

Article VI: Evaluation of sheep manure/limestone mixture for biological in-situ acid mine drainage: potential applications for permeable reactive barriers

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**EVALUATION OF A SHEEP MANURE/LIMESTONE MIXTURE FOR
BIOLOGICAL *IN-SITU* ACID MINE DRAINAGE TREATMENT:
POTENTIAL APPLICATIONS FOR PERMEABLE REACTIVE BARRIERS**

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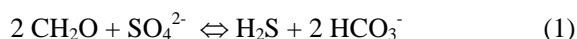
Abstract: The choice of an appropriate organic substrate as electron donor for sulphate reducing bacteria is a critical step on the performance of the biological treatment of acid mine drainage (AMD) by means of a permeable reactive barrier and constructed wetlands. The relative ability of sheep manure to sustain sulphate-reduction bacteria (SRB) activity was demonstrated in a previous study where dissolved metals were deliberately omitted. The present work evaluates sheep manure in the treatment of a synthetic acid mine drainage to ascertain the effect of dissolved heavy metals in the bacterial activity. The effluent solution was systematically analysed throughout a column experiment, at the end of which precipitates in the column were withdrawn for chemical and mineralogical analysis. Sorption onto sheep manure was examined by Fourier-transform infrared (FTIR) by comparing the general features of its spectra before and after the experiment. The results showed that although pH was rehabilitated to circum-neutral values and heavy metals were significantly depleted (with the exception of Zn), sulphate was far from being completely fixed. This marginal sulphate removal is in contrast with the results obtained in the previous study where heavy metals were excluded, presumably due to their toxic/inhibitory effect for the bacterial activity. Although a significant metal removal occurred (through pH-dependent mechanisms rather than bacterial activity), the results have emphasised that the presence of dissolved metals may pose in the biotreatment significant secondary difficulties from a toxicological viewpoint.

Key words: acid mine drainage, biodegradability, metal toxicity, permeable reactive barrier, sulphate-reducing bacteria, wetland.

1 INTRODUCTION

Acid Mine Drainage (AMD) has emerged as a widespread concern of paramount importance in the geo-environmental field. Its generation is a result of chemical and biological oxidation of sulphide minerals within mine-tailings impoundments and it is characterised by low pH and large concentrations of sulphate and dissolved heavy metals. Pollution by AMD derives from improper disposal, landfill leachates and accidental spills. Because of its negative impact on the natural ecosystems, much attention is being paid to rehabilitate AMD and minimise ecological damage.¹

The biological remediation of AMD consists of the use of sulphate-reducing bacteria (SRB), which can reduce sulphate to sulphide by the oxidation of an organic compound. The overall reaction can be represented as follows:



where CH_2O represents a simple organic carbon compound. Sulphate-reduction reaction consumes SO_4^{2-} , generates H_2S and results in an increase in alkalinity and pH. In turn, H_2S forms insoluble derivatives with a number of metallic ions.

The feasibility of using SRB to mitigate AMD has long been assessed,² although there are still some technical and economical limitations to overcome in order to carry out efficiently the microbial process in large-scale applications. Firstly, pH in AMD is commonly below 4, more acidic than the optimum range of 5 to 9 for SRB.^{3,4} Secondly, available cheap organic substrates potentially useful in a large-scale are poor effective in terms of degradability to provide carbon necessary to SRB.^{5,6} Thirdly, the time required by SRB to degrade these complex organic substrates may be too long and can make a process economically unfeasible.^{5,7,8} Fourthly, high metal concentration in AMD can be inhibitory/toxic to SRB.⁹

Ex-situ treatments can successfully overcome these obstacles by separating biological, precipitation and acid neutralisation steps thereby each one of them can be carried out in optimal conditions. Thus, SRB in a bioreactor are not directly exposed to the low pH and high metal concentrations in AMD.¹⁰⁻¹² Metals can besides be selectively precipitated and potentially recycled.¹³ Degradability of the carbon source is easily achieved by using a low-molecular-weight organic compound, such as lactate,^{4,11,14} ethanol,^{15,16} or CO_2 with H_2 ,^{12,17} although its use in a large-scale can lead to prohibitive costs.

