Science of The Total Environment, Volume 867, April 2023, 161259
DOI: 10.1016/j.scitotenv.2022.161259

Influence of sediment quality and microbial community on the functioning capacity of a constructed wetland treating alkaline leachate after 5.5 years in operation.

A. Hudson[1], J.G. Murnane[2], T. O'Dwyer[3], M. Pawlett[4], R. Courtney[1]

[1]*Department of Biological Sciences and Bernal Institute, University of Limerick*

[2] *School of Engineering, University of Limerick*

[3]*Department of Chemical Sciences and Bernal Institute, University of Limerick*

[4]*School of Water Energy and Environment, Cranfield University*

Abstract

Constructed wetlands (CWs) have been demonstrated as a cost-effective alternative to chemical treatment systems for mine waters, with the microbial communities attributed to promoting carbonation and aiding pH neutralisation. However, few data are available for the long-term use of CWs treating alkaline leachates nor the activity of microbes within them. To investigate the feasibility of CW to buffer alkaline pH, a pilot-scale wetland was implemented in 2015 to treat alkaline bauxite residue leachate. After 5.5 years, samples of supernatant water and sediment were taken at 0.5 m increments along the 11 m long wetland. Waters were analysed for pH, EC and metal(loid) content, while sediment was subjected to physico-chemical assessment and element fractionation. Microbial biomass and community were assessed by phospholipid fatty acid analysis (PLFA) and functionality by the Rapid Automated Bacterial Impedance Technique (RABIT).

Evidence presented demonstrates that the CW operating for 66 months effectively treats bauxite residue leachate, with reduced influent pH from 11.5 to 7.8. Trace element analysis revealed effective reduction in Al (94.9%), As (86.7%) and V (57.6%) with substrate analysis revealing a frontloading of elevated pH and trace element content in the first 5m of the

Published by Elsevier. This is the Author Accepted Manuscript issued with: Creative Commons Attribution Non-Commercial No Derivatives License (CC:BY:NC:ND 4.0).
The final published version (version of record) is available online at DOI:10.1016/j.scitotenv.2022.161259. Please refer to any applicable publisher terms of use.

wetland. Sediment Al, As and V were present mostly (>94% of total) in recalcitrant forms. Sediment Na was mostly soluble (48-62%), but soils were not sodic (ESP<15%). Investigations into the microbial community revealed greatest biomass was in the first 5m of the wetland, where pH, EC and metal contents were greatest. Microbial respiration using endemic *Phragmites australis* as a substrate demonstrates an ability to cycle recalcitrant carbon sources within a CW system. These novel microbial findings highlight the need for further investigation into the microbial communities in alkaline CWs.

Key words:

Constructed Wetland; Bauxite residue leachate; Microbial Community; Phragmites australis

Introduction

Production of alumina via ore digestion in sodium hydroxide (NaOH) at high temperatures and pressures (Bayer process) generates large quantities of alkaline bauxite residue (BR) as a by-product (Joyce and Björklund 2019; Balomenos *et al.* 2021). The global stockpile of BR is estimated to be in excess of 4 billion tonnes, with an annual production of 150 million tonnes (Evans 2016; Di Carlo *et al.* 2019) and its disposal and management presents a long-term challenge to operators and environmental agencies (Power *et al.* 2011). Owing to the entrained residual NaOH and other alkaline constituents, BR and associated leachates can have pH ranging between 9 and 13.2 (Burke *et al.* 2013; Higgins *et al.* 2016; Kong *et al.* 2017). In addition BR leachates can contain elevated concentrations of elements such as Al (500-1000 mg L^{-1}), As (3000-6000 µg L^{-1}), V (5000-10,000 µg L^{-1}) and Cr (65-188 µg L^{-1}) (Burke *et al.* 2012; Burke *et al.* 2013; Higgins *et al.* 2016). High alkalinity and elevated concentrations of metals can lead to concerns over the impact of potential discharges to the local environment in the event of mismanagement (Ruyters *et al.* 2011; Olszewska *et al.* 2016; Kong *et al.* 2017; Di Carlo *et al.* 2019), particularly in a post closure scenario. Soils contaminated by BR have exhibited increased pH and trace metal content (Ruyters *et al.* 2011; Lehoux *et al.* 2013). Discharge of high pH leachate from bauxite residue to the environment at Kinghorn Loch, Scotland, resulted in As contamination of sediments and cycling of As within the macrophytes present (Olszewska *et al.* 2016). Leachate neutralisation is considered a key step to reducing the content of soluble trace elements and the alkalinity risk to the surrounding aqueous ecosystems (Burke *et al.* 2013).

During the active life of a refinery, the Bauxite Residue Disposal Area (BRDA) leachates are typically neutralised and treated alongside process wastewater using conventional chemical treatment processes. However, in a post closure scenario such active treatment processes are unsustainable in the long term, requiring staff, maintenance, sludge disposal, reagents and an

undetermined lifetime that may extend to multiple decades (Chockalingam and Subramanian 2006). Passive technologies, such as constructed wetlands (CW), have shown to be feasible for a range of wastewater streams (Sheoran and Sheoran 2006; Mayes *et al.* 2009a) and combine a range of removal mechanisms including: settling, sedimentation, sorption, co-precipitation, cation exchange, photodegradation, phytoaccumulation, biodegradation, microbial activity and plant uptake (Sheoran and Sheoran 2006; Kadlec and Wallace 2009; Marchand *et al.* 2010).

Much of the current literature on CW treatment of extreme pH waters is focused on acidic drainage (e.g. Matthies *et al.* 2010; Pat-Espadas *et al.* 2018) and the efficacy of wetlands treating acid and neutral mine drainages waters has been proven up to 20 years, with the need for maintenance highlighted (Hedin 2020). The potential of CWs to also treat alkaline drainage has been shown in recent years (Mayes and Younger 2006; Mayes *et al.* 2009; Gomes *et al.* 2019), including for the treatment of BRDA leachate (Buckley *et al.* 2016; Higgins *et al.* 2017; Hua *et al.* 2019). However, much of this research has assessed CW feasibility in laboratory-based trials or short-term (<5 years) field trials and there are few data available on the long-term functioning of a CW system treating alkaline industrial leachates. Several studies have shown that pH reductions in alkaline waters were attributed to microbial activity and generation of CO_2 from respiration, as well as generation of organic acids via decomposition (Mayes *et al.* 2009; Mayes *et al.* 2009a; O'Connor and Courtney 2020). However, the functionality of such microbial populations to treat alkaline BRDA leachate in CWs and their ability to cycle endemic material as a C source is largely unknown.

In several CW studies, increases in sediment trace element content (As, Al, V, Na) was reported for both steel slag and BRDA leachate systems during their operation (Higgins *et al.* 2017; Gomes *et al.* 2019; O'Connor and Courtney 2020). Within alkaline BRDA leachates, Na^+ is the major cation of concern as it tends to be present in high concentrations and can occupy a large proportion of the adsorption sites within the wetland soil. Additionally, most

of the Na compounds formed are soluble (Hua *et al.* 2015), and the period over which a system can remove influent Na^+ before reaching saturation is unknown. Moreover, the extent to which elevated Na^+ may impede microbial functionality in the context of a wetland is undetermined. Recent studies on CW treatment of BRDA-generated alkaline bauxite residue leachate reported that while the average Na concentrations in supernatant reduced by 31% after 1 year, the inflow and outflow concentrations were similar by the 4^{th} year of operation and the total Na concentrations within the sediment had increased two-fold (Higgins *et al.* 2017; O'Connor and Courtney 2020).

The objective of this study was to measure pH reduction and element accumulation in the sediment across a single cell pilot CW treating alkaline leachate after 66 months of operation. In addition, the extent of Na front-loading within the CW sediment was measured and its potential impact on the ability of the microbial biomass to cycle carbon, and on its community structure across the length of the wetland was investigated. It is hypothesised elemental loading, pH and EC will be greatest in the first 5m of the wetland; with microbial community biomass and rates of respiration reduced in the inflow zone (0-5m) versus that nearer the outflow(5-11m). It is intended that the findings from this study can be used to make further recommendations on the long-term use of CWs for treatment of alkaline BRDA leachate.

Materials and Methods

Site Description

A pilot scale horizontal surface flow wetland was constructed at a BRDA at Aughinish Alumina Refinery located in Ireland. The CW had an area of approximately 44m^2 (4m x 11m) with soil transplanted from the local area to a depth of 200mm. The soil used had a pH = 6.7, EC 188 µS cm^{-1}, CEC 38 cmol/kg and an organic C content of 2.8%. Vegetation was dominated by *Phragmites australis* with some *Typha latifolia,* with *Juncus* stands near the outflow of the wetland. The inflow was regulated by a programmable logic controller (PLC) leachate mixing system, consisting of three 1m^3 intermediate bulk container (IBC) units: a leachate tank, a mixing tank, and a dosing tank. The neat leachate was diluted in the mixing tank to simulate potential drainage waters that could arise from the closed BRDA as a result of surface runoff and leachate: after being adjusted to a pH ca. 11, the leachate was pumped to the dosing tank, at flow rates of 10-30 l/hr in winter months and 45-55 l/h in summer months, from where it was discharged to the wetland (Residue Solutions 2007; Buckley *et al.* 2016). Seasonal flow rate variation was due to increased winter rainfall.

The system has been in operation for 66 months. Inflow and outflow pH and EC was monitored daily by field probe (monthly averages reported) over the 66-month period.

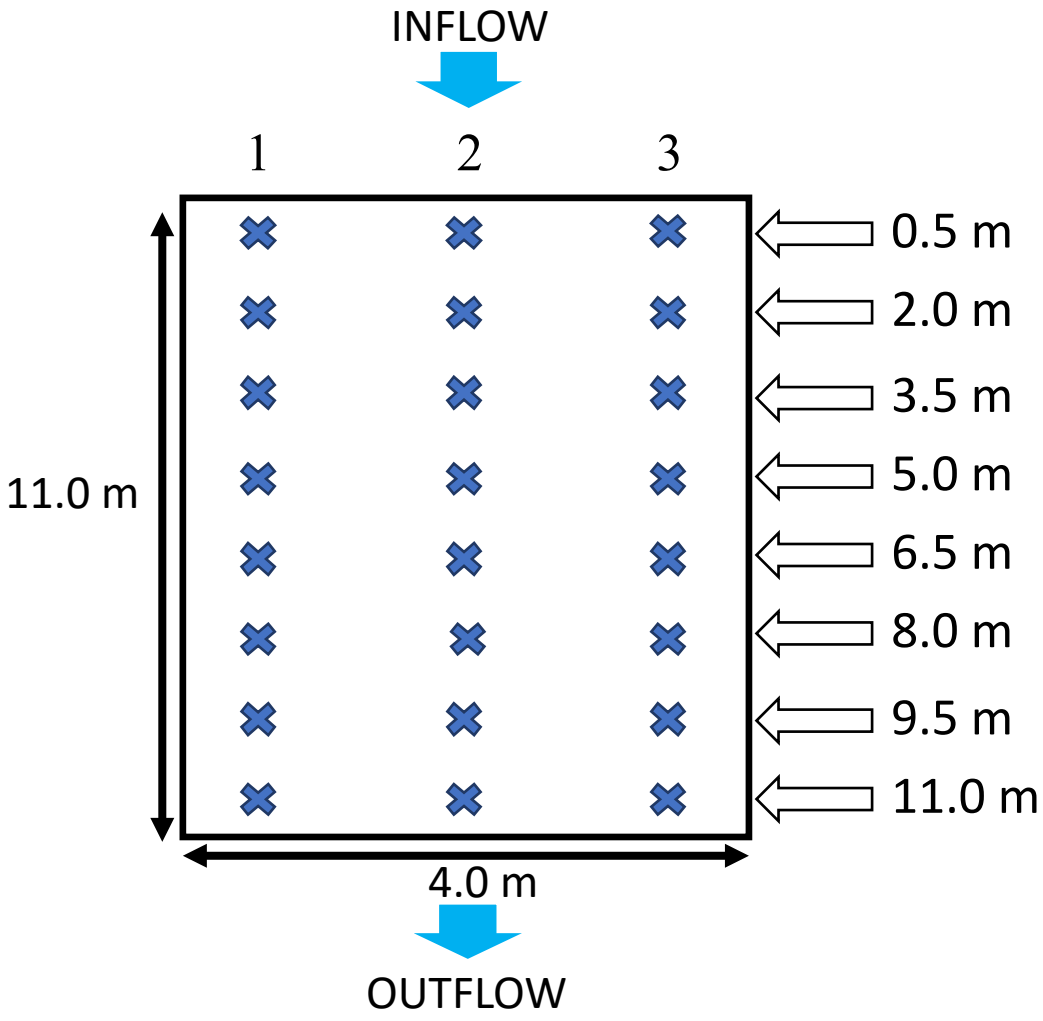
Wetland Supernatant Sample Collection

Surface water samples (1 L) were collected at 1.5m intervals from undisturbed supernatant water at the left side of the wetland (Fig 1), in the direction of outflow to inflow prior to sediment collection. Inflow and outflow pH and EC were measured at time of sampling and total Al, As, Cr, V, Na, Mg, K and Ca content in the samples was determined in the laboratory by ICP (Fitz Scientific, Drogheda, Ireland).

