

**Aerosolization of Drinking Water Metals to Indoor Air and Assessment of Human
Taste and Visual Thresholds for Manganese**

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ABSTRACT

Exposure to excess manganese via drinking water raises concerns due to potential for adverse neurological impacts, particularly in children. Manganese is ubiquitous in US groundwaters above the SMCL = 0.05 mg/L. Manganese is an essential nutrient, but exposures to elevated manganese have neurotoxic effects. Chapter 2 focuses on human senses' ability to detect manganese in drinking water. Findings indicate human senses cannot be relied upon to detect excess Mn(II) in drinking water. Mn(IV) is easily visually detected, but cannot be tasted at 10 times the SMCL. Chapter 3 is an assessment the ability of an ultrasonic humidifier to expel drinking water impurities in aerosols. The quality of the water used to charge the humidifier reservoir affects the composition of elements in the aerosols and condensate. Findings indicate condensed humidifier aerosols contain 85% of elements present in the reservoir water for a variety of water types if there is no precipitation. Waters with high concentration of hardness or iron formed precipitates that decreased the concentrations of these metals in the aerosols causing variable results for other elements that were initially present at < 1mg/L in the charge water. This indicates that humidifiers could be a source of inhalation exposure for source water contaminants.

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Chapter 1. Introduction

1.1 Manganese Chemistry

Manganese is a metallic element that is present in both soluble and insoluble form in the natural environment. Oxidized manganese is insoluble, but when reduced, manganese becomes soluble in the Mn(II) form. Manganese oxidation by oxygen is slow, but manganese can be more rapidly oxidized by microorganisms, or by chemical oxidants like ozone or chlorine dioxide (Johnson et al, 1991; Letterman, 1999).

1.2 Manganese in Drinking Water

Manganese is present in ground and surface waters in trace amounts. A survey of US ground waters performed by USGS found that 30.5% of wells tested had detectable levels of manganese with concentrations up to 28,000 µg/L, however the median manganese concentration reported was 7 µg/L (Ayotte et al, 2011). There is little seasonal or daily variation to manganese concentrations in ground waters and flushing at the tap does not significantly reduce manganese concentrations for consumers (Barbeau et al, 2011).

Manganese in surface waters does experience seasonal variation due to temperature driven stratification of the surface water, leading to precipitation of manganese into sediments under oxidizing conditions in winter months (Von Gunten et al, 1991). In summer months, manganese that was oxidized in the winter can then be reduced and aqueous once again.

1.3 Manganese Removal Techniques

Typical treatment for the removal of manganese from a drinking water involves oxidation of soluble Mn(II) to insoluble, oxidized Mn(IV). Once oxidized, manganese can be removed via the physical processes of sedimentation or filtration. Oxidation can be achieved by addition of a chemical oxidant like chlorine dioxide, chlorine, or ozone (Letterman, 1999). Point of use methods that are effective for manganese removal include reverse osmosis, ion exchange, and to a lesser extent, activated carbon. The effectiveness

of point of use treatment methods is dependent on proper maintenance by the consumer (Barbeau et al, 2011).

1.4 Human Health

Manganese serves as an important nutrient for human health. Trace amounts of manganese are necessary for mitochondrial function, bone and cartilage maintenance, and wound healing (Agency for Toxic Substances and Disease Registry (ATSDR), 2008). Humans acquire the majority of their manganese through food sources or supplements. Foods high in manganese include nuts, cooked grains, cereals, and beans (Pennington et al, 1995). The reference dose for manganese consumption is 10 mg/day for adults or 0.14 mg/kg/d (USEPA, 1996; Trumbo et al, 2001).

Manganese is also known to have significant negative health impacts when inhaled in excess. Inhalation of manganese is the prominent exposure route to induce toxic effects in humans. Excess manganese exposure is an occupational hazard for workers in manganese mines. Overexposure to manganese leads to a Parkinson's disease-like syndrome called manganism; symptoms include disturbed gait, tremors, irritability, and changes in behavior and personality (Agency for Toxic Substances and Disease Registry (ATSDR), 2008).

Manganese causes adverse neurological symptoms because it accumulates in brain tissues. Excess manganese interferes with neurotransmitter metabolism and causes oxidative stress in cerebellar tissue in rat brains, leading to motor disturbances (Erikson et al, 2004). Inhaled soluble manganese bypasses the normal metabolic removal process by entering the brain directly via the olfactory bulb in the nasal cavity (Brenneman et al, 2000). This more direct route of exposure leads to greater accumulation in brain tissues and higher probability of neurological damages (Gianutsos et al, 1997; Thompson et al, 2011). OSHA currently regulates occupational manganese exposure to a level of 5 mg/m³ (OSHA, 1998).

There is mounting evidence that elevated levels of manganese in drinking water have adverse neurological impacts on children. Woolf et al (2002) found that a child with high manganese levels in blood, hair, and urine performed far below average on memory testing. Wasserman et al (2011) found that children in Bangladesh exposed to high manganese performed poorly on memory and perceptual reasoning tests. Khan et al (2011) similarly showed that high manganese (400-500 µg/L) in drinking water correlated with externalization and internalization, markers of behavioral problems. Fell et al (1996) found that children receiving long-term parenteral nutrition were experiencing manganese toxicity resulting in brain lesions. Bouchard, M. F. et al (2011) found that for children with drinking water manganese concentrations ranging from 1-2700 µg/L, high manganese concentrations were correlated with lower IQ scores for both performance and verbal IQ. In a previous study, Bouchard, M. et al (2007) found that high manganese in drinking water was also correlated with increased hyperactivity, oppositional behavior, and ADHD. There is mounting evidence that manganese in drinking water may pose a hazard to human health.

1.5 Regulation in Drinking Water

The EPA regulates manganese as a secondary drinking water contaminant. Secondary contaminants are classified as nuisance chemicals and are not regulated due to health concerns. The World Health Organization has established a guideline value for manganese of 0.4 mg/L and remarks that manganese concentrations exceeding 0.5 mg/L would adversely affect drinking water aesthetics to the extent that individuals would not consume the water (WHO, 2006). This statement was based on a paper by Griffin (1960) reported to say that manganese causes off taste and coloration problems at 0.1 mg/L, but further examination of the paper yielded no such statement (WHO, 2003). In 2011, the WHO eliminated drinking water health based guideline for manganese with the reasoning that levels of manganese in drinking water do not pose a health concern (WHO, 2011). A Secondary Maximum Contaminant Level (SMCL) of 0.05 mg/L has been established for manganese primarily due to aesthetic concerns because manganese can cause black or brown coloration of water, and bitter metallic taste (USEPA, 2012).

Cohen, Jesse M. et al (1960a) provided a threshold value for manganese taste at 180 mg/L, which is much higher than the SMCL, however this study made use of trained panelists, and therefore may not be representative of the general population. Sly et al (1990) found that manganese deposition was occurring in the distribution system at 0.03 mg/L which is lower than the SMCL. Deposition in pipes can result in visible particulate manganese. Sly therefore recommended based on that finding that the SMCL be lowered to 0.01 mg/L. There are knowledge gaps regarding taste and visual thresholds for manganese detection which are addressed in Chapter 2 of this paper.

1.6 Inhalation Risk due to Showers and Humidifiers

Elsner and Spangler (2005) suggests that there may be a risk of high manganese exposure due to shower aerosols. Showers produce water aerosols in the inhalable particle range and therefore could provide a manganese inhalation exposure route (Yue et al, 2007).

Humidifiers also produce drinking water aerosols in the inhalable particle range so examination of humidifiers as a potential source of inhalation exposure would be critical to understanding how manganese can enter the body and have potential negative impacts on human health (Highsmith, 1992). Chapter 3 of this paper evaluates the ability of ultrasonic humidifiers to aerosolize metals, such as manganese, to indoor air. The research will provide data that can be used in evaluating if humidifiers serve as an exposure route for inhaled manganese.

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Chapter 2. Rethinking the Manganese Standard: An Evaluation of Taste and Visual Perception of Mn(II) and Mn(IV)

2.1 Abstract

The manganese secondary maximum contaminant level (SMCL) is 0.05 mg/L, reportedly due to bitter metallic taste and black-brown discoloration. This study re-visited visual and taste thresholds for Mn(II) and Mn(IV) using one-in-five and triangle tests for manganese concentrations from 0.05-506.3 mg/L. Findings indicate that Mn(II) is clear in aqueous solution at 0.05 mg/L and remains undetectable at concentrations as high as 506 mg/L. Mn(IV) is visually detectable in water at concentrations as low as 0.005 mg/L. Mn(II) and Mn(IV) do not taste significantly different than distilled water at 0.05 mg/L Mn. The population taste threshold for Mn(II) as the sulfate salt is >1000 times the SMCL; the threshold concentration is between 75-100 mg/L Mn(II) and is likely a combination of taste from both sulfate and Mn(II). Human perception, like water treatment strategies, is dependent on the redox state of manganese. Findings suggest consumers cannot detect potentially hazardous concentrations of reduced Mn(II) by taste or sight and that the bitter and metallic descriptors for 0.05 mg/L Mn are not warranted.

2.2 Introduction

The EPA currently regulates manganese in drinking water to a secondary maximum contaminant level (SMCL) of 0.05 mg/L or 50 µg/L. Secondary standards are not health based but set at a non-mandatory maximum based on primarily aesthetic considerations in order to increase consumer satisfaction and trust in drinking water (AWWA Taste and Odor Committee, 2002; Dietrich, 2006). The SMCL for manganese is reportedly based on bitter metallic taste and undesirable black or brown color, but there are limited data establishing a taste or visual threshold for manganese (USEPA, 2012). The EPA has also stated that ingesting water for 1-10 days with a manganese concentration equal to 1mg/L is not expected to cause adverse effects in children (Agency for Toxic Substances and Disease Registry (ATSDR), 2008). The World Health Organization sets no guideline for manganese because high levels in drinking water are not thought to cause health concerns. The WHO does note that a health based value of 0.4 mg/L

manganese has been established, but it is unnecessary to impose a guideline because typical drinking waters do not exceed this value (WHO, 2011).

Concentrations of manganese in ground water are variable and omnipresent. The USGS sets a health based screening level for drinking water manganese at 300 µg/L (Toccalino & Norman, 2006). The National Water Quality Assessment Program (NAWQA) tested 2,159 wells from 1992 - 2003 for manganese content which can be seen in Figure 2-1 (Ayotte et al, 2011).

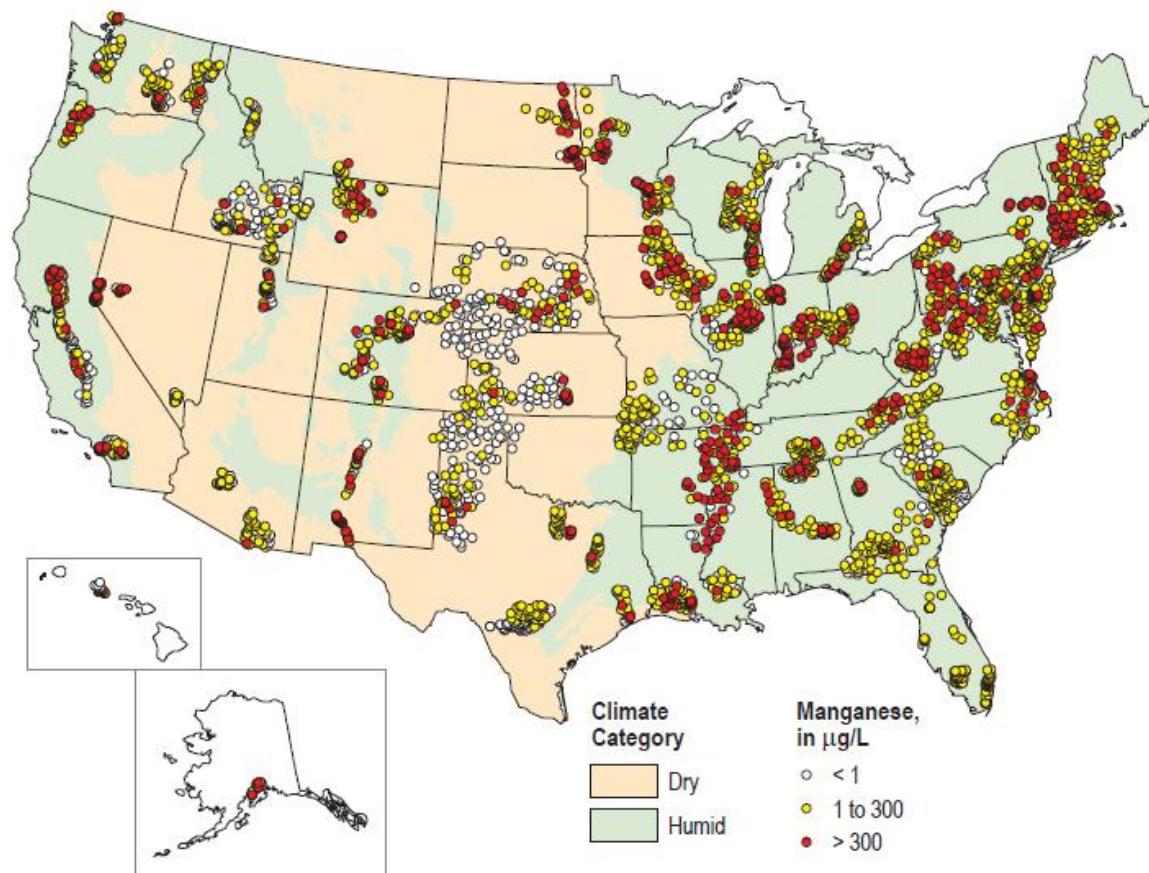


Figure 2-1: NAWQA Manganese groundwater concentrations (Ayotte et al, 2011)

It is clear that many wells exceed the 0.05 mg/L secondary standard, equal to 50 µg/L, and many wells across the United States that exceed the 300 µg/L health based screening level. The NAWQA trace metals assessment program has 4,976 samples with an overall median of 7 µg/L Mn. The 75th percentile exceeds the EPA secondary standard at 84 µg/L and the 90th percentile exceeds the health based screening level at 360 µg/L. The maximum recorded level was 28,000

$\mu\text{g/L}$, which is well above the SMCL and health based screening level. 12% of the samples exceeded the health based screening level and 31% exceeded the EPA secondary standard (Ayotte et al, 2011). The report also states that manganese concentrations were higher in sample with anoxic conditions than those with oxic conditions. This confirms that redox status has an impact on the concentration of manganese in a groundwater (Stumm & Morgan, 1996; Ayotte et al, 2011). Reduced Mn (II) has a high solubility in water whereas oxidized Mn (IV) is poorly soluble and occurs as MnO_2 particles (Stumm & Morgan, 1996). Manganese can cycle between soluble and particulate forms due to both changes in water chemistry and the activity of Mn oxidizing and reducing bacteria (Cerrato et al, 2010).

