Chlorination in a wastewater treatment plant: acute toxicityeffects of the effluent and of the recipient water body

CHLORINATION IN A WASTEWATER TREATMENT PLANT: ACUTE TOXICITY EFFECTS OF THE EFFLUENT AND OF THE RECIPIENT WATER BODY.

Cristina Pignata[1]*, Elisabetta Fea[1], Renato Rovere[1], Raffaella Degan[1], Eugenio Lorenzi[2], Margherita de Ceglia[2] , Tiziana Schilirò[1] and Giorgio Gilli[1].

[1]Department of Public Health and Microbiology, University of Torino, Via Santena 5bis, 10126 Torino, ITALY.

[2]Società Metropolitana Acque Torino s.p.a., Divisione Fognatura e Depurazione, Via Po 2, 10098 Castiglione Torinese (TO), ITALY.

***Corresponding Author:**

Cristina Pignata

Department of Public Health and Microbiology

University of Torino

Via Santena, 5bis

10126 Torino ï ITALY

tel. + 39 011 6705822

Fax + 39 011 6705874

e-mail: cristina.pignata@unito.it

ABSTRACT

This study investigates the impact of wastewater treatment plant (WWTP) effluent on the toxicity of the recipient water body and the effectiveness of the disinfection treatment applied (sodium hypochloride) to assure the compliance of both microbiological and toxicological emission limits. No toxicity was found in the majority of samples collected from the recipient river, upstream and downstream of the WWTP, using three different toxicity tests (*Vibrio fischeri, Daphnia magna, Pseudokirchneriella subcapitata*). Only three samples presented Toxic Unit (TU) values with *V. fischeri*, and one presented TU with *S. capricornutum*. The influent toxicity ranged from slightly toxic to toxic (TU = 0.68 - 4.47) with *Vibrio fischeri*, while only three samples presented TU values with the other tests. No toxicity was found in the absence of chlorination, while the mean toxicity was 3.42 ± 4.12 TU with chlorination in the effluent. Although no toxicity or very slight toxicity was found in the receiving water, its residual toxicity was higher than the U.S. EPA Quality Standard in two samples. *E. coli* concentration had a lower mean value in the chlorinated effluent: 13,993 ± 12,037 CFU/100 mL vs. 62,857 ± 80,526 CFU/100 mL for the non-chlorinated effluent. This difference was shown to be significant ($p < 0.05$). *E. coli* in ten chlorinated samples was higher than the limit established by European and Italian Legislation. The mean highest Trihalomethanes (THMs) value was found in the influent samples (2.79 ± 1.40 µg/L), while the mean highest disinfection by products (DBPs) was found in the effluent samples (1.85 ± 2.25 µg/L). Significant correlations were found between toxicity, sodium hypochlorite, THMs, DBPs, *E. coli* and residual chlorine.

In conclusion, this study highlighted that the disinfection of wastewater effluents with sodium hypochlorite determines the increase of the toxicity, and sometimes is not enough to control the *E. coli* contamination.

Keywords: Wastewater; Chlorination; Toxicity; Trihalomethanes; *Escherichia coli.*

1. Introduction

Industrial wastewater, effluent of sewage treatment plants and run-off from agriculture are major sources of surface water pollution. Wastewater is a complex mixture of various organic and inorganic compounds; in addition to the unknown products discharged into the wastewater treatment plants, other substances are formed during the treatment processes (Farrè et al. 2001; Ricco et al. 2004). Moreover, in recent years, the incidence of human-use compounds, such as pharmaceuticals and drugs, in aquatic environments has been recognized as an important issue in environmental chemistry. Some of these compounds enter the aquatic environment, mostly via the effluents of municipal sewage treatment plants, unaltered or as slightly transformed metabolites (Huerta-Fontela et al. 2008; Watkinson et al. 2009). Due to the presence of several chemical pollutants, no useful monitoring or screening of surface water can be based only on chemical analysis of a limited number of toxic compounds. Therefore, biological tests prove to be indispensable for the assessment of cytotoxic and genotoxic potential in surface water. Because of the variety of aquatic organisms and the heterogeneous condition in aquatic environments, there is no single biotest for detecting toxic and genotoxic effects. Only a set of bioassays with prokaryotic and eukaryotic organisms can be applied to estimate accurately the effects of toxicants in surface waters (Dizer et al. 2002; Persoone et al. 2003).

One of the objectives of the European Community's (EC) environmental regulations is to reduce the pollution of surface water caused by municipal waste (see the Council Directive 91/271/EEC as amended by the Commission Directive 98/15/EEC of 27 February, 1998). This requires the European Union (EU) member states to ensure that discharge of urban wastewater and its effects are monitored (Farrè et al. 2001; Mantis et al. 2005, see also Council Directive 2000/60/EC).

In order to prevent sanitary hazards related to the uses of recipient water bodies, the current Italian regulations prescribe WWTP effluent emission limits for a wide range of chemical compounds, toxicity and bacterial discharge (i.e., *Escherichia coli*). In order to

meet the bacterial discharge limit, WWTPs can introduce a wastewater disinfection step; however, disinfectants may induce chemical reactions, leading to the production of disinfection by-products (Decree Italian Law 152/2006).

The microbiological emission limit of *E. coli* is not stated by the national regulation at a general level, but it should be established by local authorities in each specific discharge licence with respect to the public health situation, and to the foreseen uses of the recipient water body. In the case of the WWTP investigated, the local authority (Piedmont Region) has evaluated the introduction of a concentration limit for *E. coli* of 20,000 CFU/100ml, while the Council Directive 2000/60/EC has introduced a concentration limit for *E. coli* of 5,000 CFU/100ml. In order to respect this limit, many WWTPs apply a wastewater disinfection process, because sometimes the *E. coli* concentration at the end of the purification process is higher than the limit established by the local authorities and by European legislation. However, disinfectants can induce chemical changes in these systems, thus resulting in changes that will not be restricted to the microbial population. One possible outcome of these chemical changes is a change of the effluent toxicity, as demonstrated by Blatchley et al. (1997), Monarca et al. (2000), Wang et al. (2007) and Wu et al. (2010). Chemical disinfectants are effective for killing harmful microorganisms in water, but they are also powerful oxidants, oxidizing the organic matter, anthropogenic contaminants, and bromide/iodide naturally present in most source waters (rivers, lakes, and groundwater). Chlorine, ozone, chlorine dioxide, and chloramines are the most common disinfectants in use today: each produces its own suite of DBPs in water, with overlapping constituents. In the 30 years since the THMs were identified as DBPs in drinking water, significant research efforts have been directed toward increasing the understanding of DBP formation, occurrence, and health effects. Although more than 600 DBPs have been reported in the literature, only a small number has been assessed either in quantitative occurrence or health-effects studies (Richardson et al. 2007). Toxicity of water disinfection and DBPs was studied intensively. Many chlorinated by-products showed doseï response

relationships with DNA and chromosome damage, cytotoxicity and apoptosis in vivo (Lu et al. 2002; Richardson et al. 2007; Yuan et al. 2006) or in vitro (Boorman et al. 1999; Lu et al. 2004; Yuan et al. 2005; Shi et al. 2009).

The aim of the present work was to investigate the impact of a WWTP effluent on the recipient water body, with particular respect to its toxicity, and to verify the effectiveness of disinfection treatment with sodium hypochlorite (NaOCl). This was to ensure the compliance with both microbiological and toxicological emission limits, using three different toxicity tests (*Vibrio fischeri, Daphnia magna, Pseudokirchneriella subcapitata*). Finally, the presence of disinfection by-products (trihalomethanes) and *E. coli* was measured to evaluate the correlation with acute toxicity and the efficiency of the chlorination process.

