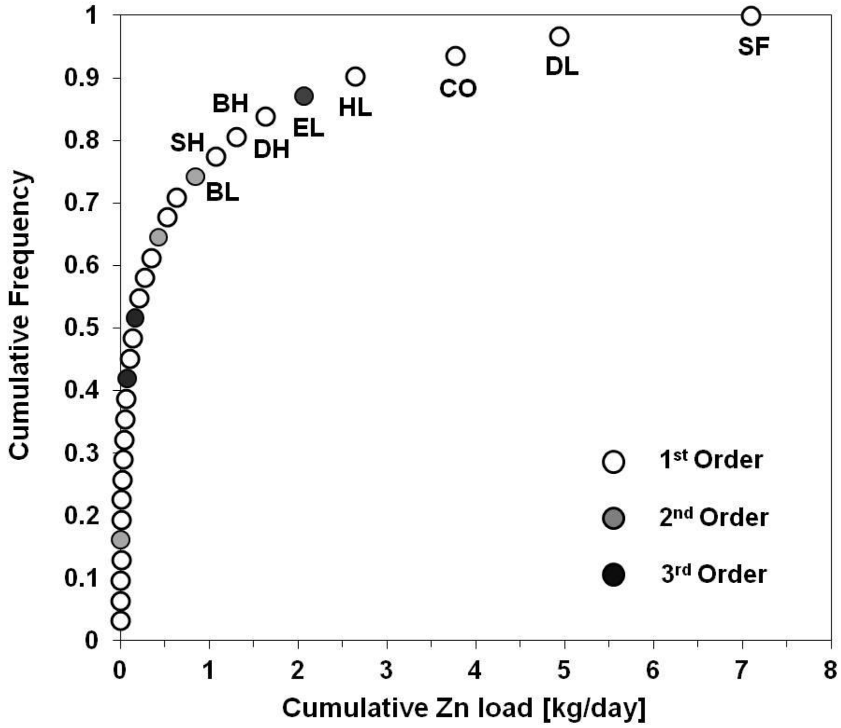

Figure 4.6 Cumulative frequency of Zn flux arising from monitored point mine water discharges. Strahler (1957) stream order derived from terrain analysis shown in colour ramp.

Although the total flux of metals arising in the Yorkshire Pennine Orefield constitutes less than 4% of the national metal mine point source pollution burden for Zn (Mayes *et* *al*., 2010), the location of the mining area in the headwaters of five major upland streams makes them particularly important. Stream order is also presented in Figure 4.7 to illustrate the relative size of the receiving streams. The vast majority of mine sites discharge into small first order headwater streams, where baseflow dilution capacity is minimal. Of the sites with highest flux, only the Eagle Level and the Shaw Water Level discharge into larger 2nd or 3rd order streams. Furthermore, all the receiving streams are of excellent hydromorphic quality, with gravel-bed substrates suitable for salmonids while the area drains a National Park and a large expanse of Special Area of Conservation (under the EU Habitats Directive) providing numerous potential stakeholder issues to consider in remedial planning (e.g. Environment Agency, 2002). The karstic nature of bedrock geology, particularly in Wharfedale and the Upper Nidd, presents additional issues with many headwater areas being ephemeral during base flow. This is the case in the upper reaches of Hebden Beck (which are entirely ephemeral) and Old Gang Beck where the river source rises from abandoned mine workings and thus has a greater bearing on in-stream water quality. The influence of mining on changing the distribution of surface water is an issue that is understudied considering the impact on water quality it can have. However, the significance of mine-induced changes on groundwater flow has been noted in other studies which have demonstrated changes in catchment drainage areas and flow regimes (e.g. Vaht *et al*., 2011) in addition to spring flow derogation (e.g. Younger *et al*., 2004).

4.4 Case Study: Hebden Beck

4.4.1 Synoptic sampling

Synoptic sampling of metal loads can yield useful information about both the relative importance of mine discharges to in-stream contamination as well as contaminant mobility. The Hebden Beck sub-catchment was chosen for repeated metal load-based surveys due to its high ambient in-stream metal concentrations (Unpublished Environment Agency data). Data from six separate sample days that represent a good range of flow conditions in the catchment are summarised in terms of the point source contribution to in-stream Zn flux at the catchment outlet under different flow conditions (Figure 4.7).

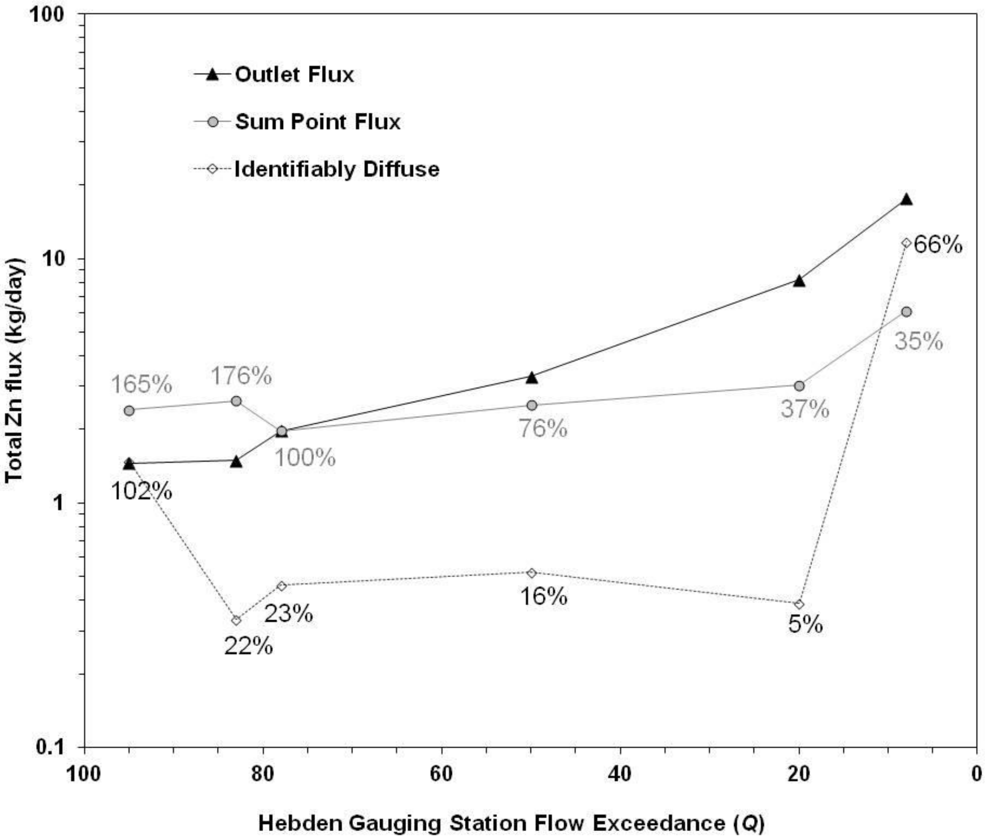

Figure 4.7 Output Zn flux with flow condition in Hebden Beck. Data also highlight the flux from point mine water discharges as well as identifiably diffuse sources from sub-catchments with large waste-rock inventories. Annotations show % contribution of point source (grey) and identifiably diffuse sources (black) as a percentage of outlet flux. Flow exceedance data derived from long term flow duration curve available at CEH (2012).

Under base flow conditions the point source dissolved Zn flux exceeds that at the catchment outlet (by up to 176%), with Duke's Level contributing between 87-98% of the point source contribution. This highlights the importance of Duke's Level as a source for in-stream Zn. The baseflow patterns also highlight that significant attenuation of dissolved Zn (~1kg Zn/day) occurs in the lower reaches of the catchment. As water flow increases, the absolute Zn flux from point sources rises, reflecting ingress of surface waters into the workings and flushing of metals from the oxidation zone as noted by other workers (Nordstrom, 2009). However, the relative contribution of the point sources to the catchment outlet flux diminishes as the importance of diffuse sources increases, but only under flow conditions greater than about Q_{50} (i.e. flow at the outlet that is exceeded 50% of the time). At the highest flow sampled (Q_8), the contribution of the point discharges to catchment outlet flux is less than 35% (of which Duke's Level contribution is 96%) and the contribution from key tributaries draining large expanses of un-vegetated waste rock and tailings (termed 'identifiably diffuse') becomes more pronounced. The Coalgrove Beck and Yarnbury sub-catchments were the main sources of Zn under high flow, both of which drain large expanses of fine spoil material which contain up to 1.2% Zn in Coalgrove (Dunham and Wilson, 1985). Such assessments are useful in targeting key sources of in-stream metal flux. As with all catchments where such exercises have been undertaken, the significance of diffuse sources under high flow is appreciable (e.g. Mighanetara *et al*., 2009; Banks and Palumbo Roe, 2010; Gozzard *et al*., 2011).

4.4.2 In stream biotic survey

Biotic interactions play an important role in the sinking of trace metals within the stream environment. In low order streams, such as Hebden Beck and its tributaries, plants, mosses and algae are able to dominate the stream bed due to the shallow stream morphology and the prevalence of low flow conditions. Biotic uptake of metals is well documented in the literature, in particularly the use of reed beds for the uptake of trace metals in mine water remediation systems, where large inventories of trace metals have been documented being sunk by reed species (Younger, 2000, Mayes *et al*., 2009). However, reed beds are usually a part of a man made, engineered system, and do not play a major role in natural attenuation in the system sampled in this study.

The baseflow samples taken that showed significant attenuation of dissolved Zn (Figure 4.7) were taken in summer months. The attenuation mechanisms likely to dominate in this system are either sorption onto the stream substrate or potentially uptake by the extensive benthic algae that dominate the in-stream biota (Fuller and Harvey, 2000). During reconnaissance surveys biofilm was observed dominating the stream bed, particularly in the upper reaches of the stream, where the stream bed has the physical features to allow biofilm to flourish. A shallow stream depth allows for a low velocity flow environment which encourages biofilm to settle (and can help to prevent scouring of the biofilm in high flow conditions), and the limestone bedrock streambed and rocks provide a solid strata for biofilm colonisation. Of particular importance was that the prevalence of observable biofilm was not limited to the streams and tributaries, with many of the point sources of mine water discharge also being heavily colonised by biofilm, as seen at the Dukes Level Discharge. Trace metal attenuation within biofilms is potentially an important natural sink, and thus spot samples were taken during a reconnaissance visit. Spot samples of digested biofilm from Hebden Beck showed Zn concentrations with a median of 1800 ppm (range 800-4100 ppm; n = 12) compared to a median of 0.47 ppm (range 0.08 - 1.28 ppm; n = 12) in the surrounding ambient water, suggesting considerable bioconcentration of dissolved Zn from the water column. A more extensive sampling campaign of the area revealed the extent of biofilm attenuation of Zn across the sub catchment. Biofilms sampled from Hebden Beck showed median uptake of 1412 mg/kg (range 609 – 4195 mg/kg; n = 27). Ambient water exhibits a median Zn concentration of 0.75mg/L, which gives an average bioconcentration factor of 1859. This level of bioaccumulation within the biofilm suggests its importance in natural attenuation of trace metals from the water column. Biofilm was collected as described in the methods chapter (sections 3.2.7.2 and 3.4.5) Upper catchment biofilm taken from the Colgrave sampling area exhibits the highest biofilm attenuated Zn concentrations (median = 2919 mg/kg; n = 9), with biofilm from Dukes level and Bolton Gill, situated further down the catchment exhibiting lower Zn concentrations (median = 1089mg/kg and 944mg/kg; n = 9, respectively). The data suggests that the higher concentrations of Zn in biofilm can be attributed to concentrations of Zn in the water column, and the two sets of data correlate well (df=10, $R^2 = 0.57$, $p < 0.05$). The data suggests there is no difference in uptake in warmer and colder months, however, during further water sampling campaigns dieback of biofilm was observed. This point raises the question of what happens to the trace metal when dieback occurs, is it in a bound state, or is it remobilised back into the water column in a mobile form, increasing its eco-toxicity . Although it is evident biofilm is a natural attenuation source for Zn, there is a lack of understanding of the mechanisms responsible for the metal attenuation, which required high resolution stream monitoring to assess. The next section of this study attempts to observe trace metal dynamics in tandem with biogeochemical processes to assess whether;

- Biofilm is directly responsible for the uptake of trace metals, via uptake of zinc with minerals known to precipitate in the biofilm, or another direct route
- Biofilm is indirectly responsible for the attenuation of trace metals, altering the in stream biogeochemical conditions and allowing for natural attenuation

processes such as abiotic precipitation of Zn minerals and/or co precipitation of Zn with other minerals.

4.4.3 Diurnal Sampling Campaign

In stream diurnal cycles of pH and temperature (Figure 4.9 (A) and (B)) were consistent with other studies (Nimick, 2005; Fuller and Davis, 1989) in conjunction with the daily photo cycle (Nimick *et al.*, 2011). The maximum pH values were observed during the afternoon (13:00 – 16:00), whilst the lowest were observed during the early hours of the morning (04:00 – 06:00), just before incident solar radiation (although impeded by cloud cover) came into contact with the stream. The same trend was observed with the temperature values, with maximum and minimum temperatures occurring at the same time as the pH maximum and minimum. Observed magnitudes in diurnal fluctuation were 103% for pH and 139% for temperature, which is similar to those found in comparable studies. During the summer flow campaign carried out by Nimick *et al*., (2005), magnitudes in change of pH and Temperature

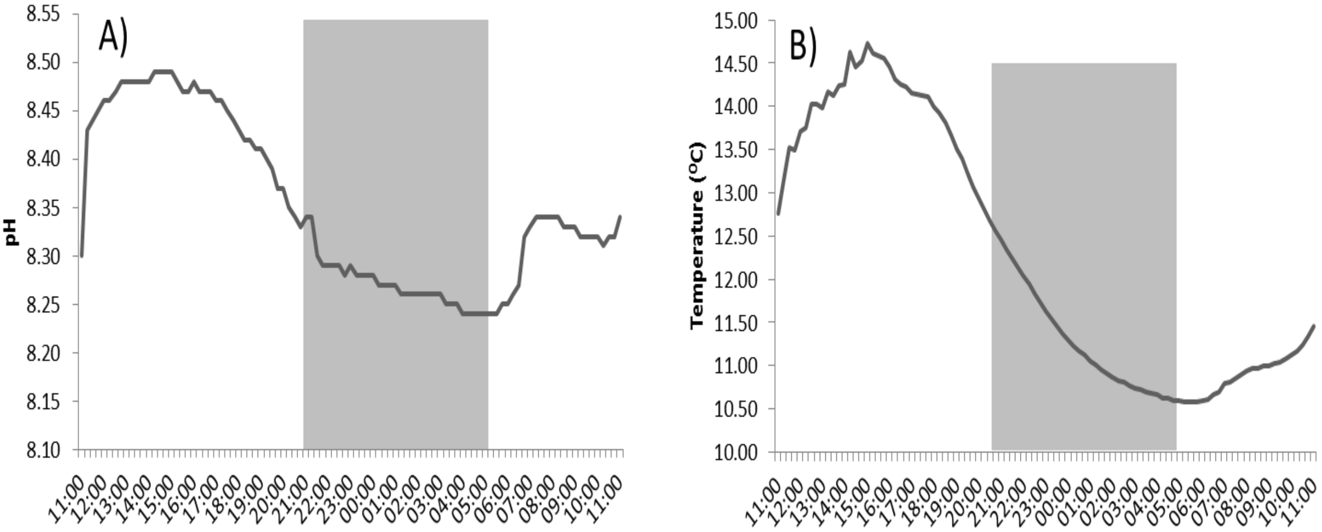

Figure 4.8 Fluctuation in pH (A) and temperature (B) over a 24 hour time period.

were 0.32 pH units and 124% respectively and summer campaign carried out by Jones *et al*. (2004) also found similar variation in magnitudes of pH (0.49 units) and temperature (176% fluctuation).

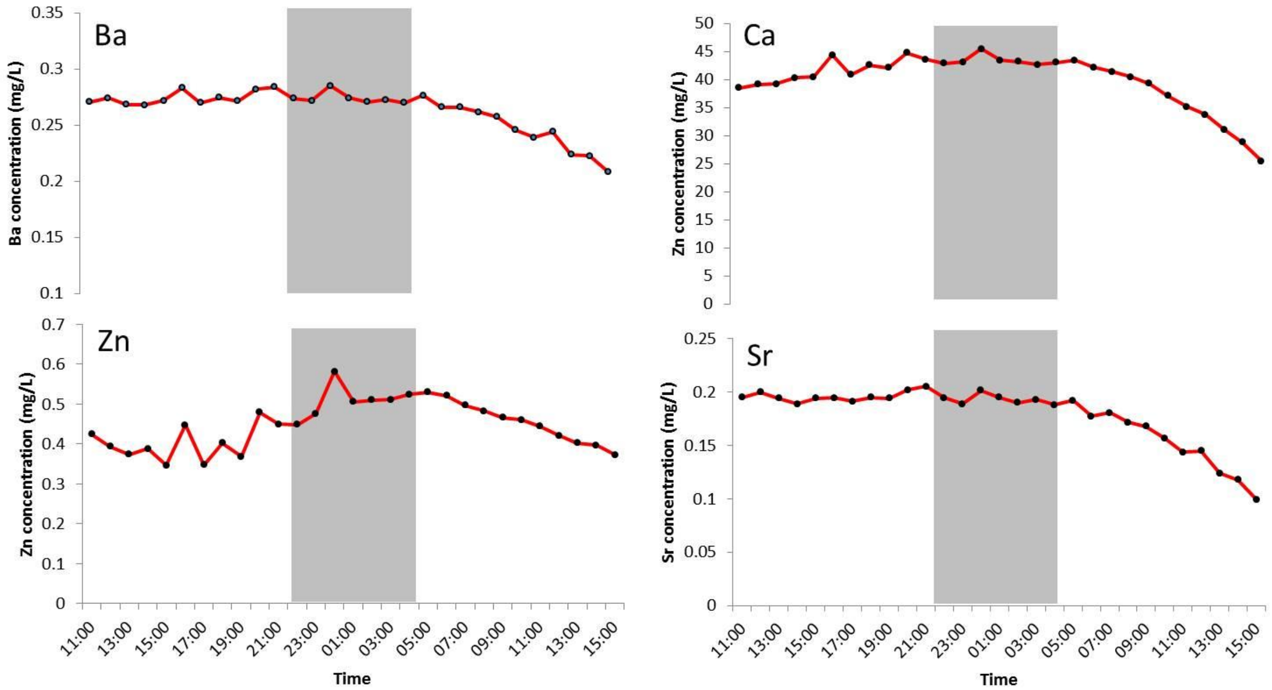

Figure 4.9 Fluctuation in element concentrations over a 24 hour time period.

Figure 4.10 shows trace elements Ba, Ca, Zn and Sr and their concentrations over a 24hour period. Zinc is the most obvious metal that is cycling, exhibiting its lowest concentrations during the afternoon (Zn_{min} = 0.34 at 15:00) and highest concentration during the night, resulting in a total fluctuation of 35%, a high fluctuation although not as pronounced as values seen in other studies (Table 4.5). Weaker signals of a diurnal cycle can be observed in Ba, Mg and Ca, which are summarised in Figure 4.10 although the exaggerated downward limb of the graph can be misleading. Sr does not seem to give any signal of a diurnal cycle.

Study	*Water pH range (SI units)*	*Zn cycling (% change between minima and maxima)*
Nimick *et al*., 2003		500 %
Nimick *et al*., 2005	Summer: 7.86 – 8.24	500 %
	Winter: 8.00 – 9.05	83 %
Nimick *et al*., 2007	8.08–8.34	172 – 196 %
Kimball *et al*., 2009	8.08 (spot reading)	800 %
This study		35 %

Table 4.5 Summary of Zn cycles between this study and others

4.4.3.1 Interference

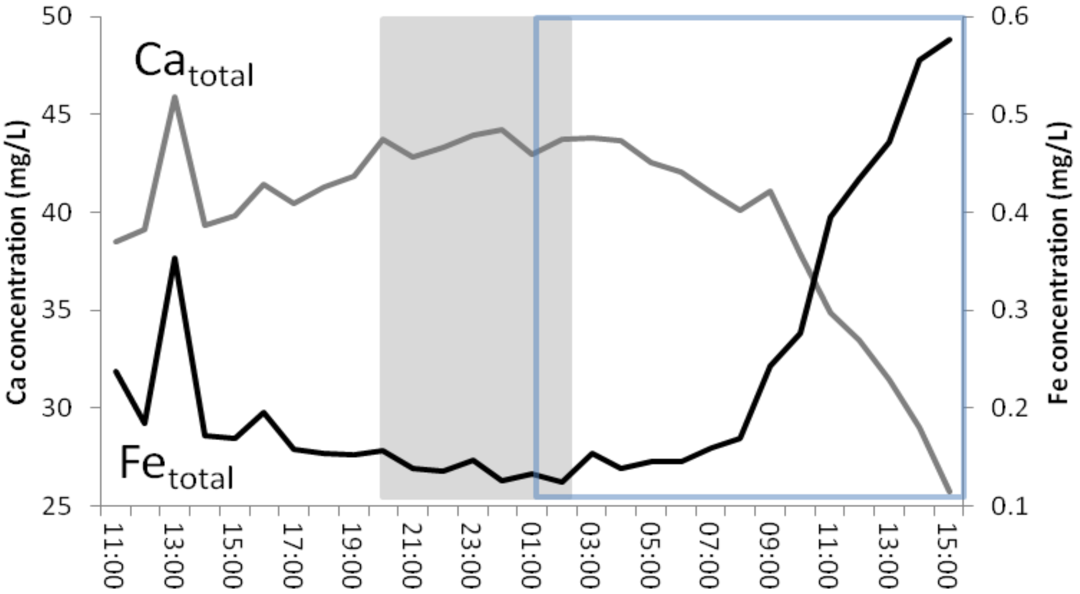

Figure 4.10 Comparison of concentration of total Calcium (Ca, Y-axis) and Iron (Fe, Z-axis) throughout a 24 hour period. Concentrations in mg/L. Shaded area denotes night time hours. Area bound in blue outline denotes rainfall.

The downward part of the cycle in trace elements can be seen to be exaggerated from around 0800 (Figure 4.10). Catchment morphology and environmental conditions can allude to the causes of this exaggeration. As previously discussed, the upper parts of the catchment are dominated by peat moorland, a potential source of iron (Mayes *et al*., 2008), and during periods of heavy rainfall, this iron can be remobilised and discharged into the streams of the catchment via surface runoff (Figure 4.11). Rain that was experienced towards the end of this sampling regime altered the flow. Given the geological features of the area, it can be assumed that surface runoff was responsible for this change in flow, and thus exaggeration of the downward cycle in diurnal dynamics of some trace elements, it should be expected that an increase in Fe concentrations should coincide with both an increase in flow and the decrease in the groundwater-sourced cations. Figure 4.10 displays this trend, with a rapid rise in the concentration of iron that is almost a mirror image to the rapid decrease in the concentration of calcium a matter of hours after the rainfall starts.

4.5 Conclusions and Management Considerations

Discharges from abandoned mines across the Yorkshire Pennine region show geochemical features similar to other Pb mining districts with carbonate-rich country rock. Key contaminants in the circum-neutral waters are Zn, Cd and Pb. An unusual geochemical characteristic of the waters are consistently high Ba and occasionally high Sr concentrations. The overall metal flux from the mine discharges is reasonably modest compared to other orefields nationally (total of 2.6 tonnes Zn/yr). However, since these abandoned mines discharge into small upland headwater streams, their relative influence on in-stream geochemistry can be more pronounced with many receiving streams in breach of aquatic life standards. The regional scale assessment provided here is useful in highlighting the small number of mine drainage systems that contribute most flux to rivers. Various remedial technologies have been trialled by workers for Fe-poor, circum-neutral pH metal mine drainage (e.g. Nuttall and Younger 2000; Piramid Consortium, 2003; Mayes *et al*. 2009), which may be suitable here, although the abandoned nature of the mine workings would necessitate passive approaches being adopted. Catchment scale assessment in the Hebden Beck highlights that point source discharges are the significant contributors to in-stream contamination under base flow conditions, under which widespread attenuation of dissolved Zn within the catchment is also observed. The extensive spoil cover in Hebden Beck however, means that diffuse metal pollution is a major issue, particularly under higher flow and remains a challenging management problem than point source remediation alone. Given biofilm has been identified as a source of natural attenuation, the opportunity has presented itself to potentially take advantage of it, and assess its potential use as a low cost, passive remediation tool to remove trace metals from the water column. However, the uptake mechanisms are not well understood and the following chapters will address this through a series of laboratory based experiments that most importantly do not take place in an external setting, allowing for individual underpinning factors in uptake mechanisms to be identified.

5

5.0 Biofilm: Batch experiments and long term flume dynamics to assess modes of Zn uptake

5.1 Introduction

Chapters 2 and 4 introduced and explained the nature of the mine drainage problem in UK catchments. To summarise;

- Assessments of the pH distribution of such discharges suggest 85% have a pH between 6 and 8, while around 5% have pH <4 (Mayes *et al.*, 2010; Jones *et al.*, 2013).
- The most common metal pollutants in these circum-neutral discharges are Zn > Pb > Cd > Cu (Jarvis and Mayes, 2012).

- The statistical distribution of both flow and Zn concentration is heavily left skewed (Mayes *et al.,* 2010). This means that while a small number of high flux discharges (usually large drainage levels) contribute a significant portion of in-stream metal loadings in affected catchments (see Duke's Level example in Chapter 4; Gozzard *et al.,* 2011), the vast majority of metal mine discharges that contribute to in-stream contamination are modest in flow rate (60% have a flow <10 L/s) and Zn concentration (55% have concentrations <1.0 mg/L: Mayes *et al.,* 2010; Jones *et al.,* 2013).

Many researchers are currently investigating low-cost technologies to strip such mobile metals from waters, notably Zn, given its ubiquity and toxicity to aquatic life at modest concentrations (Alabaster and Lloyd, 1980). These passive technologies encompass bioreactors, sorbent media as well as low-cost reactants based on recycled industrial by-products, waste, and other miscellaneous materials (Gandy and Jarvis 2012, Mayes *et al.* 2011, Warrender *et al.* 2011). While all have shown promise in various settings, the perennial ambitions to develop treatment technologies that can ally effective metal removal with low residence time, small land footprint and treatment longevity still remain elusive for many circum-neutral metal mine waters. Field assessment of biofilm Zn concentrations in circum-neutral streams draining areas of former Pb and Zn mining in North Yorkshire, UK, showed median Zn concentrations of 1800 ppm (range: 800-4100 ppm), a bioconcentration factor of up to 3500 from the water column under baseflow conditions (Chapter 4; Jones *et al*., 2013).

Studies on biofilms have found they are capable of storing large inventories of trace metals (Rogerson *et al.,* 2008). A negative charge provided by functional groups associated with acidic lipid and protein molecules allows the EPS (extracellular polysaccharides) to sequester cationic metals (Me^{2+}) from the water column (Dittrich and Sibler, 2010). Encapsulation in EPS protects the biofilm ecosystem, allowing the biofilm to modify local biogeochemical processes in semi-independent of those in the bulk water column (Bisset *et al*., 2008). During daylight hours photosynthetic microbes utilise carbon dioxide which increases pH, while during night time hours when respiration predominates carbon dioxide is released and thus the pH lowers. The diurnal cycle is highly repeatable, and is a first-order control on solid : solution ion exchange as well as pH (Rogerson *et al.*, 2010). The implications of this internal pH change are two-fold: 1) Internal changes in biofilm pH can result in chelated metals being released from the EPS (Hullebusche *et al.*, 2003). 2) processes happening in the biofilm will be invisible to analysis of ambient water, but can dominate mineral precipitation reactions (Rogerson *et al*. 2008). Further investigation by Rogerson *et al*., (2013) indicates that the chemical potential of most natural carbonate producing systems is not sufficient to allow the spontaneous precipitation of minerals such as calcite, and that processes carried out by the biota of the stream (in terms of this study this would exclusively mean the biofilm) actively lower the activation energy required to promote precipitation. Where metal ions are co-precipitating with this carbonate, as is the case in many circum-neutral mine-drainage systems, understanding of this process could be critical to understanding the pathways and fates of specific pollutants.

The capacity for metal removal exhibited by biofilm in both field and laboratory settings presents a 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 versus sorption) and permanence of metal removal in biofilms. This chapter aims to assess these processes through *in-vitro* experiments of biofilms cultured from mine-impacted streams. This serves to (1) assess the scope for culturing and maintaining biofilm under controlled conditions, (2) improve our understanding of biofilms as a control on contaminant transport in mine-affected streams, and (3) underpin approaches to harness biofilms for remediation applications at many of the circum-neutral pH metal mine discharges with a modest Zn flux.

