9-2008

Effect of Nitrification on Lead Corrosion in Chloraminated Distribution Systems

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EFFECT OF NITRIFICATION ON LEAD CORROSION IN CHLORAMINATED DISTRIBUTION SYSTEMS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Environmental Engineering and Science

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by
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December 2008

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ABSTRACT

Water treatment facilities have been shifting from using chlorination to chloramination as a primary disinfectant since 2004, when the EPA enacted the Disinfectants/Disinfection By-Products (D/DBP) Rule mandating the decrease of DBPs. After the switch to chloramination, an unexpected lead concentration increase was detected in the Washington, D. C., and Greenville, NC water systems. These increases may be associated with the switch from chlorination to chloramination. Decomposition of chloramines results in higher ammonia loading to drinking water distribution systems, which may increase nitrification. Nitrifying bacteria may facilitate lead corrosion via two mechanisms: use of nitrite or nitrate as an alternative electron acceptor and destruction of alkalinity leading to a reduction of pH.

This project explored the roles that nitrifying bacteria play in lead corrosion in drinking water distribution systems. Hypothesized lead corrosion factors provided by nitrification (the presence of nitrate, nitrite, and an acidic environment) were imposed under abiotic conditions. The effect of nitrifying bacteria on lead corrosion was also examined. The effectiveness of several lead corrosion inhibitors (orthophosphate, zinc orthophosphate, alkalinity dosing, and pH control) was examined in the presence of nitrifying bacteria and under abiotic conditions. Nitrifying bacteria were also tested for tolerance to different concentrations of chloramines.

The presence of 2 mM nitrate or nitrite significantly increased lead corrosion. Nitrate served as an electron acceptor in the corrosion process. Lead corrosion occurred concurrently with the disappearance of nitrate and formation of nitrite. Reduction of

nitrite was not quantified despite increased lead corrosion. Lead corrosion, arising from abiotic denitrification, was greater for aged coupons than for freshly cleaned coupons in the presence of nitrate. The presence of an acidic environment also significantly increased lead corrosion. When nitrifying bacteria were allowed to grow, lead corrosion factors (the presence of nitrite and an acidic environment) developed. Increased lead corrosion occurred in the presence of ammonia bio-oxidation to nitrite. Lead corrosion was higher for aged coupons than freshly cleaned coupons in biotic treatments. This suggested that the primary cause of lead corrosion for biotic treatments with a freshly cleaned coupon was the development of an acidic environment while biotic treatments with an aged coupon were susceptible to development of an acidic environment and abiotic denitrification of nitrite.

Under biotic conditions, total lead concentrations were significantly reduced for orthophosphate, pH control, and zinc orthophosphate treatments. pH control showed the greatest reduction in lead corrosion (86.9%). Zinc orthophosphate inhibited the growth of nitrifying bacteria and reduced total lead concentrations by 56.2%. Orthophosphate reduced total lead concentrations by 30.1%. Orthophosphate and alkalinity treatments also reduced total lead concentrations under abiotic conditions.

Chloramine doses as low as 0.10 mg/L Cl_2 effectively inhibited ammonia bio-oxidation to nitrite when added to an AOB culture growing in a defined medium. Chloramine doses of 0.10 or 0.25 mg/L Cl_2 were not inhibitory when added to an AOB culture following four days of growth in a defined mineral medium, in the absence of chloramine. Chloramine doses as low as 0.10 mg/L Cl_2 effectively inhibited ammonia

bio-oxidation to nitrite when added to an AOB culture growing in tap water, when the chloramine was added immediately or following eight days of growth in tap water in the absence of chloramine.

ACKNOWLEDGEMENTS

I would like to thank Dr. Yanru Yang for giving me the opportunity to work on this project, for her time and guidance, and for her dedication. I would like to especially thank Dr. Linkletter and the ENVIRON Foundation for funding this project. I would also like to thank Dr. David Freedman and Dr. Tanju Karanfil for serving on my committee and offering their assistance. Thanks go to Xiaoling Lui for teaching me many laboratory methods and skills and Bin Yang for helping with other laboratory duties. I would like to thank the faculty and staff of the department for instruction and assistance on a wide range of issues. Finally, I would like to thank my family and friends for their encouragement and support.

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ABBREVIATIONS

AOB	Ammonia Oxidizing Bacteria
DBP	Disinfection By-Products
DCWASA	District of Columbia Water and Sewer Authority
DIC	Dissolved Inorganic Carbon
DPD	N,N-diethyl-p-phenylenediamine
FDA	Food and Drug Administration
GVWD	Greater Vancouver Water District
HAA	Haloaceticacid
NOB	Nitrite Oxidizing Bacteria
THM	Trihalomethane
TTHM	Total Trihalomethanes
USEPA	United States Environmental Protection Agency

1. INTRODUCTION AND LITERATURE REVIEW

A major shift in water disinfection has been occurring over the past few years from chlorination to chloramination. Approximately 30% of major U.S. water companies are making the switch since chloramination decreases formation of hazardous disinfection byproducts (DBPs) (USEPA, 1995). This switch is due to a 1998 regulation known as the Disinfectants/Disinfection By-Products (D/DBP) Rule enacted by the United States Environmental Protection Agency (USEPA). It is expected that these guidelines will become more stringent with time as the USEPA plans to focus on specific DBPs instead of a combined count (Renner, 2004).

Disinfection with chlorine has long been plagued by DBP formation. Over 500 DBPs are currently known to originate from disinfection (Richardson, 2003). These DBPs, which include trihalomethanes (THM) and haloaceticacids (HAA), are regulated by the EPA because they are potentially carcinogenic and associated with reproductive problems (Guay et al., 2004; Richardson et al., 2000). Currently, the EPA regulates total trihalomethanes (TTHM) at 80 μg/L and HAA5 (monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid) at 60 μg/L (USEPA, 2008). Chloramination has been shown to greatly reduce the occurrence of THMs and HAAs when used as a secondary disinfectant in place of free chlorine (Guay et al., 2004; Cowman and Singer, 1996). Many utilities employ free chlorine for primary disinfection and provide chloramines for residual disinfection as a cost effective method to control DBPs (Carlson and Hardy, 1998). It is estimated that total organic halogens formed from chloramination is only 9-49% of that seen during chlorination of water

under the same conditions (Yang et al., 2007). This has made chloramination a popular alternative to chlorination for the purpose of complying with the D/DBP Rule. However, some disadvantages exist for chloramination. Chloramines can increase the likelihood of nitrification in the drinking water distribution system (USEPA, 2007b). Chloramines are also not as strong as an oxidant as chlorine (AWWA, 1999).

Despite the advantage of chloramination in generating less regulated DBPs, an unexpected lead concentration increase was noticed in two different water supply systems that may be associated with the switch of chlorination to chloramination. It was reported in 2004 by the District of Columbia Water and Sewer Authority (DCWASA) that 74 of 108 samples taken in Washington, D.C. were well above the EPA action limit of 15 ppb (District of Columbia Water and Sewer Authority, 2004). A steady increase in lead concentrations was then seen with more than 157 houses in the area having higher than 300 ppb at the tap. It is also estimated that thousands more homes are exceeding the EPA limit of 15 ppb. Sampling until mid 1999 indicated that lead was within acceptable limits (Edwards and Dudi, 2004). The DCWASA switched to chloramination in November 2000. The action level was exceeded as early as the summer of 2001. The DCWASA switches to free chlorine for one month a year. This is intended to control growth of bacteria within the distribution system. Interestingly, the number of homes exceeding the lead action level was cut in half during this time (54% to 26%). When chloramines were reintroduced as the disinfectant residual lead concentrations increased again by a factor of 13.6 (Vasquez et al., 2006). Elevated lead problems were not confirmed until August 2004 by which time consumers could have been exposed to dangerously high lead levels

for up to three and a half years (Edwards and Dudi, 2004). Elevated lead in Washington, D.C., forced the DCWASA to distribute 30,000 free water filters and offer free blood tests (Cohn, 2005).

More recently, it was discovered that two boys in Greenville, NC had high blood lead levels which was linked to tap water with a lead concentration of 400 ppb. Similar to the Washington, D.C. incident a switch from chlorine to ozone as the primary disinfectant had recently occurred with chloramines provided as the disinfectant residual (Renner, 2005).

1.1 Dangerous Health Effects of Lead

Currently, the most common use of lead is in batteries. Large amounts of lead were introduced into the environment before the USEPA banned the use of tetraethyl lead in gasoline in 1996. Lead pipes are still used in many old drinking water distribution systems. Many household bathroom fixtures contain some lead. Lead was also in many household paints until 1978 (USEPA, 2000). Solders used for drinking water distribution systems were used until they were banned in 1986 by The Safe Drinking Water Act. Of course, solders already in place throughout distribution systems could not be reclaimed. It is estimated that 70% of American households receive drinking water that comes into contact with Pb-Sn solder. The cost of massive plumbing system replacements across the nation caused no action to be taken (Reiber, 1991). Particulate and dissolved lead from these parts of the drinking water distribution system is the primary source of the contaminants (Vasquez et al., 2006).

Chronic effects including anemia, elevations in blood pressure, kidney disease leading to irreversible lead nephropathy, and detrimental effects to the nervous, immune, and cardiovascular system can occur with exposure to lead (ATSDR, 2007). Because lead is neurologically toxic, potentially carcinogenic, and very damaging to human health with especially hazardous consequences to children and infants (Renner, 2007), research is needed to elucidate the mechanisms involved in lead corrosion in such systems and to develop counteractive approaches.

1.2 Chlorine and Chloramine Chemistry

Chlorine is one of the most commonly used disinfectants applied to water distribution systems due to its ability to effectively reduce bacteriological activity. Hypochlorous acid (HOCl), a weak acid, is formed from the reaction of chlorine gas and water with complete ionization to hypochlorite (OCl^-) only occurring in alkaline solutions. Chlorine disinfectant can also be applied in salt forms such as sodium hypochlorite (NaOCl) or calcium hypochlorite ($Ca(OCl)_2$). Use of chlorine as a disinfectant was first shown by Robert Koch in 1881 and the first application of this technology to a drinking water supply occurred shortly after in 1902 with addition of calcium hypochlorite in Middelkerke, Belgium (Crittenden et al., 2005). By 1941, 85% of water supplies in the United States received chlorine for disinfection (Crittenden et al., 2005).

The potential for disinfection with chloramines was also discovered around the early 1900s. Initially, chloramines were added for taste and odor control. It was soon recognized, though, that chloramines were more stable than free chlorine in the

distribution system and use of chloramines became popular during the 1930s and 1940s. Soon after, World War II created a shortage of ammonia and use of chloramines declined until recently (USEPA, 1995). Ammonia, for generation of chloramines, is added in either the gaseous (anhydrous ammonia) or liquid form. Chloramines are produced from the reaction of free chlorine, provided by hypochlorous acid (HOCl), and ammonia in water. The hypochlorous acid immediately dissociates into OCl^-. At pHs of 7-8.5, free chlorine will react quickly with ammonia in the following reactions:

$$NH_3 + HOCl \leftrightarrow NH_2Cl + H_2O \text{ (monochloramine)} \quad (1)$$

$$NH_2Cl + HOCl \leftrightarrow NHCl_2 + H_2O \text{ (dichloramine)} \quad (2)$$

$$NHCl_2 + HOCl \leftrightarrow NCl_3 + H_2O \text{ (nitrogen trichloride)} \quad (3)$$

These reactions generate various amounts of three forms of chloramines: monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and nitrogen trichloride (NCl_3). Monochloramine is the prevalent form found for disinfection purposes due to the nature of chloramine speciation. Utilities use a Cl:N weight ratio of 3-5 and a weight ratio of 4 is widely accepted for use (USEPA, 1995). Monochloramine is dominant until a weight ratio of approximately 5 so it is the most prevalent form seen in conditions relative to disinfection (USEPA, 1995).

1.3 Lead Corrosion

The lead source of the Washington, D.C. incident was concluded to come from the old lead pipes used in the drinking water distribution system. It has been suggested that the change in chemistry caused by chloramines resulted in the increased lead leaching. Chlorination provides water with high oxidizing potential in the distribution

system. It has been shown by Schock (1989) that a layer of PbO_2 existed on the service pipes. The PbO_2 layer remained insoluble while in a highly oxidized environment. The switch to chloramination, however, provided a much less oxidized environment allowing the PbO_2 to become much more soluble. This could provide the source of the high lead levels seen in Washington, D.C. However, Greenville's drinking water system does not contain lead pipes. Instead, lead solder used to connect pipes was blamed for the incident. Lead contamination can also originate from brass, which can contain up to 8% lead, used in bathroom fixtures and fittings (Renner, 2004).

Another possibility, explored in this work, considers the effect that nitrifying bacteria could have on lead corrosion. It is well-understood that nitrifying bacteria consist of two distinct groups: ammonia oxidizing bacteria (AOB) which mediate the oxidation of ammonia to nitrite, and nitrite oxidizing bacteria (NOB) which mediate the oxidation of nitrite to nitrate. The process is collectively called nitrification. The reverse reaction occurs when chloramines degrade and reform ammonia. Systems that practice chloramination are susceptible to nitrification because a small amount of ammonia is always present due to this decomposition.

1.4 Biotic Factors Influence on Lead Corrosion

Nitrifying bacteria may facilitate lead corrosion via two mechanisms. The first mechanism involves production of protons during nitrification. The destruction of alkalinity by AOB can potentially change the pH of the environment in which the bacteria live.

$$\text{AOB: } 2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (4)$$

$$\text{NOB: } 2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (5)$$

If a small layer of water surrounding the biofilm is drastically reduced pH such that the overall pH of the water would not be much affected, these changes in local water chemistry could affect the corrosion of service lines and residential plumbing, thereby increasing the metal content of the drinking water.

The second mechanism is that nitrite produced during nitrification may be used as an electron acceptor for lead corrosion. It has been suggested that lead oxidation can occur in the presence of nitrite and nitrate through the following reactions (Uchida and Okiwaki, 1998):

$$NO_3^- + Pb + H_2O \rightarrow NO_2^- + Pb^{2+} + 2OH^- \quad (6)$$

$$2NO_2^- + 3Pb + 4H_2O \rightarrow N_2 + 3Pb^{2+} + 8OH^- \quad (7)$$

$$NO_2^- + 3Pb + 5H_2O \rightarrow NH_3 + 3Pb^{2+} + 7OH^- \quad (8)$$

Nitrifying bacteria can therefore effectively cause the corrosion of lead. These processes may also happen more quickly in the presence of nitrifying bacteria because the protons produced during nitrification can neutralize the hydroxyl ions produced in the above leaching reactions.

As can be gathered from equations 6, 7, and 8, this form of lead corrosion will produce an elevated pH. Ammonia oxidizing bacteria, performing the chemical transformations in equation 4, tend to function better under neutral conditions. The protons produced that could shut down the AOB's ability to perform nitrification could also be neutralized by the hydroxyl ions generated during lead corrosion. Therefore, the

effect of lead corrosion through the reduction of nitrate and ammonia generation through decomposition of chloramines would provide an environment conducive for the growth and functioning of AOB and NOB.

1.5 Corrosion Inhibitors

Two forms of corrosion inhibition can be utilized: passivation and neutralization. Passivation occurs through the deliberate formation of protective scales which tend to block interaction between electrolytes, anodes, and cathodes necessary for corrosion. Neutralization, however, uses more highly reactive chemicals to overcome chemical species responsible for corrosion in the water (MacQuarrie et al., 1997). The purpose of corrosion inhibitors, such as elevated alkalinity, orthophosphate, and zinc orthophosphate, is to try to form a better protective scale on pipes to reduce corrosion and reduce reactive species. However, the addition of alkalinity would also act as a neutralization approach because it would reduce available protons for corrosion.

Waters with low pH and alkalinity are usually at higher risk for corrosion. This is thought to be due to the lack of formation of a protective film of calcium carbonate on the pipes (Churchill et al., 2000). Adjustment of pH and alkalinity could provide an environment in which calcium carbonate saturation is reached and a protective film can be formed to prevent further metal corrosion. Reductions in pH or alkalinity can result in increased lead corrosion (USEPA, 2002). Increasing pH and alkalinity has been shown to decrease soluble lead concentrations (McNeill and Edwards, 2004).

Phosphate inhibitors are a popular choice for lead corrosion. Typical inhibitors include phosphates, hexametaphosphates, polyphosphate, and zinc orthophosphate

(MacQuarrie et al., 1997; Maddison et al., 2001). Inhibitors can be dosed in a variety of ways. Phosphates can be introduced as phosphoric acid or blends of phosphoric acid and zinc orthophosphate or polyphosphates (Edwards et al., 2002, Schneider et al., 2007).

Orthophosphate is a tasteless, odorless compound that is recognized as safe by the Food and Drug Administration (FDA) and USEPA and can help protect drinking water distribution systems from metal corrosion. Orthophosphate can be added by utilities as orthophosphoric acid (Schneider et al., 2007). It was first employed to prevent excess calcite precipitation from occurring within the distribution system over 60 years ago and is also commonly used for iron corrosion control (McNeill and Edwards, 2000). Orthophosphate was employed for lead control in Washington, D.C., and was able to reduce lead concentrations from 82 to 31 ppb after 3 months (USEPA, 2005). While not a lot is known about the mechanism for corrosion inhibition, it is generally thought that phosphate inhibitors tend to form protective layers of a metal-inhibitor compound on the walls of lead pipes and lead-containing fixtures within the home (District of Columbia Water and Sewage Authority, 2004a; MacQuarrie et al., 1997; Schock, 1989). Orthophosphate and dissolved carbonate compete to form complexes with lead. Orthophosphate is the preferred precipitate due to its decreased solubility over wider ranges of pH when compared to calcium carbonate. The optimum pH for orthophosphate film formation at low dissolved inorganic carbonate (DIC) levels (1 mg/L as $CaCO_3$) is about 7-8. The optimum pH is slightly affected by DIC levels of 5-25 mg/L as $CaCO_3$ (Schock, 1989). Phosphorus is also an important nutrient for many microorganisms. Increased levels of phosphorus could increase biological activity in the distribution

system (Churchill et al., 2000). Addition of orthophosphate could also increase eutriphication if water storage occurs depending on the point of introduction and pH adjustment (Schock, 1989).

Like orthophosphate, zinc orthophosphate is also a common corrosion inhibitor. Zinc is added by some utilities to further reduce corrosion (McNeill and Edwards, 2002). It was originally chosen for corrosion control in the DCWASA. This brought into question the concerns of the addition of extra metals and nutrients. While zinc is not considered a health concern for humans, increased zinc levels could have negative impacts on the biological nutrient removal wastewater processes (USEPA, 2007). Furthermore, zinc accumulation in sludge of wastewater processes poses a significant problem as Rule 503-B enforced by the EPA regulates sludge disposal containing heavy metals (USEPA, 1999). Elevated zinc levels can also negatively impact some industrial processes such as breweries (Ramaley, 1993). Discharged wastewaters with elevated zinc levels could have a toxic effect on fish.

Corrosion inhibitor addition is an accepted practice for industrial drinking water utilities. Orthophosphate was designated for use by the USEPA on an interim basis as the optimal corrosion control treatment for the Washing Aqueduct and the DCWASA in August 2004. An initial dose of 3.5mg/L as PO_4 was applied to the system. This dose was later decreased to 2.4 mg/L as PO_4. This dosage would effectively give a residual concentration of 2 mg/L as PO_4 to the distribution system. Monitoring of lead continued throughout the distribution system after this addition. It was reported by the DCWASA that samples were at or below the 15 ppb federal action level. This marked the fourth

consecutive monitoring period in which the DCWASA was in compliance with the lead action level (USEPA, 2007a). Mixed results have been shown on the comparison of different corrosion inhibition strategies. Maddison et al. (2001) found that sodium hexametaphosphate, one form of polyphosphates, was successful at controlling metal corrosion for slightly acidic and low alkalinity water in the Nova Scotia region. It was also observed that pH adjustment with sodium hydroxide had little effect on preventing metal corrosion. Churchill et al. (2000) also found that zinc orthophosphate was an effective option for corrosion inhibition of lead and copper in the Greater Vancouver Water District (GVWD) which also has a low pH and alkalinity. Interestingly, it was observed that high pH-alkalinity combinations (ranging up to pH 9 and 30 mg/L as $CaCO_3$) had increased lead corrosion when compared to low pH and alkalinity controls. McNeill and Edwards (2004) found that orthophosphate successfully reduced soluble and particulate lead concentrations. MacQuarrie et al. (1997) actually observed an increase in lead concentrations when zinc orthophosphate was applied as a corrosion inhibitor when compared to pH and alkalinity adjustment alone for standing water from the GVWD in contact with 50/50 lead-tin solder and plumbing coils. It was stated that the presence of slightly acidic conditions or a lack of alkalinity would override any corrosion inhibition from the zinc orthophosphate. The conclusion was that pH and alkalinity adjustment were the best method for corrosion inhibition. Reiber (1991) found that orthophosphates were successful at inhibiting galvanic corrosion of lead-tin solders for waters at a pH near neutral. However, this inhibition was not seen at a pH less than 6. It is thought that the

protective film formed by orthophosphate would be quickly removed below this pH (Reiber, 1991).

2. RESEARCH OBJECTIVES

This project sought to explore the role that nitrifying bacteria play in lead corrosion in drinking water distribution systems. Several factors, biotic and abiotic, were explored in this project that could be associated with the elevated lead levels seen in Washington, D.C. and Greenville, N.C. These factors included:

- pH
- Nitrite concentration
- Nitrate concentration
- Presence of nitrifying bacteria

The specific objectives included:

1) To evaluate if the presence of nitrate, nitrite, an acidic pH, or nitrifying bacteria can cause corrosion and to what extent;
2) To investigate the effect of lead corrosion inhibitors (pH control, elevated alkalinity, orthophosphate, and zinc orthophosphate) under abiotic and biotic conditions; and
3) To determine the lethal effective dose of chloramines on *Nitrosomonas europea*.

3. MATERIALS AND METHODS

The experimental design consisted of three sets of experiments that addressed each of the objectives. For experiments I and II, a biotic treatment was included in order to asses the role of ammonia bio-oxidation to nitrite in lead corrosion. The original intent was to grow the AOB culture in mineral media (section 3.3), centrifuge the culture, wash it with tap water to remove the spent media, and then add the washed culture to the reactors along with ammonia. The biotic treatments would then be compared to abiotic treatments, i.e., without cells or ammonia addition.

However, repeated attempts to use centrifugation (8000 rpm at 4°C for 5 minutes, Sorvall Evolution RC from Kendro) to concentrate and wash the cells were unsuccessful. A pellet did not form. In spite of this, attempts were made to decant off the centrate and add a small amount of liquid from the bottom of the centrifuge tube to the reactors. Regardless, no growth occurred (based on a lack of ammonia oxidation). The only method feasible for obtaining growth in the biotic treatments was to directly inoculate the reactors with the AOB culture in spent media; a 2% (v/v) inoculation was used; this was the lowest dose that allowed for discernable growth within a reasonable period of time. The disadvantage of this approach was the fact that spent media was carried over with the culture. To compensate for this, two types of abiotic controls were prepared, one with filtered spent media (since filtration removed the microbes) and one without filtered spent media added.

A description of each experiment follows.

Experiment I. The Lead Corrosion Factors Study examined the effect of various biotic and abiotic factors on lead corrosion. Lead Corrosion Factors with Fresh Coupons (Experiment I, Part A) was designed to compare the effects of nitrate, nitrite, low pH, and active nitrification in the presence of a freshly cleaned lead coupon. All treatments contained 30 mL of autoclaved tap water (obtained from the tap at the L. G. Rich Environmental Laboratory on 3/17/2008) and a freshly cleaned lead coupon. Growth media was considered to be "spent" after culture growth had occurred and the pH was reduced (see section 3.3). The proper pH of spent media was reached after the pH dye color changed from red to orange. Treatments with addition of spent media were run in quadruplicates while treatments without addition of spent media were run in triplicates. The choice between numbers of replicates was based on space available on the shaker. The experimental design is shown in Table 3-1.

Table 3-1: Design of Lead Corrosion Factors Study with Fresh Coupons (Experiment I, Part A).

No.	Treatment	Media Added	Nitrogen Added	Initial pH	Purpose
1	Abiotic	2% filtered spent media	None	7.72 ± 0.15	To test effect of abiotic lead corrosion in presence of spent media
2	Abiotic	None	None	6.70 ± 0.07	To test effect of abiotic lead corrosion in the absence of spent media
3	Biotic	2% spent media	None	7.67 ± 0.05	To test effect of biotic lead corrosion
4	Low pH	2% filtered spent media	None	6.00 ± 0.11	To test lead corrosion under acidic conditions
5	Nitrate	2% filtered spent media	2 mM	7.61 ± 0.05	To test effect of nitrate on lead corrosion in the presence of spent media
6	Nitrate	None	2 mM	6.85 ± 0.03	To test effect of nitrate on lead corrosion in the absence of spent media
7	Nitrite	2% filtered spent media	2 mM	7.68 ± 0.07	To test effect of nitrite on lead corrosion in the presence of spent media
8	Nitrite	None	2 mM	6.70 ± 0.04	To test effect of nitrite on lead corrosion in the absence of spent media

The effect of lead corrosion factors was also examined in using aged coupons (Experiment I, Part B). All treatments receiving spent media were run in quadruplicates while treatments not receiving spent media were run in triplicates. All treatments received 30 mL of autoclaved tap water (obtained from the tap at the L. G. Rich Environmental Laboratory on 3/17/2008) and an aged lead coupon. Lead coupons were aged for two weeks in autoclaved tap water prior to beginning the experiment. Coupons were cleaned and put into a 500 mL polypropylene bottle containing 250 mL autoclaved water. The experimental design is shown in Table 3-2.

Table 3-2: Design of Lead Corrosion Factors with Aged Coupons (Experiment I, Part B).

No.	Treatment	Media Added	Nitrogen Added	Initial pH	Purpose
1	Abiotic	2% filtered spent media	None	7.25 ± 0.12	To test effect of abiotic lead corrosion in presence of spent media
2	Abiotic	None	None	6.62 ± 0.03	To test effect of abiotic lead corrosion in the absence of spent media
3	Biotic	2% spent media	None	7.17 ± 0.07	To test effect of biotic lead corrosion
4	Low pH	2% filtered spent media	None	5.98 ± 0.05	To test lead corrosion under acidic conditions
5	Nitrate	2% filtered spent media	2 mM	7.32 ± 0.05	To test effect of nitrate on lead corrosion in the presence of spent media
6	Nitrate	None	2 mM	6.89 ± 0.03	To test effect of nitrate on lead corrosion in the absence of spent media
7	Nitrite	2% filtered spent media	2 mM	7.27 ± 0.05	To test effect of nitrite on lead corrosion in the presence of spent media
8	Nitrite	None	2 mM	6.95 ± 0.06	To test effect of nitrite on lead corrosion in the absence of spent media

Experiment II. The Lead Corrosion Inhibitors Study evaluated the efficiency of orthophosphate, zinc orthophosphate, alkalinity, and pH control for lead leaching control under biotic and abiotic conditions. All treatments in Experiment II were run in triplicates and received 30 mL autoclaved tap water (obtained from the L. G. Rich Environmental Laboratory on 7/10/2007) and an aged lead coupon. The experimental design for evaluating alkalinity and orthophosphate (Experiment II, Part A) is shown in Table 3-3.

Table 3-3: Design of Lead Corrosion Inhibitors Study Using Orthophosphate and Alkalinity (Experiment II, Part A).

No.	Treatment	Type	Corrosion Inhibitor	Initial pH	Purpose
1	Control	Abiotic	None	7.12 ± 0.10	To test effect of ammonia on lead corrosion
2	Control	Biotic	None	6.78 ± 0.02	To test effect of biological factors on lead corrosion
3	Orthophosphate	Abiotic	5 mg/L as PO_4 trisodium phosphate	7.21 ± 0.00	To test effect of ammonia and orthophosphate on lead corrosion
4	Orthophosphate	Biotic	5 mg/L as PO_4 (added as trisodium phosphate)	7.00 ± 0.06	To test biological effect of ammonia and orthophosphate on lead corrosion
5	Alkalinity	Abiotic	50 mg/L as $CaCO_3$ (added as sodium carbonate)	7.98 ± 0.06	To test effect of ammonia and increased alkalinity on lead corrosion
6	Alkalinity	Biotic	50 mg/L as $CaCO_3$ (added as sodium carbonate)	8.01 ± 0.01	To test biological effect of ammonia and increased alkalinity on lead corrosion

The effect of pH control on lead corrosion by nitrifying bacteria was also examined. The experimental design for Experiment II, Part B is shown in Table 3-4.

Table 3-4: Design of Lead Corrosion Inhibitor Study Using pH Adjustment Treatment (Experiment II, Part B).

No.	Treatment	Type	Corrosion Inhibitor	Initial pH	Purpose
1	Control	Abiotic	None	7.25 ± 0.12	To test effect of ammonia on lead corrosion
2	Control	Biotic	None	7.17 ± 0.07	To test effect of biological factors on lead corrosion
3	pH Adjustment	Biotic	pH maintained at 7.5 ± 0.50 (NaOH added as necessary)	7.19 ± 0.08	To test biological effect of ammonia and pH control on lead corrosion

The effect of zinc orthophosphate on lead corrosion was examined in Experiment II, Part C. Preparation of zinc orthophosphate stocks called for addition of nitric acid to achieve dissolved uniform distribution so a control in which just nitric acid was added prior to addition of the lead coupon was necessary (McNeill and Edwards, 2000). The pH of the tube was then adjusted back to neutral using NaOH. Since addition of nitric acid was necessary for preparation of the zinc orthophosphate stock, the "nitrate control" treatments received the same amount of nitric acid. Both the "nitrate control" and zinc orthophosphate treatments were returned to a neutral pH one day before beginning the experiment to ensure no effect on biological growth or lead corrosion. All treatments in this experiment received 5% filtered (abiotic treatments) or unfiltered (biotic treatments)

media. This was done in an attempt to ensure adequate biomass for growth as previous experiments with zinc orthophosphate had not grown. The experimental design for Experiment II, Part C is shown in Table 3-5.

Table 3-5: Design of Lead Corrosion Inhibitor Study Using Zinc Orthophosphate (Experiment II, Part C).

No.	Treatment	Type	Corrosion Inhibitor	Initial pH	Purpose
1	Nitrate Control	Abiotic	None	7.22 ± 0.04	To serve as a control for the Abiotic-Zinc Orthophosphate Treatment
2	Nitrate Control	Biotic	None	7.17 ± 0.12	To serve as a control for Biotic-Zinc Orthophosphate Treatment
3	Zinc Orthophosphate	Abiotic	5 mg/L as PO_4 (added as $Zn_3(PO_4)_2$)	7.10 ± 0.12	To test effect of ammonia and zinc orthophosphate on lead corrosion
4	Zinc Orthophosphate	Biotic	5 mg/L as PO_4 (added as $Zn_3(PO_4)_2$)	6.97 ± 0.02	To test biological effect of ammonia and zinc orthophosphate on lead corrosion

An overall summary of all treatments examined in Experiments I and II is presented in Table 3-6.

Table 3-6: Summary of all treatments tested during Experiments I and II.

Treatment		Experiment I																Experiment II											
		Part A								Part B								Part A						Part B			Part C		
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	1	2	3	1	2	3	4
Microbes	No (Abiotic)	x	x		x	x	x	x	x	x	x		x	x	x	x	x	x		x		x		x			x		x	
	Yes (Biotic)			x								x							x		x		x		x	x		x		x
N added or formed	none	x	x	x	x					x	x	x	x					x	x	x	x	x	x	x	x	x				
	as nitrate					x	x							x	x												x	x	x	x
	as nitrite							x	x							x	x													
	nitrite formed via AOB			x								x							x		x		x		x	x		x		x
Spent media	not added		x				x		x		x				x		x													
	present	x		x	x	x		x		x		x	x	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x
Coupon	fresh	x	x	x	x	x	x	x	x																					
	aged									x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Initial pH	neutral	x	x	x		x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	low				x								x																	
Corrosion inhibitor	none	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x					x	x		x	x		
	orthoP																			x	x									
	ALK																					x	x							
	pH maintained																									x				
	Zn orthoP																												x	x

Experiment III. The Chloramine Toxicity study was used to determine the maximum dose of chloramines that nitrifying bacteria can tolerate and continue to produce nitrite. Various doses of chloramines (0.1, 0.25, and 1.0 mg/L as Cl_2) were added either at inoculation or after moderate growth had occurred while growing in media. Each treatment received a 2% inoculum to 30 mL of growth media. This was intended to determine the dose at which nitrification can still occur while exposed to chloramines. The study was repeated in 30 mL of autoclaved tap water (obtained from L. G. Rich Environmental Laboratory on 3/17/2008) also receiving a 2% inoculum and treatments were performed in triplicates.

3.1 Experimental Setup

Reactors used for experiments were 50 mL polyethylene sterile centrifuge tubes. Polyethylene tubes were used to prevent adsorption of lead to the surface. All experiments were run in triplicates or quadruplicates. Each tube had a final liquid volume of 30 mL. Lead sheets, obtained from AMES Metal Products Company, were cut into 0.5″ x 0.5″ coupons and cleaned before introduction to the reactors by scrubbing with 400 grit sandpaper and boiling in 1% acetic acid for five minutes to ensure a fresh surface. Tubes remained on a shaker set at 100 RPM for the duration of the experiments. Tap water in this experiment was gathered from the L. G. Rich Environmental Laboratory and autoclaved prior to use. No background concentrations of nitrate, nitrite, or phosphate were detected in the tap water.

3.2 Chemicals

Sodium nitroprusside (98%), zinc orthophosphate (99.995%), and sodium salicylate (99%) were obtained from Alfa Aesar. Sodium dichlorisocyanurate (95%) was obtained from TCI. High purity nitric acid (Pb < 1ppt) and sodium carbonate (99.5%) were obtained from EMD. Sodium nitrate (99%) was obtained from Fisher. Hypochlorite (5.5% available chlorine) was obtained from J. T. Baker. Sodium nitrite (97%), ammonium chloride (99.9%), and trisodium orthophosphate (technical grade, purity not tested by manufacturer) were obtained from Mallinckrodt Chemicals. Lead sheets (98-100%) to be cut into coupons were obtained from AMES Metal Products Company through McMaster-Carr; they were cut into coupons upon receipt. All other chemicals were ACS reagent grade or equivalent.

Zinc orthophosphate stock was prepared by heating the solution to 95°C and adding 1% high purity nitric acid. Chlorine and chloramines were prepared using an N,N-diethyl-p-phenylenediamine (DPD) method. Standard Method 4500 Cl was used for generation of reagents. A full protocol is shown in Appendix C.

3.3 Bacterial Strain, Cultivation, and Generation of Spent Media

Nitrosomonas europaea (ATCC 19718) was used as a model AOB for biotic experiments because *Nitrosomonas* species are thought to be the most prevalent AOB in distribution systems in which nitrification episodes occurred (Lieu et al., 1993; Regan et al., 2002; 2003). Growth media was employed as shown in Table 3-7. One stock (1 mL) vial of frozen culture, stored at -80°C was added to a 500 mL autoclaved glass bottle containing 100 mL of growth media. The glass bottle was then closed with a screw top cap and allowed to grow in the dark for 14 days, without agitation. The pH of the growth culture was monitored by addition of 6 mg/L of phenol red. Media was considered "spent" and ready for use when the color of the pH dye changed from red to orange. Filtration of media was performed with a 13 mm Acrodisc syringe filter with PTFE membrane (0.2 μm). Tap water employed in these experiments had an alkalinity of 14 mg/L as $CaCO_3$. After a 2% inoculation from the culture media, the alkalinity increased to 32 mg/L as $CaCO_3$. A 2% inoculation was used in order to establish an ammonia concentration of 2 mM. Carry-over of phenol red into treatments from addition of spent media was not observed to significantly affect any measurements.

Table 3-7: Composition of growth media, adapted from Barnet et al., 2004.

Compound	Concentration
$(NH_4)_2SO_4$	50mM
K_2HPO_4	13mM
NaH_2PO_4	2mM
Na_2CO_3	5mM
$CaCl_2$	200µM
$MgCl_2$	750µM
$CuCl_2$	0.875µM
Fe-EDTA	16µM
Phenol Red (pH dye)	6 mg/L

3.4 Sampling Procedures

The objectives for this research required that samples be taken for several types of analyses. These analyses included total lead, soluble lead, nitrite and nitrate, pH, and ammonia. A procedure was established for each type of sample. All materials and chemicals that came into contact with the reactor during sampling were autoclaved to prevent contamination. Total lead sampling required that insoluble lead deposited on the bottom of the reactor be evenly redistributed. For this purpose, the reactors were gently shaken for ten seconds to achieve uniform distribution. A volume of 250 µL was withdrawn three times totaling 750 µL for each sample. Then, 75 µL of pure nitric acid was added to acidify the samples. The amount of samples taken and nitric acid added to samples were measured using a digital balance (±0.0001) to determine the dilution effect. Soluble lead sampling was performed using syringe filters. Sterilized Luer-Lok Tip syringes (3 mL, without needles) were obtained from BD. A volume of 500 µL was withdrawn from the reactors with the syringe. A 13mm Acrodisc syringe filter with PVDF membrane (0.45 µm) was then used for filtration of the sample. Then, 50 µL of

pure nitric acid was added to acidify the filtrate. Nitrite and nitrate samples were collected. A volume of 1 mL was filtered with a 0.2 μm syringe filter for this purpose. Another 500 μL sample was taken for use in ammonia and pH measurements.

3.5 Chemical Analyses

Lead measurements were performed using a Perkin Elmer 5000 AA Spectrophotometer. The flame spectrophotometer operates within 2 - 100 ppm and was used for lead measurements in this range (Figure A-1). The graphite furnace spectrophotometer operates within a stable calibration range of 5 - 100 ppb (Figure A-2). The graphite furnace was used to determine lead concentrations within this range. All samples requiring dilution were weighed using a digital balance to provide an exact dilution ratio.

Ammonia measurements were performed using a colorimetric method adapted from Kandeler and Gerber (1988). This method employs the use of two reagents added sequentially. These reagents include a sodium salicylate reagent, containing 0.12% sodium nitroprusside and 17% sodium salicylate, followed by 0.1% sodium dichlorisocyanurate added three minutes apart. The samples were then shaken and allowed to equilibrate for 45 minutes. A slight greenish color appears indicating the presence of ammonia. A Beckamn DU 640 Spectrophotometer operated at 690nm was used to quantify the ammonia concentration within a calibration range of 0.23 – 1.74 mg/L as N (Figure A-5). A full protocol is shown in Appendix C.

