INTERACTIONS OF CORROSION CONTROL AND BIOFILM ON LEAD AND COPPER IN PREMISE PLUMBING

by

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Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
November 2013

DEDICATION

To my lab partners (John, Amy, Greg, Wendy, Corinne, Meghan); my office D240 (Megan, Vince, Reza, Wenwen, Zaman, Hun); my friends in Halifax both in and out of the lab; my family (Mum, Dad, Elspeth, Jason, Marilyn, Maggie, David); the unbroken circle of friends at Hosannas; my friends at St. Paul's; my friends at home and around the world – without your ineffable, indefatigable love and support – none of this would have been possible. I dedicate this work to the steadfast and stalwart.

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ABSTRACT

Premise plumbing can contain copper and lead bearing fixtures, and although copper is considered primarily an aesthetic issue, the neurotoxic effects of lead present a significant public health concern. Utilities approach corrosion control in low alkalinity water by increasing the pH (>9) or adding a phosphate inhibitor at neutral pH. Phosphate inhibitors, pH and chlorine are known to affect lead and copper release through their direct action as corrosion inhibitors or oxidizing agents or through an indirect action via microbial growth. Biofilms are often an implied cause of premise plumbing corrosion, although little is known about their community structure or ability to store metals. The central hypothesis of this thesis is that biofilm contributes to lead and copper release in premise plumbing.

This thesis addresses a unique gap in corrosion literature by integrating traditional corrosion chemistry methods with microbiological and molecular biology techniques. The experiments used three distinct approaches: (i). electrochemical cell experiments to determine the key factors in decreasing lead and copper corrosion in galvanically coupled systems while maintaining microbial control (ii). galvanic macrocells using premise plumbing components to examine the unintended consequences of adding a phosphate based corrosion inhibitor and (iii). an annular reactor study to examine the impacts of two commonly applied corrosion control strategies. In the electrochemical cell experiments, the pH 9.2 with zinc orthophosphate and chlorine treatment achieved both goals: decreased lead and copper release and limited microbial growth. In galvanic macrocells experiments with premise plumbing components, zinc orthophosphate addition was shown to be positively correlated with increased bulk water bacteria, biofilm growth and biofilm community structure as measured by DGGE. Biofilm was also observed to sorb 3-29% of lead and 3-16% of copper from the bulk water. The comparison of the two corrosion control strategies showed that the majority of lead released was in the particulate form, and the results further alluded to the potentially significant role lead particles play in biofilm formation.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ANOVA	analysis of variance
APHA	American Public Health Association
ATP	adenosine triphosphate
$CaCO_3$	calcium carbonate
C_{crit}	critical chlorine concentration
CDC	Centers for Disease Control
Cl_2	chlorine
CSMR	chloride to sulphate mass ratio
Cu	copper
DGGE	denaturing gradient gel electrophoresis
DIC	dissolved inorganic carbon
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
e^-	electron
EDS	energy dispersive spectroscopy
EPS	exopolymeric substances
H^+	hydrogen
HOCl	hypochlorous acid
HPC	heterotrophic plate count
ICP-ms	inductively coupled plasma mass spectrometry
M(s)	solid metal
MAC	maximum allowable concentration
NTU	nephelometric turbidity units
O_2	oxygen
OCl^-	hypochlorite
OH^-	hydroxide
OTU	operational taxonomic units
Pb	lead
PCR	polymerase chain reaction
pK_a	acid dissociation constant
PO_4	phosphate
rDNA	ribosomal deoxyribonucleic acid
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SEC	size exclusion chromotography
TOC	total organic carbon
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WPC	World Plumbing Council

ACKNOWLEDGEMENTS

This work would not be possible without the support of my colleagues at Water Studies at Dalhousie, Heather Daurie, Jessica Campbell, Tarra Chartrand, Dalhousie Civil Engineering technicians (Brian, Brian, Mark and Blair) or the encouragement and advice offered by colleagues elsewhere at Dalhousie (especially, Dr. Margie Clow Bohan). My development as a researcher has been nurtured through opportunities with the Canadian Water Network and through accomplished professionals taking an interest in me and my work. I wish to say thank you to all of you for your support.

I gratefully acknowledge the support of this work through the NSERC Industrial Research Chair. I also wish to thank Halifax Water and particularly the J.D. Kline plant staff for their support and participation in this research. I wish to thank my supervisory committee: Dr. Truelstrup Hansen (for making the world of microbiology accessible to me), and Dr. Walsh (for acting as my engineering mentor). I also wish to thank my supervisor, Dr. Gagnon, for creating this opportunity for me in the first place and for giving me the space and support to explore my ambitions to their limit.

CHAPTER 1 INTRODUCTION

1.1 IMPETUS FOR WORK

Between 2000 and 2004, Washington, D.C., experienced dangerously high lead levels in drinking water, exceeding the action limit for lead in drinking water (Edwards & Dudi, 2004, USEPA, 2007). In the case of Washington, D.C., the utility had switched from a chlorine-based disinfection programme to a chloramine based disinfection programme, to avoid the formation of carcinogenic disinfection by-products (formed when chlorine combines with natural organic matter in water). The free chlorine previously used in the distribution system had favoured the generation of a relatively stable Pb (IV) corrosion scale on the leaded-fixtures in Washington premise plumbing. The switch to chloramines decreased the overall free chlorine in the water, changing the oxidation-reduction potential of the water. The chloramines treated water subsequently favoured the formation of the less stable Pb (II) corrosion scale. The naturally fluctuating pH of the drinking water in Washington, D.C., likely dissolved the unstable Pb (II) scale, creating an unacceptable lead release into the drinking water (USEPA, 2007). Elevated blood levels were detected in Washington's children, with infants as the most exposed portion of the population as their diet is largely reliant on water (Edwards et al., 2009). The change in disinfectant in Washington, addressed one issue of concern (decreasing disinfection by-product formation potential) but also initiated the unintended consequence of destabilizing lead corrosion scales and exposing the most vulnerable portion of the population (children and infants) to a powerful neurotoxin: lead.

This recent lead crisis in Washington, D.C. has focused the water industry's attention on the risks of lead exposure and renewed interest in premise plumbing corrosion and understanding the nuances of water chemistry and sampling protocols for effective mitigation and monitoring. In a 2008 inorganic contaminant survey of Canadian water utilities, lead was identified as a major concern for Canadian utilities. Interestingly, the survey also recorded that of the 30 participating utilities with a corrosion control program, only 15 of the utilities conducted in-home lead sampling (Knowles, et al., 2008). Similarly, in a 2001 survey of medium and large U.S. utilities, only about 60% used site specific studies to evaluate the appropriateness of phosphate based corrosion inhibitors before implementing their use in the distribution system (McNeill & Edwards, 2002). Clearly, there is a need for evidence-based guidance as well as site specific studies to help drinking water utilities address lead corrosion.

Most corrosion studies primarily focus on the chemistry involved in lead and copper corrosion control. Although biofilms are understood to be involved in lead and copper corrosion, they are not generally integrated in corrosion control studies. Biofilms are known to play a role in copper corrosion, especially in pin-hole corrosion (Critchley et al., 2004) and have been associated with lead corrosion (White et al., 2011). Biofilm bacteria produce a sticky exopolymeric substance (EPS) which allows it to adhere to surfaces and other bacteria. This EPS has been shown to adsorb natural organic matter (Wang et al., 2012) as well as inorganic components (Qin-qin et al., 2012).

1.2 THESIS HYPOTHESIS

Biofilms likely play a role as both a sink and a source for lead and copper. The available organic and inorganic components (including lead and copper) in the bulk drinking water could be absorbed by the biofilm (sink). Biofilm can be released from drinking water pipe materials during transient pressure events, and subsequently any absorbed materials would be released with it (source). Figure 1.1 describes how biofilm could act as both a sink and source for drinking water organic and inorganic components.

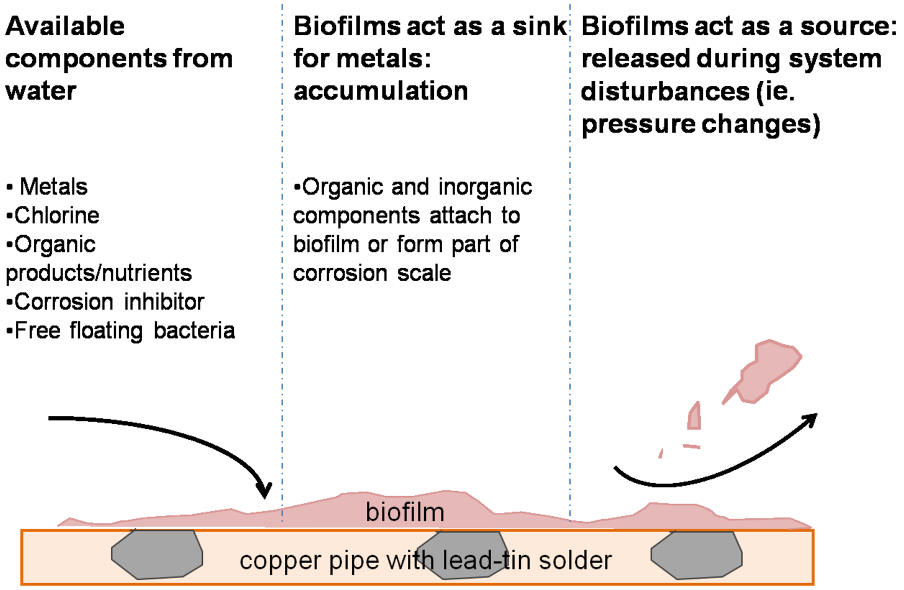


Figure 1.1 Biofilms may function as both a sink and source for metals in premise plumbing

The central hypothesis of this thesis is that biofilm contributes to lead and copper release in premise plumbing.

1.3 THESIS OBJECTIVES AND OUTLINE

By addressing the thesis hypothesis, it is anticipated that this thesis will help address a unique gap in lead and copper corrosion literature. This thesis integrates traditional corrosion chemistry methods with microbiological and molecular biology techniques to investigate the relationship between biofilm and corrosion control in premise plumbing. The experiments used three distinct approaches: 1. fundamental processes bench-scale experiments to determine the key factors in decreasing lead and copper corrosion in galvanically coupled systems 2. bench-scale experiments using real premise plumbing components to examine the unintended consequences of adding a phosphate corrosion inhibitor to Halifax Water and 3. a long term study to compare two different corrosion control schemes for the Halifax distribution system.

Chapter 1- Provides the impetus for this thesis as well as an introduction to premise plumbing, lead and copper plumbing components as well as the thesis objective and outline.

Chapter 2- Provides an overview of the health effects of lead and copper exposure as well as current regulatory environment for monitoring and controlling lead and copper exposure in Canada. Chapter 2 also provides a literature review covering the types of corrosion that affect lead and copper, the corrosivity of different agents in water, as well as corrosion control strategies.

Chapter 3- Examines the effects of chlorine, zinc orthophosphate and pH on lead release, copper release and microbial population in low alkalinity water. Details are provided from fundamental bench-scale experiments using galvanically coupled lead and copper in a controlled laboratory experiments. The experiments were evaluated over 1-d, 2-d and 4-d stagnation periods to represent worst case scenarios (i.e. stagnation of water overnight, over a long weekend). A conceptual tool designed for utilities aiming to achieve balance between microbial control and corrosion control is developed in this Chapter. This Chapter has been prepared as a journal article, a list of co-authors and this author's contribution to the manuscript is on file at the Dalhousie Faculty of Graduate Studies.

Chapter 4- Evaluates bench-scale experiments using real premise plumbing components to examine the unintended consequences of adding zinc orthophosphate as a corrosion inhibitor. The experimental design also included a representation of a benign downstream plumbing component, as a surface to develop biofilm. The effect of zinc orthophosphate on microbial activity was assessed using two different methods: a traditional plate count for enumerating heterotrophic plate count bacteria and an emerging method for measuring adenosine tri-phosphate (ATP) activity cells. The biofilm was also assessed for its ability to adsorb lead and copper from the bulk water, to estimate biofilms ability to act as a sink for metals. This Chapter has been prepared as a journal article, a list of co-authors and this author's contribution to the manuscript a is on file at the Dalhousie Faculty of Graduate Studies.

Chapter 5- Elaborates further on the biofilm analysis from Chapter 4. The biofilm was assessed using a molecular biology method, denaturing gradient gel electrophoresis

(DGGE) and DNA sequencing to examine how zinc orthophosphate influences biofilm community structure. This Chapter has been prepared as a journal article, a list of co-authors and this author's contribution to the manuscript is on file at the Dalhousie Faculty of Graduate Studies.

Chapter 6- Evaluates an annular reactor study conducted at the Halifax Water, J D Kline Water Supply Plant, to compare two commonly used corrosion control schemes used in low alkalinity water (pH 9.2 or pH 7.4 with zinc orthophosphate). The copper pipe materials (with and without lead-tin solder) were aged in annular reactors located at the JD Kline water treatment plant over the course of 1.5 yr prior to experimentation. The two corrosion control schemes were compared in the presence and absence of chlorine, and were assessed for their efficacy in controlling lead and copper. The biofilm was also collected, and the biofilm population was quantified and assessed for the concentrations of adsorbed lead and copper. The potentially significant role that lead particles may play in biofilm formation is also explored. This Chapter has been prepared as a journal article, a list of co-authors and this author's contribution to the manuscript is on file at the Dalhousie Faculty of Graduate Studies.

Chapter 7- The results of the four experimental chapters are summarized and conclusions are drawn. Recommendations for further studies are also given.

CHAPTER 2 BACKGROUND AND LITERATURE REVIEW

2.1 LEAD AND COPPER IN PREMISE PLUMBING

Premise plumbing is the portion of the distribution system located on the consumer's property. It varies from the main distribution system in terms of pipe materials, pipe diameter and temperature (National Research Council of the National Academies, 2006). Copper is a commonly used premise plumbing material, as it is flexible, available in small diameters and is appropriate for hot water fittings (WHO & WPC, 2006). Additionally, copper possesses anti-microbial properties (Osman & Cavet, 2008). Until its ban in 1986 in the United States (USEPA, 2012) and 1990 in Canada (Health Canada, 2012) lead-tin solder was an acceptable and commonly applied solder to join copper fittings. Lead is also contained in lead service lines (connecting the main distribution system to the premise plumbing) and in leaded brass fixtures (Sandvig et al., 2008).

Through corrosion processes, copper and lead can come into solution and contaminate drinking water. When lead and copper are in physical contact in a conducting solution (such as drinking water) they form a galvanic cell: the lead acts as an anode and the copper acts as the cathode. In the case of a lead-copper galvanic coupling, lead is released and copper is protected. Additionally, areas of non-uniformity or high and low oxygen may also create electrochemical cells on the lead and copper surfaces resulting in the release of metals.

Copper corrosion in drinking water plumbing presents primarily an economic nuisance (leaking pipes) or an aesthetic problem (blue water); however, exposure to high levels of copper can cause gastrointestinal disturbances (Pizarro et al., 2001). Lead ingestion poses a significant long-term health risk. Lead is a neurotoxin, and it negatively impacts the neurological development of children (Canfield et al., 2003; Zahran et al., 2009). In 2012, overwhelming evidence of the negative impacts of lead exposure in children prompted the Centers for Disease Control (CDC) to lower the level of concern for blood lead levels from 10 micrograms per deciliter to 5 micrograms per deciliter (National Centre for Healthy Housing, 2012).

2.2 REGULATIONS FOR LEAD CORROSION CONTROL

In Canada, water is regulated by individual Provinces; however Health Canada provides guidance on drinking water quality based on two tiers. In Tier 1, the recommended action limit for lead concentration in the first 1L sample following a six hour stagnation is 0.015 mg/L. If the first sample exceeds the action limit, then a second sample should be taken. Follow-up sampling (Tier 2) should take place at the exceedance sites, and requires a total of four consecutive 1L samples. (Health Canada, 2009) The Health Canada (2009) recommendation for the number of sample sites for a community sampling program is based on the United States Environmental Protection Agency (USEPA) requirements. In the United States, the number of sample sites in based on the size of the community (USEPA, 2010).

The way in which these guidelines are adopted depends on the Province and/or municipality. For example, in Nova Scotia, flushing taps prior to metal sampling is recommended, although the maximum allowable concentration is set as 0.01 mg/L (Nova Scotia Environment and Labour, 2007). Whereas, in Ontario, the Ministry of Environment recommends flushing the tap for five minutes followed by a 30 minute stagnation period. Then using a normal flow rate, two 1L samples are taken, and the higher lead concentration of the two used to determine exceedance. A third 1L sample is also taken (and pH is measured). The maximum allowable concentration is 0.01 mg/L. (Ontario Ministry of the Environment, 2009).

2.3 LITERATURE REVIEW

Corrosion is the process through which metals are returned to their naturally occurring (more thermodynamically stable) ore state (Roberge, 2008). For corrosion to occur an oxidation and reduction reaction must take place. In an oxidation, the atom loses an electron, and in a reduction, the atom gains an electron. In general terms, the oxidation (Equation 1) can be stated as:

$M(s) - e^- \rightarrow M^+$ (Equation 1)

where M, represents the metal
Adapted from Roberge (2008)

The reduction reaction, may occur through a variety of pathways: with another metal cation (Equation 2); or with other cations, such as a reaction with hydrogen (Equation 3); or in the presence of oxygen, form water (Equation 4); or hydroxide ions (Equation 5) (Roberge, 2008).

$M^+ + e^- \rightarrow M(s)$ (Equation 2)
$2H^+ + 2e^- \rightarrow H_2\ (g)$ (Equation 3)
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ (Equation 4)
$O_2 + 2H_20 + 4e^- \rightarrow 4OH^-$ (Equation 5)

Adapted from Roberge (2008)

There are many mechanisms through which corrosion can occur. Corrosion can occur uniformly across a metal surface; as a result of a specific attack corrosive agents in the water; as a result of localized non-uniform concentrations of oxygen at the surface created by crevices in the surface or microbial on the surface; or as a result of contact of dissimilar metals, where one more readily gives up an electron (the anode) (Cicek & Al-Numan, 2011).

2.3.1 Water chemistry, material choice and corrosion in premise plumbing

Specifically, in tap water, the water chemistry, additives, material choice and microbiology play a role in premise plumbing corrosion.

2.3.2 Effect of pH and alkalinity on corrosion

Acidic waters tend to be more corrosive. In low pH waters, there is an increased number of H+ ions, providing a site for reduction as in Equation 3 and 4. Subsequently, raising the pH of the water to lower solubility of the metal is a commonly used approach to decreasing corrosion of premise plumbing. Increased pH has been shown to decrease both lead corrosion (Tam & Elefsiniotis, 2009) and copper corrosion (Rahman et al., 2007). Alkalinity (measured as $CaCO_3$), acts as a buffer for pH changes, making the metal less vulnerable to acid attack. Premise plumbing in low alkalinity water is therefore particularly vulnerable to corrosion (Lytle & Schock, 2008). Low alkalinity may be naturally occurring as in the case of Eastern Canada and New England or as a result of membrane treatment processes. In the greater Vancouver Regional District, four low alkalinity treated water sources with an average pH from 6.1 (in the lowest pH water) to 8.1 (in the highest pH water) were compared. The lowest concentrations of copper and zinc were achieved in the homes receiving water with the highest pH (Knox et al., 2005). Ottawa also has a low alkalinity water source and uses increased pH to 9.2 and alkalinity to ≥ 35mg/L to successfully mitigate a lead corrosion in premise plumbing issue (Campbell & Douglas, 2008).

2.3.4 Conductivity and corrosion

Water molecules form charged clusters around any ions present in the water, and these solvated ions move through the water with an applied electrical charge. Increasing the number of ions, increases the conductance of the water. For example, sea water is more conductive than tap water (Roberge, 2008).

2.3.5 Chloride to sulphate mass ratio

The effects of the chloride and sulphate on premise plumbing have been well characterized. The chloride to sulphate mass ratio (CSMR) is used as a metric to predict the ratio of anions at the lead anode surface. At the surface sulphate combines with lead to form a lead precipitate (having a protective effect); whereas chloride combines with lead to form a soluble complex (exacerbating lead release) (Nguyen et al., 2011a). The CSMR at which the chloride attack becomes the dominant mechanism has been estimated to be 0.77 (Nguyen et al., 2011b) and 0.5 (Nguyen et al., 2010a) in low alkalinity water. Nguyen (2011c) also demonstrated that increases in CSMR that occurred within the CSMR range of 0.1-1.0 had a significant impact on increasing lead release. At CSMRs greater than 1.0, the lead releases were large, but the CSMR did not appear to be predictive of how large the lead release would be.

2.3.6 Galvanic corrosion

The type of material used in premise plumbing also plays a role in corrosion. Although fairly close in the galvanic series, copper has a positive potential; whereas lead and lead/tin solder have a negative potential. If copper and lead are connected the lead acts as the anode and corrodes, and the copper acts as the cathode and is protected (Ahmed, 2006). In premise plumbing, galvanic currents between lead and copper may occur in partial lead service line replacements (Cartier et al., 2013) and lead-tin soldered copper pipes (Nguyen et al., 2010b). Lead corrosion from galvanic corrosion can be exacerbated by the corrosive microenvironments at the anode site. As lead is released, it acts as a Lewis acid lowering the pH. In the presence of chloride anions a soluble lead chloride is formed. However, if sufficient sulphate ions are present then a less soluble lead sulphate would form, thereby decreasing lead release (Nguyen et al., 2010b). Over time, as passivation on the metal surfaces occurs, there is a decrease in corrosion currents (Reiber, 1991).

2.3.7 The effects of stagnation time on corrosion

In premise plumbing, water stagnates during periods of disuse (i.e. overnight, while consumers are away for weekends or vacation). In theory, the dissolution of lead and copper would decrease over time as surfaces passivate and equilibrium is achieved. Lytle and Schock (2000) asked what does "…equilibrium really mean in a dynamic system of oxidizing agents and reducing materials (metal pipes)." Lytle and Schock (2000) demonstrated that in stagnant systems with lead, copper and brass, the majority of the metals were released in the first 24 hours. Similarly, in Woszczynski et al. (2013), both chlorinated and chloraminated copper pipe rigs with lead-tin solder released higher concentrations of lead after a 24 hour stagnation period compared to a 30 minute stagnation period.

2.3.8 Microbial aspects of premise plumbing corrosion

Copper is a preferred plumbing material as it is easy to work with and can be made with relatively thin walls and small diameters (WHO & WPC, 2006). Copper, although necessary for some cellular functions, can also be toxic to bacteria. Some bacteria, such as *E. coli* have specific copper regulation pathways to achieve copper homeostasis (Outten, 2001). However, biofilms are well known to afford increased resistance to disinfectants, and recent evidence has also suggested that biofilms also offer increased resistance to metal toxins (Harrison et al., 2007). Fechner et al. found that freshwater biofilms exposed to lead (Fechner et al., 2012a) and copper (Fechner et al., 2012b) induced lead and copper tolerance. The production of extracellular molecular products, may produce more binding sites for toxic metals, sequestering the metals and reducing the chance that the metals will interfere with cellular metabolic functions (Harrison et al., 2007).

Microbially induced corrosion is thought to be a result of one or several mechanisms. As biofilms form on the metal pipe surface, the areas under the biofilm become anaerobic and act as an anode to the neighboring metallic surface (Cicek & Al-Numan, 2011). Also, the biofilm itself may produce acidic products and cause an acid attack on the metal (Roberge, 2008). Microbial communities have been identified as inducing blue-water corrosion in copper systems (Critchley et al., 2004) and copper pin-hole corrosion (Zumelzu et al., 2003). Although there is no definitive causative evidence linking microbial communities and lead corrosion, White et al. (2011) identified and characterized microbial communities associated with lead service line.

The effects of biofilm in corrosion are not limited to microbially induced corrosion. Bacteria from the bulk water form biofilms on the pipe surface. The bacteria adhere to the surface and to the biofilm through the secretion of exopolymeric substances (EPS). These EPS contain functional groups which provide bonding sites for cationic metal ions (Qin-qin et al., 2012) and natural organic matter (Wang et al., 2012). Feng et al. (2012) demonstrated that Pb^{2+} adsorption to EPS derived from a lactic acid bacteria depended on the pH, temperature, exposure time as well as the initial Pb^{2+} concentration and EPS concentration. Feng et al. (2012) found that the ideal pH for adsorption occurred at pH 5. At low pH levels, less Pb^{2+} would be adsorbed as H^+ likely competed for binding sites, and at high pH levels, less Pb^{2+} would be adsorbed as it likely formed insoluble hydroxyls (Feng et al., 2012). The presence of biofilm represents a storage site for both inorganic and organic matter found in the bulk water. Ginige et al. (2011) found a significant positive correlation between biomass and manganese accumulation in biofilm as well as iron accumulation in biofilm. Furthermore, biofilm can become detached from the surface through changes in water flow conditions (Garny et al., 2008). In copper pipe loops, Lehtola et al. (2006) observed that immediately after an increase in water velocity, there was an increase in biofilm detachment and copper levels.

2.3.9 Effect of chlorine on corrosion and microbial populations

Chlorine is added to drinking water as a disinfectant, and many jurisdictions (such as Nova Scotia) require that a chlorine residual is maintained throughout the distribution system. Chlorine ($pK_a = 7.6$) is a more powerful disinfectant at $pH < 7.6$ (as the HOCl species dominates) than at $pH > 7.6$ (as the OCl^- species dominates) (Fukazaki, 2006). Chlorine acts as an oxidant and has been shown to increase corrosion of lead (Lin et al., 2007) and copper (Cantor et al., 2003; Reiber, 1989), although the effects of adding chlorine does not always result in increased corrosion. Chlorine has also been shown to decrease soluble lead at pH 7.5, 8.25 and 9.0 (Edwards & Dudi, 2004). Rahman et al.

(2007) found that in stagnant water, a chlorine residual decreased copper corrosion in copper pipes. Rahman et al. (2007) suggested that the absence of chlorine residual led to microbial growth and resulted in increased copper concentrations.

2.3.10 Application of phosphate based corrosion inhibitors

Generally, the orthophosphate based corrosion inhibitors are used to passivate metal surfaces (including lead and copper) by forming a less soluble compound on the pipe surface (Schock, 1989). The composition of the corrosion product depends on the water chemistry and the other available water constituents (i.e. chloride, sulphate, calcium etc.). Orthophosphates and zinc orthophosphate have been shown to be effective as a corrosion control agent on both lead and copper surfaces. In low alkalinity water, zinc orthophosphate has been shown to be effective in controlling copper (Churchill et al., 2000) and lead (Churchill et al., 2000; McNeill & Edwards, 2004). Although generally effective in controlling uniform corrosion on a lead or copper pipe surface, mixed results have been observed when orthophosphate has been applied to a galvanic connection between lead-tin solder and copper. Nguyen et al. (2011a) observed that the addition of orthophosphate increased lead release in solder, if the concentration of sulphate in the water was less than 10 mg/L. Similar galvanic corrosion studies using zinc orthophosphate were not found in a review of the literature.

The addition of phosphate corrosion inhibitors can create some unintentional consequences. Phosphorous is a key nutrient, and phosphate addition has been shown to increase drinking water biofilm populations (Chu et al., 2005; Fang et al., 2009; Lehtola et al., 2002). Fang et al. (2009) was also able to demonstrate that although the population increased with the addition of phosphate, the exopolymeric substance production decreased, implying that the biofilm would be more readily disinfected.

Phosphate based corrosion inhibitors are widely used by drinking water utilities to control corrosion in the distribution system and in premise plumbing. A 2001 survey of American drinking water utilities estimated that 56% of utilities employed a phosphate corrosion control program (McNeill & Edwards, 2002). Orthophosphate, polyphosphate inhibitors with or without zinc are widely used, and in 2001 zinc orthophosphate was the most popular choice (McNeill & Edwards, 2002). With the use of zinc orthophosphate, recent concerns about zinc's toxicity and its presence in wastewater sludge have caused some utilities to seek low zinc or non-zinc alternatives (Schneider et al., 2007).

The selection of phosphate corrosion inhibitors should be based on site specific water chemistry as well as the characteristics of the distribution system and premise plumbing. The unintended consequences of increasing microbial activity and the downstream effects of zinc (if zinc based corrosion inhibitors are selected) must also be considered.