5.2 Methods

Generic methods and the broader justification for the approaches adopted are detailed in Chapter Three of this thesis. This section provides a brief overview of the specifics of the experimental set-up for each of the experiments detailed in this chapter.

5.2.1 Batch Experiments

Batch experiments were employed as they provided a quick method of experimenting with biofilm, in which it was easy to control external factors, in particular the removal of biotic influence, the control of light intensity, and the control of pH where of importance in these experiments. An outline of the experiments is presented in Table 5.1, which also provides the rationale for each experimental procedure.

Experiment	*Water Type*	*Wet weight Biofilm*	*Objective*	*Rationale*
Initial biofilm experiments	Synthetic water	5g	To assess the control biofilm asserts on trace metal conditions in the water column.	A ‘proof of concept’ experiment. The impact of biofilm has been seen in a variety of studies but not in such controlled conditions, this experiment compares the removal rates of biofilm against control flasks.
Light Control Experiment	Mine water from Duke’s Level	5g	To assess the impact varying light intensities had on levels of trace metal removal from the biofilm	Photosynthesis controls pH in the biofilm (chapter 2). The aim of this experiment is to assess whether different light intensities will lead to increased/decreased removal of trace metals. Increased light could theoretically increase the rates of photosynthesis and subsequently pH. This has implications for trace metal removal in two terms. Higher pH could lead to an increase in saturation indices and the precipitation of trace metal mineral species, or affect the metal uptake capacity of the biofilm.
pH Control Experiment	Mine water from Duke’s Level	5g	Assess the implications of a change in pH over a range of pH observed in natural settings	Higher pH could allow for better sorption of trace metals to the biofilm surfaces and also more bicarbonate alkalinity. This could potentially lend itself to increased removal via adsorption or precipitation of minerals
EPS test experiments	Natural with added Zn	0.1mg pure EPS	Assess EPS for its use independent of biofilm	Numerous natural and processed materials are used as sorbents for the removal of trace metals from the water column. This section will compare EPS function (independent of the biofilm) and compare to other materials used for the removal of trace metals via sorption

Table 5.1 Overview of batch experiment treatments.

5.2.2 Long Term Flume Experiment

Whilst batch experimentation with biofilm can address specific questions, there is a requirement to understand the fundamental dynamics of trace metals in the presence of biofilm in a natural environment. Whilst attempts have been made to understand these dynamics, the stochastic vagaries of natural environments (e.g. flow rate changes, differences between bulk water column and boundary water quality, diurnal cycles) make it difficult to assess exactly what variations in trace metals concentrations and biogeochemical conditions are due to the biofilm in stream systems.

To determine the effects biofilm plays on trace metals and biogeochemical conditions it was necessary to remove it from its natural environment and experiment with it in an environment which could be controlled. For this reason biofilm was allowed to colonise mesocosms situated in a controlled environment laboratory. This meant environmental conditions that are unpredictable in natural settings could be controlled with a high degree of accuracy. In this instance it was important to control temperature to maintain biofilm growth, and also maintain a regular photo-cycle and lack of atmospheric precipitation which would produce repeatable geochemical conditions over a daily timescale and maintain a steady flow rate in the flume.

The main aims for this chapter in terms of long term monitoring of the flume environment were twofold;

- Observe the colonisation of the biofilm over a long period of time and understand the practicalities of maintaining a mature biofilm, particularly with regard to nutrient requirements given the low nutrient status of many of the discharges throughout the catchments studied in chapter 4.

- Record the long term removal of trace metals from the water column. If biofilm holds the potential as a low cost, passive treatment option it will have to be able to maintain an efficient level of removal over long timescales.

Long term monitoring of the flume took place over 66 days once the biofilm had reached full maturity. Care was taken to have a set time every sampling day to exclude any potential diurnal fluctuations having an effect on the results gained. The set time was 0700, as this was around 15 minutes before the UV light came on (see Chapter 3 for full details of laboratory set up). This time was chosen based on potential fluctuations in pH, at this time the UV light had been off for 16 hours and the pH would in theory be at its most stable if it were fluctuating. It was felt this stability would in turn lead to stability in trace metal concentrations, and data acquired would not be affected (see Chapter 6).

5.2.3 Data Analysis

Boxplots are utilised to give a graphical representation of the data acquired from the batch experiments. Statistical analysis of Ba and Zn concentration data from the batch experiments were not normally distributed even after log-transformation (Kolmogorov-Smirnov: $P<0.05$), therefore the non-parametric Kruskall-Wallis test is used to test whether there is a significant difference in median Zn and Ba removal levels between different light treatments (Fowler *et al.*, 2013).

The long term flume data is presented in graphical format, as this is the best way to visualise the changes of trace metals over the long term. Initially, long term trends are shown as overall percentage losses of ions. Whilst this information is important, as it shows the overall difference in removal between the two ions, it does not utilise flow measurements in the system, and thus residence time cannot be calculated. The residence time of the system is the time it takes for a volume of water to pass through the system. The residence time (volume of reservoir / flow rate) throughout the experiment was 9.6 minutes. Calculation of the residence time allows for a more in depth comparison to be made with other systems, with residence times ranging from minutes to years, to identify the efficiency of the tested system against other systems by calculated volume adjusted metal removal rates. This assesses the efficiency of the system by calculating the metal removal of the system over a certain timescale, usually quoted in days. Volume adjusted metal removal rates have been calculated using the corresponding formula;

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

Where, A = treatment media area (m^2); Q_d = mean daily flow rate (m^3 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 (g m^{-2} day^{-1}).

To elucidate knowledge of the mechanisms governing trace metal uptake, it became apparent that saturation index data would be important to assess potential mineral precipitation. This was gained via the geochemical modelling code PHREEQC (in depth description of this process is found in chapter 3).

5.3 Results

5.3.1 Batch experiments

5.3.1.1 Initial batch experiments: Proof of biofilm influence on trace metals

Static batch experiments show biofilm is capable of high levels of Zn uptake, with an average metal removal of 81.68%. Control data (empty flask and water) show Zn is still being removed, most likely due to sorption onto glassware or precipitation from the water column (Figure 5.1).

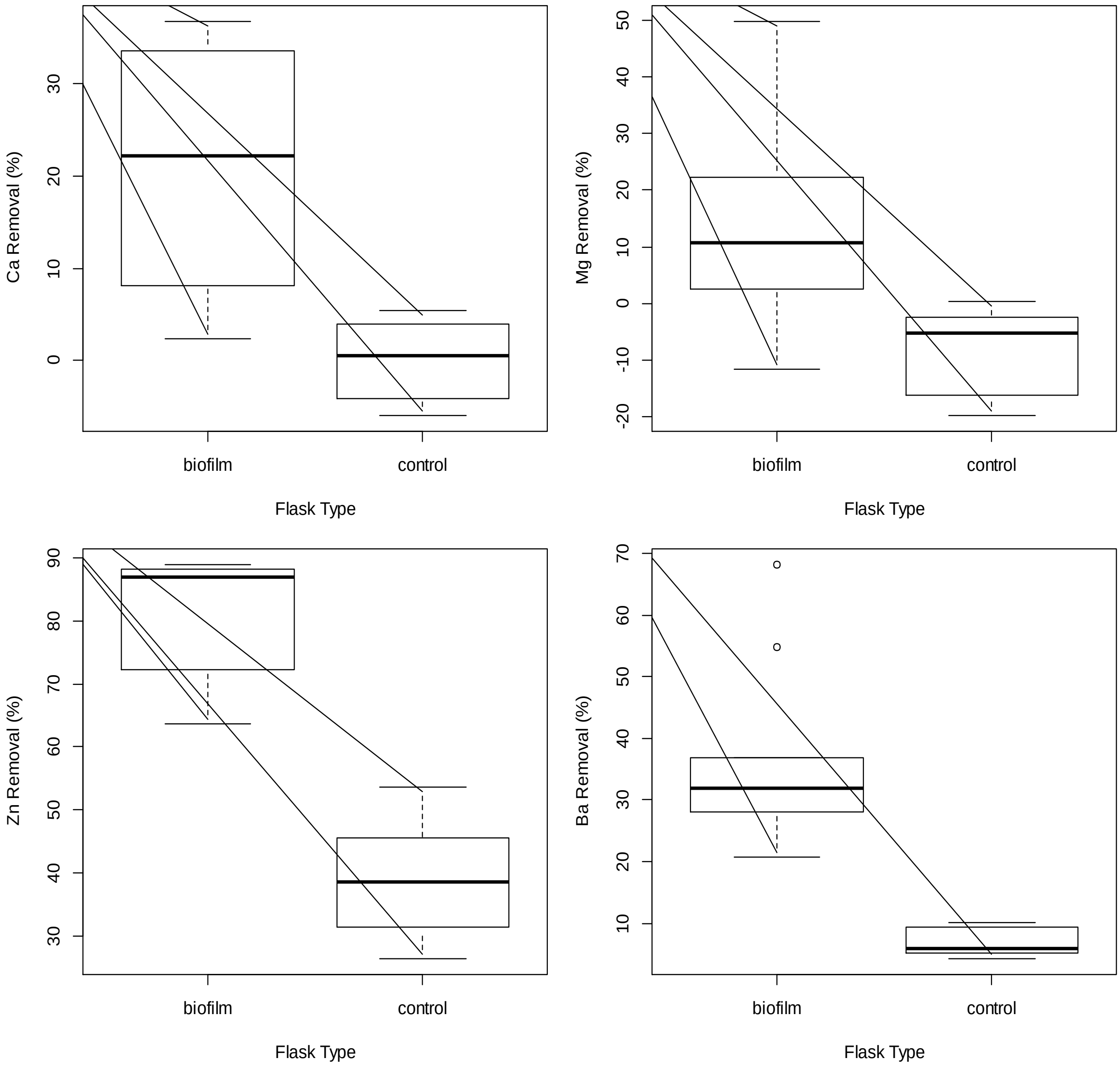

Figure 5.1 Metal removal between biofilm and control flasks (n = 8 per flask type)

However, the removal of Zn is significantly lower (Kruskal Wallis: *H:* 12.6316, df: 1, $p<0.001$) in control treatments (median of 38.92% Zn removal) than in the biofilm occupied flasks (median 81.68% Zn removal) suggesting that biofilm is responsible for the majority of Zn removal from the water column. Ba results present a similar, but less extreme pattern to the Zn uptake results and are shown in Figure 5.1. Median removal of Ba from the flask water was 31.64% (range 20 – 68%). Control data show median removal of 6.92%, which is significantly lower than the biofilm occupied flasks (Kruskal Wallis: *H:* 12.6316, df: 1, p<0.001). These results suggest the majority of uptake is attributable to biofilm. Median Ca removal was 22.25% (range 2% - 37%) in the biofilm occupied flask and 0.56% in the control flask (range -5 – 5%). The control flask data contained negative values which could be due to residual water drying on the flask during the cleaning process, to be remobilised when in the presence of water, however due to the cleaning regime detailed in Chapter 3 this is unlikely. It is more likely that, due to the negligible differences in the concentrations at the beginning and the end of the experiment, the results are small and within the analytical margin of error for the analysed ion. Mg removal between biofilm occupied and control flasks exhibited similar trends with median uptake of 10.63% (range -11% – 49%) and -5.09% (range -19% - 1%) in the biofilm and control flask respectively. Both Ca and Mg removal was significantly higher in the biofilm flask than in the control flask (Ca: (Kruskal Wallis: *H:* 10.8079, df: 1, p=0.001), Mg: (Kruskal Wallis: *H:* 9.6711, df: 1, p=0.002)).

5.3.1.2 Light

Light experiments were used to determine the effect of light intensity on the uptake of trace metals from the water column by biofilm. The photosynthetic nature of the biofilm means it relies on a light source to maintain itself; however, photosynthetic reactions also play a role in the creation of alkalinity within the biofilm and broader water column (see Chapter 2). The addition of this alkalinity can promote the precipitation of minerals such as calcite in the biofilm, although it is not known if a higher intensity of light would in turn produce more alkalinity, and thus remove more metal from the water column.

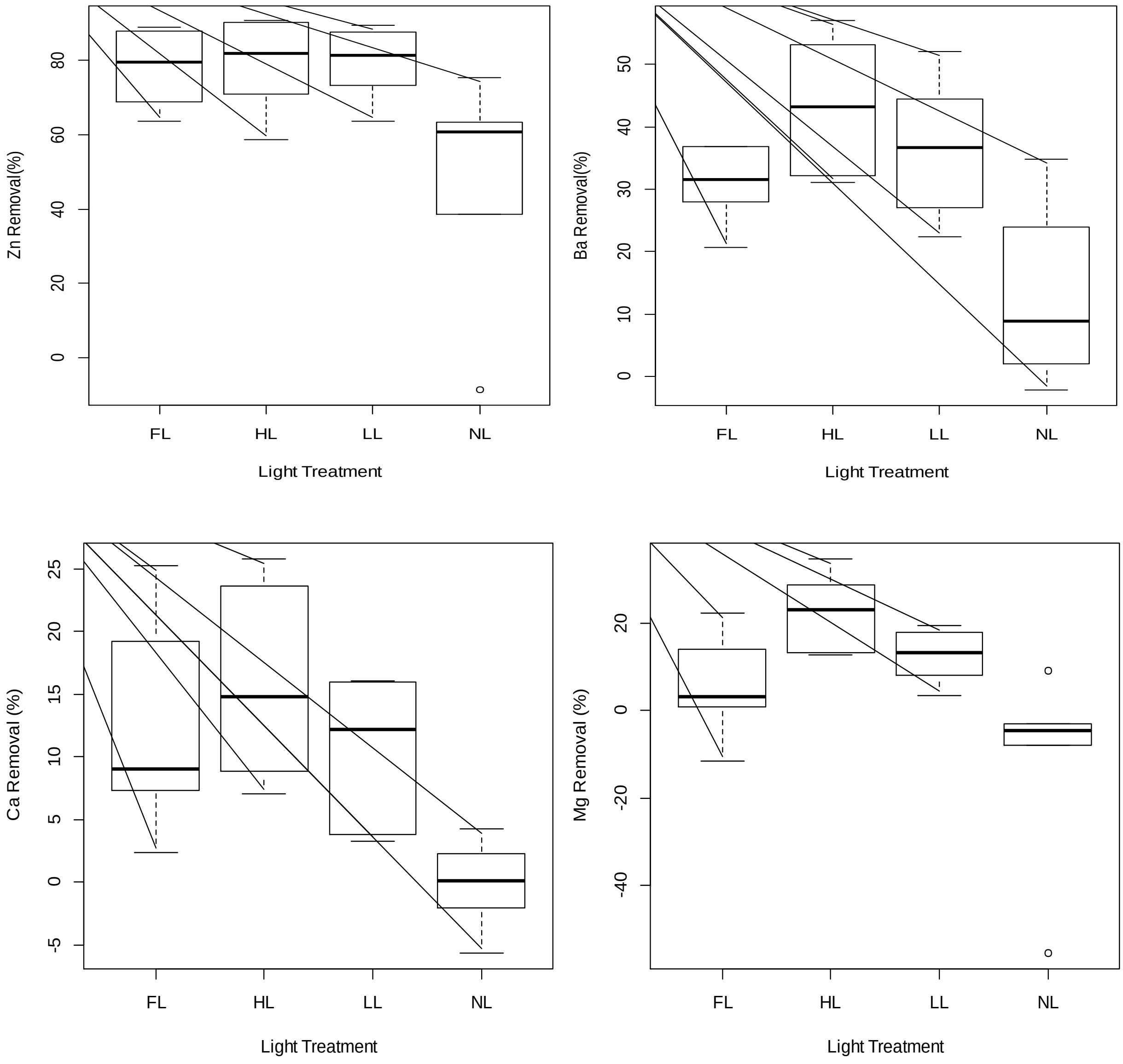

Figure 5.2 Metal removal in light experiments (n=8 per treatment). FL – full light, HL – high light, LL – low light, NL – no light.

The removal of metals via precipitation or co-precipitation is one of the potential removal mechanisms exhibited by the biofilm, however adsorptive mechanisms also need to be considered, i.e. would a higher light intensity promote more photosynthesis, thus altering the geochemical makeup of the flask water and change adsorption kinetics. There was a significant difference in Zn removal rates between the light treatments (Figure 5.2) (Kruskal Wallis: *H:* 8.46, df: 3, p = 0.037). This significance 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). Ba exhibited similar results, with a significant difference in uptake between light treatments (Kruskal Wallis: *H:* 10.8933, df: 3, p = 0.012) ascribed to the NL treatment as no significant difference was observed in the light intensities FL, HL and LL (Kruskal Wallis: *H:* 4.011, df: 2, p = 0.134). Median Ca Removal between light treatments was significantly different (Kruskal Wallis: *H:* 12.45, df: 3, p =0.006), again with the difference being ascribed to the NL treatment as no significant difference was observed in the FL, HL and LL treatments (Kruskal Wallis: *H:* 1.345, df: 2, p =0.519). Mg exhibited similar behaviour with no significant difference between the light treatments (Kruskal Wallis: *H:* 13.86, df: 3, p =0.003). The negative values observed in the NL treatments in both the Ca and Mg concentrations essentially mean more of the ion was present at the end of the experiment than at the beginning. This could be due to the loss of structural integrity of the biofilm previously described in Chapter 2, or dissolution of pre-existing Ca, Mg bearing phases within the biofilm at the start of the experiment.

5.3.1.3 pH

There was no significant difference in uptake across this pH range Zn, Ba, Ca or Mg removal (Kruskal Wallis test, p > 0.05 for all), with median Zn and Ba removal being 97% and 63% respectively (*n* = 6), and Ca and Mg removal being consistently lower with removal medians of 11% and 15% respectively. Zn has a markedly higher uptake than Ba, and given the results revealed in the light studies, it would begin to suggest Zn removal is governed by either a quicker reaction than the group two alkali earth metals, or the minerals capable of sinking the group two ions are precipitating / co-precipitating. This observation is further backed up by the poor removal of Ca, which if precipitation processes had occurred would have been, in theory, removed further. It can be ascertained from the concentration data gathered here that there is a selectivity in the sink for these metals in this system, with an order of preference (at least in percentage of initial concentration uptake) of Zn>Ba>Ca>Mg, even though a much higher concentration of Ca is available compared to the other metals at the beginning of the incubation period.

5.3.2 EPS

The high removal rates of Zn clearly indicate removal mechanisms attributable to the biofilm are responsible for the reduction in aqueous Zn concentrations, and this gives rise to the question of which parts of the biofilm are optimal points of uptake. A key question is whether the abiotic part of the biofilm, the EPS, is capable of the same removal rates as a functioning biofilm, in the absence of cells. The EPS of the biofilm was extracted to assess its potential as an adsorbent trace metal remediation tool. Results show that EPS alone is capable of zinc uptake, with average removal of 289.7µg/l from the water column observed in the test tubes (*n* = 6), which was achieved with 0.1 mg of EPS; bound zinc concentration within the EPS is therefore at equilibrium in 2.5ml of water at ~ 7.24 mg/g (in 1ml of water in equilibrium at ~ 2.87mg/g).

5.3.3 Long term Removal of Zn Ba and other Group 2 elements in the flume mesocosm

The flumes aimed to assess long term removal of Zn and other Group 2 alkali earth metals by comparing two flume systems colonised with biofilm against a sterilised control treatment. As detailed in Chapter 3, the flumes operated with recycled water sourced from Duke's Level. This allowed removal rates of metals to be assessed with long term evolution of the water (i.e. as concentrations of Zn diminished over time). Long term monitoring of the flume proceeded for 66 days and Figure 5.3 shows the systematic decline in dissolved Zn in the water column, with 78% of initial Zn removed by the biofilm treatments. The control flume (sterilised) exhibited initial Zn removal, which levels off after around 3 weeks leaving total Zn removal at 19%. Both the active (non-sterilised) flumes (number 1 and 2) show Zn removal which continues until the end of the trial.

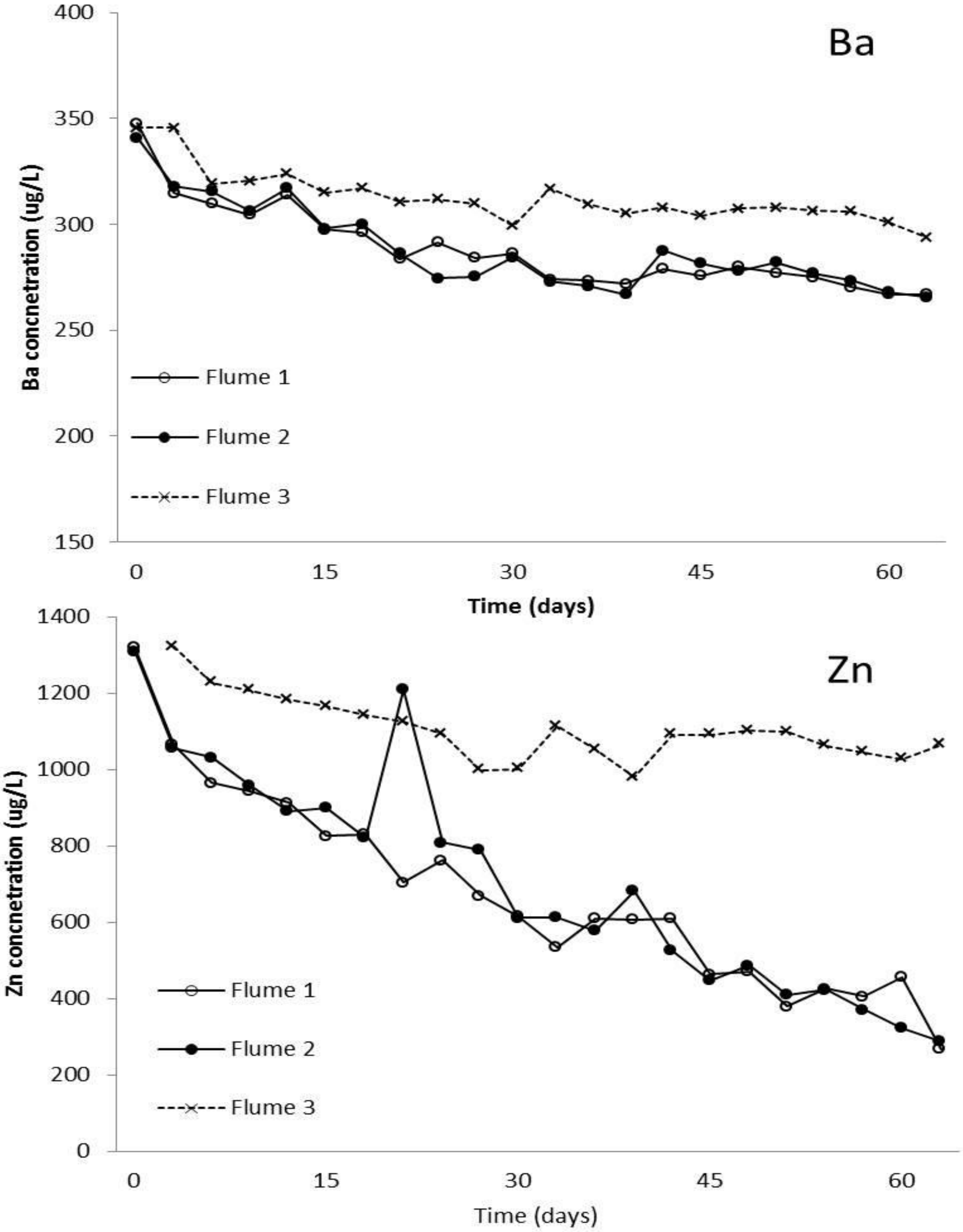

Figure 5.3 Long term removal of Ba and Zn in the biofilm mesocosm environment. A total of ~9.5m^3 of water was circulated through each flume. The abrupt spike in flume 2 after day 21 is likely to be due to entrainment of a small amount of organic debris in the sample.

Systematic decline in Ba concentration was also apparent, although not at the magnitude seen with Zn, with 23% of initial Ba was removed by the biofilm. Comparisons with the control flume are not as stark as Zn, with the control flume removing a total of 14% of Ba from the water column. A summary of the removal rates of metals in the flume are outlined in Table 5.2. Flume 1 and 2 maintain higher metal removal rates for Zn for the whole sampling campaign; whilst flume one removal rates have modest removal rates at the beginning which then level off throughout the sampling campaign. Ba exhibited a similar pattern in removal rates, albeit at lower rates, with removal rates values being an order of magnitude lower than the observed Zn removal rates. There was a lack of removal over the long term in Ca and Mg, and owing to the fact calcite and dolomite where not saturated in the PHREEQC modelling, removal rates were not calculated.

Flume	*Zn (g/m^2/day)*		*Ba (g/m^2/day)*	
	average	*max*	*average*	*Max*
1	0.2	0.67	0.01	0.08
2	0.13 (0.17*)	1.02	0.009	0.06
3 (control)	0.03	0.24	0.006	0.06

Table 5.2 Summary removal rates for Zn and Ba across the flumes. Average and maximum removal rates are presented. Minimum values are not presented (although they are included in the average calculation) due to natural fluctuations in ion concentration giving negative values. *Average removal rate with outlier at day 21 removed, the comparatively high concentration value of Zn at day 21 is due to the presence of solids in the sample upon acidifying.

Figure 5.4 shows the comparisons of metal removal and influent concentration in the flumes over the period of the study. There is no significant relationship between the influent concentration of Zn or Ba and the corresponding metal removal rate value. The lack of correlation between the influent concentration and metal removal rate suggest a concentration driven removal mechanism is not responsible for the removal of Zn or Ba, as would be expected with a precipitation reaction. The removal rate was not observed to significantly decrease over time, which also suggests the removal of metals by the biofilm is not a process that will reach a saturation point, presumably due to growth of fresh biofilm mass.

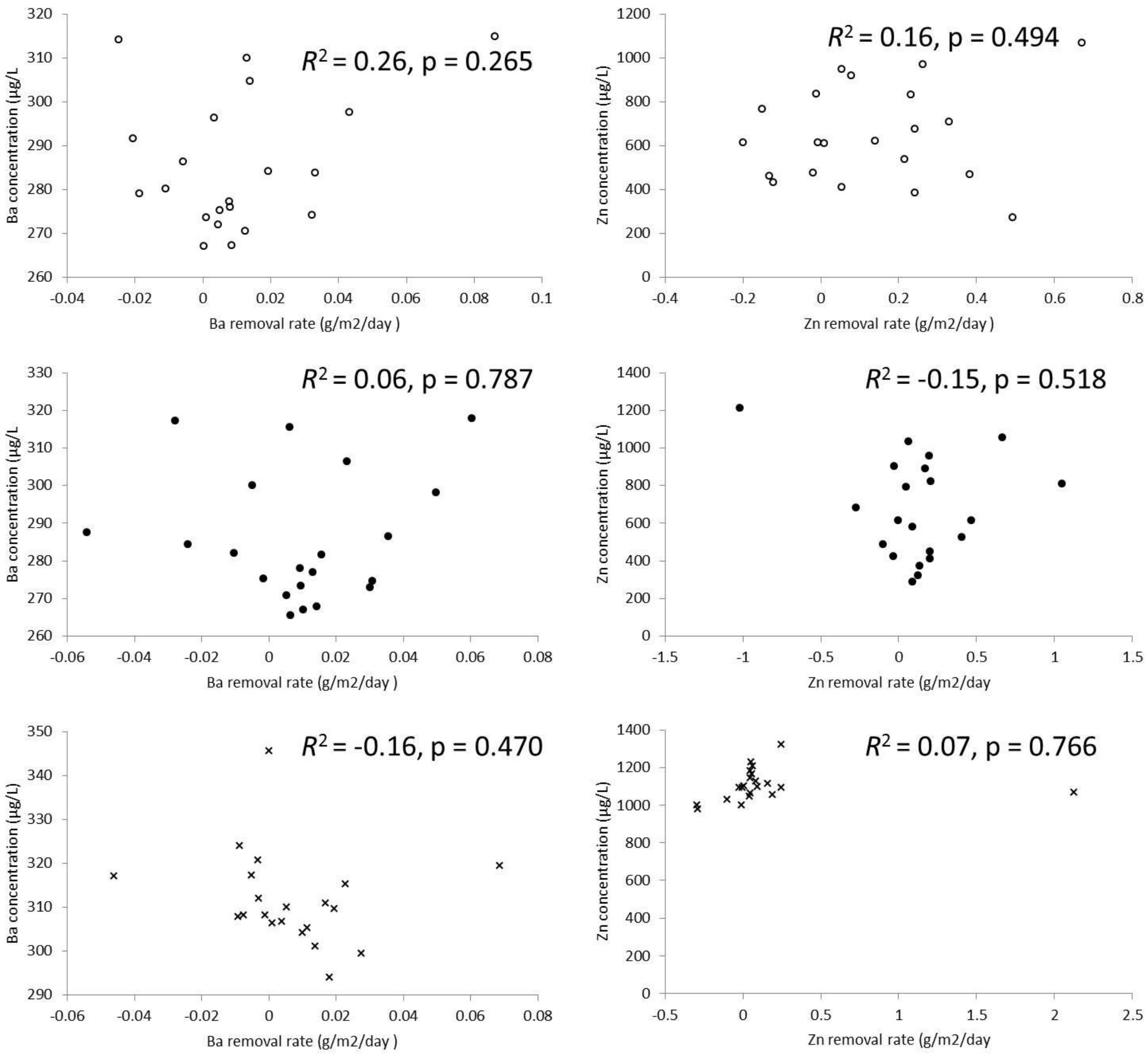

Figure 5.4 Metal removal rates versus Influent concentration in Flume 1 (open circle), Flume 2 (solid circle) and flume 3 (cross).