Manganese is an important nutrient for human health. Food and supplements are the primary exposure source for manganese in humans (Agency for Toxic Substances and Disease Registry (ATSDR), 2008). As a nutrient, manganese is necessary for mitochondria maintenance, healthy bones and cartilage, and wound healing. Manganese has the ability to cross the blood brain barrier to accumulate in the basal ganglia, globus pallidus, and striatum (Erikson et al, 2004; Agency for Toxic Substances and Disease Registry (ATSDR), 2008) when present in excess of normal levels (4-15 $\mu\text{g/L}$ in blood, 1-8 $\mu\text{g/L}$ in urine, or 0.4 – 0.85 $\mu\text{g/L}$ in serum) (Agency for Toxic Substances and Disease Registry (ATSDR), 2008). Acute and chronic exposures to excess manganese are typically due to occupation related inhalation of manganese dust. Inhaled manganese can be transported directly to the brain via the olfactory bulb (Thompson et al, 2011). Toxicity due to excess manganese exposure results in a condition called manganism with symptoms analogous to Parkinson's disease including tremor, disturbed gait, and personality changes (Agency for Toxic Substances and Disease Registry (ATSDR), 2008).

Several recent studies have assessed the impact that high manganese in drinking water can have on human health, particularly in children. In a pilot study (Bouchard, M. et al, 2007) provided evidence that there is a significant association between high levels of manganese in drinking water and elevated levels of manganese in hair (Hair-Mn) and between Hair-Mn and hyperactivity in children. The mean manganese drinking water concentrations in the study were 610 $\mu\text{g/L}$ for the high levels and 160 $\mu\text{g/L}$ for the low levels, with the children consuming the higher manganese concentrations having higher Hair-Mn which indicates that drinking water

poses a significant exposure source for manganese (Bouchard, M. et al, 2007). A later study by Bouchard, M. F. et al (2011) showed that exposure to manganese levels common in ground waters (median, 34 µg/L; range, 1-2700 µg/L) is associated with intellectual impairment in children, and that greater manganese concentrations are associated with greater impairment. They also noted that manganese intake from water and not from dietary sources was associated with high Hair-Mn content. Riojas-Rodríguez et al (2010) provided a study that showed that environmental inhalation exposure to manganese from a local mine resulted in higher Hair-Mn content inversely related to intellectual function in school aged children. In Bangladesh, high manganese concentration in drinking water has been positively and significantly associated with internalizing and externalizing behavior in children (Khan et al, 2011). In China, high manganese in drinking water has been associated with lower levels of neurotransmitters which had a negative impact on their school performance in mathematics and Chinese language (Zhang et al, 1995). These studies and others provide ample evidence that manganese in drinking water is a health concern and regulations on manganese concentration in drinking water need to be re-evaluated.

There are limited data available regarding the taste threshold for manganese. A search of the literature was performed and a single taste threshold study could be located. Cohen, J.M. et al (1960b) evaluated population taste threshold using manganous sulfate in spring water and distilled water. This study used 15-20 trained panelists and the duo-trio taste test with solutions of manganese sulfate in spring and distilled water. It is unclear which specific metal concentrations were tasted by the panel, but taste threshold frequencies were calculated for concentrations ranging from 0.05-128 mg/L Mn. The duo-trio test has a high degree of error due to a 50% chance of guessing a correct response. Multiple taste tests were performed in a single taste testing session. A cumulative plot of threshold values for individual subjects were graphed as a function of concentration. Extrapolation of the actual data allowed authors to predict concentrations at which select percentages of the human population could detect the taste of Mn. The data indicated that for Mn in spring water 50% of the human population is predicted to taste Mn at, 180 mg/L Mn with 1% of the population detecting manganese at 32 mg/L. For distilled water, a 50% population threshold of 45 mg/L Mn was predicted. The conclusion that manganese is more easily detectable in distilled water is supported by the 50% threshold values and

additional subject frequency data; 5% of the panel detected manganese at 3.6 mg/L Mn when tasted in distilled water, which is lower than the preceding 1% prediction at 32 mg/L Mn in spring water. An additional value for 5% manganese detection at 3.4 mg/L Mn is given, however it is unclear if this is in distilled or spring water and why it differs from the earlier reported value of 3.6 mg/L Mn. The final threshold distribution for manganese detection in distilled water predicts a 5% population detection of 0.9 mg/L Mn to a 95% detection of 487 mg/L Mn. Due to the variability in reported thresholds, it is worthwhile to improve our understanding of manganese detection in drinking water. A search of the literature produced no studies determining a visual population threshold for manganese detection in drinking water.

Due to the mounting evidence of negative health impacts from high concentrations of manganese in drinking water, it is important to know at what concentration manganese can be detected by the public. The objectives of this study are to determine a taste threshold for soluble, reduced Mn(II) and particulate, oxidized Mn (IV), evaluate taste descriptors for manganese, and to evaluate at what concentration Mn(II) and Mn(IV) can be visually detected.

2.3 Methods

2.3.1 *Human Subjects*

Study subjects were recruited from the Virginia Tech Civil and Environmental Engineering faculty, students and staff via e-mail in summer of 2012. Subjects were required to be 18 or older, of any health status, but not pregnant, and to abstain from food or drink for the hour before participating in a taste test. A total of 98 subjects participated in various sensory tests throughout the course of the project. Subjects were 18-61 years old. This study was approved by the Virginia Tech Institutional Review Board (IRB Protocol #09-506) and informed consent was given verbally by each subject in accordance with IRB protocol. Compensation for subjects included a snack after completing each testing session.

2.3.2 *Manganese Solutions*

Solutions for tasting were prepared daily with food grade manganese sulfate (MnSO_4). Concentrations ranged from 0.05 – 503 mg/L Mn (equivalent to 0.14 – 1382 mg/L MnSO_4) and

Mn concentrations were confirmed using atomic absorption spectroscopy (AAS), method detection limit = 0.009 mg/L Mn (USEPA, 1997), or inductively coupled plasma- mass spectroscopy (ICP-MS), minimum reporting level for Mn = 0.5, generated according to (Winslow et al, 2006) with appropriate QA-QC. Addition of NaOCl or pH adjustment to >11 then back to 6.5 using NaOH and HCl were used to oxidize Mn(II) to Mn(IV). These solutions were allowed to react for a minimum of 2 hours to insure conversion to the insoluble Mn (IV). Only the pH adjustment technique was applied to solutions that would be taste tested in order to avoid a chlorinous taste in samples.

2.3.3 Taste Testing at the SMCL

The triangle test (Mielgaard et al 1999) is used to test the ability of human subjects to detect concentrations of 0.05 mg/L Mn (II). All taste tests were conducted at room temperature. The triangle test presents the subject with three randomly, 3-digit numbered sample cups of which they must indicate one as being different from the others. The cups are 3 oz plastic white Solo® cups. Six possible combinations are used in the triangle test: AAB, ABA, BAA, BBA, BAB, ABB. Sample A is 30 mL deionized water, sample B is a 30 mL solution of deionized water and 0.05 mg/L MnSO₄. Subjects were instructed to taste the samples in the order provided. The noses of subjects were not occluded with nose clips; thus the tasting did not control for ortho and retronasal effects (Dietrich 2009). Thirty-one or thirty-three subjects participated in each triangle test. At $\alpha=0.05$, $n_{critical} = 16$ for 31 subjects or 17 for 33 subjects needed to select the correct sample in order for the Mn(II) solution to have a significantly different taste than distilled water.

Because of concern that particulate MnO₂ particles could remain in the mouth and solubilize to cause a taste (Cuppett et al. 2006; Hong et al. 2010), Mn(IV) taste test were administered using a 1-of-3 alternate forced choice test using only AAB, ABA, and BAA with two sample A as 30 mL deionized water and one sample B as a 30 mL solution of deionized water and Mn (IV). Black 5 oz plastic Solo® cups are used when testing Mn (IV) to prevent the subject from visually detecting a difference between the sample and the control. Subjects were instructed to swish samples in the cup, and consume entire sample to ensure that entire dose is received. On separate days, 0.05 mg/L Mn(IV) and 0.05 mg/L Mn(IV) solutions were tested and 31 or 33

subjects evaluated each concentration. At $\alpha=0.05$, $n_{\text{critical}} = 16$ for 31 subjects or 17 for 33 subjects needed to select the correct sample to have a significant different result.

2.3.4 Determining a taste threshold for Mn(II)

The one-in-five alternative forced choice (AFC) protocol is used to determine a taste threshold for Mn. The protocol was applied previously to evaluate the taste of copper (Cuppett et al, 2006) and iron and copper (Omur-Ozbek and Dietrich, 2011) in drinking water. Nose clips were not used; thus, the tasting included both ortho- and retro-nasal odor effects, if present. Because metals can react with lipids in the oral cavity (Mirlohi et al., 2011; Omur-Ozbek et al. 2012) and the metallic taste may linger (Cuppett et al, 2006), subjects preformed no more than two tests in one day separated by no less than one hour. Twenty three subjects participated in the threshold taste test; the taste tests were conducted at room temperature.

The 1-of-5 AFC test presents each subject with a series of five randomly numbered three-digit white sample cups of which they must indicate one as being different from the others. In this study, four of the cups contain 30 mL deionized water; the remaining cup contains a 30 mL solution of deionized water and MnSO₄ (Figure 2-2). In this study 14 concentrations of Mn(II) ranging from 0.05-506 mg/L were used. All Mn(II) solutions were clear and colorless at the given concentrations. Based on the results of the triangle test, screening taste tests in our laboratory and the Cohen et al (1960) study, 100 mg/L Mn(II) was selected as the anchor concentration and a multiplier of 1.5 was used for concentrations up to 503 mg/L and lower to 6.0 mg/L Mn(II), with addition concentrations lower than 6.0 mg/L added to define the lower concentration range. The location of the cup containing the Mn(II) solution is randomized for all tests. The subject first rinses with deionized water then proceeds to taste the samples from left to right, waiting one minute between samples, and may not re-taste a sample after moving to the next sample. The subject then indicates the one sample that they perceived to be different. If no difference is detected the subject must still choose a cup. The subjects were also asked to provide taste descriptors for samples.

Taste thresholds were determined for both the individual and the population using the one-in-five AFC data. For each subject, an individual threshold was determined by calculating the geometric

mean of the concentrations of the highest incorrectly identified sample and the lowest correctly identified sample when subjects had correct answers for all the subsequent samples after the last wrong answer. If a subject did not correctly identify three samples consecutively within the range of tested concentrations it is assumed that the subject would correctly identify the next highest concentration that would have been tested for purposes of calculating individual threshold. The next highest concentration that would have been tested is 759.5 mg/L if the multiplier of 1.5 is applied to the highest tested concentration. The group or population threshold is determined in two ways: (1) Best Estimate Threshold (BET) (2) Logistic Regression with Abbott's correction. The BET is determined by calculating the geometric mean of all individual thresholds. A logistic regression was used to determine the percent of people that can detect the taste of manganese. The logistic regression model is

$$p = \frac{1}{1 + \exp(-\beta_0 - \beta_1 * x)}$$

Where x is the concentration of manganese and p is probability that x will be detected and β_0 and β_1 are estimated from the data using maximum likelihood methods (van Aardt et al, 2001). The logistic regression was performed in R (R Development Core Team, 2011). Abbott's correction is then applied to determine the 50% population threshold in order to account for the 20% chance of guessing inherent in the one-in-five forced choice test (Abbott, 1925; Lawless, 2010). Abbott's formula is:

$$P_d = (P_{obs} - P_{chance}) / (1 - P_{chance})$$

P_d = probability of detection

P_{obs} = proportion positively identified

P_{chance} = chance of guessing correctly (1/5 for the one-in-five forced choice test)

Thus for a desired probability of detection of 50% using the one-in-five forced choice test, $P_{obs} = 0.60$.



Figure 2-2: Examples of one-in-five taste test (a) and one-in-five visual test (b). 0.05 mg/L Mn (IV) is detectable in the fourth cup in photo (b).

2.3.5 Reaction of Manganese with Saliva

Iron and copper are known to cause lipid oxidation in the oral cavity and thus this reaction was explored for manganese using the thiobarbituric acid reactive substances method (Ömür-Özbek et al, 2012). Whole saliva was collected from subjects. The ratio of saliva to test solution was 1:1 with 2 ml of each used for each test. Solutions of 9.84 mg/L Mn(II) and Mn(IV) were prepared in de-ionized water for analysis. Standard solutions were prepared using ten known concentrations of MDA standard and used to develop a standard curve. Samples and standards are vortexed, incubated in a 95°C bath, cooled, vortexed, and then centrifuged. Absorbance was then measured at 352nm using a spectrophotometer. Oxidative stress due to manganese exposure has been found in the liver, brain, and heart, but data is unavailable for lipid oxidation in saliva (Argüelles et al, 2004).

2.3.6 Visual Testing

The one-in-five alternative forced choice (AFC) test was also used to determine a visual threshold by presenting subjects with five cups, four containing deionized water, and one containing either Mn(II) or Mn(IV). In this study, four of the cups contain 250 mL deionized water; the remaining cup contains a 250 mL solution of deionized water and MnSO₄. For Mn(IV) concentrations of 0.005, 0.01, 0.03, and 0.05 mg/L Mn were tested and for Mn(II) 0.05 and 10 mg/L Mn were tested. A total of 33 subjects participated in this study. The cups are viewed under the same lighting conditions and side by side. Semi-transparent plastic cups were used in visual testing. Results are presented as percent of subjects correctly identifying the sample.

