ECOLOGICAL DEVELOPMENT OF CONSTRUCTED WETLANDS BUILT FOR TREATING MINE WATER AT TARA MINES, IRELAND[1]

Aisling D. O'Sullivan[2], Declan A. Murray and Marinus L. Otte

Abstract. Mine associated wastewater is characteristically elevated in metals and other contaminants and has been conventionally treated with costly chemical applications. The development of passive treatment systems such as wetlands, which employ both biotic and abiotic processes, has been recognized as an economically feasible, ecologically acceptable treatment technology in the last decade. Not only can constructed wetlands provide an efficient facility for treating wastewater, they can also offer ancillary benefits such as ecological niches and therefore be of educational and often recreational value to society as well. Two experimental-scale treatment wetlands were constructed at an active lead/zinc mine near Navan, Ireland in 1997 to treat water enriched with sulfate and metals. Each system comprised three 12 m^2 (2 m depth) in-series surface-flow cells *viz*., inflow, vegetated and outflow. Sulfate-reducing bacteria were indigenous in the anaerobic spent mushroom substrate used, where biological reduction of sulfate to sulfide occurred. Sulfide subsequently precipitated with metals from the water. The treatment efficiency of the wetlands was promising with concentrations of sulfate (up to 29 g m^{-2} day $^{-1}$ (69%)), lead (6.6 mg m^{-2} day $^{-1}$ (64%)) and zinc (70 mg m^{-2} day $^{-1}$ (98%)) successfully removed from the wastewater. The ecological functioning of these constructed wetlands was also demonstrated with food webs, nesting niches and refuge sites afforded by colonizing communities of macroinvertebrates, macrophytes, microorganisms and other visiting wildlife. By 15 months following construction of the treatment wetlands, 30 species of macroinvertebrates were identified in system 1 and 21 species in system 2, while 3 plant species, 3 algae species and 1 moss had also colonized the ecosystems. Sulfate reducing bacteria genera included *Desulfotomaculum, Desulfovibrio*, *Desulfococcus* and *Desulfobulbus*. Annual dieback of planted species *Typha latifolia* and *Phragmites australis* contributed substantial amounts of biomass to the ecosystems, which led to a renewal of the carbon supply that drove the biologically mediated treatment process. It is speculated that the ecological diversity of the wetlands contributed to their treatment success based on inherent ecosystem complexity.

Additional Key Words: metals, sulfate, macroinvertebrates, microbes, plants, biota

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Introduction

Water quality derived from mining operations is usually not compliant with international (Allan, 1995, Novotny, 1995) or Irish (O'Leary, 1996) discharge standards as ore processing typically results in elevated metal and sulfate concentrations. Since 1982, the decontamination of mine wastes employing biological passive treatment technologies has developed substantially in lieu of conventional chemical applications (Nawrot, 1994, Gusek and Wildeman, 2002). Typically, wetlands are incorporated into this treatment process and are referred to as 'constructed wetlands'. The success in using wetlands for treating mine wastes has been demonstrated in many capacities; the successful removal of various contaminants from wastewater, their self-renewing capabilities and the ancillary ecological benefits offered (Debusk *et al*., 1996, Beckett, 1999, O'Sullivan *et al*., 1999). Many types of constructed wetlands are discussed in detail elsewhere (Hammer, 1989, Kadlec and Knight, 1996, Mitsch and Gosselink, 2000).

Wetland ecosystems support a complex array of trophic interacting physical, chemical and biological processes. By understanding the biogeochemistry of these ecosystems, the behavior of contaminants can be biotically and abiotically controlled and treated (Dunbabin and Bowmer, 1992, Fortin *et al*., 1995). In the spent mushroom substrate used in our treatment wetlands, the rich organic material provided an organic substrate for oxidation during the simultaneous microbial reduction of sulfate to sulfide. Planted vegetation in treatment wetlands can provide essential carbon renewal for microbial operation (Dunbabin and Bowmer, 1992), physically stabilize substrates with roots and provide for microbial attachment and, attract macroinvertebrate species (Darley, 1982). Additionally, plants roots can sequester some metals from wastewater treated in constructed wetlands (Blake, 1987, Chambers and Sidle, 1991).

