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Removal of Manganese in Reservoir Tailwaters as a Function of Reservoir Operations and River Substrate.

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REMOVAL OF MANGANESE IN RESERVOIR TAILWATERS AS A FUNCTION OF RESERVOIR OPERATIONS AND RIVER SUBSTRATE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
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Doctor of Philosophy

in

The Department of Oceanography and Coastal Sciences

by
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"There is something fascinating about science. One gets such wholesome returns of conjecture out of such a trifling investment of fact." Mark Twain (1896).

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ABSTRACT

Removal of reduced manganese in aqueous systems has been described as a two-step process that incorporates a chemical or abiotic step followed by or continued with microbially mediated processes. Field and laboratory studies were conducted to describe the removal of manganese from reservoir tailwaters and partition the abiotic and microbial removal processes. Manganese removal was measured in controlled raceways and in microcosms with four sizes of substrate (cobble, 2.00 to 0.50 mm, 0.50 mm to 0.063 mm, and less than 0.063 mm). Sodium azide was used to inhibit biological uptake. An exponential decay model and a hyperbolic decay model were used to describe removal rates.

Field studies verified spatial variability in manganese adsorption to substrate exists downstream from reservoirs and adsorption occurs on a wide range of substrate sizes. Removal rates varied with discharge with a rate near 1 day^{-1} observed for low flow conditions (0.5 m^3 sec^{-1}), while the removal rate at a higher discharge (1.7 m^3 sec^{-1}) was considerably lower (0.2 day^{-1}). Higher removal rates were obtained in the raceway experiments (1.4 and 1.7 day^{-1}).

Removal rates were comparable for cobble and larger size fractions (1.9 to 0.8 day^{-1}) and were similar to raceway rates. Smaller substrate sizes displayed significantly lower removal rates (near 0.3 day^{-1}). Abiotic removal rates (sodium azide inhibition) were significantly lower than rates for uninhibited microcosms.

An exponential decay model accounted for more than 90% of the variability for most of the studies (uninhibited) but did not model abiotic alone removal very well (r^2

between 0.5 and 0.7). A hyperbolic decay model was fit to the data and resulted in an r^2 from the nonlinear regression greater than 0.95 for nearly all of the microcosms and the field data as well. Initial concentrations and removal rates developed from field and laboratory studies were used as inputs to develop a predictive model that incorporates biological processes which impact manganese removal. This model can be used as a Michaelis-Menton equation which may be more appropriate in describing the two-step manganese removal process than the exponential decay model.

CHAPTER 1
INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction

Manganese is widespread as a constituent of more than 100 minerals, occurring in oxidation states ranging from -3 to +7, and is readily cycled in aquatic systems as a function of pH, oxidation-reduction potential, and biological (microbial) processes. Understanding oxidation and reduction (redox) of manganese, thermodynamics and kinetics of redox reactions, interactions with other elements, and relationships to biological processes is necessary for describing dynamics in aquatic systems. The Mn^{+2} state is considered to be the reduced form of the oxidized, Mn^{+4} state and these two forms are generally considered to be the most important in natural waters. Manganese is introduced to the sea primarily by runoff from continents and islands in the form of dissolved, i.e., reduced Mn^{+2}, and suspended forms of oxidized manganese such as oxides and complex mineral particles (Goldberg and Arrhenius 1958; Morgenstein and Felsher 1971). Freshwater systems such as lakes and reservoirs also receive manganese in these forms from mineral weathering and runoff.

Manganese contributes to taste and odor problems in potable water supplies, staining of household plumbing fixtures, can be toxic to aquatic life in the reduced form, and its oxide forms act as a catalyst in the removal of certain heavy metals such as copper, zinc, and lead in aqueous systems. A concentration of 0.05 mg L^{-1} has been established as a secondary standard for manganese in drinking water, and Stiles (1979) estimated that approximately 40% of the utilities in the United States have difficulty in achieving this

value. Toxicity to aquatic life can occur with concentrations of reduced manganese as low as 0.3 mg L^{-1}.

Removal of manganese is a routine procedure in the water treatment industry using adsorption processes, autocatalytic reactions, and biological processes. Techniques such as adsorption to oxide coatings (Knocke et al. 1988 and 1991) and oxidation for precipitation with oxidants such as free chlorine (Knocke et al. 1988; Coffey et al. 1993) and potassium permanganate (after ozonation) (Wilczak et al. 1993) are used extensively. The presence of dissolved organic matter can interfere with oxidation processes, however, acceptable rates of Mn^{+2} oxidation can be obtained provided oxidant dosages are selected appropriately to satisfy the oxidant demand of water. "Seasoning" or "aging", which is actually the establishment of oxide coatings and bacterial populations, of filter media for Mn^{2+} removal (Griffin 1960; Weng et al. 1986) is also necessary for efficient removal. Weng et al. (1986) observed that dual-media and sand filters used for manganese removal are not always capable of reducing the concentrations below 0.05 mg L^{-1}. Removal was improved with "aged" media coated with manganese oxide and concentrations were reduced to 0.05 mg L^{-1} or less with dual-media, all sand, and activated carbon filters and to 0.08 mg L^{-1} or less for all anthracite filters. Mouchet (1992) reported that substantial improvement in iron and manganese removal was obtained with incorporation of biological processes. In addition to substrate types and extent of seasoning, surface $MnO_x(s)$ concentration, oxidation state, and pH also affect removal efficiency. Removal of zinc and manganese from mine drainages with cyanobacteria mats, (Bender et al. 1994), suggests removal mechanisms may be applicable to other manganese related problems.

The role of trace metal sequestering during manganese oxidation is also an important process that is receiving renewed attention for applications such as remediation of acid mine drainage (Harvey and Fuller 1998; Robbins 1998) and removal of lead in aquatic systems (Nelson et al. 1999). Early research followed an electrochemical theory for the formation of manganese nodules (Goldberg 1954), which suggested that the concentration of minor elements incorporated by marine biota is explained either by the direct uptake of the element or by the uptake of iron and manganese oxides with the accompanying scavenged element. Goldberg (1954) proposed as a general rule, that the longer the solid phase is in contact with the solution containing the ions to be adsorbed, the more efficient is the uptake, presuming no re-formation of the precipitate occurs. Since the hydrated oxides of manganese are negative sols (Rankama and Sahama 1950), deposits in marine waters would be expected to collect positively charged ions. Krauskopf (1956) suggested that manganese dioxide is one of the most important solid phases controlling certain trace metal concentrations in natural water environments. In fact, the oxides of Mn^{4+} have a strong adsorption or ion exchange capacity and exhibit a high affinity for cations, especially for the transition metals (Crerar and Barnes 1974; Ehrlich et al. 1973; Loganathan and Burau 1973; Varentsov and Pronina 1973). The surface chemistry of δMnO_2 in seawater, as described by Balistrieri and Murray (1982), indicates that Na, K, Mg, Ca, but not chloride and sulphate, are readily adsorbed on δMnO_2 in the pH range of natural waters. Amorphous manganese oxides and hydroxides would be effective sites for adsorption or scavenging of elements due to their large surface areas (Vuceta and Morgan 1978; Balistrieri and Murray 1982). Lion et al. (1982) found

that cadmium and lead adsorption behavior in San Francisco Bay reflected control by iron and manganese hydroxides and organic coatings on particulate matter. A general reaction describing the process was provided by Ehrlich (1975):

(1.1) $$H_2MnO_3 + X^{2+} \rightarrow XMnO_3 + 2H^+$$

where X represents the trace metal.

Manganese dynamics have been described for a variety of aquatic systems such as lakes (Mortimer 1941 and 1942; Morgan 1992; Delfino and Lee 1968 and 1971; Stauffer 1986; among others), estuaries (Graham et al. 1976; Wollast et al. 1979; Duinker et al. 1979), and fjords (Tebo and Emerson 1985). Manganese dynamics have also been described with numerous laboratory studies and have been summarized in detail in water chemistry textbooks (Stumm and Morgan 1981; among others). The fate of manganese, mobilized during depletion of dissolved oxygen in a reservoir hypolimnion has been evaluated for conceptual model construction (Gunnison and Brannon 1981; Chen et al. 1983) and described for several reservoirs (e.g., Ashby et al. 1994; Johnson et al. 1995; Herschel and Clasen 1998; and Zaw and Chiswell 1999). Information describing the fate of manganese in the release and tailwater of reservoirs is limited to a few studies (Gordon et al. 1984; Gordon 1989; Nix et al. 1991; and Dortch et al. 1992) which do not address biological processes as removal mechanisms. Development and application of one-dimensional, steady-state models to predict manganese removal in reservoir tailwaters has also been limited to a few studies (Hess et al. 1989; Dortch and Hamlin-Tillman 1995).

Manganese oxidation is both a chemical process and a microbially mediated process. Chemical oxidation in laboratory studies at pH values greater than 9.0 is well

documented (Stumm and Morgan 1981; Wilson 1980). Oxidation studies at pH values more common in surface waters (7 to 9 standard units) have also been conducted (Hem 1981). Considerably faster "oxidation" rates (e.g., hours) observed at near-neutral pH values (Hem 1981; Hess et al. 1989; and Dortch and Hamlin-Tillman 1995; and others) than autocatalytic kinetics (Stumm and Morgan 1981) or chemical equilibrium (Diem and Stumm 1984) would predict (e.g. years) imply other mechanisms such as biological processes are involved. Temperature dependence for oxidation rates in lakes (Tipping 1984) also suggested biological processes contribute to oxidation. Biologically mediated manganese oxidation and reduction has been well studied for marine (Ehrlich 1975), estuarine/fjord (Emerson et al. 1982; Rosson et al. 1984; Tebo et al. 1984; Tebo and Emerson 1985), lacustrine (LaRock 1969; Chapnick et al. 1982; Dean and Ghosh 1978; Richardson et al. 1988; Gregory and Staley 1982) and wetland environments (Ghiorse 1984a). These processes have been summarized in Ehrlich 1981; Ghiorse 1984b; Nealson et al. 1989; and Tebo et al. 1997.

Several techniques to differentiate between biotic and abiotic mechanisms of manganese removal are available. For example, manganese oxidation attributed to microbial processes is measurable using poisoned and non-poisoned experimental units (Rosson et al. 1984; Emerson et al. 1982) or with the use of radioisotopes and acidification (LaRock 1969). Oxides of manganese formed abiotically can also be determined using $CuCl_2$, (Chapnick et al. 1982). Methods for determining manganese in soils (Gambrell 1996) may also be applicable to aquatic systems, particularly streambed substrates. Determination of oxidation rates (Duinker et al. 1979; Emerson et al. 1979)

and distinguishing microbial from chemical oxidation (Wollast et al. 1979; Emerson et al. 1982; Rosson et al. 1984) may be useful in partitioning chemical and biological oxidation.

1.2 Problem Statement

Although a wealth of information exists describing chemical and biological oxidation mechanisms, techniques for differentiating these mechanisms and, processes in a variety of aquatic systems, only a limited number of studies have been conducted in reservoir tailwaters. These studies, while providing useful information, have been mostly limited to a single approach to describe manganese dynamics. Studies conducted in the tailwater region of several reservoirs have indicated that manganese removal is a first order process but removal rates vary between sites (Gordon et al. 1984; Nix et al. 1991; Gordon 1989; Hess et al. 1989; and Dortch et al. 1992). The fate of manganese (oxidation of reduced manganese and removal from the water column) in reservoir tailwaters is currently modeled with oxidation kinetics from the literature, rate constants derived from field studies, and computation of a removal rate coefficient based on transfer efficiency to the streambed and one of two substrate types (cobble or fine-grained sediment, Dortch and Hamlin-Tillman 1995). Biological processes and effects of substrate size classes from cobbles to smaller sizes on the removal of manganese are currently not addressed by the model, but are likely important mechanisms in the process.

1.3 Research Objectives

The objectives of this research were to address the basic question: What is the fate of dissolved (i.e. reduced) manganese in reservoir releases? Potential factors that can impact manganese removal in reservoir releases include discharge concentrations of

manganese, flows or velocities (i.e., exposure time), concentrations of other water quality constituents, substrate availability, chemical removal processes, and biological removal processes. Effects of substrate size and biological processes on manganese removal were emphasized in this research. The tailwater region of Nimrod Lake in central Arkansas was selected as the study location since site information and observations of manganese removal at various discharges (Nix et al. 1991) provide a background for further studies. Initial studies were conducted to characterize substrate in the tailwater region and evaluate manganese removal as a function of reservoir discharge. Additional studies then focused on chemical and microbial processes of manganese removal associated with substrate from the tailwater. Laboratory studies were conducted using substrate from a site in the reservoir tailwater that is a pool downstream from the dam and stilling basin (an area of energy dissipation and aeration via the outlet works).

Major objectives were to: 1) describe patterns in substrate characteristics and manganese removal in the tailwater (presented in Chapter 3), 2) assess the relative contribution of tailwater media (e.g., water, suspended material, substrate size fractions) on manganese removal rates (presented in Chapter 4), and 3) evaluate chemical versus microbial effects on manganese removal with assessment of the microbial community structure and determination of the contribution of microbial processes on manganese removal (presented in Chapter 4). Study results will increase the understanding of manganese removal in a flowing, freshwater system and provide a more complete description of processes that may be incorporated into predictive models such as those developed by Hess et al. 1989 and Dortch and Hamlin-Tillman 1995.

1.4 Working Hypotheses

Model development to predict manganese removal (Dortch and Hamlin-Tillman 1995) included a dependence of removal rates on substrate type or size (i.e. cobble or fine-grain sediments). However, observations of manganese concentrations that were decreasing with distance at much greater rates than kinetics would predict suggested that a different or additional mechanism (probably biological) was contributing to manganese removal. Greater changes in manganese concentrations with distance during low flow discharges than during a higher flow discharge at the same field site (Nix et al. 1991) suggested that manganese removal varied with flow or residence time (i.e., contact or exposure time with the substrate and associated biological community). Additionally, observations of oxide coatings on various sediment size fractions in the tailwater of Nimrod Lake suggested adsorption mechanisms were not limited by substrate size. These observations led to the development of two working hypotheses:

Ho1: Manganese removal rates do not vary with substrate size,

and,

Ho2: Microbial processes are not a major mechanism of manganese removal.

1.5 Research Approach

Characterization of the site and manganese removal processes (Objective 1) was conducted with field and laboratory studies. Results from previously conducted field studies (Nix et al. 1991) were used to characterize hydrologic, morphometric, and physicochemical characteristics of the tailwater region of Nimrod Lake. An additional field study to measure concentration changes with time for different discharges was

conducted for comparison to earlier studies. A detailed field study was also conducted to assess the spatial distribution of manganese on selected substrates (cobble and sediment fractions). Substrate collected from the tailwater region was used in laboratory studies employing recirculating flumes, or raceways, to evaluate manganese removal.

Manganese removal associated with substrate size fractions was described using microcosms with substrate from the tailwater of Nimrod Lake (Objective 2). Removal rates were developed from the microcosm studies and regression analysis was used to compare removal rates by size fraction. The microcosms were also used to evaluate the effects of microbial versus chemical processes on manganese removal (Objective 3) by utilizing a poison that inhibits the biological oxidation of manganese. Microbial community structure associated with selected substrate size fractions was described using a lipid analysis technique developed by Vestal and White (1989). This technique allows for determination of the total phospholipid fatty acid (PLFA) composition which can provide an estimate of microbial community biomass, and, with analysis of patterns of PLFA content, provide a measure of microbial community structure.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Much of the dynamics of manganese oxidation can be described with a review of manganese chemistry and the effects of biological processes on manganese cycling. Specific results from laboratory studies and studies in selected aquatic systems are presented to support concepts used in the development of working hypotheses of this research. A review of various modeling studies is also provided to facilitate discussions of potential application of the study results. Finally, relevance to manganese dynamics in reservoir releases during summer stratification is summarized to provide an increased understanding of manganese dynamics in an aquatic system that has been previously studied in detail by only a limited number of investigators, i.e. the tailwaters of reservoirs. The overall objective of the research is to provide new and unique information that can be applied for the improvement of descriptions and predictions of manganese dynamics in reservoir tailwaters.

2.2 Overview of Manganese Chemistry

Manganese occurs in oxidation states ranging from -3 to +7 with the +2 and +4 states generally considered to be the most important in natural waters. The +2 state is considered to be the reduced form of the oxidized, +4 state. Generally, the reduced +2 form is soluble and the oxidized +4 form is associated with insoluble compounds. Major mineral forms include manganese oxides (Mn_xO_x), manganese hydroxides ($Mn(OH)_2$), and, particularly in marine and hardwater environments, manganese carbonates ($MnCO_3$).

Manganese oxidation states fluctuate as a function of Eh, pH, dissolved oxygen concentrations, manganese concentrations, and concentrations of other anions and cations.

Effects of the oxidation/reduction (redox) potential and pH on manganese transformations have been described for waterlogged soils (Gotoh and Patrick 1972) and observations provide a general paradigm for manganese behavior that may be applicable in other systems such as anoxic zones of aquatic systems where Mn^{2+} is favored thermodynamically. The potential at which manganese oxide reduction occurs is roughly the same as that of nitrate reduction, so soluble Mn^{2+} appears in sediment and water column profiles soon after depletion of nitrate. Mn^{2+} then diffuses into the oxic zone (sediment or water depending on dissolved oxygen concentration in the water overlying the sediment) where oxidation can occur. This oxidation is accompanied by a reduction in pH and dissolved oxygen and, at sufficiently high total manganese levels, the formation of a precipitate (Pankow and Morgan 1981a). In freshwater systems, some form of oxidized manganese will be in equilibrium with Mn^{2+} at high Eh and pH values (oxidizing conditions) and some form may be in equilibrium with $MnCO_3$ at low Eh and pH (reducing conditions) Delfino and Lee (1968). Approximately ½ moles of O_2 are required in the oxidation of $MnCO_3$ based on the following equations by Bricker (1965):

Reaction	Oxygen Required (/mole $MnCO_3$)
(2.1) $MnCO_3 + ½\ O_2 = MnO_2 + CO_2$	½ mole O_2
(2.2) $8\ MnCO_3 + ½\ K_2CO_3 + 15/4 O_2 = KMn_8O_{16} + 17/2\ CO_2$	15/32 mole O_2
(2.3) $MnCO_3 + ½(1-x)O_2 + H_2O = Mn_{1-x}Mn_xO_{2-2x}(OH)_{2x} + CO_2 + xH_2$.	½(1-x) mole O_2

Utilizing the application of x-ray diffraction techniques for the identification of selected manganese minerals Bricker, (1965) was able to develop Eh-pH diagrams that provide a general relationship for oxidized and reduced forms of manganese (e.g., Figure 2.1). It is apparent that Mn_{aq}^{2+} is the predominant form of manganese for most of the Eh-pH conditions observed in surface waters. These relationships and thermodynamic calculations by Hem (1963) also indicate that above Eh + 400 mV or 0.4 V and at pH 7.8, the stability field favors insoluble manganese oxides and the concentration of Mn^{2+} should be low.

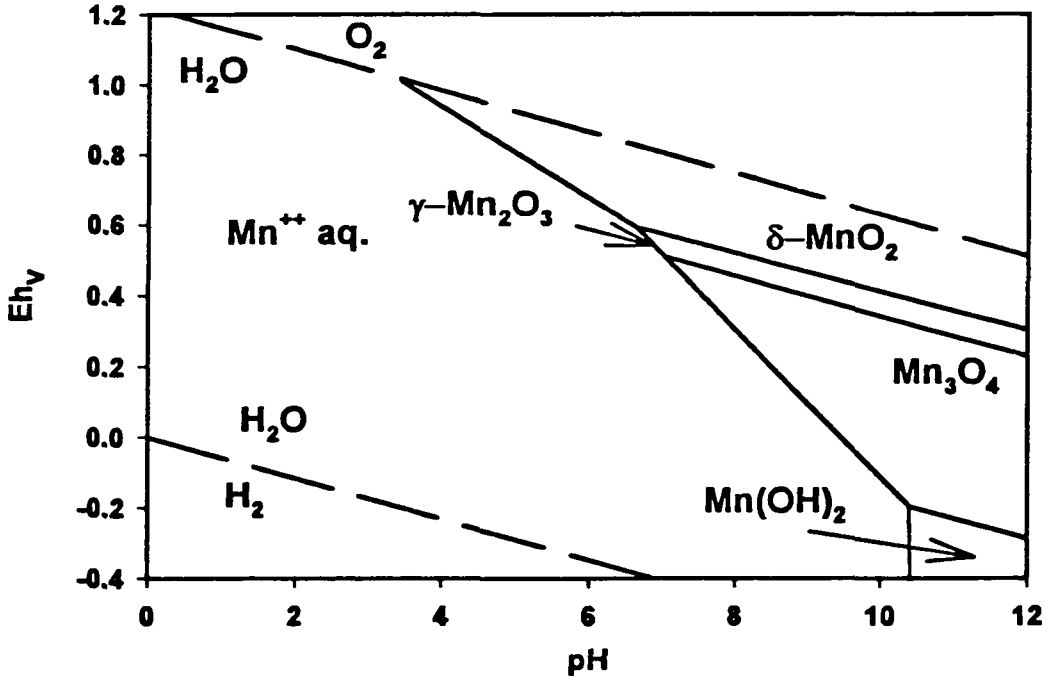


Figure 2.1. Eh-pH diagram showing stability relations among some manganese oxides at 25° C (after Bricker 1965).

The oxidation of Mn^{2+} can be considered as a two-step process as described by Morgan (1964) where first step is the sorption of soluble manganese directly onto $MnO_2(s)$ followed by a second step of slow oxidation of the sorbed Mn^{2+}. The Mn^{2+} removal rate increases with the formation of $MnO_2(s)$ and the reaction was termed autocatalytic. Specifically, once oxidation begins, the freshly formed MnO_x ($x = 1.4$-1.6) adsorbs Mn^{2+} and greatly increases the rate of the reaction (Morgan 1964). Anything that leads to changes in Eh, pH, or binding of Mn^{2+} could affect the rate of oxidation. Consequently, to predict rates of oxidation, pH, Eh, counterions, and manganese oxides present must be known. This oxidation is usually slow (not measurable on a time scale of several days below pH 8.5, Stumm and Morgan 1981) because of the high activation energy. Complexation by organic chelators, such as amino acids (Graham 1959) and anions, particularly in seawater, e.g., Cl^- (Goldberg and Arrhenius 1958) and HCO_3^- and SO_4^{2-} (Hem 1963) may contribute to the slow oxidation rate often observed.

Crerar and Barnes (1974) described the formation of deep-sea nodules as a function of pH and Eh and suggested that the solubility of Mn^{2+} decreases exponentially with increasing pH or Eh and the solubility of any manganese oxide increases exponentially with decreasing Eh and pH. Manganese precipitation occurs on the surfaces of the nodules by catalysis and also by adsorption of ionic manganese species.

Using alkalimetrtic titration curves of hydrous manganese dioxide suspensions, Morgan and Stumm (1964) and Stumm et al. (1970) were able to describe the mechanism for the drop in pH. The proposed mechanism involves the interaction of Ca^{2+} and Mn^{2+} with the hydrous manganese dioxide surface and includes the separation of a proton from

the covalent bond at the surface and the association of a solute cation with this site. The resultant reaction is:

(2.4) $Mn\text{-}OH^0 + Ca^{2+} = MnO\text{-}Ca^+ + H^+$

and the increase in H^+ lowers the pH.

Crerar and Barnes (1974) estimated the oxidation rate of manganese increases about 10^8 times and the activation energies for manganese oxidation decrease by approximately 11.5 kcal $mole^{-1}$. Crerar and Barnes (1974) concluded that catalyzing surfaces dramatically lower the activation energy (E_{act}) of the oxidation reactions by providing favorable adsorption and reaction sites, and the lowering of E_{act} enormously accelerates the rate of manganese oxidation. Crerar and Barnes (1974) describe the "growth" process for nodules with the following reactions.

(2.5) $e^- + H^+ + MnO_2(c) = MnOOH(c)$

Which provides a mechanism for the high Eh within a manganese oxide deposit, and

(2.6) $2Mn^{2+} + O_2 + 2H_2O \rightarrow 2MnO_2 + 4H^+$

which indicates that the net precipitation reaction is favored by both locally oxidizing Eh and catalytic adsorption of manganese (Crerar and Barnes 1974). Deposition would continue if there is a sufficient supply of O_2, H_2O, and Mn^{2+} flow toward the deposit, and H^+ flows away from it.

While Crerar and Barnes (1974) attributed accretion to autooxidation, they also acknowledged that Mn-fixing bacteria may also significantly accelerate accretion rates on these surfaces. Rate constants for oxidation reactions involving calcite (1.5×10^{-7} sec^{-1} g^{-1}), $Fe(OH)_3$ (7.5×10^{-7} sec^{-1} g^{-1}), and MnO_2 (2.0×10^{-6} sec^{-1} g^{-1}) are

consistent with nodule accretion rates of 10 mm per 10^6 yr (Michard 1969). Factors that contribute to oxidation rates that are faster than expected for thermodynamic reactions are discussed in detail in this chapter.

Although Mn^{2+} complexes are rare, Mn^{2+} does form a few weak complexes, however, Mn^{2+} is not significantly complexed by either humic or fulvic acids (Stiles 1979). Pankow and Morgan (1981a) observed autocatalytic oxidation of Mn^{2+} in the presence and absence of a ligand and the ligand decreased oxidation rate by decreasing free Mn^{2+} concentrations. Pankow and Morgan (1981b) also pointed out that the surfaces of metal oxides exhibited adsorptive properties towards trace metals (e.g. mineral-rich manganese nodules in marine and freshwater systems). Murray (1975) suggested that the affinity of selected metals for the surface of manganese dioxides followed the order:

$$Mg<Ca<Sr<Ba<Ni<Zn<Mn\leq Co.$$

Interaction can be characterized by its pH dependence. For example, adsorption of alkaline earth metal ions, cobalt, manganese, copper, zinc, and nickel on hydrous manganese dioxide increases as a function of pH between 2.3 and 8 standard units (Murray 1975).

Chemical modeling of trace metals (including manganese) in freshwater systems, conducted by Vuceta and Morgan (1978), focused primarily on equilibrium distribution and interactions with environmental conditions. Environmental conditions such as the type and concentration of the trace metal, types of adsorbents and the available surface area, pH, and types and concentrations of both organic and inorganic ligands were evaluated for selected trace metals. General conclusions for manganese suggested

($MnO_2(s)$) was the primary form of the precipitate and adsorption of significant amounts of trace metals was possible.

The dynamics of manganese associated with chemical oxidation processes can be described from numerous studies conducted on manganese mobilization and accumulation as concretions or nodules in association with iron and other metals. Many of these early studies were conducted in marine environments. Manganese encrustations or nodules display markedly different chemical composition which is related to the supply of reduced manganese and the chemical environment of deposition (Glasby et al. 1971; Cronan and Tooms 1969).

Early arguments for manganese dissolution from sediments to overlying waters for nodule formation were provided by Presely et al. (1967). The authors suggested that manganese appeared to be in solution either as Mn^{2+} or as a complex and study results appeared to support the concept of manganese nodule formation in deep-sea sediments through a diffusion of manganese from depth to the surface. However, Bender (1971) concluded upward diffusion does not supply a large portion of the 'excess' manganese in most manganese-rich pelagic sediments since the rate of diffusion is slow. Cronan (1972) concluded that ferromanganese oxide deposits in Lake Ontario were probably formed via both upward diffusion of iron and manganese from the sediments and in-lake circulation or movement of water enriched with iron and manganese. Both diffusion and advective transport of reduced iron and manganese (which are soluble in reduced form) are likely transport mechanisms. Stauffer (1986) suggests that the recurring patterns of Mn^{2+} in the hypolimnion of Lake Mendota, Wisconsin are best explained by microstratigraphy and

redox shifts in the upper sediments, coupled with seasonally dependent dispersion into the overlying water column. During each summer Mn^{2+} accumulation in and below the thermocline in Lake Mendota is equivalent to 2.5 years' supply from external sources. Nishri and Nissenbaum (1993) suggested that Mn-enriched carbonates were the source of manganese found in manganese oxyhydroxides on the Dead Sea coast. Concretions have also been described for freshwater systems by Harriss and Troup (1970) and the composition related to watershed chemical characteristics. In general, the manganese-rich material is poorly crystallized oxides (Bromfield 1958, via X-ray diffraction; Cronan and Tooms 1969) and oxyhydroxides (Tessier et al. 1996 via X-ray diffraction and electron microscopic analyses).

Early studies also supported the theory that adsorption processes catalyze manganese oxidation, although accumulation rates did not always agree with timelines established by Stumm and Morgan (1981), suggesting processes other than inorganic oxidation may be contributing. Hem (1963 and 1964) observed rapid precipitation of Mn^{2+} under oxidizing conditions at a pH of 8.0 on a fine sand of quartz, orthoclase, and plagioclase yet very low precipitation in the absence of the sand. Likewise, Collins and Buol (1970) observed rapid precipitation of both iron and manganese along Eh-pH gradients in quartz-sand columns and comparatively slower precipitation without catalysis. Obviously, the rate of precipitation was facilitated by the presence of a substrate. Hem (1964) observed autocatalytic deposition of manganese onto aged and aerated manganiferous sands. Gordon and Burr (1989), using columns packed with stones from the Duck River downstream from Normandy Dam, demonstrated the ability to oxidize and

remove dissolved manganese from a seep emanating from a reclaimed strip mine area. Using chert, glass marbles, and native Alabama sandstone, Gordon and Burr (1989) estimated that about 8 weeks was required for the "aging" process. Gordon and Burr (1989) attributed the "aging" to the development of a slime, implying biological effects, on manganese oxidation. Numerous other authors have demonstrated biological effects in greater detail and the topic is discussed later in this chapter.

Other surfaces or substrate types such as ferric oxyhydroxides, calcite, silica, freshly precipitated MnO_2, and manganese nodules themselves have also been implicated in precipitation of Mn^{2+} (Morgan 1967 and 1992; Morgan and Stumm 1964; Michard 1969; Jenkins 1973). Nodules also form around nuclei of pumice, altered basaltic rock or glass, clays, tuffaceous material, silica (diatom frustules or radiolarian tests), carbonate (foraminiferal, coccolith, or pteropod remains) or phosphorite (Burns and Burns 1975; Crerar and Barnes 1974), shark teeth, and whale bones, none of which contain Mn^{4+} but which can adsorb Mn^{2+}. $MnO_{x(s)}$ adsorbs Mn^{2+} and also catalyzes its oxidation, thus increasing overall removal.

In describing the rate of crystal growth of Mn_3O_4 and βMnOOH in aerated aqueous manganous perchlorate system, Hem (1981) suggests that the process is autocatalytic, but becomes pseudo-first order in dissolved Mn^{2+} activity when the amount of precipitate surface is large compared to the amount of unreacted manganese. Valence ranged from 2.67 to 3 indicating an "intermediate" oxidation.

The manganese oxidation rate may be expressed with the following equation (the Mn^{2+} terms represent thermodynamic activity of dissolved unreactive manganese and

$A_{(Mnppt)}$ represents the availability of reaction sites on the surface of the precipitated manganese dioxide).

$$\frac{-d[Mn^{2+}]}{dt} = k_1[Mn^{2+}] + k_2 A_{(Mnppt)}[Mn^{2+}] \quad (2.7)$$

The two rate constants k_1 and k_2 represent, respectively, the relatively slow precipitation reaction of dissolved manganese that is not specifically interacting with any surface, and the autocatalytic influence of an increasing availability of reaction sites as the reaction increases the area of oxide surface. When [Mnppt] becomes large and $[Mn^{2+}]$ is small, the former quantity will remain relatively constant over considerable periods of time even though $[Mn^{2+}]$ continues to decrease at a constant rate. This implies that the oxidation reaction should become pseudo-first order with respect to dissolved manganese, if other factors do not change (Hem 1981). Where the available reactive surface area is in large excess, the precipitation half-time could be as short as a few days for systems at a neutral pH and a temperature between 10 to 15 °C (Hem 1981). Using rate constants determined by Lewis (1976) from the Susquehanna River, Hem (1981) calculated half-times of about 1 day at 20 °C and about 7 days at 5 °C, at pH's near or a little below 7.0. Hem (1981) concluded that "in systems where water movement is rapid and the kinetics of precipitation are pseudo-first order, the flux rate may support accumulation of precipitate over an extensive surface area and the zone of reaction in the system may be elongated in the main direction of water flow. In a surface stream, for example, there may be manganese oxide coatings on bed material for long distances downstream from points

where Mn^{2+} is introduced." Studies by Kessick and Morgan (1975) indicated that MnOOH was the primary product of autooxidation. Many early studies indicated that these precipitates may be of biological origin (Ehrlich 1978; Mustoe 1981; Krumbein and Jens 1981; Palmer et al. 1986; Tyler and Marshall 1967; and Nealson 1977). The role of bacteria in manganese removal is discussed in the following section.

2.3 Overview of Biological Effects on Manganese Cycling

Numerous organisms have the ability to remove, oxidize, and deposit free manganous ions (Table 2.1). Microorganisms have been associated with iron and manganese minerals in fresh- and saltwater ferromanganese concretions (Burnett and Nealson 1981; Ghiorse 1980; Ghiorse and Hirsch 1982), bog ore and iron spring deposits (Caldwell and Caldwell 1980; Crerar et al. 1979; Mustoe 1981), rock varnish (Dorn and Oberlander 1981; Krumbein and Jens 1981; Taylor-George et al. 1983; Palmer et al. 1986), detrital particles in lakes and ponds (Gregory and Staley 1982; Heldal and Tumyr 1983; Jaquet et al. 1982; Klaveness 1977; Schmidt et al.1982), water-distribution systems (Marshall 1980; Ridgway et al. 1981; Ridgway and Olson 1981; and others), fjords (Emerson et al. 1982), and surface films of swamps and shallow ponds (Ghiorse and Chapnick 1983; Ghiorse and Hirsch 1982).

Early biological studies focused on bacteria associated with marine concretions. Identification and distribution of manganese oxidizing bacteria Nealson (1977), Schutt and Ottow (1977), budding bacteria Ghiorse and Hirsch (1977), and environmental conditions and time scales Thiel (1977) associated with the ferromanganese nodules were initially described for the deep sea.

Table 2.1. Partial list of Fe- and Mn-depositing organisms.

Arthrobacter	Ehrlich 1968; van Veen 1973; Bromfield and David 1976
Bacillus sp.strain SG-1	DeVrind et al. 1986; Nealson and Ford 1980
Sphaerotilus discophorus	Johnson and Stokes 1966; Ali and Stokes 1971; van Veen 1972
Leptothrix	Adams and Ghiorse 1988; Nelson et al. 1999
Citrobacter freundii (E_4)	Douka 1977
Crenothrix, Gallionella, Clonothrix	e.g., Ghiorse 1984a
Metallogenium	Jaquet et al.1982; Miyajima 1992a and 1992b
Siderocapsaceae	e.g., Ghiorse 1984a
Pedomicrobium	Zavarzin 1968; Ghiorse and Hirsch 1979
Hyphomicrobium	Tyler 1970
Thiobacillus ferroxidans	Ehrlich 1981
Pseudomonas	Okazaki et al. 1997 (P. fluorescens GB-1); Douka 1977; Jung and Schweisfurth 1979; Greene and Madgwick 1991; and others
Fungi	Thiel 1925; Ehrlich 1981
Algae	Dean and Gosh 1978; Dean and Greeson 1979; Schoemann et al. 1998
Chlamydomonas sp.	Greene and Madgwick 1991

Bacterial participation in manganese deposition around marine sediment particles has also been described as a two-step process (Ehrlich 1977; Emerson et al. 1982) yet several scenarios have been proposed (e.g., Ehrlich 1975; Tebo and Emerson 1985; Hastings and Emerson 1986; Mandernack et al.1995a). Bacteria detected on

ferromanganese nodules in deep sea sediments have the ability to oxidize Mn^{2+} after it is first bound to a Mn^{4+} oxide (Ehrlich 1963 and 1968; Ehrlich et al. 1972; Rosson and Nealson 1982). The bacteria appeared unable to oxidize free Mn^{2+} ion. In one of the earlier scenarios proposed by Ehrlich (1975), the removal of Mn^{2+} from solution by sediment involves mainly adsorption and is assumed to follow the reaction sequence of reactions 2.8 and 2.9.

(2.8) 1) $H_2MnO_3 + Mn^{2+} \rightarrow MnMnO_3 + 2H^+$ (fast)

(2.9) 2) $MnMnO_3 + 2H_2O + \frac{1}{2} O_2 \rightarrow 2H_2MnO_3$ (rate controlling for manganese accretion, accelerated by certain bacteria, Ehrlich 1963; Ehrlich et al. 1972)

Autooxidation of Mn^{2+} under conditions of the experiment was negligible. The role of bacteria in Mn^{2+} removal by sediment is to promote oxidation of adsorbed Mn^{2+} to Mn^{4+} oxide, which can then scavenge additional Mn^{2+} which, in turn, can be oxidized by bacteria. This process increases the amount of adsorption sites, i.e. Mn^{4+} oxides (i.e., A_{Mnppt} in Equation 2.7). Nelson et al. (1999) have found surface areas of biogenic manganese oxides (224 $m^2\ g^{-1}$) to be much larger than for fresh manganese oxide precipitates (58 $m^2\ g^{-1}$), and commercial manganese oxide minerals (<5 $m^2\ g^{-1}$).

In a later scenario, Tebo and Emerson (1985) demonstrated that the biologically mediated, oxidation rate of Mn^{2+} is limited by both oxygen and the concentration of microbial binding sites. Tebo and Emerson (1985) described the process as a two-step mechanism in which dissolved/reduced manganese is first adsorbed by an exchange site, E, and then oxidized with the following reactions:

(2.10) $Mn^{2+} + E \leftrightarrows Mn^{2+}\text{-}E$

(2.11) $$Mn^{2+}\text{-}E \overset{O_2}{\leftrightarrows} Mn_{oxidized} + E$$

Equation 2.10 accounts for the binding and exchange of $Mn(2^+)$ between the dissolved and solid $Mn(2^+)$ pools in the absence of oxygen. The solid pool is the $Mn(2^+)$ which is adsorbed onto the surfaces of bacteria or other inorganic particulates. Equation 2.11 describes $Mn(2^+)$ oxidation. A two-step process has also been suggested by Hastings and Emerson (1986) for marine sediments with the following reactions (as reported in Mandernack et al 1995b):

(2.12) $$3\ Mn^{2+} + \tfrac{1}{2}\ O_2 + 3H_2O \rightarrow 3\ Mn_3O_4\ (\text{hausmannite}) + 6H^+$$

which subsequently disproportionates to Mn^{4+} as MnO_2:

(2.13) $$Mn_3O_4 + 4H^+ \rightarrow MnO_2 + 2Mn^{2+} + 2H_2O$$

The sum of these two reactions is:

(2.14) $$Mn^{2+} + \tfrac{1}{2}\ O_2 + H_2O \rightarrow MnO_2 + 2H^+$$

In a third scenario, Hastings and Emerson (1986) noted that the two reactions were possible from a variety of lower valence state intermediates and were consistent with earlier findings of Hem and Lind (1983) and Hem et al. (1982). In contrast, Mandernack et al. (1995a) suggested that microbes may catalyze the direct oxidation of $Mn(2^+)$ to $Mn(4^+)$ (the combined reaction, Equation 2.14) without the formation of a discernable intermediate even when Mn^{2+} concentrations were too high to favor Mn_3O_4 disproportionation (Greene and Madgwick 1991; Takematsu et al. 1988; Mandernack and Tebo 1993). The direct mechanisms have been described by Nealson (1983) as enzymatic catalysis of specific binding by cell-associated materials. These scenarios, while different,

suggest that dissolved oxygen concentrations, substrate, and microbial activity are important in one step (e.g., Mandernack et al.1995a) or both steps e.g ., Ehrlich (1975) or Tebo and Emerson (1985).

Manganese is one of several substances that, in a reduced state, can be used as an energy source for chemolithotrophic and mixotrophic bacteria (Ehrlich 1978; Kepkay et al. 1984). Two major groups of bacteria able to catalyze manganese oxidation have been suggested by Ehrlich (1980): 1) those that act on free Mn^{2+} (some of which derive free energy) and 2) those that act only on Mn^{2+} bound to Mn^{4+} oxides (which also get free energy). The reaction for the oxidation of free ion (Mn^{2+}) would be:

(2.15) $$Mn^{2+} + 0.5\ O_2 + H_2O = MnO_2 + 2H^+$$

with a standard free energy at pH 7.0 of -68.24 kJ which would be biologically useful (Ehrlich 1978). Note that this is the same equation proposed by Hastings and Emerson (1986) as the sum of a two-step processes (see equation 2.14). The reactions for the oxidation in presence of Mn^{4+} oxide would be equations 2.8 and 2.9 above.

Ottow (1977) suggested that the accumulation of Mn^{4+} oxides may be interpreted by one of two mechanisms:

1. Accretion by enzymatic oxidation of adsorbed Mn^{2+} ions (introduced by Krauskopf (1957) and developed by Ehrlich (1963, 1966, 1975). Manganese accretion is composed of two steps, a nonbiological adsorption of Mn^{2+} by preexisting, negatively charged Mn^{2+} oxides (H_2MnO_3) and a subsequent enzymatic oxidation through Mn^{2+} oxidizing bacteria (see equations 2.8 and 2.9). In this way new adsorption sites for further adsorption reactions are produced (with affinities for Fe, Cu, Ni, Co). Such an oxidizing,

catalytic activity has been found in cell-free preparations (Ehrlich 1968) and is related to a mechanism of ATP-generation (Ehrlich 1976), or

2. Accretion by unspecific heterotrophic manganese precipitation and/or oxidation.

These bacteria (e.g., *Pseudomonas*) grow only in the presence of organic compounds.