However, these engineered actions can result impracticable in passive *in-situ* remediation technologies as permeable reactive barriers (PRB). Subsurface PRBs consist of the

emplacement of an appropriate reactive material intercepting the contaminated plume so that the material can transform or immobilise contaminants as the plume moves through it. This emerging alternative to pump-and-treat systems is attractive due to the passive nature of the treatment. An extensive overview of the chemistry and microbiology of PRB is reported by Scherer et al. (2000).¹⁸

PRB applied to the field bioremediation of AMD involves a cheap, widely available and highly effective organic substrate. However, as mentioned above, all laboratory and field studies concur that natural organic substrates are degraded only very slowly by SRB and that in continuous systems active sulphidogenesis is achieved only when they are supplemented by short chain carbon compounds or when long residence times were given (commonly in the order of days).^{7,8,14,19,20} Both requirements represent the major drawback of PRB in the AMD bioremediation and make it a costly technology. Among a large list of diverse plant and animal-derived materials,²⁰⁻²² emphasis has been focused on the identification of those likely to be cost-effective.

It is in this context that the present study describes an attempt to use sheep manure as carbon and nutrients supplier to SRB for a synthetic acid mine drainage bioremediation via sulphate reduction. The relative ability of sheep manure to promote and sustain a SRB activity has been demonstrated in a previous study comparing four different natural organic substrates in the treatment of an acidic, sulphate-rich water.²³ In that study dissolved heavy metals - commonly present in AMD at high concentrations- were deliberately omitted to focus on the sulphide generation. Sheep manure was chosen as the preferred substrate, with a sulphate reduction rate of $40,03 \text{ mg dm}^{-3} \text{ d}^{-1}$ and a sulphide generation rate of $22,02 \text{ mg dm}^{-3} \text{ d}^{-1}$ at a 9 days residence time. It may had been the most successful carbon provider because it was less lignified. Cattle manure is known to be a rich source of SRB and carbon and nutrients source and has been successfully applied,²²⁻²³ although this finding is in contrast with other published works.²¹ As a continuation of these earlier results, the present work evaluates sheep manure in the treatment of a synthetic acid mine drainage to ascertain the effect of dissolved heavy metals in the bacterial activity.

2 EXPERIMENTAL

2.1 Column experiment

The reactive mixture consisted of 25% (v/v) sheep manure (11.4 g), 70% limestone (110.0 g) and 5% creek sediment (7.1 g). This composition was similar to that tested in the mentioned previous experiment where metals were excluded from the acidic sulphate-rich water to be treated.²³ The materials were mixed and homogeneously packed in the column (internal diameter of 2.5 cm and length of 22 cm), resulting in an estimated porosity of 0.5. No inert material was added. Creek sediment served as SRB source and was collected from the anoxic zone of a local creek. The exact species of bacteria in this original inoculum were not known, but the presence of SRB was indicated by a strong H₂S odour. No enrichment of SRB was carried out. Sheep manure was the provider of the organic carbon source and was collected at a local farm near Barcelona, Catalonia (Spain). Limestone was included as pH neutralising agent and it came from a quarry (2-4 mm size). The column was covered with an opaque material to prevent light exposure and simulate conditions encountered in the aquifer.

A synthetic acidic water was used to ensure constant composition throughout the experiment. Its chemical composition was based taking into account the groundwater composition in the Agrio aquifer (Aznalcóllar, Spain), contaminated after an accidental spillage of a mine impoundment.²⁵ The chemical composition is given in Table 1. Metals and anions were added as sulphates and chlorides, and pH was adjusted to 2.80 by HCl addition. New fresh water was prepared at day 71st thereby varying slightly the input composition.

	Before 71 st day	After 71 st day
	conc (mg dm ⁻³)	conc (mg dm ⁻³)
Fe (II)	11	9
Zn	20	19
Al	10	12
Cu	16	21
Cd	3	5
SO ₄ ²⁻	1030	1282
pH	2.80	2.80

Table 1. Composition of synthetic acid mine drainage used for column experiment

This synthetic water was kept in a closed vessel with low oxygen by bubbling nitrogen and was pumped to the column by means of a multi-channel variable-speed Gilson Minipuls® peristaltic pump. All tubes and fittings were made of Teflon excluding the pump tubes, which were made of Tygon. Joints were wrapped with wax film to minimise air infiltration. Feed water was pumped, from bottom to top of the column at a flow rate of 0.072 cm³/min, which taking into account the column section and the filling material porosity (0.5) equals a flux of 0.42 m d⁻¹ (0.5 days average residence time). After 69 days of experiment, flow rate was decreased to a 0.013 cm³ min⁻¹ (2.9 days average residence time) to increase residence time and thereby to enhance bacterial activity. At day 112 flow rate was reduced again to 0.003 cm³ min⁻¹ (12.1 days average residence time). The experiment was carried out at room temperature (23±2 °C). No organic compounds were added to the water. The experiment lasted 158 days.