Ecosystems are made up of many species and function according to the interacting biophysicochemical processes of these species communities. The treatment of wastewater in the Tara Mines wetlands was based on natural responses driven by microbial, sediment and plant processes. Therefore, it was important to understand, monitor and assess the effect of ecosystem diversity on contaminant reactivity. A high biodiversity in ecosystems has been reported to enhance ecosystem performance (Naeem *et al*., 1994, Chapin III *et al*., 1997), such as treatment potential. The diverse ecosystems that established in a "self-design" nature in the treatment wetlands at Tara Mines, Ireland are reported here.

Materials and Methods

Site Description

This study was conducted at Tara Mines Ireland (53º 42' N, 06º 43' W), where the local geology comprises Lower Carboniferous calcite ($CaCO_3$) and dolomite ($CaMgCO_3$). The carbonate rocks buffer spent wastewater discharged from the mine to a pH of approximately 7.8 (O'Leary, 1996). Design specifications of the treatment wetlands are reported in detail elsewhere (O'Sullivan *et al*., 2000); while, a picture of the systems appears in Figure 1.

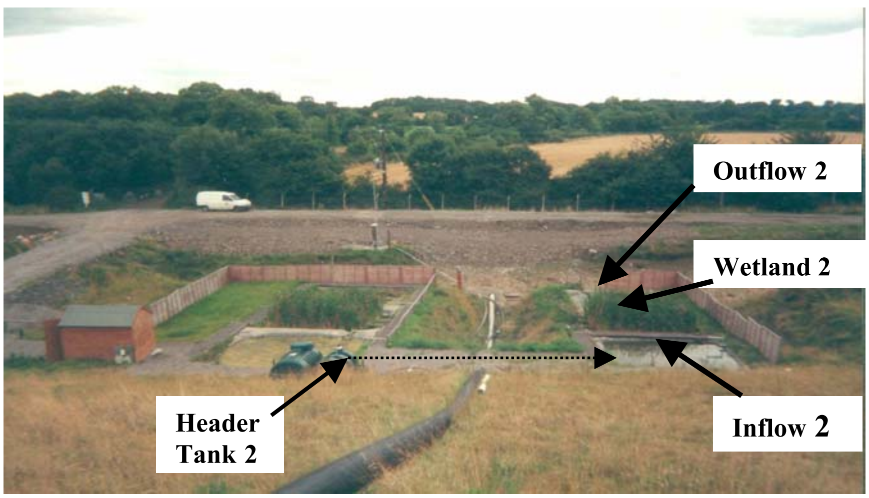


Figure 1. Constructed wetlands at Tara Mines Ireland (October 1999)

Sulfate and Metal Analyses

Sulfate was measured by Ion Chromatography using Dionex instrumentation (QIC analyzer, automated sampler and 4400 integrator) equipped with a separator column and conductivity cell. Lead and zinc were analyzed using a Unicam 929 Atomic Absorption Spectrophotometer supporting SOLAAR ATI software. The sampling techniques and sampling frequency for these contaminants are previously reported (O'Sullivan *et al*., 2000).

Macroinvertebrate Analysis

Macroinvertebrate sweep samples were obtained by dragging a 2 mm Windermere™ mesh net of 1 square foot along the sides of the cells of the treatment wetlands. Sampling was conducted twice during the baseline biological survey (January 1998) and three times between October 1998 and March 1999. Samples were preserved with 100 mL of 80% ethanol and silt was removed by wet sieving (mesh size of 500 µm). Specimens were identified to species level, primarily by two final year undergraduate students (Patrick Moran, 1998 and Maeve Rafferty, 1999). Macroinvertebrates were identified using a binocular microscope (magnification X 80) and standard identification keys of the British Freshwater Biological Association; Fitter and Manuel (1985), Pinder (1986), Elliot *et al*. (1988) and Becker *et al*. (1990).