While, bacterial oxidation is inhibited by excess organic matter (Ehrlich 1975), Li et al. (1969) and Crerar et al. (1972) have proposed that bacteria catalyze organic decay reactions of the type:

(2.16) $2H_2O + CH_2O = HCO_3^- + 5H^+ + 4e^-$,

where the carbohydrate, CH_2O, is taken as representative of typical organic matter of marine sediments. This process facilitates manganese oxide dissolution via coupled reduction reactions such as:

(2.17) $MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$.

As indicated in studies of chemical oxidation of Mn^{2+}, controlling factors include conditions of Eh, pH, amount of organic matter, and dissolved oxygen and manganese concentrations, consequently, associations of these factors with biological processes have been the subject of numerous studies. For example, Bromfield and David (1976) demonstrated that cells of *Arthrobacter sp.* rapidly adsorbed manganous ions (Mn^{2+}) from aqueous solutions from pH 5.7 - 7.5 with maximum rates at pH 6.5. However, no adsorption occurred at pH 5.4 or 7.9. Adsorption did occur between concentrations of 0.5 mM (27 mg/l) and 6 mM (329 mg L^{-1}), but was inhibited above 40 mM (2196 mg L^{-1}). Schweisfurth et al. (1977) suggested that below pH 8 and above an Eh value of +200 mV,

manganese is oxidized only by microorganisms, Mn^{2+} is oxidized only at concentrations below $3x10^{-5}$ M (1.65 mg L^{-1}), and an intracellular manganese-oxidizing protein may participate in the reaction. Unlike observations of Bromfield and David (1976) in which adsorption of manganese attributed to *Arthrobacter sp.* was not inhibited until very high concentrations were reached (e.g., > 2000 mg L^{-1}), concentrations of Mn^{2+} over 5-10 mg L^{-1} were determined to inhibit manganese oxidation by *Pseudomonas manganoxidans* (Schweisfurth et al.1977). Chapnick et al. (1982) suggested that manganese oxidation in Oneida Lake, New York was inhibited at concentrations greater than 65 μM (3.57 mg L^{-1}). Since microbial oxidation of manganese can occur under conditions that range from aerobic (Bromfield 1956; Douka 1977; Ehrlich 1968) to microaerophilic (Klaveness 1977; Uren and Leeper 1978) to anaerobic (Jung and Schweisfurth 1979) it seems likely that inhibition due to concentrations may vary by species as indicated by the varied results described above. Additionally, Rosson et al. (1984) point out that bacteria can regulate their metabolism in response to environmental conditions and the presence of a large population of a specific bacteria does not necessarily prove that microbial removal of manganese is occurring.

A variety of biological mechanisms for manganese removal, oxidation, and deposition have been proposed. Marshall (1979) proposed that mechanisms for the microbial oxidation of Mn^{2+} to higher valency forms include microbially-induced alteration to the pH and /or Eh of microhabitats, sorption of preformed oxides to cell surfaces, microbial degradation of organic-manganese complexes, the formation of insoluble protein-manganese complexes, and enzymatic oxidation of Mn^{2+}. Adams and Ghiorse

(1988) demonstrated that the oxidation state of Mn increased with age of the oxide from 3.32 in samples 11 hours old to 3.62 in samples formed over a period of 30 days with *Leptothrix discophora* as the bacteria. The involvement of cytochromes in the enzymic oxidation of Mn^{2+} and the interaction of soluble cellular constituents with the cell membrane required for Mn^{2+} oxidation and cytochrome reduction to occur have been suggested by Arcuri and Ehrlich (1980). A direct correlation found between the presence of plasmid DNA and the ability of bacteria cultured from freshwater isolates to oxidize manganese may be another possible mechanism (Gregory and Staley 1982). Okazaki et al. (1997) suggested that the manganese oxidation in *P. fluorescens* GB-1 is probably catalyzed by a protein which is part of the outer membrane or cell cover of the bacterium. The Mn^{2+} oxidizing activity of the cells first appeared in the early stationary growth phase (i.e. was dependent upon growth stage and results in a lag), the oxidation follows Michaelis-Menton kinetics (Michaelis and Menton 1913) and oxidation is inhibited by sodium azide (NaN_3). Applying the Michaelis-Menton equation to describe this process yields a generic equation:

(2.18) $$R = \frac{V_{max}}{1 + C / K_m}$$ where

R = the rate of Mn^{2+} removal,

V_{max} = the maximum rate of removal of Mn^{2+},

C = the initial concentration of Mn^{2+}, and

K_m = the concentration of Mn^{2+} at which the removal rate is half of the maximum.

Emerson et al. (1982) and Chapnick et al. (1982) refer to Mn^{2+} removal as "binding" rather than "oxidation" since oxidation implies a specific process. Mn^{2+} binding is in part the result of bacterial catalysis. Michaelis-Menton enzyme kinetics have been used by Sunda and Huntsman (1987) where $V_{max} = 1.2 * 10^{-8}$ mol liter^{-1} h^{-1} and $K_m = 1.9 * 10^{-7}$ M, Tebo and Emerson (1985 and 1986), and Tebo et al. (1997) where the latter were able to model both the Mn^{2+} binding rates in the absence of oxygen and the in situ oxidation rates as a function of Mn^{2+} concentration. Tebo and Emerson (1986) concluded that: 1) Mn^{2+} oxidation rather than binding was the rate-limiting step, and 2) the rate of Mn^{2+} oxidation was limited by the number of Mn^{2+} binding sites (or the number of Mn^{2+} -binding and -oxidizing bacteria, see Equation 2.7). Crerar and Barnes (1974) likened the net effects of microbial activity to those of adsorption: oxidation reactions are catalyzed, activation energies decreased, and local Eh elevated above that of typical seawater and suggested that a model parallel to that used to treat adsorption may be applied to biogenic catalysis.

The number, type, and distribution of manganese oxidizing bacteria are also important factors in the removal of Mn^{2+} from aquatic systems. In freshwater systems, manganese-oxidizing heterotrophic bacteria were found to comprise a significant proportion of the bacterial community of Lake Washington (Seattle, WA) and Lake Virginia (Winter Park, FL), yet the percentage of heterotrophic bacterial population that could oxidize manganese varied throughout the year and no consistent seasonal vertical patterns were found in their distribution (Gregory and Staley 1982). Emerson et al. (1982) suggested that manganese may be bound by specific bacteria which are a relatively

small fraction of the total microbial population and manganese removal activity is not simply due to the presence of high numbers of bacteria. Diem and Stumm (1984) demonstrated that Mn^{2+} catalysis increased with the addition of manganese-oxidizing bacteria. Miyajima (1992b) presented a thermodynamic evaluation of manganese oxidation by *Metallogenium sp.* that suggested the distribution of the bacterium was determined by the thermodynamic balances between oxidants (O_2, Mn-oxide) and reductants (Mn^{2+}, Fe^{2+}) in the redox transition layer of a freshwater lake.

A general description of manganese dynamics for aquatic systems that undergo wetting and drying cycles (e.g., bogs, wetlands, reservoir tailwaters) may be drawn from studies conducted by Ghiorse (1984a) that describe bacterial transformations of manganese in association with the root zones of a floating macrophyte (*Lemna* sp.) in a swamp. Results from Ghiorse (1984a) demonstrated that the accumulation of particulate manganese and iron at the surface of the swamp reflects mostly ferromanganese oxide in the surface film. Although the system was partially aerobic for most of the year, manganese reduction also occurred at the surface. Circumstantial evidence suggested that *Leptothrix* cells in the surface films were involved and increases in particulate manganese in the middle layer were probably associated with the shedding of Mn-encrusted *Leptothrix* sheaths from *Lemna* roots. Increased concentrations of dissolved manganese in the bottom water corresponded to a decline of *Lemna* at the surface and probably resulted from chemical reduction of manganese oxides associated with the *Lemna* roots as they settled to the anaerobic bottom water. Ghiorse (1984b) suggested that *Leptothrix* sp.: 1) oxidzes manganese during heterotrophic growth (late in the growth curve for

Sphaerotilus discophorus (Hajj and Makemson 1976)), 2) growth is inhibited by manganese (also observed for *Sphaerotilus discophorus* by Hajj and Makemson (1976)) and, 3) excretes a manganese-oxidizing substance which behaves like a protein with respect to heat and $HgCl_2$ sensitivity.

An excreted Mn-oxidizing protein associated with *Leptothrix* sheath material, causing manganese oxide accumulation on the exterior of the sheath, has been suggested by van Veen et al. (1978) and Adams and Ghiorse (1988). Interestingly, the sheath forming capacity is an unstable trait of *Leptothrix discophora* (Emerson and Ghiorse 1992; Ghiorse 1984b) and hence, the presence of *Leptothrix discophora* alone may not be sufficient to increase manganese oxidation or removal. Manganese deposition on the exterior of *Pedomicirobium*-like budding bacteria has also been associated with extracelluar polymers (Ghiorse and Hirsch 1979). Ghiorse (1984a) suggested that it is possible that root exudates of *Lemna* sp. (perhaps organic acids) may participate in manganese reduction by chemically reducing manganese or by stimulating heterotrophic bacteria to produce Mn-reducing end products in the root zone. Klaveness (1977) describes the morphology and distribution of *Metallogenium* in lakes and suggests that *Metallogenium* accumulates manganese in its star-shaped coenobia and the encrusted parts are not enclosing living structures. Okazaki et al. (1997) have demonstrated that *Pseudomonas fluorescens* GB-1 (rod-shaped, gram-negative bacterium) deposits manganese oxide around the cell. Precipitation of MnO_2 onto the sheaths of manganese oxidizing bacteria has also been observed by Hajj and Makemson (1976). While at the time the possible mechanism(s) for the accumulation were controversial (autooxidation or

bacterial metabolic processes), earlier work by Johnson and Stokes (1966) suggested that oxidation of manganous ions by *Sphaerotilus discophorus* was catalyzed by an inducible enzyme(s).

Organisms other than bacteria also contribute to oxidation of Mn^{2+} (see Table 2.1). Shapiro and Glass (1975) provided evidence that manganese and phosphorus limited algal growth in Lake Superior and manganese was the stimulatory agent. Dean and Ghosh (1978) and Dean and Greeson (1979), on the basis of measurements of the distribution of particulate manganese, postulated that phytoplankton were important in Mn^{2+} oxidation in Oneida Lake, New York. Phytoplankton are very efficient at taking up and accumulating Mn^{2+}, which is required as a cofactor in the water-splitting, O_2-evolving enzyme in the photosystem II (Sauer 1980; Sunda and Huntsman 1998a). Studies by Richardson et al. (1988) also support role of phytoplankton in manganese oxidation. Cycling of dissolved and particulate manganese in coastal waters has also been associated with phytoplankton blooms and the successive autotrophic and heterotrophic activities (Schoemann et al. 1998). Retention of manganese during benthic photosynthesis can be dominated by chemical precipitation and reoxidation processes that can occur on a time scale of minutes (Epping et al. 1998). The transport and intracellular accumulation of Mn^{2+} in algae (Sunda and Hunstman 1985) decreases the concentration of biologically available Mn^{2+} and may be a limiting nutrient for primary production in aquatic environments (Paerl 1982). Richardson et al. (1988) demonstrated that the oxidation of Mn^{2+} to particulate, extracellular, manganic oxides can be catalyzed by *Chlorella* sp. Indirect removal or decrease in the biologically available Mn^{2+} by phytoplankton may also result as

phytoplankton change the pH and Eh of the system. Admiraal et al. (1995) suggested that riverine phytoplankton photosynthesis promotes Mn precipitation.

2.4 Biological Oxidation Rates

Determination of oxidation rates and distinguishing microbial from chemical oxidation may be useful in partitioning chemical and biological oxidation. Manganese oxidation can be measured by using the benzidinium-HCl reagent (Feigl 1958) and the colorimetric method using formaldoxime (Morgan and Stumm 1965; Henriksen 1966) has been generally accepted by major investigators as acceptable for specific measurement of Mn^{2+}. The method, with modification, has been applied to seawater (Brewer and Spencer 1971) and pore water of anoxic estuarine sediments (Armstrong et al. 1979). A spectrophotometric method for determination of oxidized manganese using leuco crystal violet has also been developed by Kessick et al. (1972). La Rock (1969), Tebo (1983), and Rosson et al. (1984) have employed the use of radioactive tracers for measuring biological oxidation. Poisons such as sodium azide, a mixture of sodium azide, penicillin, and tetracycline, and formaldehyde were shown to be appropriate for field use in marine systems to inhibit biological oxidation (Rosson et al. 1984; DeVrind et al. 1986; Mandernack and Tebo 1993). Sodium azide, penicillin, and formaldehyde also showed limited interference when distilled water was substituted for seawater, suggesting applicability to freshwater systems, but tetracycline, mercuric chloride, and the poison mixture exhibited moderate-to-strong inhibition of manganese binding and therefore results cannot be extrapolated to freshwater system without rigorous testing of poisons Rosson et al. (1984). Using poison controls such as sodium azide, well-buffered formalin,

or mercuric chloride to inhibit biological Mn^{2+} removal measures Mn^{2+} binding or removal rate from solution, but not necessarily oxidation. Methods of directly measuring oxidation involve extraction techniques, isotopes, and colorimetric methods specific for Mn^{2+}. For example, $CuCl_2$ can be used to extract adsorbed Mn^{2+} which allows distinguishing between Mn^{2+} binding and Mn^{2+} oxidation (Chapnick et al. 1982).

Biological effects on the oxidation rates of Mn^{2+} in aquatic systems have been described from numerous studies. Initially, circumstantial evidence of biological mediation in manganese oxidation in freshwater systems (Esthwaite Water and Rostherne Mere, England) was provided by Tipping (1984) with measurements of optimal oxidation rates of Mn^{2+} at temperatures between 15-30 ^{o}C and Tipping et al. (1984) based on observed oxidation rates. Studies by Emerson et al. (1982) in a low dissolved oxygen environment indicated that the residence time of Mn^{2+} and removal to a solid phase is on the order of a few days, suggesting higher oxidation rates than would be expected based on kinetics. An average oxidation state between 2.3-2.67 indicated incomplete oxidation and only 25% of the manganese was in Mn^{4+} oxidation state. These values are well below what would be expected for MnO_2 and are consistent with the proposed two-step removal process.

In studies where anoxic water from Lake Mendota was aerated, Delfino and Lee (1968) assumed first order kinetics for manganese oxidation at a pH of 8.5 and a pMn^{2+} of 4.95 (0.6 mg L^{-1}). They calculated a quarter-life value of 10.5 days which agreed well with a value of 7 days obtained from Wilson (1980). Using data from Delfino and Lee (1968), Wilson (1980) suggested quarter-life values for Mn^{2+}, assuming first order kinetics as a function of pH and pMn^{2+}, would be 25 to 30 years for a concentration of 2 mg L^{-1}

Mn^{2+} at a pH of 7.0 to be oxidized to a level of 1.5 mg L^{-1}, if only chemical oxidation was considered. Emerson et al. (1979) concluded mean residence time of manganese with respect to oxidation in a fjord was on the order of days, which is too rapid for chemical oxidation (Stumm and Morgan 1981). Sung and Morgan (1981) demonstrated that the presence of milli-molar levels of γ-FeOOH significantly reduced the half-life of $Mn(2^{+})$ in 0.7 M NaCl from hundreds of hours to hours. Surface rate constants for colloidial MnO_2 and γ-FeOOH were comparable. Studies in Saanich Inlet, British Columbia, Canada (Emerson et al. 1982; Rosson et al. 1984; Tebo et al. 1984; Tebo and Emerson 1985); Framvaren Fjord, Norway (Tebo et al. 1984); Oneida Lake, New York (Chapnick et al. 1982); Lake Washington, Washington (Maki et al. 1987) indicated that the biological component was significant at all locations.

Chapnick et al. (1982) demonstrated a relationship between Mn^{2+} removal and substrates, bacteria, and concentration of Mn^{2+}. Binding and oxidation of Mn^{2+} was inhibited in filtered samples when particles > 0.4 μm were removed, and inhibition persisted when the particles were first ethanol treated and then reintroduced into filtered lake water. Within the range of 0-20 μM (0-1.1 mg L^{-1}) Mn^{2+}, rates of Mn^{2+} binding varied with the initial Mn^{2+} concentrations. Binding rates were -0.003 to -0.02 μmol L^{-1} h^{-1} at 0-140 hour intervals and natural concentrations of 0.48 and 1.2 μM (0.03 - 0.07 mg L^{-1}). The rate of formation of MnO_2 was -0.01 μmol L^{-1} h^{-1}, half of the initial binding rate (0-10 hours) which was similar to 35-110 interval binding rates and an order of magnitude greater than 12-62 interval binding rates and comparable to rates reported by Sunda and Huntsman (1987) for the Newport River estuary in North Carolina. Rate of

Mn^{2+} binding increased logarithmically with increasing concentration until a constant rate (saturation point) was reached at about 15-20 μM (0.82 - 1.1 mg L^{-1}) Mn^{2+}. A logarithmic fit to the data defines the equation:

(2.19) binding rate = -0.05 - 0.05 ln{Mn^{2+}}, r^2=0.96, n=6

Circumstantial evidence that the removal of manganese was bacterially mediated was evident in observed decreases in soluble Mn^{2+} and the formation of oxidized manganese (MnO_2) in the hypolimnion of Oneida Lake only for summer samples which supported metabolically active Mn-oxidizing bacteria. Mn-oxidizing bacteria were abundant in bottom water (10^3 - 10^5 CFU ml^{-1}) and numbers were temperature dependant with maximum numbers in the summer. Chapnick et al. (1982) concluded that it seems likely that bacterially mediated adsorption of the soluble Mn^{2+} accounts for much of the initial binding of soluble Mn^{2+}. Subsequent decreases in Mn^{2+} would then be due to a combination of biologically catalyzed adsorption and oxidation. As MnO_2 is formed, it will enhance the adsorption of Mn^{2+} from solution and add to the observed decreases in soluble Mn^{2+}. The adsorbed Mn^{2+} may then be oxidized. Tebo et al. (1984) have since confirmed that manganese was being oxidized and not simply bound.

Additional circumstantial evidence of bacterially mediated manganese removal in lakes is provided with an evaluation of residence times or oxidation rates. Manganese oxidation in Oneida Lake water samples occurred within ½ to 3 days at pH 8.0 (Chapnick et al.1982), a rate much greater than those observed by Stumm and Morgan (1970). Residence time of Mn^{2+} at the redox gradient in Lake Biwa, Japan was estimated by Miyajima (1992a) to be 2 to 5 days at a pH near 7 and the precipitation of manganese

(attributed to biological mediation by *Metallogenium* sp.) was consistent with Michaelis-Menton kinetics. The retention of manganese in Wahnbach reservoir in Germany has also been considered to be dependent upon the presence of manganese oxidizing bacteria, i.e., *Metallogenium personatum* (Herschel and Clasen 1998).

Johnson et al. (1995) assessed the role of biotic and abiotic mechanisms of manganese redox cycle in a freshwater dam over a 12-month period and suggested that enzymatic control of manganese oxidation was taking place with a temperature optimum of approximately 30°C. Manganese oxidation was only significant above about 19°C and oxidation was pseudo-first order and bacterially mediated. Bourg and Bertin (1994) suggested that a threshold temperature of 10°C was necessary to trigger microbially mediated reactions in river and groundwater systems.

Studies relevant to reservoir releases conducted by Gordon (1989) provided removal rate coefficients of 0.021 hr^{-1} at 1.2 m^3 sec^{-1} (43 ft^3 sec^{-1}); 0.026 hr^{-1} at 3.4 m^3 sec^{-1} (119 ft^3 sec^{-1}); and 0.028 hr^{-1} at 5.1 m^3 sec^{-1} (180 ft^3 sec^{-1}) which were useful in model development by Hess et al. (1989) and Dortch and Hamlin-Tillman (1995). Quarter-life values were 5.95 hours, 4.79 hours, and 4.53 hours (Gordon et al. 1989) which were considerably less than values of 7 to 10 days observed by Delfino and Lee (1968) but higher than uptake rates of 1-3 hours reported by Harvey and Fuller (1998) suggesting that they were within a range that is influenced by environmental conditions. Possible explanations considered were: 1) biological oxidation, and, 2) sorption on manganese coated surfaces. Mn^{2+} is oxidized as a linear function of time-of-travel at a rate of 0.41 mg L^{-1} hr^{-1} at a pH of 7.1 and a temperature of 17 °C (Gordon et al. 1984).

The oxidized Mn precipitate was quickly settled and/or sorbed upon rocks and debris resulting in a linear loss of total Mn with time-of-travel (0.35 mg L^{-1} $hour^{-1}$ at the above conditions). Of particular interest is the increase in removal rate as flow increases. The relationship between the oxidation rate for manganese and flow at the dam was log-log and could be used to predict the Mn^{2+} oxidation rate at other flows (Gordon et al.1984).

Small volume batch studies were conducted (Gordon 1989) in which slime was scraped from tailwater rocks and dispersed into solutions to evaluate effects of biological mechanisms. Increased removal of manganese indicated biologically mediated removal on rock surfaces (chert of the Fort Payne Formation) is an important component of the removal processes. A hydraulic loading rate critical to Mn removal efficiency of 1560 m^3 hr^{-1} ha^{-1} was calculated for a 4 million gallon a day load. Major conclusions from Gordon (1989) were:

1) Removal of manganese is first order with time of travel and reaction rate coefficients are a function of flow. There is no pronounced colloidal form.

2) Rock slime enhanced removal rates.

3) Hydraulic loading not mass (concentration) loading was the controlling parameter for efficiency.

2.5 Biological Interactions with Substrates

Several studies have demonstrated a relationship between substrates and bacteria that mediate manganese oxidation. In the water column, substrate size is a factor as indicated by Chapnick et al. (1982) who found that Mn-oxidizing bacteria tended to be associated with particles that are normally removed during filtration (e.g. > 0.45μm).

Nealson and Ford (1980) observed little or no manganese oxidation by a marine bacillus (strain SG-1) when it was not attached to surfaces but when it attached to a variety of surfaces (glass, sand, calcite), manganese was rapidly removed from solution indicating that substrate type may not be a factor. However, there was a difference in morphology between cells that were attached and free-swimming and implications of attached bacteria on manganese oxidation are discussed more fully later in this chapter.

Although substrate type is quite often not a factor in bacteria attachment, effects of substrate type on manganese oxidation have been observed. Wilson (1980) conducted studies with varied amounts of different substrates (montmorillonite, kaolinite, goethite, and particulate and soluble organic matter) to determined effects on Mn^{2+} oxidation rates. The study was conducted at a pH near 9.0. Study results indicated surfaces accelerate the reaction, apparently by bonding Mn^{2+}, and soluble organic materials retard the reaction by complexing the oxidizable species. Results are applicable to high concentrations (50-500 mg L^{-1}) of suspended material and the author speculates that no effect would be observed at low concentrations of suspended material. Organics at natural levels are sufficient to retard the reaction. Observations are most applicable in ocean waters and less predictable in lake waters. A suggested order of ability of substrates to accelerate the reaction of Mn^{2+} oxidation is iron hydroxides > particulate *Cyperus esculentus* (a sedge) > montmorillonite > kaolinite.

Hart et al. (1992) suggested that the primary process controlling the concentration of soluble manganese in a riverine system was the rapid adsorption of soluble manganese to existing colloidal fractions, followed by slower transfer of part of this surface-bound

manganese to the interior of the colloid. Rapid manganese removal from mine waste was possible using colloidal and particulate formation associated with concentrations of magnesium and calcium.

Bacteria interactions with substrates in streams have been observed in association with ferric hydroxide deposition with increased concentrations of iron and manganese (Ghiorse 1984a; Wellinitz, et al. 1994). Blooms of sheath-forming, ferromanganese-depositing bacteria, may thrive in iron oxide deposition zones if the iron oxide helps displace periphytic algae which normally out compete the bacteria for space and nutrients (Wellnitz and Sheldon 1995). Diversity and abundance of macroinvertebrates (Wellnitz et al. 1994), diatoms (*Leptothrix ochracea*, Sheldon and Skelly 1990) and epilithic algae (Sode 1983) are typically decreased in the presence of ferromanganese-depositing bacterial blooms. Wellnitz and Sheldon (1995) demonstrated that increased iron, not manganese, concentrations resulted in lower diatom abundance.

In a lotic environment such as a river or reservoir tailwater, the ability of bacteria to attach, and remain attached, to substrate and, the supply of materials (i.e., Mn^{2+}) to the bacteria are both major factors in determining the removal rate of Mn^{2+}. Marshall (1980) suggested that microbial deposition of manganese occurs mainly at solid surfaces in oligotrophic and mesotrophic waters and involves the adhesion of manganese oxidizing bacteria to the surface. In defining zones of nitrification for small streams and large rivers, Tuffey et al. (1974) noted that a key element is sufficient residence time to develop a bacterial population. Geesey et al. (1978) reported that sessile bacteria in small mountain streams were numerically more important than free-floating bacteria and were

associated with the upper surfaces of submerged rocks. Attached algae provided a surface for bacterial colonization. Costerton et al. (1978) have suggested that bacteria stick to submerged rocks in a fast-moving stream by means of a mass of tangled fibers of polysaccharides, or branching sugar molecules, that extend from the bacterial surface and form feltlike "glcocalyx" surrounding and individual cell or a colony of cells. To generate and maintain a glycocalyx, a bacterial cell must expend energy.

Recent advances in biochemistry have resulted in a technique that is useful in characterizing the microbial community of selected habitats (i.e. streambed substrates). A method developed by Vestal and White (1989) that uses the extraction of cellular lipids provides a quantitative and nonselective method of analysis for all viable microorganisms within complex communities. The technique has been used to evaluate physiological stress and pattern recognition of microbial community structure in periphyton communities of a stream that receives industrial wastes (Guckert et al. 1992). Interpretation of data from this technique has also been used in signature lipid biomarker analysis and provides a quantitative means to measure: 1) viable microbial biomass, 2) microbial community composition, and 3) community nutritional status (White et al. 1997).

The biological community associated with river substrate is described very well using characteristics of biofilms. Biofilms in streams less than 1.5 m deep have been estimated to be comprised of 90 to 99% bacterial biomass (Wuhrmann 1972). Substrate size may also determine the amount of bacterial growth with larger substrates having a greater part of their surface areas in growth than smaller particles (Ledger and Hildrew 1998). Removal rates of leaf leachates (Lock and Hynes 1976), ammonia (Tuffey et al.

1974), total organic carbon (Srinanthakumar and Amirtharajah 1983), and BOD (eg. Harremoës 1982 and Boyle and Scott 1984) have been attributed to biofilms in shallow streams. The importance of biofilms in describing removal processes would be reflected in the vertical structure in biofilms and mass transport associated with changes in velocity and substrate types. Gantzer et al. (1988) suggested that mass transport is faster for upper streambed biofilms than for deeper or lower streambed biofilms and that the ratio between substrate uptake rates for biofilms located at different depths in the streambed can change with stream velocity. For example, the upper portion of a streambed would provide most of the manganese removal from the water column, because the interstitial water velocities are very slow and the flux of manganese from the water column would decrease with depth. As stream velocities increase, the interstitial water velocities also increase and substrate flux into the lower streambed biofilms could approach flux rates observed for the upper streambed biofilms.

Meunier and Williamson (1981) developed a biofilm model that considers two basic processes: (1) diffusion of substrate from the bulk liquid into the biofilm; and (2) transformation of the substrate by bacteria within the biofilm. For the bacteria at depth z the potential reaction rates, r, by the Monod equation (Monod 1942), would be

$$r_d = -k_d \frac{S_{cd}}{S_{cd}+K_{sd}} X_c \qquad (2.20)$$

where r_d = the potential reaction rate of d based on concentration of S_{cd}; k_d = maximum substrate utilization rates for d; K_{sd} = half-velocity coefficients for d; S_{cd} = substrate

concentration at the differential element for d; and X_c = organism concentration within the biofilm. The Monod equation was also proposed by Williamson and McCarty (1976) as a model to describe substrate utilization by bacteria..

The movement of material (flux) into the biofilm in streambeds dominated by gravel was at a lower rate when compared to a streambed dominated by cobble (Gantzer et al. 1988). Short-term changes in water velocity affected rates in both streambeds and Gantzer et al. (1988) proposed a modified transport model to describe the process:

$$D_L d^2 \frac{S}{dx^2} - u\frac{ds}{dx} - r_b - r_f = 0 \qquad (2.21)$$

in which D_L is the longitudinal dispersion coefficient (cm^2h^{-1}), S is the water column concentration of the contaminant (mg cm^{-3}), x is the longitudinal distance down the stream channel (cm), u is the average stream velocity (cm hr^{-1}), r_b is the rate at which suspended microorganisms are reducing the contaminant concentrations (mg cm^{-3} hr^{-1}), and r_f is the rate at which attached microorganisms, the streambed biofilms, are reducing water column contaminant concentrations (mg cm^{-3} hr^{-1}).

Harvey and Fuller (1998) evaluated the effect of manganese oxidation in the hyporheic zone at three spatial scales: 1) sediment-grain scale, 2) hyporheic flow-path scale, and 3) stream-reach scale. They calculated a reach-averaged time constant ($1/\lambda_t$) for manganese uptake of 1.3 hours for the hyporheic zone and about 20% removal of dissolved manganese in the study reach. Study results indicated that uptake in the hyporheic zone at the stream-reach scale depends on the balance of chemical reaction

rates, hyporheic porewater residence time, and turnover of streamflow through hyporheic flow paths.

As described in several of the above equations, manganese removal in the streambed is directly affected by the advection of dissolved oxygen into the hyporheic zone (Harvey and Fuller 1998), the availability of dissolved organic carbon for metabolism (Findlay et al. 1993; Rutherford et al. 1995; McMahon et al. 1995), the supply of Mn^{2+} (e.g., Tebo et al. 1997), and the presence of active manganese-oxidizing bacteria (Rosson et al. 1984). Subsequent studies by Marble et al. (in press) have indicated that removal of Mn^{2+} is independent of dissolved oxygen concentrations except at very low levels and the biologically mediated process is approximately first-order and inversely proportional to H^+ concentrations.

2.6 Modeling Studies of Manganese Oxidation

One of the first attempts at modeling manganese oxidation in rivers (Hess et al. 1989) uses a one dimensional model calibrated using dissolved oxygen, biochemical oxygen demand, pH, manganese, and hydraulic data collected in the Duck River, TN. Sampling was conducted at 3 flows (1.2, 3.37, 5.15 $m^3\ sec^{-1}$ or 43, 119, and 182 $ft^3\ sec^{-1}$). The model was applied to Chattahoochee River (Buford Dam, Lake Lanier) and discrepancies between observed and predicted were attributed to inadequate pH data, precipitation of sediment particles, unsteady flow (peaking releases for hydropower generation), inaccurate rate expressions for the low pH conditions, or their combinations. This study did not consider sediment particles and biological activity and did not predict Chattahoochee River concentrations well, probably due to limited data and unsteady flow.

Manganese removal has been successfully modeled in reservoir releases using the U.S. Army Corps of Engineers tailwater model (TWQM) by Dortch et al. (1992) and Dortch and Hamlin-Tillman (1995). The model kinetics use a first order removal rate, which is highly variable natural log transformed concentrations, 0.3 to 4.45 day $^{-1}$, but is correlated with stream slope. Distinction is made for substrate type (cobble or fine-grained) with presence of cobble increasing removal rates. Biological mechanisms are suggested but not addressed and the question of annual acclimation of biota to increasing tailwater concentrations of manganese was raised. A logical next step to improve model application to reservoir tailwaters is providing information that can be used to better describe/predict variables such as substrate size effects and biological mediation.

2.7 Summary

Manganese is a transition metal that "moves" from oxidized to reduced and back to oxidized states as a function of Eh, pH, dissolved oxygen, manganese concentrations, concentrations of other elements and ligands, and biological activity (and environmental factors that influence biological activity). The cycling of manganese occurs in a wide variety of aquatic systems, involves interactions with substrates, and coprecipitation of contaminants with a potentially high level of efficiency but related to an affinity for coprecipitates.

Much of our understanding of manganese dynamics came from studies on marine concretions and studies that were expanded to freshwater systems. During these studies, it became apparent that chemical equilibria described by thermodynamics was not always observed when kinetics were considered leading to the evaluation of biological effects on

oxidation and reduction of manganese. Not only are a wide variety of organisms capable of mediating manganese oxidation, but many may derive energy from the process in a selective fashion. Although the utilization of reduced manganese is still not fully understood, a two-step process for describing the removal, adsorption, or binding of reduced manganese and subsequent oxidation at a later time is generally accepted. This process can be described with a first order exponential decay equation, a pseudo-first order equation, and/or a general form of a Monod equation or Michaelis-Menton kinetics. Incorporation of substrate interactions and mass transport processes in biofilms can be used to expand these equations for application to reservoir tailwater models.

CHAPTER 3
MANGANESE REMOVAL IN THE TAILWATER

3.1 Introduction

The removal of dissolved manganese in reservoir tailwaters has been described with several models that account for abiotic processes such as adsorption, which is related to chemical equilibrium and exposure to substrate. Combinations of conditions that influence equilibrium (e.g., pH, temperature, concentrations of manganese) and exposure to substrate (e.g., amount and size of substrate and contact area and time of water to substrate (i.e., velocity and discharge)) have been addressed in several studies designed to predict manganese removal in reservoir tailwaters (Gordon et al. 1984; Hess et al. 1989; Dortch and Hamlin-Tillman 1995). Manganese removal in these studies was described with first-order equations but removal rates were much faster than predicted by the equilibrium equations for the range of environmental conditions. Major conclusions suggested bacterial mediation, which is well documented for other aquatic systems, and effects of substrate availability. Study recommendations included more detailed assessments of substrate interactions in the removal of manganese and additional evaluation of discharge or releases on manganese removal predictions.

The objectives of these studies were to describe spatial variability and distribution of manganese accumulated on various substrate size fractions and evaluate manganese removal rates at different discharges and in controlled, laboratory studies for comparison to field observations. Manganese removal patterns in reservoir tailwaters as a function of substrate interactions and discharge were assessed with a combination of field and

laboratory studies. Spatial patterns of manganese concentrations on substrate in the tailwater of Nimrod Lake, AR were described for four size fractions of the streambed material. Lateral and longitudinal variability in manganese accumulation on streambed substrate were assessed for size fractions that provided sufficient material for analyses. Field studies were conducted at the tailwater to evaluate manganese dynamics in reservoir releases (discharge) and potential relationships between discharge and removal rates. Removal rates were calculated for two different discharges. Laboratory studies were also conducted in recirculating raceways using substrate collected from the tailwater of Nimrod Lake to describe removal rates at closer time intervals.

3.2 Field Site Description

Nimrod Lake is a reservoir on the Fourche La Fave River in west central Arkansas (Figure 3.1) and is 101 km upstream from the confluence of the Fourche La Fave River with the Arkansas River. The area of the predominantly rugged and wooded drainage basin is about 1760 km^2. Bedrock in the area is composed of shale, sandstone, and siltstone of the Atoka-Pennsylvanian Age. Soils in the area are characterized mostly as loams over silty clay or clay subsoil.

Nimrod Lake is formed by Nimrod Dam, which is operated primarily for flood control, and typically has a minimal release during stratification to provide for low flow. The centerline of the penstocks, the opening in the dam on the upstream side, is near 7.6 m above the stream bed which provides for the release of mid-depth water. Releases are discharged through two Howell-Bunger valves providing a discharge above the elevation of the tailwater which falls into a stilling basin for energy dissipation (Figure 3.2).

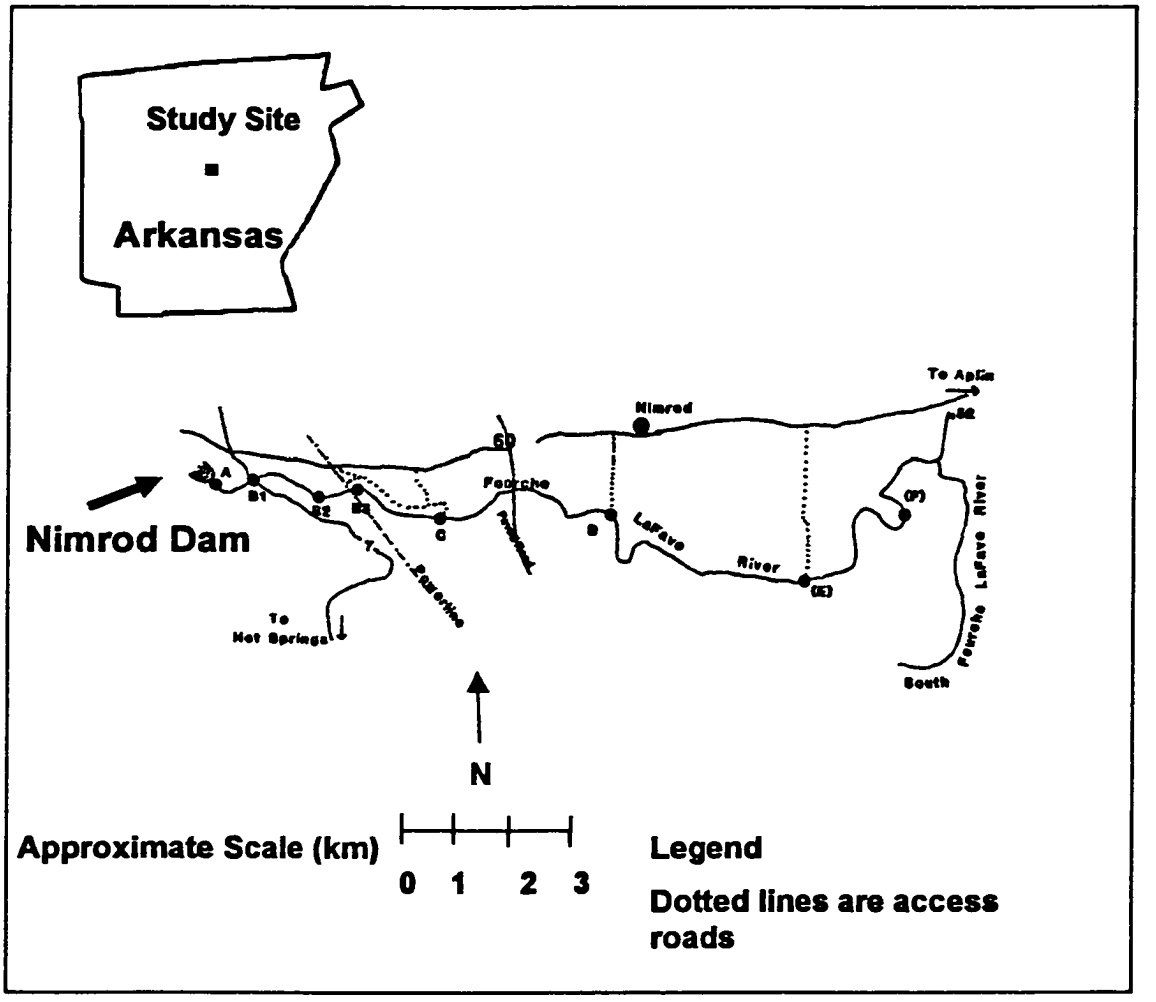


Figure 3.1. Vicinity map of Nimrod Dam and Lake and station locations in the tailwater.

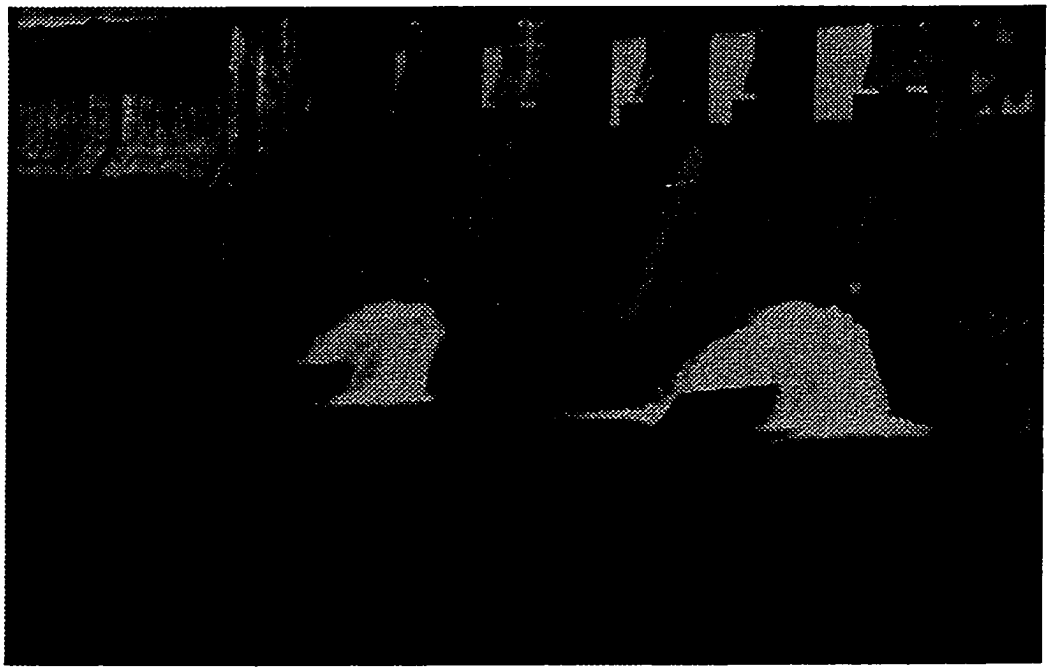


Figure 3.2. Discharge from the Howell-Bunger valves.

This type of discharge provides considerable aeration of the release (Figure 3.3) which impacts dissolved oxygen concentrations and dynamics of redox sensitive constituents such as manganese, iron, and hydrogen sulfide.