2.4 Results

2.4.1 Assessing manganese taste detection at concentrations near the aesthetic guideline

The EPA SMCL for manganese is 0.05 mg/L due to the potential for bitter metallic taste and black to brown color in pipes. The triangle testing comparison of a solution of 0.05 mg/L Mn (II) to de-ionized water found no significant difference with an $a = 0.05$; only 9 subjects out of 31 (10% female) correctly identified the Mn (II) containing sample. For 0.05 mg/L Mn(IV) there is no significant difference with an $a = 0.05$ as only 11 subjects out of 33 (36% female) correctly identified the sample. An additional test for 0.50 mg/L Mn(IV), ten times the SMCL, also found no significant difference at $a = 0.05$ as only 10 out of 33 (30% female) subjects correctly identified the sample. Thus, neither 0.05 mg/L Mn(II) or Mn(IV) could be distinguished from distilled water by taste. Since the Mn(IV) samples were highly colored at these concentrations (*vide infra*) no further taste tests were conducted for Mn (IV).

2.4.2 Determining Mn(II) Taste Threshold

Tabulated results from the 23 subjects (48% female) who participated in the one-in-five forced choice test for Mn (II) are presented in Table 2-2. Thresholds were determined for each individual (Table 2-2), and the calculated best estimate threshold (BET) is 101 mg/L Mn(II). Note that there is a wide range of individual thresholds, and that for more than half of subjects there was variability in taste perception. A subject was considered to be a variable taster if they tasted Mn(II) at a lower concentration, then could not detect Mn(II) for at least one test before positively identifying Mn(II) again. See taste subject A-94 who detected the lowest and highest Mn(II) concentration tested, but none in between, and subject A-99 who detected Mn(II) erratically in Table 2-2. The data in Table 2-2 are separated into subjects with variable and non-variable tasting patterns.

Lawless (2010) recommends using the linear portion of data for threshold analysis to perform logistic regression, thus the taste threshold was calculated based on concentrations of 0.8 mg/L Mn(II), and 8.8 mg/L to 337.5 mg/L Mn(II) for which all 23 subjects tasted all concentrations. The resulting logistic regression of the data indicates that the 50% population taste threshold for Mn (II) is 75.4 mg/L. The 5% population taste threshold was 4.5 mg/L Mn(II). The resulting

model can be seen in Figure 2-3. The estimated population parameters from the logistic regression and BET are summarized in Table 2-1.

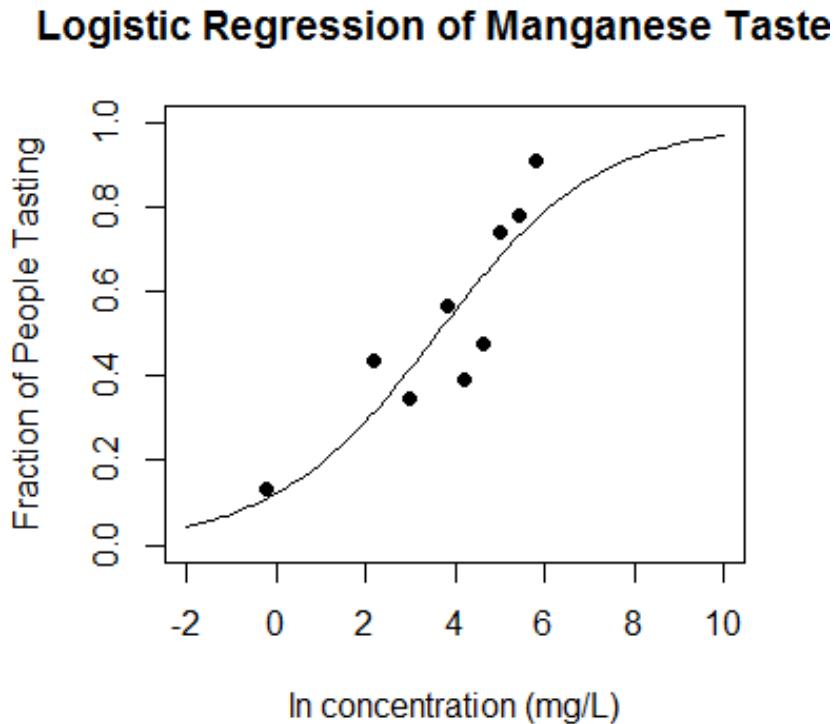


Figure 2-3: Logistic Regression for Mn (II), n=23. Model includes data from all concentrations with all 23 tasters responding in Table 2-2. Concentrations presented are 0.8 mg/L Mn(II) and 8.8 mg/L to 337.5 mg/L Mn(II). Results summarized in Table 2-1.

Table 2-1: Estimated thresholds for different percentages of the population being able to taste Mn(II) from this study

% population tasting Mn(II)	Mn (II) Concentration	
	Logistic Regression	BET method ^a
5	4.5	-
10	6.5	-
25	17.4	-
50	75.4	101
95	11134 ^b	-

- a. Only the 50% threshold can be estimated by the BET method.
- b. Substantially extrapolated beyond highest concentration of 506.3 mg/L Mn(II).

Table 2-2: Soluble Mn (II) Taste Test Results. N=23. + indicates positive detection, - indicates no detection. Blanks indicate that the subject did not complete the test. * Calculated as geometric mean of 0.8 and 8.8 mg/L

Subject	Mn (II) Concentration (mg/L)														Threshold	Non-Variable Tasters	Variable Tasters
	0.05	0.23	0.8	2.7	6.0	8.8	20.0	45.0	66.0	100.0	150.0	225.0	337.5	506.3			
A-1	-	-	-	-	-	-	-	-	+	+	+	+	+	+	54.5		
A-2	-	-	-	-	-	-	-	-	-	-	+	+	+	+	122.5		
A-3	-	-	-	-	-	-	-	+	+	+	+	+	+	+	30		
A-4	-	-	-	-	-	-	-	-	-	+	+	+	+	+	81.2		
A-5	-	-	-			+	+	+	+	+	+	+	+	+	2.66*		
A-6		-	-			-	-	-	-	-	-	+	+	+	183.7		
A-7	-	+	-			-	-	-	-	-	+	+	+	+	122.5		
A-8	+	+	-	-	+	-	-	-	+	+	+	+	+	+	54.5		
A-9	-	+	+	-	+	-	+	+	+	-	+	+	+	+	122.5		
A-10	-	+	-	-	+	+	+	+	+	+	+	+	+	+	7.3		
A-11	-	-	-	+	+	+	-	-	-	-	+	+	+	+	122.5		
A-12	-	-	+	-	-	+	-	+	-	-	-	-	+	+	275.6		
A-13	+	-	+	+	-	+	-	+	-	-	-	-	-	-	620.1		
A-14	+	-	-	-	-	-	+	+	-	+	+	+	+	+	81.2		
A-15	-	+	-	+	-	+	-	+	+	-	+	+	+	+	122.5		
A-16	-	-	-	-	+	+	-	+	-	-	+	-	+	+	275.6		
A-17	-	-	-	-		+	-	-	-	+	+	+	+	+	81.2		
A-18	-	+	-			-	+	+	+	+	+	+	+	+	13.3		
A-19	+		-			-	+	+	+	-	-	+	+	+	183.7		
A-20	+		-			-	-	-	-	-	-	-	-	+	413.4		
A-21			-			-	-	-	-	+	-	-	+	-	620.1		
A-22			-			+	+	+	-	-	+	+	+	+	122.5		
A-23			-			+	+	+	-	+	+	+	+	+	81.2		

There was no apparent correlation of individual threshold with age ($R^2 = 0.0252$). The highest and lowest threshold values were seen between age 20-23 (mean = 25.0 ± 8.55) with the highest threshold = 183.7 mg/L Mn(II) and the lowest threshold = 2.66 mg/L Mn(II). There was also no correlation between individual pH (mean = 6.1 ± 0.53) and individual threshold ($R^2 = 0.1772$). Individual mean saliva pH ranged from 6.1 to 7.7.

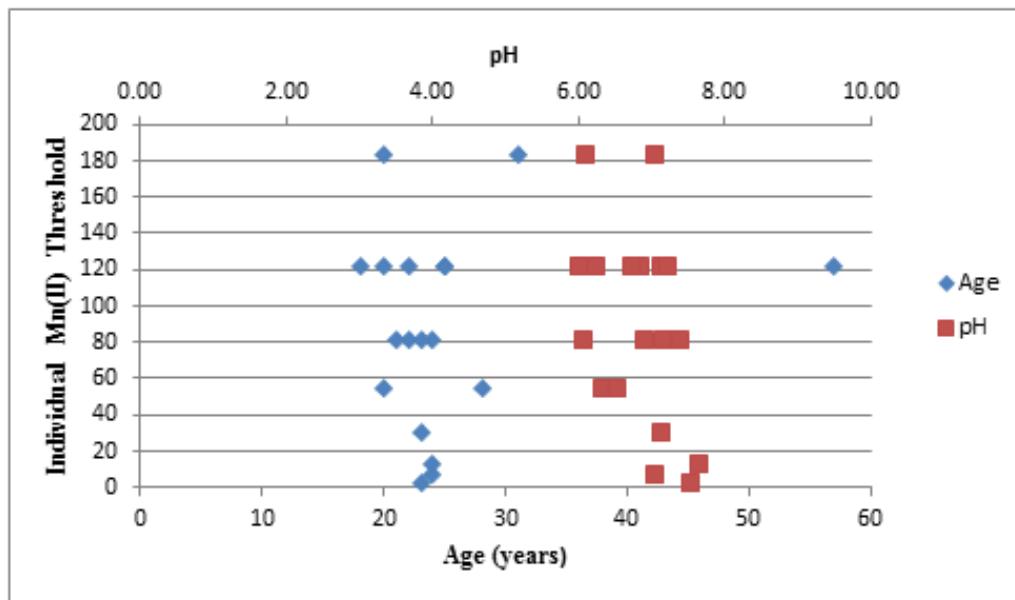


Figure 2-4: Individual Threshold by age and by pH. n=18.

Testing for salivary lipid oxidation using the TBARS method indicate that there is no lipid oxidation of Mn(II) or Mn(IV) at a concentration of 9.84 mg/L Mn. Using a t-test, Mn(II) ($p=0.055$) and Mn(IV) ($p=0.086$) showed no significant difference when compared to the control solution with an $\alpha=0.05$.

Taste descriptions for Mn (II) varied greatly. Figure 2-5 is a word cloud depicting the frequency with which descriptive words were used when describing the taste of the Mn (II) solutions. It is interesting to note that in addition to bitter and metallic, sweet was commonly used as a descriptor for manganese.



Figure 2-5: Mn(II) Taste Descriptors. Size of word correlates with frequency description was used in positively identified samples.

2.4.3 *Manganese visual recognition*

To determine appropriate concentrations for visual testing, solutions of MnO_2 are prepared and shown in Figure 2-6. In this image it is clear that Mn(IV) can be easily seen in water at higher concentrations. Mn(II) does not share this property. Table 2-3 shows the percent of people positively detecting manganese in a visual one-in-five forced choice test. Mn(IV) was detected at all concentrations, with 100% detection at ten times lower than the SMCL. Alternately, Mn(II) was not detected at 200 times the EPA SMCL. 10% detected Mn(II) at the SMCL, but 10% falls within the 20% error that can be expected due to guessing. It is also important to note that no subjects reported a visual difference in Mn(II) samples from the control even at concentrations as high as 506 mg/L during the one-in-five taste testing.

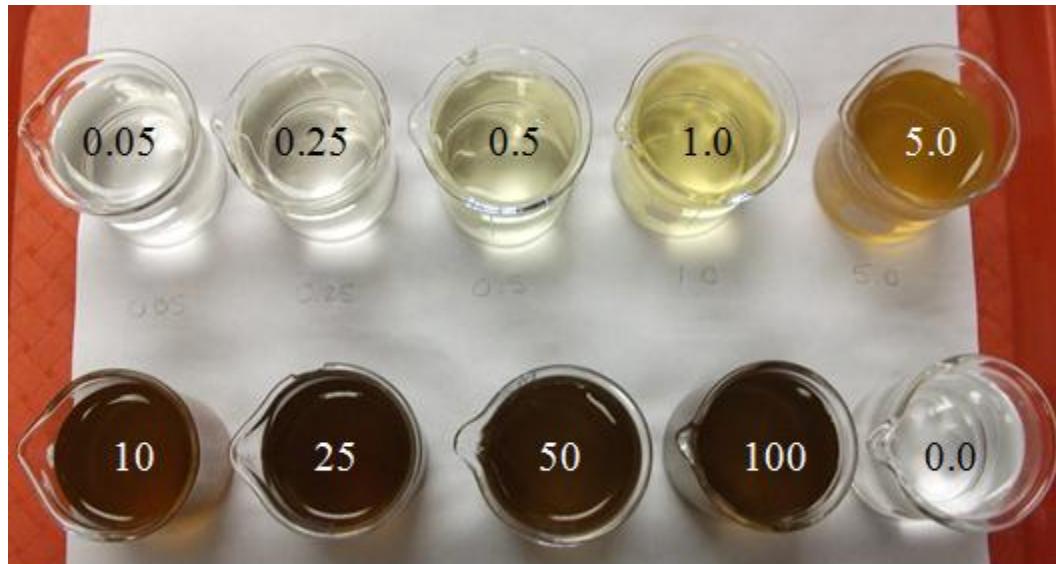


Figure 2-6: Mn(IV) in deionized water; concentrations in mg/L.

Table 2-3: Visual detection of Mn(II) and Mn(IV) from One-in-five Test. n=31 for all tests. Percentages represent n positively detecting manganese over n total. Bold entries denote EPA SMCL = 0.05 mg/L.

Concentration, mg/L Mn	Percent detected
Mn(IV), oxidized	
0.005	100
0.01	97
0.03	97
0.05	97
Mn(II), reduced	
0.05	10
10	0

2.5 Discussion

Manganese as Mn(II) or Mn(IV) has little perceptible taste at the concentration equivalent to the existing SMCL of 0.05 mg/L, although Mn(IV) has a detectable yellow color at this concentration while Mn(II) is clear and colorless. The taste detection of Mn(II) was found to vary widely between individuals. Similar variability of tasting ability was reported previously (Cohen, J.M. et al, 1960b). In this study, the lowest individual threshold was 2.66 mg/L Mn(II) while the highest confirmed threshold was 183.7 mg/L, with some subjects unable to detect manganese at concentrations as high as 506.3 mg/L Mn (II). This study also showed that for some individuals the ability to detect manganese is highly variable. A population BET of 101 mg/L Mn (II) and estimated 50% detection level at 75.4 mg/L Mn (II) indicate that the 0.05 mg/L Mn SMCL is protective of the population's ability to detect an off flavor from excess manganese. This was confirmed for both Mn(II) and Mn(IV) where no detectable difference between manganese containing water and the control at 0.05 mg/L.

