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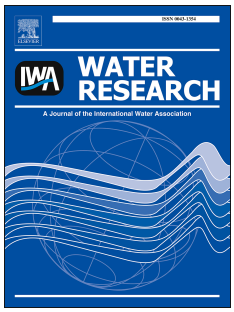

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PII: S0043-1354(18)30608-0

DOI: 10.1016/j.watres.2018.07.065

Reference: WR 13964

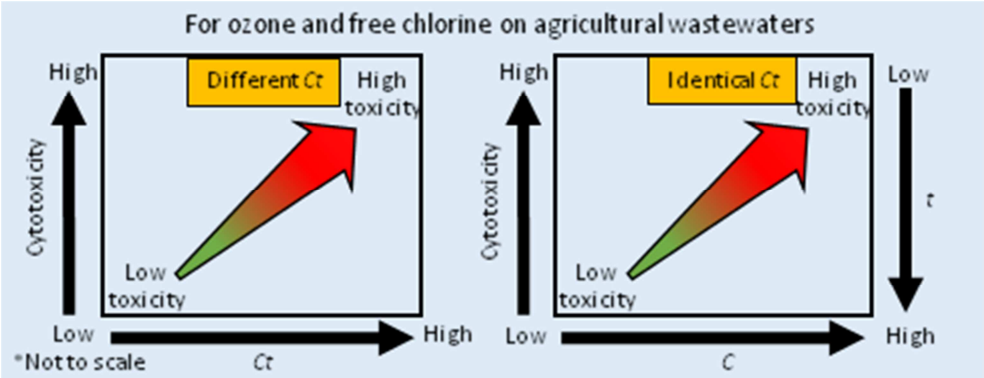
To appear in: *Water Research*

Received Date: 30 April 2018

Revised Date: 16 July 2018

Accepted Date: 26 July 2018

Please cite this article as: Dong, S., Massalha, N., Plewa, M.J., Nguyen, T.H., The impact of disinfection *Ct* values on cytotoxicity of agricultural wastewaters: Ozonation vs. chlorination, *Water Research* (2018), doi: 10.1016/j.watres.2018.07.065.



The Impact of Disinfection *Ct* Values on Cytotoxicity of Agricultural Wastewaters: Ozonation vs. Chlorination

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ABSTRACT

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Toxicity arising from toxic disinfection byproducts is an unintended result of disinfection during water reclamation. To ensure safe water reclamation treatment, it is important to develop a disinfection strategy with minimal formation of overall toxicity in the reclaimed water. The cumulative disinfectant concentration over time (*Ct*) is a useful concept for pathogen control during reuse water disinfection. We evaluated the toxicity impact of *Ct* values and different methods to achieve identical *Ct* values by ozonation or chlorination of wastewaters from four agricultural sources on mammalian cells. *N*-acetylcysteine (NAC) reactivity of the wastewater organic extracts was determined to reveal their impact on the thiol-specific biological detoxification mechanism. The results demonstrated that for two sources and for both ozonation and chlorination, higher *Ct* values enhanced cytotoxicity. The ozonated waters were at least 10% less toxic and as much as 22.4 times less toxic than either the non-disinfected controls or the chlorinated waters. Chlorination consistently induced higher cytotoxicity than ozonation by between 2.2 and 22.4 fold, respectively, and induced similar or higher cytotoxicity than the non-disinfected controls, by at most 4.4 fold. Given the same *Ct* values, the combination of high disinfectant concentration and short contact time produced finished wastewaters with higher toxicity, than the combination of low disinfectant concentration and long contact time. NAC thiol reactivity was positively and significantly correlated with mammalian cell cytotoxicity, and agreed with 80% of the cytotoxicity rank order. This suggests that the induction of cytotoxicity involved reactions with agents that acted as thiol pool quenchers. The overall results indicate that the cytotoxicity of wastewaters may increase when higher *Ct* values are applied to inactivate recalcitrant pathogens. To counteract the potential increase in cytotoxicity at high *Ct* values, for both ozonation and chlorination, lower disinfectant dose and longer contact time may be adopted.

Keywords: disinfectant exposure (*Ct*); ozone; chlorine; wastewater; thiol

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1. Introduction

Water reclamation is necessary for efficient water use (World Health Organization 2015). Worldwide 70% of the freshwater resources are used for agricultural purposes (Food and Agriculture Organization 2015), it is therefore paramount to more efficiently reuse wastewaters from agricultural sources by means of, for instance, onsite reuse. Disinfection is mandatory in this process to protect the public health. Due to its effectiveness and affordability, chlorine is currently the most widely adopted disinfectant for wastewater disinfection (Metcalf & Eddy Inc. 2013). However, chlorine is practically ineffective against several important pathogens, such as *Cryptosporidium parvum* (Korich et al. 1990). Chlorine disinfection also produces a number of regulated and unregulated disinfection byproducts (DBPs) (Crittenden et al. 2012; Richardson and Postigo 2015; Rook 1974). Ozone is a strong disinfectant against several key pathogens such as the Norwalk virus (Shin and Sobsey 2003) and *C. parvum* (Corona-Vasquez et al. 2002; Kim et al. 2007), and it is being increasingly used for wastewater disinfection (Gottschalk et al. 2010) despite its potential to form bromate, a regulated DBP, in bromide rich waters (United States Environmental Protection Agency 2006). All disinfection processes create DBPs, some of which can be toxic. With the increasing application of reclaimed waters for purposes such as recreational waters and hydroponics, to ensure the safety of consumers it is of paramount importance to determine a disinfection strategy with minimal formation of overall toxic disinfection byproducts, while achieving sufficient pathogen inactivation.