The lake has a maximum depth of 12 - 14 m and thermally stratifies in the summer with sufficient intensity to develop anoxic conditions below the thermocline, which develops near an elevation of 9 to 10 meters above the streambed. During periods of anoxia, which typically occur from July to September, concentrations of reduced metals, such as iron and manganese, increase in the hypolimnion until destratification, which occurs in late summer or fall. Typical concentrations of total iron and manganese available in the withdrawal zone (area subject to release) range between 2 to 7 mg L^{-1}, for

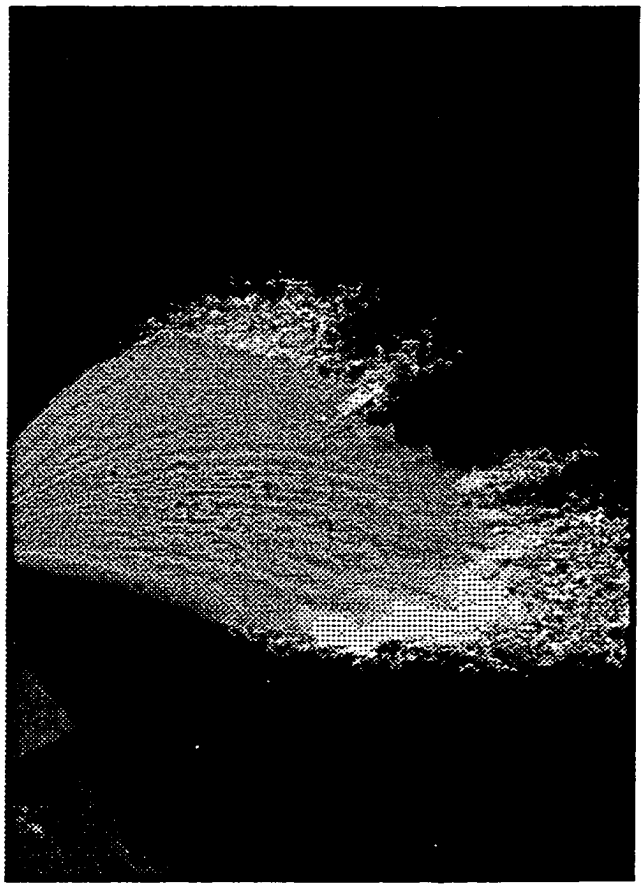


Figure 3.3. Aerial view of the release from Nimrod Dam and Lake depicting the mechanism of aeration.

iron and 2 mg L^{-1} to 4 mg L^{-1} for manganese. Under anoxic conditions, iron and manganese are in reduced forms. Concentrations in the release are near 3 and 2 mg L^{-1}, for iron and manganese respectively (Nix et al. 1991). Hydrogen sulfide production also occurs leading to occasional observations of noxious odors in the vicinity of the dam. Additional information on water quality is presented in Nix et al. (1991).

The tailwater of Nimrod Lake was selected based on the availability of information from previous studies about channel morphometry, substrate type, travel time of steady-

state releases (Nix et al. 1991) and manganese removal rates in the release (Dortch et al. 1992; Dortch and Hamlin-Tillman 1995). The site can be characterized as a series of riffles and pools. After the discharge leaves the stilling basin, it flows through a riffle area with large rocks and cobble into a deeper pool with cobble substrate that is mixed with coarse and fine grain sediments. Except for during high flows, the water leaves the pool through a narrow riffle area and then flows through a much longer pool until it reaches another riffle zone that supports emergent aquatic vegetation. The remainder of the study area has no additional riffle zones and is characterized as a pool with a mid-channel depth greater than 1 m. The series of riffles and pools provides reaeration and sedimentation which impact the dynamics of manganese removal in the tailwater.

Station A, which is approximately 100 m downstream from the stilling basin, represents the first riffle area. The area is dominated by large cobble and boulders which results in variable depths between 0.1 and 1 m at low flow. Velocities during releases are sufficient to inhibit accumulation of fine-grain sediments. Station B1, which is located at the boat ramp approximately 800 m downstream, represents the first pool area with depths ranging mostly between 0.1 and 1.5 m during low flow and decreased velocities that allow accumulation of coarse- and fine-grain sediments. At low flow, the release from this pool is primarily through a natural, cobble and boulder outlet that is about 0.5 m deep and 2-m wide. At higher flows, the entire width of the channel conveys the flow. Station B3, which is located at the powerline approximately 2.9 km downstream from the dam, is downstream of a long pool and is a riffle area that extends across the entire width of the channel and has emergent aquatic vegetation. Depths at station B3 range between 0.1

and 0.5 m at low flow. Stations C, D, and E represent downstream pool areas with relatively uniform channel morphometry and are approximately 4.7, 7.8, and 12 km downstream from the dam, respectively. Station C is the deepest area in the study reach with a maximum depth of 3 m at low flow. Depths at station D are near 1 m and near 2.5 m at station E at low flow. Depths at cross sections for selected stations are depicted in Figure 3.4.

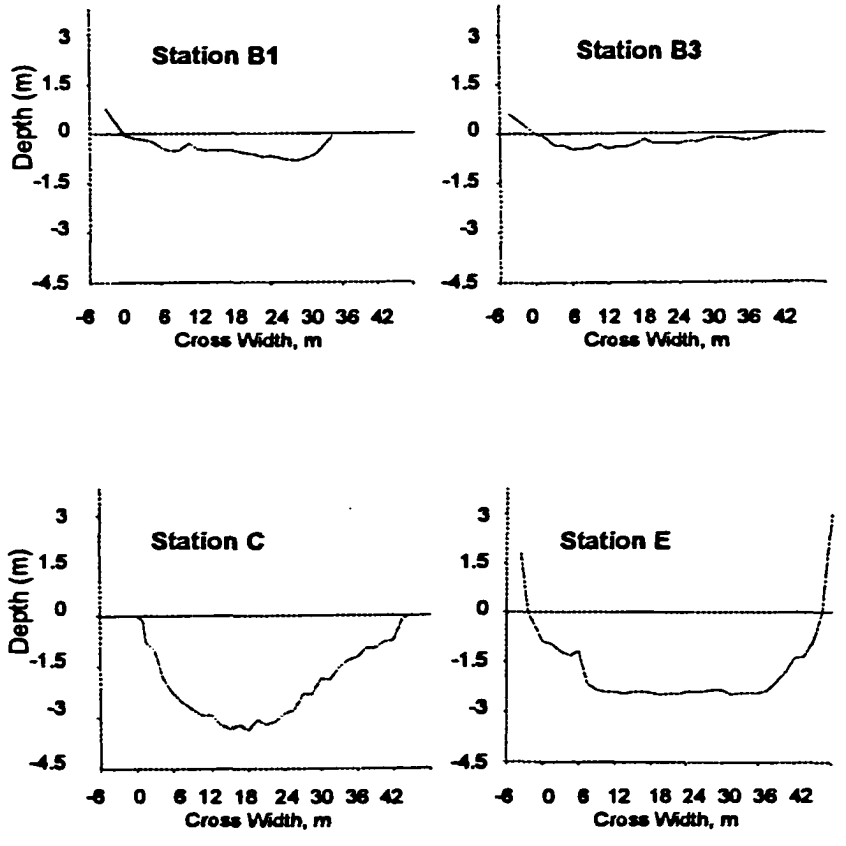


Figure 3.4. Cross sections at major stations in the tailwater of Nimrod Lake.

3.3 Methods

3.3.1 Substrate Analyses

Substrate samples were collected at stations A, B1, B3, C, and E to assess longitudinal gradients in adsorbed manganese concentrations. Substrate collection was also attempted at laterally spaced locations at several sites to describe lateral variability. Looking downstream, sampling was attempted at approximately 5 meters from the left bank (left bank), between the left bank and mid-channel (near left bank), mid-channel, between mid-channel and right bank (near right bank), and approximately 5 m from the right bank (right bank). Not all sampling yielded sufficient material for analysis. At station A, sampling was limited to collection of large cobble by grab sampling for surface coating analysis since rapid flow precludes accumulation of sand and fine-grained material. Two to four samples were collected at each lateral location at station A to assess lateral variability in surface coatings on the cobble substrate. While sample preparation included some separation of material scraped from the top and bottom of the substrate, data from both surfaces were included in the calculation of mean values by lateral site. Complete substrate sampling with an Ekman dredge was accomplished at stations B1, B3, C, and E. Substrate samples collected with the dredge were subsequently separated into selected size fractions for surface coatings analysis. Size fractions were separated in the field using standard sieves and included cobble, 2.0 mm to 0.5 mm, 0.5 mm to 0.063 mm, and less than 0.063 mm. Longitudinal variability was assessed using values measured for the cobble, 2.0 mm to 0.5 mm, and 0.5 mm to 0.063 mm size fractions.

Temperature, dissolved oxygen, pH, and specific conductance were measured in situ with a Hydrolab® multiparameter probe. Individual parameter probes were calibrated prior to use following guidelines in the operation manual (Hydrolab Corp., Houston, TX).

Substrate analyses included measurement of manganese concentrations on digested substrate samples. Samples from the cobble scrapings and each size fraction were ground and dried to a constant weight prior to analysis. Digestion for manganese analysis included block digestion of the samples in aqua regia (3:1 v/v conc HCl:conc HNO_3) and subsequent analysis on a Perkin Elmer Atomic Adsorption Spectrophotometer. Known concentrations of manganese (spikes) were added to some of the samples to estimate recovery. Concentrations were reported as dry weight and cobble scraping concentrations are not comparable to concentrations for other size fractions which incorporated parent material in the dry weight concentrations.

Scanning Electron Microscopy (SEM) was utilized for determination of the relative concentration of manganese in the surface coatings of cobble substrates. SEM analysis consisted of standardless EDX chemical analysis to determine areas relatively enriched in manganese. An Electroscan 2020 ESEM with and EDX windowless detector linked to a SUN SPARCstation 5 with IMIX analysis software (Princeton Gamma-Tech, Princeton, NY). Electron photomicrographs of selected samples were obtained with a lanthanum hexaboride electron source and a gaseous secondary electron detector (GSED). The imaging conditions employed an accelerating voltage of 20 KeV and 1.81 mA and approximately 3 to 8 Torr (400 to 933 Pa) water vapor in the sample chamber. The

environmental gas was vaporized distilled water supplied via a digitally controlled needle valve assembly. Images were collected over a period of 30 seconds.

Cores were collected from cobble samples collected from stations A, B1, B3, and E then sliced below the interface between the coating and parent material for mounting in the scanning electron microscope. A section collected from inside the center of a cobble sample from station A was also analyzed to provide a relative background manganese concentration. A sample of a bryophyte collected at station B3 that was coated was also analyzed with the SEM.

Statistical analyses were conducted using linear regression techniques in SigmaPlot (SPSS Inc, Chicago, IL) and procedures available in the Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC). Lateral and longitudinal variability of substrate size fractions were assessed with SAS procedures using general linear regression analysis (GLM) with station means comparisons conducted using the Tukey procedure (Tukey 1953). A univariate analysis was conducted with SAS procedures on data sets and the Shapiro-Wilkes statistic was used to test for normality of residuals.

Quality assurance was evaluated by examining variability of replicate samples, multiple analyses for manganese determinations associated with standard procedures for atomic adsorption analyses, and the recovery of standards, spiked samples, and concentrations in sample blanks.

3.3.2 Reservoir Release Water Quality Studies

Field studies conducted at Nimrod Lake, AR were used to evaluate manganese dynamics in reservoir releases. These studies consisted of sampling during a constant

release of two different flows to assess removal rates for dissolved manganese. Sampling sites for both studies represented both areas of pooled water and areas with riffles. Sampling design was similar to previous studies conducted by Nix et al. (1991) for comparative purposes. Sampling was conducted in the forebay of the reservoir to describe in-lake conditions, at stations A and B1 to describe conditions in the immediate tailwater, and at station B3 to describe conditions further downstream (Figure 3.1). Initial conditions of low flow (near 0.5 m^3 sec^{-1} or 17 ft^3sec^{-1}) were sampled prior to an increase in release to near 1.7 m^3 sec^{-1} or 60 ft^3sec^{-1} (referred to as high flow), which was held near constant for the remainder of the study to allow the re-establishment of steady-state conditions. The increase in release or discharge was initiated at 1500 on August 22, 1995 and held until 1700 on August 23, 1995. The increased discharge was about one third of the discharge of previous studies to provide an additional data set for model evaluation under different release conditions. Discharge records were obtained from the Little Rock District of the U.S. Army Corps of Engineers, which is responsible for operation of the dam.

Sampling was conducted at each station over a six hour period during the low flow study after a four day period of time for steady-state conditions to establish throughout the study reach. Sampling for the high flow study was conducted during a four hour period, 17 to 21 hours after the increase in release was initiated. Travel times necessary to establish steady-state conditions throughout the study reach for various discharges were determined based on estimates from the Tailwater Quality Model (TWQM) developed by Dortch et al. (1992) (Table 3.1), previous measurements using fluorescent dyes (Nix et al.

Table 3.1. Estimated travel times at various discharges in the tailwater of Nimrod Dam.

Flow (m^3 sec^{-1})	Reach	Distance (m)	Velocity (m sec^{-1})	Travel Time (Hrs)	Cumulative Travel Time (Hrs)
0.56	A-B1	914.4	0.015	16.7	16.7
	B1-B2	963.2	0.024	11.4	28.1
	B2-B3	1126.5	0.021	13.9	41.9
	B3-C	1609.3	0.015	27.5	69.4
	C-D	2735.9	0.012	69.0	138.4
1.41	A-B1	914.4	0.04	6.7	6.7
	B1-B2	963.2	0.058	4.6	11.2
	B2-B3	1126.5	0.058	5.5	16.8
	B3-C	1609.3	0.04	11	27.8
	C-D	2735.9	0.027	27.6	55.4
4.25	A-B1	914.4	0.115	2.2	2.2
	B1-B2	963.2	0.174	1.5	3.7
	B2-B3	1126.5	0.171	1.8	5.6
	B3-C	1609.3	0.122	3.7	9.3
	C-D	2735.9	0.082	9.2	18.5
5.66	A-B1	914.4	0.152	1.7	1.7
	B1-B2	963.2	0.235	1.1	2.8
	B2-B3	1126.5	0.226	1.4	4.2
	B3-C	1609.3	0.162	2.8	6.9
	C-D	2735.9	0.11	6.9	13.8

1991), and physical observations of neutrally buoyant floats. Temperature, dissolved oxygen, pH, and specific conductivity were monitored with Hydrolab® data sondes at each site prior to and during the period of increased release to describe temporal changes. An

in-lake profile of temperature, dissolved oxygen, pH, specific conductivity, and oxidation-reduction potential was conducted in the forebay region of the lake during the study to describe vertical gradients. One water sample was collected with a Van Dorn sampler from near the lake bottom for analysis of oxidized and reduced iron and manganese in the hypolimnion. This sample described hypolimnetic concentrations but would not describe initial release concentrations which would vary with discharge during stratification.

General water quality (organic carbon, chloride, nitrate, sulfate, alkalinity, and sulfide) in the tailwater was described with sample collection at each station during the high flow release. Total and dissolved organic carbon samples were collected in 50-ml polyethylene bottles and preserved with two drops of sulfuric acid to a pH of at least 2. Samples for dissolved organic carbon were filtered with 0.45 μm filters prior to acidification. Chloride, nitrate, and sulfate samples were collected in 20-ml acid-washed scintillation vials and kept on ice until analysis. Samples were transferred to the laboratory at the Wetland Biogeochemistry Institute (WBI) at Louisiana State University in Baton Rouge, Louisiana. Alkalinity and sulfide samples were collected in 500-ml amber bottles and analyzed on site. Carbon analyses were conducted using an Ionics Model 1270H analyzer with a detection limit of 1.0 mm. Chloride, nitrate, and sulfate were analyzed using a Dionex Model 2010i Ion Chromatography system with a detection limit of 0.1 ppm. Alkalinity was measured via titration (American Public Health Association 1992). Sulfide analysis consisted of extracting 5 ml of the water sample and transferring it to a 20-ml scintillation vial that contained 5 ml of an antioxidant buffer, which prevents the

oxidation of sulfide. Sulfide concentrations were then determined with a LAZAR Model IS-146 Sulfide Electrode with a detection limit of 0.01 ppm.

Water quality samples for iron and manganese concentrations (total and dissolved) were collected at each tailwater location during the low flow and high flow releases. Samples were collected in acid washed 20-ml scintillation vials. Total and total soluble metal samples (filtered with 0.45 μm filters in the field) were preserved with 3 drops of nitric acid to reduce the pH to at least 2. The samples were transferred to the WBI for analysis using a Jarrell-Ash Atom Comp Series 800 inductively coupled plasma (ICP) instrument. The detection limit on this instrument for manganese is 0.025 mg L^{-1}. On site measurements of manganous and ferrous concentrations were also conducted during the high flow event. Ferrous iron was determined in both filtered and unfiltered samples colorimetrically using ferrozine reagent. Five milliliters of sample were added to 1.0 ml of ferrozine, diluted to 10 ml of total volume with distilled, deionized water and absorbance was read at 565 nm on a Milton Roy Mini 20 spectrometer. Manganous manganese was also determined in both filtered and unfiltered samples colorimetrically using formaldoxime reagent and concentrations were compared to filtered samples analyzed with the ICP method. Five milliliters of sample were adjusted to a basic pH with 1.5 ml of 5 M NaOH prior to the addition of 0.5 ml of formaldoxime reagent. This was diluted to 10 ml of total volume with distilled, deionized water and absorbance was read at 450 nm on a Milton Roy Mini 20 spectrometer. Sample collection and analyses were provided by Dr. Robert Gambrell, Dr. Stephen Faulkner, Mr. Paul Hintze, and Ms Brenda Smith of the WBI.

Statistical analyses were conducted using linear regression techniques in SigmaPlot (SPSS Inc, Chicago, IL) and the Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC). Linear regression analysis was also conducted on natural log transformed concentration data for comparison of dissolved manganese removal rates between laboratory studies and field investigations. A univariate analysis was conducted with SAS procedures on data sets and the Shapiro-Wilkes statistic was used to test for normality of residuals.

Quality assurance was evaluated by examining variability of replicate samples, multiple analyses for manganese determinations associated with standard procedures for atomic adsorption analyses, and the recovery of standards and concentrations in sample blanks.

3.3.3 Raceway Studies

Laboratory studies to assess removal rates under controlled conditions were conducted in recirculating raceways that contained streambed substrate collected from Station B1 of the tailwater of the field site. Raceway studies included incorporation of whole substrate samples collected in November of 1997 and synthetic reservoir release water to compare results to field studies.

The raceways were filled with approximately 3000 l of deionized water that was amended with approximately 50 l of water from the tailwater. Water circulation was accomplished with submersible pumps located at one end of each raceway. The recirculating raceway system consisted of two fiberglass raceways, each with three compartments, that represent 1) shallow (< than 0.08 m deep) and high velocity, 2) deep

(~ 1 m deep) and low velocity, and 3) intermediate depth (~ 0.5 m deep) and low velocity stream reaches (Figure 3.5). The raceways are configured such that the flow originates in the shallow section, passes through the deepest section then through the intermediate depth section where it is pumped to the second raceway and travels the same route.

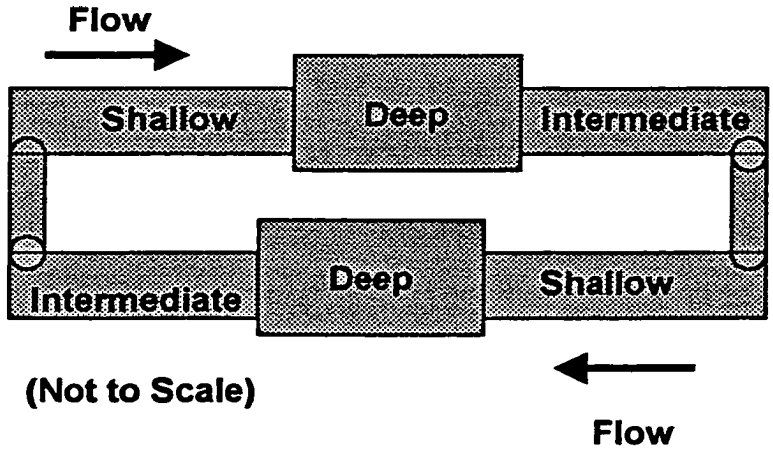


Figure 3.5. Schematic of the raceways.

Substrate was added to only one raceway and allowed to acclimate for a four month period prior to the conduct of any uptake studies. After initial water quality samples were taken on November 18, 1997 (prior to the addition of the substrate), substrate material collected from the site was added and aeration hoses were turned on to maintain adequate dissolved oxygen concentrations. The pumps were turned off after 0.5 hours of circulation for several days to allow fine material to settle out. When the water was relatively clear the pumps were turned back on and leaf packs with material collected at the field site were added to the system as a source of nutrients and organic carbon on December 16, 1997. "Unseasoned" limestone gravel was added to the raceway (February

25, 1998) during the period of acclimation for evaluation of microbial colonization. Routine water quality analyses for pH, alkalinity, phosphorus, nitrogen, iron, manganese, and selected cations and anions were conducted to monitor raceway conditions. Water level was maintained in the raceways with the periodic addition of deionized water.

Time of travel in the raceways was established with a dye study using Rhodamine WT. A dilute solution of dye was quickly added to the raceway in the shallow section and samples were collected at 5 minute intervals until well after a steady absorbance was measured on a spectrophotometer (Bausch and Lomb Spec 20). Readings were conducted at a wavelength of 520 nm which had previously determined to be optimal with a weak solution of the dye.

The addition of reduced manganese and iron was conducted on January 31, 1998 and March 1, 1998 as part of the acclimation. Reduced manganese was added as manganous sulfate, $MnSO_4$, or manganous chloride, $MnCl_2$. Reduced iron was initially added as ferrous chloride ($FeCl_2$) but ferrous ammonium sulfate, which is more soluble than ferrous chloride, was used after the initial study. Concentrated solutions were made for the addition to achieve initial concentrations of near 1 to 2 mg L^{-1} manganese and 2 to 5 mg L^{-1} iron which were comparable to concentrations observed in the field studies. Samples were collected daily after the March 1 addition of reduced manganese and iron to determine optimal sampling frequencies for removal studies.

Manganese removal dynamics were conducted in three separate studies (April, July, and August of 1998) in which reduced iron and manganese were added to the raceway to simulate the introduction of reduced metals into an aerating stream (i.e., the

tailwater). Removal of reduced manganese was evaluated by replicate measurements of total and dissolved (< 0.45μm) concentrations at a time series sufficient to describe concentration changes for approximately 2 days. Temperature, pH, and conductivity were monitored periodically throughout the study.

The raceways were drained and refilled with deionized water in June due to increases in concentrations of dissolved constituents in the routine monitoring samples. Reagent stock solutions of iron and manganese and additional leaf packs were also added. Approximately two weeks after the study conducted in early July, a leaf pack had clogged the opening to one of the sump pumps which caused a malfunction and resulted in a loss of water for most of the system. The raceways were refilled with deionized water and the study was repeated the next day (late July). NaOH was added during the late July study to increase the pH and evaluate the response in dissolved manganese concentrations.

Samples for total and soluble manganese analysis were collected with a clean, acid-washed syringe and held in clean, acid-washed scintillation vials until analysis. Samples were preserved with 3-5 drops of concentrated nitric acid. Soluble samples were filtered through a 0.45 μm membrane filter in an in-line filter holder that attached to the syringe. Approximately 5 to 10 mls of sample were passed through the filters and discarded prior to sample collection. Analysis was conducted on unfiltered samples (total) and filtered samples (dissolved) using a Jarrell-Ash Atom Comp Series 800 ICP.

Microbial biomass and community composition was determined by the analysis of membrane lipids using extraction techniques employing organic solvents (Vestal and White 1989). With this approach, total concentrations of ester-linked phospholipid fatty

acids (PLFA) are recovered from the sample and used to estimate biomass (Balkwill et al. 1989) and microbial community composition (White et al., 1999). Different functional groups, such as gram-positive and gram-negative organisms, were identified by unique patterns of fatty acids (e.g. saturated, unsaturated, and branched moieties). The fatty acids can then be attributed to specific biosynthetic pathways, which differ among various functional groups of microbes.

Substrate samples were collected from the raceway in April of 1998 to evaluate the PLFA technique and, if applicable, assess community structure in the system at the time of the uptake study. Samples collected included 2 samples of seasoned cobble and 2 cores from seasoned cobble from the shallow, high-velocity area of the raceway and from the mid-depth, low-velocity area of the raceway, 2 samples of the newly seasoned limestone from the shallow, high-velocity area of the raceway, 1 sediment sample from the deep area of the raceway, and 1 sample from the water column. The limestone had been in the raceway for approximately 4 months. Pre-cored samples were also acclimated for a period of approximately 4 months and were sampled to allow assessment of biomass and uptake per unit of surface area if adequate biomass was recovered.

Raceway samples were prepared for lipid extraction as described in White and Ringelberg (1998) and Findlay and Dobbs (1993). Lipids were quantitatively recovered from substrate materials by saturation with 7 ml of organic solvent ($CHCl_3$:MeOH, 1:2, v:v) once the overlying water was decanted. The lipid extract was then partitioned against water (via the addition of CH_2Cl_2:H_2O, 1:1, v:v) and the organic phase aspirated to a pre-packed silica column (Burdick and Jackson, Muskegon, MI) from which non-polar and

polar lipids were eluted with sequential washes of CH_2Cl_2, acetone, and methanol. The polar (methanol) eluent was dried under N_2 and then subjected to a single-step trans-esterfication to form methyl esters of the recovered phospholipid fatty acids. The PLFA were then separated, identified and quantified by capillary gas-chromatography (Hewlett Packard 6890GC) coupled to a quadrapole mass spectrometer (model 5973 mass selective detector). Detection was by electron impact ionization at 70eV.

The PLFA analysis was conducted by Dave Ringelberg and Margaret Richmond at the Environmental Laboratory located at the US Army Engineer Research and Development Center in Vicksburg, MS. Dave Ringelberg provided interpretation of the data, hierarchical cluster analysis of the sample PLFA profiles, and preparation of associated figures.

Statistical analyses were conducted using linear regression techniques in SigmaPlot (SPSS Inc, Chicago, IL) and the Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC). Linear regression analysis was also conducted on natural log transformed concentration data for comparison of dissolved manganese removal rates between laboratory studies and field investigations. A univariate analysis was conducted with SAS procedures on data sets and the Shapiro-Wilkes statistic was used to test for normality of residuals.

Quality assurance was evaluated by examining variability of replicate samples, multiple analyses for manganese determinations associated with standard procedures for atomic adsorption analyses, and the recovery of concentrations in standards and blanks.

3.4 Results and Discussion

3.4.1 Substrate Analyses

Water quality in the tailwater at the time of substrate conditions were comparable to conditions reported by Nix et al. (1991) with temperatures ranging from 27.6 to 28.7 ^{o}C, pH values between 6.7 and 6.9 standard units, dissolved oxygen concentrations between 6.8 to 7.7 mg L^{-1}, and conductivity values at 43 µmhos cm^{-1}. Conditions in Nimrod Lake indicated vertical stratification with anoxic conditions below 7 meters.

Cobble yielding sufficient material collected from scrapings was collected at 5 sites at station A to assess lateral variability. Additional cobble samples with sufficient material for analysis were collected at station B1 and station B3 and allowed an assessment of longitudinal variability in the upstream region of the tailwater. Cobble was not collected at station C due to deep water and no cobble near the bank and station D was not sampled. Cobble collected at station E was lightly coated but the material was not removable by scraping without including original rock material.

Distribution of concentrations on the cobble samples are depicted in Figure 3.6. Mean values, standard deviations, and Shapiro Wilkes values (checks for normality of distributions) for cobble scrapings at each site and station are presented in Table 3.2.

Results of univariate analyses indicated that the data were normally distributed. Analysis of material scraped from the surface of cobble suggested lateral variability in total manganese accumulation across the channel at station A ($p < 0.05$, Table 3.3). Mean concentrations ranged from 102 to 321 mg L^{-1} with higher concentrations occurring on the right bank to the mid-channel region. Although concentrations were highly

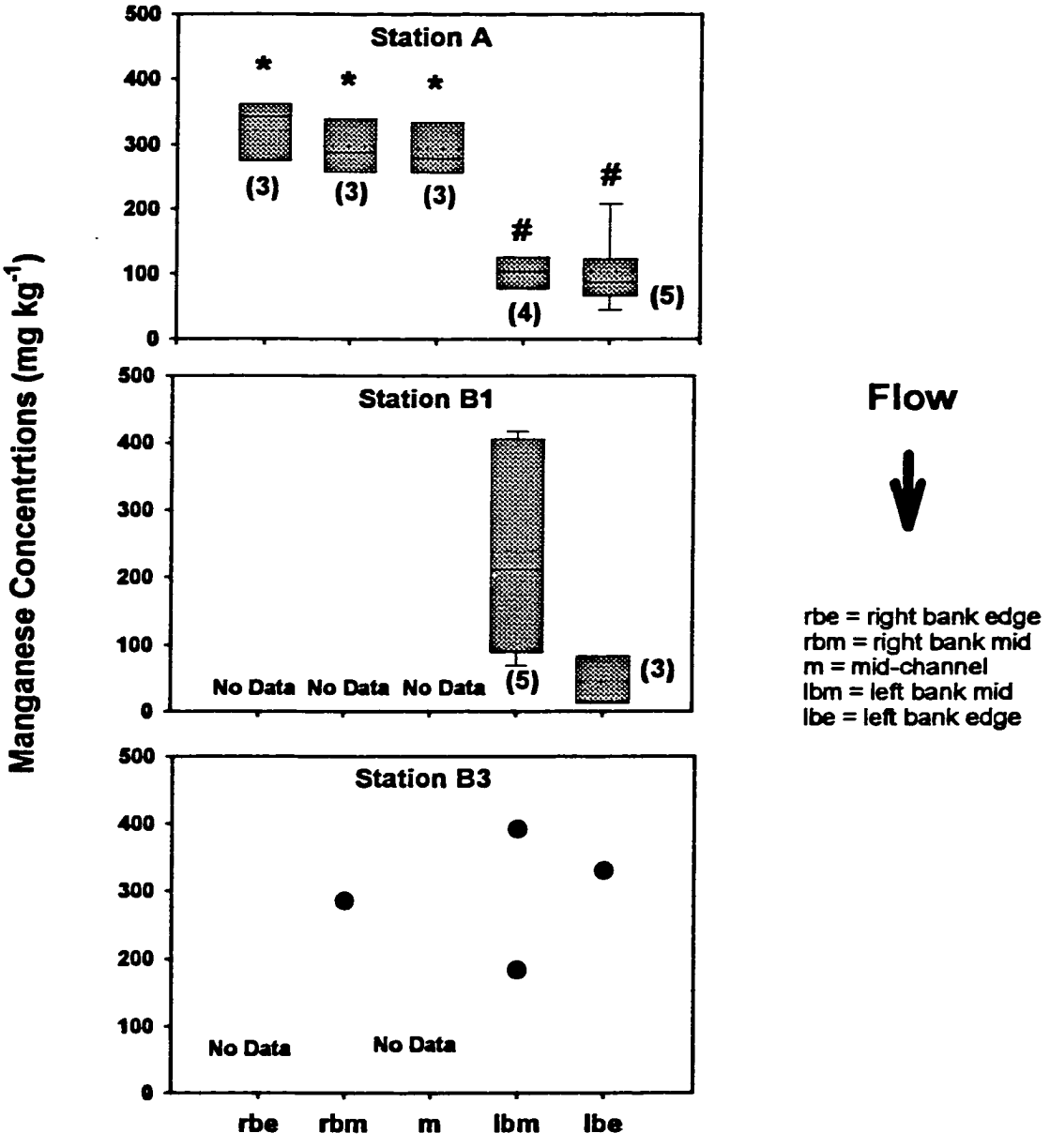


Figure 3.6. Longitudinal and lateral distribution of manganese concentrations on cobble scrapings. Numbers indicate sample size. Arrow indicates direction of flow. Boundaries of boxes indicate the 25th and 75th percentiles, the solid line within the box is the median and the dotted line is the mean, and whiskers indicate the 10th and 90th percentiles. For station A, sites with the same symbol are not significantly different ($p < 0.05$).

Table 3.2. Mean values, standard deviations, and normality values from cobble scrapings for lateral sites.

Lateral Site	n	Mean	Std Dev	Shapiro Wilkes Value W:Normal	Pr < W
Station A					
Right Bank Edge	3	321.0	61.0	0.9026	0.39
Right Bank Middle	3	297.3	54.6	0.9781	0.72
Mid-Channel	3	293.3	53.0	0.9451	0.55
Left Bank Middle	4	101.2	33.4	0.9921	0.95
Left Bank Edge	5	101.7	62.5	0.8290	0.13
Station B1					
Right Bank Edge	nd *				
Right Bank Middle	nd				
Mid-Channel	nd				
Left Bank Middle	5	239.3	164.5	0.8594	0.22
Left Bank Edge	3	44.3	53.4	0.7739	0.054
Station B3					
Right Bank Edge	nd				
Right Bank Middle	1	285	n = 1	n = 1	n = 1
Mid-Channel	nd				
Left Bank Middle	2	287.5	147.8	n = 2	
Left Bank Edge	1	330	n = 1	n = 1	n = 1

* Denotes no data.

variable at each site, higher mean concentrations in the region between the right bank and mid-channel were statistically different than mean concentrations in the left bank and left

Table 3.3. GLM test results for lateral variability on cobble scrapings from station A.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	185728.57	46432.14	15.89	0.0001
Error	13	37989.62	2922.28		
Corrected Total	17	223718.19			

Table 3.4. Tukey pairwise comparison test results for cobble scrapings from station A.

Site Comparison	Difference Between Means	Significant Comparison
rbe - rbm	23.67	
rbe - m	27.67	
rbe - lbm	219.83	Yes
rbe - lbe	219.30	Yes
rbm - m	4.00	
rbm - lbm	196.16	Yes
rbm - lbe	195.63	Yes
m - lbm	192.16	Yes
m - lbe	191.63	Yes
lbe - lbm	0.53	

bank to mid-channel region ($\alpha = 0.05$, Table 3.4). The mean concentration for station A was 202.7 mg L^{-1} (Figure 3.7).

Mean concentrations at station B1 on the left bank and left bank to mid-channel region, were 44 and 240 mg L^{-1} respectively (Figure 3.6) with a mean value of 166.2 mg/L^{-1} (Figure 3.7). At station B3, concentrations varied from 183 mg L^{-1} to 392 mg L^{-1} (Figure 3.6) with a mean value of 297.5 mg L^{-1} (Figure 3.7). Mean values at stations A and B1 were highly variable (Figure 3.7) and results of the regression analysis indicated

that mean values for the three stations were not significantly different ($p = 0.26$, Table 3.5).

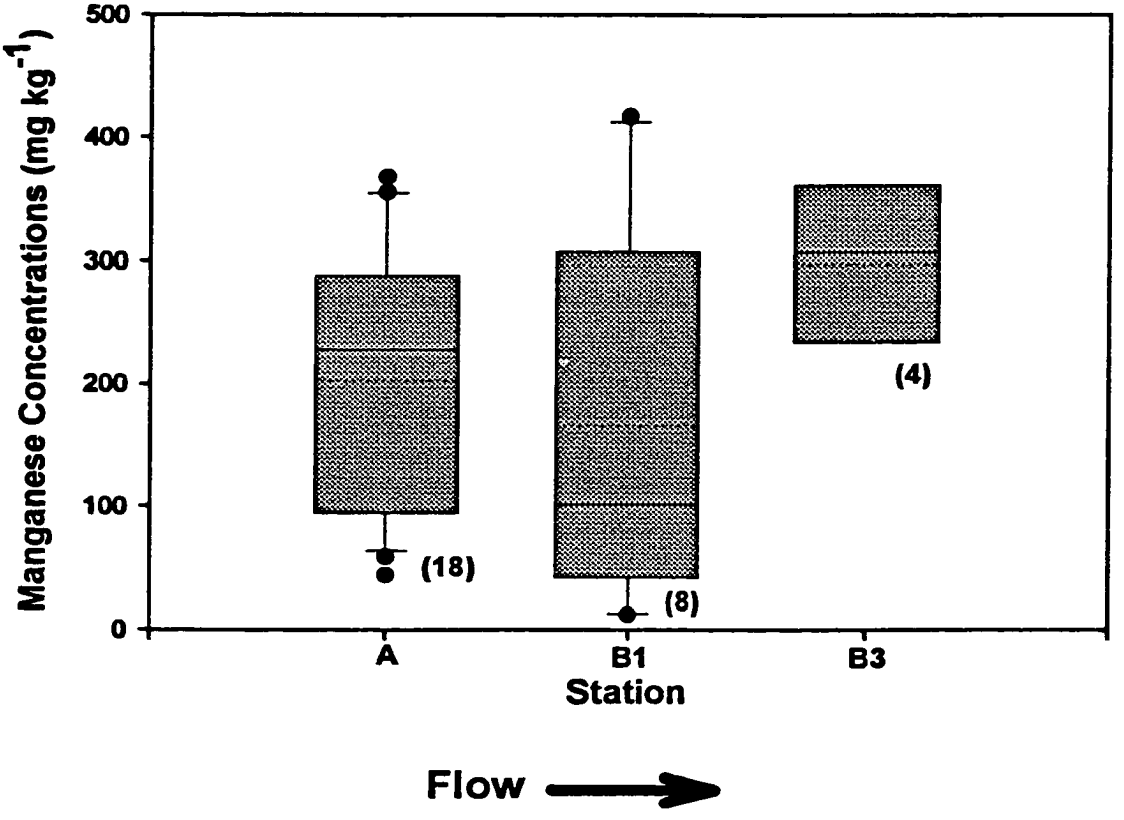


Figure 3.7. Longitudinal distribution of manganese concentrations on cobble scrapings. Numbers indicate sample size. Arrow indicates direction of flow. Boundaries of boxes indicate the 25th and 75th percentiles, the solid line within the box is the median and the dotted line is the mean, and whiskers indicate the 10th and 90th percentiles.

Table 3.5. GLM test results for longitudinal variability on cobble scrapings from stations A, B1, and B3.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	46370.79	23185.39	1.45	0.2526
Error	27	432143.23	16005.30		
Corrected Total	29	478514.01			

Substrate in the 2.00 - 0.5 mm, 0.5 to 0.063 mm, and < 0.063 mm size fractions was collected at stations at B1, B3, C, and E. Laterally spaced data for each size fraction were limited for each site and subsequently pooled for assessment of longitudinal gradients by size fraction. Mean values, standard deviations, and Shapiro Wilkes values for all substrate analyses at each station are presented in Table 3.6. In general, the data were normally distributed except at station C for the 2.00 to 0.50 mm size fraction and at station B1 for the 0.50 to 0.063 mm size fraction.

Mean concentrations of manganese on substrates between 2.00 and 0.50 mm ranged from 2 to 2.9 mg L^{-1} at stations C and E to near 7 and 8 mg L^{-1} at stations B1 and B3 (Figure 3.8). Mean concentrations were significantly different ($p < 0.05$, Table 3.7) and were higher at stations B1 and B3 than at stations C and E ($\alpha = 0.05$, Table 3.8), indicating a decrease with distance.

Mean concentrations of manganese on substrates between 0.50 and 0.063 mm ranged from 0.25 and 0.58 mg L^{-1} at stations C and E to 2.6 and 3.7 mg L^{-1} at stations B1 and B3 (Figure 3.9). As observed for the size fraction between 2.00 and 0.50 mm, concentrations were significantly different ($p < 0.05$, Table 3.9) but significant pairwise comparisons were not identified with the Tukey procedure at $\alpha = 0.05$.

Table 3.6. Mean values, standard deviations, and normality values at each station for each size fraction.

Station	Size Fraction	Mean	n	Std Dev	Shapiro Wilkes W:Normal	Pr < W
A	Cobble	202.7	18	114.7	0.8979	0.053
B1	Cobble	166.2	8	162.6	0.8353	0.069
B3	Cobble	297.5	4	88.0	0.9829	0.89
B1	2.00 - 0.50 mm	6.87	6	2.09	0.9322	0.61
B3	2.00 - 0.50 mm	8.27	2	0.53	1	n=2
C	2.00 - 0.50 mm	2.08	3	0.47	0.7500	0.0001
E	2.00 - 0.50 mm	2.90	3	0.68	0.9663	0.65
B1	0.50 - 0.063 mm	2.60	6	1.72	0.7896	0.045
B3	0.50 - 0.063 mm	3.69	2	0.69	1	n=2
C	0.50 - 0.063 mm	0.25	3	0.06	0.9355	0.51
E	0.50 - 0.063 mm	0.58	3	0.30	0.9542	0.59
B1	< 0.063 mm	30.08	4	3.73	0.7775	0.07
B3	< 0.063 mm	59.97	3	6.23	0.8222	0.17

Mean concentrations on material less than 0.063 mm were 30.1 mg L^{-1} and 60.0 mg L^{-1} at stations B1 and B3, respectively (Figure 3.10). Mean concentrations at station B3 were significantly higher than mean concentrations at station B1 ($p < 0.05$, Table 3.10). Concentrations and results for all substrate analyses are included in Appendix A. Comparisons between size fractions were not considered to be appropriate since the amount of original rock material used in calculating mass per unit dry weight is a function of the size fraction. Comparisons between size fractions were conducted in laboratory studies with uptake studies and are discussed in Chapter 4.

