

1 **ACCUMULATION AND FATE OF CONTAMINANTS (ZN, PB, FE AND S)**
2 **IN SUBSTRATES OF WETLANDS CONSTRUCTED FOR TREATING**
3 **MINE WASTEWATER**

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10 **Abstract.** Substrates of treatment wetlands, which were constructed at an operational lead-zinc min-
11 ing facility (Tara Mines) in Ireland, were analysed over a 5-year period for metal content. Lead, zinc,
12 iron and sulphate were removed from wastewater passing through the treatment systems and were sig-
13 nificantly retained in the substrates. The degree to which these contaminants are bio-available depends
14 on the biogeochemical conditions of the substrates, namely the pH, redox potential, amount of organic
15 carbon present and the contaminant loading. Various sediment fractions (water-soluble, exchangeable,
16 carbonate-bound, organic matter-bound and residual) were also analysed in these treatment wetlands,
17 in order to estimate the fate of metals and sulphur over time. Results showed that the majority of metals
18 and sulphur were retained in immobile residual forms, principally as metal-sulphides. Furthermore,
19 the neutral-alkaline pH and reducing biogeochemistry of the treatment systems suggest that metals
20 are not toxic under these conditions.

21 **Keywords:** bio-availability, metals, mining, treatment wetlands, sulphur, substrates, wastewater

22 **1. Introduction**

23 Mining activities produce wastewater with elevated concentrations of metals and
24 sulphate (Younger *et al.*, 2002). These contaminants usually exceed discharge lev-
25 els stipulated in the legislation (Novotny, 1995; O'Leary, 1996) and can be toxic
26 to a variety of organisms (Allan, 1995; Gao and Bradshaw, 1995). Heavy met-
27 als can interact with the sulphhydryl groups of amino acids (Salt *et al.*, 1995) and
28 subsequently interfere with enzyme functioning and oxygen-binding processes in
29 organisms (Kelly, 1988). Elevated sulphate concentrations can induce eutrophica-
30 tion as a consequence of anion (e.g. phosphorus) displacement (Perkins and
31 Underwood, 2001), while sulphide can be toxic to fish communities (Broderius
32 and Smith, 1977). Therefore, these contaminants are treated to render them less
33 mobile and relatively unavailable for uptake by organisms.

34 One technology employed for treating wastewater derived from mining is the
35 use of treatment wetlands (Hammer, 1989; Kadlec and Alvord, 1989; Moshiri,
36 1993; Hedin *et al.*, 1994; O'Sullivan *et al.*, 1999; Guesk and Wildeman, 2002).



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The majority of these wetlands are built to remediate waters impaired by abandoned acid mine drainage (Hedin *et al.*, 1994; Nairn and Mercer, 2000; Gusek and Wildeman, 2002), while only a few have reported treating wastewater from active mines (Dunbabin and Bowmer, 1992; Eger, 1994; O'Sullivan *et al.*, 2003). Treatment wetlands have improved substantially since their initial designs and rely on biogeochemical processes to remediate the wastewater (Huntsman *et al.*, 1978; Wieder and Lang, 1982; Hedin *et al.*, 1994; Gusek and Wildeman, 2002). Since heavy metals cannot be chemically degraded, microbial remediation (principally though immobilisation) is employed (Salt *et al.*, 1995). Under low (reducing) redox conditions, sulphate-reducing bacteria (SRB) can reduce sulphate to highly reactive sulphide (Tate, 1995; White and Gadd, 1996). Metal cations will bind with sulphide anions forming precipitates of metal-sulphides (Allen *et al.*, 1993; Yu *et al.*, 2001). Thus, both sulphate and metals can be removed from solution. However, these contaminants can also be removed from the water by other mechanisms such as adsorption and co-precipitation, depending on the biogeochemical status of the substrates (Fortin *et al.*, 1995; Wood and Shelley, 1999; Chagué-Goff and Rosen, 2001; Yu *et al.*, 2001). While vegetation is important for renewing the carbon supplies of metabolising bacteria (Dunbabin and Bowmer, 1992), it is the substrates where the majority of contaminants accumulate (Machemer *et al.*, 1993; Wood and Shelley, 1999). The extent to which metals are available for biological uptake depends on the substrate redox conditions and pH (Gambrell *et al.*, 1991; Lefroy *et al.*, 1993), the organic matter content and sulphate concentration (Miller *et al.*, 1983; Wood and Shelley, 1999; Yu *et al.*, 2001), which in turn can be affected by the activity of plant roots (Jacob and Otte, 2003).

Although contaminant removal efficiencies for treatment wetlands are reported extensively (Hedin *et al.*, 1994; Gusek and Wildeman, 2002; Younger *et al.*, 2002; O'Sullivan *et al.*, 2003), there is a dearth of information concerned with the long-term fate of the contaminants (Faulkner and Richardson, 1989; Baker *et al.*, 1991; Beining and Otte, 1996; Athay *et al.*, 2003). This is because the technology is relatively new and developing and few systems exist for more than 10 years for which there are extensive data. The study reported here examined the accumulation and speciation of metals and sulphur (Acid Volatile Sulphide (AVS)) in substrates of treatment wetlands at an active mine in Ireland, in order to assess temporal speciation. While these systems have only been in operation for about 5 years, there has already been significant accumulation of metals and sulphur in the substrates.

2. Materials and Methods

2.1. STUDY SITE

Experimental treatment wetlands were constructed at the active lead-zinc mine Outokumpu-Zinc Tara Mines, located 60 km northwest of Dublin in Ireland

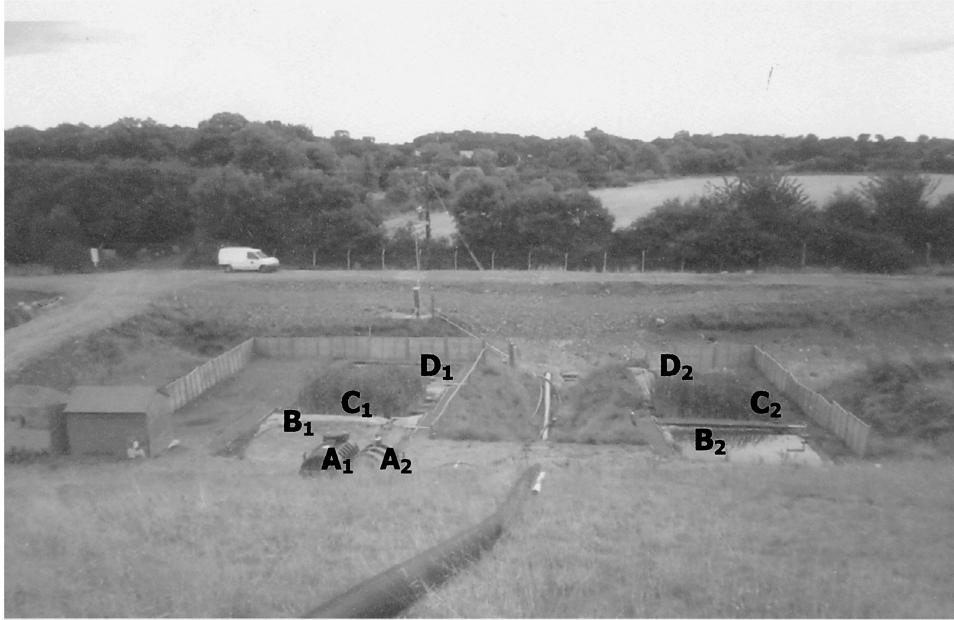


Figure 1. Experimental treatment wetlands at Tara Mines Ireland. Wastewater is stored in the header tank (A), from where it is pumped at a controlled flow rate to the inflow cell (B). From there, it overflows by gravity into the vegetated cell (C), through which the flow path is extended by four aquatic baffles until it finally reaches the outflow cell (D). The flow paths of different wastewaters are given for systems 1 (A₁-D₁) and 2 (A₂-D₂).

76 (53° 42' N, 06° 43' W). These systems were designed to remove sulphate, zinc
77 and lead from the spent mine water. This wastewater is characteristically alkaline
78 due to the buffering capacity of calcite (CaCO₃) and dolomite (CaMgCO₃) lime-
79 stone ore rock (O'Leary, 1996). Design specifications and removal rates have been
80 reported in detail elsewhere (O'Sullivan *et al.*, 2000; O'Sullivan *et al.*, 2003), while
81 a picture of the treatment wetlands is given in Figure 1.