2. Material and methods

2.1. Features of the sewage treatment plant

The considered WWTP is a consortium plant that treats civil and industrial discharges from the municipal districts of Collegno, Grugliasco, Rivoli and Villarbasse (Torino, Piedmont Region, Italy), a metropolitan area in Northern Italy, with a total population equivalent of about 400,000. The mean treated flow is around 42,000 m^3/day. The plant comprises a water and sludge treatment system. The former includes primary sedimentation, active sludge oxidation with nitrification/denitrification processes, and a section for the recovery and reutilization of treated water. The mean COD of the influent and of the effluent is 844.16 mg/L and 40.5 mg/L. In order to limit and to evaluate microbiological emissions, 15 of the 22 effluent samples were chlorinated with sodium hypochlorite (3 mg/L) at a mean dosage of 34 L/h. The final effluent was then discharged into the Dora Riparia River (one of the tributaries of the Po River, the largest Italian river) which has a mean flow rate of 26 m^3/s.

2.2. Sampling of sewage and water

Twenty-four hour composite samples of the influent (IN) and final WWTP effluent (OUT) were taken during ten different sampling events from February 2005 to November 2005 (first sampling period) and twelve different sampling events from September 2006 to May 2007 (second sampling period). On the same dates, grab samples of water were collected from the recipient river, 2 km upstream (US) and 2 km downstream (DS) of the WWTP. The samples (4 ï 14 L) were divided into four aliquots and stored in brown glass flasks at 4°C. In each sample, an aliquot of 1 L was used for the toxicological analysis, and another 200 mL aliquot was utilised for the microbiological analysis. Another 1 L aliquot was used for trihalomethanes (THMs) analysis (only during the second sampling period), and the remainder was stored at 4°C until the end of the analyses. All the analysis were performed within 24 hours from the sampling. Also grab disinfected effluent samples (100 ml) were collected for immediate analysis of the residual chlorine.

2.3. Microbiological analysis

Determination of *E. coli* was performed using the membrane filter technique (AWWA 1998), which is highly reproducible, can be used to test relatively large sample volumes, and yields numerical results more rapidly than the multiple-tube procedure. The results are expressed in Colony Forming Unit (CFU)/100 mL.

2.4. Biological assays

Microtox™ test

After the screening test, the BASIC test (90%) was applied following the procedure described in the Microtox™ manual (Azur Environmental 1995). The principle of this system is based on the evaluation of the luminous energy naturally emitted by *V. fischeri* bacteria (Azur Environmental, Carlsbad, CA, USA). Luminescence was measured at time zero and after 5, 15 and 30 minutes, and compared to the control.

The final expression of the toxic potentials of samples is the Effective Concentration at 30 minutes, EC50, showing the sample concentration factor which caused a 50% brightness decrease of the bacteria population. Each test was analysed using a MicrotoxÊ reference toxicant (phenol) as quality control.

Daphnia magna test

This test is based on the evaluation of the immobilization of 10 organisms in the presence of stress sources against a control. The dormant eggs of the crustacean and stock solution for preparation of the standard freshwater (International Organization for Standardization) medium were taken from the commercial test system, DaphToxkit F™ *magna* (MicroBioTests, Nazareth, Belgium). The hatching of ephippia and the preparation of standard freshwater were performed according to the manufacturer's instructions. The ephippia were transferred to hatching petri dishes with 50 mL pre-aerated standard freshwater, thereafter covered and incubated for 72 hours, at 20ï 22°C under continuous illumination of 6000 lux. A dilution series of treated and untreated water samples was prepared by serial 1:1 dilution with standard freshwater. Assays were carried out in 24-well plates. Five neonates were transferred into each well, which each contained a 10ml water sample. Freshwater controls were included in every test. Tests were performed in quadruplicate. The plates were covered and incubated at 20°C in the dark. After 24 hours and 48 hours of incubation, the number of dead and immobilized neonates was recorded, and the percent mortality was calculated (Cao et al. 2009). The toxic potential of the sample is expressed with EC50, showing the concentration of the sample which causes the immobilization of the 50% of the organisms against the control (OECD 1984a).

Pseudokirchneriella subcapitata test

The algal culture and stock solution for the preparation of growth media were taken from the commercial test system AlgalToxkit F™ (MicroBioTests, Nazareth, Belgium).

Water and wastewater samples were supplemented with mineral nutrients, and incubated with *P. subcapitata* at 23°C ± 2°C under constant uniform illumination (8000 lux) for 72 hours in disposable long cells in polystyrene (volume 25 mL). The test was run in triplicate for both samples and controls. Algal growth was followed by optical density (OD) at 670 nm after 24, 48 and 72 hour exposure to the samples. The algal growth inhibition was calculated from these data by integrating the mean values, from time zero to time 72 hours, for each concentration tested, including control. The toxic potential of the samples is expressed with EC50 (OECD 1984b). The toxicity test is considered acceptable when the number of algae in the control test vials increases at least by a factor of 16 during the 72 hour test period and the pH does not change by more then one unit.

Final expression of the toxicity results

The EC50 values of the three tests were subsequently converted in toxic units (TU) that are proportional to toxicity:

$TU = (1/EC50) \times 100$

Considering the hazard classification system for wastes discharge into aquatic environment described by Persoone et al. (2003) the judgment of toxicity depends on the values shown in Table 1.

Without specific information concerning the persistence of toxicity, it is recommended that effluent toxicity is limited to dilution estimates and that toxicity is assumed to be additive and conservative. For rivers, the following dilution equation should be used, assuming completely mixed conditions:

$C = (C_sQ_s + C_eQ_e)/(Q_e + Q_s)$

C = downstream toxicity concentration (TU)

C_s = upstream toxicity concentration (TU)

Q_s = upstream mean flow

C_e= effluent toxicity concentration (TU)

Q_e =effluent mean flow (U.S. EPA, 1991).

The downstream toxicity concentration (C) was calculated considering the highest TU value of the three tests applied.

2.5. Trihalomethanes analysis

Trihalomethanes (THMs), composed of disinfection by-products (DBPs) chloroform, bromoform, chlorodibromomethane, bromodichloromethane and other THMs, 1,1,1-trichloroethane, trichloroethylene, carbon tetrachloride, 1,2-dichloroethane, trichloroethylene, and tetrachloroethene, were analysed by headspace combined with gas chromatography coupled to an electron capture detector (GC-ECD) (Ottavini and Bonadonna 2000), with a detection limit of 0.1 µg/L in the samples collected during the second sampling period (Sep 2006 ï May 2007).

2.6. Residual chlorine analysis

The residual chlorine concentrations of the effluent samples were analysed as reported in the Standard Methods for the Examination of Water and Wastewater (AWWA 1998).

2.7. Statistical analysis

The statistical analyses were performed with the statistical package SPSS 17.0 (SPSS for Windows, Chicago, IL, USA) using Spearmanôs test, ANOVA, Probit regression analysis and *T*-test.

