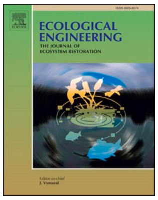
Ecological Engineering 219 (2025) 107681

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Highly efficient co-removal of zinc and manganese during passive treatment of mine drainage: Mechanisms, microbiology and application

Ilemona Cornelius Okeme [1], Pallavee Srivastava, Devin J. Sapsford [*]

School of Engineering, Cardiff University, Queen's Building, The Parade, Cardiff CF24 3AA, United Kingdom

A R T I C L E I N F O

Keywords:
Passive mine water treatment;vertical flow reactors
Limestone reactor
Co-precipitation
Adsorption
Manganese zinc co-removal
Manganese oxidising bacteria
iron oxidising bacteria
Mn-Zn oxide mineral

A B S T R A C T

Previous investigations have reported the coeval removal of zinc (Zn) and manganese (Mn) under aerobic circumneutral pH conditions during the passive treatment of mine waters, but the removal mechanisms are less well established. Here 7 months of data are presented on Mn and Zn removal from a field based passive mine water treatment trial on mine water of pH 5.42, containing Mn 1.5 mg/l, and Zn 0.8 mg/l. The final treatment train comprised two aerobic vertical flow reactors (VFRs) operated in series, the first with granite/pyrolusite media and the second with limestone media (retention times ∼19 h and ∼ 3 h respectively). Only limited Mn and Zn removal was observed in the granite/pyrolusite VFR reactor. When limestone vertical flow reactors were added to the treatment train after month 4, Mn removal was quickly established with coeval removal of Zn. The Mn concentrations decreased from a mean of 1169 μg/l and 1154 μg/l in the influents to the two limestone reactors, to 245 μg/l and 234 μg/l in the effluents, respectively. Zn concentrations decreased from a mean of 791 μg/l and 935 μg/l (influent) to 228 μg/l and 236 μg/l in the effluents, respectively, with effluent concentrations on occasion reaching as low as 15 μg/l. Detailed analyses of the precipitates bulk and surficial chemistry indicate Zn removal in association with manganese oxides (MnOx) accreted on the surface of limestone as the predominant removal mechanism. Sequential extraction data indicates a significant proportion of Zn also being associated in the "adsorbed/carbonate-associated" phase. Interestingly, based on the difference in influent and effluent concentrations, the overall ratio of Mn:Zn removal was close to 2:1 suggestive of the precipitation of a Mn—Zn oxide mineral directly from the mine water. Microbial community analyses reveal distinct structure in the accumulated ochreous sludge in the granite/pyrolusite and on the grains of limestone in the limestone reactors. The granite/pyrolusite VFR sludge was populated with iron oxidising microbes including *Gallionella* and *Pseudomonas*, of which *Pseudomonas* can also oxidize Mn. The limestone reactors were populated with several genera of Mn oxidising bacteria including *Pseudomonas* and *Leptothrix*, suggesting biotic mechanisms are important. These data reveal that Zn may be removed to concentrations was low as 15 μg/l when the Zn is co-removed with Mn within limestone reactors. This opens opportunities for this mechanism to be exploited within nature-based passive treatment systems for removal of Zn.

1. Introduction

Mining influenced waters (MIWs), including acidic, alkaline and circumneutral waters, are a major global environmental challenge as they can contain elevated concentrations of dissolved sulphate along with ecotoxic metal(loid)s such as Zn, Cu, Pb, As, and Cd (Gammons et al., 2010; Mayes et al., 2009a, 2009b; Tomiyama and Igarashi, 2022). Metal contamination from the numerous abandoned mine sites worldwide (e.g., ∼ 45,000 in North America, > 5500 in Japan, and > 10,000 in the United Kingdom) (Mayes et al., 2009a, 2009b) originates from both point and diffuse sources. These include discharges from drainage levels or adits and runoff from mine wastes like rock piles and tailings (Holanda and Johnson, 2020). The release of ecotoxic contaminants into the surface and groundwaters poses a threat to the aquatic habitat and can cause public health concerns (e.g., (Hendryx, 2015; Sun et al., 2001). Circumneutral and mildly acidic mine drainage with

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Abbreviations: MIW, Mining influenced waters;; CNMD, Circumneutral mine drainage; VFR, Vertical flow reactor; LSR, Limestone reactor; HTW, Header tank water.

[*] Corresponding author.

E-mail address: sapsforddj@cardiff.ac.uk (D.J. Sapsford).

[1] Present address: Atkinsrealis, Rutherford House, Moor Row, CA24 3JZ, UK.

https://doi.org/10.1016/j.ecoleng.2025.107681
Received 24 July 2024; Received in revised form 4 April 2025; Accepted 12 May 2025

elevated concentrations of ecotoxic metal ions can be generated either from partial to complete neutralisation of acid mine drainage (AMD), or directly from the oxidation of non-acid generating non-ferrous sulphides such as sphalerite and galena and subsequent leaching of reaction products and secondary salts (Lindsay et al., 2015; Majzlan et al., 2018). In the context of this study, As, Cd, Cu, Fe, Mn, Ni, Pb and Zn are the most commonly encountered metals/metalloids discharged from abandoned metal mines that cause statutory failures of Environmental Quality Standards in England and Wales, with Zn being the most common (Mayes et al., 2009a, 2009b).

Slightly acidic and circumneutral mine water often contains elevated concentrations of manganese and zinc (Earle and Callaghan, 1998; Pope and Trumm, 2015). Example values for mine waters from five mine waters in England and Wales range from 0.77 mg/l to 19.3 mg/l and 4.3–34 mg/l for Mn and Zn respectively (derived from flow/loading data in (Mayes et al., 2009a, 2009b)). In the mildly acidic to circumneutral pH range, the homogenous oxidation kinetics of Mn(II) is slow (relative for example to that of Fe(II) oxidation), allowing it to remain bioavailable to exert ecotoxicity in aquatic and terrestrial ecosystems (Howe et al., 2004; Neculita and Rosa, 2019). Similarly, Zn(II) often remains soluble (often as a carbonate complex) to environmentally detrimental levels within oxic circumneutral mine drainage (Fosmire, 1990; Okereafor et al., 2020). These factors make the treatment of Mn and Zn impacted mine water challenging (Katsoyiannis et al., 2004; Li et al., 2019; Tajima et al., 2022; Van Geen and Robertson, 1994). Furthermore, mildly acidic and circumneutral MIWs often contain high concentrations of major cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) which are known to compromise remedial efforts by competing for sorption sites on the reactive materials (Warrender et al., 2011). Consequently, there is a requirement for sustainable removal mechanisms that can be incorporated into treatment schemes for application to Mn and Zn impacted mine waters.

2. Technical background

Removal of metals in MIW can be achieved by active treatment technologies including chemical/alkali dosing for precipitating the metals (Sibrell et al., 2007) and active microbiological sulfidogenic bioreactors (e.g. (Holanda and Johnson, 2020)). However, active treatment technologies, are energy and/or reagent intensive, require complex operational handling including high cost of construction, along with generation of large volumes of waste with associated costs of wastes dewatering and disposal (Kennedy et al., 2023; Sheoran and Sheoran, 2006).

Passive treatment technologies typically utilise a range of nature-based and/or reactive-media based systems including various types of constructed wetlands (e.g. Gandy et al., 2016; Nyquist and Greger, 2009; Fitzsimons and Courtney, 2022), flow through bioreactors (e.g. Dean et al., 2022; Hagerty et al., 2011), settling ponds or beds with low-cost reactive media (e.g. Christenson et al., 2019; Nuttall and Younger, 2000), or high surface area adsorbents like zeolites (e.g. Hidayat et al., 2021), red mud (bauxite residue) (e.g. Gupta and Sharma, 2002), microbial biomass (e.g. Klimmek et al., 2001; Martins et al., 2004), pelletised hydrous ferric oxide (e.g. Mayes et al., 2009a, 2009b)), and biochar (e.g. James et al., 2022; Maneechakr and Karnjanakom, 2019). Examples of passive and semi-passive treatment experiments for Zn and Mn contaminated water are detailed in Table S1 (supplementary material). These reveal a range of system types which rely on a range of treatment mechanisms.