82 2.2. SAMPLE COLLECTION AND PREPARATION

83 A 2 m long, PVC corer of 5 cm internal diameter was used to sample all sub-
84 strates in triplicate from the treatment systems, while ensuring minimal disruption to
85 the natural substrate profile. Substrates were sampled at 5–10 cm depth below the
86 flooded surface in each vegetated wetland cell, of both treatment systems, on five
87 sampling dates between 1997–2000. Substrates were also sampled at the same
88 depth for each inflow cell on three of the five sampling dates and for each outflow
89 cell on two of the five sampling dates. Additionally, substrates were sampled on
90 one occasion in 2002 at 0–5 cm, 5–10 cm and 10–15 cm depth below the flooded
91 surface. Immediately upon removal from the wetland using the corer capped with a

plastic bung, samples were fractionated into increments of 5 cm (with distal ends cut and removed). Each (wet) sample was stored in airtight acid-washed polyethylene bottles, sealed with parafilmTM and kept in a cooler until returned to the laboratory. Samples from 1997–2000 were stored at 4 °C, while samples from 2000 were kept frozen until extraction and analysis. The lower storage temperature in the later sampling date was necessary for conducting a sequential extraction analysis.

Substrates were oven-dried at 60 °C until constant weight and ground using a mortar and pestle. They were then sieved through a 2 mm aluminium sieve prior to the sequential extraction for metals, *viz.*, water-soluble, exchangeable, carbonate-bound, organic matter-bound and residual forms and for Loss On Ignition (LOI). Substrates for Acid Volatile Sulphide (AVS) were retained wet in their original storage containers at 4 °C until analysed.

2.3. SEDIMENT ANALYSES 104

2.3.1. *Baseline Substrate Properties* 105

Moisture content was calculated from the weight loss between fresh and dry (dried at 60 °C for at least 6 days until constant weight) substrate samples. For estimating particle size, 5 g of dry sediment were placed in a 212 μm mesh sieve and washed with deionised water until no further particles passed through the mesh layer. Remaining particles were collected and dried at 60 °C until constant weight. The proportion of particles <212 μm was calculated from subtracting the residual fractions from the original 5 g. A calibrated portable pH meter (WTW pH 90) connected to a pH electrode (WTW Type E50) was used to measure pH. Redox potential was measured at 15 cm depth below the flooded surface using a calibrated platinum electrode coupled to a calomel reference electrode (Sentek Ag/AgCl, Type H 93) and both were connected to a mV redox meter (WTW pH90).

2.3.2. *Loss on Ignition (LOI) and Organic Matter (OM) Contribution* 117

Five grams of dried sediment, in a ceramic crucible with a perforated lid, were ashed in a muffle furnace (Carbolite[®] Model OAF 10/2) at 500 °C for 12 h (after Jespersen *et al.*, 1998). Crucibles were allowed to cool down in the furnace before the remaining sediment material was weighed using a fine balance. Organic matter (OM) was calculated as the weight loss between the dry and ashed sample. From this, organic carbon (OC) content was calculated according to Mitsch and Gosselink (2000) whereby $\text{OC} = 0.5 \times \text{OM}$. Organic matter contribution was calculated by measuring plant densities and finally expressed as g m^{-2} .

2.3.3. *Metals* 126

Samples for sequential metal extraction were analysed for various exchangeable and bound forms following the procedures described by Tessier *et al.* (1979); Otte and Wijte (1993) and Carapeto and Purchase (2000).

130 *Water-soluble:* 5 g of dried material were added to 25 ml of distilled water in
131 a 50 ml centrifuge tube, shaken intermittently at room temperature and allowed to
132 stand overnight. After centrifugation for 5 min at 3000 rpm, 10 ml of the supernatant
133 was filtered (Whatman™42 filter paper) into a vial and acidified with one drop of
134 69% HNO₃ before storing at 5 °C. The 15 ml of remaining filtrate was retained for
135 the subsequent (sequential) extraction.

136 *Exchangeable:* 25 ml of 3.2 M CH₃COONH₄ in 20% HNO₃ (pH 6.45) was
137 added to the remaining 15 ml of filtrate from the water-soluble extraction. The
138 solution was intermittently agitated for 8 h before being filtered. The filtrate was
139 acidified with one drop of 69% HNO₃ prior to storing at 5 °C, while the sediment
140 residue from the filter was washed with 3 × 10 ml aliquots of deionised water and
141 then dried at 80 °C.

142 *Carbonate-bound:* A 25 ml aliquot of 1 M CH₃COONa was added to 2 g of
143 the dried sediment residue from the exchangeable fraction in a centrifuge tube and
144 shaken intermittently for 12 h. This mixture was then filtered, acidified with one
145 drop of 69% HNO₃ and stored at 5 °C. The sediment residue from this filter was
146 washed with 3 × 10 ml aliquots of distilled water and dried at 80 °C.

147 *Organic matter-bound:* 1 g of dried sediment residue (from the carbonate-bound
148 extraction) was added to a boiling tube with 3 ml 0.02 M HNO₃ and 2 ml 30%
149 H₂O₂. After incubating in a water bath (85 °C) for 1 h, a 2 ml aliquot of H₂O₂
150 was added and left for a further h. Then, another 2 ml of H₂O₂ were added to the
151 tube and left for a further 45 min. A final 2 ml aliquot of H₂O₂ was added and left
152 for 2 h, after which the sample was allowed to cool overnight. A 10 ml aliquot of
153 3.2 M CH₃COONH₄ in 20% HNO₃ was added to the sample before diluting it to 25
154 ml with deionised water. The tube was then shaken before being filtered, acidified
155 with one drop of 69% HNO₃ and stored at 5 °C.

156 *Total:* Substrate samples for total metal analysis were digested overnight in
157 strong acid (HNO₃: HCl = 4:1) under high temperature (140 °C) and pressure
158 conditions using Teflon™ ‘bombs’ as described in Beining and Otte (1996). Extracts
159 were prepared to 10 mL with deionised water and filtered through a 0.45 µm
160 membrane (Gelman Sciences Supor® 450 Nylon Acrodisc, 25 mm diameter). All
161 extracts were acidified and stored in the dark at 5 °C. Residual metal fractions
162 were calculated from subtracting the sum of the concentrations of the sequential
163 extractions from the total concentration.

164 Standard reference soil samples and blanks were used during the initial total
165 metal analyses. The certified soil materials used were Soil Rendzina S-SP (no. 12-
166 2-09) supplied by Glen Spectra Reference Materials, England and ISE 93.1 (no. 1),
167 supplied by Vrije Universiteit, Amsterdam, the Netherlands. The mean percentage
168 deviation from the certified concentration was calculated for each element and
169 results were expressed as percentage recovery of the certified concentration. These
170 values (pooled) ± standard deviations for each element were 110% ± 10% for Zn
171 ($n = 12$) and 119% ± 19% for Pb ($n = 6$). All samples were analysed for Zn, Pb and

TABLE I

Physical and chemical substrate parameters (November 1997; before planting or flooding). Data are given for 50% Spent Mushroom Substrate (SMS) mix (v/v), which was applied at or less than this concentration in each cell

Parameter (@50% SMS content)	Unit of measurement	Value
Moisture content	%	10
Particle size (Fractions < 212 μm)	mg g^{-1}	40
Organic matter (OM)	mg g^{-1}	60
Organic carbon (OC)*	%	3
Cation exchange capacity (CEC)*	meq \% dry weight	5
pH (as a paste with distilled H_2O)	[H ion]	7.6
Eh	mV	+ 17.4
Zinc (Zn)	mg kg^{-1} ($\mu\text{mol g}^{-1}$)	52 (0.8)
Lead (Pb)	mg kg^{-1} ($\mu\text{mol g}^{-1}$)	31 (0.1)
Iron (Fe)	g kg^{-1} ($\mu\text{mol g}^{-1}$)	28 (500)

Note. Calculated from OM (after Mitsch and Gosselink, 2000).

less frequently, for Fe using a Unicam 929 Atomic Absorption Spectrophotometer (AAS) supporting SOLAAR ATI software, version 5.25 (Unicam Ltd., 1991).