3. Results

3.1. Toxicity

Tables 2 - 5 report the toxicity of the 22 different water samples in the four sampling sites. The samples collected from the Dora Riparia River, upstream (table 2) and downstream (table 5) of the WWTP, were not toxic with the three toxicity tests adopted

(Microtox™, *Daphnia magna, Pseudokirchneriella subcapitata*), not even when the chlorination process of the final effluent was started (June 2005) during the first sampling period. But, during the second sampling period, we found acute toxicity in some samples. The sixteenth sample US, and the sixteenth and eighteenth samples DS exhibited slight acute toxicity with Microtox™. Moreover, the fifteenth sample DS exhibited acute toxicity (TU = 1.55) with *P. subcapitata*. As reported in table 3, all the influent samples exhibited TUs and the toxicity ranged from slight acute toxicity to acute toxicity (TU = 0.68 - 4.47) with *V. fischeri*, while only the eleventh and the twentieth samples presented TUs values with *D. magna* (TU sample 11 = 1.09, TU sample 20 = 2.05), while the twenty-second sample presented TU = 1.18 with *P. subcapitata*. So *V. fischeri* was confirmed to have a different sensitivity in the toxicity evaluation of wastewater (Tiġler and Zagorc-Konļ an 1999; Ricco et al. 2004). As reported in table 4, TUs (*V. fischeri*) were often detected in the WWTP effluent samples ranging from 0.40 to 13.83. Using the hazard classification system reported by Persoone et al. (2003), the OUT site was classified from not toxic to hightly toxic. Moreover, four effluent samples presented TUs with *D. magna* ranging from 1.68 to 8.30, and five samples presented TUs with *P. subcapitata* ranging from 1.75 to 4.19. In two cases (the eighth and the tenth samples), the sample concentration and the inhibition of algal growth were inversely proportional. The presence of a high concentration of nutrients for algae in wastewater could have been one of the possible reasons for that. Throughout the 72 hour exposure time, the adverse effects of toxicants could have been masked by the ameliorating effects of the nutrient compounds that stimulate algae growth (Manusadġianas et al. 2003). The mean highest TUs value (*V. fischeri*) was found in the effluent samples (2.27 ± 3.65), and the results of the linear regression analysis (ANOVA) suggested that there were significant differences in the TUs between sites ($F = 7.84$ and $p < 0.001$). The post-hoc Tukey test of the ANOVA results indicated that the difference between effluent and both US and DS TU values was significant, while there was no statistical difference between the US

and DS TU values. As shown in Fig. 1, the TUs mean values were higher in the effluent samples then in the influent for the three toxicity tests applied, and this means that the toxicity generally increased in the effluent.

The evaluation of the overall toxic concentration following the ecotoxicological approach is shown in Fig. 2 (U.S. EPA 1991). Eight DS samples, taken during the disinfection period, exhibited an appreciable toxicity (C), although only the ninth and the sixteenth samples exceeded the U.S. EPA acceptance limit for acute toxicity (TU = 0.3).

In relation to the effect of the chlorination process on the toxicity of the effluent, no toxicity was found in the absence of chlorination, while the mean toxicity was 3.42 ± 4.12 TU with chlorination, considering the highest TU values of the three tests applied.

3.2. Microbiological analyses

Microbiological analyses (Tables 2 - 5) highlighted that there was generally a difference between the four sampling sites (IN: 7,622,700 ± 6,227,340 CFU/100 mL; US: 42,700 ± 23,400 CFU/100 mL; OUT: 34,700 ± 67,000 CFU/100 mL; DS: 39,000 ± 29,200 CFU/100 mL). The results of the linear regression analysis (ANOVA) suggested that these differences in *E. coli* concentration between sites were significant ($F = 31.629$ and $p < 0.0001$). The post-hoc Tukey test of the ANOVA results indicated that the difference between influent and both US and DS samples values was significant, while there was no statistical difference between the *E. coli* concentrations of the other three sites (OUT, US and DS). Microbiological analyses have highlighted the efficiency of the WWTP in the removal of *E. coli* from the influent. The mean removal was 97.83% ± 7.03% at the end of the process; however, sometimes this was not sufficient to reduce the *E. coli* concentration below 20,000 CFU/100 mL, which is the concentration limit established by the local authorities, or below 5,000 CFU/100 mL, which is the concentration limit established by the Decree Italian Law 152/2006. In relation to the effect of the chlorination process on the *E. coli* concentration of the WWTP effluent, we

found a lower mean value for the chlorinated effluent: 13,993 ± 12,037 CFU/100 mL vs. 62,857 ± 80,526 CFU/100 mL for the non-chlorinated effluent (Figure 3). This difference was shown to be significant with the *T*-test ($p < 0.05$). However, *E. coli* in ten chlorinated samples was higher than 5,000 CFU/100 mL (Decree Italian Law 152/2006).

3.3. Trihalomethanes concentration

THMs expressed as the sum of disinfection by-products (DBPs) chloroform, bromoform, chlorodibromomethane, bromodichloromethane and other THMs, 1,1,1-trichloroethane, trichloroethylene, carbon tetrachloride, 1,2-dichloroethane, trichloroethylene, and tetrachloroethene (Tables 2 - 5) were detected at all of the sampling sites at concentrations ranging from <0.10 to 7.72 µg/L. The highest mean THMs value was found in the influent samples (2.79 ± 1.40 µg/L), while the mean highest DBPs value was found in the effluent samples (1.85 ± 2.25 µg/L), and the results of the linear regression analysis (ANOVA) suggest that there were significant differences in DBPs mean concentrations between sites ($F = 5.44$ and $p < 0.01$). The post-hoc Tukey test of the ANOVA results determined that the mean DBP concentration of the WWTP effluent differs significantly from the mean DBP concentrations of the US and DS samples; however, the mean DBP concentrations of the US and DS samples are not significantly different from one another. The DBP values of the effluent exhibited a higher mean value (2.52 ± 2.52 µg/L) in the presence of chlorination, as shown in figure 4. Despite this, the t-test performed between the DBP values with and without chlorination showed that this difference was not significant (t-test, $p > 0.05$), which could be a result of the small sample size.

3.4. Residual chlorine concentration

The residual chlorine concentrations of the effluent samples (Table 4) ranged from <0.05 mg/L to 1.01 mg/L. In six samples it exceed the limit (≤ 0.2 mg/L) established by the Decree Italian Law 152/2006 for the effluent discharged into surface waters.

3.5. Comparison of toxicity, E. coli, NaOCl, residual chlorine and DBPs

Spearman correlations were calculated between toxicity and the other parameters considered in this study. Significant correlations were found for TU vs. DBPs ($r = 0.632$, $p < 0.01$), TU vs. *E. coli* ($r = 0.254$, $p < 0.05$), and DBPs vs. *E. coli* ($r = 0.570$, $p < 0.01$). These relationships become closer if one only considered the effluent site. All the data are reported in table 6.

4. Discussion

In the absence of effluent chlorination, the WWTP investigated in this study has a good efficiency in removing the influent toxicity. This evidence is confirmed by the absence of toxicity with all the tests utilized in the recipient water body both downstream and upstream of the plant discharge, except for the fifteenth sample from DS site that presented a TU value with *P. subcapitata*. Whereas, in the second sampling period (2006 - 2007), we found a low toxicity in one US sample and in two DS samples after the effluent treatment with NaOCl; the disinfection of these samples might have used the highest concentrations of sodium hypochlorite (4.58 and 5.00 mg/L). Furthermore, during the first sampling period (2005), the effluent toxicity did not change in summer, even when the disinfection had been applied. However, with the lowering of effluent temperature in October, toxicity increased significantly, showing the maximum value in the eighth sample (October 2005). This was probably due to the high temperatures observed that summer in Northern Italy. This phenomenon probably caused a high evaporation rate of oxidising volatile compounds, and minimised the formation and residence time in the water phase of disinfection by-products, as reported in the study of Matamoros et al. (2007), where it was observed that the THMs production

decreased with higher temperatures, and that this decrease could be attributed to the increase of ammonia nitrogen concentration observed during summer. Moreover, the increase in the toxicity value from summer to autumn could also depend on the change of quality of wastewater entering the plant. Ra et al. (2007) reported a seasonal variation in the toxicity which was lower in summer compared to winter, but it was due to the rainfall. The calculated toxicity (C) of the Dora Riparia was obtained by taking into account the toxicities and flow rates of both WWTP discharge and its recipient water body, and it resulted in being above the water quality standard established by U.S. EPA (1991) for acute toxicity in two samples. This result was not in accordance with the measured toxicity in the river downstream of the WWTP outlet, but it has to be considered that this was based on grab sampling, so the results are not completely representative. Moreover, the toxicity with *V. fischeri* presented a significant correlation with the NaOCl concentration, the THMs and the DBPs concentration as reported in other studies (Petala et al. 2008; Zouboulis et al. 2007; Monarca et al. 2000), but we did not find correlation with the effects on *D. magna* and *P. subcapitata.* Cao et al. (2009) found an increased mortality of neonates (*D. magna*) after chlorination, but the disinfectant dosages used were higher than 5 mg/L.