130

131 **Wetland Substrate Sample Collection**

132 The wetland was divided into 3 lanes, left (1), middle (2) and right (3) (Fig. 1), and sediment
133 samples were collected at 1.5m intervals along each lane (N=24; Fig 1). Sediment samples (1
134 kg, 0-20 cm) were divided in two; 500g used for physio-chemical analysis and 500g was
135 placed under ice, transported to the laboratory, and refrigerated until microbial analysis.
136 Leaves of *Phragmites australis* were collected randomly from across the wetland cell to be
137 utilised as food source for microbial studies.



138

Figure 1: Schematic demonstrating sample collection points within the wetland (4 m x 11 m), with distances approximately 1.5m between triplicate rows.

Sediment analysis and Sequential Extraction

Sediment samples were air-dried and sieved (<2mm) prior to analysis. Samples were analysed for pH and EC (1:5 soil solution). Element content was assessed as soluble (DI water), exchangeable (1M NH_4OAc), carbonate and residual fractions by sequential fractionation using a modified Tessier procedure (Tessier *et al.* 1979), reported by Higgins *et al.* (2017). Following the extraction process, element content (Al, As, Ca, Cr, K, Mg, Na, V) were determined by ICP-OES (Fitz Scientific, Drogheda, Ireland).

Microbial analysis

Soil microbial parameters were determined on fresh sieved (2mm) soil. Microbial biomass-C was determined using the fumigation-extraction procedure according to Jenkinson & Powlson (1976) using the KEC of 0.45 (Vance *et al.* 1987). Microbial community structure was observed using Phospholipid Fatty Acid (PLFA) profiles according to Pawlett *et al.* (2013), adapted from Frostegård *et al.* (1993). The sieved soil (approx. 7g) was freeze-dried, lipids extracted, fractionated, and the phospholipids derivatised by mild alkaline methanolysis. Fatty-acid methyl esters were subsequently separated by Gas Chromatography (Agilents, USA) using a HP-5 capillary column (Agilent Technologies: 30m length, 0.32 mm ID, 0.25 μm film). Peak areas were integrated using G2070 ChemStation and calculated as relative abundance (mol %) (Pawlett *et al.* 2013).

Indicator fatty acids included: 18:2w6,9- ectomycorrhizal fungi (Frostegård and Bååth 1996; Kaiser *et al.* 2010), the sum of i15:0, ai15:0, 15:0, i16:0, i17:0, ai17:0, cyc17:0 and 17:0- total bacteria (Frostegård and Bååth 1996), the sum of the iso and anteiso branched i15:0, ai15:0, i16:0, i17:0 and ai17:0-gram positive bacteria (Zelles 1999), the sum of 16:1w7c and 16:1w5-gram negative bacteria (Zelles 1999), and fungal to bacterial ratio- calculated using fungal

biomarker 18:2w6 divided by summed % mol of bacterial fatty acids (Frostegård and Bååth 1996). The fatty acid 16:1w5, which can be used to indicate the presence of arbuscular mycorrhizal fungi (AM) (Olsson 1999), was used with caution, as it can be present in some bacterial species (Frostegård *et al.* 2011).

Functional characteristics were observed as short term (<4h) respiration response following carbon substrate addition to the soil. Substrates were added individually to each soil (1g fresh soil) and CO_2 production quantified according to Pawlett *et al.* (2013). Substrates added were D-glucose (75 mM), α-ketoglutaric acid (9 mM)(aKG), and ground *Phragmites australis* material collected from the wetland. Glucose and α-ketoglutaric acid are easily available source of C whereas ground Phragmites is recalcitrant and native organic matter to the wetland system. Glucose and α-ketoglutaric acid were added as aliquots (500 µl). The soil's basal metabolic rate was also determined without the addition of substrates but with the addition of 500 µl deionized water. The plant material (100 mg) was added to the soil as a powder following fine grinding (pestle and mortar). CO_2 produced was quantified using the Rapid Automated Bacterial Impedance Technique (RABIT; Don Whitley Scientific, UK), using Potassium hydroxide (0.5 %) and agar (0.5%: Oxoid agar No.1) in 1ml aliquots within the impedance tubes. Changes in conductivity (microsiemens) were measured and quantified to CO_2. Microbial respiratory response rates were determined over a 4h incubation period at 25 °C.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics (v 28.0). Following implementation of Shapiro-Wilk test of normality, differences between measured values at distance through the wetland was determined individually. One way analysis of variance and Welch's analysis of variance were calculated, with further investigation using Tukey and Games-Howell post hoc tests respectively. The non-parametric Kruskall Wallis test was used

where normality was violated. The PLFA data were analysed by Principal Component Analysis (PCA), with Spearman's Rank Correlation also conducted (supplementary data). Measured parameters include pH, EC, soil microbial biomass and PLFAs, supernatant metal concentrations and sediment metal concentrations of Al, As, Ca, K, Mg, Na, V in soluble, exchangeable, carbonate-bound and residual forms. Significant differences throughout the text are reported at the 0.05 level ($p<0.05$).

Results

Supernatant Analysis

pH and Conductivity

Wetland pH and EC varied significantly spatially in the cell (Fig. 2). Inflow pH was 11.5, while wetland supernatant pH varied from pH 10 at 0.5m to pH 7.8 at 11m along its length, with the lowest value of pH 7.6 at 6.5m. There was an immediate drop in pH from 11 to 10.5 in the first 0.5m of the wetland, with a further decrease to pH 8.4 within 5m. The prime objective of the wetland system is to obtain a pH<9, which is achieved between 3.5 and 5m into the cell.

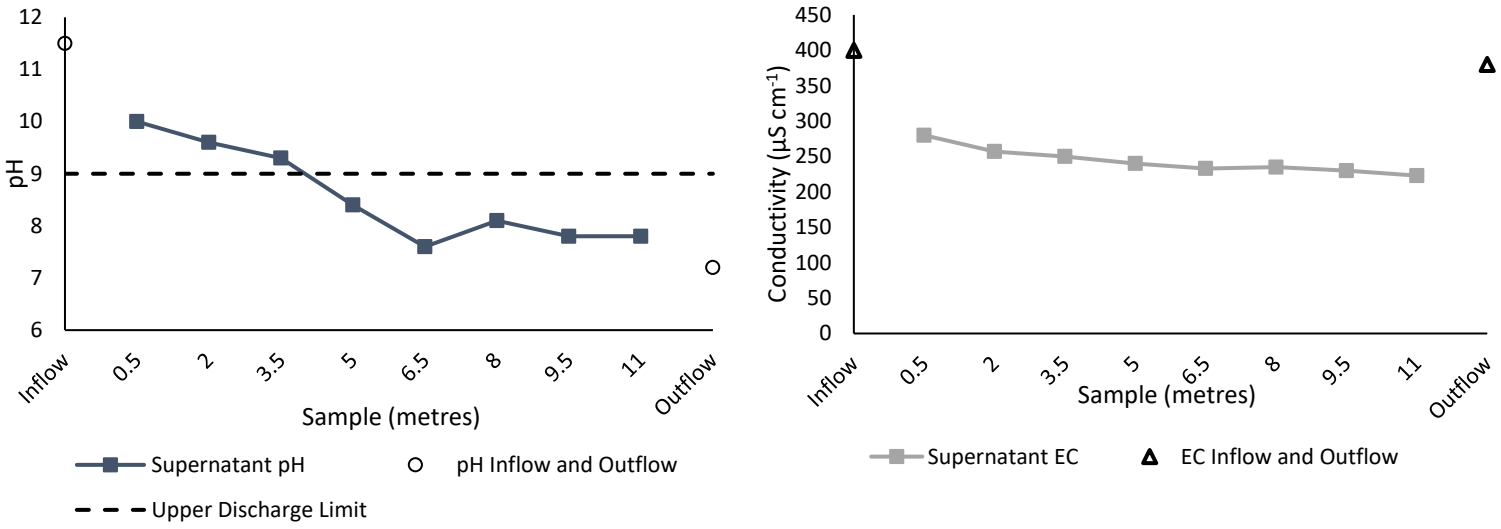


Figure 2: Wetland supernatant pH and EC (µS cm^{-1}) from inflow to outflow at 1.5m intervals across a constructed wetland treating BRDA leachate. The upper bound of pH discharge permitted under legislation is pH 9 (black dash -).

Trace Element Analysis

Reductions in supernatant concentrations of Al (94.9%), As (86.7%), Na (13%), K (50%) and V (57.6%) were observed along the length of the wetland (Table 1). Both Cr and Mg were below the limit of detection at <1 µg L^{-1} and <1mg L^{-1} respectively. There was no overall reduction in Ca concentration, which fluctuated between 2 – 6 mg L^{-1} through the wetland.

214 Na was the dominant cation, with concentrations up to 30 times greater than Ca and K.

215 Although highest Na concentrations were measured at the front sections of the wetland (up to

216 3.5m), they remained elevated along the entire length.

217 **Table 1: Trace element concentrations within supernatant samples along the length of**

218 **the constructed wetland.**

Sample (metres)	Al ($\mu g\ L^{-1}$)	As ($\mu g\ L^{-1}$)	V ($\mu g\ L^{-1}$)	Ca ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Na ($mg\ L^{-1}$)
0.5	13297	15	172	2	2	69
2	13371	24	183	2	1.2	67
3.5	11554	12	169	3.6	1.3	67
5	2402	6	181	2	1	65
6.5	1889	3	147	4	1	60
8	3590	4	130	6	2	61
9.5	788	2	72	3	1	61
11	672	2	73	2	1	60

219

220 **Substrate Analysis:**

221 ***pH and Conductivity***

222 There was a significant decrease in substrate pH along the length of the wetland, reducing

223 from average pH 8.6 at 0.5m to pH 6.4 at 11m, with the lowest pH (pH 6.0) recorded at 9.5m.

