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EVALUATION OF HEAVY METAL TOXICITY THRESHOLDS FOR THE NITRIFICATION PROCESS IN ACTIVATED SLUDGE SYSTEMS AT THE CALUMET AND STICKNEY WATER RECLAMATION PLANTS: LITERATURE REVIEW AND DATA ANALYSIS

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LIST OF ABBREVIATIONS

3,5-DCP	3,5-dichlorophenol
Ag	silver
AOBs	ammonia-oxidizing bacteria
As	arsenic
ATP	adenosine triphosphate
ATU	allylthiourea
Ca	calcium
Cd	cadmium
Co	cobalt
Cr^{+6}	hexavalent chromium
Cr^{Total}	total chromium
Cu	copper
District	Metropolitan Water Reclamation District of Greater Chicago
DO	dissolved oxygen
DTPA	diethylenetriaminepentaacetic acid
EBPR	enhanced biological phosphorus removal
EDTA	ethylenediaminetetraaceticacid
Fe	iron
HCN	hydrocyanic acid
Hg	mercury
Hg-nano	low-level mercury
HRT	hydraulic residence time
HSOM	high-strength organic materials
ICP-MS	inductively coupled plasma mass spectroscopy
ISO	International Organization for Standardization
L	liter
LAS	linear alkylbenzenesulphonate
Mg	magnesium
mg	milligram
ML	mixed liquor
mL	milliliter
mM	millimole
N_2	nitrogen
NH_3	ammonia
NH_3-N	ammonia nitrogen
NH_4	ammonium
Ni	nickel
NO_2^--N	nitrite nitrogen
NO_3^--N	nitrate nitrogen
NOBs	nitrite-oxidizing bacteria
NTA	nitrilotriacetic acid
OECD	Organisation for Economic Co-operation and Development
OUR	oxygen uptake rate
P	phosphorus

LIST OF ABBREVIATIONS (Continued)

Pb	lead
ppm	parts per million
POTW	publicly-owned treatment works
SOUR	specific oxygen uptake rate
SRT	solids retention time
TCE	trichloroethylene
USEPA	United States Environmental Protection Agency
V. fischeri	*Vibrio fischeri*
VFAs	volatile fatty acids
WRP	water reclamation plant
WS	Stickney Westside
WWTP	wastewater treatment plant
Zn	zinc
µg	microgram

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DISCLAIMER

Mention of proprietary equipment and chemicals in this report does not constitute endorsement by the Metropolitan Water Reclamation District of Greater Chicago.

EXECUTIVE SUMMARY

The Metropolitan Water Reclamation District of Greater Chicago (District) has been actively pursuing an energy neutrality goal through additional biogas production via co-digestion of external high-strength organic materials (HSOMs). The District is also exploring the potential of fermenting of these HSOMs for providing the carbon needed for enhanced biological phosphorus (P) removal (EBPR). The impact of heavy metals in these HSOMs on the activated sludge process is critical as inhibition of mainstream biological systems, especially nitrifiers, is a concern. This is the focus of this literature review and data evaluation.

This paper study included a literature review of District and non-District studies with respect to metal toxicity in the activated sludge process and an evaluation of historical plant-specific data as a first step towards developing site-specific heavy metal threshold limits for the Calumet and Stickney Water Reclamation Plants (WRPs). These limits need to be established to formulate the guidance criteria for the acceptance of HSOMs.

Based on a data survey from 239 publicly-owned treatment works (POTWs) (United States Environmental Protection Agency [USEPA], 1981), 16 heavy metals are commonly monitored due to their toxicity to the nitrification process in activated sludge and other biological processes such as anaerobic digestion. Through a literature review, inhibition mechanisms, metal toxicity measurement techniques, and dose-and-effect of heavy metals on the nitrification process or on biological treatment systems were reviewed and documented in this report. There are a number of limitations to these studies with respect to the objective of deriving District site-specific heavy metals toxicity thresholds guidance: (1) the studies conducted used various methods and measurement techniques to quantify inhibition; (2) most of the heavy metal toxicity studies assessed nitrification inhibition due to a single heavy metal and do not provide sufficient or clear guidance about the effect of combined metals; (3) almost all studies were performed at laboratory scale; (4) there could be an adaptation of the microorganisms to elevated metals whereby they can tolerate higher metal concentrations; and (5) all the studies performed used either synthetic activated biomass or activated sludge obtained from different plants.

Table 1 summarizes the heavy metal inhibition limits obtained from the USEPA's local limit guidance, non-District studies, and the District study, which clearly shows that there is wide range of inhibitory limits suggested for each metal and that these concentrations may not be applicable for the site-specific conditions at the Calumet and Stickney WRPs without further refinement through a study.

A historical data analysis was performed examining the metal and ammonia (NH_3) concentrations for the Stickney and Calumet WRP plant influent and effluent for 2012–2014. Concentration data for the eight monitored metals at the WRPs were analyzed for two scenarios: one with no heavy metals removal in primary treatment and the other with assorted percent removals of heavy metals in primary treatment. The heavy metals removal efficiencies through primary treatment are based on the previous District work performed on re-evaluation of local pretreatment limits (Monitoring and Research Department Report No. 14-58, Table AV-2). The influent metal concentrations to the aeration tanks were compared to three inhibition threshold values for both scenarios. The number of and percent of daily exceedances

TABLE 1: HEAVY METAL INHIBITION THRESHOLD LEVELS FOR NITRIFICATION PROCESS

Metal	Reference					
	USEPA, 2004[1] (mg/L)	Stasinakis et al., 2003[1] (mg/L)	Semerci et al., 2007 (mg/L)	Hu et al., 2001 (mg/L)	MWRDGC Patel et al., 2007[2] (mg/L, %)	Hartmann et al., 2013[2] (mg/L, %)
As	1.5 {0.1}	—	—	—	—	—
Cd	5.2 {1–10}	—	2–15[3]	—	10, 57.0% [15.8%]	— 10, [2%] 50, [5%] 150, [20%] 500, [45%] 4,000, [76%]
Cr^{+6}	1–10 as $(CrO_4)_2$	0.5 {≥5}	—	—	—	— 10, [52%] 50, [79%] 150, [85%] 500, [91%] 4,000, [93%]
Cr^{+3}	— {10–50}	—	—	—	—	—
Cr^{Total}	0.25–1.9 {1–100}	—	—	—	10, 89.0% [55.3%]	—

TABLE 1 (Continued): HEAVY METAL INHIBITION THRESHOLD LEVELS FOR NITRIFICATION PROCESS

Metal	Reference					
	USEPA, 2004[1] (mg/L)	Stasinakis et al., 2003[1] (mg/L)	Semerci et al., 2007 (mg/L)	Hu et al., 2001 (mg/L)	MWRDGC Patel et al., 2007[2] (mg/L, %)	Hartmann et al., 2013[2] (mg/L, %)
Cu	0.05–0.48 {1}	—	—	—	10, 68.1% [25.0%]	— 10, [0%] 50, [1%] 150, [10%] 500, [48%] 4,000, [89%]
Pb	0.5 {1–100}	—	—	—	10, 60.9% [5.9%]	—
Ni	0.25–5 {1–5}	—	—	58.7–88.05	10, 73.2% [25.9%]	— 10, [5%] 50, [13%] 150, [16%] 500, [45%] 4,000, [47%]

TABLE 1 (Continued): HEAVY METAL INHIBITION THRESHOLD LEVELS FOR NITRIFICATION PROCESS

Metal	Reference					
	USEPA, 2004[1] (mg/L)	Stasinakis et al., 2003[1] (mg/L)	Semerci et al., 2007 (mg/L)	Hu et al., 2001 (mg/L)	MWRDGC Patel et al., 2007[2] (mg/L, %)	Hartmann et al., 2013[2] (mg/L, %)
Zn	0.08–0.5 {0.3–10}	—	—	65.4–98.1	10, 8.0% [-10.75%][4]	—

[1]Total pollutant inhibition concentrations, unless otherwise indicated. Values in curly brackets represent the heavy metal inhibition threshold levels to activated sludge total (heterotrophs and nitrifiers).

[2]"%" value indicates percent inhibition to corresponding inhibitory metal concentration; values inside the square brackets were measured as percent SOUR inhibition and outside the square brackets as ammonia inhibition.

[3]50% inhibition at total Cd concentration of 2–2.5 mg/L, whereas total Cd concentration of 15 mg/L showed about 30–95% inhibition.

[4]Patel, et. al (2007) observed that 10 mg/L zinc concentration improved SOUR by 10.7% but deteriorated the nitrification rate by 8%. This was perhaps attributable to the background concentration of Zn at 4.40 mg/L which could have acclimated the Stickney activated sludge to higher concentrations of Zn.

were counted and grouped by year and for the period of 2012–2014. Nitrification efficiencies expressed as percent ammonia nitrogen (NH_3-N) removal were calculated for the three preceding and three following days as well as the days on which the daily metal concentrations exceeded the inhibitory threshold values to determine the impact on the nitrification process.

At the Stickney WRP, none of the metal concentrations exceeded their respective toxicity thresholds for nitrification for cadmium (Cd), lead (Pb), hexavalent chromium (Cr^{+6}), and nickel (Ni), while arsenic (As) and total chromium (Cr^{Total}) marginally exceeded their respective threshold values. However, for both copper (Cu) and zinc (Zn) with no removal in primary treatment, percent exceedance was 90.7 and 99.0, respectively, for the low threshold values and 7.2 and 25.0, respectively, for the high threshold values. Analysis of the data indicated that most of the exceedances were for a single metal rather than a combination of metals.

At the Calumet WRP, none of the metal concentrations exceeded their respective toxicity thresholds for nitrification for As, Cd, Cr^{Total}, Pb, Ni, and Cr^{+6}. However, for both Cu and Zn with no removal in primary treatment, percent exceedance was 17.9 and 63.5, respectively, for the low threshold values and 0.0 and 2.2, respectively, for the high threshold values. Like Stickney, analysis of the data indicated that most of the exceedances were for a single metal rather than a combination of metals.