The *Ct* (cumulative concentration of disinfectant *C* in mg/L times time *t* in minutes) is a concept based on the Chick-Watson theory (Chick 1908) that has been widely used for the disinfection of water and wastewaters for both chlorine and ozone (Stover et al. 1986; United States Environmental Protection Agency 2003). It predicts the fractional inactivation of a

pathogen given the cumulative concentration of disinfectant over time. The *Ct* value accounts for the disinfectant demands in different waters together with the disinfectant exposure duration. To achieve a given *Ct* value for pathogen inactivation, either a higher cumulative disinfectant concentration over shorter exposure time or lower cumulative disinfectant concentration over longer exposure time may be used. The *Ct* concept for microbial inactivation allows for flexibility in disinfection design by varying the chemical dosing and the disinfectant contact time. Previous research investigated the options to reduce the disinfectant usage while achieving acceptable microbial inactivation and keeping toxicity low, such as comparing a three-step chlorine dosing vs. a one-step chlorine dosing strategy (Li et al. 2017a; Li et al. 2017b). Other research investigated the effect of fixed disinfectant concentration on toxicity among varied waters (Smith et al. 2010; Yang et al. 2015). However, applications of the *Ct* concept were primarily focused on microbial inactivation (Dong et al. 2018; Hunt and Mariñas 1997; Hunt and Mariñas 1999), and the *Ct* concept in the context of disinfection-mediated toxicity production is not well understood. Owing to the potential to generate cytotoxic, genotoxic, and teratogenic DBPs which may have adverse effects upon the environment and the public health (Komaki et al. 2014; Plewa et al. 2010; Plewa and Wagner 2015; Plewa et al. 2004; Plewa et al. 2017; Richardson et al. 2007; Wagner and Plewa 2017), published research established that chlorination and ozonation could alter the toxicity of reclaimed waters due to interactions between the disinfectants and the organic and inorganic water components (Dong et al. 2016; Dong et al. 2017a; Dong et al. 2017b; Liu and Zhang 2014; Monarca et al. 2000). However, a comprehensive toxicological analysis linking the impact of varied disinfectant dose with contact time, two of the most important wastewater disinfection operational parameters, is lacking.

The objective of this research is to understand chlorination and ozonation technology for the disinfection of several sources of partially treated wastewaters concerning the toxicological response in mammalian cells to *Ct* values and different ways to achieve the same *Ct* value. The Chinese Hamster Ovary cells cytotoxicity assay was used for the toxicity analysis; the previously developed *N*-acetylcysteine thiol reactivity assay (Dong et al. 2017a; Dong 2018) was used to measure the potential impact of wastewater organic extracts on the thiol-specific biological detoxification mechanism. The results presented here will facilitate the disinfectant dose and contact time selection to minimize toxicity of the disinfected wastewater while ensuring pathogen inactivation as reflected by the applied *Ct* values.

2. Materials and methods

2.1 Wastewater sampling, processing, and characterization

Four independent sources of agricultural wastewater effluents named A, B, C, and D were collected from two vegetable farms in Illinois, USA. These farms employ closed loop recycling of water to reuse the nutrients and reduce cost. One farm was equipped with a state-of-the-art biological nitrogen removal system to better manage the seasonal nutrient surges; another farm mainly used an ebb-and-flow system with sand filtration. Due to seasonal farming activities, we were denied access to the two locations where we collected water samples A and B. Therefore we obtained samples from two other sources in the same farms instead, designated as C and D. Within one week post sample collection, samples were filtered through 1.6 μm glass fiber filters to minimize the interference of suspended solids and the loss of dissolved organic matter. The filtrates were stored at 4°C in the dark until use. Several water quality parameters were measured and are summarized in Table 1. The absorbance at 254 nm was measured by a Beckman UV−vis spectrophotometer (Beckman Coulter Life Sciences, Indianapolis, IN). The dissolved organic

carbon (DOC) was measured by a Shimadzu TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD) after filtering each sample through a 0.45 μm filter. Specific UV absorbance at 254 ($SUVA_{254}$) was calculated as the ratio of UV_{254}/DOC. Both the free chlorine (further detailed below) and ammonia nitrogen were measured using Hach kits (Loveland, CO). pH of the filtered wastewaters ranged from 6.13 to 7.05 at room temperature.

2.2 Disinfection experiments

Chlorination using free chlorine was compared with ozone.

We used Eq. (1) to calculate the *Ct* value in a completely mixed batch reactor:

$$Ct = \int_0^\tau C_L dt \quad (1)$$

where C_L is the concentration of dissolved disinfectant (free chlorine or ozone in this study), τ is the hydraulic residence time of the reactor, and the integral represents the area under the curve as depicted in Fig. 1 up to a specific time equal to the hydraulic residence time τ of this reactor. The finite middle Rieman sum was used to approximate the integral (Eq. (2)):

$$\int_0^\tau C_L dt \approx \sum_{i=1}^{n} (C_{L_i} + C_{L_{i-1}})/2\,\Delta t \quad (2)$$

Two types of chlorination experiments were carried out. For each wastewater source this entails: (1a) achieving high or low *Ct* values by a combination of high initial chlorine dose with long contact time, or low initial chlorine dose with short contact time; (2a) achieving the identical *Ct* value by a combination of high cumulative chlorine dose with short contact time, or low cumulative chlorine dose with long contact time ($\int_0^\tau C_L dt$ kept identical).

Chlorination experiments were carried out in 4 L amber glass bottles with Teflon-lined caps as approved by the U.S. EPA (United States Environmental Protection Agency 2016). Free chlorine

129 was measured using the Hach (Loveland, CO) kit based on the U.S. EPA *N*,*N*′-diethyl-*p*-
130 phenylenediamine (DPD) Method. The chlorine concentration in the stock sodium hypochlorite
131 solution (Ricca Chemical, Arlington, TX) was quantified spectrophotometrically at 292 nm using
132 a molar absorptivity of 360 M^{-1} cm^{-1} (Hussain et al. 1970). We conducted a preliminary
133 experiment to ensure that at the end of a minimum 15 min contact time and at a Cl_2 to NH_3-N
134 mass ratio of at least 8.3, free chlorine was still available, and more importantly, for the low *Ct*
135 conditions in experiment (1a) the free chlorine concentration was in compliance with actual farm
136 wastewater disinfection practice (Raudales et al. 2014). An example free chlorine concentration
137 profile as a function of exposure time is provided in Fig. 1a. At the end of the experiments, free
138 chlorine was quenched stoichiometrically (1.2 safety factor) using analytical grade sodium
139 bisulfite (Fisher Scientific, Hampton, NH).