224 Average sediment EC decreased significantly from 618 µS/cm at 0.5m to 207 µS/cm at 11m.

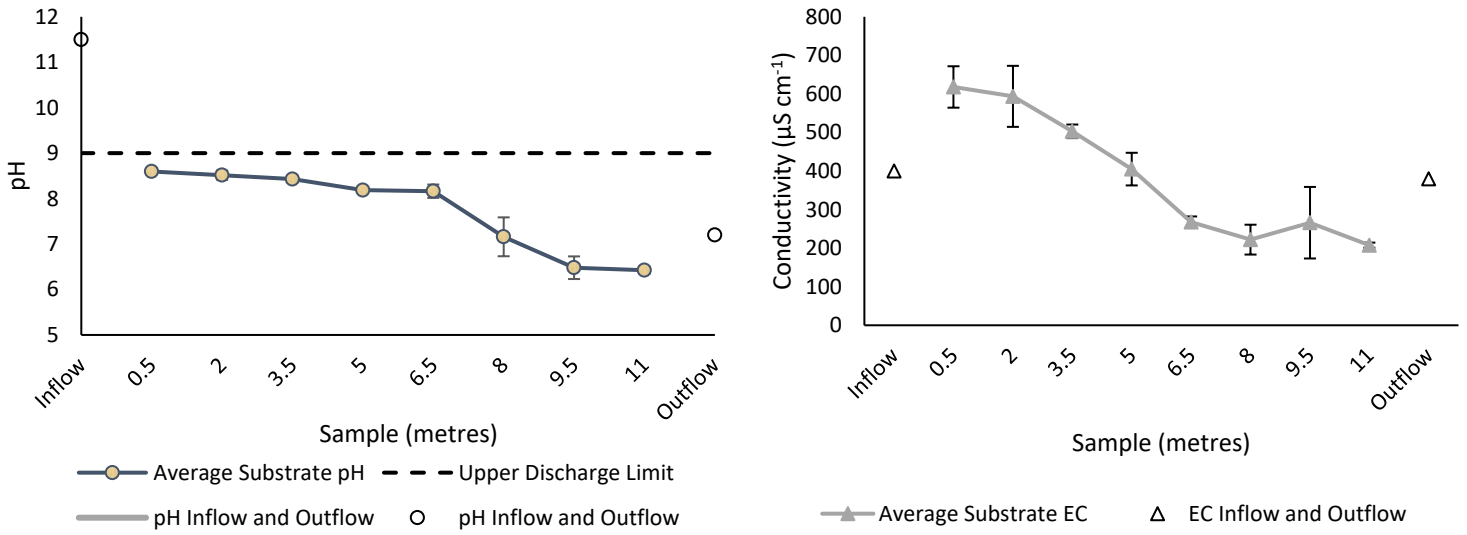


Figure 3: Average wetland supernatant pH (○) and EC (Δ, µS cm^{-1}) from inflow to outflow at 1.5m intervals along a constructed wetland treating BRDA leachate. All measurements were recorded daily over a 5.5 year period. Error bars indicate SD. The upper pH threshold of soil criteria applied to bauxite residue as proposed by Grafe and Klauber (2011) (black dash -).

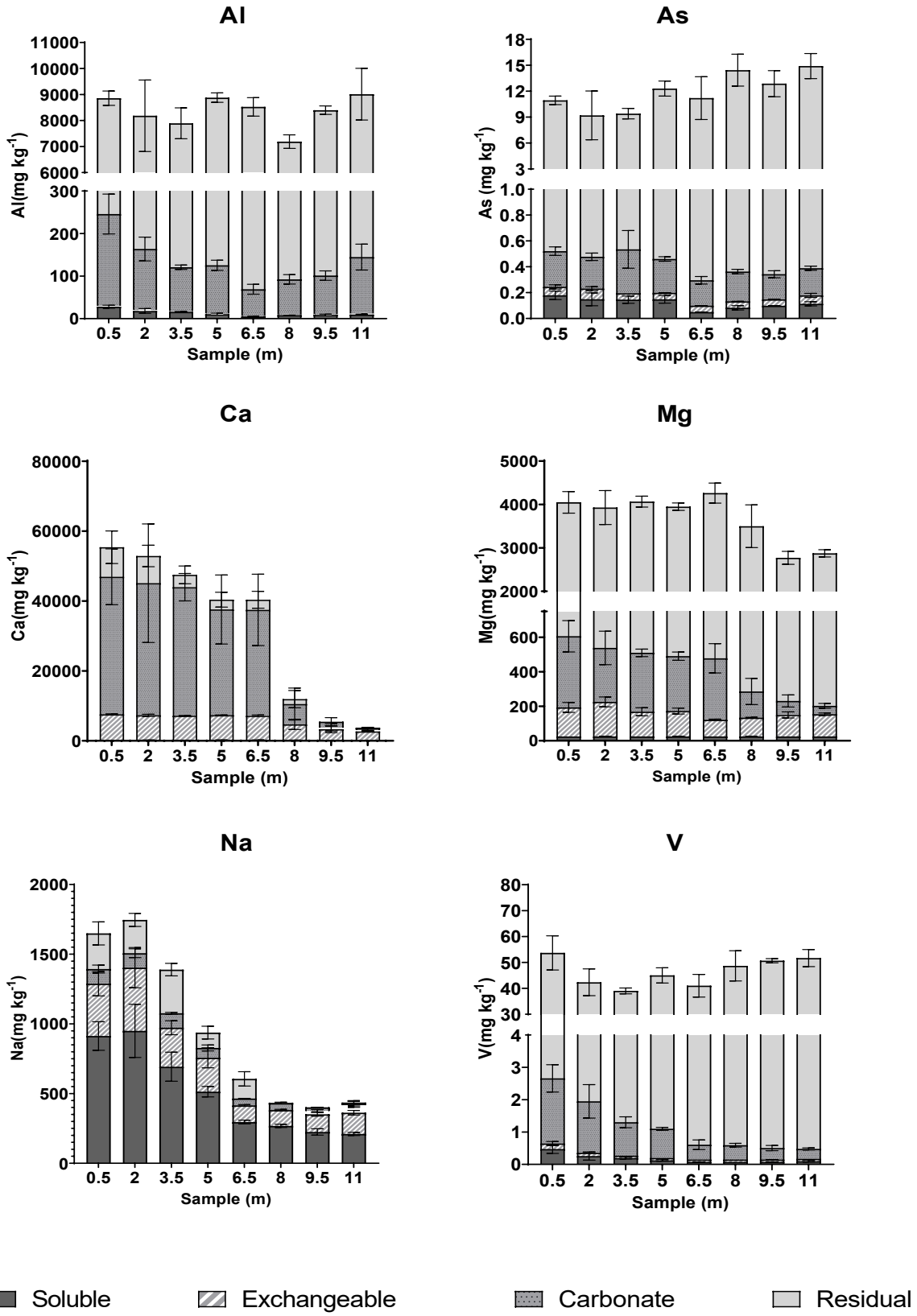
Sediment Analysis - Sequential Extraction

Total Na concentration in the upper portion (0-3.5m) of the wetland was 74% higher (p<0.05) than that measured near the outlet (Fig 4), with similar differences (p<0.05) for water soluble Na (76%) and exchangeable Na (59%).

Similarly, total Ca was 94% higher at 0.5m than at 11m (p< 0.05) and was mostly within the exchangeable and carbonate-bound fractions. The concentration of exchangeable Ca increased significantly with distance across the wetland, from 13-17% of the total Ca in the first 6.5 m of the wetland, to between 38–84% in the last 4.5 m. The opposite was observed with fraction of carbonate-bound Ca, which decreased significantly from 71-78% of the total Ca in the first 6.5 m to between 25-55% in the last 3 m.

For Al, As and V, most content was found within the remaining residual content, accounting for 97-99 % of Al, 94-97% of As, and 95-99% of V respectively. Soluble Al was significantly elevated in the upper portion of the wetland(0.5-3.5m). Carbonate Al was significantly higher at 0.5m compared to that in the later portion of the wetland from 6.5 and 9.5m ($p<0.05$). Elevated levels of soluble, exchangeable and carbonate-bound V are found in the upper portion of the wetland, with distribution of soluble and carbonate V changing significantly along the length ($p<0.05$). Similarly, As had elevated levels of soluble and carbonate-bound content in the first 5m of the wetland. Although average total content of As was 36% higher at 11m than that at 0.5m, it was not a significant change ($p>0.05$)

Total Mg content was also significantly elevated in the upper portion of the wetland, with 0.5m 70% greater than that at 11m ($p<0.05$). Of total Mg content, between 85-93% was present in a residual form that also showed significant elevation in the upper portion of the wetland ($p<0.05$). Carbonate-bound Mg also showed significant elevation in the upper wetland ($p<0.05$).



257

258 **Figure 4: Sequential fractionation of element content of wetland sediments along a**

259 **distance gradient in the wetland from inflow to outflow.**

Microbial Assessment

Microbial biomass differed significantly from 0.5m to 11m (Fig. 5), ranging from a mean biomass of 266 (3.5m) to 76 μg-C g^{-1} (6.5 m), with the values from 0.5-3.5m significantly differing to those from 5m-11m.

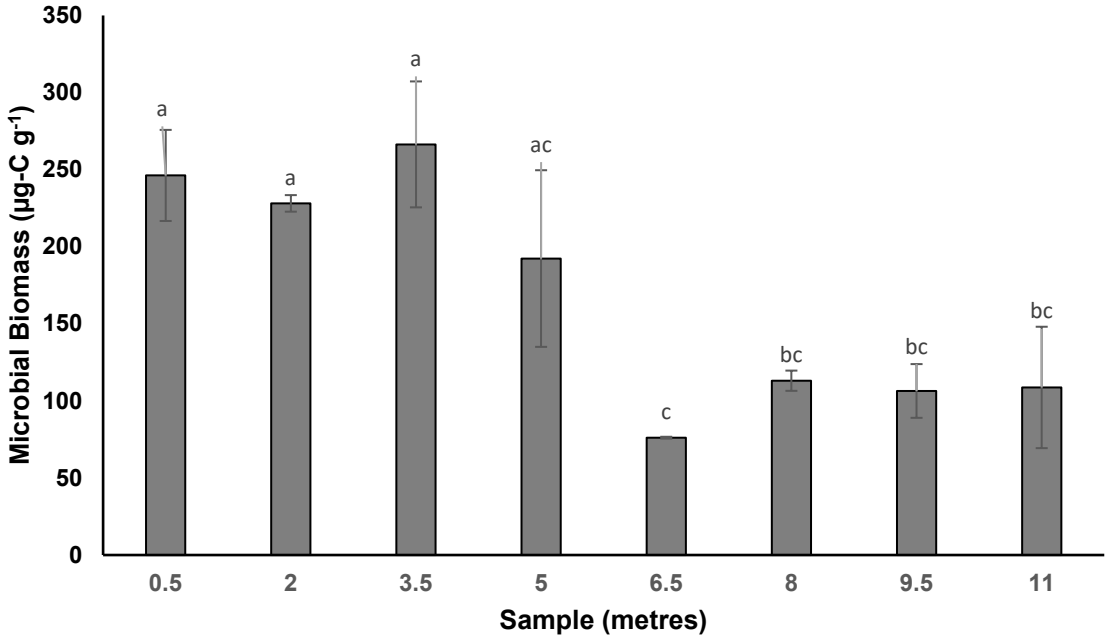


Figure 5: Microbial biomass (means =/- SE) in the sediment across the length of the wetland. Bars designated with the same letter are not significantly different (LSD; $p < 0.05$).

The first 3 principal components (PC) of the PCA generated from the PLFA data (Table S1) accounts for 59.2% of the total variation, with significant effects on the PC2 and PC3 axes (Fig. 6).

PLFAs with a positive loading (>0.8) on PC1, which accounts for 32% of the variation, include: 15:0i, 16:0i, Me17:0isomer2, 17:0i, ai 17:0, 17:0br and 17:0 (12Me), with the PLFA 18:1w7t having negative loading (>-0.8) on PC1. No other fatty acids had a strongly (>0.8) positive or negative loading on PC2 or PC3. PLFAs with the greatest loadings on PC2 and

PC3 were 20:4 (0.79) and 18:0(10Me) (-0.72), and 19:1w6 (0.6) and 18:2w6,9 (-0.79) respectively.

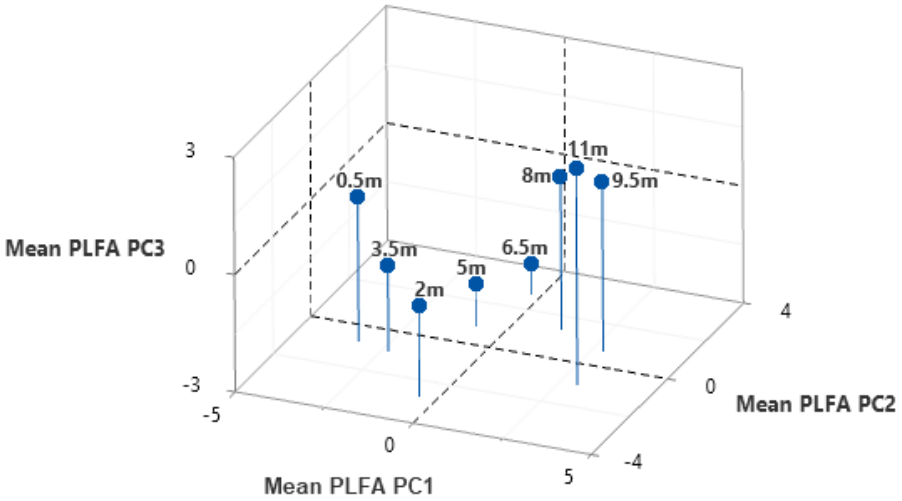


Figure 6: PCA score plot of means showing the effect of distance through the wetland on the PLFA profile. Data points are labelled with the sampling distance from 0.5-11m.