Vegetation Analysis

Planted *Typha latifolia* and *Phragmites australis* growth was quantitatively monitored over all seasons between November 1998 and March 2000. For each species, plant density (number of shoots per 1 m^2 quadrat) and, the above ground biomass (weight) of 5 plants in a representative area of the vegetated wetland cell were measured. Then, these (mean) values were calculated up for determining plant production of the total wetland area (12 m^2). Colonizing vegetation (plants, algae and mosses) was inventoried between November 1998 and October 2000. In addition, filamentous algae were identified and assigned percentage colonization cover values.

Microbiological Analysis

Sulfate reducing bacteria (SRB) were measured qualitatively (twice in autumn 1998) in substrates from each of the cells of the treatment wetlands. Selective media, specific for one type of SRB (Parkinson and Gray, 1971), were used to isolate genera following a variety of preparation techniques carried out in a laminar air-flow cabinet to maintain anaerobic conditions. Preparations techniques included (1) Enrichment procedure using Winogradsky Columns (Herbert and Gilbert, 1984), (2) Boiling Roll Tubes to grow selective SRB (Hungate, 1969), (3) Agar Shake Tube Method to provide anaerobic living conditions (Battersby, 1988) and (4) Six-fold dilution series, prior to streaking with anaerobic plates.

Colonies of each isolated SRB were placed on sterile glass slides containing 1 mL of boiled deioinised water and placed in an oven at 80 °C for 5 minutes. Bacterial cell viability was tested following exposure to these temperatures and spores were identified by phase contrast microscopy after Elliott *et al*. (1998). Spores appeared phase bright and their colony shape (rods or cocci), motility and gram staining (positive (purple) or negative (pink)) were observed. This study was conducted by Laura Wharton in conjunction with the Department of Microbiology on-campus (UCD) and is explained in detail in O'Sullivan (2001).

Results

Sulfate and Metal Removal

The treatment systems equilibrated (determined from preliminary data) by October 1998 (9 months after flooding). Sulfate was removed from the wastewater by up to 29 g m^{-2} day^{-1} in treatment wetland 2, while generally lower removal rates of up to 18 g m^{-2} day^{-1} were calculated for treatment wetland 1. Typically, more sulfate was removed after the equilibration period and this is reported in extensive detail elsewhere (O'Sullivan *et al*., 2001, O'Sullivan *et al*., 2002).

Zn and Pb removal was consistently greater following the equilibration phase. Removal of Zn in system 2 ranged between 0.5-70 mg m^{-2} day^{-1}, while for system 1, usually lower removal rates of 0.3-5.7 mg m^{-2} day^{-1} were calculated. Lead was removed by up to 6.6 mg m^{-2} $day^{-}1$ in system 2 and 5.8 mg m^{-2} day^{-1} in system 1. Metal removal from these systems is also reported previously in more detail (O'Sullivan *et al*., 2000, O'Sullivan *et al*., 2002). Metal removal rate was strongly correlated to chemical loading, as shown for Zn (r^2 = 0.99) and Pb (r^2 = 0.97) (O'Sullivan, 2001).

Macroinvertebrate Identifications

Seven macroinvertebrate species were identified in the initial 8 months following construction of the treatment wetlands, while an additional 26 taxa were identified 7 months later (Table 1). Treatment system 1 supported 30 different taxa while treatment system 2 supported 21 species. More species appeared in the non-vegetated (inflow and outflow) cells than in the wetland cells.

