Remediation of metal contaminated simulated acid mine drainage using a lab scale spent mushroom substrate wetland.

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Abstract

The performance of an innovative pilot-scale spent mushroom substrate wetland for the attenuation of Simulated Acid Mine Drainage (SAMD) similar in composition to wastewater from the Irish mining region in Avoca, Co. Wicklow was investigated. The small-scale surface flow wetland, consisting of four cells in triplicate, measuring 0.46 m in width, 0.622 m in length and 0.489 m in depth, received approximately 4.32 l/day of simulated acid mine drainage. Over a period of 800 days, average removal efficiencies of Al (99 %), Zn (99 %), Cu (99 %), Fe (97 %) and Pb (97 %) were recorded, with no removal noted for Mn. These high removal rates were found to be comparable with other published results and in most cases surpassed removal rates of published results. Despite the reduction in pH and alkalinity over the duration of the trial, the rise in sulphate concentrations and the production of ammonia within the system were of concern, especially if a similar treatment system was utilised on a large scale basis. Temperature was also found to have an effect on metal removal rates, with poor removal rates recorded at low temperature events (<1°C). The high metal removal rates of the system still make it a very attractive and environmentally sustainable remediation technology, which could be further expanded to include other passive treatment technologies, to enhance and increase the longevity of the system.

Keywords: Acid mine drainage; spent mushroom substrate; remediation.

Accepted December 12, 2020

Introduction

The bioremediation capability of Spent Mushroom Substrate (SMS) for the treatment of acidic metalliferous mine drainage has great potential, owing to the presence of dissimilatory Sulphate Reducing Bacteria (SRB) and various functional groups that have the capacity for absorbing metal ions from solution. Acid mine drainage is characterised as having low pH and high metal concentrations, typically generated from the weathering of pyrite in abandoned mining sites and is a global problem [1]. Skousen estimate that as much as 90% of AMD that reaches streams and rivers originated in abandoned mining sites.

Heavy metal influenced water is of major concern as it can cause contamination of surface and ground water [1] and have harmful effects on humans and biota [2] as is the case in the Avoca mining area in Ireland. The use of passive treatment systems are a constant area of research as they are a low cost, low maintenance method of controlling metal contaminated leachate [3]. Compost wetlands differ from aerobic wetlands in that they consist of thick anoxic sediments which encourage the growth of anaerobic bacteria such as sulphate-reducing bacteria from the Desulfovibrio species, which oxidise the sulphate present in organic matter resulting in the release of hydrogen sulphide and bicarbonate [2].

The presence of carboxylic, phenolic and phosphoryl functional groups in SMS, indicates the enormous potential of using SMS as an economic biosorbent for heavy metals through passive binding [1]. SMS is a waste material of the mushroom industry, with Europe generating over three million tonnes of SMS per annum, which presents an immense environmental challenge [4]. The aim of this study was to assess if spent mushroom substrate alone is an appropriate medium for the bioremediation of simulated metalliferous mine drainage and to identify any drivers in metal removal within the wetland system. As acid mine drainage is unstable chemically and when stored, degrades quickly, simulated AMD was utilised in this research study. SAMD has similar composition to actual AMD but is more reliant when used in research trials as it is chemically stable [5].

Materials and Methods

Wetland design

The constructed wetland was designed in triplicate with each wetland comprising of a series of 4 cells (Figure 1). All cells were produced from polypropylene, measuring 0.46 m in width, 0.622 m in length and 0.489 m in depth, reminiscent of that descried by Jamieson. The installation of an overflow pipe permitted the flow of the SAMD into succeeding cells, similar to that described by Jamieson with a polythene tunnel housing the wetland study for the duration of the trial [5]. To facilitate the overflow of SAMD into consecutive cells, the individual cells were positioned on an engineered reinforced steel frame, with a 20 cm drop facilitated between each cell. Spent mushroom substrate was collected, mixed thoroughly and filled equally

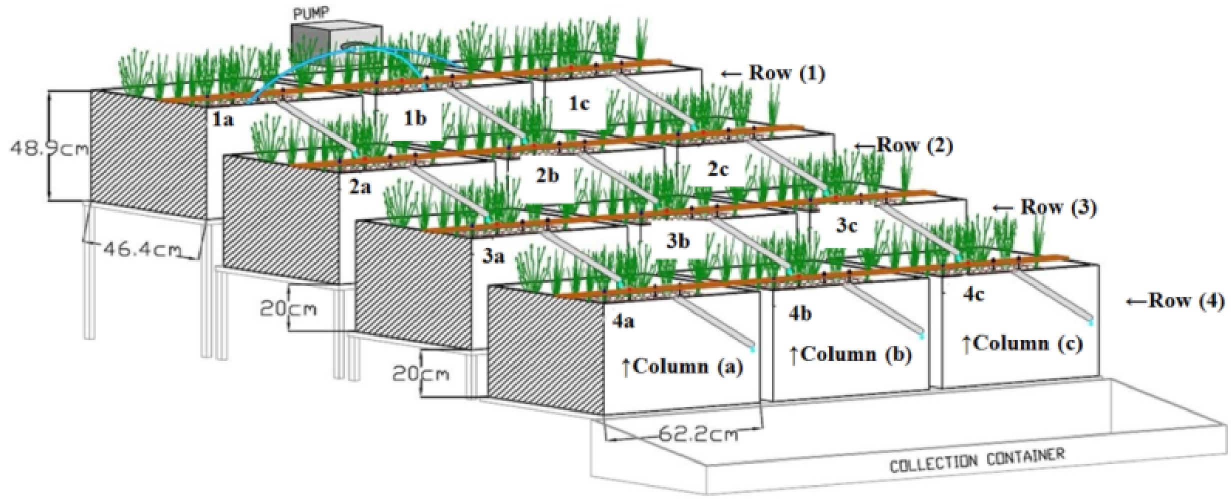

Figure 1: *Representation of constructed wetland design and cell dimensions.*

to a level of 33.4 cm in each wetland cell. As a consequence, the additional depth of 15.5 cm facilitated macrophyte seeding and emergence and the flooding of the cells. Typha latifolia, Phragmites australis and Iris pseudacorus were then transferred into the SMS wetland at a density of four rhizomes per metre squared [6]. The wetland was then submerged in water for 1 month prior to SAMD addition, to facilitate macrophyte growth and subsequent adjustment to the unaccustomed environment.