Comparison of the PLFA PC scores with soil chemical data demonstrates that PC1 was positively correlated (p<0.05) with Residual, Total K and Exchangeable K, Residual and Total V, and Residual and Total As. PC1 scores were negatively correlated (p<0.05) with Soluble, Exchangeable, Carbonate, Residual and Total Ca; Carbonate, Residual and Total K; Soluble, Exchangeable, Residual and Total Na; Supernatant pH and EC; Supernatant Total Al, As, Na and V; Sediment pH and EC; Carbonate, Residual and Total Mg and Carbonate V.

PC2 was positively correlated with Soluble K, Soluble Mg and Supernatant Ca; and negatively correlated (p<0.05) with Exchangeable Al, Carbonate Al, Exchangeable Na and Exchangeable Mg. PC3 was negatively correlated (p<0.05) with Residual Al, Total Al, and Residual Na and Soluble Ca. The strength of the PLFA correlations is displayed in Table 2.

Table 2: Correlation (p<0.05) between PLFA principal Components and Chemical Parameters. NS=not significant(p<0.05).

	PLFA PC1	PLFA PC2	PLFA PC3
Sediment pH	-0.579	NS	NS
Sediment EC	-0.699	NS	NS
Soluble Al	NS	NS	NS
Exchangeable Al	NS	-0.597	NS
Carbonate Al	NS	-0.411	NS
Residual Al	NS	NS	-0.458
Total Al	NS	NS	-0.427
Soluble As	NS	NS	NS
Exchangeable As	NS	NS	NS
Carbonate As	NS	NS	NS
Residual As	0.669	NS	NS
Total As	0.675	NS	NS
Soluble Ca	-0.567	NS	-0.524
Exchangeable Ca	-0.700	NS	NS
Carbonate Ca	-0.597	NS	NS
Residual Ca	-0.437	NS	NS
Total Ca	-0.612	NS	NS
Soluble K	NS	0.512	NS
Exchangeable K	0.427	NS	NS
Carbonate K	-0.442	NS	NS
Residual K	0.661	NS	NS
Total K	0.606	NS	NS
Soluble Mg	NS	0.426	NS
Exchangeable Mg	NS	-0.528	NS
Carbonate Mg	-0.608	NS	NS
Residual Mg	-0.543	NS	NS
Total Mg	-0.562	NS	NS
Soluble Na	-0.645	NS	NS
Exchangeable Na	-0.436	-0.453	NS
Carbonate Na	NS	NS	NS
Residual Na	-0.461	NS	-0.419
Total Na	-0.580	NS	NS
Soluble V	NS	NS	NS
Exchangeable V	NS	NS	NS
Carbonate V	-0.524	NS	NS
Residual V	0.477	NS	NS

	PLFA PC1	PLFA PC2	PLFA PC3
Total V	0.460	NS	NS
Supernatant pH	-0.778	NS	NS
Supernatant EC	-0.881	NS	NS
Supernatant Al Tot	-0.738	NS	NS
Supernatant As Tot	-0.826	NS	NS
Supernatant Ca Tot	NS	0.786	NS
Supernatant K Tot	NS	NS	NS
Supernatant Na Tot	-0.800	NS	NS
Supernatant V Tot	-0.786	NS	NS

294

295 The population biomarkers of gram-positive bacteria significantly increased across the length
296 of the wetland ($p< 0.05$). In contrast, gram negative bacteria ($p <0.05$) decreased across the
297 length of the cell. The total bacteria, ectomycorrhizal fungi and bacteria to fungal ratio did
298 not change significantly. The greatest change of all PLFAs was in 16:1w7c, the PLFA with
299 the largest %mol, which nearly halved from an average of 14.81±1.77 (0.5m) to 8.51±0.34
300 (11m).

301 The mean population sizes and significant correlations ($p<0.05$) are shown in Tables 3 and 4
302 respectively. There were no significant correlations between sediment chemical data and the
303 Bacteria to Fungal ratio. The strongest correlations were found between the gram-negative
304 bacteria and supernatant EC ($r_s = 0.905$), supernatant total Na ($r_s = 0.885$) and soluble Na ($r_s =$
305 0.851).

306

307

308

309

310

311 **Table 3: Mean relative abundance of signature PLFAs and ratios of microbial PLFAs.**

Sample Location	Total Bacteria		Gram Positive		Gram Negative		Ectomycorrhizal		Bacteria:Fungal Ratio	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
0.5m	17.63	1.12	16.59	1.04	17.55	1.20	4.33	0.58	.20	0.03
2m	18.72	0.52	17.35	0.51	17.12	0.61	3.50	0.26	.16	.006
3.5m	17.46	0.31	16.32	0.30	16.69	0.28	4.18	0.64	.19	0.17
5m	17.72	0.34	16.64	0.38	17.02	0.90	3.76	0.54	.17	0.021
6.5m	17.78	0.31	16.68	0.32	15.41	0.90	3.62	0.15	.17	.009
8m	18.98	1.71	17.86	1.68	13.38	0.49	6.01	1.75	.24	.07
9.5m	20.05	1.22	18.91	1.20	12.32	1.05	5.23	1.36	.20	0.05
11m	20.79	0.27	19.63	0.27	10.60	0.23	5.99	0.74	.22	.022

312

313 **Table 4: Correlation (p<0.05) between PLFA population biomarkers and Chemical**
314 **Parameters. NS=not significant (p<0.05).**

	Total Bacteria	Gram Positive Bacteria	Gram Negative Bacteria	Ectomycorrhizal Fungi
Sediment pH	-0.549	-0.569	0.801	-0.419
Sediment EC	-0.640	-0.649	0.809	NS
Soluble Al	NS	NS	0.494	NS
Exchangeable Al	NS	NS	NS	NS
Carbonate Al	NS	NS	NS	NS
Residual Al	NS	NS	NS	NS
Total Al	NS	NS	NS	NS
Soluble As	NS	NS	NS	NS
Exchangeable As	NS	NS	NS	NS
Carbonate As	NS	NS	NS	NS
Residual As	0.588	0.590	-0.620	NS
Total As	0.605	0.607	-0.607	NS
Soluble Ca	-0.685	-0.678	0.621	NS
Exchangeable Ca	-0.760	-0.760	0.729	NS
Carbonate Ca	-0.530	-0.534	0.810	NS
Residual Ca	NS	NS	0.507	NS
Total Ca	-0.527	-0.537	0.820	NS
Soluble K	NS	NS	NS	NS
Exchangeable K	0.437	0.434	-0.427	NS
Carbonate K	NS	NS	0.514	NS
Residual K	0.611	0.604	-0.566	NS
Total K	0.583	0.575	-0.537	NS
Soluble Mg	NS	NS	NS	NS
Exchangeable Mg	NS	NS	0.482	NS
Carbonate Mg	-0.568	-0.570	0.764	NS
Residual Mg	-0.516	-0.511	0.589	NS

	Total Bacteria	Gram Positive Bacteria	Gram Negative Bacteria	Ectomycorrhizal Fungi
Total Mg	-0.536	-0.536	0.631	NS
Soluble Na	-0.614	-0.622	0.851	NS
Exchangeable Na	NS	NS	0.614	NS
Carbonate Na	NS	NS	0.433	NS
Residual Na	-0.430	-0.439	0.682	NS
Total Na	-0.541	-0.555	0.803	NS
Soluble V	NS	NS	0.543	NS
Exchangeable V	NS	NS	NS	NS
Carbonate V	-0.503	-0.509	0.683	NS
Residual V	0.442	0.444	-0.419	NS
Total V	0.440	0.442	NS	NS
Supernatant pH	NS	NS	0.790	NS
Supernatant EC	-0.786	-0.786	0.905	NS
Supernatant Al Tot	NS	NS	0.786	NS
Supernatant As Tot	NS	NS	0.838	NS
Supernatant Ca Tot	NS	NS	NS	NS
Supernatant K Tot	NS	NS	NS	NS
Supernatant Na Tot	NS	NS	0.885	NS
Supernatant V Tot	-0.714	-0.714	0.738	NS
Microbial Biomass	NS	NS	0.483	NS

315

316 Microbial respiration decreased significantly ($p<0.05$) across the wetland for all substrates
317 (Figure 7). Principal components 1 and 2 accounted for 78.9% and 9.8% of the variation
318 respectively. All substrates were strongly negatively loaded on PC1 (>-0.8). On PC2, the
319 loadings were smaller, with glucose and water negatively loaded, -0.31 and -0.21
320 respectively, whilst aKG and plant substrates were positively loaded at 0.03 and 0.5.

321 Correlations between microbial respiration, chemical data and microbial populations reveal a
322 very strong correlation between gram negative bacteria and the plant substrate ($r_s = 0.88$).
323 Water was most strongly correlated with Soluble Ca ($r_s = 0.82$), plant to soluble Na (r_s
324 $= 0.913$), glucose to supernatant total V ($r_s = 0.786$) and aKG to supernatant EC ($r_s = 0.905$).

325

326

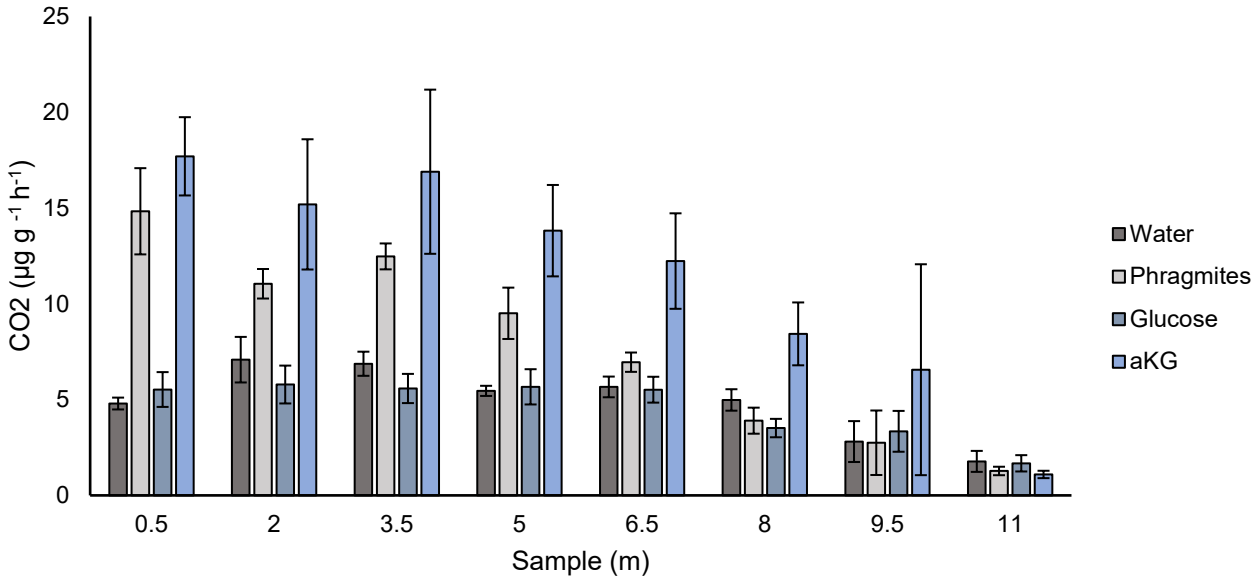


Figure 7: Rates of microbial respiration when supplied with different -CHO substrates, including water (grey stripe), *Phragmites australis* taken from the wetland (pale grey), glucose (dark blue), and α-ketoglutaric acid (pale blue).