2.2 Chemical characterisation of the sheep manure

Representative sub-samples of the sheep manure were taken and subjected to analyses for chemical characterisation. Elemental analysis (C, N, H, P, S contents) was carried out by dry combustion of a given mass of dry organic matter (CE Instruments 2100). The chemical composition is given in Table 2. Lignin and cellulose contents were determined by the method of successive extractions.²⁶ Easily available substances (EAS), which can be considered “the organic portion of a substrate that can be readily used by the micro-organisms”,¹⁰ were determined following a simplified version of a Forage Fibre Analysis Method.¹⁰

The biodegradable fraction (B) of the sheep manure can be estimated based on the lignin content (LC) following the relationship proposed by Chandler and co-workers:^{27,28}

$$B = -0.028 X + 0.830 \quad (2)$$

where B is the biodegradable fraction and X is the lignin content (LC) in the volatile solids (VS) expressed as a percent of the dry weight (LC/VS*100). VS content was determined in accordance with procedures outlined in Standard Methods.²⁹ Values of VS and B are reported in Table 2.

	Sheep manure
Lignin (LC) (g/g)	0.08
Cellulose (g/g)	0.07
EAS (g/g)	0.45
Elemental analysis	
% C	38.7
% N	2.0
% H	5.3
% S	1.4
% P	1.4
Volatile solids (g/g) (VS)	0.87
Biodegradable fraction (B)	0.57

Table 2. Composition of the evaluated sheep manure. The estimated biodegradable fraction is based on lignin and volatile solids content

2.3 Water Sampling and Analysis

During routine monitoring column effluent was sampled for pH, metals, sulphate and sulphide analysis, and flow rates were determined gravimetrically. All pH measurements were made in a sealed flow-through cell placed at the exit of the column using a Hamilton combination pH electrode coupled to a Crison GLP22 pH meter. Effluent samples for metal and sulphate analyses were filtered (0.22 μm) and acidified with concentrated HCl. Zn, Fe, Al, Cu, and Cd were measured by inductively-coupled plasma mass spectroscopy (ICP-MS) (Perkin Elmer ELAN 6000) while sulphate was measured by liquid ion chromatography (Alliance model Waters 2690) coupled to an electrical conductivity detector (Waters 996). Sulphide was analysed following the Methylene Blue method. The evolved H_2S was trapped in a zinc acetate solution to form a ZnS precipitate. The subsequent development of Methylene Blue test involved the reaction of sulphide with n,n-dimethyl-p-phenylene diamine in the presence of ferric ions, which gave rise to the characteristic blue coloration. Methylene Blue produced was measured by UV-visible spectroscopic methods (Hewlett-Packard 8453).

2.4 Solid Analysis

At the end of the experiment fractions of precipitate were retrieved from the column to be visualised using scanning electron microscopy (SEM) and analysed by energy-dispersive X-ray spectroscopy (EDS) (JEOL 6450, EDX-LINK-LZ5). Metal adsorption onto sheep manure was examined by Fourier-transform infrared (FTIR) (Perkin Elmer 1600).

3 RESULTS

3.1 Changes in Water Chemistry

Routine monitoring revealed that effluent was rehabilitated in terms of acidity, with pH increasing from 2.80 in the influent to around 7 in the effluent (Fig 1).