According Piramid Consortium , Typha latifolia and Phragmites australis grow at their optimum in the pH range of 3.5 to 10 and are heavy metal tolerant, whereas Iris pseudacorus is not tolerant of heavy metals and grows in the pH range of 6-9 [7]. However, Pérez-Sirvent reports that I. pseudacorus is capable of surviving in low pH conditions. SAMD was prepared to represent similar chemical composition to natural acid mine drainage currently being released from the abandoned mining region in Avoca, Ireland. SAMD was formulated and prepared using laboratory grade chemicals daily in accordance with Gray and O'Neill, using the succeeding proportions of cations; 150 mg L-1 Al, 130 mg L-1 Fe, 110 mg L-1 Mg, 90.0 mg L-1 Zn, 6.0 mg L-1 Mn, 5.0 mg L-1 Cu, and 1.5 mg L-1 Pb L-1. The pH of the SAMD was reduced using sulphuric acid to pH 3.1 [8].The wetland flow rate was determined using sulphate as the target influent contaminant concentration as per PIRAMID Consortium (2003), using an area adjusted removal rate of 3.5 g/m2/d. As a result, 4 l/day of SAMD was constantly passed through each individual wetland using Watson Marlow 323 digitised peristaltic pumps. The theoretical residence time of the wetland was established using the calculation, as adapted from Jamieson, which took into account the porosity of the system [9]. The duration of the trial lasted 800 days in total.

Monitoring and analysis

The pH, conductivity and oxidation reduction potential (Eh) of the cells were monitored at 5-minute intervals by electrochemical sensors at the surface of all wetland cells [10], which were fabricated and developed by EA Instruments Ltd., London. Sensor calibration was carried out at regular intervals in accordance with manufacturers specifications. The temperature of the wetland was also recorded digitally for the duration of the 800-day trial. Water samples were also collected at bi-monthly intervals from the SAMD storage tank and each wetland cell and analysed for a selection of parameters including alkalinity [11], pH, sulphate (Dionex ICS-1500 analyser), aluminium, zinc, manganese, copper, iron and lead (Varian AA240 AAS). A summary of the sampling regime is presented in Table 1.

Firstly, all samples obtained were filtered through Millipore GFC paper, followed by Millipore cellulose nitrate membrane filter paper (0.45 µm) in order to remove organic material from the solution [12] as required by various assays. Approximately 50 ml of each sample was subsequently passed through Millipore membrane filter paper (0.2 µm) as required for sulphate analysis.

Data and statistical analysis

The data was generated and graphed in Microsoft Office Excel (2016) and subsequently analysed using bivariate correlations on R© version 3.3.2 2016-10-31 (The R Foundation for Statistical Computing, 2016).

The daily average of the pH, temperature, conductivity and Eh were computed from the collected data. The results for the daily averaged data from each cell was presented in graphs of each row (a, b, c and corresponding column number 1, 2, 3, 4) and presented as cell 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 3c, 4a, 4b and 4c. The results from all other analysis are presented as averages of the three replicate rows, and presented as (cells 1,2,3,4). The data was then transferred to R© version 3.3.2 2016-10-31 (The R foundation for statistical computing, 2016) where it was subsequently analysed for collinearity or level of correlations between the main explanatory variables using the mgcv and nlme libraries (version 3.3.2) for selection for General Additive Model (GAM) with a cubic smoothing regression spline and cross validation was employed in order to identify the driving factors in metal concentration. GAMM analysis was also used to check for significant changes over time. For metal results the data was transformed (log10) when the data was skewed before analysis.

Results

The duration of the trial was 800 days long, with the wetland receiving 4.32 l/day of SAMD. Daily temperatures recorded

Table 1: *Summary of sampling regime.*

Parameter	Frequency of sampling	Method	Number of samples
pH	Every 5 minutes	Data loggers	230,400
Conductivity			
Temperature			
ORP			
Sulphate*	Twice monthly	IC	429
Metal concentrations*		AAS	6144
pH		Hand held probe	481
Ammonia	Periodically	Spectrometrically	221
*Sulphate was samples twice monthly until day 435 and then monthly thereafter. *Metal concentrations were analysis twice monthly until day 435 and then monthly thereafter.			

showed the expected seasonal distribution of data, with an exceptionally cold period recorded around day 400 of the trial, which seemed to have affected the biogeochemical processes in the wetland as discussed below.

As evident in Figure 2, the results for oxidation reduction potential for the wetland cells were recorded to be within a very reducing range of nearly -450 mV, for a prolonged period of time throughout the trial, which was probably due to the reduction of the submerged SMS substrate, resulting in the release of sulphates and the activity of various bacteria [13]. The Eh (mV) values recorded in the receiving cells (cells 1a, 1b and 1c) in the first 60 days of the trial, were in the range of a highly reducing environment. This commonly transpires in soils following the supplementation of organic materials, where the Eh values tend to decline rapidly after flooding to below -200mV [14]. Molahid reports a rapid decrease in Eh after 24 hours, in batch trial studies using submerged SMS to remove heavy metals from AMD. After approximately 60 days, the conditions changed to oxidising range, before dropping back towards a stronger reducing environment. The Eh values in the receiving cells, cell 1a, b and c, continued to fluctuate throughout the entire trial, before returning to a reducing environment towards the end of the trial, indicating that the system was still a moderately reducing environment as indicted in Figure 2. A reducing environment is known to be favourable for heavy metal reduction by sulphate reducing bacteria [15].

The results for the fourth and final tier, referred to as cells 4a, b and c, also illustrated in Figure 2 and show somewhat similar results to Figures 3 and 4 with results for the cell in row 4 revealing that these cells also remained anoxic and highly reducing until the latter end of the trial. Cell 4 b was the only cell that rose towards oxidising levels once at around day 520 and again towards the end of the trial. Cell 4 c was also found to become oxidising at the end of the trial at around day 785, with cells a maintaining a reducing environment until the end of the trial [16].