140 Two types of ozonation experiments were carried out: (1b) achieving high or low *Ct* values
141 using the same ozone mass transfer rate but with longer or shorter contact times; (2b) achieving
142 the identical *Ct* value by a combination of high ozone mass transfer rate with shorter contact time
143 or low ozone mass transfer rate with longer contact time ($\int_0^t C_L dt$ was kept identical). Ozonation
144 experiments were conducted in 4 L glass semi-batch reactors. Ozone was produced from an
145 ozone generator (E.P. Purification Inc., Champaign, IL) with a nitrogen scrubber (Airsep Inc.,
146 New York). All apparatus in contact with ozone gas or ozonated waters was ozone-resistant. The
147 dissolved ozone calibration curve was prepared by serial dilution of a stock ozone solution
148 obtained by purging ozone into cold ozone demand free phosphate buffer (pH = 7).
149 Standardization of the ozone stock followed a molar absorptivity of 2900 M^{-1} cm^{-1} at 258 nm
150 (Kilpatrick et al. 1956). Measurement of ozone in solution followed the Indigo method as
151 described elsewhere (Water Environmental Federation and American Public Health Association

2005). Different ozone mass transfer rates were achieved by conducting experiments with or without diffuser stones (Ozone Solutions, Hull, IA). An example ozone exposure profile as a function of time is shown in Fig. 1b. Ozonation experiments were stopped stoichiometrically (1.2 safety factor) using analytical grade sodium thiosulfate (Fisher Scientific, Hampton, NH) after the designed *Ct* values were achieved.

2.3 Sample concentration

The organics from the samples were concentrated by adsorption onto clean XAD resins. It is important to note that despite numerous advantages this extraction method does not consider the contribution of bromate. Fifty-five mL each of Amberlite XAD-2 (Sigma Aldrich, St. Louis, MO) and Supelite DAX-8 (Sigma Aldrich, St. Louis, MO) resins were packed above a glass wool plug in a glass chromatography column. A mixture of XAD-2 resin, an aromatic polystyrene, and XAD-8 resin, an acrylic ester was used to collect both aromatic and aliphatic compounds. The maximum ratio of water to resins was 770:1 to maximize the adsorption of organics and minimize breakthrough (Ringhand et al. 1987; Schenck et al. 1990). The samples were first acidified to pH < 2 by sulfuric acid prior to being passed through the column, to ensure protonation of carboxylic organics. We used 400 mL of optima grade ethyl acetate (Fisher Scientific, Hampton, NH) to elute the organics from the columns (Kronberg et al. 1988). Water in the ethyl acetate eluent was removed first using a separatory funnel, followed by passing the hydrophobic fraction through a column of anhydrous sodium sulfate (Fisher Scientific, Hampton, NH). The ethyl acetate eluent was reduced to 1 mL by a rotary evaporation unit (Büchi, Flawil, Switzerland) at 55 °C and further reduced to a point where the volume could not be reduced using a gentle stream of nitrogen. It should be noted that although previous research identified that the volatile fraction of DBPs are not as toxic as the semi- to non-volatile fractions (Zhu

2015), at 55 °C loss and decomposition of these volatile organics may occur (Zhang and Minear 2002). However even after these processes volatile DBPs such as the trihalomethanes are present in samples (Jeong et al. 2012). For each sample 40 µL of dimethyl sulfoxide (DMSO) was added to dissolve the organic extract. Due to the presence of non-negligible amount of organics that could not be blown dry, we measured the total volume of 40 µL of added DMSO together with the organics by means of density measurement. Specifically, we recorded the weight of the HPLC vials prior to sample addition, and for each HPLC vial containing the extracted organics and the added 40 µL DMSO, we withdrew a known volume of DMSO and organic extract mixture and recorded the change in weight. Density of the DMSO and organic extract mixture could thus be calculated, and together with the weight of the DMSO and organic extract mixture, we were able to calculate the exact final sample volume. This information was used to calculate the corresponding concentration factors in experiments compared to the original samples. The DMSO dissolved samples were stored in amber HPLC vials in darkness at −20 °C.

2.4 Chinese Hamster Ovary (CHO) cells

CHO K1 cell line AS52, clone 11−4−8 was used for all experiments (Wagner et al. 1998). The CHO cells were maintained in Ham's F12 medium containing 5% fetal bovine serum, 1% L-glutamine, and 1% antibiotics (0.25 µg/mL amphotericin B, 100 units/mL sodium penicillin G, and 100 µg/mL streptomycin sulfate in 0.85% saline) at 37 °C in an incubator with a humidified atmosphere of 5% CO_2.

2.5 CHO cell chronic cytotoxicity assay

The CHO cell chronic cytotoxicity assay quantifies the reduction in CHO cell density as a function of the concentration of samples over the course of 72 h. The metric for the cytotoxicity

assay was LC_{50}, the sample concentration that induced a cell density that was 50% of the concurrent negative controls. Detailed description to apply the assay was published (Plewa and Wagner 2009; Wagner and Plewa 2017). Up to six replicate CHO cell clones per concentration were conducted.

2.6 N-acetylcysteine (NAC) thiol reactivity assay

The *in chemico* NAC thiol reactivity assay measures the ability of the samples to react with the biologically relevant thiols. The assay was based on Ellman's test (Ellman 1959). The more thiol-reactive the samples are, the less the added NAC thiols remain, which corresponds to less color development upon addition of Ellman's reagent and can be quantified spectrophotometrically. The metric for the NAC thiol reactivity assay was EC_{50}, which was the concentration factor of the sample that induced a reduction in the NAC thiol concentration by 50% as compared to the concurrent negative controls. Details regarding this were published (Dong et al. 2017a; Dong 2018; Pals et al. 2016).

2.7 Statistical analysis

LC_{50} values were obtained through regression analysis for each concentration-response curve. Similarly, EC_{50} values were calculated through regression analyses for each concentration-response curve. We used Tukey ANOVA tests to compare the means of LC_{50} and EC_{50} values. We used Pearson's Product-Moment Correlation analysis to establish possible correlations between cytotoxicity and thiol reactivity. The power of the ANOVA test for significance was maintained at ≥ 0.8 at $\alpha = 0.05$.

3. Results and discussion

Different *Ct* values, as well as applying identical *Ct* values that are achieved through either high disinfectant concentration with low contact time or low disinfectant concentration with long contact time, were compared regarding the induction of CHO cell cytotoxicity and thiol reactivity using ozonation and chlorination technologies.

3.1 Effect of different Ct values on cytotoxicity: ozonation vs. chlorination

We compared the induced mammalian cell cytotoxicity of water samples after application of high vs. low *Ct* values to wastewaters in this section.