Nitrite and nitrate measurements were performed using a Dionex AS50 Ion Chromatograph with an AS9-HC analytical column, AG9-HC guard column, and an

ASRS-Ultra 4-mm suppressor (Figures A-3 and A-4). Sample chromatograms are shown in Appendix A for the abiotic treatment, nitrate treatment, nitrite treatment, and biotic treatment (Figures A-6, A-7, A-8, and A-9, respectively). pH measurements were performed using a pH meter.

Chlorine and chloramines were measured using an N,N-diethyl-p-phenylenediamine (DPD) method. A full protocol is shown in Appendix C. Chlorine concentrations were measured by addition of 5 mL of DPD indicator solution and 5 mL phosphate buffer followed by titration with a ferrous ammonium sulfate solution. An ammonium sulfate stock was then prepared based on the chlorine concentration. Both stocks were adjusted to a pH of 9.0-9.1, combined, and stirred for 5 minutes. The concentration of chloramines was measured by addition of 5 mL phosphate buffer solution, 5 mL DPD indicator solution, one crystal of potassium iodide, and followed by color titration with standard ferrous ammonium sulfate solution.

3.6 Data Analysis

Microsoft Excel (2003) "data analysis" function was employed for statistical analysis of results. The t-Test was used to determine statistical significance based on a p-value less than 0.1.

4. RESULTS

4.1 Lead Factors Corrosion Study: Experiment I, Part A - Fresh Coupon

Experiment I, Part A (Table 3-1) was conducted to determine if and to what extent factors imposed by nitrifying bacteria could increase lead corrosion on a freshly cleaned coupon. A replicate experiment (shown in the Appendix B) was conducted to validate results found in this section. Four treatments (nitrate, nitrite, low pH, and biotic) were examined in comparison to the control treatment (abiotic). Nitrate, nitrite, low pH, and abiotic treatments received filtered spent media while the biotic treatment received unfiltered spent media unless otherwise noted. Error bars represent standard deviation of quadruplicate reactors.

4.1.1 Nitrate Treatment

The nitrate treatment was not found to have any significant effect on the corrosion of a freshly cleaned lead coupon after 93 days. Total lead concentration did not deviate significantly from the abiotic control (Figure 4-1). Although a slow increase in total lead concentration was observed over the experimental period, the nitrate treatment was never statistically different from the abiotic control. Reduction of nitrate to nitrite has been shown to induce lead corrosion (Uchida and Okuwaki, 1998; 1999, Uchida et al., 1999). Analysis of anions in the nitrate treatment showed that a slow increase in the nitrite concentration of the nitrate treatment occurred throughout the experimental period. A significant increase in nitrite was not observed until after 96 days (Figure 4-2). Accumulated nitrite amounted to 0.36 mg NO_2-N/L after 96 days. Though a drop in the

nitrate concentration was observed after 24 days, it was not consistently reduced throughout the experimental period (Figure 4-3). The pH of nitrate treatment did not deviate significantly from the abiotic treatment over the experimental period (Figure 4-4). Soluble lead concentrations were 5.85 ± 3.21 ppb and 13.87 ± 7.74 ppb for the abiotic and nitrate treatments, respectively, after 101 days and were not statistically different.

The replicate experiment showed similar trends for the nitrate treatment. While 0.30 mg NO_2-N/L accumulated in the nitrate treatment after 80 days, the total lead concentration was not significantly higher than the abiotic treatment. These results are shown in Appendix B (Figures B-1, B-2, B-3, and B-4, and Table B-1).

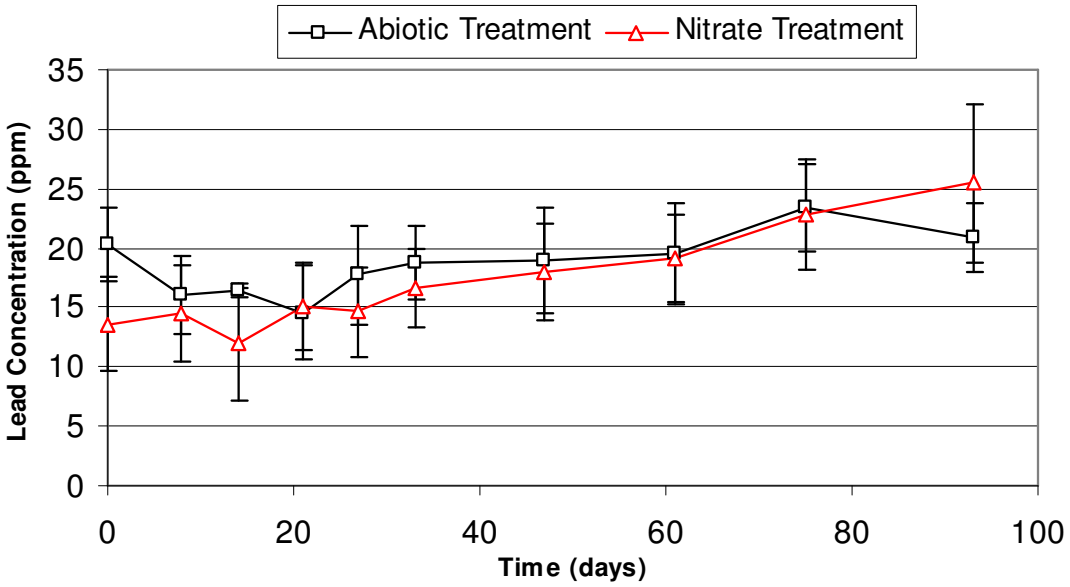


Figure 4-1: Total lead concentrations of abiotic (Experiment IA #1) and nitrate treatments (Experiment IA, #5) with a freshly cleaned coupon; error bars represent one standard deviation for averages.

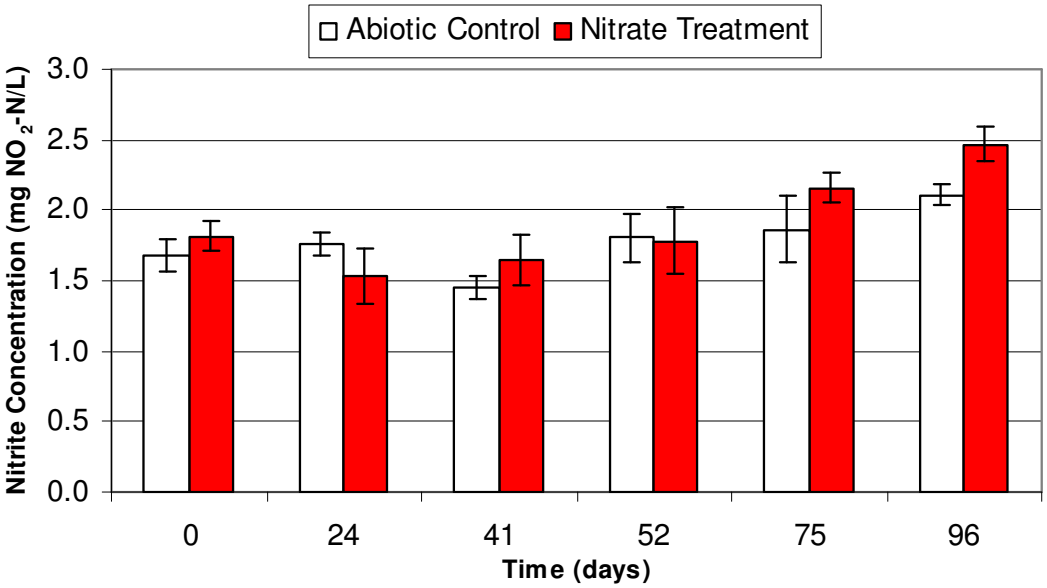


Figure 4-2: Nitrite concentrations of abiotic (Experiment IA #1) and nitrate treatments (Experiment IA, #5) with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

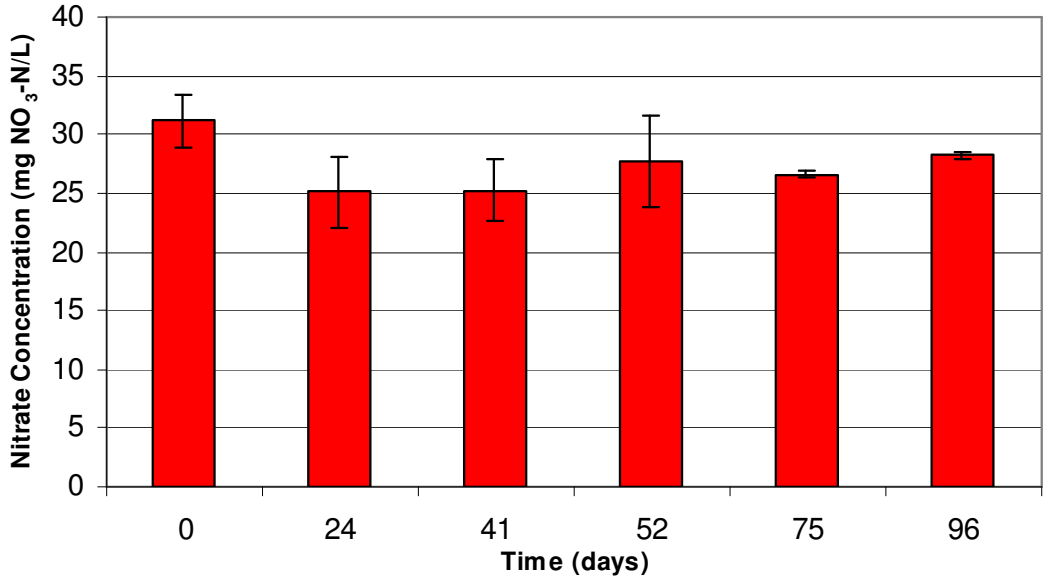


Figure 4-3: Nitrate concentration of nitrate treatment (Experiment IA #5)with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

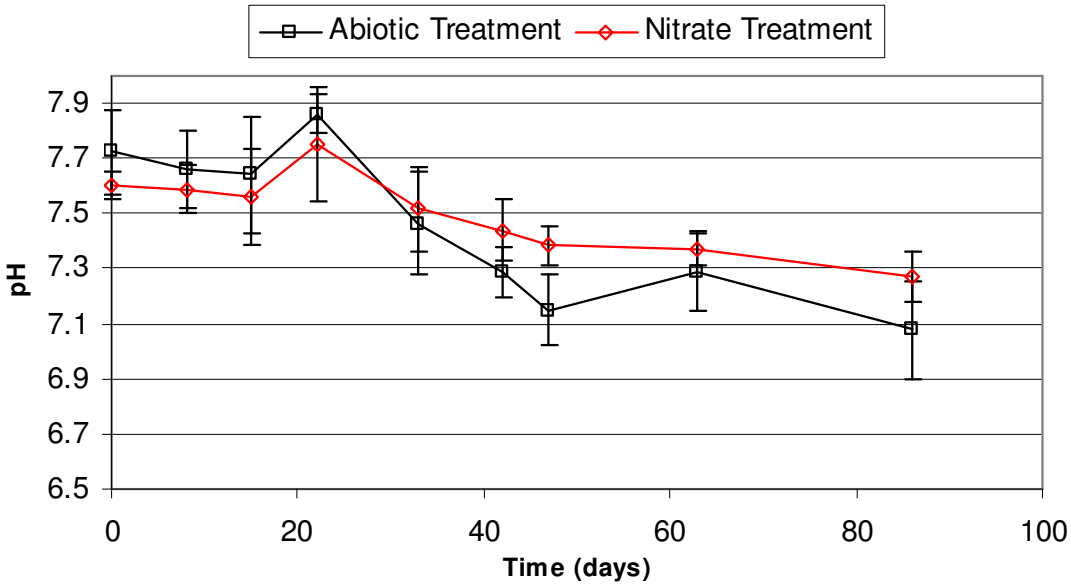


Figure 4-4: pH of abiotic (Experiment IA #1) and nitrate treatments (Experiment IA #5) with a freshly cleaned coupon; error bars represent one standard deviation for averages.

4.1.2 Nitrite Treatment

The nitrite treatment did not significantly increase lead corrosion on a freshly cleaned lead coupon. The total lead concentration was not significantly different from the abiotic control after 93 days (Figure 4-5). As with the nitrate treatment, a slow increase in lead corrosion occurred but was never statistically significant from the abiotic control. The nitrite concentration was not seen to change significantly throughout the experimental period (Figure 4-6). Nitrogen gas, the primary product of the denitrification of nitrite, was unfeasible to test for in this experiment as it was performed exposed to air, creating a large background of nitrogen gas. The pH of the nitrite treatment did not deviate significantly from the abiotic treatment (Figure 4-7). Soluble lead concentrations

were 5.85 ± 3.21 ppb and 39.4 ± 34.4 ppb for the abiotic and nitrite treatments, respectively, after 101 days and were not statistically different. These results were repeated in the replicate experiment and can be seen in Appendix B (Figures B-5, B-6, and B-7, and Table B-1).

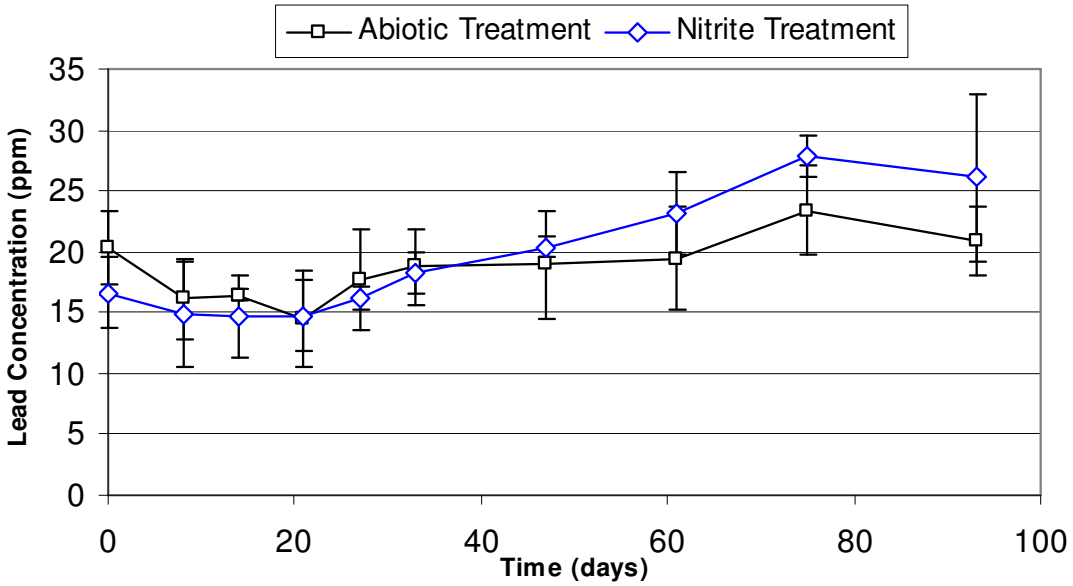


Figure 4-5: Total lead concentrations of abiotic (Experiment IA #1) and nitrite treatments (Experiment IA #7) with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

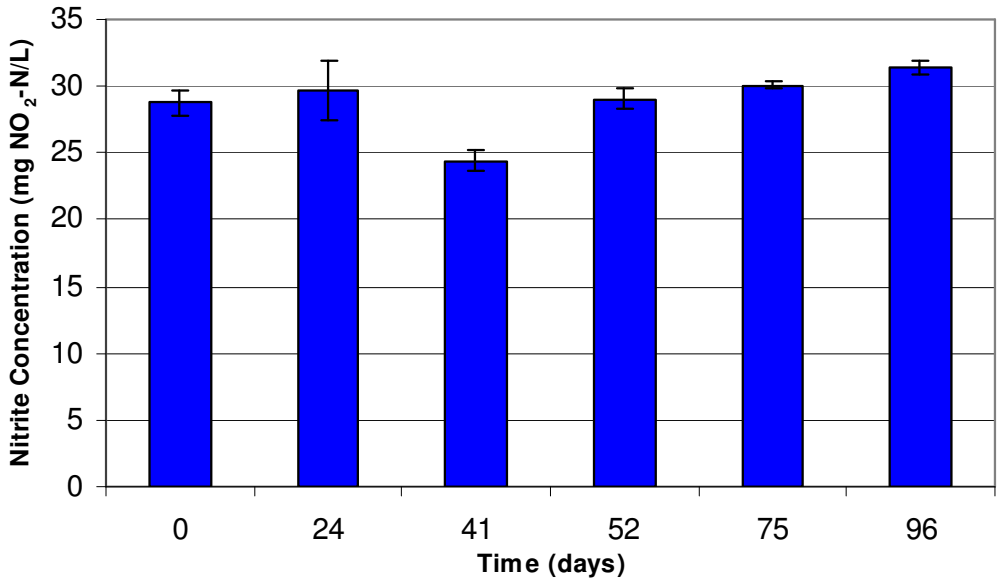


Figure 4-6: Nitrite concentrations of abiotic (Experiment IA #1) and nitrite treatments (Experiment IA #7) with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

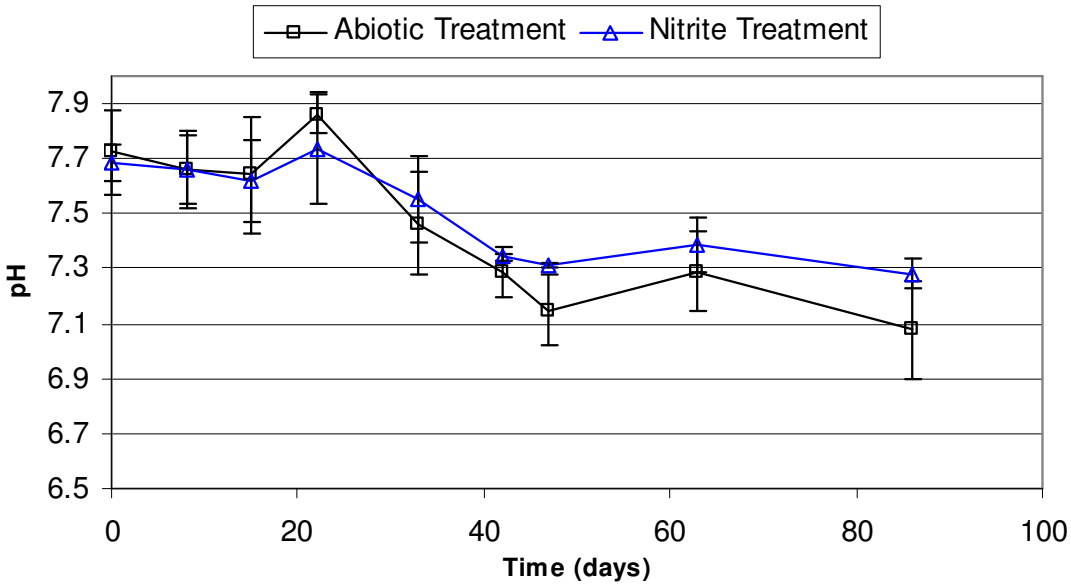


Figure 4-7: pH of abiotic (Experiment IA #1) and nitrite treatments (Experiment IA #7) with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

4.1.3 Low pH Treatment

The low pH treatment (pH maintained at 5.5 ± 0.5) had a significant impact on corrosion of the freshly cleaned lead coupon. The total lead concentration of the low pH treatment immediately increased after pH adjustment on day 0 and was 13.9 times higher than the abiotic treatment after 93 days (Figure 4-8). Soluble lead concentrations were 5.85 ± 3.21 ppb and 10,100 ± 4,040 ppb for the abiotic and low pH treatments, respectively, after 101 days and were statistically different. Readjustment of pH back to acidic conditions was performed approximately every three days for the experimental period.

Results of the replicate experiment were different with respect to total lead concentration. These results are shown in the Appendix B. While lead corrosion was seen to be greatly more significant than the abiotic control (Figure B-8), lower total lead concentrations were detected (4.3 times higher than the abiotic treatment) compared to the results shown in Figure 4-8 (13.9 times higher than the abiotic treatment). Necessary pH adjustment was performed every 1-2 days for this experiment for the 20 days. Afterword, necessary pH adjustment became less frequent (approximately every 5 days) as the pH increase was slower during this time. Soluble lead concentrations were also lower in the replicate experiment than in the first experiment (Table B-1). However, both experiments conclusively showed a vulnerability of lead coupons to an acidic pH despite differing in the amount of lead corrosion.

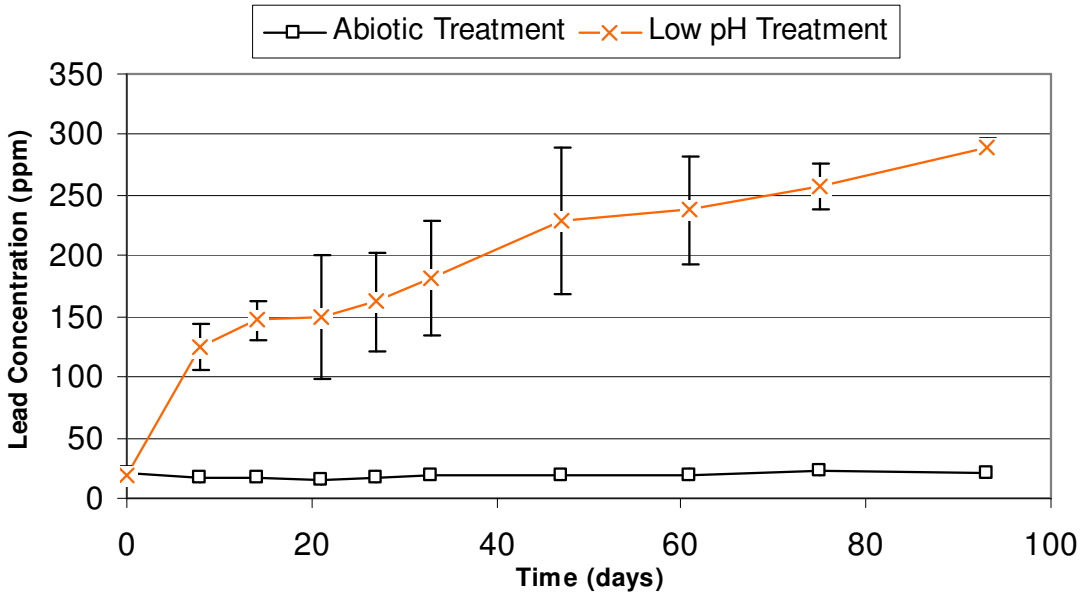


Figure 4-8: Total lead concentration of abiotic (Experiment IA #1) and low pH treatments (Experiment IA #4) with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

4.1.4 Biotic Treatment

The biotic treatment exhibited increased lead corrosion above. Total lead concentrations were significantly higher in biotic treatments as compared to abiotic treatments (Figure 4-9). Lead corrosion began increasing above that of the abiotic control after 21 days. Lead corrosion continued to increase thereafter and was 3.1 times higher than the abiotic control after 93 days. The pH of the biotic treatment decreased from 7.73 to 6.11 after the first 8 days (Figure 4-10). The pH was not seen to increase above 6.6 throughout the rest of the experiment. The consumption of ammonia during nitrification resulted in both a drop in pH and generation of nitrite. The ammonia concentration began decreasing below the abiotic control after inoculation and continued

to decrease throughout the experiment with a maximum decrease of 10.8 mg/L as NH_3-N after 86 days (Figure 4-11). The nitrite concentration increased during this time as well. Nitrite accumulation amounted to 7.4 mg/L as NO_2-N after 96 days (Figure 4-12). Ammonia consumption and nitrite formation occurred concurrently in a near stoichiometric fashion (Figure 4-13). Soluble lead concentrations were 5.85 ± 3.21 ppb and 531 ± 83.3 ppb for the abiotic and biotic treatments, respectively, after 101 days and were statistically significant.

Results of the replicate experiment were similar. Significantly higher total lead concentrations were observed in the biotic treatment as compared to the abiotic treatment (Figure B-9). The total lead concentration of the biotic treatment was 3.4 times higher than the abiotic treatment after 76 days. The pH of the biotic treatment, however, began increasing after day 33 and reached 6.76 after 69 days (Figure B-10). Ammonia consumption (Figure B-11) amounted to 7.0 mg NH_3-N/L after 69 days while nitrite production (Figure B-12) amounted to 6.8 mg NO_2-N/L after 65 days.

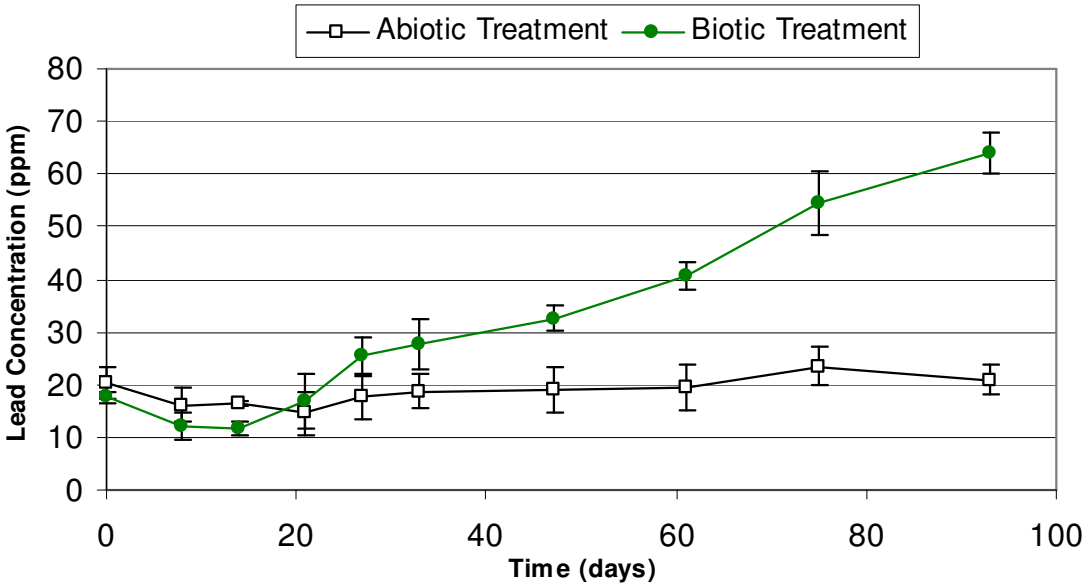


Figure 4-9: Total lead concentrations of abiotic (Experiment IA #1) and biotic treatments (Experiment IA #3) with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

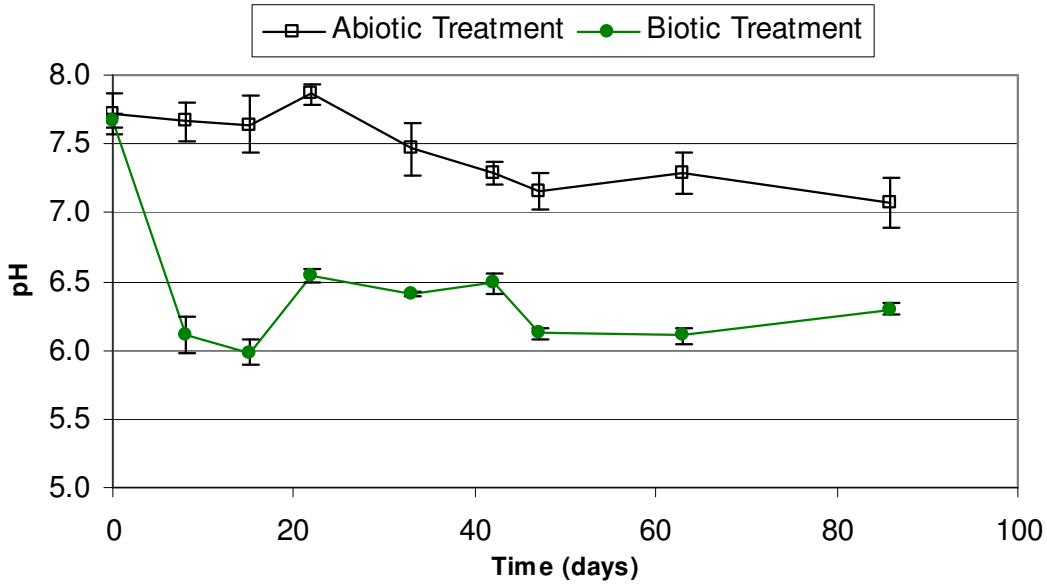


Figure 4-10: pH of abiotic (Experiment IA #1) and biotic treatments (Experiment IA #3) with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

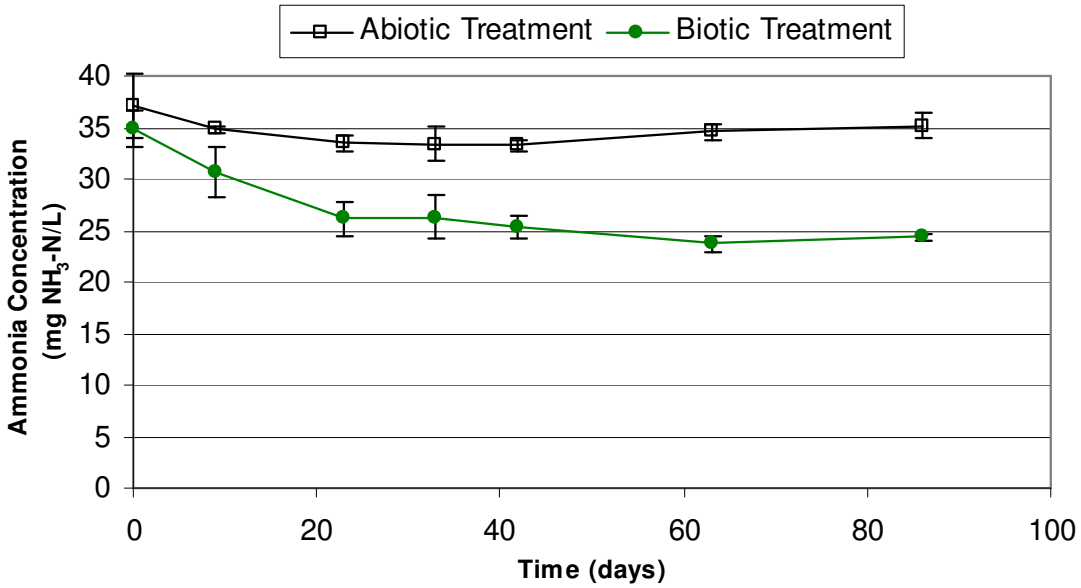


Figure 4-11: Ammonia concentrations of abiotic (Experiment IA #1) and biotic treatments (Experiment IA #3) with a freshly cleaned coupon; error bars represent one standard deviation for averages.

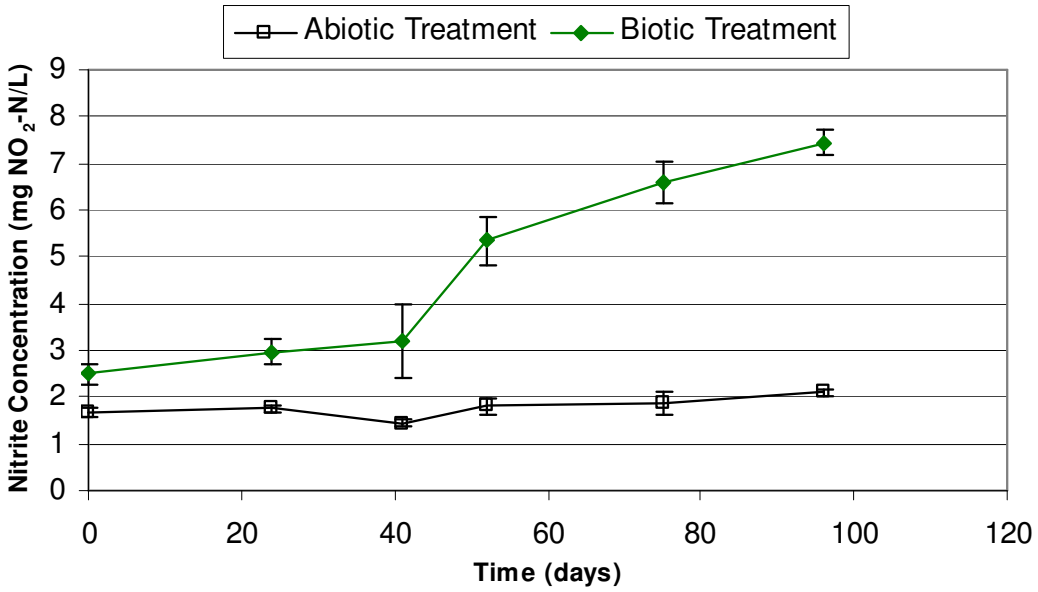


Figure 4-12: Nitrite concentrations of abiotic (Experiment IA #1) and biotic treatments (Experiment IA #3) with freshly cleaned lead coupon; error bars represent one standard deviation for averages.

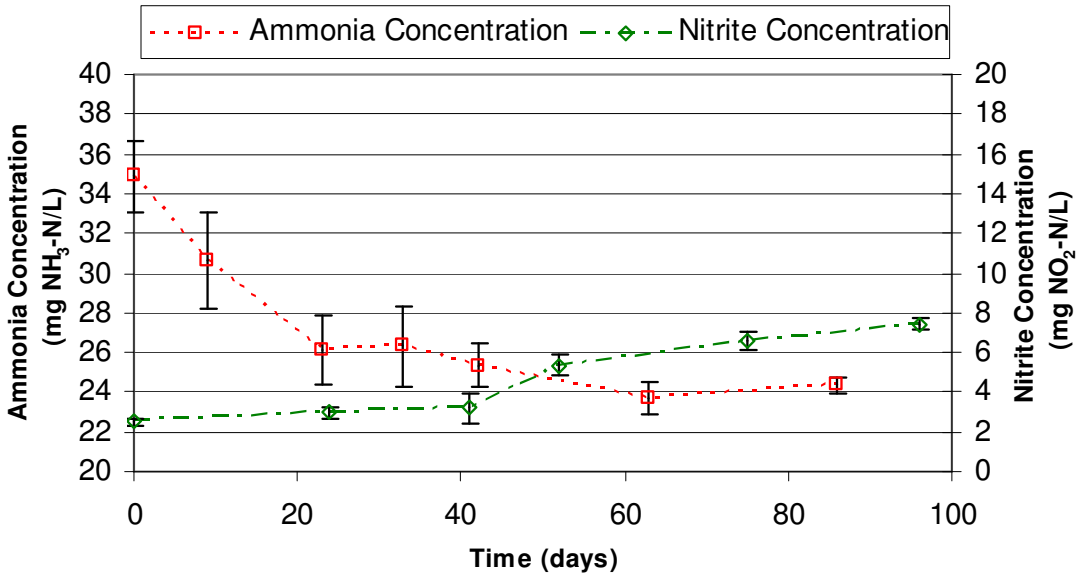


Figure 4-13: Comparison of nitrite and ammonia concentrations for the biotic treatment (Experiment IA #3) with a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

4.1.5 Nitrate Treatment without Spent Media

A concern involving the composition of the growth media for the stock nitrifying bacteria was discovered during the course of experiments. A phosphate buffer was employed in the media. Phosphate addition in this experiment was much higher than is actually used in drinking water distribution systems: 32.0 mg/L as PO_4 compared to 5 mg/L as PO_4 or less (USEPA, 2007a; Hozalski et al., 2005). An experiment was conducted to test the effect of nitrate and nitrite on lead corrosion in the presence and in the absence of spent media on a freshly cleaned coupon. Spent media (media after culture growth had occurred, explained in Chapter 3.3) was filtered (0.1 µm) and added to reactors. Nitrate and nitrite treatments with freshly cleaned coupons, as performed previously, were included to compare lead corrosion.

The presence of nitrate was found to significantly increase lead corrosion of a freshly-cleaned coupon above the abiotic treatment when spent media was not added (Figure 4-14). Lead concentrations were 3.7 times higher than the abiotic treatment without spent media after 64 days. Nitrite concentrations increased throughout the experimental period indicating abiotic denitrification of nitrate with a maximum of 2.9 mg NO_2-N/L after 68 days (Figure 4-15). Nitrate also decreased throughout the experimental period. A loss of 5.9 mg NO_3-N/L was observed after 68 days. Ammonia has been shown to be a reduction product of nitrite (Murphy, 1991). Approximately 0.15 ± 0.02 mg NH_3-N/L accumulated in the nitrate treatment without spent media after 77 days while ammonia was not detected in the abiotic treatment without spent media. The pH of both abiotic and nitrate treatments without spent media increased after day 0 and remained significantly higher than the abiotic treatment without spent media for the entire experimental period (Figure 4-16). The pH of the nitrate treatment without spent media increased to a maximum 8.85 ± 0.04 after 34 days while the abiotic treatment without spent media only increased to 8.15 ± 0.22 in the same time. The pH of the nitrate treatment without spent media remained higher than the abiotic control for the rest of the experimental period. As with previous nitrate treatments with freshly cleaned coupons, no significant total lead concentration above the abiotic treatment was observed the nitrate treatment was amended with the spent media (Figure 4-17). Soluble lead concentrations were 121 ± 7.08 ppb and 166 ± 60.7 ppb for the abiotic treatment without spent media and the nitrate treatment without spent media, respectively, after 72 days and were not statistically different.