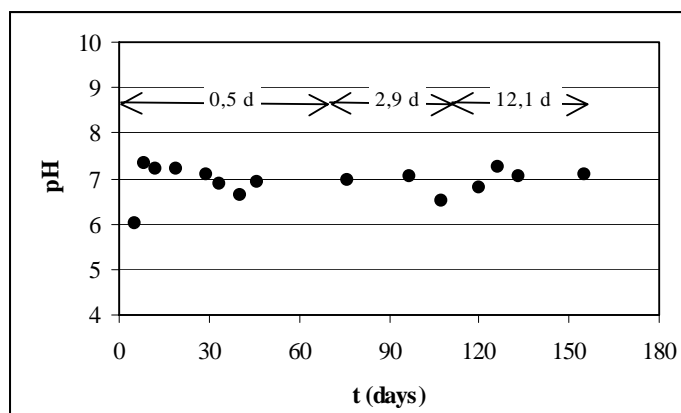


Figure 1. pH evolution over the experiment. Flow rate was decreased at day 69 and 112, the average residence time increasing from 0.5 days to 2.9 and 12.1 days respectively.

Effluent metal concentrations became mostly less than 1.2 mg dm^{-3} in 30 days for all metals excepting Zn, for which concentration stabilised at around 12.6 mg dm^{-3} (Fig 2). These concentrations remained unchanged throughout the experiment. The overall removal amounted for all metals to between 80% and 97% from the initial values, and to a 34% for Zn. No significant differences (<6%) in these retention levels were observed between the three periods at different residence times (0.5, 2.9 and 12.9 days).

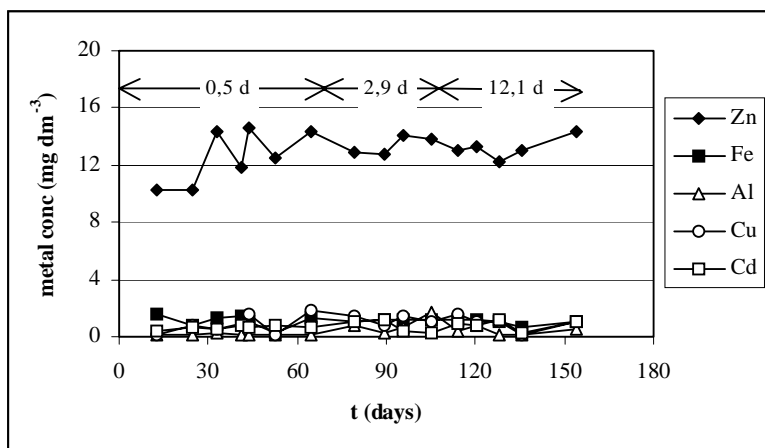


Figure 2. Metal evolution over the experiment. Flow rate was decreased at day 69 and 112, the average residence time increasing from 0.5 days to 2.9 and 12.1 days respectively.

Despite this significant metal retention, sulphate concentration was not significantly decreased from the start of the experiment with a residence time of 0.5 days (Fig 3). This finding, coupled with the undetectable sulphide concentration in the effluent ($<0,03 \text{ mg dm}^{-3}$) made evident that bacterial activity was not occurring and that mechanisms whereby metal removal took place may be more related to the pH increase than to the biogenic sulphide generation. As can be seen in Figure 3 the subsequent increases of residence times at day 69 (2.9 days residence time) and at day 112 (12.1 days residence time) to favour the enhancement of the bacterial activity did not let to any detectable increase of the sulphate reduction.

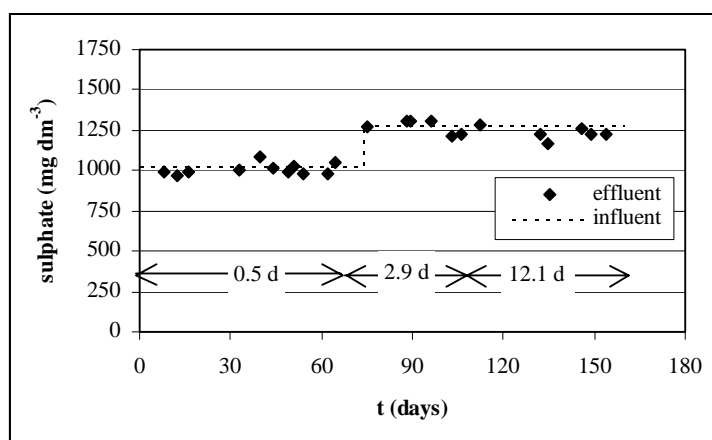


Figure 3. Sulphate evolution over the experiment. Flow rate was decreased at day 69 and 112, the average residence time increasing from 0.5 days to 2.9 and 12.1 days respectively. At day 71st new fresh water was prepared, thereby increasing the input concentration.