The apparent erratic nature of the results in this trial are consistent with reports that Eh in wetlands is very changeable and highly influenced by several factors, including hydraulic regime, presence or absence of macrophytes, type of macrophytes present and sampling depth as reported by Corbella [10] who recorded large fluctuations in Eh at 15 cm depth, even on a daily basis. O'Sullivan [14] also reports fluctuations of +300 mV in Eh values during seasonal events, such as plant die back and growth in treatment wetlands. Gerla [13] states that Eh values variable greatly from -300 to +500 mV in wetlands, being controlled by site specific conditions and other factors such as microbial activity and interactions with macrophytes.

The average pH results from the 800-day trial as monitored at 5-minute intervals were log transformed and the daily average pH reported. The results show that the pH of the receiving cells 1, a, b and c were signiicantly reduced over time ($p<0.001$) (Figure 3), following the addition of SAMD which entered the system at an average pH of 3.1 ± 0.09 (n=37). The pH of the three cells rapidly diminished over the first 200 days of the trial with 1c struggling to maintain a pH above 4. All eventually fell slightly below pH 3 towards the end of the trial. Similar results are reported by Newcombe and Brennan who report a decline in pH over time, in response to the exhaustion of the buffering capacity of the system towards the inflowing AMD used in SMS continuous flow batch trials [17].

The results show that the pH of the system was lowest in the first cells, cells 1 a, b and c and got progressively higher in each subsequent tier of the system (Figure 3). This result is similar to the results observed be Ji and Kim, who reported that the pH of a successive alkalinity producing system using SMS was lower in the samples taken closest to the inlet to the system and the effluent pH from the systems were found to reduce over time [18], as with this system.

The pH of a wetland system is crucially important in effective removal of metals. SMS has been shown to improve alkalinity and increase the pH of acid solutions, as reported by Molahid where SMS was shown to increase the pH of acid mine drainage from pH 3.5 to pH 6 after just 120 hours, in batch trial testing [19]. The buffering capacity of SMS is finite and over time the pH of solution has been shown to reduce, as stated by Grembi who report an initial increase in pH of acid mine drainage solution, remaining above pH 5 for approximately 70 days [14], followed by a decline over time after treatment in a SMS, chitin continuous flow column system. Skousen states that the pH of solution needs to be increased in order to facilitate metal precipitation as metal hydroxides, with most metals requiring a pH between 6 and 9. With this in mind, it could be considered that the addition of fresh compost to the wetland would be beneficial in providing additional support in buffering the system, with Wu stating that this is a long-known fact, that is often over looked in full scale systems [20]. The removal of the top layers of sediment may also improve the performance of wetland systems with Cheng reporting that metals were harvested from vertical flow CW by removal of the top layer of sediment and plant material [21].

The drops in pH were shown to be highly significant in all cells

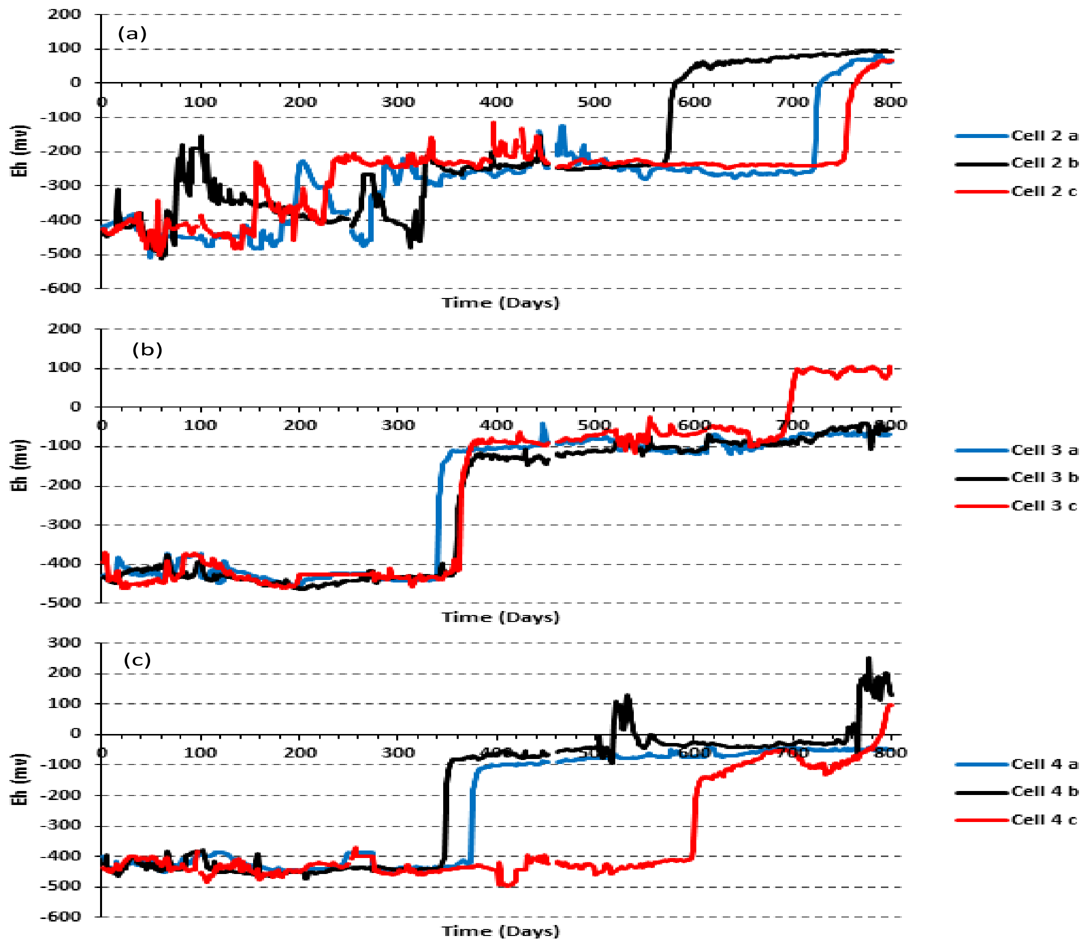

Figure 2: *Average daily Eh results from the three replicate wetland cells over an 800-day trial duration; a): Refers to daily averages for rows a, b and c for cell 2; b): Refers to daily averages for rows a, b and c for cell 3; c): Refers to daily averages for rows a, b and c for cell 4.*

over time ($p= <0.0001$) with dramatic drops in pH seem to correspond to low temperature events but this was found not to be signiicant ($p>0.05$). A decline in bicarbonate production by SRB [22] could result in a decrease in the systems buffering capacity against the low pH SAMD solution entering the system resulting in an overall reduction in pH in all cells.