It is interesting to note that manganese is different than other metals in its flavor producing ability in water. The ferrous iron population taste threshold has been found to range from 0.03-0.17 mg/L Fe(II) (Mirlohi et al, 2011; Ömur-Özbek & Dietrich, 2011) which is significantly lower than even the lowest Mn(II) threshold calculated in this study. The taste threshold for cupric ion is 0.48 mg/L (Cuppett et al, 2006) while cuprous ion is 0.61 mg/L (Ömur-Özbek & Dietrich, 2011). The high taste threshold for manganese could be related to its lack of lipid oxidation, which is necessary to produce the strong metallic flavor sensation and retronasal odors for ferrous, cupric, and cuprous ions (Ömür-Özbek et al, 2012). Ferrous ion, but not ferric ion which is flavorless, produces significant salivary lipid oxidation and retronasal odors at 10 mg/L, while Mn(II) and Mn(IV) do not cause oxidation at this concentration. Additionally, human subjects primarily describe the taste for ferrous, cupric and cuprous ion as metallic (Ömur-Özbek & Dietrich, 2011), while the descriptors for Mn (II) are much more variable. Taste descriptions from subjects in this test confirmed the USEPA (2012) descriptors of bitter and metallic flavor, but also include the sweet and salty tastes that manganese can impart for some subjects, as well as moldy, musty, yummy. Many subjects also reported that the manganese containing water tasted better than the control water. These data indicate that the taste response for Mn(II) is much different than for Fe(II), Cu (II) or Cu(I).

Using solutions of MnSO₄, the threshold for Mn was determined to be in the range of 75-100 mg/L Mn (II), or 1.37-1.8 mM Mn(II). The threshold determined in this research may not be entirely attributable to Mn(II). At these threshold concentrations, the taste solutions would contain 131- 175 mg/L SO₄⁼ and 206-275 mg/L total dissolved solids (TDS). The SO₄⁼ levels are approaching, but remain below, the aesthetic guideline of 250 mg/L SO₄⁼, which is based upon sulfate causing a salty taste as the sodium salt (European Union, 1998; WHO, 2011; USEPA 2012). Since the same value of 250 mg/L is also applied as an aesthetic guideline for chloride (European Union, 1998; WHO, 2011; USEPA 2012), using MnCl₂ for taste-testing would not allow the subjects to focus more on the taste of Mn.

TDS, or mineral content in drinking water, is acknowledged to be the major chemical factor affecting taste and likeability of drinking water when no off-flavors are present (Bruvold, W.H. & Pangborn, 1966; Burlingame et al, 2007; Gallagher & Dietrich, 2010). Aesthetic guidelines for TDS vary; typical values range from 500 mg/L TDS (USEPA, 2012) to 1000 mg/L (WHO, 2011). While TDS levels of 206-275 mg/L calculated for the Mn threshold in this study remain below the aesthetic guideline for TDS, they are within the < 450 mg/L TDS values in drinking water that consumers and trained professionals rated as tasting good to excellent (Bruvold, W. H. & Daniels, 1990). Additionally, Bruvold, W.H. (1968) reported that consumers found tap water containing approximately 85 mg/L TDS tasted better than distilled water. The interpretation that some individuals in this study may have responded to the taste of TDS is supported by the variable taste descriptors (Figure 2-5) and statements by subjects that samples with MnSO₄ tasted better than the distilled water used for comparison in the 1-of-5 AFC test.

The only known previous study on the taste threshold for Mn is that of Cohen, J.M. et al (1960b). In that research, MnSO₄ solutions for tasting were prepared in distilled water or spring water with 460 mg/L TDS. The authors concluded that “apparently manganese is more readily detected by taste in distilled water” based on calculated threshold values of 45 mg/L Mn(II) in distilled water and 180 mg/L Mn (II) in spring water. A reasonable interpretation of these results is that the taste of manganous sulfate was “masked” by the TDS in the spring water and therefore the threshold was higher. In the distilled water, the panelists were only responding to the TDS of MnSO₄.

Both this study and the Cohen, J.M. et al (1960b) studies indicate a population threshold in the range of 45-180 mg/L depending on water quality; this range would not be protective of human health. These population taste thresholds are well above even the highest recorded value of 28 mg/L Mn from the Ayotte et al (2011) survey of US groundwater trace metals and substantially greater than typical groundwater concentrations. The high population threshold and reported preference for TDS containing water are both indications that the ability for the population to detect high levels of manganese by taste should not be relied upon to protect human health.

From the findings in the visual portion of this report, high levels of oxidized Mn(IV) would be detected before consumption. Mn(IV) concentrations 10 times lower than the SMCL were accurately seen in this study. The cups containing the control however were side by side with the cup containing the sample. The distance between objects can impact the ability to detect color differences so a less direct comparison may be necessary to determine the likelihood of a consumer to detect Mn(IV) in drinking water in a household environment (Kuo, 2010). Alternately, Mn(II) was not found to be visually detectable at high concentrations. If a water with a high Mn(II) concentration is not oxidized and subsequently removed, then a person would likely not taste or see any indication that their water may have potentially toxic levels of manganese. This is especially of concern in private household wells where testing is infrequent and drinking water could contain potentially harmful levels of manganese.

2.6 Conclusions

Mn(IV) may be visually detected at concentrations lower than the SMCL. Due to the ease of Mn(IV) visual detection , if oxidation or precipitation are applied to convert Mn(II) to Mn(IV), then the resulting Mn(IV) will be removed or visualized. Mn(IV) could not be distinguished from the control by taste at 10 times the SMCL, so taste alone would not provide adequate detection of high manganese in drinking water.

The USEPA SMCL is based on total Mn. The value of 0.05 mg/L is very protective of taste of Mn(II) as only a small percentage of the population may taste 0.05 mg/L Mn(II). The SMCL

would likely protect against Mn(IV) color depending on water depth and container and viewing characteristics such as size and color of container.

Human senses cannot be relied upon to detect Mn(II) in drinking water. The 50% population taste threshold for Mn(II) was estimated to be >1000 times the EPA's current SMCL of 0.05 mg/L. Additionally, Mn(II) is visually undetectable in drinking water, even at concentrations much greater than typical groundwater concentrations. Disinfected and oxidized drinking water typically has Mn concentrations lower than the SMCL due to oxidation of Mn(II) to Mn(IV) and subsequent removal. However, groundwater that is not treated with an oxidant could result in ingestion of water with high Mn(II) concentrations. Ingestion of high levels of manganese poses a possible health hazard, so ensuring low levels of manganese in drinking water through testing is recommended.

2.7 References

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Chapter 3. Metal Concentrations in Ultrasonic Humidifiers over Time for Assorted Water Types

3.1 Abstract

Single room humidifiers are used to increase indoor relative humidity, but ultrasonic humidifiers could be a potential inhalation exposure source for metals in drinking water. Ultrasonic humidifier aerosols emitted for five different source waters were evaluated. Concentrations of metals in the reservoir increase over the course of a 12 to 14 hour humidifier experimental cycle. Concentrations of metals in the condensed aerosols decrease initially, but increase after 2 to 4 hours. Findings indicate that condensed humidifier aerosols generally contain 85-90% of metals present in the reservoir water for chlorinated, chloraminated, and dechloraminated waters. Deviations from this pattern were found in a high hardness and high iron well waters due to precipitation of select metals in the reservoir. There is little evidence to suggest that failure to remove remaining humidifier reservoir water prior to refilling the humidifier reservoir would result in higher exposures after 5 days in spite of increased concentration of metals seen in the humidifier reservoir over the course of a single cycle. Results indicate that humidifiers serve as a source of inhalation exposure to metals present in source water, with exposures increasing over the length of a humidifier cycle.

3.2 Introduction

Inhalation of drinking water contaminants via aerosols is a known phenomenon. Showers are exposure sources for volatile organic carbons like chloroform, but there is also potential for exposure to particulates or dissolved contaminants in the aerosols themselves (Jo et al, 1990; Xu & Weisel, 2002). Both humidifiers and showers produce aerosols in the inhalable range, so an examination of the potential for humidifiers to aerosolize drinking water contaminants is necessary (Highsmith et al, 1988; Highsmith, 1992; Zhou et al, 2007).

Humidifiers are often used in households when the humidity level drops below a comfortable level. In dry air (relative humidity $\leq 20\%$) there is an increase in discomfort in the eyes, lips, face and skin (Highsmith et al, 1988). Increased relative humidity (RH) can decrease discomfort associated with dry air and protect appliances and furniture from splitting, cracking or static electricity due to low RH (Reinikainen et al, 1991; Reinikainen et al, 1992; Mohan et al, 1998).

Humidifiers are also prescribed by physicians for use by patients diagnosed with respiratory illness because higher RH reduces irritation in the respiratory tract (Farrer, 2012). Humidifier use generally increases in winter months when humidity levels in households drops below an optimal comfort level of 40-50% relative humidity (Highsmith et al, 1988).

The EPA recommends use of distilled water in humidifiers with regular maintenance and cleaning, but typical consumer usage characteristics like source water quality, refilling habits, and maintenance activities for household humidifiers are not known (USEPA, 1991). There are three types of humidifier commonly used in homes: ultrasonic which aerosolize water using ultrasonic vibration, impeller which uses a fan to break water into small droplets, and evaporative, which use heat to evaporate water. Of the current top ten rated single room humidifiers, nine are ultrasonic and one is an impeller type humidifier (Consumer Reports, 2012). Highsmith et al (1988) reports that consumers have replaced most impeller or steam humidifiers with ultrasonic humidifiers due to the reduced noise, humidifying ability, and reduction in mold and bacteria problems.

The possible biological risks associated with humidifier use are well documented. There is evidence that over humidification (relative humidity >70%) can adversely impact the health of children by increasing the prevalence of dust mites and molds (Brunekreef et al, 1989). The EPA (1991) also warns against improper care or use of humidifiers because of the ability of the humidifier to aerosolize microbes. Improperly sanitized humidifiers provide an incubator for potentially pathogenic microbes which can lead to respiratory infection or humidifier fever (Edwards, 1980). Ultrasonic humidifiers disseminate microbes more readily than other humidifier types and produce an aerosol in the inhalable range of particle sizes, increasing potential exposure to pathogens (Tyndall et al, 1995).

The ability for ultrasonic humidifiers to expel respirable particles is confirmed in the literature and these particles contain not only microbes, but various minerals as well (Highsmith et al, 1988). There is evidence that 90% of the aerosols formed by an ultrasonic humidifier are in the respirable range with larger particles being produced by collisions between the smaller particles (Highsmith, 1992). This is of concern because inhalation of humidifier particles has been shown

to lead to deposition of minerals present in the source water into the lungs of exposed children (Daftary & Deterding, 2011). High mineral waters are not recommended for use in humidifiers because of the production of “white dust,” particles of dissolved minerals that remain once the water in the aerosols have dissolved (USEPA, 1991). The white humidifier dust produced are all within the respirable range of particle sizes (USEPA, 1991).

Current literature suggests that the concentration of total dissolved solids in the aerosols produced by ultrasonic humidifiers are about 90% of those in the bulk water used in the humidifier reservoir, but these data are limited to one water type and to an experimental cycle time of 0.5 hours for a single room ultrasonic humidifier (Highsmith et al, 1988). There are limited data available for different initial water qualities for single room humidifier use or a comprehensive analysis of the emission of major and minor constituents in the water over time. There is also an information gap about emissions of water constituents during long-term use when the humidifier is repeatedly refilled without discarding water remaining in the humidifier reservoir.

This study aims to fill current research gaps related to differing water qualities and usage times greater than 0.5 hours for ultrasonic humidifiers. Specifically, the objectives of this study are: (1) determine the effect of different water qualities with respect to emission of dissolved solids, specifically metals, from the humidifier reservoir into the water aerosols; (2) determine if the emission pattern of metals and non-metals are similar over time; (3) determine if the relative concentrations of metals and non-metals in the humidifier reservoir and aerosols are constant over time; (4) evaluate aerosols produced during prolonged use when the humidifier reservoir is recharged without cleaning between cycles. This information will improve understanding of inhalation exposures due to use of single room humidifiers.

3.3 Materials and Methods

3.3.1 Ultrasonic Humidifier

The humidifier model used in this study is rated in the top 10 of available tabletop single room humidifiers and rated a Best Buy by Consumer Reports, 2012 and was purchased both online and in store at a national chain retailer. The design is filter-less and has a variable output control. All experiments in this study were operated with the output set to its highest setting.

3.3.2 Test Waters

Five different source waters were tested. Water types include chlorinated municipal tap water, chloraminated municipal tap water, dechloraminated municipal tap water, high hardness well water, and high iron well water. The municipal tap waters are from surface water sources. Three liters of all municipal tap waters were collected at household taps and immediately used to fill the humidifier reservoir. The well waters were not subject to treatment, and were collected in 10 liter acid-washed Nalgene® containers from individual homes in southwest Virginia. The chloraminated municipal tap water replicates were augmented with 0.5 mg/L MnCl₂ as Mn in order to assess the impact of humidification on a metal commonly found in drinking water at low concentration (Ayotte et al, 2011). Select chemical characteristics of the five source waters are presented in Table 3-1. Triplicate experiments were performed with each water quality used to charge the reservoir of the ultrasonic humidifier. In order to evaluate the impact of failure to dispose of remaining source water when recharging the humidifier reservoir, chloraminated tap water was used to recharge the reservoir between cycles without cleaning, over the course of five 12 to 14-hour cycles. The chloraminated tap water was augmented with 0.5 mg/L MnCl₂ as Mn and 0.03 mg/L PbCl₂ as Pb to assess the impact of humidification on a potentially toxic metal concentration present in the source water.