The WWTP reached a good percentage removal of the bacterial concentration, but the disinfection process applied can be considered less effective: in eight effluent samples (four in absence of chlorination and four in presence of chlorination), *E. coli* exceeded the concentration of 20,000 CFU/100 mL (the limit established by the local authority) and exceeded the concentration of 5,000 CFU/100 mL (Decree Italian Law 152/2006) in all the effluent samples not disinfected and in eleven disinfected samples, even if the *E. coli* concentration in the effluent presented a significant correlation with the disinfectant dosage and with the residual chlorine. It is interesting to highlight that, even if the *E. coli* effluent concentrations were higher than the established limits, we observed no impact on the recipient river because the mean *E. coli* concentration upstream was 42,667 ± 23,422 CFU/100 mL. Also, the study of Gaki et al. (2007)

reported that the chlorination applied was unable to produce the required effluent standard.

THMs and DBPs in both the chlorinated and the non-chlorinated samples were acceptable under Italian legislation (Decree Italian Law 152/2006), which restricts chlorinated solvents of WWTP effluents to 1 mg/L. THMs and DBPs presented a significant correlation with the disinfectant dosage, residual chlorine and toxicity, as reported in the study by Matamoros et al. (2007), where the concentrations of THMs found were comparable with the ones reported in this study.

Regarding the hygienicï sanitary evaluation of the impact of the disinfection practice on the recipient water body, we observed that the chlorination with sodium hypochlorite seems inadequate to comply with the foreseen microbiological emission limit; moreover, it produces an increase in the toxicity of the effluent and the overcoming of the limit established by Italian Law for the residual chlorine concentration. Thompson and Blatchley (1999) studied the toxicity response of wastewater effluent samples exposed to ɔ-radiation compared with chlorinated and municipal wastewater effluent samples not disinfected. The chlorinated effluent samples often showed a statistically significant increase in toxicity as compared to those not disinfected and to the ɔ-irradiated samples. This type of disinfection system is more expensive than chlorination, so it is not as widespread. In another study, Emmanuel et al. (2004) showed that the addition of NaOCl to wastewater can reduce bacterial pollution, but highlighted considerable acute toxicity with *D. magna* (TU = 9.8 ï 116.8) and *V. fischeri* (TU = 2.47 ï 4.15). Petala et al. (2008) evaluated different ozone treatments applied to secondary effluents by combination of bioassays (*V. fischeri*) using different end-points and physicochemical parameters. The study of toxicity of pre-concentrated samples showed that ozonation may either increase or decrease the toxic potential of secondary effluents. The application of low ozone doses induced a decrease of toxicity, whereas ozone doses higher than 5.0 mg O_3/L resulted in an increase of toxicity of treated wastewater, and this was due to the formation of ozonation by-products.

Moreover Gagnè et al., (2008) evaluated the immunotoxic potential of a primary treated municipal effluent following enhanced disinfection by ozonation on freshwater mussels. They found that this disinfection process successfully reduced microbial loading, but increased the inflammatory properties of the effluent.

The studies on wastewater effluents indicated that all toxicity tests have a variable role to play in monitoring and control of water quality, and demonstrated that there is no single method that can constitute a comprehensive approach to aquatic life protection. For this reason, toxicity tests containing sensitive microorganisms should be applied in battery form, so the tests can complement each other, in addition to complementing the chemical analysis (Hemming et al. 2002; Sponza 2003).

In conclusion, this study highlighted that the disinfection of wastewater effluents with sodium hypochlorite determines the increase of the toxicity, and sometimes is not enough to control the *E. coli* contamination; the effluent toxicity after the chlorination process seems to be due to the concentration of the DBPs. The toxicity assessment of the wastewater (influent and effluent) and of the surface water provides a real approach to assess the effluent risk, and enables confirmation of the efficiency of the WWTP to remove toxic compounds. The toxicity tests can be considered as useful analytical tools for the screening of chemical analysis, and as an early warning system to monitor the WWTPs (Hernando et al. 2005). The identification of different disinfectants, such as peracetic acid, ozone or UV, and the study of the ideal concentration for reaching the toxicological and the microbiological standard for WWTP effluent seems to be a research issue that could facilitate the management of the surface water bodies.

Acknowledgment

This research was funded by Società Metropolitana Acque Torino S.p.a ï Torino - Italy.

References

American Public Health Association, American Water Works Association, Water Environmental Federation (1998). Standard Methods for the Examination of Water and Wastewater, 20th Edition. American Public Health Association 1998, Washington, DC, USA.

Blatchley, E.R., Hunt, B.A., Duggirala, R., Thompson, J.E., Zhao, J., Halaby, T., Cowger, R.L., Straub, C.M., Alle man, J.E. (1997). Effects of disinfectants on wastewater effluent toxicity. *Water Res*, 31(7),1581-1588.

Boorman, G.A., Dellarco, V., Dunnick, J.K., Chapin, R.E., Hunter, S., Hauchman, F., Gardner, H., Cox, M., Sills, R.C. (1999). Drinking water disinfection byproducts: review and approach to toxicity evaluation. *Environ Health Perspect*, 107(1), 207ï 217.

Cao, N., Yanga, M., Zhanga, Y., Hub, J., Ikec, M., Hirotsujid, J., Matsui, H., Inoue, D., Sei, K. (2009). Evaluation of wastewater reclamation technologies based on in vitro and in vivo bioassays. *Sci Total Environ*, 407, 1588-1597.

Council Directive 91/271/EEC, May 21, 1991, concerning urban wastewater treatment, OJL 135, May 30th, 1991, 40.

Decreto legislativo 3 Aprile, 2006 n. 152. Norme in materia ambientale. G.U. n. 88, April 14th, 2006, Supplemento Ordinario n. 96.

Directive 2000/60/EC, October 23, 2000, Establishing a framework for Community actions in the field of water policy, OJL 327, December 22nd, 2000, 1.

Dizer, H., Wittekindt, E., Fischer, B., Hansen, P.D. (2002). The cytotoxic and genotoxic potential of surface water and wastewater effluents as determined by bioluminescence, umu-assay and select biomarkers. *Chemosphere* 46, 225-233.

Emmanuel, E., Keck, G., Blanchard, J.M., Vermande, P., Perrodin, Y. (2004). Toxicological effects of disinfections using sodium hypochlorite on aquatic organisms and its contribution to AOX formation in hospital wastewater. *Environ Int*, 30, 891-900.

Farré, M., García, M.J., Tirapu, L., Ginebreda, A., Barceló, D. (2001). Wastewater toxicity screening of non-ionic surfactants by Toxalert® and Microtox® bioluminescence inhibition assays. *Anal Chim Acta*, 427, 181-189.