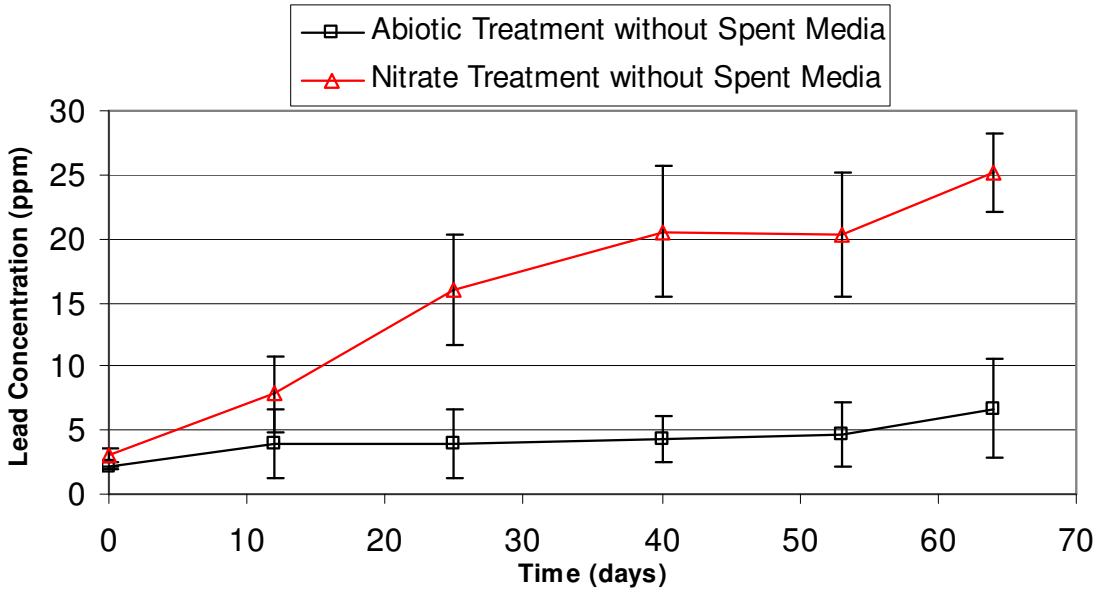


Figure 4-14: Total lead concentrations of abiotic (Experiment IA #2) and nitrate treatments (Experiment IA #6) without spent media on a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

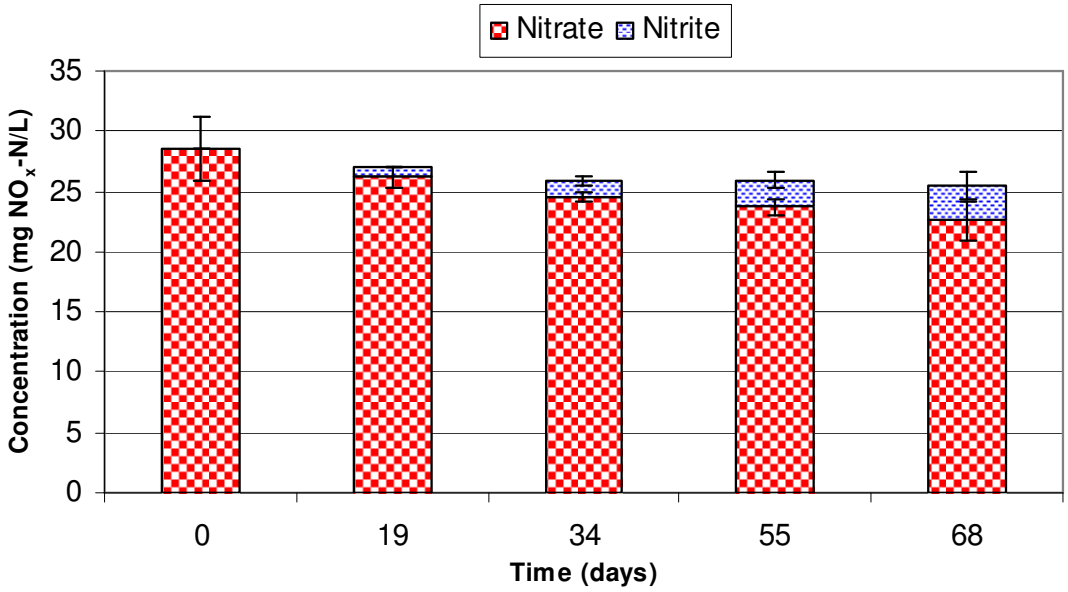


Figure 4-15: Nitrate and nitrite concentrations of the nitrate treatment (Experiment IA #6) without spent media on a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

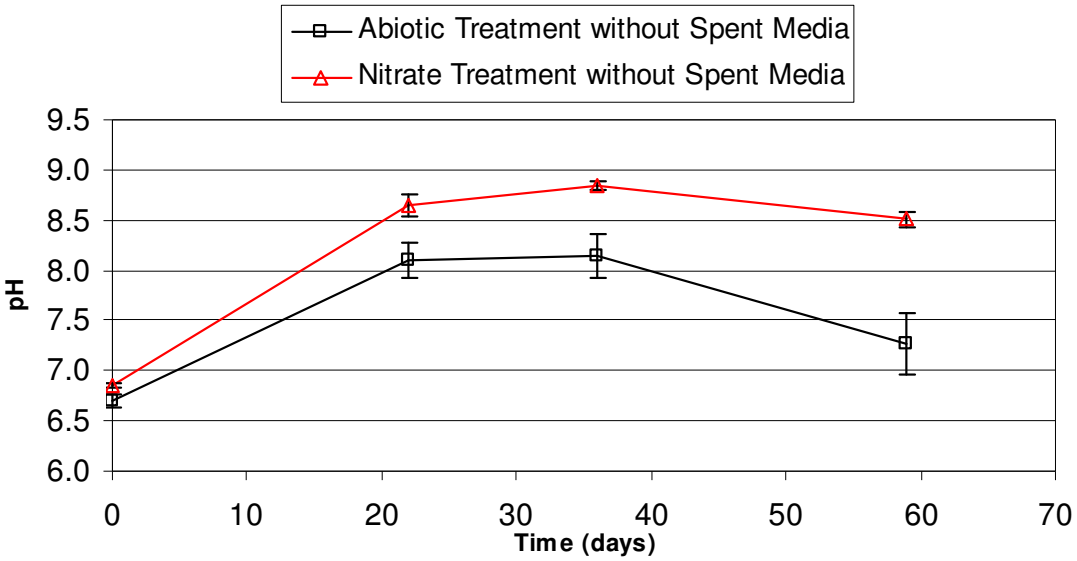


Figure 4-16: pH of abiotic (Experiment IA #2) and nitrate treatments (Experiment IA #6) without spent media on a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

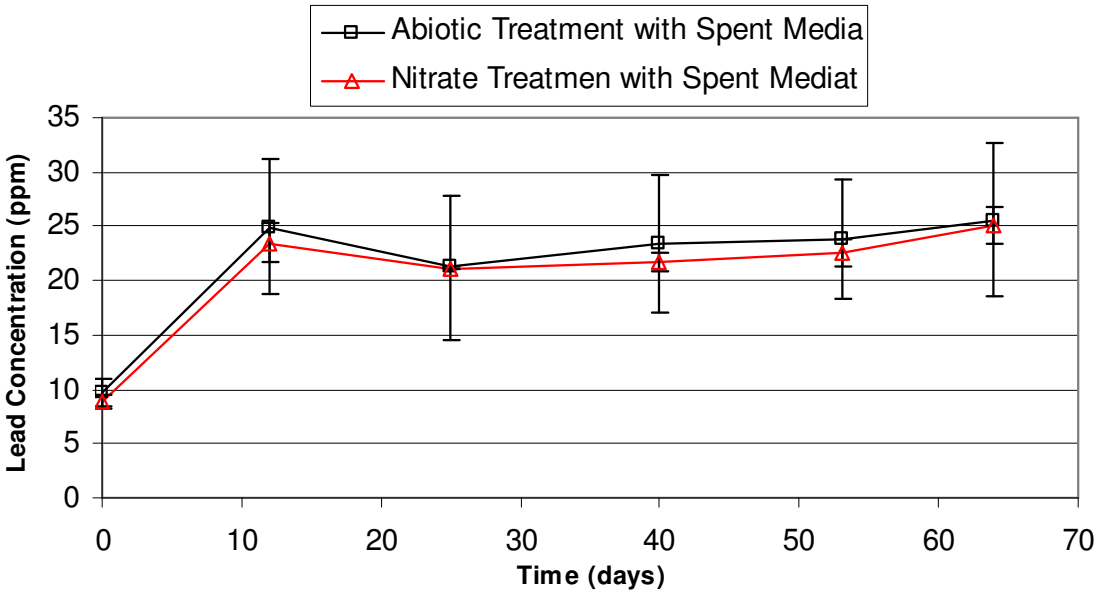


Figure 4-17: Total lead concentration of abiotic (Experiment IA #2) and nitrate treatments (Experiment IA #6) with spent media on a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

4.1.6 Nitrite Treatment without Spent Media

The presence of 2 mM nitrite significantly increased lead corrosion of a freshly cleaned coupon above the abiotic control when spent media was not added. The total lead concentration of the nitrite treatment without spent media was 11.4 times higher than that of the abiotic treatment without spent media (Figure 4-18). While increased lead corrosion was observed in the nitrite treatment without spent media, the nitrite concentration was not consistently reduced (Figure 4-19). A drop of 2.2 mg NO_2-N/L was observed after 29 days but no further drop occurred despite the increasing total lead concentration. Ammonia also accumulated in the nitrite treatment without spent media at a concentration of 0.03 mg NH_3-N/L while no significant amount of ammonia was detected in the abiotic treatment without spent media. The pH of the nitrite treatment without spent media increased above the abiotic treatment without spent media, 8.94 ± 0.35 and 8.15 ± 0.22, respectively, after 36 days and remained higher for the rest of the experimental period (Figure 4-20). As with previous nitrite treatments with freshly cleaned coupons, the total lead concentration did not increase above the abiotic treatment (Figure 4-21). Soluble lead concentrations were 121 ± 7.08 ppb and 160 ± 53.8 ppb for the abiotic treatment without spent media and the nitrite treatment without spent media, respectively, after 72 days and were not statistically different.

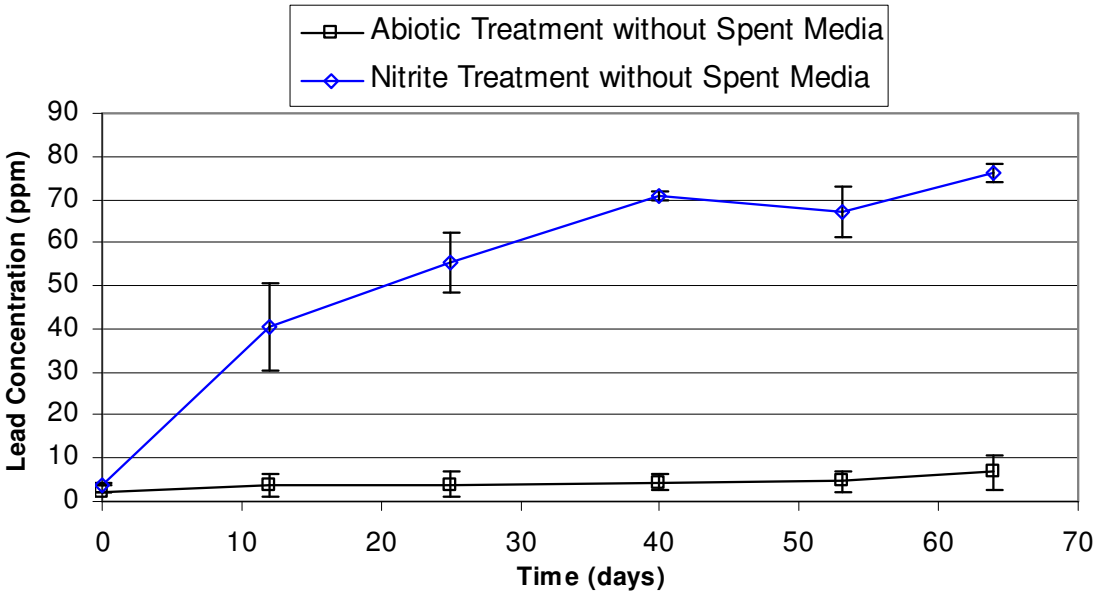


Figure 4-18: Total lead concentrations of abiotic (Experiment IA #2) and nitrite treatments (Experiment IA #8) without spent media on a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

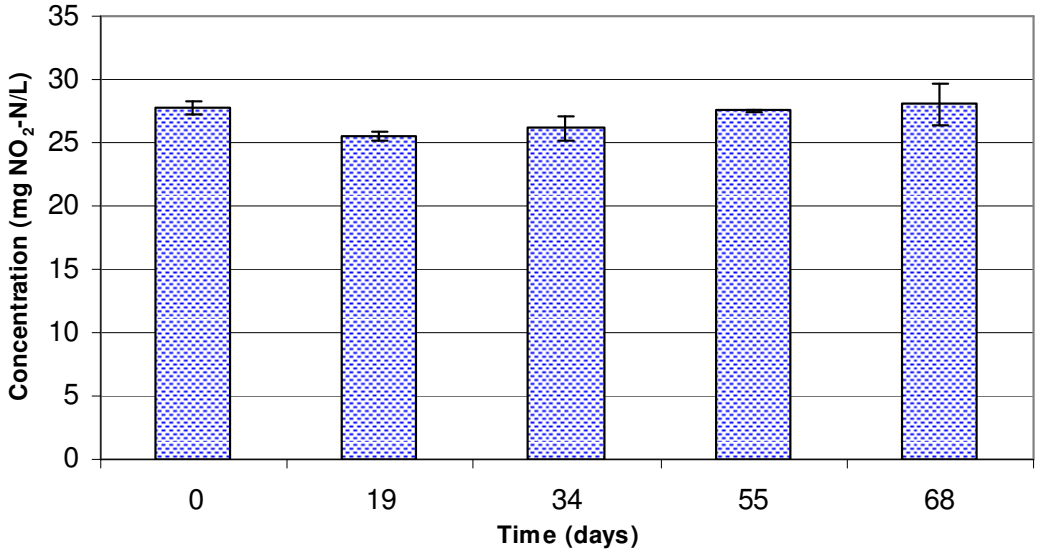


Figure 4-19: Nitrite concentrations of the nitrite treatment (Experiment IA #8) without spent media on a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

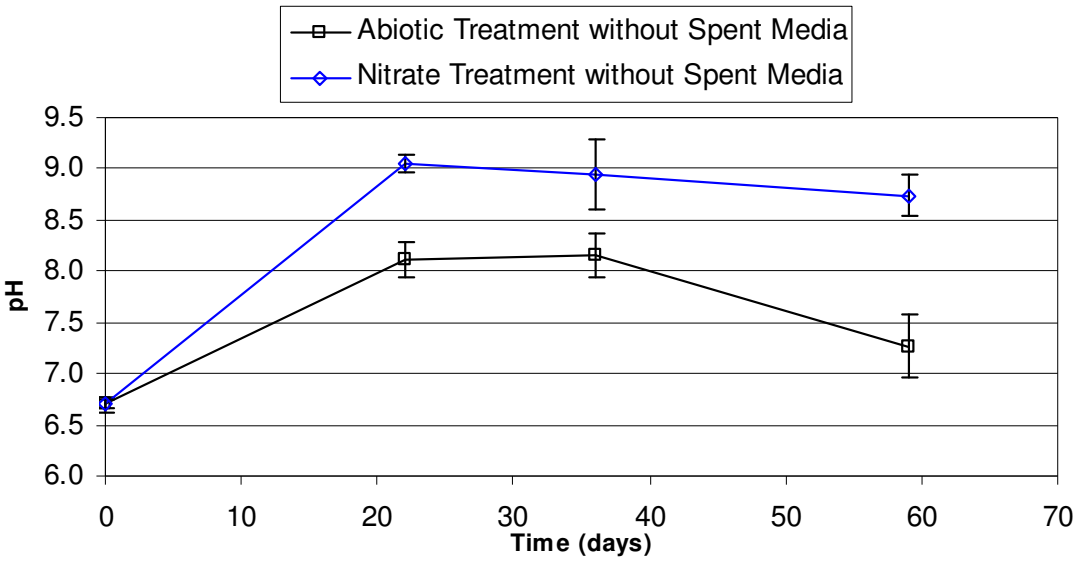


Figure 4-20: pH of abiotic (Experiment IA #2) and nitrite treatments (Experiment IA #8) without spent media on a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

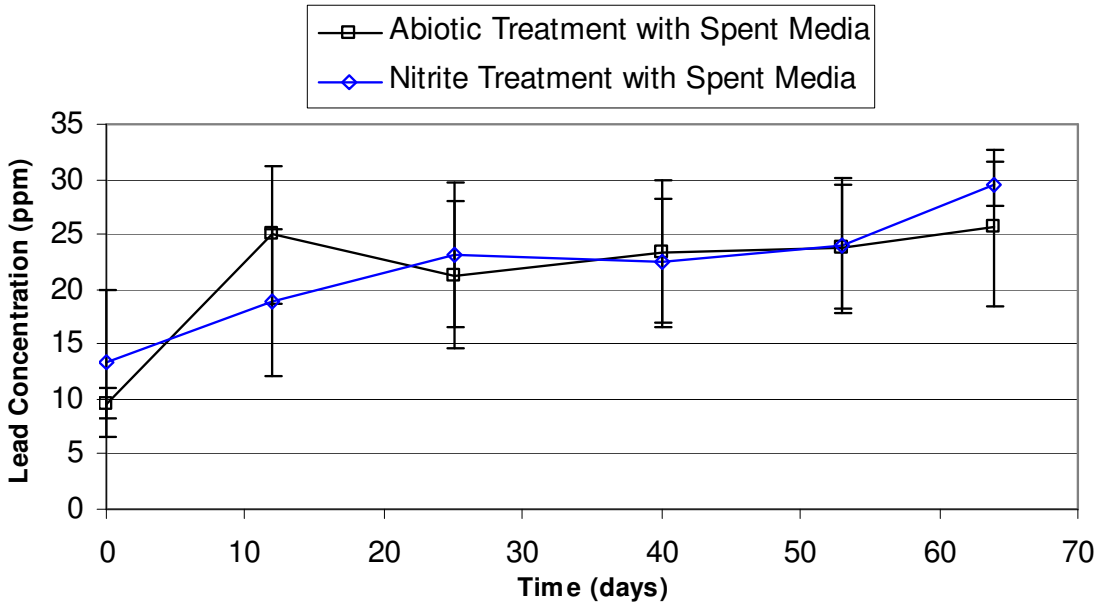


Figure 4-21: Total lead concentrations of abiotic (Experiment IA #2) and nitrite treatments (Experiment IA #8) without spent media on a freshly cleaned lead coupon; error bars represent one standard deviation for averages.

4.2 Lead Factors Study: Experiment I, Part B – Aged Pipe

Aging of lead pipe has been suggested as a method to reduce corrosion in drinking water distribution systems, especially in the presence of a corrosion inhibitor (McNeill and Edwards, 2004). Lead coupons, aged for 2 weeks in autoclaved tap water prior to beginning of the experiment, were used to determine if any difference existed in comparison to that of fresh pipe with respect to hypothesized lead corrosion factors arising from nitrification: the presence of nitrate, nitrite, a low pH, and active nitrification (biotic treatment).

4.2.1 Nitrate Treatment

The nitrate treatment was found to increase corrosion of an aged lead coupon. The presence of nitrate significantly increased lead concentrations compared to the abiotic control after 29 days (Figure 4-22). The total lead concentration increased to 3.4 times higher than the abiotic treatment after 81 days. Increasing lead concentrations coincided with an accumulation of nitrite (Figure 4-23). An initial nitrite concentration of 1.1 mg NO_2-N/L, originating from the carry-over of spent media, was detected in the nitrate and abiotic treatments on day 0 with no significant change in nitrite concentration of the abiotic treatment. The nitrite concentration rose to 2.2 mg NO_2-N/L in the nitrate treatment by day 85. Nitrate concentrations did not decrease significantly throughout the experimental period (Figure 4-24). The pH of the nitrate treatment increased to 7.57 after

15 days and remained higher than the abiotic treatment throughout the experimental period (Figure 4-25). Soluble lead concentrations were 11.9 ± 8.64 ppb and 11.0 ± 6.30 ppb for abiotic and nitrate treatments, respectively, after 89 days and were not statistically different.

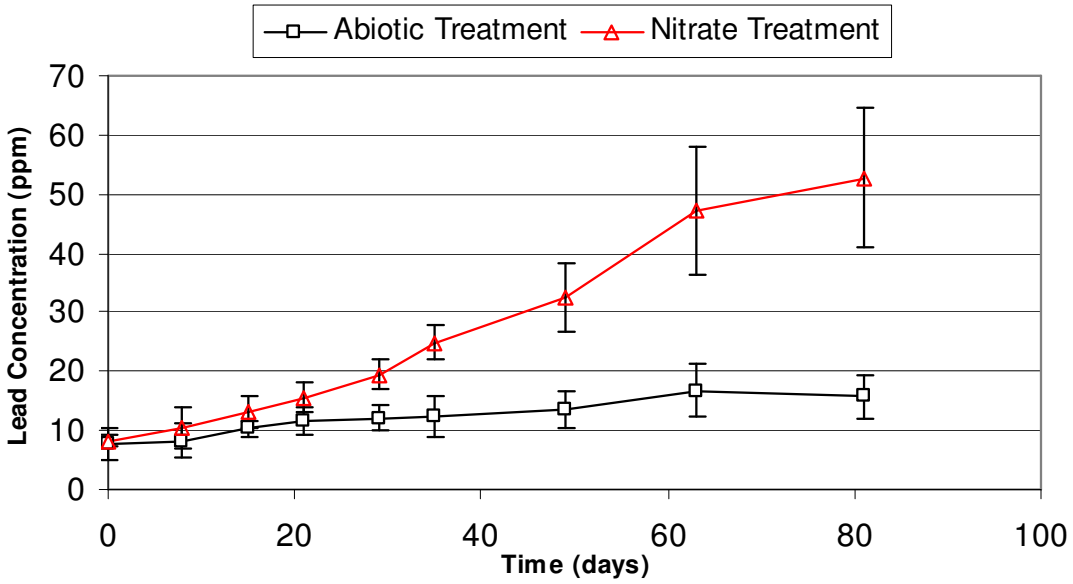


Figure 4-22: Total lead concentrations of abiotic (Experiment IB #1) and nitrate treatments (Experiment IB #5) with an aged lead coupon; error bars represent one standard deviation for averages.

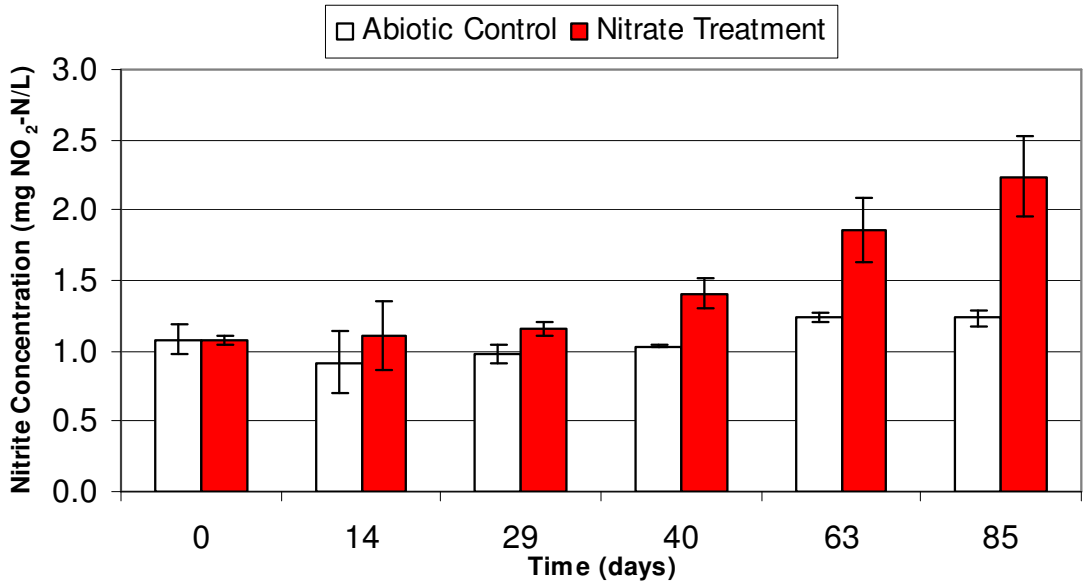


Figure 4-23: Nitrite concentrations of abiotic (Experiment IB #1) and nitrate treatments (Experiment IB #5) with an aged lead coupon ; error bars represent one standard deviation for averages.

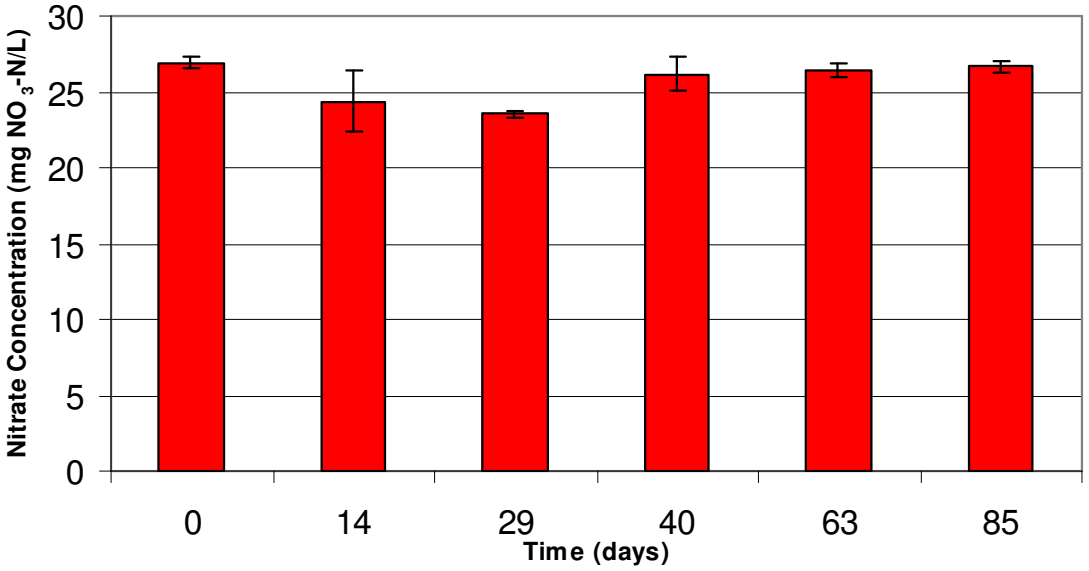


Figure 4-24: Nitrate concentration of the nitrate treatment (Experiment IB #5) with an aged lead coupon; error bars represent one standard deviation for averages.

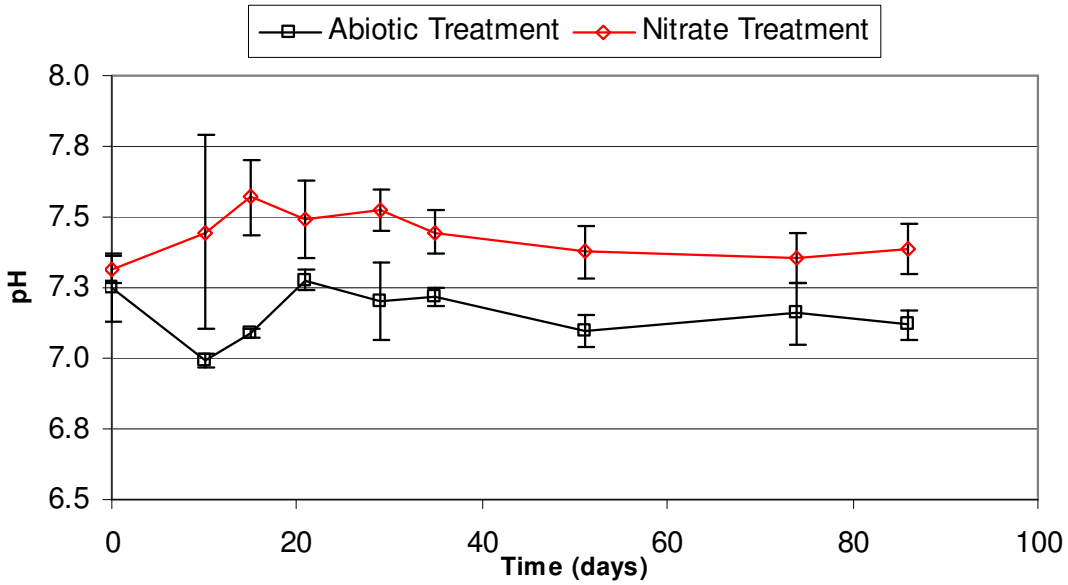


Figure 4-25: pH of abiotic (Experiment IB #1) and nitrate treatments (Experiment IB #5) with an aged lead coupon; error bars represent one standard deviation for averages.

4.2.2 Nitrite Treatment

The presence of nitrite had a significant effect on corrosion of an aged lead coupon. Lead concentrations were statistically higher than the control after day 21 and continued to increase throughout the experimental period (Figure 4-26). The total lead concentration was 4.1 times higher than the abiotic treatment after 81 days. Nitrite concentrations, however, did not decrease significantly throughout the experimental period (Figure 4-27). The pH of the nitrite treatment increased to a maximum of 7.57 after 15 days and remained higher than the abiotic control throughout the rest of the experiment (Figure 4-28). Soluble lead concentrations were 11.9 ± 8.6 ppb and 7.89 ± 2.94 ppb for the abiotic and nitrite treatments, respectively, after 89 days and were not statistically different.

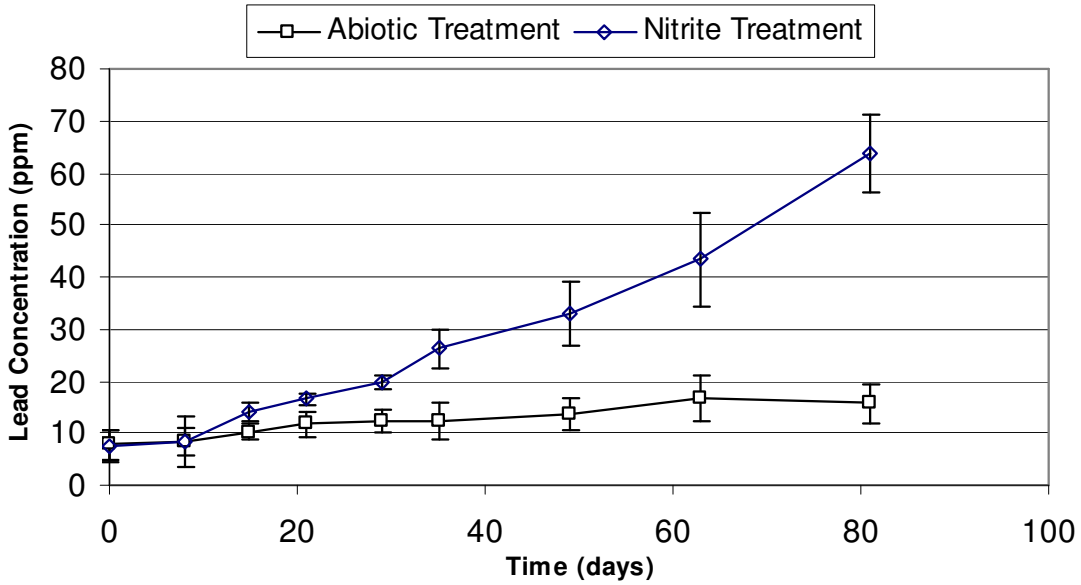


Figure 4-26: Total lead concentrations of abiotic (Experiment IB #1) and nitrite treatments (Experiment IB #7) with an aged lead coupon; error bars represent one standard deviation for averages.

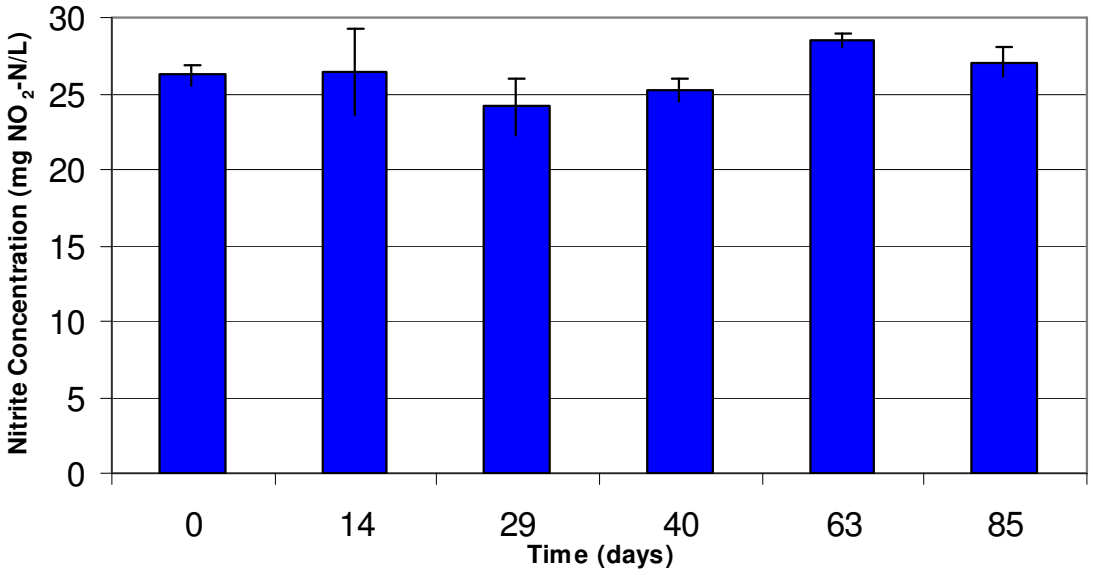


Figure 4-27: Nitrite concentrations of abiotic (Experiment IB #1) and nitrite treatments (Experiment IB #7) with an aged lead coupon; error bars represent one standard deviation for averages.

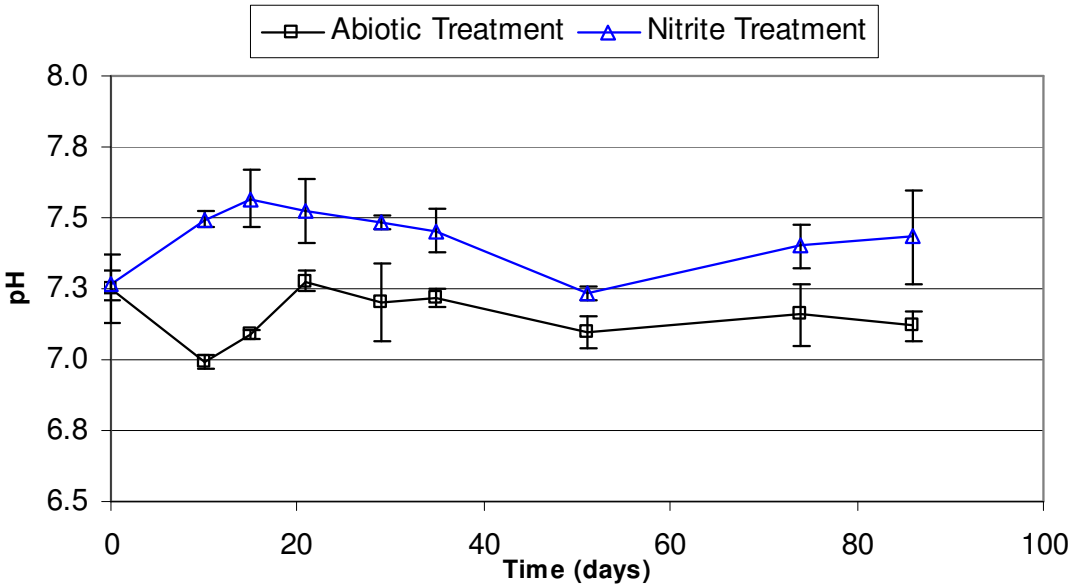


Figure 4-28: pH of abiotic (Experiment IB #1) and nitrite treatments (Experiment IB #7) with an aged lead coupon, error bars represent one standard deviation for averages.

4.2.3 Low pH Treatment

The low pH treatment had a significant effect on the corrosion of an aged lead coupon. The total lead concentration was significantly higher in the low pH treatment than in the abiotic treatment and was 6.6 times higher than the abiotic treatment after 81 days (Figure 4-29). Soluble lead concentrations were 11.9 ± 8.64 ppb and 3050 ± 2820 ppb for the abiotic and low pH treatments, respectively, after 89 days and were statistically different. Necessary pH adjustment was required every 2-3 days for the first 20 days. Afterword, necessary pH adjustment was required approximately every 5 days as the pH was slower during this time.

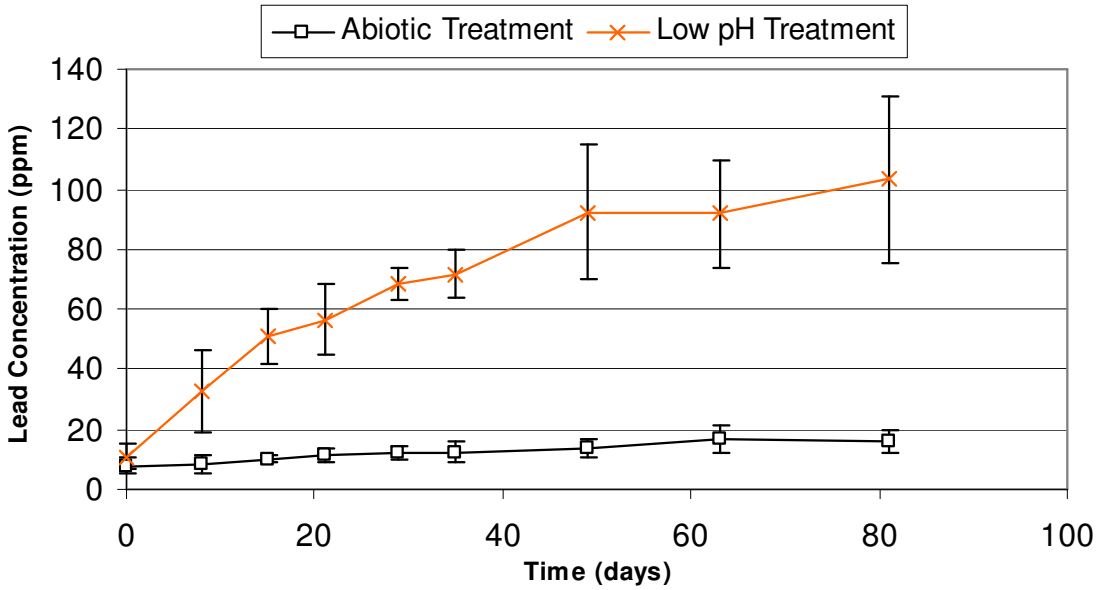


Figure 4-29: Total lead concentrations of abiotic (Experiment IB #1) and low pH treatments (Experiment IB #4) with an aged lead coupon; error bars represent one standard deviation for averages.

4.2.4 Biotic Treatment

The biotic treatment increased lead corrosion of an aged coupon. The total lead concentration of the biotic treatment increased above that of the abiotic control after 15 days (Figure 4-30). The total lead concentration continued to increase thereafter and was 5.7 times higher than the abiotic treatment after 81 days. The consumption of ammonia during nitrification resulted in both a drop of pH and generation of nitrite. The pH of the biotic treatment also decreased from 7.17 to 6.08 after 10 days (Figure 4-31). The ammonia concentration decreased below the abiotic control reaching a maximum consumption of 10.8 mg/L NH_3-N after 74 days (Figure 4-32). The pH began increasing after day 50 reaching 6.56 after 86 days. The nitrite concentration increased during this

time, reaching a maximum of 5.6 mg/L NO_2-N after 63 days (Figure 4-33). Ammonia consumption and nitrite formation occurred concurrently in a near stoichiometric fashion (Figure 4-34). Soluble lead concentrations were 11.9 ± 8.64 ppb and 320 ± 176 ppb for the abiotic and biotic treatments, respectively, after 89 days and were statistically different.