Table 3-1: Initial water quality characteristics

Water Type	TDS (mg/L total solids)	pH	Hardness (mg/L as CaCO ₃)	Alkalinity (mg/L as CaCO ₃)	Residual Chlorine (mg/L)
Chlorinated Municipal Tap	152	7.2	37.5	31.1	1.46
Chloraminated Municipal Tap	75	7.4	41.5	48.3	1.86
Dechloraminated Municipal Tap	74	7.3	44.0	42.5	n/a
High Hardness Private Well	255	7.6	333	317.5	n/a
High Iron Private Well	72	7.5	18.8	40.3	n/a

3.3.3 Water Quality Characterization and Chemical Analyses

Temperature, pH, and disinfectant residual were immediately measured when a water sample was obtained. The pH of each water was measured with an Accumet® electrode (13-620-287A) or by pH indicator strips (A011550, Sigma, MO). Temperature was measured with a Fisher Scientific Traceable® digital thermometer (14-648-45) with a range from -20 to 110°C. Total dissolved solids (TDS) were measured for each water with a Hanna Multiprobe (HI 9828, Hanna Instruments). The probe was calibrated before use with HI 9828-0 calibration solution (Hanna Instruments, Romania). Free and total chlorine concentration measurements were performed with a HACH Pocket Colorimeter™ II for chlorine. Alkalinity was measured using titration method 2320-B from Standard Methods with a 0.0193 N H₂SO₄ solution (Clesceri et al, 1998). Water samples were analyzed for metals (Na, Mg, Al, Si, Ca, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Mo, Ag, Cd, Sn, Pb) concentrations using a Thermo Electron X-Series inductively coupled plasma with mass spectrometer (ICP-MS) per Standard Method 3125-B (Clesceri et al, 1998). Samples and calibration standards were prepared in a matrix of 2% trace metal grade nitric acid by volume. The minimum reporting levels (MRL) for the metals are reported in Table 3-2. For the

purpose of this project, a major water constituent is defined as having a concentration ≥ 1000 ppb and a minor water constituent is defined as having a source water concentration < 1000 ppb.

Table 3-2: ICP-MS Minimum Reporting Levels (MRL), generated according to (Winslow et al, 2006).

Element	MRL (ppb)	Element	MRL (ppb)
Na	50	Co	0.2
Mg	2	Ni	0.2
Al	0.2	Cu	0.5
Si	10	Zn	1
P	20	As	1
Ca	50	Mo	5
V	0.1	Ag	0.1
Cr	0.2	Cd	0.2
Fe	50	Sn	1
Mn	0.5	Pb	0.05

3.3.4 Experimental Design for Assessing Humidifier Performance

For each experiment, the humidifier's upper reservoir was filled to full with three liters of source water. The humidifier outlet was attached to a glass condensation column that was vented to two 10 liter glass collection bottles connected in series to allow for recovery of water aerosols. Figure 3-1 shows the experimental apparatus.

The humidifier is operated until low water in the reservoir initiated automatic shutdown; this occurs at 12 to 14 hours. This 12 to 14 hour experimental operation period will be referred to as a cycle for the remainder of this paper. The reservoir water is sampled at 0, 1, 2 hours, followed by every 2 hours until 12-14 hours. Water samples are collected from the condensation column at the point indicated in Figure 3-1 starting at 1 hour, followed by every 2 hours up to 14 hours.

Water samples are also taken from the two 10 L glass collection bottles at the end of the humidifier cycle. If precipitates were visually detected in the humidifier reservoir an additional sample was taken and filtered using a 0.45 μm filter (09-719-2D, Fisherbrand, PA). The experiment was repeated three times for each water quality type. Three identical humidifiers were used to obtain replicates for each water type.

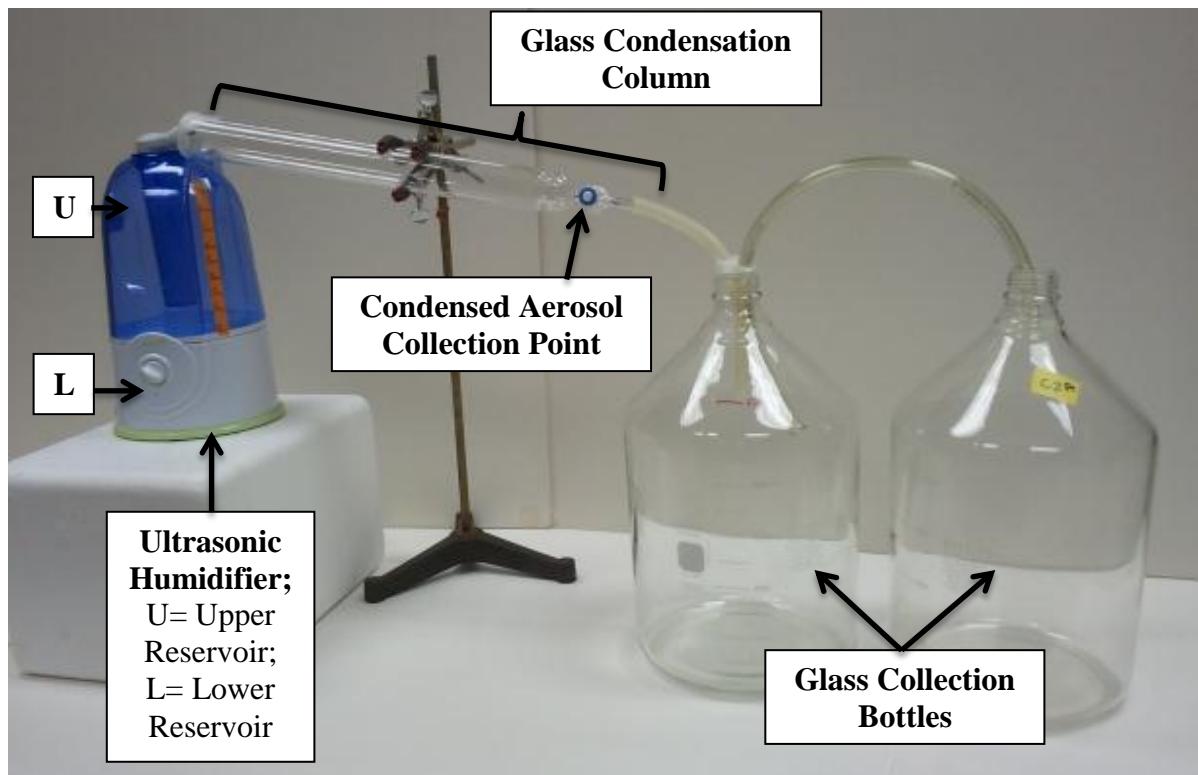


Figure 3-1: Experimental apparatus

3.3.5 Data Analyses and Statistics

Trend analysis was performed for each water type by performing a slope analysis on the percentage of metals concentration expelled by the humidifier. Percent of metal concentration expelled was calculated from the average of condensate concentration divided by reservoir concentration. The values from three humidifier cycles were then averaged, at which point a linear regression was performed on the percent values for each metal using data from 2-12 hours in Microsoft Excel (2010). The slopes from all metals were averaged, and minimum and maximum values were reported. Slopes calculated for each metal, in each ground water were compared using a one-way ANOVA with analysis in R (R Development Core Team, 2011). A

paired t-test with $\alpha = 0.05$ was used to determine if there was a significant difference over time during the 5 day extended use experiment.

3.4 Results

3.4.1 Water Quality Characterization of Source Water

Aggregate water quality parameters were measured for each source water type (Table 3-1).

While the pH for all waters were similar and ranged from 7.2 to 7.8, the TDS varied greatly from 72-225 mg/L. The high hardness well water has a hardness of 333 mg/L as CaCO₃ and other waters had hardness values ≤ 44 mg/L as CaCO₃. The chlorinated tap water and chloraminated tap water had chlorine disinfectant residuals of 1.46 and 1.86 mg/L as Cl₂, respectively. The dechloraminated, high hardness, and high iron waters were not subject to disinfection and therefore had no free chlorine residual.

Water quality data for metals and chloride are provided in Table 3-3. Major constituents are classified as those with concentrations > 1000 ppb and for all waters include the following: Ca, Cl, K, Mg, Na, and Si. Fe was included as a major metal as it was present above 1000 ppb in the high iron well water, which was the only water it was measurable in. Minor metals are classified as metals with concentrations ≤ 1000 ppb. Minor metals included are: Al, Cu, Mn, Cu, Pb, and Zn. Select metals, As, Mo, Ag, Cd, and Sn, were not included in Table 3-3 because they were below reporting limits for all waters. As expected, Ca and Mg concentrations in the high hardness water were more than six times greater than the concentration in any other water. The Na concentration in the high hardness well water was more than 5 times lower than the next highest water, chloraminated tap, and the Na concentration in the chlorinated tap and high iron well water are ~ 1.75 times the dechloraminated tap water. Cu concentrations in the chlorinated and chloraminated tap waters are higher than those measured in dechloraminated and well waters.

Table 3-3: Water Quality Data. Values given as mean \pm standard deviation in ppb for n=3 replicates.

	Chlorinated Tap	Chloraminated Tap	Dechloraminated Tap	High Hardness Well	High Iron Well
Major (ppb, > 1000)	Ca 9001 \pm 245	10677 \pm 229	10187 \pm 245	68263 \pm 3011	1456 \pm 190
	Cl *	17217 \pm 663	20850 \pm 842	8573 \pm 112	4331 \pm 58
	Fe^a BD	BD	BD	BD	4131 \pm 1213
	K 1980 \pm 78	1984 \pm 50	1347 \pm 21	1716 \pm 14	1121 \pm 11
	Mg 3991 \pm 115	4892 \pm 116	4480 \pm 91	37273 \pm 179	3548 \pm 55
	Na 17697 \pm 565	9715 \pm 128	10191 \pm 619	1808 \pm 35	18167 \pm 68
	Si 5244 \pm 158	2968 \pm 71	3760 \pm 27	5867 \pm 126	6249 \pm 65
	Al 14.40 \pm 3.88	55.30 \pm 11.46	16.30 \pm 2.20	2.12 \pm 0.15	14.95 \pm 2.34
	Cu 186.53 \pm 98.11	108.77 \pm 36.58	23.88 \pm 3.09	18.04 \pm 2.92	54.02 \pm 17.34
	Mn 514.63 \pm 37.36 ^b	529.23 \pm 6.45 ^b	509.43 \pm 2.97 ^b	1.58 \pm 0.87	364.77 \pm 39.71
	Ni 0.43 \pm 0.10	0.61 \pm 0.13	0.40 \pm 0.02	1.92 \pm 0.08	12.76 \pm 1.42
	Pb BD	29.92 \pm 2.66 ^b	BD	0.88 \pm 0.35	10.86 \pm 3.36
	Zn 7.66 \pm 4.17	35.90 \pm 5.22	104.90 \pm 7.20	8.00 \pm 1.67	338.90 \pm 22.51

* indicates no measurement taken, BD indicates below reporting limit. ^aFe is a major metal for only High Iron Well Water.

^bConcentrations augmented after water collection.

3.4.2 Humidifier Performance

The emitted aerosols were visible as a “white mist” in the glass condensation column and into the glass collection bottles. The aerosols would condense as they traveled through the condensation column and into the collection bottles. A humidifier cycle with no condensed aerosol collection was performed to confirm that collection of aerosols did not lengthen the time until automatic shutdown. Aerosols were also visible exiting the second collection bottle in series, but condensate recovery was an average of $91.0 \pm 5.1\%$ by volume. The second collection bottle was not sealed as it would allow pressure to accumulate in the system and that would have restricted aerosol production and greatly lengthened the time needed to complete a humidifier cycle. The humidifier cycle was 12-14 hours as determined by automatic shutoff induced by low water level in the humidifier reservoir. As shown in Figure 3-2, water temperature increased rapidly in the humidifier reservoir. Temperature stabilized at 44-45°C between 1.5 and 3 hours into the humidifier cycle.

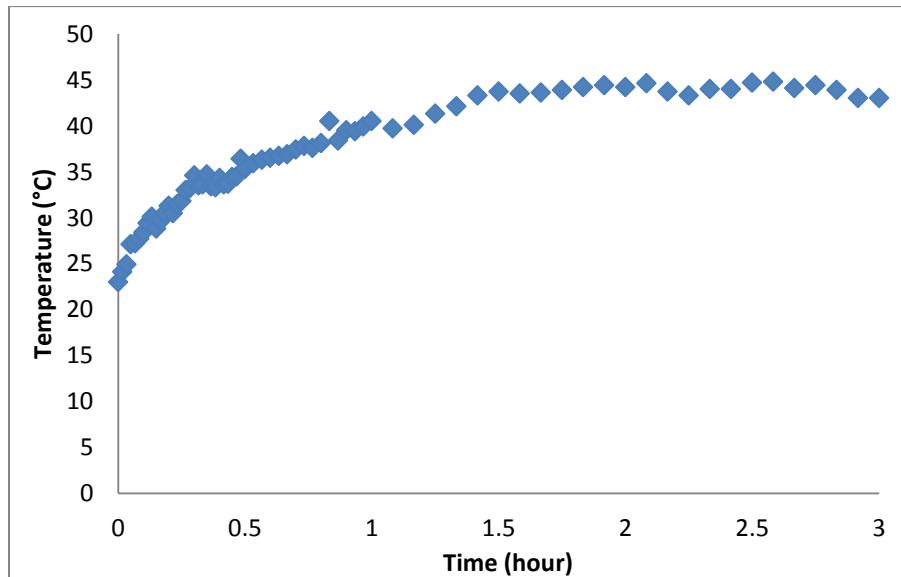


Figure 3-2: Temperature Change in Humidifier Reservoir over Time

Precipitation occurred in the humidifier reservoir during the course of experiments with both the high hardness and the high iron well waters. For the high hardness well water a milky, white precipitate formed. In the high iron well water a reddish-brown precipitate was visible. These

precipitates were not apparent in the condensed aerosols collected from the same experiments. An example of the visually detectable precipitate formed from the humidifier reservoir by the high iron well water can be seen in Figure 3-3.