For both the Stickney and Calumet WRPs, an increase or decrease in nitrification efficiency was calculated in relation to average nitrification removal efficiencies around the metal exceedance days. The total metal concentration daily exceedances above the respective threshold levels were 193 at the Stickney WRP from 2012 through 2014; however, none of these exceedances caused a severe impact on the nitrification process. Of these 193 exceedances at the Stickney WRP, only 27 exceedances coincided with a five percent or more decrease in NH_3-N removal efficiency compared to the average NH_3-N removal efficiencies of the three preceding days. Further, in 24 of the 27 exceedances, nitrification recovered within the next three days without any compromise of the NH_3-N removal efficiency. The total metal concentration daily exceedances above the respective threshold levels were 146 at the Calumet WRP from 2012 through 2014; however, none of these exceedances caused a severe impact on the nitrification process. Of these 146 exceedances at the Calumet WRP, only 12 exceedances coincided with a five percent or more decrease in NH_3-N removal efficiency compared to the average NH_3-N removal efficiencies of the three preceding days. Further, in 11 of the 12 exceedances, nitrification recovered within the next three days without any compromise of the NH_3-N removal efficiency.

Based on data analysis for both plants, it should be noted that the toxicity thresholds used in the analysis should be considered conservative, since exceedances of these thresholds at both plants generally did not result in inhibition of nitrification.

The evaluation in this study indicated the toxicity thresholds reported in the USEPA document cannot be applied directly at the Stickney and Calumet WRPs. The site-specific toxicity limits may be derived through bench- or pilot-scale tests. However, for the purpose of implementing the HSOM program in the near future, the site-specific metal toxicity limits may be estimated with the consideration of both reported thresholds and the results of the data evaluation presented in this report.

INTRODUCTION AND BACKGROUND

The Engineering department has been actively pursuing an energy neutrality goal through additional biogas production via co-digestion of external HSOMs. Generally, such wastes contain very high carbon contents that may be utilized to generate additional biogas by co-digestion and/or volatile fatty acids (VFAs) by a fermentation process for biological P removal.

The impact of HSOMs should be carefully evaluated for constituents of concern. In a broader sense, the constituents of concern include those impurities in HSOMs that can (1) inhibit digester performance, (2) result in digester failure due to toxicity, (3) result in non-compliance with the 40 Code of Federal Regulations Part 503 Standards for the Use or Disposal of Sewage Sludge, (4) inhibit the mainstream biological process, or (5) result in failure of the mainstream biological process due to toxicity. Mainstream biological processes include nitrification by autotrophic bacteria, dissolved and colloidal organic matter removal by heterotrophic bacteria, denitrification by heterotrophic bacteria, and biological P removal by phosphate-accumulating organisms. In the future, the mainstream biological process may also include N removal by heterotrophic and/or ANAMMOX® bacteria.

Fermentation of HSOMs into VFAs can certainly augment and potentially satisfy the needed VFAs for the carbon-deficit EBPR processes at the Stickney and Calumet WRPs. For this particular application, the impact of heavy metals in HSOMs on the activated sludge process is critical. Additionally, if the HSOM is used for co-digestion as suggested above, the post-digestion solids processing streams are generally recycled back to the headworks, which can exert additional stress on the mainstream activated sludge process with respect to heavy metals.

A literature review of District and non-District studies with respect to metal toxicity in the activated sludge process and an evaluation of historical plant-specific data were performed as a first step towards developing site-specific heavy metal threshold limits for these two plants. These limits need to be established to formulate the guidance criteria for the acceptance of the HSOMs.

LITERATURE REVIEW

Significance of Heavy Metals in Nitrification and Denitrification Processes

According to the USEPA (1981), it is important to study heavy metals loading to POTWs due to the (1) possible inhibitory effects on biological treatment, (2) high effluent concentrations entering the receiving water bodies, and (3) land disposal of treated biosolids.

In biological treatment of wastewater, the organic waste is treated with the aid of mixed cultures of microorganisms in either the presence or absence of oxygen. Nitrification and denitrification processes are used to minimize N loads to the receiving local water bodies. Nitrification is the process which involves conversion of NH_3-N to nitrite nitrogen (NO_2^--N) using NH_3-oxidizing bacteria (AOBs) and then to nitrate nitrogen (NO_3^--N) using NO_2-oxidizing bacteria (NOBs). Denitrification involves conversion of NO_3 to N gas (N_2) in the presence of heterotrophic facultative bacteria. The growth rate of nitrifying bacteria is very slow and highly dependent on pH, dissolved oxygen (DO), temperature, and toxic chemicals. Hence, nitrification is generally considered as the rate-limiting step in biological N removal. Inflow of inorganic and organic chemical compounds (toxic or non-toxic in nature) can have a significant impact on AOBs and NOBs resulting in inhibition or loss of nitrification capacity depending upon the types of chemical compounds and their concentrations. Nitrifying bacteria are susceptible to inhibition of inorganic wastes such as heavy metals, cyanide, free NH_3, free nitrous acid, halogenated compounds, phenols, hydantoins, mercaptans, and thiourea (Gerardi, 2002). The nitrification rate is very important in NH_3-N treatment as it determines the minimum solids retention time (SRT) required for complete nitrification during steady state conditions (USEPA, 1981).

AOBs, and nitrifiers in general, are the most sensitive microorganisms in mainstream activated sludge treatment and can be affected by a wide range of organic and inorganic compounds. They are affected by concentrations well below those that would affect aerobic heterotrophic bacteria (Metcalf and Eddy, 2014; Hu et al., 2003). Similarly, the bacteria responsible for biological P removal can also be inhibited by various compounds, though they as well are not as sensitive to inhibition as the nitrifiers (Randall and Chapin, 1997). Inhibition can be either acute (short-term) or chronic (long-term). As such, inhibition of the nitrifiers is the limiting factor towards understanding how potential toxins in processed HSOMs will affect the mainstream biological system and is the focus of our literature review and study.

Heavy Metals in Wastewater

Table 2 lists the dominant, intermediate, and less frequent heavy metal species typically found in POTWs of the United States. These are the heavy metals that are typically monitored in raw wastewater and thought of when discussing heavy metal toxicity based on a data survey from 239 POTWs (USEPA, 1981).

The toxicity of these metals mainly depends on their solubility in the wastewater along with environmental conditions such as concentration of the toxin and species plus other operating parameters such as SRT, hydraulic residence time (HRT), and other influent characteristics. Table 3 lists the concentrations of heavy metals inhibitory to nitrification and the activated sludge process based on the 2004 USEPA Local Limits Development Guidance

TABLE 2: INFLUENT HEAVY METAL SPECIES TYPICALLY PRESENT IN PUBLICLY OWNED TREATMENT WORKS

Dominant	Intermediate	Less Frequent
Cd*	As*	Aluminum (Al)
Cr^{Total}*	Iron (Fe)	Cobalt (Co)
Cu*	Manganese (Mn)	Molybdenum (Mo)
Pb*	Hg	Se
Ni*	Ag	Tin (Sn)
Zn*		

*Inhibitory concentrations are presented in this report.
Source: USEPA, 1981.

TABLE 3: INHIBITION THRESHOLD LEVELS[1]

Heavy Metal	Concentration (mg/L)[2,3]	
	Nitrification	Activated Sludge Process
As	1.5	0.1
Cd	5.2	1–10
Cr^{+6}	1–10 (as $[CrO_4]^{2-}$)	1
Cr^{+3}	—	10–50
Cr^{Total}	0.25–1.9	1–100
	1–100 (Trickling Filter)	—
Cu	0.05–0.48	1
Pb	0.5	1.0–5.0
	—	10–100
Ni	0.25–0.5	1.0–2.5
	5	5
Zn	0.08–0.5	0.3–5
	—	5–10

[1]Source: USEPA, 2004.
[2]Concentration thresholds are from different sources.
[3]Total pollutant inhibition levels, unless otherwise indicated.

(USEPA, 2004); this refined list is based on metals most common to POTWs and District facilities.

Inhibition Mechanism

Past studies have indicated that heavy metal inhibition to nitrification is mainly based on the uptake of free metal cations through a nonspecific metal inorganic transport system (Hu et al., 2001). According to the free-ion-activity model, heavy metal inhibition to nitrification can be linked to free metal cation concentrations and their transport across the cell membrane to establish a pseudoequilibrium between the bulk solution and microbial surface (Hu et al., 2002). According to Ong et al. (2010), extrapolymeric substances are responsible for biosorption of metals into the activated sludge via exchange mechanisms, complexation with negatively charged groups, adsorption, and precipitation. Heavy metals alter the cell structure of the microorganisms and in turn have an adverse effect on their metabolic activities such as respiration and growth. These metals can interact with thiol groups and may interfere and inhibit functions of the respective cations (for example, Cd with Zn or calcium [Ca]; Ni with iron [Fe]; Zn with magnesium [Mg]) (Hu et al., 2001). This will automatically lower the removal efficiency of the biological process and may affect the effluent concentrations of NH_3-N.

Metal Toxicity Measurement Techniques

Techniques such as respirometry by measuring oxygen uptake rate (OUR) and specific OUR (SOUR), adenosine triphosphate (ATP) luminescence, *Vibrio fischeri* (*V. fischeri*) bioassay, inductively coupled plasma mass spectroscopy (ICP-MS) for heavy metal analysis, pure cultures of *Nitrosomonas* and *Nitrobacter*, stress protein assay, nitrification inhibition, enzymatic inhibition, and effluent turbidity can be used to assess the toxicity of pollutants in activated sludge nitrification.

Nitrification inhibition is a toxicity bioassay used to determine the toxicity of the wastewater stream. In this method, the effect of toxic species on the conversion of NH_3 to NO_2 and NO_2 to NO_3 is measured.

Respirometry has been particularly used to assess the inhibitory effects of available toxic components on heterotrophic and nitrifying bacteria. It is considered as a more direct way for measuring sludge activity in the presence of toxic substances compared to bioluminescence. The test setup consists of a reactor cell equipped with a magnetic stirrer, thermometer and injector needle, a DO probe, and a recorder; oxygen uptake from the reactor cell is measured over time. The nitrifying organisms are selectively inhibited using allylthiourea (ATU), from that of heterotrophs. Respiration rates for nitrifiers are recorded before and after adding the wastewater sample to be tested to an activated sludge biomass. Inhibition is indicated by the 50 percent inhibition of the respiration rate of microorganisms in a given wastewater (Hartmann et al., 2013). If no change is observed in the respiration rates after adding the sample, the sample can be classified as causing no acute toxicity (Kroiss et al., 1992). On the other hand, if the respiration rate decreases (either due to gradual toxic loads or due to shock loads), the sample can be classified as causing immediate inhibition to nitrifiers.