Table 5: Correlations (p<0.05) between microbial respiration substrates, microbial populations and chemical data. NS=not significant (p>0.05).

	Water	Phragmites	Glucose	α-Ketoglutaric Acid
Sediment pH	0.577	0.843	0.537	0.583
Sediment EC	0.629	0.903	0.560	0.770
Soluble Al	NS	0.579	NS	NS
Exchangeable Al	NS	NS	NS	NS
Carbonate Al	NS	0.468	NS	NS
Residual Al	NS	NS	NS	NS
Total Al	NS	NS	NS	NS
Soluble As	NS	0.444	NS	NS
Exchangeable As	NS	NS	NS	NS
Carbonate As	NS	0.445	NS	0.431
Residual As	-0.415	-0.549	NS	NS
Total As	-0.406	-0.510	NS	NS
Soluble Ca	0.820	0.510	0.594	0.597
Exchangeable Ca	0.678	0.764	0.602	0.760
Carbonate Ca	0.514	0.766	0.504	0.478
Residual Ca	0.437	0.554	NS	NS
Total Ca	0.507	0.776	0.489	0.506
Soluble K	NS	NS	NS	NS

	Water	Phragmites	Glucose	α-Ketoglutaric Acid
Exchangeable K	-0.455	-0.448	NS	NS
Carbonate K	NS	NS	NS	NS
Residual K	NS	-0.545	NS	-0.507
Total K	NS	-0.525	NS	-0.432
Soluble Mg	NS	NS	NS	NS
Exchangeable Mg	NS	0.495	NS	0.457
Carbonate Mg	0.492	0.711	0.451	0.443
Residual Mg	0.507	0.472	NS	NS
Total Mg	0.488	0.511	NS	NS
Soluble Na	0.698	0.913	0.598	0.728
Exchangeable Na	NS	0.718	NS	0.549
Carbonate Na	NS	0.562	NS	NS
Residual Na	0.562	0.805	0.522	NS
Total Na	0.604	0.895	0.571	0.674
Soluble V	NS	0.632	NS	NS
Exchangeable V	NS	NS	NS	NS
Carbonate V	0.580	0.855	0.563	0.779
Residual V	-0.470	NS	NS	NS
Total V	-0.470	NS	NS	NS
Supernatant pH	NS	NS	NS	0.778
Supernatant EC	NS	0.857	0.762	0.905
Supernatant Al Tot	NS	0.810	NS	0.833
Supernatant As Tot	NS	0.826	NS	0.874
Supernatant Ca Tot	NS	NS	NS	NS
Supernatant K Tot	NS	NS	NS	NS
Supernatant Na Tot	NS	0.764	0.740	0.849
Supernatant V Tot	0.810	NS	0.786	0.762
Microbial Biomass	NS	0.681	NS	0.518
Total Bacteria	-0.618	-0.651	-0.424	-0.636
Gram Positive Bacteria	-0.615	-0.657	-0.433	-0.631
Gram Negative Bacteria	.572	0.880	0.529	0.622
Ectomycorrhizal Fungi	NS	NS	-0.570	NS
Bacteria:Fungal Ratio	NS	NS	NS	NS

332

Discussion

There has been increasing interest in the use of CWs for the treatment of industrial and minewater leachates over the last 40 years, owing to their potential to provide a passive, cost-effective approach for leachate remediation. Most longevity-related studies to date are over short time periods (<5 years) and focus on acidic drainage sites (Brodie *et al.* 1988; Hedin *et al.* 1994; Matthies *et al.* 2010; Pat-Espadas *et al.* 2018). Often, analysis of soil media within CWs has been overlooked, with a focus on water quality taking precedent over soil (Gill *et al.* 2014; Gill *et al.* 2017). The data in this current study demonstrates sustained treatment of alkaline leachate from a CW which has been in operation for 5.5+ years.

Supernatant

Decreases in pH, EC, Al, As, V, Na and K were observed across the length of the cell, with elevated concentrations found in the supernatant from the front portion of the wetland, nearest the inlet.

The primary objective for this study site is the reduction of the leachate pH, needing to meet a target of a pH<9 (Environmental Protection Agency 2021). Data provided in this campaign showed the pH reduction is sustained after 5.5 years, with decrease from pH 11 to 7.8 achieved. Reduction in leachate pH to below the target value of 9 by 5m into the wetland indicates that after 5.5 years, there is sufficient treatment capacity in the wetland. Previous investigations on CW treating alkaline leachate have had varied results: Higgins *et al.* (2017) and O'Connor and Courtney (2020) report sustained leachate pH reductions in bauxite residue leachate from 10.2-11.2 to below pH 9. Although Gomes *et al.* (2019) reported a reduction in steel slag leachate pH, from 12.8 to 10.5 and 10.3 in two separate wetland reedbed systems, it failed to reach the discharge threshold of pH<9. The mechanisms suggested for pH reduction in CWs include carbonation, microbial respiration and the production of organic acids and root exudates (Mayes *et al.* 2009; Higgins *et al.* 2017;

358 O'Connor and Courtney 2020). This is supported by the finding that microbial respiration
359 rates, in addition to microbial biomass, are found to be greatest in the first 5m of the wetland.

360 In addition to pH, the reduction of Al, As and V at rates of 94.9, 86.7 and 57.6% show that
361 removal rates have been sustained over a 5.5 yr operating period. An Al removal rate of
362 >90% was reported in the BRDA CW by Higgins *et al.* (2017) and O'Connor and Courtney
363 (2020). A similar rate >90% was reported in CWs treating municipal wastewater by
364 Vymazal and Krasa (2003) and Kröpfelová *et al.* (2009) as well as in domestic wastewater
365 (Lesage, (2007). The reduction in As by 86.7% is similar to the 84% reported by Higgins *et*
366 *al.* (2017) , and exceeds the 50% removal reported by Gomes (2019) and the efflux of -59%
367 reported by Kröpfelová *et al.* (2009). V removal was also greater than that found by
368 Kröpfelová *et al.* (23.8%) and Gomes *et al.* (0%) (Kröpfelová *et al.* 2009; Gomes *et al.*
369 2019).

370 Hua *et al.* (2015) proposed that a pH of 6-7 is necessary for efficient removal of trace
371 elements from BR leachate (Hua *et al.* 2015) and may account for the low rate of V reduction
372 reported in steel slag leachate (pH>10) by Gomes *et al.* (2019). Conversely, it may also be
373 responsible for the reduction of V reported in this CW.

374 It is worth noting that CW designs differ, with substrates playing a key role in providing
375 different removal properties (Wang *et al.* 2020), particularly as substrates are the host sites
376 for microbes and respiration (Truu *et al.* 2009). The main substrate in this CW is a soil-
377 organic compost mix fed with diluted 'fresh' leachate, whereas Hua *et al*. (2015) utilised
378 industrial waste materials as the substrate and seawater neutralised BR leachate which may
379 account for differing metal removal rates.

380 Importantly, several metal concentrations in the CW outflow are within drinking water
381 standards outlined under the European Union Drinking Water Regulations (*European Union*

382 *(Drinking Water) Regulations 2014*). At no point in the wetland does the Cr, Na or
383 supernatant EC exceed threshold values of 50 µg L^{-1} , 200 mg L^{-1} or 2500 µS cm^{-1}
384 respectively (*European Union (Drinking Water) Regulations 2014* ; Environmental
385 Protection Agency 2014). Additionally, supernatant As falls below the threshold of 10 µg L^{-1}
386 at 5 m into the wetland (Environmental Protection Agency 2014). In the supernatant Al at
387 11m (672µg/L) exceed the threshold for Drinking Water which is 200 µg/L but is below
388 688ug/L reported occurring in Irish groundwater (Environmental Protection Agency 2014;
389 Tedd *et al.* 2016). Additionally, when compared to regulations applied in the UK to surface
390 water discharges, the supernatant Al is below the 1mg/L maximum allowable concentration
391 for dissolved Al. Vanadium is not a measured parameter in the Drinking Water Regulations,
392 but globally river contents are low averaging 0.71µg/l, but in the US near uranium ore
393 regions where V naturally occurs can contain V concentrations between 20-220µg/l (Agency
394 for Toxic Substances and Disease Registry 2012; Kabata-Pendias and Szteke 2015). Further
395 monitoring is essential, as although it remains below the regulatory threshold, there is a
396 reduction in the rate of removal of Na in the supernatant: with a removal rate of 13% after 5.5
397 years operation in comparison to a 31% reduction reported by Higgins *et al.* (2017) after 1
398 year.

399 ## Sediments

400 Highest sediment pH was recorded at the front 6.5 m of the wetland, measuring between 8.6
401 at 0.5m and 8.1 at 6.5m: lower values were recorded for 8m, 9.5m and 11m at pH 7.2, 6.5 and
402 6.4 respectively (Fig. 2). The decreasing trend mirrors that of the supernatant pH. The higher
403 pH near the inlet reflects that soil pH has increased following additions of BR leachate
404 (Ruyters *et al.* 2011; Higgins *et al.* 2016; O'Connor and Courtney 2020). The increased
405 number of measuring intervals used further builds on the earlier work to provide a detailed
406 picture of the pH gradient across the cell: pH is elevated in sediments nearest the inlet

compared to those near the outlet, with elevated pH present approximately halfway through the cell. This demonstrates a clear front-loading effect, with the wetland displaying a potential for further pH amelioration due to the lower pH in the latter 4.5m.

The sampling transect also reveals elevated concentrations of total V, Na, Mg and soluble Al in the first 3.5 m. In addition to the pH and EC, this clearly shows a front-loading phenomena is occurring within the wetland cell. This has been reported elsewhere, with the largest concentrations of contaminants found within the first few metres of sediment nearest the inlet (Gill *et al.* 2017; Higgins *et al.* 2017; O'Connor and Courtney 2020).

Al, As and V were mostly found in the less bioavailable residual fractions of the wetland sediment (Fig. 3.2). Immobilisation and retention of Al and V in wetland sediments has been reported previously: V was found to be present in carbonate and extractable oxides, which are more recalcitrant (Mayes *et al.* 2011; Higgins *et al.* 2017; O'Connor and Courtney 2020). Hua *et al*. suggested removal of Al by precipitation required a pH<8, owing to formation of $Al(OH)_4$ between pH 8-8.6 (Hua *et al.* 2015). However, Burke *et al.* (2012)reported 99.7% of Al removed at a pH of 8.3. The pH range in this cell is from 8.6 to 6.4, with the soluble Al content of the sediment and the total in the supernatant both decreasing across the cell, which corroborates both Hua *et al.*'s and Burke *et al.*'s findings. Additionally, it has been identified that solubility of V decreases as pH decreases (Reijonen *et al*. (2016).

Ca concentrations decrease significantly across the wetland with total concentration decreasing by 93.8%. Such a trend has been reported previously in CWs treating BR leachate (Higgins *et al.* 2017; O'Connor and Courtney 2020). Most Ca was found in the first 6.5 m in carbonate (71-77%) and exchangeable (13-17%) forms. This changes to be predominantly exchangeable Ca from 6.5m to 11m, with 38-83% exchangeable and 24-55% carbonate-bound. The significant correlation between these Ca fractions and pH can be attributed to the

role of Ca in neutralising pH by forming $CaCO_3$, especially in the first 6.5 m of the wetland, as illustrated in other alkaline leachate CWs (Mayes and Younger 2006; Buckley *et al.* 2016; Higgins *et al.* 2017; Gomes *et al.* 2019; O'Connor and Courtney 2020).