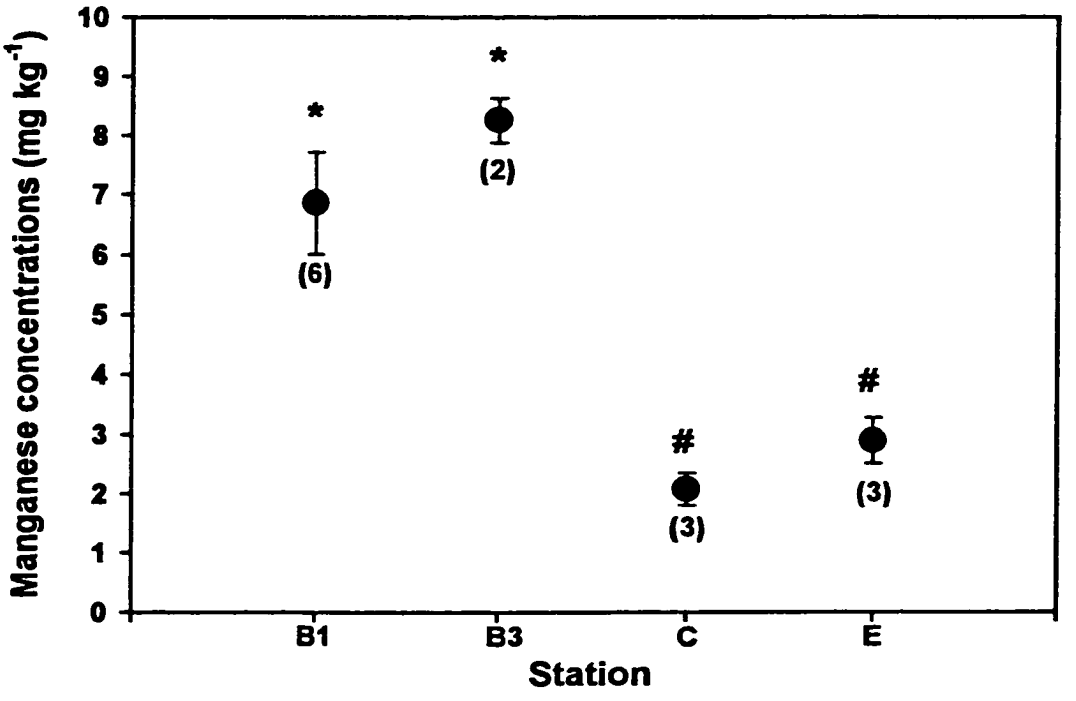


Figure 3.8. Longitudinal distribution of manganese concentrations for the 2.00 to 0.50 mm size fractions. Numbers indicate sample size. Error bars indicate the standard error. Stations with the same symbol are not significantly different ($\alpha = 0.05$).

Table 3.7. GLM test results for longitudinal variability on the 2.00 to 0.50 mm size fractions from stations B1, B3, C, and E.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	80.64	26.88	11.43	0.0014
Error	10	23.52	2.35		
Corrected Total	13	104.16			

Recovery of standard additions in the material scraped from the cobble and digested substrate in the other size fractions was relatively low for manganese (63.4 - 87.1%), but consistent, with most values between 83 and 87% and quite good for iron

(91.1 - 101.9%). In general, lower manganese recoveries were associated with the material scraped from the cobble which yielded very little mass after digestion.

Table 3.8. Tukey pairwise comparison test results for the 2.00 to 0.50 mm size fractions from stations B1, B3, C, and E.

Station Comparison	Difference Between Means	Significant Comparison
B1 - B3	1.395	
B1 - C	6.185	Yes
B1 - D	5.368	Yes
B3 - C	4.79	Yes
B3 - D	3.973	Yes
C - D	0.817	

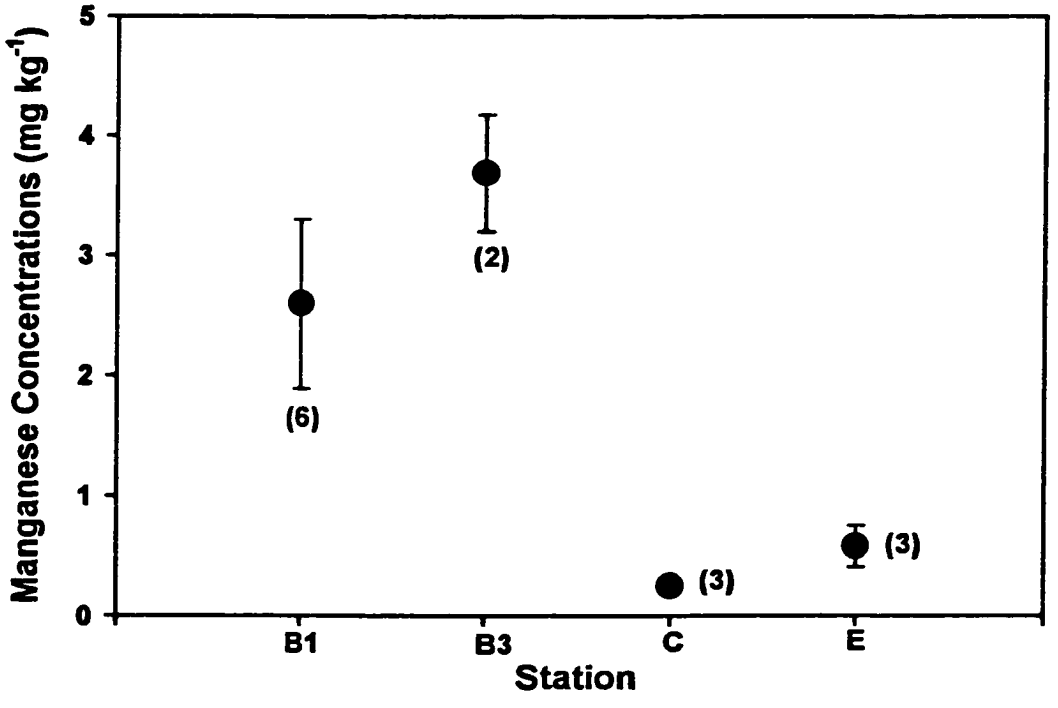


Figure 3.9. Longitudinal distribution of manganese concentrations for the 0.50 to 0.063 mm size fractions. Numbers indicate sample size. Error bars indicate the standard error. Stations were not significantly different ($\alpha = 0.05$).

Table 3.9. GLM test results for longitudinal variability on the 0.50 to 0.063 mm size fractions from stations B1, B3, C, and E.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	22.56	7.52	4.89	0.0242
Error	10	15.40	1.54		
Corrected Total	13	37.96			

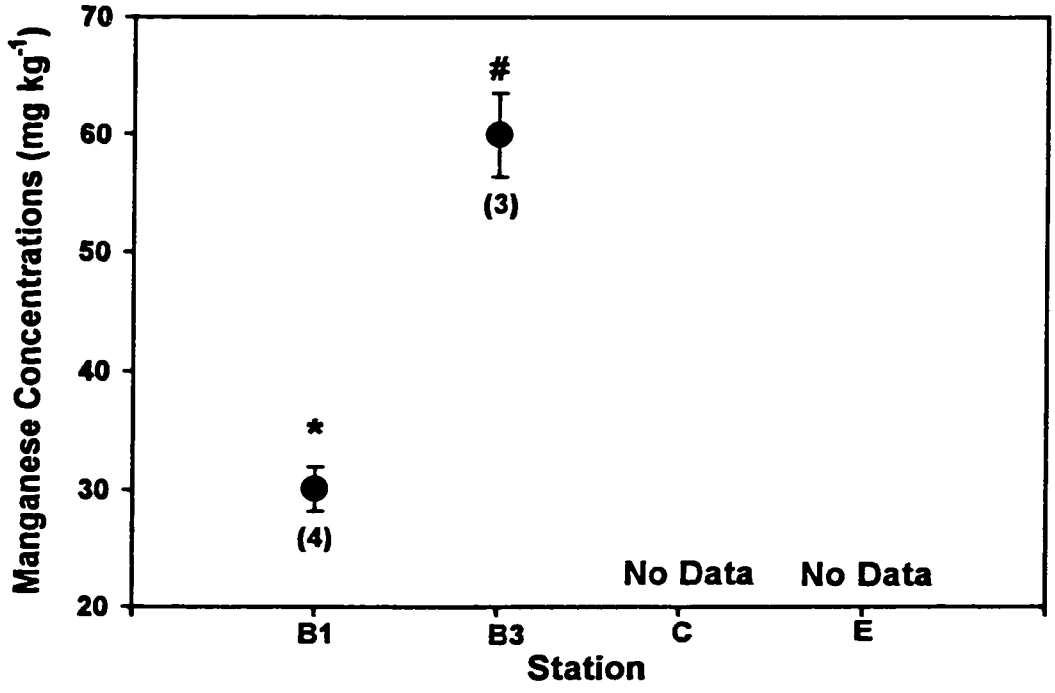


Figure 3.10. Longitudinal distribution of manganese concentrations for the less than 0.063 mm size fractions. Numbers indicate sample size. Error bars indicate the standard error. Stations with the same symbol are not significantly different ($\alpha = 0.05$).

Table 3.10. GLM test results for longitudinal variability on the less than 0.063 mm size fractions from stations B1 and B3.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1531.73	1531.73	64.16	0.0005
Error	5	119.37	23.87		
Corrected Total	6	1651.11			

SEM analysis measured relative changes in composition of surface coatings and original rock material (Figure 3.11). Manganese comprised 43 percent of the surface coating at station A (Figure 3.11 A), near 7 percent at station E (Figure 3.11 B), and was near 0 percent in the parent material (Figure 3.11 C). Manganese in the surface coating at station B1 was slightly higher (near 56 percent) than at station A. At stations B3 and C, the percent of manganese in the surface coating was near 40 and 30 percent, respectively. Although concentration data were not available from the type of analysis conducted, the relative percent contribution of manganese to the surface coating decreased with distance (Figure 3.12).

Observations at several sites and magnifications using the SEM also indicated that, in general, the substrate coating was amorphous or without crystalline structure (Figures 3.13 A, B, C, D). However, there was some evidence of crystal formation in the surface substrate as indicated in Figure 3.13 B. Bacteria were not observed, probably due to lack of preservation and preparation techniques used in the SEM analysis that were not specific for viewing bacteria. Diatoms were present at stations B1 and B3 (Figures 3.13 A and D) as was a surface coating on a sample of a bryophyte collected at station B3 (Figures 3.13 C and D).

3.4.2 Release Studies at the Nimrod Lake Tailwater

Water quality in the release is defined by conditions in the impoundment or lake formed by the dam and the depth and operation of the outlet works. A vertical profile was collected in the forebay (near-dam) region the day prior to tailwater sampling. Temperature measurements described thermal gradients and dissolved oxygen

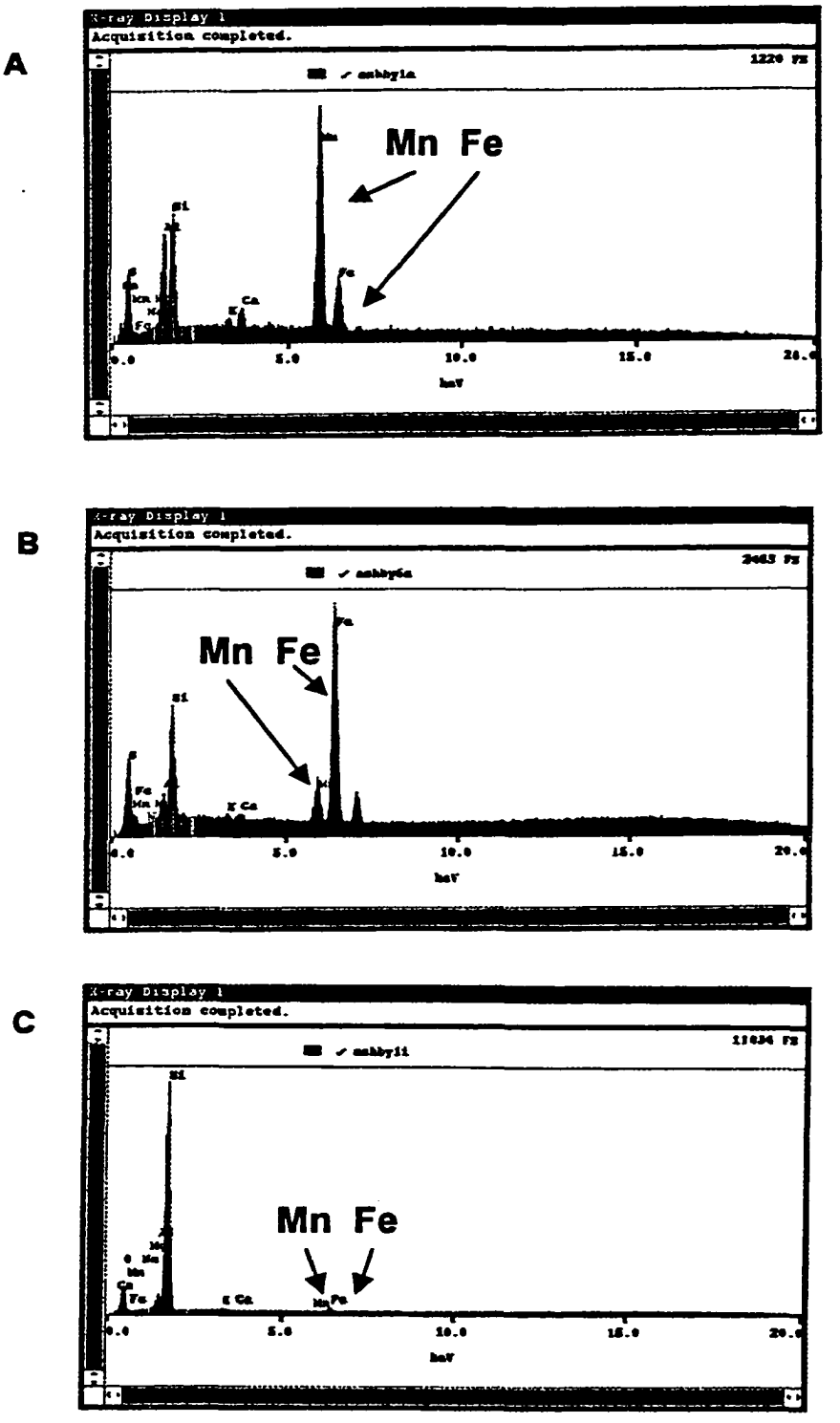


Figure 3.11. Relative elemental composition of cobble surfaces from Station A (A), station E (B), and the subsurface of the cobble from Station A (C).

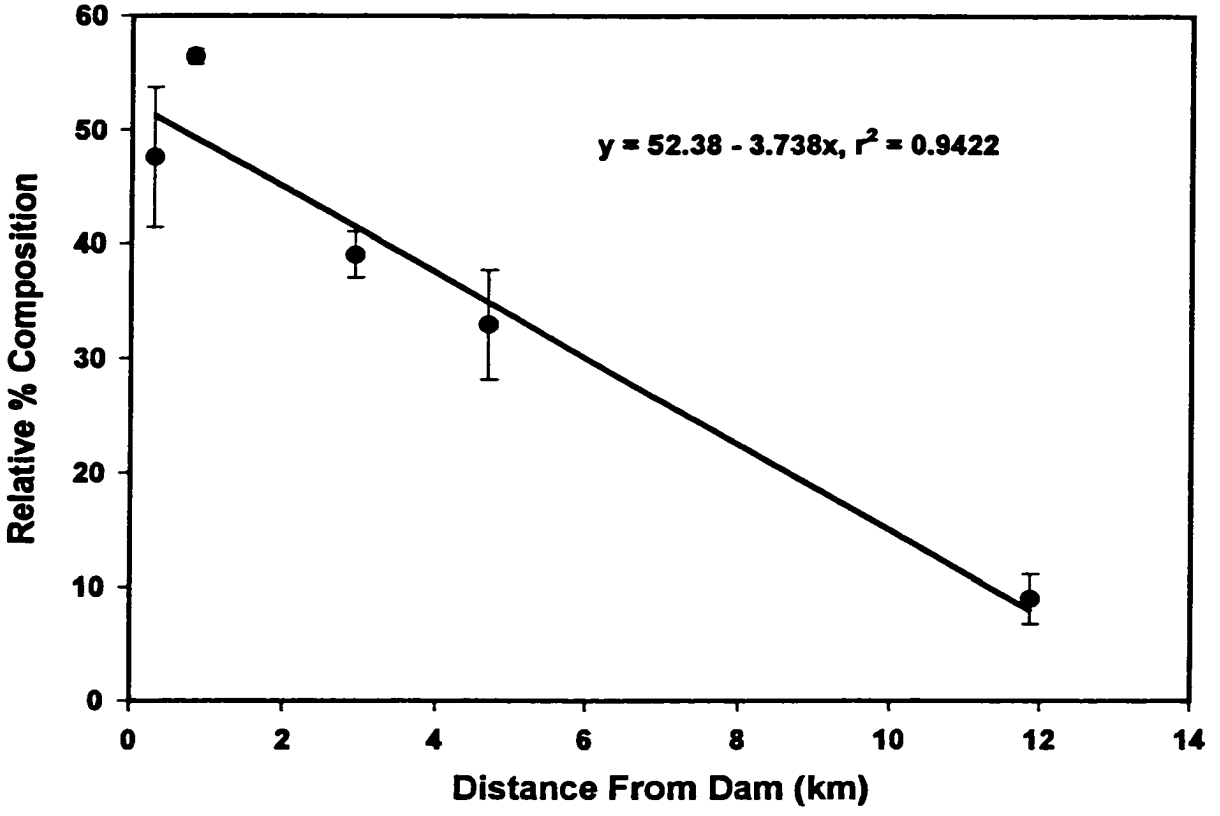
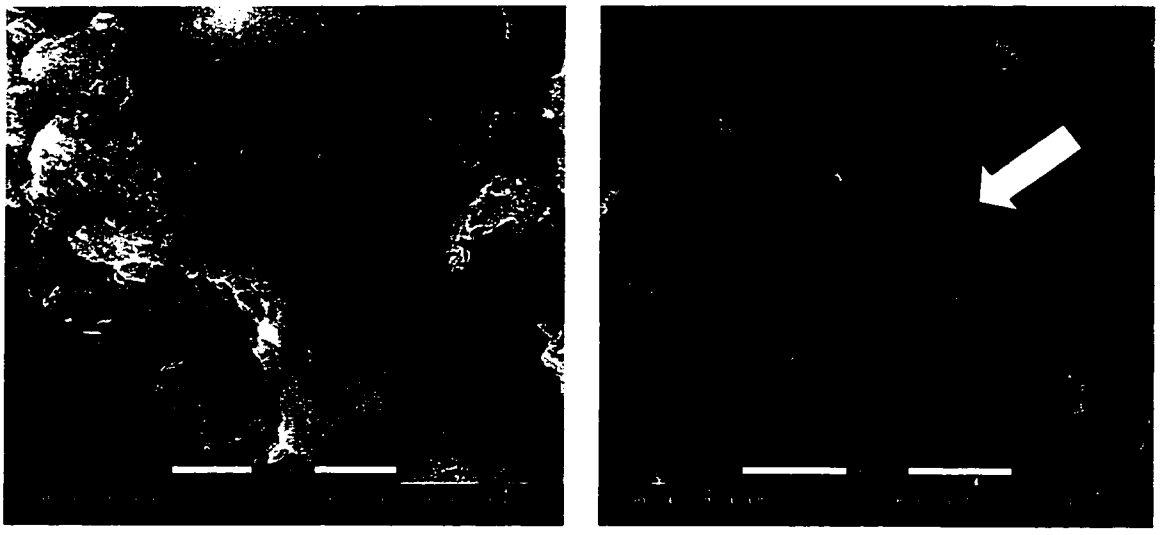
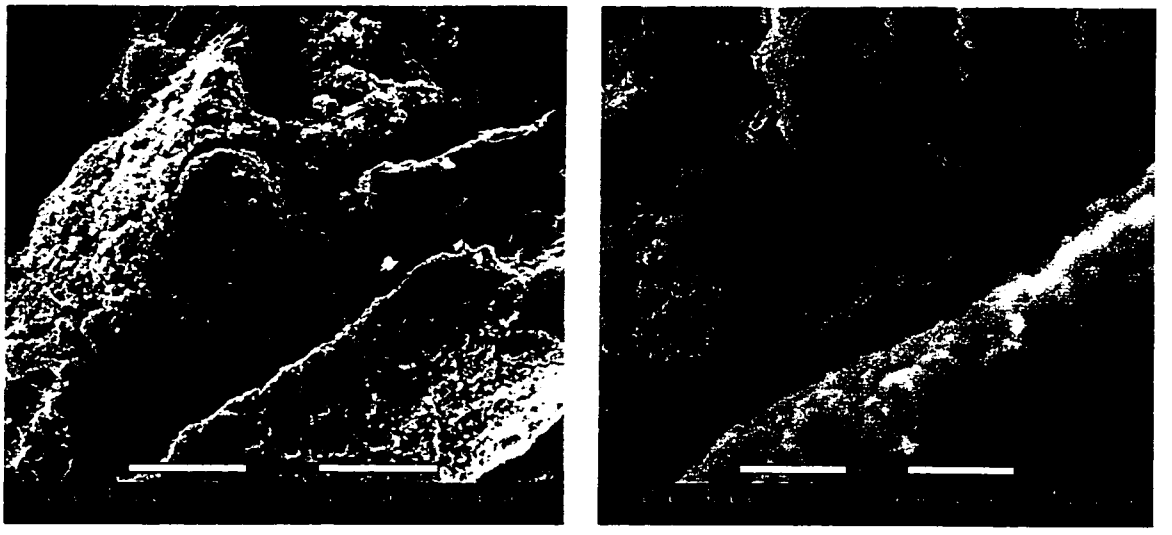


Figure 3.12. Relative percent composition of manganese on cobble surfaces as indicated by SEM analysis. (n = 2 for each site)

concentrations indicated anoxic conditions below a depth of 5 m (Figure 3.14A). Temperatures ranged from 31.3 °C in the surface to 24.7 °C at the bottom with a thermocline approximately 1 meter deep. While dissolved oxygen values were near 5 mg L^{-1} in the surface waters, concentrations decreased to less than 1 mg L^{-1} at depths greater than 4 meters (Figure 3.14B). Oxidation-reduction potential values were greater than



A Station B1 (Scale bar = 10 um) **B Station B3 (Scale bar = 45 um)**



C Station B3 (Scale bar = 250 um) **D Station B3 (Scale bar = 45 um)**

Figure 3.13. Scanning electron micrographs of various substrate surfaces from stations in the tailwater of Nimrod Dam. Figures A and B are from cobble samples and figures C and D are from the bryophyte collected at station B3.

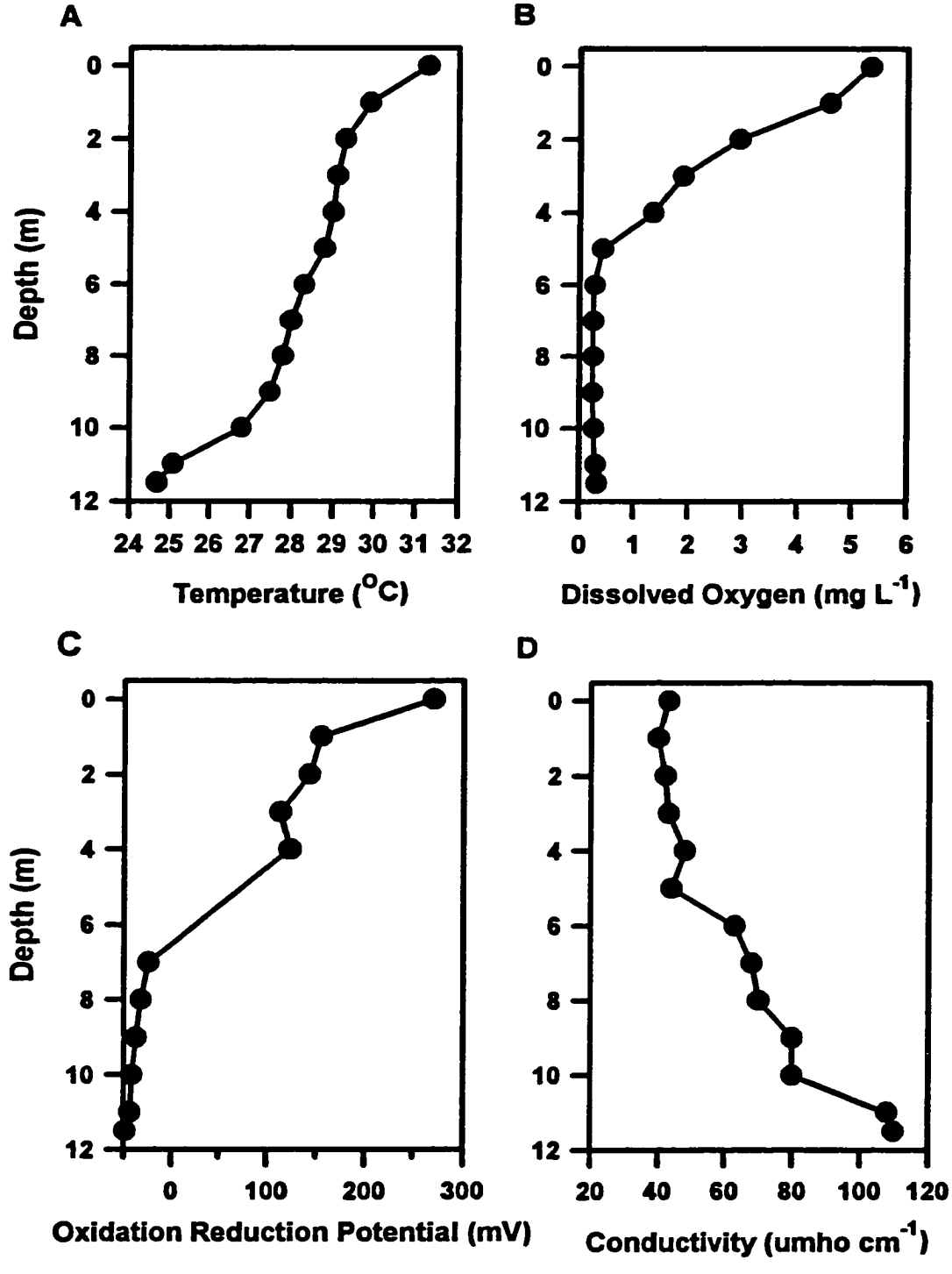


Figure 3.14. Water quality in the forebay of Nimrod Lake during the 1995 field study.

+250 mV in the surface waters (indicating oxidizing conditions for oxygen, nitrate, manganese, and iron), were between +100 and +250 mV between depths of 2 to 4 m (values conducive to reduction of nitrate and manganese), and were less than zero, e.g., negative (indicating reducing conditions for manganese and iron) at depths greater than 6 meters (Figure 3.14C). Coincident with negative oxidation reduction potential values, conductivity values increased in the bottom waters (Figure 3.14D) indicating increased concentrations of soluble constituents.

Total and dissolved manganese concentrations measured on a sample collected near the bottom of the reservoir were 7.5 and 6.5 mg L^{-1}, respectively. Total and dissolved iron were near 17 mg L^{-1}. Manganese and iron would be expected to be in reduced forms since dissolved oxygen values were near 0 mg L^{-1} and the oxidation reduction potential was negative.

Discharge indicated that high flow periods occurred in the winter and spring, prior to stratification, with a major high flow event occurring in late June and early July, during stratification (Figure 3.15). Although this major high flow event could have disrupted stratification and resultant chemical gradients, conditions observed in the lake indicated that chemical gradients existed. Releases were held near 1.7 m^3 sec^{-1} from mid-July through mid-August then decreased to near 0.5 m^3 sec^{-1} for a few days for the low flow study. Discharge was then increased to near 1.7 m^3 sec^{-1} for the high flow study. The increased discharge is still relatively low compared with winter and spring hydrographs. Travel times previously established by Nix et al. (1991) indicated that the release periods were sufficient to establish steady-state conditions for the low flow study prior to sample

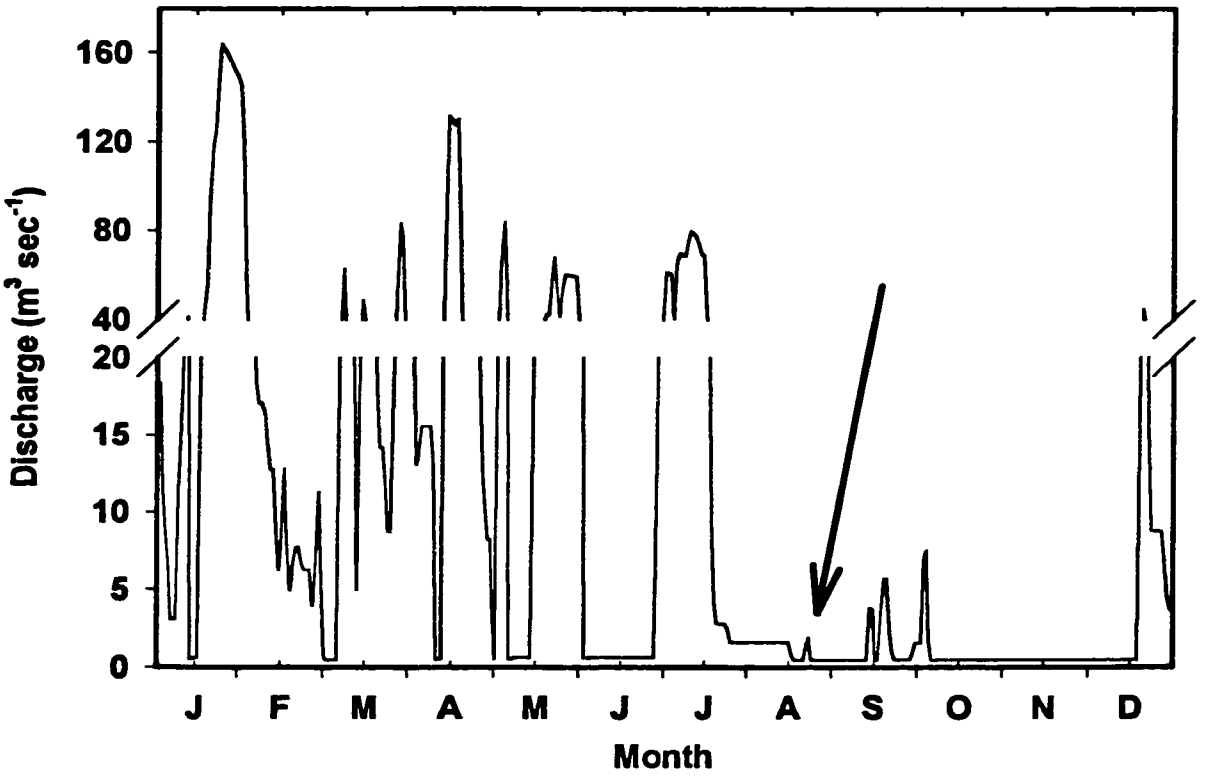


Figure 3.15. Release hydrograph for 1995 at Nimrod Dam, the arrow depicts the hydrograph for the release study.

collection but may have been insufficient for steady-state conditions to establish when samples were collected for the high flow study (Table 3.1). With the exception of occasional summer storms, typical discharges in the summer, coincident with anoxic conditions in the forebay, fall within the range of discharge observed in this study.

Water quality conditions during the release studies for selected constituents are summarized in Table 3.11. In general, little differences were observed between concentrations during the low flow and high flow discharges. Temperatures were near

27.5 °C and minor diel cycling was observed for temperature (Figure 3.16). Dissolved oxygen concentrations ranged between 5 and 7 mg L^{-1} (even at station A indicating rapid and efficient aeration). Values for pH were between 6.2 and 7.0 indicating circumneutral conditions. Sulfate concentrations were relatively low (near 0.5 mg L^{-1}). Total alkalinity was also moderately low (between 25 and 30 (mg L^{-1} as $CaCO_3$). Organic carbon concentrations, near 8 to 9 mg L^{-1}, would likely not be limiting for primary production.

Table 3.11. General water quality concentrations in the tailwater of Nimrod Lake, AR during the release studies in August of 1995.

Constituent	Approximate Concentration/Value
Temperature (°C)	27.5
Dissolved Oxygen (mg L^{-1})	6
Conductivity (µmhos cm^{-1})	5-8
Total Alkalinity (mg L^{-1} as $CaCO_3$)	25-30
Total Organic Carbon (mg L^{-1})	8-9
Dissolved Organic Carbon (mg L^{-1})	6-7
Nitrate Nitrogen (mg L^{-1})	0.25-0.50
Sulfate (mg L^{-1})	0.5
Chloride (mg L^{-1})	2.8
Sulfide (mg L^{-1})	bdl *

* Denotes concentrations below the detection limit.

Concentrations of iron at each station during the low flow and high flow release are depicted in Figure 3.17. Total and soluble (< 0.45 µm) iron concentrations were considerably less at station A (near 7.5 and 2 mg L^{-1}, respectively) than concentrations

near the bottom of the reservoir in the forebay. Lower concentrations in the immediate discharge may be attributed to dilution of bottom water with mid-depth water likely to contain lower concentrations due to the withdrawal zone and depth of the outlet works. In the tailwater, total iron concentrations decreased downstream from station A during the low flow release but remained relatively constant during the high flow release. Concentrations of soluble iron varied with distance during both low and high flow releases and a trend of decreasing concentrations was not apparent. Separate analysis using ferrozine reagent, which is specific for determining ferrous iron, indicated that measurements of soluble iron overestimated ferrous concentrations. Concentrations of ferrous iron on unfiltered samples were near 1.2 mg L^{-1} at station A and 0.5 mg L^{-1} at stations B1 and B3 during the high flow release, indicating a possible trend of decreasing concentrations with distance (Figure 3.18).

Total manganese concentrations decreased with distance during low flow but remained relatively constant during high flow (Figure 3.19). As observed for total iron concentrations, manganese concentrations at station A were less than the concentration measured in the bottom waters in the upstream impoundment indicating dilution associated with the withdrawal zone. Unlike iron, manganese was predominantly in the soluble form, and formaldoxime measurements indicated that the soluble form was indeed the reduced, manganous form. The loss or removal of manganese in the tailwater indicated a first-order removal process during the low flow release (Figure 3.20) and the removal rate was much higher during the low flow release (0.99 day^{-1}) than during the high flow release (0.17 day^{-1}) (Figure 3.21).

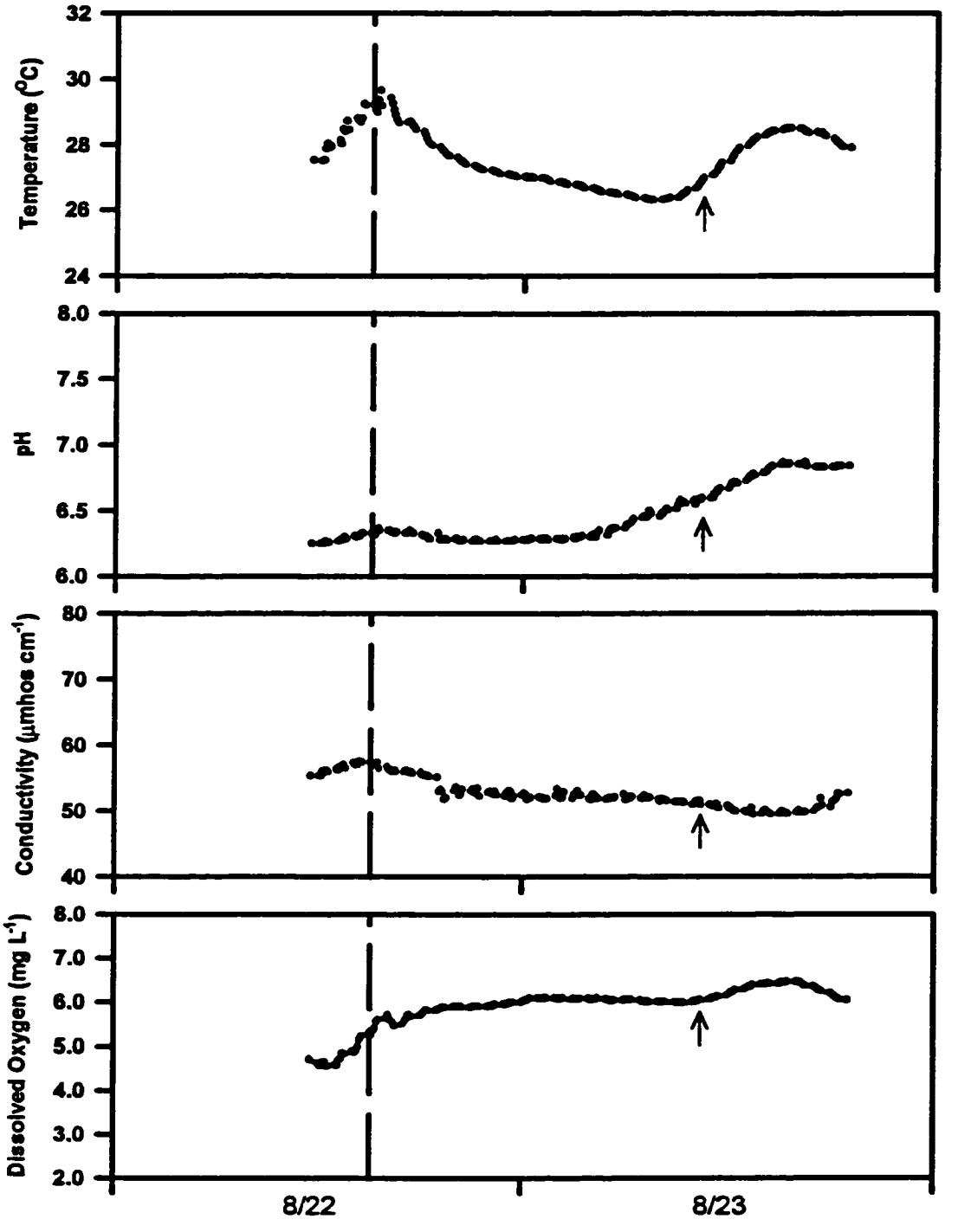


Figure 3.16. Temperature, pH, conductivity, and dissolved oxygen during the release studies. The dashed lines indicate the initiation of increased releases. The arrows denote sample collection for the high flow study. The data were collected at station B1.

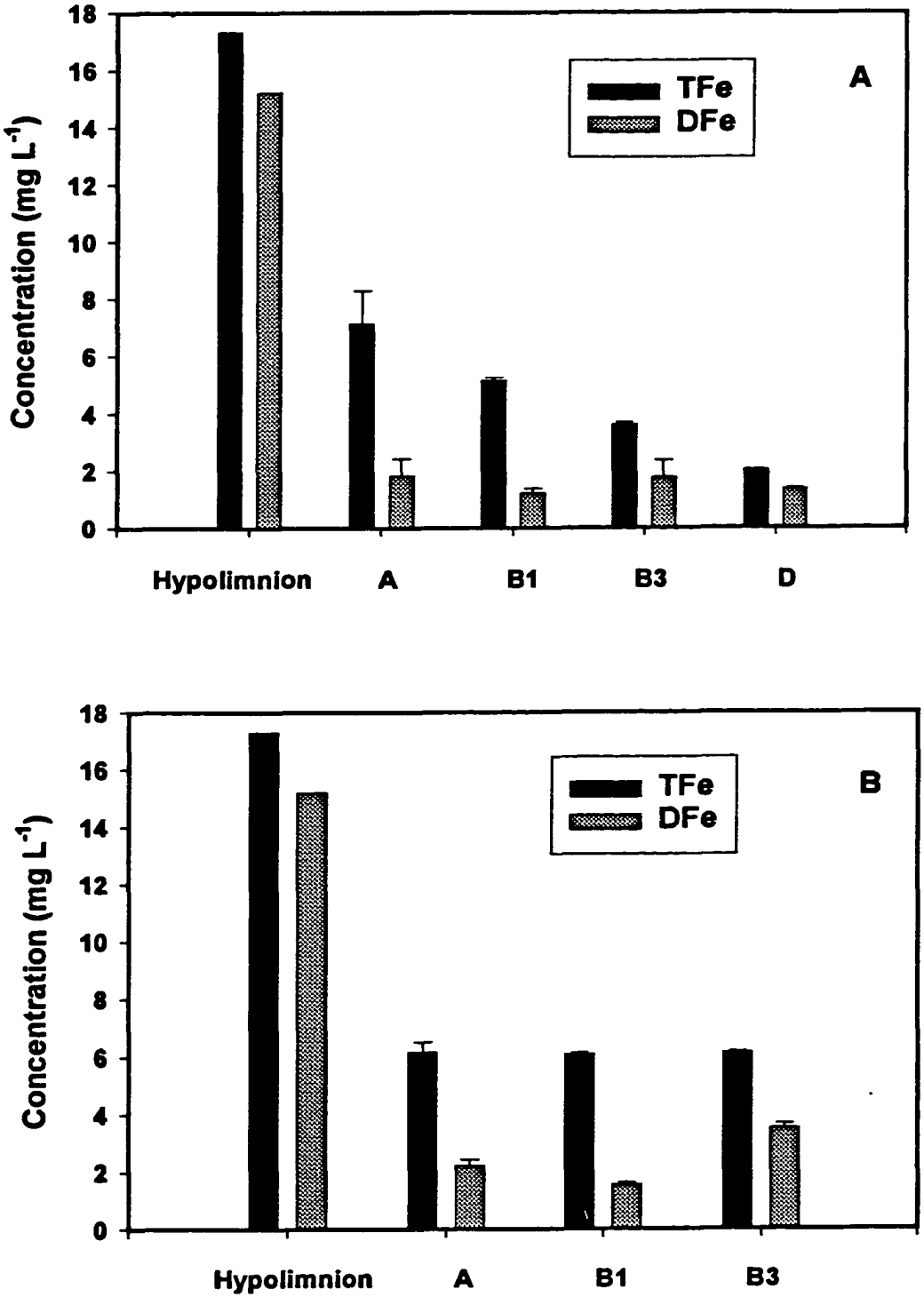


Figure 3.17. Total (TFe) and dissolved (DFe) iron concentrations in the release from Nimrod Dam during the low flow (A) and high flow (B) 1995 study (n = 2).