The primary mechanisms involved in removal of Mn and Zn during aerobic passive mine water treatment include oxidation (for Mn(II)), adsorption, precipitation and coprecipitation, influenced by a combination of abiotic and biotic processes (Abongwa et al., 2020; Ducret and Barbeau, 2024; Luan et al., 2012a; Neculita and Rosa, 2019). At circumneutral conditions, microbial oxidation of Mn(II) is reported to be the dominant process, occurring at five orders of magnitude faster than abiotic oxidation over a wide range of temperatures (Anderson et al., 2011; Li et al., 2019; Miyata et al., 2007; Tajima et al., 2022). Combined scattering and spectroscopy data have demonstrated that the Mn biooxides are typically high average oxidation state [i.e. structural Mn (IV)] layer-type oxides with very high specific surface areas and poor crystallinity, most closely resembling δ-MnO_2 or H^+-birnessite (Lanson et al., 2002; Miyata et al., 2007; Villalobos et al., 2003). The birnessite structural unit is basically a sheet of MnO_6 octahedra with a 7-Å interlayer spacing (Post, 1999) with charge-balancing cations such as H^+, Na^+, K^+, Mn^{2+}, and Ca^{2+} and water molecules occupying the interlayer spacing (Miyata et al., 2007). A similar mineral, vernadite (δ-MnO_2), can also be formed via microbial oxidation of Mn(II), has a similar structural unit, albeit with disordered layer stacking or composed of extremely thin plates (Post, 1999). Between pH 7.0–8.0, the layered δ-MnO_2 get transformed to tunnelled-MnO (TMO) (Yang et al., 2018). Zn can be removed from circumneutral mine drainage by occupying the reactive vacancies within the TMO (Tajima et al., 2022) through inner sphere complexation (Li et al., 2020). In the presence of Zn in the circumneutral drainage, Zn—Mn secondary minerals have been shown to form on the surface of the biogenic manganese oxide (Chang et al., 2014; Lefkowitz and Elzinga, 2015)$_2$ (Chang et al., 2014; Lefkowitz and Elzinga, 2015). Chalcophanite, a common weathering product of Mn-bearing base metal deposits (Post, 1999), has a structure analogous to birnessite (Post and Veblen, 1990).

In this study, mildly acidic metal mine drainage was treated using aerobic vertical flow granite/pyrolusite and limestone reactors in series. The column media was characterised via sequential extraction and a suite of analytical and microbial techniques post-treatment, aimed at generating information on Mn and Zn removal mechanisms, while assessing the effectiveness of the treatment processes. The elucidation of mechanisms involved in the co-removal of manganese and zinc from mine water has relevance to the continued development of a wide range of nature-based approaches to mine water treatment.

3. Materials and methods

3.1. Field reactor materials and operation (upstream VFR and downstream limestone reactors)

The study site is located close to the Gwindra stream (50°19′42.9”N 4°52′02.8”W) and took mine water from the Coombe Adit, an abandoned mine water drainage tunnel from a former tin and copper mine near St. Austell, in Cornwall, Southwest England. Based on previous trials of vertical flow reactors (VFRs) (Blanco et al., 2018; Dey et al., 2003; Sapsford et al., 2007; Sapsford and Williams, 2009) small column VFR with granite and pyrolusite media (0.355–0.85 mm, Universal Mineral Supplies Ltd., UK) were used to treat the mine water on site (Fig. 1a). PVC plastic columns of internal cross-sectional area of 9.5×10^{-3} m^2 were used for VFR A and VFR B. These were constructed with a bed of 6 mm granite gravel of 50 mm thickness overlain by 20 mm thickness of the MnO_2 sand. As part of initial investigations, these were fed with raw mine water from a header tank at variable flows of up to 100 ml/min for 6 months. After a pause in operation of several weeks the trial was recommenced at a consistent flow rate of 2.8 - 3 ml/min for 7 months. A column-scale limestone reactor (LSR) was added to the treatment train (Fig. 1a-c) after 100 days. The effluents from the VFRs were passed through the limestone reactors (LSR A and LSR B). These were of cross-sectional area 6.63×10^{-3} m^2, and contained a ~ 150 mm thick bed of 6 mm limestone chips. The outflow level was controlled with a swan-neck arrangement to maintain saturation of the media. Hydraulic Residence Time (HRT) estimates based on a 3 ml/min flow rate, standing water volumes and media pore volume (estimated 40 %) give are ~19 h in VFRs (~1.5 h in contact with the pyrolusite/granite bed) and ~ 3 h in the LSRs (~ 2 h in contact with the limestone bed).

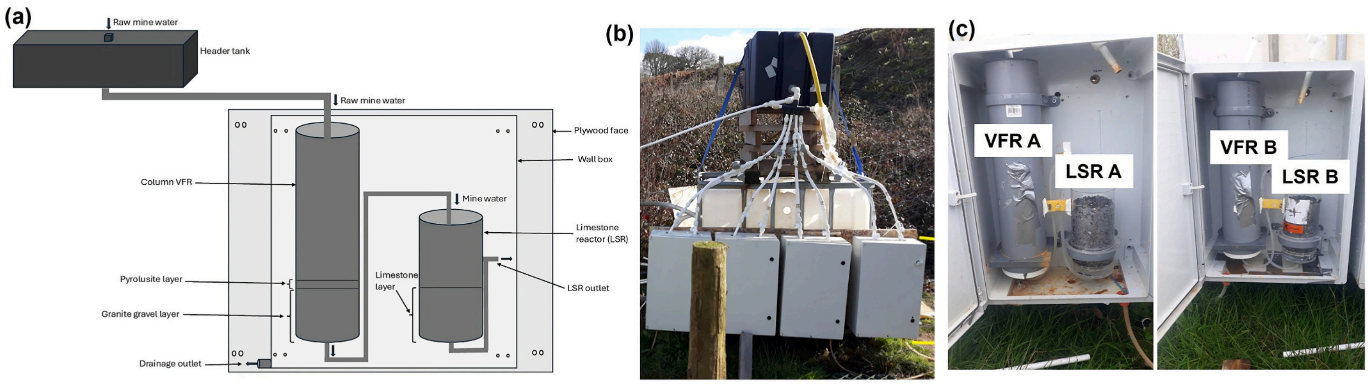

Fig. 1. (a) Schematics of the column-scale mine water treatment system trialled on site. The length and internal diameter of the VFR (vertical flow reactor) are 330 mm and 110 mm, respectively. The length and internal diameter of the LSR (limestone reactors) are 150 mm and 92 mm, respectively; (b) On-site set up of the set up with the header tank; (c) The VFR and the LSR column were housed within enclosed cases.

3.2. *Water sampling and analysis*

Various physicochemical parameters of the raw influent mine water and the effluent from both VFRs and LSRs were determined weekly. A HANNA 9828 multiparameter meter was used to determine the pH, temperature, ORP (oxidation-reduction potential), EC (electrical conductivity), and DO (dissolved oxygen) on site. Samples were taken for laboratory analyses: "total" and dissolved metals (filtered through 0.22 μm syringe filter) were preserved by acidification with HNO_3, samples were taken for anion analysis (without acidification). Elemental analyses (Fe, Zn, Mn, As, Cu, & Cd) was performed using inductively coupled plasma-mass spectrometry (analytical detection limits all <1 ppb). Anion analysis (NO_3^-, SO_4^{2-}, & Cl^-) was conducted using ion chromatography. Both analyses were performed by commercial laboratories. Alkalinity and Fe(II) analyses were carried out in the field using a HACH 16900 digital titrator following the method 2803 and phenanthroline method with HACH DR900 portable colorimeter, respectively.