2.3.4. Acid Volatile Sulphide (AVS) 174

Apparatus for AVS analysis consisted of an analytical train in which hydrogen sulphide gas was liberated from wet substrates immersed in acid (20 % HCl) in a reaction vessel (modified after Allen *et al.*, 1993). This gas was trapped as a precipitate in zinc acetate solution (through a series of washbottle flasks) and analysed colorimetrically for sulphide (after Cline, 1969). Nitrogen gas (0.4 L min^{-1}) was purged through the apparatus to exclude oxygen and therefore prevented re-oxidation of sulphide to sulphate (Larosa and Casas, 1996).

3. Results and Discussion 182

3.1. INITIAL SUBSTRATE PROPERTIES 183

Physical and chemical data for sediments analysed prior to planting and flooding are presented in Table I. Pure (100%) Spent Mushroom Substrate (SMS) has a high moisture content (e.g. 63%: O'Sullivan, 2001) and in constructed wetlands, is typically diluted in volume in order to stabilise substrates and prevent eutrophication. The 50% SMS mix offered optimal permeability combined with good plant growth in pilot mesocosm experiments (O'Sullivan, 2001) and thus, a 10% moisture content in this mix was suitable for the (anaerobic-operating) treatment wetlands at

191 Tara Mines. If the moisture content was excessive in these systems, the potential
192 for substrates to become oxygenated or short-circuiting of the wastewater could
193 occur, which is detrimental to the treatment process. A small fraction of fine sedi-
194 ment particles ($40 \text{ mg g}^{-1} < 212 \text{ }\mu\text{m}$) were measured in the substrates. This was
195 important to know since a high fraction of these sized particles could otherwise
196 lead to clogging and inhibit colonisation by biological communities. The amount
197 of carbon measured in the substrates initially seemed low at 3% (or 60 mg g^{-1}
198 organic matter (OM)). However, assuming that pure SMS contained double the or-
199 ganic matter content than that of the 50% SMS, a value of 120 mg g^{-1} is expected,
200 while other studies have reported slightly greater concentrations at 152 mg g^{-1}
201 OM in SMS produced in Ireland (Maher, 1994). The Cation Exchange Capacity
202 (CEC), a measure of the sum of exchangeable cations that a soil can hold, calcu-
203 lated initially was low at 5 meq% dry weight. (Generally, as soil organic matter
204 content increases there is an increase in the CEC, resulting from an increase in
205 the amount of exchangeable hydrogen ions). The CEC of a substrate in treatment
206 wetlands has important implications for the mobility of contaminant cations (e.g.
207 Zn^{2+} , Pb^{2+} , Fe^{2+} etc), since they can be adsorbed to soil particles in addition to
208 being precipitated.

209 Wastewater from the mine was consistently alkaline and so low pH was not really
210 a concern, despite its importance in influencing microbial activity and controlling
211 chemical kinetics. In other mine waters however, which are characteristically acidic,
212 the pH is of utmost concern as a low pH can significantly increase metal speciation
213 and bioavailability (Allan, 1995). The treatment systems reported here were clearly
214 not yet reducing during the baseline study with a redox potential of +17.4 mV.
215 Along with low pH, high redox potential can lead to metal and sulphur bioavail-
216 ability (Sidle *et al.*, 1991; Chagué-Goff and Rosen, 2001) and so it was necessary to
217 maintain flooding once the treatment process had commenced in order to keep the
218 redox low. Zinc, lead and iron baseline concentrations were slightly lower than val-
219 ues reported elsewhere for SMS (Levanon and Denai, 1994) and were below levels
220 recorded in uncontaminated waterlogged sediments (Kadlec and Knight, 1996). Q1

221 3.2. CHEMICAL CHARACTERISATION OF SUBSTRATES OVER TIME

222 3.2.1. *Temporal Accumulation of Metals*

223 Total concentrations of metals that accumulated in substrates over time are shown
224 in Figure 2. Clearly, by 1.08 years following flooding, concentrations of Zn
225 (Figures 2a and 2b) and Pb (Figures 2c and 2d) in almost all cells were above
226 the values observed at the start of the experiment (Table I) and above the average
227 background levels of $1.8 \text{ }\mu\text{mol Zn g}^{-1}$ and $0.25 \text{ }\mu\text{mol Pb g}^{-1}$ reported in uncon-
228 taminated wetland substrates (Kadlec and Knight, 1996). This may be the result of
229 metal cations rapidly adsorbing onto negative soil surfaces and is typical in newly
230 created wetlands (Machemer *et al.*, 1993; Mitsch and Gosselink, 2000). Almost a Q1
231 year later (1.83 years) concentrations had increased to more than $13 \text{ }\mu\text{mol Zn g}^{-1}$

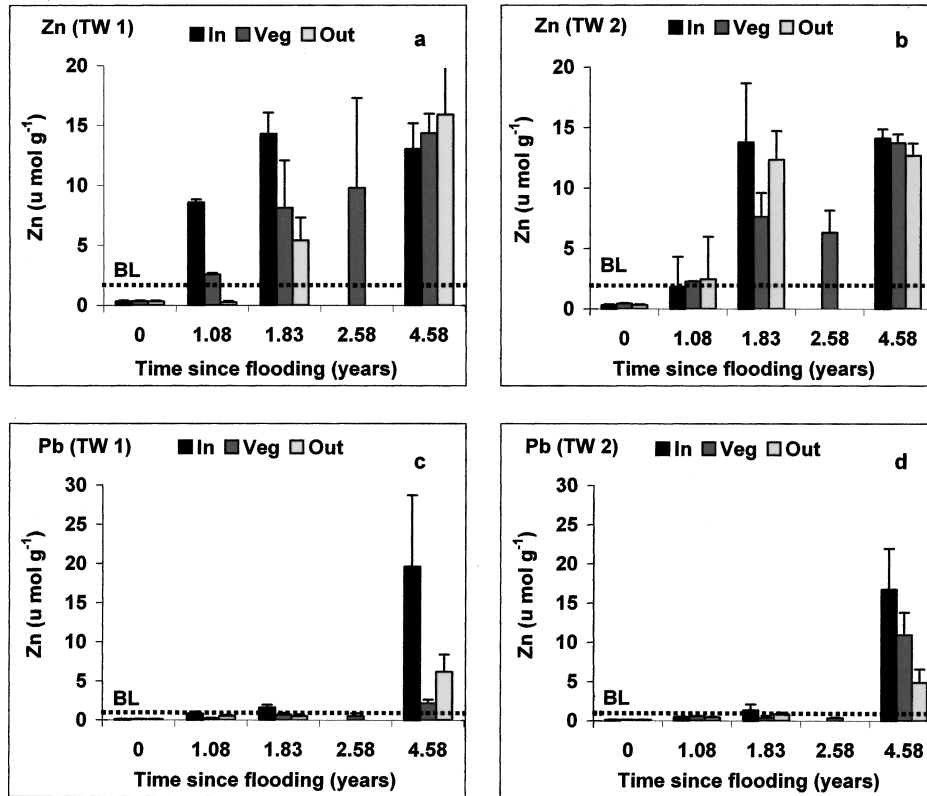


Figure 2. Total concentrations of Zn (Figures 2a and 2b) and Pb (Figures 2c and 2d) accumulated in the substrates of treatment wetlands one (TW 1; Figures 2a and 2c) and two (TW 2; Figures 2b and 2d). Concentrations are given as means + standard deviations, (number of replications, $n = 3$). IN (inflow cell), VEG (middle vegetated cell) and OUT (outflow cell). Mean Background Levels (BL) in uncontaminated wetland substrates for Zn ($1.8 \mu\text{mol g}^{-1}$) and Pb ($0.25 \mu\text{mol g}^{-1}$) are also shown as horizontal dotted lines.

and $1.3 \mu\text{mol Pb g}^{-1}$ (inflow cells) while 4.58 years after flooding, substrates of
 inflow cells had more than $16 \mu\text{mol Pb g}^{-1}$ with little change in Zn concentration.
 However, wetland and outflow cells had substantially greater Zn (in addition to
 Pb) levels at 4.58 years compared with earlier sampling dates (Figures 2a and 2b).
 Substantial Pb accumulation was not really seen until 4.58 years and this may be
 accounted for the typically lower concentrations in the wastewater during the initial
 4 years of sampling ($0.3\text{--}1.6 \mu\text{mol Pb L}^{-1}$ compared with $0.5\text{--}27 \mu\text{mol Zn L}^{-1}$;
 O'Sullivan, 2001).