The ATP luminescence method involves the use of an ATP luminescence bioassay to determine ATP content of the test organisms in activated sludge with a toxicant. Dalzell et al. (2002) evaluated the toxicity using nitrification inhibition, respirometry, ATP luminescence, enzyme inhibition, and a *V. fischeri* bioassay; however, nitrification inhibition and *V. fischeri* were found to be most sensitive bioassays based on the test results.

Heavy Metal Inhibition to Biological Treatment of Wastewater – Literature Survey

Generally, trace amounts (µg/L) of many heavy metals (Cu, Zn, Pb, Ni, cobalt [Co]) etc. may be needed as micronutrients as they can aid in enzymatic activities of the microbes (Hartmann et al., 2013). Microorganisms may acclimate at lower metal concentrations; however, shock loadings of heavy metals can cause a serious upset in biological treatment of wastewater. The toxicity of mercury (Hg), Ni, and Cd shock loads of around 20 mg/L on activated sludge depends on the metal (with Hg being the most toxic), the SRT, and the specific metal adsorption site on the microbial flocs (Ong et al., 2010). The type and extent of the upset event will indicate whether the process will recover fully or not. Past studies have indicated that most of these heavy metals cause deflocculation, as a result of an upset event due to shock loads (Kelly et al., 2002).

It is not uncommon that ligands/chelating agents such as ATU, ethylenediaminetetraaceticacid (EDTA), diethylenetriaminepentaacetic acid (DTPA), hydrocyanic acid (HCN) and ethylenediamine are used to evaluate the activity of free metal ions via formation of metal-ligand complexes (classified as labile or non-labile). Among these chelating agents, several studies have indicated that ATU and HCN inhibit nitrification due to Cu complexation at concentrations of <5 mg/L (complete inhibition) and 0.1–0.2 mg/L (50 percent inhibition), respectively, whereas EDTA and DTPA both showed lower biodegradability in municipal wastewater and were found in the effluent as high as 0.5 mg/L (Hu et al., 2003).

There is an increasing concern over the fate of silver (Ag) nanoparticles in sewage sludges. Ag nanoparticles are widely used in consumer products due to their antibacterial activity. According to a recent laboratory-scale study, the toxicity of Ag nanoparticles on nitrifying bacteria is substantially reduced once they form Ag sulfide complexes/precipitates with sulfide added to the system (Kim, et al., 2010). According to Kaegi et al. (2011), Ag nanoparticles caused strong inhibition compared to dissolved Ag or AgCl particles during growth experiments with nitrifiers.

Many researchers have evaluated possible heavy metal inhibition to biological wastewater systems. Stasinakis et al. (2003) performed an experiment to study the inhibition of Cr^{+6} addition on the activated sludge process from two continuous flow laboratory-scale activated sludge plants (Plant A as a control plant and Plant B as a test plant with no industrial wastes). Sludge samples from Plant B received different concentrations of Cr^{+6} as 0.5, 1, 3, and 5 mg/L. Inhibition to nitrification was evaluated based on the nitrification rates during various operating periods and normalized at 20°C. Experimental results showed that a Cr^{+6} concentration of 0.5 mg/L significantly inhibited nitrification causing up to a 74 percent decrease in NH_3 removal efficiency. However, removal of organic substrate was not largely impacted by Cr^{+6} addition (up to 5 mg/L), which shows that heterotrophic biomass was less sensitive than nitrifiers. When Cr^{+6} addition was terminated, nitrification was partially recovered up to 57 percent 12 days after ceasing Cr^{+6} addition. Ultimately, shock loading of 5 mg/L of Cr^{+6} for two

days resulted in significant nitrification inhibition with reduced numbers of filamentous microorganisms, whereas organic removal remained unaffected.

Semerci et al. (2007) investigated the influence of Cd speciation on possible nitrification inhibition with a nitrifier-rich biomass. Voltammetry and MINEQL+ were employed to measure free Cd^{2+} and theoretical Cd in solution, respectively. Nitrification tests were carried out in a batch reactor containing 3,000 ± 25 mg/L mixed liquor (ML) volatile suspended solids. The inhibitory effect of Cd was examined by the measurement of SOUR, Cd uptake, and ammonium (NH_4) utilization (qNH_4–N) rates in the presence and absence of Cd (1–25 mg/L). EDTA was used to adjust the Cd speciation. Experimental results showed 50 percent inhibition of nitrification bacteria occurred at a total Cd concentration of 2–2.5 mg/L. For a total Cd concentration of 15 mg/L, about 30–95 percent inhibition was observed, which showed poor correlation between total Cd and nitrifiers. Also, addition of EDTA caused a decrease in nitrification inhibition. This study showed that nitrification inhibition depended on equilibrium concentrations of free Cd^{2+}, labile (Cd_{volt}), and biosorbed Cd (Cd_{volt}) and did not correlate with the total Cd (Semerci et al., 2007).

Hu et al. (2001) evaluated the inhibitory effect of Ni (highly soluble in wastewater) and Zn (low solubility in wastewater) on nitrification. A nitrifying rich biomass was used in a continuous laboratory-scale bioreactor, and the oxidation kinetics of nitrifiers were studied using batch respirometric assays after shock loading. Ni speciation was estimated using experimental and computational tools, and metal complexing agents (e.g., EDTA, nitrilotriacetic acid [NTA], citrate, sulfate) were used to determine nitrification inhibition by various Ni fractions. It was observed that both Ni and Zn caused inhibition of NH_3 oxidation; however, both metal species did not cause inhibition to NO_2 oxidation up to total concentrations of 1.5 millimoles (mM) Ni (88.04 mg/L) and 1.5 mM Zn (98.1 mg/L). An increase in Ni and Zn doses caused a decrease in $SOUR_{NH4}$, whereas $SOUR_{NO2}$ was not adversely affected. At a dose of 1 mM each, both Ni (58.7 mg/L) and Zn (65.4 mg/L) inhibited NH_4 oxidation by approximately 30 and 60 percent, respectively (Hu et al., 2001). Addition of EDTA (above 1 mM [372.24 mg/L]) caused a reduction in Ni inhibition; however, nitrification impairment was observed at elevated metal chelating dosages. Shock loads of metal species with 1.7 mM Ni (99.8 mg/L) and 1.0 mM Zn (65.4 mg/L) were added intermittently at predetermined time intervals for 24 hours and then stopped. Being highly soluble, Ni was washed out after seven days, whereas Zn was washed out slowly due to its low solubility in the wastewater. Overall, it was observed that Ni inhibition was primarily due to the free cation species more than total aqueous concentrations.

Hartmann et al. (2013) tested the respiration inhibition of four metals (Cd, Cr, Cu, and Ni) in activated sludge samples from the Ostrava-Privoz Wastewater Treatment Plant (WWTP) and the Hermanice II WWTP. The Ostrava-Privoz WWTP has a high contribution of incoming industrial wastes from the steel industry and coking plants, whereas the Hermanice II WWTP has no industrial inflow. The Ostrava-Privoz WWTP had yearly maximum influent concentrations of 4.72 µg/L Cd, 375 µg/L Cr, 65 µg/L Cu, and 115 µg/L Ni. Activated sludge samples from both plants were collected, and 600 mL aliquots were prepared and maintained at a constant temperature with sufficient aeration during testing. The respiration tests were conducted based on the International Organization for Standardization (ISO) 8192:2007 and Organisation for Economic Co-operation and Development (OECD) 209 – Regulation EC 440 and using a Strathtox respirometer. Stock metals solutions were prepared in distilled water, and each was

diluted to 20 different concentrations as being the relevant metal concentrations causing respiratory inhibition of activated sludge. Tests were carried out in six 20-mL glass tubes in which 2 mL of synthetic sewage, diluted metal solution, and 8 mL of activated sludge were added. The test results as shown below, indicated a varying degree of respiration inhibition at different metal concentrations (4,000 mg/L, 500 mg/L, 50 mg/L, and 10 mg/L) for the sludge samples from Ostrava-Privoz and Hermanice II.

At very high concentrations (4,000 mg/L):

1. Ostrava-Privoz WWTP: $Cr^{+6} > Cu > Cd > Ni$.

2. Hermanice II WWTP: $Cr^{+6} > Cd > Cu > Ni$.

At low concentrations (10 mg/L):

1. Ostrava-Privoz WWTP: $Cr^{+6} > Ni > Cd > Cu$.

2. Hermanice II WWTP: $Cr^{+6} > Cd > Ni > Cu$.

Among all heavy metals investigated, Cr^{+6} was found to be most toxic at all dosing concentrations, whereas Ni was found to be more toxic at low concentrations. Also, it was seen that all tested heavy metals were capable of inhibiting respiration even at low concentrations. Additionally, as with many heavy metals, adaptation can significantly increase microbial tolerance to heavy metals as seen with the Ostrava-Privoz WWTP results compared to the Hermanice II WWTP results.

The ATP luminescence method involves use of an ATP luminescence bioassay to determine the ATP content of test organisms in activated sludge with a toxicant. Dalzell and Christofi (2002) used this method to determine the toxicity of Cr, Zn, and 3,5-dichlorophenol (3,5-DCP) on two sources of domestic activated sludge and one from a laboratory model plant. In this test, a specific volume of a toxicant (0.5 mL) at different concentrations was added to the activated sludge sample (2 mL) in clean polystyrene cuvettes. Cr was dosed at 12.5, 25, 50, 100, and 200 mg/L; Zn was dosed at 1,000, 3000, 10,000, and 30,000 mg/L; and 3,5-DCP was dosed at 3.125, 6.25, 12.5, 25, and 50 mg/L concentrations in addition to an individual control. The samples after incubation for 30 minutes were then analyzed to calculate IC_{20}, IC_{50}, and IC_{80}. pH was monitored at each concentration, but metal toxicant solutions were not pH adjusted. Dose response slopes were plotted, and F-tests were used to compare the slopes in assay analysis. Results of the toxicity test indicated that IC_{50} was easily calculated from dose-response data, but IC_{20} and IC_{80} values could not be estimated using statistical analysis and lay outside the concentration range. This was considered to be a limitation of this toxicity test. The IC_{50} concentrations for Cr, Zn and 3,5-DCP ranged from 21.46 to 24.07 mg/L, 11,699 to 18,655 mg/L, and 10.36 to 11.56 mg/L, respectively, for different sludges. Overall, ATP luminescence was shown to be effective for determining toxicity assays for Cr^{+6} and 3,5-DCP but not for Zn. In general, the source of activated sludge did not appear to affect inoculum response (Dalzell et al., 2002).