3.3. *Solids characterisation*

Post-operation the columns were decommissioned, and column solid samples were collected for physicochemical and microbiological analyses. The samples for microbiological analysis were retrieved aseptically and stored at −80 °C until DNA extraction. The dark coating from LSR (Supplementary Fig. 1) was obtained by gently agitating the limestone in a Nalgene HDFE bottle with DI water. A few limestone particles that still exhibited the brown-black coating were scraped using a stainless-steel chisel scraper. The scrapings thus obtained along with the ochreous solids obtained from VFRs were dried at 45 °C for 24 h followed by homogenisation using an agate mortar and pestle. The dried samples were digested in aqua regia and subjected to elemental analysis by ICP-MS (analysed at commercial laboratories). Total carbon (TC) and total inorganic carbon (TIC) were determined by Shimadzu total carbon analyser. The TC was determined by combusting the sample at 900 °C while TIC was determined by heating the samples acidified with phosphoric acid to 200 °C. Total organic carbon (TOC) was the difference between TC and TIC. Mineralogical characterisation of the samples was carried out using X-ray diffractometer (XRD, Siemens™ D5000 diffractometer, Philips PW3830 X-ray generator, PW1710 diffractometer controller) and the spectra obtained were analysed using the Match™ phase identification package. The XRD used a copper (Cu Kα) radiation source and was operated at a voltage and current of 40 kV and 30 mA, respectively, using a 2θ range of 10° to 80°, at 0.5 degrees per minute and a step size of 0.05. A Zeiss Sigma field emission scanning electron microscope (FE-SEM) with an attached energy dispersive X-ray spectroscopy (EDAX™ Octane Plus high-resolution EDS system) was used to determine the morphological and elemental characteristics. The dried samples were mounted on adhesive carbon disc mounted on a standard SEM pin-stub, and examined using the instrument in the variable pressure mode at a voltage of 15 KeV, aperture of 120 μm (in high current mode) and acquisition time of 200 s. The EDAX data was analysed using the associated EDAX (AMETEK Inc.) TEAM™ software.

A sequential extraction procedure adapted from Dold and Fontboté (2001) with modifications was used to differentiate the various phases in the solids (Table 1). An initial extraction with deionised water for water soluble phases was adapted from Dold (2003). Various phases identified by sequential extraction were water soluble (DI); carbonate associated (Carb); poorly crystalline Fe/Mn oxides and (hydroxy)oxides (Fe/Mn(H-OH)) for e.g., 2-line ferrihydrite, MnO_2; crystalline Fe/Mn oxides and (hydroxy)oxides (Cryst Fe/Mn(H-OH)) for e.g., goethite, haematite, higher order ferrihydrite; and residual phase (Res). A solid to liquid ratio of 2 mg/ml was used and the metal concentration in each fraction was determined after acidification of the extract using ICP-MS (analysed at commercial laboratories).

3.4. *Microbiological analyses*

Mine water samples along with ochre and black coating were collected aseptically and stored at −80 °C till analysis. The samples were shipped for DNA extraction, next generation sequencing (NGS) of bacterial and archaeal 16S rRNA genes, and microbiome analysis (Epistem Ltd. (Manchester, UK)). In brief, Qiagen Powersoil Pro DNA extraction kit was used to extract DNA followed by quality control on Agilent Tapestation and Nanodrop. Library preparation for sequencing was carried out using Qiagen 16S/ITS region panel kit to amplify V3-V4 and ITS regions followed by appendment of sequencing indexes. Post quality

Table 1
Summary of sequential extraction procedure used for iron and manganese rich (Dold, 2003; Dold & Fontbote, 2001).

Target Phase	Extractant used	Extraction conditions
Water soluble (DI)	Deionised water	1 h, RT
Exchangeable/ Carbonate associated (Carb)	1 M ammonium acetate, pH 4.5	2 h, RT, under shaking
Poorly crystalline Fe/Mn oxides & (hydroxy)oxides (Fe/Mn (H-OH))	0.2 M ammonium oxalate, pH 3.0	1 h, RT, under shaking
Crystalline Fe/Mn oxides & (hydroxy)oxides (Cryst Fe/ Mn(H-OH))	0.2 M ammonium oxalate, pH 3.0	2 h, 80 °C, in water bath
Residual (Res)	Aqua regia-: 1:3 solution of 67 % HNO_3 and 37 % HCl	42 min in microwave; 25 min cooling

control, the libraries were pooled and sequenced on a MiSeq Reagent Kit v3 (600 cycle). The sequence analysis was done as described in (Srivastava et al., 2022).

4. Results and discussion

4.1. Field trial results

The raw mine water was fed into a header tank from which it was fed into the column scale VFRs A and B. The effluent from these columns were fed into LSR A and B respectively from month 5 onwards. Fig. 2 (and Fig. S2) shows the water quality parameters of the raw mine water and the effluents of VFRs and LSRs. The raw mine water exhibited a mean pH of 5.42, 4.18 mg/l dissolved oxygen (DO), 13.66 mg/l as $CaCO_3$ total alkalinity, 17.47 mg/l NO_3^-, and 48.27 mg/l SO_4^{2-}. The mean total Fe, Mn, and Zn concentrations were 1.2, 1.5, and 0.8 mg/l, respectively. The detailed physicochemical characteristics of the raw mine water along with the column effluents are outlined in Table S2. The effluent from VFR exhibited a mean pH of 5.94 and increased on passing through the LSRs (LSR A pH 7.90; LSR B pH 7.88). This neutralisation of acidity may be attributed to the dissolution of the limestone in the LSR. Both VFR and LSR exhibited higher DO (> 6 mg/l) as compared to the raw mine water, which may be the result of aeration in the header tank.

The metal removal efficiency of the treatment system was determined by monitoring the metal concentrations in the effluents (Figs. 3, S3, and S4). The VFRs exhibited >95 % removal efficiency for iron and arsenic (Fig. S4). The pH (~6.0) and DO (~ 6 mg/l) conditions exhibited by the VFR have been known to be amenable for precipitation of Fe as (oxy)hydroxides (Violante et al., 2007). Under these conditions, As, if present in the system can be coprecipitated with oxyhydroxides of Fe (Violante et al., 2007). While the VFRs were effective in removing Fe and As, they were less efficient in eliminating Cd, Cu, Mn, and Zn. They removed only 40–50 % of Cd, 40–70 % of Cu, 40–50 % of Mn, and approximately 30 % of Zn. However, Mn removal, progressively improved, which may be attributed to the establishment of Manganese Oxidising Bacteria (MOB) in the columns (See section 3.3). Fig. 3 shows the changes in the metal concentration over the course of field study.

To further enhance the treatment process, effluent from VFR was fed into the LSR columns (Fig. 1) from day 100 onwards. The Mn and Zn concentrations in the effluent LSRs exhibited a decrease from 1300 and 700 μg/l to 5–15 and 50–100 μg/l (Fig. 3, S4), respectively, by the end of the study. This is a removal efficiency of 99 % (Mn) and ~ 90 % (Zn) (Fig. S5). The removal efficiencies for Cd, Cu, Mn, and Zn increased by ~35 %, ~48 %, 50 %, and 65 %, respectively as compared to VFR. The removal of Mn exhibited a marked increase from week 3 onwards (Fig. S5) which was accompanied with the appearance of black coating on the limestone (Fig. S1). The development of microbial community comprising of MOB are responsible for the enhanced removal of Mn (Christenson et al., 2019). The co-removal of Zn along with Mn may be attributed to either sorption onto the manganese oxide surfaces (Christenson et al., 2019) or coprecipitation as a Mn—Zn— mineral phase (Tum et al., 2024; Watanabe et al., 2024). The pH conditions of the LSR columns (~pH 7.90) makes abiotic homogeneous oxidation of Mn(II) unlikely, as the phenomenon is known to occur only slowly at pH < 8.5 (Katsoyiannis et al., 2004; Li et al., 2019). Autocatalysis of Mn(II) oxidation by MnOx solids and iron (oxy)hydroxides, especially 2-line ferrihydrite are well established heterogenous phenomena (Davies and Morgan, 1989; Diem and Stumm, 1984; Lan et al., 2017).