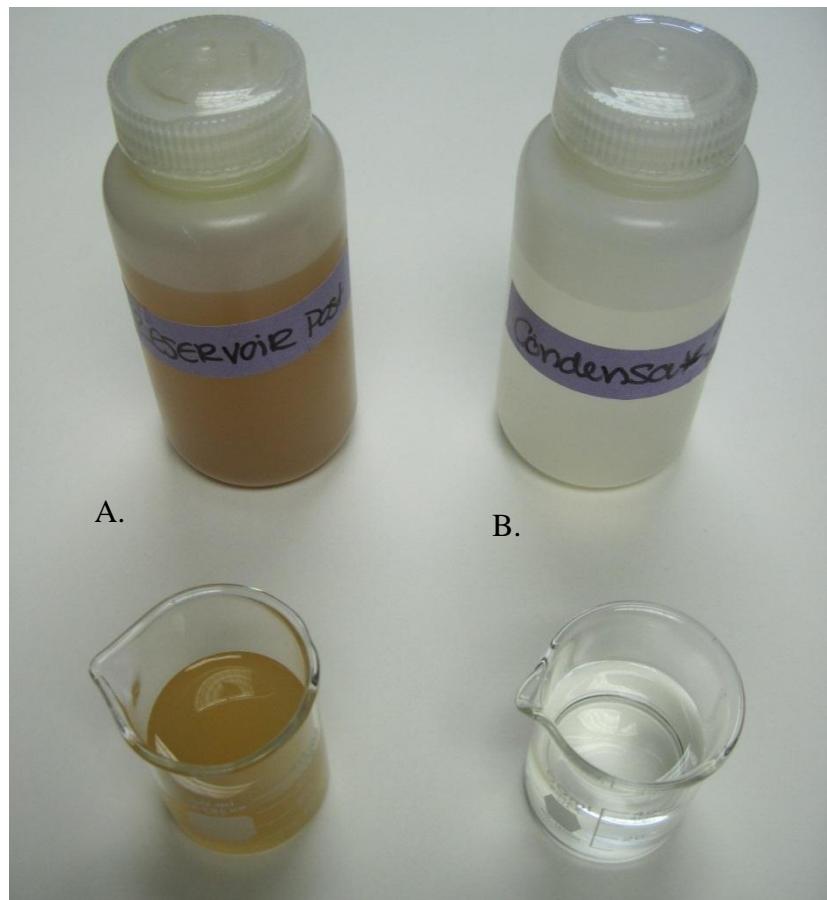


Figure 3-3. Humidifier reservoir and condensed aerosol samples from high iron well water demonstrating formation of precipitate. A.- Reservoir water, B.-condensed aerosols.

3.4.3 Comparison of Reservoir Water and Condensed Aerosols

The tap waters performed similarly in relation to the trends of both major and minor metal concentrations over time, as presented in Figure 3-6 and supplemental information (Figure A-1). For chlorinated tap water, chloraminated tap water, and dechloraminated tap water concentrations of major (Ca, Cl, Mg, K, Na, Si) and minor (Al, Cu, Mn, Ni, Pb, Zn) constituents increased over time in the humidifier reservoir. In these three waters, condensed aerosol major and minor metal concentrations both initially decrease, but after 2-4 hours they increase at a constant rate and at a slope not significantly different ($\alpha = 0.05$) from the concentration in the

reservoir through the end of the experimental cycle. This initial decrease in concentration coincides with an increase from initial room temperature water to about 45°C that is demonstrated in Figure 3-2. Overall, the condensate concentrations are ~85% of the humidifier concentration after the first 1-2 hours of humidifier operation. The residual chlorine concentrations in both the chlorinated and chloraminated tap waters were below detection limits when tested at the conclusion of their respective humidifier cycles. Chloraminated tap water and dechloraminated tap water experiments yielded similar results to chlorinated tap water. Further data is included as supplemental information.

The high hardness well water and high iron well water did not show similar trends for all metals as was seen in the municipal drinking waters tested. In Figure 3-6, c. It is apparent that the Ca concentration decreases in both the humidifier reservoir and the condensate for the high hardness well water. Since the solubility of Ca^{2+} as CaCO_3 decreases with increasing temperature, the precipitation of Ca was explored. Data for filtered humidifier reservoir water can be seen in Figure 3-6, c. The filtered sample showed lower Ca concentrations than the unfiltered humidifier reservoir sample, indicating that precipitation was occurring (Figure A-2 in supplemental information). The Mg and Na concentrations follow the trend of increasing reservoir concentration and decrease in condensate concentration followed by an increase after the first two hours of operation. The results for the minor metals in the high hardness water were also different than those seen in the municipal tap waters. In Figure 3-6, d. the Cu and Zn in the reservoir decrease over time similarly to the Ca. This is also likely due to precipitation which is also evidenced by lower concentrations of both metals in the filtered samples than unfiltered samples. Further evidence of precipitation was seen after four hours of operation, when white solids became highly visible in the humidifier reservoir. This gave the reservoir water a milky appearance, while the condensate remained quite clear in comparison.

The high iron water experienced similar precipitation effects which is visually apparent in Figure 3-3. The major metals seen in Figure 3-6, e. increase over time, and the condensed aerosol concentrations initially decrease before increasing similarly to what was seen in the municipal tap waters. The major metals in the high iron water did not appear to undergo precipitation, which is supported by the similarity in filtered and unfiltered samples. The increase in iron

concentration, followed by a decrease before increasing again, combined with the lower concentrations found in the filtered samples indicate that iron precipitated (Figure 3-4). The filtered reservoir sample is also lower than the condensed aerosol concentration for many metals indicating that there may also be particles being expelled in the humidifier aerosols, even if they were not visually apparent in Figure 3-3. There is also evidence of precipitation of additional metals (Al, Si, Cu, Mn, Ni, Pb, and Zn) in the high iron water which can be seen in the comparison of filtered to unfiltered reservoir samples in Figure 3-5 and in supplemental information (Figure A-3). Cu, Mn, and Zn also all exhibit the same pattern of increase, followed by decrease, followed by increase seen in the iron concentration. See Figure 3-6, f.

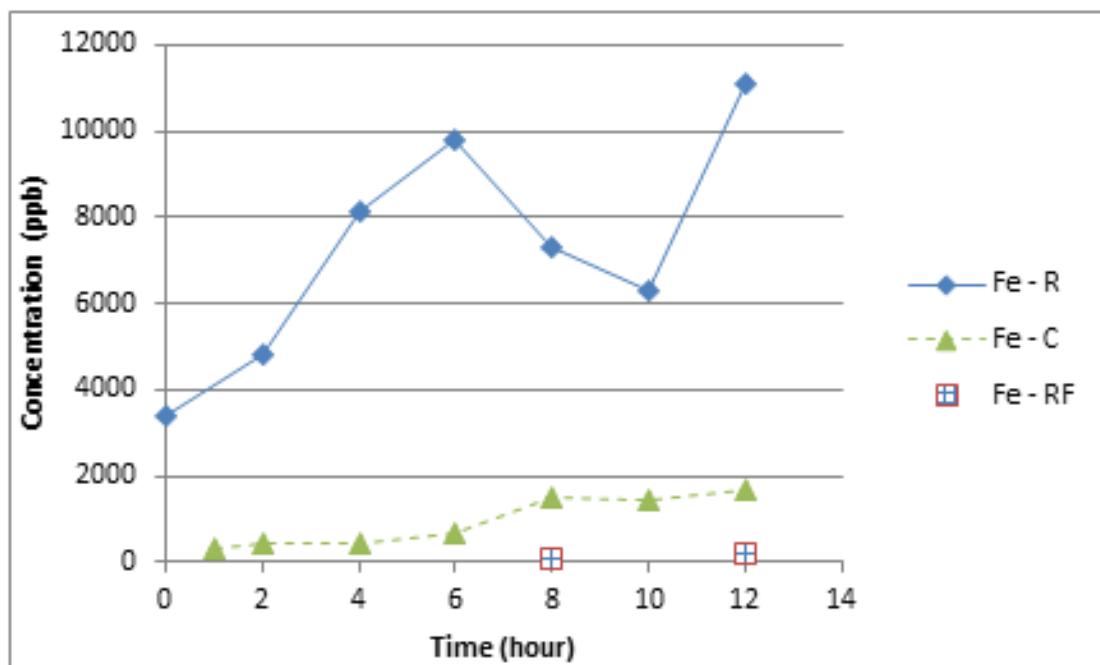


Figure 3-4. Iron concentrations for reservoir water (R), condensed aerosols (C) and for filtered reservoir water (RF) for a single replicate

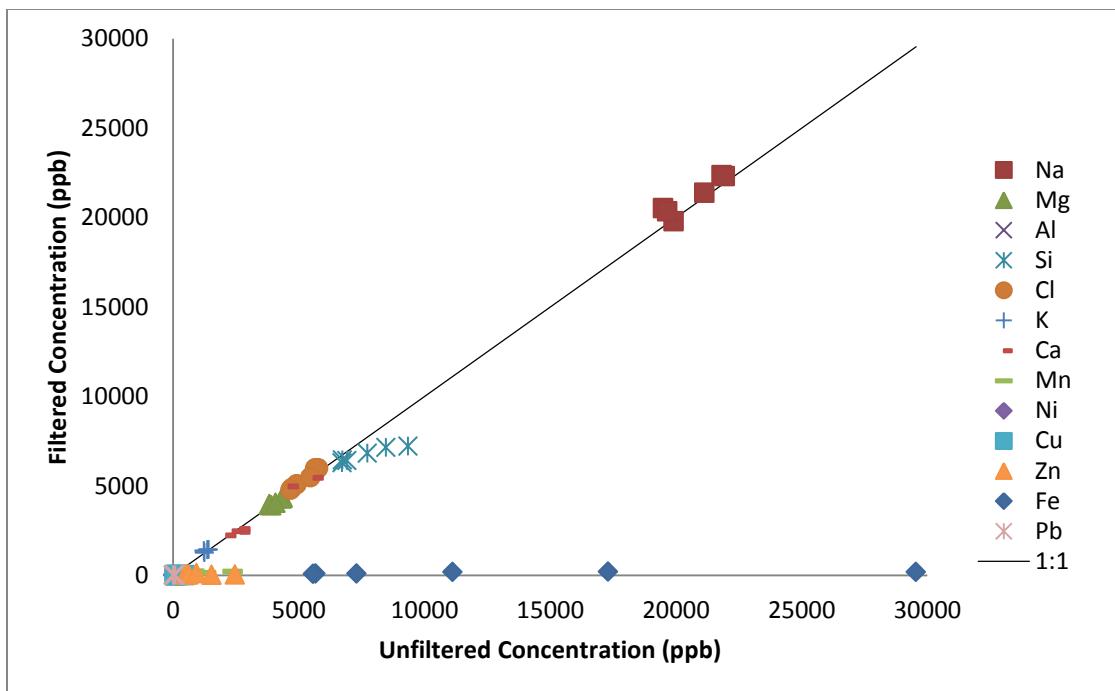


Figure 3-5. Concentrations of metals in filtered and unfiltered reservoir samples for high iron well water; Al, Si, Ni, Cu, Pb, Zn, Fe, and Mn formed precipitates.

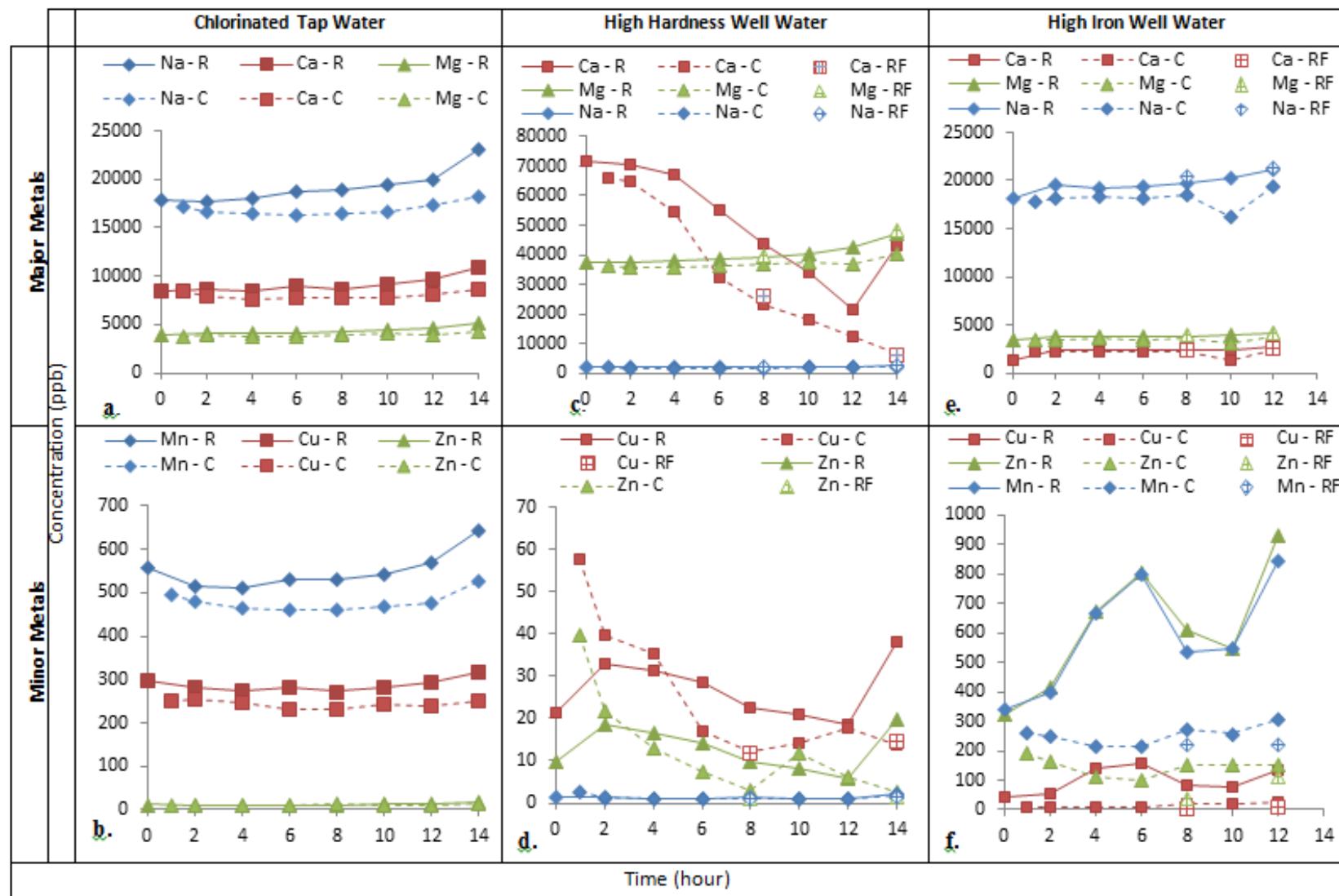


Figure 3-6: Representative Major and Minor Metal Concentrations Over Time for a Single Replicate by Water Type. R denotes reservoir sample, C denotes condensate sample, RF denotes a filtered reservoir sample

3.4.4 Patterns of Major and Minor Metal Release

Typically, 85-90 % of the metals present in the reservoir were expelled into the condensate once the temperature stabilized at 44-45 C. The amount decreased to about 70% at the end of the humidifier cycle and initially was lowered from $\geq 90\%$ as the temperature in the reservoir increased from room temperature. Examples from chlorinated and dechloraminated tap waters are presented in Figure 3-7 and Figure 3-8. Similar patterns were observed for all waters for metals that did not precipitate.