CHAPTER 3 BALANCING CORROSION CONTROL AND MICROBIAL CONTROL

3.1 ABSTRACT

Galvanic connections between lead and copper in premise plumbing can act as a source of released metals and present a significant public health concern. Low alkalinity water is particularly vulnerable to corrosion, and utilities are challenged to implement an effective corrosion control program that also effectively manages microbial water quality. Bench-scale experiments with galvanically connected lead and copper coupons were conducted in low alkalinity water (< 25 mg/L as $CaCO_3$) to examine the effects of chlorine, zinc orthophosphate and pH on lead release, copper release and microbial control. The concentrations of metals (copper and lead) and microbial activity in the bulk water were measured after a 1-, 2- and 4-d stagnation periods. The statistical analysis revealed that treatment at pH 9.2 released the least lead. The addition of zinc orthophosphate (20 mg/L) provided a greatest decrease in copper release. The addition of 1 mg/L chlorine provided a 1-3 log reduction in bulk water heterotrophic bacteria population. This work demonstrates that chlorine plays a critical role in managing microbial growth and can be a part of an effective corrosion control strategy. To achieve acceptable water, the goals of managed microbial growth and corrosion control must be considered in tandem. In this study, for both lead and copper, treatment at pH 9.2 with zinc orthophosphate and chlorine provided both a managed microbial growth and managed corrosion. This study offers a conceptual tool that can be used by utilities to evaluate pilot-scale distribution system treatment studies to select a treatment strategy that will simultaneously manage both microbial growth and corrosion.

KEYWORDS: lead, copper, corrosion control, low alkalinity, drinking water, nitric acid digestion

3.2 INTRODUCTION

Lead comes into contact with drinking water through lead service lines, lead-tin solder and brass faucets (Sandvig et al., 2008). Lead-tin solder was widely used until it was banned in the United States in 1986 (USEPA, 2012) and in Canada in 1990 (Health Canada, 2012). Although the installation of lead service line and the use of lead-tin solder for drinking water plumbing are now banned, previously installed leaded components remains a concern for older buildings. Galvanic corrosion occurs when two dissimilar metals are in electrical contact in a conducting solution, and in the case where lead and copper are connected, lead acts as the anode (releasing lead) and copper acts as the cathode (and is protected). Galvanic currents, in premise plumbing via a lead service line, through a connection between copper pipes connected to one another using a leaded-tin solder, or from lead containing brass fixtures. The release of lead and copper from household plumbing poses a public health concern. Lead ingestion poses a significant long-term health risk, particularly as it negatively affects the neurological development of children (Canfield et al., 2003; Zahran et al., 2009). Copper ingestion can cause significant (but temporary) gastrointestinal disturbances (Pizarro et al., 2001); however, the presence of copper in tap water is considered primarily an aesthetic issue.

Phosphate corrosion inhibitors, chlorine and pH are three factors that are known to have an effect on lead and copper corrosion rate through their direct action as a corrosion promoter or a corrosion inhibitor or through an implied indirect action via their effect on microbial growth.

Phosphate based corrosion control programs are employed by 56% of medium and large sized U.S. utilities, according to a 2001 survey (McNeill & Edwards, 2002). The majority of those utilities surveyed were also concerned about the effects of phosphate on microbial growth. Phosphate based corrosion inhibitors are used to passivate the metal surface by forming protective insoluble phosphate scales. Zinc orthophosphate has been shown to decrease both copper (Churchill et al., 2000) and lead corrosion rates (Churchill et al., 2000; McNeill & Edwards, 2004). However, the effectiveness of phosphate corrosion inhibitors in low alkalinity drinking water is affected by pH, particularly at pH values less than 7.2 (Gagnon & Dobrough, 2011). As well, phosphate is an essential nutrient for biological growth, and the addition of 30 µg/L phosphate has been shown to increase microbial growth by 1 log (Fang et al., 2009). Thus the efficacy of phosphate corrosion inhibitors may also be compromised by its effect on the microbial community on the surface.

Chlorine is added to distribution systems to provide residual disinfection and control microbial activity. Chlorine is a powerful oxidant and can also increase copper corrosion rates (Cantor et al., 2003; Reiber, 1989) and lead corrosion rates (Lin et al., 1997). However, the presence of chlorine has also been shown to decrease copper corrosion. Edwards and Ferguson (1993) found that the addition of 2mg/L of chlorine in soft water prevented the initiation of pits in copper. Similarly, chlorine has also been shown to decrease soluble lead by forming insoluble lead scales (Edwards & Dudi, 2004). Rahman et al. (2007) also observed a decrease in copper release in the presence of chlorine and suggested that this may be partly as a result of decreased biomass. Microbial communities have been identified as key factors for inducing "blue water" corrosion in premise plumbing (Critchley et al., 2004) and pin-hole corrosion (Zumelzu et al., 2003). Recently, active microbial communities have also been identified and characterized from lead service line, implying that biological activity may also affect lead corrosion (White et al., 2011).

Increasing the pH of the water is another commonly employed method for corrosion control, as it decreases the solubility of lead and promotes the formation of less soluble metal complexes on the pipe surface. Increased pH has been shown to decrease both lead corrosion (Tam & Elefsiniotis, 2009) and copper corrosion (Rahman et al., 2007). However, the pH of the water also affects the type of chlorine species present. As pH increases above pH 7.6, the OCl^- species dominates; whereas as pH decreases below 7.6, the more powerful disinfectant HOCl species dominates. This shift in chlorine species to the less effective OCl^- experienced at pH > 7.6 may also promote microbial growth and consequently increase corrosion rates.

Premise plumbing in low alkalinity water is particularly vulnerable to corrosion (Lytle & Schock, 2008). Although there is no universally agreed upon definition of low alkalinity, literature on this subject has considered water to be of "low alkalinity" between 20 and

50 mg/L as $CaCO_3$ (Gregory, 1990; Tam & Elefsiniotis, 2009), under 10 mg/L as $CaCO_3$ (Rahman et al., 2007) and as low as 5 mg/L as $CaCO_3$ (Boulay and Edwards 2001; Gagnon & Doubrough, 2011). Naturally occurring low alkalinity water is typical of Eastern Canada and New England in the United States. Alkalinity in water can also be decreased by membrane treatment processes. Membrane processes produce low alkalinity permeate which must then be treated to prevent the water from being corrosive to down-stream plumbing (Kettunen et al., 2000). Limited work has been completed that examines the effects of water quality and corrosion inhibitors on galvanically coupled lead and copper in low alkalinity water.

Utilities operating in low alkalinity water quality are particularly challenged to implement effective corrosion control programs. Although phosphate based corrosion control programs are widely adopted, utilities are faced with achieving a delicate balance between corrosion control and microbial control. This study examined three key elements commonly used in distribution systems as part of corrosion control programs and/or microbial control programs: phosphate based corrosion inhibitor, pH and chlorine are examined for their effect on galvanically coupled lead and copper and microbial water quality.

3.3 MATERIALS AND METHODS

3.3.1 Experimental set-up

Water for this experiment was obtained from the J.D. Kline Water Supply Plant (JDKWSP) in Halifax, Nova Scotia, Canada. Pockwock Lake is the source water for the JDKWSP, which is a direct filtration plant. Vadasarukkai et al. (2011) have provided a detailed description of the plant through a hydraulic optimization project. Filtered water was taken prior to final chlorination, pH control and phosphate addition. The alkalinity of the filtered water was measured using Standard Methods 2310B.4D Titration method for low alkalinity (APHA, 2009). Alkalinity was measured as 16.5 ± 0.2 mg/L $CaCO_3$. Dissolved inorganic carbon (DIC) was measured as 3.6 ± 0.04 mg/L using a Shimadzu TOC-VCSH TOC Analyzer (Shimadzu, Kyoto, Japan) according to manufacturer’s instructions.

Eight experimental conditions were examined. Each experimental condition was examined in duplicate. Two pH levels were used (pH 7.4 or pH 9.2) and four treatment conditions were used (control, chlorine addition, zinc orthophosphate addition, and the combination of chlorine and zinc orthophosphate). The zinc orthophosphate (Carus 3180, Carus Corporation, Peru, IL, U.S.A.) was dosed at 20 mg/L (measured as PO_4). The pH was adjusted to either 7.4 or 9.2 using sodium hydroxide. The chlorine was dosed at 1 mg/L.

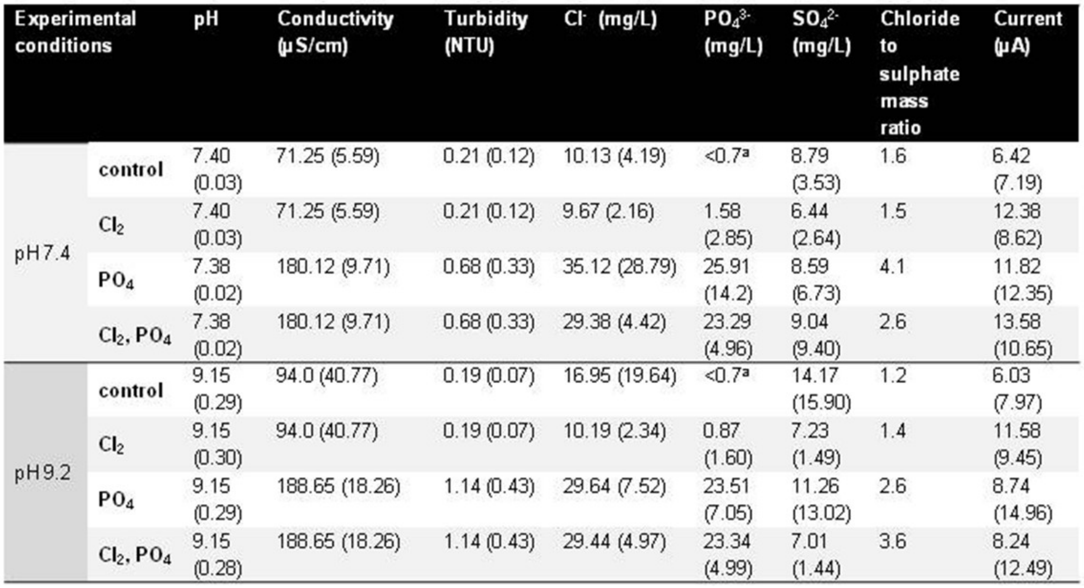
Each of the experimental conditions was examined using a unique experimental apparatus: a galvanic cell designed for this bench-scale experiment. The experiment was conducted using sixteen identical 250 mL Erlenmeyer flasks (two for each condition). Each flask had a copper coupon (76.2mm x 12.7mm x 1.68mm) and a lead coupon (38.1mm x 12.7mm x 1.74mm) that were attached using copper wire and copper alligator clips. The ratio of lead to copper was selected to ensure that a galvanic current would be measurable. A dump and fill method was used for this batch experiment, and the water was changed after a 1-d, 2-d and 4-d stagnation period. These stagnation times were used to examine worst-case scenarios for premise plumbing (e.g. stagnation over a weekend).

3.3.2 Water analysis

Following stagnation, the water was analyzed for basic water quality parameters. Temperature, pH, and conductivity were analyzed using an Accumet XL50 dual channel probe (Fisher Scientific, U.S.A.) according to manufacturer's instructions. Turbidity was analyzed using a HACH 2100N Turbidimeter (HACH, Loveland, CO, U.S.A.) according to manufacturer's instructions. Free chlorine was analyzed using a DR 400 U Spectrophotometer (HACH, Loveland, CO, U.S.A) according to manufacturer's instructions. Anions were measured using a 761 Compact IC (Metrohm, Herisau, Switzerland) using USEPA method 300.1 (USEPA, 1997). The method detection limit for chloride, sulphate and phosphate were, 0.6 mg/L, 0.5 mg/L and 0.7 mg/L respectively. The galvanic current between the copper and lead coupons was measured using a True

RM Industrial Multimeter EX530 (Extech Instruments Corporation, Waltham, MA, U.S.A). The water quality for each treatment is given in Table 3.1. Notably, the chloride to sulphate mass ratio (CSMR) is greater than 0.5 in all treatment groups, which creates a corrosive environment for lead in low alkalinity water (Nguyen et al., 2010a).

Table 3.1 Average water quality of experimental conditions at beginning of stagnation period (0-d) with the standard deviations in brackets.



Experimental conditions		pH	Conductivity (µS/cm)	Turbidity (NTU)	Cl^- (mg/L)	PO_4^{3-} (mg/L)	SO_4^{2-} (mg/L)	Chloride to sulphate mass ratio	Current (µA)
pH7.4	**control**	7.40 (0.03)	71.25 (5.59)	0.21 (0.12)	10.13 (4.19)	<0.7[a]	8.79 (3.53)	1.6	6.42 (7.19)
	Cl_2	7.40 (0.03)	71.25 (5.59)	0.21 (0.12)	9.67 (2.16)	1.58 (2.85)	6.44 (2.64)	1.5	12.38 (8.62)
	PO_4	7.38 (0.02)	180.12 (9.71)	0.68 (0.33)	35.12 (28.79)	25.91 (14.2)	8.59 (6.73)	4.1	11.82 (12.35)
	Cl_2, PO_4	7.38 (0.02)	180.12 (9.71)	0.68 (0.33)	29.38 (4.42)	23.29 (4.96)	9.04 (9.40)	2.6	13.58 (10.65)
pH9.2	**control**	9.15 (0.29)	94.0 (40.77)	0.19 (0.07)	16.95 (19.64)	<0.7[a]	14.17 (15.90)	1.2	6.03 (7.97)
	Cl_2	9.15 (0.30)	94.0 (40.77)	0.19 (0.07)	10.19 (2.34)	0.87 (1.60)	7.23 (1.49)	1.4	11.58 (9.45)
	PO_4	9.15 (0.29)	188.65 (18.26)	1.14 (0.43)	29.64 (7.52)	23.51 (7.05)	11.26 (13.02)	2.6	8.74 (14.96)
	Cl_2, PO_4	9.15 (0.28)	188.65 (18.26)	1.14 (0.43)	29.44 (4.97)	23.34 (4.99)	7.01 (1.44)	3.6	8.24 (12.49)

[a] Measured value below the detection limit

The bacterial population was also assessed during this experiment. Heterotrophic plate count bacteria were enumerated according to Standard Methods 9215C (APHA, 2009). A dilution series was used to ensure countable populations, and each dilution was plated in duplicate.

Total copper and total lead were preserved with nitric acid to pH <2 and analyzed on a X-Series 2 ICP-ms (Thermo Fisher Scientific, MA, U.S.A.). The limit of detection was 0.7 µg/L for copper and 0.4 µg/L for lead. Metal samples were analyzed using Standard Methods 3125 (APHA, 2009) and prepared according to Standard Methods 3030E (APHA, 2009), which recommends that samples with a turbidity over 1 NTU should be analyzed using nitric acid digestion.

3.3.3 Galvanic currents

The galvanic current between the lead and copper components was measured using a True RM Industrial Multimeter EX530 (Extech Instrument Corporation, Waltham, MA, U.S.A.) according to manufacturer's instructions. The galvanic current could be used to estimate the mass of lead released as a result of galvanic corrosion using Faraday's law detailed in Dudi (2004) and applied by Cartier et al. (2012).

3.3.4 Electrode corrosion scale surface analysis

Following the experiment, one copper and one lead electrode from each treatment was analyzed using energy dispersive spectroscopy (EDS) using a Hitachi S-4700 FEG scanning electron microscope. Four points on the electrode were scanned to determine elemental percent composition.

3.3.5 Statistical analysis

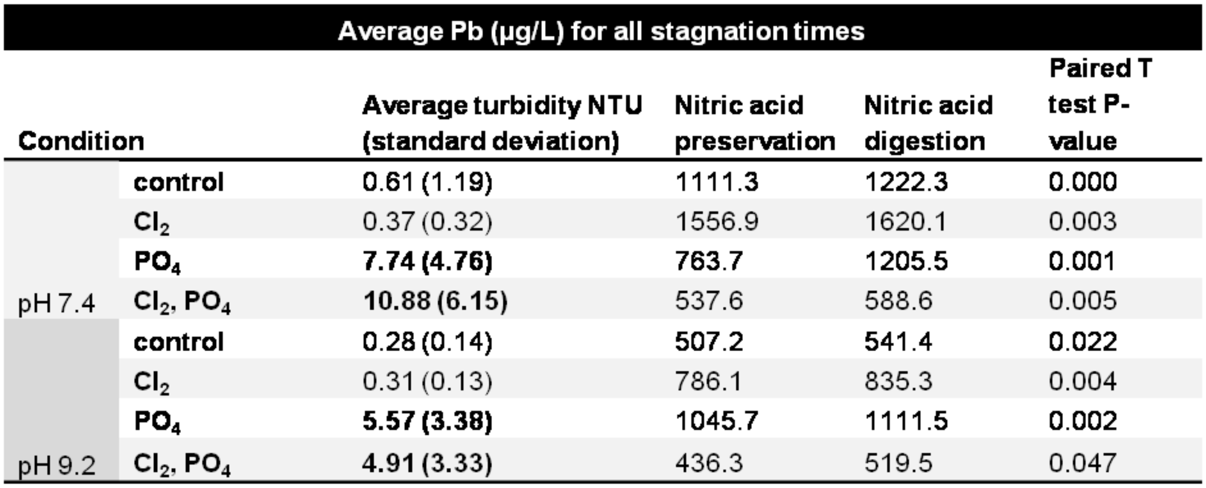
Microsoft Excel® (Microsoft, Redmond, WA, U.S.A.) was used to organize data, calculate confidence intervals. Mini-tab 16 ® (Minitab Inc., State College, PA, U.S.A.) was used to conduct Tukey's method analysis and Pearson correlation analysis. All statistical analysis was conducted at the 95% confidence level.

3.4 RESULTS AND DISCUSSIONS

3.4.1 Use of nitric acid digestion for metals analysis

As many of the samples were frequently above or near 1 NTU, a comparison of the metals analysis methods was conducted to determine if the nitric acid digestion (105˚C for 2h in 5% nitric acid) method yielded a statistically significant different amount of metal compared to samples preserved in nitric acid (herein referred to as nitric acid preservation). The treatment conditions with an average turbidity greater than 1 NTU were the samples tested with pH 7.4 with zinc orthophosphate; pH 7.4 with chlorine and zinc orthophosphate; and pH 9.2 with zinc orthophosphate conditions. A total of 158 metals samples were preserved and analyzed with and without nitric acid digestion. The two methods (nitric acid digestion and nitric acid preservation) were analyzed for all stagnation times with at least 21 samples from each experimental condition (Table 3.2).

Table 3.2 A comparison of the measured lead using nitric acid digestion method (heated at 105 ˚C for 2h) and the nitric acid preservation method. T-test conducted at 95% significant level. Average turbidity levels exceeding 1NTU in bold text.



Average Pb (µg/L) for all stagnation times

Condition		**Average turbidity NTU (standard deviation)**	**Nitric acid preservation**	**Nitric acid digestion**	**Paired T test P-value**
pH 7.4	**control**	**0.61 (1.19)**	**1111.3**	**1222.3**	**0.000**
	Cl_2	0.37 (0.32)	1556.9	1620.1	0.003
	PO_4	**7.74 (4.76)**	**763.7**	**1205.5**	**0.001**
	Cl_2, PO_4	**10.88 (6.15)**	537.6	588.6	0.005
pH 9.2	**control**	**0.28 (0.14)**	**507.2**	**541.4**	**0.022**
	Cl_2	0.31 (0.13)	786.1	835.3	0.004
	PO_4	**5.57 (3.38)**	**1045.7**	**1111.5**	**0.002**
	Cl_2, PO_4	**4.91 (3.33)**	436.3	519.5	0.047

A paired t-test (α=0.05) showed that all experimental conditions showed increased lead concentrations with the use of nitric acid digestion, regardless of turbidity. This suggests that the particulate portion of lead (captured by the nitric acid digestion method) constitutes a significant portion of the lead content for all experimental conditions. Triantifyllidou et al. (2007) demonstrated that the particulate portion of lead was underestimated when samples were preserved in 0.15% nitric acid. Therefore, the metal samples in this experiment were all analyzed using the nitric acid digestion method in order to capture more of the metal content, including the particulate content.

In experiments by Triantafyllidou et al. (2007), samples with known quantities of particulate lead were preserved in the 0.15% nitric acid for a 16 h holding time. After analysis, the samples only yielded about 20% of the total lead contained in the sample. Additionally, Triantafyllidou et al. (2007) observed that the pure lead particles settled quickly and the samples had a resulting turbidity of less than one. Similarly, Edwards and Dudi (2004) found that samples with 1 NTU of a red-coloured lead solid could contribute >1000 g/L of lead in samples. In this study, samples with consistently low turbidity (below 1 NTU) resulted in statistically significant differences in the heat acid digested lead content compared to the non-heat acid digested total metal samples. Our findings corroborate the observation that turbidity may not always act as a proxy for particulate lead, and accordingly the results in this paper will only utilize the nitric acid digestion method.

3.4.2 Galvanic currents, water chemistry and lead release

The total lead release (based on galvanic currents) and the measured lead in the bulk water phase over the course of the experiment is shown in Figure 1. The lead measured in the water phase represents a small fraction (0.4 – 2.6%) of the total lead release. The ratio of total lead (based on galvanic currents) to water phase lead was 39:1 (pH 7.4), 79:1 (pH 7.4 with chlorine), 132:1 (pH 7.4 with zinc orthophosphate), 124:1 (pH 7.4 with chlorine and zinc orthophosphate), 77:1 (pH 9.2), 130:1 (pH 9.2 with chlorine), 143:1 (pH 9.2 with zinc orthophosphate) and 231:1 (pH 9.2 with chlorine and zinc orthophosphate). This indicates that the pH 9.2 with chlorine and zinc orthophosphate treatment stored the most lead. The majority of the released lead (from galvanic currents) was assumed to have formed part of the scale on the pipe surface. This has been observed elsewhere, and the unaccounted for lead was assumed to have formed the corrosion scale on the metal surface (Cartier et al., 2012).

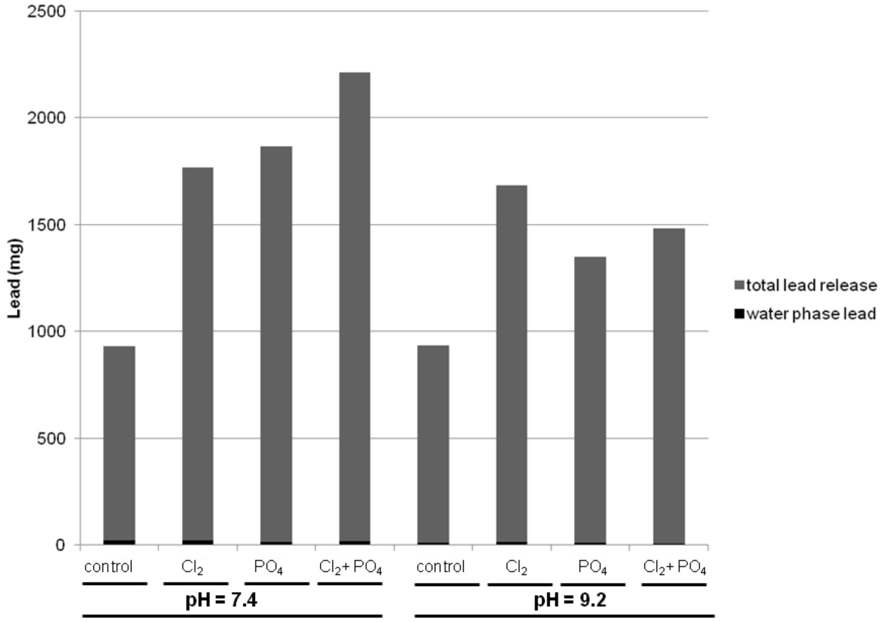
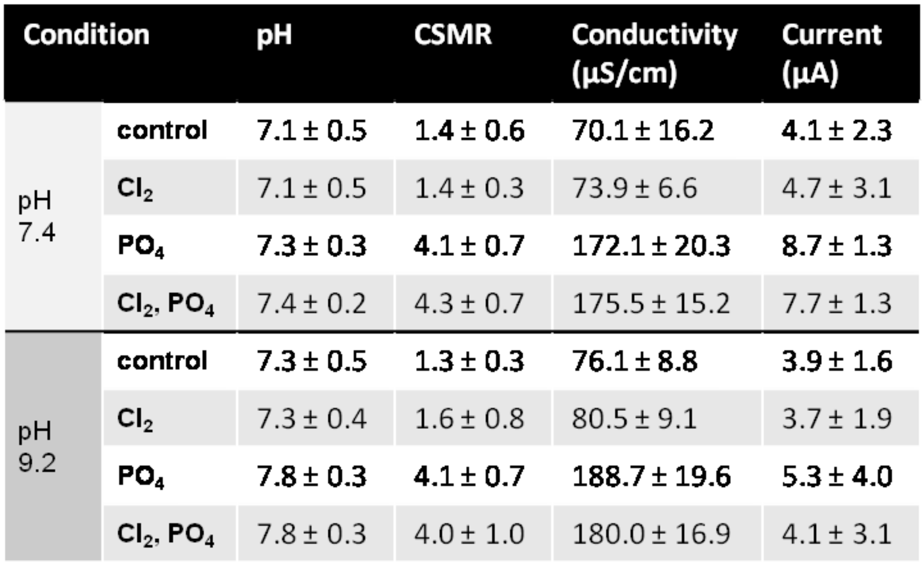


Figure 3.1 Estimated sum of total lead release and measured water phase lead for all eight treatment groups. The total lead represents the lead released through galvanic currents, estimated using Faraday's law. The water phase represents the mass of lead measured in the bulk water phase over the course of the experiment.

The addition of sodium hydroxide and zinc orthophosphate altered the mass ratios of charged anions and cations in the water, which affected both the chloride to sulphate mass ratio and the conductivity of the water. The addition of zinc orthophosphate contributed to the chloride concentration and increased the chloride to sulphate mass ratio (Table 3.3). All the chloride to sulphate mass ratios were greater than 0.5, which Nguyen et al. (2010a) previously demonstrated to be a corrosive environment for low alkalinity water. However, in this case, the increased chloride to sulphate mass ratio did not correspond to increased lead release. This observation is consistent with Nguyen et al.(2011c), who found that increases in CSMR>1 did not necessarily result in a proportionate increase in lead release. The addition of sodium hydroxide and zinc orthophosphate increased the conductivity of the water and predictably increased the measured current (Table 3.3). Interestingly, although the conductivity was the highest in the pH 9.2 with zinc orthophosphate and the pH 9.2 with chlorine and zinc orthophosphate treatments, the currents were lower than the pH 7.4 with zinc orthophosphate and the pH 7.4 with chlorine and zinc orthophosphate treatments, respectively.

Table 3.3 Average water quality of experimental conditions (all stagnation times included in average) with standard deviation



Condition		pH	CSMR	Conductivity (µS/cm)	Current (µA)
pH 7.4	**control**	**7.1 ± 0.5**	**1.4 ± 0.6**	**70.1 ± 16.2**	**4.1 ± 2.3**
	Cl_2	7.1 ± 0.5	1.4 ± 0.3	73.9 ± 6.6	4.7 ± 3.1
	PO_4	**7.3 ± 0.3**	**4.1 ± 0.7**	**172.1 ± 20.3**	**8.7 ± 1.3**
	Cl_2, PO_4	7.4 ± 0.2	4.3 ± 0.7	175.5 ± 15.2	7.7 ± 1.3
pH 9.2	**control**	**7.3 ± 0.5**	**1.3 ± 0.3**	**76.1 ± 8.8**	**3.9 ± 1.6**
	Cl_2	7.3 ± 0.4	1.6 ± 0.8	80.5 ± 9.1	3.7 ± 1.9
	PO_4	**7.8 ± 0.3**	**4.1 ± 0.7**	**188.7 ± 19.6**	**5.3 ± 4.0**
	Cl_2, PO_4	7.8 ± 0.3	4.0 ± 1.0	180.0 ± 16.9	4.1 ± 3.1

Figure 3.2 shows a plot of the water phase lead for all eight treatment groups for each of the stagnation times (1-d, 2-d, 4-d). A visual inspection of Figure 3.2 shows that the addition of sodium hydroxide (to increase the pH) was the primary factor in decreasing lead release into bulk water, and the addition of zinc orthophosphate appears to play a significant but secondary role in decreasing lead release.