4.2. Solids characterisation and metal removal mechanisms

The solids from the column reactors were subjected to chemical and mineralogical characterisation to understand the underlying mechanisms of metal removal. Mineralogical characterisation of the sludge from the VFRs exhibited the characteristic diffused peak at 35° 2θ of amorphous ferrihydrite and a small peak at 65° 2θ corresponding to 2-line ferrihydrite (Fig. 4a). The XRD spectra of scrapings from the raw limestone used as media for the LSR columns was dominated by calcite mineral phases (Fig. 4b, bottom scan). The XRD spectra of the scraping from the black coating on the column media post experiment were starkly similar to that of raw limestone (Fig. 4b). Manganese mineral phases were not detected. It is well known that amorphous and poorly crystalline Mn oxides and hydroxy(oxides) ($MnOOH/MnO_x$) are difficult to identify by XRD (Tokashiki et al., 1986) and are often referred to as Mn oxide 'wad' by XRD (Post, 1999). It is notable that manganese carbonate minerals (such as rhodochrosite ($MnCO_3$)) were also not detected despite being found in previous studies on treatment of circum-neutral mine drainage using limestone holding ponds/rock drainage system (Abongwa et al., 2020; Bamforth et al., 2006).

Fig. 5 shows the distribution of metals throughout the extraction phases present within the sludge in the VFR and the scrapings from the LSR. Fe and Mn were mostly dissolved during the extraction targeting Fe/Mn(H-OH) and crystalline Fe/Mn(H-OH), which agrees with the XRD results. The other metals like As, Pb, Cd, Ni, and Cr in the VFR sludge were predominantly associated with the Fe/Mn(H-OH) and crystalline Fe/Mn(H-OH). On the other hand, Cu and Zn in VFR A and

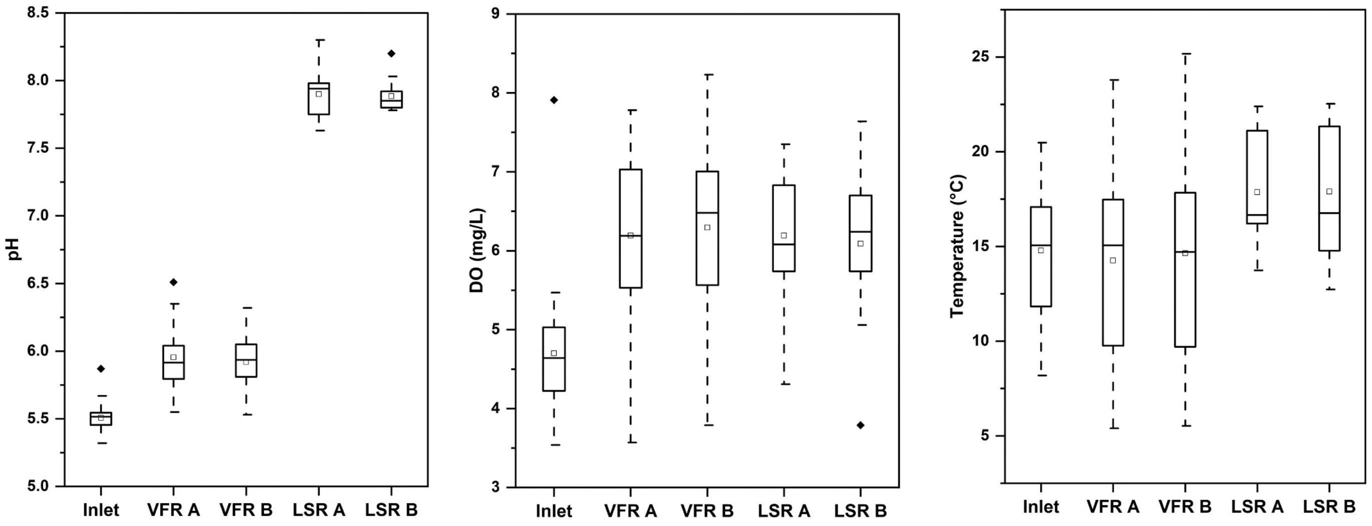

Fig. 2. Changes in physicochemical parameters pH (a), dissolved oxygen (b), and temperature (c) in comparison to the raw mine water during mine water treatment using sequential Vertical Flow Reactor (VFR) and Limestone Reactor (LSR).

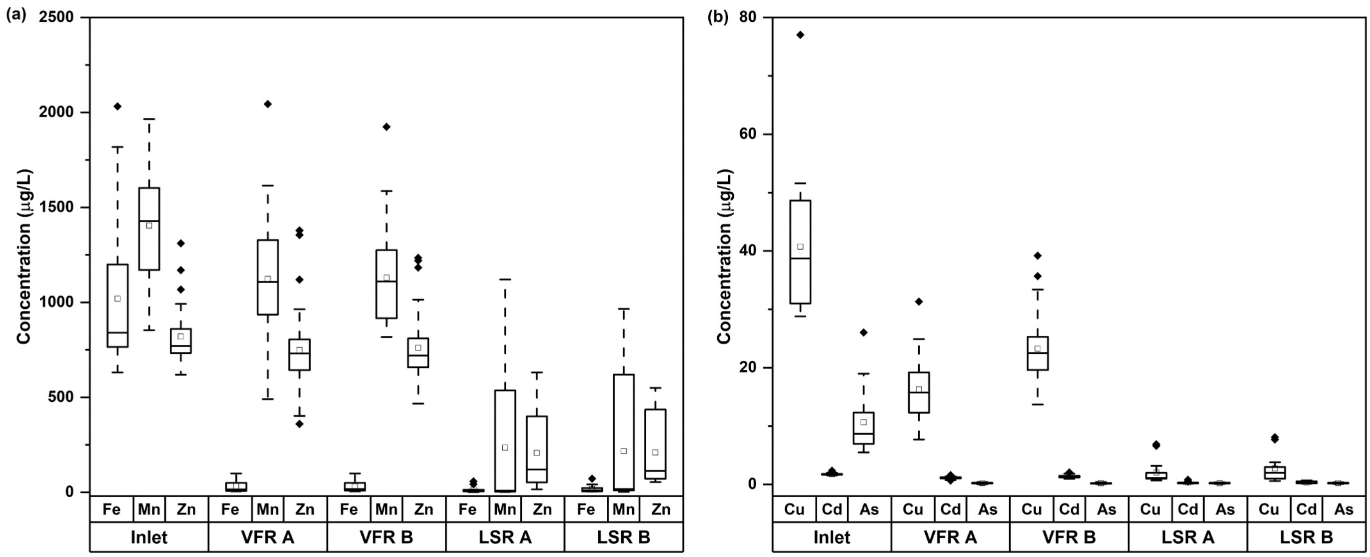

Fig. 3. Metal concentrations in the raw mine water inlet, the VFRs, and the LSRs. Note the different axes values between (a) and (b).