The system seems unable to fully recover from the low temperature event, as none of the cells regain their post freeze pH levels after the second winter at around 450 days of the trial. This could be due to the die back of sulphate reducing bacteria and sulphidogenic species [23], which result in a decline in production of bicarbonate. Ji and Kim suggest that SRB cannot survive at pH below 4, resulting in the possible reduced bicarbonate production in the system [20].

As a direct result of the chemical nature of the SAMD entering the wetland, the buffering capacity of the SMS diminished over time and this is evident in Figure 3, where the alkalinity dropped to zero in the first three cells similar to results reported by Newcombe and Brennan, who report the reduction in neutralising capacity of a continuous flow column experiment using SMS mixtures over time. The drop in alkalinity over time was shown to be highly signiicant in all cells ($p<0.0001$). This could be due to the depletion of calcium carbonate within the substrate in an effort to neutralise the pH of the incoming solution or flushing of the calcium carbonate into the subsequent cells after approximately 450 days or due to the creation of insoluble metal carbonate within the wetland system [24]. There was also a corresponding drop in alkalinity at this same point in the fourth cell. These drops in alkalinity appear to be related to a drop in temperature around that time period. This drop in temperature could be responsible for the die back of sulphate reducing bacteria and sulphidogenic species, since biochemical processes are influenced by temperature [25] as sulphate reducing bacteria function best at higher temperatures and are responsible for the production of bicarbonate through the reduction of sulphate [26]. With the rise in temperature after approximately day 450, there is a rise in alkalinity in all cells except the receiving cell, but all cells failed to recover to previous levels, possibly due to the die back of SRB, that are influenced by temperature [27]. Although the graph suggests that there may be relationship between alkalinity and temperature, correlation analysis suggest that there was no relationship between the two variables and subsequent GAMM analysis showed no significant relationship between the two.

Sulphate concentrations increased over time ($p<0.0001$) and were found to be higher than the average influent concentrations of 1964.50 mg L^{-1} SO_4 (standard error ± 42.2, n=33), as shown in Figure 4. These results are similar to those reported by Clyde [28], who report an increase in sulphate concentration in effluent from a peat biofilter system. Since SMS is known to contain

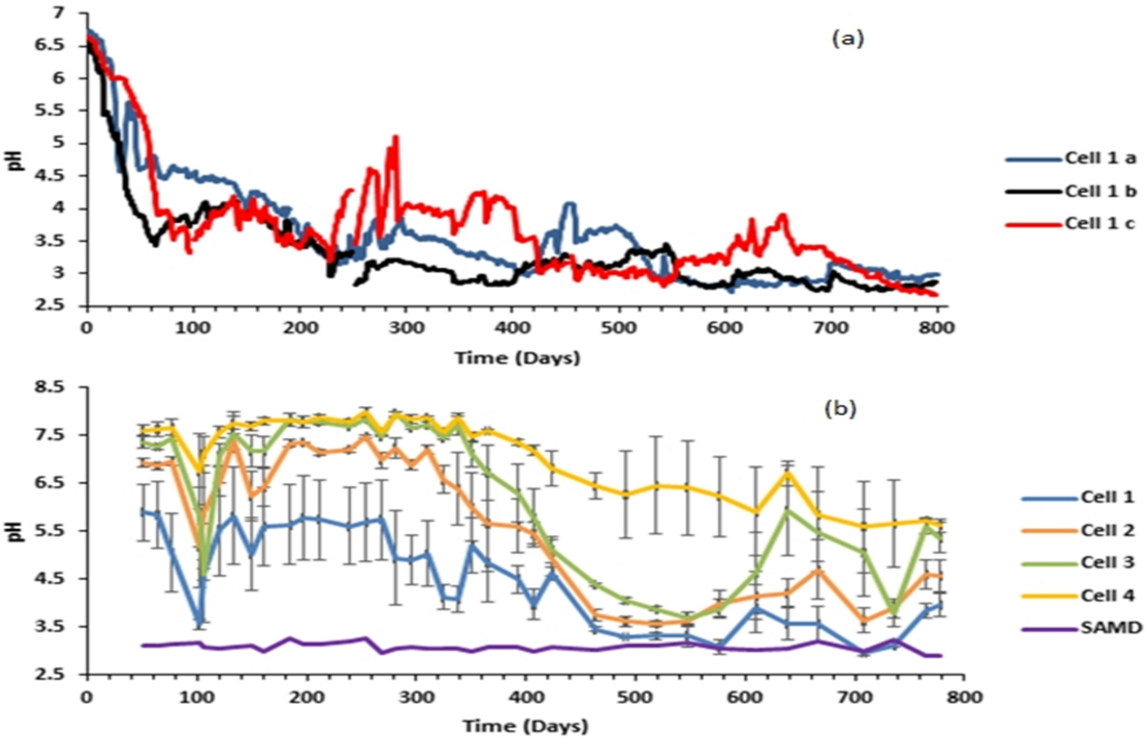

Figure 3: *a): Average daily pH results from the three replicate wetland cells (Cell 1) over an 800-day trial duration, with an average inflow pH of 3.1 ± 0.09 (n=37); b): pH of all wetland cells over time (± standard error).*

amounts of gypsum (calcium sulphate) [28], these results suggest there was a possibledissolution of gypsum contained in the SMS, resulting in a rise in sulphate concentrations.