The concentrations of Ca and Mg were also monitored during the sampling regime (Figure 5.5). Observable losses in terms of percentage loss were negligible for Ca, with a mean overall loss of 8% across the active flumes and no loss in the control flume. The same observations were made for Mg with mean overall loss of 2% across the active flumes and no loss in the control flume. Discernible losses of these metals would be indicative of a potential precipitation reaction taking place to produce calcite. This is better observed by looking at the mass loss of metals in the flume system, as opposed to efficiency loss as a percentage. The mass losses of ions in the flumes are presented in Table 5.3.

	Ca			*Mg*			*Ba*			*Zn*		
	1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
Start	1179	1173	1173	75.6	74.1	75.6	10.5	10.5	10.2	39.6	39.3	39.6
End	1062	1047	1170	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

Table 5.3 Mass loss of ions in the system (mg) across the biofilm colonised flumes (1&2) and control flume (3)

The largest loss of ions across both biofilm colonised flumes is seen in Ca (loss of 117 – 126 mg) and was expected due to the fact there are significantly more Ca ions available in solution. The next expected ion would be Mg with this logic; however, the next largest mass removal is Zn (31.5-30.6 mg/L). Ba also exhibits more loss in mass than Mg. 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 Ca>Zn>Ba>Mg.

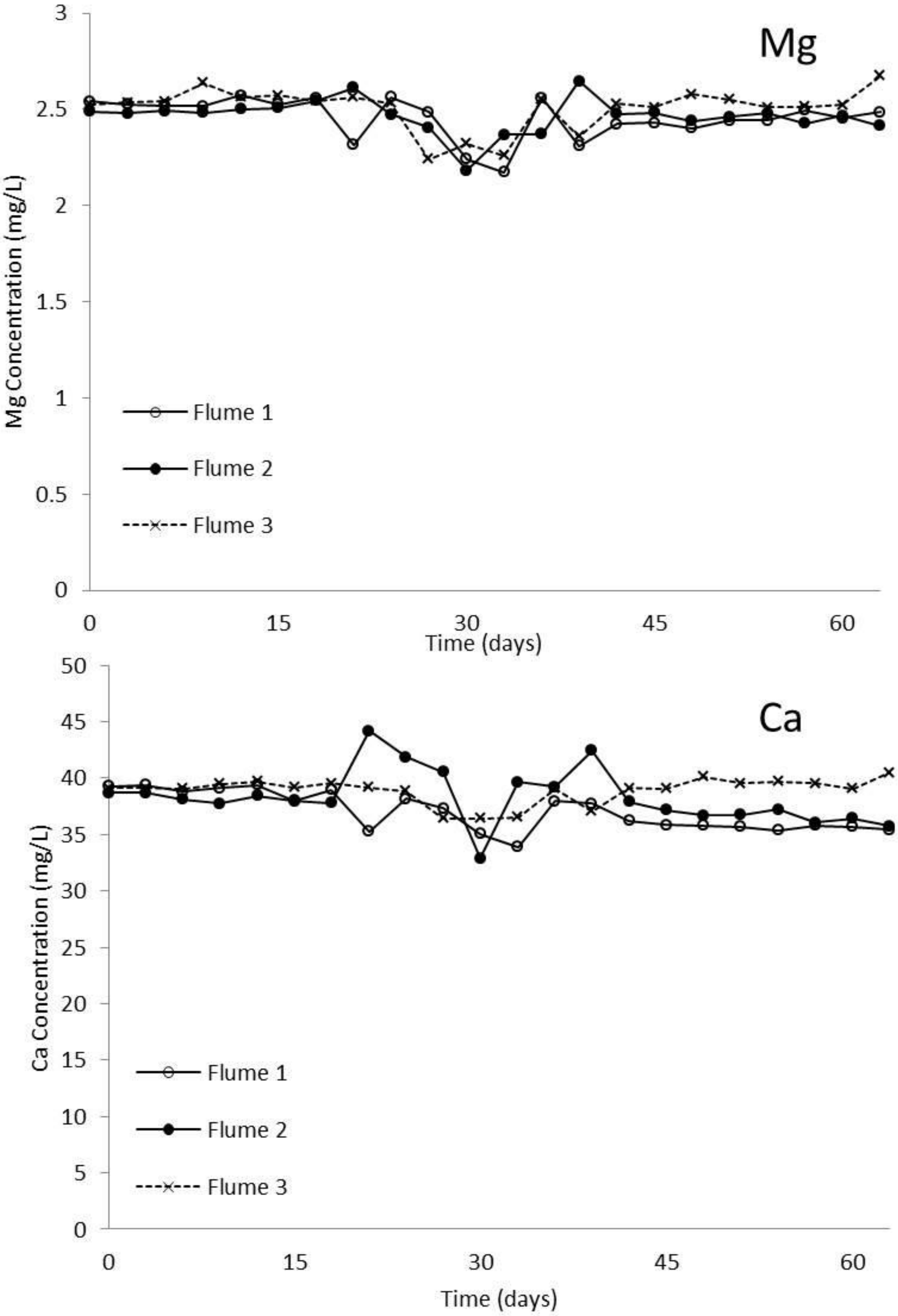

Figure 5.5 Long term monitoring of Ca and Mg concentrations in flumes

The patterns observed in the long term metal removal experiment are consistent with the proportions of abiotic and biotic removal of metals from the water column in the batch experiments. Abiotic treatments and active treatments in both the flume and batch experiments removed similar amounts of metal in terms of percentage removal. Observations of large masses of Ca in the presence of biofilm can begin to suggest that Ca could be a component of a Zn removal mechanism, potentially via precipitation of calcite. However, where there is a presence of biofilm new EPS is constantly generated, maintaining sinking of Zn and Ba over a longer period of time in the active flumes.

5.4 Discussion

5.4.1 Conclusions from batch experiments

The high levels of removal in the initial flask experiments provided a promising indication of the potential capacity of biofilms to remove metal ions from solution, especially for Zn. The difference in removal between some of the monitored elements begins to show that there are potential differences in uptake mechanisms. The stark difference between control flask and biofilm flask data alludes to these potential removal mechanisms. Zn removed from the water column could be explained by sorptive processes, as the biofilm was responsible for large removal from the water column, sorption onto glassware is also clearly evident, whilst the small amount of Ba suggests it would be more complex mechanisms responsible for it being sunk into biofilm in waters of the type used in this study.

5.4.1.1 Light experiment

These results would appear to suggest that the effect of light intensity on the uptake of trace metals is not a major control on metal removal from the water column, so long as at least some light is provided. By the end of the experiment, the No-Light biofilm was completely degraded and had a fine silt-like appearance, whereas the biofilms FL, HL, and LL are still functioning as normal with its structural integrity maintained. However, there is loss of Zn ions from solution in the No-Light flasks, indicating some chemical stability of trace metals even in degraded biofilms. The increase in Ca and Mg observed in the NL flask is likely to reflect the breaking down of the biofilm, and may induce return of Zn to solution.

5.4.1.2 pH experiment

The lack of variation in the trace metal removal rates seen in the pH experiments illustrates the stability of the biofilm over the natural range of pH observed in natural, carbonate-dominated settings (Jones *et al.,* 2013). The ability of biofilm to remove a high percentage of trace metals from the water column across a range of pH that are well documented within literature surrounding mine discharges (e.g. Chapter 4), suggests biofilm performance would not be diminished if deployed as a remediation tool. This stability could be explained by the biofilm having its own microenvironment, created by the EPS and the metabolic reactions undertaken by bacteria in the biofilm, where pH changes occur independently to those acting in the bulk water column (Bissett, 2008). Bissett (2008) illustrated that biofilm can maintain its internal pH over a range of 2 pH units in the water column; whilst water column pH ranged between 7.8 – 8.9, biofilm pH was maintained at 9.4 (in light conditions) and 7.8 (in dark conditions) in these previous studies. If there is no change outside of this pH range in the water column, it is likely that the mechanics of precipitation and adsorption will not be affected.

5.4.1.3 EPS Experiments

Sorbent	*pH*	*Zn uptake capacity (mg/g)*	*Reference*
EPS	--	2.73	This study
Flask Biofilm (dry weight)	--	2.01*	
Lignin	--	73 (30°C) 95 (40°C)	Bailey *et al*., 1999
Peat fly ash	6.3	21.4	Warrender *et al*., 2011
Compost	6.3	2.48	
Waste Shell	6.3	1.72	
BOS BFS	6.3	0.2	
Orange Peel	6-8	5.25	Kurniawan *et al*., 2006
PSA[1]	--	13.9	
PSC[2]	--	6.62	
PSS[3]	--	7.38	
Banana Peel	6-8	5.8	
Blast Furnace Slag	9	103.33	
Zeolite	6.5	3.61	
Kaolinite	6.5	1.8	
Residue Clay	7-9	13.4	
Oxidised Carbon	9	2	

Table 5.4 Comparison of metal uptake over a range of commonly used sorbents. Where the value is followed by *, it should be noted this was in a flask with a limited supply of Zn, where the removal was 98%. Field observations in Chapter 3 showed biofilm is capable of storing considerably more Zn. [1] Phosphoric acid activated pecan shell carbon. [2] Carbon dioxide activated pecan shell carbon. [3] Steam activated pecan shell carbon.

The results of the EPS experimentation give an insight into the removal efficiencies that the isolated EPS material will exert on Zn. When the results acquired in section 5.3.2 are multiplied up to a mg of metal/g of EPS measurement they compare well with other adsorbent materials used for the removal of trace metals (Table 5.4).

Sorbent materials are generally termed low cost when they are naturally abundant (e.g. bark, shell material) or is available as a by-product of industry (e.g. iron ochre, steel slag: Bailey *et al*., 1999). Although the removal exhibited by the extracted EPS looks promising, it did require a relatively long processing procedure for a very small amount of EPS to be extracted (around a week for a maximum of 2 mg in this case), and the costing of such procedures at an industrial scale are not available given the novel nature of this processing. Also, on its own it is an adsorbent and thus has a finite use before it needs to be replaced, it would only be as a part of the biofilm that the EPS would be continually replenished and thus would be reactive for a much longer period of time before it needs replacing. Whole biofilm, which would be able to maintain removal for longer than EPS alone, was observed removing a similar amount to EPS (Table 5.2). However, the finite Zn availability dictated by the nature of the batch experiment could have limited the amount of removal the biofilm is truly capable of, as much higher Zn storage in in-stream biofilm has been observed (Chapter 4). The fact that EPS would require the biotic part of the biofilm to maintain its efficiency over long periods of time mean using biofilm as a whole would be more efficient in the long term, a key factor when dealing with discharges in remote areas that may not be maintained on a regular basis. However, the information acquired provides a more in depth knowledge of how the biofilm constituents interact with the water column, and how these interactions influence trace metal mobility, and removal within an aquatic system.

5.4.1.4 Conclusions from batch experiments

Ion	*Charge Density*	*Ionic Radius*	*Experiment Summary Removal Data (median %)*		
			Proof of concept batch experiment	*Light*	*pH*
Zn^{2+}	27.9	0.074	Active (81.68)	FL (78.05)	6.5 (98.32)
			Sterile (38.92)	HL (78.98)	7.5 (96.93)
				LL (79.04)	8.5 (98.32)
				NL (48.38)	
Ba^{2+}	14.8	0.135	Active (36.40)	FL (30.09)	6.5 (46.56)
			Sterile (6.92)	HL (43.29)	7.5 (78.25)
				LL (36.57)	8.5 (59.05)
				NL (12.67)	
Ca^{2+}	20.2	0.099	Active (22.25)	FL (9.02)	6.5 (12.49)
			Sterile (0.56*)	HL (14.76)	7.5 (12.61)
				LL (12.16)	8.5 (10.51)
				NL (0.17*)	
Mg^{2+}	30.8	0.065	Active (10.63*)	FL (3.01)	6.5 (15.39)
			Sterile (-5.09*)	HL (22.95)	7.5 (11.71)
				LL (13.1)	8.5 (16.87)
				NL (-4.72*)	

Table 5.5 Summary data for biofilm batch experiment, it can be seen that Zn is preferentially removed in all experimental treatments. Values denoted with * are median values where negative values were included in the range.

While the data suggests that metal uptake is not significantly affected by changes in light intensity (table 5.5) (i.e. metal removal continues with no light present) and pH difference, it is important to note the small volumes of water used and the amount of zinc in the sample vessel was finite. The evidence in the static batch experiments suggests that the Zn uptake is only indirectly controlled by photosynthetic processes and it is likely that it is the EPS that maintains effective removal through chelation of Zn. Ba results are varied, although lower uptake rates relative to Zn are apparent throughout. There may be considerable scope for enhancing Zn removal with synthetic EPS. Determining specific metal uptake pathways in laboratory simulations and optimal operating conditions, (e.g. ambient light and temperature conditions to promote biofilm growth: Flemming and Wingender, 2010; Pedley *et al.*, 2009. Isolated bacterial strains optimised for specific metal uptake: Krishna *et al*., 2012), are going to be crucial to underpin field development of effective biofilm-based treatment systems in the future. However at the present time, there would be no benefit in terms of cost of separating the biotic and abiotic element of the biofilm at the amounts required for field remediation systems to be implemented.

5.4.2 Long term experiments

5.4.2.1 General remarks

The removal of trace metals over the long term monitoring campaign show initial promise for the use of biofilm as a remediation tool, whilst also providing an insight into the removal mechanisms that may be responsible for metal uptake from the water column. The large amounts of Zn removed in the active flumes compared to the sterile flumes show that it is the biofilm that is having a direct influence on the removal of the metal, and it is also capable of removing other metals as seen with the removal of Ba.

However the difference in rates of removal would suggest different mechanisms are exerting control on them. The removal of Ca from the flume would begin to suggest that the precipitation of calcite (or other calcium phases) plays some form of role as a control on zinc removal from the water column, although more work would be needed to further explore this (presented in Chapter 6).

5.4.2.2 Metal removal rates

To further assess the consistency and magnitude of metal removal rates, the area adjusted removal rates rely on a calculation between residence time (the time taken for water to flow through the flume system) and flow (L/s), which takes into account the metal load removed per unit surface area of biofilm per day (g $m^{-2}day^{-1}$). The mean Zn removal rates for the flumes was 0.6 g $m^{-2}day^{-1}$ (min = 0.13, max = 1.20 g $m^{-2}day^{-1}$) which was achieved with a 9.6 minute mean residence time. Comparisons of preliminary data here with other mine water treatment schemes reveal similar metal removal rates in terms of area adjusted removal rates (Table 5.6).

System	*Removal of Zn (g $m^{-2}day^{-1}$)*	*Reference*
Algal mat (pilot)	0.47	Kalin (1998)
Algal mat (field)	0.25	Kalin (1998)
Wetland system	0.37	Gillespie *et al*. 1999
Lab Wetland System	0.003	Song *et al*. 2001
Lab biofilm system	0.13 – 1.05	This study

Table 5.6 Comparison of similar Zn removal systems (Mayes *et al.*, 2009b)

Pilot and field studies utilising algal mats have been seen to achieve a similar order of magnitude of Zn removal with removal rates of 0.47 g $m^{-2}day^{-1}$ and 0.25 g $m^{-2}day^{-1}$ respectively observed (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). The comparatively small amount of Ba removed from the system is also evident in the removal rates, with the highest removal rates in terms of Ba removed in g $m^{-2}day^{-1}$ being an order of magnitude lower than the highest Zn removal rates. Metal removal rates appear to remain relatively stable in the active flumes over a range of influent concentrations. This suggests that the uptake mechanism is not likely to reach the point where is is fully saturated, as would be expected with sorbent media (e.g. Mayes *et* al., 2009b). This maintenance of removal rates again underlines the importance of the biofilm being able to produce new EPS, creating new sites for metal uptake and maintain metal uptake over the long term.

5.4.3 Management implications

Previous attempts to remove Zn from mine discharges have had mixed results. The adaptation of anoxic limestone drains to encourage smithsonite ($ZnCO_3$) precipitation has been investigated at field scale and although showing initial promise, removal rates of 10 – 30% were a product of the difficulty of maintaining narrow geochemical operating conditions under field conditions (Nuttall and Younger 2000). Much research effort has also gone in to developing bioreactors or sorbent-based systems for mine water removal. Although 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), there have been successful applications of bioreactors for circum-neutral waters. For example Gandy and Jarvis (2012) report average area-adjusted Zn removal rates of 0.4g/m^2/day in pilot 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 (e.g. Jarvis and Mayes, 2012). Sorbents, or reactive media, are another option that have been explored by many workers. Generally sorbents are capable of high metal removal rates, up to 99% removal of zinc) which initially sounds promising, however, this level of uptake can only be maintained for small volumes of water (~200L), as functional sites for metals to sink to are saturated, the 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 the biofilm is that it is a community of living organisms, so the EPS which they create, is constantly being replenished. This effectively means that sites for metal uptake are constantly being produced, resulting in higher efficiencies being maintained.

5.5 Conclusions

Biofilm holds the potential to be a low cost, passive remediation tool. Biofilms are ubiquitous in circum-neutral pH, metal-rich waters and play a crucial role in the diurnal and seasonal cycling of trace elements (e.g. Nimick *et al.,* 2011). Studies have also noted the role biofilms play in the natural in-stream attenuation of Zn (Jones *et al*., 2013). High concentrations of Zn (6100 – 7600 µg/l) have been observed elsewhere to be naturally attenuated by in-stream precipitation and co-precipitation of Zn, and the stream pH are similar to those found in this study; circum-neutral to alkaline (Podda *et al*., 2013). Further to this, the precipitation was observed on a seasonal basis, which is a strong indicator of biological mediation and a lack of inorganic precipitation, given biotic dieback in colder months. However, similar studies do point out the need to better understand mechanisms of Zn removal by biofilms, in order to fully develop them into site specific remediation options.

The laboratory studies here highlight how natural biofilm communities can be readily cultured and maintained under controlled conditions in the laboratory. Under these conditions, zinc removal was consistent and long-lived; the production of EPS appears to replenish sites for metal uptake within the biofilm. Zn removal rates are consistent with many other systems, but it is the low residence time and longevity of treatment without maintenance that are most promising for developing biofilms into a treatment option. Harnessing these low-energy biological processes in remote locations, where modest Zn concentrations impact headwater streams of otherwise high amenity value, could provide a new approach in mine water treatment. This chapter has shown observations of consistent and preferential uptake of Zn over relatively long periods of time. While there are lines of evidence that this Zn uptake encompasses both abiotic (i.e. sorption to EPS) and biologically-mediated components (i.e. the sustained removal in active treatments versus control treatments) the specific uptake mechanisms are difficult to elucidate from long term spot sampling. The next chapter considers the transient influence of biofilm on water column metal concentrations through high intensity, high resolution monitoring of the flume environment to cover features of the biofilm not currently addressed in this chapter;

- Biofilm biogeochemical processes over short timescales (24hour / diurnal)

- The permanence of trace metal removal,
- Evaluation of the scope for engineering systems at full scale

6

6.0 Transient Controls on Zinc mobility: the Influence of Freshwater Biofilms

6.1 Introduction

Observations of diurnal cycling of major labile parameters (e.g. pH, temperature, dissolved gases) in surface waters have been widely documented in recent decades (Kannan and Job, 1980; Nimick *et al*., 2011, Gammons *et al*., 2013). Fluctuations of in-stream metal concentrations have been observed in tandem with these fluctuations of master variables, with fluctuations of Zn concentration being observed as much as 500% over a diurnal cycle (Nimick *et al*., 2003, Nimick *et al*., 2005). These findings have been outlined in the literature review of this thesis, along with the current understanding of the potential controls on such fluctuations. Whilst being informative, the wide range of uncontrolled variables in field studies and with data obtained in the field based diurnal study carried out for this thesis, even with in-situ filtration of samples and alkalinity titrations make it difficult to ascertain which mechanisms are responsible for the cycling of trace metals. Combinations of physical, chemical and biological factors have been suggested as being responsible for such fluctuations (Fuller and Davis, 1989; Nimick *et al*, 2011, Gammons *et al*, 2013, Section 2.4 and 2.5). The key underlying control on in-stream cycling is considered to be the response to the solar photo cycle (Nimick and Gammons, 2011). Some efforts have already been made to try and determine the controlling mechanisms underpinning the cycling of trace elements in stream (e.g. Nimick *et al*, 2004). However, studies are universally in natural settings, where too many partially dependent variables operate simultaneously to isolate primary controls. However, there has been some attempt at controlling master variables, Morris and Meyer (2007) utilised different light chambers and observed the effects the different treatments had on Zn concentrations. Treatments applied included a sunlight chamber, a dark chamber, and two chemically treated chambers, one which inhibited photosynthesis and one which removed any biotic element in the chamber. Their experiment was only left for four hours, yet this was sufficiently long enough to observe an increase in pH and decrease in total Zn in the chamber exposed to light and no change or a decrease in pH and an increase of total Zn in the other chambers. Even though attempts at control in this study are evident, the in-stream positioning of the chambers still present numerous permutations in variables which could interfere with Zn fluctuations, and make it difficult to conclusively identify controlling factors of the cycling. The diurnal study carried out in the Chapter 4 (Section 4.4.3) outlined the issues with studying trace metal cycling in natural settings and the lack of conclusive data in the study underlined the requirement for a laboratory based study in which variables can be controlled.

Another arena of focus in this chapter lies in the ability of biofilm to withstand environmental stressors should they be implemented as part of a remediation system. As already outlined in Chapter 4, the majority of the point discharges with EQS breaches of trace metal concentrations are in remote areas, and are a part of catchments with uplands with largely peat based soils, which can cause acid-flushing events that may remobilise large inventories of metals in-stream. Studies in natural settings have shown pH unit changes of 0.2 – 0.3 (Byrne, 2009), 0.2 – 0.8 (Palumbo-Roe *et al*, 2012) and up to 1.65 (Palumbo-Roe *et al.*, 2007) due to an event which introduces acid, such as peatland run-off. The implications of lowered pH are not simple; different environments will give rise to numerous interactions which will buffer the effects of lowering pH. For example, Palumbo-Roe *et al*. (2007) found that a decrease in pH would give a negligible increase in Zn concentrations until a threshold pH was reached. When this threshold is reached, mineral dissolution conditions are promoted, and whilst carbonates are utilised buffering the solution, any metals previously incorporated in carbonates are released. It is also possible that a sizable reduction in pH would result in sorped metals becoming mobile again (Palumbo-Roe *et al.*, 2007).

The aim for this chapter concerns identifying the controls on biogeochemical factors and trace metal fluctuations exerted by biofilms. It is important to understand the short term biogeochemical cycling exerted by biofilms, and their stability and resilience to real world situations, in order to properly asses their functionality and reliability should they become a viable option for remediation. Presented are results from two experiments;

- A 96 hour laboratory diurnal study in which biogeochemical data and metal concentrations are collected to observe the control exerted by the biofilm in an environment isolated from external stressors, and other factors which could impact changes in geochemistry and trace metal concentrations (i.e. temperature, flow fluctuation, biotic influences) are controlled. For a more in depth review of the experimental procedure refer to Chapter 3.
- A simulation of an upland rainfall event. This involves flushing the flume system with acidic water, an exaggeration of a natural flow event with increase runoff from peat rich uplands. This was to give a worst case scenario to assess the stability of metals stored by the biofilm.

6.2 Methods

This chapter considers the results of two continuous flow flume experiments. These are: (a) an investigation of diurnal cycling in biofilm-colonised flumes against sterile systems, and (b) an assessment of metal release from biofilm with a simulated acid-flushing event. Generic methods detailing experimental set-up, sampling and analyses are presented in Chapter 3 of this thesis.

6.2.1 Diurnal experiment

The diurnal sampling experiments took place with the flume system reservoir filled to maximum level, and had been allowed to run for a full month to ensure the system had chance to equilibrate.

6.2.1 Acid release experiment

An experiment to determine the stability of metal sunk in the biofilm when exposed to conditions which could potentially occur in nature (acid runoff) were carried out in a laboratory setting, where all variables affecting the experiment can be controlled. The flume had been operating in a steady state for a month before the experiment commenced. For the experiment, the physical setups of the flume systems were altered to a flow-through design (Figure 6.1). In this new configuration, water from a small upland stream with a low pH (4.0 -4.5) was fed through the flume to simulate a rainfall event. This water composition is a close analogue to water derived from over-land flow entering the Hebden Beck catchment, which is surrounded by peat-rich upland. As the Hebden Beck catchment is a very representative catchment for circum-neutral mine-water affected streams in the UK, the results of this experiment are therefore highly generalizable (Section 3.2.2). The water was taken from a small stream that drained an area of peatland in the south east of the catchment (GB Grid Reference - SE 02679 64611). The actual pH of the acidic water used in the experiment was 4.14. This peat water was added to the flume without alteration, essentially representing a worst-case-scenario in which surface runoff water fully dominates the composition of water within the Beck.

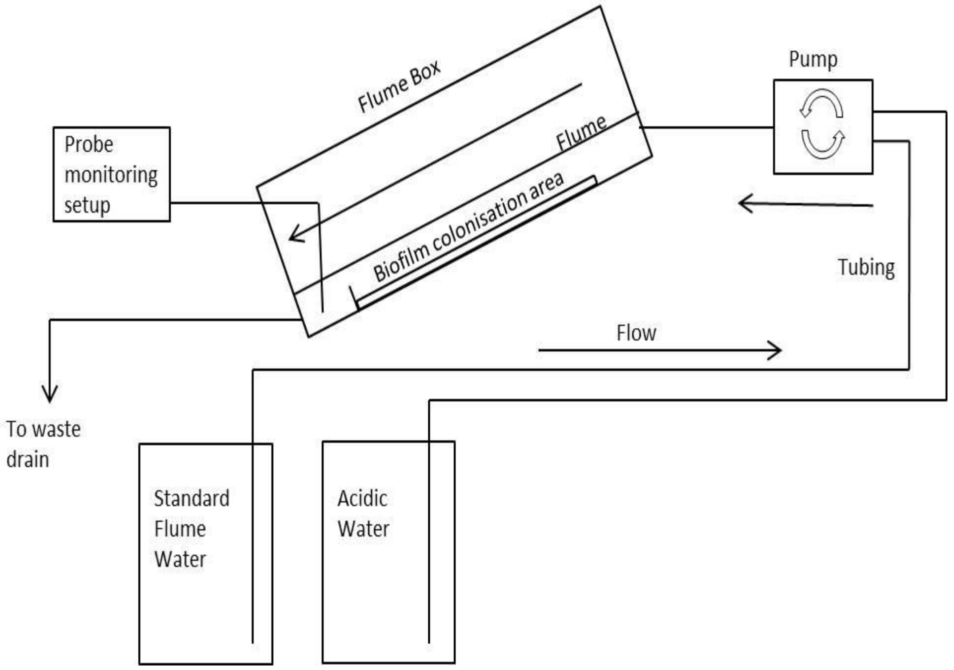

Figure 6.1 Flume set up altered for acidic experiment. Note the addition of the tank for the storage of acidic water, and the drainage from the flume going to the drain. The set up was designed so the flume water and acidic water could not mix, so the biofilm was either in a standard or an acidic environment, in order to achieve a worst case scenario conditions.

6.2.2 Geochemical Modelling

6.2.2.1 Diurnal Experiment Data

In order to assess the nature of the control on the diurnal cycle in Zn and Ba concentrations, the potential precipitation of key mineral phases have been modelled for the flume environments over the same timescales as the experiment. This was done using the geochemical code PHREEQC v.1.5.10 (Parkhurst and Appelo, 1999) with the WATEQ4F database (Ball and Nordstrom, 1991) to calculate saturation indices of relevant mineral phases. This database is particularly well-suited to assessing Zn patterns given the range of amorphous and solid phases covered in WATEQ4F.