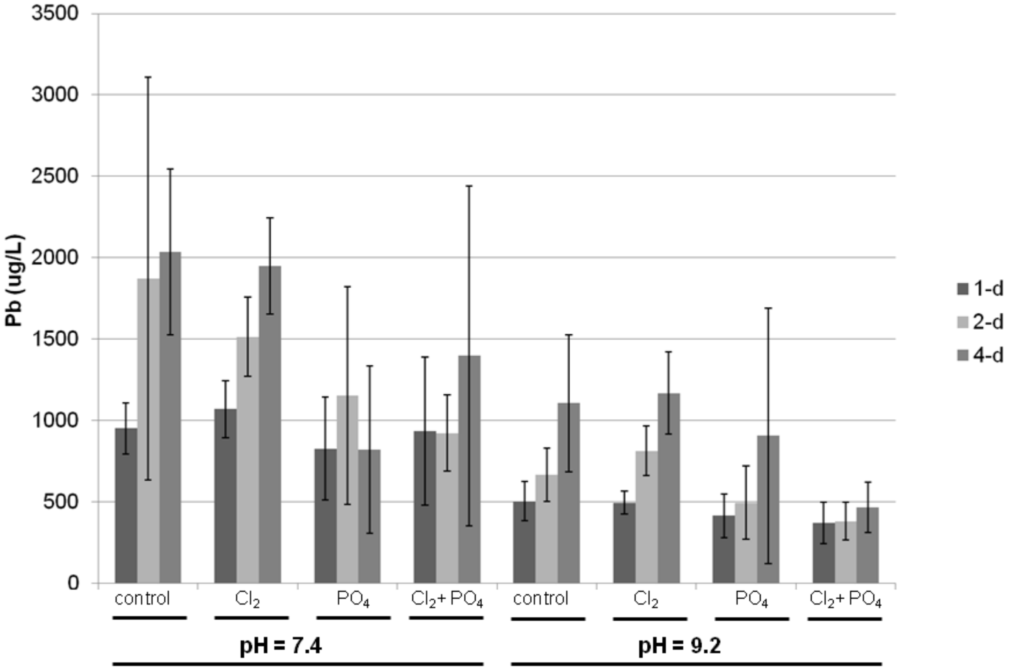
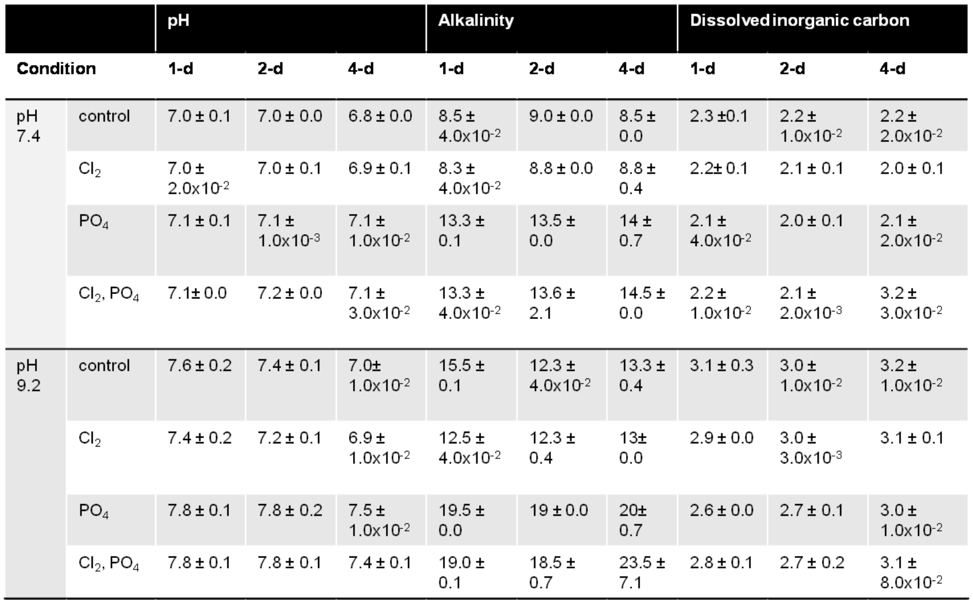


Figure 3.2 Average lead release from each experimental condition for all three stagnation times (1-d, 2-d, 4-d) with 95% confidence intervals

These observations were confirmed using Tukey's method. After the 1-d stagnation period, the pH 9.2 with chlorine and zinc orthophosphate released significantly less lead than the pH 7.4 control; pH 7.4 with chlorine; pH 7.4 with chlorine and zinc orthophosphate treatment. After the 2-d stagnation period, the pH 9.2 with zinc orthophosphate and the pH 9.2 with zinc orthophosphate with chlorine and zinc orthophosphate released significantly less lead than the pH 7.4 control treatment. After the 4-d stagnation period, the pH 9.2 with chlorine and zinc orthophosphate treatment released significantly less lead than the pH 7.4 control and the pH 7.4 with chlorine treatment.

The stagnation time affected corrosion control efficacies. Generally, lead and copper release increase with stagnation time as observed by Woszczynski et al. (2013). Lytle and Schock (2000) observed that in stagnant water systems with lead, copper and brass the majority of metal was released in the first 24 h. The increase in release over stagnation time may also be due to the changes in water chemistry over the course of the stagnation time (Table 3.4). The addition of sodium hydroxide and zinc orthophosphate contributed to the measured alkalinity in the system and subsequently created a driving force for the adsorption of atmospheric carbon dioxide, increasing dissolved inorganic carbon, and decreasing pH over the course of the experimental period (Table 3.4). The addition of alkalinity did not appear to provide additional buffering capacity. Although in this study, the pH decrease was primarily due to the macro bulk water chemistry, others have observed significant pH decreases in the microenvironments of galvanically coupled lead and copper systems (Nguyen et al., 2010b). Nguyen et al. (2010b) measured a rapid and significant pH decrease (in one case from 7.3 to 3.8 at the anodic (lead) surface in a galvanically coupled lead and copper). Additionally, Nguyen et al. (2010b) reported an increase in OH^- anions as the distance from the anode increased. The results from Nguyen et al. (2010b) suggest that the treatment conditions perceived in the bulk water may vary significantly from the treatment conditions at the anodic surface.

Table 3. 4 Average water quality of experimental conditions at beginning for all three stagnation periods (1-d, 2-d and 4-d) with the standard deviations in brackets.



		pH			Alkalinity			Dissolved inorganic carbon		
Condition		**1-d**	**2-d**	**4-d**	**1-d**	**2-d**	**4-d**	**1-d**	**2-d**	**4-d**
pH 7.4	control	7.0 ± 0.1	7.0 ± 0.0	6.8 ± 0.0	8.5 ± 4.0x10^{-2}	9.0 ± 0.0	8.5 ± 0.0	2.3 ±0.1	2.2 ± 1.0x10^{-2}	2.2 ± 2.0x10^{-2}
	Cl_2	7.0 ± 2.0x10^{-2}	7.0 ± 0.1	6.9 ± 0.1	8.3 ± 4.0x10^{-2}	8.8 ± 0.0	8.8 ± 0.4	2.2± 0.1	2.1 ± 0.1	2.0 ± 0.1
	PO_4	7.1 ± 0.1	7.1 ± 1.0x10^{-3}	7.1 ± 1.0x10^{-2}	13.3 ± 0.1	13.5 ± 0.0	14 ± 0.7	2.1 ± 4.0x10^{-2}	2.0 ± 0.1	2.1 ± 2.0x10^{-2}
	Cl_2, PO_4	7.1± 0.0	7.2 ± 0.0	7.1 ± 3.0x10^{-2}	13.3 ± 4.0x10^{-2}	13.6 ± 2.1	14.5 ± 0.0	2.2 ± 1.0x10^{-2}	2.1 ± 2.0x10^{-3}	3.2 ± 3.0x10^{-2}
pH 9.2	control	7.6 ± 0.2	7.4 ± 0.1	7.0± 1.0x10^{-2}	15.5 ± 0.1	12.3 ± 4.0x10^{-2}	13.3 ± 0.4	3.1 ± 0.3	3.0 ± 1.0x10^{-2}	3.2 ± 1.0x10^{-2}
	Cl_2	7.4 ± 0.2	7.2 ± 0.1	6.9 ± 1.0x10^{-2}	12.5 ± 4.0x10^{-2}	12.3 ± 0.4	13± 0.0	2.9 ± 0.0	3.0 ± 3.0x10^{-3}	3.1 ± 0.1
	PO_4	7.8 ± 0.1	7.8 ± 0.2	7.5 ± 1.0x10^{-2}	19.5 ± 0.0	19 ± 0.0	20± 0.7	2.6 ± 0.0	2.7 ± 0.1	3.0 ± 1.0x10^{-2}
	Cl_2, PO_4	7.8 ± 0.1	7.8 ± 0.1	7.4 ± 0.1	19.0 ± 0.1	18.5 ± 0.7	23.5 ± 7.1	2.8 ± 0.1	2.7 ± 0.2	3.1 ± 8.0x10^{-2}

However, in spite of the changes in water chemistry, the initial treatment at pH 9.2, appears to have provided a better corrosion control. This suggests that the scale formed in the pH 9.2 systems is more stable. An analysis of the corrosion scale by EDS (Table 3.5) suggests that more lead scale was present on the copper electrodes in the pH 9.2 system (average % lead atomic composition was 10.3 ± 7.0) compared to the pH 7.4 system average % lead atomic composition was 5.0 ± 4.1).

3.4.3 Copper release

In a galvanic couple, the lead anode would have a protective effect on the copper cathode. Sacrifical anodes (made of zinc) have been used to protect copper installations against corrosion in saline environments (Konsowa & El-Shazley; 2002; Rousseau et al., 2009). In this study, current was measured flowing from the anode to the cathode (Table3.3), providing cathodic protection. It follows that by decreasing corrosion at the anode, the current flowing to the cathode would decrease and so would the cathodic protection.

The average copper concentration for all eight conditions and all three stagnation times is shown in Figure 3.3.

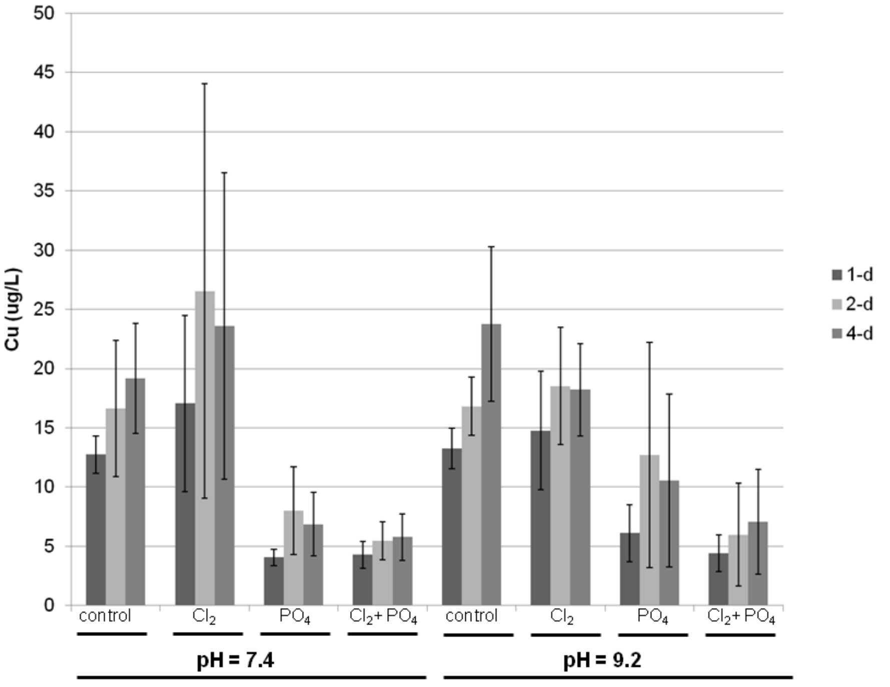


Figure 3. 3 Average copper release from each experimental condition for all three stagnation times (1-d, 2-d, 4-d) with 95% confidence intervals.

Generally, the lowest copper concentrations were observed for all treatments with the addition of zinc orthophosphate. As expected, the pH (the most significant factor in decreasing lead corrosion) did not provide a similar effect on copper. Copper concentration was compared using Tukey's method, which corroborated the visual observation that for treatments that included zinc orthophosphate at both pH levels (pH 7.4 and pH 9.2), there was a statistically significant decrease in copper release (for the 1-d stagnation only). After the 2-d stagnation period, the effects of zinc orthophosphate were less pronounced with only the pH 7.4 with zinc orthophosphate treatment; pH 7.4 with chlorine and zinc orthophosphate; and the pH 9.2 with chlorine and zinc orthophosphate treatment releasing significantly less copper than the pH 7.4 control. After a 4-d stagnation time, only the pH 9.2 with chlorine and zinc orthophosphate treatment released significantly less chlorine than the pH 7.4 with chlorine treatment and the pH 9.2 control.

Analysis of the coefficient of variation for the copper treated groups revealed more variation in the copper data in pH 9.2 treated groups compared to the pH 7.4 treated groups. This could be a combined result of less cathodic protection (decrease current from the lead anode) or improved stability of the zinc orthophosphate scale on the copper cathode at pH 7.4 compared to pH 9.2. An EDS analysis showed that both phosphate and zinc were present on both the copper and lead electrodes of the systems (at both pH 7.4 and 9.2) with added zinc orthophosphate (Table 3.5), indicating the presence of a phosphate corrosion scale.

Table 3.5 Average atomic composition (%) of surface elements, measured using EDS, of lead anode and copper cathode for all three treatment groups. P is shown in bold text. Not all percent compositions sum to 100.

			% atomic composition by element*								
Condition		**Electrode type**	**C**	**O**	**Al**	**Cu**	**Pb**	**Ca**	**P**	**Cl**	**Zn**
pH 7.4	**control**	**Lead anode**	**55.0**	**34.7**	**4.0**	**0.0**	**6.2**	**0.0**	**0.0**	**0.0**	**0.0**
		Copper cathode	53.0	20.6	0.4	30.0	4.6	0.7	0.0	0.4	0.0
	Cl_2	**Lead anode**	**31.6**	**51.3**	**5.3**	**0.6**	**11.6**	**0.0**	**0.0**	**0.0**	**0.0**
		Copper cathode	43.8	36.9	0.7	30.4	10.5	0.8	0.0	0.0	0.0
	PO_4	**Lead anode**	**26.5**	**49.3**	**0.6**	**0.0**	**11.3**	**0.5**	**6.0**	**1.6**	**2.6**
		Copper cathode	10.9	45.0		13.7	0.5	3.8	**12.5**	0.1	13.3
	Cl_2, PO_4	**Lead anode**	**23.6**	**50.7**	**3.8**	**0.0**	**9.1**	**0.8**	**10.4**	**1.5**	**11.1**
		Copper cathode	11.6	47.1	1.9	14.3	4.6	1.7	**9.2**	1.3	11.1
pH 9.2	**control**	**Lead anode**	**31.0**	**53.1**	**4.2**	**0.0**	**21.8**	**0.0**	**0.0**	**0.4**	**0.0**
		Copper cathode	40.9	16.1	0.0	39.1	3.8	0.0	0.0	0.1	0.0
	Cl_2	**Lead anode**	**22.9**	**46.3**	**14.7**	**0.0**	**14.8**	**0.0**	**1.3**	**1.1**	**0.0**
		Copper cathode	0.0	66.9	0.0	15.7	16.8	0.0	0.0	0.5	0.0
	PO_4	**Lead anode**	**35.3**	**37.7**	**3.8**	**0.0**	**25.1**	**0.8**	**7.5**	**15.6**	**2.4**
		Copper cathode	16.7	45.3	0.0	9.6	4.8	2.3	**8.8**	0.0	13.7
	Cl_2, PO_4	**Lead anode**	**29.0**	**39.6**	**0.2**	**21.1**	**5.3**	**1.7**	**4.7**	**2.6**	**4.2**
		Copper cathode	19.1	46.7	0.9	0.0	15.8	2.1	**19.0**	0.0	10.1

[a] Trace elements including Fe, K, Sr and Mg were not included in this table as they did not comprise more than 2% composition for any of the experimental conditions.

3.4.3 Heterotrophic bacteria

The average heterotrophic bacteria population (as measured by heterotrophic plate count (HPC)) for all eight experimental conditions and all three stagnation times is shown in Figure 3.4.

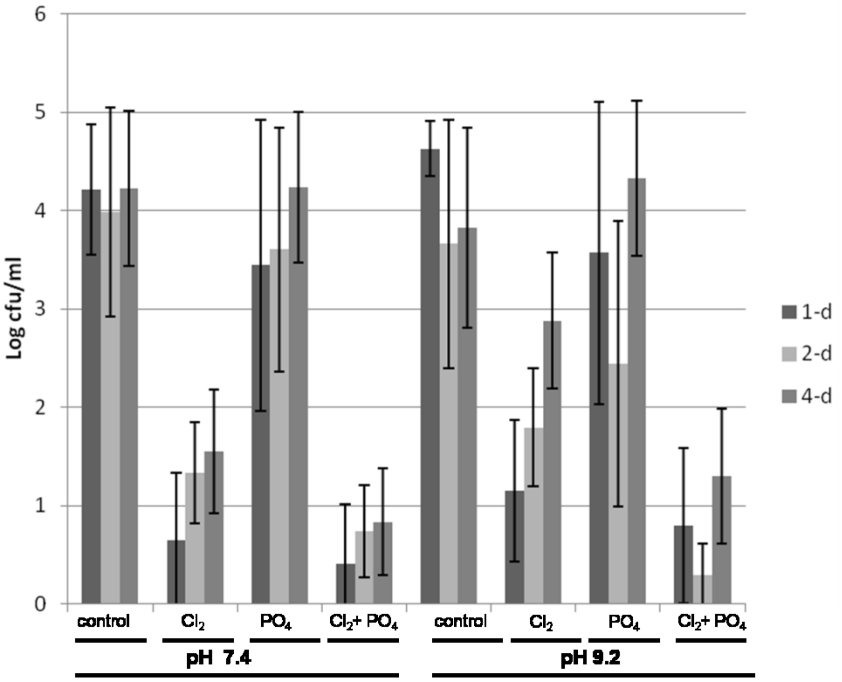


Figure 3.4 Average heterotrophic plate count for each experimental condition for all three stagnation times (1-d, 2-d, 4-d) with 95% confidence intervals

At pH 7.4, a 2-3 log reduction was observed; whereas at pH 9.2, a 1-2 log reduction was observed with the addition of chlorine. The log differences of each treatment were analyzed using Tukey's method. The addition of chlorine at pH 7.4 provided a statistically significant reduction in HPC, after the 1-, 2-, and 4-d stagnation. Similarly, at pH 9.2, the addition of chlorine also provided a statistically significant decrease in HPC, after the 1-, 2- and 4-d stagnation. However, the observed decrease in HPC population in chlorinated systems at 7.4 compared to the chlorinated systems at pH 9.2 was not statistically significant, with one exception: after the 4-d stagnation period, the pH 7.4 with chlorine treatment produced significantly less HPC population compared to the pH 9.2 with chlorine treatment. The observed disparity in chlorine efficacy is likely due to the dominance of the weaker chlorine species at the pH 9.2 treatment. Chlorine is considered to be a more effective disinfectant in the pH 7.4 range as HOCl species dominates, and HOCl species is a stronger disinfectant compared to OCl^- species (which dominates at pH 9.2) (Fukazaki, 2006).

Active microbial communities have been implied as causes of corrosion (Critchley et al., 2004; Rahman et al., 2007; Zumelzu et al., 2003). Ginige et al. (2011) observed a significant positive correlation between biomass and manganese and iron accumulation in biofilm. Rahman et al. (2007) found that copper pipes with no chlorine had the highest copper release, and microbial activity was implied as the cause of the copper release. The role of chlorine in this system is complex, as it can act both as an oxidant, a source of anions (at pH > 7.6) and can significantly reduce HPC.

It was hypothesized that if microbially induced corrosion were a significant mechanism in this system that higher metal release (lead and copper) would be observed in systems with higher HPC population. The HPC population and the metal concentrations for both copper and lead were analyzed using the Pearson correlation coefficient. The correlation between HPC and lead concentration was insignificant (Figure 3.5).

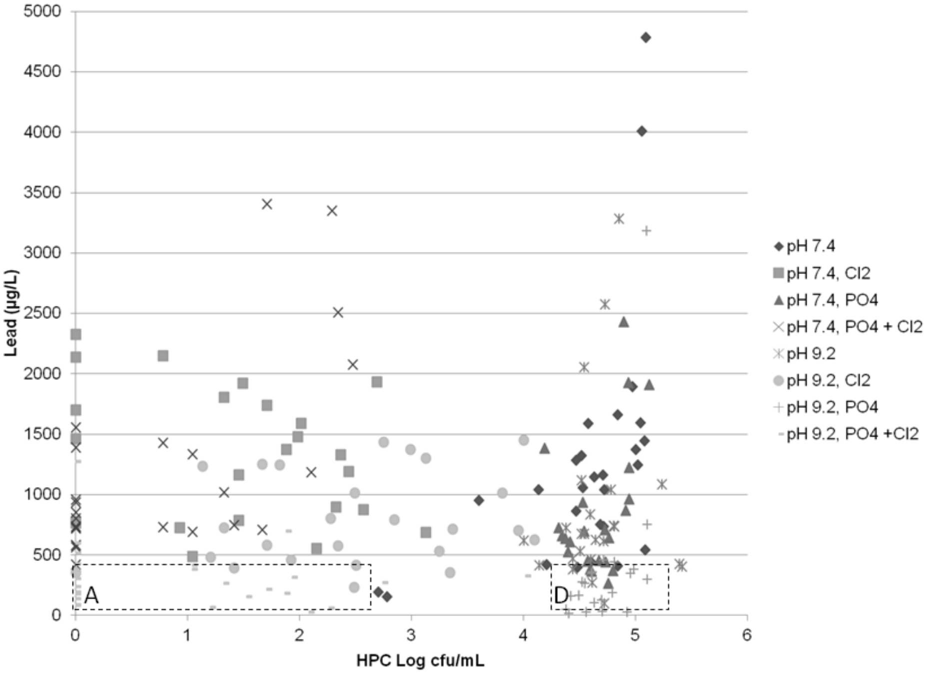


Figure 3.5 Matched data points for heterotrophic plate count bacteria in bulk water and lead concentration. Boxes are drawn to help distinguish major trends in treatments. Box A represents the pH 9.2 with chlorine, and the pH 9.2 with zinc orthophosphate and chlorine treatments. Box D represents the pH 7.4, pH 7.4 with zinc orthophosphate, pH 9.2, and pH 9.2 with zinc orthophosphate treatments.

There was a weak positive correlation between HPC and copper release (Figure 3.6), with an r value of 0.24 ($\alpha \leq 0.05$).

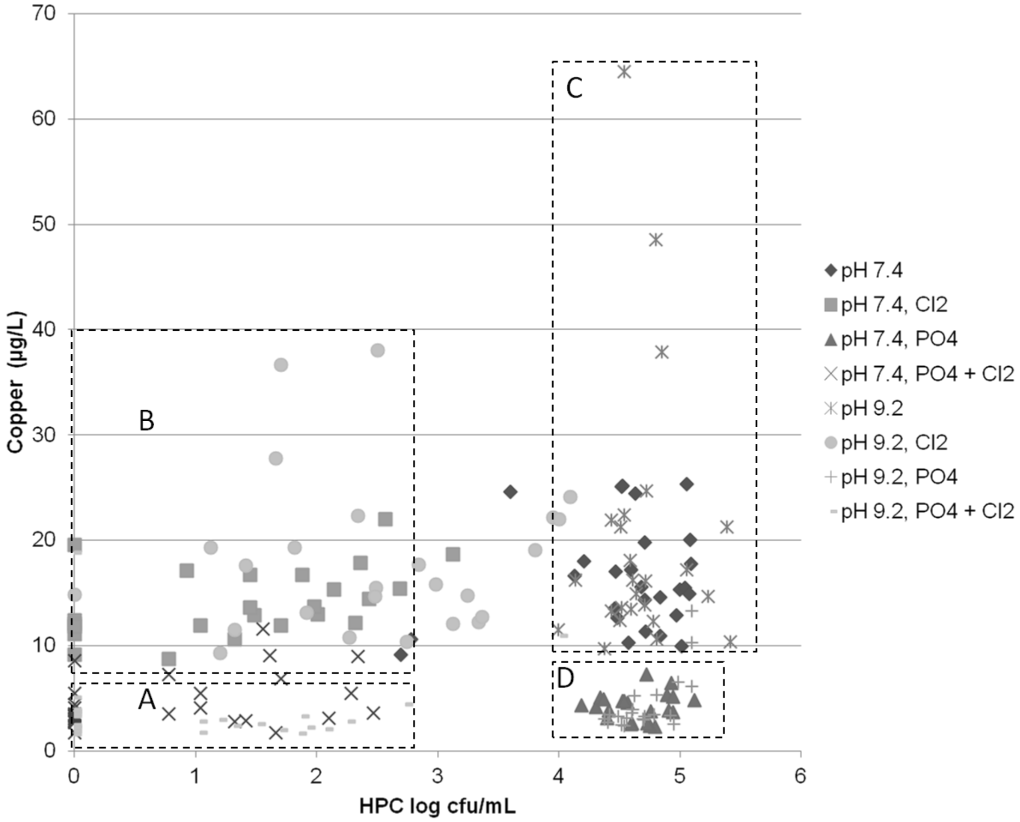


Figure 3.6 Matched data points for heterotrophic plate count bacteria in bulk water and copper concentration. Boxes are drawn to help distinguish major trends in treatments. Box B represents pH 7.4 and pH 9.2 systems treated with chlorine only. Box C represents the control groups (pH 7.4 or pH 9.2 without chlorine). Box D represents the pH 7.4 and pH 9.2 systems treated with zinc orthophosphate without chlorine. Box A represents the pH 7.4 and pH 9.2 systems treated with both zinc orthophosphate and chlorine.

3.4.4 Balancing corrosion control and microbial control

A visual examination of Figures 3.5 and 3.6 reveal several distinct trends in terms individual treatment's ability to achieve corrosion control (lowest metal releases) and microbial control (lowest HPC concentration. Boxes drawn on Figure 3.5 and 3.6 differentiate the treatment groups.

In Figure 3.5, Box A represents an effective microbial control program and corrosion control, representing treatments that provided the lowest lead release into bulk water while maintaining microbial control. Box A primarily includes the pH 9.2 with chlorine and the pH 9.2 with chlorine and zinc orthophosphate. Box D represents a system in which a comparable degree of lead corrosion control to Box A has been achieved; although microbial control has not been achieved. Box D includes the pH 7.4, pH 7.4 with zinc orthophosphate, pH 9.2, and pH 9.2 with zinc orthophosphate treatments.

In Figure 3.6, Box A represents a system where both microbial control has been achieved along with the lowest releases of copper. Box A primarily includes the pH 7.4 with zinc orthophosphate and chorine treatment, and the pH 9.2 with zinc orthophosphate and chlorine treatment. Box A represents the key operating elements of an effective corrosion control and disinfection programme. Box B represents a system where microbial control has been achieved, but corrosion control has not been achieved (as it includes some of the highest copper releases). Box B, primarily includes the pH 7.4 with chlorine, and the pH 9.2 with chlorine treatments. Box C represents a system where there is neither microbial control nor is there corrosion control. Box C primarily includes the pH 7.4 and the pH 9.2 treatment. Box D represents a system where microbial control has not been achieved but a comparable degree of copper corrosion control has been achieved as in Box A. Box D primarily includes, the pH 7.4 with zinc orthophosphate, and the pH 9.2 with zinc orthophosphate treatment.

In both Figures 3.5 and 3.6, utilities operating within Box A, would be able to achieve two critical water quality goals: maintaining microbial control and corrosion control. Figure 3.7, provides a conceptual drawing exploring the concept of managed or unmanaged microbial growth and managed or unmanaged corrosion. Figure 3.7 is divided into four operational quadrants: (A). managed microbial growth and managed corrosion; (B). managed microbial growth and unmanaged corrosion; (C). unmanaged microbial growth and unmanaged corrosion; and (D). unmanaged microbial growth and managed corrosion.