Dalzell et al. (2002) evaluated different toxicity bioassay methods: nitrification inhibition, respirometry, ATP luminescence, enzyme inhibition and *V. fischeri* bioassays. This

study was conducted to study the toxicity of a single toxicant, mixed toxicants, and real industrial wastes. An activated sludge sample from a nitrifying plant in Spain (Urduliz WWTP) was obtained for the nitrification inhibition and respirometry bioassays, whereas sludges from Whitburn WWTP (Scotland) and Weingarten WWTP (Germany) were used for ATP luminescence and enzyme bioassays, respectively. Cd, Cr, Cu, and Zn were employed as target metal species; 3,5-DCP as a common reference toxicant; and toluene and trichloroethylene (TCE) as representative organic pollutants. A mixture of heavy metals (Cd = 20 mg, Cu = 100 mg, Cr = 50 mg, Zn = 250 mg, and linear alkylbenzenesulphonate [LAS] = 500 mg) was used for a toxicity assessment using the different methods mentioned above. Each test, carried out in duplicate, consisted of five different doses of chosen toxicant. The protocol for the nitrification test was referred from Arvin et al. (1994). This test is mainly based on the effect of toxicants on the conversion of NH_4 to NO_3. Inhibition is indicated by reduction in NO_3 levels and accumulation of NH_4 (and possibly NO_2) (Dalzell et al., 2002). The formula used to calculate percent inhibition to nitrification is shown below.

$$\%\ Inhibition = 100 - \left[\frac{S_2 - S_0}{C_2 - C_0}\right] \times 100$$

Where

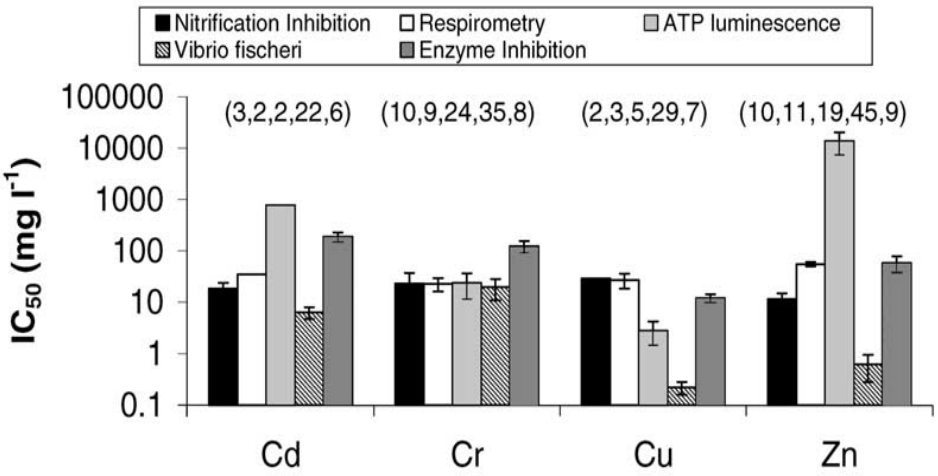
S_2 = Oxidized N concentration in tubes (sample + toxic substance) after 2 hours of incubation, mg/L
S_0 = Initial oxidized N concentration in tubes (sample + toxic substance), mg/L
C_2 = Oxidized N concentration in control tubes after 2 hours of incubation, mg/L
C_0 = Initial oxidized N concentration in control tubes, mg/L

The results of the percent nitrification inhibition were plotted as a dose-response curve (Figure 1), and the IC_{50} was then calculated for the 2-hour exposure to the toxicants. From the tests carried out, nitrification inhibition and *V. fischeri* were found to be most sensitive bioassays.

In many instances, the presence of an individual metal may not pose a threat to nitrification at low concentrations, but a combination of metals may pose adverse effects due to the possible synergistic effects. In the one study found in the literature review as performed at a District plant, Patel et al. (2007) evaluated the synergism of combined heavy metals on the nitrification process for the Calumet WRP whose final effluent showed elevated levels of NH_3-N during a plant upset in August 2006. During this upset, heavy metals such as Cd, Cr^{Total}, Cu, Ni, Pb, and Zn were detected in the raw sewage; however, their individual concentrations were not considered high enough to cause inhibition based on comparison to literature values. Hence, the possibility of a synergistic effect of the combined metals was evaluated as a part of identifying the probable causes of the upset at the Calumet WRP, because other pollutants such as toxic organic compounds were not found. Laboratory respirometry experiments were performed using ML samples from the Stickney WRP as a proxy, to evaluate the impact of a single metal (each metal concentration of 10 ppm) and metal cocktails as follows:

1. MC5 (ML + 5 ppm of each single metal).

FIGURE 1: TOXICITY ASSESSMENT RESULTS USING DIFFERENT METHODS (HALF MAXIMAL INHIBITORY CONCENTRATION VALUES) (DALZELL ET ALII, 2002)



Adapted from Dalzell et al. (2002).

2. MC1.67 (ML + 1.67 ppm of each single metal).

3. Cal MC (ML + cocktail concentrations found during the Calumet WRP upset).

The inhibition was then evaluated based on reduction in nitrification rates and OUR. Results revealed that Cr^{Total} (at total metal concentration of 10 mg/L) was observed to have the greatest inhibitory effect, whereas Zn had the least effect on nitrification and OUR. The order of inhibition by individual metals was Cr^{Total} > Ni > Cu > Pb > Cd > Zn, whereas for the metal cocktails the order was MC5 > MC1.67 > Cal MC.

There are other limited studies which investigated the effect of combined metals on nitrification under different scenarios. Cabrero et al. (1998) evaluated the effect of metal cocktails of Cu and Zn on activated sludge bacteria. Experiments were carried out using the individual metal concentrations (1, 5, 10, and 20 mg/L) and combined concentrations of 5/5, 5/10, and 10/5 of Cu/Zn as mg/L. Results indicated that a combination of the two heavy metals did not cause either synergistic or antagonistic effects on nitrification. Shuttleworth et al. (1991) evaluated the effect of Ca, Cu, Ni, and Zn on the growth of filamentous bacteria. It was observed that Cu was found to be more inhibitory than other individual metals or metal cocktails (Cu-Zn and Cu-Ni) tested, and metal cocktails exhibited synergistic effects on the growth rate of the filamentous bacteria.

There are a number of limitations to the studies reviewed: (1) the studies conducted used various methods and measurement techniques to quantify inhibition; (2) most of the heavy metal toxicity studies assessed the nitrification inhibition due to a single heavy metal and do not provide sufficient or clear guidance about the effect of combined metals; (3) many studies were performed at laboratory scale and not full scale; (4) there could be an adaptation of the microorganism to elevated metals whereby they can tolerate higher metal concentrations; and (5) all the studies performed used either synthetic activated biomass or activated sludge obtained from different plants.

Table 1 summarizes the USEPA's local limit guidance, the non-District studies, and the lone District study with respect to heavy metal inhibition limits.

PLANT DATA ANALYSIS – A TOOL FOR THE DETERMINATION OF SITE-SPECIFIC TOXICITY THRESHOLDS

Based on the literature review, there is a wide range of inhibitory limits suggested for each metal which may not be applicable for the site-specific conditions at Stickney and Calumet. As addendum to these reported limits, a historical data analysis was performed examining the metal and NH_3 concentrations for the Stickney and Calumet WRP plant influent and effluent for 2012–2014. The influent metal concentrations were compared to the literature inhibitory limits, and the effect of elevated metal concentrations on plant nitrification performance was evaluated.

Observations regarding the collected metals data are as follows:

1. At the Stickney Westside (WS) plant, low-level mercury (Hg-nano) was analyzed in addition to Hg-total. However, Hg-nano was discontinued as of April 1, 2012.

2. For all metals, analyses were performed mostly once per week; however, there were some periods where concentrations were recorded continuously.

3. All metal concentrations were expressed as total metal concentration, unless specified.

4. For raw sewage influent and outfall data, commonly encountered errors showing absent data pertained to the following:

    a. Sample analyzed past holding time.

    b. Sampling error.

    c. Plant shut down.

    d. Laboratory control standard failure.

    e. No re-run for samples due to fulfillment of sampling requirements.

    f. Inability to preserve aliquot.

    g. Test failure.

    h. Insufficiently preserved sample.

    i. Insufficient volume for analysis.

    j. MS failure.

Metal concentration data for the Stickney and Calumet WRPs' influent and effluent for the year 2012–2014 were analyzed for two scenarios: one with no heavy metals removal in primary treatment and the other with assorted percent reductions of heavy metals in primary

treatment (Table 4). The heavy metals removal efficiencies through primary treatment were derived from the Calumet and Stickney WRPs operational data and hence, the results obtained from data analysis in the latter scenario could be considered the site specific results and may be used accordingly. Based on the influent metal concentrations in raw sewage, the concentrations of metals to the aeration tanks were calculated for both scenarios.

The daily metals data were visually examined for outliers prior to use; no outliers were found. As stated, the daily aeration tank influent metal concentrations were compared against inhibitory concentrations obtained from literature, and the number of daily exceedances were counted and grouped by year and for the whole data period of 2012–2014. Nitrification efficiencies expressed as percent NH_3 removal were calculated for the three preceding and three following days as well as the days on which the daily metal concentrations exceeded the inhibitory threshold values to determine the impact on the nitrification process.