3.2 Mineralogy of precipitates

The SEM analysis of precipitates showed two predominant matrix. On one hand, an amorphous layer coating the surface of the column material (Fig 4a), composed primarily of Al, Fe, S and O, with minor amounts of Zn and Cu. It can be assumed that this layer is made up by amorphous (oxy)hydroxides of iron and aluminium serving as secondary phase for adsorption of dissolved metals such Zn and Cu. Corroded calcite fragments and secondary gypsum tabular crystals were also observed throughout the matrix. On the other hand, a second matrix consisting of an overall filamentous network comprising organic residues in a diatomite bed was also observed throughout. At the outlet of the column, spherical shaped framboids averaging 35 μm in diameter were occasionally observed (Fig 4b). From their yielded prominent Fe and S (and to a less extent Cu and Zn) peaks on EDS spectra, these spheres are interpreted to be precipitates of metallic sulphides.

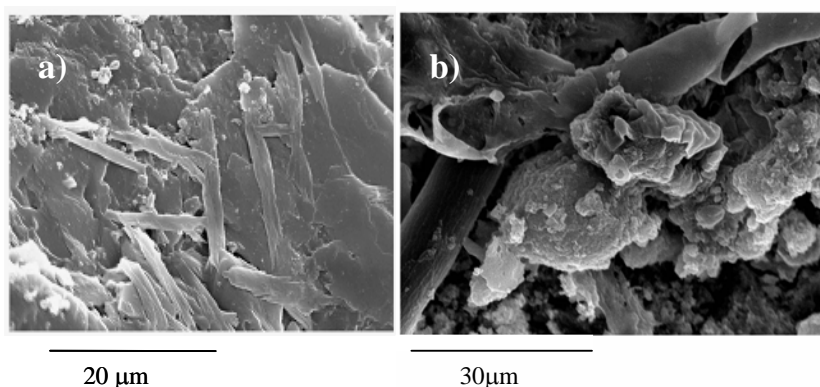


Figure 4. a) Pervasive coating layer composed by amorphous Fe- and Al- (oxy)hydroxides containing minor amounts of Cu and Zn, with secondary gypsum elongated crystals; b) Spherical shaped framboids of Fe, S, and minor amounts of Cu and Zn, interpreted to be precipitates of metallic sulphides, observed in samples from the outlet of the column.

3.3 Evaluation of the interaction between metals and sheep manure by FTIR

A comparison of FTIR spectra of sheep manure before and after experiment is given in Figure 5. The upper spectrum corresponds to the sheep manure before the experiment, while the second one corresponds to the sheep manure subjected to a metal load once the experiment concluded. The spectral assignments of the main bands for both spectra are reported in Table 3.