Total Na decreased across the length of the cell, however, content in the first 2m were approximately double that reported in baseline samples (Higgins *et al.* 2017). Soluble Na was 9% in the baseline soil, and in this campaign was found to be between 48.6 and 61.9% of the total Na present. Although the total Na has increased from the baseline of Higgins *et al* (2017) the exchangeable sodium percentage is still <15%, so the sediment is not considered sodic. Monitoring will be needed in future, as high Na concentrations can influence nutrient uptake in plants and can lead to deficiency problems (Ruyters *et al.* 2011); deficiencies are hypothesised to have a greater risk to macrophytes in treatment wetlands rather than direct toxicity (Gomes *et al.* 2019). Although there is front loading reported in the wetland, there is has were no visible indications of stress observed in the macrophytes present. The front-loading and increase in the soluble fraction over the past 5.5 years must also be considered when accounting for the rate of removal of Na in the supernatant. The release of soluble Na from the substrate to the supernatant may account for the reduced rate of removal in the supernatant when compared to the first year of operation, or may ultimately happen upon saturation of the available sorption sites. Closer investigation of this mechanism in alkaline wetlands is needed.

Microbial

Microbial biomass, respiration rates, and microbial communities, have been shown to be affected by pH (Lauber *et al.* 2009; Rousk *et al.* 2010; Urbanová *et al.* 2011) and Na concentrations (Yang *et al.* 2020) as well as salinity (Fei Xi *et al.* 2014). Elevated pH and Na in bauxite residues have been shown to result in decreased microbial biomass and activity (Courtney *et al.* 2014). In this wetland however, both microbial biomass and respiration rates

were greatest in the first 5 m of the wetland, where pH and Na content were highest. A lack of negative correlation between increased Na and microbial respiration has been noted elsewhere. Rietz and Haynes (2003) attributed it to a stress response from the microbial community. Conversely, Wong *et al.* (2008) attributed high soil microbial biomass and respiration in a high salinity treatment of soils to additional substrate becoming available due to increased solubility of soil organic matter. Additionally, the adaption of the microbial population by a shift in community tolerant of the conditions present is also possible(Wong *et al.* 2008). Further investigation is warranted to determine the species present across the wetland.

In the study of microbial community composition and function responses to emergent plant rhizospheres of an estuarine CW, Fang *et al.* (2021) found microbial abundance was greatest under *Phragmites australis* stands versus *Typha latifola* and *Cyperus alternifolius* . Root exudates and oxygen-reduction potential generated from *Phragmites australis* were suspected to be the reason for the differing abundances (Fang *et al.* 2021). The wetland vegetation within this CW comprises a *Phragmites australis* monoculture in the upper half of the wetland, which diversifies after approximately 6.5m, to include rushes, mosses and yellow flag iris (unpublished data/personal observation). Additionally, the higher biomass in the first 3.5m is counter to the findings of Gagnon *et al.* (2007) and Zhang *et al.* (2010) who reported microbial biomass was observed to increase along with species richness.

Examination of the PLFA profiles and population biomarkers and their correlations with chemical parameters reveals that gram negative bacteria in this wetland have a positive corelation with pH in both sediment and supernatant, whereas total bacteria and gram-positive bacteria has a negative correlation with pH.

Investigations of microbial communities in bauxite residue found the gram-negative bacteria (mol%) was greatest in unamended residue compared to the remediated areas where gypsum and compost amendments had been applied (Courtney *et al.* 2014). In wetlands, gram-negative bacteria have been found to be the dominant group in the rhizosphere (Chaudhry *et al.* 2005; Marchand *et al.* 2010), in addition to river sediments (Feris *et al.* 2003). Soils of monoculture stands have also been shown to contain a higher proportion of gram-negative bacteria present in their microbial communities, but that decreases as communities develop (Tscherko *et al.* 2004). The elevated gram negative biomarkers in the first 3.5m, where supernatant and substrate contain elevated pH and Na as in unamended bauxite, corresponds to the monoculture of *Phragmites australis* as per Chaudry *et al.* (2005) and Marchand *et al*. (2010). Additionally, diversification of vegetation after 6.5m, at where substrate pH falls below 8, corroborates with Tscherko *et al.* (2004)'s findings from terrestrial soils.

In addition to changes in vegetation influencing microbial communities, it has been described that pH tends to decrease with plant succession (Lauber *et al.* 2009; Rousk *et al.* 2010; Urbanová *et al.* 2011). Previous studies on bauxite residue show microbial communities are affected by restoration treatments, which are aimed to reduce alkalinity and salinity: with restoration, greater diversity of bacteria were found, along with plant communities (Schmalenberger *et al.* 2013). Organic matter and carbon content has been attributed to influencing the microbial populations in dairy water CWs, bauxite residue and estuarine systems (Nguyen 2000; Schmalenberger *et al.* 2013; Fang *et al.* 2021), and warrants further investigation in alkali treatment wetlands.

The microbial respiration investigation reveals, to the author's knowledge, for the first time the occurrence of a population of microbes within a CW treating alkaline leachates that are able to utilise endemic plant material growing on site for respiration. The microbes are able to cycle the complex material, with a greater rate than that of glucose (Fig. 5), implying the

505 presence of a complex microbial community. Similar respiration of plant residue was
506 demonstrated from samples from the northern Everglades water conservation areas reducing
507 P loading to the southern Everglades (Wright and Reddy 2007): but it must be noted such a
508 system has existed on far greater timescales for a complex microbial community to develop.

509 Primarily, the carbonation effect, which can be induced by microbial respiration, is key to
510 aiding the reduction in pH in alkaline leachates (Mayes *et al.* 2009; Mayes *et al.* 2009a;
511 Higgins *et al.* 2017; O'Connor and Courtney 2020). Recent work by Jiang *et al.* (2023)
512 highlights the role *Penicillium oxalicum* can play in reducing pH by proton neutralization and
513 Ca^{2+} exchange, as well as influencing other microbial species present in bauxite residue.
514 Additionally, the removal of trace elements in CWs is dependent on the number of sorption
515 sites available, which in surface flow wetlands, is primarily that in the sediment. Increased
516 organic matter is known to provide a greater number of removal sites and increased metal
517 retention (Deng *et al.* 2015): dead plant material contributes to the OM accumulation and
518 number of sorption sites available (Beining and Otte 1996; Beining and Otte 1997; Jacob and
519 Otte 2004; Marchand *et al.* 2010). The respiration rate produced by the microbial community
520 when fed endemic plant material provides evidence that systems can replenish their
521 adsorptive capacity somewhat.

522 **Longevity**

523 Although CWs have been proven as a sustainable method for the leachates of mine and
524 industry sites since at least the 1980s, there is a lack of evidence in the literature as to the
525 longevity over which they maintain their efficiency. Operational lifespans are generally
526 predicted to be within the 20-30 year time frame (PIRAMID Consortium 2003; Sheoran and
527 Sheoran 2006); but other sites are estimated to operate in excess of 100 (Beining and Otte
528 1996; Eger and Wagner 2002). Many projects to date have focused solely on the removal rate

529 of contaminants from waters, without accounting for the accumulation of contaminants in the
530 sediments (Gill *et al.* 2014; Gill *et al.* 2017).

531 Each site requires its own individual design dependent on local factors in order to maximise
532 lifespans, contaminant removal and reduce any leaching risks. Factors considered include:
533 substrate mixes/composition, contaminant loadings, hydraulic loading rates, retention time,
534 vegetation, climatic conditions and projected remedial work affecting influent (Nedrich *et al.*
535 2018; Khan *et al.* 2019; Hedin 2020; Khan *et al.* 2020). A potential oversight in the research,
536 design, and monitoring of CWs to date is that of the role of microbial communities present.
537 Although considered as a functional part of the system, little if any, data are available on
538 microbial communities in alkaline constructed treatment wetlands.

539 Microbial populations are inherent to the functioning of a wetland, responsible for cycling of
540 organic matter, creating additional sorption sites, and producing carbon dioxide The larger
541 biomass and respiration rate in the first 5m indicates the microbial community is not impeded
542 by the elevated pH, Na or metal contents, and is capable of utilising endemic, recalcitrant
543 carbon sources after 5.5 years of operation. Further work is required to determine a greater
544 depth of information on the microbial community present.

545 In addition to monitoring and fostering the microbial populations present, advances in
546 designs of integrated hybrid systems may also offer a potential means of increasing wetland
547 lifespans. Integrated systems have been utilised primarily for acidic mine waters (Hedin *et al.*
548 1994; Caraballo *et al.* 2011; Sapsford 2013; Hedin 2020) and sewage treatment (Kröpfelová
549 *et al.* 2009; Vymazal 2011) but aeration cascades have recently been trialled for the treatment
550 of alkaline leachates (Gomes *et al.* 2019). In steel slag leachates, decreases of pH by 1-1.5
551 units have been reported (Gomes *et al.* 2019), with active carbonation already shown as a
552 successful method for decreasing pH in bauxite residue (Higgins *et al.* 2018). Further

553 investigation is required to see if a passive cascade approach would provide similar results for
554 bauxite residue leachate.

555 **Conclusions**

556 Evidence is provided here that CWs are capable of successfully treating bauxite residue
557 leachate over a 5.5-year window of operation, obtaining an effluent pH<9 as per the operating
558 requirements. Most (>94%) Al, As and V in the substrate was present in residual forms, with
559 soluble contents greatest in the first 5m. Microbial biomass and respiration was found to be
560 greatest in the first 3.5m of the wetland. In addition, this study presents new evidence that
561 microbial populations found within this CW utilise the endemic, recalcitrant plant matter for
562 respiration in sediments containing elevated pH and metal contents. Further work is needed to
563 determine the vertical depths at which metals are retained within the substrate, the role of
564 organic matter and greater investigative emphasis on the role of microbial populations in
565 alkaline CWs.

566

567 **Acknowledgements**

568 This research was supported by funding from Aughinish Alumina.

References

Agency for Toxic Substances and Disease Registry (2012) *TOXICOLOGICAL PROFILE FOR VANADIUM*, Atlanta, Georgia: Agency for Toxic Substances and Disease Registry.

Balomenos, E., Davris, P., Sparis, D., Marinos, D., Vafeias, M., Koutsoupa, S., Koutalidi, S., Panias, D. and Karnachoritis10, I. (2021) 'Bauxite Residue Reuse Through Combined Operations: Industrial Pilot Modules', in *TRAVAUX 50, Proceedings of the 39th International ICSOBA Conference, 22-24 November 2021*.

Beining, B.A. and Otte, M.L. (1996) 'Retention of Metals Originating from an Abandoned Lead: Zinc Mine by a Wetland at Glendalough, Co. Wicklow', *Biology and Environment: Proceedings of the Royal Irish Academy*, 96B(2), 117-126.

Beining, B.A. and Otte, M.L. (1997) 'Retention of Metals and Longevity of a Wetland Receiving Mine Leachate', in *Proceedings of the 14th Annual Meeting of American Society of Mining and Reclamation*, Austin, TX, The American Society of Mining and Reclamation.

Brodie, G., Hammer, D. and Tomljanovich, D. (1988) 'Constructed Wetlands for Acid Drainage Control in the Tennessee Valley', in *Mine Drainage and Surface Mine Reclamation. Volume 1:Mine Water and Mine Waste*, Pittsburgh, PA, American Scociety of Surface Mining and Reclamation, 325-331.

Buckley, R., Curtin, T. and Courtney, R. (2016) 'The potential for constructed wetlands to treat alkaline bauxite residue leachate: laboratory investigations', *Environ Sci Pollut Res Int*, 23(14), 14115-22, available: http://dx.doi.org/10.1007/s11356-016-6582-8.

Burke, I.T., Mayes, W.M., Peacock, C.L., Brown, A.P., Jarvis, A.P. and Gruiz, K. (2012) 'Speciation of arsenic, chromium, and vanadium in red mud samples from the Ajka spill site, Hungary', *Environ Sci Technol*, 46(6), 3085-92, available: http://dx.doi.org/10.1021/es3003475.

Burke, I.T., Peacock, C.L., Lockwood, C.L., Stewart, D.I., Mortimer, R.J., Ward, M.B., Renforth, P., Gruiz, K. and Mayes, W.M. (2013) 'Behavior of aluminum, arsenic, and vanadium during the neutralization of red mud leachate by HCl, gypsum, or seawater', *Environ Sci Technol*, 47(12), 6527-35, available: http://dx.doi.org/10.1021/es4010834.