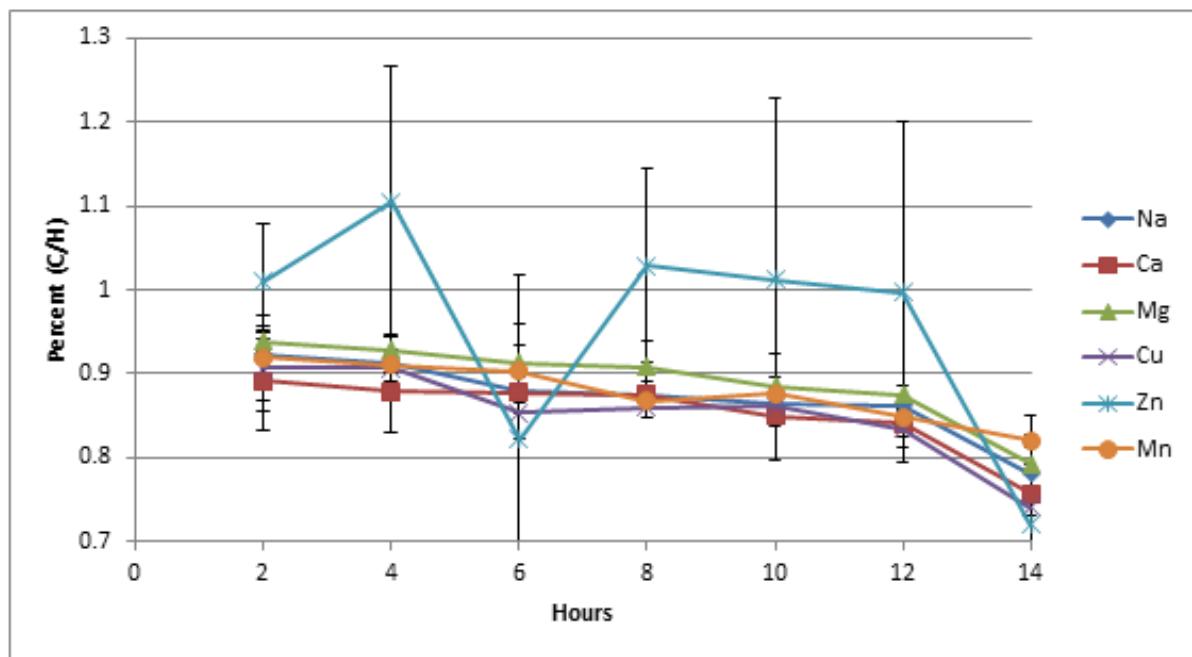


Figure 3-7. Concentration of condensed aerosols divided by reservoir concentrations over time for chlorinated tap water. Error bars represent one standard deviation.

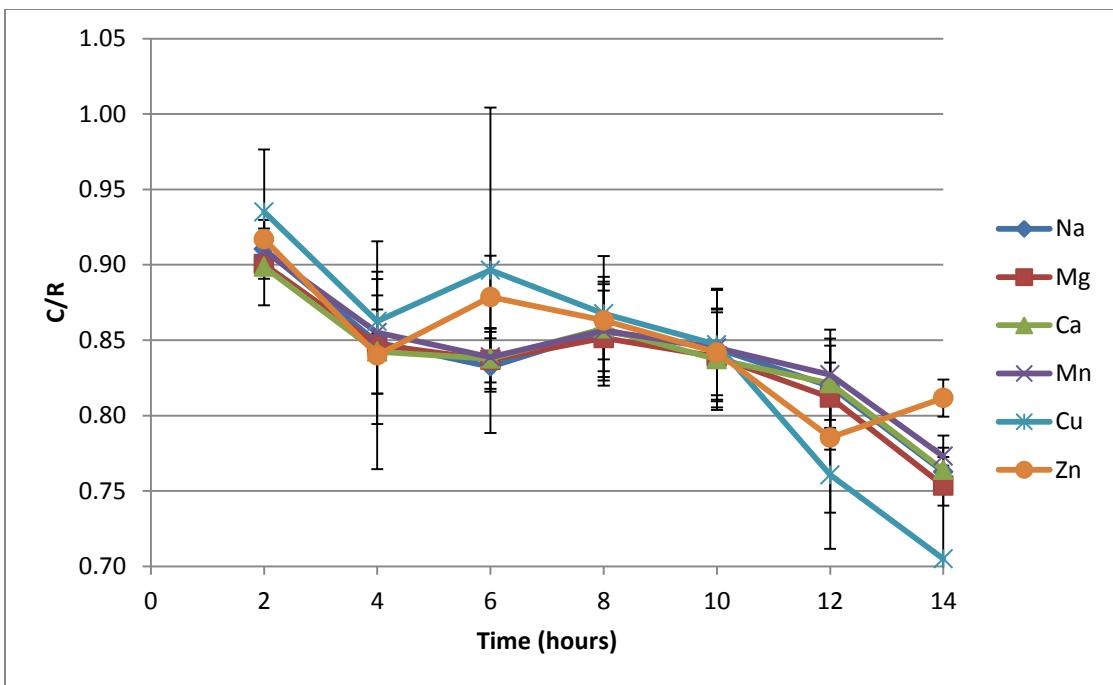


Figure 3-8. Concentration of condensed aerosols divided by reservoir concentrations over time for dechloraminated tap water. Error bars represent one standard deviation.

To analyze for trends, the data from 2-12 hours were analyzed. This data range was selected as it represented the bulk of the cycle time where the temperature was stable and it also avoided the variability in the time for completion of cycle (i.e., >12-14 hr) and the steep decline at the end of the cycle. The slopes of all metals that did not precipitate were averaged for each source water in order to develop a trend. Statistical analysis demonstrated that for all waters the mean slope values were negative. With the exception of Al, which provided the max value for chlorinated tap water, all slopes for all non-precipitating metals were negative. A one-way ANOVA showed no statistical difference in slope between the five water types with $p=0.182$ when comparing slopes of non-precipitating metals within their water type.

Water Type	Slope (hr ⁻¹)		
	Mean	Minimum	Maximum
Chlorinated Tap Water	-0.0116	-0.0767	-0.010 (0.0027)*
Chloraminated Tap Water	-0.0104	-0.0196	-0.0024
Dechloraminated Tap Water	-0.0094	-0.0326	-0.0029
High Hardness Well Water	-0.0708	-0.5037	-0.0038
High Iron Well Water	-0.0075	-0.0125	-0.0001

Table 3-4. Trend analysis for all non-precipitating metals based on the ratio of condensate/reservoir concentrations for time 2-12. *Single run of Al gave a positive slope.

3.4.5 Effect of Continuous use on Ultrasonic Humidifier Performance

To test if there is significant accumulation of metals in the reservoir if the humidifier base is not emptied after daily use a single humidifier was allowed to complete five cycles for a total of 70 hours. At the end of each 12 to 14 hour cycle the humidifier was not emptied, but the reservoir was filled with three liters of chloraminated tap water at the start of each new day. The amount of water remaining in the humidifier reservoir was 159.2 ± 27.2 mL. The initial reservoir concentrations for each day showed a net increase across all metals, but the final reservoir concentration at hour 70 was not significantly higher than any other final day concentration when evaluated with a paired t-test and an $\alpha=0.05$. Three major representative metals are presented in Figure 3-9. Three minor representative metals are presented in Figure 3-10.

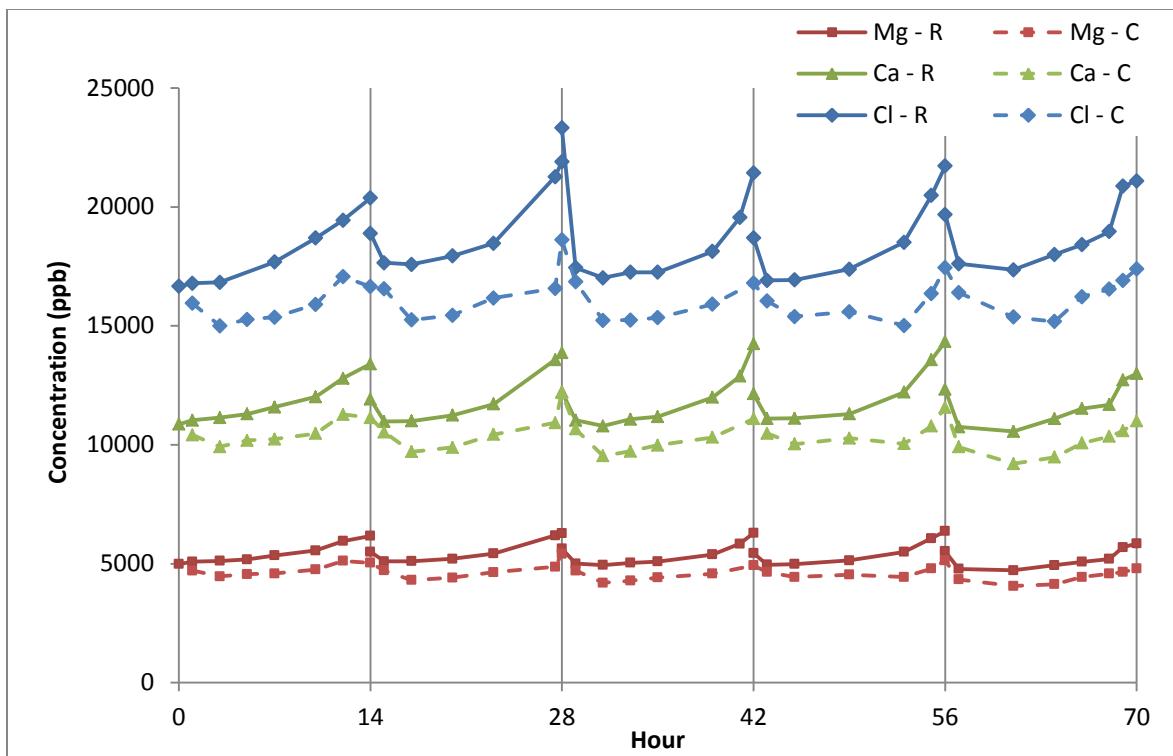


Figure 3-9: Five consecutive 14 hour cycle experimental results for selected major metals. Chloraminated tap water augmented with 500 ppb Mn and 30 ppb Pb. Vertical gridlines denote humidifier reservoir refill.

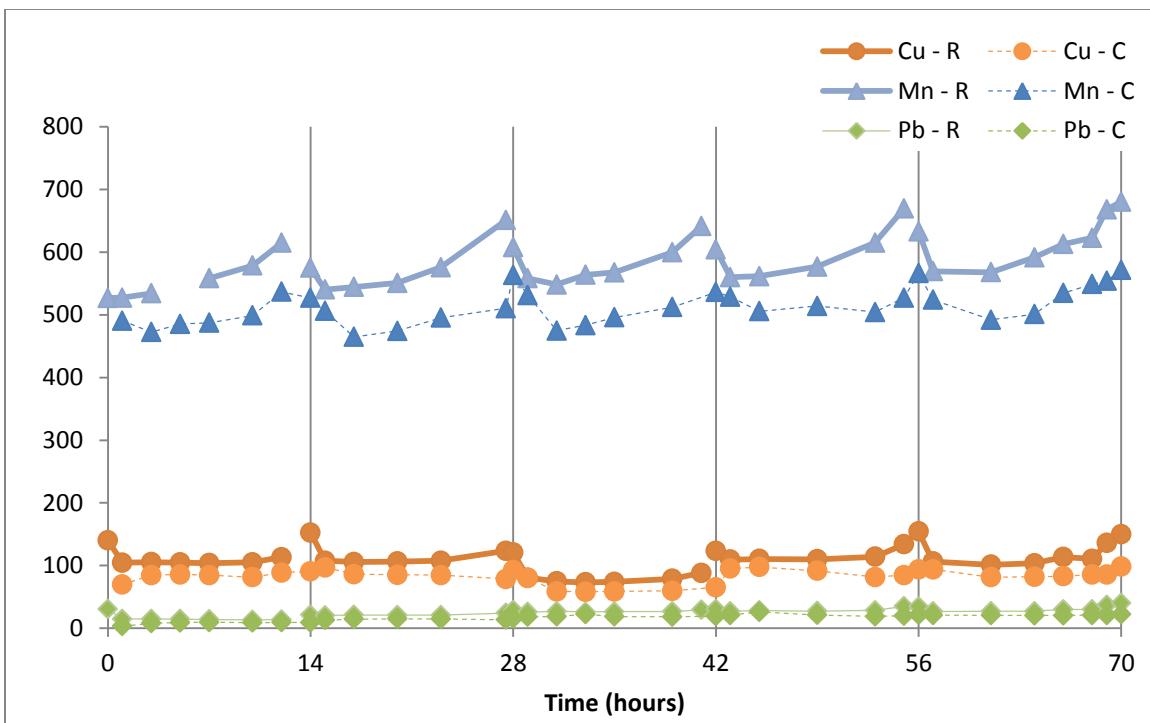


Figure 3-10. Five consecutive 14 hour cycle experimental results for selected minor metals. Chloraminated tap water augmented with 500 ppb Mn and 30 ppb Pb. Vertical gridlines denote humidifier reservoir refill.

3.5 Discussion

In all experiments, the ultrasonic humidifier produced aerosols that when condensed, contained minerals that were present in the source water. The ability of ultrasonic humidifiers to produce inhalable aerosols that contain metals indicates that they serve as an inhalation exposure source to drinking water constituents (Highsmith, 1992). The concentrations of metals in the humidifier reservoir increase over time. The concentrations in the condensate initially decrease, but between 2 and 4 hours begin to increase and maintain a concentration that is about 85-90% of the reservoir concentration, before declining at the end of the cycle to ~70% (Figure 3-7 and Figure 3-8). The 85-90% relationship between the source water and condensed aerosols found in this paper is similar to the 90% relationship for TDS reported previously for a single time point at 0.5 hr (Highsmith, 1992). This study, however, demonstrates that there is a decreasing trend in the percentage of metals expelled over time.

The concentrations of metals in the condensed aerosols decreased during the first ~2 hours of operation as can be seen in Figure 3-6. The initial decrease and subsequent stabilization and increase in the condensate concentration align with the temperature increase in the humidifier reservoir that stabilizes after 1.5 to 3 hours of operation. The concentration effect seen in the humidifier reservoir over time has precedent. In ultrasonic nebulizers used as delivery systems for aqueous drugs, increased temperature led to increased drug solution concentrations (Steckel & Eskandar, 2003).