Gagnè, F., Andrè, C., Cejka, P., Hausler, R., Fournier, M., Blaise, C. (2008). Immunotoxic effects on freshwater mussels of primary-treated wastewater before and after ozonation: A pilot plant study. *Ecotox Environ Safe*, 69, 366-373.

Gaki, E., Banou, S., Ntigkakis, D., Andreadakis, A., Borboudaki, K., Drakopoulou, S., Manios, T. (2007). Qualitative monitoring of tertiary treated wastewater reuse extensive distribution system: total coliforms number and residual chlorine concentration. *J Environ Sci Heal* A, 42, 601-611.

Hemming, J.M., Turner, P.K., Brooks, B.W., Waller, W.T., La Point, T.W. (2002). Assessment of toxicity reduction in wastewater effluent flowing through a treatment wetland using *Pimephales promelas*, *Ceriodaphnia dubia*, and *Vibrio fischeri*. *Arch Environ Contam Toxicol*, 42(1), 9-16.

Hernando, M.D., Fernandez-Alba, A.R., Tauler, R., Barcelò, D.(2005). Toxicity assays applied to wastewater treatment. *Talanta*, 65, 358-366.

Huerta-Fontela, M., Galceran, M.T., Martin-Alonso, J., Ventura, F. (2008). Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. *Sci Total Environ*, 397, 31-40.

Lu, W.Q., Chen, X.N., Yue, F., Jenter, C., Gminski, R., Li, X.Y., Xie, H., Merschï Sundermann, V. (2002). Studies on the in vivo and in vitro mutagenicity and the lipid peroxidation of chlorinated surface (drinking) water in rats and metabolically competent human cells. *Mutat Res*, 513(1ï 2), 151ï 157.

Lu, W.Q., Chen, D., Wu, X.J., Liu, A.L., Liu, H., Wu, J.J., Mersch- Sundermann, V. (2004). DNA damage caused by extracts of chlorinated drinking water in human derived liver cells (HepG2). *Toxicology*, 198(1ï 3), 351ï 357.

Mantis, I., Voutsa, D., Samara, C. (2005). Assessment of environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods. *Ecotox Environ Safe*, 62, 397-407.

Manusadǵianas, L., Balkelytā, L., Sadauskas, K., Blinova, I., Põllumaa, L., Kahru, A. (2003). Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of biotests, and correspondence between toxicity and chemical-based indices. *Aquat Toxicol*, 63, 27-41.

Matamoros, V., Mujeriego, R., Bayona, J.M. (2007). Trihalomethane occurrence in chlorinated reclaimed water at full-scale wastewater treatment plants in NE Spain. *Water Res*, 41, 3337-3344.

Microtox™ acute toxicity Basic Test. Azur Environmental (1995). Carlsbad, CA, USA.

Monarca, S., Feretti, D., Collivignarelli, C., Gazzella, L., Zerbini, I., Bertanza, G., Pedrazzani, R. (2000). The influence of different disinfectants on mutagenicity and toxicity of urban wastewater. *Water Res*, 34(17), 4261-4269.

Organization for Economic Cooperation and Development (OECD) (1984a). *Daphnia* spp. Acute immobilization and reproduction test. OECD Guideline for Testing Chemicals 202. OECD, Geneva, Switzerland.

Organization of Economic Cooperation and Development (OECD) (1984b). Algal growth inhibition test. OECD Guideline for Testing Chemicals 201. OECD, Paris, France.

Ottavini, M., Bonadonna, L. (2000). Analytical methods for drinking water. Rapporti ISTISAN 00/14, 1-223.

Persoone, G., Marsalek, B., Blinova, I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L., Kolar, B. (2003). A practical and user-friendly toxocity classification system with microbiotests for natural waters and wastewaters. *Environ Toxicol*, 18, 395-402.

Petala, M., Samaras, P., Zouboulis, A., Kungolos, A., Sakellaropoulos, G.P. (2008). Influence of ozonation on the in vitro mutagenic and toxic potential of secondary effluents. *Water Res*, 42, 4929-4940.

Ra, J.S., Kim, H. K., Chang, N.I., Kim, S.D. (2007). Whole Effluent Toxicity (WET) tests on wastewater treatment plants with *Daphnia magna* and *Selenastrum capricornutum*. *Environ Monit Assess*, 129 (1-3), 107-113.

Ricco, G., Tomei, M.C., Ramadori, R., Laera, G. (2004). Toxicity assessment of common xenobiotic compounds on municipal activated sludge: comparison between respirometry and Microtox®. *Water Res*, 38, 2103-2110.

Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., DeMarini, D.M. (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutat Res*, 636, 178ï 242.

Shi, Y., Cao, X., Tang, F., Du, H., Wang, Y., Qiu, X., Yu, H., Lu, B. (2009). In vitro toxicity of surface water disinfected by different sequential treatments. *Water Res*, 43, 218-228.

Sponza, D.T. (2003). Application of toxicity tests into discharges of pulp-paper industry in Turkey. *Ecotox Environ Safe*, 54, 74-86.

Tiġler, T., Zagorc-Konļ an, J. (1999). Toxicity evaluation of wastewater from the pharmaceutical industry to aquatic organisms. *Water Sci Tech*, 39(10-11), 71-76.

Thomson, J.E., Blatchley III, E.R. (1999). Toxicity effects of ɔ-irradiated wastewater effluents. *Water Res*, 33(9), 2053-2058.

U.S. EPA (1991). Technical support document for water quality-based toxic control. EPA/505/2-90-001, Washington, DC, USA.

Wang, L.S., Wei, D.B., Wei, J., Hu, H.Y. (2007). Screening and estimating of toxicity formation with *Photobacterium* bioassay during chlorine disinfection of wastewater. *J Hazard Mater*, 141, 289-294.

Watkinson, A.J., Murby, E.J., Kolpin, D.W., Costanzo, S.D. (2009). The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. *Sci Total Environ*, 407, 2711-2723.

Wu, Q.Y., Hu, H.Y., Zhao, X., Li, Y. (2010). Effects of chlorination on the properties of dissolved organic matter and its genotoxicity in secondary sewage effluent under two different ammonium concentrations. *Chemosphere*, 80(8), 941-946.

Yuan, J., Wu, X.J., Lu, W.Q., Cheng, X.L., Chen, D., Li, X.Y., Liu, A.L., Wu, J.J., Xie, H., Stahl, T., Mersch-Sundermann, V. (2005). Chlorinated river and lake water extract caused oxidative damage, DNA migration and cytotoxicity in human cells. *Int J Hyg Environ Health*, 208(6), 481ï 488.

Yuan, J., Liu, H., Zhou, L.H., Zou, Y.L., Lu, W.Q. (2006). Oxidative stress and DNA damage induced by a drinking-water chlorination disinfection byproduct 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in mice. *Mut Res*, 609(2), 129ï 136.

Zouboulis, A., Samaras, P., Ntampou, X., Petala, M. (2007). Potential Ozone Applications for Water/Wastewater Treatment. *Sep Sci Technol*, 42, 1433ï 1446.

FIGURE CAPTIONS

Fig. 1 TU mean values of the influent (IN) and effluent (OUT) samples.

Fig. 2 Calculated Toxicity (C) of the recipient water body (Dora Riparia River, Collegno, Torino, Italy) expressed in Toxic Unit (TU) and U.S. EPA acceptance limit.

Fig. 3 *E. coli* concentration in effluent samples chlorinated and not-chlorinated.

Fig. 4 Total THMs, DBPs, and industrial THMs in effluent samples chlorinated and not-chlorinated.