6.2.2.2 Acid Release Experimental Data Analysis

The pH was modelled to assess the implications of acidic runoff mixing with stream water, and the potential for metal release from the biofilm. Whilst the introduction of acidic water into the flume in the manner it was helped with understanding trace metal mobility under such conditions, the worst-case-scenario simulated is not representative of all acidification scenarios possible within a beck catchment. Modelling water at different ratios of stream water and acidic water helps identify what conditions would prevail as the different waters mixed, and in combination with data from other experiments allows for the understanding of how biofilms would react in natural conditions. The pH was modelled using ratios of flume water: acidic water using the MIX function in PHREEQC. Further modelling of the acidic experiment data concerned modelling the mineral phases at each mixed ratio, which gives a good insight into the precipitating environment of the flume system. This again was done using the geochemical code PHREEQC v.1.5.10 (Parkhurst and Appelo, 1999) with the WATEQ4F database (Ball and Nordstrom, 1991). These results were validated by comparing the modelled and experimental results for the worst-case-scenario.

6.3 Results

6.3.1 Diurnal Experiment

6.3.1.1 pH

The median daytime pH obtained from sensors within the flume was 8.75 in flume 1 (range 7.93-9.31, n = 43) and median night time pH was 7.99 (range 7.88 – 8.48, n = 101). Flume 2 showed a similar trend, yet had less pronounced changes in pH from daytime to night time (daytime: median = 8.71 (range = 8.29 – 9.23, n = 43) night time: median = 8.45 (range = 8.33 – 8.78, n = 101)). Flume 3 (control flume) exhibited no diurnal 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). The high pH during light conditions and the lower pH during dark conditions in biofilm colonised flumes cause the pH difference to be significantly different (Table 6.21). There is very little time between the lights coming on and the change in pH occurring. Figures 6.2, 6.3, 6.4 and 6.5 are not presented in sufficient resolution to visually observe the lag, but this was consistently around 5 minutes. This indicates a rapid transition in the photosynthetic activity in the biofilm, indicating little buffering from the ambient water or metabolic delays within the biofilm community. Diurnal changes in the field will therefore reflect gradual changes in light intensity, not buffering or lagging processes in the system.

Flume	*pH (median(range))*		*Significant difference?*
	Day	*night*	
1	8.75 (7.93-9.31)	7.99 (7.88-8.48)	Yes, P<0.001
2	8.71 (8.29-9.23)	8.45 (8.33-8.78)	Yes, P<0.001
3	8.16 (8.05-8.29)	8.13 (8.07-8.30)	Yes, P<0.01

Table 6.1 Summary flume pH data. Significant difference between day and night pH concentrations calculated via Mann-Whitney U test.

6.3.1.2 Aqueous metal concentrations

Results from the diurnal sampling campaign are shown in Figure 6.2, 6.3, 6.4 and 6.5. Zn concentrations in flumes 1 and 2 are fluctuating by up to 77% and 64% respectively. The control flume exhibited no diurnal fluctuation in Zn concentration

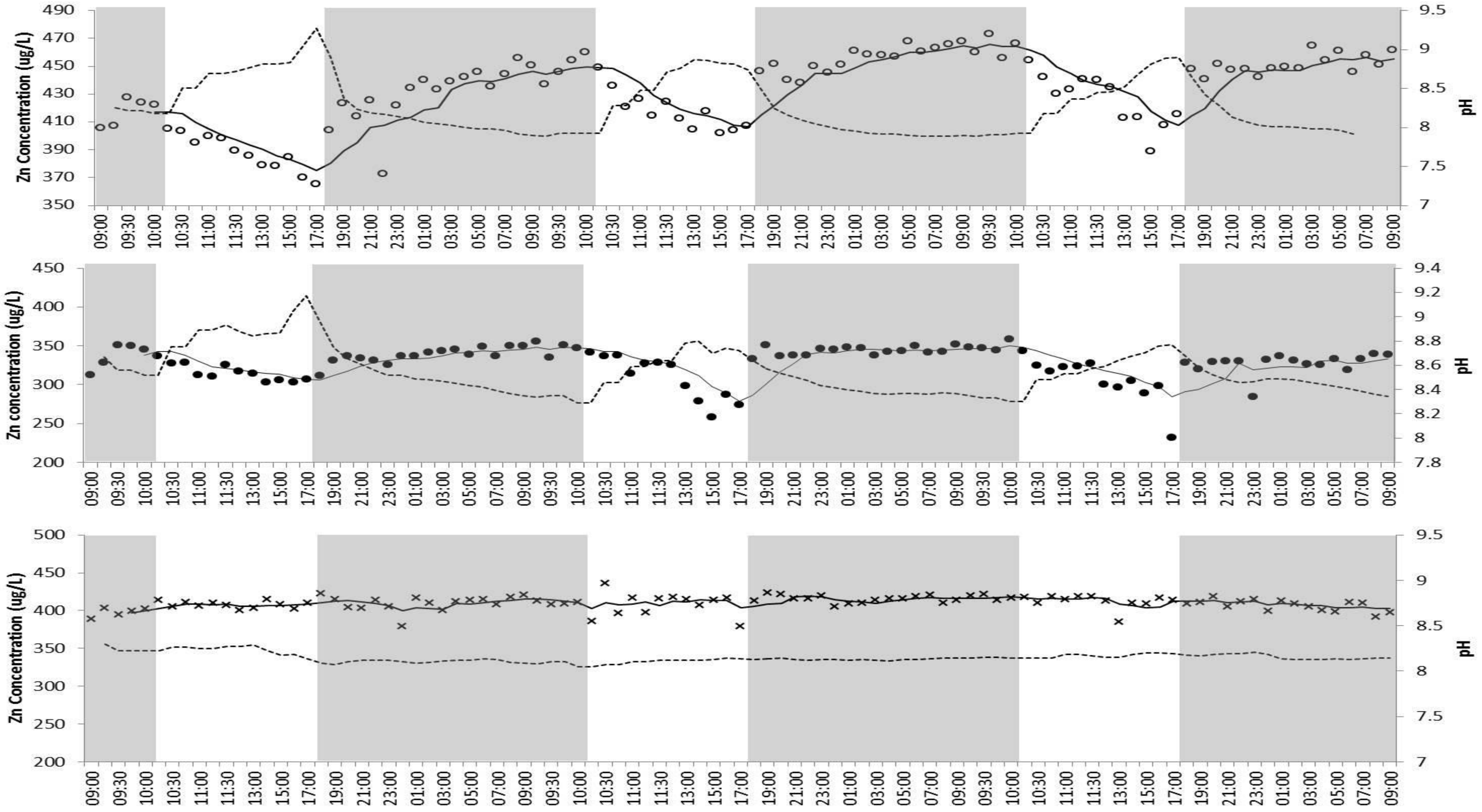

Figure 6.2 Diurnal experiment results for Zn presented for flume 1 (open circle), flume 2 (filled circle) and flume 3 (cross). pH is shown by the dashed line in all graphs. Shaded areas denote dark hours.

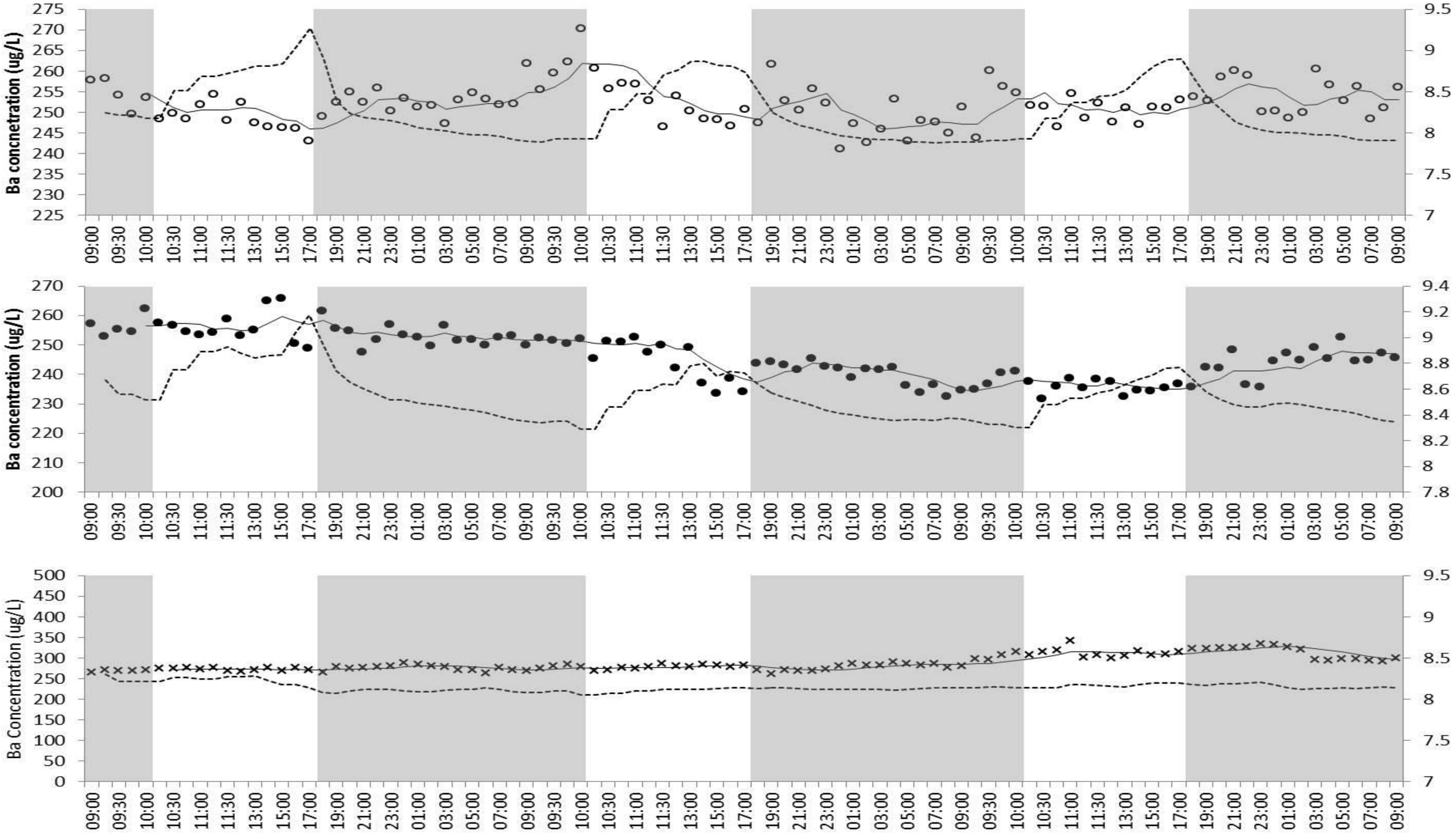

Figure 6.3 Diurnal experiment results for Ba presented for flume 1 (open circle), flume 2 (filled circle) and flume 3 (cross). pH is shown by the dashed line in all graphs. Shaded areas denote dark hours.

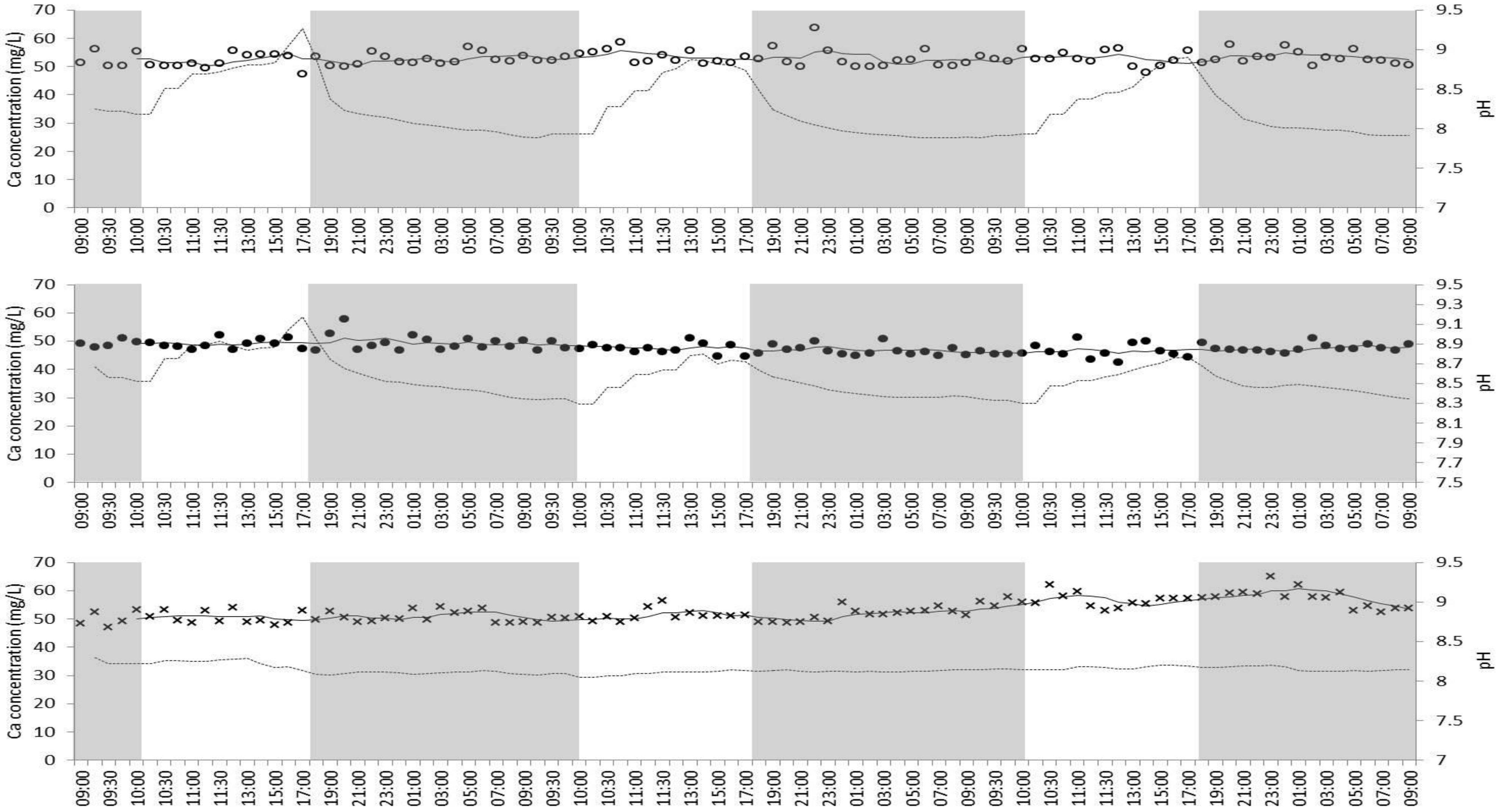

Figure 6.4 Diurnal experiment results for Ca presented for flume 1 (open circle), flume 2 (filled circle) and flume 3 (cross). pH is shown by the dashed line in all graphs. Shaded areas denote dark hours.

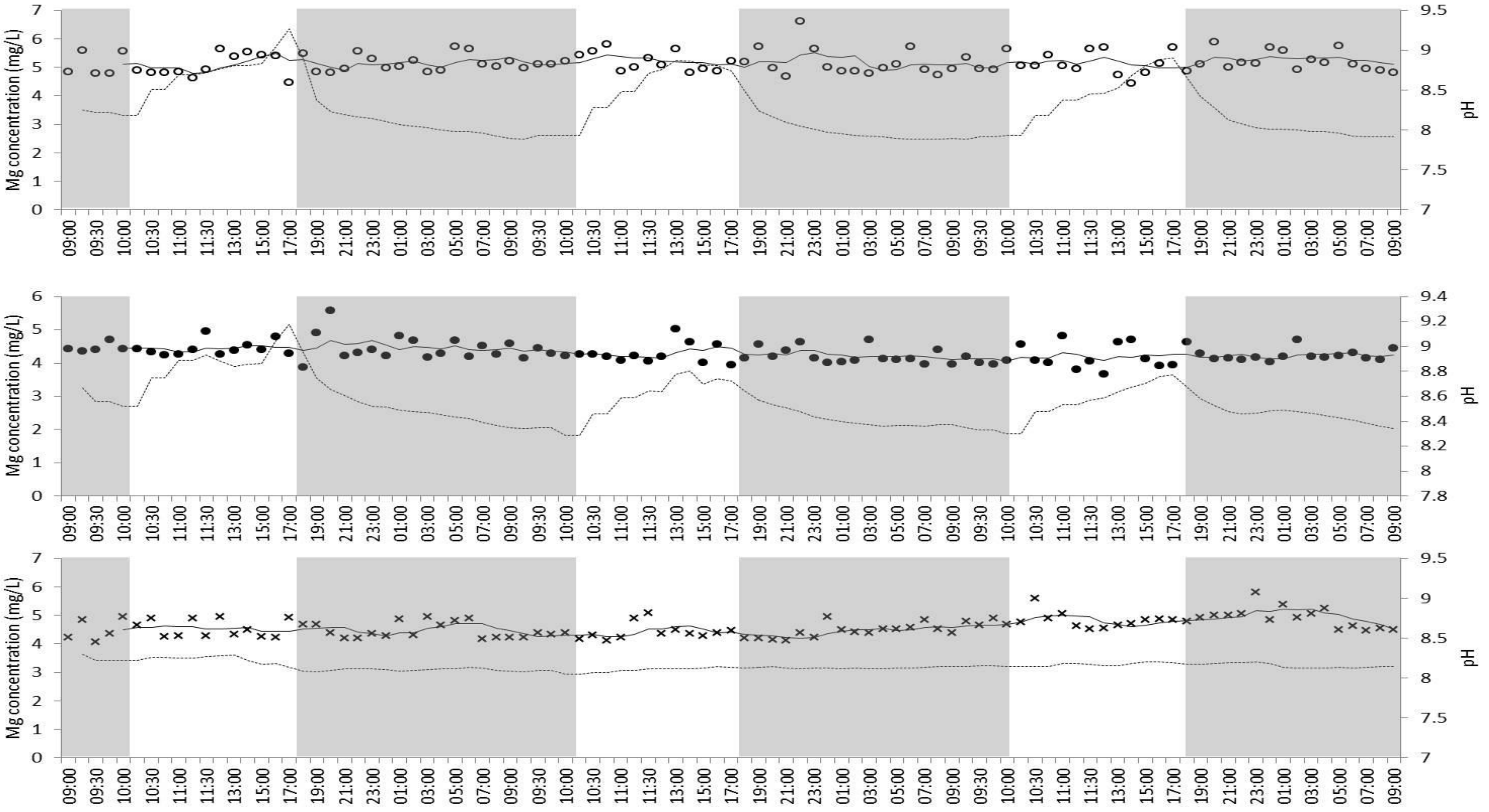

Figure 6.5 Diurnal experiment results for Mg presented for flume 1 (open circle), flume 2 (filled circle) and flume 3 (cross). pH is shown by the dashed line in all graphs. Shaded areas denote dark hours.

This again alludes to the fact that the presence of biofilm is having an effect on the aqueous concentrations of Zn. As seen in cycling of Zn in studies carried out in natural settings already referenced, the minimum and maximum concentrations of Zn are observed just before the period of light and darkness respectively (Nimick *et al.*, 2011). In flume 1 the minimum value of 365 µg/L was observed just before a period of light began, and the maximum value of 472 µg/L being observed as a period of light ended, this also occurred in the other active flume with values of 231 µg/L and 358 µg/L respectively. It should also be noted in Figures 6.2, 6.3, 6.4 and 6.5 the pH cycle and Zn cycle are mirrored, i.e. when pH reaches its highest value, Zn will reach its lowest, which suggests that the pH is exerting control over the concentrations of Zn (Pearson's correlation, $R^2 = -0.69$, $p<0.01$, df = 163).

Ba cycles are observed in flume 1, although are not apparent in flume 2, and, as expected, not apparent in the control flume (Figure 6.3). The cycle of Ba in flume 1 is not as pronounced as Zn in terms of its magnitude, with a maximum of 270 µg/L and minimum of 241 µg/L, a difference of only 29 µg/L compared with the difference of 107 µg/L observed in Zn concentrations. While there appears to be a cycle in Ba data in Flume 1 (Figure 6.3), the amplitude of this change is modest and within the detection limits of the analysis. As such, despite the significant difference (Table 6.2), further analyses would be needed to corroborate such patterns. Given there was no Ba cycle observed in Flume 2 and the Control flume, it can be concluded that if there is any Ba cycle, it is certainly not as pronounced as Zn and suggests differing mechanisms controlling their behaviour. There is a slight lag in the reaction of Ba to the photo cycle, putting it slightly out of phase with the cycle of pH. Flume 2 does not exhibit a discernible cycle in Ba concentrations as observed in flume one, which was not

Ion	*Flume*		*Median (range) (Zn, Ba - µg/L. Ca-Mg – mg/L)*	*Cycle present (Significant difference between day and night)?*
Zn	1	Day	409 (365 – 453)	Yes (P<0.001)
		Night	447 (372 – 472)	
	2	Day	314 (231 – 343)	Yes (P<0.001)
		Night	338 (284 – 358)	
	3	Day	410 (379 – 435)	No (P>0.05)
		Night	411 (379 – 423)	
Ba	1	Day	250 (243 – 260)	Yes (P<0.01)
		Night	252(241 – 270)	
	2	Day	246 (231 – 265)	No (P>0.05)
		Night	245 (232 – 262)	
	3	Day	279 (266 – 341)	No (P>0.05)
		Night	281 (260 – 334)	
Ca	1	Day	52.40 (47.30 – 58.51)	No (P>0.05)
		Night	52.39 (49.97 – 63.76)	
	2	Day	47.64 (42.55 – 52.28)	No (P>0.05)
		Night	47.36 (44.85 – 57.73)	
	3	Day	52.65 (47.91 – 62.04)	No (P>0.05)
		Night	52.61 (47.11 – 65.16)	
Mg	1	Day	5.06 (4.45 – 5.82)	No (P>0.05)
		Night	5.09 (4.67 – 6.60)	
	2	Day	4.27 (3.68 – 5.03)	No (P>0.05)
		Night	4.22 (3.87 – 5.57)	
	3	Day	4.53 (4.12 – 5.59)	No (P>0.05)
		Night	4.53 (4.07 – 5.80)	

Table 6.2 Summary trace metal data from diurnal study Illustrating maximum and minimum concentrations and if cycles were present. Ca and Mg concentrations are mg/L, Zn and Ba concentrations are µg/L. Mann Whitney U test applied to test for significant difference in night time and daytime values.

expected given the same pattern of cycle in pH observed in flume 1.Ca and Mg exhibited no diurnal fluctuation with the concentrations remaining fairly constant throughout the process (Figure 6.4 and 6.5 respectively). Ca and Mg are other ions of interest, given their likelihood to be involved in precipitation reactions presented no cycles in the active flumes. The results of the diurnal experiments are summarised in Table 6.2, where cycles are identified by a significant difference in day time and night time concentrations.

6.3.1.3 Geochemical modelling

Geochemical modelling of mineral phases over the diurnal timescale in the flumes are presented in Figure 6.6 and 6.7. Modelling data shows SI_{CaCO3} did exhibit periods of higher and lower saturation (flume 1 - max = +1.52, min = +0.67, flume 2 – max = +1.52, min = +0.67), and these fluctuations correlated well, as expected, with fluctuations in pH and Zn concentrations in both flumes. No Zn minerals are close to saturation in the flume environment, although they do exhibit diurnal cycles in their respective SI values and these results are presented in Table 6.3. No fluctuations where observed in the control flume (3). It is interesting to note the stability of SI_{BaSO4}, which could be responsible for the long term sinking of Ba in both flumes in the long term study (Chapter 5), and the apparent lack of a discernible cycle in flume 1.

Flume	Mineral	SI_{max}	SI_{min}
1	Calcite ($CaCO_3$)	+1.53	+0.31
	Barite ($BaSO_4$)	+0.42	+0.29
	$ZnCO_3{:}H_2O$	-0.29	-1.05
	Witherite ($BaCO_3$)	-1.11	-2.40
2	Calcite ($CaCO_3$)	+1.52	+0.67
	Barite ($BaSO_4$)	+0.38	+0.27
	$ZnCO_3{:}H_2O$	-0.36	-0.90
	Witherite ($BaCO_3$)	-1.15	-2.00

Table 6.3 Saturation Index information for biofilm colonised flumes as graphed in Figures 6.6 and 6.7

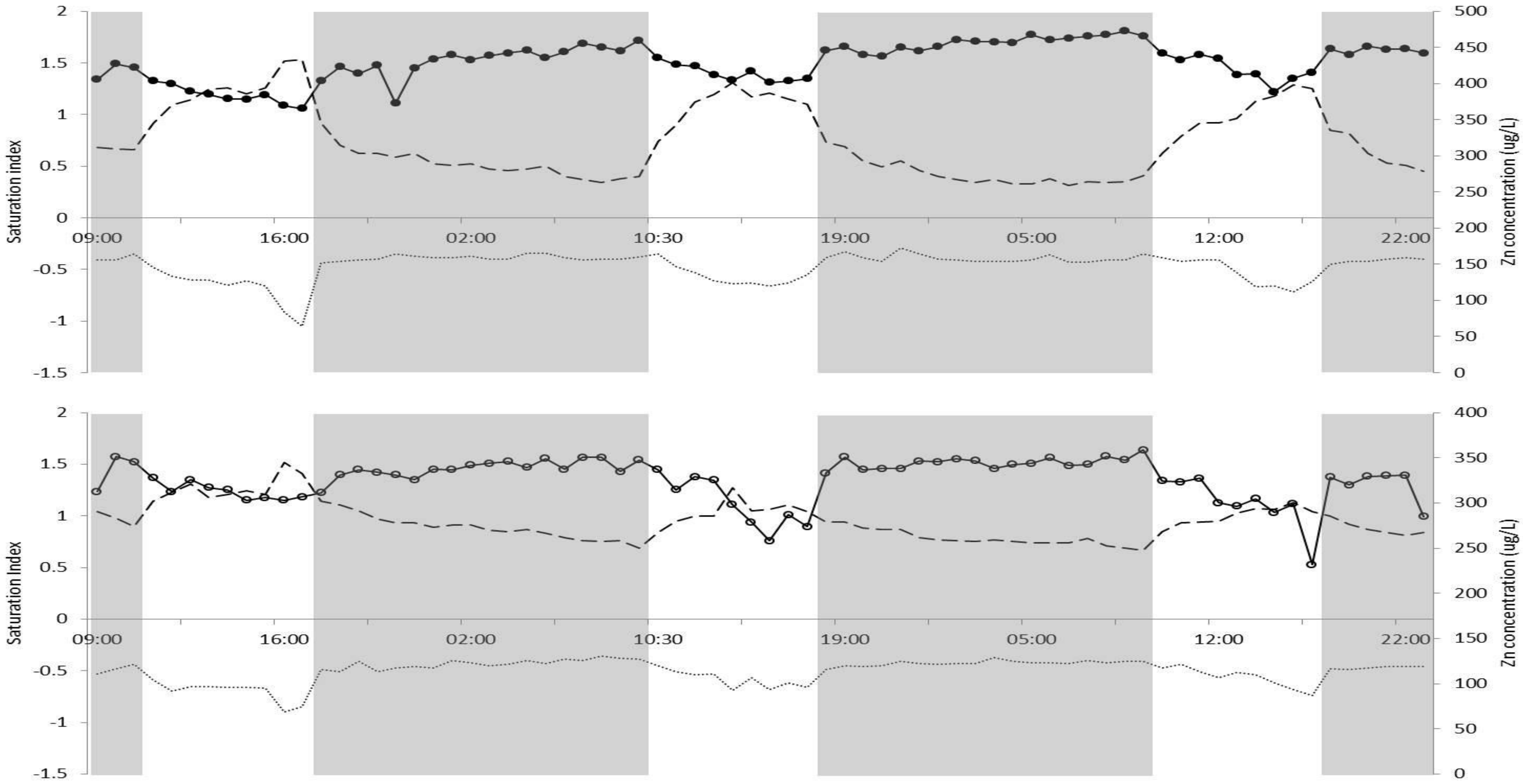

Figure 6.6 Geochemical modelling of diurnal experiment Zn data presented for flume 1 (open circle) and flume 2 (closed circle). SI data for $CaCO_3$ (long dashed line) and $ZnCO_3$:H_2O (short dashed line) are shown.