Plant Growth

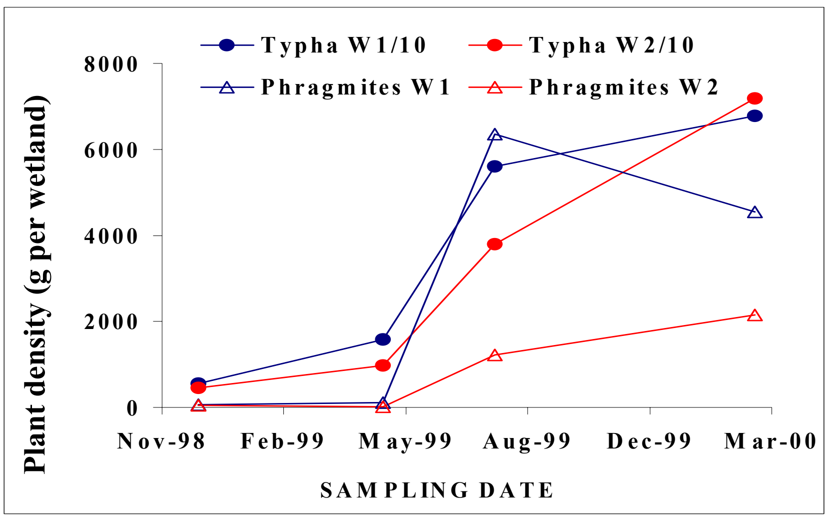
Biomass production calculations estimated the amount of organic matter that was recycled through the wetlands from plant seasonal dieback, which (visibly) typically occurred between November and mid-March. Maximum growth rates generally occurred in the summer to early autumn period, after which time the rate of growth slowed down until the start of the next growing season in early March (Figure 2). *Typha latifolia* dominated in growth and establishment initially, yet *Phragmites australis* showed an increasing vigor by the later sampling dates. Overall, *T. latifolia* contributed the most organic material to the systems; 72 kg for treatment wetland 2 by March 2000), while *P. australis* produced up to 6.5 kg for treatment wetland 1 by August 1999.

Table 1. Distribution of macroinvertebrate species in all cells of both treatment wetlands during the sampling period October 1998 and March 1999. Abbreviations; I = Inflow, W = Wetland and O = Outflow cells, TW 1 = treatment wetland 1 and TW 2 = treatment wetland 2.

Group	**Family (Sub-family)**	**Genus and sp.**	**TW 1**			**TW 2**		
			I	**W**	**O**	**I**	**W**	**O**
Mollusca	Lymnaeidae	*Lymnaea* sp.		+	+			
	Hydrobiidae	*Potamopyrgus jenkinsii*	+	+	+		+	+
Chelicerata	Hydracarina							+
Crustacea	Cladocera	*Daphnia longispina*			+		+	
	Copepoda	**Cyclops* sp.		+	+			
	Asellidae	*Ascellus aquaticus* (L.)	+	+	+	+	+	+
Ephemeroptera	Baetidae	**Cloeon dipterum* (L.)	+	+	+	+	+	+
Odonata	Coenagrionidae	*Coenagrion* sp.	+	+				
Megaloptera	Sialidae	*Sialis lutaria* (L.)			+			
Trichoptera	Limnephilidae			+				
Diptera	Chironomidae							
	(Tanypodinae)	*Procladius* sp.	+	+		+		+
		**Psectrotanypus varius*	+	+	+	+	+	+
	(Chrionominae)	*Polypedilum* sp.	+		+			+
		Glyptotendipes sp.			+			
		**Chironomus* sp.	+	+	+	+	+	+
		Microtendipes sp.	+			+		
	Tipulidae	*Tipula* sp.	+					
	Chaoboridae	*Chaoborus* sp.					+	
Coleoptera	Haliplidae	*Haliplus lineatocollis*	+	+		+		
	Dytiscidae	*Laccophilinae minutus* (L.)	+	+				+
		Agabus striolatus	+					
		Hygrotus ineaqualis						+