Table 1 Hazard classification system for wastes discharged into the aquatic environment proposed by Persoone et al. (2003).

TU	Class	Toxicity
< 0.4	Class I	No acute toxicity
0.4 < TU < 1	Class II	Slight acute toxicity
1 < TU < 10	Class III	Acute toxicity
10 < TU < 100	Class IV	High acute toxicity
TU > 100	Class V	Very high acute toxicity

Table 2 Toxicity with Microtox™, *D. magna*, *P. subcapitata*, *E. coli*, THMs concentration in the Upstream WWTP (US) sampling point.

Site and sampling	*V. fischeri* (TU)	*D. magna* (TU)	*P. subcapitata* (TU)	*E. coli* (CFU/100 ml)	THMs (µg/L)
Upstream					
1 (2005)	N.T.	N.T.	N.T.	30,000	N.D.
2 (2005)	N.T.	N.T.	N.T.	15,000	N.D.
3 (2005)	N.T.	N.T.	N.T.	33,000	N.D.
4 (2005)	N.T.	N.T.	N.T.	10,000	N.D.
5 (2005)	N.T.	N.T.	N.T.	48,000	N.D.
6 (2005)	N.T.	N.T.	N.T.	10,000	N.D.
7 (2005)	N.T.	N.T.	N.T.	150,000	N.D.
8 (2005)	N.T.	N.T.	N.T.	33,000	N.D.
9 (2005)	N.T.	N.T.	N.T.	N.D.	N.D.
10 (2005)	N.T.	N.T.	N.T.	30,000	N.D.
11 (2006)	N.T.	N.T.	N.T.	69,000	0.11
12 (2006)	N.T.	N.T.	N.T.	87,000	<0.10
13 (2006)	N.T.	N.T.	N.T.	61,000	0.24
14 (2006)	N.T.	N.T.	N.T.	37,000	0.53
15 (2006)	N.T.	N.T.	N.T.	34,000	0.56
16 (2007)	0.69	N.T.	N.T.	31,000	0.47
17 (2007)	N.T.	N.T.	N.T.	30,000	0.68
18 (2007)	N.T.	N.T.	N.T.	25,000	0.64
19 (2007)	N.T.	N.T.	N.T.	18,000	0.27
20 (2007)	N.T.	N.T.	N.T.	29,000	0.37
21 (2007)	N.T.	N.T.	N.T.	18,000	0.45
22 (2007)	N.T.	N.T.	N.T.	73,000	0.65

N.T. = not toxic; N.D. = not determined

Table 3 Toxicity with Microtox™, *D. magna*, *P. subcapitata*, *E. coli*, THMs concentration in the WWTP Influent (IN) sampling point.

Site and sampling	*V. fischeri* (TU)	*D. magna* (TU)	*P. subcapitata* (TU)	*E. coli* (CFU/100 ml)	THMs (µg/L)
Influent					
1 (2005)	1.08	N.T.	N.T.	270,000	N.D.
2 (2005)	1.18	N.T.	N.T.	1,600,000	N.D.
3 (2005)	0.76	N.T.	N.T.	1,900,000	N.D.
4 (2005)	1.46	N.T.	N.T.	12,000,000	N.D.
5 (2005)	1.70	N.T.	N.T.	9,200,000	N.D.
6 (2005)	1.08	N.T.	N.T.	13,000,000	N.D.
7 (2005)	3.86	N.T.	N.T.	4,500,000	N.D.
8 (2005)	2.63	N.T.	N.T.	12,000,000	N.D.
9 (2005)	2.44	N.T.	N.T.	11,000,000	N.D.
10 (2005)	4.47	N.T.	N.T.	8,400,000	N.D.
11 (2006)	0.88	1.09	N.T.	770,000	1.27
12 (2006)	1.05	N.T.	N.T.	980,000	3.15
13 (2006)	1.01	N.T.	N.T.	11,000,000	4.77
14 (2006)	1.14	N.T.	N.T.	12,000,000	4.60
15 (2006)	0.68	N.T.	N.T.	14,000,000	4.98
16 (2007)	1.02	N.T.	N.T.	9,800,000	1.39
17 (2007)	1.11	N.T.	N.T.	24,000,000	3.64
18 (2007)	1.15	N.T.	N.T.	11,000,000	1.86
19 (2007)	1.91	N.T.	N.T.	1,300,000	2.03
20 (2007)	3.72	2.05	N.T.	110,000	2.52
21 (2007)	1.92	N.T.	N.T.	8,700,000	1.96
22 (2007)	0.93	N.T.	1.18	170,000	1.28

N.T. = not toxic; N.D. = not determined

Table 4 Toxicity with Microtox™, *D. magna*, *P. subcapitata*, *E. coli*, NaOCl, residual Chlorine (RCHL) and THMs concentration in the WWTP effluent (OUT) sampling point.

Site and sampling	*V. fischeri* (TU)	*D. magna* (TU)	*P. subcapitata* (TU)	*E. coli* (CFU/100 ml)	NaOCl (mg/L)	RCHL (mg/L)	THMs (µg/L)
Effluent							
1 (2005)	N.T.	N.T.	N.T.	50,000	0.00	N.D.	N.D.
2 (2005)	N.T.	N.T.	N.T.	27,000	0.00	N.D.	N.D.
3 (2005)	N.T.	N.T.	N.T.	19,000	0.00	N.D.	N.D.
4 (2005)	N.T.	N.T.	N.T.	37,000	3.32	0.30	N.D.
5 (2005)	N.T.	N.T.	2.24	12,000	3.69	0.17	N.D.
6 (2005)	N.T.	N.T.	N.T.	41,000	3.88	0.17	N.D.
7 (2005)	N.T.	N.T.	N.T.	3,500	3.66	N.D.	N.D.
8 (2005)	13.83	3.53	4.17	18,000	3.52	0.62	N.D.
9 (2005)	3.17	N.T.	N.T.	18,000	1.80	0.22	N.D.
10 (2005)	5.20	1.68	3.15	8,500	2.00	N.D.	N.D.
11 (2006)	N.T.	N.T.	N.T.	2,400,000	0.00	N.D.	0.44
12 (2006)	N.T.	N.T.	N.T.	16,000	2.44	<0.05	1.51
13 (2006)	N.T.	N.T.	N.T.	17,000	0.00	N.D.	0.63
14 (2006)	N.T.	N.T.	N.T.	21,000	2.89	<0.05	0.90
15 (2006)	N.T.	N.T.	N.T.	18,000	0.00	N.D.	0.99
16 (2007)	3.31	N.T.	1.75	1,800	4.58	0.63	7.72
17 (2007)	N.T.	N.T.	N.T.	69,000	0.00	N.D.	1.05
18 (2007)	7.01	8.30	N.T.	1,700	5.00	1.01	2.85
19 (2007)	0.40	N.T.	N.T.	9,900	3.61	N.D.	0.85
20 (2007)	3.78	N.T.	N.T.	8,300	4.07	0.59	1.54
21 (2007)	5.68	N.T.	N.T.	200	3.38	0.51	5.14
22 (2007)	7.59	7.28	4.19	13,000	2.82	0.08	1.40

Table 5 Toxicity with Microtox™, *D. magna*, *P. subcapitata*, *E. coli*, THM concentration in the Downstream WWTP (DS) sampling point.