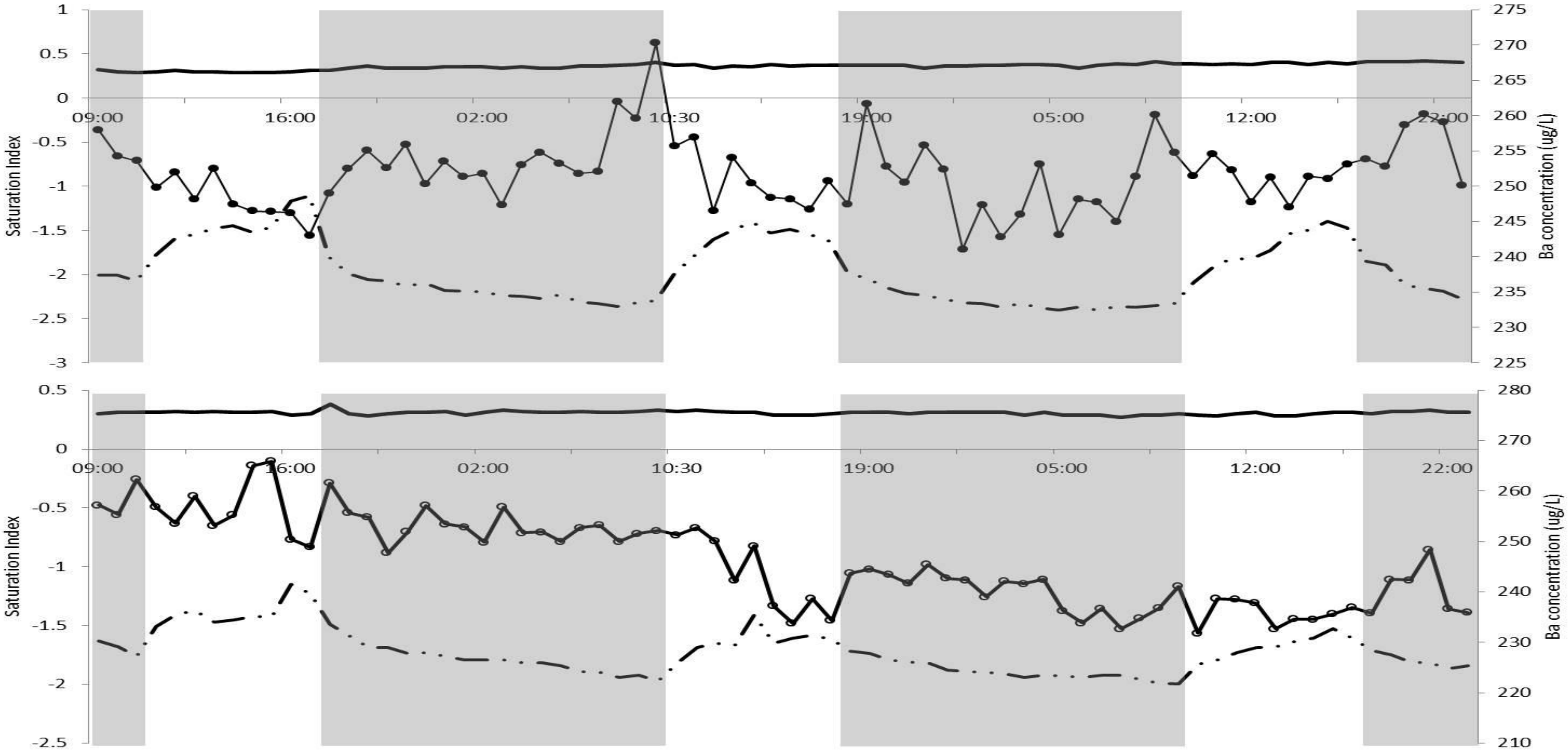

Figure 6.7 Geochemical modelling of diurnal experiment Ba data presented for flume 1 (open circle) and flume 2 (closed circle). SI data for $BaCO_3$ (solid line) and $ZnCO_3$:H_2O (dashed line) are shown

6.3.1.4 SEM Analysis

Scanning electron microscopy (SEM) analysis was performed on samples of the flume biofilm to assess the nature of the solid phase metal sinks. Indicative broad-scale SEM micrographs (e.g. upper left of Figure 6.8) show the broad fibrous structure of the biofilm with some more sub-spherical crystalline mineral precipitates which are labelled in red. Corresponding coarse-scale analysis of the biofilm via EDAX (upper right of Figure 6.8) showed the spectra to be dominated by Ca, C and O but with significant peaks of Al, Si, K, Fe, P and Zn. This would be consistent with the presence of calcite but also possible aluminosilicates (e.g. clays) and organic compounds. Interestingly, the discrete analysis of mineral precipitates (lower left and right of Figure 6.8) shows that the crystalline deposits are characterised solely by the presence of Ca, C and O, with no trace elements or Zn observed in EDAX spectra (lower right of Figure 6.8).

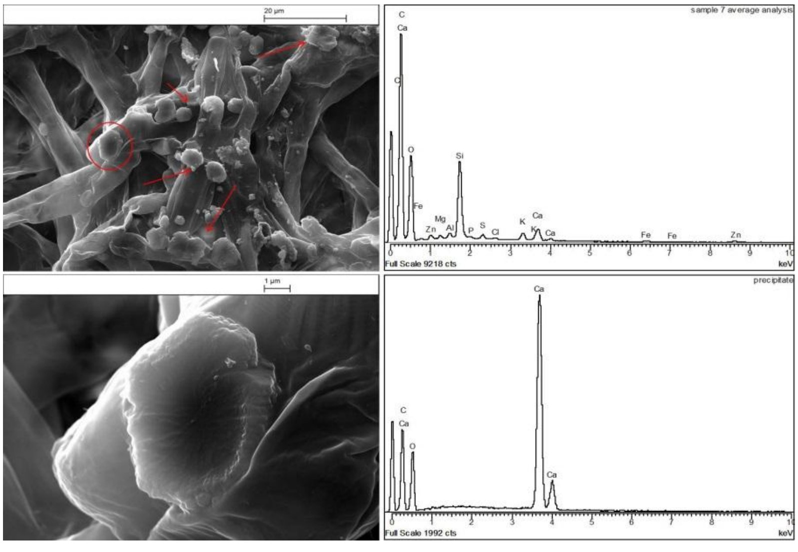

Figure 6.8 SEM of calcite precipitates within the biofilm and corresponding EDAX spectra. Biofilm taken from the flume which shows the small precipitates observed throughout the biofilm (top). Magnification and EDAX confirmation of calcite cystal (bottom).

6.3.2 Acidic experiments

6.3.2.1 Aqueous metal behaviour

Before the introduction of acidic water the pH of the flume was 7.99, and on introduction of the acidic water the pH fell abruptly, initially to 5.12 and then again to 4.19. It can be assumed that this period is remaining flume water in the system being mixed with the newly introduced acidic water, and thus buffering any immediate dramatic effects, and the inflection in pH change is in the range expected for the first dissociation of carbonic acid. The behaviour of trace metals of interest in response to the change in pH are shown in Figure 6.9. Most notable is the release of Zn from the biofilm, with release of other metals negligible. The small release of Zn observed in the control flume could be ascribed to Zn sorbed to flume surfaces, subsequently released in the presence of acidic conditions.

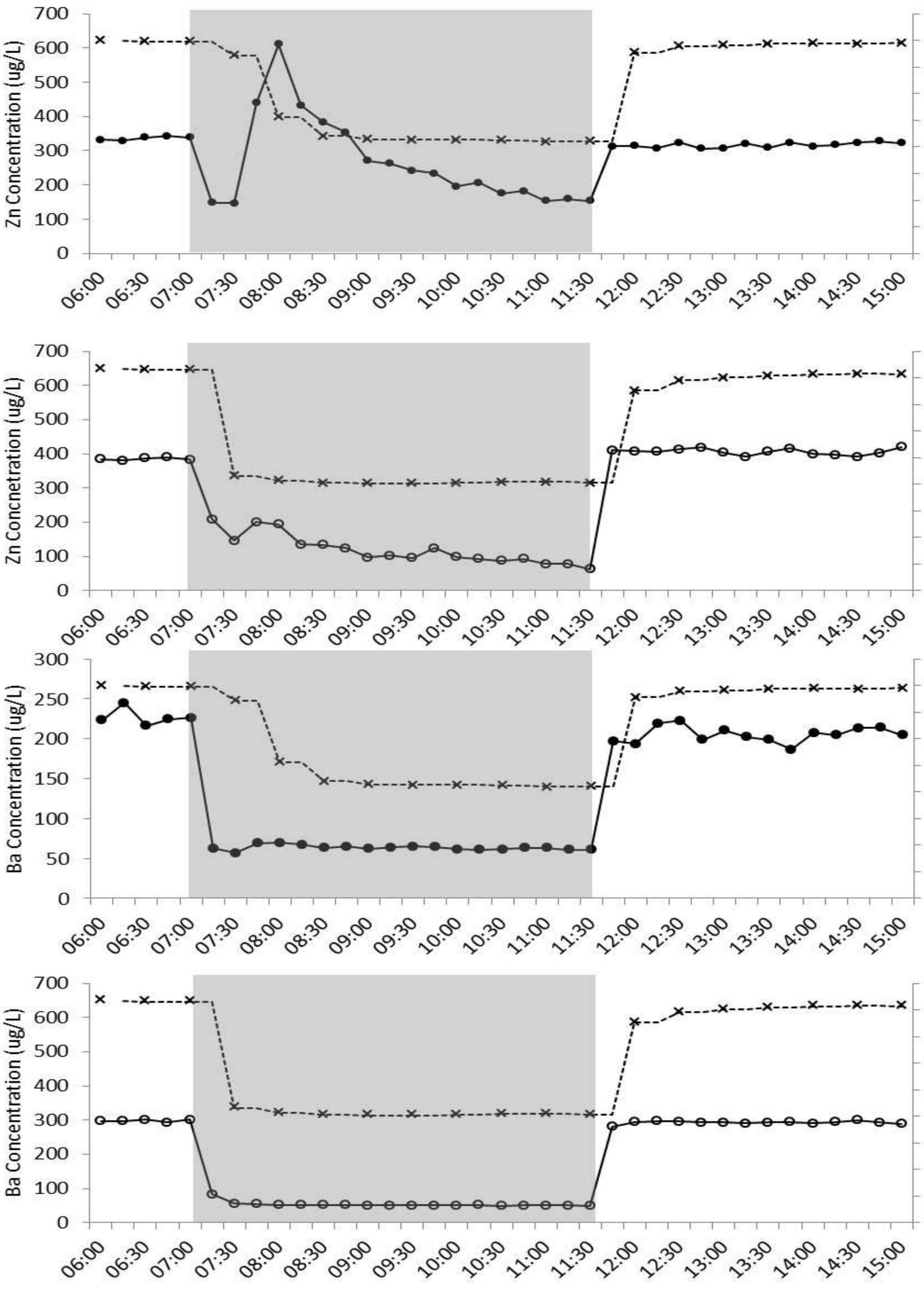

Figure 6.9 Flume response to acidic settings. Shaded area denotes acidic water exposure time. pH (crosses dashed line) and ion concentrations are shown for colonised (filled circle) and sterile (open circle) flumes.

6.4 Discussion

6.4.1 Diurnal pH fluctuations and associated control on trace metals

Fluctuations in pH are a key indicator of biological productivity in the flume. Photosynthesis during light hours will produce O_2 and remove CO_2 (which causes increases pH) and vice versa during dark hours when respiration occurs (decreasing pH) (see literature review (Chapter 2)). The fluctuations in pH observed in this study are consistent with *in situ* studies (Backstrom, *et al*., 2002; Nimick, *et al*., 2005; Morris, *et al*., 2005), and also consistent with the observed geochemical micro-environment in other calcifying biofilm mesocosms (e.g. Rogerson and Pedley, 2010). In such micro-environment studies daytime pH values are far higher than those common in the bulk water column and are apparent due to photosynthetic carbon utilisation by the biofilm (Bissett *et al*., 2008). This information is important for both adsorptive mechanisms of uptake and precipitation-reliant mechanisms of uptake as divalent cations including Zn are more likely to be removed via adsorption at circumneutral pH (Warrender *et al*., 2011). However, it must be stressed that these studies observed whole systems under ambient conditions. One of the more fundamental points of this study lies in the controlled nature of the environment which the flume system provides to observe these cycles over prolonged periods where controls on temperature, light and other external inputs such as diffuse inputs (i.e. rainfall) are in place (see Chapter 3). Given the aforementioned lack of a cycle in temperature within the flume, the fluctuations in the pH data fluctuation are solely due to the presence of biofilm. This is further illustrated by the control flume, which exhibited no pH cycle over the diurnal timescale. This rules out any abiotic factors such as degassing from carbonate saturated waters, which can cause diurnal fluctuations in pH (Liu *et al*., 2006), and illustrates that exclusively biological reactions are producing changes in pH that are consistently repeating over a diurnal timescale.

The pH change is enough to drive significant changes in saturation state of various mineral phases or sorption kinetics, as evidenced by fluctuations in trace metal concentrations (Figure 6.9). The results showed diurnal fluctuations in Zn negatively correlated with pH, therefore the pH cycle in the flume underpins the daily fluctuation of aqueous Zn concentrations (Figure 6.2). The cycling of Zn has been observed in natural settings. For example Morris *et al*. (2005) observes the Zn fluctuation in streamside experiments with results that are similar to the results found in this study. High pH and low Zn concentrations occurred during the day and low pH and high Zn concentrations found during the night, this again being ascribed to internal fluctuations in biofilm geochemistry. The results of Zn diurnal fluctuations from this study provide observations of Zn cycling where pH has been ascribed purely to the biofilm, due to the controls on the environment. Although it is clear pH fluctuations controlled by biofilm are exerting control over the aqueous Zn concentrations in the flume, the mechanism is not clear. Different diurnal patterns to Ba and the lack of resolved cycles in Ca and Mg indicate different chemical mechanisms operate for Zn.

6.4.2 Diurnal Fluctuations in SI of minerals

The results of geochemical modelling of the diurnal fluctuations in mineral precipitation within the flume give some insight into a potential transient control on trace metal mobility, and help to distinguish them from longer term removal mechanisms. Precipitation and dissolution of solid phase minerals has been identified in numerous field studies as a contributor to trace metal cycles over a diurnal timescale, particularly with calcite (Brick and Moore, 1996; Scott, *et al*., 2002; Nimick, *et al*., 2003; Nimick, *et al*., 2009) which has been observed precipitated in the flume environment (Figure 6.8).

SI_{CaCO3} can be seen to cycle within the flume, and exhibits the same characteristics of pH, with higher values throughout the day and lower values during the night with the cycle being apparent across both flumes 1 and 2. This change in SI_{CaCO3} has also been observed in situ in low order streams (Spiro and Pentecost, 1996; Montety, *et al*., 2011), although again it should be stressed that these are in-situ studies, where temperature is also argued to play a primary role (Drysdale *et al*., 2003).

The control on Zn mobility could reflect co-precipitation of Zn with calcite as demonstrated in laboratory settings (Elzinga and Reeder, 2002). The lack of Zn-dominated minerals in geochemical analyses that are supersaturated also implies that Zn could be occupying sites within a non-Zn mineral. However, the precipitation and dissolution kinetics of minerals can be slow in waters with low concentrations of constituent components of the mineral (Arivdson and Mackenzie, 1999), whereas the fluctuations (in particular in the case of Zn concentrations) are fairly rapid and dramatic in the flume environment. Indeed while peak SI_{CaCO3} values observed in the biofilm-colonised flumes approach levels where homogeneous precipitation of calcite is observed (Ford and Williams, 2008), the lack of a pronounced Ca^{2+} cycle in the flumes (Figure 6.4) suggests that calcite precipitation is of peripheral importance in controlling the observed Zn cycles. This leaves open the possibility that co-precipitation of Zn with calcite may be acting as a modest long term sink, but suggests other controls (e.g. pH dependent sorption) are affecting the concentration of Zn concentrations within the biofilm on the shorter, diurnal cycles. Ba minerals barite and witherite do not exhibit cycles in there SIs, with barite at a relatively steady +0.3 and witherite consistently in a dissolved state (SI max = -1.88) across both flumes.

6.4.3 SEM evidence

The presence of calcite in the biofilm identifies an additional potential sink for divalent metals within the biofilm, be it transient or long term. In terms of a transient sink, it provides an area in which metal ions could sorb to, although the small timescales over which environmental factors vary (e.g. pH) would mean the ion could also quickly be released. Longer term, more stable sinking of the metal ion would occur if it precipitated as its own mineral, or co-precipitated as an impurity within calcite. The precipitates identified are generally small, and were found sparsely distributed throughout the biofilm which supports the modelled aqueous data (Figure 6.8) in suggesting calcite precipitation is occurring, but that it is not particularly vigorous, in the active flumes. The absence of Zn in the crystalline deposits, suggests that the Zn sunk into the biofilm is more likely to be associated with the organic EPS matrix.

The suggestion that precipitation kinetics would not be quick enough to explain the cycling of Zn over a diurnal timescale could be explained by a quicker reaction, such as sorption mechanisms (Fuller and Davis, 1989), which could occur on pre-existing calcite precipitates which have slowly generated over time, and chelation of Zn ions into the biofilm EPS, as explained in the previous chapter. It would explain the quick fluctuation response to changes in pH within the biofilm, and this is consistent with results from the acidic experiment, as discussed below.

6.4.4 Trace metal behaviour in acidic conditions

The results gained throughout the acid flushing experiments are beneficial in two ways; (1) in providing further understanding of trace metal dynamics within the stream - biofilm environment, and (2) in assessing the permanence of Zn removal in biofilm with regards its potential use as a remediation tool.

The results of the acid flushing experiment suggest the uptake method of zinc is governed by a pH dependent adsorptive process, given the speed of the release of Zn when the biofilm was inundated with acidic water, where other ions do not show the same release, if they show a release at all (Figure 6.9). The drop in pH and the accompanying sudden rise, and subsequent gradual return, in Zn concentrations observed in the flume environment of this study (Figure 6.9), and other studies in natural settings, can be attributed to the first flush phenomenon (Younger, 2002). Observed flushing events have been attributed to snowmelt (Lundquist and Cayan, 2002) but for the purposes of this study flushing events will exclusively relate to high rainfall events, as snowmelt is not something commonly associated with UK catchments. Such a dramatic rise in the concentration of Zn could potentially breach Environmental Quality Standards, and this situation manifests itself in this experiment. When it is considered that the ambient Zn concentrations are on the borderline of breaching the aquatic standards for salmonids (300 μg/l Zn in alkalinity of 100mg/l

$CaCO_3$ waters; the flume water was between 150 and 200 mg/l $CaCO_3$), a key indicator species for water quality, any rise would effectively breach the EQS, let alone an event which effectively doubles the available zinc concentration. The bioavailability of Zn would also be dramatically increased in this system, given the lack of any discernible alkalinity, potentially rendering the EQS limit as low as 30 µg/l Zn. While this presents a simulation of an extreme geochemical change, it does suggest that acid flushing events in mine-impacted catchments could cause short term acute elevations in dissolved metal concentrations as has been sporadically observed under field conditions (e.g. Byrne *et al*., 2013; Gozzard *et al*., 2011). The implied pH dependency of the reaction raises the issue of the stability of removed metals in the biofilm when it is situated in natural settings. This simulation was done as a worst case scenario, with the acid event been run independently of any flume water and thus not been affected by the flume water's buffering capacity. Should biofilm be used as a remediation tool, it will likely be employed in area with high alkalinity, and thus the geochemical make-up of the waters will be well buffered to the effects of acidic water intrusion.

6.4.5 Geochemical modelling

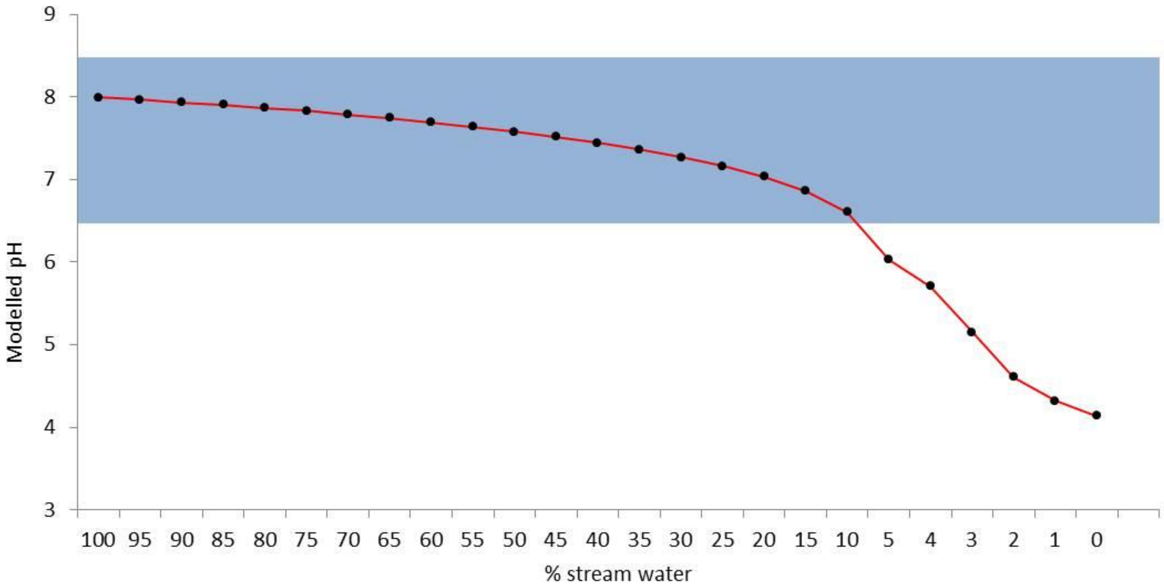

Figure 6.10 PHREEQC modelled pH for a mixture between flume water and acidic runoff water. Blue highlighted area represents pH at which uptake by biofilm is known to occur from previous experiments.

As would be expected due the acidic nature of the system during the rainfall event, the conditions for dissolution of minerals is promoted, and this was evident during PHREEQC modelling, where all minerals of interest where under-saturated. This may be very different where the drop in pH is buffered by alkalinity in the ambient water. The pH modelling carried out (Figure 6.10) illustrates the potential pH of the stream when acidic water is available at different ratios, and as the acidic water will be well buffered by the carbonate in the stream water, the pH drop is not severe until the steam to acid water ratio is in excess of 1:9. These results help to reinforce the findings from the previous chapter and help to illustrate the different mechanisms governing trace metal mobility in the flume environment and ultimately the influence biofilm exerts in streams and rivers impacted by mine drainage. The modelled SI values for the acid release experiments show even at 100% stream water, Zn minerals are under-saturated whilst calcite and barite (potentially a mineral sink for Ba) are precipitating to around a 6:4 mix of stream and acid water. SI_{Barite} has a high propensity for precipitating and generally will precipitate when Ba is in the presence of SO_4^{2-}, (Banks *et al.*, 1997). Even with under saturation of zinc minerals at 100% stream water, there is still a marked release of Zn when biofilm is exposed to acidic water, and it is interesting to note the lack of Ba released under the same forcing (given the aforementioned lack of mobility in the presence of sulphate), which again points to a mechanism removing Zn that does not rely on precipitation of minerals, but instead a biological uptake mechanism provided by the biofilm. The combination of this data, with the mass removal data from the flumes (Section 5.3.3) gives a good indication that the mechanism responsible removes ions on a preferential basis. Calcium exhibits the highest removal from the system , which is expected due to the fact there are significantly more calcium ions avialable in the water column. However, the ratios between calcium uptake and other ions uptake can help to illustrate the preferntial mechanims of uptake. On average, for every milligram of Ca sequestered , 0.26 milligrams of Zn are sequestered, which is an order of magnitude more than Ba (0.02mg of Ba per 1mg of Ca), which itself is an order of magnitude more than Mg removal (0.01mg of Mg per 1 mg of Ca). The difference in removal in term of ratios helps to illustrate the mechanims governing long term removal of Zn. Ca:Zn is order of magnitude higher than the Ca:Mg and Ca:Ba, indicating that if a precipitation rection were to be responsible for the removal of trace metals, Zn would be preferentialy removed. The presence of Calcite precipitates observed in SEM analysis show calcite precipitation throughout the biofilm, which would provide a long term sink fo Zn via co precipitation or adsorption (by providing a site for Zn to sorb to). However, the cyclng of Zn also show that shorter term dynamics play a role in the removal of Zinc, albeit in a transient nature. These removal mechanims are explained in the next section (section 6.4.6).

6.4.6 Potential Removal Mechanisms

The schematic below (Figure 6.11) outlines the mechanisms with description (Table 6.4), principles of the mechanisms and the metals that would be most probable of undergoing sinking and release in these areas

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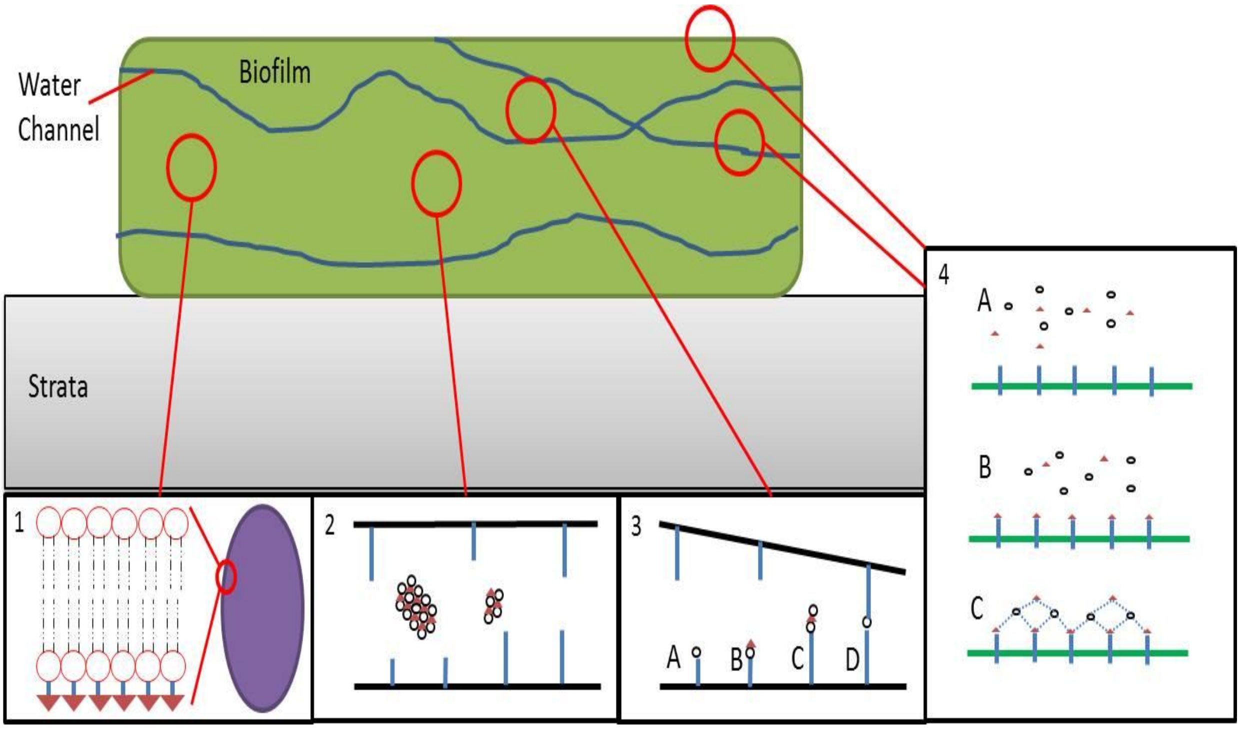

Figure 6.11 Schematic depicting processes responsible for metal removal in biofilm. Section 1: sorption to bacterial cell wall. Bacteria (purple) with magnified phospholipid bilayer (red circle/black lines), phophoryl functional groups (blue line) and Me2+ (red triangle). Section 2: homogeneous precipitation. EPS molecule (black line), negatively charged functional groups (blue line), Me^{2+} (red triangle) and Carbonate group (open circle) are depicted. Section 3: Unidentate chelation (A), Nucleation (B), Heterogeneous precipitation (C) and bidentate chelation (D), key as per section 2. Section 4: adsorption (A) and further nucleation via double layer theory (B + C). Biofilm boundary (Green line) with negatively charged functional groups (blue solid line), Me^{2+} (red triangle) and Carbonate group (open circle), and bonding between them represented by the blue dashed line.