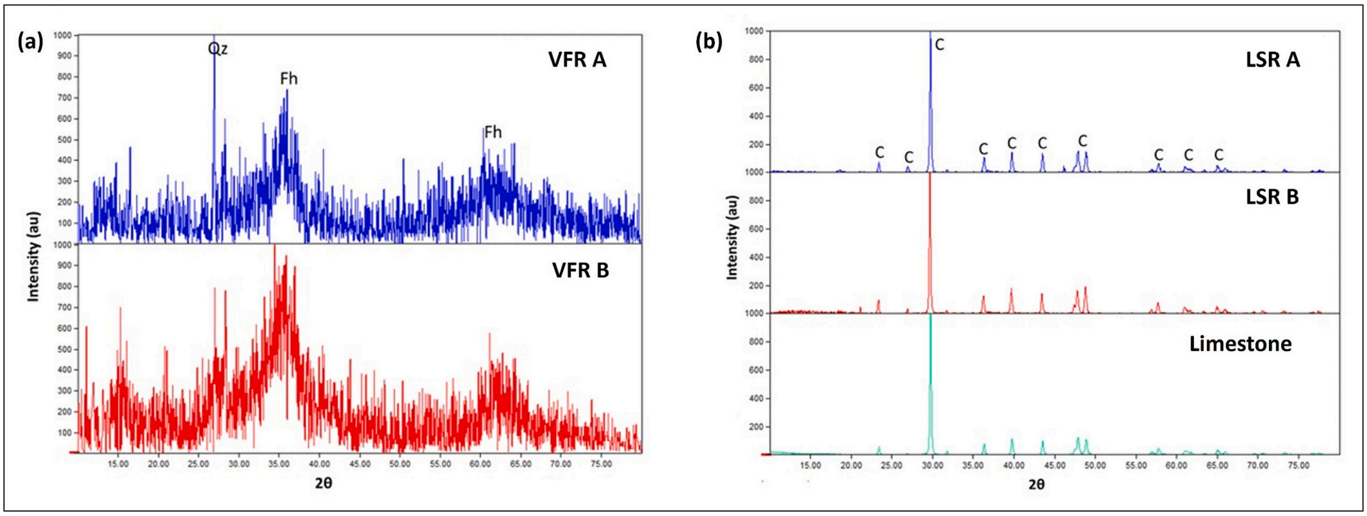

Fig. 4. X-ray diffraction spectra of the (a) sludge from the pyrolusite seeded granite VFRs; and (b) the scrapings from the limestone from the LSRs before and after the field trial. Fh: 2-line ferrihydrite; Qz: Quartz; C: calcite.

VFR B sludges were associated with the exchangeable and carbonate associated- carb), Fe/Mn(H-OH), and cryst Fe/Mn(H-OH) target fractions. As, Pb, Cd, Ni and Cr in the LSR scrapings were predominantly co-removed with Fe/Mn existing mainly in the poorly crystalline and crystalline (hydroxy)oxide fractions. Cu removal was associated with the exchangeable/ carbonate-associated (~50 %) and poorly crystalline Fe/Mn (hydroxy)oxide (~46 %) fractions. Zn on the other hand, was predominantly associated with the extraction targeting exchangeable/ carbonate- associated fraction (70 %–80 %), with another 20 %–25 % associated with the extraction targeting poorly crystalline Fe/Mn (hydroxy)oxide fractions.

To further ascertain the mechanism of Zn and Mn removal in LSR, the dark brown-black coating on the limestone chips was subjected to SEM-EDS analysis. SEM micrographs (Fig. 6a) exhibited a 'sponge-like' precipitate rich in Mn (48.4 wt%), Zn (13.3 wt%), Ca (14 wt%), and O (24.3 wt%). Similar 'sponge-like' precipitate rich in Mn and Zn were identified in studies on treatment of circumneutral mine drainage (Tan et al., 2010) and acid mine drainage (Christenson et al., 2019) using limestone leaching beds. Further EDS mapping of the dark coating directly on the limestone chip (Fig. 6b) and the scraped sponge like precipitate (Fig. 6c) demonstrates clear colocalization of Mn and Zn, with little association with Ca. This colocalization of Mn and Zn suggests coprecipitation of Zn with Mn via the process of adsorption and/or inclusion on the MnO_x surface and within the structure and/or the precipitation of a Zn/Mn oxide mineral. Various forms of MnO_x minerals (e.g. birnessite) have layered structures, with the capability of scavenging metal ions on their surfaces and withing the structure via surface and inner layer adsorption, and incorporation into vacancies or substitution for Mn within the mineral lattice structure (Nelson and Lion, 2003). These precipitated Mn minerals subsequently accelerate Mn removal via surface-catalyzed heterogeneous oxidation on Mn(III/IV) oxide surfaces (Diem and Stumm, 1984).

Interestingly, the influent/effluent concentrations for the LSRs (Fig. S4 & S5) show that the Mn to Zn removal ratio was approximately 1.9:1 (i.e. ~ 2:1). i.e. two parts Mn removing 1 part Zn. This is a much higher co-removal of Zn with Mn compared to most reported Mn ratios for Zn sorption onto manganese oxides and other treatment media (Table 2). This ratio is like that found in chalcophanite ($ZnMn_3O_7{\cdot}3H_2O$) where the mass ratio of Mn: Zn removal is approximately 5:2,hetaerolite ($ZnMn_2O_4$) where the mass ratio of Mn:Zn is approximately 1.7:1 (both