Metal contamination in soils is a cumulative process according to Miller *et al.*
 (1983) and Machermer *et al.* (1993). Retention of metals within substrates re-
 sults from; the trapping by particulate matter (by van der Waal forces), adsorp-
 tion to organic matter (via Lewis acid-base reactions) and precipitation (oxidation,
 hydrolysis, bacteria-catalysed reactions) (Gambrell *et al.*, 1991; Doyle and Otte,

245 1997; Chagué-Goff and Rosen, 2001). These abiotic processes are highly influenced
246 by soil pH, redox potential and chelating agents released during the decomposition
247 of organic matter (Gambrell *et al.*, 1991). Metal reactivity and hence bioavailability
248 can be metal-specific, yet typically metal ions are more soluble at a lower pH. This
249 may be explained by the competition between protons and metal cations for organic
250 ligands, thereby lowering the stability of metal constants (Schnoor, 1996). Under
251 soil reducing conditions, SRB convert sulphate to sulphide, which is highly reactive
252 (half-life of < 1 h), and thus binds with soluble metal cations to form precipitates.

253 Carbon concentrations increased from 30 mg g⁻¹ up to 57 g g⁻¹ in over a
254 4-year period (Table II), which contributed substantial material for SRB activity
255 in these systems. This organic carbon was derived from vegetative growth also
256 shown in Table II. Mitsch and Gosselink (2000) calculate that approximately two Q1
257 thirds of plants become fully decomposed in organically-rich substrates (sapristis) of
258 this type. The consistent alkaline-neutral pH and anaerobic status of the substrates
259 post-flooding, combined with the renewal of carbon by decomposing plants, most
260 certainly facilitated the retention of metals in the substrates resulting from the
261 reactions mentioned above. On average, 94% of Zn that was removed from these
262 treatment wetlands over time was retained in the substrates (O'Sullivan, 2001).

263 While we cannot fully characterise these metal forms from early data, it is
264 likely that they were predominantly metal-sulphides. This is based on the fact that
265 substantial sulphate was removed from solution but substantial amounts of the
266 reduced form of this compound (i.e. sulphide) were not measured in the water
267 (O'Sullivan, 2001). Reactive sulphides are therefore suspected to have bound to
268 metals relatively quickly and hence have been removed from solution concurrently.
269 Machemer *et al.* (1993) also characterised anaerobic treatment wetland substrates as
270 predominantly sulphidic. Also, other studies have reported that a longer retention
271 time in treatment wetlands increases the chance of metal-sulphide formation as
272 opposed to metal adsorption (Machemer and Wildeman, 1992; Sobolewski, 1996;
273 Chagué-Goff and Rosen, 2001). The long retention time (33 days) and maturity
274 (5 years) of the treatment wetlands at Tara Mines provides reason to concur with
275 these findings and deduce that metal precipitates exist, which are thus relatively
276 unavailable for uptake by organisms (since adsorbed metals can remobilise easier
277 than sulphide lattices; Schnoor, 1996).

278 3.2.2. *Spatial Accumulation of Metals*

279 Concentrations of metals in the inflow substrates were typically greater than in
280 substrates of the vegetated or outflow cells subsequent in the water path (Figure 2).
281 However, exceptions to this trend were seen for Zn in treatment system 1 on the 4.58
282 years sampling and a marginal difference in treatment system 2 on the 1.08 years
283 sampling. Depositional patterns of substrate metal accumulation have been reported
284 in other studies and have been attributed in part to localised fluvial processes (Sidle
285 *et al.*, 1991; Sobolewski, 1996). Generally, more metals accumulate closer to the
286 point source (Beining and Otte, 1996; Chagué-Goff and Rosen, 2001). The overall

TABLE IIa

Substrate chemical properties in treatment wetland one (TW 1) over time. Concentrations (sampled at 5–10 cm depth) are expressed as means with standard deviations in brackets below, ($n = 3$). Samples for pH and Eh were not replicated in May 1997. Organic carbon (OC) content (**values in bold**) for May '97 and Mar '02 is given in g^{-1}dw and is calculated from its OM (after Mitsch and Gosselink, 2000). IN (Inflow), VEG (vegetated) and OUT (outflow) cells—not measured

Age (Yr)	Eh (mV)	pH (-log [H+])		Total Zn		Total Pb		OM Contribution (g m^{-2}) or OC ($\text{g g}^{-1}\text{dw}$)				AVS ($\mu\text{ mol g}^{-1}$)					
		VEG	IN	OUT	IN	VEG	OUT	IN	VEG	OUT	IN	VEG	OUT	IN	VEG	OUT	
0	May '97 +15.8		8.33	9.43	0.31	0.34	0.31	0.11	0.11	0.11	0.11	-	0.03	-	-	-	-
1.08	Sept. '98 -245		8.4	8.53	8.57	2.56	0.25	0.81	0.23	0.51	-	78.4	-	-	-	-	-
1.83	Jun '99 -129		7.9	8.8	14.29	8.11	5.4	1.6	0.66	0.51	-	869.8	-	-	-	-	-
2.58	Mar '00 -315																
4.58	Mar '02 -		6.99	6.79	13.02	14.34	15.90	19.59	2.16	6.20	21	36.6	57.5	63.6	31.4	106.4	(51)
			(0.25)	(0.1)	(2.14)	(1.63)	(4.11)	(9.13)	(0.48)	(2.22)	(1)	(12)	(21.5)	(7)	(28)	(7)	(28)

TABLE IIb

Substrate chemical properties in treatment wetland one (TW 2) over time. Concentrations (sampled at 5–10 cm depth) are expressed as means with standard deviations in brackets below, ($n = 3$). Samples for pH and Eh were not replicated in May 1997. Organic carbon (OC) content (**values in bold**) for May '97 and Mar '02 is given in g^{-1}dw and is calculated from its OM (after Mitsch and Gosselink, 2000). IN (Inflow), VEG (vegetated) and OUT (outflow) cells—not measured

Age (Yr)	Eh (mV)	pH ($-\log [\text{H}^+]$)		Total Zn ($\mu\text{mol g}^{-1}$)		Total Pb ($\mu\text{mol g}^{-1}$)		OM Contribution (g m^{-2}) or OC (g g^{-1})		AVS ($\mu\text{mol g}^{-1}$)				
		VEG	IN	OUT	IN	VEG	OUT	IN	VEG	OUT	IN	VEG	OUT	
0	+18.9	8.82	8.42	0.31 (0.07)	0.34 (0.04)	0.31 (0.07)	0.31 (0.07)	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)	0.03	–	–	–
1.08	–219 (102)	9.20 (0.1)	9.13 (0.22)	1.83 (2.48)	2.24 (0.05)	2.45 (3.5)	0.48 (0.04)	0.59 (0.01)	0.42 (0.11)	–	65.1	–	–	–
1.83	–114 (21)	9.27 (0.05)	8.98 (0.21)	13.77 (4.88)	7.60 (1.98)	12.3 (2.4)	1.34 (0.75)	0.36 (0.29)	0.83 (0.22)	–	543.6	–	–	–
2.58	–244 (204)	–	–	–	6.31 (1.8)	–	–	0.34 (0.09)	–	–	1028.5	–	–	–
4.58	–	6.84 (0.06)	6.68 (0.12)	14.08 (0.78)	13.69 (0.75)	12.66 (1.02)	16.74 (5.19)	10.97 (2.85)	4.89 (1.71)	15.02 (6.8)	17.6 (9)	24.8 (8)	40 (3.5)	40.5 (15) (9.5)