Site and sampling	*V. fischeri* (TU)	*D. magna* (TU)	*P. subcapitata* (TU)	*E. coli* (CFU/100 ml)	THMs (µg/L)
Downstream					
1 (2005)	N.T.	N.T.	N.T.	20,000	N.D.
2 (2005)	N.T.	N.T.	N.T.	16,000	N.D.
3 (2005)	N.T.	N.T.	N.T.	16,000	N.D.
4 (2005)	N.T.	N.T.	N.T.	29,000	N.D.
5 (2005)	N.T.	N.T.	N.T.	39,000	N.D.
6 (2005)	N.T.	N.T.	N.T.	10,000	N.D.
7 (2005)	N.T.	N.T.	N.T.	100,000	N.D.
8 (2005)	N.T.	N.T.	N.T.	29,000	N.D.
9 (2005)	N.T.	N.T.	N.T.	N.D.	N.D.
10 (2005)	N.T.	N.T.	N.T.	26,000	N.D.
11 (2006)	N.T.	N.T.	N.T.	120,000	0.15
12 (2006)	N.T.	N.T.	N.T.	44,000	0.15
13 (2006)	N.T.	N.T.	N.T.	46,000	0.40
14 (2006)	N.T.	N.T.	N.T.	34,000	0.51
15 (2006)	N.T.	N.T.	1.55	39,000	0.65
16 (2007)	0.94	N.T.	N.T.	24,000	0.53
17 (2007)	N.T.	N.T.	N.T.	31,000	0.68
18 (2007)	0.68	N.T.	N.T.	26,000	1.03
19 (2007)	N.T.	N.T.	N.T.	12,000	0.25
20 (2007)	N.T.	N.T.	N.T.	13,000	0.38
21 (2007)	N.T.	N.T.	N.T.	19,000	0.74
22 (2007)	N.T.	N.T.	N.T.	61,000	0.44

N.T. = not toxic; N.D. = not determined

Table 6 Comparison of toxicity (TU), *E. coli,* NaOCl, THMs, DBPs and residual chlorine (RCHL) in the effluent site (OUT).

Spearman correlations	r	p
TU vs NaOCl	0.539	< 0.01
TU vs THMs	0.664	< 0.05
TU vs DBPs	0.788	< 0.01
TU vs *E. coli*	0.660	< 0.01
TU vs RCHL	0.657	< 0.01
THMs vs *E. coli*	0.850	< 0.01
THMs vs RCHL	0.865	< 0.01
NaOCl vs *E. coli*	0.631	< 0.01
NaOCl vs THMs	0.676	< 0.05
NaOCl vs DBPs	0.715	< 0.01
NaOCl vs RCHL	0.740	< 0.01
RCHL vs E. coli	0.428	< 0.05
RCHL vs DBPs	0.835	< 0.01