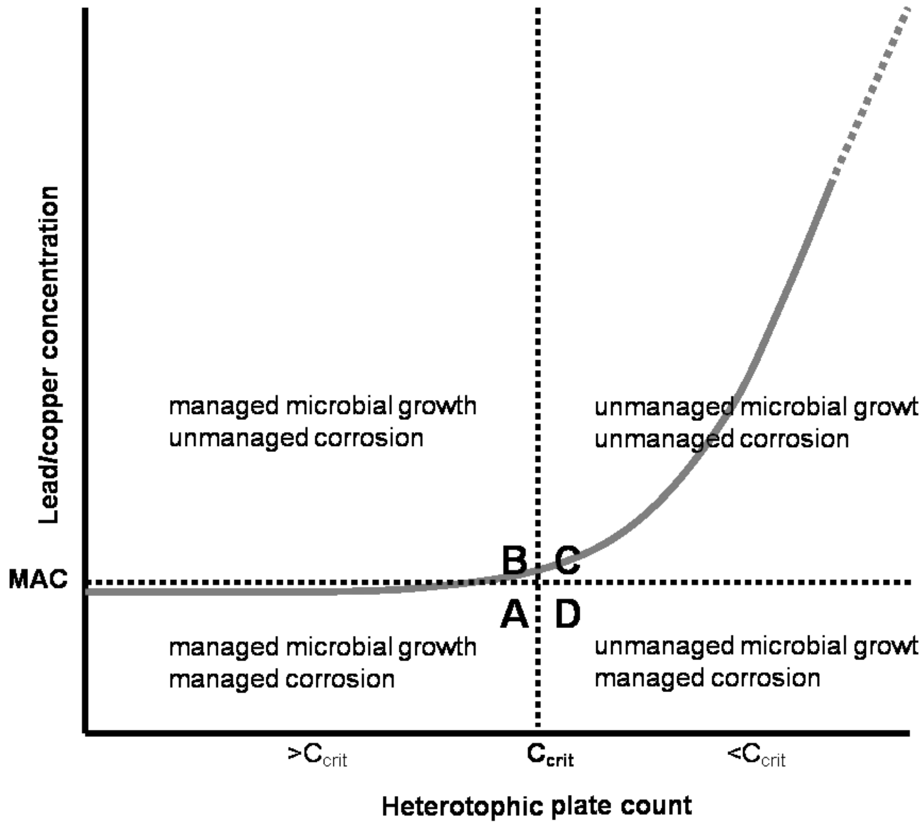


Figure 3.7 Conceptual tool to identify treatment strategies that simultaneously manage microbial growth and corrosion. The four quadrants represent A. managed microbial growth and managed corrosion; B. managed microbial growth and unmanaged corrosion; C. unmanaged microbial growth and unmanaged corrosion; and D. unmanaged microbial growth and managed corrosion. The x-axis is divided by a critical chlorine concentration (Ccrit) to maintain microbial control, and the y-axis is divided by a maximum allowable concentration (MAC) of the metal for the utilities' specific regulatory environment.

The quadrants could be defined on the x-axis as critical chlorine concentration to maintain microbial control. The concept of a critical chlorine (C_{crit}) level, to maintain microbial control of distribution system water has been explored in the literature (Huck and Gagnon, 2004; Gagnon et al., 2008). Based on water used by four diverse North American water utilities, Gagnon et al. (2008) calculated that critical chlorine concentration to maintain microbial control (HPC<500 cfu/mL) was 0.6 mg/L for chlorine and 1mg/L for chloramines. On the y-axis, the maximum allowable concentration (MAC) for the utilities' specific regulatory environment could be used to define the concept of managed corrosion. It is anticipated that these quadrants would be specific to water quality type; however the goal would be to provide utilities with a framework to consider microbial interaction with lead and copper release. The conceptual tool offered in Figure 3.7, could be used by utilities operating pilot testing systems for corrosion control schemes specific to their water.

3.5 CONCLUSIONS

- Although other literature has indicated that microbial populations can increase metal corrosion, in this case, copper corrosion was only weakly correlated to increased microbial populations. In this study, chemistry appears to play the dominant role in lead and copper corrosion. Treatment at pH 9.2 provided the greatest protection for the lead anode, and zinc orthophosphate appeared to offer some secondary protection. The copper was protected by cathodic protection and by the zinc orthophosphate scale.
- To achieve acceptable water, the goals of managed microbial growth and corrosion control must be considered in tandem. In this study, for both lead and copper, treatment at pH 9.2 with zinc orthophosphate and chlorine provided both a managed microbial growth and managed corrosion. This work demonstrates that chlorine plays a critical role in managing microbial growth and can be a part of an effective corrosion control strategy.
- The conceptual tool offered in this study can be used by utilities to evaluate pilot-scale distribution system treatment studies to select a treatment strategy that will simultaneously manage both microbial growth and corrosion.

3.6 ACKNOWLEDGEMENTS

The authors wish to thank the NSERC / Halifax Water Industrial Research Chair program for providing financial support for this research. The authors also wish to thank Karolina Smiech for her assistance in operating the experiments and Heather Daurie for her support with the metals analysis. The authors wish to acknowledge Pat Scallion and the

Institute for Research in Materials for her support and guidance with the scanning electron microscope and EDS analysis. We also acknowledge the support of the Canada Foundation for Innovation, the Atlantic Innovation Fund, and other partners which fund the Facilities for Materials Characterization, managed by the Institute for Research in Materials.

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CHAPTER 4 CONTRIBUTION OF BIOFILM TO LEAD AND COPPER STORAGE UNDER DRINKING WATER CONDITIONS

4.1 ABSTRACT

Water utilities often alter water chemistry or add phosphate based corrosion inhibitors to decrease the corrosion potential of lead and copper containing fixtures. However, phosphate is a key nutrient for biological growth and may increase growth of biofilms attached to premise plumbing. In this study, the effect of zinc orthophosphate on biofilm density and the potential for the biofilm on benign downstream plumbing to store lead and copper corrosion products was explored. Twelve galvanic macrocells, with both lead and copper components, were used to examine the effect of the addition of 1mg/L and 3mg/L zinc orthophosphate (dosed as PO_4) on biofilm density and metal accumulation in biofilms grown on a polycarbonate surface. The polycarbonate surface was used to represent a benign downstream plumbing fixture. The 1mg/L and 3mg/L zinc orthophosphate treatments significantly increased the heterotrophic plate count biofilm density on the polycarbonate coupons in the copper pipe macrocells. The addition of 3mg/L $-PO_4$ significantly increased the heterotrophic plate count biofilm density on the polycarbonate coupons in the lead pipe macrocells. The biofilms from both pipe systems were able to store both lead and copper, and sorption of the metals likely occurred through binding with charged molecules in the dissolved organic carbon fraction of the biofilm. Of the total mass of lead released through galvanic currents, only 3-9% was found in the bulk water phase and 0.1 – 2.4% was estimated to be stored in the biofilm. The remainder of the released lead is assumed to be stored as corrosion product on pipe surface. However, from the lead in the bulk water phase, 3-29 % was estimated to be exclusively removed and stored in the biofilm. Similarly, 3-16% of copper in the bulk water phase was removed exclusively by the biofilm. Biofilm represents a significant sink for corrosion products in the bulk water phase and subsequently could represent a source of stored lead and copper which could become available during a premise plumbing system disturbance (i.e. pressure change).

KEYWORDS: lead, copper, polycarbonate, premise plumbing, biofilm, corrosion control, zinc orthophosphate, galvanic corrosion

4.2 INTRODUCTION

Lead in drinking water presents a major public health concern as it has significant effects on neurological development in children (Canfield et al., 2003; Zahran et al., 2009). Copper presents mainly an aesthetic concern although high levels can cause significant gastrointestinal disturbances (Pizarro et al., 2001). Regulations and research tend to focus on the chemical aspects of corrosion control; however, there is significant evidence that biofilm also plays a major role in metals release and accumulation. Numerous studies have associated or implied that biofilms play a role in lead and copper release. The association between copper release and bacteria has been documented (Rahman et al., 2007), and other work has identified specific bacteria associated with copper corrosion (Critchley et al., 2004; Zumelzu et al., 2003). White et al. (2011) suggested that biofilms may also play a role in lead release through a study that characterized lead service line associated biofilms.

Furthermore, the application of phosphate based corrosion inhibitors by water utilities to mitigate corrosion may also increase biological activity. The phosphate acts as a corrosion inhibitor by reacting with the positively charged metal ions to form a less soluble complexed scale. Phosphorus is also an essential nutrient for cell growth, and phosphates have been demonstrated to have complex interaction with biofilm. Generally, the addition of phosphate corrosion inhibitors has been shown to increase microbial population in drinking water biofilms. Phosphate at doses as low as 0.03 mg/L-P (Fang et al., 2009), 0.01 mg/L -P (Chu et al., 2005) and 0.001 mg/L-P (Lehtola et al., 2002) have contributed to biofilm development. Although, Park et al. (2008) found that at low doses of orthophosphate (0.044 mg/L-P) microbial growth increase was dependent on the presence of sufficient levels of biodegradable organic carbon. Phosphate can also affect the adhesion process of bacteria to the surface material. Fang et al. (2009) found that although phosphorus increased biofilm population, it also decreased exoplymeric substance (EPS) production. The authors suggested that this looser biofilm structure which would have increased surface area to trap nutrients but could be more vulnerable to disinfection. The addition of phosphate has also been observed to affect the biofilm initiation process.

This increase in biofilm as a result of phosphate addition, not only has implications for microbially induced corrosion, but also for downstream benign plumbing fixtures, such as aerators. Aerators have been shown to physically trap lead particles (Triantafyllidou et al., 2007); however, aerators can also provide a surface on which biofilm can form. Aerators have been linked with nosocomial infections, where a pathogen present in low concentrations in the bulk water colonized on an aerator based biofilm (Kappenstein et al., 2000; Lavenir et al., 2008). Biofilms can also sorb both inorganic and organic constituents from the bulk water. Biofilms are complex structures in which cells are attached to a surface and to one another by exopolymeric substances. Metals can be bound to the exopolymeric substance of the biofilm through interactions with the various functional groups present, specifically the alcohol, carboxyl and amino groups found on the polymers, polysaccharides and proteins (Qin-qin et al., 2012). Biosorption of lead (II) has been observed in Ma et al. (2012), where –OH, C=O and C-O-C functional group structures were shown to provide lead (II) binding sites. The exopolymeric substances can also bind natural organic matter present in the bulk water, through a variety of mechanisms. Wang et al. (2012), found that biosorption of natural organic matter into the negatively charged functional groups of the exopolymeric substances was enhanced in the presence of divalent ions (Ca^{2+} and Mn^{2+}). The structure of the biofilm allows for sorption of metal products, and it follows that increased biofilm would lead to increased metal sorption. Ginige et al. (2011) found a significant positive correlation between biomass and both manganese accumulation and iron accumulation in the biofilm.

The potential of the biofilm to store nutrients and corrosion products has significant implications for public health. In copper pipe loops, Lehtola et al. (2006) observed that rapidly increased water velocity resulted in increased biofilm and copper release. Therefore, as a result of a transient pressure event in premise plumbing or a bulk water chemistry change biofilm could be released and its adsorbed contents consumed as drinking water.

4.2.1 Objective

The hypothesis of this Chapter is that phosphate treated biofilms will have a greater heterotrophic bacterial population, and more surface area for metal storage (specifically lead and copper). To test this hypothesis, this Chapter examines the impact of the addition of zinc orthophosphate on biofilm and the potential for biofilms to accumulate lead and copper particles on simulated benign downstream plumbing (polycarbonate surface).

4.3 MATERIALS AND METHODS

4.3.1 Experimental set-up

Twelve galvanic macrocells, described by Nguyen et al. (2010a; 2010b), including both copper and lead sections of pipe with lead-tin solder were used to generate the lead and copper particles (Figure 4.1).

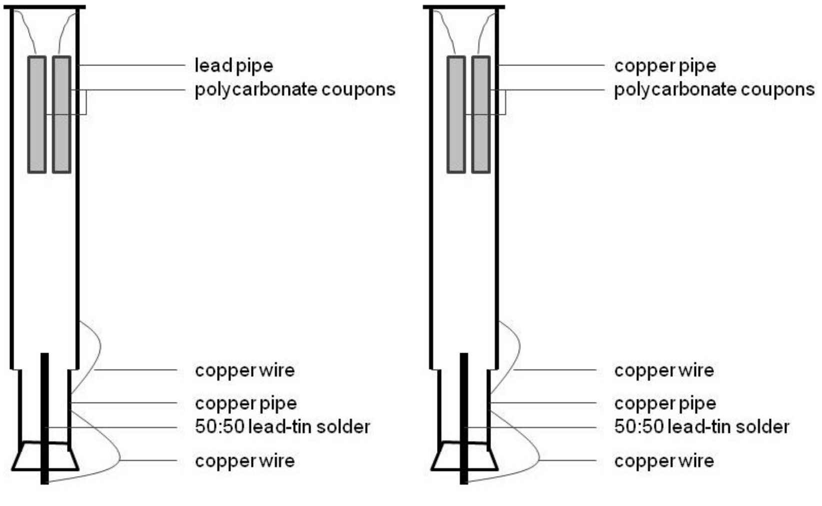


Figure 4.1 Lead and copper galvanic macrocells with polycarbonate coupons. Each galvanic macrocell contains either a large lead or copper pipe attached to a small copper pipe and 50:50 lead-tin solder. Copper wire, fastened with copper alligator clips provides an external galvanic connection.

The six lead galvanic macrocells were composed of a large lead pipe (31 cm long) attached to a smaller copper pipe (6.4 cm long). The six copper galvanic macrocells were composed of a large copper pipe (31 cm long) attached to a smaller copper pipe (6.4 cm long). All twelve galvanic macrocells had a 10 cm piece of 50-50 lead-tin solder inserted at the bottom of the smaller copper pipe through the silicone stopper. Copper wire and copper alligator clips were used to create a galvanic connection between the large section of pipe (either lead or copper) and the small copper pipe. A second galvanic connection using copper wire and copper alligator clips was also created between the small copper pipe and the lead-tin solder. To simulate a benign downstream plumbing fixture, two removable polycarbonate coupons were suspended from the top of the large pipe section using sterile neoprene fishing line. The polycarbonate coupons (Biosurface Technologies Corporation, Bozeman, MT, U.S.A) used in this experiment are generally used in annular reactors which are typically used in biofilm studies (Murphy et al., 2008; Sharp, 2001). Each polycarbonate coupon had a total surface area of 43.7 cm^2. The surface area ratio of the pipe interior surface area to the polycarbonate coupon was calculated to be 2.3:1 for the lead galvanic macrocell and 2.6:1 for the copper galvanic macrocell.

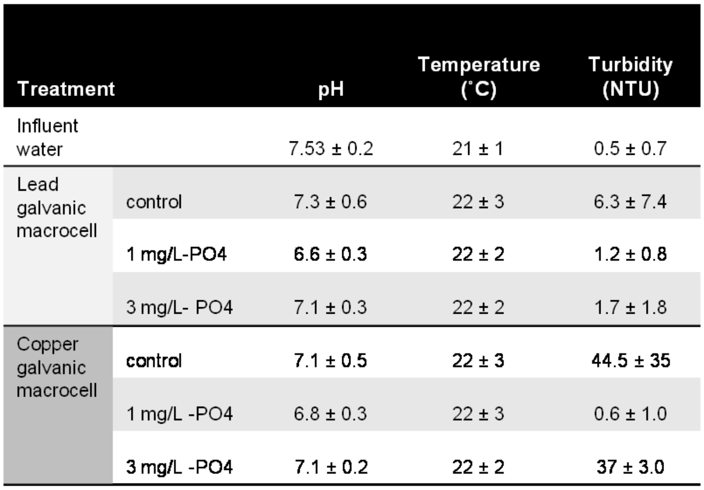
Low alkalinity water was taken post-filters from the J.D. Kline Water Supply Plant in Halifax, NS. Vadasarukkai et al. (2011) have provided a detailed description of the plant through a hydraulic optimization project. By taking water following the post-filters, it was possible to adjust to the desired pH and zinc orthophosphate dose required by the experiments. The final pH of 7.4 was selected based on average water quality maintained in the water leaving the J.D. Kline Water Supply Plant.

All twelve galvanic macrocells were acclimated for four months prior to treatment without the polycarbonate coupons. The water used during acclimation had a pH of 7.4±0.2. The water in the galvanic macrocell was replaced twice weekly, on alternating 3-d and 4-d stagnation times. The long stagnation times were selected to represent typical worst-case scenario for household plumbing (stagnation over a long weekend/holiday). The alkalinity of the filtered water was measured according to Standard Methods 2310B.4D (APHA, 2009). The alkalinity was approximately 8.5 mg/L $CaCO_3$. The dissolved inorganic carbon (DIC) of 2.5 mg/L was measured using a Shimadzu TOC-VCSH TOC Analyzer (Shimadzu, Kyoto, Japan).

Polycarbonate coupons were inserted into the pipe systems after four months of acclimation, and the coupons were left undisturbed for one month to allow for the development of biofilm. No water changes were conducted during this period. A 30 day biofilm acclimation period has typically been observed in previous biofilm studies at Dalhousie (Gagnon et al., 2004; Gagnon et al., 2005; Rand et al., 2007).

The twelve galvanic macrocells were then divided into three treatment groups. Each treatment group included two lead and two copper galvanic macrocells. During the two month treatment, the three groups of galvanic macrocells received water pH 7.4. Treatment groups included a control (no zinc orthophosphate added) or 1mg/L or 3mg/L of zinc orthophosphate (dosed as PO_4). The galvanic macrocells were treated as stagnant water systems. The water was changed after alternating 3-d and 4-d stagnation periods. Following the two month treatment period, the polycarbonate coupons were aseptically removed for biofilm analysis. The average influent water quality as well as the average effluent water quality for the six treatment groups is given in Table 4.1.

Table 4.1 Average water quality for the influent water and the effluent of the six treatments with standard deviation.



Treatment		pH	Temperature (˚C)	Turbidity (NTU)
Influent water		7.53 ± 0.2	21 ± 1	0.5 ± 0.7
Lead galvanic macrocell	control	7.3 ± 0.6	22 ± 3	6.3 ± 7.4
	1 mg/L-PO4	6.6 ± 0.3	22 ± 2	1.2 ± 0.8
	3 mg/L- PO4	7.1 ± 0.3	22 ± 2	1.7 ± 1.8
Copper galvanic macrocell	control	7.1 ± 0.5	22 ± 3	44.5 ± 35
	1 mg/L -PO4	6.8 ± 0.3	22 ± 3	0.6 ± 1.0
	3 mg/L -PO4	7.1 ± 0.2	22 ± 2	37 ± 3.0

4.3.2 Galvanic Corrosion

The galvanic current between the lead and copper components was measured using a True RM Industrial Multimeter EX530 (Extech Instrument Corporation, Waltham, MA, U.S.A.) according to manufacturer's instructions. The galvanic current could be used to estimate the mass of lead released as a result of galvanic corrosion using Faraday's law detailed in Dudi (2004) and applied by Cartier et al. (2012). The total mass of lead release was estimated based on average currents measured between the lead and copper pipes as well as between the copper pipe and lead-tin solder in each galvanic macrocell. In estimations of the total lead, lead from the one-month biofilm acclimation period was not included, as the biofilm would require several weeks to establish a stable population (Gagnon et al., 2004; Gagnon et al., 2005; Murphy et al., 2008; Rand et al., 2007).

4.3.3 Chemical matrix analysis

Anions were measured using a 761 Compact IC (Metrohm, Herisau, Switzerland) using USEPA method 300.1 (USEPA, 1997). The method detection limit for phosphate was 0.7 mg/L. Metals were measured using a X-Series 2 ICP-ms (Thermo Fisher Scientific, MA, U.S.A.). The limit of detection was 0.7 μg/L for copper and 0.4 μg/L for lead. Since the samples were highly turbid, the total copper and total lead samples were prepared according to Standard Methods 3030E (APHA, 2009), which recommends that samples with a turbidity over 1 NTU should be analyzed using a nitric acid digestion (105˚C for 2h in 5% nitric acid). The dissolved fraction of the copper and lead samples were separated using a 0.45 μm filter and preserved with nitric acid to pH<2. The metals were then analyzed according to Standard Methods 3125 (APHA, 2009).

Total organic carbon and dissolved organic carbon were measured using the Combustion Infrared Method from Standard Methods 5310B (APHA, 2009). Micro-PES, Polysulfone 0.45 μm filters (GE Water & Process Technologies, Feasterville-Trevose, PA, USA) were used for DOC. Filters for DOC analysis were selected based on Karanfil et al. (2003) and Khan and Subramania-Pillai (2007) on an Shimadzu TOC-VCSH TOC

Analyzer (Shimadzu, Kyoto, Japan). The molecular weight distribution of the dissolved organic carbon was determined using high pressure size exclusion chromatography (HPSEC) according to Lamsal et al. (2012). The samples were processed using a TSKG3000SW column with TSKgelSW guard column and silica based, hydrophilic bonded phase packing that minimizes interaction with proteinsilica media (Tosoh Biosciences, Tokyo, Japan) followed by a Perkin Elmer Series 200 Autosampler with a Perkin Elmer UV/Vis detector (Perkin Elmer, Waltham, MA, USA). Chromatograms were analyzed and characterized into a molecular weight distribution for comparison purposes. Characterization of organic matter by molecular weight distribution has provided information on the specific fraction that contributed to membrane fouling (Lamsal et al., 2012) and transformation of natural organic matter during advanced oxidation processes (Lamsal et al., 2011).

4.3.4 Biofilm collection from polycarbonate coupons

The biofilm was removed from the polycarbonate coupon, as described in detail by Gagnon and Slawson (1999). In brief, the polycarbonate coupon was placed in a sterile Secure T 177mm x 302 mm stomacher bag (Fisher Scientific, U.S.A.) containing 10 mL of sterile phosphate buffer solution (PBS).The stomacher bag containing the polycarbonate coupon was then placed in a MIX 2 Stomacher (AES Laboratories, Bruz, France) and stomached at 230 rpm for 2 minutes.

4.3.5 Microbial population analysis

Two samples were taken from the biofilm, and a dilution series was used to ensure countable populations (each dilution was analyzed in duplicate). The biofilm population was assessed using the spread plate method for heterotrophic plate count described in Standard Methods 9215C (APHA, 2009). A serial dilution was used to ensure a countable population and samples were plated in duplicate. Samples incubated at room temperature for seven days prior to enumeration. Samples of the bulk water phase were also analyzed for heterotrophic plate count bacteria in the method described above. Additionally, the bulk water phase was also analyzed for adenosine triphosphate (ATP) using the Luminultra Quench-Gone Aqueous kit ™ (Luminultra Technologies Ltd, Fredericton, NB, Canada). The ATP testing was conducted according to manufacturer's instructions.

4.3.6 Statistical analysis

Microsoft Excel® (Redmond, WA, U.S.A.) was used to organize data, calculate confidence intervals, conduct analysis of variance (ANOVA) and Tukey's method analysis. Tukey's method analysis was determined using statistical tables given by Harter (1960). Minitab ® 16 software (State College, PA, U.S.A.) was used to conduct the Pearson correlation significance testing. All statistical analysis was conducted at the 95% confidence level.

4.4 RESULTS AND DISCUSSION

4.4.1 Contribution of biofilm to removal of lead and copper from the bulk water phase

The total mass of lead released by galvanic currents was estimated using Faraday's law (Dudi, 2004), shown in Figure 4.2.

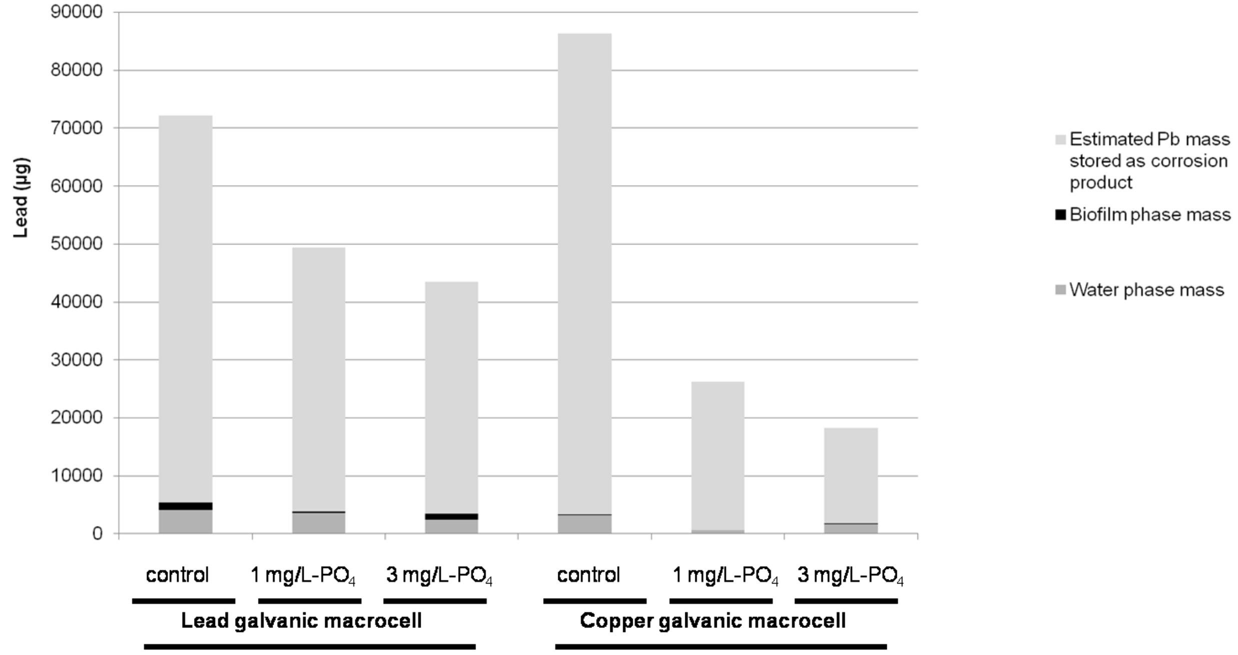


Figure 4.2 Distribution of the lead released through galvanic currents. The total mass of lead released was estimated using Faraday's law. The biofilm phase represents the total mass of lead accumulated in the biofilm (n=2 for each treatment). The water phase represents the total mass of lead measured in the bulk water phase (n≥27 for each treatment). The remainder of the lead released is assumed to be stored as a corrosion product on either the lead or copper pipe material surfaces.

In the lead galvanic macrocells, the bulk water phase could account for 6% (4155 µg), 7% (3607 µg) , 6%(2440 µg) of the lead for the control, 1 mg/L -PO_4, and 3 mg/L -PO_4 treatments, respectively. In the copper galvanic macrocells, the bulk water phase could account for 4% (3222 µg), 2% (596 µg) , 9% (1698 µg) of the lead for the control, 1 mg/L -PO_4, and 3 mg/L -PO_4 treatments, respectively.

Based on the lead and copper accumulation on the polycarbonate coupon, the mass of accumulated metal per surface area was used as a basis to estimate the contribution of biofilm to lead and copper removal. This would provide an estimate of the removal of lead and copper from the bulk water by biofilm alone (not the mineral corrosion product). The biofilm sorbed only a small portion of the total mass of lead generated by galvanic currents. In the lead galvanic macrocells, the biofilm could account for 1.7% (1200 μg), 0.6% (279 μg), 2.4% (1041 μg) of the lead for the control, 1 mg/L -PO_4, and 3 mg/L -PO_4 treatments, respectively. In the copper galvanic macrocells, the biofilm could account for 0.1% (96 μg), 0.1% (23 μg), 0.2% (45 μg) of the lead for the control, 1 mg/L -PO_4, and 3 mg/L -PO_4 treatments, respectively. Divalent metals could be 1. adsorbed by biofilm EPS or 2. precipitated with phosphate to form a lead-phosphate scale. Feng et al. (2012) observed an ideal pH (pH 5) for Pb^{2+} adsorption to lactic acid bacteria derived EPS. At low pH, H^+ ions likely competed for EPS binding sites, and at high pH the lead likely formed insoluble lead hydroxyls (Feng et al., 2012). In this study, less lead and copper were found in the biofilms in the systems receiving zinc orthophosphate treatment. This suggests that the zinc orthophosphate and biofilm EPS were in competition for available divalent metals (Pb^{2+}). The majority of the released lead generated by the galvanic current was not accounted for in either the lead mass from the bulk water phase or the biofilm phase. The majority of the released lead was assumed to be stored as a mineral corrosion product on the lead and copper pipe surfaces themselves. Cartier et al. (2012) also found that the majority of released lead (by galvanic current) could not be accounted for in the bulk water phase and concluded that the lead was stored as a corrosion product on the pipe surface.

The percentage of lead removed from the bulk water by biofilm alone was also estimated based on the mass of metal in the bulk water and the total surface area of the galvanic macrocell (Figure 4.3).