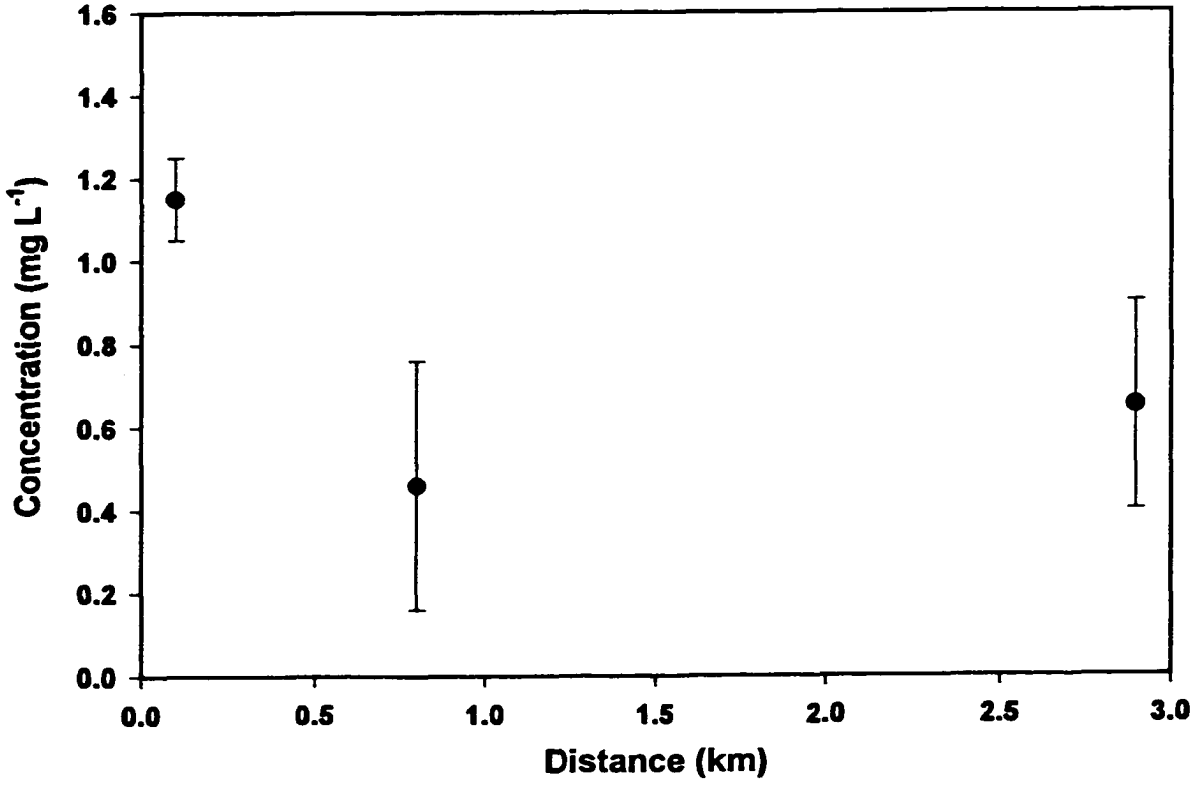


Figure 3.18. Concentrations of ferrous iron in the tailwater during the 1995 high flow release (n = 2).

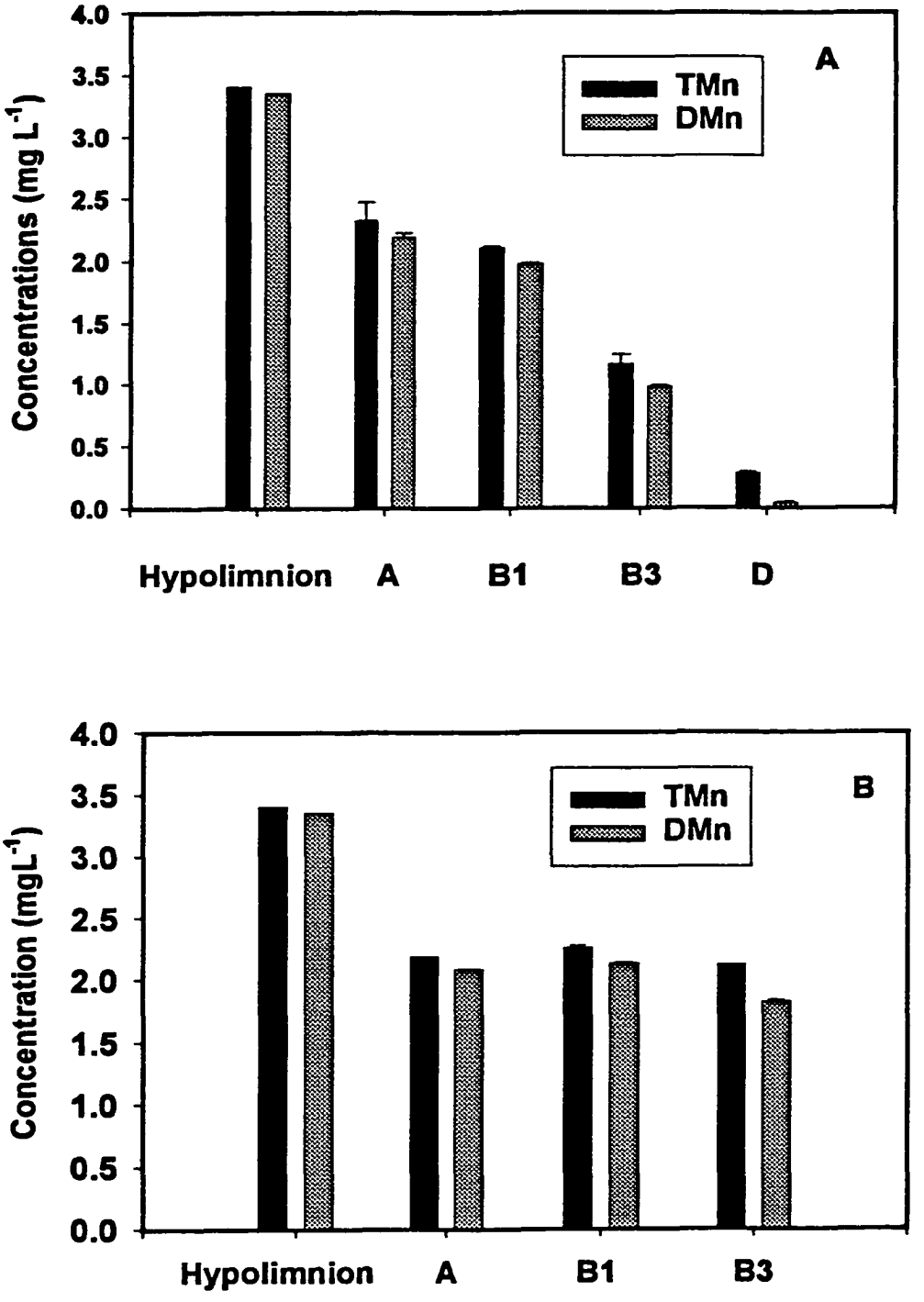


Figure 3.19. Total (TMn) and dissolved (DMn) manganese concentrations in the release from Nimrod Dam during the low flow (A) and high flow (A) 1995 study (n = 2).

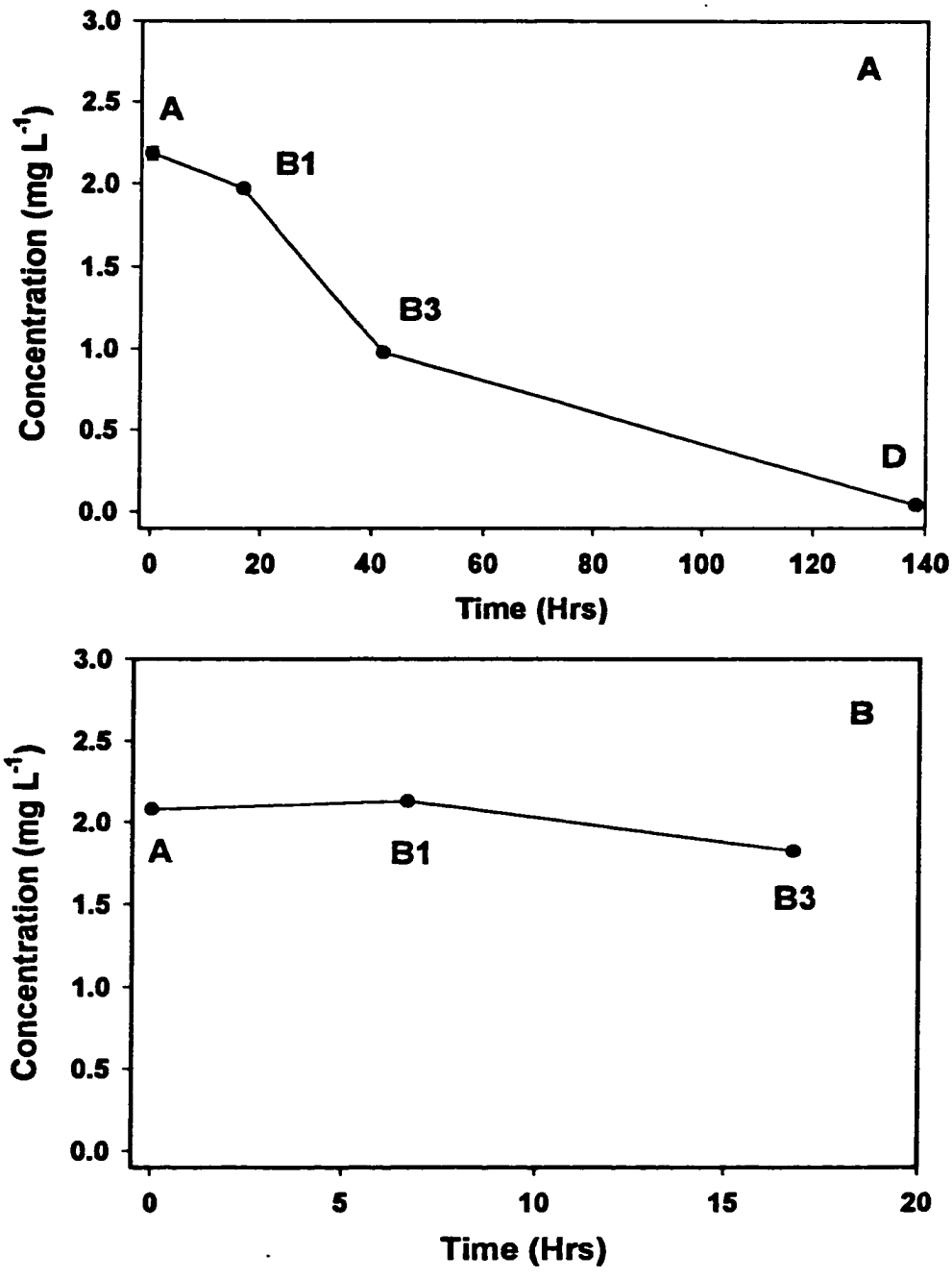


Figure 3.20. Dissolved manganese concentrations in the tailwater of Nimrod Lake during the low flow (A) and high flow (B)1995 study (n=1). Travel times were estimated from model output (TWQM).

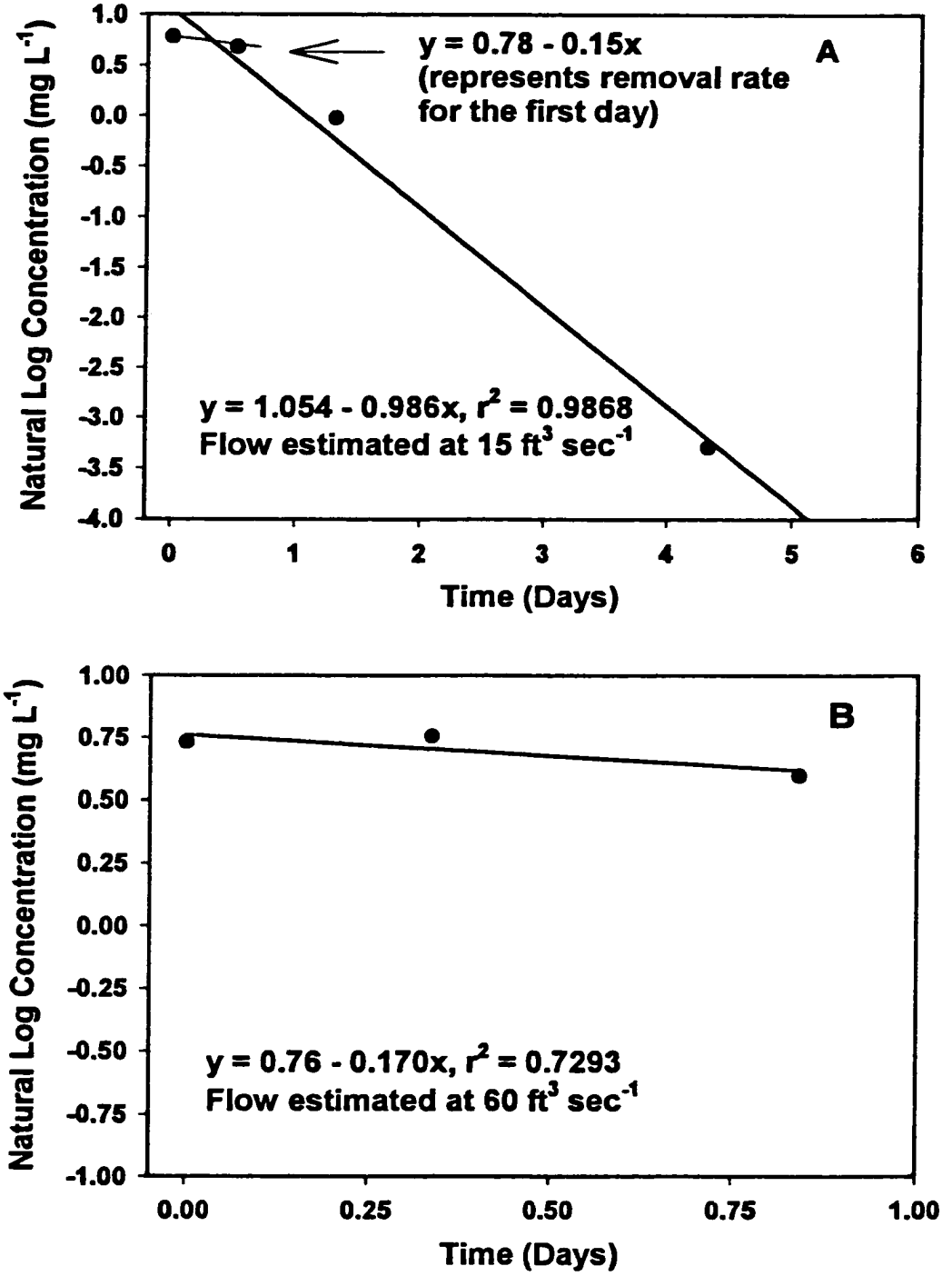


Figure 3.21. Removal rates of dissolved manganese in the release from Nimrod Dam during the low flow (A) and high flow (B)1995 study (n=1). Travel times were estimated from model output (TWQM).

3.4.3 Raceway Studies of Manganese Removal

Travel time in the raceways was estimated to be between 15 and 30 minutes based on dye study results (Figure 3.22). Base on these two estimates, average velocities would be 0.018 and 0.001 m sec^{-1} (0.06 and 0.03 ft sec^{-1}), respectively, which would be comparable to discharges of approximately 1 and 0.6 m^3 sec^{-1}. The total distance traveled in one day would be between 1.58 and 0.79 km (5,184 and 2,592 feet), respectively.

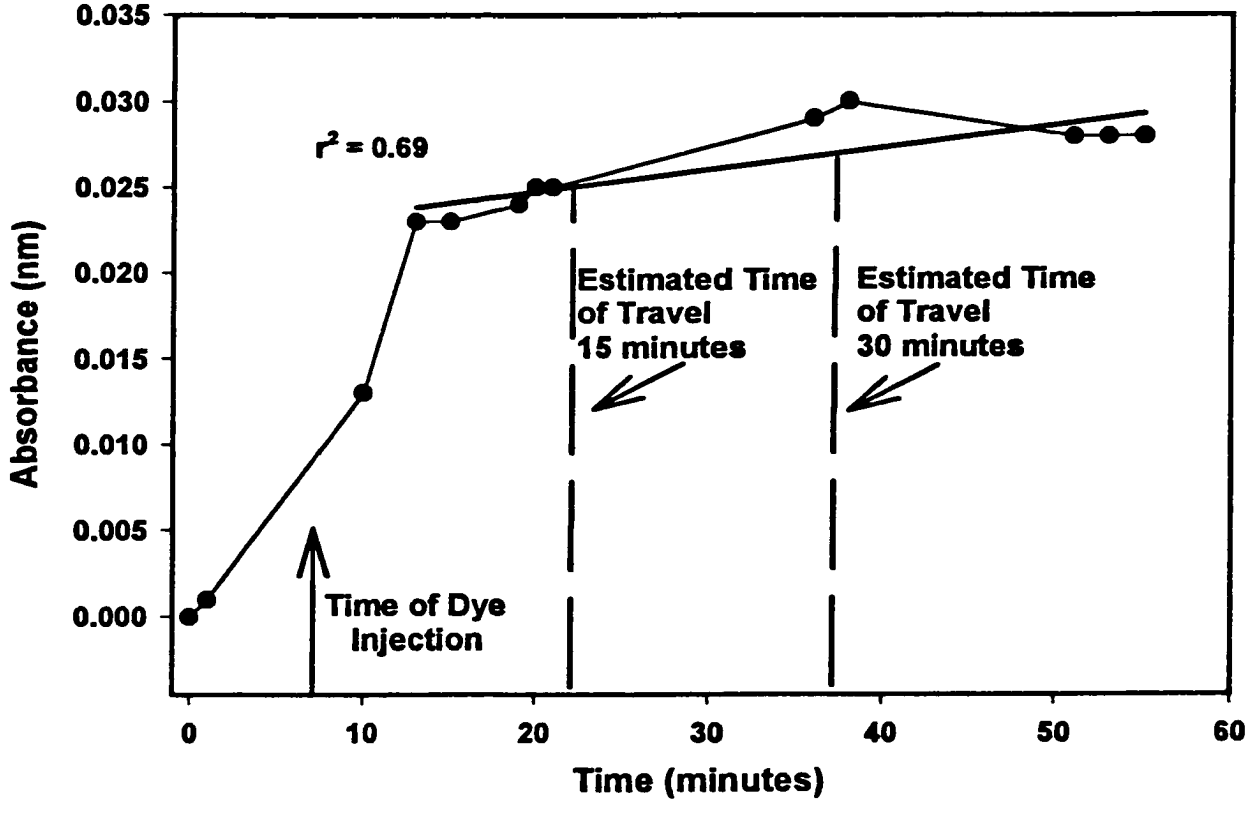


Figure 3.22. Absorbance during the dye study in the raceways. Time of travel estimated from the time of the dye injection.

Ranges of selected water quality constituent concentrations in the raceways during the period of acclimation are summarized in Table 3.12. Temperatures were between 24 and 26 °C, conductivity values remained relatively constant between 53 and 65 μmhos cm^{-1}, and pH values were between 6 and 6.6. Total and dissolved phosphorus, iron, and manganese concentrations increased in association with the addition of reduced iron and manganese stocks then decrease with time. Concentrations following the addition of the stocks in March were monitored daily for a period of several days to determine optimal sampling protocol for removal studies. Concentrations of total and dissolved manganese decreased to very low concentrations within three days with major decreases occurring during the first two days (Figure 3.23).

Table 3.12. Summary of selected water quality constituents in the raceways during the acclimation period.

Constituent	Minimum Concentration	Maximum Concentration
T. Fe (mg L^{-1})	0.05	1.175
D. Fe (mg L^{-1})	bdl *	0.3149
T. Mn (mg L^{-1})	bdl	1.035
D. Mn (mg L^{-1})	bdl	0.7554
T. Ca (mg L^{-1})	0.210	12.38
D. Ca (mg L^{-1})	0.210	13.0
T. P (mg L^{-1})	bdl	0.2315
D. P (mg L^{-1})	bdl	0.2146
pH (std units)	6.0	6.6
Conductivity (μmhos cm^{-1})	53	65
Temperature (°C)	24.0	26.0

* Denotes concentrations below the detection limit.

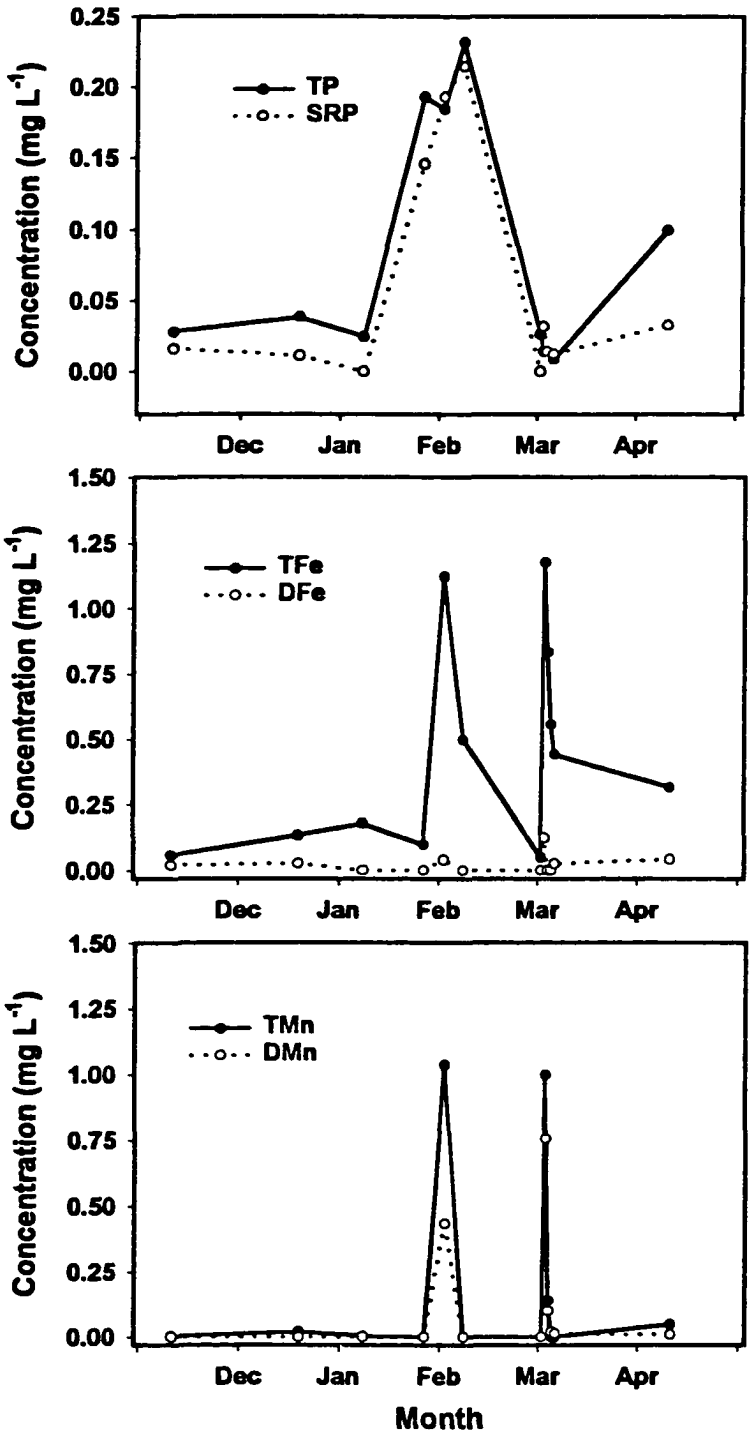


Figure 3.23. Concentrations of phosphorus, iron, and manganese in the raceways during the acclimation period.

Data for all raceway analyses are included in Appendix B and results of analyses on blanks and standards are summarized in Table 3.13. Reagent blanks were below detection limits and recovery of standards was near 100%. When replicate samples were collected, mean values were plotted with error bars representing the standard deviation.

Table 3.13. Concentrations in blanks and standards for raceway studies.

Constituent (Standard)	Blanks 3/19(1)	Blanks 3/19(2)	Blanks 4/13	Stds 3/19(1)	Stds 3/19(2)	Stds 3/19(3)	Stds 4/13
T. Cu (10 mg L^{-1})	0.0012	0.0019	0.0033	10.20	10.75	10.00	9.193
T. Zn (10 mg L^{-1})	0.0115	0.0130	0.1147	9.916	10.07	10.11	9.973
T. Cd (10 mg L^{-1})	0.0031	0.0044	0.0256	9.961	10.06	10.09	9.977
T. Pb (10 mg L^{-1})	0.0102	0.0108	0.121	10.01	10.31	10.09	9.972
T. Ni (10 mg L^{-1})	0.0026	0.0028	0.0189	10.00	10.11	10.07	9.937
T. As (10 mg L^{-1})	0.0172	0.0176	0.1439	10.01	10.29	10.14	9.984
T. Fe (10 mg L^{-1})	0.0020	0.0020	0.0119	9.863	9.837	10.10	9.925
T. Mn (10 mg L^{-1})	0.0012	0.0012	0.0033	9.814	9.783	10.14	9.957
T. Ca (10 mg L^{-1})	0.0801	0.0835	1.215	9.995	10.23	10.13	9.894
T. Mg (10 mg L^{-1})	0.0027	0.0031	0.0134	9.781	9.761	10.16	9.946
T. Al (10 mg L^{-1})	0.0455	0.0467	0.2747	10.07	10.22	10.02	9.964
T. Na (100 mg L^{-1})	0.0153	0.0342	0.1016	102.5	105.5	99.62	99.31

In general, replicate analyses indicated very low standard deviations and hence, low error introduced via sampling or chemical analysis.

Based on results of the March preliminary removal study, samples during the April removal study were collected more frequently during a 52-hour period. Total and dissolved manganese concentrations decreased with time in a curvilinear or exponential fashion, indicative of a first order removal process (Figure 3.24). Total and dissolved manganese concentrations were similar during the study. Duplicate analyses indicated minimal sampling and analytical variability. Ranges for other water quality constituents were consistent with those reported in Table 3.12 except for total and dissolved iron which were near 5 mg L^{-1} immediately following the addition of the reagent stocks.

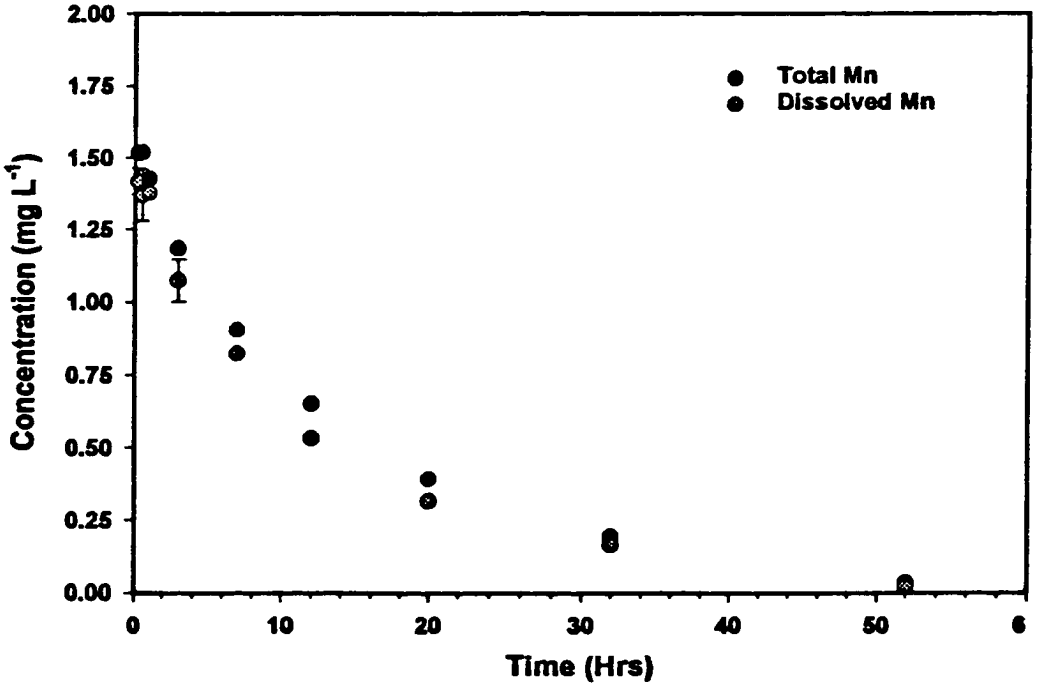


Figure 3.24. Total and dissolved manganese concentrations during the April raceway study.

Dissimilar results were observed in total and dissolved manganese concentrations when the raceway study was conducted in July after the raceways had been refilled. Instead of the curvilinear, exponential decrease in concentrations observed in the April study, concentrations of both total and dissolved manganese concentrations increased slightly and then remained relatively constant during the 52 hour period (Figure 3.25A). The study was repeated in late July (after the pump malfunction which resulted in a refilling) with results similar to the early July study. Attempts to increase pH values to 6.5-7.0 standard units (as observed in the April study) were marginally successful with values obtained near 6.5 but pH values were still mostly near 6.0 standard units. Dissolved manganese concentrations actually increased slightly following the addition of the NaOH (Figure 3.25B).

When the last raceway study was conducted in August, total and dissolved manganese concentrations decreased in a pattern similar to the concentration decrease observed in April (Figure 3.26). The pattern of concentration decrease was not quite as curvilinear as observed in April with initial concentrations during the first three hours remaining relatively constant. Following the first three hours, concentrations decreased almost linearly until the last concentration measurement, which was near the detection limit of 0.05 mg L^{-1}. Removal rates were comparable for the April and August raceway studies (Figure 3.27). Observed ranges of selected water quality constituents for each raceway study are depicted in Table 3.14. In general, lower pH values were observed for the July raceway studies and may have limited microbial removal processes during these studies.

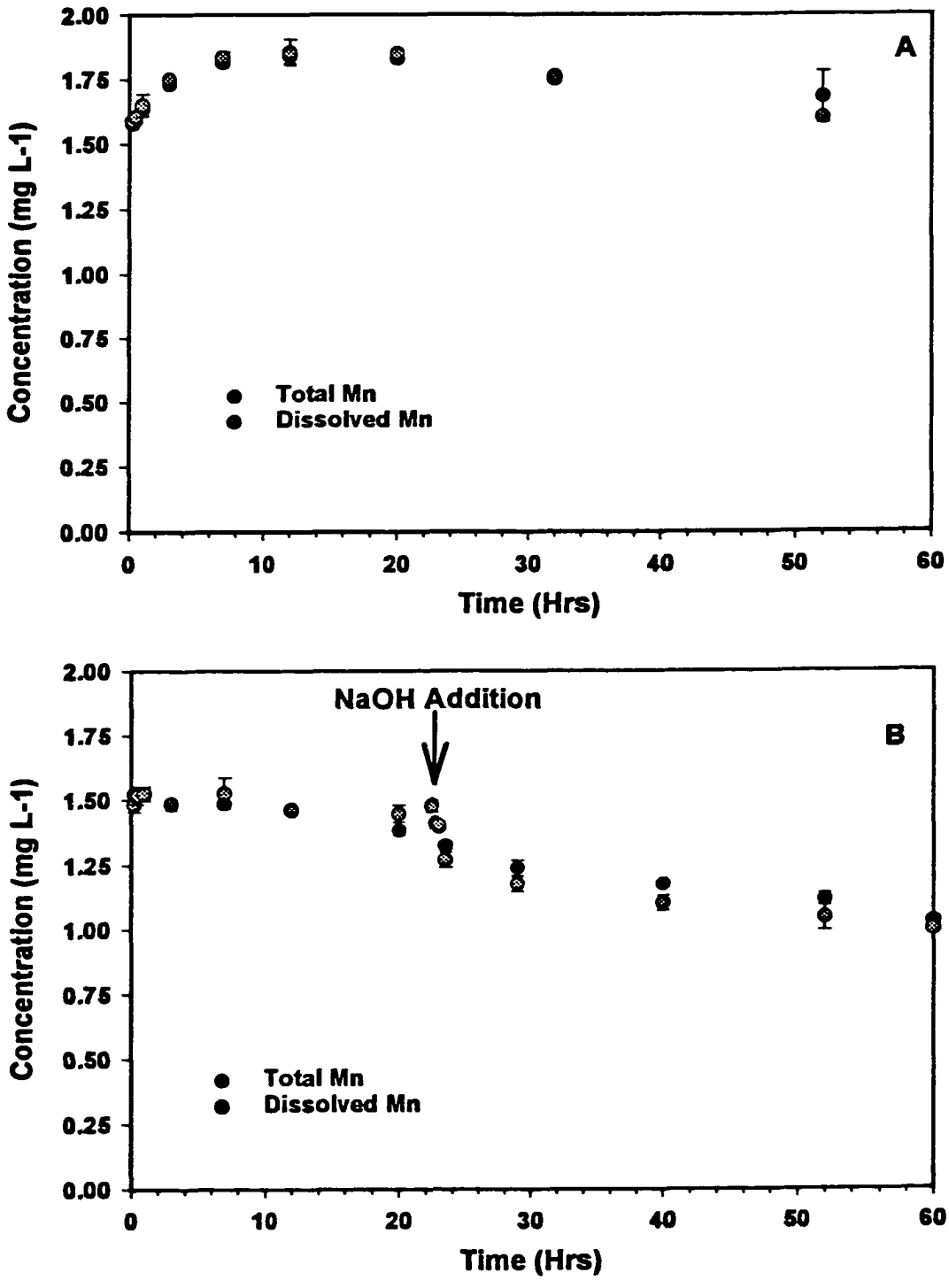


Figure 3.25. Total and dissolved manganese cncentrations during the early (A) and late (B) July raceway studies.

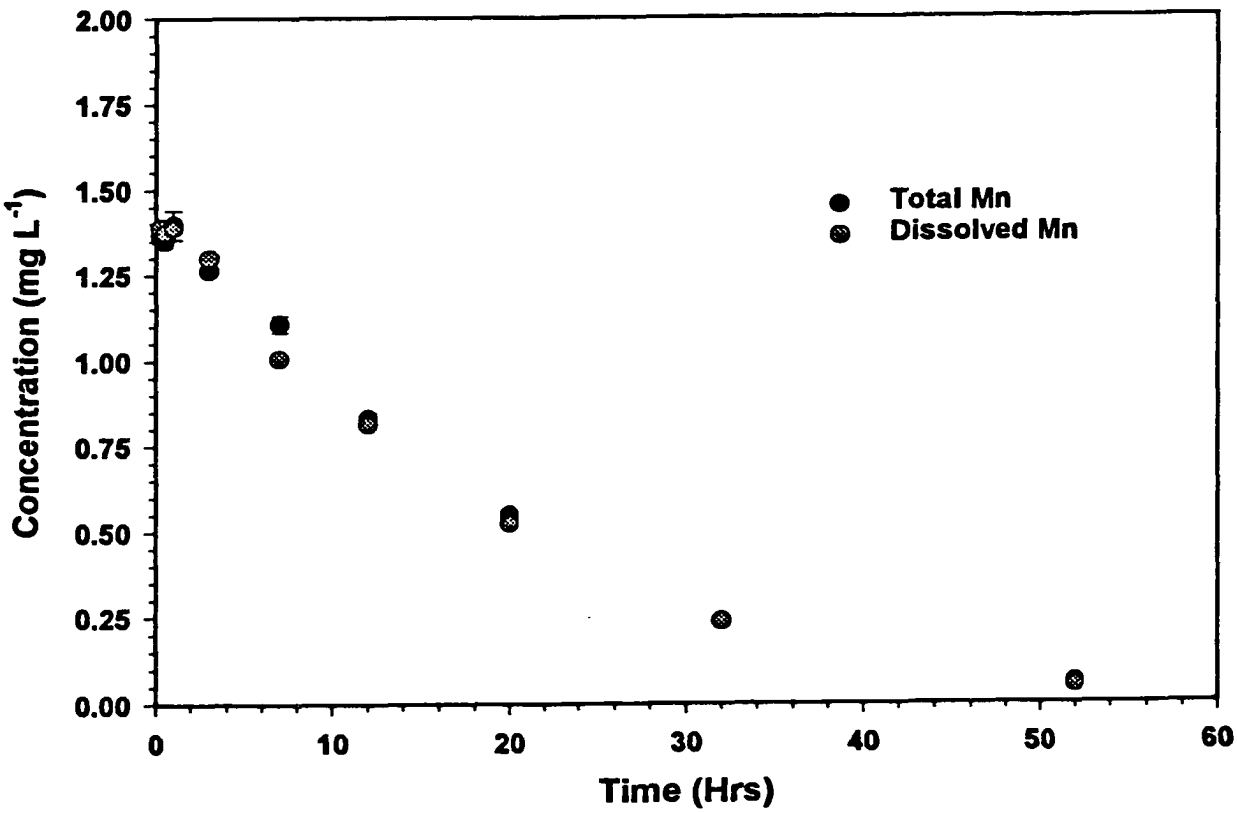


Figure 3.26. Total and dissolved manganese concentrations during the August raceway study.

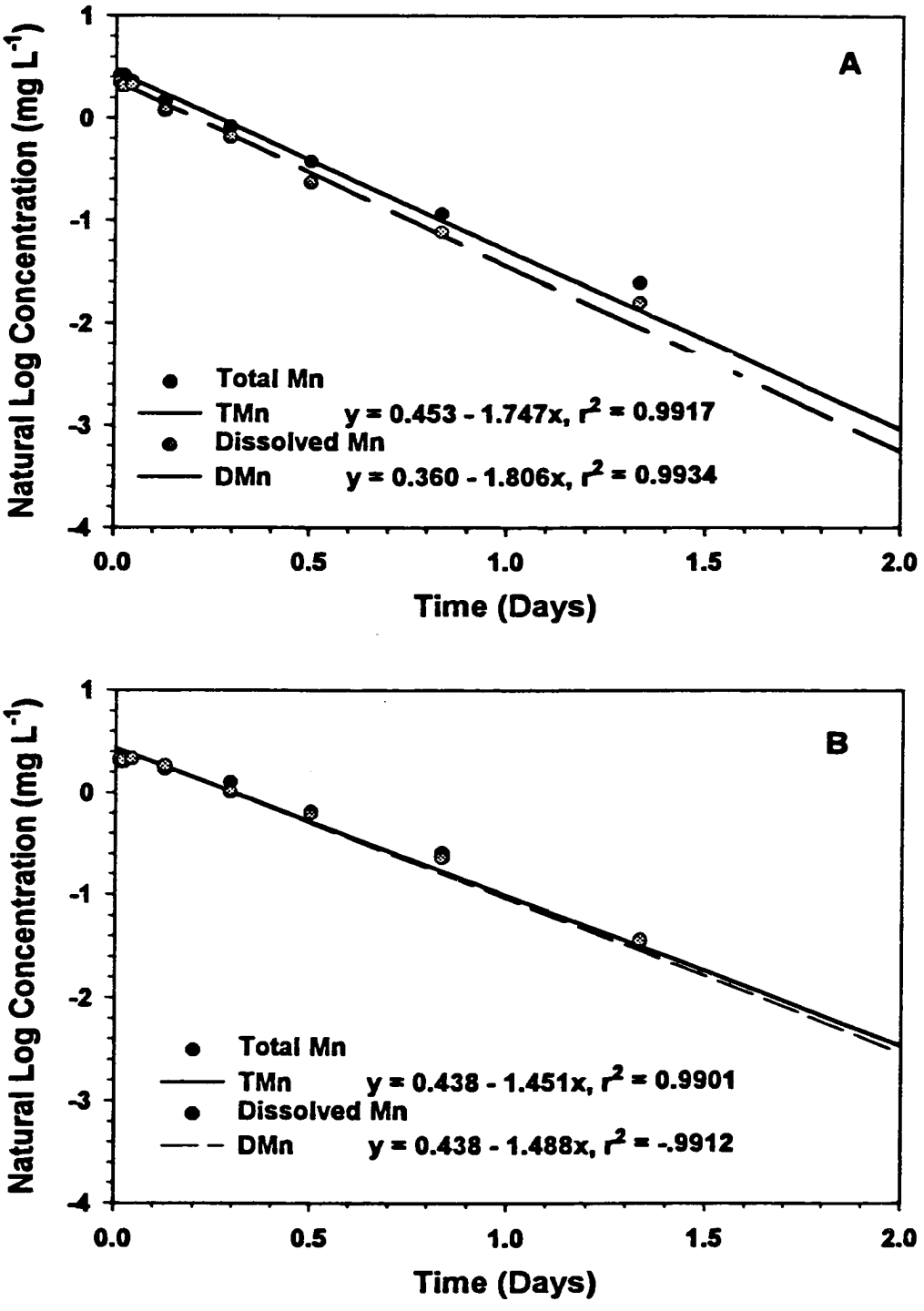


Figure 3.27. Removal rates of total and dissolved manganese concentrations during the April (A) and August (B) raceway studies.

Table 3.14. Concentration ranges for selected water quality constituents during each raceway study.

Constituent	April Study	Early July Study	Late July Study	August Study
pH (std units)	6.7 - 7.4	5.4 - 6.4	5.4 - 6.5	6.0 - 6.5
Conductivity (μmhos cm^{-1})	150 - 155	129 - 200	210 - 218	not available
T. Phosphorus ($mg\ L^{-1}$)	0.03 - 0.05	0.03 - 0.06	not available	0.04 - 0.05
Chloride ($mg\ L^{-1}$)	7.1 - 10.2	not available	not available	not available
Nitrate ($mg\ L^{-1}$)	0.61 - 0.85	not available	not available	not available
T. Calcium ($mg\ L^{-1}$)	13 - 15	14 - 22	not available	30 - 32

Assessments of microbial biomass and community structure on samples collected from the raceway following the first manganese uptake study indicated that sufficient material for analysis was available from the cobble samples but not from the surface of the pre-cored samples. Insufficient biomass was available in the sample from the water column as well. Results of the PLFA analysis are presented in Table 3.15. Maximum biomass was obtained from the seasoned- cobble collected from the shallow, high-velocity area of the raceway followed by the seasoned-cobble in the mid-depth, low-velocity area, and then comparable values were observed for the unseasoned, limestone cobble from the shallow, high-velocity area and the sediment from the deep, low-velocity area.