trend seen in the Tara Mines wetlands may be explained by two ways. Firstly, 287
since the flow rate into the treatment systems was not very high (approx. 1.5 L 288
 min^{-1}), contaminants such as Zn, Pb and S had the opportunity to precipitate out of 289
solution in the high pH and low Eh conditions even in the inflow (first) cell. This fact, 290
combined with the fact that soluble metal and sulphate concentrations were greatest 291
in the inflow cell, could have contributed to higher metal concentrations in inflow 292
substrates. Water flowing to subsequent cells was already lower in contaminant 293
concentrations. Metal removal was highly correlated with metal loading in these 294
systems (O'Sullivan *et al.*, 2003) and is reported elsewhere (Chagué-Goff and 295
Rosen, 2001). Furthermore, the same trend of metal accumulation in substrates was 296
observed for algal tissues, whereby greatest concentrations were measured in algae 297
in inflow cells compared to algae in the wetland or outflow cells (O'Sullivan *et al.*, 298
2000). The exception in system 1 at 4.58 years may suggest that the inflow cell has 299
exhausted its capacity to retain metal-sulphides. This is supported by the fact that 300
organic matter required for SRB sulphate reduction was substantially lower here 301
than in the vegetated or outflow cells (Table II) and so sulphide production could 302
have limited metal-sulphide precipitation here. Additionally, substantial amounts of 303
Pb precipitation by this sampling time could have limited zinc-sulphide formation in 304
the inflow cell (Figure 2c). Recent research is beginning to focus on quantifying the 305
spatial distribution of various metal fractions formed in similar treatment systems 306
(Athay *et al.*, 2003). 307

3.2.3. Temporal Accumulation of Sulphur 308

Sulphides can exist in various ionic forms, e.g. S^{2-} , HS^- and H_2S^- or be bound 309
to metals and can be quantified collectively as AVS (Allen *et al.*, 1993). AVS 310
concentrations measured in substrates of the treatment wetlands varied consider- 311
ably, ranging between 31 and $106.4 \mu\text{mol g}^{-1}$ (Table II). However, since back- 312
ground concentrations typically measure $<2 \mu\text{mol S}^{2-} \text{g}^{-1}$ (Broderius and Smith, 313
1977), a substantial amount of reduced sulphur accumulated over time through- 314
out these treatment wetlands. Machemer *et al.* (1993) also found in constructed 315
wetlands built to treat mine drainage that AVS concentrations increased with time 316
($40\text{--}107 \mu\text{mol g}^{-1}$) and determined that all sulphide produced was precipitated as 317
AVS. In the Tara Mines systems, hydrogen sulphide gas was evolved in later years 318
(olfactory detection) and so not all of the reduced sulphur was therefore precipi- 319
tated as AVS. Although AVS determinations were only conducted once, a significant 320
amount of bound sulphide has accumulated. Furthermore, sulphides appeared to 321
render metal cations relatively unavailable for uptake by organisms in these systems 322
as metal-sulphide precipitates formed (see below). 323

3.2.4. Metal Fractionation 324

Substrates sampled 4.58 years after flooding were analysed for metal forms by 325
sequential extractions. Concentrations of Zn, Pb and Fe were below the background 326
levels of uncontaminated wetland soils in every sediment fraction analysed, except 327

328 for the residual (e.g. sulphide-bound) forms. For this reason, only residual fractions
329 are considered here (Figure 3). These data confirm that most of the metals and
330 sulphides removed from the water were safely immobilised in the substrates and
331 were thus, unavailable for biological uptake in these systems.

332 Typically, total metal concentrations were lower with increasing depth in both
333 treatment systems, in accordance with other studies (Miller *et al.*, 1983; Sidle *et al.*,
334 1991; Chagué-Goff and Rosen, 2001; Yu *et al.*, 2001). In system 1, the outflow cell
335 had greater residual Zn concentrations in the upper (0–5 cm) substrates contrasting
336 with greater levels in the vegetated cell in the lower (5–10 cm) substrates (Figure 3a).
337 The same pattern was not seen in system 2 (Figure 3b). Inflow cells always had
338 greater concentrations of Pb than vegetated or outflow cells (Figures 3c and 3d).
339 Since Pb is more reactive than Zn, it is likely that it rapidly formed PbS in the inflow
340 cells, thus accounting for the greater residual-bound Pb in these substrates by 4.58
341 years. This rendered lower Pb concentrations available for reaction further along
342 the water pathway. Additionally, it may be speculated that sulphides available for
343 binding with Zn in the inflow cells were limited due to preferentially binding with
344 Pb, so Zn cations did not precipitate to the same degree until further along the water
345 course where they intercepted available sulphide anions. Other studies have found
346 that metals are removed from wastewater in sequence and the removal of metals
347 like Mn can be limited by the (preceding) removal of other metals including Fe, Al
348 and Zn (Lee *et al.*, 2002).

349 In system 1, between 0–10 cm depth, it is possible that some oxygenation of
350 the substrates in the vegetated cell led to less ZnS (Figure 3a) and PbS (Figure 3c)
351 formation compared with the outflow substrates. Localised oxygenation by roots
352 and rhizomes of plants like *Typha* and *Phragmites* have been reported extensively
353 (Dunbabin *et al.*, 1988; Brix and Schierup, 1990; Jacob and Otte, 2003). For residual
354 Fe, there was high spatial variability between location and depth, which is typical
355 in wetland soils (Doyle and Otte, 1997). However, residual Fe concentrations in
356 the vegetated cells were also lower than other cells (Figures 3e and 3f) and could
357 have been influenced by plant-root oxygenation. Doyle and Otte (1997) empha-
358 sised the importance of Fe oxide and organic matter fractions in wetland soils as
359 means of immobilising heavy metals, particularly in vegetated and burrowed micro-
360 environments, while others (Machemer *et al.*, 1993; Yu *et al.*, 2001) have found that
361 in anaerobic substrates where sulphate is not limiting, metal-sulphides are the pre-
362 dominant contaminant removal mechanism. Overall, metals were retained mostly
363 as residual fractions, probably as metal-sulphides and, concentrations were greater
364 in the upper few centimetres of substrates. This has important implications for
365 treatment wetlands since residual fractions are relatively unavailable for uptake by
366 organisms and, along with sulphide-metal ratios of greater than 1, therefore toxicity
367 is not likely (Allen *et al.*, 1993; Machemer *et al.*, 1993; Wood and Shelley, 1999).
368 Furthermore, it would be more practical and less destructive to the biogeochemical
369 ecosystem to harvest these upper substrates should the capacity for treatment near
370 exhaustion than to dredge the complete system.