At the applied ozone doses, ozonation lowered cytotoxicity of the non-disinfected wastewaters from two sources (A and B) by at least 2.2 times and as much as 22.4 times regardless of the selected *Ct* values ($P < 0.05$, Fig. 2). Here, we lengthened the ozone purging duration into the semi-batch reactors to create an average of 4.7 fold higher *Ct* values of ozone exposure. The prolonged disinfectant exposure may allow for longer reaction time between ozone and precursors to form byproducts (during which no significant mineralization occurred, refer to DOC values in Table S1). It is possible that at very high ozone doses beyond that required for disinfection, e.g. 4 mg O_3/ mg DOC, rather than the range of 0.31 to 0.93 mg O_3/ mg DOC in this study (highest recorded dissolved ozone concentration achieved for a given sample), the mineralization of certain fractions of organic matter may result in further lowered toxicity due to more complete destruction of these organics (Ratpukdi et al. 2010).

The impact of chlorination on cytotoxicity was specific to the water matrix. For water A, in agreement with the general trend as observed among all ozonated waters, a higher applied *Ct* value (6.8 times higher) increased the toxicity by 1.27 fold as compared to its lower *Ct* value counterpart ($P < 0.05$, Fig. 2a). We previously observed that in municipal secondary effluent

wastewater, longer chlorination contact time (e.g. 48 h vs. 15 min) resulted in formation of more haloacetonitriles that positively and significantly correlated with CHO cell cytotoxicity (Dong et al. 2016). Similarly, increased residence time for chlorination in a municipal drinking water distribution pipeline was found to have promoted the formation of *N*-nitrosodimethylamine, a carcinogenic nitrogenous DBP (Charrois and Hrudey 2007). It is likely that the longer reaction time between chlorine and organic matter led to the formation of more and/or higher concentrations of toxic byproducts. However, although consistent with certain scenarios from our previous study (Massalha et al. 2018), for water sample A neither chlorine *Ct* values produced disinfected waters with significantly different toxicity potencies than the non-disinfected controls ($P > 0.05$). One possible explanation is that water sample A did not contain sufficient precursors to generate cytotoxic DBPs.

For water sample B, a higher *Ct* value reduced the toxicity by 1.63 fold compared to the lower *Ct* value ($P < 0.05$), with both being significantly more toxic than the controls ($P < 0.05$, Fig. 2b). The observation that higher chlorine *Ct* value yielded less toxic finished waters is inconsistent with earlier research (Dong et al. 2016) and other samples within the current study, suggesting water matrix-specific toxicological responses. This difference in water matrix is reflected by the difference in the aromaticity of water B (SUVA = 2.11 $m^{-1}mg^{-1}L$) and water A (SUVA = 1.72 $m^{-1}mg^{-1}L$) (18.5% difference), despite similar DOC values (A = 14.4 mg C/L, B = 13.9 mg C/L, 3.5% difference). The result that all of the chlorinated sample B were significantly more toxic than the controls was consistent with previous work (Dong et al. 2016; Dong et al. 2017b; Massalha et al. 2018; Yang et al. 2014).

In general conditions for both ozonation and chlorination technologies, higher *Ct* values produced disinfected wastewaters with enhanced levels of mammalian cell cytotoxicity.

3.2 Effect of different ways to achieve the same Ct values on cytotoxicity: ozonation vs. chlorination

This section discusses the effect of adopting different methods to achieve identical *Ct* values, namely varying the disinfectant dose and disinfectant contact time while keeping the cumulative disinfectant dosage over time identical, on the cytotoxicity of disinfected wastewaters.

For both ozonation and chlorination, lower cumulative disinfectant concentrations over longer contact times yielded lower cytotoxicity than higher cumulative disinfectant concentrations over shorter exposure times. This trend was observed for both tested waters (C and D). For these two sources of wastewaters (C and D) for identical *Ct* values, ozonation produced cytotoxicity levels that were not significantly different from ($P > 0.05$) or even much lower than the non-disinfected controls (Fig. 3) ($P < 0.05$). These results are consistent with the observations discussed above in section 3.1 and in past research (Dong et al. 2016; Dong et al. 2017b; Massalha et al. 2018). Chlorination on the other hand increased cytotoxicity by at least 4.27 fold as compared to the negative controls (Fig. 3). At the identical *Ct* value (e.g. $Ct = 56.61$ mg min/L), the chlorination with a higher *C* and lower *t* enhanced the cytotoxicity by 4.27 fold, which was much more than the 2.79 fold induced by chlorination with a lower *C* and longer *t*. We observed that during chlorination, cytotoxicity was more associated with disinfectant dosage than contact time, likely due to higher sensitivity of the cytotoxicity to the chlorine dosage than the contact time. In water C scenario, when the free chlorine dose increased from 5 to 7 mg/L (1.4 fold increase) and the contact time reduced from 30 to 10 min (3 fold decrease), the LC_{50} of the disinfected water varied by 1.5 fold (from approximately 15 to 10 concentration factor)(Fig. 3c). For water D scenario, when the free chlorine dose increased from 6 to 8 mg/L (1.33 fold increase) and the

285 contact time reduced from 110 to 15 min (7.33 fold decrease), the LC_{50} of the disinfected water
286 only changed by 1.53 fold (from 11.74 to 7.68 concentration factor)(Fig. 3d).

287 In summary, for a given disinfectant exposure *Ct* target, lower disinfectant dosages combined
288 with longer disinfectant contact time produced the lowest induced cytotoxicity. The cytotoxicity
289 results for chlorination was more responsive to the disinfectant dosage than the disinfectant
290 contact time.

291 *3.3 Impact of wastewater organic extracts on NAC thiol-specific biological detoxification*
292 *mechanism*

293 Thiol-specific reactivity is an important metric associated with cytotoxicity (Dong et al. 2017a;
294 Dong 2018; Pals et al. 2016; Pals et al. 2017; Schultz et al. 2007; Schultz et al. 2006). In this
295 study, 80% of the cytotoxicity analyses rank order was consistent with the rank order of NAC
296 thiol reactivity assay (Fig. 4). A Pearson's correlation analysis ($r = 0.79$, $P < 0.05$) expressed a
297 significant correlation between the cytotoxicity (LC_{50}) and NAC thiol reactivity (EC_{50}) (Fig. 5).
298 This suggests that the adverse biological effects of the XAD2/8 organic extracts of the
299 wastewater samples were associated with the NAC thiol-specific soft nucleophile attacks. The
300 thiol groups are present in essentially all mammalian cells in concentrations between one to ten
301 mM, with the reduced form dominant over the oxidized form (Pastore et al. 2003). The thiol
302 moiety in these living systems exist in the forms such as amino acid *L*-cysteine and *L*-γ-
303 glutamyl-*L*-cysteinylglycine (glutathione). Glutathione is the primary intracellular tripeptide that
304 provides a thiol pool to buffer against and remediate toxicity caused by electrophiles (Meister
305 and Anderson 1983; Townsend et al. 2003). When oxidative stress is present, a cysteine thiol
306 group can be oxidized to a disulfide, or can follow stepwise reactions to produce sulfenic,
307 sulfinic, and eventually sulfonic acids (Timbrell 1999). When the cysteine thiol in glutathione is