		Coelambus confluens	+		+			
		Agabus sp. (larvae)				+		
		Ilybus sp. (larvae)	+		+		+	+
		**Dytiscus marginalis*	+	+	+	+	+	+
	Gyrinidae	*Gyrinus distinctus*		+				
Hemiptera	Notonectidae	*Notonecta glauca* (L.)	+	+	+			
	Corixidae	*Corixa punctata*	+					+
		Callicorixa praeusta			+			
		Sigara selecta/stagnalis	+		+	+		
		Sigara nigrolineata			+			
		Hesperocorixa sahlbergi			+			
		**Sigara lateralis* (Leach)	+	+	+	+	+	+
Hirudinae		**Helobdella stagnalis*					+	
		DIVERSITY (No. of taxa)	**20**	**16**	**20**	**11**	**11**	**14**

+ present in this location



* recorded in the earlier sampling (8 months following construction)

Figure 2. Plant growth in treatment wetlands 1 (W1) and 2 (W2). *T. latifolia* is expressed as one tenth of the actual value, n = 5.

Colonizing Vegetation

In addition to *Typha latifolia*, *Phragmites australis* and *Glyceria fluitans* (later died) that were planted in the treatment wetlands, other species colonized the systems in the initial months following construction. Green filamentous algal mats colonized all cells of the treatment wetlands except the inflow of system 2, to approximately a 50% cover. *Cladophora glomerata* dominated all compartments except the inflow of system 1, which was dominated by *Microspora* (Table 2). Additionally, the moss *Calliergon cuspidatum* and the pondweed *Lemna minor* colonized each wetland cell, while the grass *Poa trivialis*, a common perennial, was detected in every cell except the outflow cell of system 2. The common water starwort (*Callitriche stagnalis*) appeared in the inflow of system 1 by April 2000 but not in the other cells.

Microbiological Identification

Sulfate reducing bacteria (SRB) belonging to the genera *Desulfovibrio, Desulfotomaculum*, *Desulfococcus* and *Desulfobulbus* were isolated from the substrates in the treatment wetlands. Selective media for *Desulfosarcina* and *Desulfobacter* species were not positively colonized. The outflow cell substrates from both treatment systems contained only *Desulfovibri*o, which was isolated from every cell, while *Desulfococcus* was not isolated from substrates in any cell of treatment system 1. Most colonies were characteristically rod-shaped and gram negative, with a few indicating cocci formation. All colonies displayed distinct motility and *Desulfotomaculum* was the only genera to indicate positive sporulation. More detailed results are documented elsewhere (O'Sullivan, 2001).

Table 2. Location of colonizing vegetation in the treatment wetlands monitored between October 1998 and October 2000. Abbreviations; I = Inflow, W = Wetland and O = Outflow cells.

Type	**Family**	**Species**	**Occurrence**	
			SYSTEM #	**CELLS**
Algae	Charophyceae	Chara species	**1**	**I**
	Chlorophyceae (Oedogoniales)	Cladophora glomerata	**1**	**I, W, O**
			2	**W, O**
	Chlorophyceae (Ulotrichales)	Microspora species	**1**	**I, O**
Bryophyta	Amblystegiaceae	*Calliergon cuspidatum	**1**	**W**
			2	**W**
Angiosperma	Lemnaceae	Lemna minor	**1**	**W**
			2	**W**
	Ranunculaceae	*Ranunculus tripartitus	**2**	**W**
		Poa trivialis	**1**	**I, W, O**
			2	**I, W**
		Callitriche stagnalis	**1**	**I**

* recorded in the earlier sampling (8 months following construction)

Discussion

Sulfate and Metal Removal

Lower sulfate removal seen prior to equilibration of the systems may be explained in part by the initial biogeochemical changes occurring in the systems, which is typical of newly created wetlands (Mitsch and Gossleink, 2000). These changes would have ultimately affected the redox status and therefore the chemistry of the wetland substrates, particularly sulfate dynamics (Engler and Patrick, 1973, Lefroy *et al*., 1993). Sulfate reducing bacterial activity was not apparently inhibited during winter, possibly due to mild temperatures (8-10°C in water; typically higher in substrates) characteristic of the Irish Atlantic climate. Generally, greater amounts of sulfate and metals were removed from the water as the systems became more mature and ecologically complex with time, especially between November and March (non-growing period). Biologically reduced substrates, coupled with increased organic material renewal, may account for generally more favorable sulfate removal rates seen in non-growing seasons (O'Sullivan *et al.,* 2000).