The rise in sulphate concentrations could also be attributed to the release of sulphate from the substrate [29]. The rise in sulphate concentrations imply that sulphate reducing bacteria found it hard to survive the reduction in pH, as suggested by Ji and Kim [30]. The results indicate that there was a strong negative relationship ($p<0.001$) between sulphate concentrations and alkalinity. This result would be expected, since alkalinity is produced with the bacterial reduction in sulphate, since there was a rise in sulphate concentration [30], it follows, that there was a decrease in alkalinity. The results from cell 1 show that sulphate concentrations were lowest in this cell towards the end of the trial and this could be due to the fact that the reduction potential of these cells had returned to a reducing environment. Similar results were reported by Newcombe and Brennan indicting that SRB metabolism was inhibited by the presence of oxygen in a SMS system amended by chitin for the removal of heavy metals from wastewater.

From the results in Figure 4, it can be seen that the levels of ammonia in cell 1 decrease over time ($p<0.0001$) while the levels in cell 4 increase ($p<0.001$). This result indicates the bacteria in the system were using the SMS substrate as a carbon source for metabolism. A similar result was observed by Newcombe and Brannan, who report a steady supply of ammonium in the effluent from a SMS and chitin amended passive treatment system for the removal of heavy metals from AMD. The results indicate that there was more ammonia produced in cell 4, which is possibly due to the fact that it remained under reducing conditions until near the end of the trial, since ammonium is a by-product of bacterial degradation of organic material under reducing conditions [32]. It has been reported that SMS can leach nutrients [33] and as a result, the releasing of ammonia, organics and other salts from SMS could result in the accumulation of nutrients in cell 4 and potentially cause eutrophication of nearby waterways [34]. Alternative ammonia removal strategies such as vertical subsurface flow wetlands [31-33] and the use of hydroponics have been shown to have excellent nutrient removal rates and may be required if an SMS wetland was employed.

Metal concentration recorded in SMS wetland

The results for Al removal show that the wetland system was capable of removing an average of 99 % of the aluminium entering through the addition of SAMD, which contained an average of 149.16 mg L-1 Al. However, the results show that Al slowly increased over time in all cells ($p<0.0001$) indicating that the sediment may be becoming saturated with Al. The results from Figure 5 suggest that Al concentrations were influenced by the low temperature event around 93 days and also around day 407 in cells 2 and 3. Although there appears to be a trend between temperature and Al concentration, as with most of the other metals tested, results of GAMM analysis reveal that temperature was not a significant driving factor in Al concentrations in the system [34].

These results are comparable to published results, with high removal efficiency for Al being reported by Morari who state that planted constructed wetlands used in the treatment of municipal wastewater were capable of removing 96% of Al from solution. Molahid report SMS as being very capable of removing Al from AMD solution when using batch testing, reporting a removal ate of 94%. Aluminium has been shown to form aluminium hydroxide in pH’s greater than 5, resulting in