Caraballo, M.A., Macías, F., Rötting, T.S., Nieto, J.M. and Ayora, C. (2011) 'Long term remediation of highly polluted acid mine drainage: A sustainable approach to restore the environmental quality of the Odiel river basin', *Environmental Pollution*, 159(12), 3613-3619, available: http://dx.doi.org/10.1016/j.envpol.2011.08.003.

Chaudhry, Q., Blom-Zandstra, M., Gupta, S.K. and Joner, E. (2005) 'Utilising the Synergy between Plants and Rhizosphere Microorganisms to Enhance Breakdown of Organic Pollutants in the Environment (15 pp)', *Environmental Science and Pollution Research - International*, 12(1), 34-48, available: http://dx.doi.org/10.1065/espr2004.08.213.

Chockalingam, E. and Subramanian, S. (2006) 'Studies on removal of metal ions and sulphate reduction using rice husk and Desulfotomaculum nigrificans with reference to remediation of acid mine drainage', *Chemosphere*, 62(5), 699-708, available: http://dx.doi.org/10.1016/j.chemosphere.2005.05.013.

Courtney, R., Harris, J.A. and Pawlett, M. (2014) 'Microbial Community Composition in a Rehabilitated Bauxite Residue Disposal Area: A Case Study for Improving Microbial Community Composition', *Restoration Ecology*, 22(6), 798-805, available: http://dx.doi.org/10.1111/rec.12143.

Deng, L., Zeng, G., Fan, C., Lu, L., Chen, X., Chen, M., Wu, H., He, X. and He, Y. (2015) 'Response of rhizosphere microbial community structure and diversity to heavy metal co-pollution in arable soil', *Applied Microbiology and Biotechnology*, 99(19), 8259-8269, available: http://dx.doi.org/10.1007/s00253-015-6662-6.

Di Carlo, E., Chen, C.R., Haynes, R.J., Phillips, I.R. and Courtney, R. (2019) 'Soil quality and vegetation performance indicators for sustainable rehabilitation of bauxite residue disposal areas: a review', *Soil Research*, 57(5), 419-446, available: http://dx.doi.org/10.1071/sr18348.

Eger, P. and Wagner, J. (2002) 'THE USE OF WETLANDS TO REMOVE NICKEL FROM MINE DRAINAGE - IS PERPETUALTREATMENT REALLY POSSIBLE?', in *2002 National Meeting of the American Society of Mining and Reclamation*, Lexington, KY, ASMR, 798-817.

Environmental Protection Agency (2014) 'Drinking Water Parameters. Microbiological, Chemical and Indicator Parameters in the 2014 Drinking Water Regulations 2014. An overview of parameters and their importance.'.

Environmental Protection Agency (2021) 'Industrial Emissions Licence P0035-07', 64.

European Union (Drinking Water) Regulations 2014, Dublin: Stationary Office, available: https://www.irishstatutebook.ie/eli/2014/si/122/made/en/print?q=S.I.+No.+122&years=2014# [accessed.

Evans, K. (2016) 'The History, Challenges, and New Developments in the Management and Use of Bauxite Residue', *Journal of Sustainable Metallurgy*, 2(4), 316-331, available: http://dx.doi.org/10.1007/s40831-016-0060-x.

Fang, J., Dong, J., Li, C., Chen, H., Wang, L., Lyu, T., He, H. and Liu, J. (2021) 'Response of microbial community composition and function to emergent plant rhizosphere of a constructed wetland in northern China', *Applied Soil Ecology*, 168, 104141, available: http://dx.doi.org/https://doi.org/10.1016/j.apsoil.2021.104141.

Fei Xi, X., Wang, L., Jun Hu, J., Shu Tang, Y., Hu, Y., Hua Fu, X., Sun, Y., Fai Tsang, Y., Nan Zhang, Y. and Hai Chen, J. (2014) 'Salinity influence on soil microbial respiration rate of wetland in the Yangtze River estuary through changing microbial community', *Journal of Environmental Sciences*, 26(12), 2562-2570, available: http://dx.doi.org/https://doi.org/10.1016/j.jes.2014.07.016.

Feris, K.P., Ramsey, P.W., Frazar, C., Rillig, M.C., Gannon, J.E. and Holben, W.E. (2003) 'Structure and seasonal dynamics of hyporheic zone microbial communities in free-stone rivers of the estern United States', *Microbial Ecology*, 46(2), 200-215, available: http://dx.doi.org/10.1007/bf03036883.

Frostegård, A. and Bååth, E. (1996) 'The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil', *Biology and Fertility of Soils*, 22(1-2), 59-65, available: http://dx.doi.org/10.1007/bf00384433.

Frostegård, Å., Tunlid, A. and Bååth, E. (1993) 'Phospholipid Fatty Acid Composition, Biomass, and Activity of Microbial Communities from Two Soil Types Experimentally Exposed to Different Heavy Metals', *Applied and Environmental Microbiology*, 59(11), 3605-3617, available: http://dx.doi.org/doi:10.1128/aem.59.11.3605-3617.1993.

Frostegård, Å., Tunlid, A. and Bååth, E. (2011) 'Use and misuse of PLFA measurements in soils', *Soil Biology and Biochemistry*, 43(8), 1621-1625, available: http://dx.doi.org/10.1016/j.soilbio.2010.11.021.

Gagnon, V., Chazarenc, F., Comeau, Y. and Brisson, J. (2007) 'Influence of macrophyte species on microbial density and activity in constructed wetlands', *Water Science and Technology*, 56(3), 249-254, available: http://dx.doi.org/10.2166/wst.2007.510.

Gill, L.W., Ring, P, Higgins, N.M.P. and Johnston, P.M. (2014) 'Accumulation of heavy metals in a constructed wetland treating road runoff', *Ecological Engineering*, 70, 133-139.

Gill, L.W., Ring, P., Casey, B., Higgins, N.M.P. and Johnston, P.M. (2017) 'Long term heavy metal removal by a constructed wetland treating rainfall runoff from a motorway', *Science of the Total Environment*, 601-602, 32-44.

Gomes, H.I., Mayes, W.M., Whitby, P. and Rogerson, M. (2019) 'Constructed wetlands for steel slag leachate management: Partitioning of arsenic, chromium, and vanadium in

waters, sediments, and plants', *Journal of Environmental Management*, 243, 30-38, available: http://dx.doi.org/https://doi.org/10.1016/j.jenvman.2019.04.127.

Gräfe, M. and Klauber, C. (2011) 'Bauxite residue issues: IV. Old obstacles and new pathways for in situ residue bioremediation', *Hydrometallurgy*, 108(1), 46-59, available: http://dx.doi.org/https://doi.org/10.1016/j.hydromet.2011.02.005.

Hedin, R., Watzlaf, G. and R, N. (1994) 'Passive treatment of acid mine drainage with limestone', *Journal of Environmental Quality*, 23(6), 1338-1345.

Hedin, R.S. (2020) 'Long-term Performance and Costs for the Anna S Mine Passive Treatment Systems', *Mine Water and the Environment*, 39(2), 345-355, available: http://dx.doi.org/10.1007/s10230-020-00676-9.

Higgins, D., Curtin, T., Burke, I. and Courtney, R. (2018) 'The potential for constructed wetland mechanisms to treat alkaline bauxite residue leachate: carbonation and precipitate characterisation', *Environmental Science and Pollution Research*, 25(29), 29451-29458.

Higgins, D., Curtin, T. and Courtney, R. (2017) 'Effectiveness of a constructed wetland for treating alkaline bauxite residue leachate: a 1-year field study', *Environmental Science and Pollution Research*, 24(9), 8516-8524, available: http://dx.doi.org/10.1007/s11356-017-8544-1.

Higgins, D., Curtin, T., Pawlett, M. and Courtney, R. (2016) 'The potential for constructed wetlands to treat alkaline bauxite-residue leachate: Phragmites australis growth', *Environ Sci Pollut Res Int*, 23(23), 24305-24315, available: http://dx.doi.org/10.1007/s11356-016-7702-1.

Hua, T., Haynes, R.J. and Zhou, Y.F. (2019) 'Removal of Al, Ga, As, V and Mo from alkaline wastewater using pilot-scale constructed wetlands', *Environ Sci Pollut Res Int*, 26(34), 35121-35130, available: http://dx.doi.org/10.1007/s11356-019-06490-3.

Hua, T., Haynes, R.J., Zhou, Y.F., Boullemant, A. and Chandrawana, I. (2015) 'Potential for use of industrial waste materials as filter media for removal of Al, Mo, As, V and Ga from alkaline drainage in constructed wetlands--adsorption studies', *Water Res*, 71, 32-41, available: http://dx.doi.org/10.1016/j.watres.2014.12.036.

Jacob, D.L. and Otte, M.L. (2004) 'Influence of Typha latifolia and fertilization on metal mobility in two different Pb–Zn mine tailings types', *Science of the Total Environment*, 333(1-3), 9-24, available: http://dx.doi.org/10.1016/j.scitotenv.2004.05.005.

746 Jenkinson, D.S. and Powlson, D.S. (1976) 'The effects of biocidal treatments on metabolism
747 in soil—I. Fumigation with chloroform', *Soil Biology and Biochemistry*, 8(3), 167-
748 177, available: http://dx.doi.org/https://doi.org/10.1016/0038-0717(76)90001-8.

749
750 Jiang, Y., Qin, X., Zhu, F., Zhang, Y., Zhang, X., Hartley, W. and Xue, S. (2023) 'Halving
751 gypsum dose by Penicillium oxalicum on alkaline neutralization and microbial
752 community reconstruction in bauxite residue', *Chemical Engineering Journal*, 451,
753 139008, available: http://dx.doi.org/https://doi.org/10.1016/j.cej.2022.139008.

754
755 Joyce, P.J. and Björklund, A. (2019) 'Using Life Cycle Thinking to Assess the Sustainability
756 Benefits of Complex Valorization Pathways for Bauxite Residue', *Journal of*
757 *Sustainable Metallurgy*, 5(1), 69-84, available: http://dx.doi.org/10.1007/s40831-019-
758 00209-x.

759
760 Kabata-Pendias, A. and Szteke, B. (2015) 'Trace Elements in Abiotic and Biotic
761 Environments', available: http://dx.doi.org/10.1201/b18198.

762
763 Kadlec, R. and Wallace, S. (2009) *Treatment Wetlands*, Boca Raton: CRC Press, Taylor and
764 Francis Group.

765
766 Kaiser, C., Frank, A., Wild, B., Koranda, M. and Richter, A. (2010) 'Negligible contribution
767 from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2ω6,9 and
768 18:1ω9', *Soil Biology and Biochemistry*, 42(9), 1650-1652, available:
769 http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2010.05.019.

770
771 Khan, U.A., Kujala, K., Nieminen, S.P., Raisanen, M.L. and Ronkanen, A.K. (2019)
772 'Arsenic, antimony, and nickel leaching from northern peatlands treating mining
773 influenced water in cold climate', *Sci Total Environ*, 657, 1161-1172, available:
774 http://dx.doi.org/10.1016/j.scitotenv.2018.11.455.

775
776 Khan, U.A., Kujala, K., Planer-Friedrich, B., Räisänen, M.L. and Ronkanen, A.-K. (2020)
777 'Long-term data reveals the importance of hydraulic load and inflow water quality for
778 Sb removal in boreal treatment peatlands', *Ecological Engineering*, 148, 105785,
779 available: http://dx.doi.org/10.1016/j.ecoleng.2020.105785.

780
781 Kröpfelová, L., Vymazal, J., Švehla, J. and Štíchová, J. (2009) 'Removal of trace elements in
782 three horizontal sub-surface flow constructed wetlands in the Czech Republic',
783 *Environmental Pollution*, 157(4), 1186-1194.

784
785 Lauber, C.L., Hamady, M., Knight, R. and Fierer, N. (2009) 'Pyrosequencing-Based
786 Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the
787 Continental Scale', *Applied and Environmental Microbiology*, 75(15), 5111-5120,
788 available: http://dx.doi.org/doi:10.1128/AEM.00335-09.