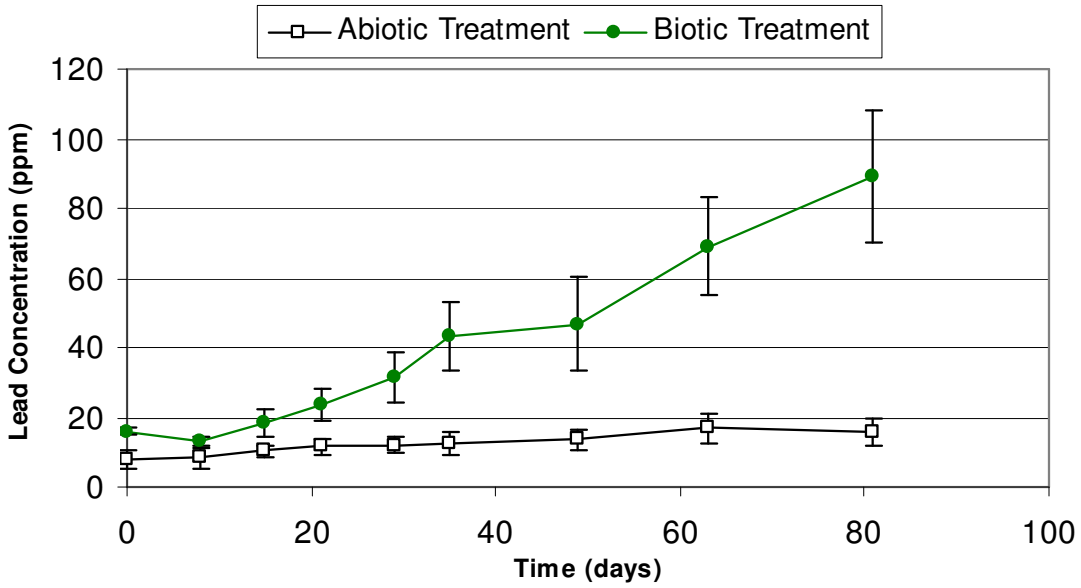


Figure 4-30: Total lead concentrations of abiotic (Experiment IB #1) and biotic treatments (Experiment IB #3) with an aged lead coupon; error bars represent one standard deviation for averages.

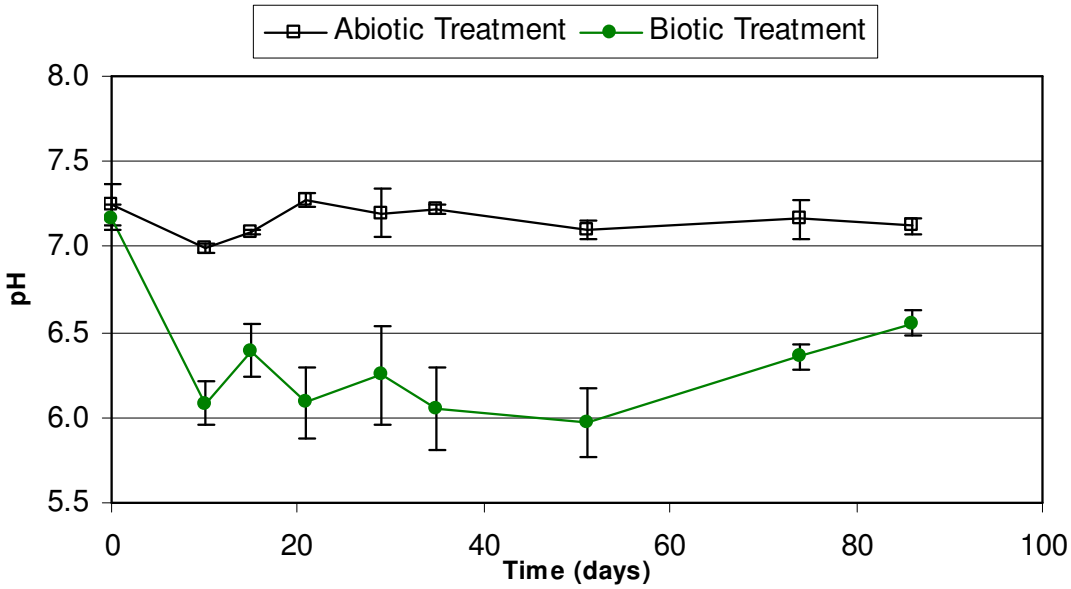


Figure 4-31: pH of abiotic (Experiment IB #1) and biotic treatments (Experiment IB #3) with an aged lead coupon; error bars represent one standard deviation for averages.

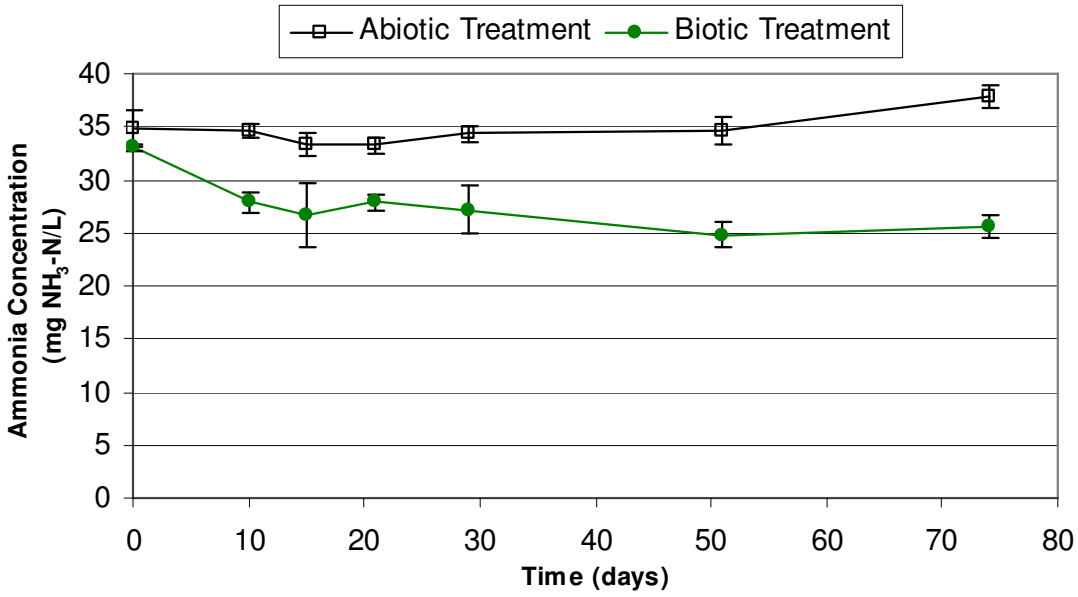


Figure 4-32: Ammonia concentration of abiotic (Experiment IB #1) and biotic treatments (Experiment IB #3) with an aged lead coupon; error bars represent one standard deviation for averages.

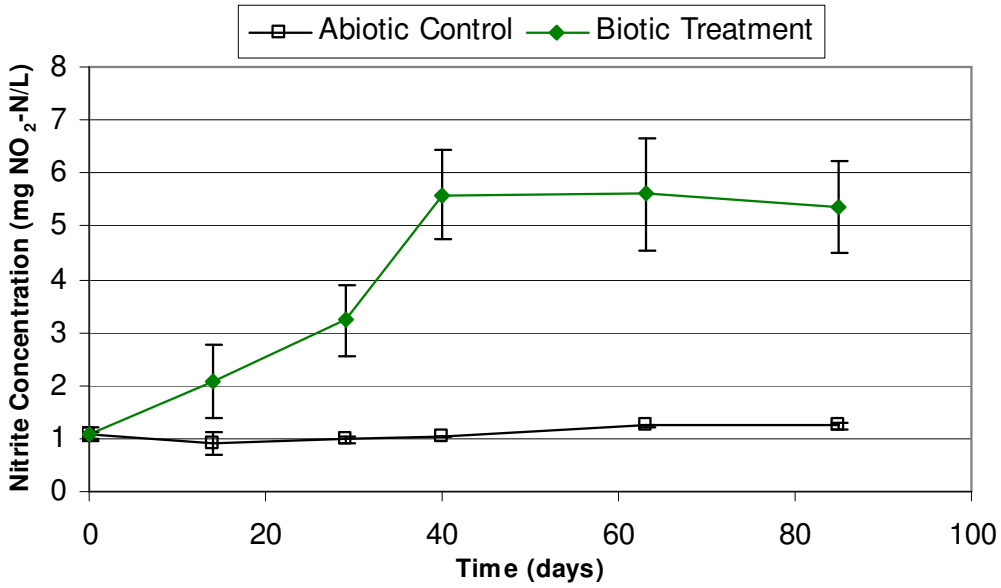


Figure 4-33: Nitrite concentrations of abiotic (Experiment IB #1) and biotic treatments with an aged lead coupon (Experiment IB #3); error bars represent one standard deviation for averages.

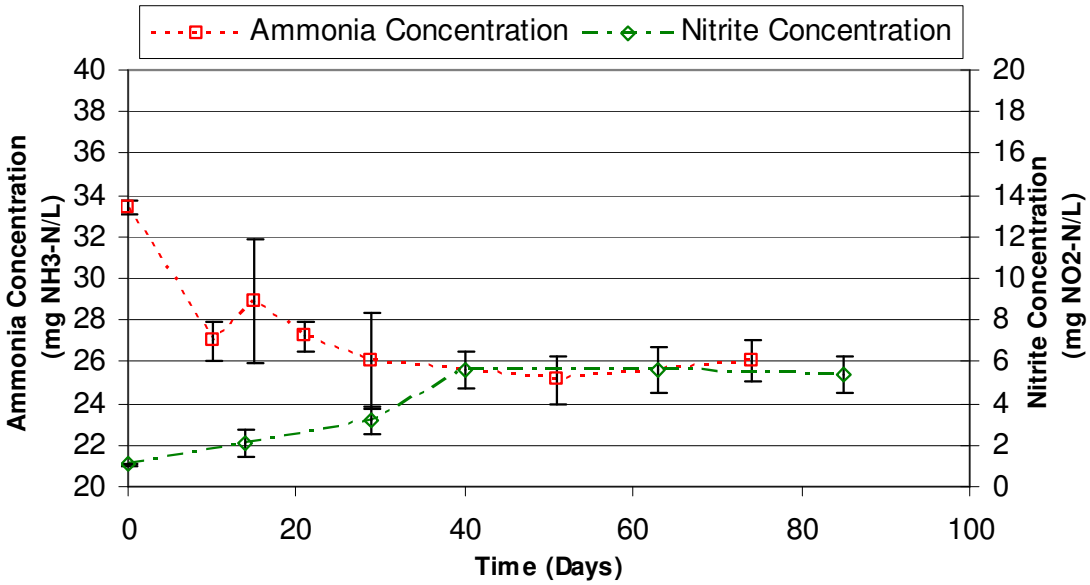


Figure 4-34: Comparison of nitrite and ammonia concentrations for the biotic treatment with a freshly cleaned lead coupon (Experiment IB #3); error bars represent one standard deviation for averages.

4.2.5 Nitrate Treatment without Spent Media

The presence of nitrate significantly increased corrosion of an aged lead coupon above the abiotic treatment when not amended with spent media (Figure 4-35). The total lead concentration was 5.1 times higher than the abiotic treatment without spent media after 55 days. The nitrite concentration increased throughout the experimental period indicating abiotic denitrification of nitrate (Figure 4-36) with 6.0 mg NO_2-N/L accumulation by day 63. Nitrate also decreased throughout the experimental period in a stoichiometric fashion, by 9.0 mg NO_3-N/L after 63 days. A small amount of ammonia, 0.18 ± 0.07 mg NH_3-N/L, accumulated after 58 days in the nitrate treatment without spent media while ammonia was not detected in the abiotic treatment without spent media. The nitrate treatment without spent media, again, had significantly higher total lead concentrations than the nitrate treatment with spent media (Figure 4-37). The pH of the nitrate treatment without spent media was significantly higher than the abiotic treatment without spent media for the entire experimental period (Figure 4-38). Soluble lead concentrations were 23.0 ± 21.6 ppb and 142 ± 84.2 ppb for the abiotic treatment without spent media and the nitrate treatment without spent media, respectively, after 63 days and were statistically different.

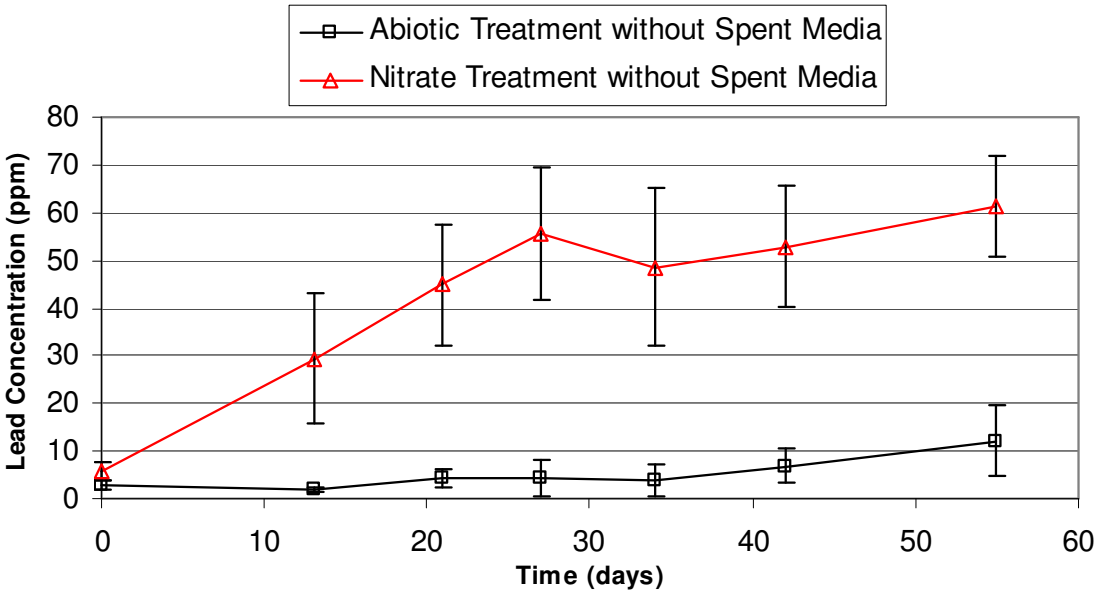


Figure 4-35: Total lead concentrations of abiotic (Experiment IB #2) and nitrate treatments without spent media on an aged lead coupon (Experiment IB #6); error bars represent one standard deviation for averages.

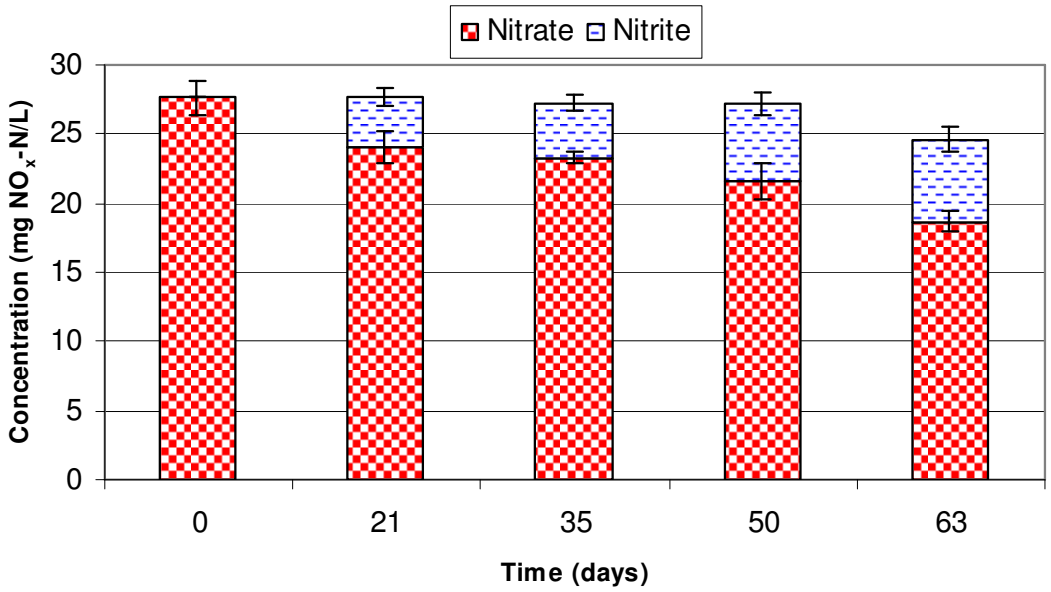


Figure 4-36: Nitrate and nitrite concentrations of the nitrate treatment without spent media on an aged lead coupon (Experiment IB #6); nitrite and nitrate were never detected in the abiotic treatment without spent media (Experiment IB #2) ; error bars represent one standard deviation for averages.

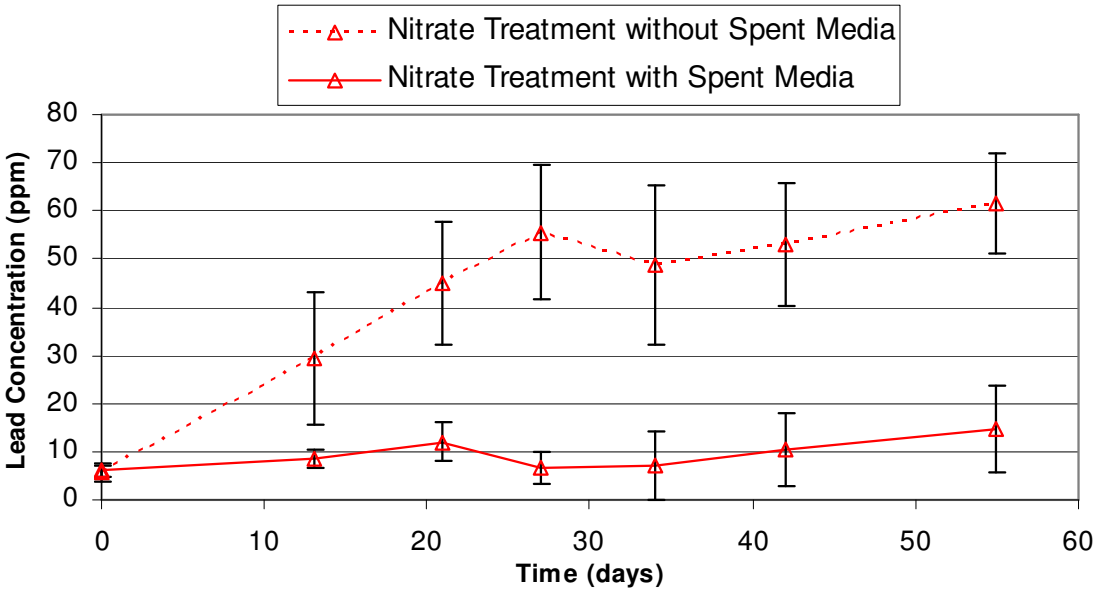


Figure 4-37: Total lead concentrations of nitrate treatment with spent media (Experiment IB #5) and nitrate treatment without spent media (Experiment IB #6) on an aged lead coupon; error bars represent one standard deviation for averages.

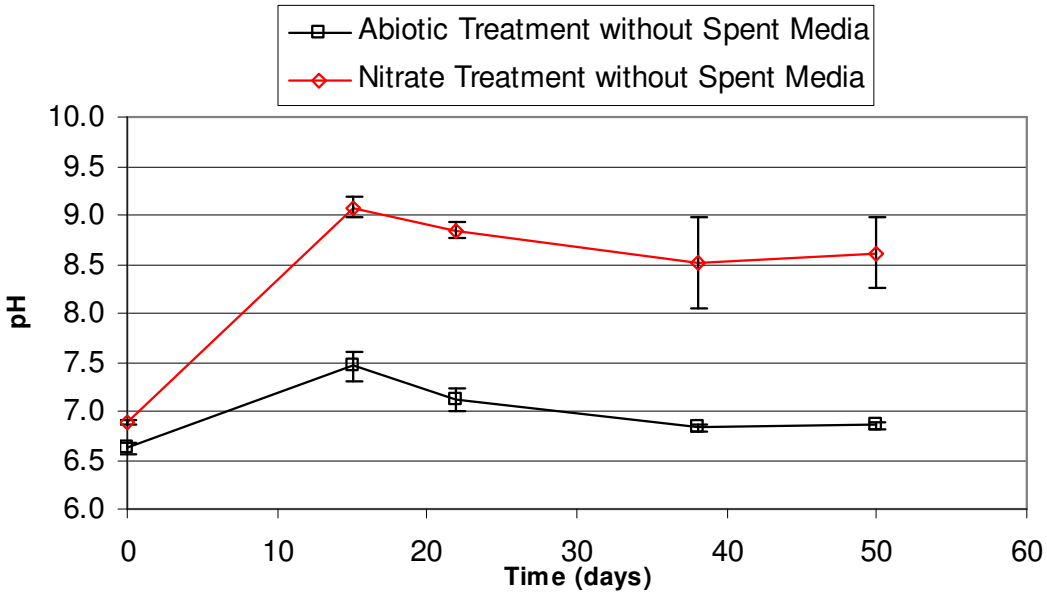


Figure 4-38: pH of the abiotic (Experiment IB #2) and nitrate treatments without spent media (Experiment IB #6); error bars represent one standard deviation for averages.

4.2.6 Nitrite Treatment without Spent Media

The presence of nitrite significantly increased corrosion of an aged lead coupon when spent media was not added. The total lead concentration increased rapidly after beginning the experiment and was 6.9 times higher than the abiotic treatment without spent media after 55 days (Figure 4-39). The nitrite concentration did not decrease (Figure 4-40). Ammonia accumulated at a concentration of 0.18 ± 0.04 mg NH_3-N/L after 58 days in the nitrate treatment without spent media while no ammonia detected in the abiotic treatment without spent media. The nitrite treatment without spent media had significantly higher total lead concentrations than the nitrite treatment with spent media (Figure 4-41). The pH of the nitrite treatment without spent media was significantly higher than the abiotic treatment without spent media for the entire experimental period (Figure 4-42). The soluble lead concentrations were 23.0 ± 21.6 ppb and 125 ± 47.3 ppb for the abiotic treatment without spent media and the nitrite treatment without spent media, respectively, after 63 days and were statistically different.

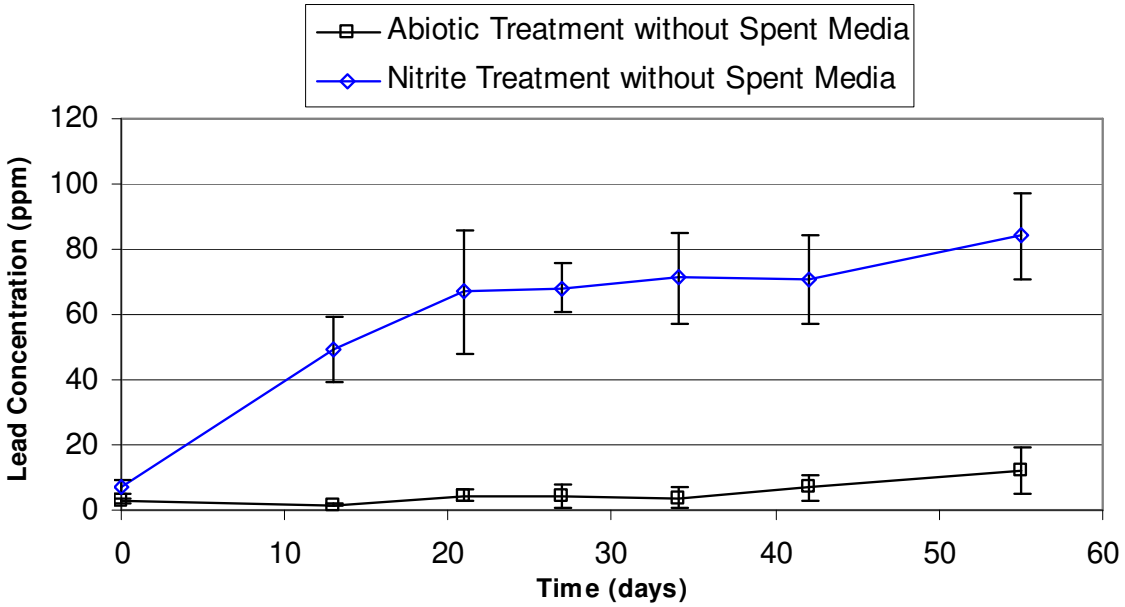


Figure 4-39: Total lead concentrations of abiotic (Experiment IB #2) and nitrite treatments without spent media on an aged lead coupon (Experiment IB #8); error bars represent one standard deviation for averages.

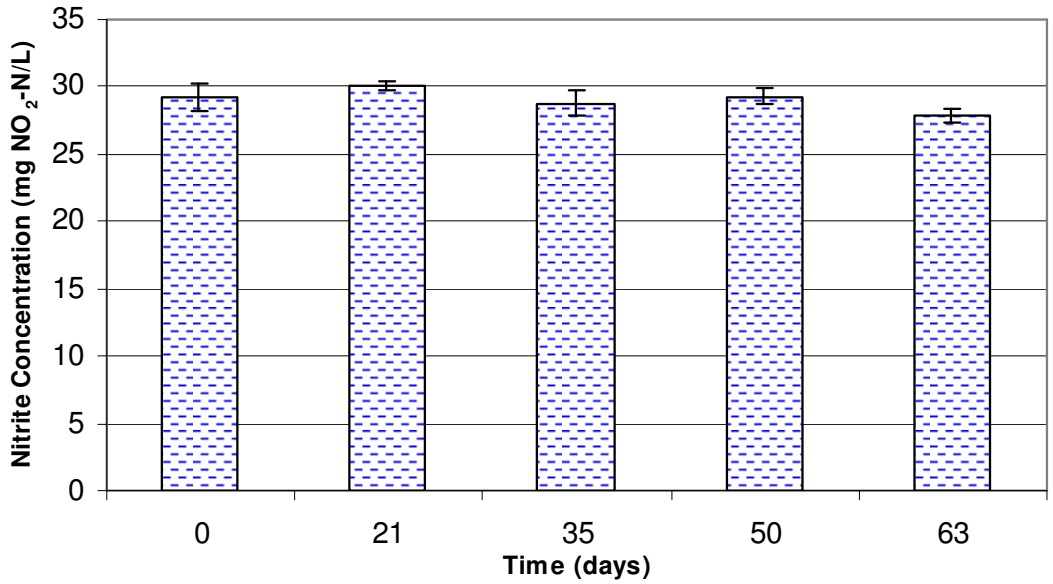


Figure 4-40: Nitrite concentrations of the nitrite treatment without spent media on an aged lead coupon (Experiment IB #8); nitrite and nitrate were never detected in the abiotic treatment without spent media (Experiment IB #2); error bars represent one standard deviation for averages.

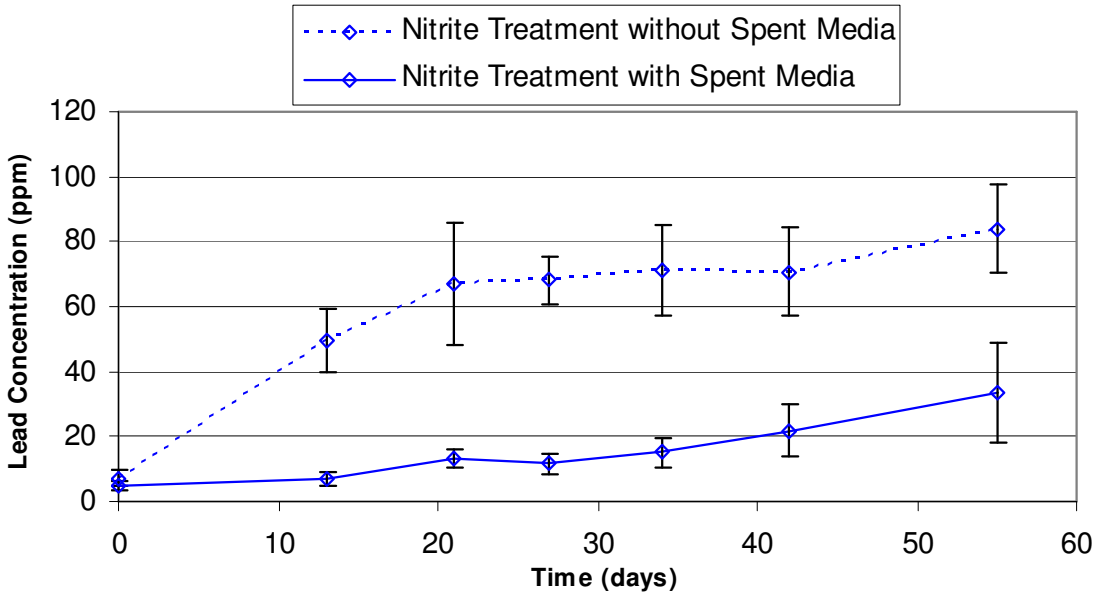


Figure 4-41: Total lead concentrations of nitrite treatment with spent media (Experiment IB #7) and nitrite treatment without spent media (Experiment IB #8) on an aged lead coupon; error bars represent one standard deviation for averages.

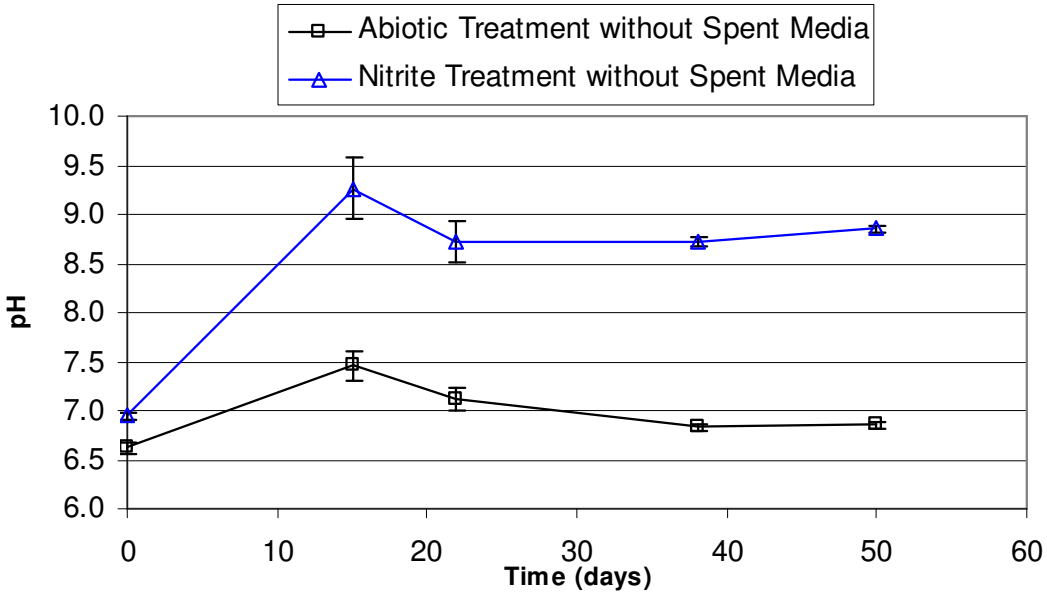


Figure 4-42: pH of the abiotic (Experiment IB #2) and nitrite treatments without spent media (Experiment IB #8); error bars represent one standard deviation for averages.

4.3 Lead Corrosion Inhibitor Study

The effect of several different lead corrosion inhibitors (orthophosphate, alkalinity, pH control, and zinc orthophosphate) were examined under nitrifying conditions. Experimental designs are shown in Experiment II Part A for orthophosphate and alkalinity treatments, Experiment II Part B for the pH adjustment treatment, and Experiment II Part C for the zinc orthophosphate treatment. Each inhibitor was also examined in an abiotic environment. Aged coupons were used for this study.

4.3.1 Orthophosphate

Orthophosphate was observed to reduce lead corrosion under abiotic and biotic conditions. This is shown in Figure 4-43 and Figure 4-44. Orthophosphate dosing significantly reduced the total lead concentration by an average of 37.9% for abiotic treatments and 30.1% for biotic treatments compared to controls after 76 days. Lead corrosion inhibition was only considered significant between days 47 - 60 for the biotic-orthophosphate treatment while the abiotic-orthophosphate treatment was considered significant after day 25. No significant difference was observed for soluble lead concentrations in abiotic controls or abiotic-orthophosphate treatments as they were 79.7 ± 7.97 ppb and 94.8 ± 49.7 ppb, respectively. However, the biotic-orthophosphate treatment had significantly higher soluble lead concentrations than biotic controls with 441 ± 22.7 ppb and 262 ± 94.9 ppb, respectively. The pH of both the abiotic control and abiotic-orthophosphate treatments remained very similar over the experiment (Figure 4-45). The pH of the biotic control began increasing more quickly after 76 days than the biotic orthophosphate treatment. No significant difference was noted between the

ammonia consumption (Figure 4-46) and nitrite production (Figure 4-47) of biotic controls and the biotic-orthophosphate treatment. Also, no difference was observed in ammonia or nitrite concentrations in abiotic controls and abiotic-orthophosphate treatments. However, nitrite was completely converted to nitrate in one of the three biotic control replicates after 97 days (Figure 4-48). Two of three biotic-orthophosphate replicates similarly converted nitrite to nitrate after 97 days.

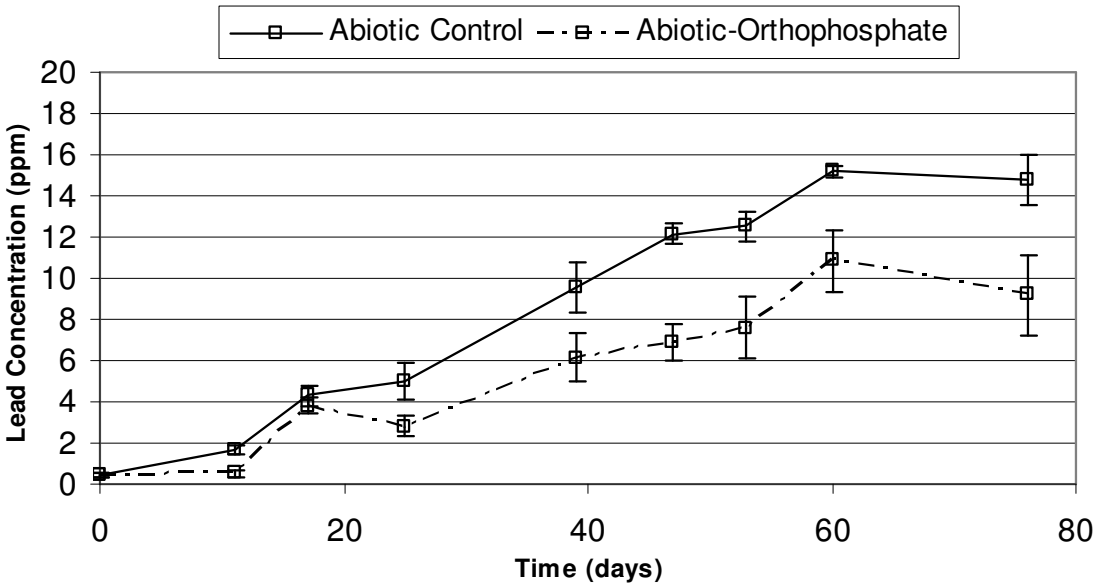


Figure 4-43: Total lead concentrations of abiotic control (Experiment IIA #1) and abiotic-orthophosphate treatments (Experiment IIA #3); orthophosphate dose of 5 mg/L PO_4; error bars represent one standard deviation for averages.

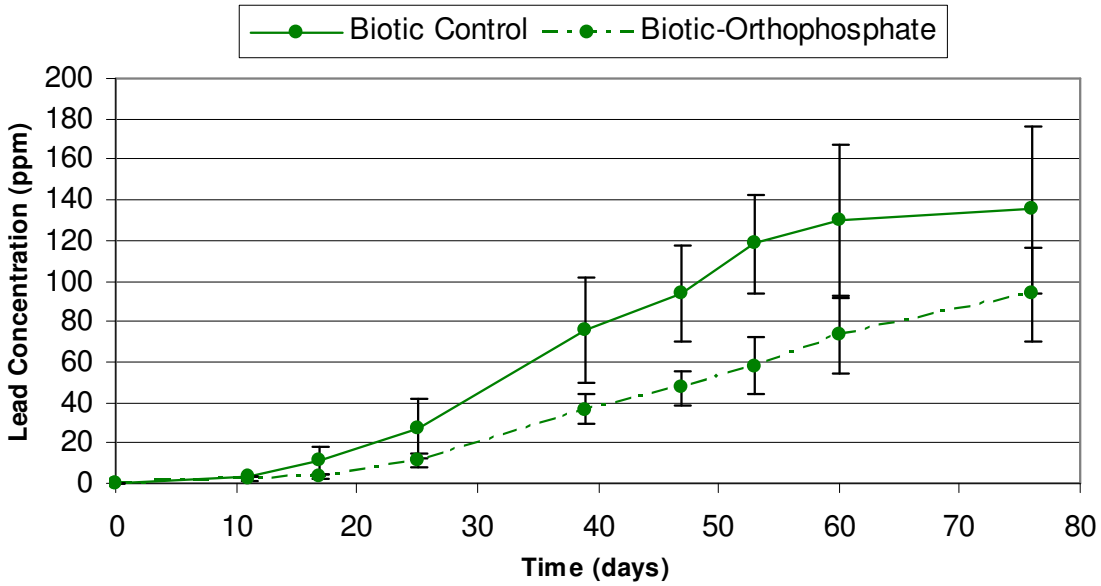


Figure 4-44: Total lead concentrations of biotic control (Experiment IIA #2) and biotic-orthophosphate treatments (Experiment IIA #4); orthophosphate dose of 5 mg/L PO_4; error bars represent one standard deviation for averages.