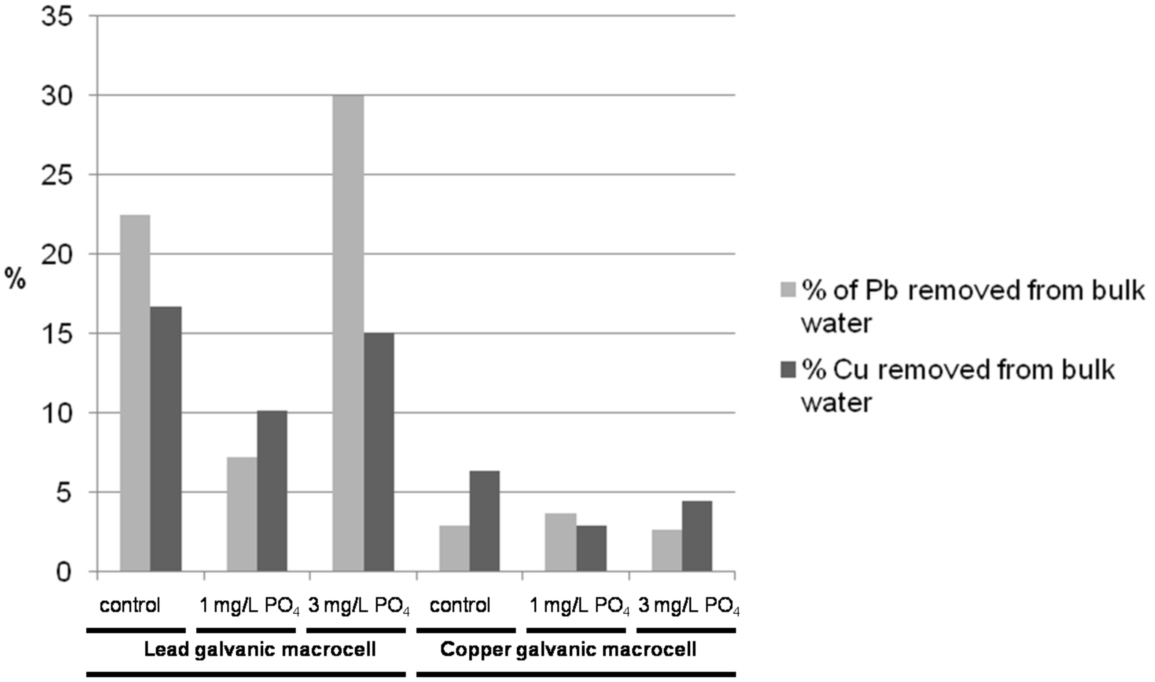


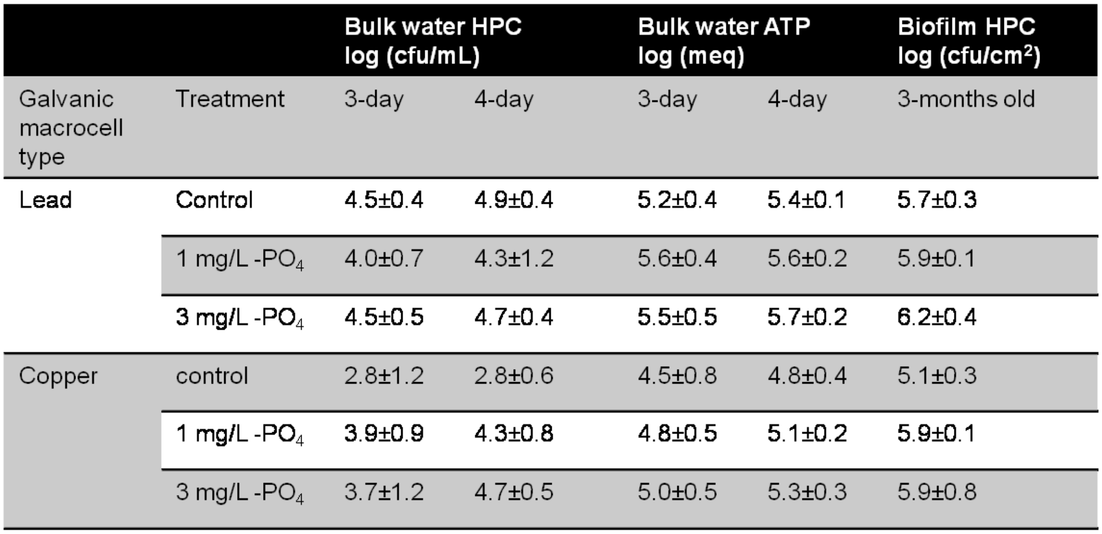
Figure 4.3 The estimated percent of lead and copper mass removed from the bulk water phase by the biofilm.

In the lead galvanic macrocells, the lead removal from bulk water phase by the biofilm was estimated to be 22%, 7%, 29% for the control, 1 mg/L -PO_4, and 3 mg/L -PO_4 treatments, respectively. In the copper galvanic macrocells, lead removal from the bulk water phase by the biofilm was estimated to be 3%, 4%, and 3% for the control, 1 mg/L -PO_4, and 3 mg/L -PO_4 treatments, respectively. Similarly, copper was also removed from the bulk water by biofilm. In the lead galvanic macrocells, the copper removal from bulk water phase by the biofilm was estimated to be 16%, 10%, 15% for the control, 1 mg/L -PO_4, and 3 mg/L -PO_4 treatments, respectively. In the copper galvanic macrocells, copper removal from the bulk water phase by the biofilm was estimated to be 6%, 3%, and 4% for the control, 1 mg/L -PO_4, and 3 mg/L -PO_4 treatments, respectively. Biofilm clearly plays an important role in metal storage, as it has the potential to sorb lead and copper from the bulk water.

4.4.2 Effect of zinc orthophosphate on heterotrophic biofilm population

In the bulk water phase, the average heterotrophic plate counts and ATP measurements are shown in Table 4.2.

Table 4.2 Estimates of microbial activity in the bulk water using heterotrophic plate count (HPC) and ATP counts for both the 3-d and 4-d stagnation time, and estimates of the biofilm density as measured as heterotrophic plate count (HPC) after one month acclimation and two months of treatment.



		Bulk water HPC log (cfu/mL)		**Bulk water ATP log (meq)**		**Biofilm HPC log (cfu/cm^2)**
Galvanic macrocell type	Treatment	3-day	4-day	3-day	4-day	3-months old
Lead	Control	4.5±0.4	4.9±0.4	5.2±0.4	5.4±0.1	5.7±0.3
	1 mg/L -PO_4	4.0±0.7	4.3±1.2	5.6±0.4	5.6±0.2	5.9±0.1
	3 mg/L -PO_4	4.5±0.5	4.7±0.4	5.5±0.5	5.7±0.2	6.2±0.4
Copper	control	2.8±1.2	2.8±0.6	4.5±0.8	4.8±0.4	5.1±0.3
	1 mg/L -PO_4	3.9±0.9	4.3±0.8	4.8±0.5	5.1±0.2	5.9±0.1
	3 mg/L -PO_4	3.7±1.2	4.7±0.5	5.0±0.5	5.3±0.3	5.9±0.8

With increased phosphate dose the heterotrophic plate count bacteria population and ATP activity increased. The Pearson correlation coefficient for phosphate dose and heterotrophic plate count was significant ($\rho = 0.281$, $P<0.05$) as was the correlation coefficient for phosphate dose and ATP ($\rho = 0.311$, $P<0.05$). Similarly, in biofilm zinc orthophosphate addition increased the heterotrophic plate counts (Table 1). An ANOVA revealed that the mean biofilm population was different by treatment. Tukey's method was used to determine which differences were statistically significant ($\alpha=0.05$). The biofilm grown on the polycarbonate coupon in the copper control galvanic macrocell had significantly less HPC than all other treatments. The biofilm grown on the polycarbonate coupon in the lead control galvanic macrocell had significantly less HPC than the lead pipe with 3mg/L -PO_4. This trend of increased HPC population is consistent with the findings of others (Chu et al., 2005; Fang et al., 2009; Lehtola et al., 2002), who found that phosphorus addition increased the microbial population. Lauderdale et al. (2011) observed that the phosphate addition addressed the nutrient limitations of a biofilter-associated biofilm, which resulted in less EPS production, but increased HPC population (with increased metabolic activity inferred from increased ATP).

Both lead and copper were shown to accumulate in the biofilm, and it appears that the biofilms in the lead galvanic macrocells remove more lead and copper from the bulk water phase. This could be a result of the increased biofilm density in the lead galvanic macrocells, although no significant correlations between increased biofilm density and metal concentration were observed. This lack of significance may be a result of the limited sample size of this experimental design, as others have found statistically significant correlations between biofilm density and divalent metal storage (Ginige et al., 2011). However, evidence of a plausible mechanism for sorption of available copper and lead were observed. The dissolved organic carbon content of the biofilm was analyzed using size exclusion chromatography (SEC). In this study, molecular weight distribution analysis of the dissolved organic content in the biofilm attached to the polycarbonate coupons is presented in Figures 4.4 and 4.5. Specifically, Figure 4.4 shows the molecular weight distribution of organic material in the lead galvanic macrocells, and Figure 4.5 shows similar data for the copper galvanic macrocells.

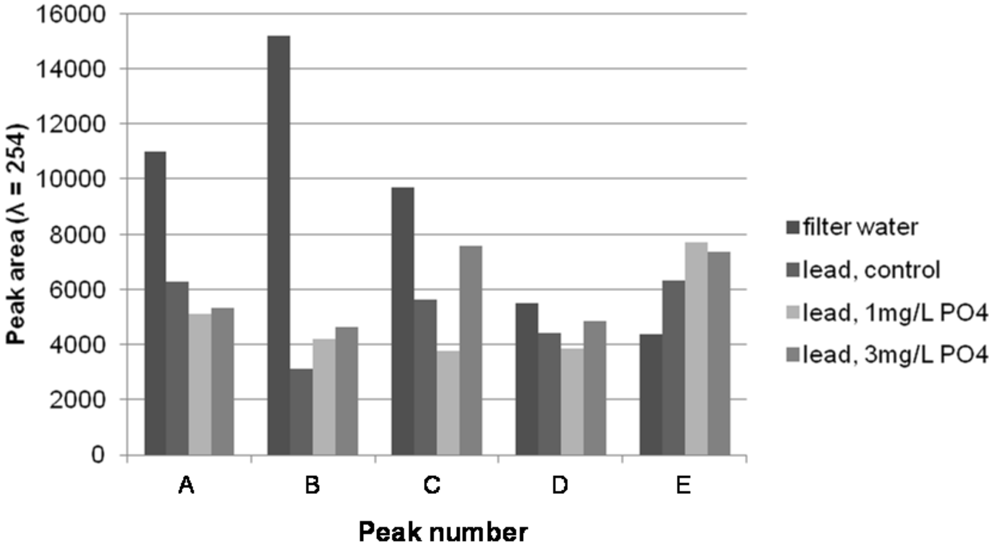


Figure 4.4 Area associated with chromatography peaks in the SEC chromatogram in the lead macrocells. The average molecular size (Da) with standard deviation is Peak A: 1360 ± 105, Peak B: 780 ± 91 , Peak C: 550± 92, Peak D: 420 ± 76, Peak E: 260 ± 64.

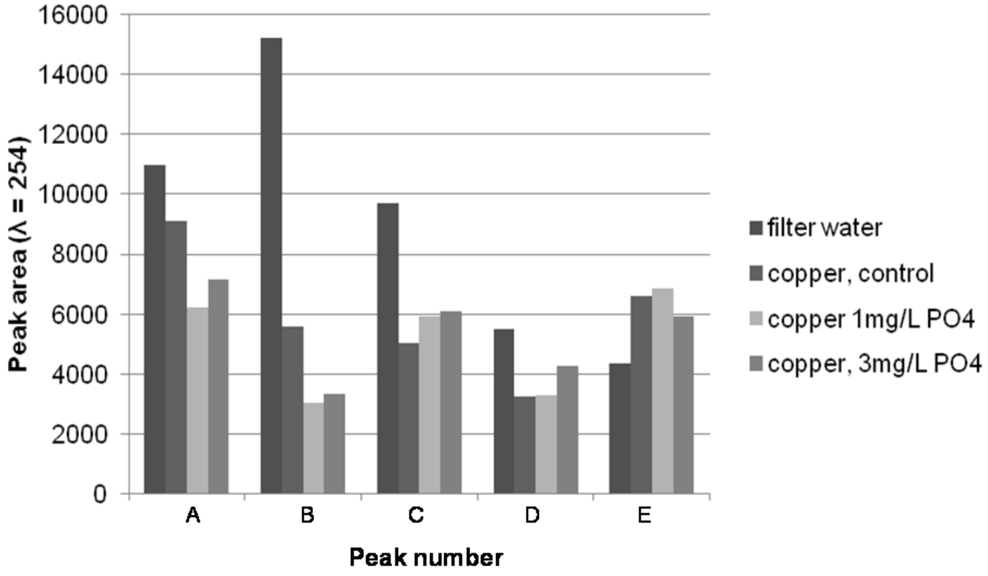


Figure 4.5 Area associated with chromatography peaks in the SEC chromatogram in the copper macrocells. The average molecular size (Da) with standard deviation is Peak A: 1360 ± 105, Peak B: 780 ± 91 , Peak C: 550± 92, Peak D: 420 ± 76, Peak E: 260 ± 64.

Based on previous work on this water supply (Montreuil, 2011), Peak A consisted largely of hydrophobic material; whereas Peaks D and E largely consisted of hydrophilic material. Hydrophilic material has been associated with many of the functional groups present in both EPS and soluble microbial products that have been shown to bind divalent metals (Wang & Zhang, 2010; Wang et al., 2012). Although limited in sample size, more hydrophilic material was identified in the biofilms from the polycarbonate coupons in the lead galvanic macrocells. The presence of more hydrophilic material may explain why the biofilms on the polycarbonate coupons in the lead galvanic macrocells absorb significantly more lead (Figure 4.6) even though similar amounts of lead were released through galvanic currents (Figure 4.2).

4.4.3 Accumulation of lead particles in biofilm

In the biofilm accumulated on the polycarbonate, the surface density of both the total and dissolved lead fraction is given in Figure 4.6. In the lead galvanic macrocells, the majority of the biofilm associated lead is in the total (or particle) fraction. Lead particles have been shown to play a dominant role in several recent drinking water studies conducted at neutral pH with a phosphate based corrosion inhibitor (Camara et al., 2013; Woszczynski et al., 2013; Cartier et al., 2012). Lead particles are a critical concern as they have been shown to be trapped in aerators, providing a significant source of lead ingestion in a case study involving a child with lead poisoning (Triantafyllidou et al., 2007). In the copper galvanic macrocells, the piece of lead-tin solder acted as the only source of lead for the system, and Figure 4.6 shows that the solder contributed to both the particulate and dissolved fraction of lead. A visual inspection of Figure 4.6 reveals that the dissolved fraction of lead appears to be equal across all six conditions. A Tukey’s analysis revealed that there is no significant difference between the mass of dissolved lead in any of the six treatment systems. This suggests that the dissolved fraction of lead, in this study, primarily comes from the lead-tin solder.

4.4.4 Contribution of benign downstream plumbing to lead and copper removal from bulk water phase

The polycarbonate coupons in this experiment were used to represent downstream benign plumbing fixtures. Based on the biofilm associated lead densities shown in Figure 4.6, the total mass lead accumulated on the polycarbonate coupons inside the lead galvanic macrocells was 367 µg, 85 µg and 318 µg for the control, 1mg/L – PO_4 and 3 mg/L – PO_4 treatments, respectively. The total mass of lead accumulated on the polycarbonate coupons of the copper galvanic macrocells was 27 µg, 6.4 µg and 13 µg for the control, 1mg/L – PO_4 and 3 mg/L – PO_4 treatments, respectively. Based on the biofilm associated copper densities shown in Figure 4.7, the total mass of copper accumulated on the polycarbonate coupons inside the lead galvanic macrocells was 4.0 µg, 4.5 µg and 4.0 µg for the control, 1mg/L – PO_4 and 3 mg/L – PO_4 treatments , respectively. The total mass of copper accumulated on the polycarbonate coupons inside the copper galvanic macrocells was 23 µg, 26 µg and 52 µg for the control, 1mg/L – PO_4 and 3 mg/L – PO_4 treatments, respectively.

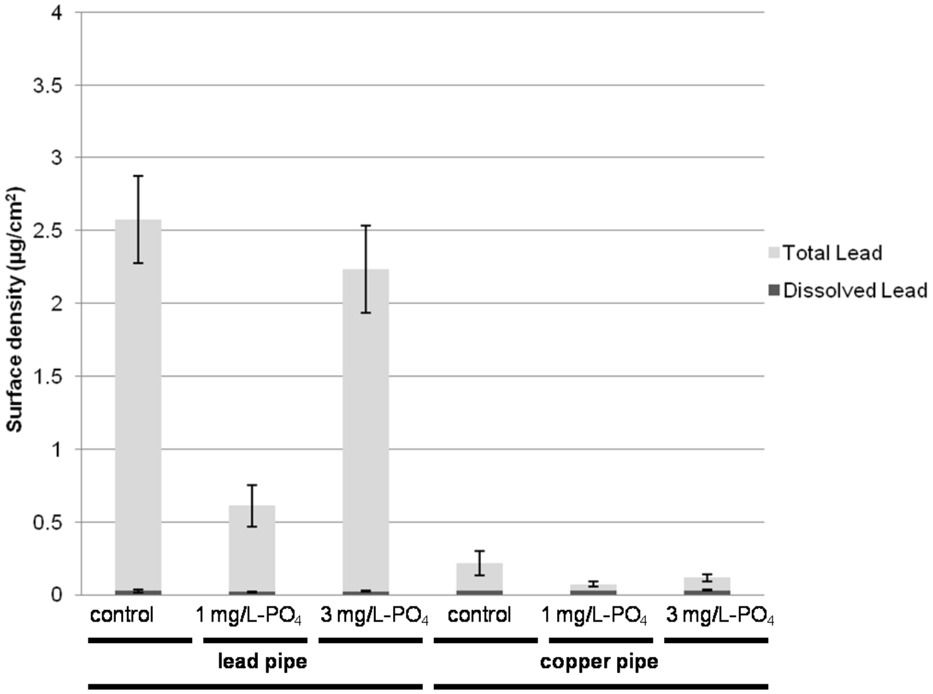


Figure 4.6 Surface density of the total and dissolved fractions of the lead accumulated in the biofilm of the polycarbonate coupon, given in µg/cm^2. Error bars represent the 95% confidence interval.

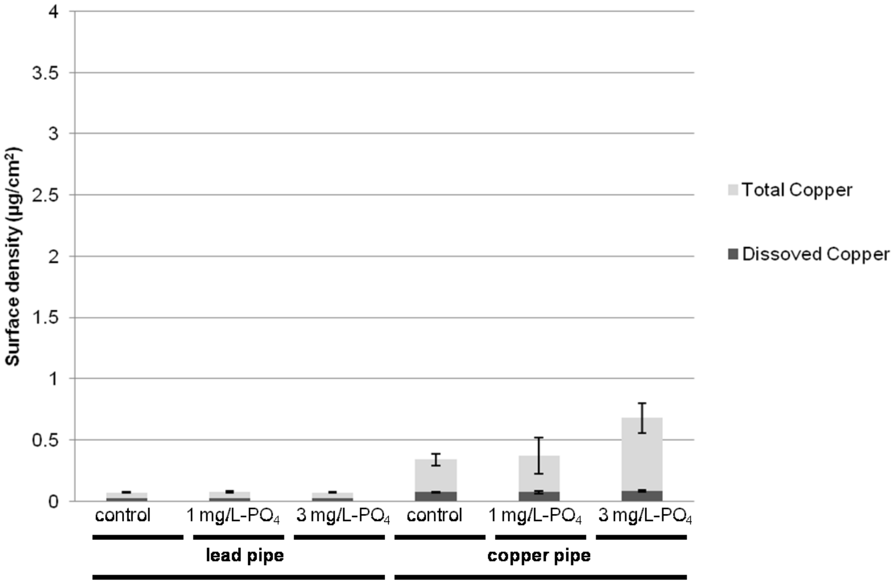


Figure 4.7 Surface density of the total and dissolved fractions of the copper accumulated in the biofilm of the polycarbonate coupon, given in µg/cm^2. Error bars represent the 95% confidence interval.

Although the total mass of lead and copper accumulated in the biofilm over the two month treatment period in this study is small, it does demonstrate that upstream corrosion products (lead and copper) can accumulate in downstream benign plumbing fixture associated biofilm. In the experience at a German hospital, reported by Kappstein et al. (2000), measures were put in place to control biofilms such as a weekly aerator cleaning to remove biofilms to avoid pathogen accumulation (and infection risk to patients). In systems where lead and copper particles are present, it may be prudent to explore similar best practices to not only control the microbial risk at plumbing fixtures but also to manage metal accumulation in these biofilms.

4.5 CONCLUSIONS

- The majority of available lead generated by galvanic currents was not detectable in the bulk water phase or biofilm phase and was assumed to be a part of the corrosion scale.

- The addition of zinc orthophosphate increased the biofilm population on the polycarbonate surfaces. Utilities adding zinc orthophosphate as means of corrosion inhibitor may in fact be increasing biofilm density in the distribution system.

- Lead-tin solder provided a source of both particulate and dissolved lead, and lead-tin solder appears to be the primary source for the dissolved fraction of lead observed in biofilms.

- Notably, in the lead pipe systems, lead particles constituted the majority of the lead in the biofilm.

- In this study, the biofilm associated with the benign polycarbonate surface was shown to accumulate lead and copper available from the bulk water in stagnant systems. Biofilms were shown to remove lead in the range of 3-29% and copper in the range of 3-16% from the bulk water phase. This indicates that metals released from upstream plumbing materials (distribution system, service lines etc.) may accumulate in biofilms in benign downstream plumbing fixtures. Best practices should be explored to manage biofilms (not simply for the microbial risk), but for the risk of accumulated lead and copper particles

4.6 ACKNOWLEDGEMENTS

The authors wish to thank Matthew P. King and Karolina Smiech for their contribution to the initial operation of the experiment, Krysta Montreuil and Amina Stoddart for their guidance with the size exclusion chromatography and Heather Daurie for analyzing the metal samples. The authors also acknowledge the National Sciences and Engineering Research Council of Canada (NSERC) and the Halifax Water industrial chair for their financial support for this research. The authors also wish to thank the Atlantic Innovation Fund and Halifax Water for their support of the Clean Water Technology Laboratory at Dalhousie University and the Canada Foundation for Innovation for their financial support of the ICP-ms.

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CHAPTER 5 IMPACT OF ZINC ORTHOPHOSPHATE ON LEAD AND COPPER INFLUENCED DRINKING WATER BIOFILMS

5.1 ABSTRACT

Phosphate based corrosion inhibitors are widely used by water utilities to decrease corrosion from copper and lead containing plumbing components. However, phosphate is a key nutrient for bacterial growth and could promote biofilm formation in premise plumbing. Bench-scale stagnant water galvanic macrocells, with lead and copper plumbing components, were fed with drinking water containing three levels of a common corrosion inhibitor, zinc orthophosphate, at concentrations of 0 (control), 1 and 3 mg/L -PO_4. The stagnant water systems were changed twice per week (after 3-d and 4-d). Polycarbonate coupons were suspended into the bulk water and were used as an inert, non-corrosive material (representing a benign downstream plumbing fixtures) to enable biofilm formation. Community profiling, using denaturing gradient gel electrophoresis (16S rDNA PCR-DGGE) revealed that phosphate dose was the primary influence on biofilm community diversity. Pipe material also contributed to microbial diversity, but to a lesser extent than the phosphate. In general, community diversity increased with increasing heterotrophic plate count (HPC) population that in turn rose in response to the elevated phosphate concentrations. Partial 16s rDNA sequences obtained from DGGE gel bands were used to identify the dominant bacterial taxa found in these autochthonous biofilms. The identified taxa are ubiquitous in soil and/or aqueous environments. Major phylogenetic groups were Verrumicrobia, Firmicutes, Bacteroidetes and α-Proteobacteria. The increase in size and diversity of biofilm communities as a result of phosphate treatment further highlights the challenges of a phosphate corrosion control program.

KEYWORDS: lead, copper, zinc orthophosphate, drinking water, biofilm ecology, PCR-DGGE

5.1 INTRODUCTION

Biofilms are often implied as the cause of premise plumbing corrosion, and there is evidence that microbial communities contribute to copper corrosion (Critchley et al., 2004; Pavissich et al., 2010; Rahman et al., 2007; Zumelzu et al., 2003). Evidence linking biofilms and lead corrosion has been limited in the literature; however, White et al. (2011) identified and characterized a biofilm community isolated from a lead service line suggesting that biofilm may play a role in lead corrosion. The species identification does not imply causality of lead corrosion; rather it acknowledges the presence of microorganisms in a corroding environment.

In an effort to mitigate corrosion, water utilities employ phosphate based corrosion inhibitors to decrease corrosion of distribution system and premise plumbing materials including lead and copper. Phosphate is also a key nutrient for bacteria and has been found to increase drinking water biofilm population at exposure levels as low as 0.03 mg/L (Fang et al., 2009), 0.01 mg/L (Chu et al., 2005) and 0.001 mg/L (Lehtola et al., 2002). This increased biofilm population not only has implications for lead and copper bearing pipes and fixtures, but also for other downstream benign plumbing fixtures such as aerators. Aerators have been shown to be sites of biofilm growth, where bacteria present in the bulk water can colonize the surface (Kappenstein et al., 2000; Lavenir et al., 2008), and metals can become subsequently sorbed to the exopolymeric substances (EPS) of the biofilm (Qin-qin et al., 2012). Increased phosphate may increase biofilm growth, which may in turn foster a more diverse biofilm community. Phosphate addition has been linked to increased biofilm diversity, as has been reported by Jang et al. (2012). Multi-species biofilms have been shown to be more resistant to disinfection than single species biofilms (Simões et al., 2010). Thus, understanding how the addition of phosphate corrosion inhibitors affects drinking water distribution system biofilms warrants further investigation and consideration by water utilities.

Molecular tools such as finger printing the microbial community using PCR amplified 16S rRNA genes followed by denaturing gradient gel electrophoresis (DGGE) can be used to identify shifts in biofilm community structure as a result of environmental changes in drinking water (Jang et al., 2012). Individual bands of amplified 16S rDNA can subsequently be excised from DGGE gels and the DNA can be sequenced to identify taxonomic units in the biofilm. Others have used this approach to determine microbial water quality changes in a simulated drinking water distribution system (Williams et al., 2004), and in a real drinking water distribution system (McCoy & VanBriesen, 2012). In the work of the latter group, it was demonstrated that shifts between the dominant subclasses (Alpha, Beta, and Gamma) of Proteobacteria were linked with seasonal and water quality changes.

This study used culture-based and culture-independent methods to investigate the impact of zinc orthophosphate and the presence of metal particles on biofilm diversity developing in a simulated domestic plumbing system consisting of a mixture of lead, copper and benign downstream plumbing fixtures (i.e. aerator). The work was conducted under controlled laboratory environment using a procedure that was originally developed for understanding lead release (Nguyen et al., 2010a; Nguyen et al., 2010b). The water used for this corrosion study was sampled from a local water utility.

5.2 MATERIALS AND METHODS

5.2.1 Experimental set-up

To generate lead and copper, twelve galvanic macrocells were constructed as described by Nguyen et al. (Nguyen et al., 2010a; Nguyen et al., 2010b). The galvanic macrocells included either a large lead or copper section of pipe, which was connected to a shorter copper section and a piece of lead-tin solder. Six galvanic macrocells were composed of a large lead pipe (31 cm long) attached to a smaller copper pipe (6.4 cm long). The other six galvanic macrocells were composed of a large copper pipe (31 cm long) attached to a smaller copper pipe (6.4 cm long). All twelve galvanic macrocells had a 10 cm piece of 50-50 lead-tin solder inserted at the bottom of the smaller copper pipe through the silicone stopper at the bottom of the small copper pipe piece. Copper wire and copper alligator clips were used to create a galvanic connection between the large section of pipe (either lead or copper) and the small copper pipe. A second galvanic connection using copper wire and copper alligator clips was created between the small copper pipe and the lead-tin solder. The lead and copper galvanic macrocell design is shown in Figure 5.1.

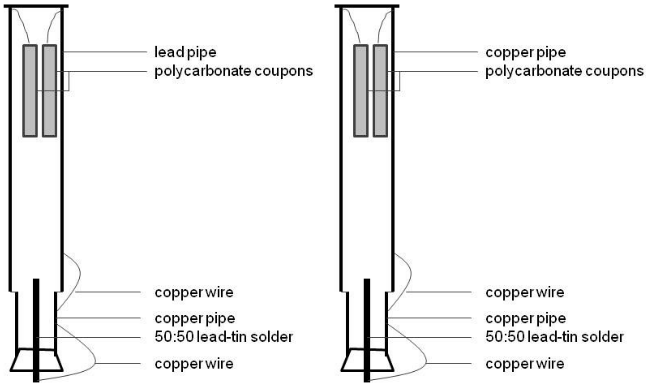


Figure 5.1 A lead (left) and copper (right) galvanic macrocell (details in Nguyen et al., 2010a; Nguyen et al., 2010b) with suspended removable polycarbonate coupons for biofilm sampling.