Figure 1

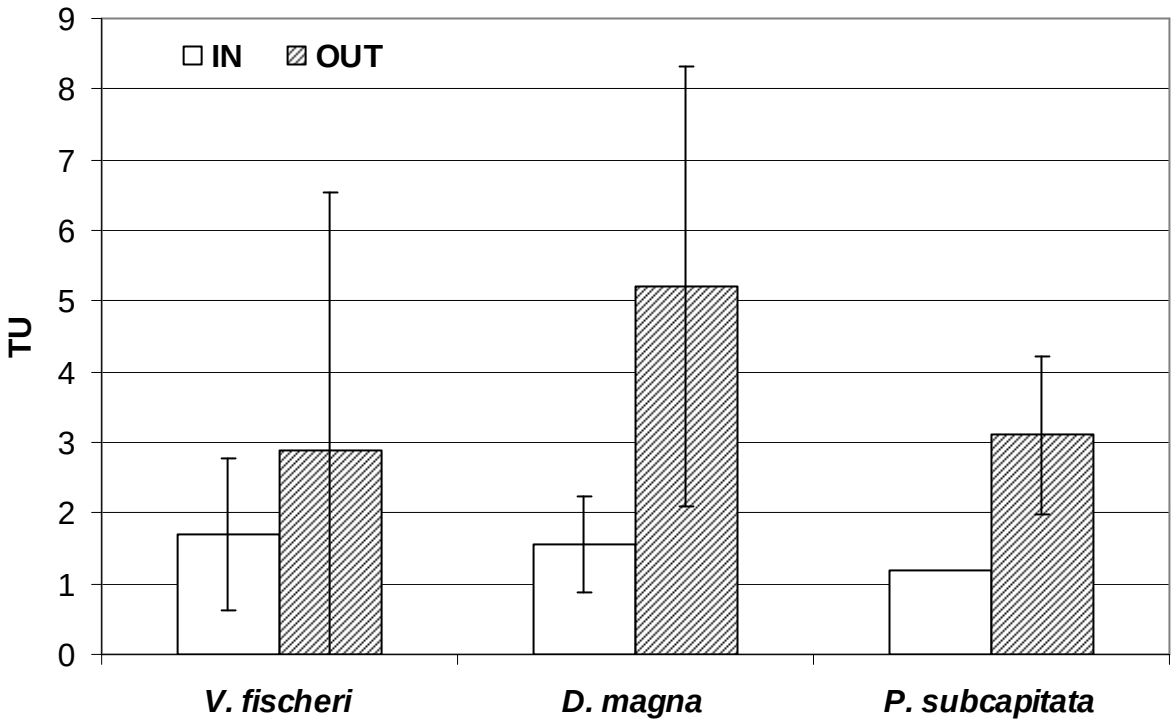


Figure 2

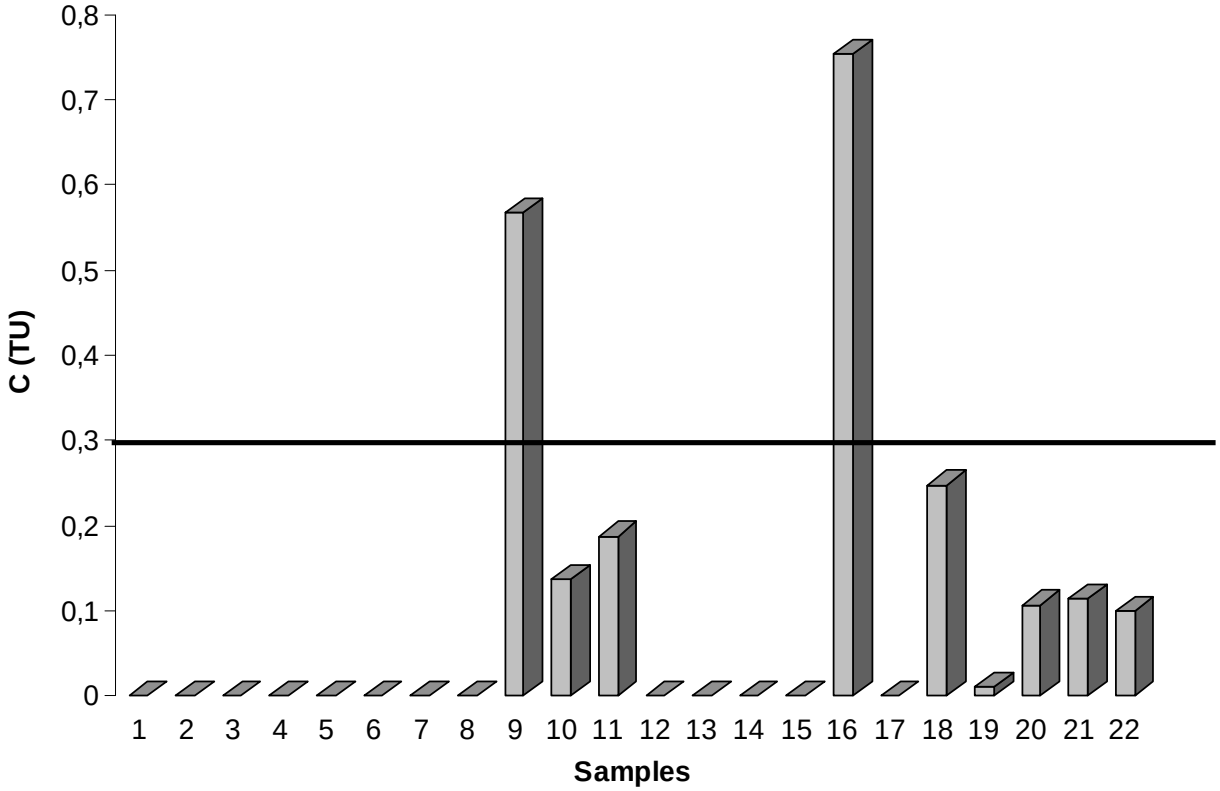


Figure 3

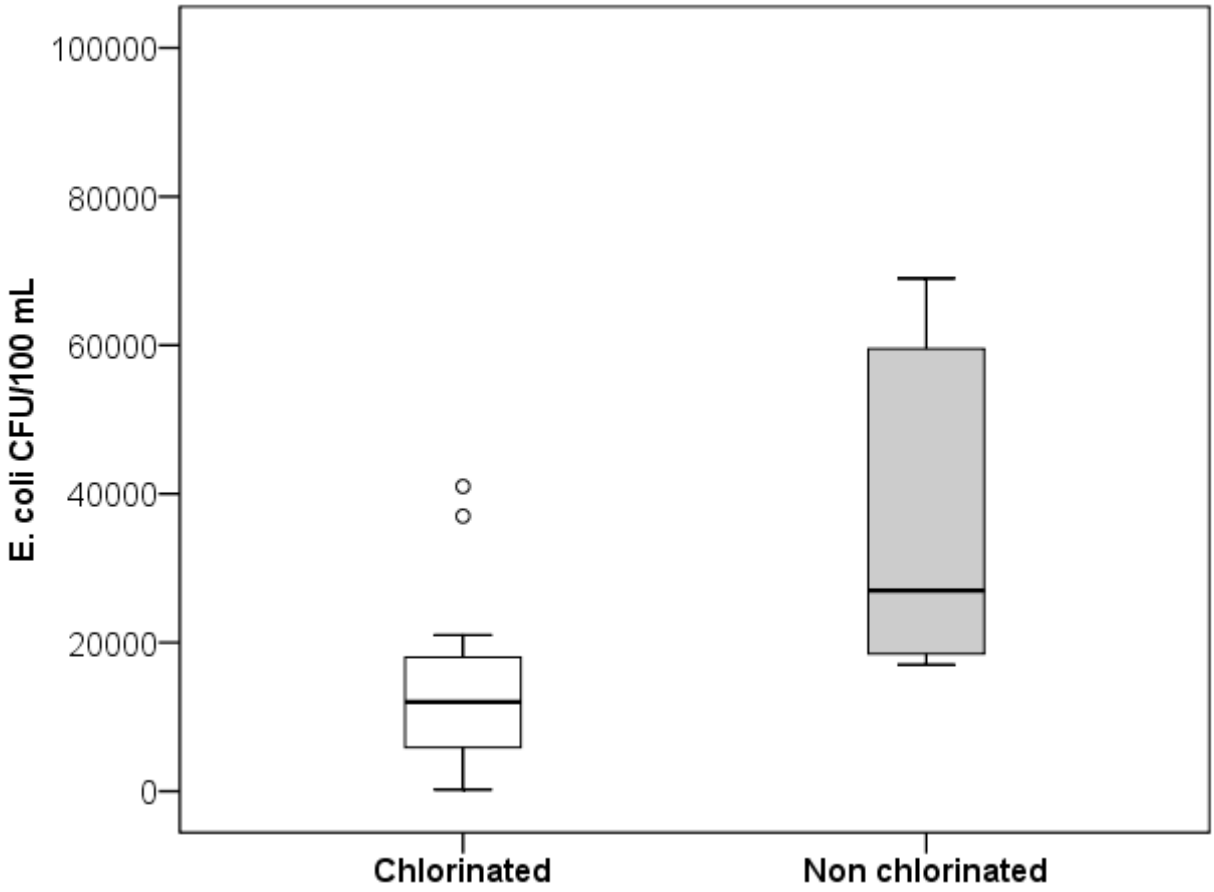
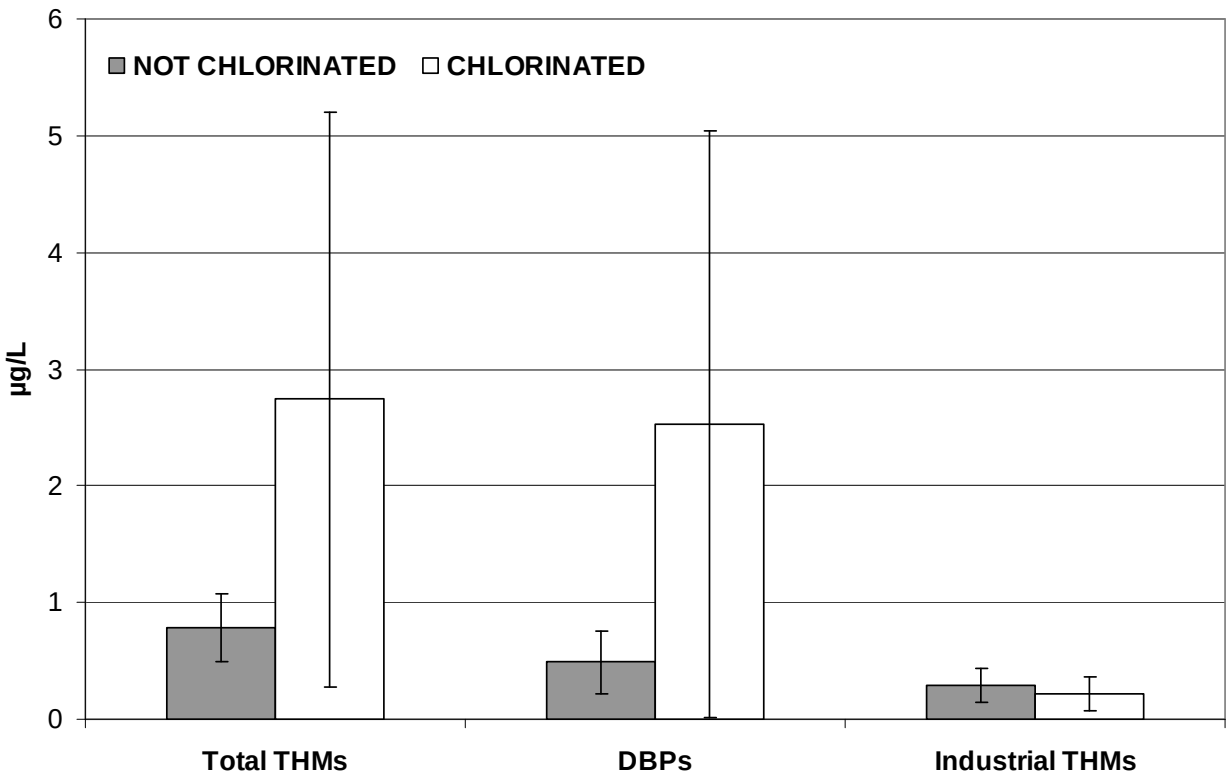

100000
80000
60000
40000
20000
0
E. coli CFU/100 mL
Chlorinated
Non chlorinated

Figure 4


NOT CHLORINATED
CHLORINATED
µg/L
0
1
2
3
4
5
6
Total THMs
DBPs
Industrial THMs