789

Lehoux, A.P., Lockwood, C.L., Mayes, W.M., Stewart, D.I., Mortimer, R.J.G., Gruiz, K. and Burke, I.T. (2013) 'Gypsum addition to soils contaminated by red mud: implications for aluminium, arsenic, molybdenum and vanadium solubility', *Environmental Geochemistry and Health*, 35(5), 643-656, available: http://dx.doi.org/10.1007/s10653-013-9547-6.

Lesage, E., Rousseau, D.P.L., Meers, E., Tack, F.M.G. and De Pauw, N. (2007) 'Accumulation of metals in a horizontal subsurface flow constructed wetland treating domestic wastewater in Flanders, Belgium', *Science of the Total Environment*, 380(1-3), 102-115, available: http://dx.doi.org/10.1016/j.scitotenv.2006.10.055.

Marchand, L., Mench, M., Jacob, D.L. and Otte, M.L. (2010) 'Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: A review', *Environmental Pollution*, 158(12), 3447-3461, available: http://dx.doi.org/10.1016/j.envpol.2010.08.018.

Matthies, R., Aplin, A. and Jarvis, A. (2010) 'Performance of a passive treatment system for net-acidic coal mine drainage over five years of operation', *Science of the Total Environment*, 408(20), 4877-4885.

Mayes, W.M., Aumonier, J. and Jarvis, A.P. (2009) 'Preliminary evaluation of a constructed wetland for treating extremely alkaline (pH 12) steel slag drainage', *Water Science and Technology*, 59(11), 2253-2263.

Mayes, W.M., Batty, L.C., Younger, P.L., Jarvis, A.P., Kõiv, M., Vohla, C. and Mander, U. (2009a) 'Wetland treatment at extremes of pH: A review', *Science of the Total Environment*, 407(13), 3944-3957, available: http://dx.doi.org/10.1016/j.scitotenv.2008.06.045.

Mayes, W.M., Jarvis, A.P., Burke, I.T., Walton, M., Feigl, V., Klebercz, O. and Gruiz, K. (2011) 'Dispersal and attenuation of trace contaminants downstream of the Ajka bauxite residue (red mud) depository failure, Hungary', *Environ Sci Technol*, 45(12), 5147-55, available: http://dx.doi.org/10.1021/es200850y.

Mayes, W.M. and Younger, P.L. (2006) 'Buffering of Alkaline Steel Slag Leachate across a Natural Wetland', *Environmental Science & Technology*, 40(4), 1237-1243, available: http://dx.doi.org/10.1021/es051304u.

Nedrich, S., Chappaz, A., Hudson, M.L., Brown, S.S. and Burton Jr., G.A. (2018) 'Biogeochemical controls on the speciation and aquatic toxicity of vanadium and other metals in sediments from a river reservoir', *Science of the Total Environment*, 612, 313-320.

Nguyen, L.M. (2000) 'Organic matter composition, microbial biomass and microbial activity in gravel-bed constructed wetlands treating farm dairy wastewaters', *Ecological*

Engineering, 16(2), 199-221, available: http://dx.doi.org/10.1016/s0925-8574(00)00044-6.

O'Connor, G. and Courtney, R. (2020) 'Constructed wetlands for the treatment of bauxite residue leachate: Long term field evidence and implications for management', *Ecological Engineering*, 158, 106076, available: http://dx.doi.org/10.1016/j.ecoleng.2020.106076.

Olsson, P.A. (1999) 'Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil', *FEMS Microbiology Ecology*, 29(4), 303-310, available: http://dx.doi.org/10.1111/j.1574-6941.1999.tb00621.x.

Olszewska, J.P., Meharg, A.A., Heal, K.V., Carey, M., Gunn, I.D., Searle, K.R., Winfield, I.J. and Spears, B.M. (2016) 'Assessing the Legacy of Red Mud Pollution in a Shallow Freshwater Lake: Arsenic Accumulation and Speciation in Macrophytes', *Environ Sci Technol*, 50(17), 9044-52, available: http://dx.doi.org/10.1021/acs.est.6b00942.

Pat-Espadas, A.M., Loredo Portales, R., Amabilis-Sosa, L.E., Gómez, G. and Vidal, G. (2018) 'Review of constructed wetlands for acid mine drainage treatment.', *Water*, 10(11), 1685.

Pawlett, M., Ritz, K., Dorey, R.A., Rocks, S., Ramsden, J. and Harris, J.A. (2013) 'The impact of zero-valent iron nanoparticles upon soil microbial communities is context dependent', *Environmental Science and Pollution Research*, 20(2), 1041-1049, available: http://dx.doi.org/10.1007/s11356-012-1196-2.

PIRAMID Consortium (2003) *Engineering guidelines for the passive remediation of acidic and/or metalliferous mine drainage and similar wastewaters. European Commission 5th Framework RTD Project no. EVK1-CT-1999-000021 "Passive in-situ remediation of acidic mine / industrial drainage" (PIRAMID).* available: http://www.imwa.info/piramid/files/PIRAMIDGuidelinesv10.pdf [accessed 2019-10-31].

Power, G., Gräfe, M. and Klauber, C. (2011) 'Bauxite residue issues: I. Current management, disposal and storage practices', *Hydrometallurgy*, 108(1), 33-45, available: http://dx.doi.org/https://doi.org/10.1016/j.hydromet.2011.02.006.

Reijonen, I., Metzler, M. and Hartikainen, H. (2016) 'Impact of soil pH and organic matter on the chemical bioavailability of vanadium species: The underlying basis for risk assessment', *Environmental Pollution*, 210, 371-379, available: http://dx.doi.org/https://doi.org/10.1016/j.envpol.2015.12.046.

Residue Solutions (2007) *Residue Management Sustainability Review Aughinish Alumina Limited*, Brisbane, Queensland, available:

https://epawebapp.epa.ie/licences/lic_eDMS/090151b280195475.pdf [accessed 11.1.2022].

Rietz, D.N. and Haynes, R.J. (2003) 'Effects of irrigation-induced salinity and sodicity on soil microbial activity', *Soil Biology and Biochemistry*, 35(6), 845-854, available: http://dx.doi.org/10.1016/s0038-0717(03)00125-1.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R. and Fierer, N. (2010) 'Soil bacterial and fungal communities across a pH gradient in an arable soil', *The ISME Journal*, 4(10), 1340-1351, available: http://dx.doi.org/10.1038/ismej.2010.58.

Ruyters, S., Mertens, J., Vassilieva, E., Dehandschutter, B., Poffijn, A. and Smolders, E. (2011) 'The Red Mud Accident in Ajka (Hungary): Plant Toxicity and Trace Metal Bioavailability in Red Mud Contaminated Soil', *Environmental Science & Technology*, 45(4), 1616-1622.

Sapsford, D.J. (2013) 'New perspectives on the passive treatment of ferruginous circumneutral mine waters in the UK', *Environmental Science and Pollution Research*, 20(11), 7827-7836.

Schmalenberger, A., O'Sullivan, O., Gahan, J., Cotter, P.D. and Courtney, R. (2013) 'Bacterial Communities Established in Bauxite Residues with Different Restoration Histories', *Environmental Science & Technology*, 47(13), 7110-7119, available: http://dx.doi.org/10.1021/es401124w.

Sheoran, A.S. and Sheoran, V. (2006) 'Heavy metal removal mechanism of acid mine drainage in wetlands: A critical review', *Minerals Engineering*, 19(2), 105-116.

Tedd, K., Coxon, C., Misstear, B., Daly, D., Craig, M., Mannix, A. and Williams, T.H. (2016) *Assessing and Developing Natural Background Levels for Chemical Parameters in Irish Groundwater*, available: https://www.epa.ie/publications/research/water/research-183-assessing-and-developing-natural-background-levels-for-chemical-parameters-in-irish-groundwater.php [accessed.

Tessier, A., Campbell, P.G.C. and Bisson, M. (1979) 'Sequential extraction procedure for the speciation of particulate trace metals', *Analytical Chemistry*, 51(7), 844-851, available: http://dx.doi.org/10.1021/ac50043a017.

Truu, M., Juhanson, J. and Truu, J. (2009) 'Microbial biomass, activity and community composition in constructed wetlands', *Science of the Total Environment*, 407(13), 3958-3971, available: http://dx.doi.org/10.1016/j.scitotenv.2008.11.036.

Tscherko, D., Hammesfahr, U., Marx, M.-C. and Kandeler, E. (2004) 'Shifts in rhizosphere microbial communities and enzyme activity of Poa alpina across an alpine chronosequence', *Soil Biology and Biochemistry*, 36(10), 1685-1698, available: http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2004.07.004.

Urbanová, M., Kopecký, J., Valášková, V., Ságová-Marečková, M., Elhottová, D., Kyselková, M., Moënne-Loccoz, Y. and Baldrian, P. (2011) 'Development of bacterial community during spontaneous succession on spoil heaps after brown coal mining', *FEMS Microbiology Ecology*, 78(1), 59-69, available: http://dx.doi.org/10.1111/j.1574-6941.2011.01164.x.

Vance, E.D., Brookes, P.C. and Jenkinson, D.S. (1987) 'An extraction method for measuring soil microbial biomass C', *Soil Biology and Biochemistry*, 19(6), 703-707, available: http://dx.doi.org/https://doi.org/10.1016/0038-0717(87)90052-6.

Vymazal, J. (2011) 'Constructed wetlands for wastewater treatment: Five decades of experience', *Environmental Science and Technology*, 45(1), 61-69.

Vymazal, J. and Krása, P. (2003) 'Distribution of Mn, Al, Cu and Zn in a constructed wetland receiving municipal sewage', *Water Science and Technology*, 48(5), 299-305, available: http://dx.doi.org/10.2166/wst.2003.0336.

Wang, Y., Cai, Z., Sheng, S., Pan, F., Chen, F. and Fu, J. (2020) 'Comprehensive evaluation of substrate materials for contaminants removal in constructed wetlands', *Science of the Total Environment*, 701, 134736, available: http://dx.doi.org/10.1016/j.scitotenv.2019.134736.

Wong, V.N.L., Dalal, R.C. and Greene, R.S.B. (2008) 'Salinity and sodicity effects on respiration and microbial biomass of soil', *Biology and Fertility of Soils*, 44(7), 943-953, available: http://dx.doi.org/10.1007/s00374-008-0279-1.

Wright, A.L. and Reddy, K.R. (2007) 'Substrate-Induced Respiration for Phosphorus-Enriched and Oligotrophic Peat Soils in an Everglades Wetland', *Soil Science Society of America Journal*, 71(5), 1579-1583, available: http://dx.doi.org/10.2136/sssaj2007.0095.

Yang, C., Wang, X., Miao, F., Li, Z., Tang, W. and Sun, J. (2020) 'Assessing the effect of soil salinization on soil microbial respiration and diversities under incubation conditions', *Applied Soil Ecology*, 155, 103671, available: http://dx.doi.org/10.1016/j.apsoil.2020.103671.

Zelles, L. (1999) 'Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review', *Biology and Fertility of Soils*, 29(2), 111-129, available: http://dx.doi.org/10.1007/s003740050533.

Zhang, C.-B., Wang, J., Liu, W.-L., Zhu, S.-X., Ge, H.-L., Chang, S.X., Chang, J. and Ge, Y. (2010) 'Effects of plant diversity on microbial biomass and community metabolic profiles in a full-scale constructed wetland', *Ecological Engineering*, 36(1), 62-68, available: http://dx.doi.org/https://doi.org/10.1016/j.ecoleng.2009.09.010.



https://dspace.org/

Influence of sediment quality and microbial community on the functioning capacity of a constructed wetland treating alkaline leachate after 5.5 years in operation

Hudson, A.; Murnane, J. G.; O'Dwyer, Thomas F.; Pawlett, Mark; Courtney, Ronan G.

2023-04-01
Elsevier
https://doi.org/10.1016/j.scitotenv.2022.161259
Hudson A, Murnane JG, O'Dwyer T, et al., (2023) Influence of sediment quality and microbial community on the functioning capacity of a constructed wetland treating alkaline leachate after 5.5 years in operation. Science of the Total Environment, Volume 867, April 2023, Article number 161259

Downloaded from a DSpace repository. https://dspace.org/