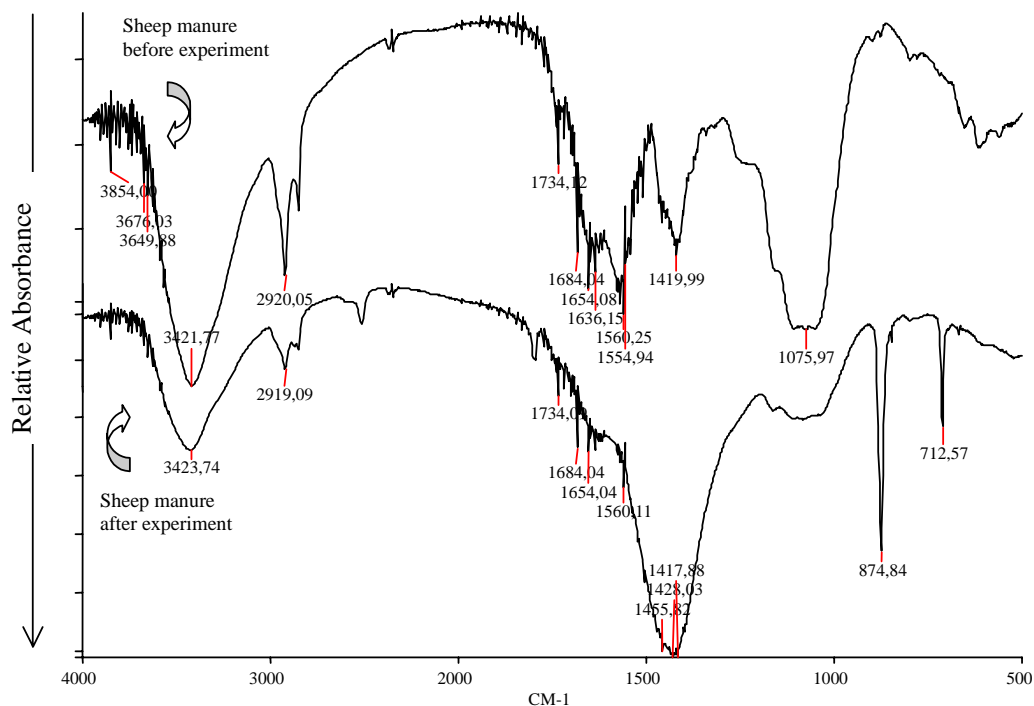


Figure 5. Comparison of FTIR spectra of sheep manure before and after experiment prepared in KBr disks.

Sheep manure ($\nu \text{ cm}^{-1}$)	Metal loaded-sheep manure ($\nu \text{ cm}^{-1}$)	Assignments
3421.77	3423.74	O-H, N-H, COO-H stretchings
2920.05	2919.09	C-H asymmetric, C-H stretching (aliphatic)
1654.08	1654.04	C=O stretching (esters, polypeptides, quinones)
1560.25	1560.11	C-N stretching (amide II)
---	1428.03	C=O stretching (inorganic carbonate)
1419.99	1417.88	C-H deformation of CH_2 or CH_3 groups
1075.97	1075.97	C-O stretching of polysaccharide
---	874.84	C-O stretching (inorganic carbonate)
---	712.57	C-O stretching (inorganic carbonate)

Table 3. Assignments of peaks appeared in spectra before and after experiment

The spectrum of raw sheep manure exhibited two predominance bands at 3421.77 (ascribed to the O-H, N-H and COO-H stretchings)^{30,32} and 1075.97 cm⁻¹ (C-O stretching of polysaccharide),^{30,32} two distinct peaks at 1560.25 cm⁻¹ (C-N stretching of amides)³² and 1419.99 cm⁻¹ (C-H deformation of CH₂ or CH₃ groups)³² a shoulder at 1654.08 cm⁻¹ (C=O stretching of carboxyl^{30,33,34} or C=C in aromatic structure³² in esters, polypeptides or quinones), and a well-pronounced peak at 2920.05 cm⁻¹ (C-H asymmetric, C-H stretching). This spectrum resembled that reported for pig manure³², the main difference being that peak at 1650 cm⁻¹ had not become a main peak in our spectra. Peaks at the region 1730-1750 cm⁻¹ attributable to oxidised structures (COOH groups)^{32,35} typical in mature compost were weakly observable.

The presence of metals modified the spectrum in the following manner: (1) The 3421.77 and 1075.97 cm⁻¹ bands decreased, indicating involvement of O-H and COO-H groups in metal biosorption; (2) a predominant broad peak at 1440 cm⁻¹ appeared, attributable to C=O stretching of inorganic carbonate of limestone;³⁵ (3) two narrow sharp peaks at 874.84 and 712.57 cm⁻¹ appeared, which were also probably due to C-O stretching of inorganic carbonate.³⁵ Peaks in the region 1119 and 619 cm⁻¹ typically attributable to inorganic S=O and S-O stretchings³⁵ were not observed, suggesting that sulphate sorption onto compost did not occur.

4 DISCUSSION

The results of this study indicated that although pH was rehabilitated to circum-neutral values suitable for SRB activity³⁶ due to calcite dissolution and heavy metals were significantly depleted (with the exception of Zn), sulphate was far from being completely reduced. This low sulphide generation suggests that metal retention can not attributed to bacterially mediated sulphate reduction but to other mechanisms, namely metal (oxy)hydroxide precipitation and sorption onto organic matter surfaces.