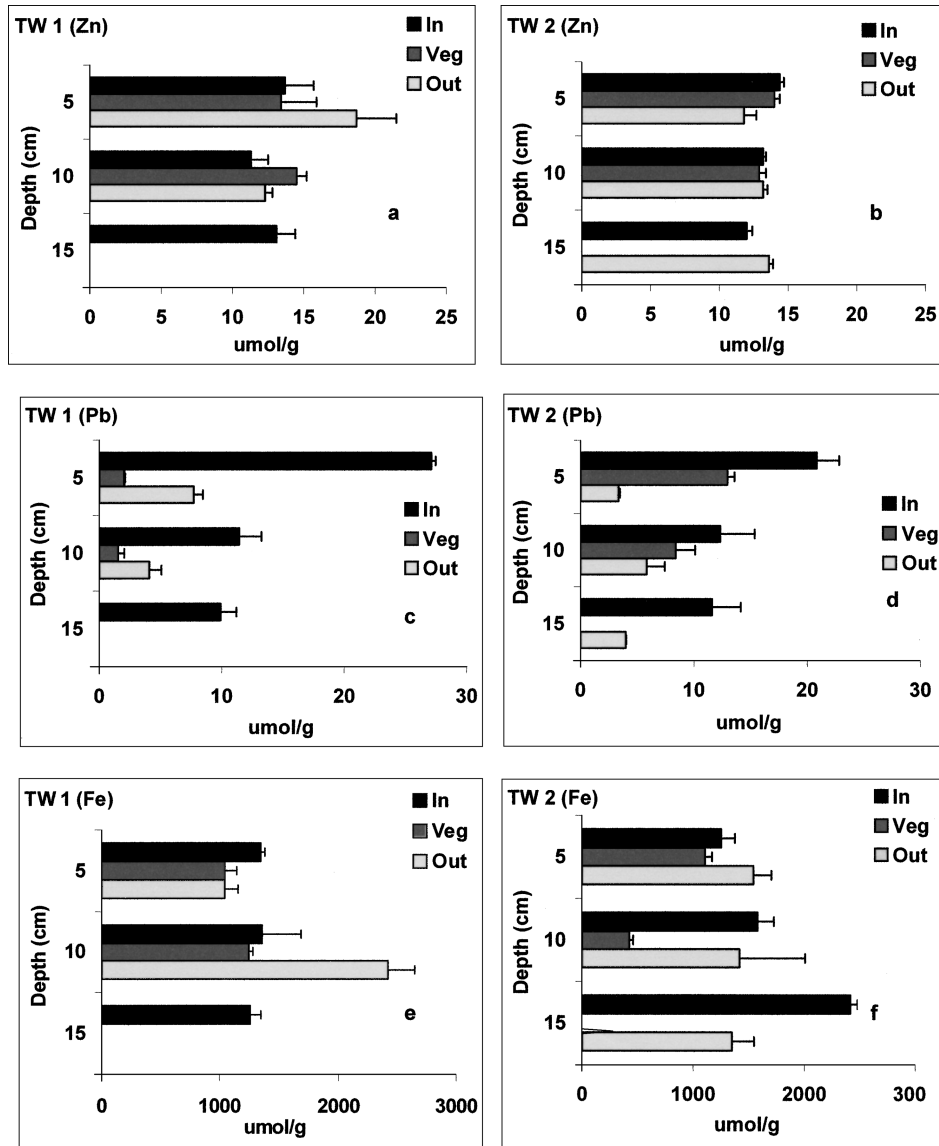


Figure 3. Residual metal concentrations determined from the sequential extraction of substrates sampled at 0–5 cm (5), 5–10 cm (10) and 10–15 (15) cm depth after 4.58 years in treatment wetlands one (TW 1) and two (TW 2), (all other fractions were below the levels in uncontaminated wetland substrates). Data are given for Zn (Figures 3a and 3b), Pb (Figures 3c and 3d) and Fe (Figures 3e and 3f). No data are available for VEG and OUT at 15 cm in TW 1 or for VEG at 15 cm in TW 2. Concentrations are given as means + standard deviations ($n = 3$). IN (inflow cell), VEG (middle vegetated cell) and OUT (outflow cell).

371 3.2.5. Biogeochemical Processes

372 A conceptual model of the biogeochemical processes occurring within a cell of the
 373 treatment systems at Tara Mines is shown in Figure 4. Contaminants entered the
 374 system as dissolved or suspended forms and travelled through the water and sub-
 375 strates by various paths, where they were immobilised in the substrates or absorbed
 376 by vegetation. Characteristically, plants did not absorb many metals. Rather, they
 377 excluded them at the root zone or sequestered them on their surface tissues as in
 378 the case of filamentous algae (O'Sullivan, 2001).

379 Particulate matter can weakly bind water-soluble metals, probably by van der
 380 Waal forces but low concentrations of these forms were measured in the substrates
 381 reported here (data not shown). Typically exchangeable metal fractions adsorb onto
 382 substrate surfaces but these forms are easily exchangeable with other cations de-
 383 pending on adsorption kinetics and equilibria. Low concentrations of these metal
 384 forms were also measured in the systems reported here after 4.58 years (data not
 385 shown). With the high alkalinity of the water at Tara Mines and possible dissolution
 386 of limestone from the substrate, carbonates could also bind metals (Miller *et al.*,
 387 1983). However, low carbonate metal fractions were measured in the 4.58 year-old
 388 substrates (data not shown). Metals are more strongly organically-bound through
 389 Lewis acid-base reactions when soils have a high humic content. This is due to the
 390 attraction of the metal cation to the negatively-charged organic soil surface and is

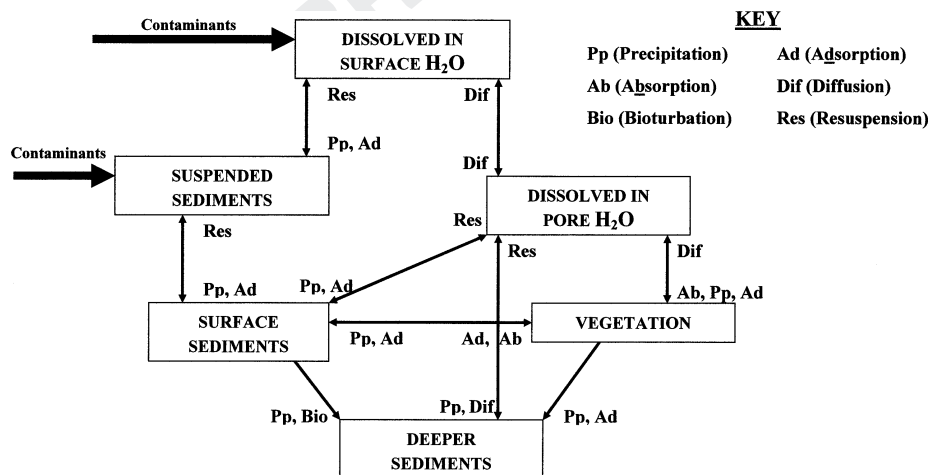


Figure 4. Conceptual model of the complex fate and transport of contaminants within a cell of a treatment wetland at Tara Mines, Ireland. The image shows the state variables (boxes) where contaminants can end-up, the various transport paths between these states (narrow arrows; some are bi-directional) and the modes of transport (abbreviated from the key) by which contaminants can travel. Metals are retained in the substrate by adsorption (exchangeable forms) and precipitation (carbonate and sulphide bound forms) as determined from sequential extraction.

commonly referred to as Cation Exchange (CE). This explains the greater opportunity for metal removal by complexing in organically-rich substrates (Miller *et al.*, 1983; Chagué-Goff and Rosen, 2001). The CE capacity (CEC) probably increased with time in the vegetated Tara Mines systems due to an increase in organic matter renewed cyclically (Table II) and thus, enhanced the potential for metal removal. However, since the substrates from the initial samplings were not subjected to sequential extractions, it is not possible to determine in what forms the contaminants were retained. Despite this, the fact that contaminants were removed relatively quickly from the water (O'Sullivan *et al.*, 2003) combined with the observations of Machemer *et al.* (1993) in similar treatment wetlands, it may be speculated that most of the contaminants were initially adsorbed relatively strongly to the soil surfaces. Since the substrates were organically-rich and the redox potential was consistently low, it is most likely that contaminants were originally organically-bound. It is possible that exchangeable forms became more strongly bound and or precipitated as the wetlands matured (became progressively more reduced) in accordance with other observations (Machemer *et al.*, 1993).

Predominantly, adsorption and precipitation reactions catalysed the removal of metals and sulphate from the wastewater. This was strongly facilitated by the alkaline pH and reducing substrates (Table II). A greater amount of residual (e.g. sulphide) precipitation compared to adsorption accounted for overall contaminant removal according to the substrate sequential extraction analyses, as reported by others (Machemer and Wildeman, 1992; Machemer *et al.*, 1993). Furthermore, the majority of contaminants were retained in the upper 15 cm of substrates compared with deeper substrates in a once-off analysis (O'Sullivan, 2001). Typically, metals do not migrate to much deeper sediments (Miller *et al.*, 1983; Sidle *et al.*, 1991; Chagué-Goff and Rosen, 2001) but substrate macroinvertebrates can induce higher metal concentrations even at depth as a result of bioturbation (Doyle and Otte 1997).