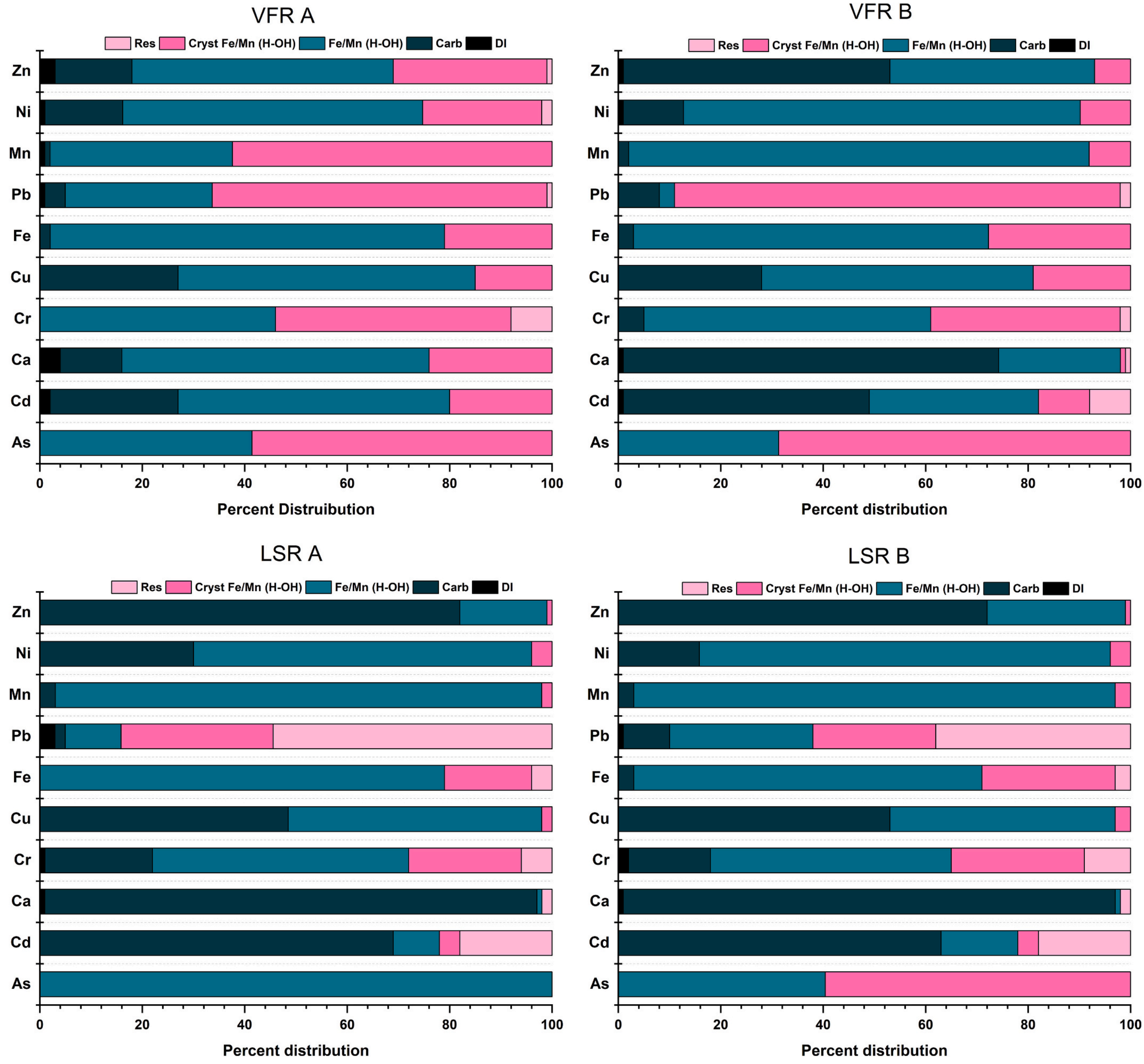

Fig. 5. Comparison of distribution of metals in various sequential extraction phases from the VFR sludges (VFR A & VFR B) and the dark coating on the limestone chips from LSR A & LSR B. (Res: Residual; Cryst Fe/Mn (H-OH): Crystalline Fe/Mn oxides & hydroxy(oxides); Fe/Mn (H-OH): Poorly crystalline Fe/Mn oxides & hydroxy(oxides); Carb: Exchangeable/ Carbonate associated; DI: Water soluble), see Table 1 for full details of stages.

minerals found in mine weathering environments (Post, 1999). The ratio is also similar to woodruffite ($ZnMn_3O_7{\cdot}2H_2O$) where the ratio is 2.5:1. Taking the treatment data, SEM-EDS, XRD, and sequential extraction data together it is postulated that the exceptional removal of Zn may be due to precipitation of a Mn—Zn oxide mineral (speculatively hetaerolite) directly from the mine water on the surface of the limestone, with additional removal through sorption occurring on mineral surfaces (cf. (Silva et al., 2010)). It is also interesting to note from Table 2, that the other study that came close to the removal ratio seen in the present study was a similar configured reactor, treating mine water of broadly comparable quality (Christenson et al., 2019). Recently, similar findings of zinc removal with manganese oxides precipitated on limestone during passive circumneutral mine water has been reported by (Tum et al., 2024) and (Watanabe et al., 2024). These authors reported woodruffite (a mixed Zn and Mn(III)/(IV) bearing oxide) as the mineral product.

It is noteworthy that Zn removal was not noted in the VFR media despite the upper surface comprising MnO_2 sand (see Fig. 1a). The sorption edge reported for δMnO_2 was shown experimentally to lie between pH 7–8 for similar conditions as reported here (Catts and Langmuir, 1986). Thus, it is posited that the excellent co-removal of Zn (and probably incorporation into the mineral) involves adsorption of Zn onto the growing MnO_2 surface. As this adsorption is pH dependent, it is suggested that it is the elevated pH achieved by the limestone reactors was key for the observed high co-removal of the Zn with Mn in this study. Tajima et al. (2022) demonstrate that abiotic coprecipitation reactions can induce Zn—Mn compounds to form including hetaerolite and woodruffite on the δ-MnO_2 surface and conclude that the pH is an important controlling factor.

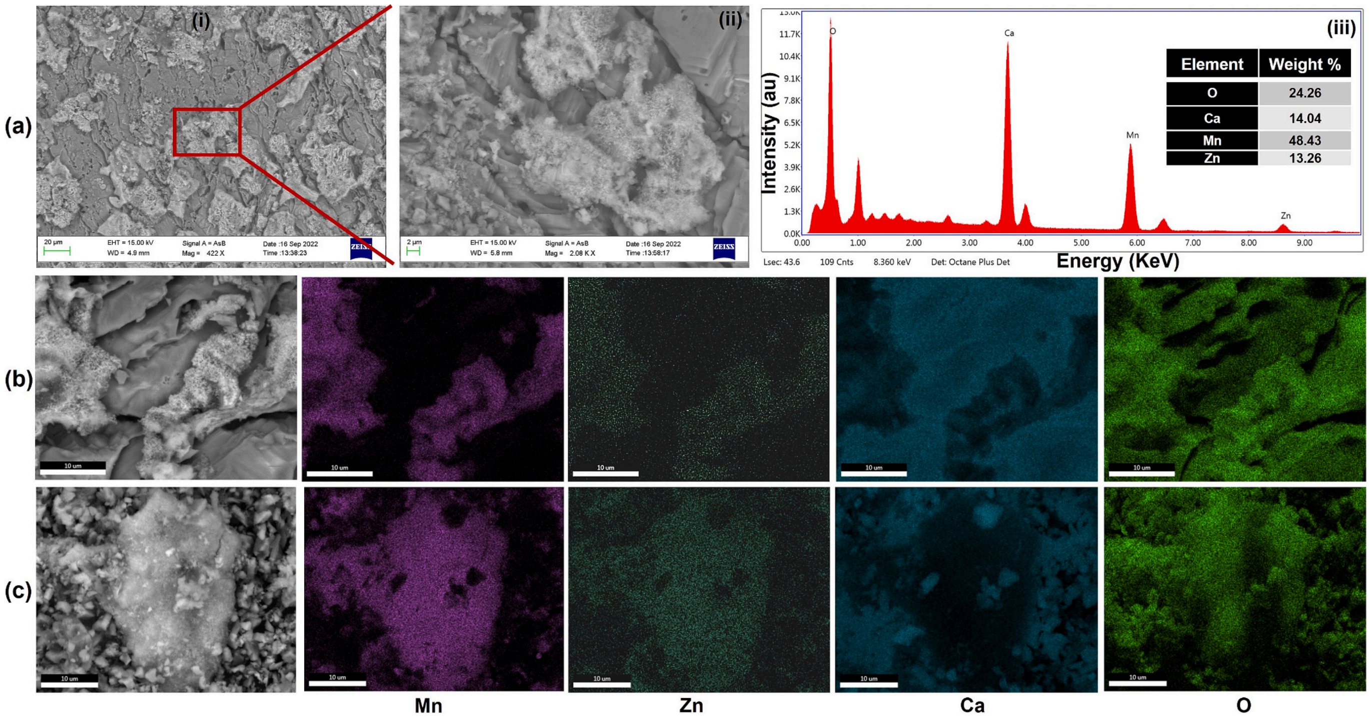

Fig. 6. (a) SEM micrographs ((i)& (ii)) of the dark sponge like precipitate on the limestone chips from the limestone reactors alongside the associated EDS spectrum and composition with characteristic emission peaks (iii); (b) & (c) Backscattered electron micrographs (grey) and the corresponding elemental map of the dark sponge like precipitate on the surface of the limestone chip (b) and scrapings (c) showing the distribution of Mn, Zn, Ca, & O.

Table 2
Comparison of Mn to Zn removal ratios by various studies on sorption media for treatment of metal contaminated wastewater (data recalculated from maximum loadings obtained, or from mean treatment data).