The initial concentrations of the metals in the source water appear to have little impact on the proportion of metals that are expelled unless those metals are present at concentrations that allow for precipitation. The potential for precipitation is an important distinction seen when comparing the five different water types. If precipitation occurs, as it did in the high hardness and high iron waters in Figure 3-6, the concentration of the metals in the humidifier reservoir does not follow the pattern of an increase in concentration over time. The concentrations of metals in the humidifier reservoir increase and decrease somewhat erratically. As total concentration of a metal increases in the reservoir it is more likely to approach its solubility limit in solution so the chance for precipitation to occur can increase over the course of the operation of the humidifier. Additionally, research has shown that ultrasonic vibrations increase the rate of calcium carbonate precipitation proportional to ultrasonic intensity (Nishida, 2004). The dependence on ultrasonic intensity suggests that further testing of household humidifiers at different output rates may be necessary.

There is evidence of a concentrating effect of the metals in the humidifier reservoir over time. Due to the relationship between the reservoir concentration and the condensate concentration, it is possible for the final condensate concentration to exceed the initial source water concentration. This is especially important to note for metals like lead and manganese with known toxic effects when inhaled because higher exposures could result in negative health impacts (Agency for Toxic Substances and Disease Registry (ATSDR), 2008). The evidence that 85-90% of source water metals are aerosolized except in cases of precipitation, when combined with information from previous studies finding that

aerosols produced by ultrasonic humidifiers are within the inhalable particle range, indicate that single room humidifiers are a source of inhalation exposure to aerosolized metals (Highsmith, 1992; Mohan et al, 1998). This exposure can be expected to be 85-90% of humidifier reservoir concentration over time, and can potentially exceed the concentrations found in the source water as both the humidifier and condensate concentrations increase over time.

There is not significant evidence that neglecting to empty the lower reservoir between reservoir refills will result in a build-up of major or minor metals over the course of 5 days. This is likely due the 3 liters of source water used to refill the humidifier reservoir diluting the metals present in the average 159.2 ± 27.2 mL of water remaining in the reservoir from the previous cycle. Examination of the reservoir concentration curves at the start of each 14 hour cycle support this assumption. The curves show a decrease in metals concentration in the humidifier reservoir during the first two hours after refilling. This should not indicate that there is no harm in neglecting to properly empty and clean the humidifier reservoir as there are also potential health concerns due to growth and dispersion of bacteria in ultrasonic humidifiers (Baur et al, 1988; USEPA, 1991; Tyndall et al, 1995).

3.6 Conclusion

Initial water quality does have an impact on the concentrations of major and minor metals in the humidifier reservoir and condensed aerosols. Typically, both major and minor metals increase in concentration in the reservoir over time and initially decrease but experience an overall increase in condensed aerosols. This indicates that if potentially hazardous metals like manganese or lead are in the source water, it will be expelled as an aerosol at similar concentrations.

Overall, the condensate concentrations are 85-90% of the humidifier reservoir concentration at the corresponding time. One important exception is in the case of precipitation. If precipitation of minerals occurs in the reservoir the concentrations

present in the humidifier reservoir and condensed water aerosols do not follow typical patterns. Major and minor minerals behave similarly.

There does not appear to be a sustained concentrating effect on minerals over the course of 70 hours with four refills of the reservoir after the initial fill. Source waters with lowest possible levels of metals and other impurities should be used in ultrasonic humidifiers to reduce inhalation exposure and protect human health.

3.7 References

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Appendix A. Supplemental Information

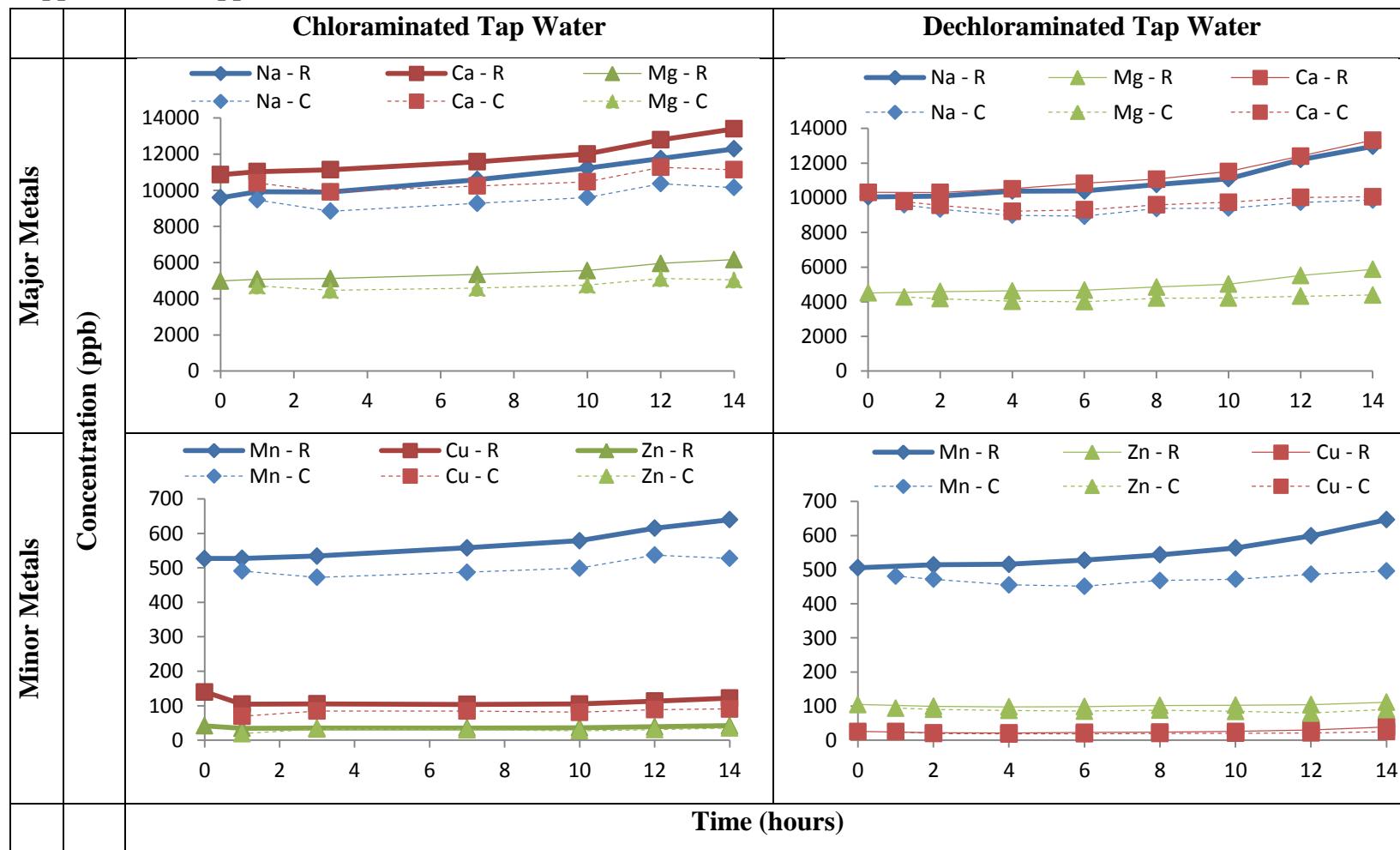


Figure A-1. Representative Major and Minor Metal Concentrations Over Time for a Single Replicate by Water Type. R denotes reservoir sample, C denotes condensate sample.

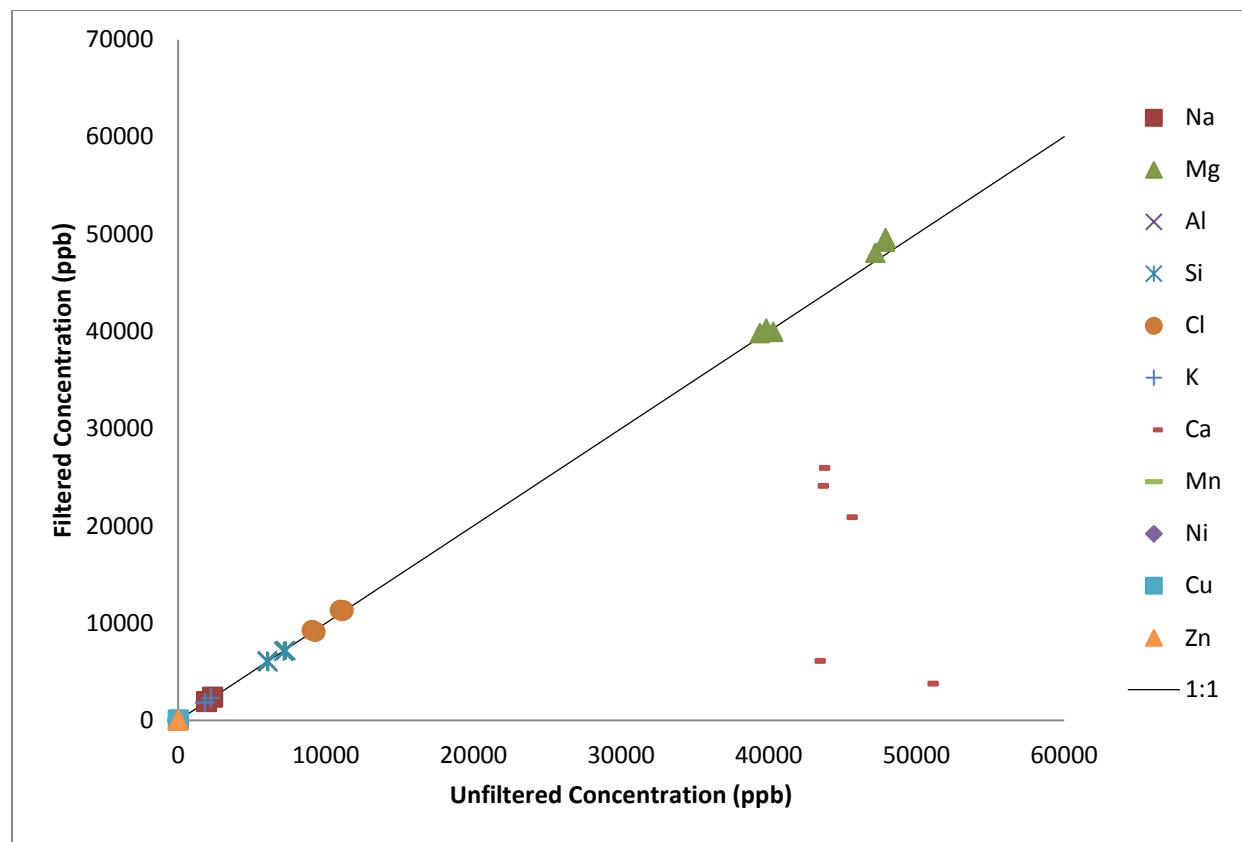


Figure A-2. Concentrations of metals in filtered and unfiltered reservoir samples for high hardness well water.

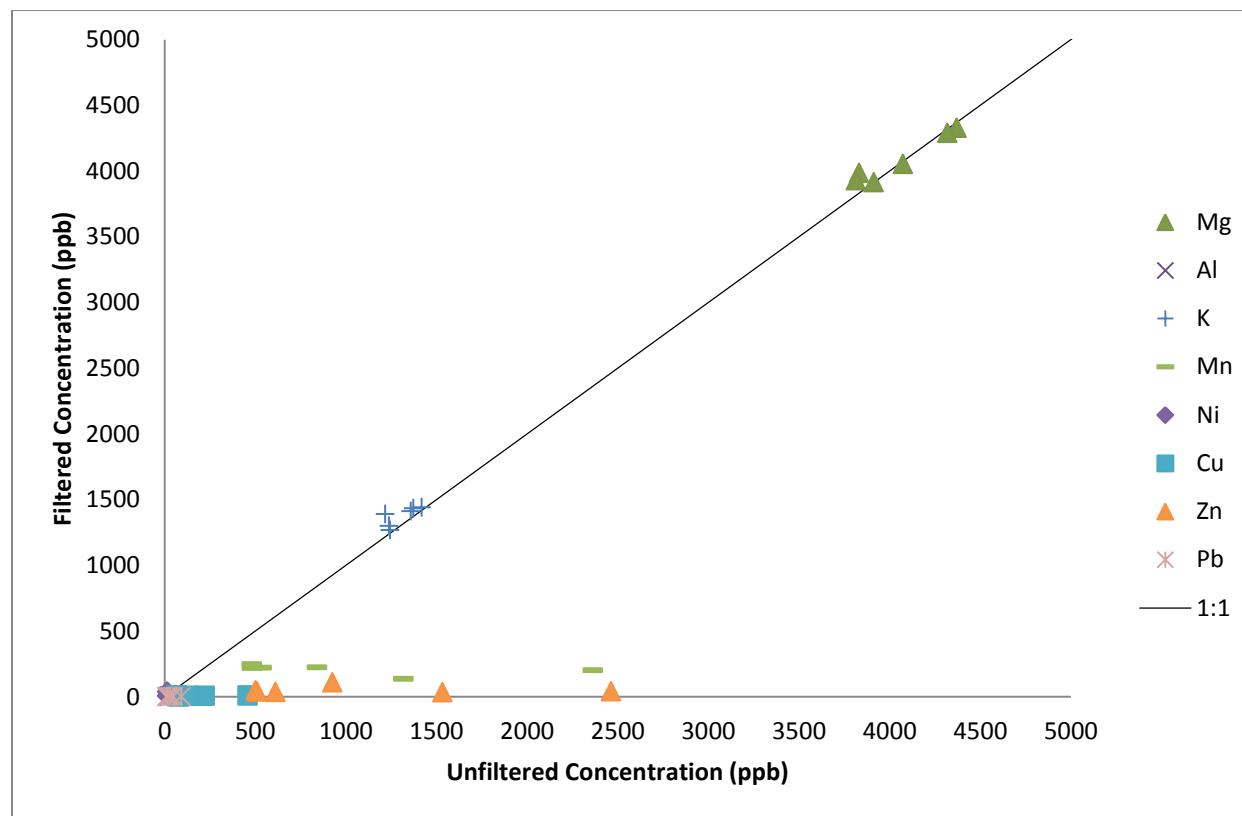


Figure A-3. Concentrations of metals in filtered and unfiltered reservoir samples for high iron well water; metals with concentrations < 5000 ppb.