Two removable polycarbonate coupons (43.7 cm^2) were suspended from the top of the large pipe section using neoprene fishing line. The polycarbonate coupons (Biosurface Technologies Corporation, Bozeman, MT, U.S.A.) were used to simulate a plumbing polycarbonate surface on which particles and biofilm from a premise plumbing system could accumulate. The polycarbonate coupons used in this experiment are typically used in biofilm studies carried out in annular reactors simulating the distribution system (Murphy et al., 2008).

The J.D. Kline Water Supply Plant is a direct filtration facility that uses anthracite/sand filters and chlorine disinfection. A detailed plant description is provided in Vadasarukkai et al., 2011. Low alkalinity drinking water was obtained from the J.D. Kline Water Supply Plant in Halifax, NS, Canada. Water was sampled following filtration (pH≈5.8, turbidity < 0.1 NTU) and prior to final chlorination and chemical adjustment. By using water prior to final chemical adjustment, it was possible to manipulate the phosphate dose for the purposes of this experiment. The pH adjustment was conducted in order to achieve a final pH typical of the final water quality employed by Halifax Water at the J.D. Kline Water Supply Plant. The water used during acclimation was chemically adjusted using sodium hydroxide to a pH of 7.4 (alkalinity of approximately 8.5 mg/L (as $CaCO_3$) and a dissolved inorganic carbon (DIC) concentration of 2.5 mg/L).

All twelve galvanic macrocells were acclimated for four months prior to treatment (without the polycarbonate coupons). The water in the galvanic macrocells was replaced twice weekly (alternating 3-d or 4-d stagnation times) with freshly processed filtered water from the J.D. Kline Water Supply Plant, adjusted to pH 7.4. The 3-d and 4-d stagnation time was chosen as a worst case scenario for corrosion in household plumbing (ie. water standing in pipes over a weekend or holiday). Similarly long stagnation periods of 2-d and 3-d have been used elsewhere (Nguyen et al., 2010a; Nguyen et al., 2010b) Following this four month acclimation period, two polycarbonate coupons were inserted into each galvanic macrocell. The polycarbonate coupons were left undisturbed to develop a biofilm over the course of one month. No water changes were conducted during this acclimation period. A 30 day biofilm acclimation period has typically been observed in previous biofilm studies at Dalhousie (Gagnon et al., 2004; Gagnon et al., 2005; Rand et al., 2007). Following the acclimation period and the polycarbonate coupon biofilm development period, the twelve galvanic macrocell systems were divided into three treatment groups. Each treatment group included two lead galvanic macrocells and two copper galvanic macrocells. During the two month experimental period, the three treatment groups received pH adjusted water containing 0, 1 or 3 mg/L of zinc orthophosphate (reported as phosphate). The water in the galvanic macrocells was replaced twice weekly (alternating 3-d or 4-d stagnation times). The pipe sections were treated as stagnant water systems. The water quality for all six treatments is given in Table 5.1 below.

Table 5.1 Average water quality for the influent water and the average effluent water quality six treatments with standard deviation. For the treatment groups, the pH, temperature and turbidity represent the water quality following stagnation, and the phosphate represents the initial concentration of phosphate added.

Treatment		pH	Temperature (˚C)	Turbidity (NTU)	Phosphate (mg/L)
Influent water		7.53 ± 0.2	21 ± 1	0.5 ± 0.7	nd
Lead galvanic macrocell	control	7.3 ± 0.6	22 ± 3	6.3 ± 7.4	nd
	1 mg/L-PO4	6.6 ± 0.3	22 ± 2	1.2 ± 0.8	0.8 ± 0.1
	3 mg/L-PO4	7.1 ± 0.3	22 ± 2	1.7 ± 1.8	3.6 ± 0.3
Copper galvanic macrocell	control	7.1 ± 0.5	22 ± 3	44.5 ± 35	nd
	1 mg/L - PO4	6.8 ± 0.3	22 ± 3	0.6 ± 1.0	0.8 ± 0.1
	3 mg/L - PO4	7.1 ± 0.2	22 ± 2	37 ± 3.0	3.6 ± 0.3

-nd indicates that the parameter was not detected (below the detection limit)

5.2.2 Metal and phosphate analysis

Since the biofilm samples were turbid, nitric acid digestion (105 ˚C for 2 h in 5% nitric acid) was used to prepare the samples for total lead and copper analysis according to Standard Methods 3030E (APHA, 2009). The lead and copper were then analyzed on a on an X-Series 2 ICP-MS (Thermo Fisher Scientific, MA, U.S.A.) following Standard Methods 3125 and 3030E (APHA, 2009). The limit of detection was 0.4 µg/L and 0.7 µg/L for lead and copper, respectively. Phosphate was measured using a 761 Compact IC (Metrohm, Herisau, Switzerland) using US EPA method 300.1 (USEPA, 1997), and the detection limit for phosphate was 0.7 mg/L.

5.2.3 Biofilm collection from polycarbonate coupons

The polycarbonate coupons were aseptically removed at the end of the two-month zinc orthophosphate treatment period. The polycarbonate coupons used for microbiological analysis were placed in separate sterile Secure T 177 × 302 mm stomacher bags (Fisher Scientific, Nepean, ON, Canada) containing 10 mL of sterile phosphate buffer solution (PBS) and stomached at 230 rpm for 2 minutes in a MIX 2 Stomacher (AES Laboratories, Bruz, France), as described by Gagnon and Slawson (1999). Biofilm samples were successfully collected from 11 of the 12 available polycarbonate coupons as samples from one coupon from the lead galvanic macrocells treated with 3 mg/L -PO_4 were lost.

5.2.4 Microbial analysis

5.2.4.1 Biofilm quantification

The culturable biofilm was quantified using the spread plate method to obtain the heterotrophic plate count as described in Standard Methods 9215C (APHA, 2009). Two samples were taken from each of the biofilm preparations, serially diluted with suitable dilutions plated in duplicate on R2A agar (Becton Dickenson and Co., MD, USA). Following a 7-day incubation period at room temperature, the colonies were enumerated and results reported as log colony forming units per surface area of the polycarbonate surface (log CFU/cm^2),

5.2.4.2 DNA Extraction from biofilm

Biofilm samples were stored frozen at -20°C until DNA extraction. Samples were thawed at room temperature and centrifuged at 3400 × *g* for 15 minutes at room temperature. The supernatant was decanted and genetic material was extracted from the pellet using the PowerSoil DNA extraction kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following manufacturer's protocols. The resulting DNA samples were then subsequently stored at -20°C until further analysis.

5.2.4.3 PCR amplification of the 16S rRNA V3 region from the biofilm microbes

From the extracted biofilm DNA, the V3 region of the 16S bacterial rRNA gene was amplified through polymerase chain reaction (PCR) according to previously described conditions (Schafer & Muyzer, 2001). Briefly, 40 μL reactions were prepared consisting of 1× iTaq amplification buffer (Bio-Rad Laboratories (Canada) Ltd., Mississauga, ON), 200 nM of each dNTP, 500 nM of each primer, 2 mg/mL BSA, and 2U iTaq polymerase (Bio-Rad Laboratories (Canada) Ltd.). The primers used for generating the DGGE fragments were the bacterial-specific 341F (5'-CCT ACG GGA GGC AGC AG-3') forward primer and 518R (5'-ATT ACC GCG GCT GCT GG-3') reverse primer, with a 40 bp GC-clamp (5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G-3') added to the 5' end of the forward primer. Five micro litres of the biofilm DNA sample was added to each PCR reaction tube. The 16S rDNA V3 region was amplified using the touchdown cycling routine outlined by Muyzer et al. (1993). Here, the samples were held at 94°C for 5 minutes, followed by 20 cycles of 1 min at 94°C, 1 min at 65°C-55°C (touchdown -0.5°C cycle^{-1}), and 3 min at 72°C, followed by 15 cycles of 1 min at 94°C, 1 min at 55°C, and 3 min at 72°C, followed by 7 min final extension at 72°C. The PCR product was verified through gel electrophoresis on a 1.5% agarose gel. The community 16S rDNA amplicons were stored at -20°C until electrophoresis.

5.2.4.4 DGGE Run Conditions and Analysis

PCR products were separated by denaturing gradient gel electrophoresis (DGGE) using a Cipher DGGE System (DGGE-2401; CBS Scientific Company Inc., Del Mar, CA, USA). A 6% polyacrylamide gel, with a 40-60% denaturing gradient (where 100% denaturing gradient is defined as 40% formamide v/v and 42.8% of urea w/v) in 1× TAE buffer was cast. After 2 h of polymerization, the gel was moved to the electrophoresis tank preheated to 60°C, the wells were rinsed with the running buffer (1× TAE buffer), and the gel was run at 10 V for 10 minutes. Then, 20 μL of the biofilm sample PCR product was mixed with a 10× gel-loading solution (2 μL 50% (v/v) glycerol, 0.25% (w/v) bromophenol blue, and 0.25% (w/v) xylenecyanole) and added to separate wells of the gel. All biofilm samples were run in duplicates and contained in the same gel, so no standards were run. Once loaded, the gel was run at 200 V for 5 min, then 100 V for 14 h. The gels were then separated from the glass plates and stained with 30 mL of 1× SYBR Gold nucleic acid gel stain (Life Technologies Inc., Burlington, ON, Canada). The stained gel was photographed with an ImageMaster VDS-CL bio-imaging system (Amersham Pharmacia

Biotech Ltd., Piscataway, NJ, USA). Banding patterns were analyzed in GeneTools software (Synoptics Ltd., Cambridge, UK) using the rolling disk method for background subtraction. Cluster analysis was performed with GeneDirectory software (Synoptics Ltd.). First, a similarity matrix was calculated through profile comparison using Pearson's product moment correlation coefficient. A dendrogram was then produced using the complete linkage algorithm and a 0.8% threshold. Phylogenetic diversity was calculated using operational taxonomic units (OTUs), which are classifications that represent species-level or higher phylogenetic identities, but cannot be confirmed without DNA sequence information. OTU richness was calculated as the total number of bands identified per sample in the computational fingerprint analysis.

5.2.4.5 DGGE Fragment Sequencing and Analysis

After imaging, the DGGE gel was transferred to a Dark Reader transilluminator (Clare Chemical Research Inc., Dolores, CO, USA) for band excision. Representative bands were excised from the DGGE gel using sterile tweezers, transferred to a 200 µL PCR tube, and incubated overnight in 40 µL of 1× TE buffer (10 mM Tris-HCl, 1 mM EDTA) at 4°C. The eluted DNA was amplified through PCR using the conditions described above, except the forward primer without the GC-clamp was used. The PCR amplicons were cleaned using an UltraClean PCR cleanup kit (MoBio Laboratories Inc.) and sent to Bio-Basic Inc. (Markham, ON, Canada) for Sanger sequencing using the 341F primer as the sequence primer. Sequence matches were identified using the Naïve Bayesian classifier feature of the Ribosomal Database Project Release 10 database (Wang et al., 2007). All of the DGGE sequences obtained were run through the classifier function, but only those sequences with family-level identification exceeding 90% were used for constructing the phylogenetic tree. Multiple sequence alignments were made using neighbour-joining algorithm in ClustalX 2.1 (Larkin et al., 2007), and phylogenetic trees were constructed using NJplot (Perrière & Guoy, 1996).

5.2.4.6 Data organization and statistical analysis

Microsoft Excel ® (Redmond, WA, USA) was used to organize the data and conduct Tukey's method analysis with statistical tables given by Harter (1960). Mini-Tab 16 ®

(State College, PA, USA) was used to conduct Pearson correlation significance testing. All statistical analysis was conducted at the 95% confidence level.

5.3 RESULTS AND DISCUSSION

5.3.1 The influence of zinc orthophosphate and pipe environment on diversity

The dendrogram generated from the DGGE fingerprint similarity calculated using Pearson's product moment correlation coefficient is given in Figure 5.2.

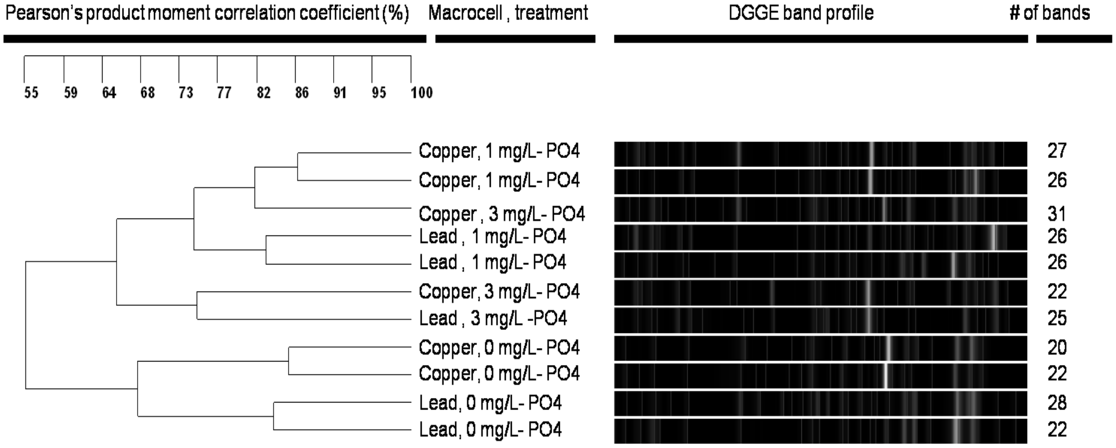


Figure 5.2 Dendrogram of biofilm DGGE profiles for all six treatment groups (lead and copper galvanic macrocells treated as control, 1 mg/L-PO_4 or 3 mg/L-PO_4). Two samples for each treatment group were analyzed, except in the case of the lead galvanic macrocell with 3 mg/L-PO_4 (as that sample was lost). The Pearson's product moment correlation coefficient (%), DGGE band profile and the number of bands in each profile is shown.

Generally, the presence or absence of zinc orthophosphate had the greatest effect on species diversity. This resulted in the control (0 mg/L -PO_4) treatments being dissimilar from both the 1 mg/L and 3mg/L -PO_4 treated systems (55% DGGE profile similarity). Within the control treatment group, biofilms from the duplicate coupons suspended in the same pipe material were similar to each other. This meant that the biofilms grown in the copper galvanic macrocell had a DGGE profile similarity of 86% while the biofilms grown in the lead galvanic macrocell exhibited a similarity of 84%. In contrast, the similarity between the biofilms grown in the copper and lead galvanic macrocells was 68%, indicating that exposure to different metals caused a change in the biofilm community composition in the control treatment.

Similarly for the 1 mg/L -PO_4 treatment group, a higher DGGE profile similarity was observed for biofilms formed in the same pipe material systems, with DGGE profile similarities of 82% and 86% for biofilms grown in the copper galvanic macrocells and the lead galvanic macrocells, respectively. The DGGE profile similarity between the biofilm in the copper galvanic macrocells and lead galvanic macrocells was only 74% for this phosphate treatment, again suggesting that the metal exposure influenced the community diversity.

The impact of metal on biofilm community composition appeared to diminish as the zinc orthophosphate corrosion inhibition concentration was increased to 3 mg/L -PO_4. For biofilms in the copper galvanic macrocells, a low DGGE profile similarity score of 65% was observed among the duplicate samples. One of the biofilm samples from the copper galvanic macrocell exhibited a DGGE profile similarity of 82% to the biofilms from the 1 mg/L -PO_4 treatment copper treatment, while the other demonstrating 74% DGGE profile similarity to a biofilm sample from the lead galvanic macrocell treated at 3 mg/L -PO_4.

In summary, in this experiment, it appears that both metal type and zinc orthophosphate concentration influenced the biofilm community composition, but as the concentration of zinc orthophosphate increased the effect of the metal type on community structure became less pronounced.

5.3.2 Impact of zinc orthophosphate on quantifiable biofilm

As the zinc orthophosphate concentration was increased, the culturable microbial population (here reported as the heterotrophic plate count) increased (Figure 5.3).

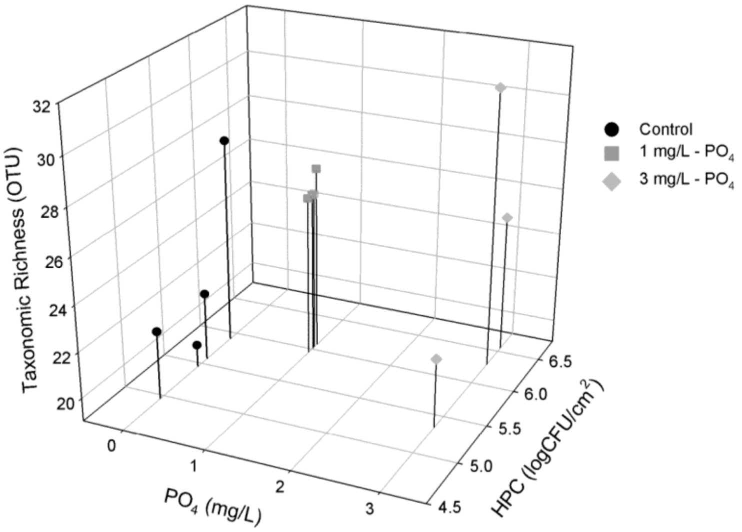


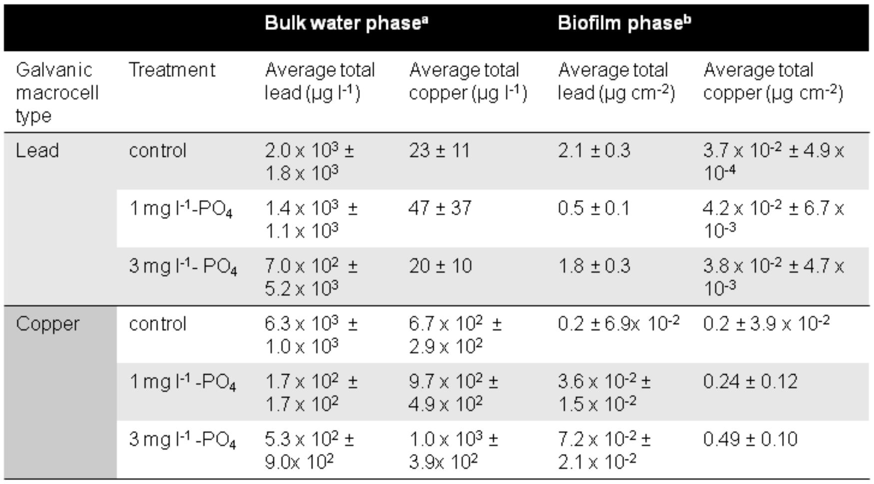
Figure 5.3 A 3-dimensional plot of zinc orthophosphate dose (as PO_4) vs. heterotrophic plate count (HPC) and Taxonomic richness (OTU richness) for all six treatment groups (lead and copper galvanic macrocells treated as control, 1 mg/L -PO_4 or 3 mg/L -PO_4. Two samples for each treatment group were analyzed, except in the case of the lead galvanic macrocell 3mg/L -PO_4 as that sample was lost. Each data point has a projection line to the x-y plane.

The average heterotrophic plate count was significantly higher in the 3mg/L- PO_4 treatments than in the control treatments for both the lead and copper galvanic macrocells. The greater biofilm biomass (biofilm HPC counts) correlated with higher taxonomic richness in the biofilm (Figure 3). The Pearson correlation showed a significant ($\rho = 0.727$, $P<0.05$) relationship between the biofilm heterotrophic plate count and taxonomic richness. Jang et al. (2012) observed that the diversity of biofilms grown on stainless steel and cast iron coupons increased with the addition of phosphate. Moreover, Fechner et al. (2012b) reported that phosphate exposure was an important factor in increasing copper tolerance in freshwater biofilms. The phosphate induced changes in community structure, size and taxonomic richness observed in this study and elsewhere may have significant implications for water utilities. Elevated resistance to sodium hypochlorite disinfection has, for example, been shown for multi-species biofilms in comparison to single species biofilms (Simões et al., 2010).

5.3.3 Influence of lead and copper on quantifiable biofilm

In the lead galvanic macrocells, the average lead concentrations were 2.0 x 10^3 µg/L, 1.4 x 10^3 µg/L and 7.0 x 10^2 µg/L and the average copper concentrations were 23 µg/L, 47 µg/L and 20 µg/L in the bulk water of the control, 1 mg/L and 3mg/L -PO_4 treated systems, respectively (Table 1). In the copper galvanic macrocells, the average lead concentrations were 6.3 x 10^2 µg/L, 1.7 x 10^2 µg/L, 5.3 x 10^2 µg/L and the average copper concentrations 6.7 x 10^2 µg/L, 9.7 x 10^2 µg/L, 2.0 x 10^2 µg/L in the bulk water of the control, 1 mg/L and 3mg/L -PO_4 treated systems, respectively (Table 5.2).

Table 5.2 Lead and copper concentrations (with standard deviations) in bulk water phase and biofilm phase for the galvanic macrocells treated as either controls, or with 1 mg/L-PO_4 or 3 mg/L-PO_4.



		Bulk water phase[a]		**Biofilm phase**[b]	
Galvanic macrocell type	Treatment	Average total lead (µg l^{-1})	Average total copper (µg l^{-1})	Average total lead (µg cm^{-2})	Average total copper (µg cm^{-2})
Lead	control	2.0 x 10^3 ± 1.8 x 10^3	23 ± 11	2.1 ± 0.3	3.7 x 10^{-2} ± 4.9 x 10^{-4}
	1 mg l^{-1}-PO_4	1.4 x 10^3 ± 1.1 x 10^3	47 ± 37	0.5 ± 0.1	4.2 x 10^{-2} ± 6.7 x 10^{-3}
	3 mg l^{-1}- PO_4	7.0 x 10^2 ± 5.2 x 10^3	20 ± 10	1.8 ± 0.3	3.8 x 10^{-2} ± 4.7 x 10^{-3}
Copper	control	6.3 x 10^3 ± 1.0 x 10^3	6.7 x 10^2 ± 2.9 x 10^2	0.2 ± 6.9x 10^{-2}	0.2 ± 3.9 x 10^{-2}
	1 mg l^{-1} -PO_4	1.7 x 10^2 ± 1.7 x 10^2	9.7 x 10^2 ± 4.9 x 10^2	3.6 x 10^{-2} ± 1.5 x 10^{-2}	0.24 ± 0.12
	3 mg l^{-1} -PO_4	5.3 x 10^2 ± 9.0x 10^2	1.0 x 10^3 ± 3.9x 10^2	7.2 x 10^{-2} ± 2.1 x 10^{-2}	0.49 ± 0.10

a. The bulk water phase data shows the average of the 3-d and 4-d metal concentrations. For bulk water phase sample, n ≥ 18.
b. For the biofilm phase data, n = 2.

There was no significant (P>0.05) correlation between metal concentration in the bulk water phase and the biomass (biofilm HPC counts). Similarly, there was no significant (P>0.05) correlation between metal concentration in the bulk water and taxonomic richness. The accumulated metal content in the biofilms ranged between 0.1 to 0.5 µg/cm^2 and 0.1 to 2.1µg/cm^2 for copper and lead, respectively (Table 1). The concentrations of total lead and copper entrapped in the biofilm did not exert a significant

($P>0.05$) influence on biomass (HPC) or on taxonomic richness. Therefore, at the lead and copper concentrations observed in this study do appear to have an inhibitory effect on biomass or taxonomic richness.

Although the diversity of the biofilms was affected by both phosphate dose and the dominant metal in the water, as evidenced through the DGGE profile similarity, the biomass was not. Biofilms attained biomass densities which were approximately 1 log higher than those found in another biofilm study using similar source water on polycarbonate coupons in annular reactors (Murphy et al., 2008). It is possible that the species were selected for by the presence of metals and/or lead and copper tolerance was induced. Freshwater biofilms exposed to lead (Fechner et al., 2012a) and copper (Fechner et al., 2012b) enriched environments were shown to induce tolerance to both lead and copper, respectively. Fechner et al. (2012a) demonstrated that freshwater biofilms exposed to 100 µg/L Pb induced lead and zinc tolerance, and exposures of up to 2.07 g/L of the metals did not cause full inhibition of biofilm growth.

5.3.4 Identification of the phyla associated with drinking water biofilm

Of the DGGE gel bands excised for sequencing, only eight bands gave suitable results for phylogenetic classification, which were compared against 24 species found within the families of the obtained sequences (Figure 5.4).

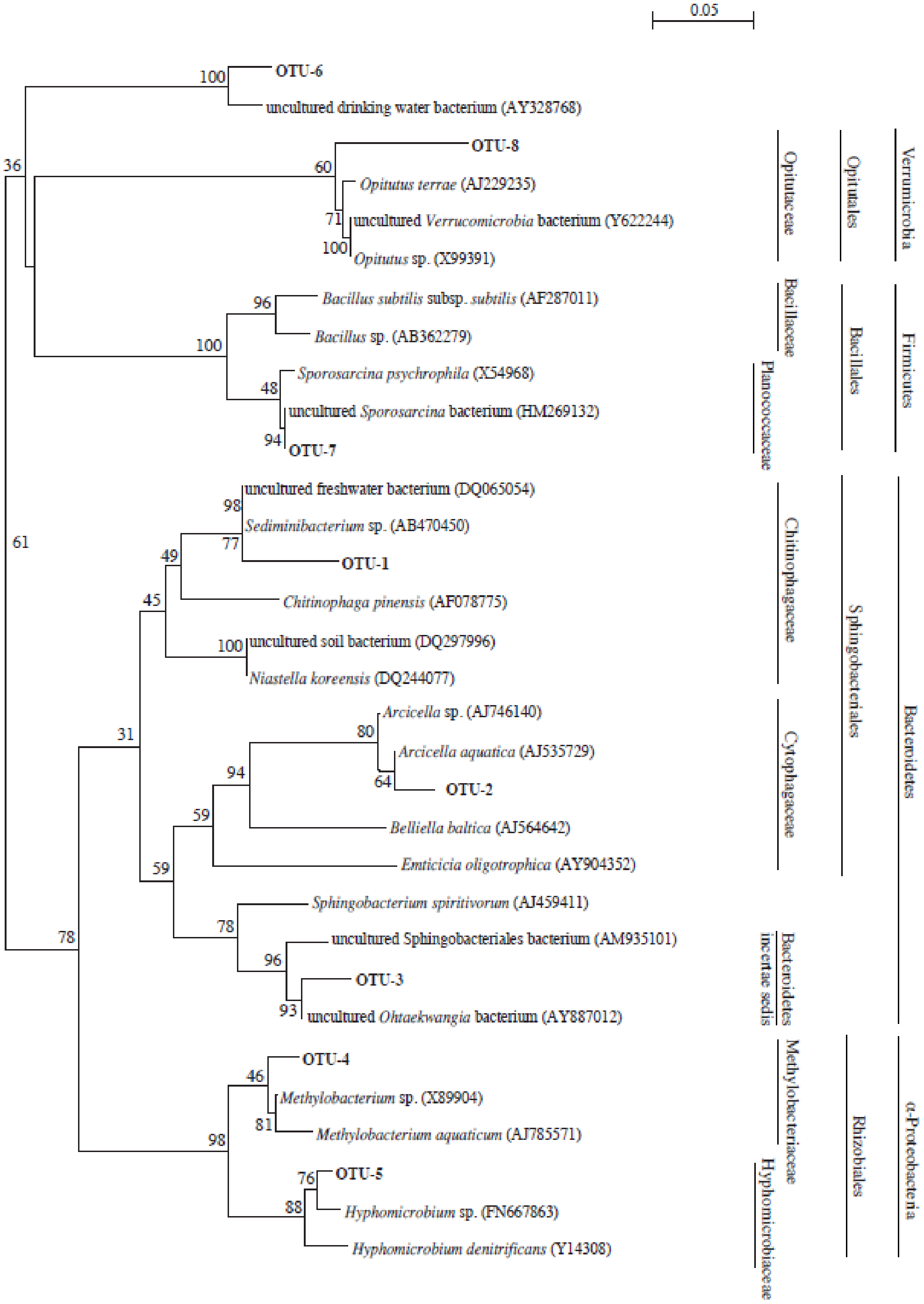


Figure 5.4 A bootstrapped (n=100) phylogenetic neighbour joining tree of 16S rDNA sequences. The BLAST database accession number is given in brackets.