Sorbent	Approximate Mn: Zn removal ratio	Reference
γ-MnO_2 nanomaterial for sorption of zinc from aqueous solution	29:1	Dinh et al. (2016)
Mn oxide modified- Palm kernel cake derived biochar for adsorption of metal cations (Fe^{3+}, Fe^{2+}, Ca^{2+}, and Zn^{2+})	97:1	Maneechakr and Karnjanakom (2019)
Ozone precipitated MnO_2 for sorption of Zn^{2+} from aqueous solution	47:1	Contreras-Bustos et al. (2016)
Synthetic Mn oxide for adsorption of Cu, Cd, Pb, and Zn	25:1	Della Puppa et al. (2013)
Mn oxide modified diatomite for adsorption of Zn^{2+} from aqueous solution	42:1	Caliskan et al. (2011)
Hydrous MnO_2 for sorption of Pb^{2+}, Cd^{2+}, and Zn^{2+} from aqueous solution	29:1	Su et al. (2010)
Hydrous MnO_2 (δ-MnO_2) for sorption of heavy metal ions including Zn^{2+}	24:1	Loganathan and Burau (1973)
Limestone leaching beds for treatment of coal mine drainage removing Mn and other trace element like Ni and Zn	6:1	Christenson et al. (2019)
Sequential pyrolusite seeded Vertical Flow Reactor and Limestone reactor for treatment of circumneutral mine drainage for co-removal of Mn and Zn, along with other metals like Fe, As, Cu, and Cd	2:1	Present study

4.3. *Role of microbiology in metal removal*

Abiotic homogeneous oxidation of Mn(II) at circumneutral pH is slow and the rates may increase by several orders of magnitude by bacteria-mediated oxidation (Morgan, 2005; Tebo et al., 2005). The sludge/sediment that developed over time in the VFRs along with the dark coating on the limestone chips from the LSRs and header tank water (HTW) were subjected to metagenomic analysis to gain insights into the microbial processes involved in metal removal. Although the samples were sequenced for both bacterial and archaeal 16S rRNA gene, only bacteria were detected. The microbiology of VFR samples was distinct from LSR and HTW (Fig. 7). A total of 12 phyla were identified in the samples. The diversity and abundance of microbes in HTW was low and limited to only *Firmicutes* (67 %) and *Bacteroidetes* (33 %). The sediment samples from VFR and LSR exhibited a higher diversity, with VFR exhibiting 7 different phyla, and LSR exhibiting 6 phyla.

Firmicutes (60 %) was the predominant phylum in VFR followed by *Bacteroidetes* (22 %), *Proteobacteria* (11 %), and *Actinobacteria* (4 %) (Fig. 7a). VFR was fed with aerated mildy acidic ferruginous mine water which would explain the presence of neutrophilic aerobic iron oxidising bacteria (IOB) like *Gallionella* (9 %) and the micro-aerobic IOB *Pseudomonas* (8 %) (Fig. 7b). *Gallionella* (p_*Proteobacteria*) are known to grow autotrophically and/or mixotrophically with Fe(II) as the electron donor (Hallbeck and Pedersen, 1990). *Gallionella* spp. are obligate chemolithoautotrophic iron oxidizers that derive energy from the oxidation of Fe(II) CO₂ as a carbon source under microaerophilic conditions (Hallbeck and Pedersen, 1990; Hanert, 1992). These bacteria are well-established contributors to iron cycling in groundwater and mine-impacted systems, and their presence in the VFRs aligns with Fe removal and ferric oxyhydroxide formation observed in the solids. At circumneutral pH, they form characteristic twisted stalks encrusted with Fe(III) minerals (See Fig. S6), although at pH < 6.0 such stalks are not typically observed (Fabisch et al., 2016).

Various species of *Pseudomonas* (p_*Proteobacteria*) have been shown to oxidize Fe(II) under micro-aerobic conditions (Sachan and Singh,

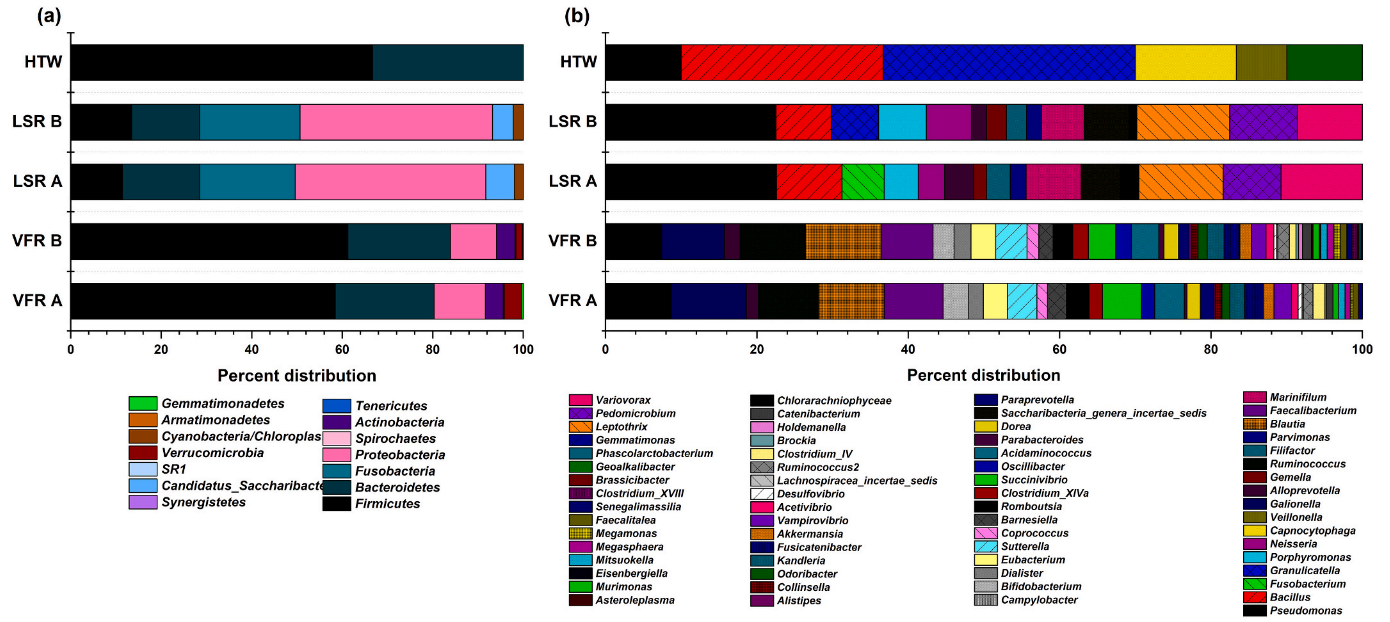

Fig. 7. Composition of the microbial community developed in the HTW, VFRs, and the LSRs at phylum (a) and genus (b) level during passive mine water treatment using a pyrolusite seeded granite vertical flow reactor and limestone reactor, operated sequentially. (HTW: Header Tank Water; VFR: Vertical Flow Reactor; LSR: Limestone Reactor).

2020; Sudek et al., 2009) and Mn(II) oxidation (Okazaki et al., 1997; Parikh and Chorover, 2005; Villalobos et al., 2003). *Pseudomonas* spp. found in both VFR and LSR are metabolically versatile facultative heterotrophs, capable of aerobic respiration and known to oxidize both Fe (II) and Mn(II) (Okazaki et al., 1997; Parikh and Chorover, 2005; Villalobos et al., 2003). Mn oxidation by *Pseudomonas putida* and related strains has been demonstrated in multiple systems, typically via indirect enzymatic pathways such as multicopper oxidases (Geszvain et al., 2016; Tebo et al., 2005). These are generally considered heterotrophs (Emerson and Merrill Floyd, 2005; Luan et al., 2012b). Their detection in both reactor types suggests their role in both initial Fe removal and subsequent Mn oxidation processes.