It should be noted that the biological P removal process was partially or fully implemented at both plants during the study period of 2012–2014; thus, the aerated portion and nitrification capacity of the batteries were reduced. The reduced aeration capacity may have an adverse impact on nitrification efficiency during the stressful operating conditions such as when the heavy metal concentrations are higher.

Results and Discussion

While a number of sources were reviewed, for the purpose of this study the concentrations provided in Table 3 are those most commonly reported, widely used, and judged to be authentic based on a USEPA document that lists the nitrification toxicity thresholds for heavy metals (Local Limits Development Guidance Appendices, 2004). For this table, if the inhibition threshold for a particular metal is given as a range for nitrification and/or the activated sludge process, then the stricter range was used to calculate three threshold values: the lowest and highest values and the average of the range values. For example, if the activated sludge process inhibition threshold for a particular metal is lower than the nitrification inhibition threshold, the activated sludge threshold is used, e.g. Cd and Cr^{+6}. Thus, all three inhibition threshold values (low, average and high) presented in Table 5 were used to determine the number of exceedances by comparing them against the daily metal concentrations for both plants.

Stickney Water Reclamation Plant. Table 6 documents the percent exceedances above the inhibition thresholds for each metal for the combined period of 2012–2014.

Table 6 indicates that none of the metal concentrations exceeded their respective toxicity thresholds for Cd, Pb, Cr^{+6}, and Ni. However, for Cu and Zn, concentrations were above their threshold limits for a significant number of days, while for As and Cr^{Total} concentrations were above their threshold limits for a limited number of days. Table 7 shows the number of days above the toxicity thresholds for Cu, Zn, As, and Cr^{Total} during each year and for the total period of 2012–2014. Table 8 shows the number of days on which any combination of two, three, or all four metals exceeded the toxicity thresholds for Cu, Zn, As, and Cr^{Total} during each year and for the total period of 2012–2014. Tables 6 through 8 indicate that most of the metal exceedances were for a single metal rather than a combination. Figures 2, 3, 4, and 5 depict the exceedances with the specific dates in relation to the threshold values for Cu, Zn, As, and Cr^{Total}, respectively.

TABLE 4: FRACTIONAL REMOVAL EFFICIENCIES OF HEAVY METALS THROUGH PRIMARY TREATMENT AT THE CALUMET AND STICKNEY WATER RECLAMATION PLANTS[1]

Metal	Calumet WRP	Stickney WRP
Arsenic (As)	0.00	0.00
Cadmium (Cd)	0.15	0.25
Chromium, Total (Cr^{Total})	0.20	0.25
Chromium, hexavalent (Cr^{+6})	0.00	0.00
Copper (Cu)	0.25	0.23
Lead (Pb)	0.24	0.25
Mercury (Hg)	0.10	0.04
Nickel (Ni)	0.09	0.16
Silver (Ag)	0.07	0.08
Zinc (Zn)	0.32	0.28
Selenium (Se)	0.25	0.07

[1]The removal efficiencies for all metals were adopted from a previous report entitled "Re-evaluation of Local Pretreatment Limits" (Monitoring and Research Department Report No. 14-58, Table AV-2).

TABLE 5: INHIBITION THRESHOLDS FOR METALS[1]

Metal	Low (mg/L)	Average (mg/L)	High (mg/L)
Arsenic (As)[2]	0.1	0.8	1.5
Cadmium (Cd)[3]	1	5.2	10
Chromium VI (Cr^{+6})	1	5.5	10
Chromium, Total (Cr^{Total})	0.25	1.08	1.9
Copper (Cu)	0.05	0.27	0.48
Lead (Pb)	0.5	—	—
Nickel (Ni)	0.25	0.38	0.5
Zinc (Zn)[4]	0.08	0.29	0.5

[1]Source: USEPA, 2004. Based on lower range, three values (low, average, and high values) were determined and used for evaluation.

[2]Inhibition threshold for As for activated sludge process (0.1 mg/L) is lower than the nitrification inhibition threshold (1.5 mg/L). Hence, both values and an average thereof were considered to estimate the number of days that exceeded the inhibition thresholds.

[3]The inhibition threshold for Cd for the activated sludge process ranges 1–10 mg/L, whereas for nitrification it is 5.2 mg/L. Hence, all values were considered for evaluation.

[4]The inhibition threshold range for Zn for nitrification is 0.08–0.5 mg/L, and for the activated sludge process is 0.3–5 mg/L.

TABLE 6: PERCENT OBSERVATIONS ABOVE TOXICITY THRESHOLDS FOR THE PERIOD OF 2012–2014 AT THE STICKNEY WATER RECLAMATION PLANT[1]

	Low Threshold		Average Threshold		High Threshold	
Metal	No Removal in Primaries	% Removal in Primaries[2]	No Removal in Primaries	% Removal in Primaries[2]	No Removal in Primaries	% Removal in Primaries[2]
As	1.5	1.5	—	—	—	—
Cd	—	—	—	—	—	—
Cr^{Total}	2.6	0.5	—	—	—	—
Cu	90.7	77.8	17.5	12.4	7.2	3.6
Pb	—	—	—	—	—	—
Ni	—	—	—	—	—	—
Zn	99.0	95.3	50.5	46.4	25	15.1
Cr^{+6}	—	—	—	—	—	—

[1]Percent exceedances were calculated as the ratio of number of observations above the respective threshold to total numerical observations for a particular period multiplied by 100.

[2]Removal efficiencies used for all metals listed above were different: As (0%), Cd (25%), Cr^{Total} (25%), Cu (23%), Pb (25%), Ni (16%), Zn (28%), and Cr^{+6} (0%). These values were taken from a previous report entitled "Re-evaluation of Local Pretreatment Limits" (Monitoring and Research Department Report No. 14-58, Table AV-2).

"—" indicates no concentration data was above the toxicity threshold concentration for nitrification.

TABLE 7: NUMBER OF DAYS ABOVE THRESHOLD CONCENTRATIONS WITH AND WITHOUT REMOVAL IN THE PRIMARIES FOR COPPER, ZINC, ARSENIC, AND CHROMIUM-TOTAL AT THE STICKNEY WATER RECLAMATION PLANT[1]

Year	Low Threshold		Average Threshold		High Threshold	
	No Removal in Primaries	% Removal in Primaries[2]	No Removal in Primaries	% Removal in Primaries[2]	No Removal in Primaries	% Removal in Primaries[2]
			Cu			
2012	43 (50)	30 (50)	5 (50)	3 (50)	3 (50)	2 (50)
2013	65 (73)	54 (73)	11 (73)	8 (73)	5 (73)	1 (73)
2014	68 (71)	67 (71)	18 (71)	13 (71)	6 (71)	4 (71)
2012−14	176 (194)	151 (194)	34 (194)	24 (194)	14 (194)	7 (194)
			Zn			
2012	49 (52)	46 (52)	16 (52)	13 (52)	7 (52)	5 (52)
2013	72 (73)	70 (73)	37 (73)	36 (73)	20 (73)	8 (73)
2014	69 (71)	67 (71)	44 (71)	40 (71)	21 (71)	16 (71)
2012–14	190 (192)	183 (192)	97 (192)	89 (192)	48 (192)	29 (192)
			As			
2012	0 (50)	0 (50)	0 (50)	0 (50)	0 (50)	0 (50)
2013	0 (73)	0 (73)	0 (73)	0 (73)	0 (73)	0 (73)
2014	3 (72)	3 (72)	0 (72)	0 (72)	0 (72)	0 (72)
2012–14	3 (195)	3 (195)	0 (195)	0 (195)	0 (195)	0 (195)
			Cr^{Total}			
2012	1 (50)	0 (50)	0 (50)	0 (50)	0 (50)	0 (50)
2013	0 (73)	0 (73)	0 (73)	0 (73)	0 (73)	0 (73)
2014	4 (71)	1 (71)	0 (71)	0 (71)	0 (71)	0 (71)
2012–14	5 (194)	1 (194)	0 (194)	0 (194)	0 (194)	0 (194)

[1]Numbers outside the parentheses indicate total numerical count of observations that exceeded the threshold values out of total numerical count of observations in the parentheses.

[2]Removal efficiencies used for all metals listed above were different: As (0%), Cr^{Total} (25%), Cu (23%), and Zn (28%). These values were taken from a previous report entitled "Re-evaluation of Local Pretreatment Limits" (Monitoring and Research Department Report No. 14-58, Table AV-2).

TABLE 8: NUMBER OF DAYS (WITH AND WITHOUT PRIMARY REMOVAL[1]) WHEN TWO OR MORE METALS (COPPER, ZINC, ARSENIC, AND CHROMIUM-TOTAL) EXCEEDED THE THRESHOLD CONCENTRATIONS AT THE STICKNEY WATER RECLAMATION PLANT[1]

	Low Threshold		Average Threshold		High Threshold	
Year	No Removal	% Removal	No Removal	% Removal	No Removal	% Removal
	Number of days above threshold concentration for two metals					
2012	43 (50)	30 (50)	5 (50)	3 (50)	3 (50)	2 (50)
2013	65 (73)	54 (73)	11 (73)	7 (73)	5 (73)	1 (73)
2014	65 (71)	64 (71)	17 (71)	12 (71)	6 (71)	4 (71)
	Number of days above threshold concentration for three metals					
2012	1 (50)	—	—	—	—	—
2013	—	—	—	—	—	—
2014	6 (71)	4 (71)	—	—	—	—
	Number of days above threshold concentration for all four metals					
2012	—	—	—	—	—	—
2013	—	—	—	—	—	—
2014	1 (71)	—	—	—	—	—

[1]Primary treatment removal efficiencies used for the respective metals were Cu (23%), Zn (28%), As (0%), and Cr^{Total} (25%), which were adopted from a previous report entitled "Re-evaluation of Local Pretreatment Limits" (Monitoring and Research Department Report No. 14-58, Table AV-2).

[2]Numbers outside the parentheses indicate total numerical count of observations that exceeded the threshold values out of total numerical count of observations in the parentheses.