Community composition was evaluated using different fatty acid (PFLA) functional groups (e.g., normal saturate (nsat) which are ubiquitous, terminally branched

saturate (terbsat) which indicate Gram-positive bacteria, monounsaturate (mono) which indicate Gram-negative bacteria, branched monounsatrate (brmono) which also indicate Gram-negative bacteria, mid-chain branched saturate (midbrsat) which indicate actinomycetes, and poly unsaturate (poly) which indicate microeukaryotes) Table 3.15. In general, community compositions of the seasoned cobble in the high and low velocity areas were considered to be closely related and quite distinct from either the unseasoned cobble or the sediment (Figure 3.28). The samples for the two seasoned cobbles displayed a diverse community composition and showed evidence of Gram-negative bacteria, Gram-positive bacteria, possible iron-reducing bacteria, and microeukaryotes. The Gram-negative bacterial component was the largest. The unseasoned cobble showed a less diverse community structure, a lesser biomass, and an even greater Gram-negative bacterial presence than for the seasoned cobble. Community composition of the sediment sample also showed a strong Gram-negative bacterial presence but was distinct from both seasoned and unseasoned cobble samples with a very strong actinomycete type input. The geomorphology and hydrology in a stream can be major determinants of interactions between surface and interstitial habitats and would influence the biological community structure (Chafiq et al. 1996). Storey et al. (1999) suggested that the biofilm in interstitial habitats will occupy a variety of microniches, allowing a great diversity of microbial types, and promoting the activity of some otherwise poor competitors. These types of interactions, coupled with a seasonal supply of reduced manganese from the reservoir release, could provide conditions conducive to exploitation by manganese oxidizing bacteria.

Table 3.15. Results of the PLFA analysis conducted on substrate from the raceway.

Description	Area in Raceway	Biomass (pmole)	Community Composition (PLFA functional group, %)					
		avg (s.d.)	nsat (s.d.)	terbrsat (s.d.)	mono (s.d.)	brmon (s.d.)	midbrsat (s.d.)	poly (s.d.)
Core	Shallow High-velocity	90 (104)	65.1 (4.0)	5.2 (7.3)	23.8 (6.7)	0.0 (0.0)	5.9 (3.3)	0.0 (0.0)
Core	Mid-depth Low-velocity	56 (40)	84.8 (19.2)	0.8 (1.1)	13.0 (16.2)	0.0 (0.0)	1.4 (1.9)	0.0 (0.0)
Seasoned Cobble	Shallow High-velocity	36100 (26187)	32.6 (8.9)	7.9 (2.8)	47.2 (6.8)	1.4 (0.9)	4.5 (0.5)	6.3 (0.3)
Seasoned Cobble	Mid-depth Low-velocity	20284 (14544)	34.4 (4.2)	6.0 (1.2)	48.4 (6.1)	2.1 (0.5)	5.9 (0.4)	3.3 (0.8)
Unseasoned Cobble	Shallow High-velocity	11737 (684)	27.9 (2.1)	5.3 (0.8)	56.6 (1.7)	0.0 (0.0)	6.2 (0.2)	4.0 (0.2)
Sediment		12755 (n=1)	24.8	6.8	51.9	1.8	14.7	0.0
Water Column		5 (n=1)	100.0	0.00	0.0	0.0	0.0	0.0

s.d. Denotes the standard deviation.
nsat = normal saturate terbsat = terminally branched saturatepoly = polyunsaturate
mono = monounsaturate brmono = branched monounsaturate
poly = polyunsaturate midbrsat = mid-chain branced saturate

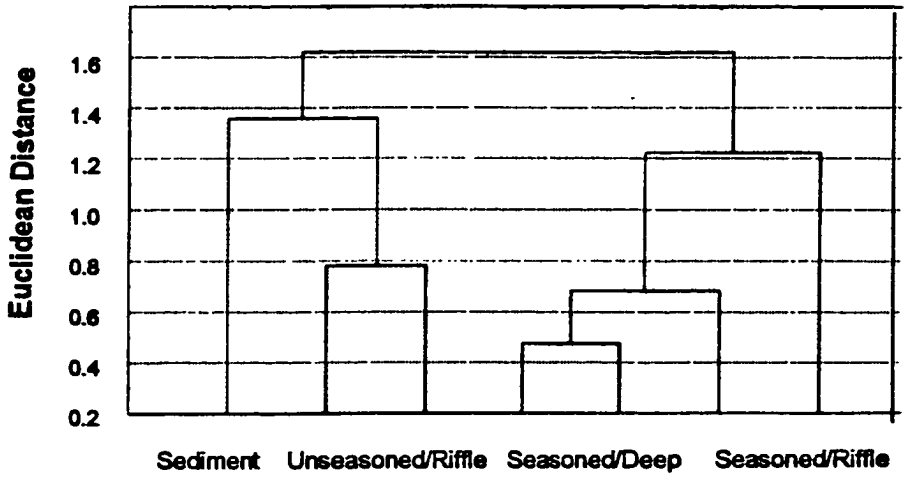


Figure 3.28. Hierarchical cluster analyses of PLFA profiles from the raceway samples.

3.5 Summary and Conclusions

The removal of manganese in reservoir tailwaters can initially be described by observations of manganese accumulation on substrate. Observations from the field studies indicated that manganese accumulation occurs on different substrate size fractions and the accumulation varies laterally (at least in the immediate headwater) and decreases with distance. Lateral variability would be expected if accumulation processes are a function of the supply of manganese (concentration, hydraulic loading rate, and exposure time). If the concentrations in the release are well-mixed (as suggested by lateral measurements of other water quality constituents), then exposure time (as a function of velocity or the hydraulic loading rate) would be the primary determinant of accumulation. Different discharges and channel morphometry (including slope) provide heterogeneity to the system and may account, in part, to the highly variable spatial distribution observed for manganese concentrations on substrate in the tailwater.

Longitudinal decreases in concentrations on substrates would be expected since there is removal of manganese from the water column to the bed material as the release moves downstream. This decreases the supply of manganese with distance. Decreased concentrations with distance were most pronounced for relative concentrations as determined by SEM analysis on cobble surfaces and on extractions from intermediate grain sizes (2.0 - 0.50 mm and 0.50 - 0.063 mm). Observations of decreased concentrations on substrate with distance from the dam confirm an earlier hypothesis by Hem (1981) that gradients in accumulation exist with distance from the source. With the exception of the < 0.063 mm size fraction, concentrations tended to be similar for each size fraction in the first 3 km of the

tailwater (station A to station B3) suggesting that there is a defined zone where removal is relatively consistent with time even though discharge would vary as a function of reservoir operations each year. Lower manganese concentrations in the < 0.063 mm size substrate in the upstream region than in the downstream region is likely related to the occurrence of higher flows in the upstream region which would keep the smaller sized material in suspension longer and result in downstream transport until velocities decreased sufficiently to allow settling and accumulation. Observations of increased particulate iron downstream indicated that resuspension occurs which could also allow increased transport of smaller particles (i.e., < 0.063 mm) further downstream.

The supply of manganese in the release increases as concentrations in the hypolimnion of the upstream impoundment increase and varies as a function of reservoir operations that affect the withdrawal zone. Additionally, as discharge increase, velocities tend to increase, reducing exposure time, yet more substrate is exposed to the release water by increased movement into the river bed (hyporheic zone) and an increase in the wetted area of the river bed. An increase in the removal rate of manganese as the net effect of increased discharge has been suggested (Gordon et al. 1984; Dortch et al. 1992) indicating that increased exposure to substrate more than compensates for decreased exposure time associated with increased flow. Gantzer et al. (1988) describes the process with mass transfer equations that account for an increase in the flux (supply) of a selected constituent (e.g., manganese) to deeper substrates (more substrate) as flow increases. Conclusions by Gordon (1989) that the hydraulic loading rate is critical to removal rates and increases in flow result in increases to the substrate also support the concept of increased removal as discharge increases. This

concept is also consistent with Goldberg (1954) who suggested that the longer the solid phase (i.e., substrate) is in contact with the solution, the more effective the uptake. Since flows vary from near immeasurable to very high during all discharge conditions, a tailwater with a sufficient area for low velocities to occur would provide sufficient removal time if enough substrate (and the associated microbial community) were available.

Individual effects of processes such as exposure time and supply on manganese removal are difficult to assess but can be further described from field studies conducted in 1995 that measured manganese removal as a function of time or distance at two separate discharges (0.5 and 1.7 m^3 sec^{-1}, referred to as "low" flow and "high" flow respectively) for comparison to previously conducted studies at the same site. Manganese removal measured during the low flow field study indicated a first order removal process occurs but there is a lag period during the first 18 hours. This lag, while possibly a function of limited sampling or imprecise estimates of travel times, was most pronounced during the "high" flow release. The observed removal rate for the low flow study (0.986 day^{-1}) was in agreement with those of Dortch et al. (1992), who report removal rates ranging from 0.70 day^{-1} for similar flows (0.71 m^3 sec^{-1}) to 1.81 day^{-1} for higher flows (5.66 m^3 sec^{-1}). However, the lower rate observed for the "high" flow study (0.17 day^{-1}) and a similar rate calculated for the lag period of the low flow study (0.15 day^{-1}) were considerably lower than those observed by Dortch et al. (1992) suggesting conditions were not conducive for increased kinetics observed during other studies.

Comparability between the two relatively low removal rates observed for the first day of each study suggests that manganese removal in tailwaters follows a pseudo-first order

process. Observations by Hem (1981) for water treatment systems also suggest a pseudo-first order process that is a function of substrate availability and supply of reduced manganese. Furthermore, Dortch and Hamlin-Tillman (1995) observed a wide range of removal rates (0.03 to 4.45 day^{-1}) at various reservoir tailwaters, suggesting that site characteristics may also contribute to removal processes. However, first order reactions have been successfully used to describe the manganese removal process in association with substrates in natural waters (Wilson 1980) and reservoir tailwaters (Gordon et al. 1984; Hess et al. 1989; and Dortch et al. 1992) when site characteristics such as stream depth and channel slope are included in estimates of removal rates (Dortch and Hamlin-Tillman 1995). These models do not include a mechanism for biological mediation which most likely accounts for the rapid kinetics relative to autocatalytic reactions described thermodynamically for higher pH systems (Morgan 1964).

Manganese removal rates observed in the April and August raceway studies (1.747 day^{-1} and 1.451 day^{-1}, respectively) were in closer agreement with rates observed by Dortch et al. (1992) for the Nimrod tailwater at a higher flow rate near 5.66 m^3 sec^{-1} than the rates observed in the 1995 field studies. The initial lag period observed in the 1995 field studies was also observed by Dortch et al. (1992), but not in the April and August raceway studies. The lag in removal observed in field studies is not readily explainable but could be the result of sampling techniques that do not match sample collection with the actual time of travel since the lag was most pronounced for sampling conducted in this way. Another possible explanation for the observed lag is the coincident higher velocities in the immediate tailwater and concurrent approximate lag time of 0.8 days at most discharges. Even though

manganese accumulation was observed on the substrate in the immediate tailwater, indicating removal, the exposure time of manganese to substrate is at the lowest for the study reach since this is the region where maximum velocities occur. Lag times of manganese removal in the immediate tailwater were also observed at other sites investigated by Dortch et al. (1992).

Raceway studies conducted in July resulted in no, or limited manganese removal (after pH manipulations) indicating that unsuitable conditions for manganese removal can exist. While exact causes of little to no manganese removal during the July studies were not identified, the major potentially contributing water quality condition was lower pH values observed in the July study. The effect of pH on manganese removal (i.e., oxidation) is well documented but slow rates are attributed to higher pH values. The low rates observed in July under low pH conditions (e.g., < 5.5) may actually be attributed to inactivity of the biological community at the lower pH. Another probable contributor to low removal rates observed in July is the stress on the biological system associated with draining and refilling the raceways and the possible introduction of excessive amounts of chlorinated water. Insufficient acclimation by the biota, as suggested by Gordon and Burr (1989) and Dortch and Hamlin-Tillman (1995), may also have contributed to observations of no or limited removal during the July studies.

Microbial community composition was more distinct between seasoned cobble and sediment (i.e., substrate size) than between areas with different velocities, suggesting substrate size has a greater impact on biotic structure than velocities. While the microbial communities from each of the sampling areas indicated potential manganese oxidizers, the greater potential for removal appears to be in the community associated with the larger

substrate. Although microbial populations were observed for all substrate sizes, the greater abundance on larger substrates indicates a potential for substrate size limitation, as observed by Chapnick et al. (1982) for particles less than 0.4 μm, and/or a substrate size preference, as observed by (Ledger and Hildrew 1998). Substrate influences on microbial populations (both size and distribution) likely have a profound impact on the manganese removal in tailwaters.

Accumulation patterns of manganese on tailwater substrates and observed removal rates support first order removal reactions which allow predictive capabilities for describing downstream concentrations. Observations of accumulation on different size fractions indicates that substrate availability is probably not a limiting factor, however, high removal rates or kinetics, indicate that biological mechanisms (most likely associated with substrates) are important and could vary with substrate. Increased removal rates with increased discharge is likely the result of increased supply of manganese to the substrate and the biological community. Although removal rates have been estimated based on site characteristics such as stream depth, channel slope, and substrate size, results from the raceway studies and varied rates observed at the same field site suggest that other mechanisms (e.g., biological processes) may be used to estimate removal rates and would certainly be related to kinetic mechanisms that may better describe the removal of manganese in reservoir tailwaters.

CHAPTER 4
EFFECTS OF SUBSTRATE SIZE AND MICROBIAL PROCESSES ON MANGANESE REMOVAL

4.1 Introduction

Manganese removal is modeled quite well as a first order removal process (Dortch and Hamlin-Tillman 1995). However, removal rate coefficients, developed from field studies and modeled with stream depth, slope, substrate type (cobble versus fines) and water temperature, do not describe contributions of substrate size fractions to manganese removal or biological processes that contribute to kinetics that exceed predictions based on thermodynamics. In addition, biological mediation in manganese oxidation or removal has been described as a two-step process (e.g., Ehrlich 1963; Ehrlich et al. 1972; Emerson et al. 1982; Chapnick et al. 1982; Tebo and Emerson 1985) as a function of "binding" sites. It follows that prediction of removal rates may be more accurately described by incorporating substrate affects and biological processes into equations describing manganese removal. For example, the use of the Michaelis-Menton equation (Michaelis and Menton 1913) to further describe the kinetics and predict removal rates (Sunda and Huntsman 1987; Tebo and Emerson 1985 and 1986; Miyajima 1992a; Tebo et al. 1997) may be possible.

The objectives of this study were to identify the contribution of substrate size fractions and biological processes on manganese removal in reservoir tailwaters. Manganese removal rates associated with substrate size fractions collected from the tailwater of Nimrod Lake, AR were evaluated in poisoned and unpoisoned microcosms.

An assessment of the microbial biomass and community structure was also used to describe biological conditions associated with each substrate size fraction. Study results provided removal rates for each size fraction and allowed the partitioning of abiotic and biotic manganese removal. Results were used in assessing an existing first-order removal model and utilization of a negative hyperbolic function that provided a better fit to the data and allowed the use of a Michaelis-Menton type equation to describe manganese removal.

4.2 Methods

4.2.1 Microcosm Studies

For microcosm studies, substrate samples were collected with an Ekman dredge at Station B1 in the tailwater of Nimrod Lake, AR (see Figure 3.1), and separated in the laboratory using sieves. Size fractions included cobble and substrate in 3 separate size classes between 2.0 mm to 0.50 mm, 0.50 to 0.063 mm, and less than 0.063 mm. The study was conducted with triplicate experimental units. For the cobble size fraction, three approximately equal size cobbles were used for each microcosm. Approximately equal aliquots of material were used for the other size fractions. Water collected at the site was spiked with $MnSO_4$ to obtain an initial concentration of reduced manganese near 3.5 mg L^{-1} and approximately 800 ml was immediately added to pre-cleaned, acid-washed 2 L beakers containing the substrates, or beakers without substrate that represented the water column (referred to as amended tailwater). Half of the experimental units received 1 ml of 1.5 M sodium azide to inhibit microbial uptake of reduced manganese (referred to as poisoned) and half did not receive the sodium azide (referred to as unpoisoned). Sampling

was conducted at selected intervals for the next 66 hours to describe removal rates of reduced manganese. All experimental units were held near 20 °C in a water bath and lightly mixed with air bubbles from an in-house air supply. The experiment was conducted in a room equipped with timers for the lighting system which provided a period of no lights from 2030 to 0600 hours. The pH of each experimental unit was measured at the initiation of the study and periodically throughout the study. The initial pH of the amended tailwater added to each microcosm was 7.1 and the poisoned microcosms remained relatively constant at a pH of 6.9 to 6.8 while the pH of the unpoisoned microcosms remained near 6.4 to 6.5. Although the pH was about a half of a standard unit lower in the unpoisoned microcosms, the pH in both treatments was considered to be within the range that allows microbial processes for removal. Samples for total and soluble manganese analysis were collected with a clean, acid-washed syringe and held in clean, acid-washed scintillation vials until analysis. Samples were filtered through a 0.45 μm membrane filter in an in-line filter holder that attached to the syringe and the first 5-10 mls were used to rinse the filter and then were discarded. Samples were preserved with 3-5 drops of concentrated nitric acid. Analysis was conducted using a Jarrell-Ash Atom Comp Series 800 ICP. Reagent blanks and standards or standard curves were analyzed for all chemical analyses and results indicated near detection limits for the blank and near 100% recovery for the standards.

4.2.2 Analysis of Microbial Community Structure

Microbial biomass and community composition was determined by the analysis of membrane lipids using extraction techniques employing organic solvents (Vestal and

White 1989). With this approach, total concentrations of ester-linked phospholipid fatty acids (PLFA) are recovered from the sample and used to estimate biomass (Balkwill et al., 1989) and microbial community composition (White et al., 1999). Different functional groups, such as gram-positive and gram-negative organisms, were identified by unique patterns of fatty acids (e.g. saturated, unsaturated, and branched moieties). The fatty acids can then be attributed to specific biosynthetic pathways, which differ among various functional groups of microbes.

The analysis of the microbial biomass and community composition in the tailwater of Nimrod Lake was conducted on substrate samples collected at Station B1 (see Figure 3.1) and used in the microcosm study described above. Substrates for each size fraction used in the microcosm uptake study were collected upon completion of the studies. Differential sample processing was conducted to insure maximum recovery of microbial biomass for the analysis. For the 2.00 to 0.50 mm and the 0.50 to 0.063 mm size fractions, the overlying water was decanted and approximately 2 g of wet substrate (1.4 - 1.6 g, dry weight) was collected for subsequent processing. Concentrations were reported per gram of dry weight. For the unpoisoned, amended water and the < 0.063 mm substrate, the samples were centrifuged and subsequent analysis was conducted on solids collected by centrifugation. Approximately 30 mL of the amended water and the < 0.063 mm substrate for the poisoned microcosms were collected for extraction in separatory funnels. Approximately 30 mL of water from each microcosm containing cobble was collected for extraction in separatory funnels.

Substrates used in the microcosm studies were prepared for lipid extraction as described in White and Ringelberg (1998) and Findlay and Dobbs (1993). Lipids were quantitatively recovered from substrate materials by saturation with 7 ml of organic solvent ($CHCl_3$:MeOH, 1:2, v:v) once the overlying water was decanted. The lipid extract was then partitioned against water (via the addition of CH_2Cl_2:H_2O, 1:1, v:v) and the organic phase aspirated to a pre-packed silica column (Burdick and Jackson, Muskegon, MI) from which non-polar and polar lipids were eluted with sequential washes of CH_2Cl_2, acetone, and methanol. The polar (methanol) eluent was taken to dryness under N_2 and then subjected to a single-step trans-esterfication to form methyl esters of the recovered phospholipid fatty acids. The PLFA were then separated, identified and quantified by capillary gas-chromatography (Hewlett Packard 6890GC) coupled to a quadrapole mass spectrometer (model 5973 mass selective detector). Detection was by electron impact ionization at 70eV.

The PLFA analysis was conducted by Dave Ringelberg and Margaret Richmond at the Environmental Laboratory located at the US Army Engineer Research and Development Center in Vicksburg, MS. Dave Ringelberg provided interpretation of the data, hierarchical cluster analysis of the sample PLFA profiles, and preparation of associated figures.

4.2.3 Data Analysis

Statistical analyses of data for selected studies were conducted using linear regression techniques in SigmaPlot (SPSS Inc, Chicago, IL) and the Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC). Univariate analysis was conducted with

SAS procedures on data sets and Shapiro-Wilkes statistics were used to test for normality on residuals. Linear regression analysis (with a no intercept model) and linear contrasts (SAS procedures) were used to compare dissolved manganese removal rates by treatment (nonpoisoned and poisoned) and size fraction. Individual regressions within treatments for each size fraction were also used to compare treatments and removal rates between size fractions with a mixed linear regression model using a SAS procedure with the Tukey adjustment. PLFA patterns were examined by hierarchical cluster analysis (Ward's Method) to determine similarities between samples using Statistica, version 5.1 (Statsoft, Inc., Tulsa, OK). The PLFA data were transformed using an arcsin square root function on mole percentage values. Values were reported in pmol PLFA $gram^{-1}$ for the 2.00 to 0.50 mm and the 0.50 to 0.063 mm size fractions and in pmol PLFA ml^{-1} for the extract from the cobble, the < 0.063 mm, and the amended water. These different methods of reporting PLFA results precluded quantitative comparisons between substrate sizes.

4.3 Results and Discussion

4.3.1 Microcosm Studies

Dissolved manganese concentration responses were quite similar to responses of total manganese concentrations, and, consequently, were used to describe concentration responses for manganese in the microcosm studies. Data are compiled in Appendix C. Regressions are plotted for natural log transformed concentrations and time in days for comparison to raceway and field studies. In general, the residuals were normally distributed (Table 4.1) although the distributions for the < 0.063 mm size fraction in the poisoned microcosms were "marginally" significant (i.e., $p < 0.10$). Individual regressions

were also significant ($p < 0.05$) for all of the unpoisoned microcosms and the poisoned microcosms with the 2.00 mm to 0.50 mm and cobble size fractions, indicating slopes greater than zero and removal of dissolved manganese with time (Table 4.1). Regressions for the poisoned microcosms for the 0.50 mm to 0.063 mm, < 0.063 mm, and the amended water were not significant and indicated no removal of manganese with time. Removal rates for dissolved manganese ranged from 1.910 to 0.049 day^{-1} for the unpoisoned microcosms and from 0.732 to 0.007 for the poisoned microcosms (Table 4.1). Additional discussion of removal rates is included later in this chapter.

Individual regressions for both treatments in the cobble size fraction and the unpoisoned treatments in the 2.00 mm to 0.50 mm and 0.50 mm to 0.063 mm size fractions were significantly different ($p < 0.05$), Table 4.2, indicating differences in removal rates for the three replicate microcosms in each respective size fraction. Individual regressions within treatments for each substrate size fraction were not significantly different ($p < 0.05$) for the amended water, the < 0.063 mm size fraction, the poisoned 0.05 mm to 0.063 mm, and the poisoned 2.00 mm to 0.05 mm size fractions (Table 4.2). Regressions that were not significantly different within a treatment indicated that manganese removal rates were not different for that treatment and size fraction.

Differences in poisoned and unpoisoned microcosms were observed and results of regression analyses and linear contrasts indicated significant differences ($p < 0.05$) between treatments for all size fractions and in the amended water sample (Table 4.3). When each individual regression within each treatment was used (PROC MIXED using SAS procedures) the treatments were still significantly different ($p < 0.05$) for each size

Table 4.1. Summary of univariate and regression analyses of manganese removal in unpoisoned and poisoned microcosms.

Size Fraction and Treatment	Shapiro-Wilkes Value Pr < W	Regression Value p < 0.05	Rate day^{-1} Natural Log Concentration
Cobble - Unpoisoned	0.9692	0.0001	-1.605
Cobble - Unpoisoned	0.4854	0.0001	-1.478
Cobble - Unpoisoned	0.9355	0.0017	-1.396
Cobble - Poisoned	0.108	0.0002	-0.732
Cobble - Poisoned	0.3845	0.0106	-0.290
Cobble - Poisoned	0.1518	0.01	-0.247
2.00 - 0.50 mm - Unpoisoned	0.9471	0.0016	-0.760
2.00 - 0.50 mm - Unpoisoned	0.0863	0.0001	-1.910
2.00 - 0.50 mm - Unpoisoned	0.8248	0.0008	-1.850
2.00 - 0.50 mm - Poisoned	0.9323	0.0067	-0.203
2.00 - 0.50 mm - Poisoned	0.657	0.0019	-0.087
2.00 - 0.50 mm - Poisoned	0.8241	0.0002	-0.144
0.50 - 0.063 mm - Unpoisoned	0.7243	0.0002	-1.830
0.50 - 0.063 mm - Unpoisoned	0.4637	0.0001	-1.594
0.50 - 0.063 mm - Unpoisoned	0.9284	0.0014	-1.584
0.50 - 0.063 mm - Poisoned	0.4651	0.0634	-0.120
0.50 - 0.063 mm - Poisoned	0.1365	0.0877	-0.093
0.50 - 0.063 mm - Poisoned	0.1825	0.1345	-0.073
< 0.063 mm - Unpoisoned	0.4886	0.0003	-0.340
< 0.063 mm - Unpoisoned	0.4387	0.002	-0.334
< 0.063 mm - Unpoisoned	0.4273	0.0011	-0.310
< 0.063 mm - Poisoned	0.0607	0.5244	-0.032
< 0.063 mm - Poisoned	0.0251	0.3031	-0.050
< 0.063 mm - Poisoned	0.0567	0.3264	-0.040
Water Column - Unpoisoned	0.2214	0.0002	-0.193
Water Column - Unpoisoned	0.8969	0.0099	-0.049
Water Column - Unpoisoned	0.8229	0.0124	-0.133
Water Column - Poisoned	0.5387	0.6627	-0.007
Water Column - Poisoned	0.976	0.2203	-0.010
Water Column - Poisoned	0.5283	0.1598	-0.023

fraction (Table 4.4). Differences between treatments indicated that removal of dissolved manganese is affected by biological processes that can be inhibited with the addition of sodium azide. Furthermore, substrate size (including the amended water) had no effect on differences between treatments.

Table 4.2. Summary of linear contrasts for within treatment regressions for each substrate size.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Cobble - Unpoisoned	2	1.2985	0.6493	7.15	0.0024
Cobble - Poisoned	2	1.8257	0.9129	10.05	0.0003
2.00 mm - 0.50 mm Unpoisoned	2	8.8269	4.4135	48.64	0.0001
2.00 mm - 0.50 mm Poisoned	2	0.2239	0.1120	1.23	0.3031
0.50 mm - 0.063 mm Unpoisoned	2	0.8238	0.4119	3.54	0.0396
0.50 mm - 0.063 mm Poisoned	2	0.0309	0.0154	0.13	0.8763
< 0.063 mm Unpoisoned	2	0.0015	0.0007	0.03	0.9749
< 0.063 mm Poisoned	2	0.0010	0.0005	0.02	0.9829
Amended water, no substrate - Unpoisoned	2	0.0214	0.0107	3.17	0.0541
Amended water, no substrate - Poisoned	2	0.0007	0.0003	0.11	0.8965

Table 4.3. Summary of linear contrasts between treatments for each size fraction.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Cobble	1	15.9959	15.9959	176.08	0.0001
2.00 mm -0.50 mm	1	26.3810	26.3810	290.75	0.0001
0.50 mm - 0.063 mm	1	39.4188	39.4188	338.57	0.0001
< 0.063 mm	1	1.400	1.400	47.83	0.0001
Amended water, no substrate	1	0.2676	0.2676	79.21	0.0001

Table 4.4. Summary of mixed regression analysis between treatments for each size fraction.

Substrate Size	DF	Difference	Std. Err.	t	Pr >|t|
Cobble	28	-0.7273	0.1194	-6.09	0.0001
2.00 mm - 0.50 mm	28	-1.0526	0.1906	-5.52	0.0001
0.50 mm - 0.063 mm	28	-1.2490	0.0682	-18.31	0.0001
< 0.063 mm	28	-0.1664	0.0089	-29.88	0.0001
Amended Water	28	-0.1007	0.0132	-7.62	0.0001

Differences between manganese removal rates for the poisoned microcosms between size fractions were significant ($p < 0.05$) for all comparisons using linear contrasts within each size fraction except for the 2.00 mm to 0.50 mm versus the 0.50 mm to < 0.063 mm, the 2.00 mm to 0.50 mm versus the < 0.063 mm, and the 0.05 mm to < 0.063 mm versus the < 0.063 mm size fractions (Table 4.5). Similar results were observed when regression analysis using the mixed model was applied except that a significant difference was observed for the 0.50 to < 0.063 mm versus the < 0.063 mm size fractions (Table 4.6). Differences in removal rates by size on poisoned substrate

indicate that substrate size has an affect on manganese removal with highest rates occurring on the cobble. Lower rates, associated with the 2.00 to 0.05 mm size fraction, were not significantly different than the smaller size fractions and the amended water and likely near zero since the smaller size fractions were not significantly different from zero (see Table 4.1).

Differences between manganese removal rates for the unpoisoned microcosms between size fractions were significant ($p < 0.05$) using linear contrasts for all comparisons except for the cobble versus the 2.00 mm to 0.50 mm size fractions (Table 4.7). Results of regression analysis using the mixed model indicated that removal rates for the cobble and the 0.5 to 0.063 mm size fraction and the 2.00 to 0.50 mm and the 0.50 to 0.063 mm size fractions were also not significantly different (Table 4.8).

Table 4.5. Summary of linear contrasts between size fractions for poisoned microcosms.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Cobble vs 2.00 - 0.50 mm	1	2.600	2.600	106.97	0.0001
Cobble vs 0.50 - 0.063 mm	1	2.447	2.447	100.65	0.0001
Cobble vs < 0.063 mm	1	3.098	3.098	127.46	0.0001
Cobble vs Amended water	1	5.008	5.008	206.05	0.0001
2.00 - 0.50 vs 0.50 - < 0.063 mm	1	0.0023	0.0023	0.10	0.7573
2.00 - 0.50 vs < 0.063 mm	1	0.0218	0.0218	0.90	0.3461
2.00 - 0.50 mm vs Amended water	1	0.3912	0.3912	16.10	0.0001
0.50 - < 0.063 vs < 0.063 mm	1	0.0384	0.0384	1.58	0.2120
0.50 - < 0.063mm vs Amended water	1	0.4540	0.4540	18.68	0.0001
< 0.063 mm vs Amended water	1	0.2283	0.2283	9.39	0.0029

Table 4.6. Summary of mixed model comparisons between size fractions for poisoned microcosms.

Substrate Size	DF	Difference	Std. Err.	t	Pr >|t|
Cobble vs 2.00 - 0.50 mm	28	-0.4432	0.0854	-5.19	0.0001*
Cobble vs 0.50 - 0.063 mm	28	-0.3822	0.0800	-4.78	0.0001*
Cobble vs < 0.063 mm	28	-0.4215	0.0793	-5.32	0.0001
Cobble vs Amended water	28	0.5978	0.0793	7.54	0.0001
2.00 - 0.50 vs 0.50 - < 0.063 mm	28	0.0609	0.03397	1.79	0.0835*
2.00 - 0.50 vs < 0.063 mm	28	0.0217	0.0323	0.67	0.5074*
2.00 - 0.50 mm vs Amended water	28	0.1546	0.324	4.78	0.0001*
0.50 - < 0.063 vs < 0.063 mm	28	-0.0393	0.0118	-3.34	0.0024
0.50 - < 0.063mm vs Amended water	28	0.2156	0.0119	18.06	0.0001
< 0.063 mm vs Amended water	28	0.1763	0.0056	31.59	0.0001

* Denotes no significant interactions

Table 4.7. Summary of linear contrasts between size fractions for unpoisoned microcosms.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Cobble vs 2.00 - 0.50 mm	1	0.2263	0.2263	2.10	0.1512
Cobble vs 0.50 - 0.063 mm	1	0.5110	0.5110	4.73	0.0322
Cobble vs < 0.063 mm	1	20.94	20.94	193.99	0.0001
Cobble vs Amended water	1	32.7197	32.7197	303.8	0.0001
2.00 - 0.50 vs 0.50 - < 0.063 mm	1	1.4173	1.4173	13.13	0.0005
2.00 - 0.50 vs < 0.063 mm	1	16.8154	16.8154	155.76	0.0001
2.00 - 0.50 mm vs Amended water	1	27.5041	27.5041	254.77	0.0001
0.50 - < 0.063 vs < 0.063 mm	1	27.9965	27.9965	259.33	0.0001
0.50 - < 0.063mm vs Amended water	1	41.4086	41.4086	383.56	0.0008
< 0.063 mm vs Amended water	1	1.3082	1.3082	12.12	0.0029

Table 4.8. Summary of mixed model comparisons between size fractions for unpoisoned microcosms.

Substrate Size	DF	Difference	Std. Err.	t	Pr >|t|
Cobble vs 2.00 - 0.50 mm	28	-0.1179	0.2080	-0.57	0.5753*
Cobble vs 0.50 - 0.063 mm	28	0.1395	0.1118	1.25	0.2226*
Cobble vs < 0.063 mm	28	-0.8824	0.0897	-9.84	0.0001
Cobble vs Amended water	28	1.2245	0.0902	13.57	0.0001
2.00 - 0.50 vs 0.50 - < 0.063 mm	28	0.2574	0.1995	1.29	0.2076*
2.00 - 0.50 vs < 0.063 mm	28	-0.7645	0.1880	-4.07	0.0004
2.00 - 0.50 mm vs Amended water	28	1.1065	0.1883	5.88	0.0001
0.50 - < 0.063 vs < 0.063 mm	28	-1.0219	0.0678	-15.08	0.0001
0.50 - < 0.063mm vs Amended water	28	1.3639	0.0684	19.93	0.0001
< 0.063 mm vs Amended water	28	0.3421	0.0149	22.92	0.0001

* Denotes no significant interactions

Dissolved manganese concentrations in the microcosms with cobble size substrate decreased in a curvilinear, exponential pattern (Figure 4.1). Removal rates within and between treatments were different ($p < 0.05$, Tables 4.2 and 4.3 or 4.4, respectively) and removal rates for the unpoisoned cobble were greater than rates observed for the poisoned cobble. The rate of decrease was similar in both poisoned and unpoisoned microcosms for the first 5 - 10 hours but while concentrations continued to decrease to near the detection limit in the unpoisoned microcosms, concentrations in the poisoned concentrations remained relatively constant well above the detection limit.

Rates for the unpoisoned cobble ranged from -1.4 to -1.6 day^{-1} (Figure 4.2), which is comparable to rates observed in the raceway and field studies described in Chapter 3. Linear regression analyses of log transformed data accounted for 88 to 99 % of the

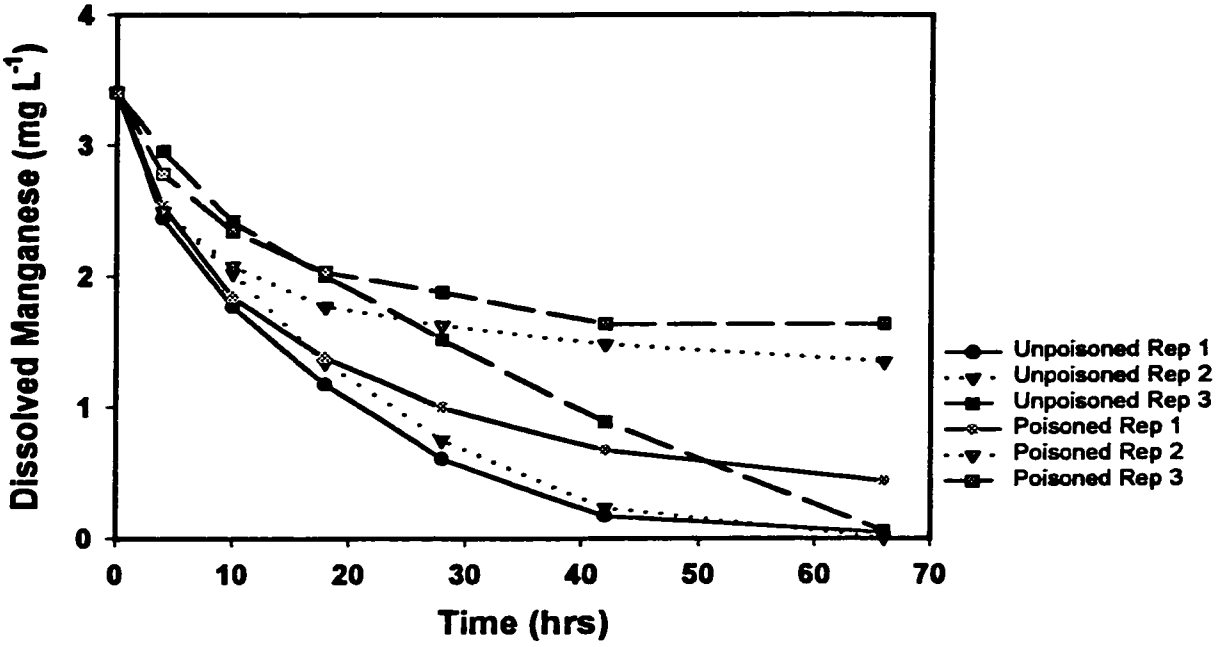


Figure 4.1. Manganese concentrations in the microcosms with cobble substrate.

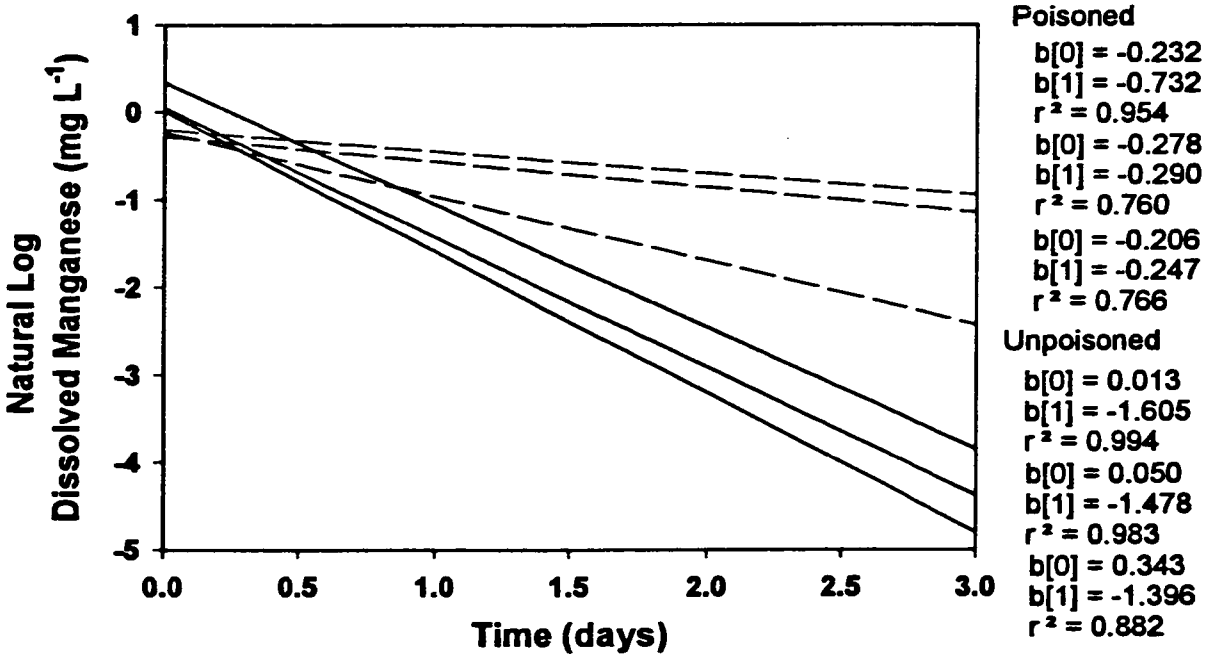


Figure 4.2. Linear regressions (no intercept) for the poisoned (dashed line) and unpoisoned (solid line) cobble substrates.

variability. In the poisoned microcosms, removal rates of -0.25 to -0.76 day^{-1} were lower than those observed for unpoisoned cobble and regression analysis accounted for 76 to 95 % of the variability. Unlike the removal observed for the unpoisoned cobble, concentrations did not decrease to the detection limit and, in general, removal greatly decreased after a period of approximately 10 - 20 hours indicating substrate limitation.

Removal rates for the unpoisoned cobble were not significantly different ($p > 0.05$) than removal rates for the 2.00 to 0.50 mm size fraction (Tables 4.7 and 4.8) or the 0.50 to 0.063 mm size fraction (Table 4.8). Removal rates were significantly greater for the cobble than for the remaining, smaller size fractions. In the poisoned microcosms, removal rates for the cobble were significantly greater than removal rates for all other size fractions (Tables 4.5 and 4.6).

Results indicate that, for cobble, manganese removal can occur for about 10-15 hours until all of the oxide coating that is providing sites for manganese binding is utilized. The biological community (when active or uninhibited) likely maintains the removal processes by providing additional substrate as exterior coatings. Removal for cobble only substrate was comparable to size fractions between 2.00 to 0.50 mm and 0.50 mm to 0.063 mm suggesting similar removal rates would be expected in streams with coarse sediments but not cobble.