depleted or overwhelmed by oxidizing agents, adverse biological responses may be induced (Meister and Anderson 1983; Townsend et al. 2003). Many halogenated compounds such as chlorinated acyls are known to react with the thiol group, consuming the available thiols and forming substances such as thiol esters (Solomons 1996). Previous research on DBPs such as alkyl halides suggested that the thiol group on NAC reacted with these soft electrophiles and therefore NAC may possess the potential to reflect the relative toxicity of these individual compounds (Pals et al. 2016; Pals et al. 2017). Additionally, thiol reactivity was found to correlate with toxicity among several halo-carbonyl compounds (Schultz et al. 2007). It is therefore likely that the organic extracts contained these thiol-reactive DBPs, resulting in the observed relationship between cytotoxicity and thiol reactivity. We recently demonstrated a good correlation between thiol reactivity and CHO cell cytotoxicity (Dong 2018).

Although these DBPs and other agents isolated from the water samples are associated with the interaction of biological thiols, further study is necessary to elucidate the precise molecular mechanisms and cellular targets of the individual DBPs. Since thiol-specific reactivity may reduce the cellular defenses against reactive toxic agents, understanding their direct cellular target molecules and toxicogenomic characteristics responsible for the toxicity requires additional research. However, the NAC thiol reactivity demonstrated the adverse impact of wastewater organic extracts on the biological thiol-specific detoxification mechanism.

3.4 Implications for ozonation and chlorination practice

For water reclamation disinfection operations, it is desirable to develop engineering designs to minimize the overall cytotoxicity of the finished water. The results from this work suggest that design efforts should be made to lengthen the disinfectant contact time while keeping the disinfectant dose to a minimum, regardless of the disinfectant choice (between ozone and

chlorine). Unfortunately, it is well developed that certain pathogens of concern, such as *Giardia*, may require relatively high disinfectant exposure *Ct* values (Crittenden et al. 2012; Jarroll and Hoff 1988). As demonstrated in this work, high disinfectant exposure produced significantly higher levels of induced cytotoxicity. This presents a conundrum in that these more recalcitrant pathogens could be ineffectively inactivated due to insufficient disinfectant exposure if one is to simply pursue low toxicity in the disinfected water. A previous research suggested a three-step disinfection approach rather than the traditional one-step chlorine dosing method (Li et al. 2017b) as the former improved inactivation while reducing the generation of total organic halogen. In combination with the current investigation, one strategy to overcome such a problem is the disinfectant boosting method, where disinfectant is applied at lower *Ct* values, and at the end of a disinfectant exposure period another adequate amount of low *Ct* value boost disinfectant is applied. This strategy has the combined benefit of meeting the designed cumulative disinfectant exposure to inactivate the resistant pathogens while ensuring a minimum level of cytotoxicity in the disinfected reclaimed water. It is also important that other guidelines are followed depending on the end use of the disinfected water. For instance, if the reclaimed water is to be used for plant irrigation and cultivation the disinfection process must not damage plant roots, especially at the beginning of the exposure period when the disinfectant concentration is the highest.

Ozone consistently reduced or did not increase the cytotoxicity in all of the analyzed wastewaters. This is consistent with previous research (Blatchley et al. 1997) and suggests that ozonation may be a preferred technology compared to chlorination from the point of view of disinfection and toxicity minimization. If mandated for residual disinfectant maintenance in distribution pipelines, ozonation may serve as a primary disinfectant, after the dissipation or consumption of which only very low dosage of chlorine is needed to solely fulfill the residual

354 requirement. This strategy combines the advantage of lowering toxicity during ozonation with
355 the advantage of low chlorine dosage plus long contact time. In order to protect the environment
356 and the public health these strategies should be considered with specific water reuse
357 requirements and technologies.

358 **4. Conclusion**

359 With this research, we demonstrated that:

360 • Higher disinfectant exposure in general resulted in higher levels of mammalian cell
361 cytotoxicity in agricultural wastewaters.
362 • Given the identical disinfectant exposure, lower disinfectant concentration combined with
363 longer contact time was found to produce lower toxicity than higher disinfectant
364 concentration combined with shorter contact time.
365 • Disinfectant boosting method, where disinfectant is applied at lower *Ct* values, and at the end
366 of a disinfectant exposure period another low *Ct* value boost disinfectant is applied to satisfy
367 the total *Ct* design, may minimize toxicity.
368 • NAC thiol reactivity suggested that thiol-specific nucleophilic attacks by the reactive and
369 toxic wastewater organic extracts may initiate adverse biological impacts.
370 • Ozonation may be a preferred technology compared to chlorination from the point of view of
371 disinfection and toxicity minimization.

372

Acknowledgement

We would like to acknowledge the USDA and USDA/EPA grants on human health impacts of water reuse (THN, MJP), the U.S. Army Engineer Research and Development Center and the Army Environmental Quality Technology program, CESU W9132T-16-2-0005 (MJP). BARD, the United States - Israel Binational Agricultural Research and Development Fund, Vaadia-BARD Postdoctoral Fellowship Award No. FI-552-16, and the Israeli Council for Higher Education are acknowledged for supporting Dr. Nedal Massalha. We thank E.P. Purification Inc. and Professors J. Gary Eden and Sung-Jin Park for providing the ozone generator. Marika Maggos is acknowledged for some of the solid phase extraction work.

Notes

[Δ] These authors contributed equally.

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Table 1

Water characteristics of the tested wastewaters.