The major OTUs present were found within the phyla Verrumicrobia, Firmicutes, Bacteroidetes and α-Proteobacteria. Since not all bands were excised from each gel (due to physical and visualization constraints) conclusions should not be drawn about the distribution of OTUs in individual biofilms. Generally, the four phyla represented are commonly associated with freshwater environments. The Verrumicrobia phylum are considered to be ubiquitous in the environment, and are associated with soil (Chin et al., 2002), drinking water (Martiny et al., 2005) and the human intestine (Wang et al., 2005). The Firmicutes phylum has previously been associated with the culturable portion of a phosphate treated drinking water biofilms (Jang et al., 2012). The Bacteroidetes phylum has also been associated with the culturable portion of a drinking water biofilm (Martiny et al., 2005). Within the Bacteroidetes phylum, *Arcicella* spp. has been isolated from freshwater samples and has been shown to form biofilm (Furuhata et al., 2008). The α-Proteobacteria, has been identified as a ubiquitous phyla in simulated drinking water distribution systems (Williams et al., 2004) and in drinking water distribution systems (McCoy & VanBriesen, 2012).Within the α-Proteobacteria phylum, *Methylobacterium* spp. are well characterized, and have been shown to be hydrophilic and possess a negative surface charge (Simões et al. 2010b), which may create sites for biosorption of divalent metals. *Methylobacterium* spp. has also been shown to produce quorum sensing inhibitors (Simões et al., 2007), which could have implications for the formation of multispecies biofilms by interfering with biofilm formation by other bacteria. *Methylobacterium* have also been associated with the biodegradation of haloacetic acids (HAAs) which are an important class of undesirable disinfection by-products (Zhang et al., 2009). The unclassified drinking water bacterium (GenBank accession number AY328768) was also identified by Williams et al. (2004) in a lab-based drinking water pipe loop study. Unclassified bacteria from drinking water samples are not uncommon. Poitelon et al. (2009) sequenced 16S rDNA from bacteria living in chlorinated drinking water band reported that between 6.3 and 36.5% of sequenced bacteria were of unknown phylogenetic affiliation.

5.4 CONCLUSIONS

- Phosphate dose influenced biofilm diversity more than metal type
- Heterotrophic plate count biofilm density is a predictor for species richness/diversity in this system
- The bacteria identified were generic freshwater bacteria.
- Addition of phosphate based corrosion inhibitors increased the amount of biofilm produced in a simulated premise plumbing system. Hence addition should be applied with that trade-off in mind (corrosion control vs. biofilm control)
- The results of this study warrant further investigation to understand the implication of phosphate and metal exposure at the flow-through and/or pilot scale level.

5.5 ACKNOWLEDGEMENTS

The authors thank Mike Schock and especially Colin White at the US EPA for their insight and encouragement to continue with this research and Drs. Martin Kalmokoff and Greg Benzanson at Agriculture and Agri-Food Canada for their technical support and guidance in developing and interpreting the DGGE results. The authors also thank John Bergese, Matthew P. King and Karolina Smiech for their contribution to operating the experimental apparatus, as well as Dr. Corinne Krentz for her help in optimizing the DGGE process. The authors also acknowledge the National Sciences and Engineering Research Council of Canada (NSERC) and the Halifax Water industrial chair for their financial support for this research. The authors also thank the Atlantic Innovation Fund and Halifax Water for their support of the Clean Water Technology Laboratory at Dalhousie University and the Canada Foundation for Innovation for their financial support of the ICP-MS.

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CHAPTER 6 ENTRAINMENT OF PARTICULATE LEAD IN DRINKING WATER BIOFILMS

6.1 ABSTRACT

Few corrosion control studies consider the role of biofilm in terms of its interactions with released lead particles. This study used annular reactors (outfitted with either copper coupons or copper coupons with 50-50 lead-tin solder) to compare two corrosion control treatments: (a) pH 7.4 with zinc orthophosphate (3mg/L –PO_4) and (b) pH 9.2. Both treatments were compared in the absence and presence of chlorine (0.2 mg/L). The majority of the released lead was in the particulate form, and particulate lead was associated with zinc orthophosphate and the presence of chlorine. Notably, in the presence of chlorine, the zinc orthophosphate system supported more biofilm growth which could be a result of increased surface area due to the increased presence of lead particles and/or a result of increased nutrients from phosphate. The results allude to the potentially significant role that lead particles may play in biofilm formation.

KEYWORDS: lead, copper, particles, zinc orthophosphate, pH, corrosion control, drinking water, biofilm

6.2 INTRODUCTION

Lead in drinking water presents a major public health concern, as lead is a neurotoxin with significant impacts on the neurological development of children (Canfield et al., 2003; Zahran et al., 2009). Copper in drinking water presents mainly an aesthetic concern, although high levels of copper can cause gastrointestinal disturbance (Pizarro et al., 2001). Lead and copper can be present as components of drinking water materials. In premise plumbing, lead may be present as a lead service line, in lead bearing solder or brass faucets (Sandvig et al., 2008). Copper is a widely used plumbing material, noted for

its workability (WHO & WPC, 2006) and natural antimicrobial properties (Osman & Cavet, 2008).
There are two major approaches to corrosion control which include adding a corrosion inhibitor such as phosphate to form a passivating scale on the metal surface or to raise the pH of the water to decrease the solubility of the metal. Both of these approaches are based in water chemistry.

A 2001 survey of medium and large U.S. utilities estimated that 56% used a phosphate based corrosion control strategy (McNeill & Edwards, 2002). Phosphate likely plays two major roles in corrosion control, (a) contributes to the mineral scale and (b) could contribute to microbial growth. Phosphate reacts with dissolved lead to form a lead-phosphate scale on the surface. Studies employing phosphate corrosion inhibitors have cited the dominance of lead particles compared to dissolved lead in the system (Camara et al., 2013; Cartier et al., 2012; Woszczynski et al., 2013; Wang et al., 2012). Particularly, orthophosphate has been shown to provide effective decreases in released soluble lead across a range of pH levels (7.2-9.5) (Edwards & McNeill, 2004).

In the literature, phosphate has been shown to contribute to drinking water biofilm growth (Chu et al., 2005; Fang et al., 2009). Interestingly, a majority of the medium and large sized utilities surveyed in 2001 were aware of the potential biological impact of a phosphate corrosion control program (McNeill & Edwards, 2002). Biofilms produce exopolymeric substances which possess charged functional groups that could provide binding sites for metals (Qin-qin et al., 2012). The increased biofilm density could provide increased storage potential for metal species. Drinking water biofilms have been shown to sorb divalent metals such as manganese (Ginige et al., 2011; Burger et al., 2008; Rygel, 2006), and it is plausible that lead could also be adsorbed to biopolymers as it has been observed in isolated bacteria exopolymeric substances as in Feng et al. (2012). However, there is relatively limited evidence concerning lead storage in drinking water biofilms. Biofilms have been demonstrated to be a factor in inducing blue water corrosion (Critchley et al., 2004) and pin-hole corrosion (Zumelzu et al., 2003) in copper pipes under drinking water conditions. Although biofilms have not been directly linked as a cause of lead corrosion, recent work by White et al. (2011) has identified bacterial communities associated with lead service line scale.

Another approach to controlling metal release, involves increasing the pH to reduce the solubility of lead (Schock, 1989). Edwards & McNeill (2002) found that pH 9.5 (with or without added corrosion inhibitor) was effective in mitigating lead release compared to treatments with neutral pH with phosphate corrosion inhibitor. Lytle and Schock (2005) showed that the pH of the water favoured different speciation of lead-scales. PbO_2 was shown to be a major component of these scales, and it was relatively stable in the presence of chlorine (an oxidant) and stable water chemistry. The PbO_2 scale is capable of dissolving and reattaching. In the absence of a corrosion inhibitor, high pH system stability is dominated by the solubility chemistry of dissolved lead.

Given that neutral pH with phosphate corrosion inhibitors appear to be dominated by lead particles and high pH systems with no phosphate inhibitors appear to be dominated by dissolved lead species, an experiment was devised to examine how these two approaches impacted microbial growth. These experiments compare two commonly used corrosion control approaches in the presence and absence of a chlorine residual: (a) pH 7.4 with a zinc orthophosphate corrosion inhibitor and (b) pH 9.2.

6.3 MATERIALS AND METHODS

6.3.1 Site description

The study was carried out at the J.D. Kline Water Supply Plant in Halifax, Canada. J.D. Kline is a direct dual media filtration plant, and produces an average of 90 ML/day (Halifax Regional Municipality, 2010). The raw water is extremely low alkalinity (<1mg/L) and the treated water has an alkalinity of approximately 20 mg/L (Halifax Regional Municipality, 2011). A detailed description of the J.D. Kline plant is provided in Vadasarukkai et al. (2011).Water was taken from water treatment plant's filter effluent (prior to final disinfection, pH adjustment and corrosion inhibitor addition). The filter effluent (pH 5.8, turbidity < 0.1NU) was used to feed the experimental system.

6.3.2 Annular reactor operation

Annular reactors have been widely used to evaluate drinking water distribution system biofilm growth in both laboratory (Camper et al., 2003; Murphy et al., 2008) and field settings (Camper et al., 2003; Jang et al., 2011; Rand et al., 2007; Sharp et al., 2001). The operating conditions were selected based on a 1 inch diameter copper pipe diameter. The annular reactor (Biosurfaces Technologies Inc., Bozeman, MT) was operated at approximately 74 rpm which corresponds to a shear stress of $0.25 N/m^2$, according to the manufacturer. The hydraulic retention time was adjusted to six hours to simulate the six hour stagnation time recommended by both the U.S. EPA and Health Canada prior to lead and copper sampling. Each annular reactor has 20 removable coupons for biofilm sampling. Prior to use, the annular reactors were cleaned with anti-bacterial soap, rinsed in deionized water, and soaked in 70% ethanol for 24 hours, and allowed to dry. All tubing used in the experiment was new, and was cleaned with 70% ethanol for 2 hours. The annular reactors have been acclimated for 12 months, in aggressive water (pH 5.5 – 7.0) to accelerate coupon aging.

6.3.3 Experimental design

Four annular reactors were used in this experiment. Two annular reactors had copper coupons, and the other two annular reactors contained copper coupons with approximately 0.25 g of 50-50 lead-tin solder added. The copper coupon and the copper coupon with lead-tin solder are shown in the photo. To the author’s knowledge, the addition of lead-tin solder to an annular reactor copper coupon is a unique application of this apparatus.

The four annular reactors were divided into two treatment groups. Each treatment group included one annular reactor with copper coupons and one annular reactor with copper coupons with lead-tin solder (Figure 6.1).

Figure 6.1 A copper coupon (top) and a copper coupon with added lead-tin solder (bottom) used in annular reactors

The two different corrosion control strategies used in this study were selected based on the approaches used by two Canadian cities with low alkalinity water. Halifax Water's J.D. Kline Water Supply Plant uses phosphate based corrosion control (using a zinc ortho/polyphosphate blend) (Knowles, 2011), and the City of Ottawa uses a pH adjustment (pH raised to 9.2) based approach (Campbell & Douglas, 2008). Two corrosion control treatments were compared in this study: (a) pH 7.4 with zinc orthophosphate (3mg/L $-PO_4$) corrosion inhibitor (hereafter referred to as zinc orthophosphate treatment) or (b) pH 9.2 (hereafter referred to as pH 9.2 treatment). Zinc orthophosphate (Carus 3180, Carus Corporation, Peru, IL) was used to provide the phosphate dose, and the average phosphate dose was 8 ± 9 mg/L-PO_4, and the average residual following the six hour hydraulic retention time was 6 ± 5 mg/L-PO_4. Sodium hydroxide was used to adjust the pH to the desired level. Both corrosion control treatments (zinc orthophosphate treatment and the pH 9.2 treatment) were compared in the absence and presence of chlorine (0.2 mg/L residual). The bulk water chemistry of the annular reactor effluent was measured twice weekly. The bulk water chemistry from the effluent of each reactor, following the six hour hydraulic retention time, is shown in Table 6.1.

Table 6.1 Average values with standard deviation for water quality of bulk water for each treatment

		pH		Temperature (˚C)		Turbidity (NTU)		Chlorine residual (mg/L)	
treatment	material	chlorine	no chlorine	chlorine	no chlorine	chlorine	no chlorine	chlorine	no chlorine
Zinc orthophosphate	copper	7.2 ± 0.2	7.1 ± 0.1	16.7 ± 3.9	15.1 ± 1.6	26.6 ± 30.2	57.4 ± 115.3	0.56 ± 0.65	<0.01
	copper with lead-tin solder	7.1 ± 0.2	7.1 ± 0.1	16.6 ± 3.8	15.2 ± 1.9	33.8 ± 35.7	<0.01	1.25 ± 1.58	<0.01
pH 9.2	copper	7.9 ± 0.4	7.5± 0.2	17.0 ± 3.9	15.1 ± 1.7	14.2 ± 31.3	1.7 ± 1.1	0.3 ± 0.43	<0.01
	copper with lead-tin solder	7.9 ± 0.3	7.6 ± 0.2	16.9 ± 3.7	14.7 ± 1.6	4.5 ± 10.0	1.7 ± 1.1	0.65 ± 1.67	<0.01

6.3.4 Sample collection and water quality analysis

Sterile conical flasks were used to collect annular reactor effluent. Analysis for pH and temperature were conducted using a dual channel pH/ionmeter (Accumet Excel XL25, Fisher Scientific, Waltham, MA). Turbidity was analyzed using a turbidimeter (2100N Turbidimeter, HACH, Loveland, CO). Free chlorine was analyzed using the DPD method using a spectrophotometer. Phosphate was measured using ion chromatography (761 Compact IC, Metrohm, Herisau, Switzerland) using USEPA method 300.1 (USEPA, 1997). The method detection limit for phosphate was 0.7 mg/L.

6.3.5 Biofilm collection

The coupons were acclimated for a minimum 30 days to ensure a stable biofilm population. A 30 day acclimation period for biofilm growth has been typically observed in biofilm studies at Dalhousie (Gagnon et al., 2004; Gagnon et al, 2005; Rand et al., 2007). Biofilm was gently scrapped from the coupons into sterile 50 mL conical tubes using a sterilized rubber policemen and rinsing with 10 mL of sterilized phosphate buffered solution (PBS). After rinsing the coupon was sprayed with 70% ethanol, allowed to air dry and returned to its original slot in the annular reactor.

6.3.6 Microbiological enumeration

The culturable portion of the bulk water and biofilm were enumerated using the spread plate method for heterotrophic plate count described in Standard Methods 9215C (APHA, 2005). Samples were serially diluted in PBS and plated in duplicate on R2A agar (Becton Dickenson and Co., MD). The plates were incubated in the dark for seven days at room temperatures. The colony forming units (cfu) were enumerated and reported as $\log_{10}$cfu/mL for the bulk water samples and $\log_{10}$cfu/cm^2 for the biofilm samples.

6.3.7 Lead and copper analysis

The total and dissolved fractions of lead and copper were measured using inductively coupled plasma mass spectrometry (ICP-ms) (X-Series 2 ICP-ms, Thermo Fisher Scientific, MA). The limit of detection was 0.7 µg/L for copper and 0.4 µg/L for lead. Bulk water total lead and copper samples were collected and preserved with nitric acid ($pH<2$) and stored in the refrigerator prior to analysis. Immediately after collection, a portion of the bulk water sample was passed through a 0.45µm filter to obtain the dissolved portion of the metal. Following filtering, the dissolved sample was preserved with nitric acid ($pH<2$) and stored in the fridge prior to analysis. The biofilm samples were prepared for analysis using Standard Methods 3030E (APHA, 2005), which recommends that samples with a turbidity over 1-NTU be analyzed using nitric acid digestion (105˚C for 2h in 5% nitric acid).

6.3.8 Scanning electron microscopy and energy dispersive spectrometry (EDS)

A scanning electron microscope (Hitachi S-4700, Hitachi Ltd, Tokyo, Japan) with an energy dispersive X-ray analysis system with an electron backscatter diffraction system(Oxford Inca EDS, Oxford Instruments plc, Abingdon, U.K.) was used to provide an atomic and weight percent composition of the corrosion product on the metal surface.

6.3.9 Statistical analysis

Means were compared using ANOVA and Tukey's method with a 95% level of significance. All other statistical analysis were calculated using a 95% level of significance. Data was organized using a Microsoft Excel (Microsoft, Redmond, WA) and statistical analysis was performed using Minitab 16 ® statistical analysis software (Minitab Inc., State College, PA).

6.4 RESULTS

The total and dissolved copper in the bulk water is shown for each of the treatments in Figure 6.2. Figure 6.2 shows that in the absence of chlorine the zinc orthophosphate treatment released less total copper. In the presence of chlorine, the pH 9.2 treatment released less total copper.

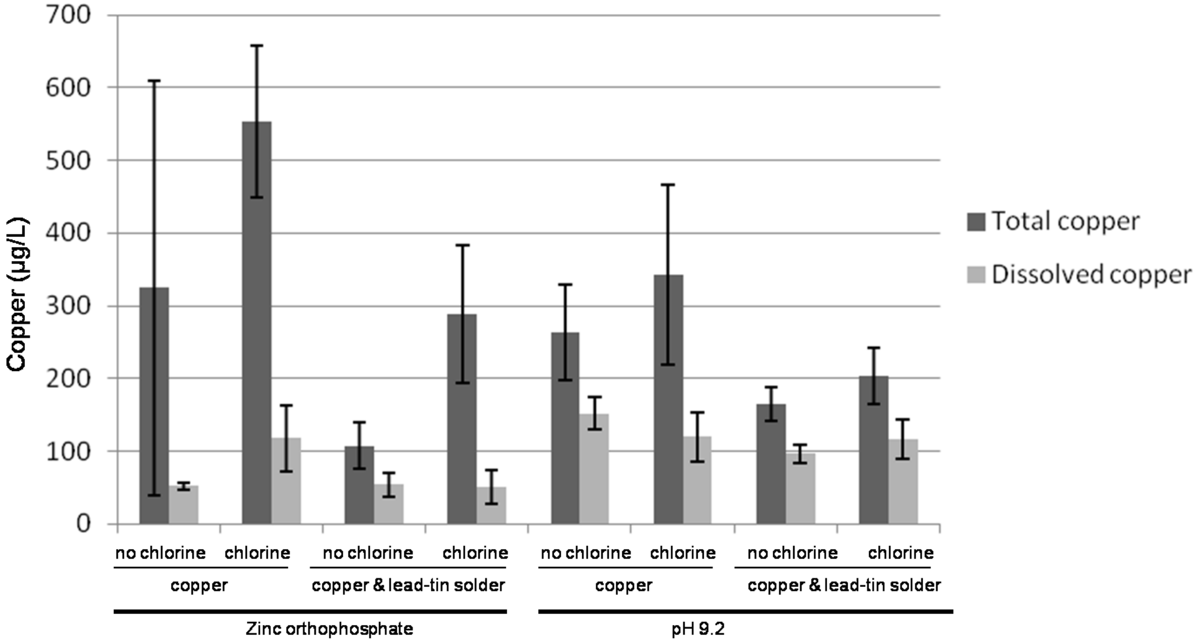

Figure 6.2 Total and dissolved copper for each of the treatments. The error bars represent the 95% confidence interval.

A Tukey's analysis revealed that in the absence of chlorine, the observed differences were not significant. However, in the presence of chlorine, the zinc orthophosphate treatment on copper released significantly more copper than all other treatments. It should be noted that since the copper coupons with lead-tin solder had less surface area (due to the presence of solder) and would experience cathodic protection, less copper release would be expected. The percentage of dissolved copper was generally higher in the pH 9.2 treatments compared to the zinc orthophosphate treatments.

The total and dissolved lead in the bulk water is shown for each treatment in Figure 6.3. Figure 6.3 shows that the same trend appears as with copper. In the absence of chlorine zinc orthophosphate released less lead, and in the presence of chlorine pH 9.2 released less lead.

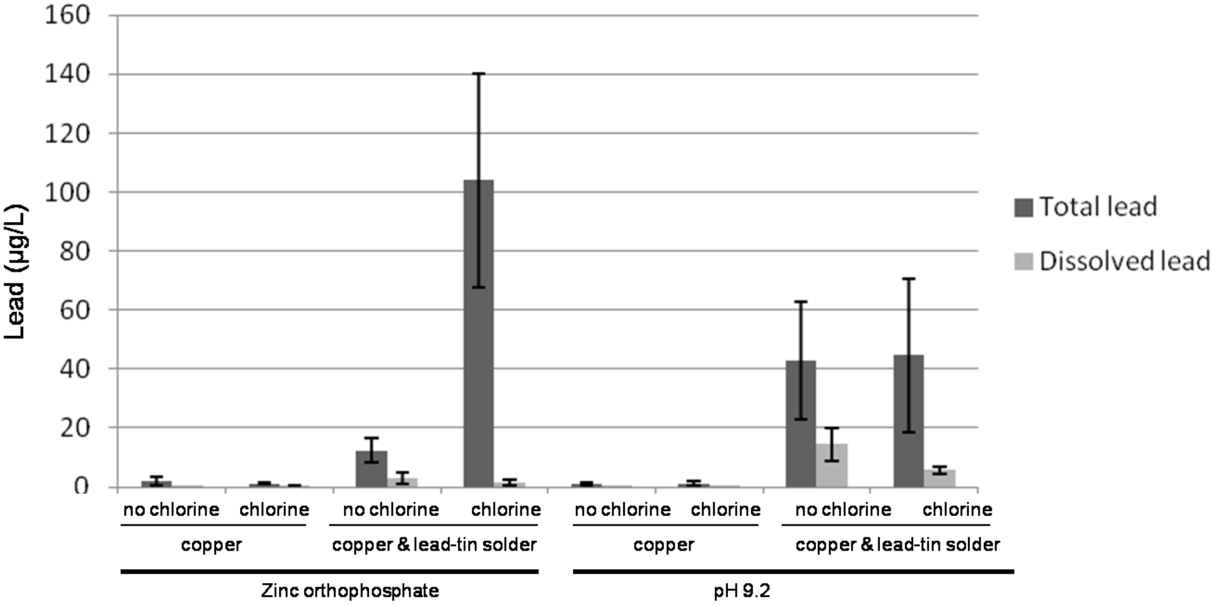

Figure 6.3 Total and dissolved lead for each of the treatments. The error bars represent the 95% confidence interval

These observations were corroborated with a Tukey's analysis that showed that in the absence of chlorine, the pH 9.2 treatment on copper with lead-tin solder released the most lead. In the presence of chlorine, the zinc orthophosphate treatment on copper with lead-tin solder released the most lead.

The percentage of dissolved lead was higher in the pH 9.2 treatments compared to the zinc orthophosphate treatments. There was significantly more dissolved lead in the bulk water of the pH 9.2 copper with lead-tin solder and no chlorine treatment than any of the other treatments. The particulate form of lead was estimated by subtracting the dissolved fraction from the total fraction of the metal as in Woszczynski et al. (2013). In the zinc orthophosphate copper with lead-tin solder treatment, the percent of lead in the bulk water that was in the particulate form was 77% without chlorine and 98% with chlorine. In the pH 9.2 copper with lead-tin solder treatment, the percent of lead in the particulate form was 66% without chlorine and 87% with chlorine.

The average concentration of heterotrophic plate count bacteria in the bulk water is given in Figure 6.4.

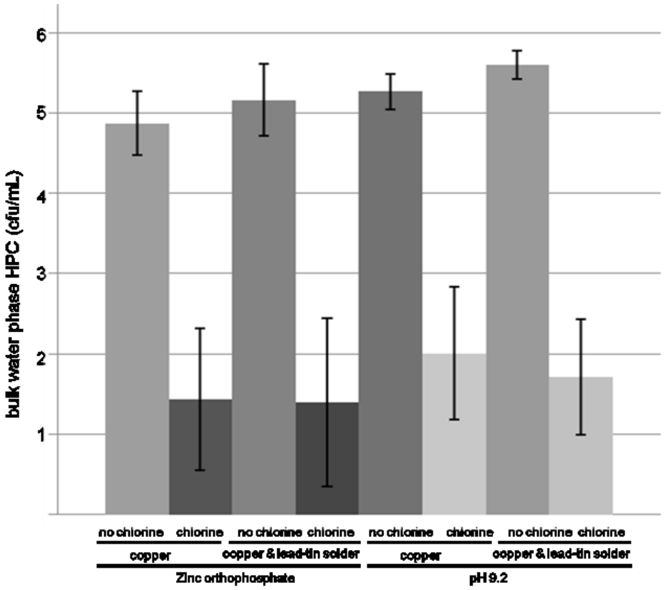

Figure 6.4 Bulk water phase heterotrophic plate count bacteria for each of the treatments. The error bars represent the 95% confidence intervals. For all treatments n=9, except for the pH 9.2 copper with chlorine treatment where n=8 and the pH 9.2 copper with lead-tin solder with chlorine treatment where n=10.

Generally, in the presence of chlorine, the average concentration was significantly lower (3.5-3.7 log) compared to treatments without chlorine. A visual inspection of Figure 6.4 shows that in the absence of chlorine, the treatments at pH 9.2 had higher average concentrations, although these observed differences were not significant. Similarly, in the presence of chlorine, the pH 9.2 treatments had higher average concentrations of heterotrophic plate count bacteria, although these observed differences were not significant.

The average number of biofilm bacteria (measured as heterotrophic plate count bacteria on the surface area of the coupon) is shown in Figure 6.5.

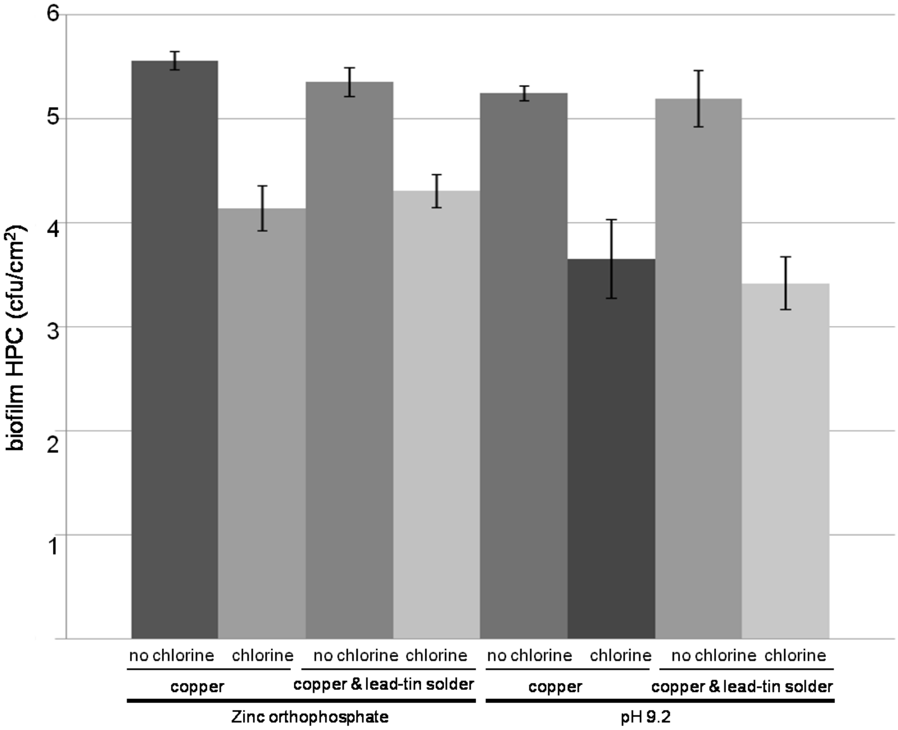

Figure 6.5 Biofilm heterotrophic plate count bacteria, measured on the surface area of the coupons, for each of the treatments. The error bars represent the 95% confidence intervals. For all treatments n=8, except the zinc orthophosphate with chlorine treatment where n=7 and the pH 9.2 copper with lead-tin solder with no chlorine treatment where n=6.

Generally, in the presence of chlorine, the average concentration was significantly lower (1.3-1.8 log) compared to the treatments without chlorine. A visual inspection of Figures 6.4 and 6.5 reveals the opposite trend observed in the bulk water compared to the biofilm. The higher heterotrophic bacteria counts are associated with the zinc orthophosphate treatment. Examining the biofilm in the absence of chlorine, the only significant differences were that the zinc orthophosphate copper coupon had a higher density of biofilm bacteria than the pH 9.2 copper coupon with lead-tin solder. In the presence of chlorine, the zinc orthophosphate copper with lead-tin solder had a significantly higher biofilm bacteria density than either of the treatments at pH 9.2.