Results similar to those observed in the cobble microcosms were observed for the 2.00 to 0.50 mm size fraction (Figures 4.3 and 4.4) with a greater exponential rate of concentration decrease for the unpoisoned microcosms than for the poisoned microcosms. In the poisoned microcosms, the initial, rapid rate of decrease occurred primarily during

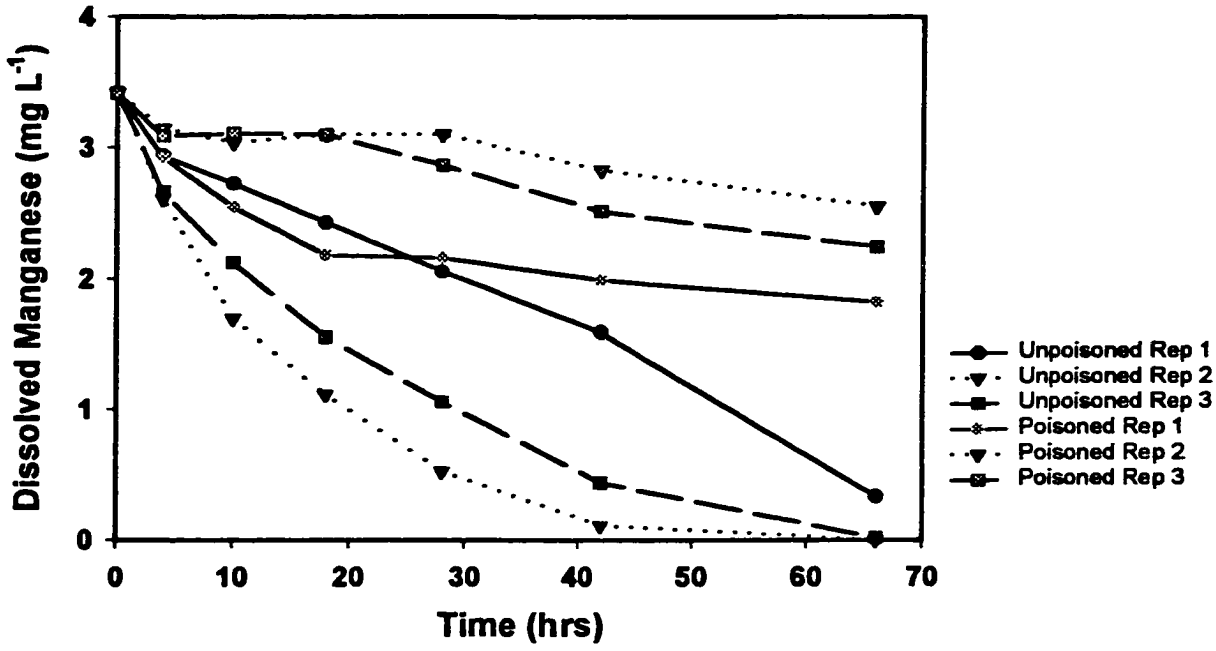


Figure 4.3. Manganese concentrations in microcosms with the 2.00 - 0.50 mm size fraction substrate.

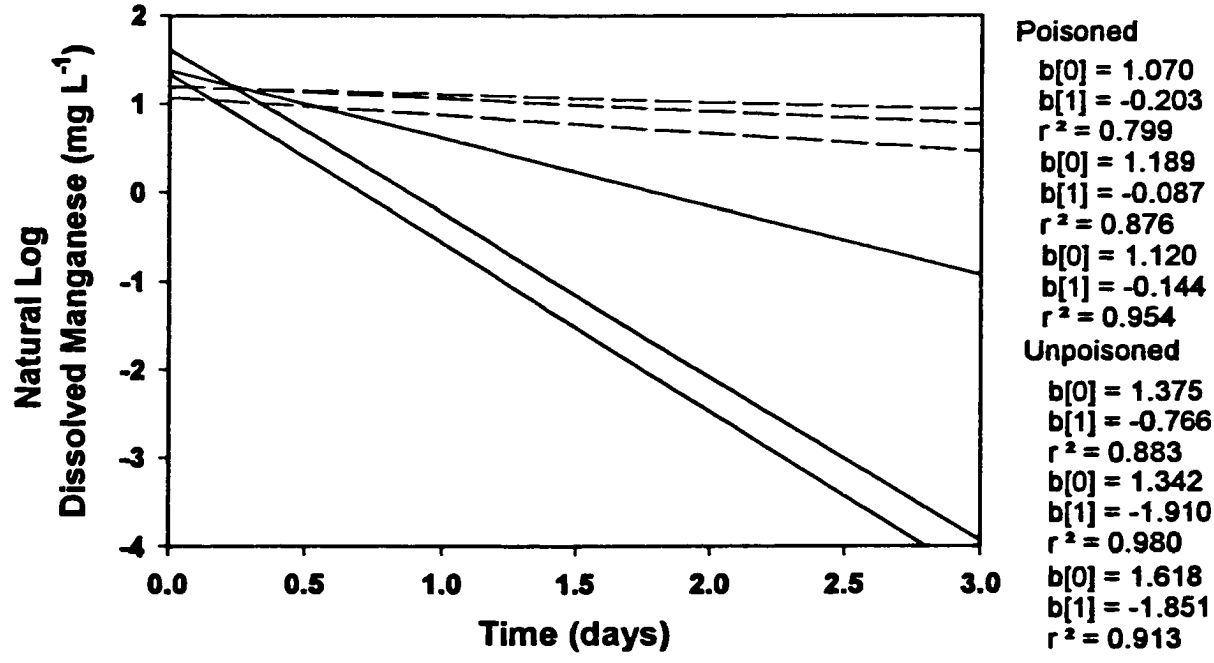


Figure 4.4. Linear regressions (no intercept) for the poisoned (dashed line) and unpoisoned (solid line) 2.00 - 0.50 mm size fraction substrate.

the first 5 hours, although decreased concentrations in one microcosm continued until near 20 hours, demonstrating variability between microcosms within treatments. Removal rates in the poisoned microcosms were relatively low ranging from -0.09 to -0.2 day^{-1}. However, regression analysis accounted for 80 to 95 % of the variability. Variability within treatments was also apparent for the unpoisoned microcosms with the occurrence of a lower removal rate (-0.76 day^{-1}) for one microcosm compared to removal rates of -1.91 and -1.85 day^{-1} for the other two microcosms although the regressions accounted for 88 to 98% of the variability. Removal rates in the unpoisoned microcosms were comparable to rates observed for unpoisoned cobble (Tables 4.7 and 4.8) and the 0.50 to 0.063 mm size fraction (Table 4.8). Removal to near detection limit concentrations was observed for the unpoisoned microcosms during the 66 hours of observation.

Concentration decreases observed for the 0.50 to 0.063 mm size fractions (Figures 4.5 and 4.6) were less variable and the deviation in concentration decreases between treatments was apparent at about 10 hours after initiation of the experiment. Removal rates for the poisoned microcosms were relatively low, ranging from -0.12 to -0.07 and not significantly different from 0 suggesting no removal ($p > 0.05$, Table 4.1). Regression analysis accounted for only 39 to 53 % of the variability indicating a different model may be more appropriate. Removal rates observed for the unpoisoned microcosms (1.8 to 1.6 day^{-1}) were signifcantly higher ($p < 0.05$) than rates observed for poisoned microcosms, comparable to rates observed for the 2.00 to 0.50 mm size fractions (Table 4.8), and regression analysis explained 89 to 95 % of the variability. Removal to near detection

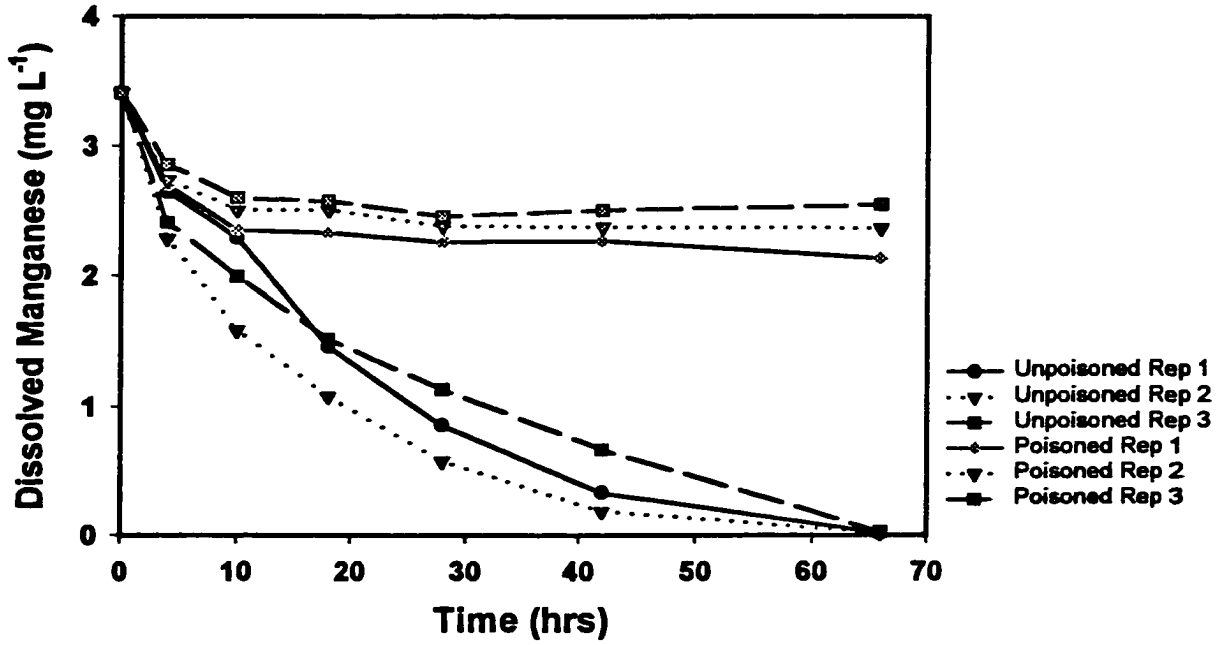


Figure 4.5. Manganese concentrations in microcosms with the 0.50 - 0.063 mm size fraction substrate.

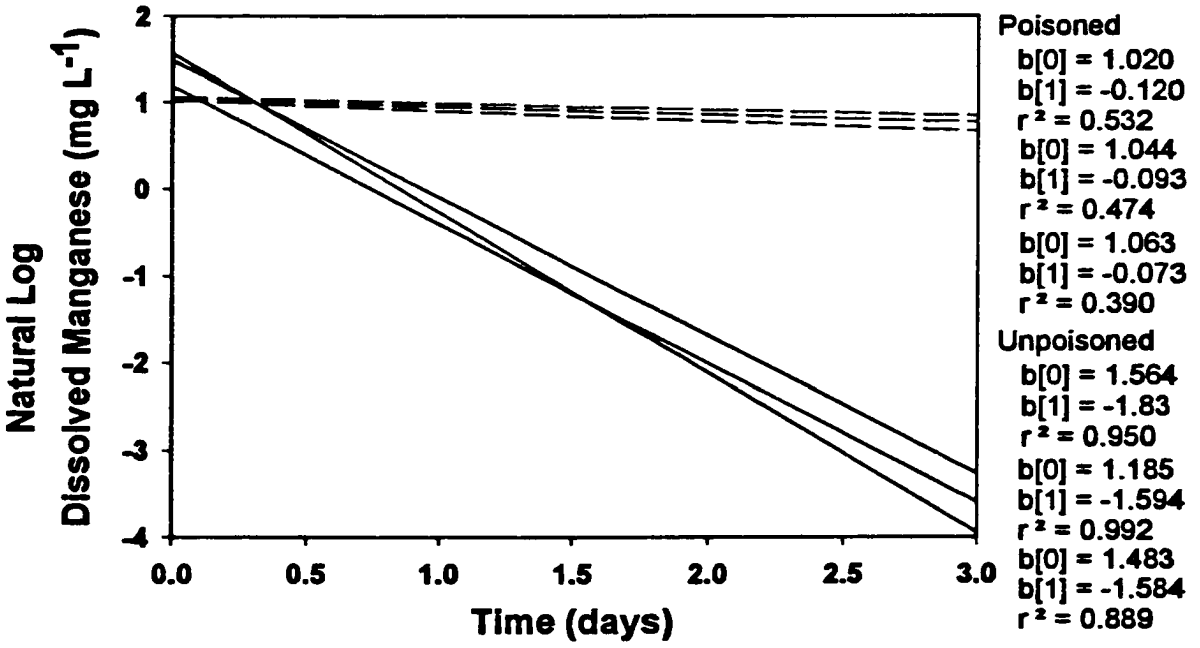


Figure 4.6. Linear regressions (no intercept) for the poisoned (dashed line) and unpoisoned (solid line) 0.50 - 0.063 mm size fraction substrate.

limit concentrations was observed for the unpoisoned microcosms during the 66 hours of observation.

Manganese removal in the < 0.063 mm size fraction microcosms was similar to observations of other size fractions with a rapid decrease in both poisoned and unpoisoned microcosms in the first 5 hours followed by a much lower rate of decrease for the unpoisoned microcosms and essentially no decrease occurred after this time in the poisoned microcosms (Figures 4.7 and 4.8). Although concentrations continued to decrease in the unpoisoned microcosms, concentrations did not decrease to the detection limit as observed for larger substrate size fractions. Incomplete removal of manganese in the poisoned microcosms indicates substrate limitation, which may also be limited in the unpoisoned microcosms by "decreased" biological processes (although continued observations may have demonstrated complete removal after an extended period of time). Removal rates in the poisoned microcosms were not significantly different ($p > 0.05$, Table 4.1) from 0, indicating no removal, and regression analysis explained only 9 to 19 % of the variability indicating that another model may be more appropriate. Although removal rates were relatively low (-0.34 to -0.31 day^{-1}) for the unpoisoned microcosms, they were significantly different from 0 ($p < 0.05$, Table 4.1), indicating removal but at a lower rate than the rate observed for cobble, yet comparable to the 2.00 to 0.50 mm size fraction (Table 4.8).

In the microcosms with the amended water (representing release water), decreases in dissolved manganese concentrations occurred primarily in the first five hours and then remained relatively constant until 40 hours when concentrations in the unpoisoned

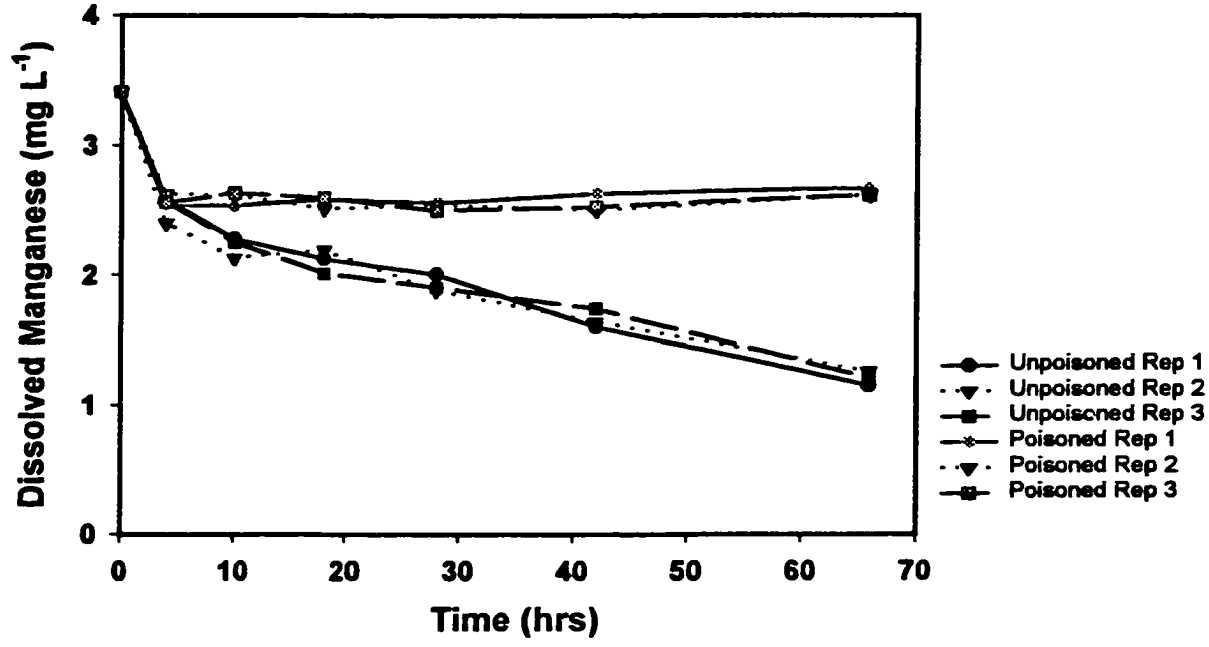


Figure 4.7. Manganese concentrations in microcosms with the < 0.063 mm size fraction substrate.

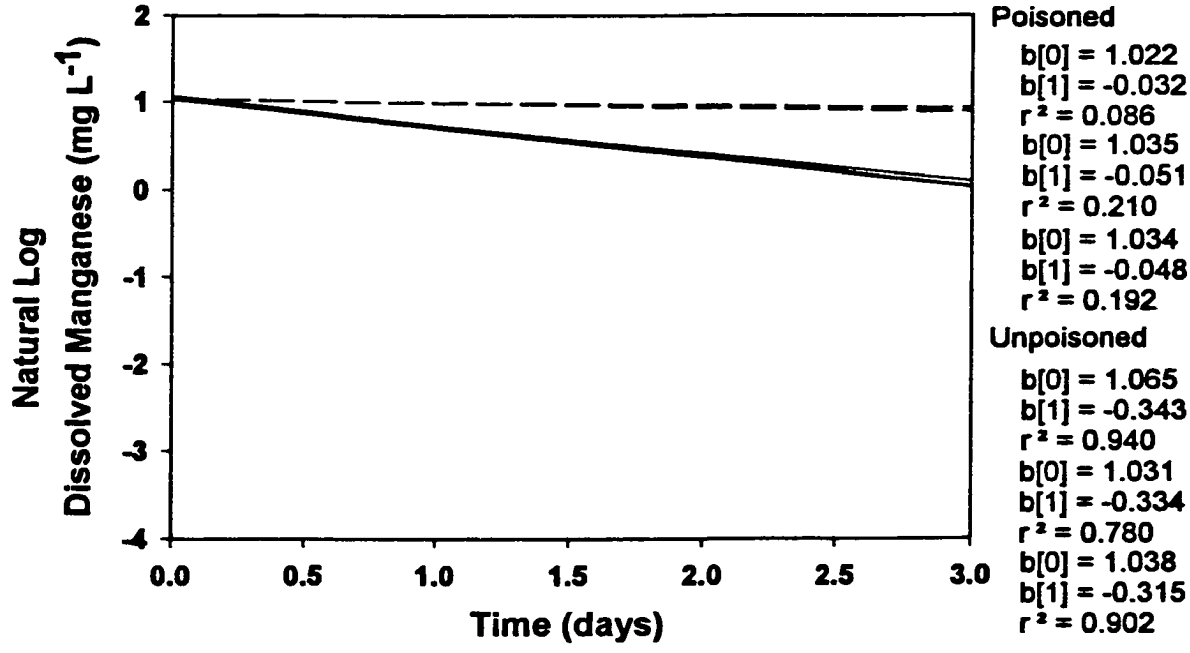


Figure 4.8. Linear regressions (no intercept) for the poisoned (dashed line) and unpoisoned (solid line) < 0.063 mm size fraction substrate.

microcosms decreased by about 30% over the duration of the study (Figures 4.9 and 4.10). After an initial decrease during the first five hours, dissolved manganese concentrations in the poisoned microcosms remained relatively constant and the regressions for both treatments were not significantly different from 0 ($p > 0.05$, Table 4.1). As observed for smaller size fractions, regression analysis explained only 4 to 35 % of the variability for the unpoisoned microcosms indicating a different model may be more appropriate. However regression analysis for the unpoisoned microcosms for the larger size fractions explained 49 to 95 % of the variability.

Daily removal rates were calculated with an exponential decay function of the regression slope for day 1 and day 2 using the maximum and minimum regression for each size fraction and treatment class that were significantly different from 0 ($p < 0.05$, Table 4.1) and results are presented in Table 4.9. For the larger size fractions (cobble, 2.00 to 0.50 mm, and 0.50 to 0.063 mm), removal on the first day was between 53 and 85 % with mostly greater that 95 % removal occurring by the second day. These removal rates are comparable to observations of others (Gordon 1989; Hem 1981; Hess et al. 1989; and Dortch and Hamlin-Tillman 1995) and half-lifes of 1-7 days (Lewis 1976) and quarter-life values near 0.2 and 0.25 days (Gordon et al. 1989). A higher rate of manganese removal (93 % in the initial 10 minutes) has been observed by Kepkay (1985). Manganese removal in the < 0.063 mm size fraction (fines) was at a rate about half of that observed for larger size fractions (near 27 % for day 1 and 48 % for day 2, Table 4.9). Removal rates in the poisoned microcosms with the cobble and the 2.00 to 0.50 mm substrates ranged from 22 - 52 % and 8 - 18 %, respectively, for the first day and from 39 - 77 % and 16 to 34 %,

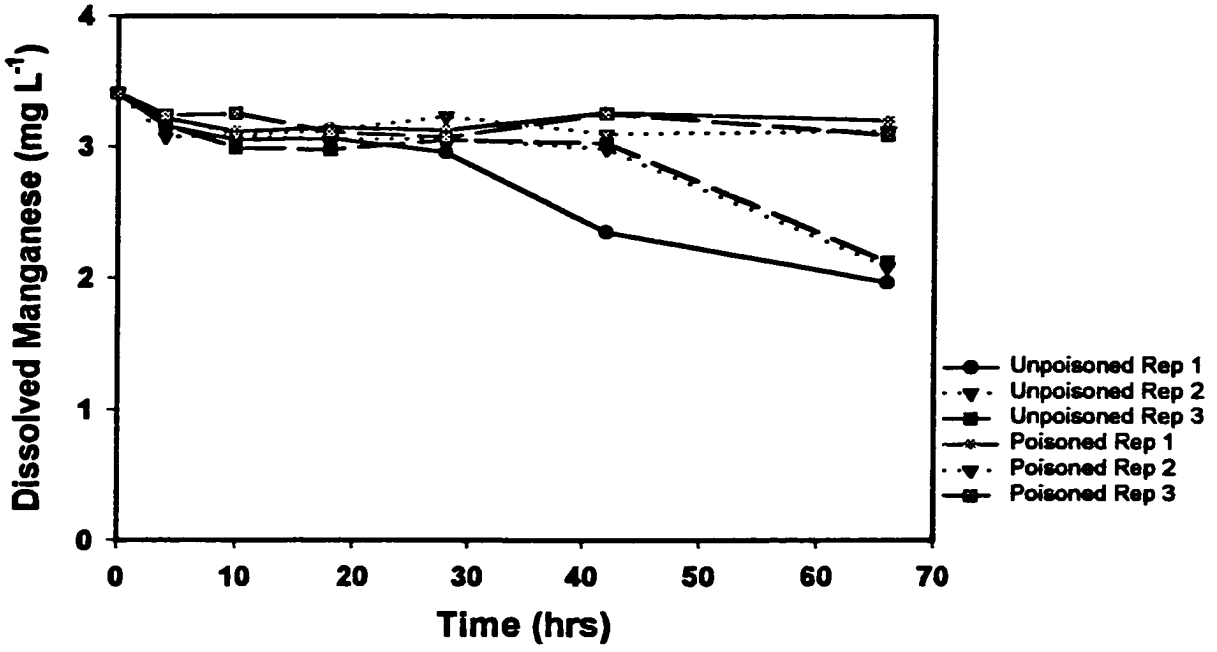


Figure 4.9. Manganese concentrations in microcosms with only amended water from the Nimrod Lake tailwater.

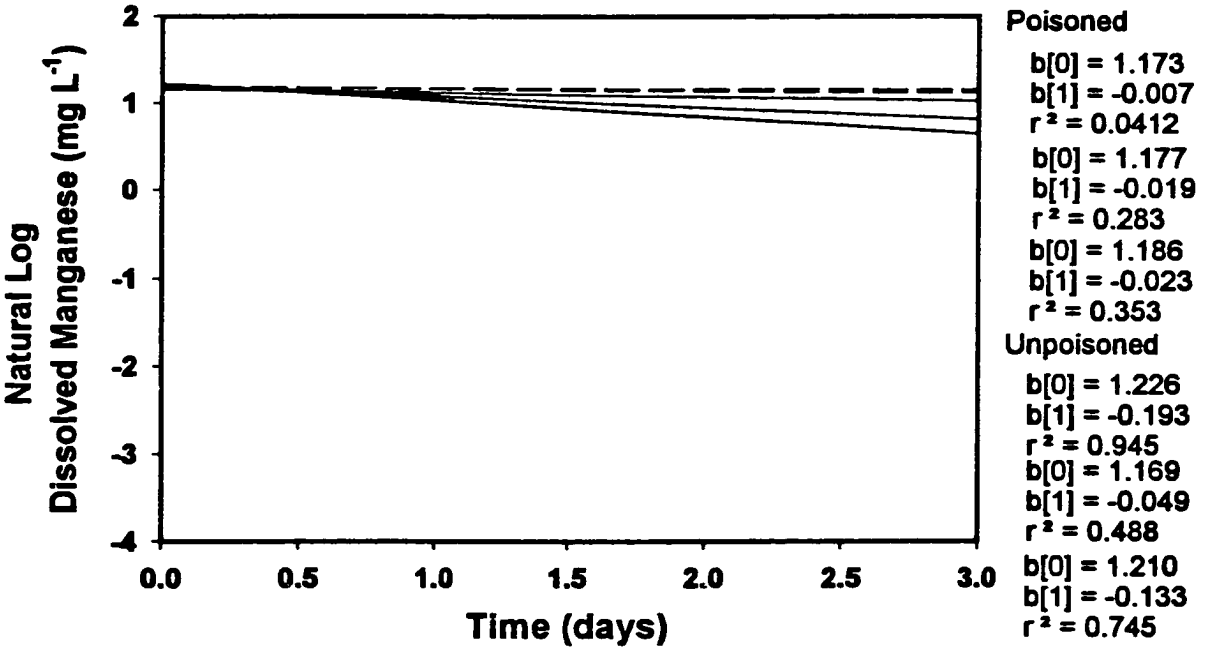


Figure 4.10. Linear regressions (no intercept) for the poisoned (dashed line) and unpoisoned (solid line) amended water from the Nimrod Lake tailwater.

Table 4.9. Maximum and minimum removal rates of manganese for microcosms.

Microcosm	Rate	% Removed Day 1	% Removed Day 2
Unpoisoned Cobble	-1.396	75.24	93.87
	-1.605	79.91	95.96
Unpoisoned 2.00 - 0.50 mm	-0.76	53.23	78.13
	-1.91	85.19	97.81
Unpoisoned 0.50 - 0.063 mm	-1.584	79.48	95.79
	-1.83	83.96	97.43
Unpoisoned < 0.063 mm	-0.31	26.66	46.21
	-0.34	28.82	49.34
Unpoisoned Amended Water	-0.049	4.78	9.34
	-0.193	17.55	32.02
Poisoned Cobble	-0.247	21.89	38.98
	-0.732	51.91	76.87
Poisoned 2.00 - 0.05 mm	-0.087	8.33	15.97
	-0.203	18.37	3.37

respectively, for the second day, indicating substantial removal in the absence of biological processes if sufficient substrate exists.

Nonlinear regression analyses indicated that a hyperbolic decay function with 3 coefficients would provide a better fit for both the unpoisoned and poisoned data. The following equation was used to describe each regression for the microcosms:

(4.1) $f = y0 + ((a*b)/(b+x))$

where: f = concentration, x = time, and y0, a, and b are coefficients calculated with input of concentration and time. This equation, with the coefficients calculated for each microcosm, improved the fit of the model to the data for nearly all of the microcosms (Table 4.10). This model did not provide an improved fit for 2 of the regressions in the poisoned microcosms with the < 0.063 mm size substrate and 1 regression in the poisoned microcosm with amended water only. Examples of the curves fit are depicted in Figure 4.11.

4.3.2 Microbial Community Analysis

Differences in community structure were observed between the four size fractions of cobble used in the microcosm uptake studies (Figure 4.12). The three size fractions representing substrate of <0.063 mm diameter to 2 mm in diameter contained unique and distinct microbial communities from those observed in the tailwater (referred to as a control in Figure 4.12) and cobble samples. This differentiation likely represents a difference between adhered and bulk phase populations since the aqueous portion of the cobble was analyzed and not the rock. PLFA profiles for the other three size fractions are illustrated in Figure 4.13. The upper two size fractions, 2.0 - 0.5mm and 0.5 - 0.063mm, showed showed a greater abundance of the 18 carbon PLFA monounsaturate 18:1w7c, compared to the < 0.063 fraction which showed a greater relative percentage of the 16 carbon moiety, 16:1w7c. This is a clear taxonomic difference, but unfortunately one difficult to describe due to the ubiquitous distribution of both of these acids among Gram-negative bacteria. The presence of either PLFA is conducive to the presence of

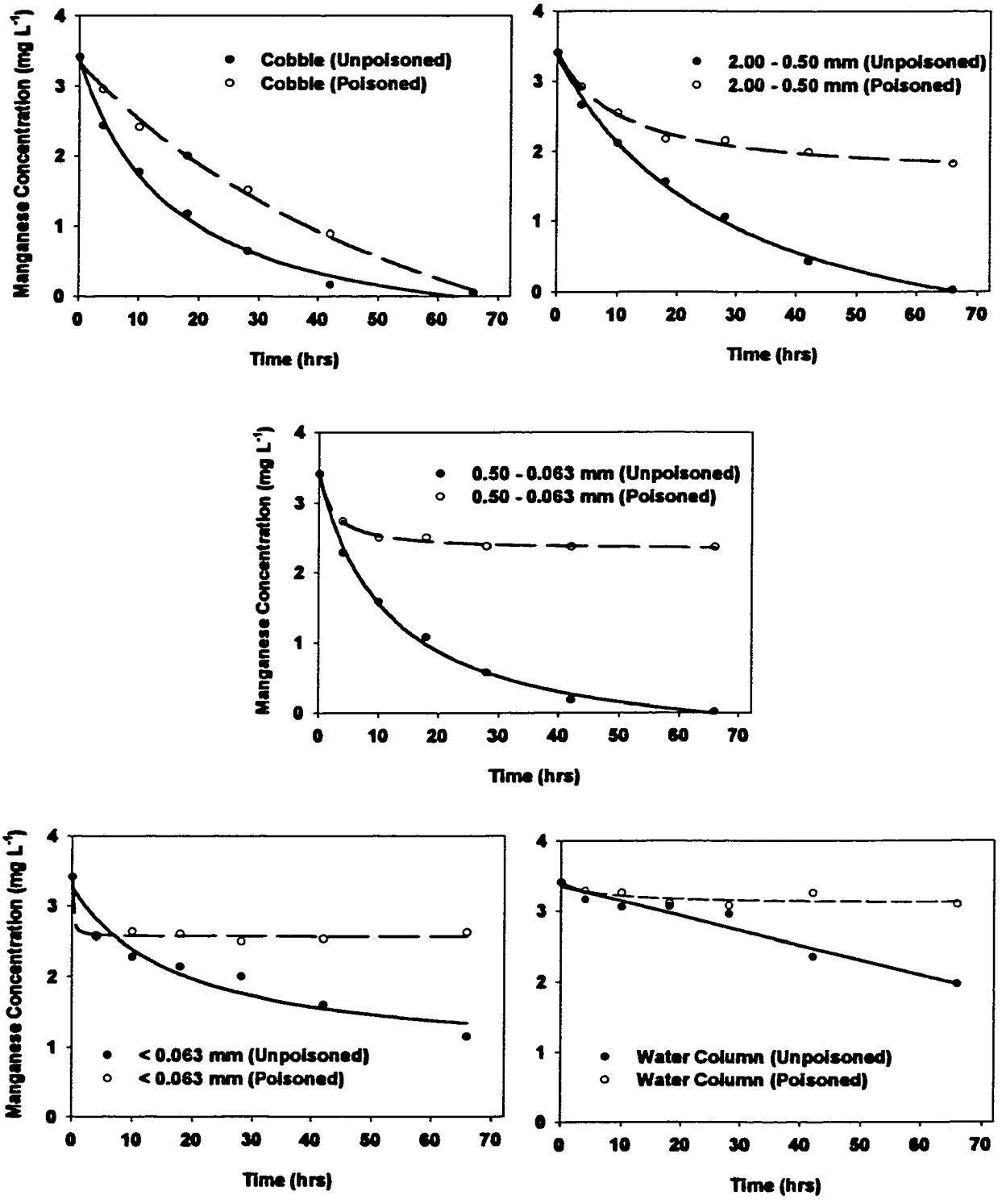


Figure 4.11. Examples of hyperbolic decay model (Equation 4.1) fit to data from unpoisoned and poisoned micrcosms.

Table 4.10. Comparison of exponential decay model and hyperbolic decay model for each microcosm.

Microcosm	r^2 for Exponential Decay	r^2 for Hyperbolic Decay
Amended Water (Unpoisoned)	0.945	0.9729
Amended Water (Unpoisoned)	0.488	0.8843
Amended Water (Unpoisoned)	0.745	0.8682
Amended Water (Poisoned)	0.041	0.2095
Amended Water (Poisoned)	0.283	0.7938
Amended Water (Poisoned)	0.353	0.6866
< 0.063 mm (Unpoisoned)	0.940	0.9380
< 0.063 mm (Unpoisoned)	0.780	0.9160
< 0.063 mm (Unpoisoned)	0.902	0.9506
< 0.063 mm (Poisoned)	0.086	0.0972
< 0.063 mm (Poisoned)	0.211	0.1459
< 0.063 mm (Poisoned)	0.192	0.9761
0.50 - 0.063 mm (Unpoisoned)	0.950	0.9917
0.50 - 0.063 mm (Unpoisoned)	0.992	0.9967
0.50 - 0.063 mm (Unpoisoned)	0.889	0.9815
0.50 - 0.063 mm (Poisoned)	0.532	0.9898
0.50 - 0.063 mm (Poisoned)	0.474	0.9943
0.50 - 0.063 mm (Poisoned)	0.390	0.9825
0.50 - 2.00 mm (Unpoisoned)	0.883	0.9859
0.50 - 2.00 mm (Unpoisoned)	0.980	0.9951
0.50 - 2.00 mm (Unpoisoned)	0.913	0.9968
0.50 - 2.00 mm (Poisoned)	0.799	0.9929
0.50 - 2.00 mm (Poisoned)	0.876	0.8619
0.50 - 2.00 mm (Poisoned)	0.954	0.9434
Cobble (Unpoisoned)	0.994	0.9961
Cobble (Unpoisoned)	0.983	0.9962
Cobble (Unpoisoned)	0.882	0.9969
Cobble (Poisoned)	0.954	0.9994
Cobble (Poisoned)	0.760	0.9980
Cobble (Poisoned)	0.766	0.9967

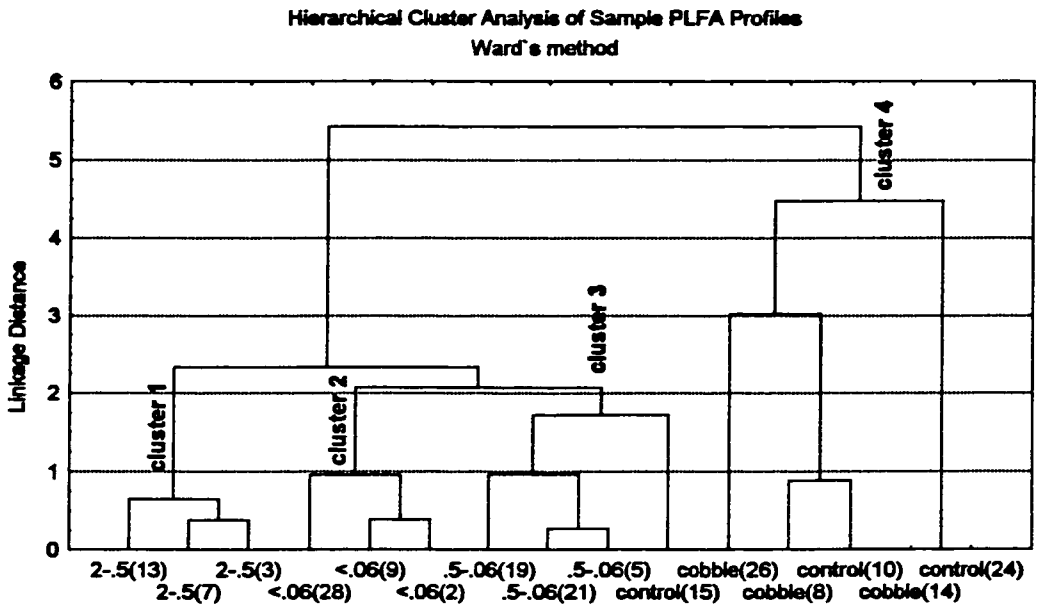


Figure 4.12. Hierarchical cluster analyses for the different size fractions used in the microcosm study.

manganese oxidation, in that both are predominant in the cell membranes of bacteria such as *Pseudomonas fluorescense* and *Leptothrix discophora* (manganese oxidizing bacteria).

The 2.0 - 0.5 mm size fraction showed additional unique characteristics. The relative percentages of 16:0 and 18:0, palmitic and stearic acids, were depleted whereas cyclopropyl PLFA (cy17:0 and cy19:0) and methyl branched PLFA (10me16:0) were enhanced. This taxonomic difference is interesting since the conversion of 16:0 to 10me16:0, 16:1w7c to cy17:0 and 18:1w7c to cy19:0 all involve the presence of a transmethylation enzyme suggesting a predominance of a single genera of bacteria. The taxonomic differences described above indicate that the different size fractions support different microbial communities.

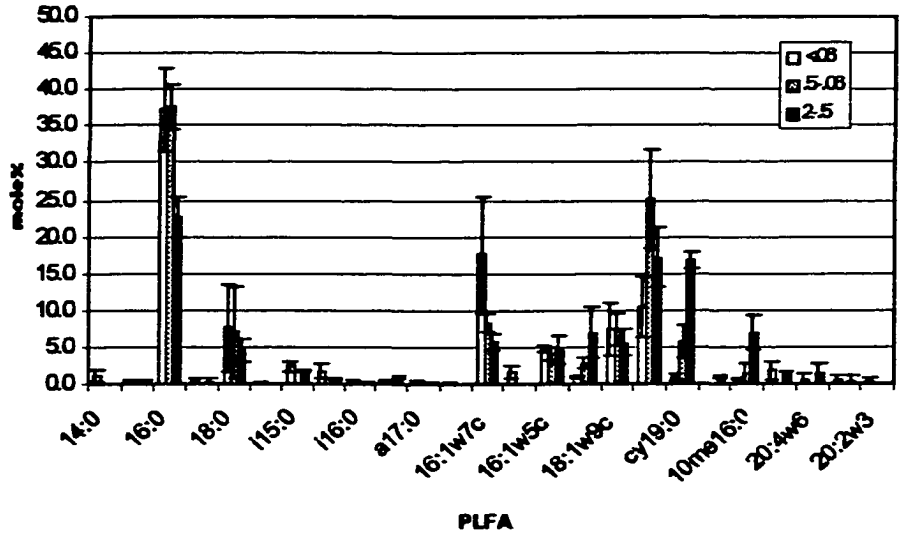


Figure 4.13. Microbial community composition associated with selected size fractions in the microcosm study.

The presence of sodium azide had no effect on total PLFA concentration (Figure 4.14). Microbial biomass in the poisoned samples equaled and often exceeded that observed in the corresponding un-poisoned samples. Consequently, it was not possible to normalize manganese removal rates with biomass. Sodium azide inhibits the transfer of e^-'s to oxygen during cellular respiration and, as a result, should inhibit cell growth as well. However, sodium azide may also interfere with enzyme activity (it is often used as a medicine preservative) and it may not result in cell lysis. If phospholipase activity is hindered and/or cell membranes retain their integrity, then membrane phospholipids will remain intact and the assay will record a biomass for a viable but non-metabolically active population. This may well be the situation encountered in this study. If so, then the

recorded biomass at the end of the study (66 hours) indicates that there was little to no growth in the extant (un-poisoned microbial populations). Although the use of sodium azide appears to have hindered the use of the PLFA assay in relating microbial biomass to manganese removal, it does not interfere with taxonomic descriptions.

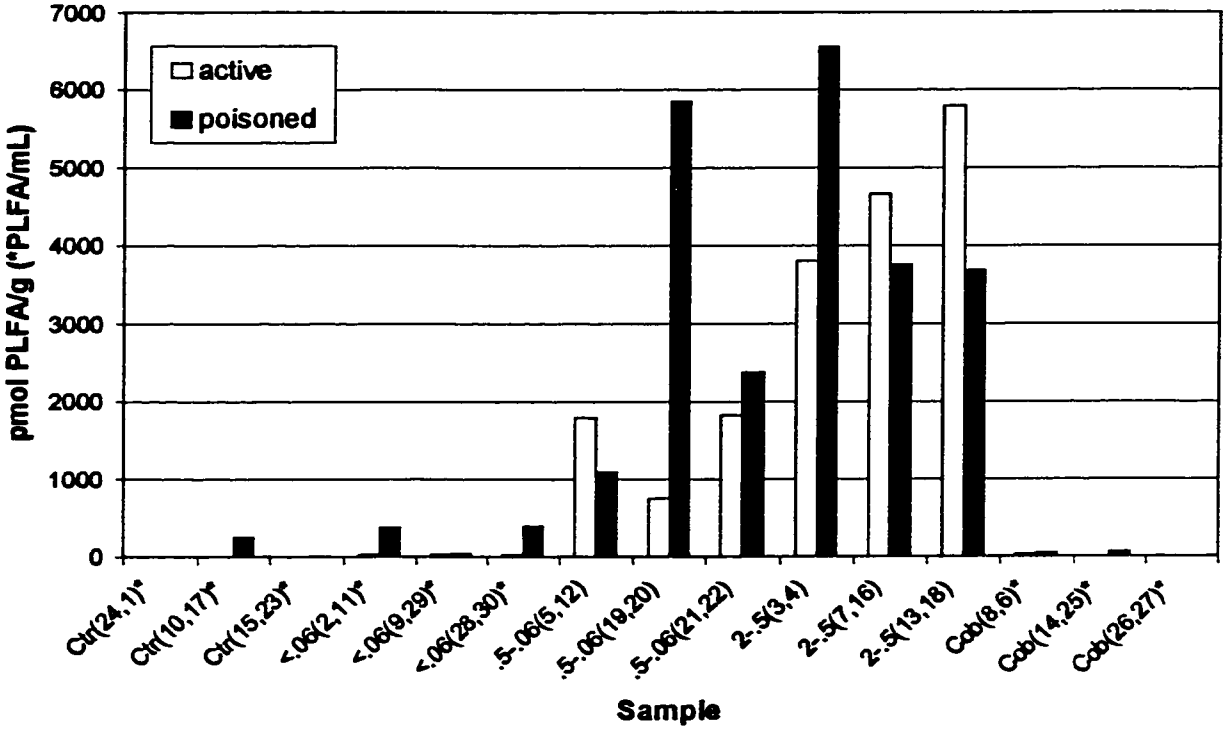


Figure 4.14. Viable biomass estimates after completion of the microcosm study.
* Denotes concentration per ml for volumetric extractions.