Section	*Mechanism*	*Description*
1	Sorption to bacteria cell wall	The phospholipid bi-layer has been identified as playing an important role in the removal of Zn via sorption (Toner *et al*., 2005). Toner *et al*, show that 85% of the zinc sorption to the biofilm can be attributed to Zn-Phosphoryl complexes, and postulate that these complexes are likely to take place at the phospholipid bilayer of gram negative bacteria as they provide the phosphoryl group in the Zn – Phosphoryl complex. This was found in a synthetic single species biofilm *Pseusomonas putine*, and although the findings are consistent with other studies the authors point out that testing of natural microbial biofilms is needed to asses that the results corroborate (Toner *et al*., 2005 and references therein)
2	Homogenous nucleation and precipitation	The numerous microorganisms that inhabit the biofilm are responsible for the creation of microenvironments with different conditions over small spaces of the biofilm (Sutherland, 2001; Harrison *et al*., 2007). These microenvironments could in theory promote the spontaneous homogenous precipitation of minerals that would not precipitate in the bulk water column, although the high energy requirements of homogenous precipitation mean heterogeneous precipitation is presumed to be the predominant form of precipitation (Misra, 2012)
3 (A,B,C)	Nucleation and heterogeneous precipitation process	As stated in the previous section the creation of microenvironments throughout the biofilm matrix allows for geochemical controls on trace metal mobility that are independent of the bulk water geochemistry. Nucleation of precipitates occurs when, for example, a divalent metal ion that is chelated (unidentate) would still allow for an anion such as carbonate to bond to it and the process would continue, creating a mineral precipitate.
3 (A,D)	Chelation	Ions can be sunk to the biofilm matrix via chelation. The EPS provides many negatively charged functional groups to which divalent ions can co-ordinate with. This chelation renders the ion immobile, and there are various levels of stability to a co-ordination, divalent ions will chelate in a unidentate (A) or bidentate (D) manner, with the latter being more stable (Morris *et al*, 2013). Rogerson *et al* (2009) suggests chemoselectivity based on the ionic radius of ions, with larger radii (lower charge density) being preferentially chelated. .
4	Boundary adsorption	Areas where the bulk water and the biofilm mass interface will provide the opportunity for mobile ions to adsorb to the surface of the biofilm, removing metals from the water column. Of course, this is pH dependent, and the dynamic nature of the bulk water column will mean this process of removal and release is fairly fluid. The internal water channels of the biofilm do provide the opportunity for potentially more stable sequestration of ions through an adsorptive mechanism, however. If, in a specific area there is sufficient sorption of divalent ions to the negatively charged biofilm, in effect the area will have a positively charges surface owing to a spare electron available from the sequestered ion, this theory is known as double layer theory (Rogerson, 2008). This in turn would allow for a bond to form between the said ion and an anion (such as a carbonate ion) and thus begin the process of the precipitation of a mineral (Decho, 2010).

Table 6.4 Summary of processes governing trace metal mobility in biofilms as depicted in Figure 6.11

6.4.7 Where are metals sequestered?: Insights into differences in Zn and Ba removal mechanisms

The magnitude of the diurnal cycle and the nature of the Zn release would suggest a fast reaction governs the mobility of Zn within the biofilm, and that it is pH dependent given the relationship with pH in the diurnal study and the dramatic release of Zn in the acidic release experiment, and a pH dependent sorption and release mechanism is the most widely accepted theory on the control of Zn mobility (Nimick *et al*., 2003). Historically, pH dependent sorption and release mechanisms have been documented exerting controls on trace metal concentrations in natural settings. Fuller and Davis (1989) ascribed the cycling of arsenate to an adsorption and desorption mechanism on iron oxy-hydroxides, whilst Brick and Moore (1996) have documented the adsorption and desorption of Zn from streambed sediments, albeit due to changes in ORP. More recently, this behaviour has also been documented in other studies where a small change in pH, due to light, has resulted in fluctuations in zinc uptake and release, however the majority of these studies ascribe sorption to in-stream sediments and other strata, the fact that these processes essentially rely on a negatively charged surface and a supply of cations means that these processes will be responsible for the removal and release of Zn within the biofilm.

Biofilm EPS will, in theory, provide extra negatively charged areas for Zn to bond to, or co-ordinate with, via chelation. It is likely that only small amounts of zinc will be temporarily removed in this manner, due to selectivity processes outlined by (Rogerson *et al*., 2008). Rogerson *et al.* (2008) showed biofilms associated with large calcite precipitates (tufa's) where capable of storing a large inventory of ions, and that these ions were chelated chemoselectively ordered according to their charge and ionic radii, with larger radii and thus less dense ions being preferred to chelate. In the case of this study, it can be assumed Ba would be the ion that is preferentially removed via this mechanism, as it is the ion with the largest ionic radius. As this method of removal affords the chelated ion some form of stability in a bidentate chelation, and is held in a relatively stable EPS geochemical environment, it would not be as prone as surface sorption mechanisms to fluctuating pH. Adsorption and unidentate chelation can lead to more stable removal of Zn and Ba, by means of co-precipitation and precipitation of minerals. Unidentate bonding of Zn or Ba would allow for homogenous precipitation mechanism as a site for nucleation has been provided utilising only one of the electrons, where adsorption also provides an area or potential nucleation via the formation of an "electrical double layer" (Figure 6.12). The predominance of heterogeneous precipitation processes in combination with the chemoselectivity of chelation processes in biofilms (Table 6.4) would suggest predominantly larger ions removed via precipitation processes, in this case Ba preferred over Zn. This is evident in the literature, with group 2 metals being identified as being easily nucleated, particularly in the case of Ca nucleation (Rogerson *et al.*, 2009). Precipitation of a mineral such as calcite could still play a part in the long term removal or Zn and Ba, as it could incorporate the ions into its lattice structure, as had been observed in various laboratory studies with Zn, Ba and other ions being incorporated into artificially precipitated calcite (Elzinga *et al*., 2006; Shaki *et al*., 2005).

The behaviour of Zn and Ba in the presence of biofilm would suggest that it is capable of metal removal in streams across a wide range of pH, and the modelling of potential in stream pH suggests that this uptake is stable up to a point in high flow conditions, a point already shown to be important in catchments with peat uplands at the top of their respective catchments. The potential implementation options utilising biofilm as a remediation option are outlined in the following sections.

6.5 Management Implications

The ability to quantify the short term dynamics of trace metals in the presence of biofilm is of key importance should they be used as a remediation tool to remove trace metals from the water column. Previously this thesis has discussed the need to understand trace metal dynamics to underpin monitoring regimes to accommodate times at which trace metals will be at their maximum and minimum concentrations.

The diurnal mesocosm data gained in this study correlates well with previous experiments (referenced throughout Section 2.5), and although the referenced work does not take place in the highly-controlled environment that was utilised through this study, the similarity in geochemical and trace metal processes observed helps to underpin broader applicability of the findings in this study. Whilst work has shown the fluctuations in Zn concentrations in the water column, mechanisms are not as well documented, with lists of potential mechanisms being described. The consensus is that the process is either adsorptive/chelate (Podda *et al*., 2013; Flemming and Wingender, 2010) or some form of mineral precipitation mechanism acting to remove metals (Rogerson *et al*, 2008; Pedley 2013) although little is done on understanding the stability of the metals within the biofilm structure.

Documenting the fluctuations and understanding the changes in trace metal mobility is an important factor in the step of utilising biofilms for remediation of trace metals.

However, there are numerous practical considerations that warrant discussion for up-scaling biofilm systems for removing metals at remote mine sites.

6.5.1 Feasibility of field site implementation: scaling up and geochemical stability issues

This section of the chapter will focus on findings of Chapter 5 and 6, combining the results to assess the viability of a field based biofilm remediation system. Whilst the results of the metal removal rates presented in Chapter 5 are promising, and the results of this chapter give a good insight into the removal processes, it must be remembered that these findings are at a laboratory level. To transfer the high rates of metal removal observed in a laboratory to a real world setting, in field remediation systems (as outlined in sections 6.4.8.1 and 6.4.8.2), the size of the flume environment would have to be increased to accommodate higher flows and thus increased metal loads. Table 6.5 presents calculations based on removal rate data (both average removal rates and maximum removal rates – the latter assuming systems could be optimised with further experimentation and refinement of set-up – see section 5.2.2 for removal rate calculation) presented throughout this chapter and Chapter 5, and metal load data from point discharges in Chapter 4. This provides a preliminary insight into the size of biofilm treatment system that would be required to remediate point discharges monitored from the reconnaissance chapter (Chapter 4) with biofilm remediation systems. These scenarios are based on an assumed target Zn removal rate of 90% which is frequently quoted for similar passive systems such as wetlands (e.g. Pilon-Smits and Pilon, 2002).

Location	*discharged Zn kg / day*	*90% target removal (g)*	*Avg removal rate (m^2)*	*High removal rate area (m^2)*
Lanshaw Level	0.0075	6.75	36.49	6.43
Yarnbury Reservoir	0.011	9.9	53.51	9.43
Spence Level	0.0114	10.26	55.46	9.77
Yarnbury Cockur Pond nr Powder House	0.013	11.7	63.24	11.14
Billybank	0.0133	11.97	64.70	11.40
Yarn Wash Dam	0.025	22.5	121.62	21.43
Gunnerside Gill discharge	0.0315	28.35	153.24	27.00
Ray Gill Level	0.0348	31.32	169.30	29.83
How Lead Level	0.0424	38.16	206.27	36.34
Wonderful Level	0.0634	57.06	308.43	54.34
Cock Hill Level	0.0704	63.36	342.49	60.34
Parkes Level	0.0881	79.29	428.59	75.51
Colgrave Beck	9.55E-02	85.95	464.59	81.86
Yorke Level	0.1055	94.95	513.24	90.43
Shaw Beck	0.2111	189.99	1026.97	180.94
Bunton Level	0.228	205.2	1109.19	195.43
Devis Hole Level	0.3216	289.44	1564.54	275.66
Eagle Level	0.438	394.2	2130.81	375.43
Hard Level	0.5775	519.75	2809.46	495.00
Cononley mine	1.123	1010.7	5463.24	962.57
Duke's Level	1.1706	1053.54	5694.81	1003.37
Sir Francis Level	2.1516	1936.44	10467.24	1844.23

Table 6.5 Selected Zn loads from Chapter 4, and s calculated to remove 90% of the metal loading with the area of biofilm required (at average (0.185 g/m2/day) and high (1.02 g/m2/day) removal rates) to achieve the 90% removal target.

The results of this calculation illustrate at a large number of point discharges the areas of biofilm remediation systems are such that they could be readily engineered and maintained in upland valleys with limited flat land area in riparian areas, even at the average removal rates. If high removal rates could be maintained the area required would be even smaller, and this is ideal given the isolated environment the majority of point source discharges monitored during this study where found in. For the primary drainage levels monitored across the Yorkshire Pennine Orefield (e.g. Duke’s Level, Sir Francis Level, Cononley Mine), the required size approaches or exceeds 1000m^2 even at the more optimistic removal rate. At such sites it is likely that more capital intensive approaches would be more suitable given land areas required. Such approaches have been trialled by numerous workers and could include vertical flow bioreactor systems (e.g. Gandy and Jarvis, 2012) such as the system recently installed at Force Crag mine in Cumbria (RESTOR (2014). As such, the potential niche for biofilm systems is likely to be in polishing applications or for the large number of low Zn flux mine discharges that are apparent in many mining settings (see Chapter 4 and Mayes *et al.*, 2010).

6.5.2 Modelling evidence: potential pH permutations and Geochemical Stability

An advantage of utilising the biofilm at point discharges is the relative stability of the geochemical conditions of the water, which would render any impacts from runoff in high flow negligible. The buffering capacity of typical waters sampled is illustrated in Figure 6.10, where pH was modelled based on the ambient pH of the flume water, and the lowest pH observed in the acidic release experiment. The high bicarbonate alkalinity of the ambient flume water, a characteristic feature of all mine discharges seen throughout this study (see Chapter 4), has a high buffering capability with respect to acidic conditions. This high buffering capacity allows for a circum-neutral pH to be maintained with up to 90% acidic water in the system, where the pH is reduced to 6.60 from the ambient (100% stream water) pH of 7.99. The combination of data gathered in Chapter 5 (flask pH experiment) and the modelling data acquired in this chapter illustrates both the buffering capacity of waters with similar constituents and the ability of biofilm to remove metals at the lowest pH. In context, the biofilm would still be capable of 98% Zn removal at this pH, as evidenced by the flask experiments (Chapter 5).

The saturation indices of what would be considered to be key mineral species controlling trace metal mobility in the flume environment are shown in Figure 6.12. Knowledge of mineral precipitation within the biofilm is important for the long term removal of metals (Chapter 5). Ambient stream water has $SI_{calcite}$= +0.41, theoretically sufficient for the heterogeneous precipitation of calcite in the flume environment. A positive SI is maintained from 100% to 75% stream water, however anything below a 70% stream water will promote the dissolution of calcite ($SI_{calcite} \leq -0.04$). Barite exhibits a similar trend with 100% stream water giving $SI_{barite} = + 0.22$, and positive values continue until a 60% stream water, and anything below this level of stream water gives $SI_{barite} \leq -0.04$ although given the observed lack of Ba released from the flume, even in 100% acidic water environment, it could be suggested that barite precipitates are not responsible for the short term transient storage of metals from the flume environment. Zn minerals can be seen to not be precipitating at any point throughout the simulation, even in 100% stream water conditions, with the saturation indices of $ZnO(a)_{max}$= -1.22 and $ZnCO_3{:}H_2O_{mzx}$= -0.70.

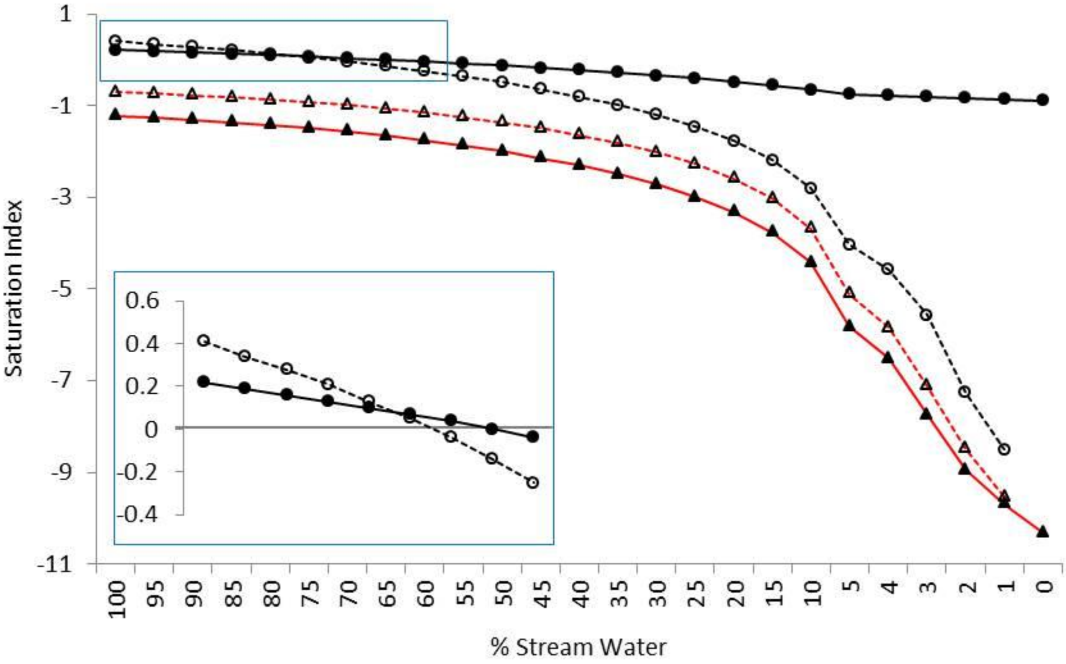

Figure 6.12 PHREEQC modelled saturation indices for Barite (Filled circle) Calcite (Open circle), ZnO (Open Triangle) and ZnCO^3:H$_2$O (filled triangle) across the whole range of Stream water to acidic water ratios.

6.5.3 Use in borderline EQS breaches to meet Water Framework Directive Targets

Throughout the catchments sampled in Chapter 4, many of the levels and adits had concentrations that where either slightly above, or slightly below EQS limits. Biofilm based remediation options could be implemented at such sites to help remove traces metals below these EQS limits, and thus observing the Water Framework Directive aim of having a good chemical and ecological status. Previous systems used to remove metals involve wetland systems, which have yielded good results in the removal of Zn, examples of which are summarised in Mayes *et al*. (2009). However, a distinct drawback of such systems is the large areas they take up in order to give them a high impact on the Zn concentrations in the catchment, for example wetland remediation schemes are multi acre remediation schemes, with a considerable engineering of the land to maintain suitable flows to keep the system alive and efficient metal removal rates (EPA, 2013). These systems are ideal for environments with the land available for such environmental engineering to take place; however, in the majority of UK catchments this is not possible, given the remoteness of many of the point discharges, and the small area of land available to implement such schemes. A biofilm based system would get around these difficulties, and be suitable for the types of water shown. As shown in Table 6.5, biofilm systems could feasibly be implemented with a small land footprint, and require minimal maintenance as was demonstrated in Section 3.3.11.

6.5.4 Use as a polishing step in remediation systems

There are certain high metal (Podda *et al*., 2013), or extreme pH (Mayes *et al*., 2009) conditions which would not be suitable for standalone biofilm treatment. Although bacterial populations have been observed developed in stream and taking an active role in promoting the removal of Zn, it is noted however, that other hydrological mechanisms play an important role in removal of Zn (Medas *et al*., 2009). In such extreme conditions, it is likely that active means are required to first remove the majority of metals or reduce/increase the pH prior to biofilm treatment. Utilising biofilm post-active treatment would serve two purposes:

- Remove any residual metal that remains in the water column.
- Provides preliminary indicator of how ecologically sound water is (Lear and Lewis, 2009, Burns and Ryder 2001)

6.5.5 Potential field units

There is also the possibility that the biofilm may be manipulated to constantly remove metals, and still maintain its passive nature, which would be of importance again given the isolated nature of the point discharges. The results of the diurnal experiments gave observations of higher concentrations of metals at night, and lower concentrations of metals during the day, so metal concentrations will be lower in well-lit conditions. Utilising a remediation system which uses the flowing water of the discharge and solar panels to power a UV light that is permanently on at low UV levels, or used to extend winter daytime light hours (a full strength UV light permanently on would denature the biofilm) to maintain biofilm growth will enable the constant lowering of metal concentrations (which, as already stated in the previous section, will help borderline EQS breach discharges meet WFD targets), and thus potentially increase the average, and higher, removal rates previously observed. If the increased removal rates can be maintained via utilising a light source would also mean the area of the remediation system could be reduced through the layering of biofilm surfaces within the remediation system.

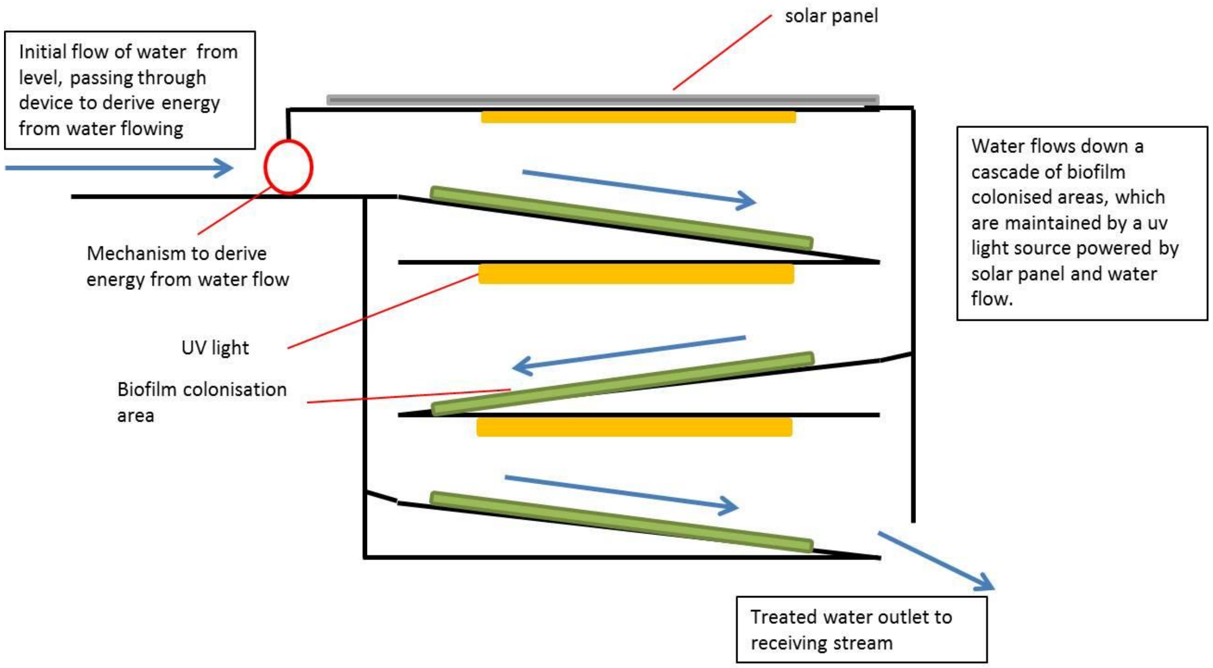

Figure 6.13 Schematic of potential biofilm field units.

6.6 Conclusions

The understanding of trace metal mobility and diurnal cycling of such trace metals in biofilms is important for both the understanding of in stream processes and the potential use of biofilm as a remediation option. This chapter addresses the diurnal fluctuations of Zn, a predominant and ubiquitous pollutant in mine impacted catchments, and Ba, a pollutant that receives less attention than Zn, but had still been illustrated as being a pollutant due to previous mining activity which can have an adverse effect in aquatic environments (Frančišković-Bilinski *et al*., 2007) and also Ca and Mg, through which precipitation reactions could be key in controlling Zn and Ba mobility. Whilst the phenomenon of diurnal cycles is well documented (Nimick *et al*., 2011 and Chapter 2 and references therein), there appears to have been little research carried out identifying the control individual variables exert on trace metal concentrations. This study has reproduced the solute cycling observed in mine drainage affected streams under controlled laboratory settings with flumes colonised with biofilms. These studies have identified the control biofilm exerts on trace metal concentrations, in isolation of the other variables that could affect metal mobility under field conditions. These findings will help to inform a manner of management implications. The fluctuation in Zn concentrations here show peaks during the night, and lows during the days, a trait which is prevalent in diurnal studies with Zn as an ion of interest. As already outlined the majority of monitoring programs are conducted during normal working hours, and the findings in this thesis would suggest this need to be addressed in at least one of two ways;

- Deploying passive samplers to provide more accurate short term averages of in-stream contaminant concentrations (Davison and Zhang., 1994) in strategic, high pollution, catchments.
- Use of models to account for diurnal cycling significance (Kurz *et al.*, 2013)

Both of these points need to be considered at stream to catchment level for the implementation of a successful remediation of trace metals that does not over or underestimate the concentrations of pollution, and thus over or underestimate the cost of remediation strategies.

This chapter also assessed the viability of biofilm as a potential remediation tool. Biofilm is low cost and passive; two facts illustrated by the ubiquitous nature of biofilm naturally occurring in many mine impacted catchments (see Chapter 4). However, the stability of metals removed by biofilms is relatively understudied and the tests carried out in this chapter have illustrated the dynamics of trace metal mobility when exposed a relevant stressor, decreased pH. Decreased pH is associated with runoff from peat uplands, a common feature of catchments impacted by historic mining, and is particularly pertinent given the implications of climate change, and the likely increased frequency of rainfall events (DEFRA, 2009). The stability testing of the biofilms has illustrated they would be able to cope with stresses of increased pH in the majority of cases; the difference between bulk water geochemistry and internal biofilm geochemistry provided by the EPS is responsible for such independence (Chapter 2). However, due to the unpredictability of intense, long term storms (such as those experienced in 2012), the strategic placement of remediation programs would be recommended in areas which are more resilient to changes in geochemistry, i.e. well buffered point sources of metals, such as adits and levels. This would particularly suit catchments in this thesis, where strategic remediation systems could target single point sources of trace metals, particularly those of modest Zn flux, which were common in all catchments studied and key contributors to in-stream pollution.

7

7.0 Conclusions and Future work

7.1 General Aims and Success of Thesis

This chapter addresses the aims of the thesis and outlines how the aims were met with conclusions drawn from each chapter. The objectives set out in the introduction are presented below (Figure 7.1), with responses as to how these objectives have been achieved (and where in the thesis they are addressed), with subsequent sections outlining the conclusions drawn

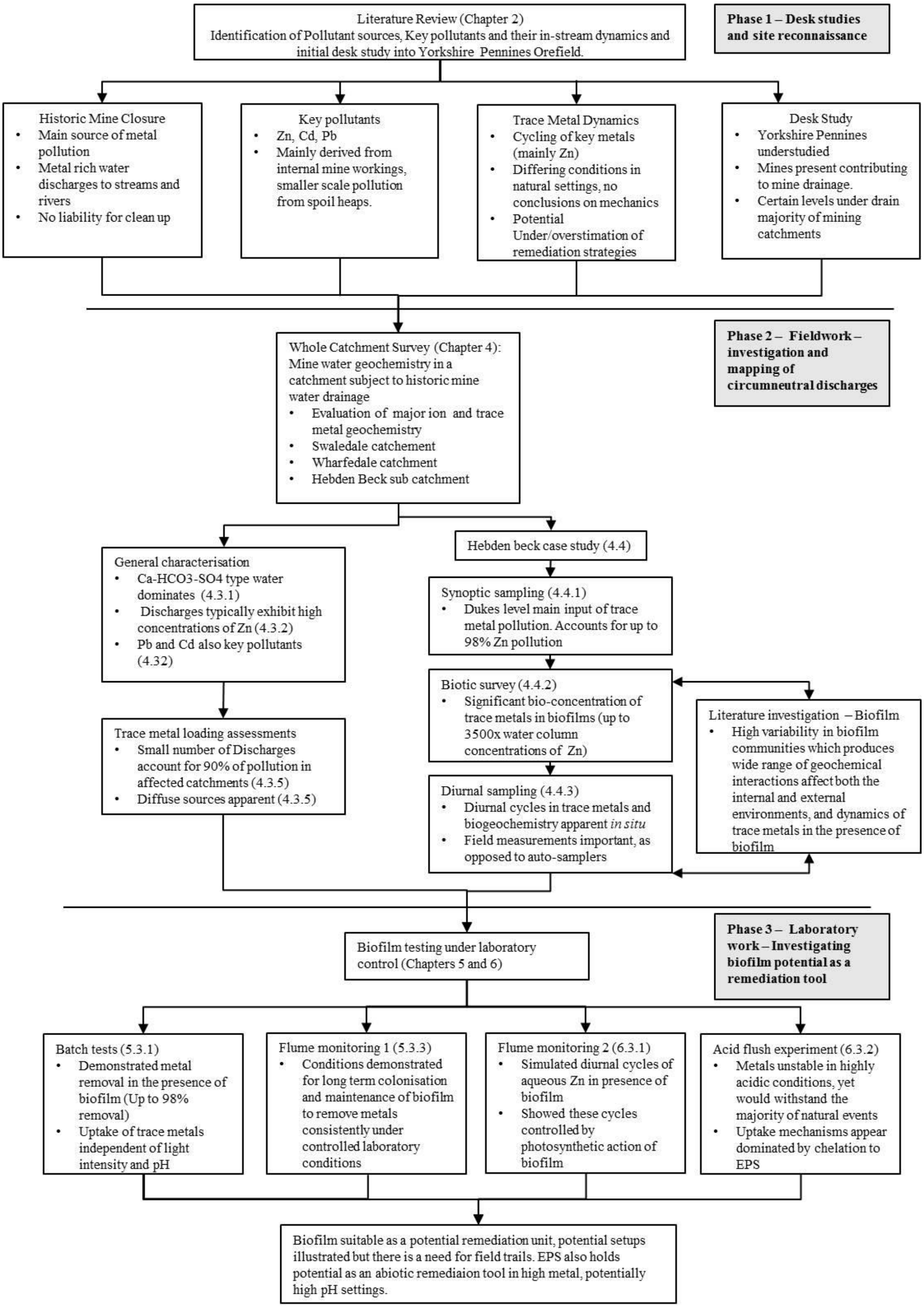

Figure 7.1 Flow chart summarising the completed aims and objectives of the thesis. Chapters relevant to section are shown in brackets.

7.2 Fieldwork conclusions

The work carried out in the process of completing this thesis has investigated trace metal dynamics in mine impacted streams located in carbonate catchments situated in the Yorkshire Pennines. The Yorkshire Pennines are an area in which industrial scale mining took place throughout the 19 and early 20th centuries, and when the mines became uneconomical, they were abandoned leaving a network of mining artefacts which drained metal rich water into receiving streams. Despite the rich mining history of the area, it is poorly studied relative to other catchments throughout the UK (and under-represented in national prioritisation exercises: Mayes *et al*., 2009), and was subsequently monitored during a field work sampling campaign. After assessment of the area utilising maps and online resources to identify potential mine discharges, the fieldwork sampling campaign focused on sampling metal rich discharges and their dynamics at three levels; catchment (Wharfedale and Swaledale), sub-catchment (Hebden beck catchment), and individual stream level (Hebden Beck). The conclusions of findings at these levels are outlined in the sections below.

7.2.1 Catchment level

At the catchment level, data was collected for 20 point discharges of metal rich water drained from abandoned mine workings which had previously not been monitored. A combination of metal concentration and flow data illustrated how up to 7.5kg/day of Zn is entering the system from such discharges. The majority of the pollution derived from these point sources was attributed to one or two main point discharges in each catchment, with Duke’s Level (Wharfedale) and Sir Francis Level (Swaledale) being responsible for 90% of the pollution in their respective catchments. The streams receiving the metal rich discharge were usually first order streams, with excellent hydromorphic quality making them ideal salmonid habitats, however, the hydrology of first order streams can limit the dilution of metal loads.