FIGURE 2: CONCENTRATIONS ON THE DAYS ABOVE TOXICITY THRESHOLD FOR COPPER AT THE STICKNEY WATER RECLAMATION PLANT

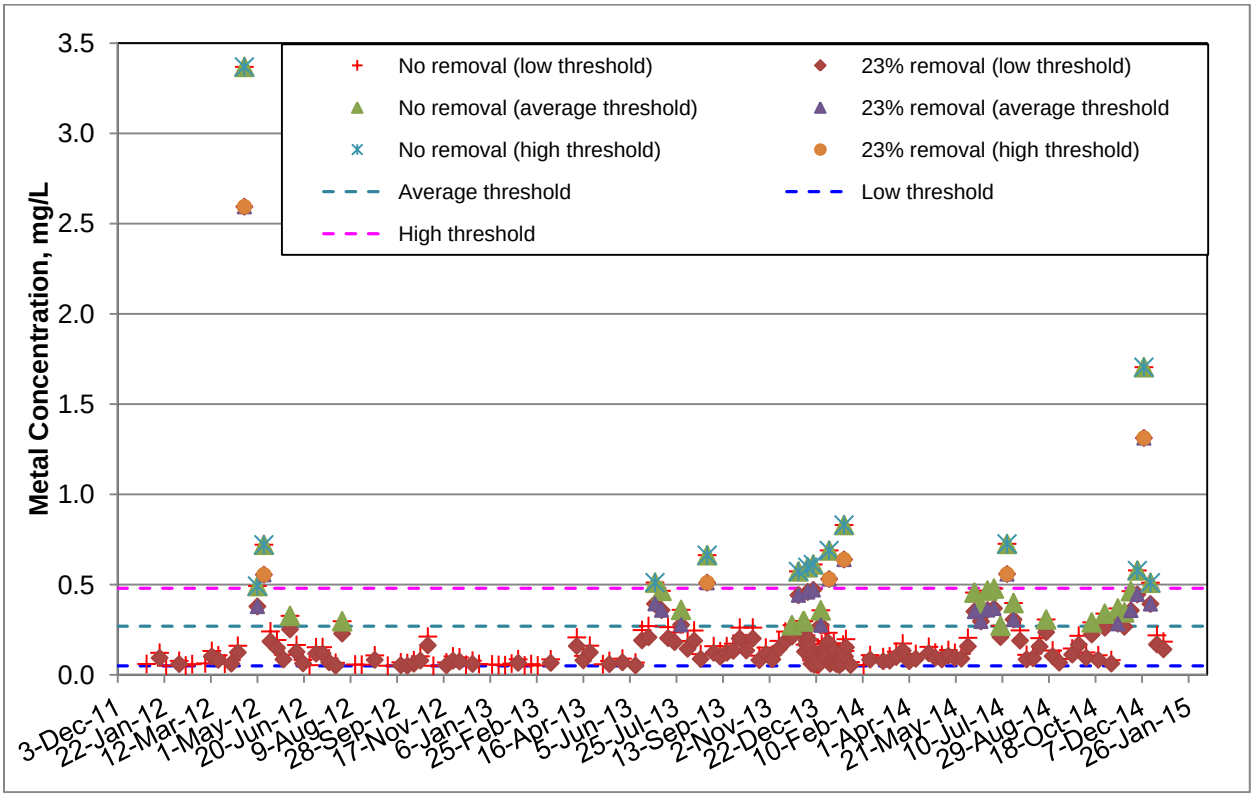


FIGURE 3: CONCENTRATIONS ON THE DAYS ABOVE TOXICITY THRESHOLD FOR ZINC AT THE STICKNEY WATER RECLAMATION PLANT

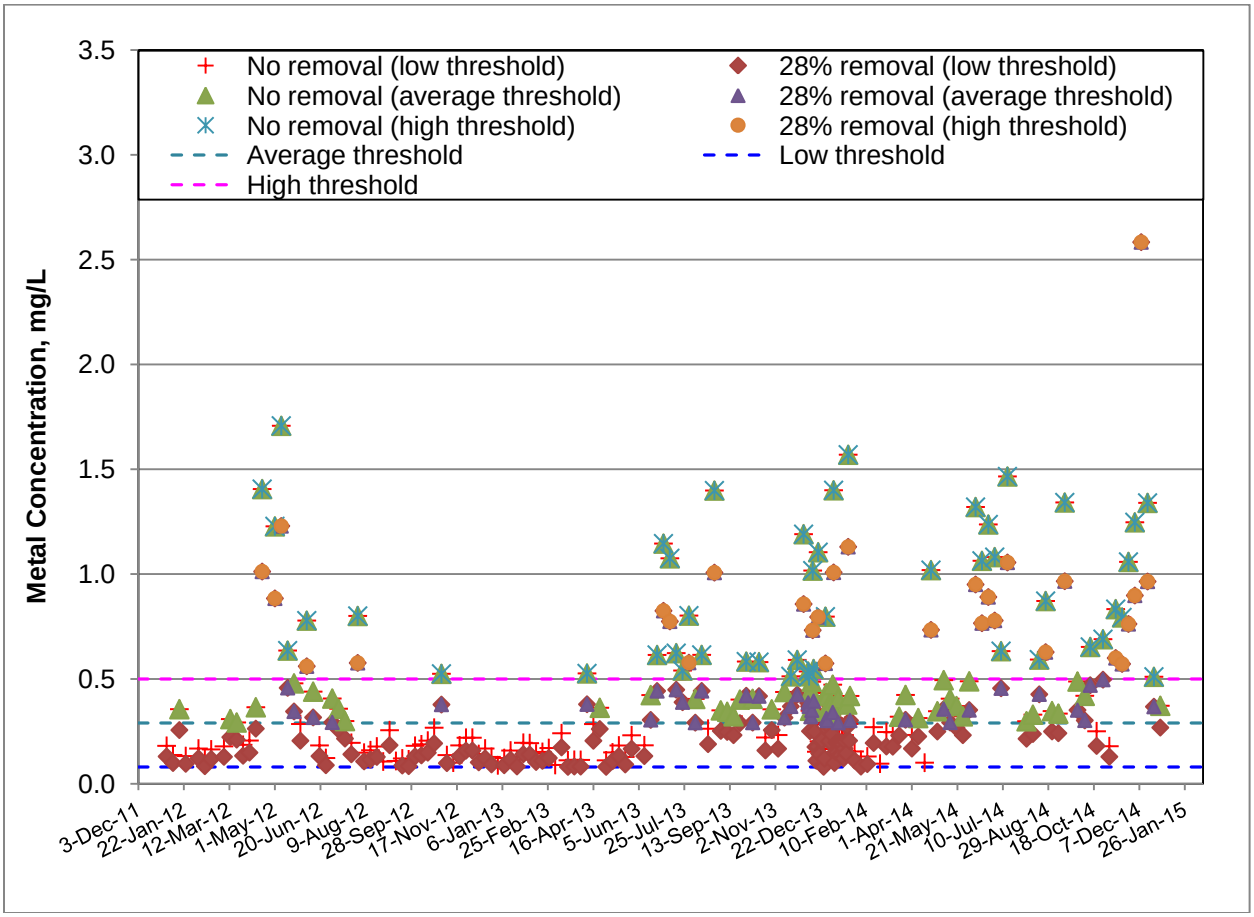


FIGURE 4: CONCENTRATIONS ON THE DAYS ABOVE TOXICITY THRESHOLD FOR ARSENIC AT THE STICKNEY WATER RECLAMATION PLANT

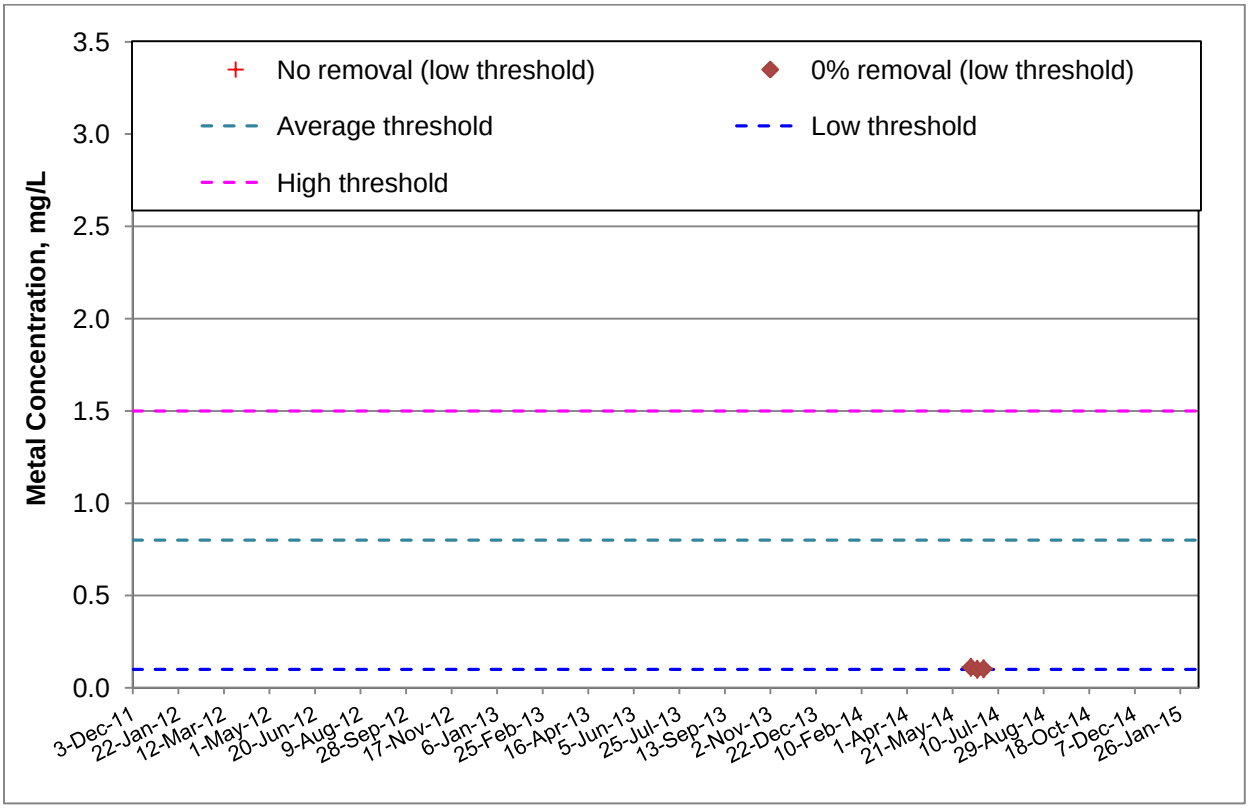
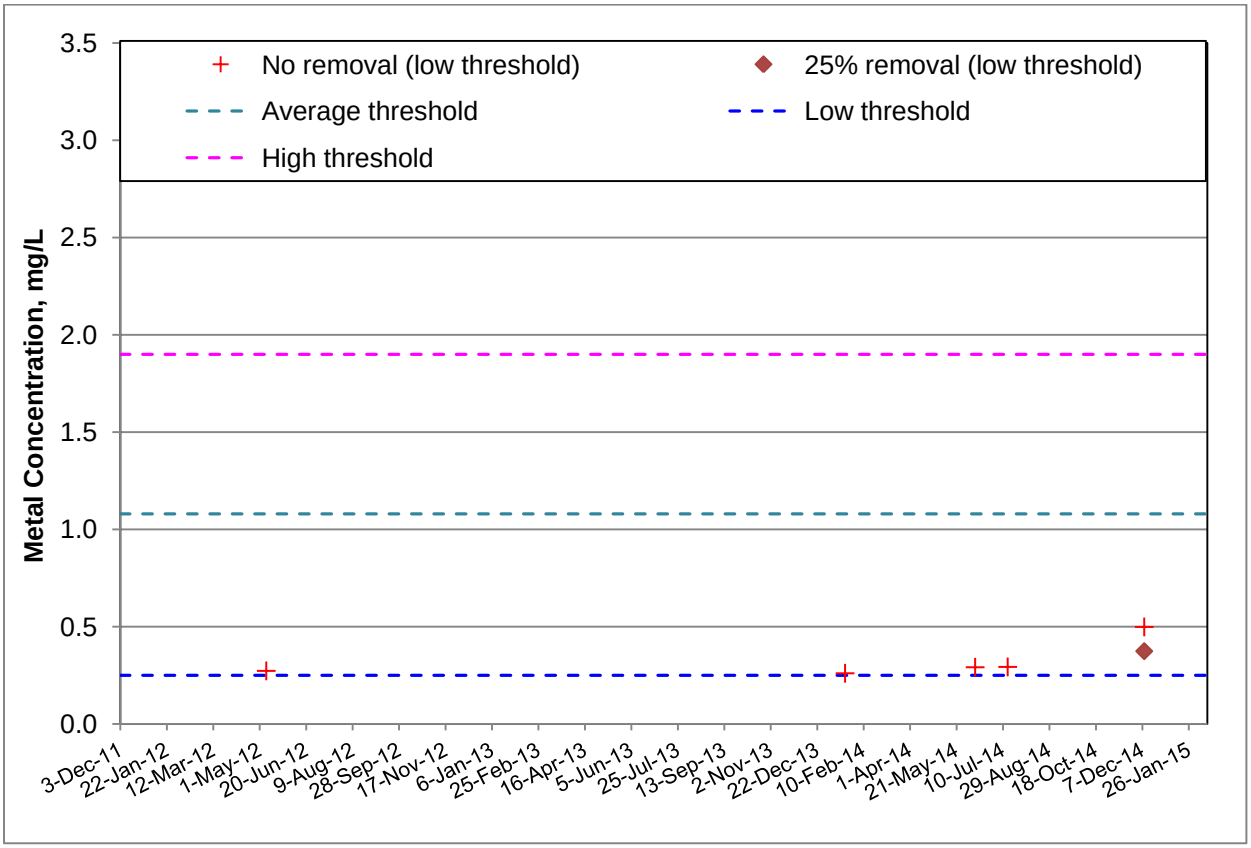


FIGURE 5: CONCENTRATIONS ON THE DAYS ABOVE TOXICITY THRESHOLD FOR CHROMIUM-TOTAL AT THE STICKNEY WATER RECLAMATION PLANT



Calumet Water Reclamation Plant. Table 9 documents the percent exceedances above the toxicity thresholds for each metal for the combined period of 2012–2014.

Table 9 indicates that none of the metal concentrations exceeded their respective toxicity thresholds for As, Cd, Cr^{Total}, Pb, Ni, and Cr^{+6}. However, a significant number of days displayed concentrations above the threshold limits for Cu and Zn. Table 10 indicates the number of days on which the toxicity thresholds for Cu and Zn were exceeded during each year and for the total period of 2012–2014. Table 11 shows the number of days on which both metals exceeded the toxicity thresholds for Cu and Zn during each year and for the total period of 2012–2014. Tables 9 through 11 indicate that most of the metal exceedances were due to a single metal. Figures 6 and 7 depict the exceedances with the specific dates in relation to the threshold values for Cu and Zn, respectively.

Both the Stickney and Calumet WRPs showed elevated Cu and Zn concentrations above the respective toxicity thresholds. Additionally, the SWRP had elevated Cr^{Total} and As concentrations above the respective toxicity thresholds. For all other metal species, the number of occurrences above the toxicity thresholds were either none or very few. Additionally, more than one metal exceedance on the same day was infrequent at both the plants. Nitrification efficiencies in terms of NH_3-N removal at both plants were determined for the days that had the elevated metal concentrations higher than the respective threshold values; similarly average NH_3-N removals were determined for three days before and after the days that had the elevated metal concentrations. An increase or decrease in nitrification efficiency was calculated in relation to the average nitrification removal efficiency of the three previous days to evaluate the impact on these exceedances on nitrification.

The metal concentration daily exceedances above the respective threshold levels were 146 at the Calumet WRP from 2012 through 2014; however, none of these increases caused a severe impact on the nitrification process. Of these 146 exceedances at the Calumet WRP, only 12 exceedances coincided with a five percent or more decrease in NH_3-N removal efficiency compared to the average NH_3-N removal efficiencies of the three preceding days. Further, of these 12 incidences at the Calumet WRP, only one exceedance (on April 1, 2014, due to Zn) had a 7.91 percent decrease in NH_3-N removal efficiency compared to the average NH_3-N removal efficiency of the three preceding days, followed by another decrease of 2.75 percent during the three following days; in the remaining 11 of the 12 exceedance events, nitrification recovered within the next three days without any compromise of NH_3-N removal efficiency.