Sample	UV_{254} (cm^{-1})	DOC (mg C/L)	SUVA ($m^{-1}mg^{-1}L$)	NH_3-N (mg N/L)	pH
Water A	0.248	14.4	1.72	0.45	7.05
Water B	0.294	13.9	2.12	7.2	6.13
Water C	0.219	16.5	1.33	0.49	6.63
Water D	0.231	12.3	1.88	0.28	6.77

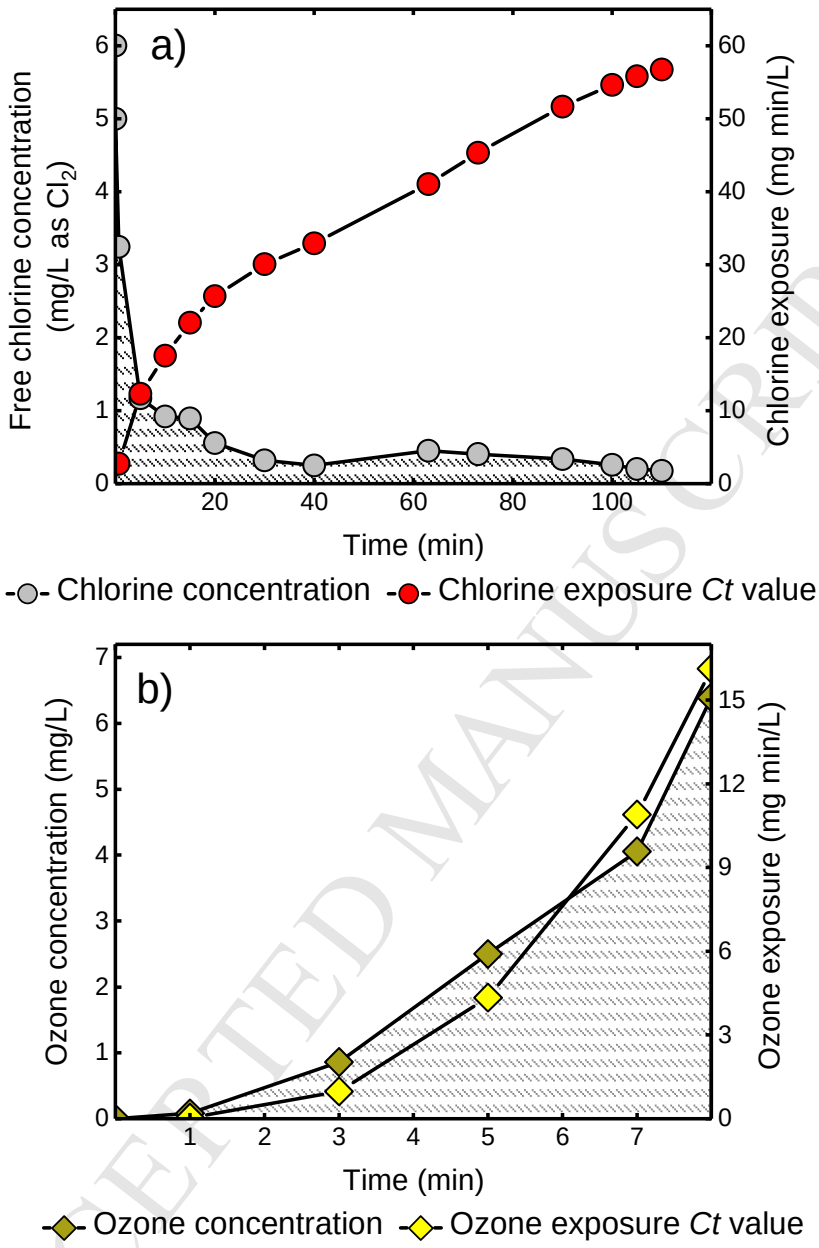

Fig. 1. Disinfectant consumption and *Ct* curves as a function of time for an example a) chlorination experiment and b) ozonation experiment. Ozonation experiments were stopped stoichiometrically using sodium thiosulfate after the designed *Ct* values have been achieved.