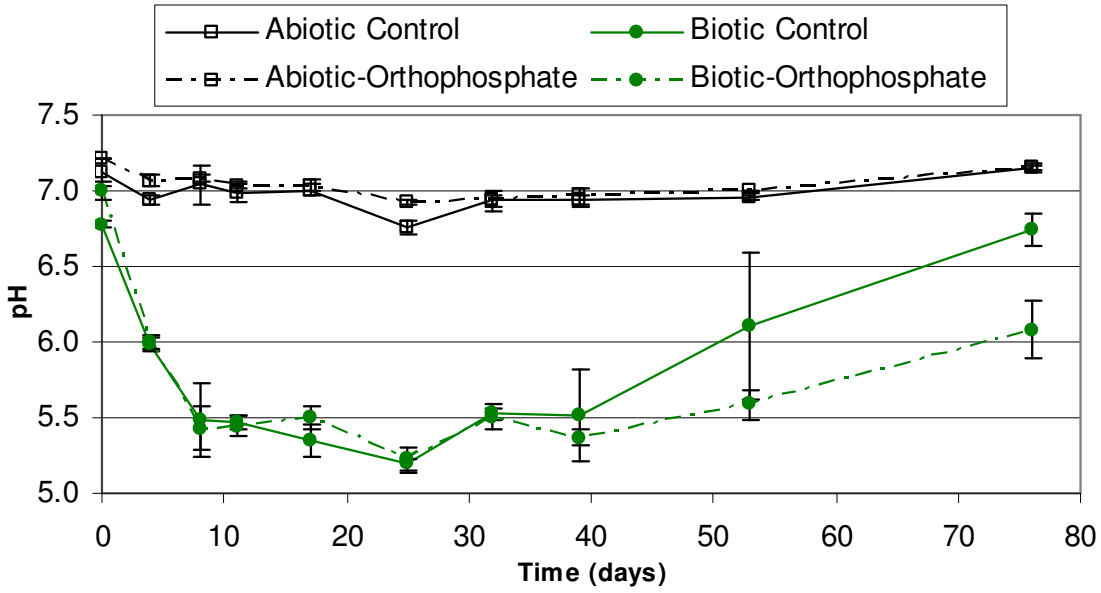


Figure 4-45: pH of abiotic control (Experiment IIA #1), abiotic-orthophosphate (Experiment IIA #3), biotic control (Experiment IIA #2), and biotic-orthophosphate treatments (Experiment IIA #4); orthophosphate dose of 5 mg/L PO_4, error bars represent one standard deviation for averages.

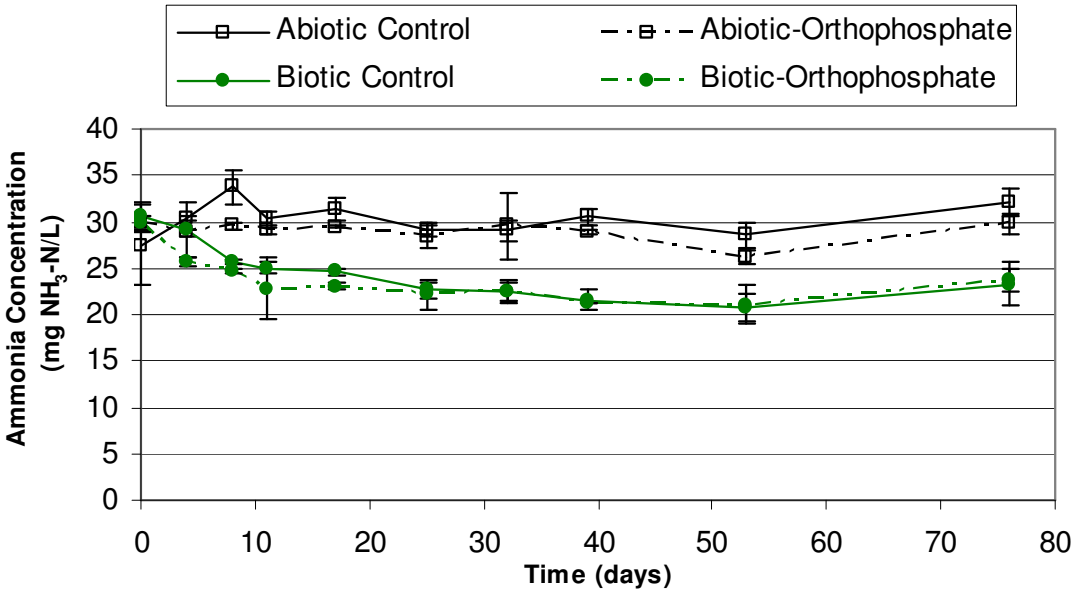


Figure 4-46: Ammonia concentrations of abiotic control (Experiment IIA #1), abiotic-orthophosphate (Experiment IIA #3), biotic control (Experiment IIA #2), and biotic-orthophosphate treatments (Experiment IIA #4); orthophosphate dose of 5 mg/L PO_4; error bars represent one standard deviation for averages.

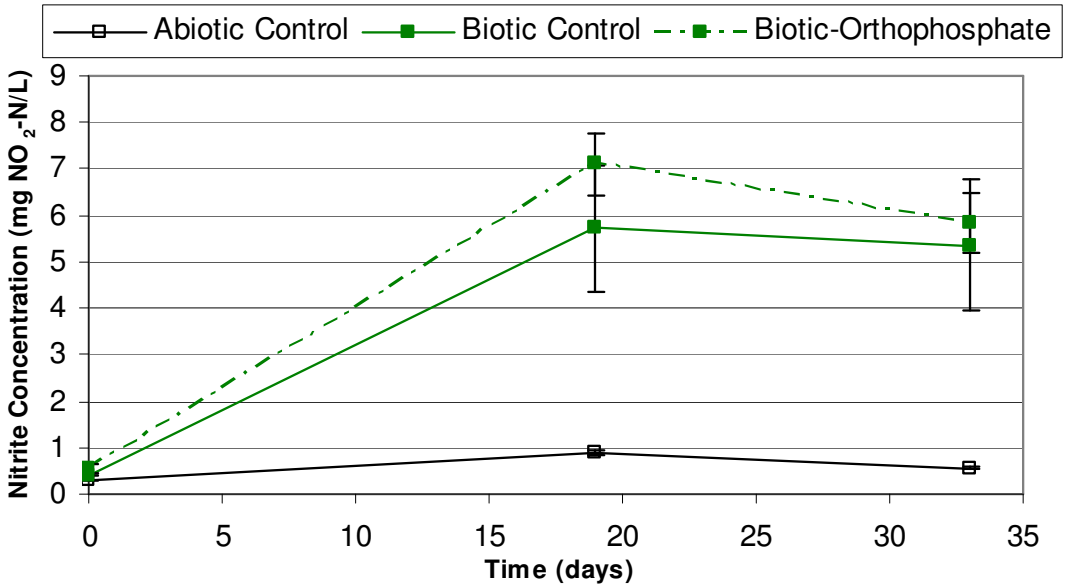


Figure 4-47: Nitrite concentrations of abiotic control (Experiment IIA #1), biotic control (Experiment IIA #2), and biotic-orthophosphate treatments (Experiment IIA #4); orthophosphate dose of 5 mg/L PO_4; error bars represent one standard deviation for averages.

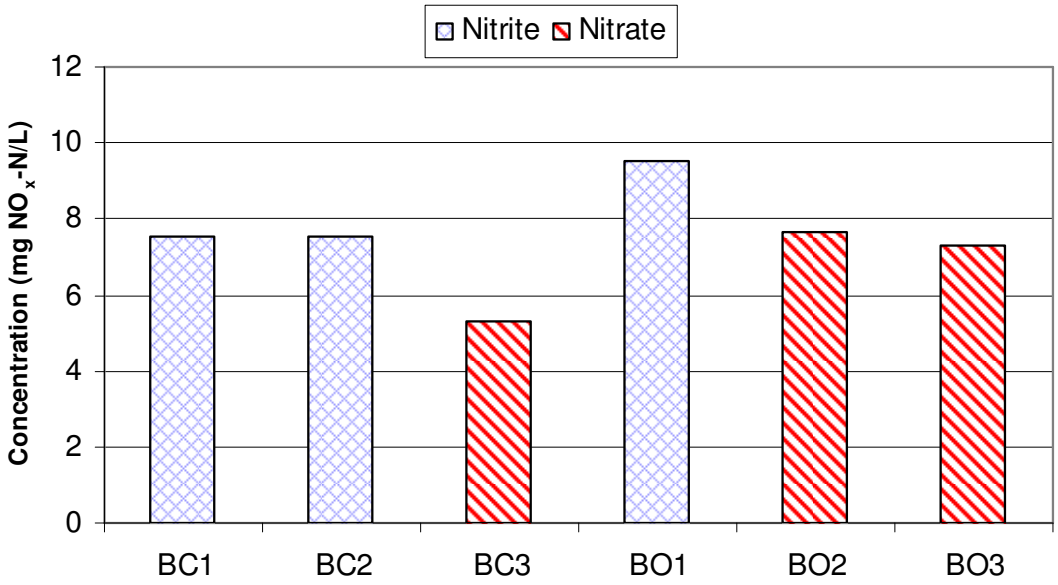


Figure 4-48: Nitrite or nitrate concentration of biotic control replicates (BC1, BC2, BC3) (Experiment IIA #2) and biotic-orthophosphate replicates (BO1, BO2, BO3) (Experiment IIA #4) after 93 days; orthophosphate dose of 5 mg/L PO_4.

4.3.2 Alkalinity

Alkalinity dosing with 50 mg/L as $CaCO_3$ effectively reduced lead corrosion under abiotic conditions (Figure 4-49). The biotic alkalinity treatment, however, did not significantly decrease lead corrosion during the experimental period (Figure 4-50). The large variance between replicates made this result statistically insignificant and p-values were below 0.15 for days 11–60. Abiotic-alkalinity treatments reduced the total lead concentration by 30.9% compared to abiotic controls after day 76. Soluble lead concentrations for abiotic-alkalinity treatments were not significantly different compared to abiotic controls as they were 64.3 ± 7.81 ppb and 79.7 ± 7.97 ppb, respectively. Biotic-alkalinity treatments were, however, higher than biotic controls with 444.0 ± 82.2 ppb and 262.4 ± 94.9 ppb of soluble lead, respectively.

The pH of alkalinity treatments was much higher at day 0 than the controls, 8.0 and 7.1, respectively (Figure 4-51). The pH of the abiotic-alkalinity treatment slowly decreased to 7.5 after which no further change was observed. The biotic-alkalinity remained in the growth phase for a longer period of time due to the increased buffering and reached a minimum of 5.5 after 39 days. The pH of both the biotic control and biotic-alkalinity treatment increased to near that of the abiotic control after 97 days. The biotic-alkalinity treatment consumed more ammonia than the biotic controls after 32 days, 11.5 mg NH_3-N/L and 6.6 mg NH_3-N/L, respectively (Figure 4-52). Increased nitrite production was also observed in the biotic-alkalinity treatment than biotic controls, 11.3 mg NO_2-N/L and 5.4 mg NO_2-N/L, respectively (Figure 4-53).

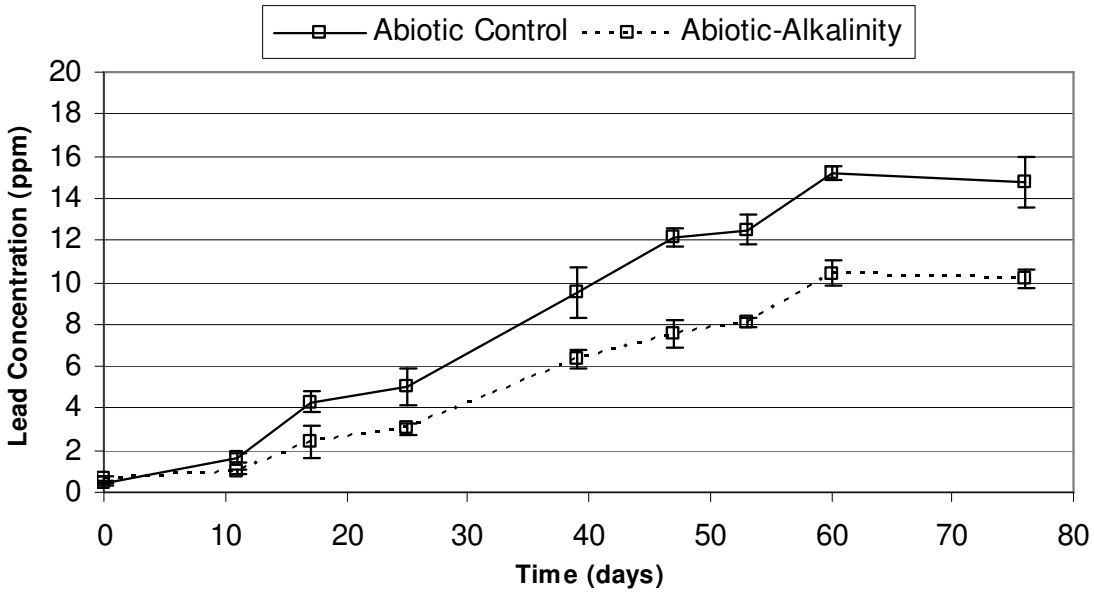


Figure 4-49: Total lead concentrations of abiotic control (Experiment IIA #1) and abiotic-alkalinity treatments (Experiment IIA #5); alkalinity dose of 50 mg/L $CaCO_3$; error bars represent one standard deviation for averages.

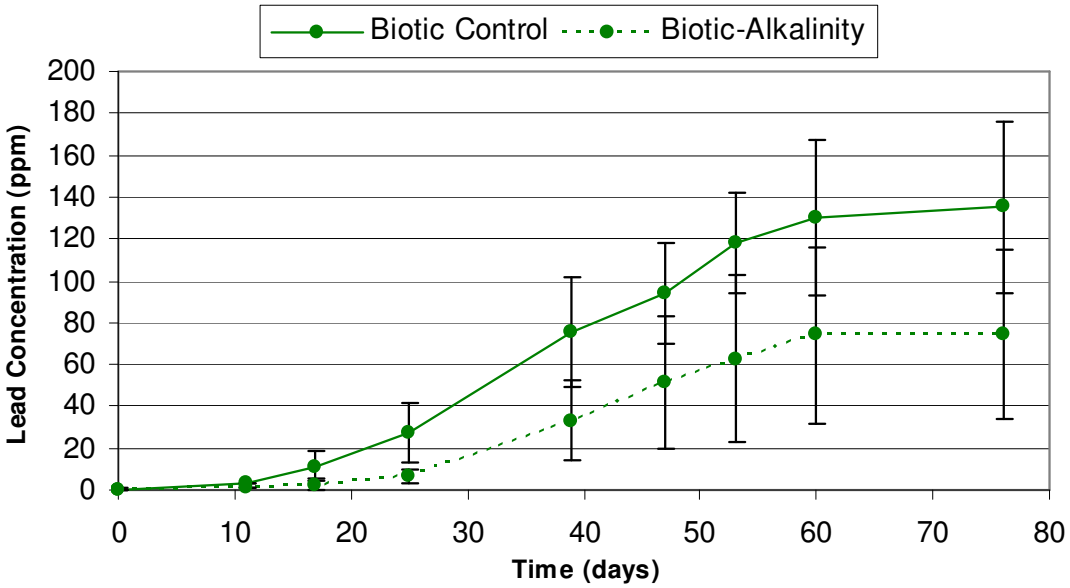


Figure 4-50: Total lead concentrations of biotic control (Experiment IIA #2) and biotic-alkalinity treatments (Experiment IIA #6); alkalinity dose of 50 mg/L $CaCO_3$; error bars represent one standard deviation for averages.

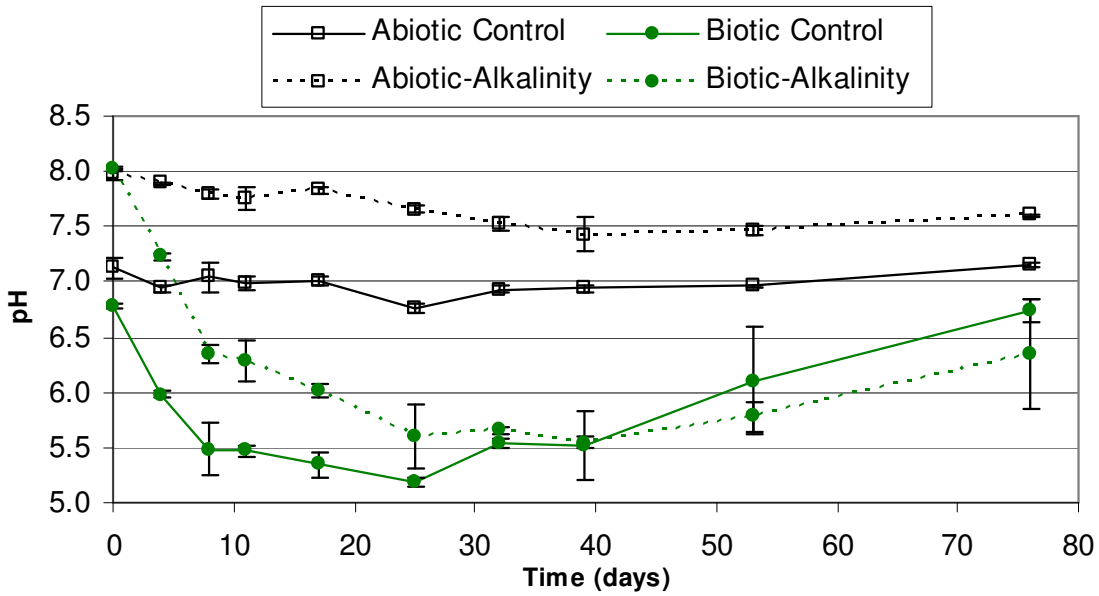


Figure 4-51: pH of abiotic control (Experiment IIA #1), abiotic-alkalinity (Experiment IIA #5), biotic control (Experiment IIA #2), and biotic-alkalinity treatments (Experiment IIA #6); alkalinity dose of 50 mg/L $CaCO_3$; error bars represent one standard deviation for averages.

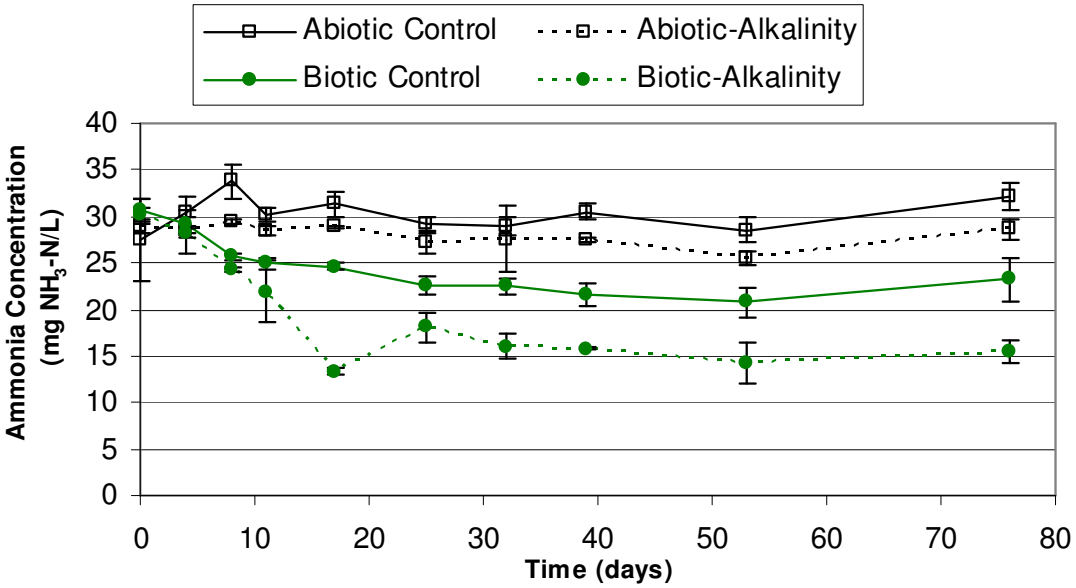


Figure 4-52: Ammonia concentration of abiotic control (Experiment IIA #1), abiotic-alkalinity (Experiment IIA #5), biotic control (Experiment IIA #2), and biotic-alkalinity treatments (Experiment IIA #6); alkalinity dose of 50 mg/L $CaCO_3$; error bars represent one standard deviation for averages.

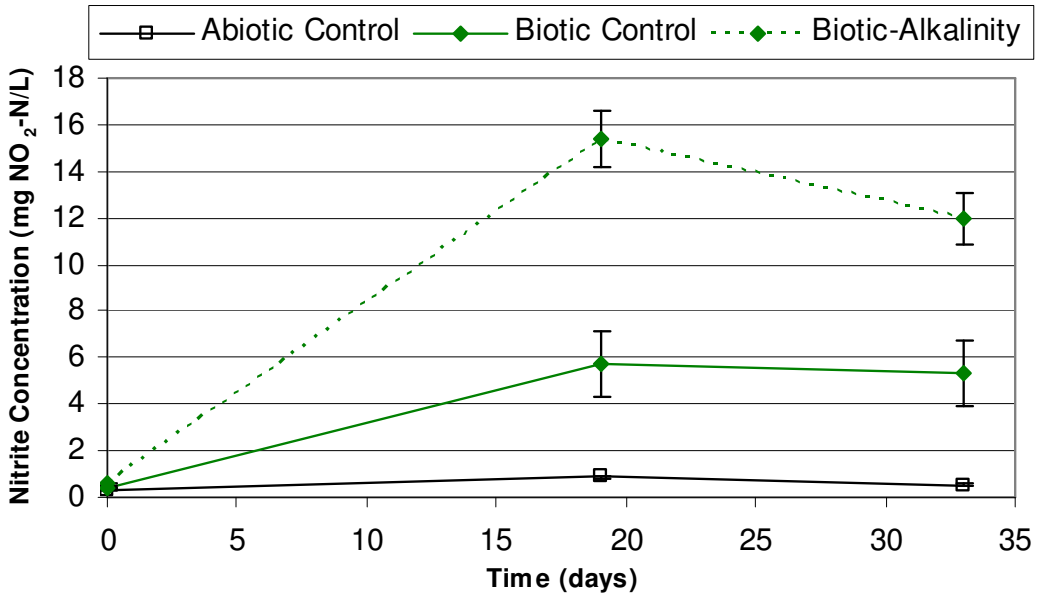


Figure 4-53: Nitrite concentrations of abiotic control (Experiment IIA #1), biotic control (Experiment IIA #2), and biotic-alkalinity treatment (Experiment IIA #6), alkalinity dose of 50 mg/L $CaCO_3$; error bars represent one standard deviation for averages.

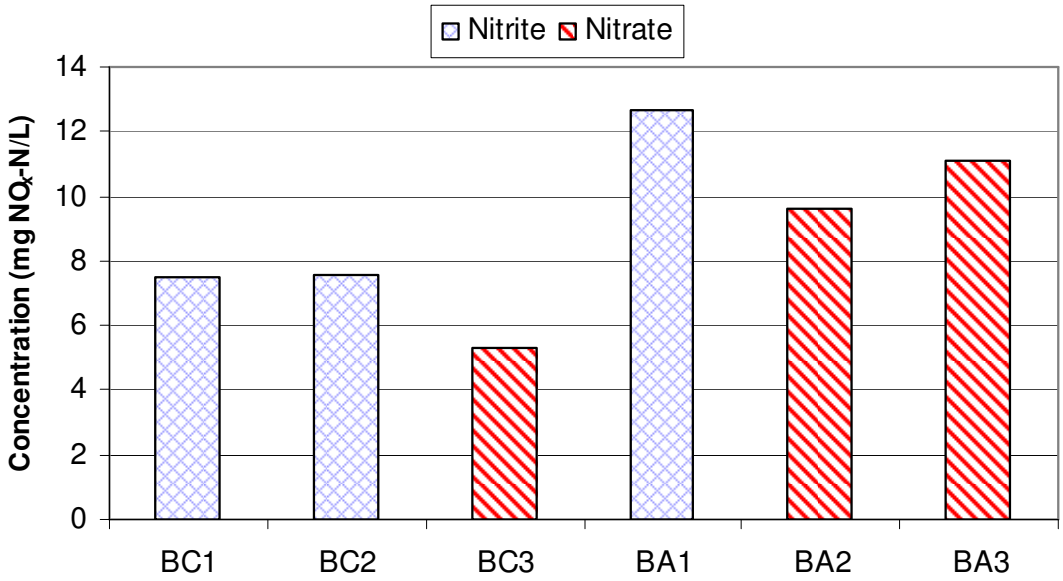


Figure 4-54: Nitrate or nitrite concentration of biotic control replicates (BC1, BC2, BC3) (Experiment IIA #2) and biotic-alkalinity replicates (BA1, BA2, BA3) (Experiment IIA #6) after 97 days, alkalinity dose of 50 mg/L $CaCO_3$.

As with the biotic-orthophosphate treatment, two of three biotic-alkalinity replicates converted all nitrite to nitrate after 97 days (Figure 4-54).

4.3.3 pH Adjustment

Addition of sodium hydroxide for pH control significantly reduced total lead concentrations. pH of the biotic-pH adjustment treatment was maintained at 7.5 ± 0.5 for the entire experiment. Lead corrosion in the biotic-pH adjustment treatment was significantly reduced after day 21 and continued to be for the rest of the experimental period. The biotic-pH adjustment treatment reduced the total lead concentration by 86.9% compared to the biotic control after 81 days (Figure 4-55). Soluble lead concentrations were 11.9 ± 8.64 ppb, 90.3 ± 78.3 ppb, and 320 ± 176 ppb for the abiotic control, biotic-pH adjustment treatment, and the biotic control, respectively, after 89

days. The soluble lead concentration of the biotic-pH adjustment treatment was statistically different from the biotic control.

The pH of the biotic control decreased rapidly reaching a minimum of 5.75 after 34 days. Addition of sodium hydroxide to the biotic-pH adjustment treatment maintained the pH from 6.5 to 7.5 for the entire experiment. The biotic-pH adjustment treatment consumed almost all available ammonia after 24 days (Figure 4-56). Nitrite concentrations increased much higher than those of the biotic control after 85 days, 26.7 mg NO_2-N/L versus 5.4 mg NO_2-N/L, respectively (Figure 4-57).

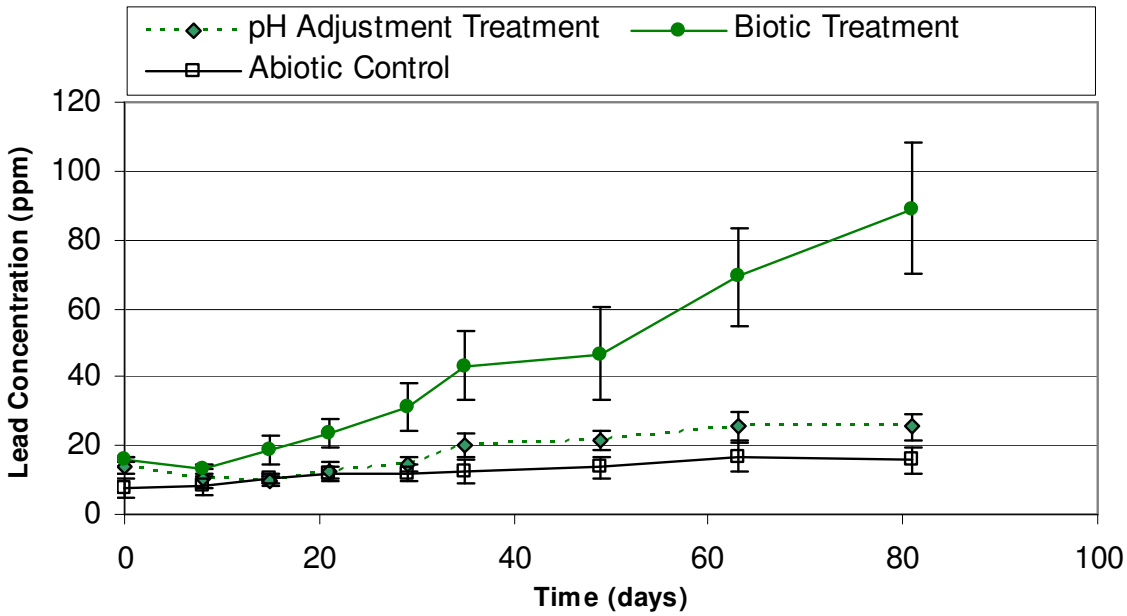


Figure 4-55: Total lead concentrations of abiotic control (Experiment IIB #1), biotic control (Experiment IIB #2), and high pH treatment (Experiment IIB #3), pH maintained at 7.5 ± 0.5; error bars represent one standard deviation for averages.

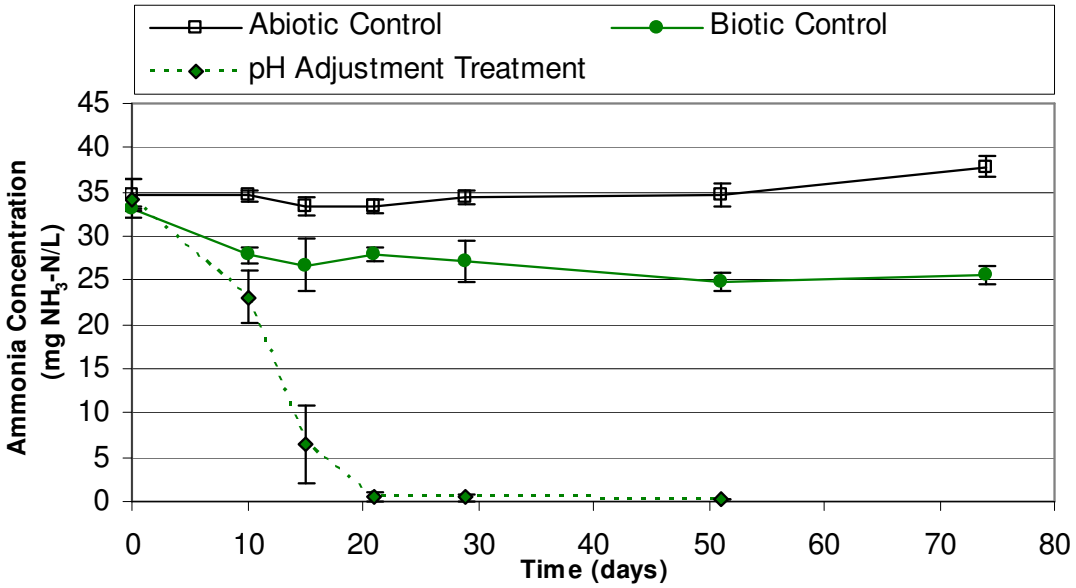


Figure 4-56: Ammonia concentrations of abiotic control (Experiment IIB #1), biotic control (Experiment IIB #2), and high pH treatment (Experiment IIB #3), pH maintained at 7.5 ± 0.5; error bars represent one standard deviation for averages.

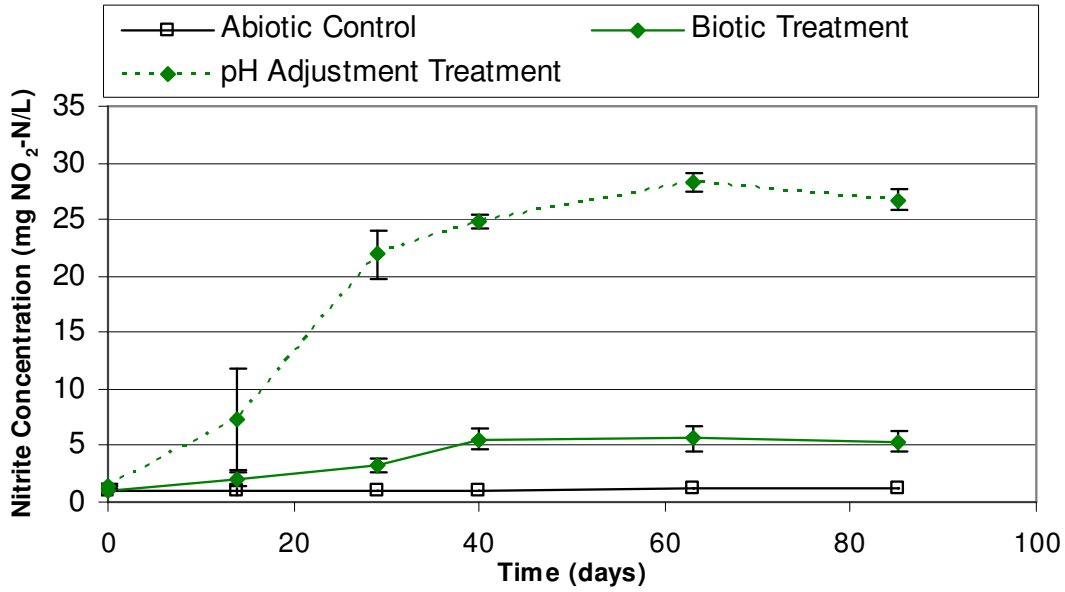


Figure 4-57: Nitrite concentrations of abiotic control (Experiment IIB #1), biotic control (Experiment IIB #2), and high pH treatment (Experiment IIB #3), pH maintained at 7.5 ± 0.5; error bars represent one standard deviation for averages.

4.3.4 Zinc Orthophosphate

Lead corrosion was not reduced significantly under abiotic conditions (Figure 4-58). Day 78 had a p-value of 0.16. The total lead concentration was, however, reduced by 56.2% under biotic conditions after 78 days (Figure 4-59). Soluble lead concentrations were 10.6 ± 0.33 ppb and 46.4 ± 19.3 ppb for the abiotic-nitrate control and abiotic-zinc orthophosphate treatment, respectively, after 99 days and were statistically different. Soluble lead concentration for the biotic-nitrate control and the biotic-zinc orthophosphate treatment were 160 ± 31.1 ppb and 26.3 ± 14.7 ppb, respectively, after 99 days and were also statistically different. The pH of the biotic-nitrate control and the biotic-zinc orthophosphate treatment decreased to 6.14 and 6.26, respectively, after 13 days (Figure 4-60). The pH of the biotic-nitrate control dropped more quickly than the biotic-zinc orthophosphate treatment. The biotic-zinc orthophosphate treatment experienced a lag in pH drop. Similarly, a lag in ammonia consumption also occurred for the biotic-zinc orthophosphate treatment when compared to the biotic-nitrate control (Figure 4-61). An increase of 0.6 mg NO_2-N/L was detected in the biotic-zinc orthophosphate treatment after 92 days while an increase of 10.8 mg NO_2-N/L occurred in the biotic-nitrate control (Figure 4-62).

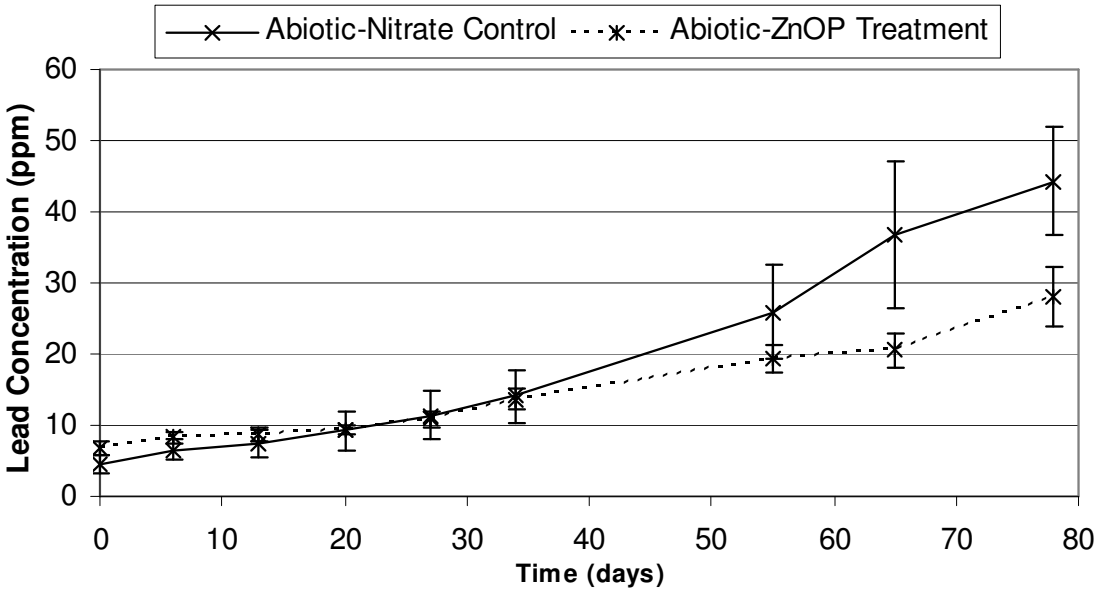


Figure 4-58: Total lead concentrations of abiotic-nitrate control (Experiment IIC #1) and abiotic-zinc orthophosphate (ZnOP) treatments (Experiment IIC #3), zinc orthophosphate dose of 5 mg/L PO_4; error bars represent one standard deviation for averages.

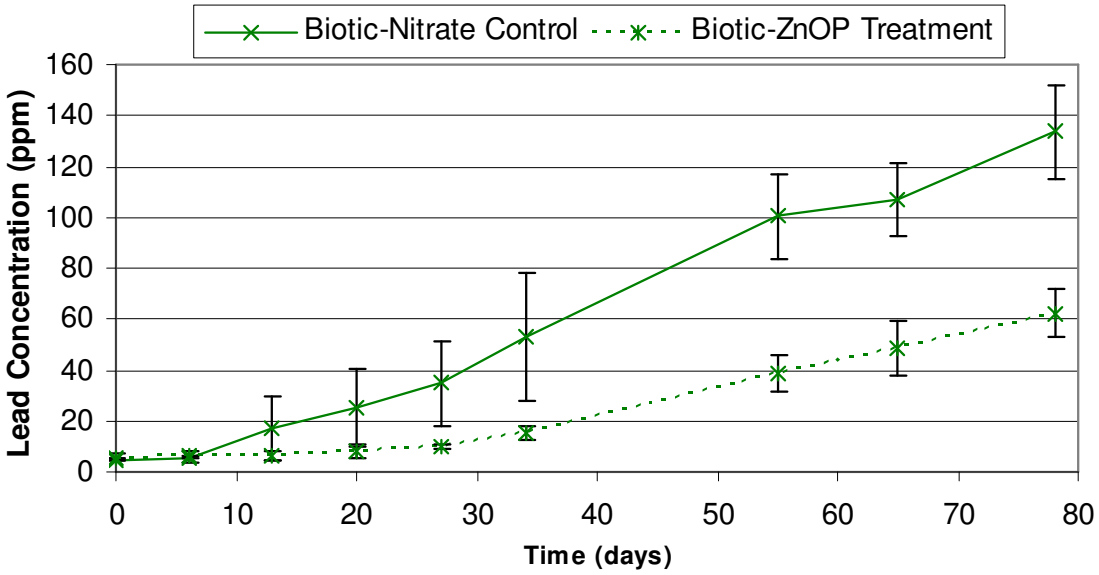


Figure 4-59: Total lead concentrations of biotic-nitrate control (Experiment IIC #2) and biotic-zinc orthophosphate (ZnOP) treatments (Experiment IIC #4), zinc orthophosphate dose of 5 mg/L PO_4; error bars represent one standard deviation for averages.