Removal of zinc and lead generally improved after the equilibration phase and removal was greater in system 2 than in system 1. These trends may be attributed to biogeochemical stabilization and maturation of the treatment wetlands over time and to higher concentrations of metals in the water supplied to system 2, respectively. Metal removal as a function of sampling date was significant, yet this was most likely due to variable chemical loading rather than effects of season. This explanation is supported by the strong correlation between chemical loading and removal seen for these metals.

Macroinvertebrate Community Diversity

A substantial increase in the species numbers present in the treatment wetlands during the later sampling compared to the first sampling may be due to greater stabilization and colonization of the wetlands over time.

Colonizing fauna were characteristic of a slow flowing, shallow and nutrient-rich habitat (Table 1). The species *Cloeon dipterum* and *Ascellus aquaticus* are indicative of beta-mesosaprobity (a zone of minor organic enrichment) according to Elliot *et al*. (1988), which was probably due to nutrient release by the spent mushroom substrate. The Coleopteran *Agabus* *striolatus* is typical of fen Carr regions. Therefore, occurrence of this species was typical for alkaline water. Many predatory species, particularly from the carnivorous families Dytiscidae and Chrionomidae, contributed substantially to the species diversity of the treatment wetlands. Lower macroinvertebrate diversities observed in the inflow and wetland cells of system 2 may be explained by the relatively higher zinc concentrations (see O'Sullivan *et al*., 2001) measured at the same time in the inflow cell of system 2 (Armitage, 1979), in which only half the number of Chironomids were recorded compared with the inflow cell in system 1.

Members of the sub-family Chironominae, which constituted the majority of the Chironomids in the wetlands are considered relatively tolerant to heavy metal contamination (Pinder, 1986). These carnivores can live in metal-enriched water since they ingest little sediment or algal material, by comparison to their counterpart detritivores and herbivores (Kelly, 1988). The absolute numbers and diversity of both detritivores and herbivores were however substantial, particularly amongst the Corixidae. The detritivorous (crustacean) Isopod *Ascellus aquaticus* was located in every compartment. Wickham *et al*. (1987) noted that a high crustacean to insect population ratio is typical of water uncontaminated by heavy metals. These results may suggest that successful removal of metals and sulfate from the wastewater led to colonization by some relatively intolerant species. While estimating the effects of heavy metals on invertebrate communities, it is important to consider the reactivity of the metal and the species it may affect. For instance, sediment-ingesting benthic faunas are vulnerable to toxicity from sediments while other species may not be (Kelly, 1988). In alkaline waters, metal toxicity to organisms can be reduced due to the precipitation of metals from solution, rendering them relatively unavailable.

Vegetation Density and Organic Matter Contribution

Anaerobic substrates did not appear to impede the growth of rhizomous *T. latifolia* and *P. australis*. Apparently, these species produce more secondary shoots during their initial stages of growth and this may have helped in their rapid establishment in the treatment wetlands (Mitsch and Gosselink, 2000). Some initially dominant species (e.g. *T. latifolia*) can become less important in constructed wetlands as an ecosystem matures and become replaced by other successful species (e.g. *P. australis*) according to Mitsch and Gosselink (2000). A complete mortality of *Glyceria fluitans* probably resulted from insufficient light interception due to shading effects from the other 2 emergent species (Naeem *et al*., 1994).

Substantial organic matter contributed by the plants, particularly by *T. latifolia*, was important in the wetland ecosystems since the microbial driven treatment processes relied on sufficient organic carbon. For every mole of sulfate biologically reduced, 2 moles of carbon are oxidized through microbial metabolism. Growth rates for both *Typha* and *Phragmites* are promising, for ensuring sufficient organic material in treating the wastewater in the future when other organic sources in the substrate may become exhausted.