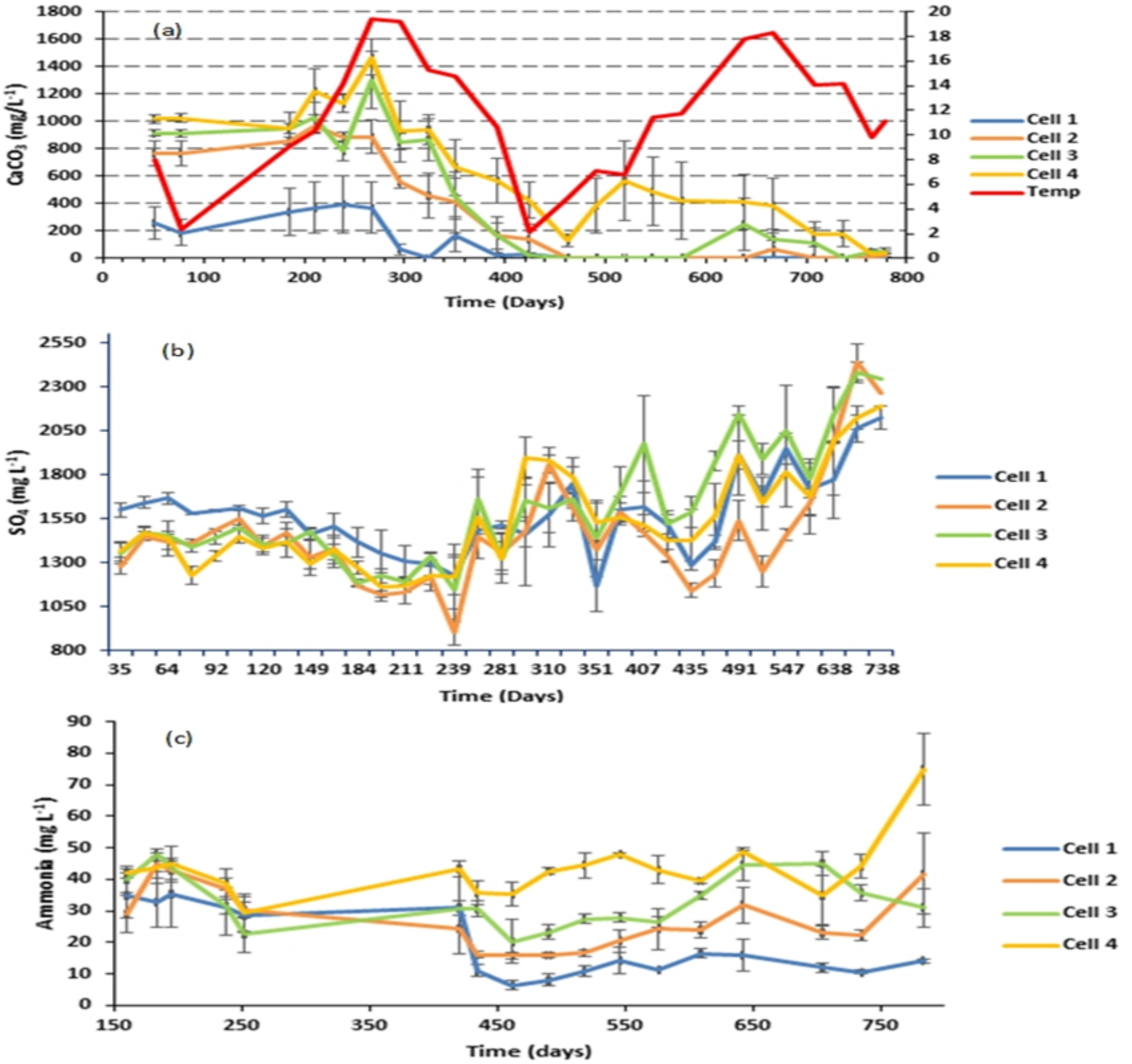

Figure 4: *Effect of SMS constructed wetland on; a): alkalinity values over time; b): sulphate values over time (with an average inflow concentration of 1964.50 mg L-1 SO4); c): ammonia concentration over time.*

precipitation [35]. Although pH was shown not to be significant driver of Al concentration in the wetland, it has been stated that pH is an important factor in the removal of Al from solution, with resuspension occurring over pH 9. At approximately pH 4, Al exits as Al3+ and allows for binding to organic matter and phosphate, with the latter being an insoluble compound [33], XRD results from this study support this statement, since AlPO4 compounds were identified in the sediment from the wetland system which were not present in the starting material (data not shown).

In relation to zinc, it is not biodegradable and can bio-accumulate and enter the food chain. Zinc concentrations were constantly low in the wetland cells throughout the trial, apart from cell 1 ($p>0.05$), with an increase in zinc noted again during the temperature drop event (Figure 5). Most zinc removal in wetlands is credited to hydrous metal oxides of manganese and iron precipitating under reducing conditions. Zinc is also known to interact with hydrogen sulphide to form zinc sulphide [36] this is supported by the results from XRD analysis of sediment from this study, which identifies ZnSO4 and ZnS in the majority of sediments tested (Data not shown).

As highlighted in Figure 5, the SMS wetland had the capacity to remove 99 % of Zn from the SAMD solution. These results are comparable to published results for Zn removal using constructed wetlands, with Gill reporting removal efficiencies of 86% for Zn and 95% removal when sampling discrete storm events [37]. The results show that there were two periods throughout the trial when removal efficiency dropped, these times correspond to low temperature events. The results from GAMM analysis presented in Table 2 reveals that temperature was a significant factor in influencing Zn concentration in the effluent from cell 1 ($p<0.001$). The average Zn effluent concentration from cell 4, with the exception of extreme temperature events, was found to be 0.06 mg L-1 and this value is within the range of the permitted reference values set out in the surface water regulations (S.I. No. 272 of 2009) of between 5 µg-100 µg L-1 for freshwaters [38].

With the addition of 5.26 mg L^{-1} of manganese to the constructed wetland, an increase in manganese concentration was recorded in all cells over the course of the trial ($p<0.0001$) with the exception of cell 1 ($p>0.05$), as illustrated in Figure 5, resulting in no Mn removal efficiency the end of the experimental trial. The outflow from the system was found to be greater than the