Precipitation of new phases is supported by SEM and EDS analysis of precipitates, which showed a pervasive coating layer composed by amorphous Fe- and Al- (oxy)hydroxides containing minor amounts of Cu and Zn (Fig 4a). This layer is similar in morphology and composition to that described in previous works.²⁰ Associations of metals such as Zn, Cu, Cd and As onto Fe-Al-(oxy)hydroxides correlates with findings of other studies, where the precipitates from the column were subjected to sequential chemical analysis.^{20,37,38}

At the outlet of the column, SEM analysis detected occasional Fe(Zn,Cu)S spherical framboids, indicating that bacterially generated H₂S had locally occurred (Fig 4b). Metallic sulphides have been observed in previous laboratory experiments^{20,39} and in field applications

in full-scale permeable reactive barriers^{40,41} and artificial wetlands.⁴² The significance of these occasional framboids in our experiment will be discussed below. Secondary gypsum fine spindle-shaped aggregates or larger size tabular crystals not containing significant concentration of metals were observed throughout the matrix. Their presence are in accordance with other investigators.³⁹

Heavy metals can also be removed by adsorption to the organic matter, as reported by numerous published works.^{7,43} Sorption onto sheep manure was examined in this study by FTIR by comparing the general features of its spectra before and after the experiment. Hence, from the positions and characteristics of the peaks and bands, information of the structure of possible surface groups involved in the sorption can be obtained in a diagnostic manner. In the presence of metals, a broad band in the C-H deformation region at 1420 cm^{-1} appeared, coupled to a decrease of O-H and C-O bands, indicating involvement of these groups in metal sorption. To date, few studies have applied spectroscopic techniques to study metal sorption on organic matter in this context. There remains much uncertainty in quantify the contribution of precipitation, co-precipitation and adsorption in the metal removal, and they are often reported in the literature only in a descriptive manner.

Zn exhibited a particular behaviour. Its removal from solution occurred to a lesser extent (effluent concentrations of 12.6 mg dm^{-3}) than that of the rest of metals (the concentration of which fell to a maximum of 1.2 mg dm^{-3}) by any of the speculated mechanisms. The mentioned removal mechanisms are not efficient for Zn. Figure 6a shows the species distribution diagram as a function of pH for the system Zn(II)-CO₃-SO₄-H₂O for a total Zn(II) concentration of 20 mg dm^{-3} (0.31 mM) in a carbonate and sulphate medium similar to that encountered in the column. For comparative purposes, species distribution diagram for Cu is shown in Figure 6b.

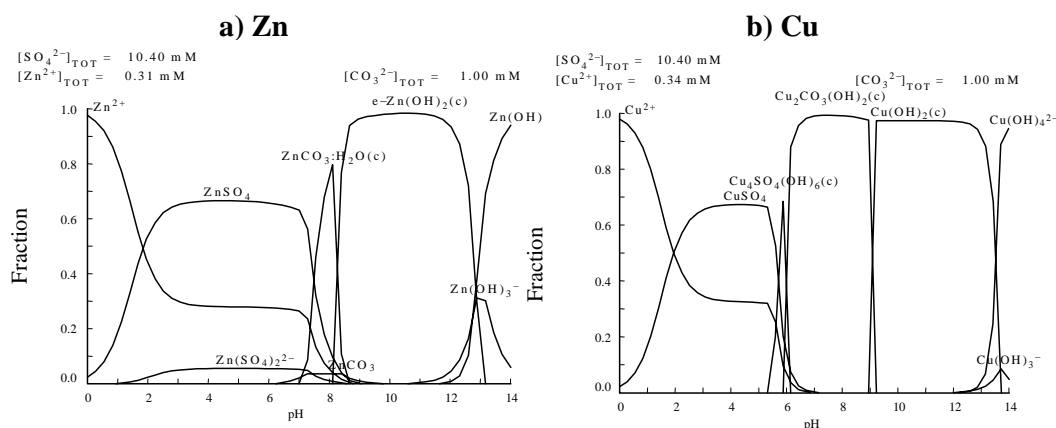


Figure 6. Species distribution diagram as a function of pH for the system a) Zn(II)-CO₃-SO₄-H₂O and b) Cu(II)-CO₃-SO₄-H₂O (from Medusa and Hydra equilibrium calculation codes⁴⁴).