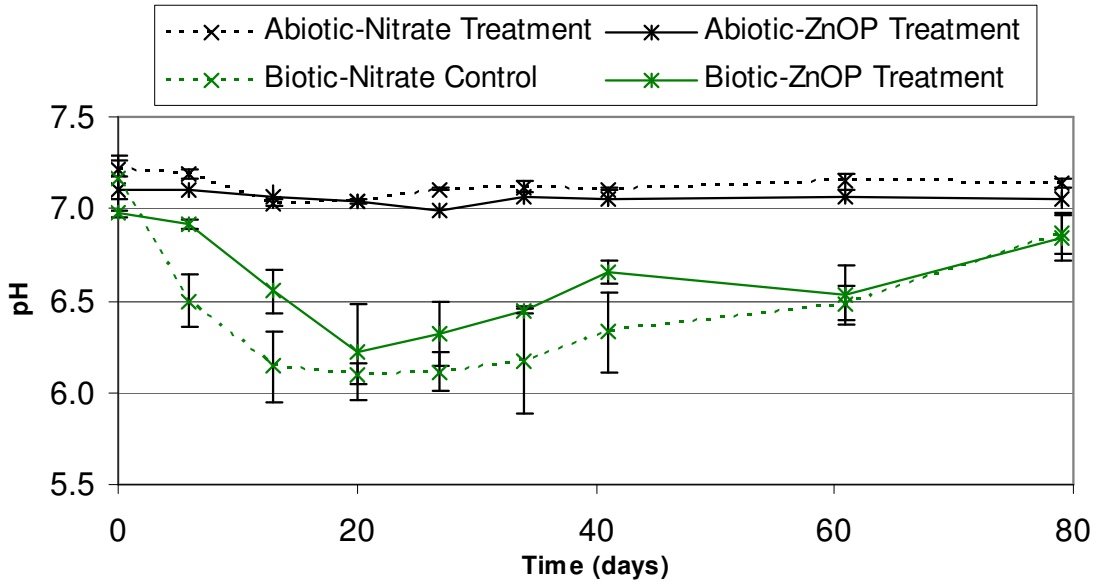


Figure 4-60: pH of abiotic-nitrate control (Experiment IIC #1), abiotic-zinc orthophosphate (ZnOP) (Experiment IIC #3), biotic-nitrate control (Experiment IIC #2), and biotic-zinc orthophosphate (ZnOP) treatments (Experiment IIC #4), zinc orthophosphate dose of 5 mg/L $PO_4$4 error bars represent one standard deviation for averages.

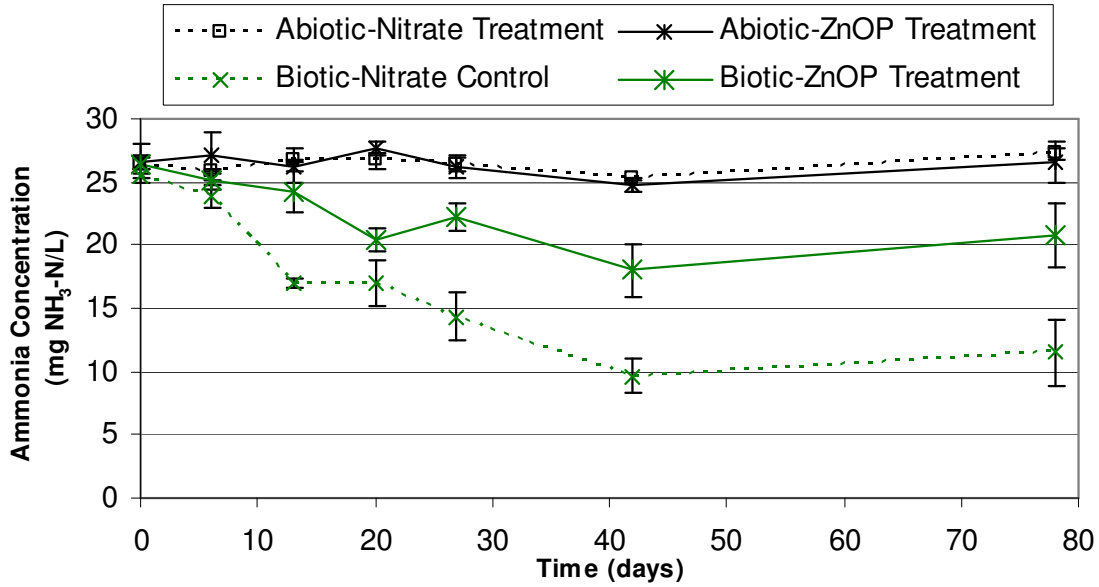


Figure 4-61: Ammona concentrations of abiotic-nitrate control (Experiment IIC #1), abiotic-zinc orthophosphate (ZnOP) treatment (Experiment IIC #3), biotic-nitrate control (Experiment IIC #2), and biotic-zinc orthophosphate (ZnOP) treatments (Experiment IIC #4), zinc orthophosphate dose of 5 mg/L PO_4; error bars represent one standard deviation for averages.

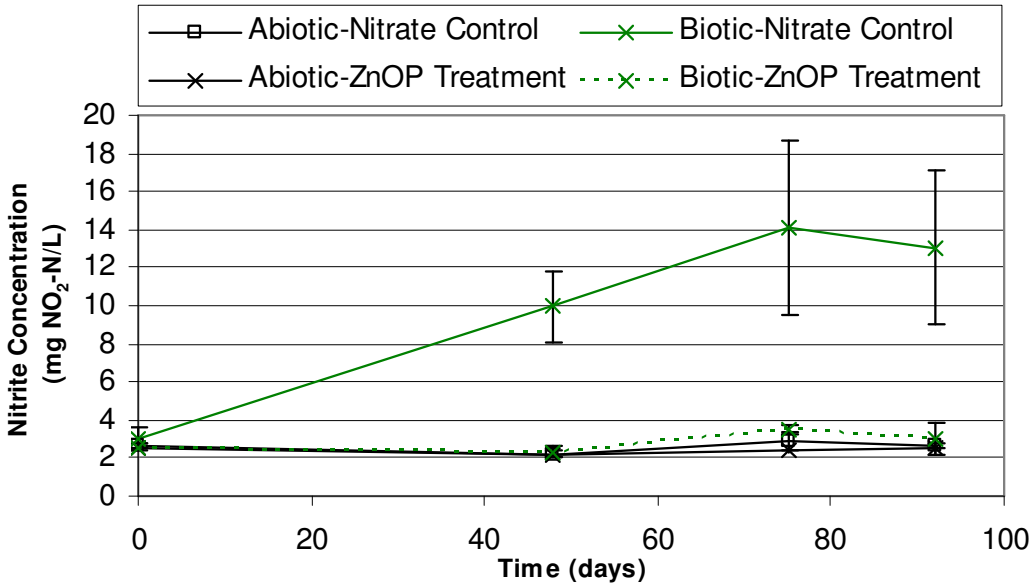


Figure 4-62: Nitrite concentrations of abiotic-nitrate control (Experiment IIC #1), abiotic-zinc orthophosphate (ZnOP) (Experiment IIC #3), biotic-nitrate control (Experiment IIC #2), and biotic-zinc orthophosphate (ZnOP) treatments (Experiment IIC #4), zinc orthophosphate dose of 5 mg/L PO_4; error bars represent one standard deviation for averages.

4.4 Chloramine Toxicity Study

A chloramines toxicity study was performed to determine the disinfectant dose at which *Nitrosomonas europaea* growth becomes inhibited. Nitrite production was monitored to determine growth activity. This study was performed in either 100 mM ammonia growth media (to allow bacteria the optimum environment for growth) or tap water.

4.4.1 Growth Media Study

All treatments receiving a chloramine dose of 0.1, 0.25, and 1.0 mg/L as Cl_2 on day 0 did not deviate significantly from the abiotic control in either nitrite production or pH. The pH of the abiotic control and all biotic treatments receiving a dose on day 0

remained between 8.1 and 8.3 for the entire experiment (Figure 4-63). Nitrite concentrations of treatments receiving a chloramine dose on day 0 did not differ significantly from the abiotic control after 16 days (Figure 4-64). The biotic control, which received no chloramines, grew rapidly. The pH of the biotic control began decreasing after inoculation and reached a minimum of 6.0 after 16 days and nitrite concentrations amounted to 128 mg/L NO_2-N after 16 days.

A second set of treatments received doses of chloramines (0.1, 0.25, and 1.0 mg/L as Cl_2) after 4 days. This was intended to allow time for biomass to be generated before receiving a chloramines dose. Biotic treatments receiving 0.1 or 0.25 mg/L as Cl_2 on day 4 produced the same amount of nitrite as the biotic control after 16 days (Figure 4-66). The pH of the biotic control and biotic treatments receiving 0.1 and 0.25 mg/L as Cl_2 reached a minimum of 6.0 after 16 days (Figure 4-65). The biotic treatment receiving 1.0 mg/L as Cl_2 reached a pH of 7.60 after 4 days. The pH remained between 7.6 and 7.7 after day 4 and did not deviate for the rest of the experimental period. The biotic treatment receiving 1.0 mg/L as Cl_2 on day 4 had only produced 31.8 mg/L NO_2-N by day 16.

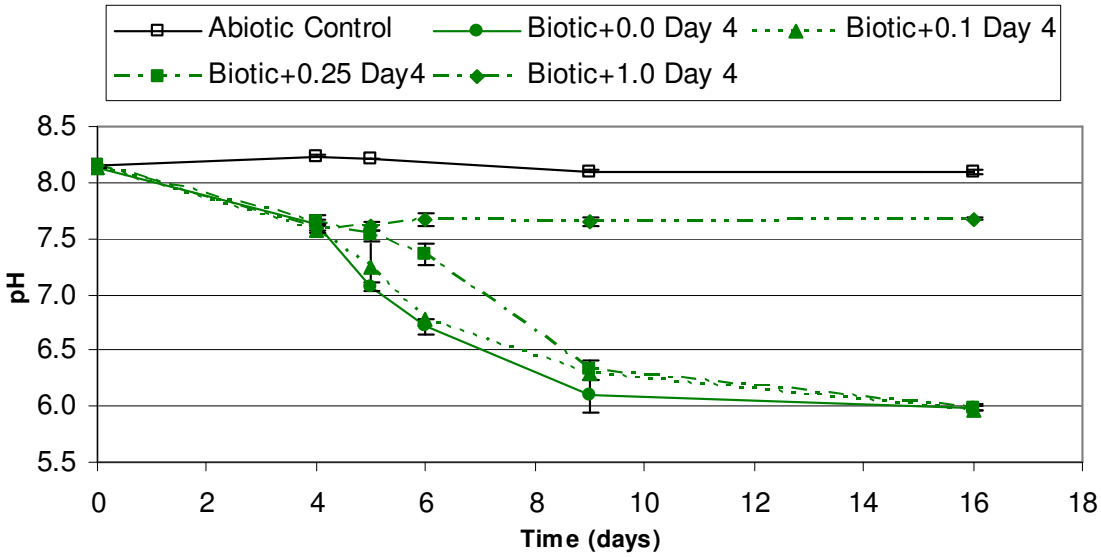


Figure 4-63: pH of abiotic control, biotic control (0 mg/L Cl_2), biotic + 0.1 mg/L Cl_2 (initial), biotic + 0.25 mg/L Cl_2 (initial), and biotic 1.0 mg/L Cl_2 (initial); error bars represent one standard deviation for averages.

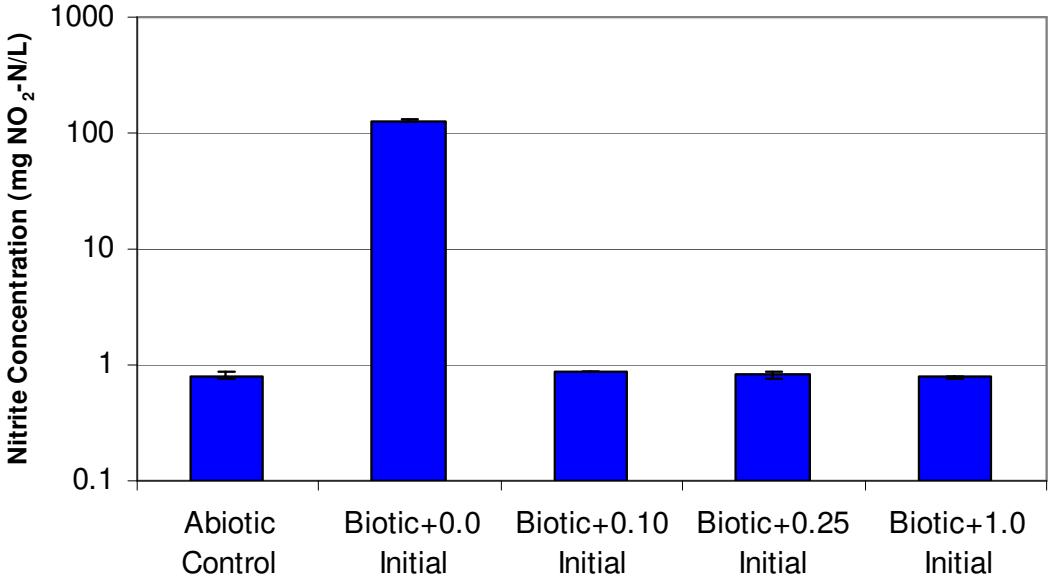


Figure 4-64: Nitrite concentrations of abiotic control, biotic control (0 mg/L Cl_2), biotic + 0.1 mg/L Cl_2 (initial), biotic + 0.25 mg/L Cl_2 (initial), and biotic 1.0 mg/L Cl_2 (initial) after 16 days; error bars represent one standard deviation for averages.

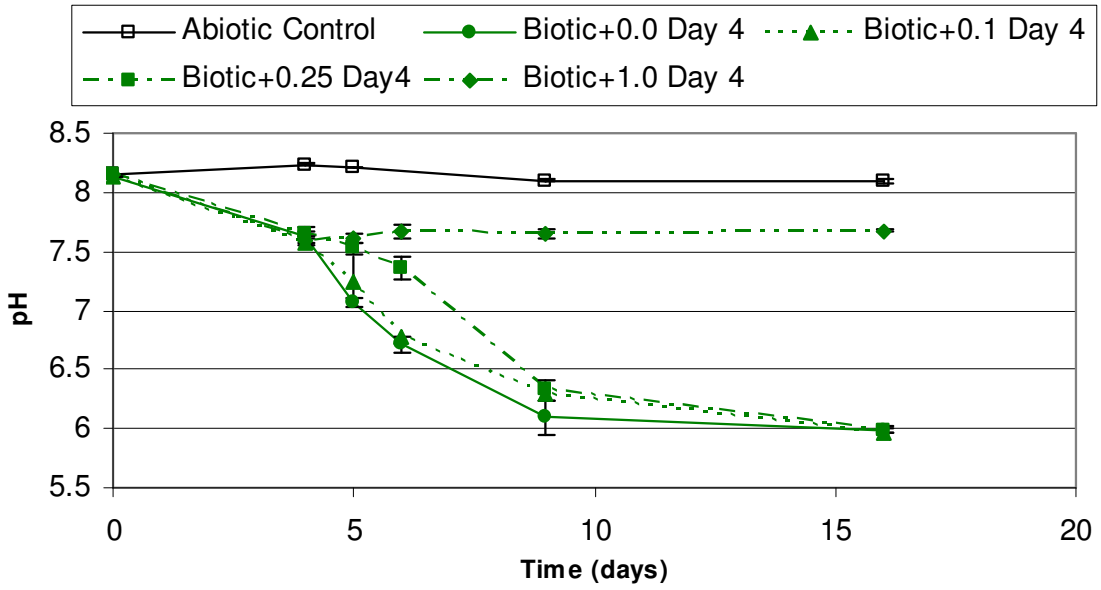


Figure 4-65: pH of abiotic control, biotic control (0 mg/L Cl_2), biotic + 0.1 mg/L Cl_2 (day 4), biotic + 0.25 mg/L Cl_2 (day 4), and biotic 1.0 mg/L Cl_2 (day 4); error bars represent one standard deviation for averages.

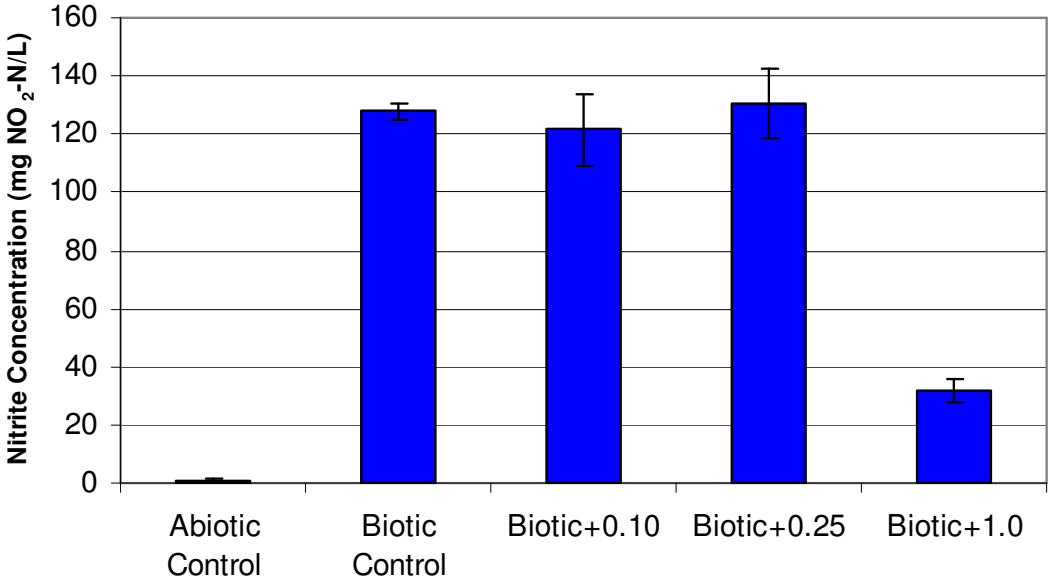


Figure 4-66: Nitrite concentrations of abiotic control, biotic control (0 mg/L Cl_2), biotic + 0.1 mg/L Cl_2 (day 4), biotic + 0.25 mg/L Cl_2 (day 4), and biotic 1.0 mg/L Cl_2 (day 4) after 16 days; error bars represent one standard deviation for averages.

4.4.2 Tap Water Study

A similar study was performed to investigate the toxicity of chloramines to *Nitrosomonas europaea* in tap water. Chloramines were applied either initially (day 0) or after 8 days of incubation at concentrations of 0.10, 0.25, 0.50, or 0.75 mg/L as Cl_2. Tubes receiving chloramines on day 8 and the biotic control were adjusted with NaOH to a pH of 7.6 before dosage.

All tubes receiving chloramines on day 0 did not undergo any significant amount of nitrification. The biotic control, which received no chloramines, grew rapidly. The pH of the abiotic control, biotic control, and biotic treatments receiving chloramines on day 0 are shown in Figure 4-67. The pH of the biotic treatment dropped rapidly after 8 days while no treatments receiving any dose of chloramines changed significantly over the experimental period. Furthermore, the nitrite concentration of the biotic treatment increased to 4.2 mg NO_2-N/L after 35 days while no biotic treatments receiving a dose of chloramines deviated significantly from the abiotic control (Figure 4-68).

A second set of tubes was allowed to grow for 8 days before being dosed with chloramines. The pH of the biotic control and biotic treatments receiving chloramines were adjusted to 7.6 before dosage (Figure 4-69). All biotic tubes had produced 0.30 mg NO_2-N/L after 8 days. The pH of the biotic control and the biotic treatment receiving 0.10 mg/L as Cl_2 dropped rapidly after pH adjustment and reached 6.27 after 13 days.

Biotic treatments receiving 0.25, 0.50, and 0.75 mg/L as Cl_2 decreased in pH much more slowly, reaching 7.02 after 35 days. The nitrite concentration in the biotic control increased by 2.95 mg NO_2-N/L after 35 days (Figure 4-70). No significant increase in nitrite concentrations occurred after day 8 in any treatment receiving chloramines.

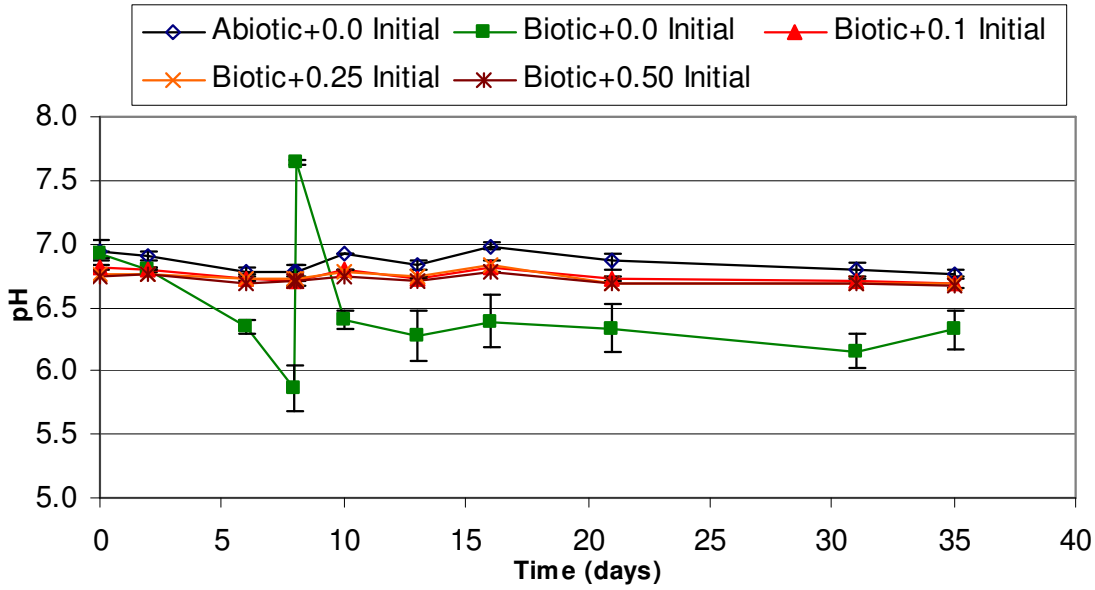


Figure 4-67: pH of the abiotic control, biotic control (0 mg/L Cl_2), and biotic treatments receiving 0.10, 0.25, and 0.50 mg/L as Cl_2 on day 0 (pH readjustment performed on day 8); error bars represent one standard deviation for averages.

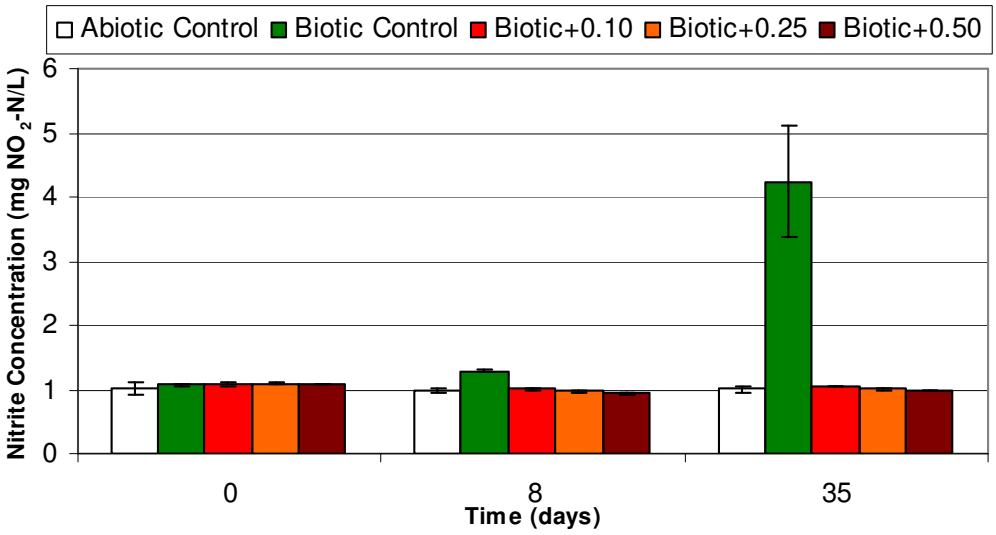


Figure 4-68: Nitrite concentrations of the abiotic control, biotic control (0 mg/L Cl_2), and biotic treatments receiving 0.10, 0.25, and 0.50 mg/L as Cl_2 on day 0; error bars represent one standard deviation for averages.

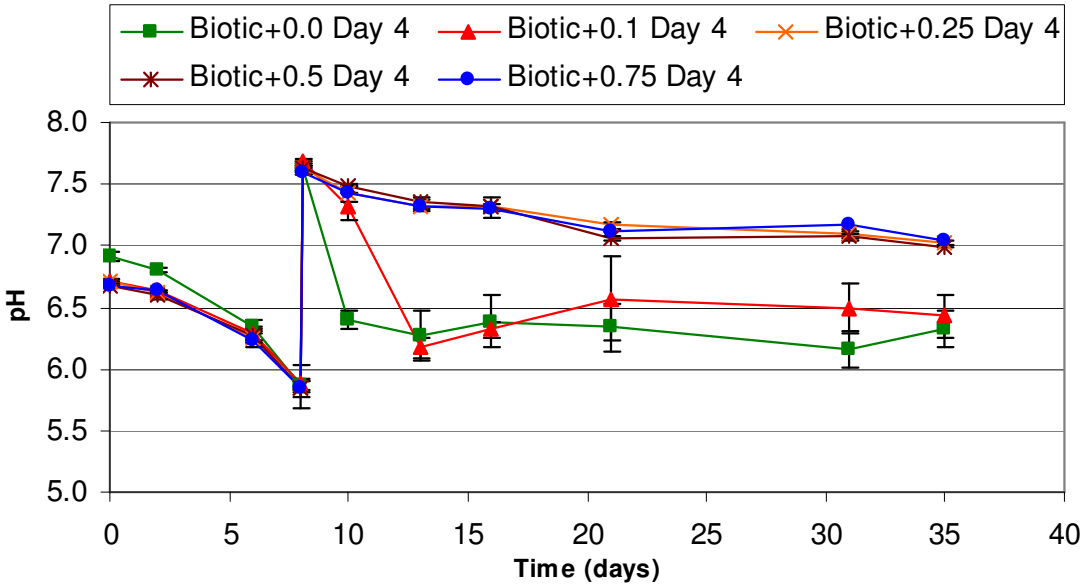


Figure 4-69: pH of the biotic control (0 mg/L Cl_2) and biotic treatments receiving 0.10, 0.25, 0.50, and 0.75 mg/L as Cl_2 on day 8 (pH readjustment performed on day 8); error bars represent one standard deviation for averages.

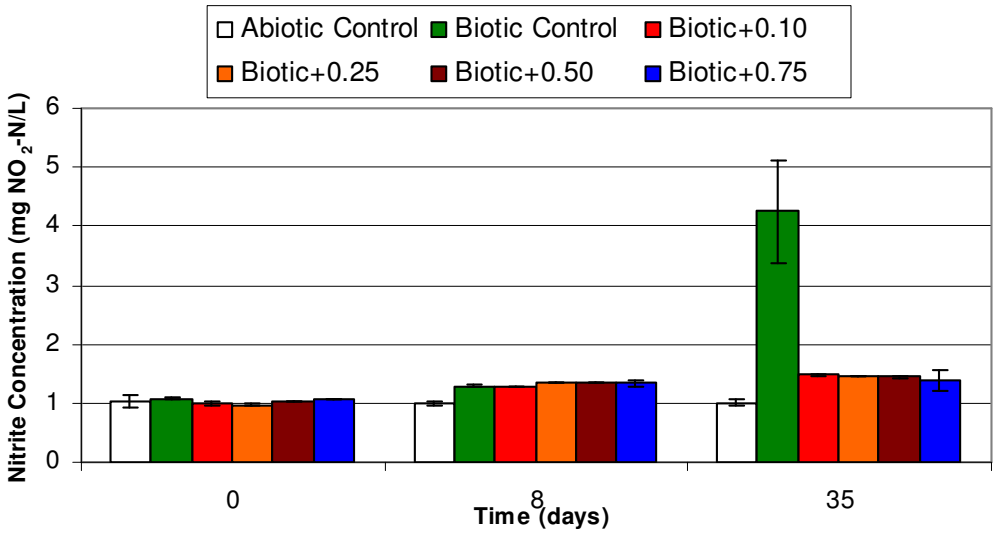


Figure 4-70: Nitrite concentrations of the abiotic control, biotic control (0 mg/L Cl_2), and biotic treatments receiving 0.10, 0.25, 0.50, and 0.75 mg/L as Cl_2 on day 8; error bars represent one standard deviation for averages.

4.5 Summary of Results

Experiments I and II examined the effect of several parameters on lead corrosion, as described in sections 4.1-4.3. A summary of these results is shown in Table 4-1. Abiotic denitrification of nitrate to nitrite occurred concurrently with lead corrosion in nitrate treatments. Increased lead corrosion occurred in the nitrite treatments but abiotic denitrification of nitrite was not quantified. Lead corrosion was also significant in low pH treatments. Hypothesized abiotic lead corrosion factors arising from ammonia bio-oxidation to nitrite did significantly increase lead corrosion. When ammonia bio-oxidation to nitrite occurred, lead corrosion factors were imposed by nitrifying bacteria. Lead corrosion was also significant under biotic conditions. However, lead corrosion in the biotic treatment with a freshly cleaned coupon was primarily due to the development of acidic conditions while lead corrosion in the biotic treatment with an aged coupon could have been caused by the presence of nitrite and the development of a low pH.

All lead corrosion inhibitors effectively reduced lead concentrations under biotic conditions, abiotic conditions, or both. Alkalinity and pH adjustment significantly increased the extent of ammonia bio-oxidation to nitrite while zinc orthophosphate significantly inhibited it. Despite increased activity of nitrifying bacteria, lead corrosion was still significantly reduced in the pH adjustment treatment suggesting that the development of an acidic pH was a large contributor to lead corrosion in these experiments.

Experiment III examined the effect of a range of chloramine doses added either at the time of inoculation or after some growth occurred, as described in section 4.4. A

summary of these results is shown in Table 4-2. When added immediately after inoculation, chloramine doses as low as 0.10 mg/L as Cl_2 significantly inhibited ammonia bio-oxidation to nitrite. However, chloramine doses of 0.10 and 0.25 mg/L as Cl_2 were not inhibitory when added to AOB cultures in a defined mineral medium after four days of growth while a chloramine doses of 1.0 mg/L as Cl_2 was inhibitory. A chloramine doses as low as 0.10 mg/L as Cl_2 was inhibitory when added to AOB cultures in tap water after eight days of growth.

Table 4-1: Summary of Results for Lead Corrosion (Experiments I and II)

Experiment	Treatment	Media Added	Lead Corrosion in Comparison to Abiotic Control
I, Part A	Fresh Coupon, Nitrate, abiotic	2% filtered spent media	Not significantly different
I, Part A	Fresh Coupon, Nitrate, abiotic	None	Significantly higher
I, Part A	Fresh Coupon, Nitrite, abiotic	2% filtered spent media	Not significantly different
I, Part A	Fresh Coupon, Nitrite, abiotic	None	Significantly higher
I, Part A	Fresh Coupon, Low pH, abiotic	2% filtered spent media	Significantly higher
I, Part A	Fresh Coupon, Biotic, AOB	2% spent media	Significantly higher
I, Part B	Aged Coupon, Nitrate, abiotic	2% filtered spent media	Significantly higher
I, Part B	Aged Coupon, Nitrate, abiotic	None	Significantly higher
I, Part B	Aged Coupon, Nitrite, abiotic	2% filtered spent media	Significantly higher
I, Part B	Aged Coupon, Nitrite, abiotic	None	Significantly higher
I, Part B	Aged Coupon, Low pH, abiotic	2% filtered spent media	Significantly higher
I, Part B	Aged Coupon, Biotic, AOB	2% spent media	Significantly higher
II, Part A	Aged Coupon, Orthophosphate	Abiotic	Significantly reduced vs. no orthophosphate
II, Part A	Aged Coupon, Orthophosphate	Biotic	Signficantly reduced vs. no orthophosphate
II, Part A	Aged Coupon, Alkalinity	Abiotic	Signficantly reduced vs. no ALK addition
II, Part A	Aged Coupon, Alkalinity	Biotic	Not significantly different vs. no ALK addition
II, Part B	Aged Coupon, pH Adjustment	Biotic	Significantly reduced vs. no pH adjustment
II, Part C	Aged Coupon, Zinc Orthophosphate	Abiotic	Not significantly different vs. no ZnPO4
II, Part C	Aged Coupon, Zinc Orthophosphate	Biotic	Signficantly reduced vs. no ZnPO4

Table 4-2: Summary of Results for Chloramine Inhibition of Nitrification (Experiment III)

Chloramine Dose	Day Dose was Added	Nitrite Production in Comparison to Biotic Control
0.10 mg/L as Cl_2, in media	0	Significantly lower
0.25 mg/L as Cl_2, in media	0	Significantly lower
1.0 mg/L as Cl_2, in media	0	Significantly lower
0.10 mg/L as Cl_2, in media	4	Not significantly different
0.25 mg/L as Cl_2, in media	4	Not significantly different
1.0 mg/L as Cl_2, in media	4	Significantly lower
0.10 mg/L as Cl_2, in tap water	0	Significantly lower
0.25 mg/L as Cl_2, in tap water	0	Significantly lower
0.50 mg/L as Cl_2, in tap water	0	Significantly lower
0.10 mg/L as Cl_2, in tap water	8	Significantly lower
0.25 mg/L as Cl_2, in tap water	8	Significantly lower
0.50 mg/L as Cl_2, in tap water	8	Significantly lower
0.75 mg/L as Cl_2, in tap water	8	Significantly lower

5. DISCUSSION

Lead corrosion was significantly increased in the presence of nitrate. The formation of nitrite and consumption of nitrate occurred concurrently with lead corrosion. Furthermore, lead corrosion occurred concurrently with a significant increase in pH. An electron balance (Equation 6) shows that 1 mg NO_3-N/L will serve as an electron acceptor (yielding nitrite) for oxidation of 14.8 mg/L of lead. For a fresh coupon, expected amounts of lead corrosion from the measured nitrite accumulation are compared with measured total lead concentrations of the nitrate treatment without spent media (section 4.1.5) in Table 5-1. Theoretical lead oxidation, based on nitrate reduction to nitrite, was higher than measured total lead concentrations. Formation of a 0.15 mg/L NH_3-N was also detected and would account for 36.4% of the total lead corrosion. During the course of experiments, "white scale" was observed to form on lead coupons. When shaken prior to sampling, this scale typically flaked off and uniformly distributed in the tube. However, some amount of "white scale" remained on the lead coupons after shaking. This remaining scale could have been oxidized lead which collected on the surface of the coupon and did not contribute to total lead measurements. This could be a source of error between theoretical lead oxidation and measured total lead concentrations.

Table 5-1: Comparison of expected and actual lead corrosion of the nitrate treatment without spent media (fresh coupon), calculated by stoichiometry of nitrate reduction.

Day	Amount of Nitrite Accumulation (mg/L NO_2-N)	Theoretical Lead Oxidation (mg/L)	Measured Lead Concentration, subtracting the control (mg/L)	Theoretical/Measured
19	0.81	12.0	8.3	1.5
34	1.31	19.4	14.8	1.3
55	2.20	32.6	16.5	2.0
68	2.86	42.3	18.4	2.3

A similar comparison was made for the nitrate treatment without spent media with an aged coupon (section 4.2.5). Again, lead corrosion occurred concurrent with nitrite accumulation. Lead concentrations based on nitrite accumulation are compared with actual lead concentrations for the nitrate treatment without spent media in Table 5-2. This comparison suggests that lead corrosion arose from the reduction of nitrate to nitrite. Continuous reduction of nitrate occurred after day 35 while no further increase in the total lead concentration was detected. A small amount of ammonia also accumulated (0.18 mg NH_3-N/L). This reduction would account for 11.1% of the total lead corrosion and was considered negligible. A noticeable amount of "white scale" had formed on the surface of lead coupons in the nitrate treatment without spent media by this time. No such scale was observed on lead coupons in the abiotic treatment without spent media. This "white scale" could be a contributing source of error between the theoretical and actual lead concentrations if the scale hindered corroded lead from being completely distributed in the tap water.

Table 5-2: Comparison of expected and actual total lead concentrations of the nitrate treatment without spent media on an aged lead coupon, calculated from stoichiometry of nitrate reduction.

Day	Amount of Nitrite Accumulation (mg/L NO_2-N)	Theoretical Lead Oxidation (mg/L)	Measured Lead Concentration, subtracting the control (mg/L)	Theoretical/Measured
21	3.56	52.7	40.4	1.3
35	3.98	58.9	46.8	1.3
50	5.62	83.2	46.1	2.3

Nitrate treatments were also performed in the presence of spent media so comparison could be made with the biotic treatment. The presence of nitrate did not significantly increase lead corrosion on a freshly cleaned coupon when amended with spent media on a fresh coupon (Section 4.1.1). A statistically significant amount of nitrite accumulation did not occur until after 96 days. The lack of lead corrosion was attributed to the large amount of phosphate (32 mg/L) carried over with the spent media. Orthophosphate has been shown to inhibit lead corrosion at concentrations as low as 3.1 mg/L as PO_4 in a laboratory stagnation experiment (McNeill and Edwards, 2004) and as low as 2.4 mg/L as PO_4 in pipe loop experiments conducted by DCWASA (2006).

However, statistically significant lead corrosion was observed for the nitrate treatment amended with spent media and an aged coupon (Section 4.2.1). Lead concentrations based on nitrite accumulation are compared with actual lead concentrations in

Table 5-3. Actual lead concentrations were higher than would be expected assuming that corrosion arose from only the reduction of nitrate to nitrite. The cause of this is unclear. A significant increase in ammonia from the reduction of nitrite was not detected due to the large amount of background ammonia carried over in the spent media. Further lead corrosion could have been caused by reduction of nitrite to nitrogenous

gases. Formation of 1.0 mg/L of di-nitrogen would be required to account for the different between theoretical and actual lead concentrations observed by day 81.

Table 5-3: Comparison of expected and actual total lead concentrations of the nitrate treatment with spent media on an aged lead coupon, calculated from stoichiometry of nitrate reduction.