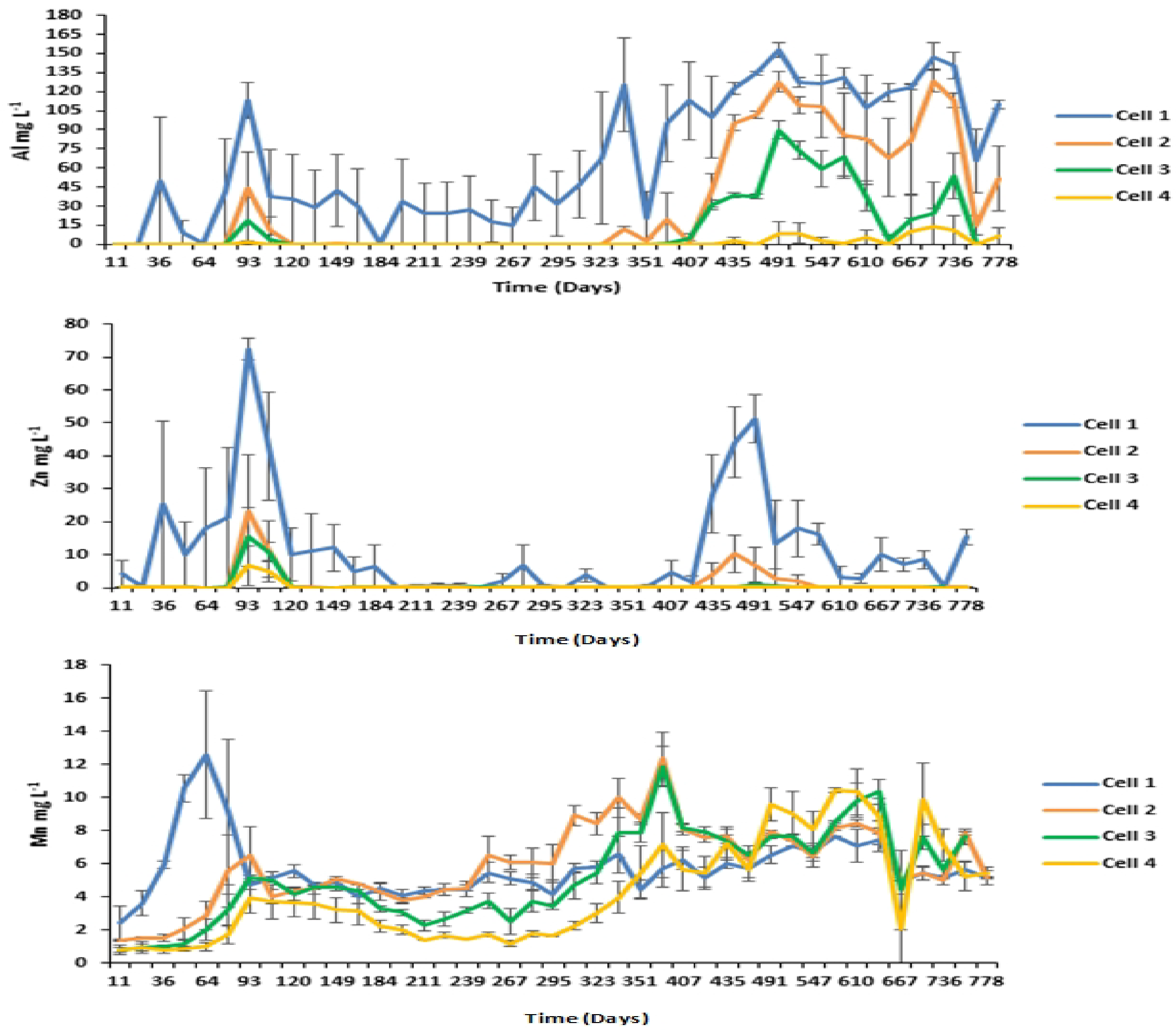

Figure 5: *Weekly average results from the SMS wetland over time for; a): Al concentrations (with an average inflow concentration of 149.16 mg L-1 of Al), b): Zn concentrations (with an average inflow concentration of 80.19 mg L-1 of Zn) and 5c) ; c):Mn concentrations (with an average inflow concentration of 5.26 mg L-1 of Mn).*

Table 2: *Results from general additive models (GAMM) for weekly metal concentration in relation to the treated effluent, exiting cell 1 and cell 4 of the limestone channel system over an 800-day trial period.*

Metal	Significant variable	edf	F-value	P	R-sq.(adj)	AIC
Mn*	ORP	2.4	36.26	<3.84e-12	0.68	160.5
Zn**	Temp	2.2	7.4	0.001	0.22	304
n = 41						
* GAMM for Mn concentration in relation to the treated effluent exiting cell 4, final effluent exiting the system. ** GAMM for Zn concentration in relation to the treated effluent, exiting cell 1.						

inflow to the system after 337 days. This elevation may be caused by the SMS substrate being reduced, which resulted in the subsequent release of the 0.40 g kg^{-1} Mn typically found in SMS.

Although the results indicate that Mn was not retained within the system, it is evident that Mn was retained in the sediment at depths below 15 cm (data not shown). The sediment metal profile also indicates the possibility of Mn leaching from the top 10 cm of the sediment since, for the most part, the top 10 cm of sediment contained less [37-39] Mn than the starting material. This would indicate that Mn was retained in the sediment and then was leached from the top 10 cm of sediment. Mn leaching from SMS has also been confirmed by Vasquez, with an average of 97% of Mn leached from SMS during sequential extraction experiments. Skousen reports that since Mn is present in several oxidation states, removal can be very inconsistent and requires pH 9 and above. In saturated soils dominated by high Al and low pH, Mn has been shown to become displaced into the surrounding solution [32]. The initial concentration of Cu in the SAMD entering the wetland was 5 mg L^{-1}, and for the duration of the trial, copper concentrations remained appreciably low ($p<0.001$), as illustrated in Figure 5, with the exception of cells 1, 2 and 3 on one occasion, which occurred during extreme freezing events when temperatures reached below 1˚C. Although a relationship with temperature and concentration was observed at very low temperatures, no significant relationship was identified with subsequent GAMM analysis ($p>0.05$). Since no Cu sulphide compounds were found upon analysis with XRD and high DOC conditions found within the system (Data not shown), it can be presumed that Cu ions were removed from solution as insoluble organic metal complexes, as suggested by Reddy and DeLaune [29]. The SMS wetland was found to have a greater than 99% removal rate from the final effluent from the system and this high removal rate is supported by Gill, who report removal rates of 88% from a constructed wetland system treating motorway run off and Cu removal rates of 80% reported by Arivoli using constructed wetlands [40].

Like the trends noted for Cu and Zn concentrations in the wetland, during low temperature events, the Fe concentrations were appreciably high (Figure 6). Although there appears to be a relationship between temperature and Fe concentration in the effluent, GAMM analysis shows there the relationship was not signiicant ($p>0.05$). The results indicate that there was a signiicant rise in Fe in all cells over time ($p<0.0001$). Reddy and DeLaune (2008) reported that Fe (II) concentrations are inclined to rise with declining Eh values over time [41], but in some cases can be constrained by nitrate concentrations, which may have been a factor in this study. There was no relationship found between Fe concentrations and Eh in this study ($p>0.05$) and therefore by estimating the end products of Fe reduction in the soluble and exchangeable phase, the total reduction rates may be undervalued [32]. The average Fe removal from the wetland, cell 4, was shown to be greater than 97% from the system (Figure 6). This result, shows better removal rates than published results for other constructed wetlands designed for the removal of Fe, with Arivoli removal rates of 74% Fe in planted constructed wetlands possibly due to design differences and differences in substrates used.

The rise in Pb concentration in the system over time was found to be signiicant ($p<0.05$). The wetland system was shown to have 97% removal efficiencies from the outlet effluent from cell 4, but removal efficiencies in cell 1 were found to be reduced during low temperature events, yet temperature was found not to be a significant driver in Pb concentration (Figure 6). Gill report removal efficiencies of 31% of Pb from motor way run off treated in a constructed wetland, but earlier studies show a removal efficiency of 86% when sampling inlet and out effluent of discrete storm events [27-30]. Despite the fact that the system was found to have a removal efficiency of greater that 97%, the