4. Conclusions

Treatment wetlands function by inducing biogeochemical change leading to contaminant removal from the wastewater. Removal of contaminants is determined by dynamic processes including adsorption, absorption and precipitation in substrate, vegetation and water fractions. Sequential extractions can quantify the various metallic forms in substrates and thus, provide an indication of metals available for uptake by organisms (Miller *et al.*, 1983; Sidle *et al.*, 1991; Yu *et al.*, 2001). The constructed wetlands at Tara Mines were engineered specifically to provide a biogeochemistry conducive to sulphate reduction and subsequent metal-sulphide formation. Although previous results showed significant removal of these contaminants from the water, their accumulation and fate were not fully ascertained. This study found that the majority of contaminants were retained as residual metal forms (e.g. immobile ZnS, PbS and Fe_(n)S), while other more mobile fractions

431 were present at concentrations lower than background levels of uncontaminated
432 wetland soils. This was attributed largely to the prevailing alkaline pH, reducing
433 substrate conditions and carbon and sulphate availability, which facilitated metal-
434 sulphide formation over time (Machemer and Wildeman, 1992; Machemer *et al.*,
435 1993). It is possible that adsorption to organic matter preceded metal-sulphide for-
436 mation, since cation exchange reactions resulted in a 'sponge' effect typically seen
437 in newly created wetlands. However, with a characteristically long detention time
438 and persistently alkaline pH, residual metal forms such as sulphides formed in the
439 systems reported here. This has important implications in the long-term opera-
440 tion of such treatment systems as contaminants are removed from water and ren-
441 dered quite immobile under these conditions. In other studies, metal bio-availability
442 was correlated to pore water concentrations and not to substrate concentration
443 (Wickham *et al.*, 1987; Yu *et al.*, 2001). Pore water metal concentrations
444 ($<1 \mu \text{mol Zn L}^{-1}$; $<0.3 \mu \text{mol Pb L}^{-1}$) in the systems at Tara Mines were ex-
445 tremely low compared with concentrations in the inflow water (O'Sullivan, 2001)
446 and sulphide concentrations in the pore water were also not high despite substantial
447 AVS formation, thus reinforcing the fact that these ions were rapidly removed from
448 solution. Therefore, it might be speculated that these systems are not potentially
449 toxic to organisms. Furthermore, since most contaminants were retained in the up-
450 per layer, potential harvesting of the accumulated metals may not be of detriment to
451 the established ecosystem. Therefore, should these systems reach a capacity, albeit
452 limited, there is scope to renew their potential by removing the upper substrate layer.

453

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459

References

- 460 Allan, R.: 1995, 'Diffuse Sources of Pollution by Toxic Metals', in W. Salomons, U. Forstner and
461 D. Hyman (eds.), *Heavy Metals: Problems and Solutions*, Springer-Verlag, Berlin, Germany, pp.
462 33–52.
- 463 Allen, H., Gongmin, F. and Baolin, D.: 1993, 'Analysis of Acid Volatile Sulphide (AVS) and Simulta-
464 neously Extracted Metals (SEM) for the Estimation of Potential Toxicity in Aquatic Sediments',
465 *Environ. Toxicol. Chem.* **12**, 1441–1453.
- 466 Athay, D. Nairn, R. and Strevett, K.: 2003, 'Mine Drainage Treatment Wetland Substrate Analysis', in
467 R.I. Barnhisel (eds.), *Proceedings of the 20th Meeting of the American Society for Surface Mining
468 and Reclamation*, American Society for Mining and Reclamation, Billings, MT., pp. 2–16.

- Baker, K., Fennessy, S. and Mitsch, W.: 1991, 'Designing Wetlands for Controlling Coal Mine Drainage: An Ecologic-Economic Modelling Approach', *Ecol. Econ.* **3**, 1–24. 469–470
- Beining, B. and Otte, M.: 1996, 'Retention of Metals Originating from an Abandoned Lead-Zinc Mine by a Wetland at Glendalough, Co. Wicklow', *Biol. Environ.* **96(B2)**, 117–126. 471–472
- Brix, H. and Schierup, H.H.: 1990, 'Soil Oxygenation in Constructed Reed Beds: The Role of Macrophyte and Soil-Atmosphere Interface Oxygen Transport', in P. Cooper and B. Findlater (eds.), *Constructed Wetlands in Water Pollution Control*, WRc Swindon, Wiltshire, UK, pp. 53–65. 473–475
- Broderius, S. and Smith Jr, L.: 1977, 'Relative Toxicity of Free Cyanide and Dissolved Sulfide Forms to the Fathead Minnow (*Pimephales promelas*)', *J. Fish Res. Board Can.* **34**, 2323–2332. 476–477
- Carapeto, C. and Purchase, D.: 2000, 'Use of Sequential Extraction Procedures for the Analysis of Cadmium and Lead in Sediment Samples from a Constructed Wetland', *Bull. Environ. Contam. Toxicol.* **64**, 51–58. 478–480
- Chagué-Goff, C. and Rosen, M.: 2001, 'Using Sediment Chemistry to Determine the Impact of Treated Wastewater Discharge on a Natural Wetland in New Zealand', *Environ. Geol.* **40**, 1411–1423. 481–482
- Cline, J.: 1969, 'Spectrophotometric Determination of Hydrogen Sulfide in Natural Waters', *Limnol. Oceanography* **14**, 454–458. 483–484
- Doyle, M. and Otte, M.: 1997, 'Organism-Induced Accumulation of Iron, Zinc and Arsenic in Wetland Soils', *Environ. Pollut.* **96**, 1–11. 485–486
- Dunbabin, J. and Bowmer, K.: 1992, 'Potential Use of Constructed Wetlands for Treatment of Industrial Wastewaters Containing Metals', *Total Environ.* **111**, 151–168. 487–488
- Dunbabin, J., Pokorny, J. and Bowmer, K.: 1988, Rhizosphere Oxidation by *Typha dominigensis* Pers. in Miniature Artificial Wetland Filters for Metal Removal from Wastewater, *Aquatic Bot.* **29**, 303–317. 489–491
- Eger, P.: 1994, 'Wetland Treatment for Trace Metal Removal from Mine Drainage: The Importance of Aerobic and Anaerobic Processes', *Water, Sci. Technol.* **29(4)**, 249–256. 492–493
- Fortin, D., Davis, B., Southam, G. and Beveridge, T.: 1995, 'Biogeochemical Phenomena Induced by Bacteria Within Sulfidic Mine Tailings', *J. Indust. Microbiol.* **14**, 178–185. 494–495
- Faulkner, S. and Richardson, C.: 1989, 'Physical and Chemical Characteristics of Freshwater Wetland Soils', in D. Hammer (ed.), *Constructed Wetlands for Wastewater Treatment*, Lewis Publishers, Chelsea, MI, pp. 41–72. 496–498
- Gambrell, R., Wiesepape, J., Patrick Jr, W. and Duff, M.: 1991, 'The Effects of pH, Redox, and Salinity on Metal Release from a Contaminated Sediment', *Water, Air Soil Pollut.* **57–58**, 359–367. 499–500
- Gao, Y. and Bradshaw, A.: 1995, 'The Containment of Toxic Wastes: II; Metal Movement in Leachate and Drainage at Parc Lead-Zinc Mine, North Wales', *Environ. Pollut.* **90(3)**, 379–382. 501–502
- Gusek, J. and Wildeman, T.: 2002, 'A New Millennium of Passive Treatment of Acid Rock Drainage: Advances in Design and Construction Since 1988', in D. Barnhisel and M. Collins (eds.), *Proceedings of the 21st Meeting of the American Society for Surface Mining and Reclamation*, American Society for Mining and Reclamation, Lexington, KY, 9th–13th June 2002, pp. 935–951. 503–506
- Hammer, D.: 1989, 'Constructed Wetlands for Wastewater Treatment, Municipal, Industrial and Agricultural', in *Proceedings from the First International Conference on Constructed Wetlands for Wastewater Treatment*, Lewis Publishers, Chelsea, MI. 507–509
- Hedin, R., Nairn, R. and Kleinmann, R.: 1994, 'Passive Treatment of Coal Mine Drainage', *Information Circular # 9389*, Bureau of Mines, US Department of the Interior, pp. 34. 510–511
- Huntsman, B., Solch, J. and Porter, M.: 1978, 'Utilization of *Sphagnum* Species Dominated Bog for Coal Acid Mine Abatement Drainage', *Abstracts of the 91st Annual Meeting of the Geological Society of America*, Toronto, Ontario, Canada, pp. 322. 512–514
- Jacob, D. and Otte M.: 2003, 'Conflicting Processes in the Wetland Plant Rhizosphere: Metal Retention or Mobilization?', *Water, Air Soil Pollut. Focus* **3**, 91–104. 515–516
- Jespersen, D., Sorrell, B. and Brix, H.: 1998, 'Growth and Root Oxygen Release by *Typha Latifolia* and its Effects on Sediment Methanogenesis', *Aquatic Bot.* **61**, 165–180. 517–518