From equilibrium calculations, it is clear that, at the prevalent effluent pH 7, almost all Zn is expected to remain in solution, the high content of sulphate in the water giving the ZnSO₄(aq) complex as the predominant species, with minor concentrations of Zn²⁺ and ZnSO₄²⁻(aq), and that none solid phase would appear. A pH of 7.8 is necessary to precipitate (as ZnCO₃ and substantially as Zn(OH)₂). Its difficulty to be depleted from solution lies thus on the dominance of these different Zn species between the acidic and circum-neutral aquatic environment. Difficulties in removing Zn from circum-neutral mine waters have previously been reported, and research to overcome them have led to the development of new technologies.^{45,46} In contrast to Zn behaviour, Cu is expected to precipitate as Cu₂CO₃(OH)₂ at pH 7 (Fig 6b). A similar pattern is found for Cd present in this study.

However, this is in strong contrast with the effluent Zn concentration obtained in the column, with outflow concentrations around 12.6 mg dm⁻³ (which from a total concentration of 20 mg dm⁻³ corresponds to a 37% retention). As advanced before, adsorption of Zn onto organic matter as an additional mechanism responsible of the metal removal has been reported,⁴² which would explain the partial removal of Zn from de inflow.

Concerning the SRB activity in this experiment, sheep manure could not promote any detectable sulphate reduction. This finding is in contrast with the results obtained in a previous study with a similar system where dissolved heavy metals were excluded to focus on the sulphide generation, where an average bacterial sulphate reduction rate around 57 mg dm⁻³ d⁻¹ was attained at a residence times of 9 days.²³

The lack of bacterial sulphate reduction found in the present work may be due to the adverse effect of dissolved heavy metals in the bacterial activity. Indeed, both toxic (causing death) and inhibitory (causing a reduction in metabolic activity) effects of dissolved heavy metals on SRB has been reported.^{9,47} Although their interaction is acknowledged quite complex, mechanisms of inhibition/toxicity are postulated to be biochemical (by deactivating enzymes, denaturing proteins and competing with essential cations)⁴⁷ and/or physical (by precipitating metal sulphide on the bacterial cell and therefore acting as a barrier that prevents bacterial activity) in nature.⁹ Table 4 presents a simplified summary reported by Utgikar and co-workers⁴⁷ of the characteristic toxic concentration of the heavy metals involved in this study to pure strains and mixed cultures of SRB.

Metal	SRB strain	Toxic concentration
		(mg dm ⁻³)
Cu	<i>Desulfovibrio</i> strains	2-50
	Mixed culture	4-20
Zn	<i>Desulfovibrio</i>	13
	<i>desulfuricans</i>	
	Mixed culture	20-40
Cd	Strain L-60 ^a	54
	Mixed culture	>4-20
Mixture (Cr, Ni, Cu, Cd, Zn, Pb)	Mixed culture	20

^aResembles *Desulfosarcina*

Table 4. Characteristic toxic concentrations of the heavy metals to SRB⁴⁶

It is clear that metal content in the inflow of our experiment exceeded the toxic threshold of a multi-metallic solution, rendering the acidic water highly toxic to SRB. Results showed, however, that this metal content is mostly removed by the reactive material, not through bacterial activity but by other mechanisms, as mentioned above. This removal has been reported to take place significantly at the entrance zone of the column,²⁰ after which the achieved concentrations are well below the toxic concentrations and conditions are thus favourable for bacterial activity from a toxicological viewpoint. This observation is corroborated by the fact that the occasional framboids (metallic sulphides) were localised at the end of the column. Similar findings were obtained in previous works.²⁰

Residence time is reported to be a key factor in the performance of such systems, although in this experiment the subsequent increases of residence times at day 69 (2.9 days residence time) and at day 112 (12.1 days residence time) did not let to any detectable increase of the sulphate reduction. This is not attributable to a limited degradability of sheep manure but to the toxic effects of metals on SRB.

It can be summarised that while results from the previous study noted the importance of considering degradability and residence time, results from the present study have emphasised that the presence of dissolved metals may pose significant additional difficulties from a toxicological viewpoint. With regard to field PRB applications, two considerations must be noted: 1) the mentioned constraints imply long residence times (>12 days) for a significant sulphate reduction to occur, which in turn involves an enhancement of the width barrier and may entail prohibitive costs; and 2) organic matter degradability tests should be performed with the inflowing water containing, not only sulphate but the metal concentrations expected to be treated.

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