Day	Amount of Nitrite Accumulation (mg/L NO_2-N)	Theoretical Lead Oxidation (mg/L)	Measured Lead Concentration, subtracting the control (mg/L)	Theoretical/Measured
29	0.17	2.5	7.5	0.3
40	0.38	5.6	27.6	0.2
63	0.62	9.2	30.4	0.3
85	1.00	14.8	37.0	0.4

More lead corrosion occurred for an aged lead coupon than a freshly cleaned lead coupon in nitrate treatments. Lead corrosion for aged and fresh coupons is shown in Table 5-4. A study performed by Abd El Rehim and Mohamed (1998) showed that nitrate was able to increase the dissolution of the lead passive layer (composed of PbO) instead of progressing to a "trans-passive" region of Pb_3O_4 and PbO_2.

Table 5-4: Comparison of lead corrosion in nitrate treatments for aged and fresh coupons at end of experimental period.

			Lead Corrosion	

Media Added	Section	Lead Corrosion for Fresh Coupon, subtracting the control (mg/L)	for Aged Coupon, Subtracting Abiotic Treatment (mg/L)	Aged/Fresh
Filtered spent media	Fresh: 4.1.1 Aged: 4.2.1	4.6 (Not Significant)	32.0 (Significant)	N/A[a]
None	Fresh: 4.1.5 Aged: 4.2.5	18.4 (Significant)	49.3 (Significant)	2.68

[a] N/A = not applicable, since the denominator was not statistically significant.

For a freshly cleaned lead coupon, the pH of nitrate and nitrite treatments with spent media was never significantly different than the abiotic control. No significant lead corrosion occurred in these treatments. However for aged coupons, lead corrosion in the nitrate and nitrite treatments with spent media occurred concurrently with increases in pH. This is consistent with Equations 6 and 7 which show abiotic denitrification would increase pH. A similar trend was observed for nitrate and nitrite treatments without spent media. Lead corrosion and increase in pH occurred in both treatments for aged and fresh coupons.

The presence of nitrite also significantly increased lead corrosion. However, a significant consumption of nitrite was never detected. The major reduction product of nitrite, nitrogen gas, was not feasible for measurement in this study as it was performed exposed to air. The background nitrogen gas concentration would make detection of small amounts of nitrogen gas infeasible. For a freshly cleaned coupon, lead corrosion was significantly increased in the nitrite treatment without spent media (section 4.1.6) while no trend in nitrite concentrations was observed. A small amount of ammonia (0.14 mg/L NH_3-N) was detected but was considered a negligible source of lead corrosion only

accounted for 8.3% of the total lead corrosion. A reduction of 3.1 mg/L NO_2-N would be required to account for the 69.5 mg/L total lead concentration measured after 64 days. For an aged lead coupon, the nitrite treatment without spent media (section 4.2.6) produced similar results to those seen with the freshly cleaned lead coupon. Again, the nitrite concentration did not decrease despite significantly increased lead corrosion. The total lead concentration after 55 days (71.8 mg/L) would require a reduction of 3.2 mg/L NO_2-N to nitrogen gas. A small amount of ammonia was again detected but only accounted for 11.1% of the total lead corrosion and was considered negligible. When amended with spent media, the nitrite treatment with a freshly cleaned coupon (section 4.1.2) did not significantly increase lead corrosion. However for an aged coupon, the nitrite treatment with spent media (section 4.2.2) did significantly increase lead corrosion. Again, no trend in nitrite concentration was observed despite increased lead corrosion. A reduction of 2.2 mg/L NO_2-N would be necessary for the total lead concentration measured at the end of the experimental period. The reduction of nitrite with lead was not quantified in this study. Huang and Zhang (2006) reported the reduction of nitrite (1.4 mM) with zero valent iron to di-nitrogen and ammonia. Kielemoes et al. (2000) reported that nitrate and nitrite (0.07 mM) could oxidize metallic iron through reduction to ammonium under anaerobic conditions.

The low pH treatment increased lead corrosion for fresh and aged coupons. Lead corrosion for low pH treatments are shown in
Table 5-5. Results were inconclusive with respect to a difference between fresh and aged coupons in low pH treatments. However, lead corrosion was sensitive to acidic conditions.

Table 5-5: Lead corrosion for low pH treatments, all treatments received spent media.

Coupon Type	Section	Lead Corrosion for Fresh Coupon, Subtracting Abiotic Treatment (mg/L)	Statistically Significant?
Fresh	4.1.3	269	Yes
Fresh	Appendix B	70.5	Yes
Aged	4.2.3	87.4	Yes

The biotic treatment showed increased lead corrosion. Active nitrification resulted in the presence of nitrite and a low pH. For a fresh coupon, lead corrosion was increased in the low pH treatment while the nitrite treatment did not significantly increase lead corrosion. This suggests that most, if not all, lead corrosion in the biotic treatment with a fresh coupon (section 4.1.4) could be attributed to the development of an acidic environment provided by nitrification and not from abiotic denitrification of nitrite. The biotic treatment with an aged coupon (section 4.2.4) also underwent nitrification resulting in the presence of nitrite and an acidic environment. As shown previously for an aged lead coupon, the low pH treatment and the nitrite treatment did significantly increase lead corrosion. Since both the nitrite and low pH treatments were shown to increase lead corrosion on aged coupons, both were possible contributors in the biotic treatment. Furthermore, lead corrosion in the biotic treatment with an aged coupon was 1.39 times higher than with a fresh coupon. Therefore, the primary lead corrosion factor for the biotic treatment with a freshly cleaned coupon was the low pH while the aged coupon could be affected by low pH and abiotic denitrification.

Orthophosphate, zinc orthophosphate, and pH adjustment treatments significantly reduced lead corrosion under biotic conditions. Alkalinity and orthophosphate treatments significantly reduced lead corrosion under abiotic conditions. Each inhibitor displayed interesting characteristics in the presence of nitrifying bacteria.

Orthophosphate significantly reduced lead corrosion for abiotic and biotic treatments (section 4.3.1). Orthophosphate is thought to form complexes with lead which create a more protective film (USEPA, 2004). Orthophosphate could also increase biological activity as it is a necessary nutrient for growth for many organisms, including nitrifying bacteria (Churchill et al., 2000). Though more nitrite was produced on average in the biotic-orthophosphate treatment than the biotic control, the difference was not statistically significant. Soluble lead concentrations were higher in the biotic-orthophosphate treatment than in the biotic control. This higher soluble lead concentration in the biotic-orthophosphate treatment existed despite having a lower total lead concentration. This data conflicts with findings in a study done by McNeill and Edwards (2004) in which orthophosphate decreased soluble lead concentrations of pipes of various ages. However, the McNeill and Edwards study was performed under abiotic conditions with pipe aged for up to 3 years.

Due to addition of spent media, orthophosphate dosing only accounted for a 16% increase in total phosphate. This small increase in phosphate, however, did impact lead corrosion. A much more significant decrease in lead corrosion would be expected to occur in a comparison between tap water receiving no inhibitor and receiving 5 mg/L as PO_4. Orthophosphate has been shown to inhibit lead corrosion at concentrations as low as

3.1 mg/L as PO_4 in a laboratory stagnation experiment (McNeill and Edwards, 2004) and as low as 2.4 mg/L PO_4 in pipe loop experiments conducted by DCWASA (2006).

Alkalinity dosing of 50 mg/L as $CaCO_3$ significantly reduced lead corrosion under abiotic conditions (section 4.3.2). Alkalinity dosing reduced total lead concentrations by 45.1% under biotic conditions but was not statistically significant due to the large variance between replicates. Calculated p-values were less than 0.15 for days 11 – 60 for the biotic-alkalinity treatment. Alkalinity has been suggested to precipitate tough scales on pipes which inhibit lead corrosion by protecting pipe from chemical attack by lead surface passivation (Maddison et al., 2001). Like orthophosphate dosing, elevated alkalinity has been shown to decrease lead corrosion (McNeill and Edwards, 2004). Alkalinity can also provide more buffering capability and hence reduce lead corrosion by maintaining a non-acidic environment (Churchill et al., 2000). Alkalinity was seen, though, to increase growth of nitrifying bacteria. Increased ammonia consumption and nitrite production occurred in the biotic-alkalinity treatment. Nitrifying bacteria, which are autotrophic, consume carbonate as a carbon source. Alkalinity was thought to promote growth through two possible mechanisms: pH buffering and additon of more carbonate as a carbon source. pH buffering from alkalinity dosing influenced biotic-induced lead corrosion in two ways. Firstly, the pH of biotic-alkalinity treatments was not in the low pH region (5.0 – 6.0) for as long as the biotic control. This suggests that the ability for alkalinity to buffer the tap water could have reduced overall lead corrosion in nitrifying distribution systems. Secondly, the formation of the scale from alkalinity dosing could have also provided a more protective surface against lead

corrosion. However, the extended growth period allowed more nitrite to accumulate which could have increased lead corrosion.

The control of pH under nitrifying conditions significantly reduced the total lead concentration (section 4.3.3). The nitrite concentration was observed to be very high in the biotic-pH adjustment treatment. This was thought to be caused by the maintenance of pH within the near neutral (6.5 to 7.5) range. Growth continued to occur until nearly all ammonia was consumed as long as pH was maintained. Lack of pH control was the major contributor to the cessation of growth in the biotic control. The ability of an acidic environment to significantly impact corrosion of a lead coupon was already shown to occur in Experiment I, Part B. When the pH was not maintained, growth stopped before all ammonia was consumed, as in the biotic control. While the nitrite concentration was much higher in the biotic-pH adjustment treatments than the biotic controls, lead corrosion was inhibited. This supports the results that an acidic environment is an important lead corrosion factor. As shown earlier, nitrite was able to significantly increase lead corrosion on an aged coupon. Despite removal of acidity as a corrosion factor, higher lead concentrations were observed in the biotic-pH adjustment treatment than the abiotic control. This also suggests that while pH control was able to effectively reduce the amount of lead corrosion it alone would not completely eliminate all corrosion arising from nitrification.

Zinc orthophosphate significantly reduced lead corrosion under biotic conditions (section 4.3.4). Biotic-zinc orthophosphate treatments were not observed to undergo as much nitrification as the biotic-nitrate control. Very little nitrite production occurred in

the biotic-zinc orthophosphate treatment. Furthermore, less ammonia consumption occurred in biotic-zinc orthophosphate treatment than in the biotic-nitrate control. These findings suggested that zinc effectively inhibited growth of *Nitrosomonas europaea*. Zinc inhibition of nitrifying bacteria was observed by Mertens et al. (2007) in which nitrification by *Nitrosospira* sp. was reduced by 20% at zinc concentrations of 5 to 150 μM. Zinc orthophosphate treatments in this study received 80 μM of zinc.

A statistically significant decrease in lead corrosion did not occur in the abiotic-zinc orthophosphate treatment. As with orthophosphate, zinc orthophosphate had a higher soluble lead concentration than the control under abiotic conditions. A study by McNeill and Edwards (2004) found that particulate lead concentrations were increased for zinc orthophosphate compared to orthophosphate. One possible explanation for this was that the zinc phosphate precipitate initially lowered the soluble phosphorous concentration allowing greater vulnerability to lead corrosion as compared to orthophosphate. It was also recently found by Schneider et al. (2007) that changing from zinc orthophosphate to orthophosphoric acid did not affect lead corrosion rates. Schneider suggested that the decision between orthophosphate and zinc orthophosphate was largely an operational consideration due to the effect of zinc loading on wastewater treatment facilities.

Chloramine doses as low as 0.10 mg/L as Cl_2 were capable of stopping nitrification when added on day 0 in either the growth media (section 4.4.1) or tap water study (section 4.4.2). This was thought to be due to the small amount of biomass present when the chloramine dose was applied. Nitrifying bacteria are autotrophic indicating that

they produce small amounts of biomass. The vulnerability of nitrifying bacteria to such low chloramine doses could be due to this. After allowing 4 days of growth to occur in growth media, chloramine doses of 0.10 and 0.25 mg/L as Cl_2 were tolerated and nitrification continued. A dose of 1.0 mg/L as Cl_2, however, still stopped nitrification. This data suggests that development of biomass is an important factor to tolerance of chloramine doses by nitrifying bacteria. A study by Wolfe et al. (1990), which examined water sources at different places on a chloraminated drinking water distribution, found that ammonia-oxidizing bacteria were 13 times more resistant to monochloramines than free chlorine. Furthermore, they found that 1.0 mg/L monochloramine only inactivated 99% of ammonia-oxidizing bacteria and suggest that a residual of chloramine at this dose could still allow a nitrification episode to occur. Another study by Pintar and Slawson (2003) found that AOB activity was detected, yet inhibited, at residual monochloramine concentrations of 0.2-0.6 mg/L, typically low concentrations, in a bench-scale distribution system. Biofilm was able to develop. A free chlorine burn resulted in short-term cessation of AOB activity but rebounded to prechlorination levels at monochloramine concentrations of 0.1-0.2 mg/L.

6. CONCLUSIONS AND RECOMMENDATIONS

Several conclusions were drawn from this study and are shown below.

- The presence of nitrate and nitrite increased lead corrosion. Abiotic denitrification of nitrate to nitrite occurred concurrently with lead corrosion. Despite increased lead corrosion in nitrite treatments, abiotic denitrification of nitrite was not quantified.
    - Lead corrosion was significantly higher than the control for freshly cleaned and aged coupons in nitrate and nitrite treatments not amended with spent media.
    - Lead corrosion was significantly higher than the control for aged coupons in nitrate and nitrite treatments amended with spent media.
    - Lead corrosion was not significantly different than the control for freshly cleaned coupons in nitrate and nitrite treatments amended with spent media.
- Significant lead corrosion occurred in low pH treatments for freshly cleaned and aged coupons with spent media present.
- As a result of ammonia bio-oxidation to nitrite, lead corrosion was significantly increased in biotic treatments.
    - Lead corrosion in the biotic treatment with a freshly cleaned coupon was primarily attributed to the development of a low pH.
    - Lead corrosion in the biotic treatment with an aged coupon was attributed to the presence of nitrite and development of a low pH.

- Under abiotic conditions, orthophosphate and alkalinity treatments significantly reduced lead corrosion (with spent media present).
- Under biotic conditions, orthophosphate, pH adjustment, and zinc orthophosphate treatments significantly reduced lead corrosion (with spent media present).
- Chloramine doses as low as 0.10 mg/L Cl_2 effectively inhibited ammonia bio-oxidation to nitrite when added to an AOB culture growing in a defined medium.
- Chloramine doses of 0.10 or 0.25 mg/L Cl_2 were not inhibitory when added to an AOB culture following four days of growth in a defined mineral medium, in the absence of chloramine.
- Chloramine doses as low as 0.10 mg/L Cl_2 effectively inhibited ammonia bio-oxidation to nitrite when added to an AOB culture growing in tap water, when the chloramine was added immediately or following eight days of growth in tap water in the absence of chloramine.

Recommendations for future research include:

- Centrifugation process for washing of cells: Phosphate inhibition of lead corrosion, due to carry-over of spent media, was shown to be significant. A harvesting process for cells, which includes washing to remove spent media, should be developed to remove the carry-over of spent media as a complicating factor.
- A study of corrosion factors on lead coupons at different ages: Lead corrosion was observed to be more significant for lead coupons aged in tap water for 2 weeks than for freshly cleaned coupons for some treatments. A study of lead

corrosion factors on coupons of different ages could better elucidate the effect of nitrate and nitrite on aged coupons.

- A study of lead corrosion at additional pH levels: Lead corrosion was significant at a pH range of 5.5 ± 0.5. Evaluation of corrosion over a range of pH values would better show the extant to which low pH increases lead corrosion.
- Examination of lead corrosion in pipe loop experiments: Pipe loop studies have been employed by the DCWASA (USEPA, 2006). Nitrification could be imposed on a pipe loop study to better resemble actual drinking water distribution systems and further investigating the effect of chloramines on nitrifying bacteria. A pipe loop study was designed for the DCWASA and could be used as an example system (USEPA, 2004).
- Measurement of gaseous reduction products from reduction of nitrite: Measurement of di-nitrogen (and perhaps nitric oxide and nitrous oxide) could be a better method for determining a mass balance for the abiotic denitrification of nitrite with lead.

APPENDICES

Appendix A: Calibration Curves and Sample Chromatograms

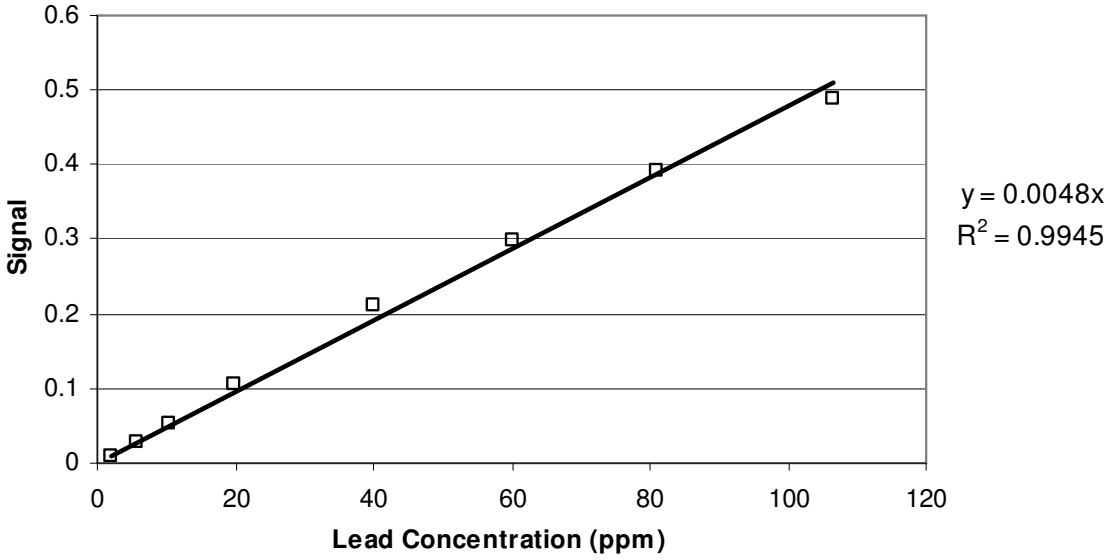


Figure A-1: Sample calibration curve for lead concentrations (2–100 ppm).

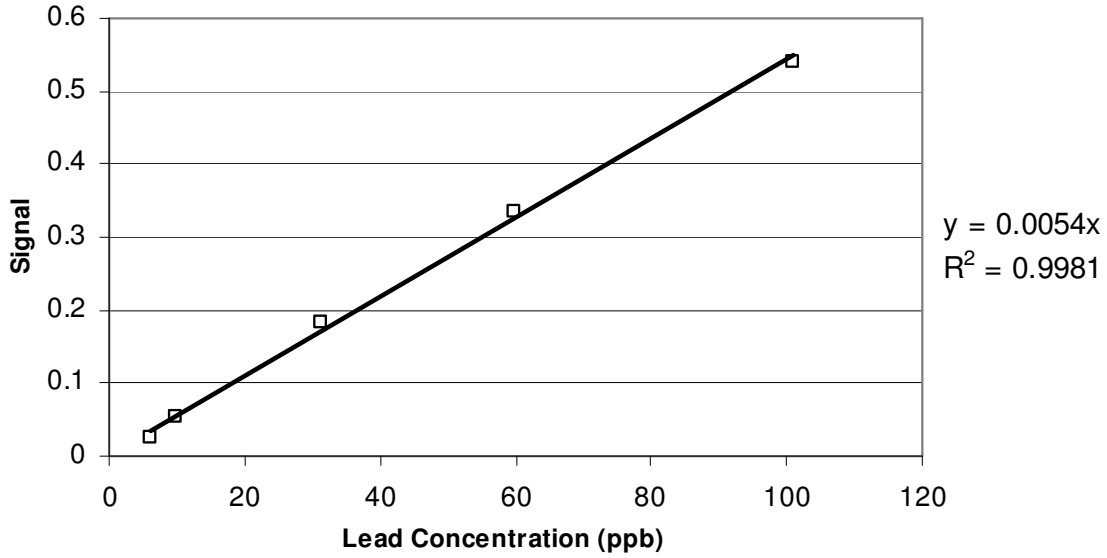


Figure A-2: Sample calibration curve for lead concentrations (5–100 ppb).

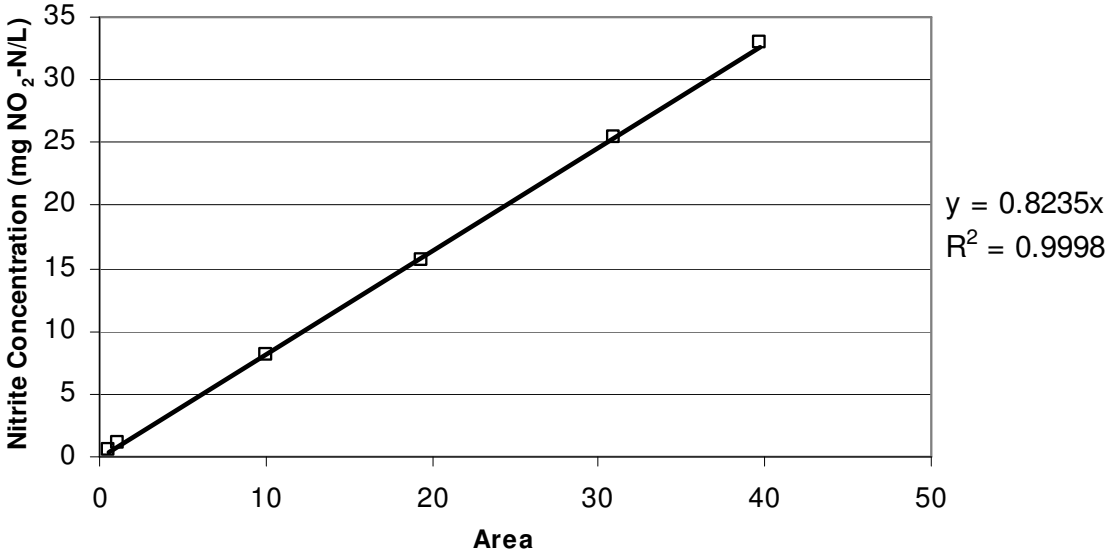


Figure A-3: Sample calibration curve for nitrite concentrations (0.50–32.9 mg NO_2-N/L).

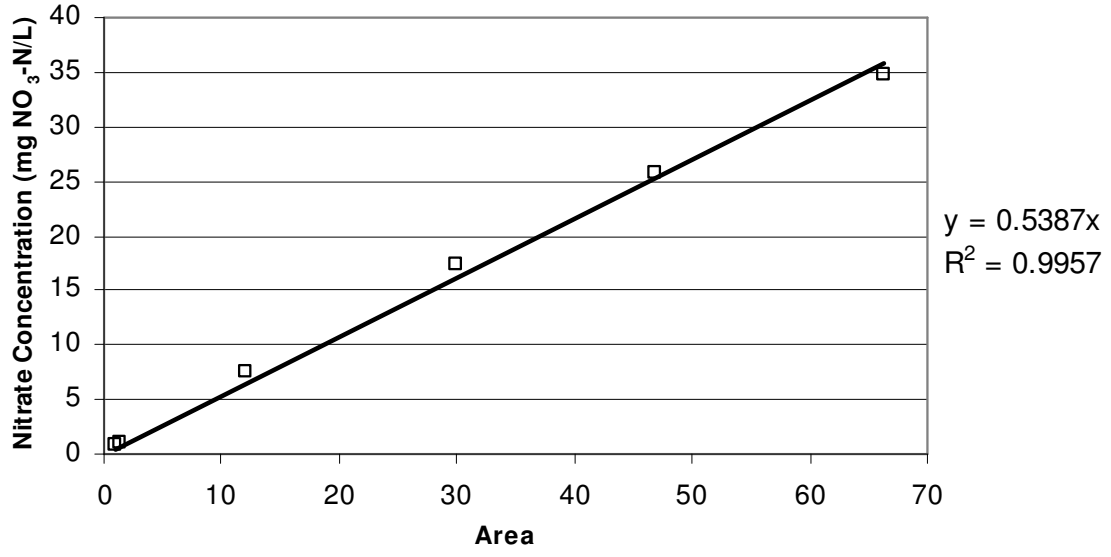


Figure A-4: Sample calibration curve for nitrate concentrations (0.50-34.7 mg NO_3-N/L).

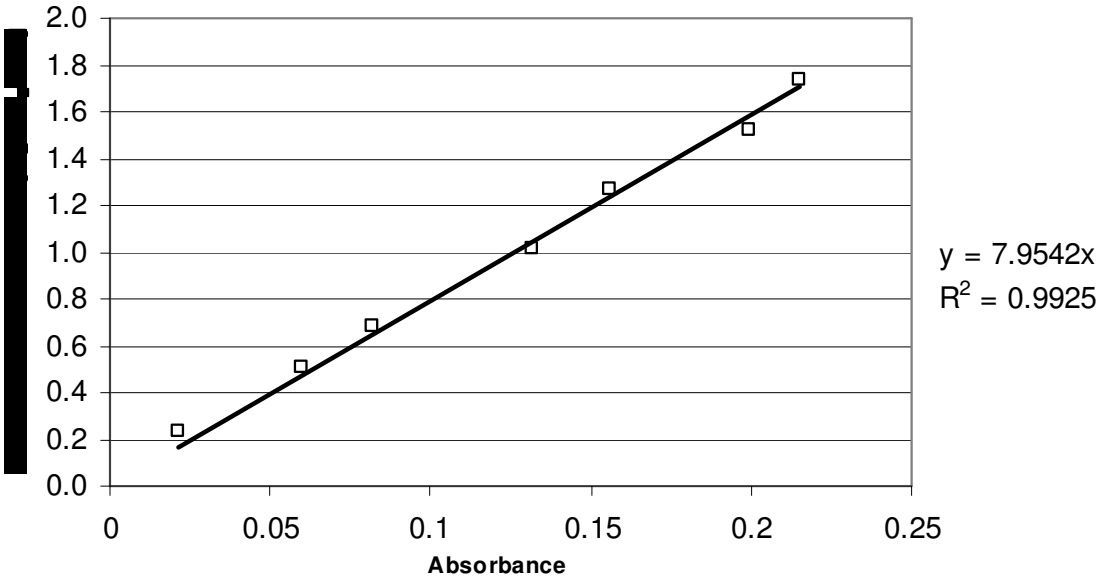


Figure A-5: Sample calibration curve for ammonia concentrations (0.23-1.74 mg NH_3-N/L).

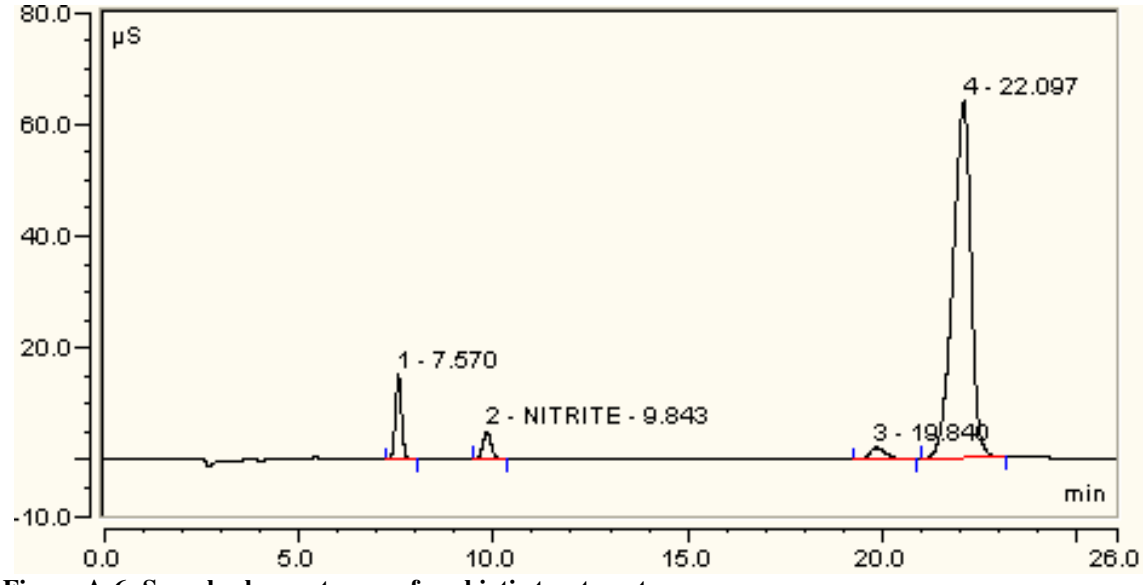


Figure A-6: Sample chromatogram for abiotic treatment.

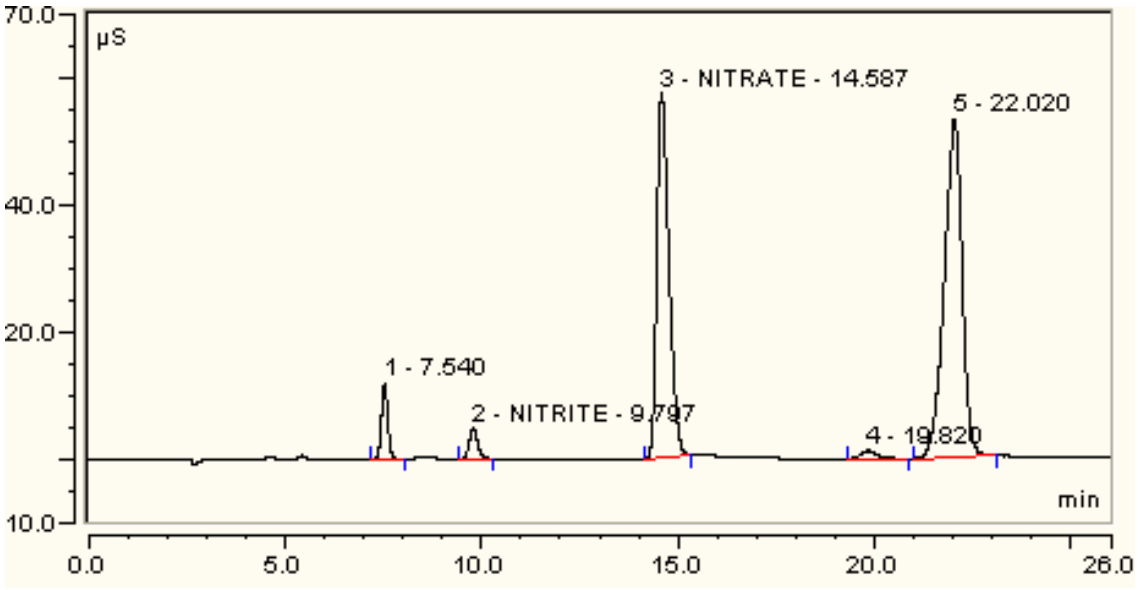


Figure A-7: Sample chromatogram for nitrate treatment.

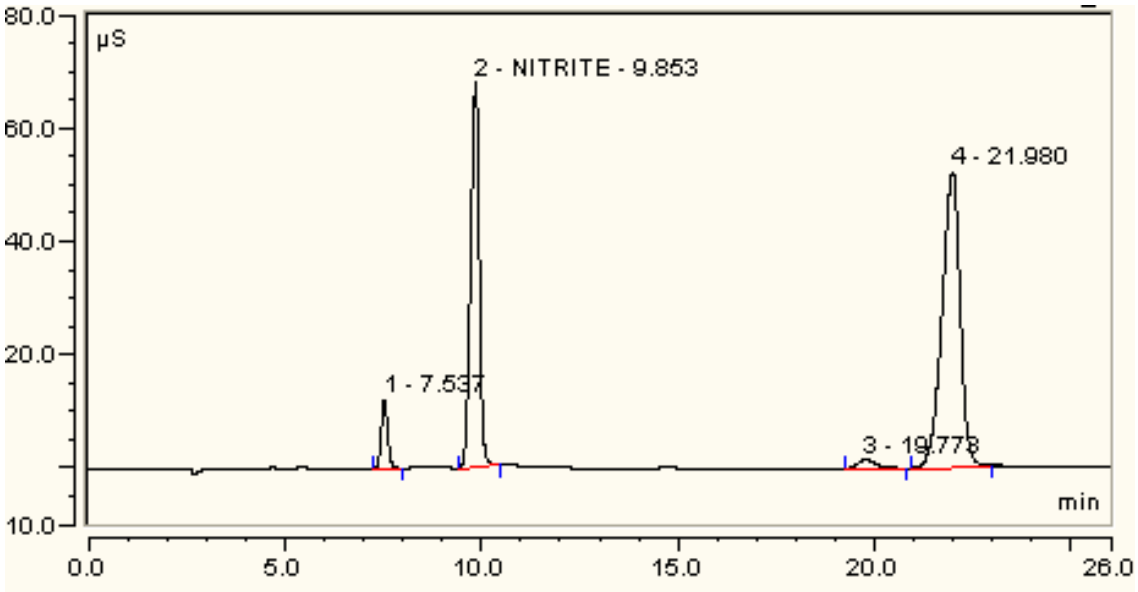


Figure A-8: Sample chromatogram for nitrite treatment.

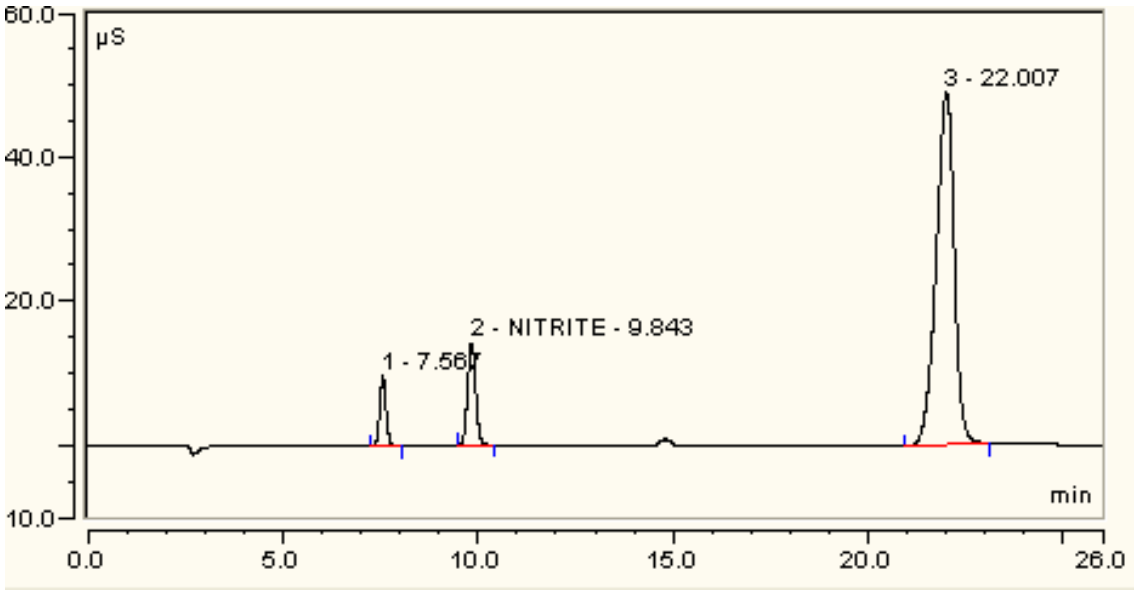


Figure A-9: Sample chromatogram for biotic treatment.