- 519 Kadlec, R. and Alvord, H.: 1989, *Treatment Wetlands*, Lewis Publishers, CRC Press, FL.
- 520 Kelly, M.: 1988, *Mining and the Freshwater Environment*, Elsevier, London, UK.
- 521 Larosa, B. and Casas, A.: 1996, 'A Comparison of Sampling Handling and Analytical Methods
522 for Determination of Acid Volatile Sediment in Sediment', *Marine Chem.* **52** (3–4), 211–
523 220.
- 524 Lee, G., Bigham, G. and Faure, G.: 2002, 'Removal of Trace Metals by Coprecipitation with Fe, Al
525 and Mn from Natural Waters Contaminated with Acid Mine Drainage in the Ducktown Mining
526 District, Tennessee', *Appl. Geochem.* **17**, 569–581.
- 527 Lefroy, R., Samsor, S. and Balir, G.: 1993, 'The Dynamics of Sulfur, Phosphorus and Iron in Flooded
528 Soils as Affected by Changes in Eh and pH', *Aust. J. Soil Res.* **32**, 493–508.
- 529 Levanon, D. and Danai, O.: 1994, 'Chemical, Physical and Microbiological Considerations in Recy-
530 cled Spent Mushroom Substrate', in *Proceedings of the Spent Mushroom Substrate Symposium*,
531 Philadelphia, PA, pp. 17–24. **Q3**
- 532 Machemer, S., Reynolds, J., Laudon, L. and Wildeman, T.: 1993, 'Balance of S in a Constructed
533 Wetland Built to Treat Acid Mine Drainage, Idaho Springs, Colorado, USA', *Appl. Geochem.* **8**,
534 587–603.
- 535 Machemer, S. and Wildeman, T.: 1992, 'Adsorption Compared with Sulfide Precipitation as Metal
536 Removal Processes from Acid Mine Drainage in a Constructed Wetland', *J. Contamin. Hydrol.*
537 **9**, 115–131.
- 538 Maher, M.: 1994, 'The Use of Spent Mushroom Substrate (SMS) as an Organic Manure and Plant
539 Substrate Component', in *Proceedings of the Spent Mushroom Substrate Symposium*, Philadelphia,
540 PA, pp. 117–124.
- 541 Miller, W., McFee, W. and Kelly, J.: 1983, 'Mobility and Retention of Heavy Metals in Sandy Soils',
542 *J. Environ. Quality* **12**(4), 579–584.
- 543 Moshiri, G.: 1993, *Constructed Wetlands for Water Quality Improvement*, Boca -Raton, Lewis Pub-
544 lishers, London, UK.
- 545 Nairn, R. and Mercer, M.: 2000, 'Alkalinity Generation and Metals Retention in Successive Alkalinity
546 Producing Systems', *Mine, Water Environ.* **19**, 124–133.
- 547 Novotny, V.: 1995, 'Diffuse Sources of Pollution by Toxic Metals and Impact on Receiving Waters, in
548 W. Salomons, U. Forstner and D. Hyman (eds.), *Heavy Metals: Problems and Solutions*, Springer-
549 Verlag, Berlin, Germany pp. 33–52. **Q4**
- 550 O'Leary, W.: 1996, 'Wastewater Recycling and Environmental Constraints at a Base Metal Mine and
551 Process Facilities', *Water, Sci. Technol.* **33**(10–11), 371–379.
- 552 O'Sullivan, A.: 2001, 'Constructed Wetlands for Passive Biological Treatment of Mine Tailings Water
553 at Tara Mines, Ireland.', *Ph.D. Thesis*, Department of Botany, Division of Biosciences, University
554 College Dublin, Ireland, pp. 229.
- 555 O'Sullivan, A., Murray, D., McCabe, O. and Otte, M.: 1999, 'Wetlands for Rehabilitation of Metal
556 Mine Wastes', *Biol. Environ.* **99**(B1), 11–17.
- 557 O'Sullivan, A., Murray, D. and Otte, M.: 2000, 'Rehabilitating Mine Tailings Water Using Constructed
558 Wetlands', in L. Daniels and S. Richardson (eds.), in *Proceedings of the 17th Annual Meeting of
559 the American Society for Surface Mining and Reclamation*, Tampa, FL, pp. 438–445.
- 560 O'Sullivan, A., Murray, A. and Otte, M.: 2003, 'Constructed Wetlands for Treating Processed Mine
561 Water—an Irish case study', in G. Spiers, P. Beckett and H. Conroy (eds.), in *Proceedings of the
562 3rd International Mining and Environment Meeting*, Canadian Land Reclamation Association,
563 Sudbury, Ontario, Canada, pp. 739–747.
- 564 Otte, M. and Wijte, A.: 1993, 'Environmental Variation Between Habitats and Uptake of Heavy Metals
565 by *Urtica Dioica*', *Environ. Monitor. Assess.* **28**, 263–275.
- 566 Perkins, R. and Underwood, G.: 2001, 'The Potential for Phosphorus Release Across the Sediment-
567 Water Interface in an Eutrophic Reservoir Dosed with Ferric Sulphate', *Water Research* **35**(6),
568 1399–1406.

- Salt, D., Blaylock, M., Kumar, N., Dushenkov, V., Ensley, B., Chet, I. and Raksin, I.: 1995, 'Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment Using Plants', *Biotechnology* **13**, 468–473. 569–571
- Schnoor, J.: 1996, 'Modeling Trace Metals', in J. Schnoor and A. Zehnder (eds.) *Environmental Modeling: Fate and Transport of Pollutants in Water, Air and Soil*, Wiley, New York, pp. 381–451. 572–574
- Sidle, R., Chambers, J. and Amacher, M.: 1991, 'Fate of Heavy Metals in an Abandoned Lead-Zinc Tailings Pond: II. Sediment', *J. Environ. Quality* **20**, 752–758. 575–576
- Sobolewski, A.: 1996, 'Metal Species Indicate the Potential of Constructed Wetlands for Long-Term Treatment of Metal Mine Drainage', *Ecol. Eng.* **6**(4), 259–271. 577–578
- Tate, R.: 1995, 'The Sulphur and Related Biogeochemical Cycles', in R. Tate (ed.) *Soil Microbiology*, Wiley, New York, pp. 359–381. 579–580
- Tessier, A., Campbell, P. and Bisson, M.: 1979, 'Sequential Extraction Procedure for the Speciation of Particulate Trace Metals', *Anal. Chem.* **51**(7), 844–851. 581–582
- Unicam Ltd.: 1991, *SOLAAR Data Station Software*, version 5.25. Unicam Ltd., Division of Analytical Technical Incl., Cambridge, UK. 583–584
- White, C. and Gadd, G.: 1996, 'Mixed Sulphate-Reducing Bacterial Cultures for Bio-Precipitation of Toxic Metals: Factorial and Response-Surface Analysis of the Effects of Dilution Rate, Sulphate and Substrate Concentration', *Microbiology* **142**, 2197–2205. 585–587
- Wickham, P., Van de Walle, J. and Planas, D.: 1987, 'Comparative Effects of Mine Wastes on the benthos of an acid and an alkaline pond', *Environ. Pollut.* **44**, 83–99. 588–589
- Wieder, K. and Lang, G.: 1982, 'Modification of Acid Mine Drainage in Freshwater Wetland', in B. R. McDonald (ed.), *Proceedings of the Symposium on Wetlands of the Unglaciaded Appalachian Region*, WV University, Morgantown, WV, pp. 43–53. 590–592
- Wood, T. and Shelley, M.: 1999, 'A Dynamic Model of Bioavailability of Metals in Constructed Wetland Sediments', *Ecol. Eng.* **12**, 231–252. 593–594
- Younger P., Banwart S., Hedin R.: 2002, *Mine Water: Hydrology, Pollution, Remediation*. Kluwer Academic, London, UK, pp. 442. 595–596
- Yu, K-C., Tsai, L-J, Chen, S-H. and Ho, S-T.: 2001, 'Chemical Binding of Heavy Metals in Anoxic River Sediments', *Water Res.* **35**(17), 4086–4094. 597–598

Queries

- Q1. Au: This reference is not listed.
- Q2. Au: What does Focus stand for?
- Q3. Au: This reference is not cited.
- Q4. Au: This reference is not cited.

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