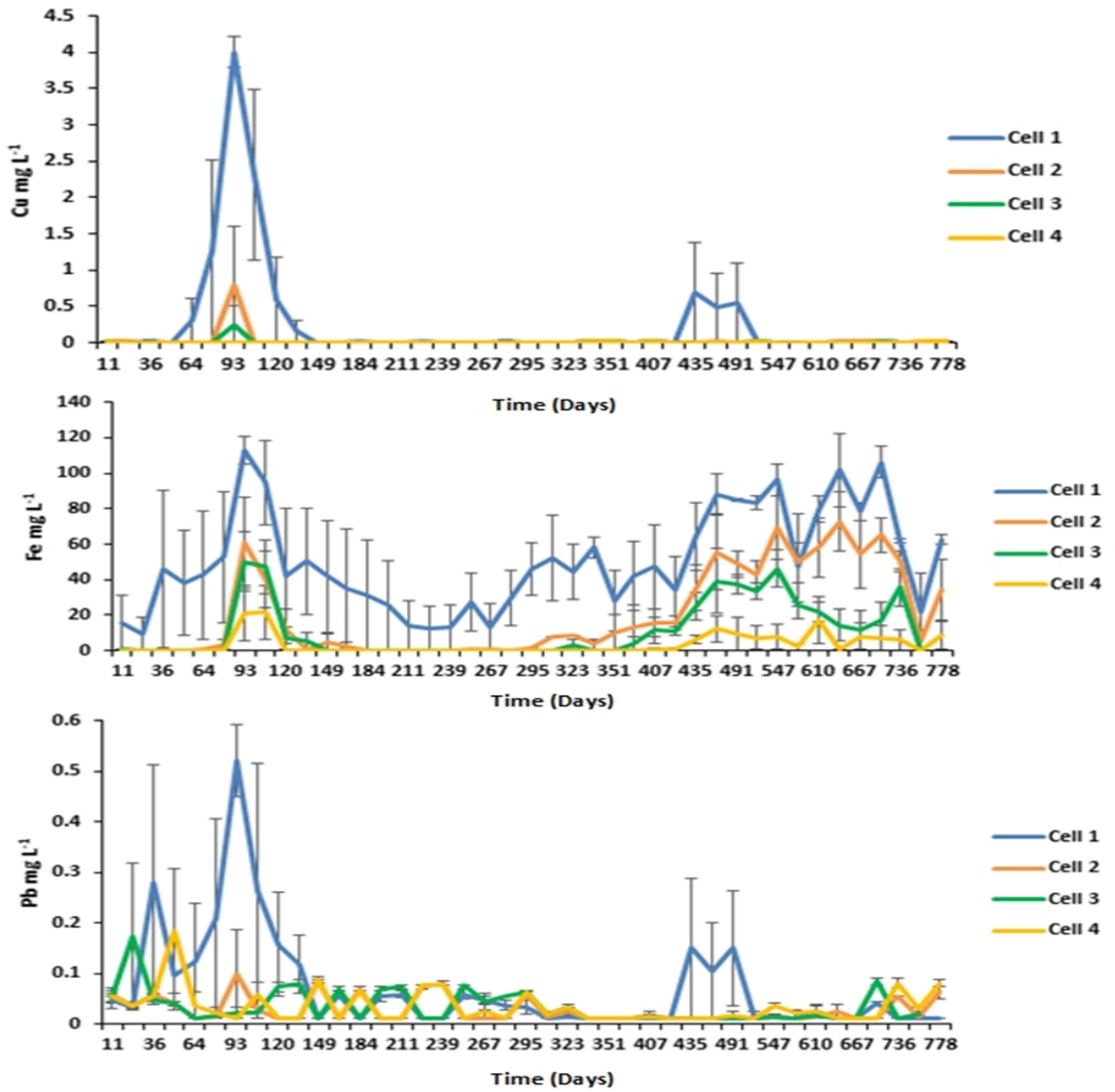

Figure 6: *Effect of SMS constructed wetland over time on; a): Cu concentrations (with an average inflow concentration of 5.64 mg L-1 of Cu); b): Fe concentrations (with an average inflow concentration of 130.24 mg L-1 of Fe); c): Pb concentrations (with an average inflow concentration of 0.92 mg L-1 of Pb).*

effluent concentration from cell 4 contained an average of 0.03 mg L-1 which is above the permitted reference set out in the surface water regulations (S.I. No. 272 of 2009) of 7.2 µg L-1 for freshwaters [42].

Lead can be removed from solution by sorption to organic material or following microbial sulphate reduction, by precipitation as sulphide minerals [43] however no lead sulphide compounds were found upon sediment analysis using XRD (data not shown), therefore, it can be presumed that Pb ions were removed from solution as insoluble organic metal complexes, as suggested by Skousen.

From the results, it is evident that the constructed wetland was indeed capable of removing metals from solution. Highly signiicant correlations between Al and Fe (p=0.001) and Zn and Fe (p=0.0001) are recorded, indicating possible co-precipitation with Shim reporting the co-precipitation of Fe with other metals in AMD. The results indicate that metal sulphides were formed in the sediment, resulting in the formation of insoluble metal sulphide, and their precipitation [44].These results also suggest that metal oxides and metal carbonates were also formed as both were found in the sediment after the trial, similar to reports by Gill. The removal of metals was also achieved by interaction with phosphate contained in SMS [45], with the formation of iron phosphate and aluminium phosphates as reported by Azam and Finneran, Karjalainen [38] and possibly binding to organic matter as stated by Gill.

Discussion and Conclusions

With high metal removal efficiency for most of the metals tested, the results indicate that the use of a SMS compost wetland for the treatment of AMD can be an effective means of remediating metal burdened wastewater. The wetland performed well in terms of dissolved metal concentrations in the effluent from the system, proving that SMS has the capability to retain heavy metals. The results also suggest that SMS can release metals such as Mn, suggesting an additional alternative approach may be needed to remove these metals, such as chemical treatment technologies.

The pH of the system slowly decreased as time progressed, with most metals requiring a pH between 6 and 9 to precipitate as hydroxides, the addition of fresh compost to the wetland should be considered and could be beneficial in providing additional buffering to the system. Furthermore, the high ammonia concentrations in the outlet from the system would need to be addressed if this system was employed on a large-scale basis. The addition of a vertical flow wetland could be beneficial, as it has been proven to be effective in the removal of ammonia from solution.

Funding

The authors would like to acknowledge the EPA for funding this research (S.N. Jordan, 2007-FS-WRM-12-S5) as part of the Science, Technology, Research and Innovation for the Environment (STRIVE) Programme 2007-2013.

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