Appendix B: Replicate Experiment Data

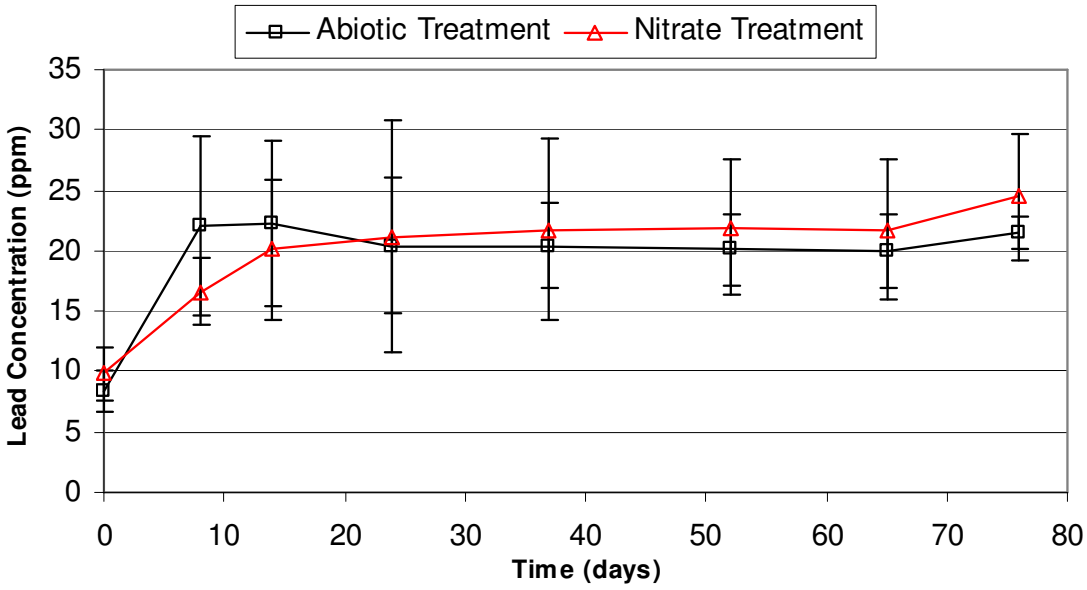


Figure B-1: Total lead concentrations of abiotic and nitrate treatments with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

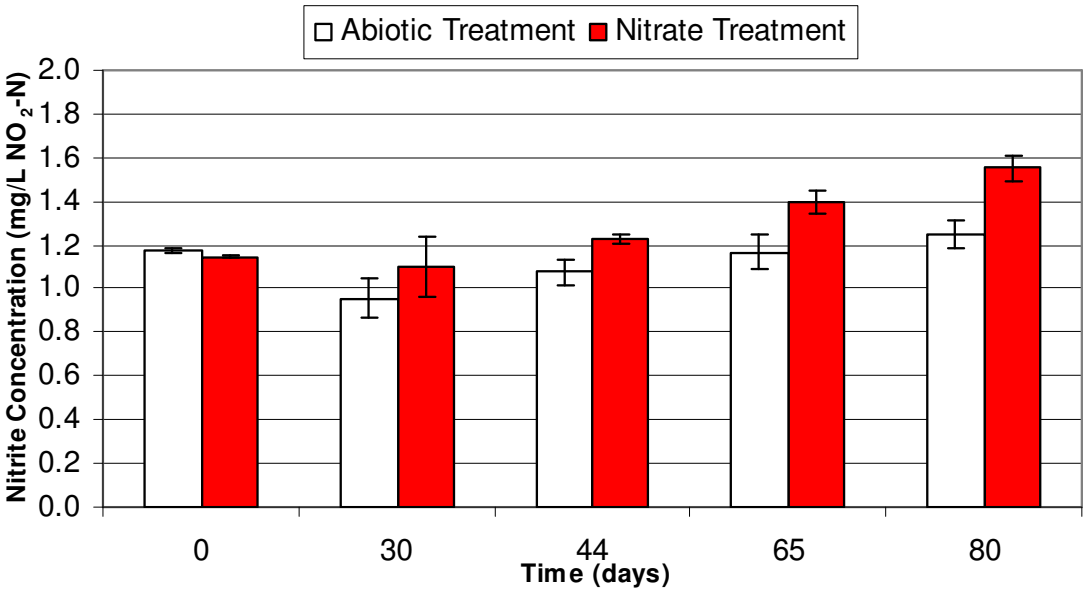


Figure B-2: Nitrite concentrations of abiotic and nitrate treatments with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

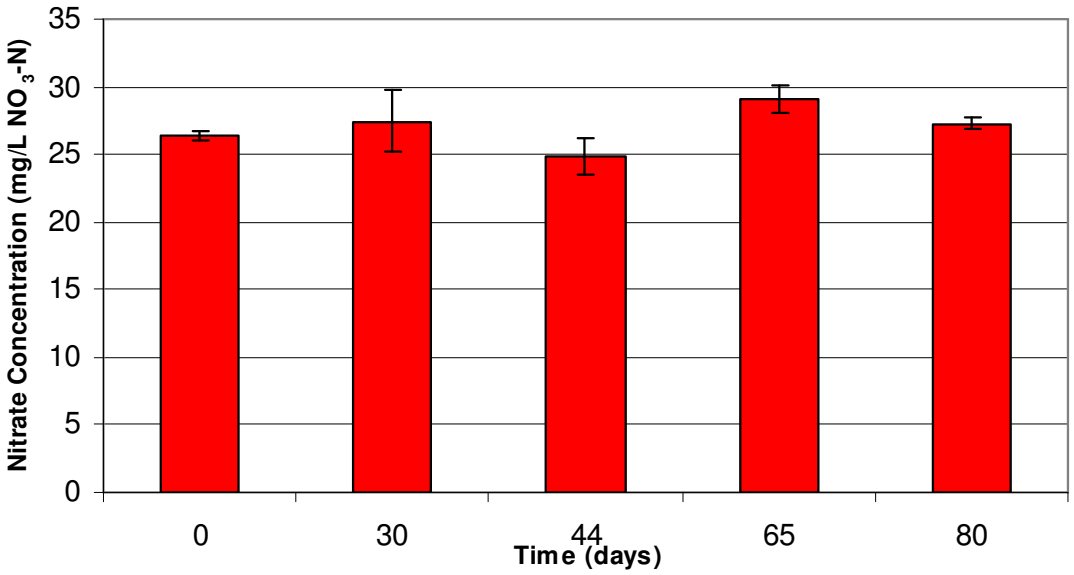


Figure B-3: Nitrate concentration of nitrate treatment with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

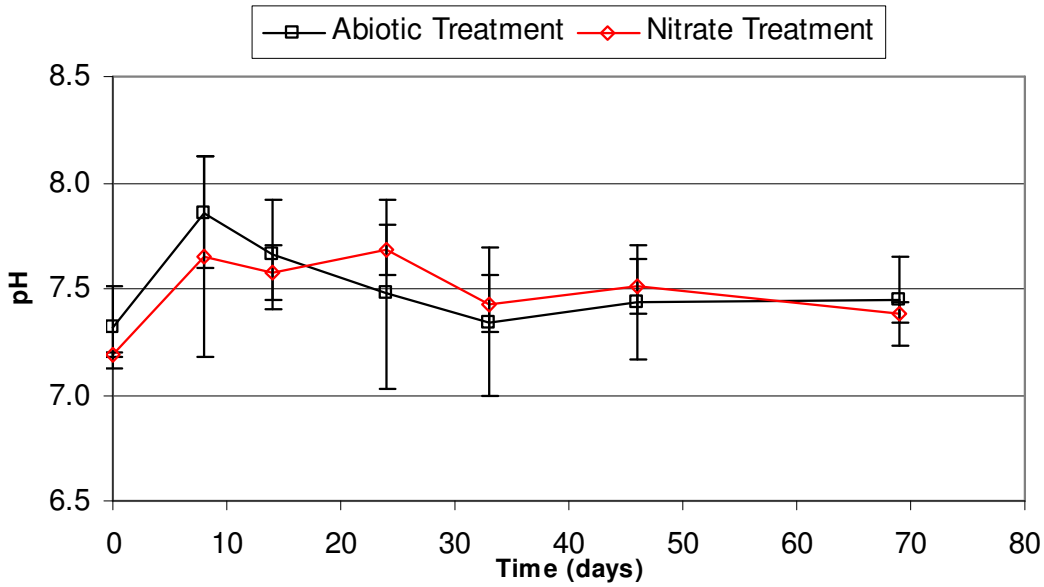


Figure B-4: pH of abiotic and nitrate treatments with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

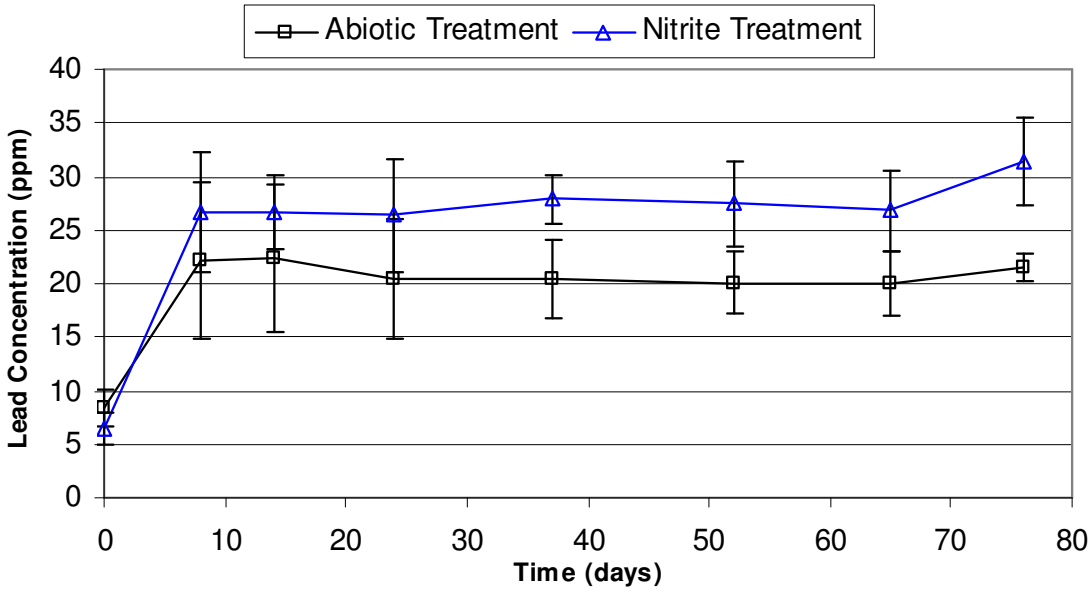


Figure B-5: Total lead concentrations of abiotic and nitrite treatments with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

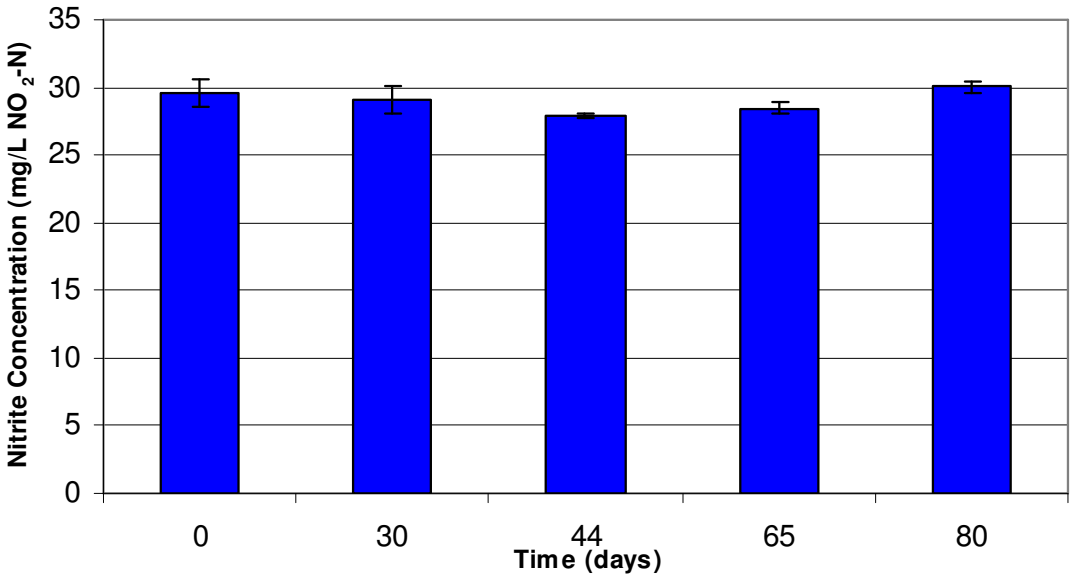


Figure B-6: Nitrite concentrations of abiotic and nitrite treatments, replicate experiment, error bars represent one standard deviation for averages.

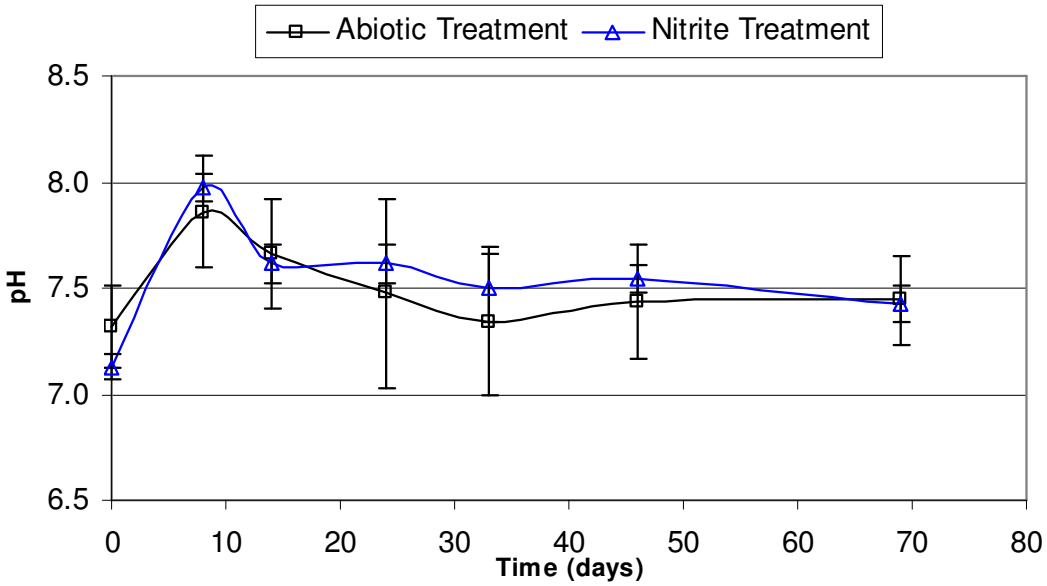


Figure B-7: pH of abiotic and nitrite treatments with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

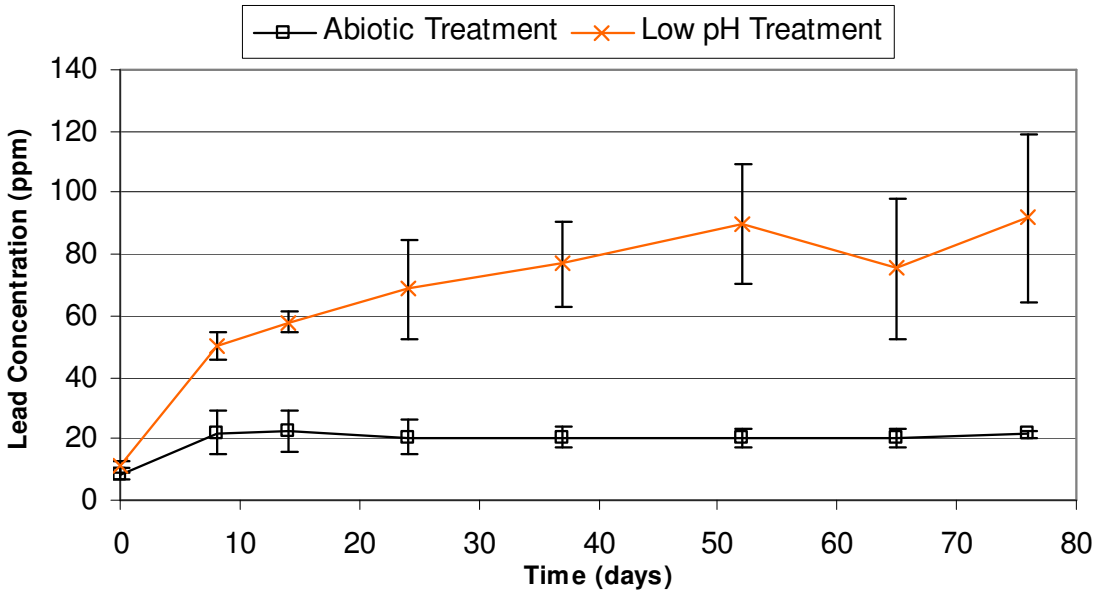


Figure B-8: Total lead concentrations of abiotic and low pH treatments with a freshly cleaned coupon, replicate experiment, error bars represent one standard deviation for averages.

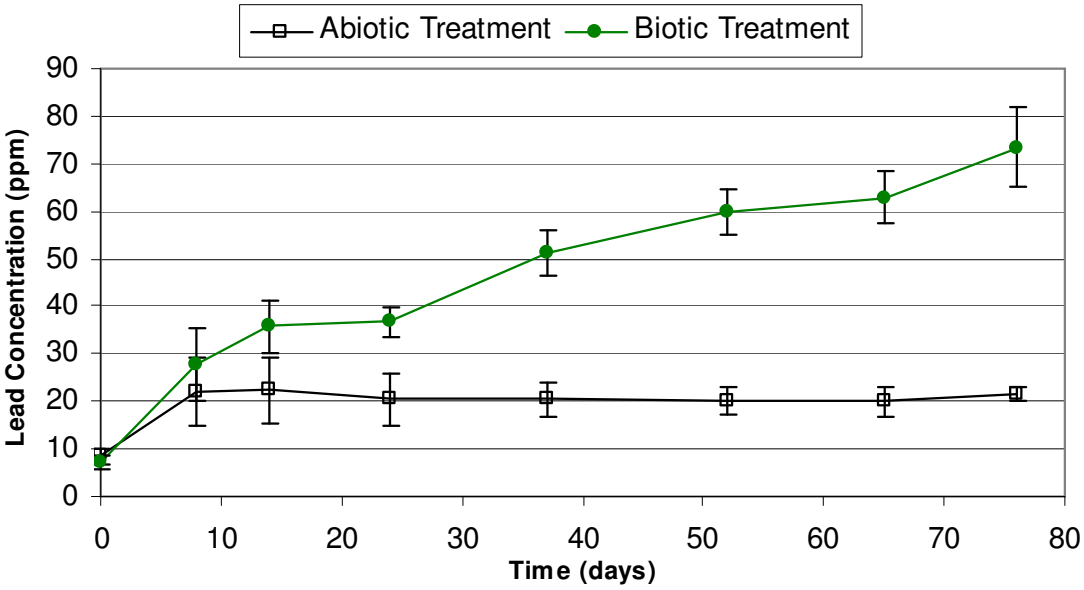


Figure B-9: Total lead concentrations of abiotic and biotic treatments with freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

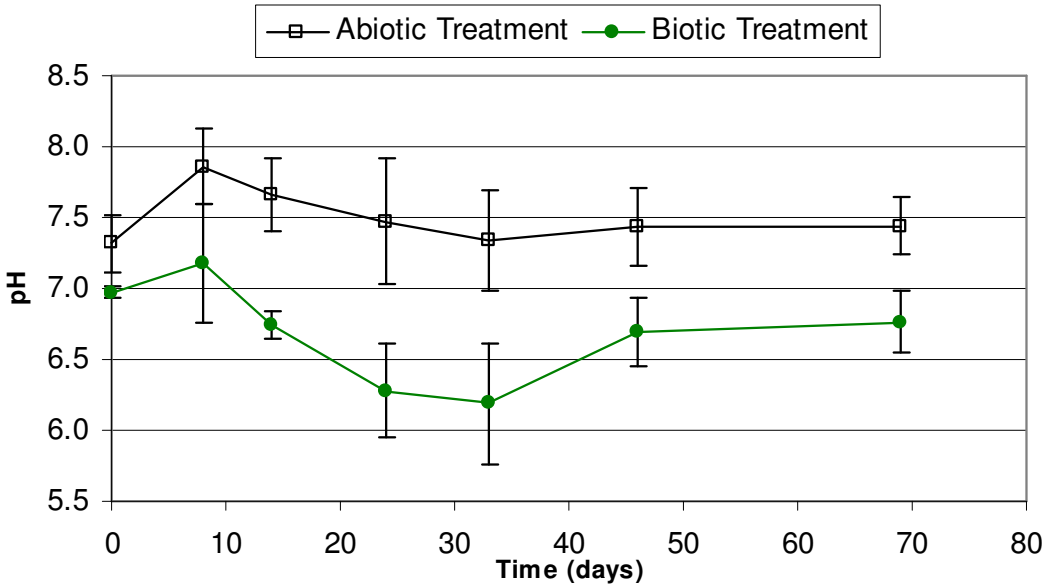


Figure B-10: pH of abiotic and biotic treatments with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

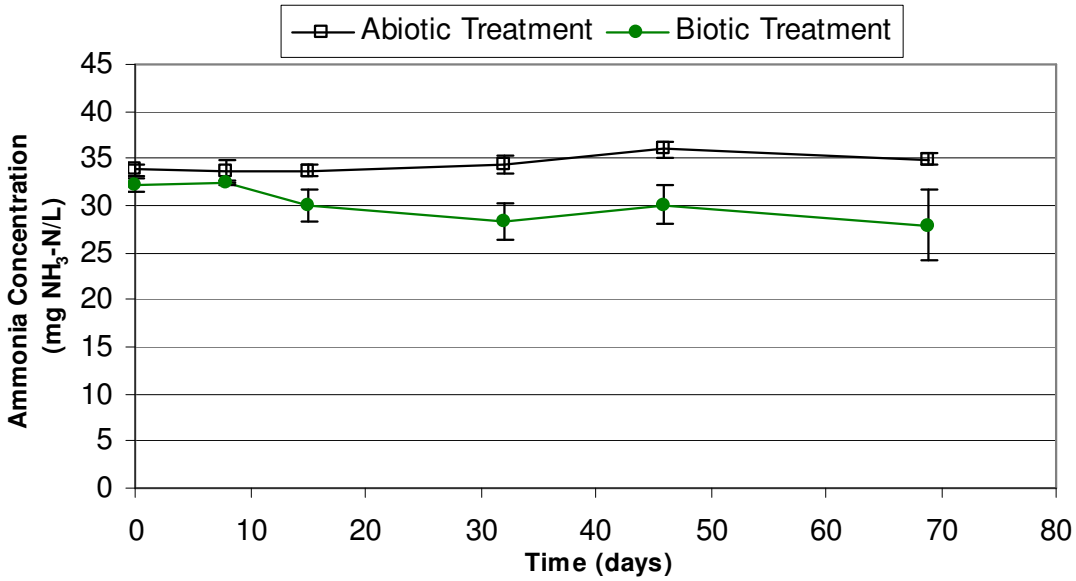


Figure B-11: Ammonia concentrations of abiotic and biotic treatments with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

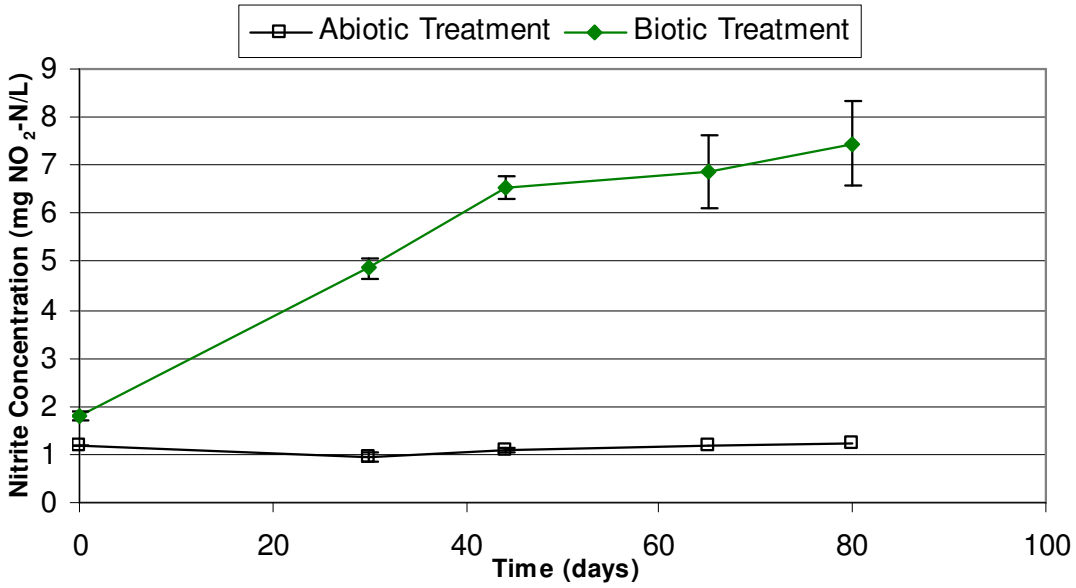


Figure B-12: Nitrite concentrations of abiotic and biotic treatments with a freshly cleaned lead coupon, replicate experiment, error bars represent one standard deviation for averages.

Table B-1: Soluble lead concentrations for the Lead Factors Study - Replicate Experiment after 84 days.

Treatment	Soluble Lead Concentration (ppb)	Standard Deviation
Abiotic	6.28	1.34
Nitrate	5.83	2.81
Nitrite	33.1	19.3
Low pH	1800	1130
Biotic	312	115

Appendix C: Experimental Protocols and Reagents

B.1 STANDARD PROCEDURE FOR MEASURING MONOCHLORAMINE / DICHLORAMINE

Version 1, Sep 2001

Adapted from standard method 4500-Cl F.

SAFETY PRECAUTIONS

The following protocol calls for the storage, use and disposal of chemicals, which may present a physical hazard (fire, explosion, etc) or health hazard (toxic, carcinogenic, etc.). Anyone conducting research, which follows this protocol is required to abide by the following standard operation procedures (SOP):

Chemical labels-Carefully read the labels of all hazardous chemicals before they are used in the protocol. Before transferring a chemical to another container, the new container must be clearly labeled with the chemical name.
Material Safety Data Sheets (MADSs)- The MSDSs for hazardous chemical s used in this protocol are located in the folders labeled "MSDS" in the lab. Additional information can be obtained from the MSDS computer station located in the computer lab. Anyone using this protocol must familiarize themselves with the hazardous properties of the chemicals by reviewing the MSDSs.
Personal Protective Equipment- any personal protective equipment recommended on the chemical container labels or MSDSs (gloves, aprons, goggles, etc) is required to be used during handling of the chemicals in this protocol.
Containment Devices-Any containment devices recommended on the chemical container labels or MSDSs (chemical fume hood, glove box, explosion-proof refrigerator, etc.) is required to be used during storage and active handling of the chemicals in this protocol.
Chemical Waste- Any chemical waste generated as a result of procedures described in this protocol are required to be disposed of in compliance with the federal, state, and local environmental regulations. Accumulation in a labeled waste container specific for a class of chemicals is the preferred method. Evaporation in a chemical fume hood is not an option. Waste chemicals, no matter how seemingly innocuous, may not be poured down the drain to the sanitary sewer unless specific permission is obtained from the health safety officer.

APPARATUS

a. pH Meter
b. Titration vessel – Use a 200-mL beaker.
c. Magnetic stirrer.
d. Pipets- volumetric 0.5-mL, 1-mL and 20-mL.
e. Flasks- volumetric, 100- and 1000-mL.
f. Burets- borosilicate glass, 10-mL.

REAGENTS

a. phosphate buffer solution :

1) Dissolve 800 mg disodium ethylenediamine tetraacetate dihydrate (EDTA) in 100mL DDW. Use 100- mL volumetric flask for this purpose.

2) Dissolve 24g anhydrous Na2 HPO4 and 46g anhydrous KH2PO4 in 1000-mL volumetric flask having initially approx. 500mL DDW in it.

3) Combine 100mL EDTA solution (generated at step 1) with 1000-mL volumetric flask.

4) Dilute to 1000mL with DDW.

*5) Add 20mg HgCl2 to prevent mold growth. In addition, interference from trace amounts of iodide in the reagents can be negated by the addition of 20mg HgCl2 to the solution. (Caution: HgCl2 is toxic-take care to avoid ingestion.) * for health perspective, not using HgCl2 anymore.

b. monochloraimine stock

1) Make up chlorine stock

Transfer 1mL solution from stock sodium hypochlorite solution (NaOCl, available chlorine is 5.0%) and dilute to 100mL.The concentration of chlorine should be about 500mg/L. Check the stock concentration by doing a 1:200 dilution.

2) Make up NH3 stock

Weigh 67.3mg (NH4)2SO4 into 100-mL volumetric flask and dilute to 100mL. (If the chlorine stock concentration was not 500mg/L, use equation

(chlorine concentration mg/L)*67.3mg/(500mg/L)=the weight of (NH4)2SO4 mg, then do dilution as mentioned above)

3) Transfer chlorine stock and NH3 stock generated at step 1 and 2 into 250-mL Erlenmeyer flask, respectively. Adjust pH of each of the solution to 9.0-9.1 using (1N, 0.2N)HCl solution and (1N, 0.2N) NaOH solution.

4) Add Cl2 solution to NH3 solution and stir for about 5 min.

The concentration of monochloramine should be about 250mg/l, as Cl2/L.Check the monochloramine stock concentration by doing a 1:100 dilution.

c. Standard ferrous ammonium sulfate (FAS) titrant:

1) Dissolve 1mL 1+3H2SO4 in 1000-mL volumetric flask, which initially may have approx. 500 mL DDW in it.

2) Dissolve 1.106 g Fe(NH4)2(SO4)2·6H2O in it and dilute to 1L with DDW.

d. N,N-diethyl-p-phenylenediamine (DPD) indicator solution:

1) Dissolve 8mL 1+3H2SO4 and 200mg disodium EDTA in a 1000-mL volumetric flask, which initially may have approx. 500mL DDW in it.

2) Dissolve 1.1g anhydrous DPD sulfate to the flask and dilute to 1000mL.

e .Potassium iodide, KI, crystals

PROCEDURE

a. Prepare sample by taking 20mL sample and diluting to 100mL with DDW (1:5 dilution).
b. Dilute FAS solution by taking 20mL sample and diluting to 100mL with DDW (1:5 dilution).
c. Take 10mL 10,000mg/L phosphate dibasic buffer solution, 5mL phosphate buffer solution and 5mL DPD indicator solution into the titration beaker, respectively (If sample is added before buffer, test does not work).
d. Add 100mL sample. If sample turns to red, this indicates the presence of free chlorine. The free chlorine concentration can be titrated by diluted FAS solution until the color is discharged (Reading A).
e. Add one very small crystal of KI (about 0.5 mg) and mix to dissolve.
f. Continue titrating until red color is discharged again (Reading B).
g. Add several crystals KI (about 1g) and mix to dissolve. Let stand for 2 min and continue titrating until red color is discharged (Reading C).
*The quantities given below are suitable for concentration of total chlorine up to 5 mg/l. If monochloramine exceeds 5 mg/l, use a smaller sample and dilute to a total volume of 100mL.
*pH control: For accurate results careful pH control is essential. At the proper pH of 6.2 to 6.5, the red colors produced may be titrated to sharp colorless end points.

CALCULATIONS
To calculate monochloramine:
For a 100-mL sample, 1.00mL standard FAS titrant =1.00mg Cl as Cl2/L.
The monochloramine concentration is B-A.
The dichloramine concentration is C-B.

B.2 Colorimetric Determination of Ammonium

Adapted from Kandeler and Gerber (1988)

Reagents:

(1) Sodium dichlorisocyanurate

-Add 0.1 g sodium dichlorisocyanurate to 50 mL DDI water

(2) Sodium salicylate

-Add 5.666 g sodium salicylate and 0.08 g sodium nitroprusside to 100 mL DDI water

(3) Sodium hydroxide

-Add 0.5 g sodium hydroxide to 50 mL DDI water

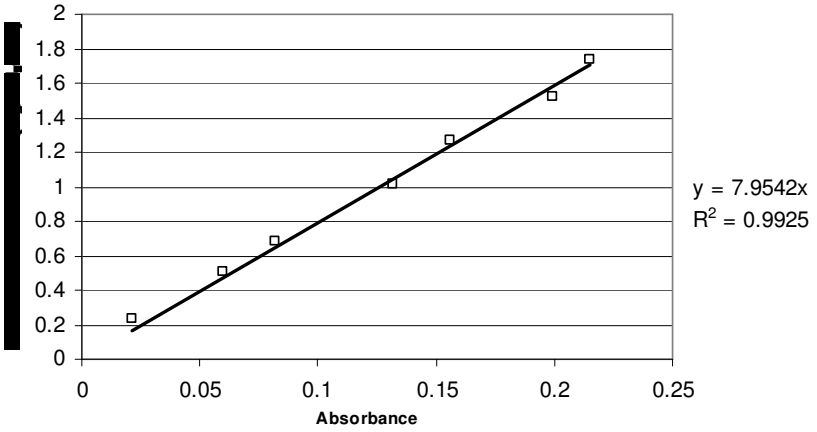
Notes: Store reagents in dark when not in use.
Reagents (1) and (2) need to be remade daily.

Equipment:

Spectrophotometer, operated at 690 nm

Calibration:

There is a stable calibration range of 0.2 – 2.0 mg NH_4^+-N/L. A sample calibration curve is provided below.



Procedure:

1) Dilute samples to a volume of 0.5 mL and ammonium concentration of 0.2 – 2.0 mg NH_4^+-N/L.
2) Add 0.25 mL of sodium salicylate reagent to all samples.
3) Wait 3 minutes, then add 0.1 mL sodium dichlorisocyanurate reagent to all samples.
4) Immediately add 0.1 mL sodium hydroxide to all samples.
5) Cap samples and shake well. Wait 45 minutes.
6) Pour sample into Q-vat, place Q-vat in spectrophotometer

Kandeler E and Gerber H. (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils*. 6:68-72.

Appendix D: t-test Data

t-Test: Two-Sample Assuming Unequal Variances **Nitrate Treatment vs. Abiotic Treatment**	
t Stat	0.987717204
P(T<=t) one-tail	0.189603358
t Critical one-tail	2.131846782
P(T<=t) two-tail	0.379206716
t Critical two-tail	2.776445105

t-Test: Two-Sample Assuming Unequal Variances **Low pH Treatment vs. Abiotic Treatment**	
t Stat	74.08819237
P(T<=t) one-tail	9.94484E-08
t Critical one-tail	2.131846782
P(T<=t) two-tail	1.98897E-07
t Critical two-tail	2.776445105

t-Test: Two-Sample Assuming Unequal Variances **Nitrite Treatment vs. Abiotic Treatment**	
t Stat	0.871679486
P(T<=t) one-tail	0.216289248
t Critical one-tail	2.131846782
P(T<=t) two-tail	0.432578496
t Critical two-tail	2.776445105

t-Test: Two-Sample Assuming Unequal Variances **Biotic Treatment vs. Abiotic Treatment**	
t Stat	5.836383236
P(T<=t) one-tail	0.005010884
t Critical one-tail	2.353363435
P(T<=t) two-tail	0.010021767
t Critical two-tail	3.182446305

t-Test: Two-Sample Assuming Unequal Variances **Nitrate Treatment without Spent Media vs.** **Abiotic Treatment without Spent Media**	
t Stat	5.87156646
P(T<=t) one-tail	0.013901151
t Critical one-tail	2.91998558
P(T<=t) two-tail	0.027802302
t Critical two-tail	4.30265273

t-Test: Two-Sample Assuming Unequal Variances **Nitrite Treatment without Spent Media vs.** **Abiotic Treatment without Spent Media**	
t Stat	21.89084876
P(T<=t) one-tail	0.014530672
t Critical one-tail	6.313751514
P(T<=t) two-tail	0.029061345
t Critical two-tail	12.70620473

Figure D-1: t-Test for Lead Corrosion Factors Study with Fresh Coupon of total lead concentrations after 93 days.

t-Test: Two-Sample Assuming Unequal Variances **Nitrate Treatment vs. Abiotic Treatment**	
t Stat	4.999878976
P(T<=t) one-tail	0.018875638
t Critical one-tail	2.91998558
P(T<=t) two-tail	0.037751277
t Critical two-tail	4.30265273

t-Test: Two-Sample Assuming Unequal Variances **Low pH Treatment vs. Abiotic Treatment**	
t Stat	7.482709101
P(T<=t) one-tail	0.008697687
t Critical one-tail	2.91998558
P(T<=t) two-tail	0.017395374
t Critical two-tail	4.30265273

t-Test: Two-Sample Assuming Unequal Variances **Nitrite Treatment vs. Abiotic Treatment**	
t Stat	9.522294191
P(T<=t) one-tail	0.001228108
t Critical one-tail	2.353363435
P(T<=t) two-tail	0.002456216
t Critical two-tail	3.182446305

t-Test: Two-Sample Assuming Unequal Variances **Biotic Treatment vs. Abiotic Treatment**	
t Stat	6.512328683
P(T<=t) one-tail	0.011388311
t Critical one-tail	2.91998558
P(T<=t) two-tail	0.022776623
t Critical two-tail	4.30265273

t-Test: Two-Sample Assuming Unequal Variances **Nitrate Treatment without Spent Media vs.** **Abiotic Treatment without Spent Media**	
t Stat	6.019492274
P(T<=t) one-tail	0.013252907
t Critical one-tail	2.91998558
P(T<=t) two-tail	0.026505814
t Critical two-tail	4.30265273

t-Test: Two-Sample Assuming Unequal Variances **Nitrite Treatment without Spent Media vs.** **Abiotic Treatment without Spent Media**	
t Stat	7.820670649
P(T<=t) one-tail	0.007979717
t Critical one-tail	2.91998558
P(T<=t) two-tail	0.015959433
t Critical two-tail	4.30265273

Figure D-2: t-Test for Lead Corrosion Factors Study with Aged Coupon of total lead concentrations after 81 days.

t-Test: Two-Sample Assuming Unequal Variances Nitrate Treatment vs. Abiotic Treatment	
t Stat	0.987882886
P(T<=t) one-tail	0.213670971
t Critical one-tail	2.91998558
P(T<=t) two-tail	0.427341942
t Critical two-tail	4.30265273

t-Test: Two-Sample Assuming Unequal Variances Low pH Treatment vs. Abiotic Treatment	
t Stat	5.169838624
P(T<=t) one-tail	0.007021022
t Critical one-tail	2.353363435
P(T<=t) two-tail	0.014042043
t Critical two-tail	3.182446305

t-Test: Two-Sample Assuming Unequal Variances Nitrite Treatment vs. Abiotic Treatment	
t Stat	3.044080583
P(T<=t) one-tail	0.027843763
t Critical one-tail	2.353363435
P(T<=t) two-tail	0.055687526
t Critical two-tail	3.182446305

t-Test: Two-Sample Assuming Unequal Variances Biotic Treatment vs. Abiotic Treatment	
t Stat	12.33648095
P(T<=t) one-tail	0.000573704
t Critical one-tail	2.353363435
P(T<=t) two-tail	0.001147408
t Critical two-tail	3.182446305

Figure D-3: t-Test for Lead Corrosion Factors Study with Fresh Coupon - Replicate Experiment for total lead concentrations after 76 days.

t-Test: Two-Sample Assuming Unequal Variances Abiotic Control vs. Abiotic-Orthophosphate Treatment	
t Stat	2.722653232
P(T<=t) one-tail	0.036193636
t Critical one-tail	1.637744352
P(T<=t) two-tail	0.072387273
t Critical two-tail	2.353363435

t-Test: Two-Sample Assuming Unequal Variances Abiotic Control vs. Abiotic-Alkalinity Treatment	
t Stat	6.112236581
P(T<=t) one-tail	0.012869046
t Critical one-tail	1.885618083
P(T<=t) two-tail	0.025738093
t Critical two-tail	2.91998558

t-Test: Two-Sample Assuming Unequal Variances Biotic Control vs. Biotic-Orthophosphate Treatment	
t Stat	1.54297725
P(T<=t) one-tail	0.110258706
t Critical one-tail	1.637744352
P(T<=t) two-tail	0.220517411
t Critical two-tail	2.353363435

t-Test: Two-Sample Assuming Unequal Variances Biotic Control vs. Biotic-Alkalinity Treatment	
t Stat	1.818802047
P(T<=t) one-tail	0.071538042
t Critical one-tail	1.533206273
P(T<=t) two-tail	0.143076083
t Critical two-tail	2.131846782

t-Test: Two-Sample Assuming Unequal Variances Biotic Control vs. Biotic-pH Adjustment Treatment	
t Stat	5.653981635
P(T<=t) one-tail	0.01494325
t Critical one-tail	1.885618083
P(T<=t) two-tail	0.0298865
t Critical two-tail	2.91998558

t-Test: Two-Sample Assuming Unequal Variances Abiotic-Nitrate Control vs. Abiotic-Zinc Orthophosphate Treatmen	
t Stat	1.864565643
P(T<=t) one-tail	0.079553973
t Critical one-tail	1.637744352
P(T<=t) two-tail	0.159107946
t Critical two-tail	2.353363435

t-Test: Two-Sample Assuming Unequal Variances Biotic-Nitrate Control vs. Biotic-Zinc Orthophosphate Treatment	
t Stat	3.409913507
P(T<=t) one-tail	0.021076684
t Critical one-tail	1.637744352
P(T<=t) two-tail	0.042153368
t Critical two-tail	2.353363435

Figure D-4: t-Test for Lead Corrosion Inhibitors Study for lead concentrations after 76 days (Orthophosphate and Alkalinity Treatments), 81 days (pH Adjustment Treatment), or 78 days (Zinc Orthophosphate Treatment).

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