7.2.2 Sub catchment level

The Hebden Beck catchment provided the ideal model sub-catchment to monitor trace metal dynamics. The surrounding environment is representative of many other catchments in the UK, featuring limestone country rock, and a peat-based upland. The addition of a flow monitoring station at the bottom of the catchment meant quantification of metal loadings from point discharges could be accurately calculated, as well as the diffuse contribution from other sources. Again, the importance of Duke's Level in terms of Zn contribution is illustrated, contributing between 87% and 98% of the point source pollution in the sub catchment. One of the more importance findings of this work was the prevalence of biofilm in the main stream, and its tributaries. Samples of biofilm were analysed for trace metals, as they were identified as a potential natural attenuation source for trace metals. Biofilm was seen as important given the lack of any identifiable mineral precipitates seen in-stream, such as Fe and Mn oxides, which are mineral sinks well noted in the literature. Biofilms sampled from Hebden Beck showed median uptake of 1412 mg/kg, with an average bio concentration factor of 1859, this level of bioaccumulation within the biofilm suggests its importance in natural attenuation of trace metals from the water column.

7.2.3 Hebden Beck - stream

The identification of natural attenuation such as this, and literature surrounding biofilms, led to identifying the need to understand biofilm interactions over the short term to assess their use as a remediation tool or as part of monitored natural attenuation in mine-impacted streams. It was understood biofilm played an important role in removing metals from the stream, however, only theories of metal removal mechanisms were available from laboratory investigations, with no tangible benefit to how they would interact in a real world setting. The constituents of biofilm are known to be made up of bacteria and algae, and metabolic reactions taking place within the biofilm release and remove carbon dioxide from the water column via photosynthesis, subsequently causing increases and decreases in pH (Nimick *et al*, 2011; Gammons *et al*., 2013). It was not known whether the photosynthesis directly removed metal, or indirectly removed the metal via changes in geochemistry caused by the fluctuations in pH allowing for precipitation to occur. For these reason a diurnal sampling regime was carried out in the Hebden Beck, with the aim of observing diurnal changes in trace metal concentrations and geochemical parameters. There was an observable difference in Zn concentrations, which exhibited a daytime minimum of 0.34 mg/L and a night time maximum of 0.60 mg/L. There was no discernible cycle in Ba, Ca or Sr. Although the initial part of the exercise was successful, i.e. observing the upward limb of a diurnal cycle, rain interference altered the flow substantially enough to alter flow conditions to the point where dilution exaggerated the downward limb of the diurnal cycle.

7.3 Laboratory Conclusions

The prevalence of biofilm, and it ability to attenuate large amounts of metal from the water column presented the opportunity to assess it for use as a remediation tool for use at point discharges of metal rich waters from abandoned mines. It would be passive and would require very little maintenance which would be ideal given the isolated nature of many of the discharges sampled in the fieldwork sections of this thesis. However, the lack of conclusive data in the diurnal sampling exercises presented the need to assess the short term dynamics of trace metals and water geochemistry in the presence of biofilm in a laboratory setting, where variables (such as rainfall, temperature and light) can be precisely controlled.

To assess the potential of biofilm as a remediation tool, and to assess short term fluctuations of trace metals and geochemistry in the water column, a series of batch experiments were conducted, using biofilm in flasks which had different treatments applied to them. A flume set up was also utilised to assess long term removal of trace metals from the flume environment, as well as diurnal fluctuations in the flume water. The conclusions from these three experiments are outlined in the sections below.

7.3.1 Assessing biofilm for use as a remediation tool: batch experimentation and long term removal of trace metals

7.3.1.1 Batch Experiments

The first stage in assessing the potential of biofilm for remediation, and also understanding the mechanisms of trace metal removal was to utilise batch experiments with different treatments applied to the flasks in which biofilm and metal rich circumneutral pH water was placed (from Duke's Level). Initial batch experimentation focussed on proving biofilm was responsible for the removal of trace metals from the water column, and to achieve this objective metal removal in flasks with biofilm and sterilised flasks was compared. The results showed biofilm flasks removed significantly more Zn and Ba than the sterilised flasks. Light treatments where applied to assess the metal removal in differing light levels, to identify whether biofilm-mediated photosynthesis was directly responsible for the removal of trace metals. Zn removal was not significantly different across the light treatments, suggesting the removal mechanism for Zn was not directly linked to photosynthetic reactions. Metal removal in flasks kept in completely dark conditions was significantly lower than the light flasks, attributed to the biotic element of the biofilm dying and the integrity of the biofilm being reduced sufficiently for it to break down. This highlighted the need for a light source to be constantly available should the biofilm be used as a remediation device in a field setting. The last treatment assessed biofilms ability to removal metals across the range of pH observed during the catchment studies, to assess if metal removal was altered across the pH range. There was no significant difference in any metal removal across the pH range, illustrating how biofilm would be usable across all the sampled discharges of the field characterisation.

7.3.1.2 Long term removal and subsequent suitability as a remediation tool

Whilst being useful for answering the specific questions presented, the finite amount of metal available in the flasks meant these experiments did not address the dynamics long term removal of trace metal removal from the water column. Flumes were constructed and biofilm was allowed to colonise within them, which would allow for the monitoring of water geochemistry over long periods of time. Flumes were constructed in a laboratory where conditions (light, temperature, external inputs (e.g. rainfall)) could be precisely controlled, and biofilm colonisation was allowed in two flumes, whilst one was left sterilised to act as a control. This was done to give the best opportunity to allow for the underpinning factors involved in metal removal to be understood. The flumes built where all built to a precise specification, which allowed for the calculation of metal removal rates, a common measure of comparison of remediation systems. Overall removal in the biofilm colonised flumes over a 60 day period was 78% (Zn) and 23% (Ba), starkly different to overall removal observed in the control flume (19% removal of Zn and 14% removal of Ba). Significant Ca and Mg removal was not observed in the flumes, which began to suggest in stream precipitates were not responsible for the removal of Zn or Ba. However, observations under an SEM did show small, sporadic calcite precipitates spread throughout the biofilm, which are capable of incorporating trace metals into their crystal lattice, and adsorption to their surfaces, which could be acting as a long term sink within the biofilm. This is further backed up by the fact the biofilm has a geochemical environment independent of the water column, with concentration of ions in the biofilm matrix, which would allow for the maintenance of precipitates.

Removal rates of trace metals in the flume environment are presented in Chapter 5. Removal rates calculated for the flumes allowed for the comparison against other systems, and the biofilm compared well against other similar systems, such as wetlands, albeit with a much shorter residence time. Metal removal rates appear to remain relatively stable in the active flumes over a range of influent concentrations, which suggests that the uptake mechanism is not saturable, as would be expected with sorbent media, again ideal for a remediation system that would receive very little maintenance given its location. In terms of the suitability of biofilm as a remediation tool, the nutrient requirements of the biofilm were also assessed, and it was capable of surviving in low nutrient conditions for the length of the time it was in the flume and used for this study (around 15 months). The water used in the flume had the same nutrient composition as many of the discharges in the Yorkshire Pennine Orefield, another beneficial characteristic of the biofilm as it would be able to flourish without needing the addition of a growth nutrient / organic amendment.

7.3.2 Short Term Fluctuations in trace metal concentrations and geochemistry in the presence of biofilm.

The final aim of this thesis was to assess three final issues surrounding biofilms and their influence on trace metal concentrations and geochemistry of surrounding water which were not covered previously;

- Biofilm biogeochemical processes over short timescales (24hour / diurnal)
- The permanence of trace metal removal,
- Evaluation of the scope for engineering systems at full scale

The flumes were monitored over a 96 hour period to assess the diurnal biogeochemical process and fluctuations in trace metal concentrations, with the aim of understanding mechanisms of trace metal removal. The controlled nature of the flume set-up meant that any changes would be wholly ascribable to the biofilm. Fluctuations in pH are evident, and statistically different pH was recorded during the daytime (maximum average pH – 8.75) and night time (minimum average pH 7.99) periods of the day. Changes of this magnitude were not observed in the control flume (maximum average pH – 8.16, minimum average pH – 8.13).

Fluctuations were also observed in trace metal concentrations, in particular Zn. In flume 1 the minimum value of 365 µg/L was observed during the day time, and the maximum value of 472 µg/L being observed during the night time. This pattern also occurred in the other active flume with values of 231 µg/L observed during the day time minimum and 358 µg/L during the night time maximum. No cycle was apparent in the control flume. Ba gave observable cycles only in flume 1 although it was not as apparent as with Zn concentrations, with a maximum of 270 µg/L and minimum of 241 µg/L, a difference of only 29 µg/L compared with the difference of 107 µg/L observed in Zn concentrations. No cycle was apparent in the control flume. Ca and Mg did not cycle over the length of the experiment in the biofilm colonised flumes or the control flumes. These changes that occurred in the flume environment can be ascribed wholly to the biofilm which is colonised within the flume. These results allow us to draw conclusions about the nature of mechanism that are responsible for the diurnal dynamics of trace metals in the flume environment. The fact that pH and Zn exhibit maximum values and minimums in opposition with each other suggest a pH dependent mechanism is responsible for the diurnal cycle in Zn. The lack of any cycles in Ca data would suggest that this mechanism does not depend on a transient calcite precipitation mechanism, with periods of dissolution and precipitation being responsible for the subsequent release and uptake of Zn. This would indicate that the mechanism most important mechanism for controlling Zn concentrations over diurnal timeframes is an adsorption/release mechanism onto the biofilm surfaces and EPS molecules. This is further evidenced by SEM/EDAX spectra that show no Zn peak in close ups of calcite precipitates within the biofilm, yet do show Zn peaks in the wider scans with include areas of biofilm with no calcite precipitates. The prevalence of a positive SI in the flume for the mineral calcite, would suggest that the concentrations of ions available and the sluggishness of precipitation mechanisms that this precipitation is a longer term process. No other minerals are present precipitating in the flume environment, which rules out attenuation processes such as Fe and Mn oxides being responsible for metal removal or diurnal cycles of trace metals.

7.3.3 Permanence of metal removal: experimentation and implications for field remediation systems

The use of a biofilm remediation system would require it to be resilient to external influences in natural settings. The catchments previously monitored in the field experiments usually had a peat upland, which, during high rainfall events has been illustrated lowering pH of the water within the catchments. To assess the resilience of biofilm to such conditions the flume set up was altered to simulate a rainfall event, in which acidic water was passed through the biofilm flume and control flume to assess any release that may occur. The water passed through had a pH of 4.14, and was not mixed with flume water to ensure a worst case scenario was achieved, as it was felt less dramatic events could be calculated from the data after the experimentation. The results would also provide more information on the stability of metals and thus, more insight into removal mechanisms. Zn exhibited the most dramatic response to acidic water being introduced to the flume, and concentrations where observed going higher than previously observed ambient concentrations. The mechanism is very quick and very dramatic when compared to the other ions observed. There is a slight rise in the Zn in the control flume, which is ascribed to Zn which has sorbed to flume surfaces, although this in itself can illustrate the amount of Zn that is being stored by the biofilm. There is a negligible release of Ba in the biofilm flume, and no release in the control flume. The same patterns as seen in Ba concentrations are observed in Ca and Mg concentrations. The modest change in Ca values again suggests that carbonate sinks for Zn are not particularly prevalent in the system. This experiment should be considered to be a binary exercise i.e. the flume was either subject to acidic water or flume water, with no gradual changes being able to be performed. To this end, geochemical modelling was performed to observed changes in pH at different ratios of stream water and acidic water. It was found that at a 90% acidic water 10% flume water mixture the pH was still high enough (6.60) to maintain metal removal of 90%+ (as observed in the flask experiment where 98% removal was achieved at a pH of 6.5). This information, combined with the knowledge from characterising the geochemical nature of levels and adits (well-buffered and resilient to environmental change) and characterisation of biofilm (able to regulate their internal environment independent of the water column), allows for the conclusion that biofilm would be resilient to environmental change if deployed at a point discharge of mine impacted water in a carbonate catchment.

7.4 Future Work

This study has characterised catchments with circumneutral pH streams, impacted with mine drainage from historic mine workings, and assessed mechanisms of metal removal by biofilm and biofilm suitability for a low cost, passive remediation option. The following sections are lines of future work which could carry on research into biofilms and their interactions with contaminated waters.

7.4.1 Creation and installation of biofilm units in pilot scale studies

The results presented here are from biofilms held in laboratory settings, and whilst the results are promising, it is important to gain an understanding of the efficiency of metal removal by biofilms in natural settings. This could be achieved through the development of a biofilm remediation system (as described in section 6.5.5), and utilising it in pilot scale studies to assess how it functions in a real world setting. Maintenance of the biofilm over a long period of time would be a key consideration, as deterioration of areas of biofilm would lead to poor metal removal efficiencies. Also assessing the removal rates over a seasonal timescale would be required to assess the longevity of the field units. Natural assemblages of biofilm that dominate the streambed in Hebden Beck where observed dying away in the colder winter months. Field units would have to overcome this. The heat generated by the proximity of the UV light could aid this, although care must be taken not to overheat the biofilm, as well as the temperature stability of levels and adits which come from well insulted bedrock sources.

7.4.2 Development of biofilms "tailored" to specific waters

The isolation of bacteria, and subsequent signal organism biofilms, is a prevalent topic in the literature (Singh *et al*., 2006). The isolation of individual factions of the biofilm could allow for a reduction in the variation of EPS constituents. This could be used in the identification of specific EPS molecules which preferentially sequester pollutants, and subsequently be used to in different water types. This will improve the range of biofilm remediation systems, and potentially allow for them to be applied across a range of water types (Roeselers *et al*., 2008).

7.4.3 Further development of EPS units

The ability of EPS being able to removal trace metals from the water column, independent of the biofilm, presents the opportunity to create a new, sorbent based media with the potential to remove metals in extreme conditions where biofilm colonies would not survive. Further investigations into the use of EPS as a sorbent based media would have to resolve how to immobilise it, allowing water to pass through it without washing the EPS away, and also the longevity of such a system. This could provide an opportunity to develop a range of “ready to go” remediation systems that would be well utilised in isolated upland catchments.

7.4.4 Catchment studies

Further investigation into trace metal mobility in catchments would lead to better understanding of mobility on a variety of scales. Whilst this thesis has been primarily concerned with the sinking of trace metals into biofilms, further studies could investigate the prevalence and impact of non-biotic sinks for trace elements. At a sub-catchment scale, this would help to inform specific remediation systems (i.e. if the water chemistry allows for the natural attenuation of Zn, can the mechanism be promoted and enhanced as a remediation tool?). At the wider catchment scale, natural sinks could be mapped, and the amount of trace metal they are storing over time, and their stability examined, with a view to predicating the amount stored which could potentially be released should the right geochemical conditions allow. Detailed field monitoring over full hydrological and seasonal cycles would also be useful to provide empirical evidence of the transient importance of biofilms as both sinks (e.g. during baseflow) and sources (e.g. during dieback or under high flow, acid flush events) of metals at a catchment scale.

8

8.0 Appendix

During the time spent studying for this PhD, work that contributes to this thesis has been published in peer reviewed journals. These papers are presented over the following pages.

8.1 Appendix 1

Mine water geochemistry and metal flux in a major historic Pb-Zn-F orefield, the Yorkshire Pennines, UK

8.2 Appendix 2

Controls on zinc uptake from circum-neutral mine drainage in freshwater biofilms

8.3 Appendix 3 – Flume data (Long term loss)

	Zn			Ba			Ca			Mg		
	Flume 1	Flume 2	Flume 3	Flume 1	Flume 2	Flume 3	Flume 1	Flume 2	Flume 3	Flume 1	Flume 2	Flume 3
0	1321.9328	1312.0544		347.6202	340.87501		39.284714	38.705439	39.133152	2.5422501	2.4891463	2.5275318
3	1065.8816	1057.3334	1322.6459	314.6809	317.84267	345.48318	39.395462	38.677372	39.101	2.5268045	2.4789508	2.5352
6	965.86159	1033.3316	1228.9323	309.74555	315.42607	319.22579	38.787804	38.13954	39.069563	2.5186272	2.4936282	2.5430261
9	945.01674	958.39263	1208.2896	304.4793	306.51574	320.4745	39.098952	37.722977	39.46379	2.5164897	2.4827835	2.6369679
12	914.74337	892.0298	1183.5141	313.96641	317.15034	323.79295	39.380712	38.435528	39.718258	2.5721582	2.5001596	2.5634305
15	826.57161	901.75366	1166.214	297.47881	298.107	315.11152	38.009374	37.933017	39.152227	2.5266321	2.5078846	2.5726137
18	831.11111	822.22127	1143.8991	296.22967	300.01882	317.08429	38.941361	37.78113	39.541503	2.5601795	2.5435585	2.5453736
21	705.4506	1211.7942	1125.9287	283.61326	286.41102	310.67165	35.291999	44.190242	39.175445	2.3163406	2.6116213	2.5631951
24	762.91879	809.88059	1094.0052	291.4833	274.60389	311.79389	38.181156	41.854454	38.87188	2.5646636	2.4749184	2.531034
27	670.43152	791.4412	999.14323	284.06976	275.18657	309.81033	37.314007	40.5646	36.407246	2.4870926	2.4058619	2.241429
30	617.35164	613.16444	1002.0642	286.2476	284.39831	299.34687	35.080536	32.868781	36.452142	2.2420269	2.1830935	2.3207613
36	535.24541	613.56649	1114.7855	273.95616	272.88299	316.92041	33.88658	39.639883	36.507908	2.1723931	2.3697224	2.2603152
39	611.18277	579.17362	1053.5714	273.54798	270.8851	309.48763	37.973928	39.20755	39.068	2.5614129	2.3722614	2.5519158
42	608.0204	682.99898	980.66135	271.80938	267.01318	305.0861	37.72583	42.428421	37.045547	2.3097162	2.64554	2.3590674
45	611.051	527.02955	1091.935	278.92035	287.65538	307.94976	36.201488	37.925646	39.125444	2.4245916	2.4758455	2.5278341
48	464.89306	449.37599	1092.8281	275.88591	281.62232	304.0899	35.868757	37.157154	39.058761	2.4310507	2.4820552	2.5104257
51	472.60834	488.20091	1102.4717	280.03802	278.02937	307.57333	35.788115	36.679644	40.111952	2.4017421	2.4403726	2.579573
54	380.53211	410.7512	1099.3763	277.03453	281.98015	307.96772	35.673041	36.760659	39.546936	2.4437665	2.4607505	2.5523801
57	427.23188	424.06253	1063.3921	275.15702	276.97997	306.46044	35.391555	37.210265	39.723199	2.443504	2.4804872	2.510725
60	406.87147	372.02738	1045.9338	270.35525	273.36135	306.09756	35.782262	36.06098	39.565769	2.4957204	2.4279859	2.5135639
63	456.86663	323.53068	1028.9858	267.08188	267.95473	300.83725	35.711993	36.442106	39.062677	2.4561643	2.4688285	2.5216792
66	268.49558	289.32104	1067.0057	266.99963	265.5334	293.92082	35.436004	35.756671	40.513527	2.4864153	2.4145838	2.6745094

Zn and Ba concentrations in µg/L, Ca and Mg in mg/L

8.4 Appendix 4 – Flume diurnal Data

			Flume 1			
Time	pH	Zn	Ba	Ca	Mg	CB
09:00	8.25	405.3589	257.9217	51.24932	4.849237	-4.12
09:15		406.8492	258.1662	56.29789	5.607353	
09:30	8.22	427.1089	254.2599	50.31727	4.777444	-8.33
09:45		423.7788	249.5902	50.37695	4.788491	
10:00	8.18	422.1578	253.6241	55.33973	5.574761	-2.02
10:15		404.8101	248.5468	50.68042	4.896197	
10:30	8.51	403.3823	249.8079	50.35161	4.811938	-4.76
10:45		394.9448	248.5026	50.31865	4.825793	
11:00	8.69	399.6843	251.9799	51.10407	4.835661	-6.2
11:15		398.1091	254.3595	49.43384	4.628415	
11:30	8.72	389.1561	248.1506	51.01195	4.933362	-8.35
12:00	8.81	385.2148	252.5077	55.79075	5.642396	-2.41
13:00	8.81	378.5928	247.5	54.18982	5.3916	-8.58
14:00	8.81	378.1918	246.5149	54.27	5.533457	-0.07
15:00	8.86	384.2113	246.3968	54.21565	5.440521	-2.48
16:00	9.23	369.5278	246.23	53.89221	5.412867	-1.82
17:00	9.31	365.2184	243.0463	47.30389	4.466221	-9.44
18:00	8.48	403.7435	249.0434	53.62686	5.473545	-2.49
19:00	8.27	423.2903	252.4601	50.29342	4.846973	-6.49
20:00	8.2	413.6272	255.0479	49.98284	4.803193	-6.3
21:00	8.18	425.3861	252.5544	50.79455	4.938933	-6.27
22:00	8.15	372.4785	255.9018	55.53281	5.569144	1.85
23:00	8.14	421.4781	250.3184	53.44359	5.308345	-6.36
00:00	8.08	434.1329	253.5205	51.73219	4.975213	-4.49
01:00	8.05	439.7406	251.3354	51.2434	5.041863	-7.42
02:00	8.04	432.8397	251.8011	52.6428	5.242001	-6.63
03:00	8.01	438.5646	247.3045	51.17039	4.849645	-6.97
04:00	7.99	441.9968	253.0565	51.76896	4.90856	-6.89
05:00	7.97	445.5844	254.8039	56.88416	5.723608	-1.78
06:00	7.99	435.3397	253.2363	55.55636	5.645697	-5.18
07:00	7.93	443.8712	251.8262	52.38753	5.104538	-5.9
08:00	7.91	455.7307	252.0553	51.97336	5.033417	-6
09:00	7.89	450.3638	261.9595	53.75049	5.229371	-1.6
09:15		436.7344	255.4746	52.18083	4.975413	
09:30	7.93	445.3441	259.5642	52.3035	5.09866	-3.96
09:45		454.1354	262.2133	53.56497	5.115845	
10:00	7.93	459.6703	270.2978	54.54064	5.224066	-2.73
10:15		448.6118	260.8275	55.25319	5.434052	
10:30	8.28	435.6281	255.6859	56.24404	5.559834	-0.34
10:45		420.6701	257.123	58.50903	5.816739	
11:00	8.48	426.1254	256.9767	51.41348	4.870811	-5.88
11:15		413.9638	252.8546	51.90184	5.01179	
11:30	8.71	424.0656	246.5156	54.15103	5.315447	-2.42
12:00	8.81	412.1662	254.0502	52.20744	5.087454	-4.52
13:00	8.93	404.4195	250.4639	55.63017	5.642018	-0.68
14:00	8.8	417.1764	248.4212	51.03103	4.821755	-5.13
15:00	8.84	401.688	248.1971	51.81783	4.937357	-4.01

16:00	8.78	403.5779	246.682	51.28987	4.877312	-4.42
17:00	8.69	406.6528	250.7002	53.53757	5.208804	-3.12
18:00	8.29	445.9712	247.4441	52.68694	5.201885	-4.09
19:00	8.2	451.1077	261.6255	57.2149	5.734924	-0.94
20:00	8.12	440.0359	252.7912	51.65352	4.963912	-3.72
21:00	8.07	437.6433	250.5063	50.08129	4.670035	-5.01
22:00	8.03	449.7528	255.7976	63.76316	6.603039	7.13
23:00	7.99	445.0778	252.3801	55.73852	5.653162	-0.04
00:00	7.95	450.8759	241.0926	51.66816	4.992241	-5.86
01:00	7.95	460.8577	247.3796	50.00926	4.868107	-5.38
02:00	7.91	458.1628	242.7851	49.96898	4.865083	-6.23
03:00	7.93	457.7506	246.0525	50.21877	4.779092	-7.77
04:00	7.9	456.5422	253.1658	52.12094	4.985244	-2.88
05:00	7.89	467.5524	243.0947	52.39133	5.1201	-2.99
06:00	7.89	460.2934	248.1575	56.22253	5.716559	-1.09
07:00	7.88	462.6209	247.7233	50.56478	4.91619	-4.43
08:00	7.9	465.5327	244.979	50.32672	4.725806	-8.17
09:00	7.89	467.6072	251.3366	51.31382	4.95614	-5.97
09:15		459.4785	243.8118	53.88856	5.358568	
09:30	7.91	472.754	260.0868	52.68517	4.938698	-3.25
09:45		455.6166	256.3494	51.80848	4.91609	
10:00	7.93	465.9247	254.7244	56.26256	5.63442	-1.47
10:15		453.806	251.7724	52.59381	5.054968	
10:30	8.18	441.7601	251.4945	52.61382	5.066986	-4.39
10:45		429.9535	246.5066	54.75049	5.421235	
11:00	8.37	432.8405	254.5947	52.72115	5.049176	-1.91
11:15		440.1516	248.6205	51.93169	4.957424	
11:30	8.45	439.8934	252.2459	55.99961	5.636068	-3.08
12:00	8.47	434.4171	247.7762	56.57104	5.693942	-0.01
13:00	8.58	412.4988	251.214	49.94591	4.732101	-5.4
14:00	8.79	413.3133	247.0489	47.9194	4.445503	-8.04
15:00	8.83	388.5062	251.3897	50.3766	4.826653	-4.72
16:00	8.94	407.2697	251.1049	52.05549	5.136763	-4.45
17:00	8.86	415.4137	253.1475	55.79311	5.704746	-0.65
18:00	8.44	447.7056	253.8731	51.44856	4.88021	-4.41
19:00	8.4	440.3206	252.8067	52.45993	5.106805	-3.97
20:00	8.16	451.5478	258.6818	57.85614	5.88739	2.36
21:00	8.1	447.2488	260.1971	51.88453	4.991777	-4.27
22:00	8.05	447.7872	259.0827	53.39252	5.175568	-5.39
23:00	8.01	442.0733	250.1443	53.36591	5.147085	-2.11
00:00	8.01	448.3124	250.4499	57.61693	5.700836	1.68
01:00	8.01	449.3906	248.6057	55.15796	5.589101	-3.21
02:00	7.99	448.0549	249.9681	50.34592	4.920171	-7.15
03:00	7.97	464.1892	260.5368	53.21322	5.269387	-4.11
04:00	7.99	454.0451	256.7775	52.73357	5.164994	-3.19
05:00	7.93	460.876	252.958	56.16345	5.760191	0.44
06:00	7.91	445.4563	256.3492	52.43976	5.104381	-7.17
07:00	7.91	457.4262	248.5302	52.07948	4.946223	-5.54
08:00	7.91	450.7807	251.1723	51.02377	4.896527	-1.97
09:00	7.91	461.3849	256.045	50.47795	4.820917	-6.25

Flume 2						
	pH 4	Zn	Ba	Ca	Mg	CB
09:00	8.67	312.3444	257.216	49.16045	4.430128	-4.91
09:15		328.8414	252.8562	48.00125	4.347894	
09:30	8.56	351.4164	255.3205	48.35523	4.392256	-11.36
09:45		349.756	254.5801	51.0799	4.699777	
10:00	8.52	345.3027	262.2843	49.75663	4.427302	-3.85
10:15		337.1359	257.4337	49.36895	4.433404	
10:30	8.75	327.6325	256.8033	48.50316	4.336512	-10.5
10:45		328.493	254.7167	48.05996	4.247931	
11:00	8.89	312.5803	253.5525	47.20443	4.274293	-10.32
11:15		310.8122	254.3314	48.50552	4.406287	
11:30	8.93	325.825	259.0152	52.27705	4.948665	-5.72
12:00	8.83	317.1382	253.1846	47.14277	4.27454	-9.95
13:00	8.85	314.7648	255.2663	49.26649	4.387876	-8.06
14:00	8.87	303.0704	265.0519	50.74015	4.534492	-6.06
15:00	8.86	305.8434	265.901	49.27944	4.39464	-6.82
16:00	9.23	302.8851	250.4454	51.50581	4.793237	-6.55
17:00	9.12	306.7693	248.882	47.32252	4.279947	-10.55
18:00	8.78	311.2889	261.567	46.72222	3.8701	-2.2
19:00	8.71	331.1524	255.6437	52.58763	4.903362	-2.7
20:00	8.6	336.6768	254.8267	57.72703	5.571034	1.62
21:00	8.61	333.8322	247.7287	47.14869	4.229543	-8.09
22:00	8.51	331.0135	251.9226	48.40201	4.309795	-11.15
23:00	8.53	325.5499	257.0933	49.537	4.413399	-7.11
00:00	8.5	337.1835	253.4265	46.81034	4.224582	-10.83
01:00	8.48	337.0033	252.8293	52.30509	4.814359	-4.85
02:00	8.48	341.6239	249.8058	50.53375	4.691065	-7.36
03:00	8.46	344.02	256.8326	46.97964	4.167926	-1.39
04:00	8.44	345.8067	251.7349	48.06358	4.281515	-9.48
05:00	8.43	339.2702	251.8202	50.78591	4.687111	-8.11
06:00	8.41	349.1904	249.9586	47.90709	4.201603	-10.87
07:00	8.37	337.1761	252.6857	50.00058	4.529865	-7.06
08:00	8.36	350.3805	253.2479	48.09722	4.276777	-8.56
09:00	8.34	350.5305	249.9567	50.21014	4.595762	-5.25
09:15		355.762	252.4593	46.68014	4.155476	
09:30	8.35	334.7018	251.5657	49.93152	4.450942	-6.15
09:45		350.6988	250.6105	47.56562	4.283808	
10:00	8.29	347.3814	252.1283	47.35899	4.215528	-9.02
10:15		341.8168	245.58	48.71822	4.25663	
10:30	8.46	336.8747	251.2788	47.64791	4.267358	-7.93
10:45		338.3499	251.115	47.6301	4.206562	
11:00	8.59	314.4022	252.7126	46.19036	4.077515	-9.94
11:15		327.239	247.7423	47.49924	4.214441	
11:30	8.64	328.9141	250.0175	46.28328	4.062173	-10.31
12:00	8.63	325.449	242.2548	46.78416	4.208139	-9.55
13:00	8.93	298.2684	249.1091	51.15941	5.033414	-2.99
14:00	8.67	278.8768	237.1826	49.17165	4.643116	-6.33
15:00	8.73	258.2036	233.7602	44.75633	4.009682	-10.39
16:00	8.75	287.0962	238.6428	48.78322	4.555713	-6.3
17:00	8.7	273.7963	234.321	44.62427	3.953538	-11.7
18:00	8.58	333.144	243.7264	45.664	4.150755	-10
19:00	8.56	350.809	244.4811	49.09121	4.567544	-5.91
20:00	8.51	336.6689	243.4437	47.12948	4.202568	-8.43