The metal concentration daily exceedances above the respective threshold levels were 193 at the Stickney WRP from 2012 through 2014; however, none of these increases caused a severe impact on the nitrification process. Of these 193 exceedances at the Stickney WRP, only 27 exceedances coincided with a five percent or more decrease in NH_3-N removal efficiency compared to the average NH_3-N removal efficiencies of the three preceding days. Further, of these 27 incidences at the Stickney WRP, the exceedance on January 8, 2014, due to Cu and Zn, had a 5.42 percent decrease in NH_3-N removal efficiency compared to the average NH_3-N removal efficiency of the three preceding days, followed by another decrease of 3.07 percent during the three following days. The exceedance on January 16, 2014, due to Cu and Zn, had a 6.41 percent decrease in NH_3-N removal efficiency compared to the average NH_3-N removal efficiency of the three preceding days, followed by another decrease of 7.93 percent during the

TABLE 9: PERCENT OBSERVATIONS ABOVE TOXICITY THRESHOLDS FOR THE PERIOD OF 2012–2014 AT THE CALUMET WATER RECLAMATION PLANT[1]

	Low Threshold		Average Threshold		High Threshold	
Metal	No Removal	% Removal[2]	No Removal	% Removal[2]	No Removal	% Removal[2]
As	—	—	—	—	—	—
Cd	—	—	—	—	—	—
Cr^{Total}	—	—	—	—	—	—
Cu	17.9	11.5	0.4	—	—	—
Pb	—	—	—	—	—	—
Ni	—	—	—	—	—	—
Zn	63.5	40.9	5.7	5.2	2.2	1.7
Cr^{+6}	—	—	—	—	—	—

[1]Percent exceedances were calculated as the ratio of number of observations above the respective threshold to total numerical observations for a particular period multiplied by 100.

[2]Primary treatment removal efficiencies used for all metals listed above were different: As (0%), Cd (15%), Cr^{Total} (20%), Cu (25%), Pb (24%), Ni (9%), Zn (32%), and Cr^{+6} (0%). These values were taken from a previous report entitled "Re-evaluation of Local Pretreatment Limits" (Monitoring and Research Department Report No. 14-58, Table AV-2).

"—" indicates no concentration data was above the toxicity threshold concentration for nitrification.

TABLE 10: NUMBER OF DAYS ABOVE THRESHOLD CONCENTRATIONS WITH AND WITHOUT PRIMARY REMOVAL FOR COPPER AND ZINC AT THE CALUMET WATER RECLAMATION PLANT[1]

Year	Low Threshold		Average Threshold		High Threshold	
	No Removal	% Removal[2]	No Removal	% Removal[2]	No Removal	% Removal[2]
			Cu			
2012	18 (52)	12 (52)	1 (52)	0 (52)	0 (52)	0 (52)
2013	6 (53)	3 (53)	0 (53)	0 (53)	0 (53)	0 (53)
2014	18 (129)	12 (129)	0 (129)	0 (129)	0 (129)	0 (129)
2012–14	42 (234)	27 (234)	1 (234)	0 (234)	0 (234)	0 (234)
			Zn			
2012	46 (52)	37 (52)	7 (52)	2 (52)	2 (52)	1 (52)
2013	32 (53)	17 (53)	0 (53)	0 (53)	0 (53)	0 (53)
2014	68 (125)	40 (125)	7 (125)	4 (125)	3 (125)	2 (125)
2012–14	146 (230)	94 (230)	14 (230)	6 (230)	5 (230)	3 (230)

[1]Numbers outside the parentheses indicate total numerical count of observations that exceeded the threshold values out of total numerical count of observations in the parentheses.

[2]Primary treatment removal efficiencies used for all metals listed above were different: Cu (25%) and Zn (32%). These values were taken from a previous report entitled "Re-evaluation of Local Pretreatment Limits" (Monitoring and Research Department Report No. 14-58, Table AV-2).