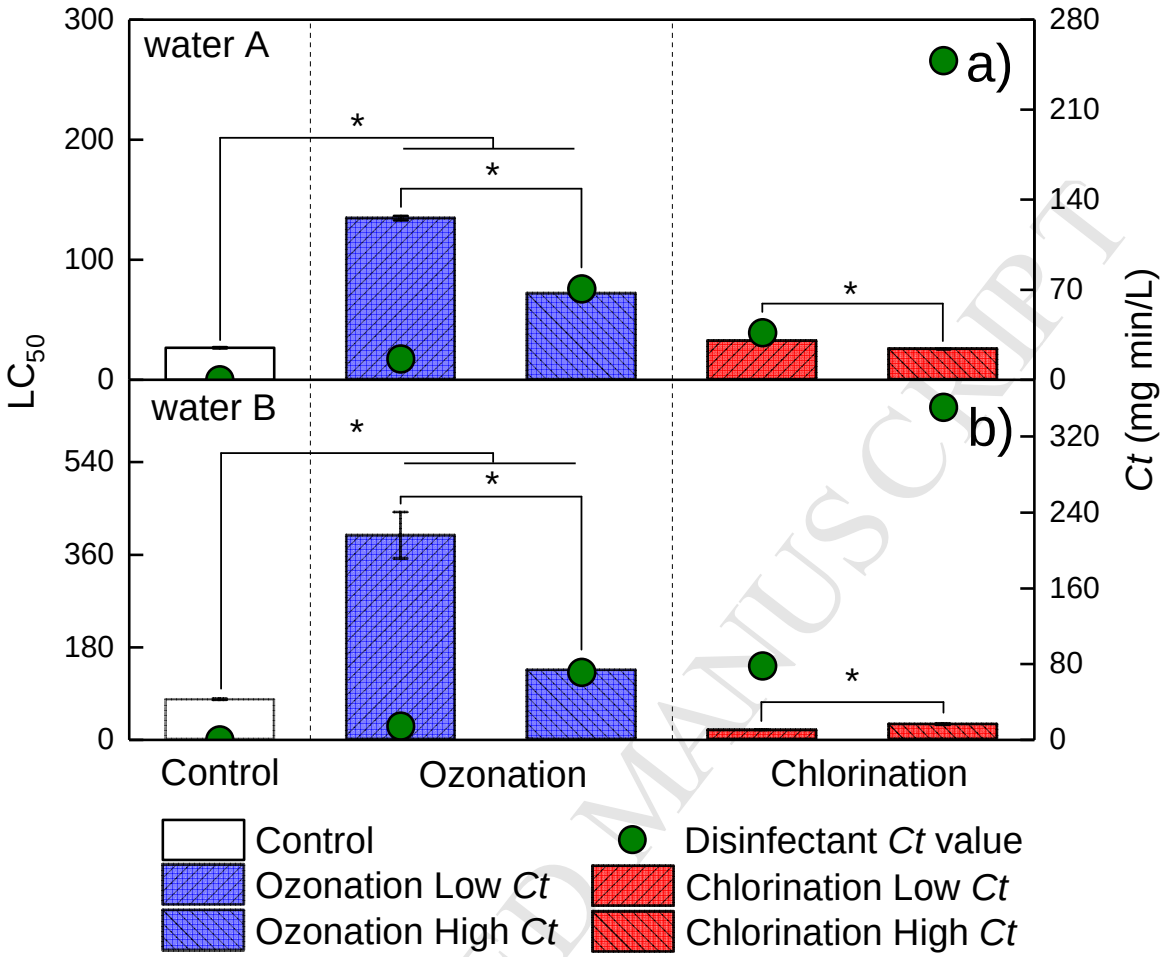

Fig. 2. Cytotoxicity of all samples for the varied *Ct* experiments expressed in LC_{50} values (left y-axis) of sample concentration factors. The top panel (labeled a)) corresponds to wastewater source A, and the bottom panel (labeled b)) corresponds to wastewater source B. Green dots represent the corresponding *Ct* values for each sample (right y-axis). Error bars represent standard error of the mean of up to six replicates. Asterisks indicate significant difference in means from ANOVA tests ($\alpha = 0.05$).

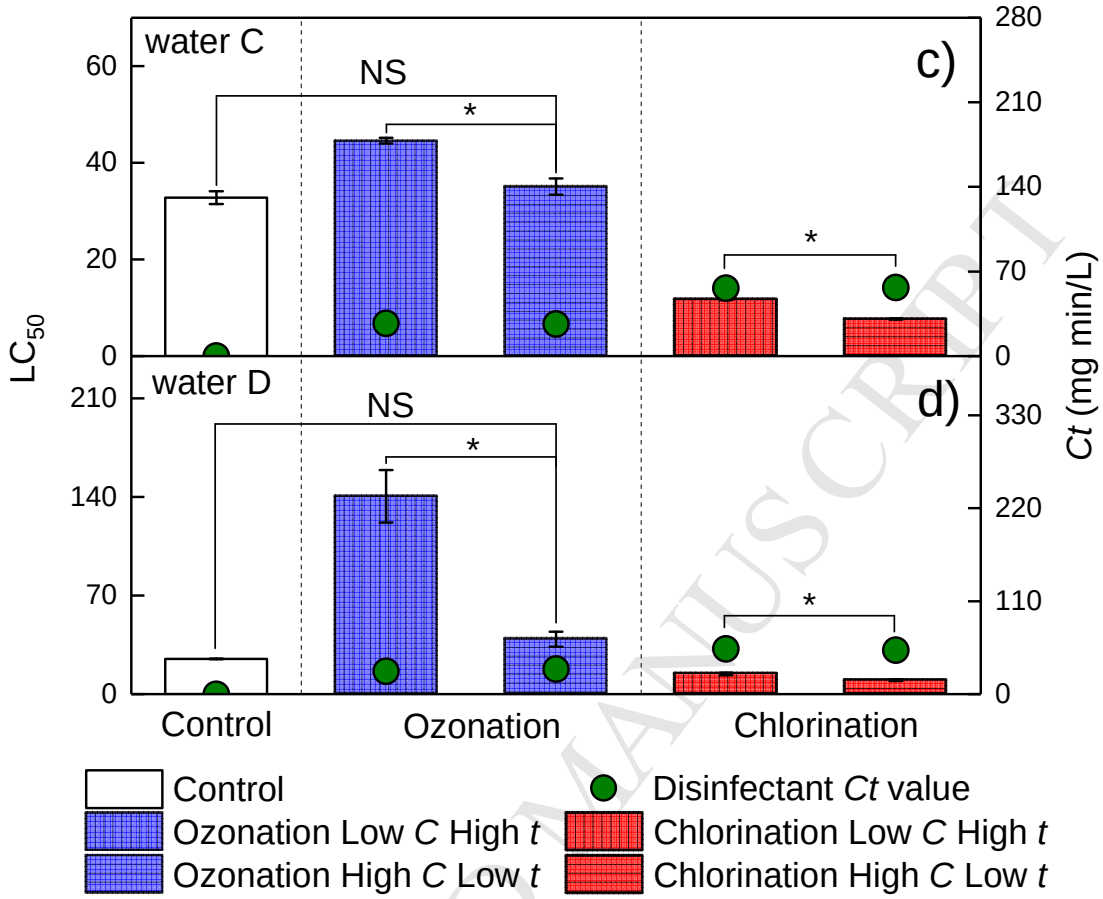

Fig. 3. Cytotoxicity of all samples for the identical *Ct* experiments expressed in LC_{50} values (left y-axis) of sample concentration factors. The top panel (labeled c)) corresponds to wastewater source C, and the bottom panel (labeled d)) corresponds to wastewater source D. Green dots represent the corresponding *Ct* values for each sample (right y-axis). Error bars represent standard error of the mean of up to six replicates. Asterisks indicate significant difference in means from ANOVA tests ($\alpha = 0.05$), NS stands for Not Significant at $\alpha = 0.05$.