4.4 Summary and Conclusions

Separation of substrate by size fractions and measuring manganese removal in poisoned and unpoisoned microcosms allowed assessment of the contribution of different size fractions and biological processes. Results indicated that if there is sufficient substrate and an active biological community, near complete removal of manganese (at an intial concentration representative of reservoir tailwaters) can occur in about 2 days. The removal can be modeled with an exponential decay equation which accounts for greater than 90% of the variability. Although the removal is described with a first-order reaction, inhibition of biological removal processes demonstrated a two-step process was occurring. Initial removal (within approximately 10 hours) was comparable in both poisoned and unpoisoned microcosms (step one) and then only maintained at the same exponential rate with biological processes (step two). A two-step removal process has been described with adsorption and subsequent oxidation reactions by several investigators (e.g., Morgan 1964; Crerar and Barnes 1974, Hess et al.1989) and Hem (1981) who includes a second-order term that accounts for substrate precipitation. The two-step model has also been described with biological processes of "binding" and development of exterior coatings on bacteria followed by oxidation of the reduced manganese for cell metabolism (e.g., Ehrlich 1977; Emerson et al. 1982; Tebo and Emerson 1985; Hastings and Emerson 1986; Mandernack et al. 1995a).

Greater removal rates observed for the cobble and larger substrate size fractions and much higher biomass associated with the 2.00 to 0.50 mm size fraction and the 0.50 to 0.063 mm size fraction than with the < 0.063 mm size fraction supports the conceptual

model of removal dependency on substrate size and the microbial community. Although it was not possible to measure or estimate the surface area of all size fractions, Ledger and Hildrew (1998) demonstrated that biomass distribution was a function of substrate size with greater populations occurring on larger substrate sizes and this may account for similar observations in this study. PLFA extraction methods used on the cobble and small size fractions (< 0.063 mm and amended tailwater) precluded assessment of biomass contributions at higher and smaller size fractions.

Removal rates, generally observed for substrates greater than 0.063 to 0.50 mm, were comparable to those of field studies (e.g., Dortch and Hamlin-Tillman 1995). There were observations of lower removal rates (near -0.7 day^{-1}) in these size classes for both the poisoned cobble size fraction and the unpoisoned 2.00 to 0.50 mm size fraction that still accounted for greater than 75% of the manganese removal within 2 days. These rates were comparable to those observed in field studies at Nimrod Lake conducted in 1995 and described in Chapter 3.

The use of a hyperbolic decay equation to describe manganese removal provided an even better fit for most of the data, especially for the poisoned microcosms. This type of equation resembles the Michaelis-Menton equation and uses coefficients that could be used to represent microbial activity or uptake, or, increasing substrate area, which are likely mechanisms that account for the observed patterns in manganese removal. Conditions with very limited substrate availability and/or microbial mediation, (e.g., small size fractions (< 0.063 mm and the amended water) and microbial inhibition (poisoned))

were not described very well with the hyperbolic decay equation unless there was an initial removal (most likely attributed to some substrate availability).

Microbial community analysis and biomass estimation with the PLFA extractions indicated a high potential for the presence of Gram-negative bacteria with characteristics of species capable of oxidizing reduced manganese. There was evidence that a single genera was dominant on the 2.00 to 0.50 mm size fraction. Biomass estimates were variable between size fractions, yet similar removal rates observed for the larger size fractions suggests that sufficient biomass was present and removal was not limited under conditions of the study. In a different approach using addition of substrate to established larger microbial populations, LaRock (1969) demonstrated that manganese uptake increases linearly as the inoculum increased. Similar uptake rates observed for the larger size fractions with greatly different microbial biomass suggests that there is a point in which some factor other than microbial biomass limits the rate of manganese removal. In other words, as long as there is a sufficient microbial population, manganese removal will occur at the rates observed in the microcosm study. Additional studies are required to determine the maximum rate of manganese removal and what factors (e.g., environmental conditions, microbial community structure and size) contribute to maximum removal rates.

Results indicated that manganese removal is comparable for size fractions greater than 0.063 mm that support an active microbial community. Chapnick et al. (1982) reported limited manganese uptake on substrates less than 0.45 μm so there is likely a relationship between size and microbial preference for size as described by Ledger and Hildrew (1998) that could be used to further differentiate. However, since most reservoir

tailwaters will have substrate of various sizes and will likely contain material greater than 0.063 mm, substrate limitation for abiotic removal will probably not be the limiting factor and biological processes will indeed be important. Additional studies to describe biological rates of manganese removal and the associated increased in manganese oxide coatings should be conducted for incorporation into the hyperbolic decay equation.

CHAPTER 5
SUMMARY AND CONCLUSIONS

The objectives of this study were to describe the fate of manganese in reservoir releases and determine the contribution of abiotic and biological processes to the removal of manganese in the tailwater. Spatial and temporal patterns in the distribution of manganese during selected releases and on different substrate sizes were measured in a reservoir tailwater (Nimrod Lake), and manganese removal rates were assessed in raceway and microcosm studies. Study results were then incorporated into an assessment of existing models used to predict the fate of manganese in tailwaters and compared to predictions from a hyperbolic decay model that functions like a Michaelis-Menton equation and can be used to account for biological processes in manganese removal.

The removal of manganese from reservoir tailwaters is a combination of abiotic adsorption and biological processes that are related to substrate availability and environmental conditions that affect the biological processes. Biological mediation accounts for kinetic removal rates in excess of predictions based on thermodynamics and stoichiometric reactions. Removal rates can be described with first-order reactions, but the actual removal process is described by a two-step process that includes an initial binding of manganese to existing substrate that may actually be a chemical (i.e., abiotic) process and continued binding to biologically produced substrates (e.g., ferromanganese coatings on selected bacteria). Several investigations have documented the abiotic removal (step one) as function of environmental conditions such as manganese

concentrations, dissolved oxygen concentrations, oxidation-reduction potentials, and pH (e.g., Morgan 1964; Stumm and Morgan 1981). Early chemical investigations often focused on oxidation states and mineral formation (e.g., Bricker 1965) and often described the actual process as a two-step process (e.g., Morgan 1964). In general, the second step has also been described to occur in two steps, initial binding by bacteria and subsequent oxidation at a later time (e.g., Ehrlich 1975; Tebo and Emerson 1985; and Hastings and Emerson 1986). The distinction is oxidation versus removal where removal does not describe oxidation state, but merely removal or a decrease in concentration from the water column. These processes result in temporal and spatial removal of manganese from reservoir tailwaters.

Efforts to predict manganese removal in rivers and reservoir tailwaters have focused on first order (Gordon 1989; Dortch and Hamlin-Tillman 1995) and pseudo-first order reactions (Hem 1981) that have incorporated various environmental conditions (concentration, pH, dissolved oxygen concentrations) and tailwater conditions such as slope, substrate type, and discharge. While some early models were applied with less success at sites (Hess et al.1989), recent modifications to early first order models have been applied at other sites with more success when site characteristics are incorporated into removal rate coefficients (Dortch and Hamlin-Tillman 1995). However, these modifications did not include removal affected by biological processes which is often the major mechanism of removal if substrate for adsorption is limited. In other words, application of first order removal equations may be appropriate, but the mechanism(s) that result in the first order removal have not been adequately considered or incorporated into

model development. Since conditions in rivers and reservoir tailwaters are conducive to development of microbial populations (i.e., sufficient nutrient supply, appropriate pH, and adequate substrate), observations of manganese removal in the field may be attributed to biologically mediated processes, yet described with simple, first order reactions. Agreement for half-life (1-7 days, Lewis 1976) and quarter-life (4-6 days) removal of manganese compared to years in abiotic settings also supports the concept of biological mediation. Furthermore, comparability between sites also suggests that a range of environmental conditions, common to rivers and tailwaters, results in comparable removal rates and allows simplification of descriptive reactions and models.

Evaluation of manganese concentration distributions on substrate from the tailwater of Nimrod Dam and Lake on the Fourche La Fave River in Arkansas indicated lateral variability in adsorption onto cobble size substrate in the region immediately downstream from the dam, an extended area of accumulation for approximately 3 kilometers, and a general tendency for a linear decrease with distance from the dam. Manganese accumulation was observed on selected substrate size fractions that include cobble, 2.00 to 0.50 mm, 0.50 mm to 0.063 mm, and less than 0.063 mm substrates. Limited sampling of each substrate size for all downstream sites indicated that manganese removal was not limited by substrate size. Studies of removal rates for selected sizes of substrate indicated that removal rates were greater for larger substrate size fractions with minimal removal for substrate sizes between 0.50 and 0.063 mm and less than 0.063 mm. Therefore, the null hypothesis (Ho1: Manganese removal rates do not vary with size) is rejected and substrate size does influence manganese removal. However, given the

comparability of removal rates for cobble and the larger size fractions, implications of substrate influences on manganese removal in reservoir tailwaters may be important for tailwaters that have mixed substrate sizes and less important for tailwaters that are dominated by fine material (e.g., mud or silt). These results are also consistent with Chapnick et al. (1982) in which removal of manganese was minimal at sizes less than 0.45 μm.

Greater removal rates in unpoisoned microcosms than in poisoned microcosms indicated that Ho2 (Microbial populations are not a major mechanism of manganese removal.) is false and, indeed, microbial populations are major contributors to manganese removal. While this conclusion is not surprising, given the body of literature that describes biologically mediated manganese removal, only a limited number of studies have been conducted to differentiate the contribution of microbial processes on manganese removal, and even fewer studies have attempted to incorporate measurements of biological processes into manganese removal process in reservoir tailwaters.

Bacterial removal of manganese is accomplished with binding of the aqueous manganese via proposed mechanisms such as the production of enzymes (Arcuri and Ehrlich 1980) and proteins (Van Veen et al. 1978; Adams and Ghiorse 1988) and alteration of conditions such as Eh and pH in a microhabitat (Marshall 1979). The binding of the manganese (and iron and other heavy metals sequestered in the process) results in extracellular formation of ferromanganese oxides on sheaths and other structures which are amorphous and have a large surface area that provides additional substrate for continued binding in an autocatalytic fashion.

Detailed studies in the microcosms provided evidence that manganese oxidizing bacteria were present on the tailwater substrate, particularly for the intermediate sized substrates and that biomass was not critical for efficient manganese removal as long as sufficient biomass was present. Attempts to culture manganese oxidizing bacteria were conducted with samples collected from the field an a first generation of bacteria were produced, confirming the presence of manganese oxidizing bacteria associated with the substrate. The bacteria are mostly all attached to substrate in highly structured assemblages that often consist of microcolonies of single bacterial taxa (Costerton and Lappin-Scott 1995). Ledger and Hildrew (1998) have observed an increase in biofilm quantities as substrate size increases, suggesting habitat preferences exist. As suggested by Rosson et al. (1984) and Emerson et al. (1982), large populations or a high percentage of active biota are not necessarily indicative of a high potential for removal. LaRock (1969) however demonstrated that removal increased linearly with an increase in biota. These concepts support the use of a Michaelis-Menton equation in the description of manganese removal which could account for biological production of substrate to continue the removal.

A conceptual model for manganese removal can be developed from the literature and results of the studies described in previous chapters. First, a two-step removal process is clear both from the literature and assessment of regressions of manganese removal in poisoned microcosms that indicated a definite divergence from the exponential removal was observed for unpoisoned microcosms. The removal process can be expressed as a first-order reaction since the deposition of manganese oxide coatings on the

exterior of the bacteria provides a source of substrate for continued adsorption (step two). The presence of manganese oxidizing bacteria on cobble and substrates > 0.063 mm combined with sufficient exposure time (varied flow) provides sufficient contact time (as hypothesized by Goldberg (1954)) for efficient removal. Expanding the pseudo-first order reaction by using a Michaelis-Menton equation to describe the production of additional manganese oxide for continued manganese removal would allow a more accurate method for describing the removal process in tailwaters.

As recipients of usually oxygenated inflows that can have elevated concentrations of reduced manganese (from the upstream impoundment), reservoir tailwaters would be ideally suited for manganese removal since water quality conditions typically meet conditions conducive to biological mediation of the removal. For example, dissolved oxygen is not usually limiting, pH, while too low for autooxidation, is appropriate for microbial habitat, and contact time with the biological community is often sufficient in enough areas of the tailwater at various discharge rates to accomplish efficient removal.

The hyperbolic decay equation that fit the microcosm data was used with manganese concentrations observed in the Nimrod Lake tailwater region in 1995 (Figure 5.1). Two different removal rates were observed with the greater removal rate (1.054 day^{-1}) observed for the lower discharge. While the equation fit for manganese removal at the lower discharge was quite good ($r^2 = 0.953$), the fit for the higher discharge accounted for only 57% of the variability. The data for these studies were limited and additional studies with more robust sample collection should be conducted to evaluate application of the model. Data from the raceway studies were also used to evaluate the

hyperbolic decay equation and r^2 values of 0.998 and 0.995 indicated a very good fit. Initial concentrations, removal rates, and half-lifes were substituted into the hyperbolic model for the coefficients (a, y0, and b, respectively) and were able to described the observed data for the raceway studies (Figure 5.2) reasonably well but failed to predict complete removal.

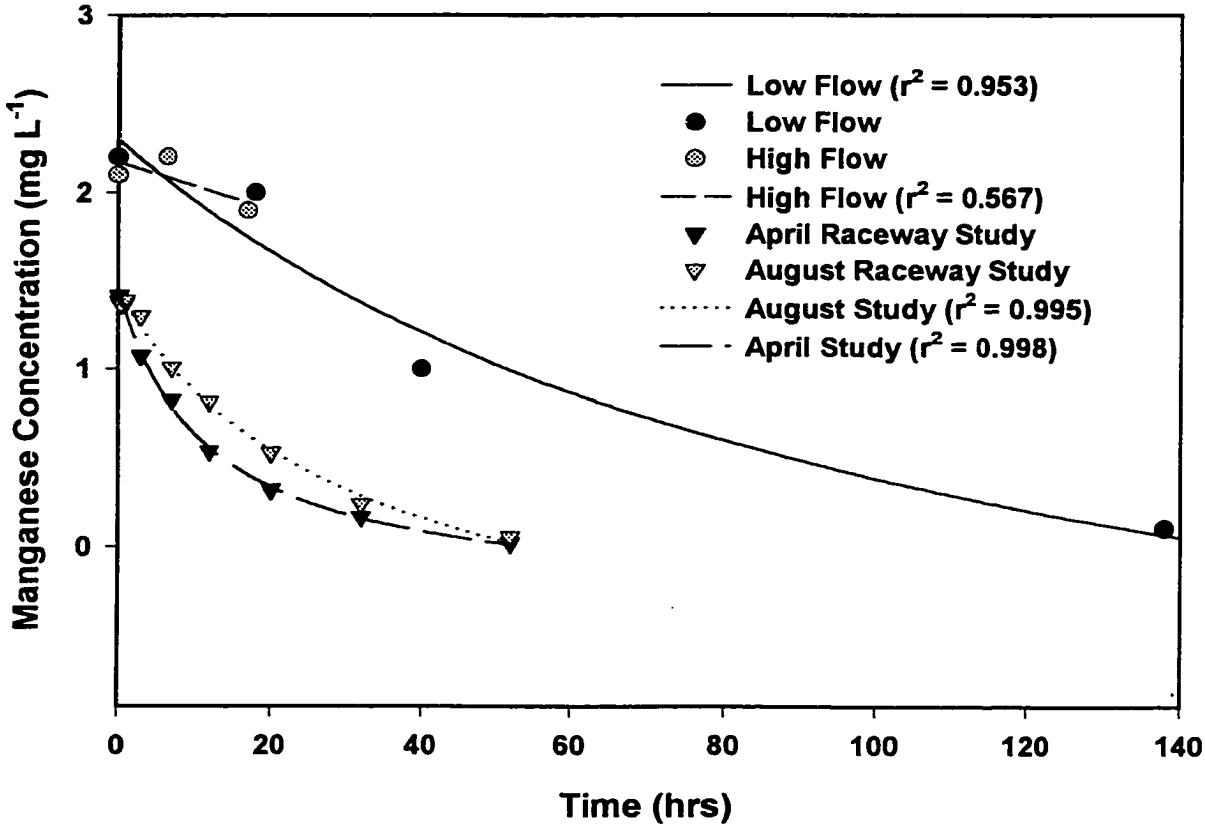


Figure 5.1. Hyperbolic decay model fit of manganese removal to the 1995 tailwater study at Nimrod Lake and the raceway studies using substrate from the tailwater of Nimrod Lake.

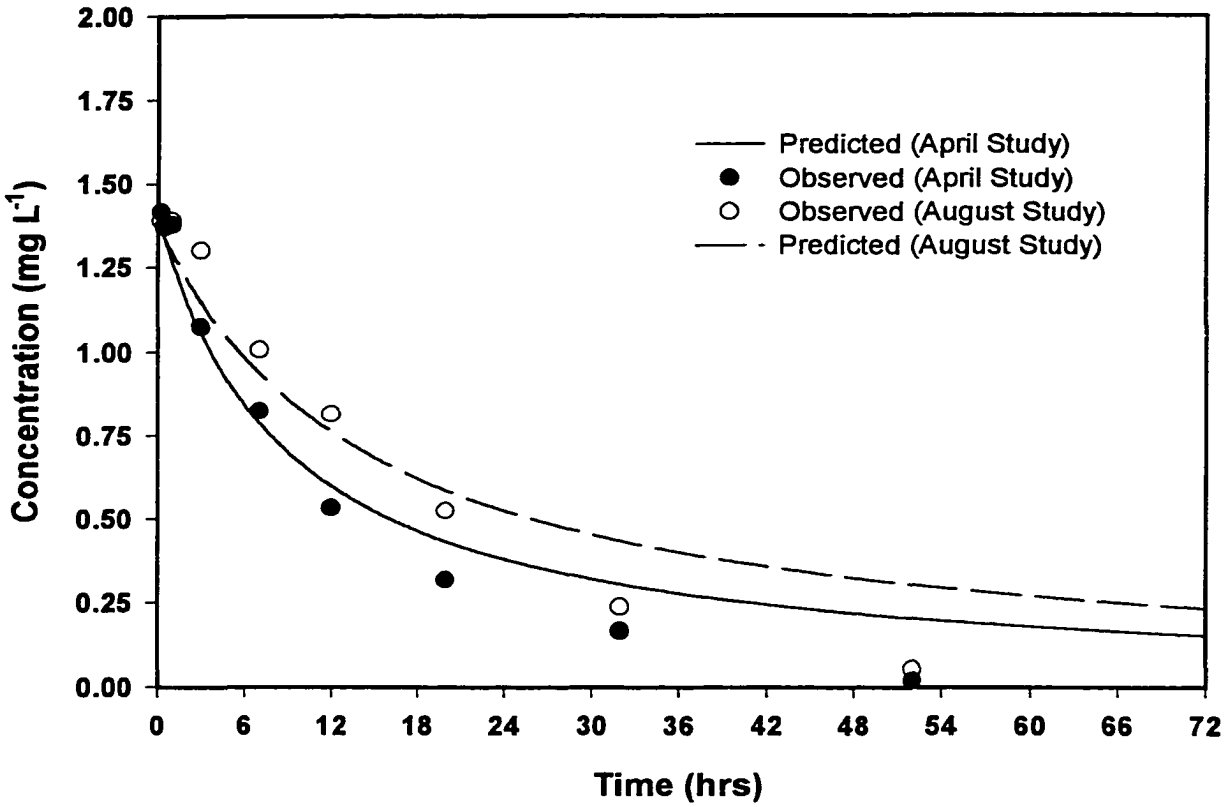


Figure 5.2. Observed manganese concentrations and predicted values using the hyperbolic decay model and coefficients derived from the raceway studies.

Additional research should be conducted to develop methods that would allow determination of microbial activity and increased production of oxide coatings associated with manganese removal. Recent work by Dong et al. (2000), has indicated that measurements of increased substrate availability can be accomplished and a Langmuir adsorption isotherm, which is very much like a Michaelis-Menton equation since it uses terms for adsorption on a per unit quantity of material basis, can be used to describe the sequestering of heavy metals associated with manganese removal if the surface area of the

substrate is measured. This approach could be used to calculate or estimate coefficients in the hyperbolic decay equation, particularly those estimates of "binding" or removal rates and half-lifes, thus improving applications of the model.

Variability at a site or between sites has not been completely evaluated but study results indicate that site characteristics such as exposure to biota and substrate (affected by discharge features such as velocity) are likely contributors as uncertainties about how and why bacteria adsorb the manganese from the water column. It seems likely that utilization of enzymes and proteins and/or the manipulation of microhabitats to facilitate the adsorption and subsequent oxidation of Mn^{2+} as an alternative energy source is a reasonable explanation that is also worthy of further investigation since manganese removal is widespread in nature, used in the water treatment industry, and is applicable for heavy metal sequestering.

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APPENDIX A: CONCENTRATIONS OF MANGANESE ON SUBSTRATES IN THE TAILWATER OF NIMROD LAKE

Substrate Type	Station	Lateral Site	Conc (mg/kg)	Mean (Lateral Site)	Mean (Station)
Cobble	A	lbe	74	101.7	202.6777778
		lbe	44.2		
		lbe	87.9		
		lbe	94.4		
		lbe	208		
		lbm	95.8	101.175	
		lbm	111		
		lbm	58.9		
		lbm	139		
		m	249	293.3333333	
		m	352		
		m	279		
		rbm	248	297.3333333	
		rbm	288		
		rbm	356		
		rbe	343	321	
		rbe	368		
		rbe	252		
	B1	lbe	106	44.33333333	166.175
		lbe	12		
		lbe	15		
		lbm	212	239.28	
		lbm	96.2		
		lbm	69.2		
		lbm	417		
		lbm	402		
	B3	lbe	330		297.5
		lbm	183	287.5	
		lbm	392		
		rbm	285		
2.00 - 0.50 mm	B1	lbe	6.08	5.18	6.87
		lbe	4.69		
		lbe	4.76		
		lbm	7.4	8.56	
		lbm	8.3		
		lbm	9.99		
	B3	lbm	7.89		8.265
		rbm	8.64		
	C	lbe	2.35		2.08
		lbe	1.54		
		lbe	2.35		

2.00 - 0.50 mm	E	lbm	3.04		2.896666667
		m	3.49		
		rbm	2.16		
		rbm			
0.50-0.063 mm	B1	lbe	1.44		2.596666667
		lbe	1.31		
		lbe	1.11		
		lbm	2.2		
		lbm	4.84		
		lbm	4.68		
	B3	lbm	4.18		3.69
		rbm	3.2		
	C	lbe	0.27		0.253333333
		lbe	0.19		
		lbe	0.3		
	E	lbm	0.91		0.583333333
		m	0.51		
		rbm	0.33		
< 0.063 mm	B1	lbe	26.7		30.075
		lbe	27		
		lbm	33.5		
		lbm	33.1		
	B3	lbm	52.8		59.96666667
		rbm	64.1		
		rbm	63		

APPENDIX B: TOTAL AND DISSOLVED MANGANESE CONCENTRATIONS FROM THE RACEWAY STUDIES

Date	Description	Total Mn (mg/l)	Dissolved Mn (mg/l)
11/18/1997	Initial Raceway Quality Before Substrate Added	0.003	bdl *
12/19/1997	After Substrate Was Added	0.019	bdl
01/07/1998	After Leaves Were Added	0.004	bdl
01/25/1998	During Acclimation	bdl	bdl
01/31/1998	After Initial Addition of Fe and Mn	1.035	0.4326
02/06/1998	During Acclimation	bdl	bdl
03/01/1998	Before Addition of Fe and Mn on 3/1/98	bdl	bdl
03/01/1998	2 Hours After Addition of Fe and Mn on 3/1/98	0.9969	0.7544
03/03/1998	Followup to 3/1/98 Addition of Fe and Mn	0.1378	0.1002
03/04/1998	Followup to 3/1/98 Addition of Fe and Mn	bdl	0.0188
03/05/1998	Followup to 3/1/98 Addition of Fe and Mn	bdl	0.0125
04/08/1998	Followup to 3/1/98 Addition of Fe and Mn	0.0484	0.009
04/22/1998	First Removal Study		
	Before Addition of Mn Rep 1	0.006	bdl
	Before Addition of Mn Rep 2	0.005	bdl
	15 Minutes Rep 1	1.517	1.449
	15 Minutes Rep 2	1.515	1.384
	30 Minutes Rep 1	1.517	1.432
	30 Minutes Rep 2	1.519	1.306
	1 Hour Rep 1	1.423	1.385
	1 Hour Rep 2	1.429	1.369
	3 Hours Rep 1	1.18	1.127
	3 Hours Rep 2	1.19	1.022
	7 Hours Rep 1	0.904	0.828
	7 Hours Rep 2	0.905	0.819
	12 Hours Rep 1	0.653	0.525
	12 Hours Rep 2	0.651	0.541
	20 Hours Rep 1	0.393	0.323
	20 Hours Rep 2	0.391	0.311
	32 Hours Rep 1	0.196	0.174
	32 Hours Rep 2	0.197	0.155
	52 Hours Rep 1	0.033	0.02
	52 Hours Rep 2	0.035	0.019
Early July	Before Addition of Mn Rep 1	0.02	bdl
	Before Addition of Mn Rep 2	0.02	bdl
	15 Minutes Rep 1	1.58	1.59
	15 Minutes Rep 2	1.58	1.59
	30 Minutes Rep 1	1.6	1.61
	30 Minutes Rep 2	1.59	1.6
	1 Hour Rep 1	1.62	1.62
	1 Hour Rep 2	1.65	1.68
	3 Hours Rep 1	1.74	1.76
	3 Hours Rep 2	1.72	1.74
	7 Hours Rep 1	1.81	1.85
	7 Hours Rep 2	1.82	1.82

	12 Hours Rep 1	1.82	1.89
	12 Hours Rep 2	1.82	1.85
	20 Hours Rep 1	1.82	1.84
	20 Hours Rep 2	1.84	1.86
	32 Hours Rep 1	1.76	1.75
	32 Hours Rep 2	1.76	1.75
	52 Hours Rep 1	1.61	1.59
	52 Hours Rep 2	1.75	1.61
Late July	Before Addition of Mn Rep 1	0.0809	bdl
	Before Addition of Mn Rep 2	0.0344	bdl
	15 Minutes Rep 1	1.518	1.462
	15 Minutes Rep 2	1.525	1.502
	30 Minutes Rep 1	1.509	1.494
	30 Minutes Rep 2	1.536	1.541
	1 Hour Rep 1	1.545	1.539
	1 Hour Rep 2	1.506	1.51
	3 Hours Rep 1	1.497	1.497
	3 Hours Rep 2	1.474	1.469
	7 Hours Rep 1	1.477	1.484
	7 Hours Rep 2	1.491	1.568
	12 Hours Rep 1	1.464	1.464
	12 Hours Rep 2	1.453	1.457
	20 Hours Rep 1	1.37	1.425
	20 Hours Rep 2	1.395	1.469
	22.5 Hours (after addition of NaOH) Rep 1	1.473	1.49
	22.5 Hours (after addition of NaOH) Rep 2	1.483	1.459
	22.75 Hours Rep 1	1.42	1.397
	22.75 Hours Rep 2	1.398	1.423
	23 Hours Rep 1	1.392	1.395
	23 Hours Rep 2	1.402	1.413
	23.5 Hours Rep 1	1.313	1.249
	23.5 Hours Rep 2	1.335	1.29
	29 Hours Rep 1	1.216	1.155
	29 Hours Rep 2	1.258	1.195
	40 Hours Rep 1	1.171	1.122
	40 Hours Rep 2	1.178	1.082
	52 Hours Rep 1	1.097	1.011
	52 Hours Rep 2	1.132	1.082
	60 Hours Rep 1	1.019	0.998
	60 Hours Rep 2	1.04	0.9979
August	Before Addition of Mn Rep 1	0.0021	bdl
	Before Addition of Mn Rep 2	0.0041	bdl
	15 Minutes Rep 1	1.351	1.381
	15 Minutes Rep 2	1.385	1.39
	30 Minutes Rep 1	1.361	1.348
	30 Minutes Rep 2	1.348	1.402
	1 Hour Rep 1	1.428	1.38
	1 Hour Rep 2	1.369	1.399
	3 Hours Rep 1	1.255	1.282

	3 Hours Rep 2	1.272	1.318
	7 Hours Rep 1	1.001	1.008
	7 Hours Rep 2	1.033	1.004
	12 Hours Rep 1	0.8126	0.8184
	12 Hours Rep 2	0.8425	0.8101
	20 Hours Rep 1	0.5486	0.5329
	20 Hours Rep 2	0.5535	0.5172
	32 Hours Rep 1	0.2376	0.2376
	32 Hours Rep 2	0.2385	0.2357
	45 Hours Rep 1	0.0571	0.0514
	45 Hours Rep 2	0.0608	0.0552

APPENDIX C: DATA FOR THE MICROCOSM STUDY

Number	Description	rep #	round #	TFe	TMn	DFe	DMn
10	Amended Water	1	1	3.664	3.384	2.35	3.172
10			1			2.316	3.144
10			2	3.507	3.271	2.278	3.055
10			3	3.483	3.309	2.255	3.061
10			4	3.668	3.302	2.253	2.956
10			5	3.337	2.916	1.852	2.349
10			6	3.147	2.192	2.272	1.965
24	Amended Water	2	1	3.555	3.306	2.254	3.075
24			2	3.403	3.221	2.262	3.084
24			3	3.435	3.27	2.247	3.055
24			4	3.32	3.176	2.275	3.06
24			5	3.402	3.229	2.333	2.984
24	* late analysis for dissolved		6	3.165	2.234	2.51	2.08
15	Amended Water	3	1	3.547	3.276	2.307	3.163
15			2	3.597	3.379	2.336	2.992
15			3	3.55	3.382	2.289	2.976
15			4	3.534	3.34	2.283	3.053
15			5	3.583	3.319	2.399	3.029
15			6	3.147	2.281	2.357	2.121
23	Amen. Water (Poisoned)	1	1	3.575	3.33	2.28	3.217
23			2	3.492	3.319	2.273	3.118
23			3	3.478	3.306	2.294	3.143
23			4	3.394	3.272	2.288	3.126
23			5	3.403	3.336	2.361	3.26
23			6	3.346	3.294	2.296	3.201
17	Amen. Water (Poisoned)	2	1	3.54	3.289	2.34	3.23
17			2	3.528	3.347	2.38	3.09
17			3	3.461	3.328	2.331	3.122
17			4	3.377	3.296	2.378	3.231
17			5	3.316	3.254	2.261	3.097
17			6	3.318	3.287	2.238	3.122
1	Amen. Water (Poisoned)	3	1	3.559	3.252	2.371	3.238
1			2	3.621	3.412	2.44	3.253
1			3	3.446	3.284	2.266	3.112
1			4	4.087	3.363	2.312	3.072
1			5	3.441	3.356	2.342	3.253
1			6	3.251	3.207	2.223	3.093
8	Cobble	1	1	3.551	2.696	2.381	2.44
8			1	3.635	2.748	2.402	2.455
8			2	3.502	2.02	2.351	1.766
8			3	3.349	1.315	2.313	1.173
8			4	3.338	0.7418	2.309	0.6051
8			5	3.109	0.2592	2.285	0.1684
8			6	2.94	0.0439	2.267	0.0031
26	Cobble	2	1	3.538	2.718	2.297	2.491
26			2	3.543	2.187	2.358	2.024
26			3	3.36	1.51	2.315	1.338

26			4	3.283	0.925	2.226	0.7459
26			5	3.172	0.34	2.234	0.2307
26			6	2.954	0.0071	2.235	0
14	Cobble	3	1	3.656	3.097	2.327	2.953
14			2	3.584	2.715	2.36	2.414
14			3	3.416	2.218	2.369	2.003
14			3	3.398	2.181	2.32	1.944
14			4	3.389	1.726	2.248	1.513
14			5	3.207	1.102	2.275	0.8855
14			6	2.982	0.1447	2.201	0.0516
6	Poisoned Cobble	1	1	3.494	2.635	2.375	2.537
6			2	3.391	1.983	2.349	1.839
6			3	3.285	1.46	2.331	1.375
6			4	3.32	1.068	2.288	0.9955
6			5	3.231	0.7517	2.197	0.6675
6			6	3.097	0.4953	2.216	0.4324
25	Poisoned Cobble	2	1	3.423	2.636	2.242	2.496
25			2	3.358	2.206	2.202	2.075
25			3	3.327	1.895	2.241	1.766
25			4	3.352	1.714	2.328	1.621
25			5	3.292	1.542	2.289	1.479
25			6	3.187	1.41	2.264	1.342
27	Poisoned Cobble	3	1	3.618	2.83	2.385	2.779
27			2	3.467	2.43	2.341	2.341
27			3	3.412	2.141	2.332	2.028
27			4	3.35	1.945	2.341	1.877
27			5	3.363	1.852	2.232	1.633
27			6	3.235	1.73	2.247	1.632
3	2-0.5 mm	1	1	3.722	3.211	2.351	2.932
3			2	3.482	2.952	2.346	2.722
3			3	3.448	2.657	2.374	2.426
3			4	3.308	2.275	2.29	2.055
3			5	3.194	1.804	2.235	1.59
3			6	3.037	0.4744	2.177	0.3298
7	2-0.5 mm	2	1	3.582	2.706	2.285	2.607
7			2	3.439	1.972	2.254	1.695
7			3	3.313	1.278	2.27	1.113
7			4	3.242	0.7208	2.261	0.521
7			5	3.137	0.2532	2.184	0.1077
7			6	2.999	0.0698	2.18	0
13	2-0.5 mm	3	1	3.648	2.949	2.266	2.662
13			2	3.708	2.386	2.342	2.122
13			3	3.377	1.77	2.278	1.555
13			4	3.285	1.226	2.221	1.054
13			5	3.14	0.5906	2.206	0.4334
13			6	2.932	0.1057	2.148	0.0143
4	Poisoned 2-0.5 mm	1	1	3.765	3.125	2.41	2.92
4			2	3.803	2.832	2.481	2.546
4			3	3.485	2.391	2.285	2.179

4			4	3.45	2.203	2.346	2.156
4			5	3.332	2.097	2.329	1.988
4			6	3.144	1.872	2.173	1.818
16	Poisoned 2-0.5 mm	2	1	3.626	3.296	2.316	3.137
16			2	3.577	3.343	2.265	3.034
16			3	3.435	3.245	2.298	3.093
16			4	3.379	3.183	2.333	3.095
16			5	3.465	3.031	2.363	2.823
16			6	3.346	2.726	2.248	2.552
18	Poisoned 2-0.5 mm	3	1	3.641	3.311	2.338	3.082
18			2	3.583	3.306	2.322	3.101
18			3	3.611	3.273	2.318	3.093
18			4	3.467	2.98	2.312	2.859
18			5	3.378	2.662	2.286	2.511
18			6	3.163	2.275	2.285	2.242
21	0.5-0.063 mm	1	1	3.781	3.01	2.225	2.647
21			2	3.522	2.567	2.247	2.289
21			3	3.5	1.766	2.272	1.457
21			4	3.351	1.09	2.255	0.8546
21			5	3.242	0.4954	2.205	0.3286
21			6	3.047	0.1046	2.217	0.0174
19	0.5-0.063 mm	2	1	4.045	2.832	2.26	2.286
19			2	3.84	2.052	2.237	1.581
19			3	3.777	1.405	2.287	1.078
19			4	3.448	0.8304	2.27	0.5745
19			5	3.45	0.381	2.268	0.1831
19			6	3.14	0.133	2.217	0.0174
5	0.5-0.063 mm	3	1	3.985	2.68	2.373	2.408
5			2	3.762	2.332	2.409	2.067
5			2	3.48	2.126	2.242	1.919
5			3	3.498	1.807	2.266	1.516
5			4	3.383	1.358	2.236	1.132
5			5	3.307	0.8189	2.312	0.6632
5			6	3.094	0.1126	2.333	0.0265
12	Poisoned 0.5-0.063 mm	1	1	3.939	3.054	2.422	2.693
12			2	3.82	2.753	2.288	2.346
12			3	3.556	2.499	2.3	2.33
12			4	3.608	2.443	2.301	2.258
12			5	3.54	2.457	2.329	2.267
12			6	3.382	2.329	2.227	2.131
22	Poisoned 0.5-0.063 mm	2	1	3.859	3.09	2.325	2.738
22			2	3.643	2.8	2.245	2.503
22			3	3.567	2.68	2.359	2.504
22			4	3.478	2.581	2.283	2.378
22			5	3.451	2.549	2.266	2.372
22	* late analysis for totals		6	3.72	2.57	2.231	2.363
20	Poisoned 0.5-0.063 mm	3	1	3.739	3.105	2.369	2.856
20			2	3.613	2.872	2.273	2.598
20			3	3.647	2.845	2.322	2.573

20			4	3.526	2.706	2.279	2.455
20			5	3.42	2.595	2.286	2.502
20			6	3.398	2.58	2.357	2.544
2	<0.063 mm	1	1	4.134	3.054	2.291	2.583
2			2	3.985	2.826	2.261	2.28
2			3	3.853	2.505	2.261	2.124
2			4	3.573	2.218	2.292	2.002
2			5	3.6	1.993	2.227	1.598
2			6	3.35	1.415	2.117	1.152
9	<0.063 mm	2	1	4.144	3.06	2.38	2.402
9			2	3.719	2.596	2.267	2.131
9			3	3.745	2.49	2.302	2.191
9			4	3.64	2.255	2.234	1.877
9			5	3.614	2.002	2.271	1.639
9			6	3.557	1.48	2.299	1.255
28	<0.063 mm	3	1	4.003	3.009	2.302	2.569
28			2	3.918	2.687	2.289	2.251
28			3	3.666	2.409	2.26	2.01
28			4	3.577	2.169	2.249	1.9
28			5	3.659	1.959	2.36	1.735
28	* late analysis for totals		6	4.04	1.51	2.283	1.205
11	Poisoned < 0.063 mm	1	1	4.068	3.066	2.316	2.544
11			2	3.82	2.875	2.361	2.535
11			3	3.799	2.822	2.32	2.584
11			4	3.573	2.756	2.303	2.558
11			5	3.696	2.87	2.374	2.629
11			6	3.596	2.803	2.379	2.672
29	Poisoned <0.063 mm	2	1	4.052	3.078	2.34	2.624
29			2	3.909	2.94	2.294	2.621
29			3	3.746	2.838	2.281	2.512
29			4	3.58	2.747	2.316	2.543
29			5	3.739	2.881	2.321	2.502
29			6	3.533	2.739	2.278	2.622
30	Poisoned <0.063 mm	3	1	4.094	3.108	2.316	2.56
30			2	3.844	2.895	2.292	2.634
30			3	3.815	2.864	2.292	2.596
30			4	3.631	2.746	2.219	2.497
30			5	3.723	2.852	2.294	2.529
30			6	3.547	2.728	2.264	2.621
N.063				5.248	4.47	2.241	2.78
N-F-D-1						2.15	1.81
N-F-D-2						2.18	1.881
N-F-D-3						2.203	1.962
N-F-T-1				3.074	2.076		
N-F-T-2				2.978	2.109		
N-F-T-3				3.046	2.205		
N-I				3.818	3.414	2.317	3.409
NIM 1				3.787	2.34	2.402	2.311

VITA

Steven Lane Ashby was born December 24, 1955, in Denver, Colorado. He attended Harrison Central High School in Lyman, Mississippi, and graduated in 1974. He completed an Associate Degree at the Jefferson Davis Campus of the Mississippi Gulf Coast Community College in 1976. He attended the University of Southern Mississippi in Hattiesburg, Mississippi, where he received an undergraduate degree in environmental science in 1979.

Upon completion of his undergraduate degree, he accepted a position as a physical scientist with the Waterways Experiment Station, a research laboratory for the Corps of Engineers located in Vicksburg, Mississippi. After being married in 1979, he and his wife moved to Wisconsin to establish a field station for water quality research on reservoirs. In 1983, he and his wife and daughter relocated to Elberton, Georgia, to establish a second research facility at a reservoir on the Savannah River. He completed his master of science degree in zoology in 1987 studying sediment chemistry in reservoirs under Dr. Jim Schindler. He and his wife and two daughters returned to Vicksburg, Mississippi, in 1987 where he has remained employed as a research hydrologist for the Environmental Laboratory of the U.S. Army Engineer Research and Development Center. He will receive the degree of Doctor of Philosophy from the Department of Oceanography and Coastal Sciences in December of 2000.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Steven Ashby

Major Field: Oceanography and Coastal Sciences

Title of Dissertation: Removal of Manganese in Reservoir Tailwaters As a Function of Reservoir Operations and River Substrate

Approved:

Robert P. Stambell
Major Professor and Chairman

Dean of the Graduate School

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Date of Examination:

October 2, 2000