TABLE 11: NUMBER OF DAYS (WITH AND WITHOUT PRIMARY REMOVAL) WHEN BOTH COPPER AND ZINC EXCEEDED THRESHOLD CONCENTRATIONS AT THE CALUMET WATER RECLAMATION PLANT[1]

Year	Low Threshold		Average Threshold		High Threshold	
	No Removal	% Removal[2]	No Removal	% Removal[2]	No Removal	% Removal[2]
	Number of days above threshold concentration for two metals					
2012	18 (52)	12 (52)	1 (52)	—	—	—
2013	6 (53)	3 (53)	—	—	—	—
2014	18 (125)	12 (125)	—	—	—	—

[1]Numbers outside the parentheses indicate total numerical count of observations that exceeded the threshold values out of total numerical count of observations in the parentheses.

[2]Primary treatment removal efficiencies used for all metals listed above were Cu (25%) and Zn (32%). These values were taken from a previous report entitled "Re-evaluation of Local Pretreatment Limits" (Monitoring and Research Department Report No. 14-58, Table AV-2).

FIGURE 6: CONCENTRATIONS ON THE DAYS ABOVE TOXICITY THRESHOLD FOR COPPER AT THE CALUMET WATER RECLAMATION PLANT

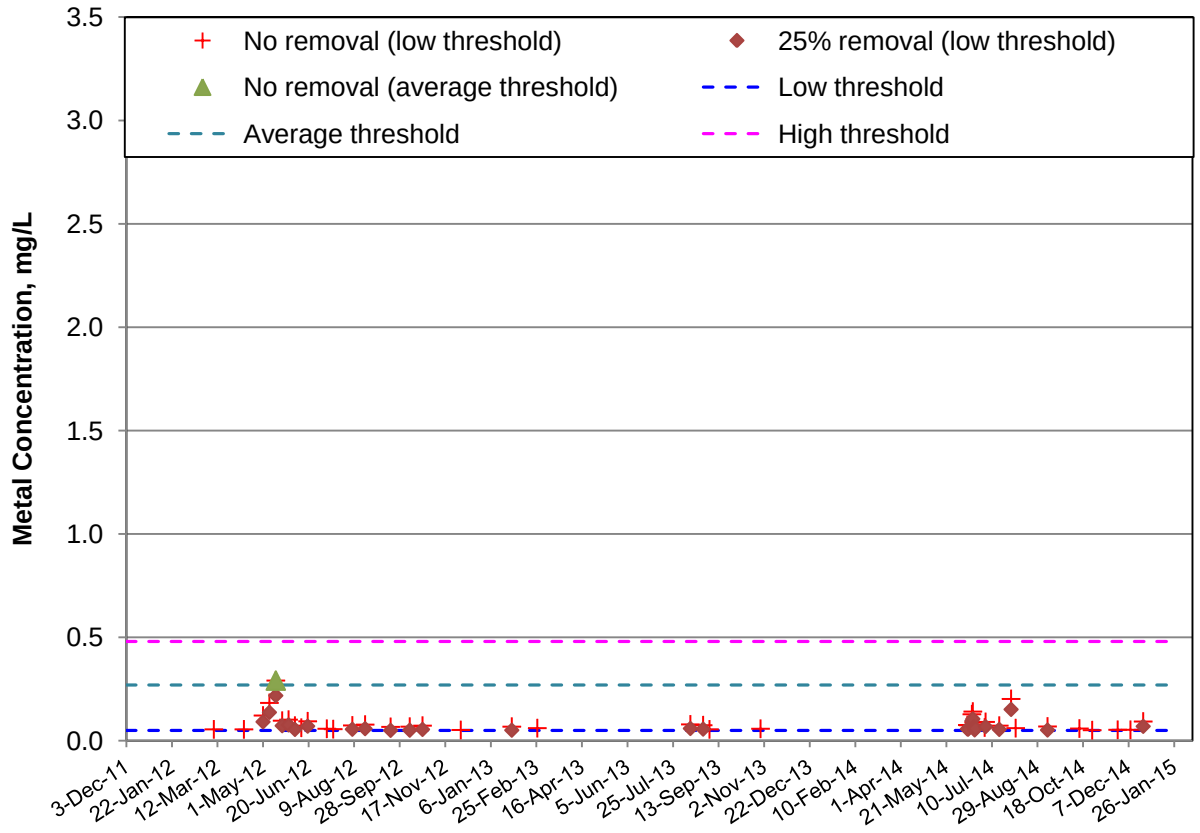
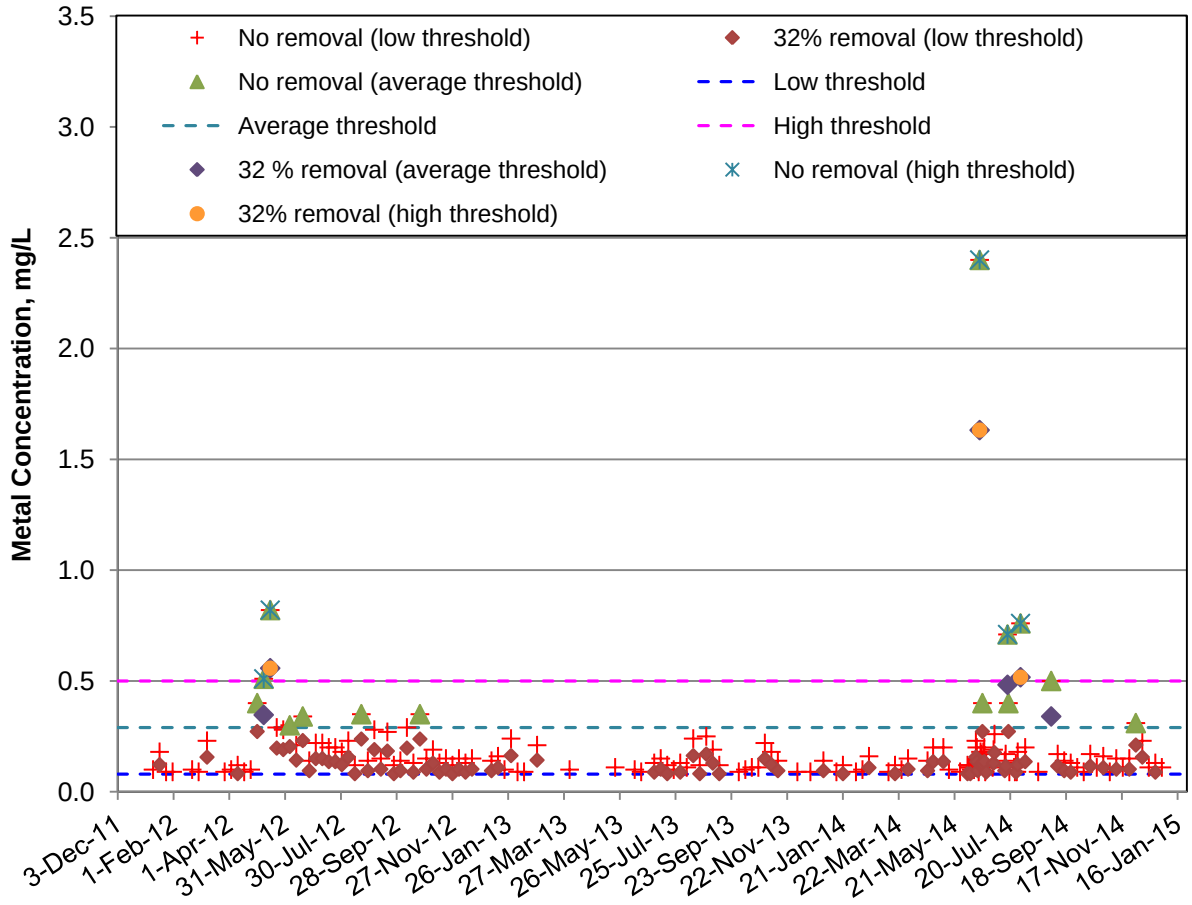


FIGURE 7: CONCENTRATIONS ON THE DAYS ABOVE TOXICITY THRESHOLD FOR ZINC AT THE CALUMET WATER RECLAMATION PLANT



three following days. The exceedance on August 19, 2014, due to Cu and Zn, had a 5.20 percent decrease in NH_3-N removal efficiency compared to the average NH_3-N removal efficiency of the three preceding days followed by another decrease of 0.76 percent during the three following days; in the remaining 24 of the 27 exceedance events, nitrification recovered within the next three days without any compromise of NH_3-N removal efficiency.

It should be noted that for both plants the operating conditions were similar (e.g. HRT, SRT, ML suspended solids, and NH_3 loading) over the course of the monitoring period (i.e. three days before and after the exceedance).

Data Analysis Summary Statement

Based on data analysis for both plants, it should be noted that the toxicity thresholds used in the analysis should be considered conservative, since exceedances of these thresholds at both plants generally did not result in inhibition of nitrification.

Recommendation

For the purpose of implementing the HSOM program in the near future, the site-specific metal toxicity limits may be estimated with the consideration of both reported thresholds and the results of the data evaluation presented in this report.

The routine total metal analyses on primary effluent at both plants should be added at a frequency of twice per month since no historic metal data are available on primary effluent. It is also recommended to further analyze the soluble portions for select metals (As, Cd, Cr^{Total}, Cr^{+6}, Cu, Pb, Ni, and Zn) in raw sewage, primary effluent, and final effluent. These data will be useful for investigating any future nitrification upsets at both plants as well as to corroborate the results obtained from the below suggested future work to help establish accurate and reasonable site-specific metal toxicity thresholds.

Future Work

Due to the urgent need of the site-specific metal toxicity thresholds, it is prudent and pragmatic at this time to follow the guidance from this report. However, it is also necessary to perform bench-scale toxicity testing through respirometry for both plants and develop dose-response relationships to obtain toxicity thresholds for metals due to the lack of study in this area at the Stickney and Calumet plants. The metals identified as having the highest occurrence of exceedances should be evaluated first followed by the remaining metals and combination of metals thereafter. The dose ranges in the respirometry testing will be based on the plant influent and primary effluent data and thresholds identified in this report. Metal speciations will be used in the study as total and soluble portions.

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