<u>Colonizing Vegetation</u>

Inevitably, other plant species native of the adjacent grassy areas (e.g. *Poa trivialis* and *Ranunculus tripartitus*) established at the margins of each cell, having encroached from the peripheries (Table 2). Plant invasion in wetlands will occur by natural wetland invaders in a 'self-design' process until a natural dominance is established (Mitsch and Gosselink, 2000). This phenomenon appears to be in accordance with the laws of ecosystem dynamics and complexity as identified by Chappin III *et al*. (1997).

Although algal growth was not purposely planned for, it can be a natural component of an ecosystem and can thus contribute to nutrient cycling within treatment wetlands. Algal colonization was especially of interest since some types can remove metals from mine-polluted waters (Sladeckova and Matulova, 1998) as well as support microbial (and invertebrate) attachment on their surfaces (Boyd, 1978).

<u>Microbiological Identification</u>

The alkaline pH of the wastewater, biologically reducing substrate environment, carbon supplied from substrates and vegetation collectively supported growth of specific genera of SRB, which use organic compounds for growth (anabolism) and as an energy source (catabolism) in the reactions they catalyze (Ledin and Pederson, 1996). Activity of these bacteria is highly related to the availability of suitable electron acceptors (i.e. sulfate in the absence of oxygen) and donors (carbon) in spatially and geochemically distinct substrate zones (Fortin *et al*., 1995). However, *Desulfosarcina* and *Desulfobacter* were not identified in the substrates, yet it is reported that these genera may be restricted to marine environments (Fortin *et al*., 1995). It is also possible that they required different growing conditions (i.e., temperature, pH) than those prevalent in Tara Mines treatment wetlands.

Greater diversity of SRB occurred in the inflow and wetland substrates compared to the outflow cells in each system. In the inflow cells, greater sulfate concentrations may have led to greater bacterial diversity since their populations are related to sulfate concentrations (Awadallah *et al*. 1988, Fortin *et al*., 1995). In the wetland substrates, this difference may be accounted for by more organic material present initially in the substrates and additionally provided through plant seasonal dieback (Dunbabin and Bowner, 1992).

Conclusions

Ecosystems are made up of many different species, growing and functioning at various trophic levels. All these species from the microorganism level to the algal, plant, macroinvertebrate and animal levels interact with and relate to each other in developing such a complex system. Therefore, each species level is somewhat dependent on another trophic (usually lower) level of species functioning for survival and functioning. The diversity of each of the trophic levels outlined above has been examined in the wetlands constructed for treating wastewater at Tara Mines in Ireland. It has been shown that a moderate degree of diversity has almost 'self-developed' in these ecosystems and it is this diversity that invariably has contributed to the remediation of the wastewater through a multitude of complex biological, chemical and physical interrelating processes.

As the treatment wetland systems matured and stabilized with time, they were also more ecologically diverse and complex. Greater numbers and densities of plant, algae and macroinvertebrate colonizers were seen in later sampling dates. Greater sulfate and metal removal rates were also seen in these systems with time and especially when plants were in a non-growing stage. Annual plant dieback contributes more carbon to the metabolic performance of the microbial community under appropriate reducing conditions in the substrate. Therefore, a functioning microbial population, which is supported by higher species trophic levels such as vegetation, led to removal of sulfate (up to 29 g m^{-2} day^{-1}), lead (6.6 mg m^{-2} day^{-1}) and zinc (70 mg m^{-2} day^{-1}) contaminants through metal-sulfide precipitation following biological reduction of sulfate. Some authors have emphasized that ecosystem performance, such as elemental cycling including removal of nutrients from water, is strongly dependent on ecosystem complexity (Naeem *et al*., 1994), which in turn is determined from ecosystem diversity (Chappin III *et al*., 1997). This discussion would strengthen the case that a diverse ecosystem hierarchy in constructed wetlands built for wastewater treatment is crucial to treatment performance sustainability per se.

Aspects of the interacting biogeochemical treatment processes within these wetland ecosystems are currently being elucidated and quantified using a systems dynamic model, which will be reported at a later date.

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