21:00	8.5	337.9712	241.6886	47.62632	4.3818	-6.86
22:00	8.45	338.064	245.4451	49.92637	4.632579	7.78
23:00	8.42	346.1982	242.7056	46.60392	4.141713	-8.63
00:00	8.41	345.5295	242.3016	45.50198	4.021818	-9.1
01:00	8.39	348.6447	239.0328	44.84548	4.028941	-11.7
02:00	8.38	346.9122	242.0982	45.7188	4.072205	-10.06
03:00	8.36	337.9005	241.6303	50.89125	4.702683	-3.85
04:00	8.36	342.6564	242.5014	46.48117	4.130745	-10.12
05:00	8.37	343.9973	236.2831	45.51572	4.097664	-9.89
06:00	8.36	349.8985	233.7973	46.27126	4.11797	-9.93
07:00	8.36	341.4246	236.726	45.04029	3.973168	-12.28
08:00	8.39	342.549	232.6323	47.54372	4.414058	-8.05
09:00	8.35	351.6213	234.7497	45.25251	3.975509	-9.48
09:15		348.4624	234.9859	46.49042	4.20442	
09:30	8.33	347.3847	236.7872	45.34641	4.016552	-8.12
09:45		344.2134	240.7523	45.37911	3.971534	
10:00	8.3	358.8626	241.0876	45.7435	4.078424	-9.03
10:15		343.8194	237.6659	48.36001	4.566658	
10:30	8.48	324.3586	231.778	46.35976	4.077426	-9.71
10:45		316.984	236.0843	45.37375	4.019509	
11:00	8.53	323.1922	238.6877	51.32704	4.814071	-3.54
11:15		323.9474	235.5659	43.55487	3.807279	
11:30	8.57	327.2407	238.523	45.75294	4.061924	-11
12:00	8.61	299.9731	237.8101	42.54519	3.675212	-14.85
13:00	8.66	296.6362	232.6895	49.40586	4.628466	-4.98
14:00	8.69	304.7963	234.6152	49.93693	4.704344	-5.92
15:00	8.72	289.4572	234.546	46.49845	4.128265	-9.13
16:00	8.8	298.9974	235.5946	45.38131	3.918438	-11.11
17:00	8.74	231.5559	236.9107	44.39892	3.937338	-8.48
18:00	8.61	328.6286	235.7378	49.58299	4.628575	-6.69
19:00	8.55	319.8025	242.4555	47.22264	4.292916	-8.63
20:00	8.5	329.4698	242.3412	47.0103	4.132529	-7.97
21:00	8.46	330.0566	248.4115	46.83981	4.146289	-9.83
22:00	8.46	330.5073	236.6208	46.71138	4.111477	-6.44
23:00	8.47	284.7443	235.8755	46.29314	4.169246	-8.89
00:00	8.5	332.0174	244.7149	45.83348	4.036141	-10.76
01:00	8.48	336.6797	247.3831	47.16955	4.190915	-9.88
02:00	8.48	331.7818	244.9227	51.0799	4.709068	-4.55
03:00	8.45	326.5539	249.3035	48.30444	4.193692	-8.66
04:00	8.44	326.2119	245.6032	47.34633	4.180817	-9.32
05:00	8.42	333.4052	252.6442	47.23436	4.212635	-9.81
06:00	8.4	319.4157	244.7998	49.09237	4.315764	-6.01
07:00	8.37	333.4029	244.8512	47.70175	4.154798	-8.49
08:00	8.35	339.609	247.4033	46.87743	4.104391	-9.38
09:00	8.34	339.3678	245.7316	48.88003	4.453791	-5.77

Flume 3						
	pH 1	Zn	Ba	Ca	Mg	CB
09:00	8.3	389.0048	264.9917	48.41066	4.236587	-0.98
09:15		403.1457	271.0943	52.38501	4.82922	
09:30	8.22	394.6453	270.0044	47.11065	4.073986	-8.44
09:45		399.5427	269.2046	49.12992	4.350882	
10:00	8.22	402.5843	270.7055	53.3672	4.941807	-7.11
10:15		413.9802	275.3598	50.78735	4.645498	
10:30	8.26	404.9193	274.8972	53.26441	4.892528	-2.05
10:45		411.0383	276.2341	49.5648	4.264497	
11:00	8.25	406.0548	273.7781	48.68611	4.279404	-4.43
11:15		410.1993	276.7503	53.11199	4.897751	
11:30	8.27	407.2397	269.1781	49.28127	4.279054	-4.64
12:00	8.28	400.5427	266.6553	54.09346	4.936697	-0.64
13:00	8.29	403.0163	270.3326	49.09175	4.319166	-4.46
14:00	8.16	415.17	277.0739	49.44052	4.480267	-3.29
15:00	8.19	407.7187	268.961	47.90155	4.246056	-4.98
16:00	8.17	402.6831	277.2629	48.62585	4.229346	-4.2
17:00	8.1	410.2253	271.2975	53	4.915582	-1.29
18:00	8.07	422.81	265.2942	49.83993	4.676483	-3.71
19:00	8.08	414.8748	279.5583	52.81693	4.676809	-0.4
20:00	8.12	404.6252	274.3677	50.58979	4.377283	-2.88
21:00	8.12	403.6897	277.4631	48.88587	4.193461	-5.69
22:00	8.12	413.6532	278.3354	49.26133	4.201228	-3.76
23:00	8.12	404.7873	281.1655	50.29772	4.354444	-5.27
00:00	8.09	379.0363	288.9828	50.07346	4.276073	-4.47
01:00	8.09	416.9659	283.8097	53.87104	4.860012	-0.14
02:00	8.1	409.7433	281.2376	49.7027	4.294202	-2.28
03:00	8.12	400.7244	279.5012	54.34123	4.946434	-2.62
04:00	8.12	411.715	270.1666	52.16033	4.661005	-3.29
05:00	8.12	413.7035	270.5176	52.78831	4.820083	-3.69
06:00	8.15	414.8825	264.0722	53.91455	4.884577	-1.56
07:00	8.1	407.8536	276.1091	48.81018	4.183043	-4.49
08:00	8.09	418.0194	271.7858	48.81774	4.236209	-8.13
09:00	8.08	420.4454	268.3289	48.9781	4.236731	-3.37
09:15		413.0007	274.7899	48.642	4.215476	
09:30	8.1	407.7206	281.2412	50.54076	4.3964	-3.16
09:45		408.6555	285.1725	50.33817	4.335155	
10:00	8.05	411.1094	277.9884	50.76821	4.395167	-2.48
10:15		385.489	268.9259	49.14662	4.172808	
10:30	8.07	435.6732	271.2421	50.91671	4.300145	-2.45
10:45		396.4556	276.3613	49.07706	4.11605	
11:00	8.1	416.77	275.6594	50.18418	4.232125	-2.67
11:15		397.4427	279.5056	54.35215	4.88514	
11:30	8.12	415.9493	286.9912	56.56133	5.080885	1.87
12:00	8.12	417.8855	280.4502	50.65927	4.353618	-2.8
13:00	8.12	414.8127	279.4294	52.29066	4.504514	-0.08
14:00	8.12	406.8809	283.6592	51.06647	4.365661	-3.61
15:00	8.14	413.5299	283.3659	50.99086	4.26878	-3.11
16:00	8.14	416.6304	279.3295	51.05338	4.390092	-2.93
17:00	8.13	379.1959	282.3073	51.27219	4.475843	-2.05
18:00	8.13	413.2019	271.276	48.93206	4.194191	-3.81
19:00	8.14	423.4954	260.7206	48.89239	4.195416	-4.08
20:00	8.14	421.6158	270.4955	48.70065	4.153046	-4.66

21:00	8.12	415.7331	269.763	49.08694	4.128133	-4.68
22:00	8.12	415.538	269.8781	50.67638	4.382879	-3.63
23:00	8.13	419.6322	272.5086	49.1563	4.23186	-3.66
00:00	8.12	405.2749	280.9621	55.98278	4.958707	-0.24
01:00	8.12	409.2624	287.0349	52.85326	4.501224	-0.93
02:00	8.13	410.4482	282.0318	51.51433	4.40382	-1.43
03:00	8.11	414.0283	282.9195	51.55076	4.376352	-2.19
04:00	8.12	415.8649	291.1002	52.22059	4.522172	-4.97
05:00	8.13	415.8649	285.7122	52.61099	4.531452	-0.52
06:00	8.13	418.5733	282.2338	52.9692	4.567867	-2.49
07:00	8.14	420.382	285.6807	54.57484	4.851557	0.18
08:00	8.14	410.4482	277.1189	52.79127	4.53291	-2.12
09:00	8.14	414.0283	280.855	51.27323	4.396441	2.37
09:15		420.0175	297.2681	56.11237	4.781125	
09:30	8.15	421.1728	296.9215	54.60663	4.652539	1.17
09:45		414.2022	307.3965	57.73111	4.886444	
10:00	8.14	416.8993	314.3143	55.84166	4.681046	1.12
10:15		418.1572	307.0943	55.79163	4.762359	
10:30	8.14	410.1902	314.7305	62.03875	5.589561	6.67
10:45		418.6677	318.8564	58.19343	4.909197	
11:00	8.18	414.8504	341.1021	59.60086	5.070398	4.38
11:15		418.2855	302.4253	54.67759	4.616907	
11:30	8.17	418.8243	308.3129	53.10296	4.51235	-2.77
12:00	8.14	412.7612	300.7863	53.71276	4.554768	-0.27
13:00	8.16	384.4909	306.4604	55.56737	4.645665	-0.1
14:00	8.2	410.2584	316.5157	55.49447	4.720594	0.91
15:00	8.2	408.5527	307.7891	57.2557	4.848822	3.36
16:00	8.2	417.043	310.1646	57.28738	4.871198	1.75
17:00	8.19	414.1248	315.1629	57.29828	4.831188	1.74
18:00	8.16	408.785	321.9183	57.53905	4.777157	0.55
19:00	8.18	410.6408	323.2997	57.92718	4.911299	2.4
20:00	8.19	419.1489	325.017	59.13529	5.014722	3.16
21:00	8.19	405.1963	324.305	59.33822	5.016768	3.67
22:00	8.2	412.0425	327.3401	58.77206	5.047218	3.22
23:00	8.21	414.6219	334.8013	65.16147	5.80101	7.49
00:00	8.15	399.815	333.1528	57.95332	4.844158	1.69
01:00	8.12	412.7109	326.3433	62.0336	5.387591	5.05
02:00	8.13	408.6934	320.956	57.7194	4.927006	4.83
03:00	8.13	405.5264	296.5131	57.49468	5.068675	0.23
04:00	8.13	400.749	294.7447	59.38235	5.240677	2.71
05:00	8.14	398.585	298.3823	52.988	4.501885	-2.24
06:00	8.12	411.3695	297.485	54.70245	4.640758	-0.66
07:00	8.15	409.8798	293.8731	52.3492	4.454027	-2.05
08:00	8.14	391.6736	291.9465	53.70392	4.551906	-3.48
09:00	8.14	397.539	300.4657	53.68227	4.481911	0.45

Zn and Ba concentrations in µg/L, Ca and Mg in mg/L. CB – Charge balance.

8.5 Appendix 5 – Flume SI data

	Flume 1		
	CaCO3	ZnCO3:H2O	BaCO3
09:00	0.68	-0.41	-2.01
09:30	0.67	-0.41	-2.01
10:00	0.66	-0.35	-2.07
10:30	0.91	-0.48	-1.78
11:00	1.09	-0.57	-1.59
11:30	1.14	-0.6	-1.55
12:00	1.24	-0.6	-1.48
13:00	1.26	-0.65	-1.45
14:00	1.2	-0.61	-1.52
15:00	1.26	-0.66	-1.46
16:00	1.52	-0.91	-1.17
17:00	1.53	-1.05	-1.11
18:00	0.92	-0.44	-1.8
19:00	0.7	-0.42	-1.99
20:00	0.62	-0.41	-2.06
21:00	0.62	-0.4	-2.07
22:00	0.59	-0.35	-2.13
23:00	0.62	-0.37	-2.1
00:00	0.52	-0.39	-2.18
01:00	0.51	-0.39	-2.19
02:00	0.52	-0.37	-2.2
03:00	0.47	-0.4	-2.24
04:00	0.46	-0.4	-2.25
05:00	0.47	-0.34	-2.27
06:00	0.5	-0.34	-2.23
07:00	0.4	-0.39	-2.31
08:00	0.37	-0.41	-2.33
09:00	0.34	-0.4	-2.36
09:30	0.38	-0.4	-2.32
10:00	0.4	-0.38	-2.3
10:30	0.74	-0.35	-1.99
11:00	0.9	-0.47	-1.79
11:30	1.12	-0.53	-1.6
12:00	1.19	-0.61	-1.5
13:00	1.31	-0.64	-1.41
14:00	1.17	-0.63	-1.53
15:00	1.21	-0.66	-1.49
16:00	1.15	-0.63	-1.55

17:00	1.1	-0.55	-1.62
18:00	0.73	-0.39	-1.99
19:00	0.69	-0.33	-2.04
20:00	0.55	-0.39	-2.15
21:00	0.49	-0.42	-2.21
22:00	0.55	-0.29	-2.24
23:00	0.46	-0.35	-2.28
00:00	0.4	-0.4	-2.32
01:00	0.37	-0.41	-2.33
02:00	0.34	-0.42	-2.37
03:00	0.37	-0.42	-2.33
04:00	0.33	-0.42	-2.38
05:00	0.33	-0.41	-2.4
06:00	0.38	-0.36	-2.37
07:00	0.31	-0.43	-2.4
08:00	0.35	-0.43	-2.36
09:00	0.34	-0.41	-2.37
09:30	0.35	-0.41	-2.35
10:00	0.41	-0.35	-2.33
10:30	0.62	-0.39	-2.09
11:00	0.79	-0.42	-1.92
11:30	0.92	-0.41	-1.82
12:00	0.92	-0.41	-1.83
13:00	0.96	-0.53	-1.73
14:00	1.13	-0.67	-1.54
15:00	1.18	-0.66	-1.5
16:00	1.29	-0.72	-1.4
17:00	1.25	-0.62	-1.47
18:00	0.85	-0.45	-1.85
19:00	0.82	-0.42	-1.89
20:00	0.62	-0.42	-2.12
21:00	0.53	-0.4	-2.16
22:00	0.51	-0.39	-2.19
23:00	0.45	-0.4	-2.28
00:00	0.48	-0.55	-2.27
01:00	0.49	-0.54	-2.25
02:00	0.43	-0.55	-2.27
03:00	0.43	-0.54	-2.28
04:00	0.43	-0.54	-2.28
05:00	0.39	-0.55	-2.35
06:00	0.38	-0.56	-2.32
07:00	0.36	-0.56	-2.36
08:00	0.31	-0.57	-2.39
09:00	0.34	-0.55	-2.35

	Flume 2		
	Calcite	ZnCO3:H2O	BaCO3
09:00	1.04	-0.53	-1.63
09:30	0.98	-0.48	-1.68
10:00	0.9	-0.44	-1.76
10:30	1.14	-0.59	-1.51
11:00	1.23	-0.7	-1.41
11:30	1.31	-0.65	-1.37
12:00	1.18	-0.65	-1.47
13:00	1.21	-0.66	-1.45
14:00	1.24	-0.66	-1.42
15:00	1.21	-0.67	-1.43
16:00	1.52	-0.9	-1.15
17:00	1.41	-0.85	-1.23
18:00	1.14	-0.49	-1.49
19:00	1.11	-0.51	-1.59
20:00	1.05	-0.41	-1.69
21:00	0.97	-0.51	-1.69
22:00	0.93	-0.47	-1.74
23:00	0.93	-0.46	-1.73
00:00	0.89	-0.47	-1.76
01:00	0.91	-0.4	-1.79
02:00	0.91	-0.42	-1.79
03:00	0.86	-0.45	-1.79
04:00	0.85	-0.44	-1.82
05:00	0.87	-0.4	-1.82
06:00	0.83	-0.43	-1.84
07:00	0.79	-0.39	-1.89
08:00	0.76	-0.4	-1.9
09:00	0.75	-0.36	-1.94
09:30	0.76	-0.38	-1.92
10:00	0.69	-0.39	-1.97
10:30	0.84	-0.45	-1.82
11:00	0.95	-0.51	-1.69
11:30	1	-0.54	-1.65
12:00	1	-0.53	-1.67
13:00	1.27	-0.69	-1.41
14:00	1.05	-0.57	-1.65
15:00	1.06	-0.68	-1.61
16:00	1.11	-0.62	-1.58
17:00	1.04	-0.66	-1.62
18:00	0.94	-0.49	-1.72

19:00	0.94	-0.45	-1.74
20:00	0.88	-0.46	-1.79
21:00	0.87	-0.45	-1.81
22:00	0.87	-0.41	-1.82
23:00	0.79	-0.43	-1.88
00:00	0.77	-0.44	-1.89
01:00	0.76	-0.43	-1.9
02:00	0.75	-0.43	-1.91
03:00	0.77	-0.37	-1.94
04:00	0.75	-0.41	-1.92
05:00	0.74	-0.42	-1.93
06:00	0.74	-0.42	-1.94
07:00	0.74	-0.43	-1.92
08:00	0.78	-0.4	-1.92
09:00	0.71	-0.42	-1.96
09:30	0.69	-0.41	-1.99
10:00	0.67	-0.41	-2
10:30	0.85	-0.47	-1.83
11:00	0.93	-0.44	-1.79
11:30	0.94	-0.51	-1.73
12:00	0.95	-0.57	-1.69
13:00	1.03	-0.52	-1.68
14:00	1.07	-0.54	-1.64
15:00	1.06	-0.62	-1.61
16:00	1.13	-0.68	-1.53
17:00	1.04	-0.74	-1.61
18:00	1	-0.48	-1.71
19:00	0.92	-0.49	-1.75
20:00	0.87	-0.47	-1.81
21:00	0.84	-0.46	-1.82
22:00	0.81	-0.46	-1.87
23:00	0.84	-0.46	-1.84
00:00	0.87	-0.72	-1.79
01:00	0.87	-0.71	-1.8
02:00	0.89	-0.71	-1.82
03:00	0.85	-0.71	-1.83
04:00	0.83	-0.71	-1.85
05:00	0.81	-0.69	-1.85
06:00	0.79	-0.7	-1.9
07:00	0.76	-0.68	-1.92
08:00	0.74	-0.67	-1.93
09:00	0.73	-0.66	-1.96

Flume 3			
	Calcite	ZnCO3:H2O	BaCO3
09:00	0.65	-0.6	-2.01
09:30	0.61	-0.58	-2.02
10:00	0.71	-0.58	-1.98
10:30	0.7	-0.58	-1.98
11:00	0.63	-0.57	-2.01
11:30	0.66	-0.58	-1.99
12:00	0.71	-0.59	-1.98
13:00	0.68	-0.58	-1.97
14:00	0.55	-0.56	-2.09
15:00	0.57	-0.57	-2.08
16:00	0.55	-0.58	-2.09
17:00	0.53	-0.57	-2.15
18:00	0.48	-0.56	-2.18
19:00	0.5	-0.57	-2.17
20:00	0.52	-0.58	-2.13
21:00	0.52	-0.58	-2.12
22:00	0.51	-0.57	-2.13
23:00	0.54	-0.58	-2.11
00:00	0.5	-0.61	-2.14
01:00	0.52	-0.57	-2.15
02:00	0.48	-0.57	-2.16
03:00	0.58	-0.58	-2.1
04:00	0.55	-0.57	-2.13
05:00	0.57	-0.57	-2.12
06:00	0.59	-0.56	-2.11
07:00	0.48	-0.58	-2.16
08:00	0.51	-0.56	-2.14
09:00	0.46	-0.57	-2.2
09:30	0.5	-0.58	-2.15
10:00	0.45	-0.58	-2.12
10:30	0.47	-0.55	-2.2
11:00	0.49	-0.57	-2.17
11:30	0.57	-0.57	-2.12
12:00	0.52	-0.56	-2.14
13:00	0.52	-0.57	-2.15
14:00	0.53	-0.57	-2.12
15:00	0.54	-0.57	-2.11
16:00	0.54	-0.56	-2.11
17:00	0.53	-0.61	-2.12
18:00	0.51	-0.57	-2.15

19:00	0.52	-0.56	-2.15
20:00	0.52	-0.56	-2.13
21:00	0.51	-0.57	-2.15
22:00	0.52	-0.57	-2.14
23:00	0.51	-0.56	-2.14
00:00	0.57	-0.58	-2.12
01:00	0.53	-0.57	-2.13
02:00	0.52	-0.57	-2.13
03:00	0.51	-0.57	-2.14
04:00	0.56	-0.56	-2.09
05:00	0.53	-0.57	-2.13
06:00	0.55	-0.56	-2.12
07:00	0.56	-0.56	-2.11
08:00	0.56	-0.57	-2.12
09:00	0.49	-0.57	-2.17
09:30	0.56	-0.56	-2.1
10:00	0.57	-0.57	-2.08
10:30	0.61	-0.57	-2.08
11:00	0.63	-0.57	-2.01
11:30	0.6	-0.56	-2.03
12:00	0.55	-0.57	-2.1
13:00	0.6	-0.6	-2.06
14:00	0.63	-0.57	-2.01
15:00	0.63	-0.57	-2.04
16:00	0.64	-0.56	-2.02
17:00	0.63	-0.57	-2.02
18:00	0.62	-0.57	-2.03
19:00	0.63	-0.57	-2.02
20:00	0.64	-0.56	-2.01
21:00	0.64	-0.58	-2.02
22:00	0.65	-0.57	-2
23:00	0.71	-0.57	-1.98
00:00	0.6	-0.58	-2.04
01:00	0.6	-0.57	-2.07
02:00	0.55	-0.58	-2.1
03:00	0.6	-0.58	-2.09
04:00	0.6	-0.58	-2.1
05:00	0.56	-0.58	-2.09
06:00	0.55	-0.57	-2.11
07:00	0.56	-0.57	-2.09
08:00	0.58	-0.59	-2.08
09:00	0.55	-0.59	-2.1

8.6 Appendix 6 – Acidic experiments data

	biofilm		
	Zn	Ba	pH
06:00	331.3455	223.3679	7.99
06:15	328.6046	244.9209	
06:30	338.7298	216.6045	7.97
06:45	342.2525	224.3174	
07:00	339.029	225.9997	7.96
07:15	148.142	62.64362	
07:30	147.2965	57.01304	7.44
07:45	439.0612	68.89504	
08:00	611.3922	69.83191	5.12
08:15	432.8208	67.43224	
08:30	383.7628	63.23772	4.4
08:45	354.3807	64.86226	
09:00	270.0388	62.23336	4.29
09:15	263.1994	63.96628	
09:30	241.8938	65.02122	4.27
09:45	234.3407	64.67779	
10:00	195.2485	61.79038	4.26
10:15	206.1103	60.93098	
10:30	175.5652	61.78819	4.25
10:45	182.1631	63.05969	
11:00	153.4739	62.99914	4.19
11:15	158.4039	60.89552	
11:30	153.9366	60.808	4.22
11:45	312.8814	197.1334	
12:00	314.9505	193.6769	7.55
12:15	307.4345	219.082	
12:30	323.4609	222.9968	7.78
12:45	306.2153	198.7738	
13:00	306.8095	210.3319	7.83
13:15	320.7375	202.5676	
13:30	308.2935	198.5897	7.87
13:45	324.0088	186.5228	
14:00	313.157	207.5721	7.9
14:15	317.1965	204.6557	
14:30	323.6921	213.4554	7.88
14:45	327.3021	213.9481	
15:00	322.6073	204.3929	7.91

	control		
	Zn	Ba	pH
06:00	383.972	297.2691	8.35
06:15	380.5276	297.9786	
06:30	386.6097	301.132	8.32
06:45	389.4382	294.0717	
07:00	383.6342	301.1208	8.31
07:15	208.9327	84.10421	
07:30	145.5195	56.48361	4.32
07:45	200.4568	54.80088	
08:00	193.453	52.80175	4.13
08:15	134.7395	52.34946	
08:30	133.9531	51.9578	4.05
08:45	123.3439	52.61418	
09:00	96.14397	50.73323	4.04
09:15	101.7675	51.2539	
09:30	95.93042	50.67256	4.04
09:45	123.6494	51.27137	
10:00	97.70911	50.21452	4.05
10:15	92.63964	52.45115	
10:30	87.40938	50.03751	4.09
10:45	92.96103	51.30763	
11:00	77.86984	50.8317	4.09
11:15	77.0656	50.65394	
11:30	62.00737	49.90419	4.05
11:45	409.952	281.5368	
12:00	408.2951	294.7555	7.52
12:15	406.4896	297.7199	
12:30	412.6515	295.793	7.91
12:45	419.0936	293.2588	
13:00	403.7745	293.962	8.01
13:15	391.0716	290.8642	
13:30	406.267	293.6463	8.08
13:45	415.81	294.9012	
14:00	399.9486	290.9292	8.13
14:15	397.2742	294.6569	
14:30	391.3823	300.0989	8.16
14:45	401.9545	293.0883	
15:00	419.3109	289.0845	8.13

8.7 Appendix 7 – Acidic Experiment Modelling

Percentage Stream water	PHREEQC pH	$CaCO_3$	$BaSO_4$	ZnO(a)	$ZnCO_3$:H20
100	7.99	0.41	0.22	-1.22	-0.7
95	7.964	0.34	0.19	-1.26	-0.73
90	7.935	0.28	0.16	-1.31	-0.77
85	7.903	0.21	0.13	-1.36	-0.82
80	7.868	0.13	0.1	-1.42	-0.87
75	7.83	0.05	0.07	-1.49	-0.93
70	7.788	-0.04	0.04	-1.56	-0.99
65	7.743	-0.14	0	-1.65	-1.07
60	7.693	-0.25	-0.04	-1.75	-1.15
55	7.639	-0.36	-0.08	-1.86	-1.25
50	7.58	-0.49	-0.12	-1.99	-1.36
45	7.515	-0.64	-0.17	-2.14	-1.49
40	7.444	-0.8	-0.22	-2.3	-1.64
35	7.363	-0.99	-0.28	-2.49	-1.81
30	7.271	-1.2	-0.34	-2.72	-2.02
25	7.164	-1.46	-0.4	-2.99	-2.27
20	7.032	-1.77	-0.48	-3.32	-2.59
15	6.859	-2.19	-0.56	-3.77	-3.03
10	6.602	-2.8	-0.65	-4.42	-3.68
5	6.027	-4.03	-0.75	-5.82	-5.11
4	5.71	-4.57	-0.78	-6.52	-5.84
3	5.148	-5.57	-0.8	-7.74	-7.12
2	4.606	-7.25	-0.83	-8.94	-8.49
1	4.317	-8.5	-0.86	-9.69	-9.53
0	4.14		-0.89	-10.32	

9

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