The average total lead density on the coupon surface is given in Figure 6.6. In a visual inspection of Figure 6.6, it appears that less lead is stored in the biofilm in the absence of chlorine, although statistical analysis reveals that this observation is not significant.

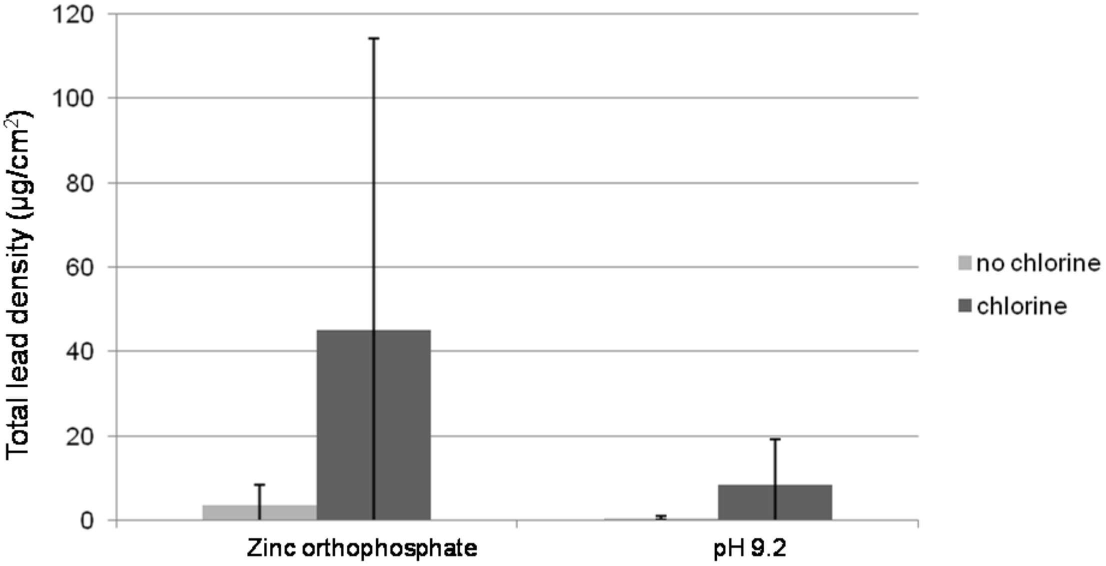

Figure 6.6 Total lead density in biofilm, measured on the surface area of the coupon, for the annular reactors with lead-tin solder. The error bars represent the 95% confidence intervals

Dissolved lead analysis was only performed for the no chlorine treatments. The dissolved fraction of the metal comprised less than 1% for the zinc orthophosphate copper with lead-tin solder treatment and the pH 9.2 copper with lead-tin solder treatment. An analysis of the biofilm-corrosion product on the surface of the coupon was analyzed by EDS (Table 6.2). In the treatments with zinc orthophosphate, both zinc and phosphate were detected on the surface of those coupons, indicating the presence of a phosphate containing corrosion scale.

Table 6.2 Average atomic composition (%) of surface elements, measured using EDS, from the coupons of all treatments. For the copper coupons with lead-tin solder analysis was taken from the border area between solder and copper surface. Trace elements were not included in this table, such as Mn, S and Na , since they did not comprise more than 1% composition for any of the experimental conditions.

			% atomic composition													
treatment	material	disinfection	C	O	Al	Si	P	Cl	Ca	Cr	Fe	Cu	Zn	Pb	Sn	Rh
Zinc orthophosphate	copper	no chlorine	7.2	34.1	5.0	0.5	4.9	0.4	0.4		2.9	32.2	12.3			
		chlorine	4.9	32.2	4.7	1.1	7.7	0.6	1.1	1.7	12.7	25.7	8.1			
	copper with lead-tin solder	no chlorine	24.3	28.9	1.4	0.3	2.7	2.1		0.9	6.1	3.4	3.3	10.5	26.4	4.9
		chlorine	4.4	22.5	2.5		1.0	0.3			2.8	24.3	2.9	7.5	32.5	
pH 9.2	copper	no chlorine	11.1	31.3	3.7	1.5	0.3	0.6			0.6	50.6				
		chlorine	35.6	25.1	2.9	1.2		0.6	0.4		2.1	34.9				
	copper with lead-tin solder	no chlorine		31.1	1.2	0.7					1.4	9.5		15.6	41.2	
		chlorine	8.4	27.5	1.4			1.2	0.3	1.1	23.9	20.4		7.5	14.6	

6.5 DISCUSSION

6.5.1 The majority of copper and lead is in the particulate form

In this study, the majority of lead detected in the bulk water and in the biofilm was in the particulate form. The importance of lead particles has been noted by several recent studies (Camara et al., 2013; Cartier et al., 2012; Wang et al., 2012; Woszczynski et al., 2013). In the study conducted by Woszczynski et al. (2013), an alternating flow-through and stagnant copper pipe system with lead-tin solder was treated with zinc orthophosphate and chlorine. The majority of lead samples were found to be in the particulate form (Woszczynski et al., 2013). A recent survey of Halifax homes served by cast iron mains with full or partial lead service lines revealed that a relationship between iron particles and lead particles (Camara et al., 2013). Experimental work by Camara (2012) used finished water from the same water treatment plant as in this study. The finished water, with a pH of 7.4, treated with 0.5 mg/L PO_4 and 1.0 mg/L chlorine was used to conduct adsorption experiments, which showed that particulate lead sorbed to particulate iron.

In this study, more dissolved lead was observed in the bulk water at the pH 9.2 treatment compared to the zinc orthophosphate treatment. Cartier et al. (2012) observed that the particulate fraction of lead released from brass fixtures increased in the presence of orthophosphate. Cartier et al. (2012) attributed this increase in particulate lead to the mechanism of orthophosphate. The dissolved lead would react with orthophosphate to form the scale but would not react with the particulate lead. Edwards and Dudi (2004) also observed that the addition of phosphate decreased soluble lead at pH 7.5, 8.25, and 9.

Additionally, in this study, the proportion of lead in the dissolved form was greater in the treatments without chlorine. Chlorine, a powerful oxidant, was observed to decrease soluble lead at pH 7.5, 8.25 and 9.0 (Edwards & Dudi, 2004). Edwards and Dudi (2004) also observed that the insoluble solids took longer to form at higher pH levels, and suggested that this was because chlorine is a less powerful oxidant at $pH>7.6$. The results suggest that in this system, chlorine oxidation created more stable corrosion scales. In combination with chlorine, zinc orthophosphate treatment provided even greater stability than increased pH.

6.5.2 Biofilm analysis

In the absence of chlorine, there were in the order of 10^5 cfu/mL heterotrophic plate count bacteria in the bulk water for all treatments. Chlorine suppresses microbial growth and provides disinfection, and decreased heterotrophic plate counts were observed in both the bulk water and biofilm heterotrophic plate counts. In the bulk water, although not significantly different, the higher heterotrophic plate counts observed in the pH 9.2 treatments (Figure 6.4) would be expected as chlorine is a more powerful oxidant at pH <

7.6 (Fukazaki, 2006). In the biofilm, the opposite trend was observed (Figure 6.5); the biofilm density (as measured by heterotrophic plate counts) was significantly higher in the zinc orthophosphate (copper with lead-tin solder) treatments than the biofilm density in either of the pH 9.2 treatments.

Two plausible explanations could help reconcile these apparently opposite trends: 1. The presence of phosphate offers a metabolic advantage to the biofilm bacteria or 2. The increased surface area created by lead particles offers more sites for biofilm formation.

Phosphate is a key nutrient and has been shown in the literature to increase biofilm density in drinking water biofilms (Chu et al., 2005; Fang et al., 2009). The addition of phosphate may have provided additional nutrients to the biofilm bacteria mitigating the effectiveness of chlorine on the biofilm. Phosphate has also been shown to increase diversity in drinking water biofilms (Jang et al., 2012), and multi-species biofilms have demonstrated increased disinfection resistance compared to single-species biofilms (Simões et al., 2010).

In this study, surface area of the coupons (copper with lead-tin solder) would have increased with increased presence of lead particles adhered to the biofilm-corrosion product complex on the surface. The highest levels of measured lead particles were observed in the zinc orthophosphate treatment in the presence of chlorine (Figure 5). These particles (visualized in Camara, 2012) could potentially provide surfaces for biofilm adhesion.

Figure 6.7 was created to illustrate how lead particles could encourage microbial growth and depicts the four treatments used in this study: in part A, zinc orthophosphate (no chlorine); in part B, zinc orthophosphate (with chlorine); in part C, pH 9.2 (no chlorine); in part D, pH 9.2 (with chlorine).

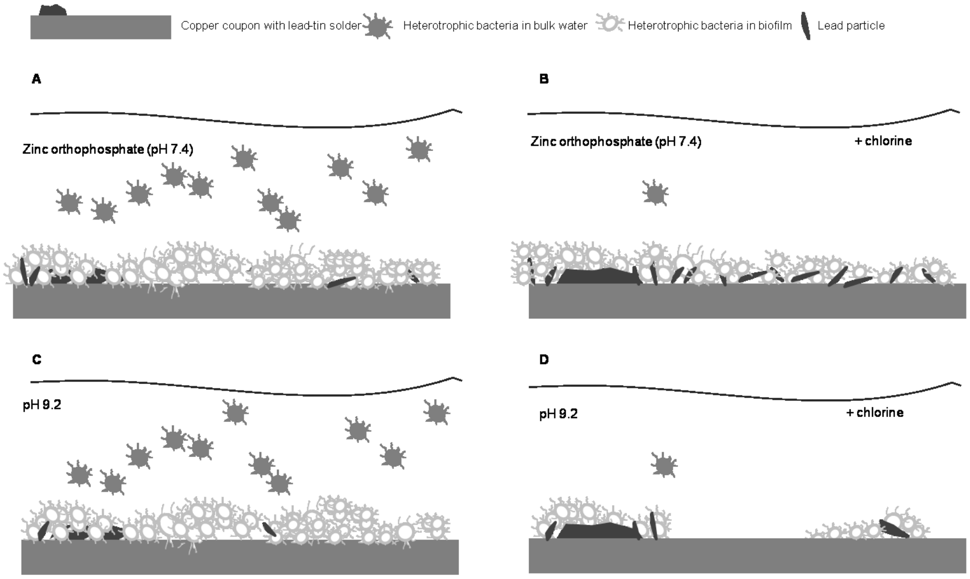

Figure 6.7 A conceptual drawing of the role of lead particles on biofilm formation in the four different treatments conditions on copper coupons with lead tin solder: zinc orthophosphate with no chlorine treatment (A); zinc orthophosphate with chlorine treatment (B); pH 9.2 with no chlorine (C); and pH 9.2 with chlorine (D).

In the zinc orthophosphate treatment, lead is primarily present in its particulate form as been observed elsewhere (Camara et al., 2013; Cartier et al., 2012; Woszczynski et al., 2013). The presence of chlorine further decreases soluble lead (Edwards & Dudi, 2004). This results in zinc orthophosphate with chlorine treatment (Figure 6.7 B) having the most lead particles followed by the zinc orthophosphate (no chlorine) treatment (Figure 6.7A), pH 9.2 with chlorine (Figure 6.7 D) and the pH 9.2 without chlorine (Figure 6.7 C) with the least. In the presence of chlorine, microbial growth is inhibited in the bulk water and fewer initial bacterial cells are available to colonize the surface. The lead particles could provide sheltered areas and increased surface area (i.e. opportunity to attach) for the free floating bacteria in the bulk water. Others have observed that cast iron provided the highest density of heterotrophic plate count bacteria compared to plastic surfaces (Camper et al., 2003; Ollos et al., 2005). It should be noted that Camper et al. (2003) implied and Ollos et al. (2005) and Murphy et al. (2008) showed that factors such as disinfectants could play a dominant role in determining biofilm population density over pipe material. In this study, it appears that the pipe surface played an important role in influencing biofilm density.

In the absence of chlorine (Figure 6.7A and Figure 6.7C), microbial growth is uninhibited. The difference in available surface area may not play a significant role, as once the biofilm has been initiated on the surface there is a constant supply of new bacteria and no inhibition of growth on the surface other than the natural nutrient and growth limitations of the biofilm itself. Essentially, the biofilm is capable of unfettered growth.

A third explanation should also be considered. The addition of zinc orthophosphate may have also decreased the toxic effects of lead and copper (by binding the dissolved portion), creating a more conducive growth environment.

The three proposed explanations (phosphate enhanced growth, increased surface area from lead particles and decreased toxicity through lead and copper binding with phosphate) are not mutually exclusive. Further studies that included particle size analysis would help elucidate which of these three mechanisms played the dominant role.

The two proposed explanations (phosphate enhanced growth and increased surface area from lead particles) are not mutually exclusive. The presence of phosphate corrosion inhibitors may further enhance the growth potential of biofilms. Further experiments would be required to determine which of these two mechanisms plays the dominant role.

6.6 CONCLUSIONS

Lead particles appeared to play a dominant and important role in the four treatments used in this study. As expected, more released lead particles were observed in the zinc orthophosphate systems than the pH 9.2 system with the presence of chlorine increasing the particulate fraction in both. In the presence of chlorine, the zinc orthophosphate system also supported more biofilm which could be a result of increased surface area due to the increased presence of lead particles and/or a result of increased nutrients from the phosphate.

Other work has addressed the complex interaction and interplay of orthophosphates, chlorine and pH on lead corrosion. This new contribution attempts to describe the complex relationship between lead corrosion and biofilm accumulation. The results from this work allude to the potentially significant role that lead particles may play in biofilm formation and warrants further investigation.

6.7 ACKNOWLEDGEMENTS

The authors thank the NSERC/Halifax Water Industrial Research Chair program for providing financial support and gratefully acknowledge the participation of the JD Kline plant staff. The authors thank John Bergese, Jessica Campbell, Heather Daurie, and Brian Kennedy at Dalhousie University for their assistance with set-up, operation and analysis. The authors acknowledge Pat Scallion and Dalhousie's Institute for Research in Materials for support with the EDS analysis. We also acknowledge the support of the Canada Foundation for Innovation, the Atlantic Innovation Fund, and other partners which fund the Facilities for Materials Characterization, managed by the Institute for Research in Materials.

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CHAPTER 7 CONCLUSION

This thesis integrated traditional corrosion chemistry methods with microbiological and molecular biology techniques to investigate the relationship between biofilm and corrosion control in premise plumbing. The central hypothesis of this thesis was that biofilm contributes to lead and copper release in premise plumbing. This hypothesis was tested using three distinct experimental approaches: 1. electrochemical cell experiments to determine the key factors in decreasing lead and copper corrosion in galvanically coupled systems 2. galvanic macrocells made of premise plumbing components to examine the unintended consequences of adding a phosphate corrosion inhibitor to Halifax Water and 3. annular reactors to compare two different corrosion control schemes for the Halifax distribution system. The key findings from these three experimental approaches are summarized in Table 7.1.

Table 7.1 Key Findings

Chapter	Experimental approach	Key Findings
Chapter 3: Balancing corrosion control and microbial control	Electrochemical cells	•pH 9.2 provided the greatest protection for the lead anode, and zinc orthophosphate appeared to offer some secondary protection. •Copper was protected by cathodic protection and by the zinc orthophosphate scale. •pH 9.2 with zinc orthophosphate and chlorine provided both a managed microbial growth and managed lead and copper corrosion. •Chlorine plays a critical role in managing microbial growth and can be a part of an effective corrosion control strategy. •Conceptual tool was developed to help utilities evaluate pilot-scale distribution system treatment studies to select a treatment strategy that will simultaneously manage both microbial growth and corrosion.
Chapter 4: Contribution of biofilm to lead and copper storage under drinking water conditions	Galvanic macrocells	•The majority of available lead generated by galvanic currents was not detectable in the bulk water phase or biofilm and was assumed to be a part of the corrosion scale. •Zinc orthophosphate increased the biofilm population on the polycarbonate surfaces. •In the lead macrocells, lead particles constituted the majority of the lead in the biofilm •Biofilms removed 3-29% of lead and 3-16% of copper from the bulk water phase •Metals from upstream plumbing materials may accumulate in biofilms in benign downstream fixtures.
Chapter 5: Impact of zinc orthophosphate on lead and copper influenced drinking water biofilms	Galvanic macrocells	•Phosphate dose influenced biofilm diversity more than metal type •Heterotrophic plate count biofilm density was a predictor for species richness/diversity •The bacteria identified were generic freshwater bacteria. •Addition of zinc orthophosphate increased the amount of biofilm
Chapter 6: Uptake of particulate lead in drinking water biofilms	Annular reactors	•More released lead particles were observed in the zinc orthophosphate systems than the pH 9.2 system with the presence of chlorine increasing the particulate fraction in both •In the presence of chlorine, the zinc orthophosphate system also supported more biofilm which could be a result of increased surface area due to the increased presence of lead particles and/or a result of increased nutrients from the phosphate •Lead particles may play a significant role in biofilm formation

7.1 Testing the hypothesis

The hypothesis that biofilm contributes to lead and copper release in premise plumbing was demonstrated in two ways: 1. biofilm acted as a storage site for released lead and copper and 2. lead particles provided surface area to support increased biofilm growth.

The majority of lead released by galvanic currents was not accounted for in the bulk water, as seen in Chapters 3 and 4. The released lead likely precipitated as a lead scale onto the pipe surface (observed in Chapter 3 and 6) or was sorbed into the biofilm (observed in Chapters 4 and 6). Evidence suggests that only 0.4-2.6% (Chapter 3) and 0.1-2.4% (Chapter 4) of lead released by galvanic currents was found in the bulk water. The biofilm analysis in Chapter 4 further suggested that biofilm alone was able to sorb 3-29% of lead and 3-16% of copper from the bulk water. Additionally, downstream inert plumbing fixtures may act as accumulation sites for metals from upstream processes. Therefore, if there is a source of lead or copper upstream, there is a risk of accumulation in downstream plumbing biofilms.

In Chapter 3, a conceptual tool was developed to identify treatments that could effectively manage both corrosion and microbial growth. The conceptual tool is shown again as Figure 7.1 where quadrant A represents managed microbial growth and managed corrosion; quadrant B represents managed microbial growth and unmanaged corrosion; quadrant C represents unmanaged microbial growth and unmanaged corrosion; and quadrant D represents unmanaged microbial growth and managed corrosion.

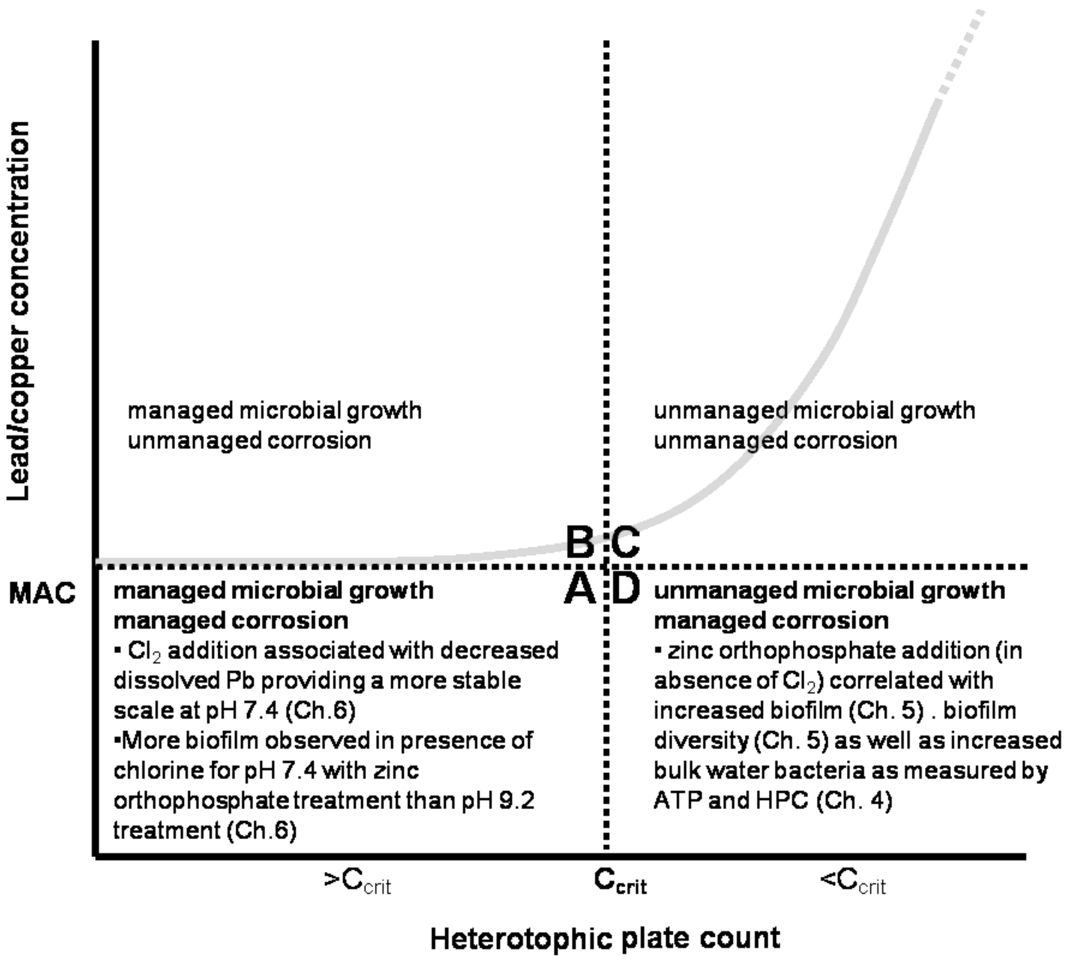

Figure 7.1 Achieving operational ideals in bulk water, with implications of biofilm explored in this thesis shown in quadrants A and D. The dashed lines representing the critical chlorine concentration (C_{crit}) and the maximum allowable concentration (MAC) for metals separate the four operational quadrants.

This tool could be used to evaluate various corrosion and microbial control schemes at the flow-through/pilot scale for a utility's specific water type. The effects of these selected conditions should also be evaluated in terms of their effect on biofilm.

Chapter 4 and 5 examined biofilm under stagnant treatment conditions that would be categorized in quadrant D, managed corrosion (zinc orthophosphate treatment) and unmanaged microbial growth (no chlorine). The addition of zinc orthophosphate not only positively correlates to increased microbial growth in the bulk water as measured by ATP and HPC (Chapter 4) but also increased biofilm as measured by HPC (Chapter 5). The increased biofilm (as measured by HPC) was associated with increased biofilm diversity (Chapter 5). Given that diversity in biofilms is an important factor in increased disinfectant resistance, the effects of zinc orthophosphate on biofilm diversity are an important long-term effect for utilities to consider.

Chapter 6 explored biofilm under flow-through conditions in both quadrant A and quadrant D. In quadrant A, managed corrosion (zinc orthophosphate treatment or pH treatment) and managed microbial growth (chlorine addition), more lead particles were observed for both treatments compared to quadrant D. Within quadrant A, more lead particles were observed in the zinc orthophosphate system compared to the pH 9.2 system. Additionally in quadrant A, in the zinc orthophosphate treated system, more biofilm was observed compared to the pH 9.2 treated system. The role of particles in biofilm formation developed in Chapter 6 is shown again here as Figure 7.2.
This increased biofilm was proposed to be a result of (i) increased phosphate nutrient and/or (ii) increased surface area for biofilm formation due to the increased presence of lead particles.

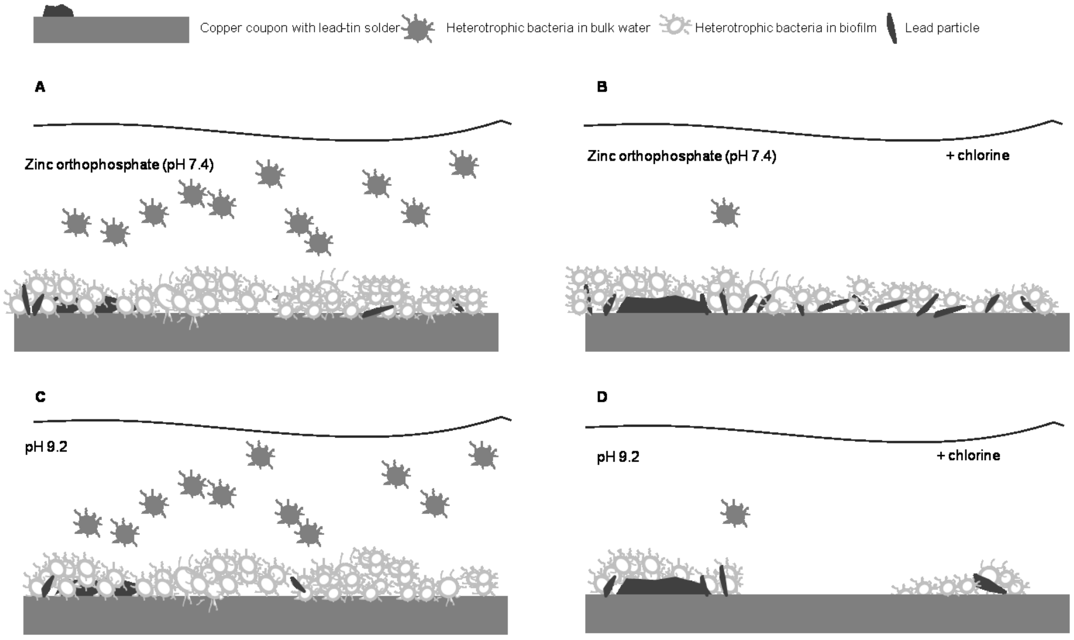

Figure 7.2 Lead particles may provide surface area for biofilm growth

The results from Chapter 3 and 4 suggest that biofilm provides a site for lead and copper storage, and the results from Chapter 6 suggest that particulate lead, may provide surface area for biofilm growth. Given the role that biofilms play in lead and copper storage and the potential for corrosion control additives to affect biofilm diversity, utilities considering a corrosion control and microbial control scheme must also consider the effects on biofilm.

7.2 Recommendations

7.2.1 Understanding the role particles play in creating surface area for biofilm growth

Lead particles have been associated with neutral pH water with phosphate corrosion inhibitors in this thesis and elsewhere in the literature. This study also suggested that the presence of lead particles may also increase the available surface area for biofilm formation. However, these experiments were not specifically designed to observe this phenomena. The potential for lead particles to be released with biofilm (and provide a significant source of lead ingestion in drinking water) and this newly identified role as substrate for biofilm growth warrants further study. Bench-scale experiments could be designed specifically to associate the surface area created by lead particles and biofilm formation potential at various levels of pH, phosphate and chlorine.

7.2.2 Improve the understanding of the relative stability of lead sorbed to biofilm vs. lead in a corrosion scale

In this study, lead and copper were shown to accumulate in biofilm. Since changes in water quality and flow patterns can affect corrosion scales and biofilm stability, it would be important to understand the water chemistry and operational conditions (pressure) that would ensure the greatest stability to both these storage sites for metals.

7.2.3 Understanding the potential of phosphate addition to prevent uptake of lead and copper by biofilm

If as a result of Recommendation 7.2.2, corrosion scales are shown to be more stable than biofilms as a site for lead storage, optimization of phosphate dose should consider the pH at which stable mineral scales are formed and biofilm sorption of lead is minimized. Lead can be bound to a premise plumbing surface by sorption to the biofilm or by precipitation and deposition as a corrosion scale. Phosphate based corrosion inhibitors may have additional value as phosphate would compete with the biofilm for lead binding. Experiments examining this competition between phosphate and biofilm for lead binding should be executed at several pH levels, including low pH levels (pH<4) as pH in the microenvironment at the anodic surface may be significantly different than the pH in the bulk water.

7.2.4 Understanding the effect of phosphate dose on biofilm community diversity at the pilot/full scale

Further studies should be undertaken to observe how phosphate dosage affects biofilm community structure at the full scale under real drinking water operating conditions. Experiments should be designed to specifically elucidate the effect of increased diversity as a result of phosphate corrosion inhibitors on the disinfectant resistance of biofilms in the Utility's specific water type. Since diversity has implications for biofilm disinfection resistance, diversity will be an important factor to consider when implementing a corrosion control and microbial control strategy.

7.2.5 Validation of conceptual tool by Halifax Water

The conceptual tool, developed in Chapter 3, could be validated by Halifax Water. The model could be validated by populating it with real water quality data from routine in-home sampling. The conceptual tool offered in Chapter 3 could be used by utilities to evaluate their specific water type at the pilot-scale. Experiments should include the ability to examine biofilm growth and metal storage potential. Utilities could use the conceptual tool to identify a strategy that simultaneously achieves both treatment goals: managed microbial growth and managed corrosion.

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