The black coating on the limestone comprised of *Proteobacteria* (42 %), *Firmicutes* (12 %), *Bacteroidetes* (16 %), *Candidatus_Saccharibacteria* (5 %), and *Cyanobacteria* (2 %) (Fig. 7a). In a study on passive treatment of coal mine drainage, the three bacterial phyla of Mn(II) oxidising bacteria (MOB) identified were *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* (Santelli et al., 2010). In another study on limestone reactors used for Mn removal from coal mine drainage, *Proteobacteria* accounted for 47 % of all the bacterial sequences (Chaput et al., 2015). The influent for LSR had low iron and arsenic (removed in VFR), but relatively high concentrations of Mn and Zn. Contact with the limestone in the LSR increased the pH (to ~7.90) making the environment amenable for MOB to thrive. Thus, the pre-dominant genera observed in LSR were *Pseudomonas* (p_*Proteobacteria*) (23 %), followed by *Leptothrix* (p_*Proteobacteria*) (12 %), *Variovorax* (p_*Proteobacteria*) (10 %), *Pedomicrobium* (p_*Proteobacteria*) (8 %), and *Bacillus* (p_*Frimicutes*) (8 %) (Fig. 7b). Several species of *Bacillus, Pseudomonas, and Leptothrix* have been reported to bring about direct oxidation of Mn(II) through a 2-step enzyme facilitated process (Gounot, 1994; Tebo et al., 2005). Species of *Leptothrix* have also been shown to bring about indirect oxidation of Mn(II) via formation of free radical or oxidant (Gounot, 1994) and were identified in a passive treatment system of similar chemistry (Okibe et al., 2023). These organisms have been identified in the sediment present at ferromanganese caves (Carmichael et al., 2013) and Mn contaminated ponds (Santelli et al., 2014). They have also been identified in bacterial communities present in the biologically active limestone treatment beds for remediation of coal mine drainage (Santelli et al., 2010). The organisms identified here have all been previously implicated in manganese cycling, with varied metabolic strategies. For example*Pseudomonas* species, well represented in both VFR and LSR, are facultative heterotrophs with known capacity to oxidize both Fe(II) and Mn (II)(Okazaki et al., 1997; Tebo et al., 2005). (Watanabe et al., 2024) studied the microbial community from mine water treatment systems similar to the one described herein, with limestone media used to remove Mn and Zn from circumneutral mine water. These studies demonstrated the possible function of heterotrophic and chemolithoautotrophic Mn(II) oxidizers in the removal of Mn and Zn onto limestone during mine water treatment. The potential role of chemolithoautotrophic MOB in the system reported herein remains a topic for future study.

Importantly, the microbial data in this study are not presented as an ecological survey but as functional evidence of microbially mediated geochemical processes that underpin the treatment efficiency observed. The enrichment of Mn-oxidising bacteria in the LSRs directly supports the biogenic origin of the MnOx precipitates observed via SEM-EDS and explains the markedly enhanced Mn and Zn removal. Thus, the microbial community structure is likely integral to explaining the system's functionality (cf. Christenson et al., 2019). The organisms identified have been widely implicated in Mn and Fe oxidation in natural and engineered systems, and their metabolic attributes (ranging from chemoautotrophy to heterotrophy) align with the reactor redox and pH conditions. These insights reinforce the importance of fostering active MOB communities to optimize Mn and Zn removal in aerobic passive treatment systems, particularly where Fe has been pre-stripped and pH elevation is achieved via limestone contact.

4.4. Implications for treatment of mildly acid and circumneutral MIW containing Mn, Zn and other divalent metals

The use of inexpensive passive limestone-based treatment technology has proven to be effective in the removal of Mn and Zn from mildly acidic mine drainage under aerobic conditions, this finding is in common with recent studies (Okibe et al., 2023; Tum et al., 2024; Watanabe et al., 2024). As found in this study, the scavenging capability of Mn oxides/oxyhydroxides formed in a limestone treatment system is very important in the further removal of Mn(II) and trace metals (such as Zn, Cd, Cu) via adsorption/coprecipitation and possibly precipitation of

Zn—Mn oxides such as hetaerolite/chalcophanite or woodruffite (Okibe et al., 2023; Tum et al., 2024; Watanabe et al., 2024)). Furthermore, the efficient upfront removal of Fe using the VFR technology is an advantage, as this prevents reduction in metal removal efficiency due to armouring of the limestone by Fe oxyhydroxides, and MnO_x reductive dissolution/oxidation to Mn(II) by Fe(II) (Outram et al., 2017; Villinski et al., 2003).

Passive treatment technologies for removal of Zn from mildly acidic and circumneutral mine waters rely on a range of mechanisms including anaerobic sulphate reduction and adsorption (See Table S1). The low effluent concentrations of dissolved Zn achieved by the VFR-LSR system (mean of 228 μg/l and 236 μg/l – see Table S2 and low of 15 μg/l Fig. S4f) demonstrate that aerobic passive treatment systems are capable of polishing Zn concentrations to low levels. The treatment residues left by passive treatment systems ultimately have to be disposed of or recycled (Jouini et al., 2019; Orden et al., 2021; Rakotonimaro et al., 2017; Vasquez et al., 2022). Anaerobic systems leave a reduced solid phase (Coulton et al., 2003; du Preez, 2021), which releases bound metals during waste stability testing (Rakotonimaro et al., 2017; Royer-Lavallée et al., 2020), and being mixed with organic matter may make metal recovery more challenging (Dang et al., 2013; Fermoso et al., 2015). Anaerobic systems can also produce odorous H_2S during operation ((Johnson and Hallberg, 2005) and the references therein). Thus extending the list of technologies that can operate aerobically and still achieve low effluent Zn concentrations is potentially a promising development for future passive treatment. This study presents data that indicates that passive nature-based treatment systems that harness the precipitation/coprecipitation/sorption of Zn with Mn could be a useful addition to the toolbox of aerobic techniques, albeit restricted to mine waters with an excess of manganese to zinc.

5. Conclusions

This research provides data on Mn and Zn removal in aerobic vertical flow reactors (VFR) with granite/pyrolusite and limestone media. Limited removal of Mn along with minimal Zn was observed in the granite/pyrolusite VFR. In contrast, the limestone VFRs exhibited rapid Mn removal with commensurate Zn removal. Surficial analyses with SEM-EDS suggest that Zn removal is mainly due to its association with MnOx on the limestone surface, with sequential extraction data also indicating significant Zn in the "exchangeable/carbonate-associated" phase. The Mn removal ratio was approximately 2:1, a much higher removal of Zn than typically seen by sorption on MnOx, implying coprecipitation or precipitation of a Mn—Zn oxide mineral from the mine water, possibly hetaerolite, woodruffite or chalcophanite. Microbial analyses revealed distinct communities in the reactors: the granite/pyrolusite VFR was populated with iron-oxidising microbes such as *Galionella* and *Pseudomonas* (the latter also oxidizes Mn), while the limestone VFRs harboured Mn-oxidising bacteria like *Pseudomonas* and *Leptothrix*. This suggests that both biotic and abiotic mechanisms accelerate Mn oxidation and Zn removal. These findings indicate that Zn can be reduced to very low concentrations (as low as 15 μg/l) with only a slight excess of Mn, suggesting that low target effluent concentrations can be achieved in the absence of anaerobic sulphate-reducing conditions and/or high pH. This study further highlights the potential of aerobic passive treatment systems to be effectively self-treating for Zn where Mn(II) co-occurs as a contaminant, requiring in this case only a slightly higher concentration of Mn to enable Zn removal. Further exploration is now required to investigate the mineralogy of the precipitates and longevity of the treatment system.

CRediT author statement

Ilemona Cornelius Okeme: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Investigation, Writing - Review & Editing; **Pallavee Srivastava:** Writing - original draft, Visualization, Methodology, Formal Analysis, Data Curation; **Devin J Sapsford:** Conceptualization, Methodology, Writing - Original Draft, Visualization, Supervision, Funding acquisition, Project administration.

CRediT authorship contribution statement

Ilemona Cornelius Okeme: Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pallavee Srivastava:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Devin J. Sapsford:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank The Coal Authority for permitted access to the trial site at the Coombe adit discharge. Thanks to Dr. Julia Kennedy, Dr. Joe Dean, and Mr. Jeff Rowlands for their valuable assistance in site visits and sample collection. Thanks to Jeff Rowlands for technical support. Thanks to Geochemic Ltd. and Chemtech environmental for ICP-MS and IC analysis. Thanks to Epistem Ltd. for sequencing services. This work was funded by the Welsh European Funding Office Smart Expertise programme under the METAL-SoLVER project (grant number: 82347 with support from The Coal Authority, Natural Resource Wales, and Geochemic Ltd.) and Engineering and Physical Sciences Research Council (grant number: EP/T03100X/1).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2025.107681.

Data availability

Data will be made available on request.

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