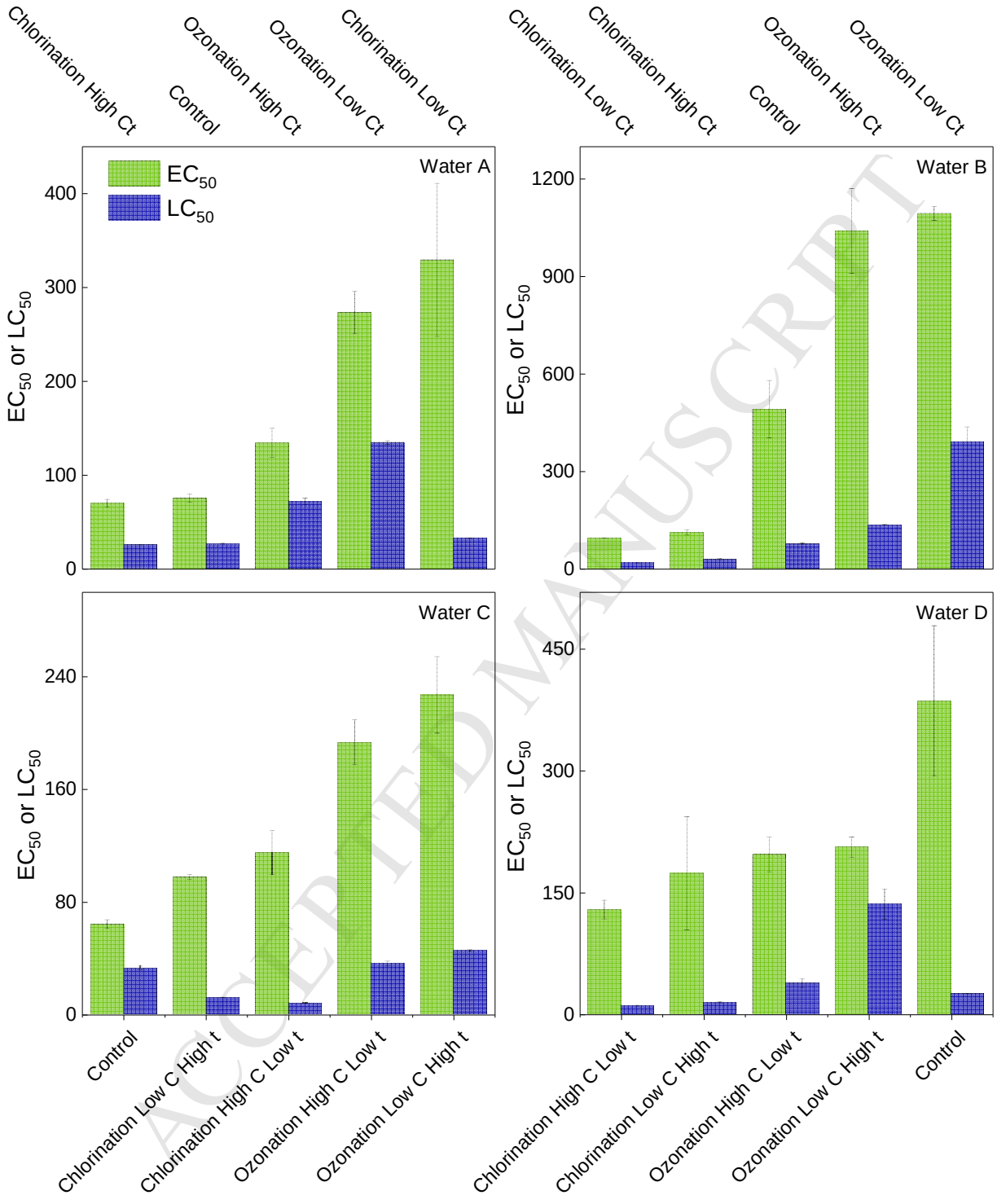

Fig. 4. Comparison of cytotoxicity (expressed in LC_{50} values) and NAC thiol-specific reactivity (expressed in EC_{50} values) of the XAD organic extracts of wastewater sample A, B, C, and D, with and without ozonation or chlorination following various disinfection regimes. Error bars represent standard error of the mean of up to six replicates.

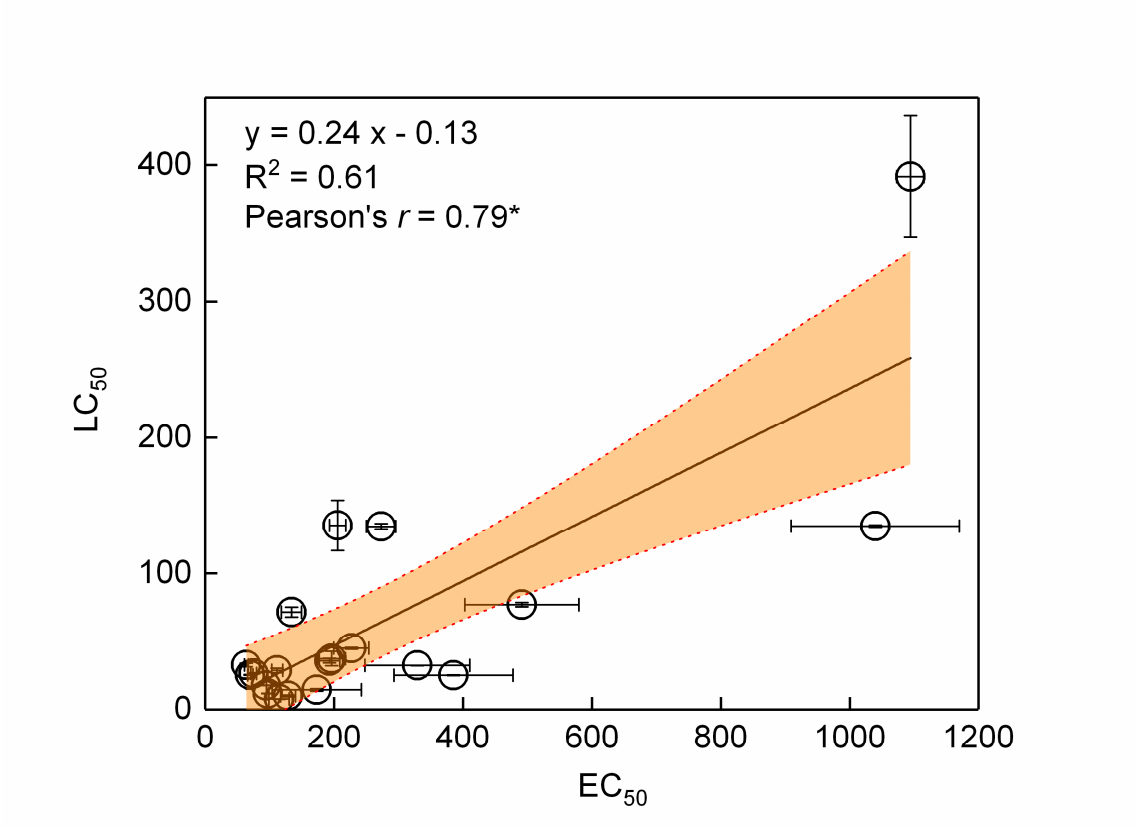

Fig. 5. Cytotoxicity LC_{50} values vs. NAC thiol reactivity EC_{50} values. Error bars represent standard error of the mean of six replicates. The shaded area surrounding the linear regression line represents the 95% confidence interval.

Highlights

- Ozonated wastewaters were less toxic than chlorinated wastewaters
- Ozone and chlorine toxicity increased with increasing disinfectant exposure (*Ct*)
- Ozone and chlorine low *C* with long *t* induced less toxicity than high *C* with low *t*
- Multipoint disinfectant injection at low dose preferred over single point high dose
- Thiol-specific attacks positively correlated with cytotoxicity